Tomas Bata University in Zlín
Faculty of Technology

Habilitační práce

Faktory ovlivňující funkční vlastnosti tavených sýrů

Factors affecting the functional properties of processed cheeses

Autor: **Ing. Richardos Nikolaos Salek, Ph.D.**

Obor: Technologie potravin

Zlín, 2021

Vydala **Univerzita Tomáše Bati ve Zlíně** v edici **Habilitation Thesis.**
Publikace byla vydána v roce 2021.

Klíčová slova: *přírodní sýr; tavený sýr; tavená sýrová omáčka; tavící soli; procesní parametry; stupeň zralosti; skladování*.

Keywords: *natural cheese; processed cheese; processed cheese sauce; emulsifying salts; maturity degree; storage*.

ABSTRACT

The scope of the current work was to explain the importance of emulsifying salts, natural cheese (type and maturity degree) and selected technological properties during processed cheese production. Firstly, principles of emulsifying salts action in the system or processed cheese were described. The work was focused on phosphate- and citrate-based emulsifying salts. The role of solely applied phosphates and citrate (sodium salts) also was discussed. Additionally, more complicated systems consisted of binary and ternary mixtures of phosphate and/or citrate emulsifying salts were also described. Moreover, in the habilitation thesis are described basic/general natural cheese-making processing steps. In addition, selected natural cheese varieties (Edam, Mozzarella, Swiss-type, Cheddar and white brined cheeses, respectively), specific producing steps and their typical characteristics are presented, since these varieties are among the most applicable cheese types during the industrial production of processed cheese. Furthermore, the impact of natural cheese maturity degree (or in other words its intact casein content) on the resultant processed cheese properties (mainly textural and rheological characteristics) is also mentioned. On the whole, the effect of divergent cheese varieties on the above-mentioned properties of processed cheese is also described. Furthermore, the impact of selected target processing parameters (dry matter content, fat in dry matter content) and specific technological characteristics (melting temperature, holding time, speed of agitation) on processed cheese properties was discussed. On the whole, the habilitation thesis aimed to summarize the existing knowledge in the field of characteristics of raw materials for the production of processed cheeses, production technology of these products and factors influencing the consistency (a parameter described mainly by textural and rheological properties) of processed cheeses. Based on the results of the current thesis, it is possible to provide a more comprehensive point of view of the importance of the composition of a mixture of raw ingredients (including natural cheese, emulsifying salts – phosphates and/or citrates) and processing parameters in influencing the textural and rheological properties of processed cheeses.

ABSTRAKT

Záměrem této práce bylo vysvětlit význam tavicích solí, přírodního sýra (druh a stupeň zralosti) a vybraných technologických parametrů při výrobě taveného sýra. Byly popsány principy působení tavicích solí ať již v modelovém systému mléka, tak v reálné matrici taveného sýra. Práce byla cílena na tavicí soli na bázi fosforečnanů a citronanů. Diskutována byla také role samostatně aplikovaných sodných solí fosforečnanů a citronanů. Dále byly popsány složitější systémy sestávající z binárních a ternárních směsí fosforečnanových a/nebo citronanových tavicích solí. V habilitační práci jsou dále popsány obecné kroky výroby přírodního sýra. Kromě toho byly v textu představeny vybrané druhy přírodních sýrů (konkrétně Edam, Mozzarella, sýr švýcarského typu, čedar a bílé sýry zrající v solném nálevu), specifické kroky výroby a jejich typické vlastnosti. Dále byl zmíněn dopad stupně zralosti přírodního sýra, nebo jinými slovy obsahu intaktního kaseinu, na výsledné vlastnosti taveného sýra (zejména texturní a reologické vlastnosti). Byl také popsán vliv odlišných druhů sýrů na výše uvedené vlastnosti taveného sýra. Dále byl diskutován vliv vybraných cílových procesních parametrů (obsah sušiny, obsah tuku v sušině) a specifické technologické charakteristiky (teplota tavení, doba výdrže tavicí teploty a rychlost míchání taveniny v průběhu tavicího procesu) na vlastnosti taveného sýra. Habilitační práce jako celek měla za cíl shrnout dosavadní znalosti v oblasti charakteristiky surovin, technologie výroby a faktorů ovlivňujících konzistenci tavených sýrů (parametr popsaný zejména texturními a reologickými vlastnostmi). Výsledky této práce poskytují komplexnější pohled na důležitost složení směsí surovin (včetně přírodního sýra, výběru tavicích solí – fosforečnanů a/nebo citronanů) a procesních parametrů, tedy faktorů, které mohou následně významně ovlivnit texturní a reologické vlastností konečných výrobků, tavených sýrů.

„*Ἓν οἶδα ὅτι οὐδὲν οἶδα*“
„ *I know one thing, that I know nothing*“
Socrates

„*The current work is dedicated to my wife for her never-ending patience and unlimited love*“

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincerest gratitude to anyone who contributed in any way to my personal and professional growth, and guided me in my life and helped me in order to fulfil the current work. Furthermore, I would like to express my deepest thanking to prof. Ing. František Buňka, Ph.D., for his valuable comments and advice in conducting the scientific experiments, evaluating obtained data and writing the work. I would also like to express my wholeheartedly thanking and appreciation to doc. MVDr. Michaela Černíková, Ph.D., for her help and cooperation during our common scientific activities. Additionally, I would like to extend my thanks to my friends, colleagues, technicians and students of the Department of Food Technology, Faculty of Technology at Tomas Bata University in Zlín, for their help and support. Last but not least, I would like to extend my great thanks to my family, wife and daughter, who respected the time required for this work and have supported me for this path that I have chosen.

TABLE OF CONTENTS

List of selected publications which are part and parcel of the habilitation

A. The impact of emulsifying salts composition and natural cheese on the functional properties of processed cheese and similar products.

A1. Nagyová, G., Buňka, F., **Salek, R. N.**, Černíková, M., Mančík, P., Grůber, T., & Kuchař, D. (2014). Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. *Journal of Dairy Science*, *97*(1), 111-122. https://doi.org/10.3168/jds.2013-7210

A2. Salek, R. N., Černíková, M., Nagyová, G., Kuchař, D., Bačová, H., Minarčíková, L., & Buňka, F. (2015). The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. *International Dairy Journal, 44*, 37-43. https://doi.org/10.1016/j.idairyj.2014.12.009

A3. Salek, R. N., Černíková, M., Maděrová, S., Lapčík, L., & Buňka, F. (2016). The effect of different composition of ternary mixtures of emulsifying salts on the consistency of processed cheese spreads manufactured from Swiss-type cheese with different degrees of maturity. *Journal of Dairy Science, 99*(5), 3274-3287. https://doi.org/10.3168/jds.2015-10028

A4. Salek, R. N., Černíková, M., Pachlová, V., Bubelová, Z., Konečná, V., & Buňka, F. (2017). Properties of spreadable processed Mozzarella cheese with divergent compositions of emulsifying salts in relation to the applied cheese storage period. *LWT – Food Science & Technology, 77*, 30-38. https://doi.org/10.1016/j.lwt.2016.11.0198

A5. Salek, R. N., Černíková, M., Lorencová, E., Pachlová, V., Kůrová, V., Šenkýřová, J., & Buňka, F. (2020). The impact of Cheddar or white brined cheese with various maturity degrees on the processed cheese consistency: A comparative study. *International Dairy Journal*, *111*. https://doi.org/10.1016/j.idairyj.2020.104816

A6. Salek, R. N., Vašina, M., Lapčík, L., Černíková, M., Lorencová, E., Li, P., & Buňka, F. (2019). Evaluation of various emulsifying salts addition on selected properties of processed cheese sauce with the use of mechanical vibration damping and rheological methods. *LWT – Food Science & Technology, 107*, 178-184. https://doi.org/10.1016/j.lwt.2019.03.022

B. The impact of selected processing parameters on the functional properties of processed cheese

B1. Černíková, M., Nebesářová, J., **Salek, R. N.**, Řiháčková, L., & Buňka, F. (2017). Microstructure and textural and viscoelastic properties of model processed cheese with

different dry matter and fat in dry matter content. *Journal of Dairy Science*, *100*(6), 4300-4307. https://doi.org/10.3168/jds.2016-12120

B2. Černíková, M., **Salek, R. N.**, Kozáčková, D., & Buňka, F. (2018). The effect of different agitations and temperature maintainings on viscoelastic properties of full-fat processed cheese spreads. *LWT – Food Science & Technology, 89,* 244-247. https://doi.org/10.1016/j.lwt.2017.10.054

B3. Přikryl, J., Hájek, T., Švecová, B., **Salek, R. N.**, Černíková, M., Červenka, L., & Buňka, F. (2018). Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions. *LWT – Food Science & Technology, 87*, 266-271. https://doi.org/10.1016/j.lwt.2017.08.093

B4. Černíková, M., **Salek, R. N.**, Kozacková, D., Běhalová, H., Luňaková, L., & Buňka, F. (2017). The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads. International Dairy Journal, 66, 84-90. https://doi.org/10.1016/j.idairyj.2016.11.007

B5. Pluta-Kubica, A., Černíková, M., Dimitreli, G., Nebesářová, J., Exarhopoulos, S., Thomareis, A. S., **Salek, R. N.**, & Buňka, F. (2021). Influence of the melt holding time on fat droplet size and the viscoelastic properties of model spreadable processed cheeses with different compositions. *International Dairy Journal*, *113*. https://doi.org/10.1016/j.idairyj.2020.104880

B6. Černíková, M., Nebesářová, J., **Salek, R. N.**, Popková, R., & Buňka, F. (2018). The effect of rework content addition on the microstructure and viscoelastic properties of processed cheese. *Journal of Dairy Science*, *101*(4), 2956-2962. https://doi.org/10.3168/jds.2017-13742

Introduction

The term “processed cheese” characterizes a dairy product produced by heating a mixture of various cheese types/varieties of different degrees of maturity in the presence of appropriate emulsifying salts (mostly sodium phosphate, polyphosphates, citrates and/or their combinations), usually under reduced pressure (vacuum) with constant stirring, commonly in the temperature range of 90 – 100 °C, until a smooth and homogenous compact mass is formed with desired textural and organoleptic properties. Optional dairy (butter, anhydrous milk fat, skim milk powder, whey powder, coprecipitates, caseinates, etc.) and non-dairy (water, vegetables, spices, flavorings, colorings, salt, hydrocolloids, preservatives, etc.) ingredients can be added into the blend (Guinee et al., 2004; Kapoor & Metzger, 2008). The discontinuous production of processed cheeses includes the following steps: (i) determining the composition of ingredients (with respect to the desired parameters of the final product); (ii) placing the determined amounts of ingredients and additives into the melting equipment and the actual melting process is run (at a usual temperature of 85 to 105 °C with a holding time of several minutes); and (iii) packaging in different materials (Guinee et al., 2004; Mizuno & Lucey, 2007). Furthermore, emulsifying salts play a key-role during the manufacturing of processed cheese. In particular, they possess the ability to sequester calcium from the casein matrix by exchanging sodium ions, which results in the conversion of insoluble calcium paracaseinate into the more soluble sodium paracaseinate (Guinee et al., 2004; Kapoor & Metzger, 2008). Thus, within the matrix sodium paracaseinate acts as a “true” emulsifier at the oil-in-water interface. The control and stabilization of the pH level and an influence on the formation of a final structure after cooling are some of the additional effects of emulsifying salts (Dimitreli & Thomareis, 2009; El-Barky et al., 2011; Guinee et al., 2004). Nevertheless, not all emulsifying salts have the same calcium ion-exchange ability. The phosphate ion-exchange ability increases with increasing P_2O_5 content in the following order: monophosphate < diphosphate < triphosphate < polyphosphate (Buňka et al., 2013, Guinee et al., 2004; Shirashoji et al., 2006). In addition, El-Bakry et al. (2011) and Mizuno & Lucey (2005) stated that trisodium citrate presents better calcium chelating ability and casein peptization properties than do sodium mono- and diphosphates. Furthermore, the consistency of processed cheese can be influenced by many factors, including the following: (i) raw material composition – the type and chemical profile of the natural cheese applied (dry-matter, fat, protein, and calcium ion contents, and maturity degree), composition and concentration of emulsifying salts, addition of other optional dairy and non-dairy ingredients and also the pH of the mass to be melted; and (ii) processing and storage conditions – agitation speed, target melting temperature and holding time, cooling rate and also storage temperature. Nowadays, hydrocolloids, regularly are applied during the production of processed cheese, are important components affecting the consistency of processed cheese (Dimitreli & Thomareis, 2007; Shirashoji, et al., 2006).

1. Current state of the examined topic

1.1 Processed cheese characteristics, production and trends

1.1.1 Historical background

Natural cheese varieties/types are known and appreciated as a valuable food component since ancient Roman times (more than 2,000 years ago). Processed cheese could be characterized as a "new-born" dairy product, originating in the 20th century. Moreover, the idea of processed cheese was though based on natural cheese (Tamine, 2011). The production of processed cheese started in Europe and might be dated near to the early 1890s. It may be proposed, that the concept (or idea) of processed cheese originated from the search of new strategies for a more "*sophisticated*" manufacture technique of cheese, in order to extend the shelf-life of natural cheese, by-passing traditional preservation methods (including air-drying or smoking techniques). Likewise, another possible reason could be the desire to develop a new dairy product characterized by milder taste and more stable structure (Tamine, 2007; 2011). According to Guinee et al. (2004) the idea of processed cheese stemmed from a typical Swiss traditional dish, called "*Fondue*". For the preparation of which natural cheese is melted (together with the application of heat) in the presence of wine, in which tartrate from the wine provided an emulsifying effect. However, Jan Hendrikzoon was the first who established a thermal treatment for canned Gouda-cheese in 1899 (Kammerlehner, 2012). In addition, the Swiss researchers Walter Gerber and Fritz Stettler closely followed traditional fondue preparation and, after several experiments with tartaric acid, added sodium citrate prior to heat-processing of the shredded raw material (natural cheese) to achieve a stable but modified final product (Tamine, 2011). Industrial production of processed cheese began in Europe and the USA between 1910 and 1920. The first processed cheese was developed by the Swiss scientists mentioned earlier; Walter Gerber and Fritz Stettler in Thun, Switzerland (1911). At this certain production attempt, natural Emmentaler cheese was applied in order to produce a heat-treated cheese (known as "*Schachtelkäse*") by the addition of sodium citrate (as an emulsifying salt). Nearly at the same time period in the USA (1917), independently, James Lewis Kraft, an entrepreneur, inventor and industrialist started working with pieces of Cheddar cheese, citrates and monophosphates. Hence, in 1916, he published a patent describing the method of heating cheese and its emulsification in the presence of alkaline salts. However, these early attempts were of limited success in order to manufacture a good-quality processed cheese. The process became widespread by the 1930s, due to the appearance of polyphosphate emulsifying salts on the world market (Fox, 2000; Hui, 2005; Tamine, 2007; 2011; Zerfiridis, 2001).

1.1.2 Processed cheese classification

Processed cheese is a multicomponent dairy complex matrix also described as stable oil-in-water emulsion/system (Chen & Liu, 2012; Hanaei et al., 2015; Lee et al., 2004). In a physicochemical point of view, processed cheese could be characterized as a dispersion of fat droplets in a concentrated, gelled protein network (Hasenhuetl & Hartel, 2008). Therefore, the multilateralism of the above-mentioned system (processed cheese) derives from the fact that it can contain plethora of interacting components and relatively high amount of water content (Marchesseau et al., 1997). Thus, the processed cheese matrix can be formed by blending shredded natural cheese (of different types/varieties and maturity degrees) in the presence of appropriate amount and type of emulsifying salts (mainly sodium salts of phosphates, polyphosphates and/or citrates), under partial vacuum, constant stirring and upon heat treatment; resulting in the constitution of a homogeneous and smooth mass with desired functional and organoleptic properties (Buňka, et al., 2009; Guinee, 2011; Sádlíková et al., 2010; Zerfiridis, 2001). Additionally, the application of heat during the processing can inactivate starter culture microorganisms and other bacteria, including also enzymes, all present in the natural cheese applied; resulting in the shelf-life extension of the final product (Tamine, 2007; 2011).

However, there is no specific European Union legislation on processed cheese. For a more complete oversight on the legislation of processed cheese and related products within the European Union, it is necessary to look at a selection of Member States. In addition, processed cheese according to international standards is grouped by the following characteristics: composition, water content and consistency. According to these characteristics, exist three main categories (mainly in the USA): processed cheese blocks, processed cheese foods and processed cheese spreads. Moreover, additional subcategories can be processed cheese slices and smoked processed cheese (Tamine, 2007; 2011). Furthermore, according to the decree 397/2016 Collection of Laws, edited by the Czech Ministry of Agriculture, defining the requirements for milk and milk products, ice creams and edible fats and oils, processed cheese (*tavený sýr* – in Czech) is defined as a dairy product which was thermally treated in the presence of emulsifying salts and at least 51 % (w/w) of the dry matter content of processed cheese shall originate from natural cheese. In addition, if the product contains more than 5 % (w/w) of lactose, must be designated as processed cheese product (*tavený sýrový výrobek* – in Czech).

1.1.3 Manufacture principles and steps in processed cheese manufacture

In general, processed cheese production protocol is characterized by complexity and thus, it is primarily influenced by the chemical interactions occurring between the applied ingredients of dairy origin and the emulsifying salts utilized. On the contrary, the principal technological operations of processed cheese manufacture are rather characterized as "simple"; however, they require skillful and professional handling in order to control all the used ingredients, their concentrations in the blend and processing parameters/conditions.

The basic theory of processed cheese manufacture is based on the change of the state of casein protein from the coarsely dispersed calcium-paracaseinate which is present in the natural cheese, due to application of heat, stirring and the presence of particular salts (as emulsifying/peptizing agents), into a homogeneous free-flowing condition (the sol state) with desired functional and organoleptic properties.

The basic technological steps for processed cheese production could be summarized by the following operations:

- natural cheese/cheeses selection,
- formulation of the blend,
- blending,
- shredding,
- emulsifying salts addition,
- processing (thermal treatment),
- packaging,
- cooling,
- storage of the final product.

Moreover, in a simplified point of view, the principles of processed cheese manufacture could be described by two stages/phases. During the first stage/phase, present caseins are liberated and dispersed, by the action of emulsifying salts and then could serve as "*true*" emulsifiers. This phase is also known by the term "ion-exchange phenomenon". During the second stage/phase, the new developed protein network is established, in which the proteins are hydrated and the fat is emulsified. This phase is also known as the "creaming phase".

In addition, the release of caseins is caused by the action of emulsifying salts, during which the ion-exchange phenomenon occurs. Polyvalent anions (especially phosphates, polyphosphates and citrates) and monovalent cations (especially sodium, but in some cases also potassium and/or aluminum) are applied as emulsifying salts. Multivalent anions have more intensive affinity to bivalent ions (in the case of processed cheese are calcium ions) in comparison to monovalent ions. Therefore, when e.g. sodium salt of phosphate is added into the mixture of the raw materials, in the presence of sufficient amount of water, under heat treatment and agitation, the ion-exchange of calcium to sodium ions will occur. Moreover, the insoluble calcium paracasein (from the natural cheese protein network) is transformed into the more soluble sodium paracasein and the latter might be dispersed in the processed cheese melt. Therefore, the dispersed proteins (caseins) within the system could effectively act as

emulsifiers. In particular, the first role of emulsifying salts is to adjust the environment of the raw materials mixture in a manner that the caseins will enhance their emulsifying properties. Hence, this attribute is defined as the "main role" of emulsifying salts (Carić et al., 1985; Guinee et al., 2004; Kapoor & Metzger, 2008; Kawasaki, 2008; McIntyre, et al., 2017; Mizuno & Lucey, 2005a; 2005b; Shirashoji et al., 2006; Templeton & Sommer, 1936).

Furthermore, emulsifying salts (salts of phosphates, polyphosphates and citrates) are sometimes labelled as emulsifiers (substances presenting surface activity). However, this is not the exact (or correct) definition because these substances do not have an amphiphilic character. In general, the term emulsifying salts/agents, is the most appropriate for use (Carić et al., 1985; Guinee et al., 2004).

Moreover, during processing (so called also "melting" process) under heat treatment (90 – 100 °C) and mechanical stirring in the presence of water, polyvalent anions (especially phosphates) start binding onto the caseins via calcium ions resulting in the incensement of the hydrophilic character of the proteins. Caseins possess the ability to bind water and caseins modified in the above-mentioned way are more effective in terms of binding water. In addition, parallel, the fat is emulsified by dispersed caseins and partially by the residues of original lipoprotein membranes (covered fat droplets before processing). Caseins with bonded phosphates have more intensive negative charge leading to their dispersion. Generally, the addition of emulsifying salts can also increase the pH value from approximately 5.0 – 5.5 (in the mixture of the raw materials) to the target values which are in the range of 5.6 – 6.0 (typical for spread-type processed cheese), something which is also supported by the raising of caseins negative charge. These phenomena (proteins hydration and fat emulsification) cause the increase of viscosity in the resultant melt. Additionally, during holding time (generally a process lasting several minutes) under a target melting temperature and during the cooling of the hot melt, the new structure is established – the new hydrated protein network is developed, in which the fat is emulsified. The above-mentioned processes are named as the "creaming" process. There are many interactions, which participate in the process of the final network structure formation and thus, supporting creaming (new calcium bridges, phosphates-calcium complexes, hydrophobic interactions, hydrogen bonds and/or disulfide bonds are taking place). The establishment of the new protein network can be observed as the intensive increase of melt viscosity (Awad et al., 2004; Berger et al., 2002; Bowland & Foegeding, 2001; Buňka et al., 2013; Hoffmann, 2012; Fu et al., 2018; Kalliapan & Lucey, 2011; Kapoor et al., 2007; Kapoor & Metzger, 2008; Kawasaki, 2008; Lucey et al., 2003; Lucey et al., 2011; Mizuno and Lucey 2005b, 2007).

The successful melt creaming for the establishment of the network (final product) with desirable properties for the consumers requires some time, in which a target melting temperature is maintained under continuous stirring. In particular, during the first phase, the ion exchange phenomenon and casein dispersion occur by the activity of emulsifying salts together with heating and stirring. Furthermore, caseins are hydrated, fat is emulsified and the new protein network starts to develop. During the prolonging of the processing time, the viscosity of the melt is increasing due to interactions connected with caseins chains in the newly formed matrix, resulting in the decrease of the fat droplets (up to the maximum available viscosity under actual conditions). The heat treatment and holding time under the target melting temperature is stopped when the melt possesses the appropriate properties for the manufacture

of a final product with desirable properties for the customers. However, when the processing time is extended, the aggregation between the proteins (increasing number of interactions between caseins) will continue and from a certain point the fat and later also the water phases will be released from the matrix. Thus, the developed system will become unstable with lower values of viscosity. Nevertheless, this undesirable phase is commonly labeled as "over creaming" and is a significant quality defect. (Kawasaki, 2008; Lee et al., 2003; Shirashoji et al., 2006).

Cheese ripening (mainly the proteolysis) can affect the new protein network establishment. When high amount of short-chained caseins and low concentration of intact casein are available (proteolysis is very intensive), the danger of an unstable protein matrix development would exist, resulting in an undesirable final product in which fat or even also water might be released (Buňka et al., 2013; Brickley et al., 2007; Carić 7 Kaláb, 1997; Salek et al., 2016).

According to Lee et al. (2003), the creaming process (the formation of a new network) is mainly described by the interactions of caseins. The authors supported their statement in an experiment with full-fat processed cheese and also with a sample with no fat content. In both products, the creaming phenomenon occurred. On the other hand, the presence of fat significantly influenced the protein matrix and therefore the consistency of the final product. Generally, fat can disturb the continuity and the compactness in such way that decreases the number of interactions between caseins. The latter can lead to the development of a processed cheese with lower viscosity (more spreadable). The proteins interact with fat – emulsify it – and in those places cannot interact with each other (caseins) and a compact network is obtained. A similar effect on the continuity and the compactness of the protein matrix possesses water. Also the cooling rate of the processed cheese has a significant effect on the network formation. When the cooling rate is too fast, the viscosity of the products decreases especially because the interactions between proteins take place especially when the melt is hot (Awad et al., 2002; Bowland & Foegeding, 2001; Guinee, 2003; Kapoor & Metzger, 2008; Lee et al., 2003; Lucey et al., 2011; Piska & Štětina, 2004).

It could be concluded that the final structure of processed cheese is affected by the following factors:

- the effectiveness of ion-exchange and proteins dispersion (including the actual pH-value and its impact on the negative charge of caseins);
- the intensity of protein hydration;
- the intensity of fat emulsification;
- the number of interactions between caseins.

Moreover, the addition of other ingredients (optional) can contribute to the increase of the above-mentioned interactions among proteins. On the other hand, the presence of other ingredients can also disrupt the continuity and the compactness of the developed casein network.

2. Natural cheese in processed cheese production

2.1 The effect of natural cheese type and maturity degree on the properties of processed cheese.

The basic ingredient for the production of processed cheese is natural cheese. Therefore, by the selection of this main raw material, it is possible to influence the consistency of the final processed cheese product. During the selection of the natural cheese, it is necessary to take into account the type of natural cheese (variety), the degree of maturity, its composition (dry matter, fat, protein, and calcium contents, respectively), the pH value and the required properties (organoleptic or functional) of the final product.

Natural cheese varieties which are predominantly applied in various countries of the world vary (including mainly Cheddar, Dutch-type, Swiss-type, Mozzarella-type (or other pasta-filata cheeses) and white brined cheeses). In general, in the English-speaking countries (e.g., Britain, USA, Canada, Australia, and New Zealand) the basic natural cheese as the main raw material for processed cheese manufacture is commonly Cheddar and Mozzarella cheese. On the other hand, in regions around the Mediterranean basin, Balkan, the Near and Middle East, white brined cheeses belong among the most preferable and consumed natural cheese types and are utilized as the basic ingredient for the production of processed cheese and related products (Černíková et al., 2017; Moatsou & Govaris, 2011; Salek et al., 2020). For the past years several studies have been performed in which divergent natural cheese varieties were implemented for the production of processed cheese and similar products. In particular, Abdel-Hamid, El-Sharaby & Awad (1999); Awad et al. (2002) used *Ras* cheese, Adhikari et al., (2009); Biswas et al. (2014); Brickley et al. (2007); Guinee & O'Kennedy (2009; 2012); Rafiq & Ghosh (2017), Salek et al. (2020); Hassan et al. (2007); Fu & Nakamura (2020); Fu et al. (2018); Janevski et al. (2012); Shirashoji et al. (2010; 2016; 2006); Guinee & O'Callaghan (2013); Kommineni et al. (2012); Fagan et al. (2007); Hoffmann et al. (2012) applied *Cheddar* cheese, Chavhan, Kanawja, Khetra & Puri (2015); Seth & Bejwa (2015); Salek et al. (2017); Chen & Liu (2012) used *Mozzarella* type cheese, Cunha & Viotto (2010); Cunha, Alcandara & Viotto (2012); Nogueira et al. (2018); Ferrão et al. (2018); Torres et al. (2017); Belsito et al. (2017); Alves et al. (2007) used *Requeijão cremoso* cheese, Černíková et al. (2017); Salek et al. (2016; 2017; 2020a; 2020b); Hauerlandová et al. (2014); Nagyová et al. (2014); Macků et al. (2009); Buňka et al. (2004; 2013); Weiserová et al. (2011); Schädle et al. (2020); Přikryl et al. (2018); Sádlíková et al. (2010); Piska & Štětina (2004) utilized *Edam* cheese, Stangieski, Weiss & Kaczmarek (2019); Weiss, Stangieski, Baranowska & Rezler (2018); Dimitreli & Thomareis, (2004; 2007; 2008); Fu & Nakamura (2020); Fu et al. (2018); Mozuraityte et al. (2019) applied in their studies *Gouda* cheese, Chatziantoniou, Thomareis & Kontominas (2015, 2019); Kontou, et al. (2019) used a *Myzithra*-type whey cheese, Hanna (1999) applied *Halloum* cheese, Kaminarides & Stachtiaris (2000); Topcu et al. (2020) utilized in their work *Kasseri/Kashar* (pasta-filata) cheese, Ghods Rohani & Rashidi, (2019), Kontou, et al. (2019) used *Feta* cheese, Salek et al. (2016) used *Swiss*-type cheese, Burgos, et al. (2020) used goat cheese, Yılmaz et al. (2011) applied white cheese.

Proteolysis is one of the principal biochemical changes during cheese ripening leading to physical, chemical, flavor, and functional changes in natural cheese. Proteolysis occurs when various enzymes hydrolyze proteins in cheese during ripening. The sources of enzyme for proteolysis originate from chymosin and bovine pepsin in rennet residual, indigenous and endogenous heat-stable proteinases in milk, and various proteinases from starter and non-starter bacteria (McSweeney &Fox, 1997). In other words, during the ripening process of natural cheeses, the phenomenon of proteolysis occurs, where the casein (*para*-casein) component is hydrolyzed into peptides and free amino acids. In particular, proteolysis of casein occurs by the action enzymes involving the residual activity of native milk enzymes, rennet and the exogenous enzymatic system of acidic and non-acidic lactic acid bacteria. The ratio of cleaved and practically non-cleaved (intact) casein is an important criterion influencing the consistency of processed cheeses (Kapoor & Metzger, 2008). The term intact casein in cheese refers to the casein that has not been hydrolyzed during the ripening process, which is used as a non-specific method to measure cheese proteolysis. Intact casein content in the final product has been also correlated to cheese rheological and functional properties. Experimentally, intact casein is determined by the difference between total protein and the pH 4.6-soluble protein using nitrogen-based quantifications. Furthermore, intact casein and total protein contents can provide indirect essential information for processed cheese manufacturing as they both can determine its functional and textual properties (Kapoor & Metzger, 2008). Furthermore, it is generally accepted that the intact casein content of natural cheese is inversely related to the age of the natural cheese. Hence, as a natural cheese is matured, its intact casein content (Kapoor et al., 2007).

Moreover, Guinee et al. (2004) stated that "*young*", unripe natural cheese containing 70-95 % of intact casein is suitable for the production of sliceable processed cheese products, whilst medium-ripened cheese with 60-75 % of intact casein is suitable for the manufacture of spreadable processed cheese products. Thus, if an immature raw material with a low degree of proteolysis is used, the processed cheese product obtained is characterized by a stiffer, gummier consistency and an empty taste. On the other hand, the advantage of using this kind of raw material (unripen natural cheese) is the reduction of raw material costs. In addition, by using a ripened natural cheese with a high degree of proteolysis, an easily melting mixture is obtained and the final processed cheese product has a fine and spreadable consistency. During a longer maturing process, plethora of sensory active substances are formed, giving to the developed processed cheese melt a full and distinctive aroma (Guinee et al., 2004; Guinee, 2003; Lee & Klostermeyer, 2001; Swenson, et al., 2000). In practice, a mixture of more or less matured natural cheeses is usually applied during the production of processed cheese. If the raw material composition contains older, very mature natural cheeses with excessively hydrolyzed proteins, the addition of intact casein (in the form of young cheeses or curd) is usually included, which will help to form a stable protein matrix. Otherwise, there is a risk of an unstable to liquid-like consistency of the product. This phenomenon would occur due to the low binding of water and its release and the inability to form a stable protein matrix. The effect of increasing the degree of proteolysis of natural cheese (Cheddar natural cheese was the basic raw material) on the textural and viscoelastic properties of processed cheese was demonstrated in a study by Brickley et al. (2007). The degree of proteolysis of Cheddar increased most rapidly in the first 28 days after its production, which was in line with the highest decrease in the firmness of the

processed cheese produced. Piska & Štětina (2004) produced processed cheeses from mixtures of natural cheeses with different degrees of proteolysis. It was found that the sample showing the highest stiffness, adhesiveness and gumminess was made from natural cheese with a low proteolysis index (ratio of water-soluble nitrogen at pH 4.6 to total nitrogen). On the contrary, the values of these textural parameters were the lowest for the product manufactured from the raw material with a higher proteolysis index.

Additionally, Bunka et al. (2013) studied the dependence of natural cheese (Edam block; 2, 4 and 8 weeks of maturity) maturity level on selected textural properties (hardness, cohesiveness and relative adhesiveness) of spreadable processed cheese (40 %, w/w, dry matter, 50 % w/w fat in dry matter contents) during a 30 day storage period. The above-mentioned authors stated that hardness of processed cheese decreased when a raw material with higher level of maturity was applied for the production of the examined samples. Furthermore, with the raising maturity degree of the cheese, the values of processed cheese cohesiveness slightly decreased. On the other hand, the obtained values of relative adhesiveness of the processed cheese samples increased. Furthermore, significant changes in the relative adhesiveness values were reported when cheese with 8-week maturity was used. A possible explanation of this could be due to a shortening length of casein fractions caused by the rising intensity of proteolytic changes during the ripening of the utilized raw material (natural cheese) (Brickley et al., 2007; Piska & Štětina,2004). The results are in accordance to the findings reported by Brickley et al. (2007) and Guinee et al. (2004). In general, the values processed cheese relative adhesiveness should increase with the rising maturity level of the used natural cheese. Moreover, according to the work of Hladká et al. (2014) model samples of processed cheeses (40 % w/w dry matter content and 50 % w/w fat in dry matter content; Edam cheese block was applied as the main raw material; 50 % w/w dry matter, 30 % w/w fat in dry matter) were homogeneous regardless of the maturity degree of cheese (the maturity of the applied raw material was in the range of 1–16 weeks). Moreover, the latter authors developed processed cheese samples without emulsifying salts addition. The highest values of hardness were observed in the products made of cheese with 1–2 weeks of maturity. Moreover, with the increasing degree of cheese maturity (4–12 weeks), the hardness of the tested samples was gradually decreasing. Additionally, the hardness of processed cheese made of natural cheeses with maturity of 12–16 weeks did not differ. Nevertheless, the lowest values of hardness were observed in the samples examined on the production day and this parameter was significantly increasing with the prolonging of the storage period. In addition, other series of studies performed by Salek et al. (2016; 2017; 2020) examined the effect of various natural cheese varieties and their degree of maturity on selected textural and rheological properties of model processed cheese samples. The above-mentioned authors applied Mozzarella-type (42 % w/w dry matter content; 35 % w/w fat in dry matter content; 0, 2, 4 weeks of maturity), Swiss-type (60% w/w dry matter content, 30% w/w fat in dry matter content; 4, 8, 12, and 16 weeks of maturity), Cheddar (dry matter content 62 % w/w, fat in dry matter content, 50 % w/w; 4, 8, 12 and 16 weeks of maturity) and White brined cheeses (dry matter content 48 % w/w, fat in dry matter content 38 % w/w; 2, 4, 8, 16 and 24 weeks of maturity) respectively, as the basic raw material in the formulation of the resultant processed cheese samples. The hardness of the samples obtained decreased with the rising maturity degree of the natural cheese used (regardless of the applied cheese variety). Moreover, the monitored values of the gel strength

and interaction factor decreased with the increasing maturity degree of the cheese used. According to Kapoor & Metzger (2008), the reported decrease in the gel strength values could be probably due to fewer number of interactions within the processed cheese matrix. In general, it could be stated that a more rigid end-product can be expected with higher gel strength values. The intensity of rigidity of the processed cheese samples has an analogous relationship to the intensity of the gel strength; the higher the gel strength of the sample, the more firmer the product that can be expected (Salek et al., 2016; 2017). Moreover, higher values of gel strength were reported for the processed cheese samples produced with Cheddar cheese in comparison to those made from White brined cheese. According to Salek et al., (2020) from the comparison of processed cheese samples manufactured from two different natural cheeses (Cheddar and White brined cheese) of the same maturity level (4-, 8- and 16-week maturity), it can be seen that the White brined processed cheese samples were significantly less hard that those made of Cheddar cheese. Moreover, this important difference in the hardness values development could be due to different chemical composition (including pH, calcium content, NaCl content, residual lactose content and mainly the amount of intact casein) and cheesemaking process of the applied natural cheese (Piska & Štětina, 2004). As it was mentioned previously, the level of intact casein present in the natural cheese can influence the properties of the resultant processed cheese. Furthermore, the structural network of processed cheese is mainly formed by intact casein and thus, a high level of intact casein could be a potential indicator for abundant interactions between the present proteins and fat. According to the literature, processed cheese produced from natural cheese with lower intact casein level is soft, whereas processed cheese made from natural cheese with a higher level of intact casein is firm. On the same token, probably the level of intact casein in the utilized White brined cheese was lower in comparison to that in Cheddar cheese, resulting in significant differences in their values of hardness. On the whole, from the reported results of rheological and hardness analyses it could be concluded that the processed cheese gel strength and hardness development follow a contiguous trend which is not affected by the type of applied natural cheese.

In addition, Burgos et al. (2020) evaluated the effect of the maturity degree of natural goat cheese on the textural, rheological and organoleptic properties of processed cheese products. Furthermore, the developed processed cheeses were formulated using goat cheeses with 10, 20 and 40 days of ripening. In particular, the hardness, adhesiveness and complex modulus (G*) of the tested samples decreased as the maturity degree of the utilized natural cheese (raw material) increased, which is probably associated with the intact casein content. According to the latter authors, increasing the amount of natural goat cheese having hogher amount of intact casein in the formulation can influence the complex modulus of the resultant end product. Finally, a general statement could be reported that as the maturity degree of natural cheese applied in process cheese manufacture increased, the hardness of the resultant processed cheese decreased (Kapoor, et al., 2007; Piska & Štětina, 2003).

Active acidity (pH value) has a significant effect on the textural and rheological properties of processed cheeses. In particular, these properties are mainly influenced by the type and concentration of emulsifying salts used and the pH value of the applied natural cheese. Moreover, processed cheese with low pH values (in the range of 4.8 - 5.2) can be expected to have a stiffer consistency than less acidic processed cheeses with higher values of pH (pH> 6.0), which are usually more spreadable under otherwise identical circumstances (Awad, et al.,

2004). The closer the pH value of the processed cheese is to the value of the isoelectric point of casein (pI ≈ 4.6), the more attractive protein interactions occur, resulting in a stiffer product (Lee, et al., 2004). Marchesseau et al. (1997) investigated the effect of increasing pH on the rheological properties of processed cheeses with a constant content of emulsifying salts. The result was decreasing values of loss and elastic moduli of elasticity, indicating a decrease in viscosity and elasticity of the tested processed cheese with an increasing pH.

Moreover, Lee & Klostermeyer (2004) also achieved the same results in processed cheese spreads with reduced fat content (12.0 % w/w of fat). In contrast, Swenson et al. (2000) found a decrease in the firmness of processed cheese containing only 0.6 % w/w of fat with a decreasing pH. However, these different results are probably due to the analysis of different types of matrices (e.g. different processing equipment and conditions, chemical composition of the matrix). Moreover, factors such as natural cheese calcium and phosphorus contents and salt-moisture-content can influence the functional properties of processed cheese. According to Kapoor at al. (2006) high calcium and low phosphorus content in natural cheese can influence the processed cheese melting properties, viscous modulus and apparent viscosity. Moreover, decreased levels of calcium content can affect the rheological properties of processed cheese in such a way that the resultant end product will present lower values in fracture test, strain and hardness. In general, such products are characterized as soft and brittle in texture (Guinee & O'Kennedy, 2009). In addition, the salt-in-moisture content of natural cheese can influence the proteolysis degree. In particular, an increase in the salt-in-moisture content can result in a decreased degree of proteolysis. Thus, the hardness of the resultant processed cheese should be higher, whereas the values of cohesiveness will be lower. Furthermore, according to Biswas et al. (2015) processed cheese produced from Cheddar cheese with high calcium and phosphorus contents and high salt-in-moisture content is harder with lower tendency to flow.

3. Emulsifying salts in processed cheese production

3.1 The role of emulsifying salts within the processed cheese matrix

Emulsifying salts (sometimes also called emulsifying agents) are more commonly known in the dairy industry as "*melting*" salts and are of great importance during the production of processed cheese and similar products (processed analogues or processed cheese sauces). Emulsifying salts are ionic compounds made up of monovalent cations (sodium, potassium) and polyvalent anions (phosphates, polyphosphates and/or citrates) (Chen & Liu, 2012; Buňka et al., 2014). The most commonly applied emulsifying salts are sodium citrates, sodium hydrogen monophosphates, diphosphates and polyphosphates. Nowadays, during the industrial production of processed cheese, emulsifying salts are rarely used as individual compounds, whereas they are applied rather in the form of phosphate and phosphate-citrate blends (binary, ternary or even quaternary mixtures) (Chen & Liu, 2012; Hasenhuetl & Hartel, 2008). Nevertheless, emulsifying salts cannot be characterized as "*true*" emulsifiers (low molecular-weight surfactants) since they do not indicate any surface-activity properties. In the narrow sense, emulsifying salts cannot be used for the preparation of oil-in-water (O/W) or water-in-oil (W/O) emulsions, whereas they play important role in modifying the emulsifying activity of the present surface-active proteins (mainly caseins from the applied natural cheese which is the main raw material) within the processed cheese matrix. (Carić et al., 1985; Hasenhuetl & Hartel, 2008; Lucey, 2002; Tamine, 2007; 2011; Zehren & Nusbaum, 2000). In addition, caseins possess calcium binding ability, which has the effect of reducing their solubility and thus enhancing their emulsifying properties. Furthermore, emulsifying salts present higher affinity for calcium than do the caseins, and thus, are able to improve the solubility and emulsifying ability of the caseins. In general, two types of emulsifying salts exist; those that bind calcium relatively „*weakly*", and those that bind calcium more „*strongly*". "*Weak*" emulsifying salts have a modest effect on the emulsifying properties of the caseins, leading to the formation of a softer processed cheese with relatively large fat droplets. On the other hand, "*strong*" emulsifying salts give a greater improvement in the emulsifying capacity and result in a firmer processed cheese with smaller droplets of fat (Hasenhuetl & Hartel, 2008). According to the European Union legislation (Commission Regulation No. 1333/2008) the maximum permitted level of emulsifying salts (expressed as P_2O_5) in processed cheese is up to 20,000 mg/kg (or mg/L). In general, depending on the nature of the applied emulsifying salt, the latter are added in an amount ranging from 1 to 3 % (w/w) (Tamine, 2007; 2011). The essential role of emulsifying salts is to solubilize calcium paracaseinate, sequester calcium and thus, dispersing the present proteins. Calcium in the calcium-paracaseinate complex of natural cheese is removed by the ion-exchange properties of the emulsifying salts; solubilizing the paracaseinate, usually as sodium paracaseinate (Fox, 2000; Hui, 2005). Moreover, pH adjustment and calcium sequestering are among the main functions of emulsifying salts during processed cheese production. Emulsifying salts possess the ability to sequester calcium from the casein matrix; by exchanging sodium ions, resulting in the conversion of the insoluble calcium paracaseinate into the more soluble sodium paracaseinate (Guinee et al., 2004; Chen & Liu, 2012; Kapoor, et al., 2007; Salek et al., 2015). Within the matrix, sodium paracaseinate acts as an emulsifier

leading to the stabilization of the oil-in-water interface. Some of the additional properties of emulsifying salts are the control and stabilization/adjustment (an upward trend) of the pH level and an effect on the formation of the final product structure after cooling (Dimitreli & Thomareis, 2009; El-Bakry et al., 2011). Moreover, application of the appropriate blend of emulsifying salts increases (due to their buffering capacity) the pH from the typical values in the range of ~4.6 – 5.5 for natural cheese to values ranging from 5.6 to 6.2 in the spreadable processed cheese. Furthermore, the increase in pH extends the calcium-sequestering ability of the emulsifying salts and the negative charge on the paracaseinate. The dispersed hydrated paracaseinate contributes to the emulsification of the free fat by coating the surfaces of the dispersed free fat globules, resulting in the formation of an artificial (recombined) membrane. The high water-binding capacity of the paracaseinate enhances the developed emulsion stability as it leads to higher values of viscosity of the aqueous phase and thus a reduction in the collision frequency of the emulsified particles is observed. However, not all emulsifying salts have the same calcium ion-exchange ability. The phosphate ion-exchange ability increases with increasing P_2O_5 content in the following order: monophosphate < citrates ≈ diphosphate < triphosphate < polyphosphate (Shirashoji et al., 2006; Buňka et al., 2013). According to El-Bakry et al. (2011) and Mizuno & Lucey (2005), trisodium citrate (TSC) presents better calcium chelating ability and casein peptization properties than do sodium monophosphates and diphosphates. Moreover, on a mole for mole basis, phosphates possess higher calcium chelating ability than citrates (Dimitreli & Thomareis, 2009) The ion-exchange ability increases with the increasing length of the polyphosphate chain, resulting in better casein dispersion and better fat emulsification and water stabilization, which leads to better crosslinking of the matrix network in the final product (Chen & Liu, 2012; El-Bakry et al., 2011; Shirashoji et al., 2010; Molins, 1991). Nevertheless, absence of emulsifying salts (during heating and shearing of the applied mixture of various ingredients), could lead to the formation of an undesired inhomogeneous mass (Guinee et al., 2004; Buňka et al., 2014). In particular, heating (to temperatures utilized during processed cheese production) and stirring of the used natural cheese together with the other ingredients of the formula (in the absence of emulsifying salts); might result in the membrane destruction of the emulsified fat globules, leading to their clustering into larger units. Moreover, the combined effect of low pH and high processing temperatures application would cause aggregation and contraction of the casein molecules, resulting in water release followed by the separation of hydrophilic and hydrophobic phases. The latter phenomenon is a defect known also under the term oiling-off (Guinee et al., 2004). Furthermore, another important property of emulsifying salts is their bacteriostatic effects. Traditional manufacture of processed cheese normally involves processing temperatures in the range of 70 – 95 °C, which are lower than those which are used over sterilization. Hence, processed cheese might contain viable microbial spores (e.g. *Clostridium* genus) mainly from the utilized raw materials. Nevertheless, spore germination during storage could lead to serious technological problems (or defects – such as blowing of the packaging material, protein putrefaction and off-flavors). Generally, bacterial spoilage can be eliminated by the addition of preservatives. In addition, some emulsifying salts possess bacteriostatic properties. Particularly, polyphosphates can inhibit many microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium sporogenes* and some *Salmonella* spp.). Moreover, monophosphates and diphosphates have been found to inhibit the growth of *Clostridium botulinum* (depending on the levels of moisture, NaCl and pH

of the processed cheese). However, on the contrary, citrates do not possess bacteriostatic properties, and may be even degraded by bacteria and thus reducing the shelf-life of the final processed cheese product (Carić & Kaláb, 1997; Buňková, et al., 2008; Fox et al., 2000; Tanaka et al., 1986; Zerfiridis, 2001).

3.1.1 Phosphate emulsifying salts

Phosphates are the salts derived from phosphoric acid (H_3PO_4). Phosphates constitute a large group of compounds, in which the anion consists of PO_4 tetrahedra which may be linked together by the sharing of corners. The phosphate series begins with single PO_4 group, which may exist as the triply charged monophosphate (named also as orthophosphate) anion PO_4^{-3}, or may participate in molecules where one, two or three of the four oxygens are covalently bonded to other atoms. Additionally, the PO_4 group is a tetrahedron formed by four oxygen atoms surrounding a phosphorus atom. The present oxygen molecules may constitute a bridge between the phosphorus atom and other atoms (including also other phosphorus atoms). Therefore, a long series of two- or three-dimensional phosphates can originate through P-O-P linkage. A term often used in relation to polyphosphates (including also diphosphates) is that of "condensed phosphates". The origin of the term lies in some of the industrial processes used in the manufacture of polyphosphates, which involve elimination of water at high temperatures. Under very specific conditions, monophosphates or longer-chain phosphates having terminal OH groups lose water between hydroxyl groups. The dehydrated tetrahedra are thereby brought together through a sharing of corners (are condensed) in order to form polymers. Similar processes may result in two monophosphate molecules coming together to form a diphosphate (previously named as pyrophosphates), or a monophosphate and a diphosphate may link into a triphosphate, and so on. Moreover, the term ultraphosphate includes any phosphate having a tridimensional structure. On the other hand, in processed cheese industry, linear (two-dimensional) polyphosphates are used (Molins, 1991).

In addition, sodium, potassium, ammonium, calcium, magnesium and sodium aluminum salts of phosphates can be used in the food industry. In particular, in the processed cheese industry, sodium salts of phosphates are predominantly utilized. Potassium salts of phosphates are generally not applied due to possible bitter flavor of the end-products (Guinee et al., 2004; Kapoor & Metzger, 2008; Mayer, 2001). In some countries, the sodium aluminum salt of phosphate is permitted and some recent papers showed that bitter flavor could not be a problem and pointed out lower sodium content (Chavhan et al. 2015;Nogueira et al., 2018; Schatz et al., 2014).

3.1.2 The effect of individual emulsifying salts on processed cheese properties

Phosphate and polyphosphate salts with varying number of linearly bonded phosphate units in the molecule possess different properties. The first property that is significantly influenced by the above-mentioned factor is the ability to exchange ions. The capability of sodium phosphate-based emulsifying salts to exchange sodium and calcium between emulsifying salts and a natural cheese casein structure is affected by two factors: (i) the length of phosphate-based salt; and (ii) the target temperature during processed cheese production.

When longer phosphate-based salt is used and higher target melting temperature applied, the ion-exchange ability and subsequently the casein dispersion are enhanced (Buňka et al., 2013; Carić & Kaláb, 1997; Gupta et al., 1984; Molins, 1991; Nagyová et al., 2014; Sádlíková et al., 2010; Salek et al., 2015; Templeton & Sommer, 1936; Weiserová, et al., 2011).

The ion-exchange activity of sodium phosphate-based emulsifying salts is also affected by the actual pH of the mixture of the raw materials, the latter in turn might be influenced by the selection of (i) natural cheese type; (ii) other ingredients; (iii) and also by the applied emulsifying salts (type and/or concentration). In general, when the pH value of the mixture of the raw materials is low, the ion-exchange ability of sodium phosphate-based emulsifying salts is enhanced. The pH-values of aqueous solution (1 % w/v) prepared using different sodium phosphate-based ES (commonly applied by industrial practice) are presented in Table 1 and could be ordered as follows:

$$Na_2H_2P_2O_7 < NaH_2PO_4 < (NaPO_3)_n < Na_2HPO_4 < Na_5P_3O_{10} < Na_4P_2O_7 < Na_3PO_4$$

In Table 1, the pH-values of processed cheese manufactured with the individual sodium phosphate-based ES (in concentration of 3 % w/w) are also presented. The order of pH-values of processed cheeses produced is the same (as in the case of the aqueous solutions of 1 % w/v). It should be added that the actual pH-value of the raw material mixture influences also the aggregation processes of caseins. The caseins aggregation is more intensive, when the pH-value of the mixture is near to its isoelectric point (pH ≈ 4.6). Furthermore, excessive aggregation of caseins and "*poor*" ion-exchange ability of the used emulsifying salts and therefore, insufficient dispersion of proteins could lead to unsatisfactory fat emulsification and protein hydration. On the whole, it could be reported that the selection of proper emulsifying salts is very important for reaching the target pH-value of the final product (Carić & Kaláb, 2004; Guinee et al., 2004; Marchesseau et al., 1997; Lee & Klostermeyer, 2001; Molins, 1991; Muslow et al., 2007; Nagyová et al., 2014; Shirashoji et al., 2016; Sádlíková et al., 2010; Salek et al., 2015).

Table 1
Ranges of pH values* of aqueous solutions (1 % w/v) and final processed cheese samples (3 % w/w) manufactured by the utilization of selected sodium phosphate emulsifying salts (Nagyová et al., 2014; Sádlíková et al., 2010; Salek et al., 2015).

Chemical formula	Name	P_2O_5 content	pH value of aqueous solution (1 % w/v)	pH value of the final processed cheese (3 % w/w)**
NaH_2PO_4	monosodium phosphate	59.15	4.5	4.9
Na_2HPO_4	disodium phosphate	50.00	9.1	6.5
Na_3PO_4	trisodium phosphate	49.94	11.9	6.9
$Na_2H_2P_2O_7$	disodium dihydrogen phosphate	63.95	4.1	4.7
$Na_4P_2O_7$	tetrasodium diphosphate	53.38	10.2	6.7
$Na_5P_3O_{10}$	pentasodium triphosphate	57.88	9.7	6.6
$(NaPO_3)_n$***	sodium polyphosphate	69.61	5.9	5.4

* values are expressed as mean; the standard deviations for aqueous solutions and processed cheese samples were in range of 0.01 – 0.03 and 0.02 – 0.06, respectively,

** model spread-type processed cheese with 40 % (w/w) dry matter and 50 % (w/w) fat in dry matter produced using Edam cheese,

*** mean length for sodium polyphosphate was at the level of $n \approx 20$

With respect to the above-mentioned relations and all references presented above, the ion-exchange ability of sodium phosphate-based emulsifying salts could be presented by the following order:

$NaH_2PO_4 < Na_2HPO_4 < Na_3PO_4 < Na_2H_2P_2O_7 < Na_4P_2O_7 < Na_5P_3O_{10}$ < sodium polyphosphates ($n \leq 9$) < sodium polyphosphate ($n > 9$).

The use of sodium phosphate-based emulsifying salts is not limited only by the pH-value adjustment of the final products but some of these salts possess also buffering capacity and are able to stabilize the pH of the processed cheese. The buffering capacity of sodium phosphate-base emulsifying salts is lower when the length of phosphates and polyphosphates is decreasing. Therefore, monophosphates and partially diphosphates are evaluated as substances with higher buffering ability (Carić & Kaláb, 2004; Guinee et al., 2004; Lucey et al., 2011; Muslow et al., 2007).

The length of phosphate-based emulsifying salts influences also the ability to support the casein network (gel) formation. Diphosphates are evaluated as very effective substances for dairy gels forming, followed by triphosphates. According to some literal sources, it was reported that also units consisting of calcium with diphosphates or triphosphates also help to establish stable casein networks, due to the support to hydrophobic interactions between the proteins. On the other hand, polyphosphates are considered that do not support gel forming, due to their bonding with caseins and thus increasing their negative charge (Buňka et al., 2013; Carić & Kaláb, 2004; El-Bakry et al., 2011; Guinee et al., 2004; Lu et al., 2008; Lucey et al., 2011; Mizuno & Lucey, 2007; Muslow et al., 2007; Shirashoji et al., 2010, 2016).

The effect of individual (sodium) phosphate-based emulsifying salts on the properties of processed cheese has been studied in detail for last years. On the other hand, the most of the referenced studies are not directly comparable because different manufacturing conditions were applied (different raw materials, divergent target dry matter and fat in dry matter values, processing parameters – target temperature and holding time, agitation speed). On the other hand, indirectly, the observed trends could be compared.

In the work of Nagyová et al. (2014) are depicted the results of hardness values of processed cheese samples with solely applied nine emulsifying salts (target parameters: 40 % w/w dry matter content; 50 % w/w fat in dry matter content), which were produced under the same conditions. Each emulsifying salt was added in an amount of 2 % w/w. In general, the hardness of the resultant model processed cheese samples increased with the rising number of phosphate units in the sodium phosphate and polyphosphate molecule. According to the latter authors, the hardness increased between samples with sodium polyphosphates in comparison with products in which was used pentasodium triphosphate. Moreover, hardness of processed cheeses also increased when the number of phosphates units in sodium polyphosphates raised up to $n \approx 13$ (the mean value of the polyphosphate length). Therefore, the hardness of the samples, in which sodium polyphosphate with $n \approx 13$, $n \approx 20$ and $n \approx 28$, remained practically similar. When the emulsifying salts were used individually, the effect on the hardness of the samples was controlled and explained by the ability of the certain phosphate and polyphosphates texchanging ions. Furthermore, monophosphates are substances (low-molecular) with the ability to bind onto caseins and increase their hydration. Diphosphates cause the gel formation of caseins and act as cross-linking agents, while bonding with calcium ions. The latter mentioned phenomenon leads also to the reduction of charge repulsion, which can help to facilitate hydrophobic interactions between the hydrophobic segments of the present proteins. On the other hand, excessive amounts of diphosphates could bond too much amount of calcium, making it unavailable for cross-linking. Moreover, long-chain polyphosphates can strongly bind calcium and intensively support the dispersion of caseins. However, it is accepted that they do not support gel formation (due to their multiple negative charges). In addition, it could be reported that emulsifying salts are not used in practise as sole ingredients but as mixtures of several phosphate salts, sometimes also in the combination with citrate salts.

3.1.3 The effect of binary and ternary mixture of emulsifying salts on processed cheese properties

As it was mentioned above, under industrial conditions, mixtures of individual phosphates and also citrates are commonly applied during the production of processed cheese. Mixtures for commercial production of processed cheese contain two or even more individual types of emulsifying salts.

El-Bakry et al. (2011) reported the influence of the binary mixture consisting of disodium phosphate (DSP) and trisodium citrate (TSC). Moreover, the current authors reported that the firmness of processed cheese increased when the relatively amount of TSC raised (within the mixture of emulsifying salts). Moreover, Weiserová et al. (2011) studied the effect of six binary mixtures contained DSP, tetrasodium diphosphate (TSPP), pentasodium triphosphate (PSTP) and sodium salt of polyphosphate in which the mean amount of phosphate units was $n \approx 20$ (POLY20). The hardness of the processed cheese samples (target parameters: 40 % w/w dry matter content; 50 % w/w fat in dry matter content) in which the above-mentioned six binary mixtures (DSP:POLY20, TSPP:POLY20, PSTP:POLY20 and TSPP:PSTP) were applied increased, when the relative amount of phosphate with higher ion-exchange capability raised. Furthermore, a different trend of hardness development was observed when the binary mixtures of DSP:TSPP and DSP:PSTP were studied. In samples produced using DSP:TSPP or DSP:PSTP, the maximum values of hardness were reported, when the ratio of DSP and TSPP or DSP and PSTP ranged in the range of 1:1 – 2:3. These findings leaded to the conclusion that the ion-exchange ability (and subsequently the intensity of caseins dispersion) is not the only role of emulsifying salts. Weiserová et al. (2011) suggested the hypothesis that emulsifying salts especially in some ratios can influence the creaming process and generally, the new protein network forming.

Buňka et al. (2013) continued with the ternary mixture of emulsifying salts consisted of DSP, TSPP and POLY20. In particular, in samples when the relative amount of POLY20 was lower than 60 %, processed cheese hardness rapidly increased (in comparison with products with the same relative amount of POLY20), when the mutual ratio of DSP and TSPP was in the interval of 1:1 – 2:3. When the ratio of DSP and TSPP was different or (<2:3 or >1:1) the hardness of processed cheese decreased. The effectiveness of the specific combination DSP:TSPP was increasing, when the relative amount of POLY20 was decreasing. The hardest processed cheese were obtained when the ratio of DSP:TSPP was 2:3 and POLY20 was not included. However, in samples with the relative amount of POLY20 > 60 %, the significant influence of the specific ratio of DSP:TSPP was not observed (Buňka et al., 2013).

Nagyová et al. (2014) followed up with a research study, in which they used sodium salts of polyphosphates with different mean number of phosphate units ($n \approx 5$ – POLY05; $n \approx 9$ – POLY09; $n \approx 13$ – POLY13; $n \approx 20$ – POLY20; $n \approx 28$ – POLY28) in ternary mixtures composed also with TSPP and DSP. Generally, the trend of the influence of the ternary mixtures composition, described by Buňka et al. (2012) only for POLY20 (wit DSP and TSPP), on the firmness of processed cheese remained unchanged also for the other polyphoshate with 5 – 28 units of phosphates linearly bonded in the molecules. When the relative amount of the above mentioned phosphates were ≤ 60 %, the effect of the DSP:TSPP combinations 1:1 – 2:3 still existed regardless of the polyphoshate used. However, the absolute values of hardness were

affected. When POLY05 or P0LY09 were added, the absolute values of the model processed cheeses hardness were slightly higher with the application of ternary mixtures with the relative amount of polyphosphate < 50 % compared to those samples with POLY13, POLY20 and POLY28 (excluding for the amount of polyphosphates was zero). The latter mentioned phenomenon was observed not only in products, where the combination of DSP and TSPP was at 1:1 – 2:3, but also in samples with the same ratio consisting of DSP and PTPP. With the application of the relative amount of polyphosphate ≥ 60 %, the hardness of the processed cheeses decreased in relation to the decreasing number of phosphate units.

Furthermore, the explanation of the above-mentioned results in processed cheese samples manufactured using the binary and ternary mixtures of monophosphates, diphosphates and polyphosphates could be based on the specific properties of the individual phosphates or their mutual synergic relationship (Buňka et al., 2013; El-Bakry et al., 2011; Lu et al., 2008; Mizuno & Lucey, 2005b, 2007; Molins, 1991; Nagyová et al., 2014; Shirashoji et al., 2010, 2016; Weiserová et al., 2011; Salek et al., 2015). In particular, when zero or the low relative amount of polyphosphates (< 10 %) were in the mixtures of emulsifying salts composed of monophosphates and diphosphates (1:1 – 2:3), the strong gel formation of caseins was probably affected by the presence of diphosphates. Thereafter, small molecules of monophosphates have the ability to permeate among cross-linked proteins and thus strongly bind water. A sufficiently short distance between caseins supports their hydrophobic associations resulting in the increase of the final processed cheeses hardness. However, zero or low levels of polyphosphates do not provide caseins a strong negative charge resulting in the repulsion of proteins. Moreover, a low relative amount of diphosphates does not provide sufficient concentration of cross-linking agents and a strong gel cannot be developed. On the contrary, a high relative amount of diphosphates could lead to very effective binding of calcium, which could negatively affect the formation of diphosphate-calcium cross-links (bridges) between caseins more difficult. In addition, a relative low level of monophosphate permeating among cross-linked caseins is probably not sufficient to bind water.

When the relative amount of polyphosphates is higher (≤ 60 %), the effect of polyphosphates on the processed cheese network could be more effective. Hence, their ability to provide caseins multiple negative charges could diminish the effect of the specific ratio of monophosphates with diphosphates. Furthermore, the rising relative amount of polyphosphates, could result in the decrease of the hardness of processed cheese when the specific ratio of monophosphates with diphosphates (1:1 – 2:3) is decreasing. When the relative amount of polyphosphates is > 60 %, the effect of polyphosphate on the caseins network formation is predominant. In addition, polyphosphates strongly disperse caseins, leading to their hydration. Hence, the dispersed caseins emulsify the fat present within the matrix. The above-mentioned phenomena lead to the production of processed cheese with similar values of hardness regardless of the ratio of monophosphates with diphosphates.

When the mean number of phosphate units linearly bonded in the polyphosphates decreases (in the case when the relative amount of polyphosphates is below 50 %), the hardness of the processed cheese could increase. The explanation could be based on the different ability of polyphosphates (with a varying mean number of phosphate units) to give caseins multiple negative charges. In general, short-chain polyphosphates (the mean number of phosphate units n < 10) cause a lower intensity on the negative charge on caseins, leading to a higher intensity

of hydrophobic interactions among the dispersed caseins and thus increase of hardness of the processed cheese might be expected. When the relative amount of polyphosphates is above 60 % (regardless of the length), the influence on the processed cheese hardness is related to the mean number of phosphate units (which are linearly bonded in the polyphosphate). In particular, processed cheese hardness slightly increases with the increasing phosphate units in the molecule (up to $n \approx 13$). In addition when the length is higher than 13 ($n \geq 13$) the values of processed cheese hardness are similar (regardless of the polyphosphate length).

Among the factors which could affect the consistency of processed cheese, are the type and the maturity degree of the applied natural cheese, the total concentration of emulsifying salts applied and also the length of the storage time. Buňka et al. (2013) and Salek et al. (2016; 2017; 2020) utilized different types of natural cheese with divergent maturity degree (Dutch-type cheese, matured 2 – 8 weeks; Swiss-type cheese, matured 4 – 16 weeks; Mozzarella-type cheese, matured up to 4 weeks; Cheddar-type cheese, matured 4 – 16 weeks; and white brined type cheese, matured 2 – 24 weeks). In particular, the absolute values of the processed cheese hardness varied in dependence to the applied natural cheese. On the other hand, the general trend (as it was previously described) of the influence of the phosphate-based ternary mixtures of emulsifying salts composition on the hardness of processed cheese remained unaffected. In general, the increasing degree of maturity (in other words the intact casein concentration decreased), the hardness of the resultant processed cheese decreased, whereas the general trend of functionality of emulsifying salts (the mixtures) was not influenced. Moreover, processed cheese hardness increased over the storage time (up to 60 days), without significant effect on the general trend of functionality of emulsifying salts.

3.1.4 Other properties of phosphate emulsifying salts

Phosphates possess an inhibitory effect on the growth of some microorganisms. As far as the growth inhibition of gram-positive bacteria is concerned, the inhibitory effect of phosphates is dependent on the length of their chain. The effect of long-chain phosphates is more significant than the inhibitory effect of short-chain phosphates. Moreover, the antimicrobial effect is also influenced especially by temperature and pH value of the environment. As it was described in the subchapter 6.2.1, long-chain polyphosphates have a high affinity for bivalent metal ions. These ions are essential for maintaining the integrity of the cell wall of gram-positive bacteria because they form transverse bridges between the molecules of teichoic acids in the cell wall. Binding of bivalent ions can also be manifested by the unavailability of these ions for some essential physiological processes of the growth. Cleavage of the above-mentioned ions will result in bactericidal or bacteriolytic effects. When spore-forming bacteria are exposed to the effects of polyphosphates, the spore germination process is suppressed. The bactericidal or bacteriolytic effect of polyphosphates were studied in model laboratory conditions but also in real processed cheese with similar results. The inhibitory effect of phosphates on gram-negative bacteria are rarely described in the literature. (Buňková & Buňka, 2017; Lorencová et al., 2012; Velazquez et al., 2001).

The hydrolysis of phosphates in food systems is gaining attention because of its implication in the possible loss of functional phosphate properties. Phosphate hydrolysis in foods could occur due to causes as varied as enzyme activity, high temperature, pH-value and

also a result of bacteria metabolisms. Generally, the linkages between phosphate units in linear polyphosphates is more stable at 25 °C in comparison to temperature above 70 °C. On the other hand, when the temperature of the environment is < 25 °C, the hydrolysis could slowly run. Linear polyphosphates are relatively stable in alkaline or neutral solution, their hydrolysis could be accelerated by decreasing pH to acid area. The latter mentioned relationship is important, because the optimal pH-value of spreadable processed cheeses generally range in the interval of 5.6 – 6.0 (see chapter 9) and for processed cheese block and slices slightly lower pH-values (in many cases 5.0 – 5.5) are required. Polyphosphate hydrolysis proceeds usually by scission of terminal phosphate groups rather than by middle group link rupture. On the other hand, the studies described also "random cleavage in the middle" of linear polyphosphates rarely exist. As a result of hydrolysis, breakdown of polyphosphate is accompanied by a continuous built up of monophosphate, which is the end product of the hydrolytic process. Generally, the hydrolysis rate raises with the increase of the number of phosphate units in the linear polyphosphate molecule. Increasing water content, concentration of bivalent ions (especially calcium cation), storage temperature and actual pH-value could also accelerate the hydrolysis rates (Carić & Kaláb, 1997; Guinee et al., 2004; Lucey et al., 2011; Molins, 1991).

3.2 Citrate emulsifying salts

Citrates are the salts obtained from citric acid. To obtain citrate salts the acidic hydrogen atoms must be replaced by cations from the tribasic citric acid. Particularly, the H^+ ions of citric acid are neutralized with Na^+ resulting into three types of salts: mono-, di-, and trisodium citrates, respectively. The most important emulsifying salt applied in the manuafcture of processed cheese is the trisodium citrate (TSC). In addition, one percent aqueous solution of TSC had a pH-value 8.4 ± 0.1 and the pH value of processed cheese (target parameters: 40 % w/w dry matter content; 50 % w/w fat in dry matter content; solely applied TSC in amount of 2 % w/w.) was 6.4 ± 0.1. Monosodium and disodium citrates are not applied as sole ingredients because the final product might be acidic with poor textural properties and oiling-off could be expected. Additionally, citrates are applied in blends for processed cheese pH modification. TSC is used for the manufacture of processed cheese in slices or sliceable processed cheese (block-type). Moreover, potassium or ammonium citrates are applied in the production of processed cheese with reduced sodium content. Nevertheless, at high levels extensive bitterness of the final processed cheese can be reported. TSC presents better calcium ion-exchange ability and casein dispersion properties compared to sodium monophosphates and diphosphates. However, TSC is not able to bind on caseins and therefore, is not directly part of the novel protein network. In general, the main role lies on the ability of ion-exchange ability (Dimitreli & Thomareis, 2009; El-Bakry et al., 2011; Lu, Shirashoji & Lucey, 2008; Mizuno & Lucey, 2005, Shirashoji et al., 2006; Molins, 1991; Salek et al., 2015; Tamine, A. Y., 2011).

The development of hardness in processed cheese samples, depending on the composition of emulsifying salt mixtures consting of DSP, TSPP and TSC was studied by Salek et al. (2015). The specific ratio of DSP:TSPP (approximately 1:1–2:3) was once again identified, leading to a significant increase in the hardness of the tested processed cheese samples. However, the influence of this specific ratio rapidly decreased, when the proportion of TSC in the mixture of

emulsifying salts increased. Nevertheless, the influence of this specific ratio (DSP:TSPP) on the hardness of the processed cheese samples even at 40 % levels of TSC was insignificant. On the other hand, if the proportion of TSC was higher than 40 % (in the ternary mixture of emulsifying salts), the hardness of the samples increased with the increasing proportion of TSPP and TSC and with the decreasing levels of DSP (Salek et al., 2015).

In addition the latter authors reported the development of hardness values in processed cheese samples, depending on the composition of the emulsifying salts in ternary mixtures composed of DSP, TSC and POLY20. It could be stated that the increasing level of TSC and POLY20 (and with the reducing level of DSP in the mixture), the hardness of the processed cheese samples increased (Salek et al., 2015). Furthermore, in the same study were also presented the values of hardness of processed cheese samples composed by the ternary mixtures of emulsifying salts of TSC:TSPP:POLY20. When the proportion of POLY20 was at zero levels in the ternary mixtures of emulsifying salts, firmer processed cheese samples were reported than samples composed of TSC and TSPP (at a ratio 1:1). In addition, any deviation from this ratio resulted in significant hardness decrease. Hence, this phenomenon was only observed in the absence of POLY20 (in the mixture of emulsifying salts). On the contrary, with the increasing proportion of POLY20, the hardness of the processed cheese samples increased. Moreover, at constant levels of POLY20 the hardness of the samples slightly decreased as the level of TSC decreased (Salek et al., 2015).

The explanation of the above-mentioned results in processed cheese samples developed by the application of ternary mixtures of monophosphates, diphosphates, polyphosphates and trisodium citrate could be based on the specific properties of the individual phosphates and/or citrates or their mutual synergic relationship (El-Bakry et al., 2011; Lu et al., 2008; Mizuno & Lucey, 2005b, 2007; Shirashoji et al., 2010; Salek et al., 2015). The development of processed cheese hardness with the use of ternary mixtures containing TSC could be explained by the ability of the mixture of emulsifying salts to disperse casein. Generally, when a mixture of emulsifying salts with more intensive ability to disperse casein was applied, the harder processed cheese was produced. (Buňka et al., 2013; Lu et al., 2008; Mizuno & Lucey, 2007; Shirashoji et al., 2010). In case of TSC and TSPP binary mixtures, a specific ratio was observed (1:1) which led to the increase of the hardness of the processed cheese samples. However, there is no clear explanation about the interactions among TSC and TSPP and their involvement in the development of the casein network. TSC does not participate in the creation of new networks, and therefore the effect of diphosphates on casein crosslinking would not be influenced by the presence of TSC (Kalliapan & Lucey, 2011; Lu et al., 2008; Mizuno & Lucey, 2005). Additionally, a possible explanation may be that diphosphates are effective on enhancing casein proteins gel formation ability only when their concentration is at some optimum level relatively to the protein content (Mizuno & Lucey, 2007). In general, TSC is applied in mixtures of trade emulsifying salts and is used in the production of block-type and processed cheese in slices (Gupta, et al., 1984; Carić & Kaláb, 1997; Guinee et al., 2003; Mizuno & Lucey, 2005a, 2007; Shirashoji et al., 2006).

Salek et al. (2015, 2016, 2017; 2020) used different types of natural cheese with varying maturity degrees. The absolute values of processed cheese hardness were dependent on the natural cheese type applied. On the other hand, the general trend of the effect of the ternary mixture composition (DSP, TSPP, POLY20 and TSC) on the processed cheese hardness was

not affected. Therefore, when the maturity degree increased, the hardness of processed cheese decreased, but the general trend of the functionality of ternary mixtures (including also TSC) of emulsifying salts was not affected. In addition, processed cheese hardness increased with the prolonging of the storage time (up to 60 days), with no significant impact on the general trend of functionality of the ternary mixtures of emulsifying salts (Dimitreli & Thomareis, 2009; El-Bakry et al., 2011; Lu et al., 2008; Salek et al., 2015, 2016, 2017; Shirashoji et al., 2006).

Citrates can present some inhibitory effect on microorganisms which can cause spoilage of processed cheese (mainly due to production of gas). On the whole, the antimicrobial effect of polyphosphates, orthophosphates or monophosphates is characterized as better in comparison to citrates (Buňková & Buňka, 2017).

4. Selected factors affecting the functional properties of processed cheese

4.1 The impact of natural cheese and dairy fat source on the textural and rheological properties of processed cheese

Many types of natural cheeses could be used during the production of processed cheeses. Different geographical regions (countries) usually use various types of natural cheese. Hence, Dutch-type, Swiss-type, Cheddar-type, Mozzarella-type chesses are commonly applied in the European countries. However, white-brined cheeses are frequently used in the regions of Near East and Middle East. For processed cheese structure and consistency is not important only the type of natural cheese, but also "age" of natural cheese so-called degree of maturity (Guinee et al., 2004; Carić et al., 1985; Kapoor et al., 2007; Kapoor & Metzger, 2008; Salek et al., 2015, 2016, 2017). Many biochemical and microbiological changes are taking place during the natural cheeses ripening. These biochemical and microbiological changes are responsible for organoleptic and physicochemical properties of natural cheeses, which are crucial for the processed cheese properties. In the work of Brickley et al. (2007, 2008), Buňka et al. (2013, 2014), Salek et al. (2016, 2017) was reported that, when the maturity degree of natural cheese increases, the hardness of the resultant processed cheese is decreasing. Furthermore, more mature natural cheese with more hydrolyzed protein chains entail better meltability and fullness of aroma in the final processed cheese. However, there is a risk of a sharp to pungent taste and above all, instability of the final product, because short protein chains are not able to bind water sufficiently. In addition, there is a risk of fat release (oiling-off), because short protein chains do not have the required emulsifying capacity (Berger et al., 2002; Guinee et al., 2004; Piska & Štětina, 2004). In practice, this problem is usually solved by processing raw material with a higher level of intact (non-hydrolyzed, or very slightly hydrolyzed) casein. Young natural cheeses (less mature) are cheaper compared to middle or high maturity cheeses. These young natural cheese result in lower values of meltability in processed cheese, but the resulting network of processed cheeses is stable without a tendency to release water. Consistency of processed cheese manufactured from young raw material is from stiff to very firm (solid). Therefore, a larger proportion of younger natural cheese is applied for the production block-type processed cheese or processed cheeses intended for slicing (Guinee et al., 2004; Brickley et al., 2007; Lu et al., 2007; Salek et al., 2016). Moreover, for the manufacture of spread-type processed cheese it should be used a more mature natural cheese (as the main raw material).

Additionally, processed cheese manufacturers apply different sources of milk fat (containing various amounts of surfactants, such as phospholipids, lipoproteins, glycoproteins). In case of butter and anhydrous milk fat, is possible (due to the technology of their production) to except extremely small amounts of surfactants. On the other hand, in case of cream and natural cheeses, fat is in the form of fat globules (with lipoproteins membranes containing surfactants). These surface-active substances can cooperate over fat re-emulsification during processed cheese production and their concentration can affect final product rheological and textural characteristics. In addition, the influence of dairy fat source with various surface-active compounds content on the viscoelastic properties of processed cheeses was studied by

Černíková et al. (2018b). Natural cheese with various level of fat (ingredient containing fat globules coated in surface-active compounds) and butter (fat without surface-active compounds) were used in different ratios to reach 50% w/w fat content in the final product. According to the above-mentioned authors the effect of various fat content originating from natural cheese was not significant. The latter observation is important for processed cheese producers, because they can utilize the basic raw material (natural cheese) with different fat in dry matter content without consistency defects.

4.2 The effect of melt pH on the textural and rheological properties of processed cheese

Spreadable processed cheeses have pH optimum in range 5.6 – 6.0, processed cheese blocks are characterized pH 5.0 – 5.5 (Berger et al., 2002) and cheese sauces are determined pH in value 6.5 – 7.0. This means that a lower pH of processed cheese than the optimum (specify for each type of product) occurs to the solid or even crumbly consistency of processed cheeses (Marchesseau et al., 1997; Lee & Klostermeyer, 2001; Muslow et al., 2007; Barth et al., 2017), which exhibited a harder and more brittle appearance than processed cheeses with higher pH (6.0 – 6.8), that showed a more liquid texture, resembling a very viscous liquid (Barth et al., 2017). Higher pH than optimum value can cause not only a watery texture but there exists also the risk of undesirable microflora development. Therefore, it is necessary to carefully consider the choice of emulsifying salts for each type of product and choose specific combination for the achievement of the optimal pH. If the raw materials have low pH (e.g. curd), it is absolutely essential to choose emulsifying salts which will increase the pH of the system and vice versa, if more mature natural cheeses are used for production, it is advisable to apply emulsifying salts adjusting the pH to a more acidic range. Barth et al. (2017) studied how pH influences the hydrolysis of sodium polyphosphate in dairy matrices and the structure of processed cheeses. The latter authors stated that hydrolysis of sodium polyphosphates increased with the decreasing pH value. In processed cheeses with initial pH of 5.2 and 5.6, the final pH remained practically constant, because the higher rate of hydrolysis (at these pH values) increased the percentage of monophosphates (buffering agents), which are able to maintain unaffected pH values. Decreasing the pH value caused the rising of hardness, adhesiveness, and gumminess of the processed cheese. Higher pH (above 6.0) values resulted in a concentrated protein emulsion with “long” texture and less adhesiveness. The negative charges of the caseins allow the formation hydrogen bonds and electrostatic interactions, which enhance the absorption and binding of water by the protein matrix. The long chain polyphosphates added multiple negative charges to the caseins and thus, the hardness of the matrix decreased.

However, emulsifying salts do not affect the consistency only due to pH value. Many studies established specific ratios of emulsifying salts. Furthermore, each emulsifying salt has specific properties and in selected/specific ratios can influence the consistency of the developed processed cheese differently (Mizuno & Lucey, 2005, 2007; Shirashoji et al., 2006, 2010; Sádlíková et al., 2010; El-Bakry et al., 2011; Weiserová et al., 2011; Buňka et al., 2013; Nagyová et al., 2014; Salek et al. 2015, 2016, 2017). In these studies, the authors described the use of individual emulsifying salts, binary and ternary mixtures of emulsifying salts and their effect on processed cheese textural and rheological properties. Additionally, the total concentration of the applied emulsifying salts can influence the consistency of processed

cheese. Buňka et al. (2014) stated that the hardness of processed cheese increases with the rising concentration of emulsifying salts in the mixture. The same statement was also reported by Mozuraityte et al. (2019).

4.3 The impact of target parameters, holding time, agitation speed, melting temperature, cooling rate and storage time on processed cheese textural and rheological properties

Target parameters

A very large group of factors affecting the consistency of processed cheeses are target parameters, especially dry matter content, fat in dry matter content, non-fat dry matter, percentage of protein content in dry matter content, and pH. As a source of protein could be applied nature cheeses which are also source of fat, curd, caseins, and other ingredients describe in previous chapter. Similarly, it can be talk about fat sources. It can be used milk fat in different form as butter, cream, natural cheeses, and anhydrous milk fat and then could be used non-dairy fat sources as different type of oils. It is also mentioned above. About target parameters are not many comparable works to be done in the last 20 years. Articles which exist about that (Dimitreli & Thomareis 2004; 2007; 2008), Bayarri et al. (2012), Guinee & O'Callaghan (2013), Chatziantoniou et al. (2015), Lee et al. (2015) and Černíková et al. (2017a), are relatively difficult to compare to each other and to look for clear trends in influencing the consistency of processed cheese, because individual experiments were usually performed under different conditions (in terms of final processed cheese parameters (especially dry matter and fat in dry matter content) and the composition of the raw material mixture, as well as in terms of the process parameters used in production of processed cheese model samples.
The dry matter content and the fat in dry matter content are one of the most important parameters. The limits or ranges of these parameters are usually incorporated to the food law. These are also the basic parameters according to which processed cheese are often divided in terms of consistency into processed cheese blocks, processed cheeses intended for slicing, processed cheeses with a quarry, spreadable processed cheeses, or processed cheese sauce.
Lee et al. (2015) studied the influence of protein content (10 – 20 % w/w) and fat content (0 – 40 % w/w) on viscoelastic properties of model samples of processed cheeses made from sweetly (rennet) precipitated casein, melting temperature 85 °C, stored for 24 hours. With increasing protein content and decreasing fat content (constant ratio protein: water, changeable dry matter content) growing processed cheese firmness. Influence of protein content was found out more significant in comparison to fat content. Guinee & O´Callaghan (2013) prepared for their work processed cheeses from Cheddar and defatted cheese at a melting temperature 80 °C. Final processed cheeses hade different contents of fat (14 – 33 % w/w) and different contents of protein (25 – 12 % w/w). Dry matter content (46 – 47 % w/w) was constant. Cheeses were stored for a maximum of 4 days. The firmness decreased with increasing fat content (reducing ratio protein: fat). Dimitreli & Thomareis, (2004; 2007; 2008) examined viscosity, textural and viscoelastic properties of processed cheeses (manufactured from Gouda at a melting temperature 80 °C) with different dry matter content (38 – 62 % w/w), protein content (11 – 30 % w/w) and fat content (12 – 23 % w/w). All cheeses were storied only 24 hours. The

firmness of processed cheeses decreased with the decreasing dry matter content and protein content and with increasing fat content. Bayarri et al. (2012) and Chatziantoniu et al. (2015) sold specific processed cheeses made from whey cheese and at the same time examined the influence of the content components on the consistency of commercially available processed cheeses. Both authors presented increasing firmness with decreasing fat content. Černíková et al. (2017a) studied consistency and microstructure model processed cheese with two dry matter content (35 % and 45 % (w/w)), two fat in dry matter content (40 % and 50 % (w/w)), melting temperature 86 °C, and storage 14 days. When assessing the effect of one parameter (e.g. dry matter content), the other parameter (in this case the fat content) was constant and vice versa. It was found that with increasing dry matter content (with constant fat in dry matter content) and decreasing fat in dry matter content (while maintaining dry matter content) the gel strength was increased. At the same time, an interaction factor (*z*) increased, it means the number of interacting structural units in the protein network was higher. Furthermore, it has been shown that with increasing fat in the dry matter content (at a constant dry matter content), the diameter of the fat globules increased and at the same time the firmness of the processed cheeses decreased. The protein to fat ratio was ~ 1: 0.85 for model processed cheeses with 40 % (w/w) fat in dry matter content and ~ 1: 1.30 for products with 50 % (w/w) fat in dry matter content. The relatively lower amount of proteins, especially caseins, which act as emulsifiers in processed cheeses, caused a lower degree of fat emulsification, which was reflected in increasing of the fat globule's diameter. Larger fat globules can disrupt the continuity of the protein matrix much more than smaller fat globules and produce a softer, more spreadable processed cheese. On the other hand, processed cheeses containing a larger number of smaller fat globules give rise firmer and less spreadable processed cheese (Kapoor & Metzger, 2008; Lee et al., 2003). Increasing the dry matter content (with a constant fat content in the dry matter) and thus increasing the relative concentration of proteins, which increase the emulsifying ability of the system, was reflected in a decrease in the diameter of the fat globules and at the same time an increase in firm stiffness of processed cheese. Lee et al. (2015) also established that with increasing protein content reach the fat globules lower size. Increasing of apparent viscosity with rising protein concentration in model samples described also Lenze et al. (2019), who proved the necessity minimally 15 % (w/w) of protein to reach an apparent viscosity over then 1.0 $Pa \cdot s^{-1}$ at time approximately 200 minutes.

So, with increasing nitrogenous substances (under the same condition) grow firmness and decrease spreadability. This fact is explained by rising intensity of protein network, because nitrogenous substances are primarily "network building or supporting stuffs" and they need to meet other protein molecules to increasing the number of interactions with following protein aggregation and gel (network) formation. Lenze et al. (2019) also assumed that increasing protein concentration including para-caseinate can better and quickly adsorb on the stuffs surface at the oil-water interface, thereby decrease interfacial tension and facilitate emulsion stabilization. On the other hand, under the same condition, increasing water and in water soluble saccharides, or fat content, or other water content ingredients is protein matrix much more damaged three-dimensional network in processed cheese, which lead to less solidity and more spreadable processed cheese.

Holding time

Sutheerwattananonda et al. (1997) studied the effect of the holding time 0 – 15 minutes at the melting temperature 65.5 °C on the distribution of fat globules in processed cheese blocks and observed the decreasing fat globules size (the emulsification improved) in the first five minutes holding time at the melting temperature. The reduction of fat globules size had a consequence harder processed cheese and was stopped after 5 minutes holding time. Further extension of holding time did not have the significant effect on the size of fat globules. Bowland & Foegeding (1999) studied the effect of processing time on the consistency of processed cheese blocks prepared from rennet casein. Authors chose 10, 20, and 30 minutes holding time, 80 °C melting temperature and constant agitation speed (first three minutes 300 rpm (only rennet casein) and, than other ingredients were added and samples were mixed at 2000 rpm). The strength of the processed cheese gel increased with the prolonged melting time. On the other hand, Swenson et al. (2000) studied the effect of holding time (0 – 20 minutes) on the final consistency of fat-free processed cheese spread. They used lower melting temperature, specifically 75 °C, than Bowland & Foegeding (1999), the agitation speed was constant but not exactly mentioned. In this case, the opposite result, the extending holding time cause decreasing firmness of processed cheese product, was found. Lee et al. (2003) described the viscosity changes of the hot melt. In this work, the viscosity riced for the first 25 minutes and then, after reaching the local maximum, the viscosity of hot melt slowly fell to the end of the measurement (50 minutes). The final consistency of cooled products did not evaluate. Norohna et al. (2008a) investigated the effect of different agitation speed in range 100 – 1500 rpm at the point of 80 °C with a constant endurance 2 minutes on the consistency of melted cheese blocks. The final consistency of processed cheese block was stiffer with increasing agitation speed. Similar result published Přikryl et al. (2018), who stated rising value of complex modulus with prolonged holding time (0, 5, 10 minutes) at melting temperature 80 °C and 90 °C and constant agitation speed 1500 rpm. Articles mentioned-above studied separately one of the important processing parameters, holding time, or melting temperature, or influence of special ingredients.

A comprehensive work, which would consider the both effect of the mixing speed and the holding time of the melting temperature was published by Černíková et al. (2017b, 2018c). Different in these articles was in usage processed cheese samples with various fat in dry matter content (40 % and 50 % (w/w)).

Černíková et al. (2017b) dealt the combined effect of the agitation speed of the knives of the production equipment (1000, 1500 and 3000 rpm) and the holding time (0 – 20 minutes) at a melting temperature of 90 °C on the consistency of spreadable processed cheeses with 35 % (w/w) dry matter content and 40 % (w/w) fat in dry matter during 60 days of storage. The holding time of the melting temperature was divided into shorter intervals than in previous publications. First, a holding time of 0 minutes was selected, when the melting process was completed after reaching the melting temperature. Furthermore, the melting temperature endurance was extended to 1, 3, 5, 7, 9, 11, 13, 15, 17 and 20 minutes. After reaching the melting temperature, the melt was constantly stirred at the set speed during the mixing. The monitored residence time intervals were chosen to map the effect of blending time more precisely on the consistency of processed cheeses. It was found that both parameters significantly affected the consistency of processed cheeses. As the melting temperature

continued, the firmness of processed cheeses generally increased, similarly to Bowland & Foegeding (1999). The exception was the lowest endurance times (one day after manufacturing), when model samples produced with an endurance of one minute showed lower stiffness compared to samples produced without any endurance, which were not reported by previous authors. A downward trend in stiffness (weakening of the protein matrix) for one minute was observed for all three mixing speeds used. The decreasing complex modulus which determinates hardness of processed cheese, was supported by the detected decrease in gel strength (A_F) and interaction factor (z). With a further increase in the holding time of the melting temperature above 3 minutes the stiffness of the model samples increased with increasing holding time for all observed knife speed intensities (1000, 1500 and 3000 rpm). There was exception in 3000 rpm 1 day after storage. In this case was noticed further decrease of complex modulus value. However, there was no significant difference between processed cheese production at the agitation speeds of 1000 and 1500 rpm (melting temperature 90 °C 1 and 3 minutes). A similar trend in rising the strength and coherence of processed cheese was observed by Sutheerawattananonda et al. (1997) and Bowland & Foegeding (1999). However, Černíková et al. (2017b, 2018c) used higher melting temperature than above-mentioned authors Sutheerawattananonda et al. (1997) who applied 65.5 °C and Bowland & Foegeding (1999) who used 80 °C which is closer to typical industry melting temperature. The increase in firmness of processed cheeses is explained by Sutheerawattananonda et al. (1997) by reducing the size of fat globules, where a larger number of small fat globules disrupt the continuity of the protein matrix less intensively compared to the presence of a smaller number of order of magnitude larger fat globules. Simultaneously with the decrease in the size of fat beads, the authors found that the stiffness of the monitored samples also increases with increasing holding time. However, the above-mentioned authors also stated that the reduction in the size of the fat globules stops after about 5 minutes. Therefore, in this matrix, where the increase in stiffness occurred even with longer melting temperature durations (in addition to the effect of decreasing the size of fat globules), other interactions can be expected (especially more intensive hydration of caseins and increase in the number of their bonds), which contribute to increasing stiffness and are supported by more extensive melt mechanical stress (Bowland & Foegeding, 1999; Lee et al., 2003). The biggest and rapid increase of z-factor was observed et the highest used agitation speed (3000 rpm) from 10^{th} minutes. Similar trend initial firmness decrease up to 3th minutes and following increase of firmness (determined G*), as in work Černíková et al. (2017b) who research semi-fat processed cheeses, was noted by processed cheese samples with higher fat in dry matter content (full fat processed cheeses) in study Černíková et al. (2018c).

Agitation speed

As it was mentioned above, not only the holding time, but also the agitation speed fundamentally affected the consistency of the model processed cheeses. In the literature exist two different conclusions. Noronha et al. (2008a) reported more rigid final products processed cheese block with increasing agitation speed. They used melting temperature 80 °C, agitation speed 100 – 1500 rpm (750, 1125 and 1500 rpm in Stehpan and 100 or 200 rpm in Blentech cooker) and constant holding time 2 minutes. On the other hand, Černíková et al. (2017b, 2018c) recorded softest samples were made at 1500 rpm, then at 1000 rpm agitation speed.

With using the highest agitation speed (3000 rpm) were prepared the most solid spreadable processed cheese samples. The difference in results may be due to the type of processed cheese blocks versus spreadable processed cheeses, various melting temperature, lower mixing speeds and shorter holding time.

Melting temperature

Processed cheeses can be produced by many melting temperatures in ranges 85 – 110 °C (Kapoor & Metzger, 2008) or 72 – 145 °C (Tamine, 2011). Some authors described also lower temperature 65 °C, Sutheerawattananonda et al. (1997). Higher temperatures (135 – 145 °C) are used especially during continuous production in compare to lower melting temperature in discontinuous equipment. Generally, for processed cheese blocks can be used lower temperature than for spreadable processed cheeses. But it is also possible to produce block processed cheeses by the higher melting temperature with adaptation other production conditions such as type of melting equipment and agitation speed (Guinee, 2003; Guinee et al., 2004; Tamine, 2011).

Influence of consistency by melting temperature was studied by Kaláb et al. (1987) and Dimitreli & Thomareis (2004), who report that when the melting temperature increases, the viscosity of melt decreases and the size of the fat globules decreases, which is accompanied by an increase in the stiffness of the final product. On the other hand, Swenson et al. (2000) studied the effect of temperature in the range of 60 – 90 °C on the consistency of defatted processed cheese spreads. Their work showed decreasing in the firmness of fat-free processed cheeses, with increasing melting temperature in the range from 60 – 80 °C. The increasing firmness of these fat-free processed cheese spreads was observed at melting temperature 90 °C. An increase of processed cheese spread firmness produced at 90 °C was observed also by Přikryl et al. (2018) in all batches produced (control samples without biologically active substances as well as samples containing rutin and quercetin) compared to samples produced at 80 °C. This conclusion was valid for the defatted processed cheeses and also for full fat processed cheeses with 50 % (w/w) fat in dry matter content. Similar, Mozuraityte et al. (2019) studied different creaming time (1.5, 3.0, 4.5 minutes) at various creaming temperature (45, 55, 65 °C), the final melting temperature (95 °C) was holding 6 minutes, but authors stated that, the creaming temperature was not crucial for the final consistency of processed cheese product. In this case is important to say, that in chapter 6 is creaming describe like something different.

Cooling rate and storage time

The final consistency (described by textural and rheological characteristics) of processed cheese can be influenced by the cooling rate. Piska & Štětina (2004) reported that higher cooling rate can lead to the less firm, more spreadable and with elevated stickiness processed cheese. In addition, high cooling rate of processed cheeses provides more extensively presence of the β´ polymorph compare to the β polymorph which is usually observed in bulk milk fat (Ramel & Marangoni, 2017). The above-mentioned authors also reported that the presence of protein and/or other ingredients can led to higher changes of the β´ polymorph to β polymorph (Ramel & Marangoni, 2017). On the other hand, when the conditions and other parameters (including

the dry matter content, fat in dry matter content, melting temperature, holding time and agitation speed) are the same, lower cooling rate provides rise to stiffer products final processed cheese products. In the same token, higher cooling rate is required for the production of spreadable processed cheese, while relatively low cooling rate is suggested for the manufacture of block-type processed cheese (Muslow et al., 2007). In the case of processed cheese spreads and especially processed cheese sauces, lower rate of cooling can cause the product to remain at temperatures suitable for the growth of microorganisms for a longer period of time, and this may lead to undesired multiplication of contaminating microflora (Görner & Valík, 2004).

Processed cheese storage, together with some other processing parameters (agitation speed, holding time, melting temperature), one of the most important factors affecting the textural and rheological properties, and thus also the structure of processed cheeses. The possible sources of processed cheese changes (like appearance, structure, color and flavor) during storage are e.g. loss of water, hydrolysis of polyphosphates, changes in ionic equilibria, fat polymorphism, formation of crystals, oxidation, non-enzymatic browning, enzyme activity, interactions with packaging materials (for other changes than consistency see chapter 10). The intensity of changes occurring during with the prolonging of processed cheeses storage period can be induced by ingredients applied, selected processing parameters, packaging type and storage conditions.

In the work of Černíková et al. (2017b, 2018c) the basic trends, the effects of agitation speed and holding time at a melting temperature, did not change during the two months of storage time. However, there was an increase in the absolute values of the elastic and loss moduli of the model processed cheese samples, a result as corresponding to the findings of other studies (Awad et al., 2002; Weiserová et al., 2011; Nagyová et al., 2014 and Salek et al., 2015), respectively decrease of processed cheese elasticity (Younis et al., 1991). The explanation of this phenomenon in particular the possible hydrolysis of emulsifying salts – diphosphates and polyphosphate, the possible change in the dissociation of the salts or other compounds present, the decrease in the pH values of processed cheeses and also the polymorphism of milk fat and ongoing changes in its crystalline form where β´ polymorph change to β polymorph (Awad et al., 2002; Muslow et al., 2007; Dimitreli & Thomareis, 2009; Shirashoji et al., 2010; Weiserová et al., 2011; Nagyová et al., 2014). Increasing dry matter content during processed cheese storage period (due to water evaporation in dependence on permeability of packaging material) could be other reason to increase the firmness of processed cheese (Fox at al., 2004; Mohammadi & Fadaei, 2018). Weiss et al. (2018) wanted to prepare mathematical models as predictive tool for the assessment of changes in quality parameters during the storage of spreadable processed cheese at 8, 20 and 30 °C. During 4 monthly the storage at 8 °C was found only minor no significant changes. At the storage temperature 20 °C were identified dynamic changes which significantly decreased quality of processed cheese product, but the highest rate of changes was registered at the storage temperature 30 °C. Reactions in the processed cheeses led to the negative taste, smell and changes in consistency and authors recommended the approximate shelf-life limit for spreadable cheeses stored at 8, 20 and 30 °C without significant changes in its quality on the 49, 28 and 4 days (Weiss et al., 2018). Which does not fully correspond to the commonly storage condition for processed cheeses, because processed cheeses can be stored also at a room temperature for 4 months or 6 months in a refrigerator. Also, Schär & Bosset (2002) described processed cheese as “semi-preserved food” with a

limited shelf-life, they allege that products without bacterial spoilage retain their quality for 4 – 12 months at room temperature and under the lower storage temperature for long time, because lower temperature cause more slowly changes in quality processed cheeses.

4.4 The effect of rework addition on processed cheese textural and rheological properties

Rework (sometimes also reported as a pre-cooked cheese) is a processed cheese that has already been produced and the creaming process has previously occurred. Manufacturers apply rework as a raw material during the production processed cheese. In addition, exist different types of rework. In particular, rework is produced in the industry either intentionally (expected) or unintentionally. Hence, intentionally means that the production of processed cheese was targeting the development of rework or the rework is the residual amount of processed cheese in production apparatus. The term unintentionally rework describes a processed cheese originally intended for the retail market network, however it was not "*released*" in the market, due to some deficiencies (incorrect dry matter content and/or fat in dry matter content or because of unsuitable packaging, or excessive stickiness on the packaging material). Rework is usually applied as fresh (a processed cheese residue in the production apparatus), 3 or up to 14 days after manufacture (it could be intentionally or unintentionally produced processed cheese) in a level up to 20 % (w/w), depending on the type of rework and also the desired target parameters of the final processed cheese (Kaláb et al., 1987; Guinee et al., 2004). The reasons for rework application are on the one hand economic and on the other hand to increase processed cheese viscosity, increase hardness, improve meltability and possibility of emulsifying salts reduction (rework contains emulsifying salts) (Lauck, 1972; Kaláb et al., 1987; Pluta et al., 2000; Kapoor & Metzger, 2008; Černíková et al., 2018a). According to Kaláb et al. (1987) are available three types of rework: (i) the fresh rework (produced and rapidly frozen immediately after production), (ii) the regular rework from previous manufacture batches and (iii) the so-called hot melt, simulating processed cheese subjected to extreme stress conditions (cooled from 82 °C to 4 °C in 5 hours). Furthermore, another rework type according to Mayer (1973) is referred as over-creamed. In general, each type of rework can be utilized for various reasons and in various levels. Hence, the first type of rework (fresh) can be applied in an amount ranging from 1 to 2 % (w/w) in order to increase the creaming of processed cheese spreads, containing higher level of matured natural cheese. In addition, the second type of rework is used for processed cheese blocks or block-type processed cheese in concentration ranging from 2 up to 30 % (w/w), to increase hardness and elasticity. Finally, the third type of rework (over-creamed) is applied in a quantity less than 1 % (w/w), because it has an extraordinarily strong creaming effect and could easily lead to deficiencies the final processed cheese (over-creaming). However, Lauck (1972) recommended that the rework addition should be between 2 to 15 % (w/w). Guinee et al. (2004) evaluated the application of individual rework types in processed cheese production. Furthermore, additional heating of rework can cause a higher degree of temperature-induced dehydration and aggregation of the para-casein (mainly the third type of rework) and increase the elasticity of the final product. Moreover, the emulsifying salts in rework can create more effective dispersion resulting in faster hydration, higher degree of emulsification (effective dispersion of fat) leading to product viscosity increase. The impact of different rework amount on the viscoelastic properties and

microstructure of spreadable processed cheeses was reported in the work of Černíková et al. (2018a). The later authors applied rework at a range of 0 – 20 % (w/w) using real industrial conditions. The rework was prepared from the same raw material (natural cheese) as processed cheeses and was added to the blend of ingredients 3 days after the production day and was stored at 6 ± 2 °C. The consistency of processed cheeses without rework was characterized as soft (samples reported the lowest values of elastic and loss moduli and the largest size of fat droplets) compared to samples prepared without addition of rework. The processed cheese firmness increased with the quantity of rework and simultaneously, the size of fat droplets gradually decreased, which means increasing emulsification properties with the further rework addition. Higher concentration of rework from 10.0 to 20.0 % (w/w) did not provide significant changes in the processed cheese consistency, express by viscoelastic properties (complex modulus; G*) and also the median of fat droplet size was smaller than in the case of lower rework addition. Moreover, the addition of rework affected the intensity of the emulsification of the present fat, which was demonstrated by the reduction of the fat droplets size. As the degree of fat emulsification grew, the processed cheese firmness increased, corresponding with the findings presented by Kapoor & Metzger (2008), Lee et al. (2015) and Černíková et al. (2017a). However, these results are contradict to those reported by Kaláb et al. (1987), who used block-type processed cheese with higher dry matter content.

In addition, Fu et al. (2018) used four types of rework (pre-cooked cheeses) in amounts 1.5 % (w/w) with different agitation speeds [400 or 1500 rpm (rounds per minute)] and total stirring times (10 or 30 minutes), PCLS (400 rpm, 10 min), PCLL (400 rpm, 30 min), PCHS (1500 rpm, 10 min), PCHL (1500 rpm, 30 min), the final melting temperature was 90 °C and rework was used 24 hours after production and was stored at 5 °C. The viscosity of products with short time mixing were smooth without any increasing of viscosity (the creaming effect did not occur). The addition of long time (30 minutes) rework (PCLL, PCHL) influenced the viscosity of processed cheese samples which increased and the creaming effect was enhanced during processing. The application of PCHL showed higher increase probably due to advanced agitation speed. This study showed that the rework in amount less than 1.5 % (w/w) produced with longer mixing time had a capacity to increase the viscosity (to induce the creaming effect) of processed cheese manufacturing only for 15 minutes, due to fine-stranded network. Lenze et al. (2019) stated that rework addition [5 and 10 % (w/w)] dramatically accelerated the creation of the new structure. During creaming was observed very short initial phase (to 10 minutes), when exponential phase started earlier, and the apparent viscosity of the matrix increased very sharply. Plateau phase was shorter and in higher level of apparent viscosity in comparison to samples developed without rework addition. Rework addition in amount 10 % (w/w) caused faster structure formation than 5 % (w/w) supplement (Lenze et al., 2019).

4.5 The impact of hydrocolloids and food additives addition on the textural and rheological properties of processed cheese

Hydrocolloids can be classified also as biopolymers. In addition, hydrocolloids include two categories of substances –proteins and polysaccharides, that are widely used for their physicochemical characteristics, primarily water binding, increasing of system viscosity (thickening), ability to create gel structures, stabilizing foams, emulsions, or dispersions and

decreasing water release (control of the syneresis phenomenon), secure temperature stability, control release of flavors etc. Hydrocolloids can be also applied for end-product price reduction. Even though they are added in very low level [commonly less than 1.0 % (w/w)], they can have a significant influence on the textural/rheological and sensory properties of the final food matrix (Lu et al. 2007). Hydrocolloids can be used in dairy systems (not only in processed cheeses) separately or in mixtures of these (Swenson et al., 2000; Vega et al., 2005; Černíková et al., 2008; Macků et al., 2008; Chatziantoniou et al., 2019).

Proteins are present in processed cheeses by caseins or caseins fragments, sometimes by the use of whey proteins. Sometimes may be encountered the application of other animal-origin proteins (gelatin) or plant-origin proteins (isolated from soybean and pea) into processed cheese or their imitations. Furthermore, gelatin in an amount of 2 % (w/w) was applied by Swenson et al. (2000) for the production of processed cheese free of fat. The consistency of the latter products was more firm compared to full-fat processed cheese (a reference sample). Native whey protein isolate and polymerized whey protein isolate in an amount of 2, 4, 6 % (w/w) were investigated as partial fat replacements in processed cheese. Thus, the final structure of these products was different (Nastaj et al., 2020).

In addition, the second large group of hydrocolloids which are used in the food industry are polysaccharides. In particular, processed cheese manufacturers can use polysaccharides extracted from plants (starch and pectin; native or modified), from seeds (guar or locust been gum), tree exudates (arabic, karaya, tragacanth gums) or polysaccharides from algae sources (agar, carrageenan, furcellaran, alginate). The last group of polysaccharides, which can be used in processed cheese production, originate from microorganisms (xanthan gum). Hydrocolloids have to comply with food law, which content different requirements in EU (e.g. Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives, as amended) and in USA (requirements Code of Federal regulation). In both case all of ingredient must be healthy and used in permission amount (Europa, 2008; USA, 2020). Locust bean gum, guar gum, xanthan gum, gum karaya, tragacanth, carrageenan, carboxy-methyl-cellulose, sodium alginate, propylene-glyco-alginate, gelatin are allowed as additives to the spreadable processed cheeses in concentration up to 0.8 % (w/w) due to specific interaction with caseins proteins (USA, 2020). Most of them are used in limited amount up to 0.8 % (w/w) (Chatziantoniou et al., 2019). Processed cheese containing xanthan gum, guar gum, locust bean gum and mixtures of xanthan and guar gums showed less consistensy and solid structure, while their spreadability and smoothness was higher (compared to control samples). Furthermore, largest fat globules and higher levels of spreadability were established in the samples with xanthan gum addition. On the other hand, the smallest fat globules were observed in the samples prepared with a mixture of locust bean gum and κ-carragenan, which also presented lower values of spreadability and exhibited the highest hardness. Simmilar results were reported in samples containing mixtures of locust bean and xanthan gums. In both cases were noted synergistic effects resulting in increased firmness and decreased spreadability. In case of partial substitution of κ-carragenan with guar or xanthan gums, were produced samples with decreased hardness and increased smoothness and spreadability (Chatziantoniou et al., 2019). There are many studies focused on application 0.1 – 1.0 % (w/w) of carrageenan into processed cheese. The authors observed that with the increasing carrageenan level up to 1.0 % (w/w), the hardness of the processed cheese increased (Ribeiro et al., 2004; Gustaw & Mleko, 2007; Černíková et

al., 2008). In addition, 0.2 % (w/w) of carrageenan can cause a reduction in processed cheese stickiness onto the packaging material (Gustaw & Mleko, 2007). Černíková et al. (2008) reported that a concentration of 0.05 % (w/w) was too low for effective increase of processed cheeses hardness. However, higher concentrations (0.15 % and 0.25 % (w/w) caused significant increase of model processed cheese hardness in comparison to the control sample prepared without carrageenan addition. This fact was previously presented by Bourriot et al. (1999). Probably exists a minimum concentration of carrageenan for the effective formation of a carrageenan network. Moreover, this limiting amount is in dependence with many factors (such as strength of the protein matrix, degree of protein hydrolysis, pH, ionic environment, etc). On the other hand, Syrbe et al. (1998) brought out the information about possible destabilization and disintegration of the developed system with further increasing of carrageenan concentration. Carrageenan in an amount of 0.5 – 3.0 % (w/w) can also be applied in order to reduce the natural cheese content addition during the processed cheese manufacture (Williams & Phillips, 2009).

As a stabilizer for low fat processed cheeses can be applied also the konjac glucomannan (gum), locust bean gum, guar gum etc. (Swenson et al., 2000; Da Silva et al., 2016). Da Silva et al. (2016) used 0.5 % (w/w) of konjac glucomannan or konjac flavor in processed cheese samples with fat reduction (25, 50, 75, 100 %). The highest value of hardness was reported for samples with 50 % fat reduction. The effect of fat replacement by inulin on the physicochemical properties of processed cheese was studied by Sołowiej et al. (2015), who observed decreased hardness and adhesiveness of low-fat processed cheese in comparison to control full-fat processed cheese. On the other hand, Giri et al. (2017) reported decreasing values of spreadability with the increasing concentration of inulin [tested level were within the range of 0 – 8 % (w/w)].

Furthermore, another hydrocolloid applied in processed cheese production is pectin. Gels created by low esterified pectin are dependent on many factors such as, solubility and concentration of pectin, pH of the system, content and availability bivalent cations (especially Ca^{2+} ions). Affinity of pectin chain to calcium grows with the decreasing degree of esterification and increasing concentration of polymer (May, 2000; Löfgren et al., 2007). Moreover. the addition of carbohydrates can facilitate gel formation although the concentration of Ca^{2+} ions might be low (Fu et al., 1999). The gel strength increases with rising calcium content (Lootens et al. 2003). The rising pectin content resulted in the rose of processed cheese hardness, whereas this relation (processed cheese rigidity on pectin concentration) was found not to be linear. (Macků et al., 2008).

In addition, many types of starch exist which can be used as ingredients into processed cheese. Starches are usually applied in processed cheese as texture modifiers. Some producers take advantage of starch in order to replace part of the used natural cheese or for the production of processed cheese analogues. Mounsey & O´Riordan (2001) described lower meltability in the final processed cheese after application of various types of starch with low amount of amylose [wheat, potato, rice starch – in a total concentration of 3 % (w/w)]. They explained that the meltability decrease was resulted by partial protein dehydration and more intensive protein interactions. According to Mounsey & O´Riordan (2001) is important not only the source of starch, but also its structure. In particular, starch with low content of amylose can cause lower values of hardness, when used in an amount of 3 % (w/w). On the other hand, when this type

of starch will be applied in higher concentrations [more than 5 % (w/w)], the result could be increasing hardness of processed. Trivedi et al. (2008a) reported increasing values of complex modulus and viscosity in dependence with starch type and amount. The same authors also evaluated the possibility of partial replacement of natural cheese with cheaper ingredients like starch. The effect of tapioca and potato starch on the mechanical properties and microstructure of processed cheese with different types of casein network were evaluated by Fu & Nakamura (2018). According to the latter study, tapioca starch addition into processed cheese with random type of casein structure led to a dispersed casein network structure, so the firmness of the final product was higher. Moreover, the addition of potato starch led to the creation of a fine-stranded structure (from initial random structure) of the casein network in the resultant processed cheese. Hence, these samples presented higher values of hardness.

Hosseini-Parvar et al. (2015) focused on the effect of basil seed gum on the textural, rheological, and microstructural properties of processed cheese. In addition, basil seed gum is a novel surface-active hydrocolloid showing promising stabilizing and emulsifying properties and can be used as a functional ingredient in the food industry. The increasing level of basil seed gum led to a more elastic character of the processed cheese (the values of elastic and loss moduli increased). In general, the basil seed gum addition probably contributed to the emulsification of fat.

Lactose and sucrose are among the substances commonly used in the processed cheese production, for example in the form of whey, or other ingredients during the manufacture flavored processed cheese (e.g. chocolate, when producers usually use sugar). Macků et al. (2009) described the addition of glucose, galactose, lactose, and sucrose in processed cheeses samples also containing pectin. In conclusion, they reported that the addition of 1.0 % (w/w) of saccharide can cause the reduction of the gel stiffness, regardless of its reducing or non-reducing properties, structure (aldose versus ketose; monosaccharide versus disaccharide), or steric arrangement (epimers). Additionally, with the increasing lactose content, the firmness of the tested processed cheese decreased (Macků et al., 2009). However, high lactose content can cause the crystallization of lactose having a negative result on the consistency of the final processed cheese (Kapoor & Metzger, 2008).

Generally, the addition of emulsifiers supports the emulsification of fat. Thus, with the addition of an emulsifier the fat droplets become smaller, resulting in increased hardness and decreased values of spreadability of the tested processed cheese. Buňka et al. (2007) described the effect of 0.25 % (w/w) monoacid-glycerols (with low chain fatty acidy) addition on organoleptic properties of processed cheese. However, the above-mentioned authors did not observe significant effect on the processed cheese consistency. On the other hand, monoacid-glycerols with C16 and C18 have an impact on the flavor of the final processed cheese and increasing gel strength was also observed. Moreover, Awad et al. (2002) reported rising hardness of processed cheeses when emulsifiers were applied for the production of block-type processed cheese. Lee et al. (1996) stated that the influence of emulsifiers is not depending only on their emulsifying ability, but also on other properties (ionogenic or non-ionogenic emulsifiers). Furthermore, ionogenic emulsifiers can modify the charge of the protein fractions in processed cheese, a phenomenon which can influence the finally consistency of the processed cheese.

Therefore, many authors fortified processed cheeses or processed cheese analogues by so-called biological active (bioactive) substances. The latter can improve the nutritional characteristics of processed cheeses and similar products. Bioactive ingredients can be applied in a dry form, extracts or essential oils and include polyphenols, or other ingredients possessing antioxidant capacity. In particular, flavonoids (rutin, quercetin) were used in the work of Přikryl et al. (2018). Furthermore, carrot paste was used by Mohamed et al. (2016), apricot pulp by Mohamed & Shalaby (2016), tomato juice by Mehanna et al. (2017). Alga *Chlorella vulgaris* as a source of antioxidant, protein, fatty acid, fiber, vitamins, and minerals was applied by Tohamy et al. (2018). Moreover, Sadek et al. (2017) applied probiotics into processed cheese samples. All the above-mentioned ingredients could affect the processed cheese consistency in a different manner. The application of tomato juice caused a higher phase separation especially of oil and a less stable product was obtained (Mehanna et al., 2017). Furthermore, Přikryl et al. (2018) applied rutin and quercetin in an amount 0.5 % (w/w), which significantly decreased the gel strength of the resultant processed cheese (when the holding time was 1 min at the melting temperature of 80 °C). Additionally, higher temperature (90 °C) caused lower values of the complex modulus in all examined times, in comparison with processed cheese without rutin or quercetin addition. In the study of Sohli et al. (2020) processed cheeses were fortified with different levels of asparagus powder, which is a rich source in flavonoids and other phenolic compounds. In particular, the asparagus powder was added in an amount of 0.5; 1.0; 1.5 % (w/w) resulting in a more elastic processed cheese, with increased hardness and decreased values of spreadability.

Moreover, Belsito et al. (2017) and Ferrão et al. (2018) utillized galactooligosaccharides, respectively xyloologosaccharides into a unique type of processed cheese, the so called *requeijão cremoso*. The addition of galactooligosaccharides in an amount ranging from 1.5 to 4.0 % (w/w) led to lower values of complex modulus and gel strength. The lowest values of G* were observed for samples with 1.5 % (w/w) galactooligosaccharide addition. On the contrary, processed cheeses with 3.0 % (w/w) addition showed the most compact structure (investigated by scanning electron microscopy) with reduced size of fat globules (Belsito et al., 2017). Also, the addition of xyloologosaccharide in an amount of 3,3 % (w/w) (to low fat and low salt content processed cheese) resulted in a denser and more compact structure, with increasing apparent viscosity, values of elastic and complex moduli (Ferrão et al., 2018).

AIM OF THE HABILITATION THESIS

The main aim of the habilitation thesis was to evaluate the influence of selected factors affecting the functional properties (mainly in terms of consistency) of processed cheese during storage. The work was focused on factors that are important for industrial scale production of processed cheese and whose effect on the consistency of processed cheeses was not sufficiently up to now described in the literature.

Hence, in order to achieve the main objective of the thesis, it was necessary to establish the following sub-objectives:

- Evaluate the effect of various phosphate-based and/or citrate-based emulsifying salts application (as sole ingredients) on the functional properties of processed cheese and processed cheese sauces,
- To deal with changes in the consistency of processed cheeses depending on the different composition of ternary mixtures of phosphate and/or citrate emulsifying salts,
- To describe the effect of various natural cheese (different types and degrees of maturity) application in processed cheese production and describe their influence on processed cheese functional properties,
- To evaluate changes in the consistency of processed cheeses during their storage,
- To describe the effect of various levels of dry matter content and fat content in dry matter content on the consistency of processed cheese,
- To evaluate the impact of selected processing parameters (holding time and speed of agitation) on the consistency of spreadable processed cheese. In particular, to describe the effect of increasing holding time of the melt at the so-called melting temperature on the consistency of processed cheese manufactured at different regimes of agitation speed of knives,
- To investigate the effect of rework addition (at divergent levels) on the rheological and textural properties of processed cheese.

MAIN RESULTS OF THE WORK

A. The impact of emulsifying salts composition and natural cheese on the functional properties of processed cheese and similar products

Processed cheese can be manufactured by mixing natural cheese (of various types and degrees of maturity), water, emulsifying salts, and other optional dairy/non-dairy ingredients, commonly under vacuum, in the presence of heat and shear. The desired compact structure of processed cheese is obtained by the addition of appropriate emulsifying salts. Hence, the ability of emulsifying salts to sequester calcium from the casein matrix (exchanging Na^+ for Ca^{2+}) and the pH adjustment cause protein hydration and dispersion, and the casein present acts as the "true" emulsifier within the matrix (El-Bakry et al., 2011, Kapoor & Metzger, 2008, Lee et al., 2003, Lee & Klostermeyer, 2001; **A1**; **A2**; **A3**; **A4**; **B2**; **B5**; **B6**). However, the ion-exchange ability is not identical for all emulsifying salts. Therefore, the phosphate ion-exchange ability increases with the increasing content of P_2O_5 (Buňka et al., 2014, Shirashoji et al., 2006; **A1**; **A2**; **A3**; **A4**; **A5**). The consistency (a critical processed cheese property described by textural and rheological parameters) of processed cheese can be affected by many factors, including the type, composition, and chemical profile of the natural cheese utilized (dry matter, fat, protein, and calcium ion content, and maturity degree), the type and concentration of emulsifying salts, the presence and concentration of ions (especially calcium, sodium, and potassium), other optional dairy and nondairy ingredients, the pH of the mass to be melted, the processing and storage conditions (processing and storage temperature, stirring speed, time and temperature of the fusion, and cooling rate) and a possible use of some hydrocolloids (Shirashoji et al., 2006; Dimitreli & Thomareis, 2007; Gustaw & Mleko, 2007). Given the huge range of processed cheeses in terms of their consistency, flavor and possibilities of use in gastronomy, it is clear that the choice of the final parameters of the product and the derived raw material composition should be the first step in determining the final consistency of the product.

Furthermore, as it was mentioned above, the essential role of emulsifying salts is the exchange of sodium ions for calcium ions in the casein matrix (gel) of the cheese; insoluble calcium paracaseinate transformes into more soluble sodium paracaseinate, whose molecules (chains) can move within the melt system and thus enhance fat emulsification and water binding (Guinee et al., 2004; Shirashoji et al., 2006; Muslow et al., 2007). The ability of individual emulsifying salts to support the exchange of sodium for calcium ions can vary. Generally, the ability to support ion exchange occurs in the following order (considering of sodium salts): long polyphosphates (> 10 phosphorus atoms in a molecule) > short polyphosphates (< 10 phosphorus atoms in a molecule) > triphosphates > diphosphates > monophosphates (**A1**; **A2**; **A3**; El-Bakry et al., 2011). In addition, citrates might support ion-exchange to a similar extent that to of monophosphates. However, El-Bakry et al. (2011) and Mizuno & Lucey (2005) stated that citrates support ion exchange to a greater extent than monophosphates. The role of polyphosphates in processed cheese manufacture with different chain lengths was described in the study **A1**. Sodium salts of polyphosphate with different mean lengths of chain are often

used under real industrial practice. Furthermore, chains of different length could affect the intensity of the exchange of sodium ions for calcium ions and thus, casein dispersion (Mizuno & Lucey, 2007; Lu et al., 2008; Sádlíková et al., 2010). A different number of phosphorus atoms in a linear chain could also affect the creaming process by means of interactions with casein fractions of varying intensity. Moreover, the use of polyphosphates with different chain lengths can also affect the pH of the product (Lu et al., 2008). Based on the above-mentioned statements, the aim of the study **A1** was to observe selected textural parameters (large deformation properties) of model samples of processed cheese with the addition of ternary mixtures containing DSP, TSPP, and polyphosphates with different mean lengths ($n \approx 5$, 9, 13, 20, and 28).The current study would provide valuable information about the role of phosphate-based emulsifying salts in processed cheese properties, something that was missing from the scientific literature. Furthermore, in the second part of the current work (**A1**), TSPP was replaced with PSTP. The samples were produced without and with pH adjustment (to achieve pH values typical of processed cheese spreads, in the range of 5.60 – 5.80). Thus, with a low content of polyphosphate, hardness of the processed cheese increased and cohesiveness and relative adhesiveness decreased at a ratio of DSP to TSPP approximately 1:1 to 3:4. An increasing amount of polyphosphate (in the ternary mixture) led to a decrease in hardness of the processed cheese at this specific ratio. With the relative amount of polyphosphates reaching ≥ 60 %, the influence of this specific ratio became insignificant. This trend was observed in all ternary mixtures; the only differences were found in the absolute values of the textural parameters of the processed cheeses examined. Replacing TSPP with PSTP did not affect the general trend either. However, the absolute values of hardness of model samples with the addition of PSTP were lower compared with that in which TSPP was used. Duration of the storage period increased hardness of the processed cheeses. In the samples in which the pH was intentionally increased, hardness and cohesiveness decreased and relative adhesiveness increased slightly. However, the reverse trend was observed in samples in which pH was decreased. The more significant the pH adjustment was, the more noteworthy changes were observed. However, pH adjustment did not affect the value of the specific ratio of DSP:TSPP and DSP:PSTP and its general influence on textural parameters of the processed cheeses. The influence of the specific ratio of DSP:TSPP and DSP:PSTP and the general trend concerning the dependence of composition of the ternary mixtures of phosphate emulsifying salts on the textural parameters of processed cheeses cannot be attributed only to the effect of phosphates on the dispersion of casein. In addition, the dispersion of casein is closely related to the processed cheese matrix formation. Hence, an intensive dispersion of casein allows caseins to develop their emulsifying and hydrating abilities and thus, stabilize the fat and water present in the mixture. Increasing the range of protein hydration and fat emulsification results in higher intensity of casein crosslinking. A more rigid processed cheese will occur with a greater number of cross linkages in its matrix (**A1**; **A2**). Over the past few years, several studies (Awad et al., 2002; Weiserová et al., 2011; Buňka et al., 2012, 2013) have shown the dependence of textural parameters of processed cheeses on the composition of binary and ternary mixtures of phosphate emulsifying salts (consisting mainly of disodium phosphate, tetrasodium diphosphate, and sodium salt of polyphosphate). In these studies, a specific ratio of disodium phosphate to tetrasodium diphosphate was determined (approximately 1:1 to 3:4), at which hardness of the processed cheeses increased rapidly but cohesiveness and adhesiveness

decreased. The influence of this specific ratio decreased with an increasing relative amount of sodium salt of polyphosphate. When the amount of sodium salt of polyphosphate exceeded 60 %, the influence of this specific ratio became insignificant. However, existing studies are limited to linear-chain polyphosphates with mean length (n; the number of phosphorus atoms bound in a linear molecule of polyphosphate) of about 20 (Sádlíková et al., 2010; Weiserová et al., 2011; Buňka et al., 2012, 2013).

In addition, as it was mentioned above trisodium citrate presents better calcium chelating ability and casein peptization properties than do sodium mono- and diphosphates (El-Bakry et al., 2011; Mizuno & Lucey, 2005). The effect of the individual phosphates and composition of phosphate binary and ternary mixtures on textural properties of processed cheese has been previously studied (Buňka et al., 2013, Dimitreli & Thomareis, 2009, El-Barky et al., 2011, Lu et al., 2008, Sádlíková et al., 2010, Shirashoji et al., 2006, Weiserová et al., 2011). Processed cheeses in which the content of DSP is predominant have softer consistency. On the other hand, when polyphosphates are dominant in the binary and ternary mixtures, the hardness of the product increases (Buňka et al., 2013, Weiserová et al., 2011).

Nevertheless, in the above-mentioned studies the effect of TSC on processed cheese consistency was not evaluated, thus providing space for further scientific work. To the best of our knowledge, we have evaluated the effect of TSC on the processed cheese properties. Moreover, a specific ratio of DSP to TSPP exists that significantly affects textural parameters of processed cheese (**A1)**. In the latter study it was found that when the polyphosphate content was at low levels (less than 60 %) and the ratio of DSP to TSPP ranged from 1:1 to 3:4, the hardness of the resultant processed cheeses increased, while their cohesiveness and relative adhesiveness decreased. Based on the results in work **A1,** a study providing information about how ternary mixtures containing parallel phosphate and citrate emulsifying salts influence textural properties of processed cheese was performed (**A2**). The impact of the ternary mixtures composition on textural parameters of processed cheese and on the casein micelle dispersion (in a simplified model milk system) was also studied. Moreover, the impact of a specific ratio of DSP:TSPP and TSC:TSPP on processed cheese hardness was also described. Hence, at constant content of POLY20 ≤ 60 % or TSC ≤ 40 % in the emulsifying salt ternary mixtures a rapid increase in hardness of the product was observed, especially at a specific ratio of DSP to TSPP of approximately 1:1 to 2:3. When the content of POLY20 or DSC was absent in the ternary mixture, the products consisting of TSC and TSPP in a range of approximately 1:1 were the hardest (among samples with the binary mixture of TSC and TSPP). Also the hardness of all processed cheese samples increased with the increasing storage period.

Furthermore, processed cheese with diverse textural and rheological characteristics and alternative functional properties may be manufactured as a result of the use of different types (phosphate, citrate, or both) and combinations of emulsifying salts. In practice nowadays, the individual application of emulsifying salts is very rare. In fact, emulsifying salts are applied in ternary or even more componential mixtures (Guinee et al., 2004; Kapoor & Metzger, 2008; **A2**. Generally, the effect of different composition of ternary mixtures of the individual sodium salts of phosphates (especially disodium hydrogenphosphate, tetrasodium diphosphate, and sodium salt of polyphosphate) has been described in the papers by Weiserová et al. (2011) and

Buňka et al. (2012, 2013), but only for Dutch-type cheese as the main raw material for the processed cheese samples tested.

On the whole, it could be concluded that the type and composition of applied emulsifying salts can affect the rheological and textural properties of processed cheese and similar products. In general, when the tested emulsifying salts were applied as sole ingredients the storage and loss moduli decreased (parameters describing the consistency of processed cheese) in the following order: POLY20 > TSPP ≈ TSC > DSP. This specific trend was also confirmed by observing of the processed cheese hardness. With respect to our studies (**A2; A3; A4; A6**), we are able to infer that the general trend of the effect of the ternary mixtures composition is the same when different natural cheeses are used as raw material.

Moreover, significant parameters influencing the textural and rheological properties of processed cheeses are the type and the maturity degree of the utilized natural cheese. To the best of our knowledge, a series of studies providing information about how the type and maturity degree of natural cheese (other than Edam type cheese) can affect the properties of processed cheese was performed. Swiss-type cheese is a group of hard or semi-hard cheeses in texture, with desired propionic acid fermentation caused by propionic acid bacteria (especially *Propionibacterium freudenreichii* ssp. *freudenreichii* and *Propionibacterium freudenreichii* ssp. *shermanii*). Therefore, their flavor is characterized as sweet and nut-like. This is due to free fatty acids, peptides, amino acids, carbonyls, or their mutual interactions (Beuvier et al., 1997; Bouton et al., 2009). However, processed cheese manufacture using Swiss-type cheese as the main raw ingredient in the available literature is scare. Under real industrial practice, Swiss-type cheese is often used as part of the raw material for processed cheese manufacture. On the other hand, the individual usage of Swiss-type cheese in processed cheese production was described in the study **A3**. Moreover, the influence of different maturity degrees of Swiss-type cheese associated with different combinations of emulsifying salts ternary mixtures affecting processed cheese consistency was also reported. According to the findings in the work **A3** the application of the binary mixture of DSP:TSPP (in a ratio of 1:1) resulted in products with the highest values of hardness (regardless of the maturity degree of the Swiss-type cheese applied). Furthermore, the hardness of the samples obtained decreased with the rising maturity degree of the Swiss-type cheese used (regardless of the emulsifying mixture applied). However, on the contrary, the hardness of all processed cheese samples increased with prolonging the storage period. Admittedly, the results of texture profile analysis corresponded to those of the rheological analysis. The highest overall rigidity (G*), gel strength, and interaction factor values were found in the samples prepared with DSP:TSPP (1:1), followed by the samples prepared with POLY20, TSPP, TSC, and DSP, respectively. The monitored values of the gel strength and interaction factor decreased with the increasing maturity degree of the Swiss-type cheese used. The intensity of rigidity of the processed cheese samples has an analogous relationship to the intensity of the gel strength; the higher the gel strength of the sample, the more inflexible the product that can be expected.

In the same token, the combined effect of Mozzarella cheese storage period and different emulsifying salts (type and/or composition) on the textural and rheological characteristics of spread-type processed cheese during its storage was studied **(A4)**. Furthermore, during the

storage of Mozzarella cheese (a pasta-filata cheese), complex biochemical events determine its final quality and acceptance. Proteolysis is the major phenomenon that occurs during cheese aging (besides glycolysis and lipolysis) that greatly affects the physical characteristics of nearly all cheeses. Generally, cheeses show similar proteolytic trends. On the other hand, differences in cheese nature and manufacturing processes influence the proteolytic pattern. In the case of processed cheese, rheological and textural properties are influenced by the age of the applied cheese and/or also by specific technological operations during cheese manufacturing. Hence, more intensive proteolytic reactions result from an increasing cheese maturity level. The combined impact of the Mozzarella cheese age and different ternary mixtures of emulsifying salts on the textural and viscoelastic properties of processed cheese were evaluated in the study **A4**. According to the latter research paper (**A4**), the increasing storage period of the processed cheese samples resulted in an increase in hardness. On the contrary, the hardness of the samples decreased with expanding Mozzarella cheese storage time. Model samples with diverging properties were obtained by the application of different types of ternary mixtures of emulsifying salts. The hardest samples were those comprised of DSP:TSPP (1:1). However, when DSP or TSPP were replaced by TSC, this ratio was not observed. The rising amount of POLY20 in the mixtures led to a decrease in the samples' hardness (up to ≥ 50%). The results obtained from the rheological analysis were in accordance to those of the hardness analysis. Hence, the ratio of DSP:TPSS resulted in processed cheese with the highest values of gel strength and interaction factor. Moreover, with increasing Mozzarella cheese storage periods the values of gel strength and interaction factor decreased.

Last but not least, a research providing direct comparison of the viscoelastic properties of processed cheese produced under identical processing parameters and similar experimental design from two technologically very different varieties of natural cheese (Cheddar cheese and white brined cheese, respectively), and additionally with various degrees of maturity, was performed **(A5)**. The impact of the Cheddar cheese and white brined cheese maturity and different compositions of ternary mixtures of emulsifying salts on the hardness and gel strength of processed cheese during 60 days of storage was investigated in the latter work. Hence, with raising the storage period of the processed cheese samples, an increase in hardness was observed. On the other hand, the hardness and gel strength of the samples decreased with prolonging of cheese ripening period for both tested natural cheeses (Cheddar and white brined cheeses, respectively) used as the basic raw material. The hardest samples were those composed of DSP:TSPP (1:1). However, when the relative amount of DSP and TSPP (in the ratio of 1:1) were replaced by TSC or POLY20, the influence of the latter mentioned ratio diminished. Furthermore, higher values of hardness and gel strength were reported for the processed cheese samples produced with Cheddar cheese in comparison with those made from white brined cheese.

In conclusion, four types of natural cheese (Dutch type – **A1**; **A2**; Swiss-type – **A3**; Mozzarella-type – **A4**; Cheddar-type – **A5**; and white brined-type – **A5**, respectively), which are used through the whole world, were tested. In all examined cheese varieties/types the mechanisms of function of the above mentioned ternary mixtures were practically very similar regardless of the ripening period of the cheese utilized. Moreover, with raising storage period of the processed cheese samples, an increase in hardness was observed. On the other hand, the

hardness and gel strength of the samples decreased with prolonging of cheese ripening period for all applied natural cheeses which used as the main raw material. Hence, the hardest samples were those composed of DSP:TSPP (1:1). However, when the relative amount of DSP and TSPP (in the ratio of 1:1) were replaced by TSC or POLY20, the influence of the latter mentioned ratio diminished cheese were performed (**A1; A2; A3; A4; A5**).

Processed cheese sauces are novel cheese products and commercially can be found in many forms (frozen, semi-liquid, shelf-stable dry mixtures). Processed cheese sauces can serve as flavor enhancers, dipping sauces, act as the main attractiveness in many dishes or help to intensify or round out an appetizer flavor profile. However, at the moment there are no standards of identity or definitional legal for processed cheese sauce. Thus, the latter dairy products can be manufactured by applying many ingredients such as natural cheese, cheese powder, processed cheese and other ingredients of dairy or non-dairy origin. Processed cheese sauces and processed cheeses could be described as stable oil in water emulsions. Hence, their manufacture could be realized by mixing natural cheese, milk fat, water, emulsifying salts, other optional ingredients, commonly under vacuum in the presence of heat (temperature range of 85 up to 110 °C) and constant shear (**A3; A6; B4; B5;** Shalaby et al., 2017). It could be stated that the hardness of the processed cheese sauce samples was affected by the applied type of emulsifying salt, pH of the melt and storage period. Furthermore, it was found that the samples prepared with DSP:TSPP (1:1) and POLY20 (without pH adjustment) presented the higher values of hardness. The latter phenomenon was also confirmed by the increasing samples complex viscosity and storage modulus. On the contrary, the remaining tested samples showed lower hardness, storage modulus and complex viscosity values. The hardness of all examines samples increased with the increasing storage period (**A6**).

B. The impact of selected processing parameters on the functional properties of processed cheese.

As it was reported above (**A1; A3; A4; A5; A6**) the basic raw materials for the production of processed cheese (and/or processed cheese sauces) are natural cheeses of Dutch- and Swiss-type, whereas in the Anglo-American countries it is usually Cheddar and Mozzarella cheeses, respectively. In areas of the Near and Middle East white brined cheeses are also widely used. Other dairy raw materials (butter, cream, anhydrous milk fat, curd, milk powder, whey powder) and non-dairy raw materials (water, flavoring agents, and hydrocolloids) as well as emulsifying salts (especially sodium salts of phosphates, polyphosphates and citrates) are added to this cheese-base of different maturity degrees. Ingredients are usually heated under constant agitation at a temperature of 90–110 °C until a homogenous mass with desired properties is developed (Kapoor & Metzger, 2008, Khetra et al., 2015, Muslow et al., 2007).

One of the most important and very critically evaluated parameters of processed cheeses is their consistency, which can be, in the form of blocks, slices, spreads, or sauces (Kapoor & Metzger, 2008). Consistency of processed cheese and can be affected by three main groups of factors: (i) the composition of the raw material mixture, (ii) processing parameters during the

production of processed cheese (especially the speed of agitation, holding time, the target temperature during the melting process and the cooling rate), and (iii) the storage temperature and length (Bayarri et al., 2012, Dimitreli & Thomareis, 2004, Kapoor & Metzger, 2008, Khetra et al., 2015, Muslow et al., 2007, Schatz et al., 2014, Subramanian et al., 2006). In general, it could be stated that the processing parameters during the production of processed cheese (and similar products) are of great significance.

In the industry, the consistency of processed cheese regularly is evaluated by sensory analysis. However, the instrumental evaluation using small (e.g., dynamic oscillation rheometry) or large (e.g., texture profile analysis) deformations or their combinations is increasing for the past few years (Lee et al., 2003; Kapoor & Metzger, 2008; Buňka et al., 2013; 2014). Rheological parameters measured in the area of small or large deformations are given mainly by the microstructure of processed cheeses and mutual bonds between the individual components (especially the properties of the protein network and its interactions with other components; Hosseini-Parvar et al., 2015; da Silva et al., 2016). To explain the nature of the current state of consistency, it is therefore useful to have the data about the mechanical properties of the processed cheese and also its microstructure. The microstructure of processed cheeses may be studied by several methods, the most common of which are optical microscopy (Hladká et al., 2014; da Silva et al., 2016), scanning electron microscopy (Noronha et al., 2008; Cunha et al., 2010), transmission electron microscopy (Lee et al., 2003), and confocal laser scanning microscopy (Lee et al., 2015).

Although the final parameters of processed cheeses (especially dry matter, fat in dry matter, and fat-free dry matter content) affect their consistency to a large extent, they have not been given sufficient attention in the literature over the past 10 years. One of the few studies, by Lee et al. (2015), dealt with the effect of protein content (10 – 20 % w/w) and fat content (0 – 40% w/w) on the viscoelastic properties of model samples of processed cheeses made from rennet casein (melting temperature = 85 °C) and stored for 24 h. With the increasing protein content and decreasing fat content (constant protein-to-water content, variable dry matter content), the rigidity of the processed cheeses increased. A more significant effect of the protein content was observed compared with the fat content. In the work **B1** was evaluated the effect of different dry matter contents (35 and 45 % w/w) and fat in dry matter contents (40 and 50 % w/w) on the textural and viscoelastic properties and microstructure of model processed cheeses made from real ingredients regularly used in the dairy industry. Apart from the basic chemical parameters, textural and viscoelastic properties of the model samples were measured and scanning electron microscopy was realized. With increasing dry matter content, the rigidity of the products increased and thus the size of the fat globules in the model samples of the processed cheeses decreased. With increasing fat in dry matter content, the rigidity of the processed cheeses decreased and the size of the fat globules increased.

However, full-fat products with a significantly higher fat content are also commonly produced. The question therefore remains whether the trends observed in **B1** also apply to full-fat processed cheeses. In particular, in work **B2** was evaluated the effect of the holding time at a given melting temperature, and agitation speed, on the consistency of full-fat processed cheeses. It was found that, after an initial decrease in the firmness of the processed cheeses

(during holding times of up to 3 min), the firmness of the products increases significantly as the holding time is extended at the melting temperature. As the storage period extended to 60 days, the firmness of the processed cheeses also increased, regardless of either the applied holding time at the melting temperature, or the agitation speed. Although the effect of extended holding times at the given melting temperature on the consistency of semi-fat and full-fat processed cheeses seems clear under the given conditions, this cannot be said of the effect of agitation speed, which remains unclear and requires further studies.

The work **B3** described the effect of melting temperature and holding time on the rheological properties of spreadable processed cheese. The values of G* significantly increased with the increase of holding time. Elevated temperature of melting also caused the increase of G^*. The higher levels of observed processing parameters led to development of denser structure and therefore the model processed cheese became more rigid (**B1**).

Furthermore, in the work **B4** were studied the viscoelastic properties of model processed cheese spreads at various agitation speeds and holding times at the melting temperature over 60 days cold storage. Under the conditions of the experiment mentioned above it was found that at all the agitation speeds tested (1000, 1500 and 3000 rpm) the firmness of the samples increased steadily from the 3rd to the 20th minute of the holding time. The most striking increase was observed in the model processed cheeses manufactured at 3000 rpm, especially from the 10th minute of the holding time onwards. However, a clear trend in the development of viscoelastic properties of the observed samples depending on the agitation speed could not be determined. This trend changed according to the particular holding time of the melt at the melting temperature. During the 60 day storage period, the firmness of all the observed processed cheeses increased. In recent years, other works have been published which attempt to contribute to the clarification of, in particular, the role of agitation speed and holding time at a given melting temperature on the consistency of the resulting processed cheese (Shirashoji et al., 2016; **B4**). The conclusion drawn as a result of the studies conducted for both these works was the same, namely that when the holding time at the given melting temperature is extended, the firmness of the processed cheeses increases. From the article **B4**, it can be assumed that this trend is not linear. Furthermore, it was demonstrated that the effect of agitation speed on the consistency of the product is not clear, and depends significantly on the holding time. During short holding times (up to 3 min), processed cheeses produced at lower agitation speeds (1000 rpm) were more solid than those products produced at higher speeds (1500 and 3000 rpm). However, as the holding time was extended, this trend changed significantly.

The effect of processing parameters, such as holding time of the melt, on the consistency of processed cheese spreads has been studied extensively. Swenson et al. (2000) investigated fat-free processed cheese (with 40 %, w/w, dry matter content) and stated that, the longer the holding time, the lower the firmness of the product. However, Bowland & Foegeding (2001) examined the effect of processing time (10, 20 and 30 min) on the viscoelastic properties of model processed cheese (49.5–52.5 %, w/w, dry matter; 51.4–54.5 %, w/w, fat in dry matter) over a decreasing temperature regime from 25 °C to 80 °C (to determine sample solidification). The authors concluded that there was no relationship when the small strain analyses (G′, G′′, G* and δ) were performed at temperatures lower than 80 °C. Moreover, Lee et al. (2003) found

that the apparent viscosity of spreadable processed cheese melt containing 50 % (w/w) dry matter and 50 % (w/w) fat in dry matter rose until 25 mins of processing at 80 °C and then decreased. Furthermore, the studies **B1**, **B2** and **B4** investigated the effect of holding time of the melt in a selected temperature on the viscoelastic properties of processed cheese with 35 % (w/w) dry matter and 40 % (w/w) and 50 % (w/w) fat in dry matter content. The conclusion in these studies was that the firmness of processed cheese decreased up to the 3rd minute of holding time but then increased significantly (the maximum holding time applied was 20 min). Nevertheless, the above-mentioned results are contradictory and the effect of holding time on the consistency of processed cheese spreads with different dry matter and fat in dry matter contents remains unclear. Especially, the effect of holding times below 10 min (in close gaps within the holding time range) on spreadable processed cheese samples (with different dry matter and fat in dry matter contents; produced under identical processing protocol) viscoelastic properties described by the complex modulus and phase shift was missing from the existing scientific literature. In general, it is accepted that the short duration of the holding time is economically advantageous. According to the results presented in the work **B5**, were studied six different types of model spread-type processed cheese prepared and stored for 30 days stated that the viscoelastic properties depend on the holding time, time of storage and dry matter and fat in dry matter contents. For most of the produced spread-type processed cheese, it was demonstrated that, on the 1st, 14th and 30th day of storage, G* (a measure of consistency) decreased in the first 2 or 3 min of the holding time and gradually increased afterwards. In the most cases of dry matter and fat in dry matter contents, prolonging the holding time from the 3rd min up to the 10th min and storage for 30 days increased the G* in all samples examined. Also, G* increased with increasing dry matter content at constant fat in dry matter and also with decreasing fat in dry matter. The same dry matter content and increasing fat in dry matter content caused decreasing value of G*. Nevertheless, inverse relationships were observed in the case of the phase shift evaluation. In addition, most of the spread-type processed cheeses produced exhibited more elastic than viscous consistency (solid-like behavior). It could be concluded that dry matter and fat in dry matter contents, holding time and length of the storage time affected the rheological properties of the processed cheese samples. In particular, increasing fat content reduced the values of complex modulus, resulting in more soft processed cheese final products. Moreover, the processed cheeses with low dry matter content were more viscous than the samples with higher level of dry matter content. This information may be relevant to industry practice. Moreover, longer holding times of the melt can result in smaller diameter of milk fat droplets in the final product. However, a significant decrease in size was observed after 2 or 3 min. Furthermore, the size of milk fat droplets decreased as the dry matter content increased and the fat in dry matter content decreased. In general, from an economic point of view, shorter holding times could be evaluated as more advantageous for producers of processed. In addition, processed cheese formulation can have an impact on final product price, as higher dry matter content can result in higher processed cheese price. Comparing processed cheeses with the same dry matter content, the higher the fat in dry matter content, the lower the price of the final product.

In addition, the work **B6** described the impact of rework content (utilized rework "*age*" of 72 h) on the consistency of processed cheese. It was discovered that a lower quantity of added

rework, up to 10.0% (w/w), caused a gradual increase in the firmness of the processed cheese; simultaneously, the size of the fat droplets gradually decreased, which results primarily from the improvement of the emulsification properties as the amount of rework added increases. As the rework concentration in the raw material composition increased further (from 10 – 20 % w/w), the firmness of the processed cheese no longer increased, and the median size value of the fat droplets, which was smaller than that in samples with less added rework, also no longer differed significantly. Additionally, the term rework is describing a processed cheese that has already been processed once and in which creaming has already occurred; it is used as a raw material for the production of processed cheese. Therefore, its consistency is affected by all of the aforementioned factors. Rework is created in the industry either (1) intentionally (production of processed cheese for rework or residue of processed cheese in production equipment) or (2) unintentionally (production of processed cheese originally intended for the market network but ultimately not released for market; for example, due to unsuitable packaging or incorrect dry matter or fat in dry matter content). Rework is usually used fresh (processed cheese residue in production equipment) or 3 to 14 days old (processed cheese unsuitable for the market network due to unsuitable fat or dry matter content, or an incorrect packaging weight. The reasons for using rework can be economic, but also to increase viscosity (with the increasing age of the rework and with its increasing concentration) after production, increase firmness, improve meltability, or reduce emulsifying salt content, since rework already contains emulsifying salt (Lauck, 1972; Meyer, 1973; Kaláb et al., 1987; Pluta et al., 2000; Kapoor & Metzger, 2008).

In general, it could be concluded that the factors including the dry matter and fat in dry matter contents, holding time and length of the storage time influence the rheological and textural properties of the processed cheese. In particular, increasing fat content reduced the values of complex modulus, resulting in "*softer*" (or more spreadable) processed cheese final products. Moreover, the processed cheeses with low dry matter content were more viscous than the samples with higher level of dry matter content. Finally, based on our results it was found that the applied agitation speed during the production of processed cheese affects the firmness of the samples.

CONTRIBUTION TO SCIENCE AND PRACTICE

The principal contributions of the current habilitation thesis to science and practice could be summarized in the following points:

- It was found that when the phosphate and citrate emulsifying salts were applied individually the hardness of the model processed cheeses increased in the following order: POLY20 > TSPP ≈ TSC > DSP (regardless of the type of natural cheese applied and its maturity degree or length of the storage time).
- It has been documented that the basic trend of the dependence of the consistency of processed cheeses on the composition of mixtures (including binary or ternary mixtures) of phosphate and citrate emulsifying salts (consisting of sodium hydrogen phosphate, sodium diphosphate, sodium polyphosphate and sodium citrate) is not significantly affected by the natural cheese applied, maturity degree or length of storage time. In all studied combinations of all the above-mentioned factors, the existence of specific ratios of sodium hydrogen phosphate and sodium diphosphate was identified, as well as sodium diphosphate and sodium citrate, which significantly increase the hardness of processed cheeses.
- It was found that with a low content of polyphosphate, hardness of the processed cheese increased and cohesiveness and relative adhesiveness decreased at a ratio of DSP to TSPP around 1:1 to 3:4. Moreover, the increasing amount of polyphosphate (in the ternary mixture) led to a decrease in hardness of the processed cheese (at this specific ratio). With the relative amount of polyphosphates reaching ≥ 60 %, the influence of this specific ratio became insignificant. This trend was observed in all ternary mixtures; the only differences were found in the absolute values of texture parameters of the processed cheeses.
- The influence of different compositions of ternary mixtures of phosphate and citrate emulsifying salts on the consistency of processed cheeses made from Dutch-type, Swiss-type, Mozzarella-type, Cheddar-type and white brined-type cheeses was described. It has been shown that the choice of a particular natural cheese will significantly affect the consistency of processed cheeses and must be taken into account when "modeling" the raw material composition formula. Furthermore, it has been found that as the maturity degree of natural cheeses (for all types of the tested natural cheeses) increases, the hardness of processed cheeses decreased, regardless of the applied phosphate and citrate emulsifying salts applied (sole, binary or ternary mixtures). In addition, the maturity degree of the applied natural cheese affects hardness "absolute" values development, whereas the main trends remain unaffected.
- The effect of different dry matter and fat content in dry matter on the consistency of processed cheeses was described (values of dry matter and fat in dry matter content which are typical for the Central Europe region). In particular, increasing fat content reduced the values of complex modulus, resulting in more soft processed cheese final products. Moreover, processed cheese with lower values of dry matter content were more viscous than the samples with higher level of dry matter content. Furthermore, the size of milk fat

droplets decreased as the dry matter content increased and the fat in dry matter content decreased.

- The effect of the longer holding time at the so-called melting temperature on the consistency of processed cheeses has been documented for such a range of stirring speeds in the production protocol as is typical for the production of spreadable processed cheeses. The existence of a limit time for the developed melt to remain at a specific melting temperature has been proved, until which the hardness of the final processed cheeses decreases. It has been found that if the hardness of processed cheeses is to be increased only by extending the residence time of the melt at the melting temperature (assuming a constant level of other factors affecting the consistency of these products), then the total residence time must be more than 3 minutes. Furthermore, the latter phenomenon was also identified in processed cheese samples with various fat in dry matter level.
- It has been found that the hardness of processed cheeses increases during cold storage, with these changes being most intense during the first 14 days of storage.
- The effect of rework addition on the viscoelastic properties of model processed cheeses samples was evaluated. The addition of rework (in spread-type processed cheese) up to 10.0 % (w/w) increased the hardness and elastic character of the processed cheese. However, with the further addition of rework, the consistency of the processed cheeses did not differ significantly.
- The obtained results could be used as models describing the dependence of processed cheese functional properties (mainly textural and rheological) on the composition of mixtures of emulsifying salts for the production of final products with different consistency. Under real industrial conditions the obtained models could be applied as a description of the samples textural and viscoelastic properties arising from the type and maturity degree of the utilized natural cheese in combination with the applied type and concentration of emulsifying salts. From the obtained data could be proposed an appropriate mixture of emulsifying salts, a suitable type and maturity level of natural cheese in order to produce final processed cheese products with desirable consistency and organoleptic properties.

CONCLUSIONS

Taking into account that processed cheese is one of the youngest dairy products and also due to the complexity of the processed cheese matrix (system), there is still insufficient relevant information in the scientific literature about the effect of individual factors on the consistency of processed cheeses. Hence, this provides enough "space" not only for the producers but also for the researchers in order to perform high-quality research studies to understand the exact course of reactions/phenomena evolved in the production of processed cheeses (or similar products). The presented habilitation thesis aimed to summarize the existing knowledge in the field of characteristics of raw materials for the production of processed cheeses, production technology of these products and factors influencing the consistency (a parameter described mainly by textural and rheological properties) of processed cheeses. Based on the results of the current thesis, it is possible to provide a more comprehensive point of view of the importance of the composition of a mixture of emulsifying salts (phosphates and/or citrates) in influencing the textural and rheological properties of processed cheeses. The habilitation thesis also dealt with the use of different natural cheeses with divergent maturity degrees and how this factor for industrial practice affects the consistency of processed cheeses. Last but not least, the development of the consistency of processed cheeses manufactured from different raw materials, with different compositions of emulsifying salts and cheeses produced under different processing conditions was described. Changes in the consistency of processed cheeses occurring during their storage (under refrigeration conditions) have also been reported.

LIST OF REFERENCES

Adbel-Hamid, L. B., El-Shabrary, S. A., Awad, R. A. (2000). Chemical properties of processed Ras cheese spreads as affected by emulsifying salt mixtures. *Journal of Food Processing and Preservation*, 24, 191–198.

Adhikari, K., Cole, A., Grün, I., Heymann, H., Hsieh, F. -H., & Huff, H. (2009). Physical and sensory characteristics of processed cheeses manufactured by extrusion technology. *Journal of the Science of Food and Agriculture*, 89(8), 1428–1433.

Aguilera, J.M, & Stanley, D.W. Microstructural principles of food processing and engineering. 2nd Ed. SciTech Book News. 2000, vol. 24143. ISSN:0196-6006.

Alichanidis, E., & Polychroniadou, A. (2008). Characteristics of major traditional regional cheese varieties of East-Mediterranean countries: a review. *Dairy Science and Technology*, 88(4-5), 495–510.

Aljewicz, M, & Cichoz, G. Protective effects of Lactobacillus cultures in Dutch-type cheese-like products. (2015). *LWT-Food Science and Technology*, 63, 1, 52–56.

Al-Nabulsi, A. A., Osaili, T. M., AbuNaser, R. A., Olaimat, A. N., Ayyash, M., Al-Holy, M. A., Kadora, K. M., & Holley, R. A. (2020). Factors affecting the viability of Staphylococcus aureus and production of enterotoxin during processing and storage of white-brined cheese. *Journal of Dairy Science*, 103(8), 6869–6881.

Alves, R. M. V., Van Dender, A. G. F., Jaime, S. B. M., Moreno, I., & Pereira, B. C. (2007). Effect of light and packages on stability of spreadable processed cheese. *International Dairy Journal*, 17(4), 365–373.

Amagliani, G; Giammarini, C; Omiccioli, E; Brandi, G; Magnani, M. (2007) Detection of *Listeria monocytogenes* using a commercial PCR kit and different DNA extraction methods. *Food Control*,18, 9, 1137–1142.

Awad, R. A., Abdel-Hamid, L. B., El-Shabrawy, S. A., & Singh, R. K. (2002). Texture and microstructure of block type processed cheese with formulated emulsifying salt mixtures. *LWT-Food Science and Technology*, 35(1), 54–61.

Awad, R. A., Abdel-Hamid, L. B., El-Shabrawy, S. A., & Singh, R. K. (2004) Physical and sensory properties of block processed cheese with formulated emulsifying salt mixtures. *International Journal of Food Properties*, 7, 429–448.

Barth, A. P., Tormena, C. F., Wiotto, W. H. 2017. pH influences hydrolysis of sodium polyphosphate in dairy matrices and the structure of processed cheese. *Journal of Dairy Science*, 100, 8735–8743.

Bayarri, S., Carbonell, I., Costell, E., 2012. Viscoelasticity and texture of spreadable cheeses with different fat contents at refrigeration and room temperatures. *Journal of Dairy Science*, 95 (12), 6926–6936.

Belsito, P. C., Ferreira, M. V. S., Cappato, L. P., Cavalcanti, R. N., Vidal, V. A. S., Pimentel, T. C., Esmerino, E. A., Balthazar, C. F., Neto, R. P. C., Tavares, M. I. B., Zacarchenco, P. B., Freitas, M. Q., Silva, M. C., Raices, R. S. L., Pastore, G. M., Pollonio, M.

A. R., & Cruz, A. G. (2017). Manufacture of Requeijão cremoso processed cheese with galactooligosaccharide. *Carbohydrate Polymers*, 174, 869–875.

Berger, W; Klostermeyer, H; Merkenich, K, & Uhlmann, G. (2002) Processed cheese manufacture. A Joha Guide, Ladenburg: BK Giulini Chemie GmbH & Co. OHG.

Beuvier, E., Berthaud, K., Cegarra, S., Dasen, A., Pochet, S., Buchin, S., & Duboz, G. (1997). Ripening and quality of Swiss-type cheese made from raw, pasteurized or microfiltered milk. *International Dairy Journal*, *7*(5), 311–323.

Bisig, W; Frohlich-Wyder, M,T; Jakob, E; Wechsler, D. Comparison between Emmentaler PDO and generic Emmental cheese production in Europe. (2010). *Australian Journal of Dairy Technology*, 65, 3, 206–213.

Biswas, A. C., Muthukumarappan, K., Marella, C., & Metzger, L. E. (2015). Understanding the role of natural cheese calcium and phosphorous content, residual lactose and salt-in-moisture content on block-type processed cheese functional properties: Cheese hardness and flowability/meltability. *International Journal of Dairy Technology*, 68(1), 44–53.

Bourriot, S., Garnier, C., Doublier, J. L., 1999. Micellar casein–kappa carrageenan mixtures. I. Phase separation and ultrastructure. *Carbohydrate polymers*, 40 (2), 145–157.

Bouton, Y., Buchin, S., Duboz, G., Pochet, S., & Beuvier, E. (2009). Effect of mesophilic lactobacilli and enterococci adjunct cultures on the final characteristics of a microfiltered milk Swiss-type cheese. *Food Microbiology*, *26*(2), 183–191.

Bowland, E. L., Foegeding, E. A., 2001. Small Strain Oscillatory Shear and Microstructural Analyses of a Model Processed Cheese. *Journal of Dairy Science*, 84, 2372–2380.

Brickley, C. A., Auty, M. A. E., Piraino, P., & McSweeney, P. L. H. (2007). The effect of natural Cheddar cheese ripening on the functional and textural properties of the processed cheese manufactured therefrom. *Journal of Food Science*, 72(9), C483–C490.

Buňka, F., Doudová, L., Weiserová, E., Kuchař, D., Ponížil, P., Začalová, D., Nagyová, G., Pachlová, V., & Michálek, J. (2013). The effect of ternary emulsifying salt composition and cheese maturity on the textural properties of processed cheese. *International Dairy Journal*, 29(1), 1–7.

Buňka, F., Hrabě, J., & Kráčmar, S. (2004). The effect of sterilisation on amino acid contents in processed cheese. *International Dairy Journal*, 14(9), 829–831.

Buňka, F., Pavlínek, V., Hrabě, J., Rop, O., Janiš, R., Krejčí, J., 2007. Effect of 1–monoglycerides on viscoelastic properties of processed cheese. *International Journal of Food Properties*, 10, 819–828.

Buňka, F; Doudová, L; Weiserová, E; Černíková, M; Kuchař, D; Slaviková, S; Nagyová, G; Ponížil, P; Gruber, T; Michálek, J. (2014). The effect of concentration and composition of ternary emulsifying salts on the textural properties of processed cheese spreads. *LWT-Food Science and Technology*, 58, 1, 247–255.

Buňka, F; Kříž, O; Veličková, A; Buňková, L; Kráčmar, S. (2009). Effect of acid hydrolysis time on amino acid determination in casein and processed cheeses with different fat content. *Journal of Food Composition and Analysis*, 22, 3, 224–232.

Buňková, L., Buňka, F., 2017 Microflora of processed cheese and the factors affecting it. *Critical Reviews in Food Science and Nutrition*, 57, 2392–2403.

Buňková, L; Pleva, P; Buňka, F; Valášek, P; Kráčmar, S. (2008). Antibacterial effects of commercially available phosphates on selected microorganisms. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 56, 5, 19–24.

Burgos, L., Pece, N., & Maldonado, S. (2020). Textural, rheological and sensory properties of spreadable processed goat cheese. *International Journal of Food Studies*, *9*.

Carić, M., & Kaláb, M. Processed cheese products. Cheese: Chemistry, Physics and Microbiology, Vol. 2, Major Cheese Groups, 2nd ed. P.F. Fox, ed., Chapman & Hall, London, 1997, 467-505. ISBN 0-412-535106

Carić, M., Gantar, M., & Kaláb, M. (1985). Effects of emulsifying agents on the microstructure and other characteristics of process cheese — A review. *Food Microstructure*, 4, 297–312.

Černíková, M., Buňka, F., Pavlínek, V., Březina, P., Hrabě, J., & Valášek, P. (2008). Effect of carrageenan type on viscoelastic properties of processed cheese. *Food Hydrocolloids*, 22 (6), 1054–1061.

Černíková, M., Nebesářová, J., Salek, R. N., Popková, R., & Buňka, F. (2018a). The effect of rework content addition on the microstructure and viscoelastic properties of processed cheese. *Journal of Dairy Science*, 101, 2956–2962.

Černíková, M., Nebesářová, J., Salek, R. N., Řiháčková, L., & Buňka, F. (2017b). Microstructure and textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content. *Journal of Dairy Science*, 100(6), 4300–4307.

Černíková, M., Nebesářová, J., Salek, R. N., Řiháčková, L., & Buňka, F. (2017a). Microstructure, textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content. *Journal of Dairy Science*, 100, 4300–4307.

Černíková, M., Pachlová, V., Holas, O., Moudrá, K., Slintáková, K., & Buňka, F. (2018b). The effect of dairy fat source on viscoelastic properties of full–fat processed cheese spreads. *European Journal of Lipid Science and Technology*, 120, 1700319.

Chandan, R. C, Kilara, A., & Shah, N. (2009). Dairy processing and quality assurance. ISBN: 9780813827568.

Chatziantoniou, S. E., Thomareis, A. S., & Kontominas, M. G. (2015). Effect of chemical composition on physico-chemical, rheological and sensory properties of spreadable processed whey cheese. *European Food Research and Technology*, *241*(6), 737–748.

Chatziantoniou, S. E., Thomareis, A. S., & Kontominas, M. G. (2019). Effect of different stabilizers on rheological properties, fat globule size and sensory attributes of novel spreadable processed whey cheese. *European Food Research and Technology*, *245*(11), 2401–2412.

Chavhan, G. B., Kanawjia, S. K., Khetra, Y., & Puri, R. (2015). Effect of potassium-based emulsifying salts on sensory, textural, and functional attributes of low-sodium processed Mozzarella cheese. *Dairy Science & Technology*, *95*(3), 265–278.

Chen, L., & Liu, H. (2012). Effect of emulsifying salts on the physicochemical properties of processed cheese made from Mozzarella. *Journal of Dairy Science*, 95(9), 4823–4830.

Commissiion, The Codex Alimentarius. CXS 283 - Codex General Standard for Cheese. Codex Alimentarius. 2009, 1.

Commission, The Codex Alimentarius. CXS 265 - Codex Standard for Edam. Codex Alimentarius. 2009, 1.

Commission, The Codex Alimentarius. CXS 269 - Codex Standard for Emmental. Codex Alimentarius. 2009, 1.

Costa Dias, M.A, Sant'ana, A.S, Cruz, A.G, Faria, J.D.A.F, Fernandes De Oliveira, C.A, & Bona, E. (2012). On the Implementation of good manufacturing practices in a small processing unity of Mozzarella cheese in Brazil. *Food Control*, 24, 199–205.

Costabel, L., Pauletti, M. S., & Hynes, E. (2007). Proteolysis in Mozzarella Cheeses Manufactured by Different Industrial Processes. *Journal of Dairy Science*, *90*(5), 2103–2112.

Cunha, C. R., & Viotto, W. H. (2010). Casein peptization, functional properties, and sensory acceptance of processed cheese spreads made with different emulsifying salts. *Journal of Food Science*, *75*(1), C113–C120.

Cunha, C. R., Alcântara, M. R., & Viotto, W. H. (2012). Effect of the type of emulsifying salt on microstructure and rheological properties of "Requeijão Cremoso" processed cheese spreads. *Journal of Food Science*, *77*(8), E176–E181.

Da Silva, D. F., De Souza Ferreira, S. B., Bruschi, M. L., Britten, M., Matumoto–Pintro, P. T. (2016). Effect of commercial konjac glucomannan and konjac flours on textural, rheological and microstructural properties of low fat processed cheese. *Food Hydrocolloids*, 60, 308–316.

De Angelis, M, & Gobbetti, M. (2004). Environmental stress responses in *Lactobacillus*: A Review. *Proteomics*, 4, 106–122.

Dimitreli, G., & Thomareis, A. S. (2004). Effect of temperature and chemical composition on processed cheese apparent viscosity. *Journal of Food Engineering*, 64(2), 265–271.

Dimitreli, G., & Thomareis, A. S. (2007). Texture evaluation of block-type processed cheese as a function of chemical composition and in relation to its apparent viscosity. *Journal of Food Engineering*, 79(4), 1364–1373.

Dimitreli, G., & Thomareis, A. S. (2008). Effect of chemical composition on the linear viscoelastic properties of spreadable-type processed cheese. *Journal of Food Engineering*, 84(3), 368-374.

Dimitreli, G., & Thomareis, A. S. (2009). Instrumental Textural and Viscoelastic Properties of Processed Cheese as Affected by Emulsifying Salts and in Relation to Its Apparent Viscosity. *International Journal of Food Properties*, 12,1, 261–275.

Döüsterhöft, E M; Van Den Berg, G. (2010). Dutch-type Cheeses. Cheese Problems Solved. 230–245.

El-Bakry, M; Duggan, E; O'Riordan, E.D; O'Sullivan, M (2011). Effect of chelating salt type on casein hydration and fat emulsification during manufacture and post-manufacture functionality of imitation cheese. *Journal of Food Engineering*, 102, 2, 145–153.

Faccia, M, Trani, A, & Di Luccia, A. (2009). Short Communication: Relationships between milk quality and acidification in the production of table Mozzarella without starters. *Journal of Dairy Science*, 92, 9, 4211–4217.

Fagan, C. C., Everard, C., O'Donnell, C. P., Downey, G., Sheehan, E. M., Delahunty, C. M., O'Callaghan, D. J., & Howard, V. (2007). Prediction of processed cheese instrumental texture and meltability by mid-infrared spectroscopy coupled with chemometric tools. *Journal of Food Engineering*, 80(4), 1068–1077.

Ferrão, L. L., Ferreira, M. V. S., Cavalcanti, R. N., Carvalho, A. F. A., Pimentel, T. C., Silva, H. L. A., Silva, R., Esmerino, E. A., Neto, R. P. C., Tavares, M. I. B., Freitas, M. Q., Menezes, J. C. V., Cabral, L. M., Moraes, J., Silva, M. C., Mathias, S. P., Raices, R. S. L., Pastore, G. M., & Cruz, A. G. (2018). The xylooligosaccharide addition and sodium reduction in requeijão cremoso processed cheese. *Food Research International*, 107, 137–147.

Ferrari, E; Gamberi, M; Manzini, R; Pareschi, A; Persona, A; Regattieri, A. (2003). Redesign of the Mozzarella cheese production process through development of a microforming and stretching extruder system. *Journal of Food Engineering*, 59, 1, 13–23.

Flasarová, R; Pachlová, V; Buňkova, L; Menšíková, A; Georgová, N; Drab, V; Buňka, F. (2016). Biogenic amine production by *Lactococcus Lactis* subsp. *Cremoris* strains in the model system of Dutch-type cheese. *Food Chemistry*, 194, 68–75.

Fox, P. F. Cheese and Fermented Milk Foods (3rd Edition). Volume 1: Origins and Principles (728 Pp.) Volume 2: Procedures and Analyses (330 Pp.). International Dairy Journal. 1997, vol. 7, 5, 360-361. ISSN:0958-6946.

Fox, P.F.; Guinee, T.P.; Cogan, T.M.; McSweeney, P.L.H. Fundamentals of Cheese Science. Gaithersburg, USA: Aspen Publication, 2000.

Fox, P.F; McSweeney, P.L.H. (1996). Proteolysis in cheese during ripening. *Food Reviews International*. 1996, vol. 12, no. 4 s. 457-509. ISSN:8755-9129.

Fox, P.F; Uniacke-Lowe, T; MsSweeney, P.L.H; O'Mahony, J.A. Dairy Chemistry and Biochemistry, Second Edition. 2015. ISBN: 3319148915.

Fu, J. T., Rao M. A., 1999. The influence of sucrose and sorbitol on gel–sol transition of low–methoxyl pectin + Ca^{2+} gels. *Food Hydrocolloids*, 13, 371–380.

Fu, W. & Nakamura, T. (2018). Effect of starch on the mechanical properties and microstructure of processed cheeses with different types of casein network structures. *Food hydrocolloids*, 79, 587–595.

Fu, W., & Nakamura, T. (2020). Moisture content impact creaming effect and microstructure of processed cheese containing different textural starches. *International Dairy Journal*, 105.

Fu, W., Watanabe, Y., Inoue, K., Moriguchi, N., Fusa, K., Yanagisawa, Y., Mutoh, T., & Nakamura, T. (2018). Effects of pre-cooked cheeses of different emulsifying conditions on mechanical properties and microstructure of processed cheese. *Food Chemistry*, 245, 47–52.

Gernigon, G; Schuck, P; Jeantet, R. (2010). Processing of Mozzarella cheese wheys and stretchwaters: A Preliminary Review. *Dairy Science & Technology*, 90, 1, 27–46.

Ghods Rohani, M., & Rashidi, H. (2019). Improving the characteristics of spreadable processed cheese using Konjac and Xanthan gums. *Journal of Food Processing and Preservation, 43*(12).

Giri, A., Kanawjia, S. K., Singh, M. P. (2017). Effect of inulin on physico–chemical, senzory, fatty acid profile and microstructure of processed cheese spread. *Journal of Food Science and Technology*, 54(8), 2443–2451.

Görner, F., Valík, Ľ., 2004. Aplikovaná mikrobiológia poživatín. Bratislava: Malé centrum. 528 pp. ISBN 80–967064–9–7.

Gorrasi, G; Bugatti, V; Tammaro, L; Vertuccio, L; Vigliotta, G; Vittoria, V. (2016). Active coating for storage of Mozzarella cheese packaged under thermal abuse. *Food Control*, 64, 10–16.

Guidone, A; Zotta, T; Matera, A; Ricciardi, A; De Filippis, F; Ercolini, D; Parente, E. (2016). The microbiota of high-moisture Mozzarella cheese produced with different acidification methods. *International Journal of Food Microbiology*, 216, 9–17.

Guinee, T. P, Carić, M, & Kaláb, M. (2004). Pasteurized Processed cheese and substitute/imitation cheese products. Cheese: Chemistry, Physics and Microbiology, 2, 349–394.

Guinee, T. P., & O'Callaghan, D. J. (2013). Effect of increasing the protein-to-fat ratio and reducing fat content on the chemical and physical properties of processed cheese product. *Journal of Dairy Science*, 96(11), 6830–6839.

Guinee, T. P., & O'Kennedy, B. T. (2012). Reducing the level of added disodium phosphate alters the chemical and physical properties of processed cheese. *Dairy Science & Technology*, *92*(5), 469–486.

Guinee, T.P. (2011). Pasteurized Processed Cheese Products. *Encyclopedia of Dairy Sciences*, 805-813. ISBN: 978-0-1237-4407-4.

Gupta, S. K., Karahadian, C., & Lindsay, R. C. (1984). Effect of emulsifier salts on textural and flavour properties of processed cheese. *Journal of Dairy Science*, 67, 764–778.

Gustaw, W., & Mleko, S. (2007). The effect of polysaccharides and sodium chloride on physical properties of processed cheese analogs containing whey proteins. *Milchwissenschaft*, 62, 59–62.

Hanaei, F., Cuvelier, G., & Sieffermann, J. M. (2015). Consumer texture descriptions of a set of processed cheese. *Food Quality and Preference*, 40, 316–325.

Hanna, S. S. (1999). The use of halloum cheese for the production of processed cheese. *International Journal of Dairy Technology*, 52, 3, 117–118.

Hasenhuettl, G.L, & Hartel, R.W. (2008). Food Emulsifiers and their Applications: Second Edition, 1-426. ISBN: 9780387752839.

Hassan, A. N., Awad, S., & Mistry, V. V. (2007). Reduced fat process cheese made from young reduced fat Cheddar cheese manufactured with exopolysaccharide-producing cultures. *Journal of Dairy Science*, 90(8), 3604-3612.

Hauerlandová, I., Lorencová, E., Buňka, F., Navrátil, J., Janečková, K., & Buňková, L. (2014). The influence of fat and monoacylglycerols on growth of spore-forming bacteria in processed cheese. *International Journal of Food Microbiology*, 182-183, 37–43.

Hayaloglu, A. A. (2017). Cheese varieties ripened under brine. In Cheese (pp. 997-1040). Elsevier.

Hladká, K., Randulová, Z., Tremlová, B., Ponížil, P., Mančík, P., Černíková, M., Buňka, F., 2014. The effect of cheese maturity on selected properties of processed cheese without traditional emulsifying agents. *Lebensmittel-Wissenschaft und Technologie – Food Science and Technology*, 55, 650–656.

Hoffmann, W., Gärtner, J., Lück, K., Johannsen, N., & Maurer, A. (2012). Effect of emulsifying salts containing potassium on the quality of block-type processed cheese. *International Dairy Journal*, 25(1), 66–72.

Hosseini–Parvar, S. H., Matia–Merono, L., & Golding, M. (2015). Effect of basil seed gum (BSG) on textural, rheological and microstructural properties of model processed cheese. *Food Hydrocolloids*, 43, 557–567.

Hui, Y.H. Handbook of Food Science, Technology, and Engineering. 2005, vol. 148. ISBN: 9781466507876.

Hui, Y.H; Evranuz, E.Ö; Chandan, R.C. (2012). Handbook of animal-based fermented food and beverage Technology. ISBN: 9781439850237.

Isolini, D., Bachmann, H. P., Butikofer, U., Jakob, E., & Fröhlich-Wyder, M. T. (2011). Swiss-Type Cheeses. *Encyclopedia of Dairy Sciences*, 712–720.

Janevski, O., Hassan, A. N., & Metzger, L. (2012). Application of salt whey in process cheese food made from Cheddar cheese containing exopolysaccharides. *Journal of Dairy Science*, 95(7), 3609–3616.

Johnson, M. E., & Lucey, J.A. (2006). Major technological advances and trends in cheese. *Journal of Dairy Science*, 89, 4, 1174–1178.

Kaliappan, S., & Lucey, J.A. (2011). Influence of mixtures of calcium-chelating salts on the physicochemical properties of casein micelles. *Journal of Dairy Science*, 94, 9, 4255–4263.

Kaminarides, S., & Stachtiaris, S. (2000). Production of processed cheese using kasseri cheese and processed cheese analogues incorporating whey protein concentrate and soybean oil. *International Journal of Dairy Technology*, 53, 2, 69–74.

Kammerlehner, J. Käsetechnologie. 2012, Verlug Reisinger, Kuenstlerpresse W Bode, Frising. ISBN 978-00-037724-2.

Kapoor, R. & Metzger, L.E. (2008). Process Cheese: Scientific and Technological Aspects – A Review. *Food Science and Food Safety*, 7, 194–214.

Kapoor, R; Metzger, L.E., Biswas, A.C., & Muthukummarappan, K. (2007). Effect of natural cheese characteristics on process cheese properties. *Journal of Dairy Science*, 90, 4, 1625-1634.

Kawasaki, Y. (2008). Influence of „creaming" on the properties of processed cheese and changes in the structure of casein during cheese making. *Milchwissenschaft*, 63, 149–153.

Kommineni, A., Amamcharla, J., & Metzger, L. E. (2012). Effect of xylitol on the functional properties of low-fat process cheese. *Journal of Dairy Science*, 95(11), 6252–6259.

Kontou, V., Dimitreli, G., & Raphaelides, S. N. (2019). Elongational flow studies of processed cheese spreads made from traditional greek cheese varieties. *Lwt*, *107*, 318–324.

Lauck, R. M., inventor; Stauffer Chemical Co., assignee. 1972. Using salvage cheese in preparing pasteurized process cheese. New York. US patent 3, 697, 292.

Law, B.A; Tamine, A.Y. Technology of Cheesemaking. 2010. ISBN: 9781444323740.

Lee, S. K., Buwalda, R. J., Euston, S. R., Foegeding, E. A., & Mckenna, A. B. (2003). Changes in the rheology and microstructure of processed cheese during cooking. *Lebensmittel-Wissenschaft und Technologie*, 36, 339–345.

Lee, S. K., Klostermayer, H., & Anema, S. G. (2015). Effect of fat and protein–in–water concentration on the properties of model processed cheese. *International Dairy Journal*, 50, 15–23.

Lee, S.K., & Klostermeyer, H. (2001). The effect of pH on the rheological properties of reduced-fat model processed cheese spreads. *Food Science and Technology*, 34, 5, 288–292.

Lee, S.K., Anema, S. & Klostermeyer, H. (2004). The influence of moisture content on the rheological properties of processed cheese spreads. *International Journal of Food Science and Technology*, 39, 763–771.

Lenze, S., Wolfschoon–Pombo, A., Schrader, K., & Kulozik, U. (2019). Effect of the compositional factors and processing conditions on the creaming reaction during process cheese manufacturing. *Food and Bioprocess Technology*, 12, 575–586.

Löfgren, C., & Hermansson, A. M., 2007. Synergistic rheological behaviour of mixed HM/LM pectin gels. *Food Hydrocolloids*, 21, 480–486.

Lootens, D., Capel, F., Durand, D., Nicolai, T., Boulenguer, P., & Langendorff, V. (2003). Influence of pH, Ca concentration, temperature and amidation on the gelation of low methoxyl pectin. *Food Hydrocolloids*, 17, 237–244.

Lorencová, E., Vltavská, P., Budinsky, P., & Koutný, M. (2012). Antibacterial effect of phosphates and polyposphates with different chain length. *Journal of Environmental Science and Health*, 47, 2241–2245.

Lu, Y., Shirashoji, N., & Lucey, J. A. (2007). Rheological, textural and melting properties of commercial samples of some of different types of pasteurized processed cheese. International *Journal of Dairy Technology*, 60 (2), 74–80.

Lu, Y., Shirashoji, N., & Lucey, J. A. (2008). Effects of pH on the textural properties and meltability of pasteurized process cheese made with different types of emulsifying salts. *Journal of Food Science*, 73, 363–369.

Lucey, J. A., Johnson, M. E., & Horne, D. S. (2003). Perspectives on the basis of the rheology and texture properties of cheese. *Journal of Dairy Science*, 86, 2725–2743.

Lucey, J. A., Maurer-Rothmann, A., & Kaliappan, S. (2011). Functionality of ingredients: emulsifying salts. In Tamine, A. Y. (Ed.) Processed cheese and analogues. John Wiley & Sons, Ltd, Chichester, 110–132.

Lucey, J.A. (2002). Formation and Physical Properties of Milk Protein Gels. *Journal of Dairy Science*, 85, 2, 281-294.

Macků, I., Buňka, F., Voldánová, B., & Pavlínek, V. (2009). Effect of addition of selected solid cosolutes on viscoelastic properties of model processed cheese containing pectin. *Food Hydrocolloids*, 23(8), 2078–2084.

Marchesseau, S., Gastaldi, E., Lagaude, A., & Cuq, J.L. (1997). Influence of PH on Protein Interactions and Microstructure of Process Cheese. *Journal of Dairy Science*, 80, 8, 1483-1489.

Marth, E.H, & Steele, J.L. Applied Dairy Microbiology. 2001. Boca Raton: CRC Press, 2., ed. ISBN 978-0824705367.

May, C. D., 2000. Pectins. In Handbook of hydrocolloids. Eds. Phillips, G.O. & Williams, P.A. Woodhead Publishing Limited and CRC Press: Boca Raton, 169–188. ISBN 0–8493–0850–X

McIntyre, I., O´Sullivan, M., & O´Riordan D. (2017). Monitoring the progression of calcium and protein solubilisation as affected by calcium chelators during small-scale manufacture of casein-based food matrices. *Food Chemistry*, 237, 597–604.

McMahon, D., & Oberg, J. (2011). Pasta-filata cheeses: Low-moisture part-skim Mozzarella (pizza cheese). *Encyclopedia of Dairy Sciences*, 737–744.

McSweeney, P.L.H. (2004). Biochemistry of cheese ripening. *International Journal of Dairy Technology*, 57, 2-3, 127–144.

McSweeney, P.L.H. Cheese Problems Solved. 2007. ISBN 978-1-84569-060-1.

McSweeney, P.L.H., & Sousa, M.J. (2000). Biochemical Pathways for the production of flavour compounds in cheeses during ripening: A Review. *Lait*, 80, 3, 293–324.

Mehanna, N. S., Hassan, F. A. M., El–Messery, T. M., Mohamed, A. G., 2017. Production of functional processed cheese by using tomato juice. *International Journal of Dairy Science*, 12, 155–160.

Miloradovic, Z., Smigic, N., Djekic, I., Tomasevic, I., Kljajevic, N., Nedeljkovic, A., & Miocinovic, J. (2018). The influence of NaCl concentration of brine and different packaging on goat white brined cheese characteristics. *International Dairy Journal*, 79, 24–32.

Mizuno, R., & Lucey, J. A. (2005a). Effects of emulsifying salts on the turbidity and calcium-phosphate-protein interaction in casein micelles. *Journal of Dairy Science*, 88 3070–3078.

Mizuno, R., & Lucey, J. A. (2007). Properties of milk protein gels formed by phosphates. *Journal of Dairy Science*, 90, 4524–4531.

Mizuno, R., & Lucey, J. A., (2005b). Effects of two types of emulsifying salts on the functionality of nonfat pasta filata cheese. *Journal of Dairy Science*, 88 3411–3425.

Moatsou, G., & Govaris, A. (2011). White brined cheeses: A diachronic exploitation of small ruminants milk in Greece. *Small Ruminant Research*, 101(1-3), 113–121.

Mohamed, A. G., & Shalaby, S. M. (2016). Texture, chemical properties and sensory evaluation of a spreadable processed cheese analogue made with apricot pulp (*Prunus armeniaca* L.). *International Journal of Dairy Science*, 11, 61–68.

Mohamed, A. G., Shalaby, S. M., & Gafour, W. A. (2016). Quality characteristics and acceptability of an analogue processed spreadable cheese made with carrot paste (*Daucus carota* L.). *International Journal of Dairy Science*, 11, 91–99.

Molins, R.A. Phosphates in food. Boca Raton: CRC Press. 1991. 261 s. ISBN 0-8493-4588-X.

Mounsey, J.S., & O´Riordan, E.D. (2001). Characteristics of Imitation Cheese Containing Native Starches. *Journal of Food Science*, 66 (4), 586–591.

Mozuraityte, R., Berget, I., Mahdalova, M., Grønsberg, A., Øye, E. R., & Greiff, K. (2019). Sodium reduction in processed cheese spreads and the effect on physicochemical properties. *International Dairy Journal*, 90, 45–55.

Muslow, B. B., Jaros, D., & Rohm, H. (2007). Processed cheese and cheese analogues. In Tamine, A. Y. (Ed.) Structure of Dairy Products. Blackwell Publishing Ltd., Oxford, 210–235.

Nagyová, G., Buňka, F., Salek, R. N., Černíková, M., Mančík, P., Grůber, T., & Kuchař, D. (2014). Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. *Journal of Dairy Science*, 97(1), 111–122.

Nastaj, M., Terpiłowski, K., & Sołowiej, B. G. (2020). The effect of native and polymerised whey protein isolate addition on surface and microstructural properties of processed cheeses and their meltability determined by Turbiscan. I*nternational Journal of Food Science and Technology*, 1–9.

Nogueira, E. B., Costa-Lima, B. R. C., Torres, F, Regazone, A. V., Melo, L., Franco, R. M., & Cortez, M. A. S. (2018). Effect of potassium-based emulsifying salts on the sensory and physicochemical parameters of low-sodium spreadable processed cheese.

Noronha, N., Duggan, E., Ziegler, G. R., O'Riordan, E. D., & O'Sullivan, M. (2008b). Investigation of imitation cheese matrix development using light microscopy and NMR relaxometry. *International Dairy Journal*, 18, 641–648.

Noronha, N., O'Riordan, E. D., & O'Sullivan, M., (2008a). Influence of processing parameters on the texture and microstructure of imitation cheese. *European Food Research and Technology*, 226 (3), 385–393.

O'Sullivan, D.J., McSweeney, P.L.H., Cotter, P.D., Giblin, L., & Sheehan, J.J. Compromised. *Journal of Dairy Science*, 99, 4, 2625–2640.

Oberg, E.N., Oberg, C.J., Motawee, M.M., Martini, S., & McMahon, D.J. (2015). Increasing stringiness of low-fat Mozzarella string cheese using polysaccharides. *Journal of Dairy Science*, 98, 7, 4243–4254.

Pachlová, V., Buňka, F., Buňkova, L., Weiserová, E., Budinský, P., Žaludek, M., & Kráčmar, S. (2011). The effect of three different ripening/storage conditions on the distribution of selected parameters in individual parts of Dutch-type cheese: Cheese ripened/stored under three different conditions. *International Journal of Food Science & Technology*, 46, 1, 101–108.

Pachlová, V., Buňka, F., Flasarová, R., Válková, P., & Buňková, L. (2012). The effect of elevated temperature on ripening of Dutch type cheese. *Food Chemistry*, 132, 4, 1846–1854.

Pisano, M.B., Scano, P., Murgia, A., Cosentino, S., & Caboni, P. (2016). Metabolomics and microbiological profile of Italian Mozzarella cheese produced with buffalo and cow Milk. *Food Chemistry*, 192, 618–624.

Piska, I., & Štětina, J. (2004). Influence of cheese ripening and rate of cooling of the processed cheese mixture on rheological properties of processed cheese. *Journal of Food Engineering*, 61(4), 551–555.

Pluta, A., Ziarno, M., & Smolińska, A. (2000). Możliwości zastosowania hydrokoloidów w produkcji serów topionych. *Przemysl Spożywczy,* 5, 42–44.

Přikryl, J., Hájek, T., Švecová, B., Salek, R. N., Černíková, M., Červenka, L., & Buňka, F. (2018). Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions. *LWT - Food Science and Technology*, 87, 266–271.

Rafiq, S. M., & Ghosh, B. C. (2017). Effect of potato incorporation on the physico-chemical, textural and sensory properties of processed cheese. *Journal of Food Measurement and Characterization*, *11*(2), 776–780.

Ramel, P. R., & Marangoni, A.G. (2017). Characterization of the polymorphisms of milk fat within processed cheese products. *Food Structure*, 12, 15–25.

Ramel, P. R., & Marangoni, A.G. (2018). Processed cheese as a polymer matrix composite: A particle tool kit for the replacement of milk fat with canola oil in processed cheese. *Food Research International*, 107, 110–118.

Ribeiro, K. O., Rodrigues, M. I., Sabadini, E., & Cunha, R. L. (2004). Mechanical properties of acid sodium caseinate– κ–carrageenan gels: Effect of co–solute addition. *Food Hydrocolloids*, 18, 71–79.

Rowney, M.K., Hickey, M.W., Roupas, P., Everett, D.W. (2003). The Effect of homogenization and milk fat fractions on the functionality of Mozzarella cheese. *Journal of Dairy Science*, 86, 3, 712–718.

Rubio-Texeira, M. (2006). Endless versatility in the biotechnological applications of *Kluyveromyces* LAC genes. *Biotechnology Advances*, 24, 2, 212–225.

Sadek, Z. I., Refaat, B. M., Adb El–Shakour, E. H., Mehenna, N. S., Hassan, M. S., 2017. Biocontrol of processed cheese by incorporation of probiotic bacteria and its metabolites. *International Journal of Dairy Science*, 12, 93–104.

Sádlíková, I; Buňka, F; Budinský, P; Barbora, V; Pavlínek, V; Hoza, I. (2010) The effect of selected phosphate emulsifying salts on viscoelastic properties of processed cheese. *LWT - Food Science and Technology*, 43, 8, 1220–1225.

Salek, R. N., Černíková, M., Lorencová, E., Pachlová, V., Kůrová, V., Šenkýřová, J., & Buňka, F. (2020). The impact of Cheddar or white brined cheese with various maturity degrees on the processed cheese consistency: A comparative study. *International Dairy Journal*.

Salek, R. N., Černíková, M., Maděrová, S., Lapčík, L., & Buňka, F. (2016). The effect of different composition of ternary mixtures of emulsifying salts on the consistency of processed cheese spreads manufactured from Swiss-type cheese with different degrees of maturity. *Journal Of Dairy Science*, *99*(5), 3274–3287.

Salek, R. N., Lorencová, E., Míšková, Z., Lazárková, Z., Pachlová, V., Adámek, R., Bezděková, K., & Buňka, F. (2020). The impact of Chios mastic gum on textural, rheological and melting properties of spread-type processed cheese during storage. *International Dairy Journal*, 109.

Salek, R. N; Černíková, M; Nagyová, G; Kuchař, D; Bačová, H; Minarčíková, L; Buňka, F. (2015). The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. *International Dairy Journal*, 44, 37–43.

Schädle, C. N., Eisner, P., & Bader-Mittermaier, S. (2020). The combined effects of different fat replacers and rennet casein on the properties of reduced-fat processed cheese. *Journal of Dairy Science*, 103(5), 3980–3993.

Schatz, K., Hoffmann, W., Schrader, K., Maurer, A. 2014. Effect of emulsifying salts containing potassium on the melting properties of block-type dairy cheese analogues. International *Journal of Dairy Technology* 67, 202 – 210.

Seth, K., & Bajwa, U. (2015). Effect of acidulants on the recovery of milk constituents and quality of Mozzarella processed cheese. *Journal of Food Science and Technology*, *52*(3), 1561–1569.

Shalaby, S. M., Mohamed, A. G., & Bayoumi, H. M. (2017). Preparation of a Novel Processed Cheese Sauce Flavored with Essential Oils. *International Journal of Dairy Science*, *12*(3), 161–169.

Shirashoji, N., Aoyagi, H., Jaeggi, J. J., & Lucey, J. A. (2016). Effect of tetrasodium pyrophosphate concentration and cooking time on the physicochemical properties of process cheese. *Journal of Dairy Science*, 99(9), 6983–6994.

Shirashoji, N., Jaeggi, J. J., & Lucey, J. A. (2010). Effect of sodium hexametaphosphate concentration and cooking time on the physicochemical properties of pasteurized process cheese. *Journal of Dairy Science*, 93(7), 2827–2837.

Shirashoji, N; Jaeggi, J.J; Lucey, J.A. (2006). Effect of trisodium citrate concentration and cooking time on physicochemical properties of pasteurized process cheese. *Journal of Dairy Science*, 2006, 89, 15–28. ISSN 0022-0302.

Solhi, P., Azadmard–Damirchi, S., Hesari, J., Hamishehkar, H. (2020). Effect of fortification with asparagus powder on the qualitative properties of processed cheese. *International Journal of Dairy Technology*, 73, 1, 226–233.

Sołowiej, B., Glibowski, P., Muszyński, S., Wydrich, J., Gawron, A., Jeliński, T., 2015. The effect of fat replacement by inulin on the physicochemical properties and microstructure of acid casein processed cheese analogues with added whey protein polymers. *Food Hydrocolloids*, 44, 1–11.

Sousa, M. J., Ardö, Y., & McSweeney, P. L. H. (2001). Advances in the study of proteolysis during cheese ripening. *International Dairy Journal*, *11*(4-7), 327–345.

Stangierski, J., Weiss, D., & Kaczmarek, A. (2019). Multiple regression models and Artificial Neural Network (ANN) as prediction tools of changes in overall quality during the storage of spreadable processed Gouda cheese. *European Food Research and Technology*, 245(11), 2539–2547.

Sutheerawattananonda, M., Fulcher, R. G., Martin, F. B., Bastian, E. D., 1997. Fluorescence image analysis of process cheese manufactured with trisodium citrate and sodium chloride. *Journal of Dairy Science*, 80 (4), 620–627.

Swenson, B. J., Wendorff, W. L. & Lindsay, R.C. (2000). Effects of ingredients on the functionality of fat-free process cheese spreads. *Journal of Food Science*, 65, 822–825.

Syrbe, A., Bauer, W. J. Klostermeyer, H., 1998. Polymer science concepts in dairy systems – an overview of milk protein and food hydrocolloid interaction. *International Dairy Journal*, 8, 179–193.

Tamine, A.Y. (2007). Structure of Dairy Products. 2007,1–288. ISBN: 978-1-4051-2975-6.

Tamine, A.Y. Processed cheese and analogues. Ames: Wiley-Blackwell, 2011. Society of dairy technology. ISBN 978-1-4051-8642-1.

Tanaka, Y; Takei, T; Aiba, T; Masuda, K; Kiuchi, A; Fujiwara, T. (1986). Development of synthetic lung surfactants. *Journal of Lipid Research*, 27, 5, 475–485.

Templeton, H. L, Sommer, H. H., 1936. Studies on the emulsifying salts used in processed cheese. *Journal of Dairy Science*, 19, 561–572.

Tohamy, M. M., Ali, M. A., Shaaban, H. A. G., Mohamad, A. G., Hasanain, A. M., 2018. Production of functional spreadable processed cheese using Chlorella vulgaris. *Acta Scientiarum. Polonorum Technologia Alimentaria*, 17 (4), 347–358.

Topcu, A., Bulat, T., & Özer, B. (2020). Process design for processed Kashar cheese (a pasta-filata cheese) by means of microbial transglutaminase: Effect on physical properties, yield and proteolysis. *LWT - Food Science and Technology*, 125.

Torres, F. R., Esmerino, E. A., Carr, B. T., Ferrão, L. L., Granato, D., Pimentel, T. C., Bolini, H. M. A., Freitas, M. Q., & Cruz, A. G. (2017). Rapid consumer-based sensory characterization of requeijão cremoso, a spreadable processed cheese: Performance of new statistical approaches to evaluate check-all-that-apply data. *Journal of Dairy Science*, 100(8), 6100–6110.

Trivedi, D., Bennett, R. J., Hemar, Y., Reid, D. C. W., Lee, S. K., Illingworth, D., 2008a. Effect of different starches on the rheological and microstructural properties of (I) model processed cheese. *International Journal of Food Science and Technology*, 43, 2191–2196.

Trivedi, D., Bennett, R. J., Hemar, Y., Reid, D. C. W., Lee, S. K., Illingworth, D., 2008b. Effect of different starches on the rheological and microstructural properties of (II) commercial processed cheese. *International Journal of Food Science and Technology*, 43, 2197–2203.

Vega, C., Dalgleish, D. G., Goff, H. D., 2005. Effect of κ–carrageenan addition to dairy emulsions containing sodium caseinate and locust bean gum. *Food Hydrocolloids*, 19, 187–195.

Velazquez, L. D., Escudero, M. E., and De Guzman, A. M., 2001. Antibacterial effects of different food-related phosphates using Aeromonas hydrophila. *Journal of Food Protection*, 64, 195–200.

Weimer, B.C. (2007). Improving the flavour of cheese. ISBN 978-1-84569-007-6.

Weiserová, E., Doudová, L., Galiová, L., Žák, L., Michálek, J., Janiš, R., & Buňka, F. (2011). The effect of combinations of sodium phosphates in binary mixtures on selected texture parameters of processed cheese spreads. *International Dairy Journal*, 21(12), 979-986.

Weiss, D., Stangierski, J., Baranowska, H. M., & Rezler, R. (2018). Kinetic models of quality parameters of spreadable processed Gouda cheese during storage. *Food Science and Biotechnology*, *27*(5), 1387-1394.

Williams, P.A. Woodhead Publishing Limited and CRC Press: Boca Raton, 169–188. ISBN 0–8493–0850–X

Yun, J.J; Kiely, L.J; Kindstedt, P.S; Barbano, D.M. (1993). Mozzarella Cheese: Impact of coagulant type on functional properties. *Journal of Dairy Science*, 76, 12, 3657–3663.

Yvon, M; & Rijnen, L. Cheese Flavour Formation by Amino Acid Catabolism. *International Dairy Journal*. 2001, vol. 11, no. 4 s. 185-201. ISSN:0958–6946.

Zehren, V.L; & Nusbaum, D.D. Process cheese. 2nd ed. Madison, Wisconsin, USA: Cheese Reporter Publishing, Co, Inc., 2000.

Zerfiridis, G.K. Technology of dairy products (In Greek). Thessaloniki: Giachoudis publishing, Edit^{2nd}, 2001.

LIST OF USED SYMBOLS AND ABBREVIATIONS

DSP – disodium phosphate

TSPP – tetrasodium disphosphate

PSTP – pentasodium triphosphate

TSC – trisodium citrate

POLY5 – sodium salt of polyphosphate in which the mean amount of phosphate units was n ≈ 5

POLY9 – sodium salt of polyphosphate in which the mean amount of phosphate units was n ≈ 9

POLY13 – sodium salt of polyphosphate in which the mean amount of phosphate units was n ≈ 13

POLY20 – sodium salt of polyphosphate in which the mean amount of phosphate units was n ≈ 20

POLY28 – sodium salt of polyphosphate in which the mean amount of phosphate units was n ≈ 28

AUTHOR 'S PUBLISHING ACTIVITIES[a]

Author identifiers:

***ORCID**: 0000-0001-5129-5329; **RESEARCHERID**: E-2500-2015;*

***SCOPUS ID**: 55921210200*

1. Nagyová, G.; Buňka, F.; **Salek, R. N. (20)**; Černíková, M.; Mančík, P.; Grůber, T.; Kuchař, D.(5). Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. *Journal of Dairy Science*, 2014, *97*. ISSN 0022-0302.
2. **Salek, R. N. (45)**; Černíková, M.; Nagyová, G.; Bačová, H.; Mynarčíková, L.; Buňka, F. The impact of ternary mixtures composed of sodium phosphate and citrate salts on processed cheese spreads hardness. In *Sborník příspěvků z XVI. Konference mladých vědeckých pracovníků s mezinárodní účastí*. Brno: Veterinární a farmaceutická univerzita, 2014. ISBN 978-80-7305-670-4.
3. **Salek, R. N. (40)**; Buňka, F.; Černíková, M.; Nagyová, G.; Kuchař, D.; Bačová, H.; Mynarčíková, L. Ternární směsi tavicích solí obsahující citronan sodný a jejich vliv na tvrdost modelových tavených sýrů. In *Celostátní přehlídka sýrů a konference mléko a sýry*. Praha: Vysoká škola chemicko-technologická v Praze, 2014. ISBN 978-80-7080-909-9.
4. Lapčík, L.; Lapčíková, B.; Otyepková, E.; Otyepka, M.; Vlček, J.; Buňka, F.; **Salek, R. N. (5)**. Surface energy analysis (SEA) and rheology of powder milk dairy products. *Food Chemistry*, 2015, *174*. ISSN 0308-8146.
5. Buňka, F.; **Salek, R. N. (30)**; Černíková, M.; Kuchař, D. Vliv složení ternárních směsí tavicích solí s citronanem sodným na tvrdost modelových tavených sýrů. In *Hygiena a technologie potravin XLIV. Lenfeldovy a Höklovy dny Brno*. Brno: Veterinární a farmaceutická univerzita Brno, 2014. ISBN 978-80-7305-728-2.
6. **Salek, R. N. (35)**; Černíková, M.; Nagyová, G.; Kuchař, D.; Bačová, H.; Mynarčíková, L.; Buňka, F. The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. *International Dairy Journal*, 2015, *44*. ISSN 0958-6946.
7. Nagyová, G.; Buňka, F.; **Salek, R. N. (20)**; Černíková, M.; Bačová, H.; Kráčmar, S. The effect of individual phosphate emulsifying salts and their selected binary mixtures on hardness of processed cheese spreads. *Potravinárstvo*, 2013, *7*. ISSN 1338-0230.
8. **Salek, R. N. (35)**; Černíková, M.; Maděrová, S.; Lapčík, L.; Buňka, F. The effect of different composition of ternary mixtures of emulsifying salts on the consistency of processed cheese spreads manufactured from Swiss-type cheese with different degrees of maturity. *Journal of Dairy Science*, 2016, *99*. ISSN 0022-0302.
9. Černíková, M.; Nebesářová, J.; **Salek, R. N. (10)**; Řiháčková, L.; Buňka, F. Srovnání různých metod přípravy vzorků tavených sýrů pro elektronovou mikroskopii. In *Hygiena a technologie potravin - XLV. Lenfeldovy a Höklovy dny*. Brno: Veterinární a farmaceutická univerzita Brno, 2016. ISBN 978-80-7305-782-4.

10. Černíková, M.; Nebesářová, J.; **Salek, R. N. (20)**; Řiháčková, L.; Buňka, F. Microstructure and textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content. *Journal of Dairy Science*, 2017, *100*. ISSN 0022-0302.
11. Burešová, I.; **Salek, R. N. (30)**; Varga, E.; Masaříková, L.; Bureš, D. The effect of Chios mastic gum addition on the characteristics of rice dough and bread. *LWT- Food Science and Technology*, 2017, *81*. ISSN 0023-6438.
12. **Salek, R. N. (35)**; Burešová, I.; Kráčmar, S.; Lorencová, E.; Zálešáková, L.; Dabash, V. Evaluation of selected physicochemical parameters of extra virgin olive oil commercialized in the Czech market and stored during a period of 5 months. *Potravinárstvo*, 2017, *11*. ISSN 1338-0230.
13. Černíková, M.; **Salek, R. N. (25)**; Kozáčková, D.; Běhalová, H.; Luňáková, L.; Buňka, F. The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads. *International Dairy Journal*, 2017, *66*. ISSN 0958-6946.
14. **Salek, R. N. (35)**; Černíková, M.; Pachlová, V.; Lazárková, Z.; Konečná, V.; Buňka, F. Properties of spreadable processed Mozzarella cheese with divergent compositions of emulsifying salts in relation to the applied cheese storage period. *LWT- Food Science and Technology*, 2017, *77*. ISSN 0023-6438.
15. Černíková, M.; **Salek, R. N. (25)**; Kozáčková, D.; Běhalová, H.; Luňáková, L.; Buňka, F. Vliv vybraných technologických parametrů na konzistenci tavených sýrů. In *Hygiena a technologie potravin – XLVII. Lenfeldovy a Höklovy dny*. Brno: Veterinární a farmaceutická univerzita Brno, 2017. ISBN 978-80-7305-793-0.
16. Přikryl, J.; Hájek, T.; Švecová, B.; **Salek, R. N. (10)**; Černíková, M.; Červenka, L.; Buňka, F. Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions. *LWT- Food Science and Technology*, 2018, *87*. ISSN 0023-6438.
17. Černíková, M.; **Salek, R. N. (25)**; Kozáčková, D.; Buňka, F. The effect of different agitations and temperature maintainings on viscoelastic properties of full-fat processed cheese spreads. *LWT- Food Science and Technology*, 2018, *89*. ISSN 0023-6438.
18. Černíková, M.; Nebesářová, J.; **Salek, R. N. (10)**; Popková, R.; Buňka, F. Vliv přídavku nátavku na viskoelastické vlastnosti a mikrostrukturu tavených sýrů. In *Výsledky přehlídek a sborník příspěvků konference Mléko a sýry*. Praha: Vysoká škola chemicko-technologická v Praze, 2018. ISBN 978-80-7592-030-0.
19. Lazárková, Z.; Sumczynski, D.; **Salek, R. N. (10)**. Effect of cooking and germination on antioxidant activity, total polyphenols and flavonoids, fiber content, and digestibility of lentils (Lens culinaris L.). *Journal of Food Processing and Preservation*, 2018, *42*. ISSN 0145-8892.
20. Červenka, L.; Hájek, T.; **Salek, R. N. (15)**; Černíková, M.; Velichová, H.; Buňka, F. Addition of rutin/quercetin mixture to spreadable processed cheese: Antioxidant and textural characteristics. *Potravinárstvo*, 2018, *12*. ISSN 1338-0230.
21. Pachlová, V.; Buňková, L.; Flasarová, R.; **Salek, R. N. (5)**; Dlabajová, A.; Butor, I.; Buňka, F. Biogenic amine production by nonstarter strains of Lactobacillus curvatus and Lactobacillus paracasei in the model system of Dutch-type cheese. *LWT- Food Science and Technology*, 2018, *97*. ISSN 0023-6438.

22. Černíková, M.; Nebesářová, J.; **Salek, R. N. (10)**; Popková, R.; Buňka, F. The effect of rework content addition on the microstructure and viscoelastic properties of processed cheese. *Journal of Dairy Science*, 2018, *101*. ISSN 0022-0302.
23. **Salek, R. N. (35)***; Vašina, M.; Lapčík, L.; Černíková, M.; Lorencová, E.; Li, P.; Buňka, F. Evaluation of various emulsifying salts addition on selected properties of processed cheese sauce with the use of mechanical vibration damping and rheological methods. *LWT-Food Science and Technology*, 2019, *107*. ISSN 0023-6438.
24. Lorencová, E.; **Salek, R. N. (30)***; Černošková, I.; Buňka, F. Evaluation of force-carbonated Czech-type lager beer quality during storage in relation to the applied type of packaging. *Food Control*, 2019, *106*. ISSN 0956-7135.
25. Buňka, F.; Buňková, L.; Pachlová, V.; Černíková, M.; Míšková, Z.; **Salek, R. N. (5)**; Pleva, Pavel. Spolupráce při vývoji nové řady mléčných výrobků. LACRUM Velké Meziříčí, s.r.o., 2019. 1.
26. Buňka, F.; Černíková, M.; Míšková, Z.; Pachlová, V.; **Salek, R. N. (5)**; Buňková, L.; Pleva, P. Spolupráce při inovaci technologických procesů výroby mléčných výrobků. LACRUM Velké Meziříčí, s.r.o., 2019. 1.
27. Lorencová, E.; **Salek, R. N. (30)***; Černíková, M.; Buňková, L.; Hýlková, A.; Buňka, F. Biogenic amines occurrence in beers produced in Czech microbreweries. *Food Control*, 2020, *117*. DOI: 10.1016/j.foodcont.2020.107335.
28. **Salek, R. N. (50)***; Lorencová, E.; Míšková, Z.; Lazárková, Z.; Pachlová, V.; Adámek, R.; Bezděková, K.; Buňka, F. The impact of Chios mastic gum on textural, rheological and melting properties of spread-type processed cheese during storage. *International Dairy Journal*, 2020, *109*. DOI: 10.1016/j.idairyj.2020.104755.
29. **Salek, R. N.(40)**; Černíková, M.; Lorencová, E.; Pachlová, V.; Kůrová, V.; Šenkýřová, J.; Buňka, F. The impact of Cheddar or white brined cheese with various maturity degrees on the processed cheese consistency: A comparative study. *International Dairy Journal*, 2020, *111*. DOI: 10.1016/j.idairyj.2020.104816.
30. Pluta-Kubica, A.; Černíková, M.; Dimitreli, G.; Nebesářová, J.; Exarhopoulos, S.; Thomareis, A. S.; **Salek, R. N.(20)**; Buňka, F. Influence of the melt holding time on fat droplet size and the viscoelastic properties of model spreadable processed cheeses with different compositions. *International Dairy Journal*, 2021. DOI: 10.1016/j.idairyj.2020.104880.
31. Polášek, Z., **Salek, R. N.(25)**, Vašina, M., Lyčková, A., Gál, R., Pachlová, V., & Buňka, F. (2021). The effect of furcellaran or κ-carrageenan addition on the textural, rheological and mechanical vibration damping properties of restructured chicken breast ham. *LWT- Food Science and Technology*, 2021, *138*. DOI: 10.1016/j.lwt.2020.110623.
32. Lorencová, E., **Salek, R. N (30)**., Buňková, L., Szczybrochová, M., Černíková, M., & Buňka, F. (2021). Assessment of biogenic amines profile in ciders from the Central Europe region as affected by storage time. *Food Bioscience*, 41. DOI: 10.1016/j.fbio.2021.100957

* Corresponding author

[a] The list was generated on April 2021

SELECTED PUBLICATIONS WHICH ARE PART AND PARCEL OF THE HABILITATION THESIS

Research paper 1

A1

Nagyová, G., Buňka, F., Salek, R. N., Černíková, M., Mančík, P., Grůber, T., Kuchař, D.

Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese.

Journal of Dairy Science. 2014, 97, 111-122. ISSN: 00220302.

J. Dairy Sci. 97:111–122
http://dx.doi.org/10.3168/jds.2013-7210

Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese

G. Nagyová,* F. Buňka,*[1] R. N. Salek,* M. Černíková,* P. Mančík,* T. Grůber,† and D. Kuchař†
*Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlin, T. G. Masaryka 5555, 760 01, Zlin, Czech Republic
†Department of Research and Development, Fosfa PLC, Hraniční 268, 691 41 Břeclav-Poštorná, Czech Republic

ABSTRACT

The objective of this study was to describe the dependence of textural properties (hardness, cohesiveness, and relative adhesiveness) of processed cheese spreads on the proportion of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), and sodium salts of polyphosphate in ternary mixtures of emulsifying salts. Sodium salts of polyphosphate with different mean lengths (n ≈ 5, 9, 13, 20, and 28) were used. Pentasodium triphosphate (PSTP) was used instead of TSPP in the second part of the study. Products with and without pH adjustment were tested (the target pH value was 5.60–5.80). Textural properties of the processed cheese were observed after 2, 9, and 30 d of storage at 6°C. Hardness of the processed cheese with a low content of polyphosphate increased at a specific DSP:TSPP ratio (~1:1 to 3:4). This trend was the same for all the polyphosphates used; only the absolute values of texture parameters were different. The same trends were observed in the ternary mixtures with PSTP, showing lower final values of hardness compared with samples containing TSPP. Hardness and cohesiveness decreased and relative adhesiveness increased in the samples with increased pH values and vice versa; the main trend remained unchanged.
Key words: processed cheese, emulsifying salt, polyphosphate, textural property

INTRODUCTION

Processed cheese can be characterized as a viscoelastic matrix, the basic material of which consists of cheeses at different stages of maturity. It is made by using a wide range of dairy (e.g., cream, butter, anhydrous milk fat, curd, milk powder, whey powder, caseinates) and nondairy ingredients and additives (e.g., hydrocolloids, coloring, sensory active mixtures), which are applied to modify the content (e.g., DM content, fat content, protein content) or functional properties of the product (e.g., firmness, meltability). Key components for the production of processed cheeses are emulsifying salts (**ES**), usually sodium salts of phosphates, polyphosphates, or citrates. The discontinuous production of processed cheeses includes (1) determining the composition of ingredients (with respect to the desired parameters of the final product); (2) placing the determined amounts of ingredients and additives into the melting device and the actual melting process (at a usual temperature of 85 to 105°C with a dwell time of several minutes); and (3) packaging in different wrapping materials (Guinee et al., 2004; Mizuno and Lucey, 2007).

The essential role of ES is the exchange of sodium ions for calcium ions in the casein matrix (gel) of the cheese; insoluble calcium paracaseinate changes into more soluble sodium paracaseinate, whose molecules (chains) can move within the melt system and thus enhance fat emulsification and water binding (Guinee et al., 2004; Shirashoji et al., 2006; Muslow et al., 2007). The ability of individual ES to support the exchange of sodium for calcium ions can vary. Generally, the ability to support ion exchange occurs in the following order (considering sodium salts): citrates ≈ monophosphates < diphosphates < triphosphates < short polyphosphates (<10 phosphorus atoms in a molecule) < long polyphosphates (>10 phosphorus atoms in a molecule) (Guinee et al., 2004; Mizuno and Lucey, 2005a, 2007). El-Bakry et al. (2011) stated that citrates support ion exchange to a greater extent than monophosphates.

However, ES also affect the process of gel formation in the cooling matrix of the melt and thus enhance the formation of the final structure of the processed cheese. The process of forming the final matrix during cooling and subsequent storing is called creaming and it covers a wide range of different interactions: calcium bridges, disulfide bridges, hydrophobic interactions, electrostatic interactions, hydrogen bonds, calcium-phosphates complexes (bridges), and so on (Horne, 1998; Mizuno and Lucey, 2005a, 2007). Individual ES are able to influence gel formation in different ways. Diphosphates and triphosphates are considered to be substances directly supporting gel formation, and this is especially true when they are at an optimal concentration with respect to the other components in the mixture (Mizuno and

Received July 1, 2013.
Accepted September 22, 2013.
[1]Corresponding author: bunka@ft.utb.cz

Lucey, 2007; Buňka et al., 2013). According to Kaliappan and Lucey (2011) and Weiserová et al. (2011), specific interactions exist between monophosphates and diphosphates (at a ratio of approximately 1:1 to 3:4) that strongly support gel formation. Weiserová et al. (2011) emphasized that specific interactions also occur between monophosphates and triphosphates that influence the properties of processed cheeses. On the other hand, polyphosphates are thought to inhibit gel formation. Within the conditions of processed cheeses, polyphosphates bind to casein fractions and give them a strong multiple negative charge. More intensively charged casein fractions repel each other, which inhibits the formation of some of the above-mentioned bonds (mainly hydrophobic interactions; Mizuno and Lucey, 2007; Shirashoji et al., 2010; Buňka et al., 2013).

Processes such as the exchange of sodium ions for calcium ions and formation of the final matrix (creaming), and thus the roles of the individual phosphates in these processes, are closely related. According to Mizuno and Lucey (2005a,b) and Shirashoji et al. (2010), higher ion-exchange ability is linked to better casein dispersion in the melt. According to these authors, greater casein dispersion also leads to a more intensive formation of mutual bonds during the creaming process. On the other hand, longer polyphosphates make the negative charge of caseins more intensive and thus weaken the gel (Shirashoji et al., 2010; Buňka et al., 2013). A balance exists between these 2 processes in the melt that seem to be contrary to each other, which is reflected in the final quality of the gel. However, the above-mentioned processes are much more complex because ternary and quaternary mixtures of ES are often used in practice and therefore the mutual interactions between ES must also be taken into consideration (Awad et al., 2002; Weiserová et al., 2011; Buňka et al., 2012).

Over the past few years, several studies (e.g., Awad et al., 2002; Weiserová et al., 2011; Buňka et al., 2012, 2013) have shown the dependence of texture parameters of processed cheeses on the composition of binary and ternary mixtures of phosphate ES (consisting mainly of disodium phosphate, tetrasodium diphosphate, and sodium salt of polyphosphate). In these studies, a specific ratio of disodium phosphate to tetrasodium diphosphate was determined (approximately 1:1 to 3:4), at which hardness of the processed cheeses increased rapidly but cohesiveness and adhesiveness decreased. The influence of this specific ratio decreased with an increasing relative amount of sodium salt of polyphosphate. When the amount of sodium salt of polyphosphate exceeded 60%, the influence of this specific ratio became insignificant. The phenomena were not affected by the maturity stage of the raw material (Dutch-type cheese; maturity stage within the range of 2 to 8 wk) or the concentration of the ES (2–3% wt/wt; Kapoor et al., 2007; Weiserová et al., 2011; Buňka et al., 2012, 2013).

However, existing studies are limited to linear-chain polyphosphates with mean length (n; the number of phosphorus atoms bound in a linear molecule of polyphosphate) of about 20 (Sádlíková et al., 2010; Weiserová et al., 2011; Buňka et al., 2012, 2013). On the other hand, sodium salts of polyphosphate with different mean lengths of chain are often used in practice. Chains of different length could affect the intensity of the exchange of sodium ions for calcium ions and thus casein dispersion (Mizuno and Lucey, 2007; Lu et al., 2008; Sádlíková et al., 2010). On the basis of the available literature, this hypothesis has not yet been proved experimentally. The role of polyphosphates with different chain lengths in mixtures with triphosphates, diphosphates, and monophosphates during the process of casein dispersion has not been described either. A different number of phosphorus atoms in a linear chain could also affect the creaming process; for example, by means of interactions with casein fractions of varying intensity. Moreover, the use of polyphosphates with different chain lengths can also affect the pH of the product (Lu et al., 2008). Finally, no studies have dealt with the influence of different compositions of ternary mixtures of ES containing triphosphate on the texture parameters of processed cheeses.

The first aim of this study was to compare selected texture parameters (hardness, cohesiveness, and relative adhesiveness) of model processed cheeses made with ternary mixtures of phosphate ES with the addition of sodium salts of polyphosphate with different mean lengths. The second aim was to observe the influence of the replacement of pentasodium triphosphate with tetrasodium diphosphate in ternary mixtures of ES on the textural properties of model processed cheeses. The above-mentioned parameters were observed (1) with nonadjusted pH of the processed cheeses arising from the interactions of the ES mixtures, and (2) with adjusted pH values of the samples (target pH values in the range from 5.60 to 5.80), which correspond to standard pH values of processed cheese spreads. The third aim was to study the link between the development of selected texture parameters of model processed cheeses related to different composition of ternary mixtures of phosphate ES and the effect of these mixtures on dispersion of casein micelles in the model milk system.

MATERIALS AND METHODS

Processed Cheese Manufacturing

Composition of the ingredients (Dutch-type cheese blocks, ~50% wt/wt DM content; ~30% wt/wt fat in

DM content, 7-wk maturity; butter ~82% wt/wt DM content; ~80% wt/wt fat content; and water) for the production of model processed cheese spreads was designed to reach the target values of DM content and fat in DM content of processed cheeses of 40 and 50% (wt/wt), respectively. First, ternary mixtures of disodium phosphate (**DSP**), tetrasodium diphosphate (**TSPP**), and 5 sodium salts of polyphosphate (**Pxx**) with different mean length (n ≈ 5, 9, 13, 20, and 28; designated **P05**, **P09**, **P13**, **P20**, and **P28**, respectively) were used as ES, which resulted in 5 ternary mixtures of DSP:TSPP:Pxx (for P05, P09, P13, P20, and P28). Another 5 ternary mixtures (DSP:PSTP:Pxx) were formed by using pentasodium triphosphate (**PSTP**) instead of TSPP in the mixtures. All 10 ternary mixtures of sodium salts of phosphates (DSP:TSPP/PSTP:Pxx) were applied in percentage proportions with increments of 20% together with some specific ratios with 50% of some salts (100:0:0; 80:20:0; 60:40:0; 50:50:0; 40:60:0; 20:80:0; 0:100:0; 80:0:20; 60:20:20; 40:40:20; 20:60:20; 0:80:20; 60:0:40; 40:20:40; 20:40:40; 0:60:40; 50:0:50; 30:20:50; 20:30:50; 0:50:50; 40:0:60; 20:20:60; 0:40:60; 20:0:80; 0:20:80; 0:0:100; 26 variants in total). Each combination was made 3 times for a total of 780 lots (26 variants × 10 types of ternary mixtures × 3 productions). The ES were supplied by Fosfa PLC (Břeclav-Poštorná, Czech Republic). All ternary mixtures were applied at the total concentration of 3% (wt/wt) of the ES (the amount calculated based on the total weight of the melt).

Subsequently, model samples with pH adjusted to reach the optimal range for pH of the processed cheese spreads were made; the target value was pH 5.60 to 5.80. The pH values were adjusted by adding NaOH or HCl (1 mol/L). The model samples were produced the same way as the products without any pH adjustment. The calculated amount of acid or alkali (the amount based on a calibration model made in the pilot study; unpublished data) was added to the manufacturing equipment at 85 to 86°C (approximately 30 to 50 s before reaching the melting temperature). The addition of water was decreased by the calculated amount of NaOH or HCl (to reach a constant DM content and fat in DM content). A total of 780 lots (26 variants × 10 types of ternary mixtures × 3 productions) were made in this way (with pH adjustment).

The model samples were produced using a Vorwerk Thermomix TM 31-1 blender cooker (Vorwerk & Co., GmbH, Wuppertal, Germany). The same equipment was also used for the production of processed cheeses in the work of Lee et al. (2004) and Macků et al. (2008). The melting temperature of 90°C was kept for 1 min (the total melting time of 10–12 min). The hot melt was poured into cylindrical polypropylene pots (52 mm in diameter; 50 mm high) and sealed with aluminum lids. Within 2 h after the production, the samples were cooled to 6 ± 2°C, and stored at that temperature until the analyses were started. The products were tested on d 2, 9, and 30 of storage (6 ± 2°C; d 1 was the day of the production).

Dry matter content (ISO, 2004) and pH (measured by direct insertion of a spear electrode into the model samples; pHSpear, Eutech Instruments, Oakton, Malaysia) were determined in the model samples. Each variant of the sample was measured 9 times.

Texture Analysis

Textural properties of the model samples were assessed by 2 sequential penetration events by means of TA.XT Plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK). A cylindrical probe 20 mm in diameter was used (strain of deformation 25%, probe speed 2 mm/s). According to the force–deformation curve describing the dependence of the force needed (N) on time (s), the following texture parameters were determined: hardness, cohesiveness, and relative adhesiveness (Cunha and Viotto, 2010; Weiserová et al., 2011; Bayarri et al., 2012; Cunha et al., 2013). Each parameter was measured 9 times.

Preparation of the Model Milk Samples and Measurement of Turbidity

Evaluation of the intensity of casein dispersion is based on the method of measuring optical density ("turbidity") developed by Kaliappan and Lucey (2011). It is assumed that the lower the optical density, the greater the casein dispersion that has been achieved. Skim milk powder (Moravia Lacto PLC, Jihlava, Czech Republic) stirred in deionized water; an amount corresponding to 5% (wt/vol) was used as the model milk system. After careful stirring, dissolving (~1 h of stirring at a laboratory temperature 22 ± 1°C), and addition of sodium azide (0.2% wt/vol), the pH of the system was adjusted to reach 5.80 ± 0.01 (HCl at 1 and 0.1 mol/L was added for fine adjustment of pH) and kept overnight (~18 h) at 22 ± 1°C to stabilize the environment. Subsequently, 10 ternary mixtures of phosphate ES (DSP:TSPP/PSTP:Pxx) were applied in percentage proportions with increments of 20% and some specific ratios with 50% of some salts (the same ratios as during the production of processed cheeses; 26 variants in total). Each combination was produced 3 times for 780 model milk systems in total (26 variants × 10 types of ternary mixtures × 3 productions). The ternary mixtures of ES were added at a total concentration of 0.3% (wt/vol). After the addition of ES, the mixtures were

stirred for 10 min and the pH was adjusted to reach the value of 5.80 ± 0.01 (NaOH or HCl at 1 and 0.1 mol/L were used for fine adjustments). The mixture was stirred at room temperature (22 ± 1°C) for another 50 min. Subsequently, the optical density was measured at λ = 700 nm (UV-VIS Spectrophotometer, UV Mini 1240, Shimadzu, Duisburg, Germany). The results were expressed with respect to the optical density of the milk system without the addition of ES, the pH of which was adjusted to reach 5.80 ± 0.01 (100%; after ~18 h). Each variant (including the control sample) was measured 9 times.

For comparison of pH of individual phosphate salts used, model samples consisting of deionized water or dairy model system (skim milk powder in deionized water 5% wt/vol) with tested individual phosphate salts (1% wt/vol) were also produced.

Statistical Analysis

The results of chemical and texture analysis and optical density measurements were subjected to nonparametrical ANOVA by Kruskal-Wallis and Wilcoxon tests (Unistat 5.5 software; Unistat, London, UK). The significance level used in the tests was 0.05.

RESULTS

Basic Chemical Analysis

Dry matter content, which was determined in all variants of the model samples tested, ranged from 40.56 to 41.22% (wt/wt). This range is acceptable in showing consistency of DM content in the individual variants of processed cheeses. Dry matter content is an important parameter that influences the textural properties of processed cheeses, and maintaining a consistent DM content is important for ensuring the comparability of the individual samples (Lee et al., 2004).

The pH value of processed cheeses is controlled by the ingredients used for their production and by the ES, the composition of which plays a key role in affecting this parameter. Table 1 shows pH values of the model solutions of the individual ES in water and in milk systems (5.0% wt/vol of skim milk powder in deionized water). The pH of the model solutions decreased significantly ($P < 0.05$) with an increasing number of phosphorus atoms in the phosphate molecule. The range of pH values in the samples with deionized water (4.21–10.09) was wider compared with that in the milk system (6.22–8.65; comparing the concentration of 1.0% wt/vol). This difference could be explained by the buffer capacity of the milk system (Mizuno and Lucey, 2005a; Kaliappan and Lucey, 2011).

The pH values of the processed cheeses with the addition of the individual phosphate ES are shown in Table 1 (d 2 after production, stored at 6°C). The highest pH values were reached when using DSP, TSPP, or PSTP separately ($P < 0.05$) compared with the processed cheeses with the addition of other ES. A slightly lower pH value compared with the previous products (5.95 ± 0.02; $P < 0.05$) was observed in the samples made with P05. The pH values of the samples with addition of P09, P13, P20, or P28 ES did not show a significant difference ($P \geq 0.05$) and ranged from 5.34 to 5.45. The development of pH values of the processed cheeses with the ES applied separately was in accord with the trend of changes in pH of the model solutions (Table 1).

Figure 1 illustrates the dependence of pH of the model samples of processed cheeses (after 2 d of storage at 6°C) without any pH adjustment on the composition of ternary mixtures of ES. It shows the pH values of products made using ternary mixtures containing DSP:TSPP:P05 or DSP:TSPP:P28. When using only DSP with TSPP (without polyphosphates), the pH of the processed cheeses was high (pH >6.40; $P < 0.05$). With an increasing proportion of polyphosphates,

Table 1. The pH values of 1.0% (wt/vol) of the individual emulsifying salts (ES) in deionized water, of the model milk system (5.0% wt/vol of skim milk powder in deionized water) with 0.3% (wt/vol) or 1.0% (wt/vol) of the individual ES, and of the model processed cheese with 3.0% (wt/wt) of the individual ES (mean ± SD; n = 9)

Emulsifying salt	Code	1.0% ES in deionized water	0.3% ES in model milk system	1.0% ES in model milk system	Processed cheese with 3.0% ES[1]
Disodium phosphate	DSP	9.43 ± 0.01	6.56 ± 0.01	7.20 ± 0.01	6.45 ± 0.02
Tetrasodium diphosphate	TSPP	10.06 ± 0.02	6.68 ± 0.01	8.62 ± 0.01	6.63 ± 0.03
Pentasodium triphosphate	PSTP	9.93 ± 0.02	6.49 ± 0.03	7.89 ± 0.02	6.41 ± 0.03
Sodium salt of polyphosphate[2]					
(n ≈ 5)	P5	8.46 ± 0.02	6.30 ± 0.01	6.98 ± 0.01	5.95 ± 0.02
(n ≈ 9)	P9	7.14 ± 0.01	6.17 ± 0.01	6.46 ± 0.01	5.44 ± 0.02
(n ≈ 13)	P13	6.82 ± 0.01	6.15 ± 0.01	6.45 ± 0.01	5.38 ± 0.03
(n ≈ 20)	P20	5.93 ± 0.01	6.13 ± 0.02	6.26 ± 0.01	5.36 ± 0.01
(n ≈ 28)	P28	4.27 ± 0.02	6.14 ± 0.01	6.25 ± 0.01	5.35 ± 0.02

[1]pH values after 2 d of storage at 6°C.

[2]Where n indicates the length of the polyphosphate.

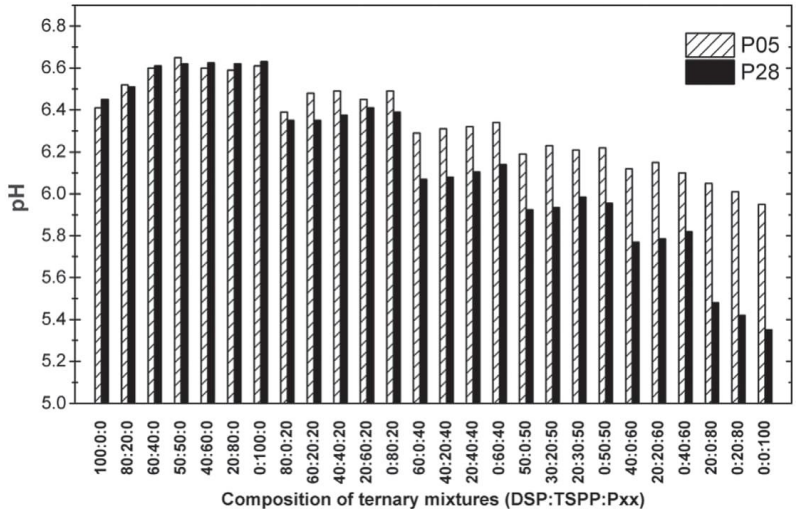

Figure 1. The dependence of pH values of the model samples (stored for 2 d at 6°C) on the relative amount (x-axis; %) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), and sodium salts of polyphosphate (P05, sodium salts of polyphosphate with medium length, n ≈ 5; P28 = sodium salts of polyphosphate with medium length, n ≈ 28) in a ternary mixture of emulsifying salts. The results are expressed as means (n = 9).

the pH decreased gradually ($P < 0.05$). The slowest decrease in pH of the processed cheeses (with an increasing proportion of polyphosphate) was observed in samples with the ternary mixtures containing P05. On the other hand, pH values of the samples containing P28 decreased fastest ($P < 0.05$; Figure 1). The development of pH values of processed cheeses with other ternary mixtures with TSPP (DSP:TSPP:P09, DSP:TSPP:P13, and DSP:TSPP:P20; data not shown) was analogous and values ranged within the interval defined by pH values of DSP:TSPP:P05 and DSP:TSPP:P28. The replacement of TSPP with PSTP did not lead to any significant differences in pH values of the model samples ($P \geq 0.05$; data not shown) when comparing corresponding samples (e.g., processed cheeses made using ternary mixtures of DSP:TSPP:P09 and DSP:PSTP:P09 at a ratio of 40:20:40 stored for 9 d at 6°C).

The real pH values in the model processed cheeses with pH adjustment (to reach the intended target pH of 5.60–5.80) ranged from 5.65 to 5.81 (data not shown). With respect to production from cheese and butter, the interval achieved can be considered acceptable. During a 30-d storage period at 6°C, a slight decrease in pH of the model samples occurred. In most samples, the decrease was between 0.1 and 0.2 pH units ($P < 0.05$).

Effect of the Composition of Phosphate Ternary Mixtures on Texture Parameters

Figure 2A–C shows the values of hardness of the model samples for the ternary mixtures of ES of DSP:TSPP:P05 after 2 (panel A), 9 (panel B), and 30 (panel C) d of storage at 6°C. A content of P05 ≤60% in the ternary mixtures of ES led to a rapid increase in hardness of the products at a specific ratio of DSP to TSPP of approximately 1:1 to 3:4 (Figure 2). Any deviation of the ratio of DSP to TSPP (with a constant content of P05) resulted in a rapid decrease in hardness. The influence of the specific ratio of DSP to TSPP decreased with an increasing proportion of P05 in the ternary mixtures ($P < 0.05$). With an increasing proportion of P05 >60%, the ratio of DSP to TSPP did not have a significant influence on hardness of the samples ($P \geq 0.05$; comparing the model processed cheeses with a constant storage period). This general development concerning the dependence of hardness of the model processed cheeses on the composition of ternary mixtures of ES was observed regardless of the storage period of the model samples (Figure 2). With an extending storage period, an increase in the absolute values of hardness of the processed cheeses was observed ($P < 0.05$; comparing the samples made using a constant ratio of DSP:TSPP:P05).

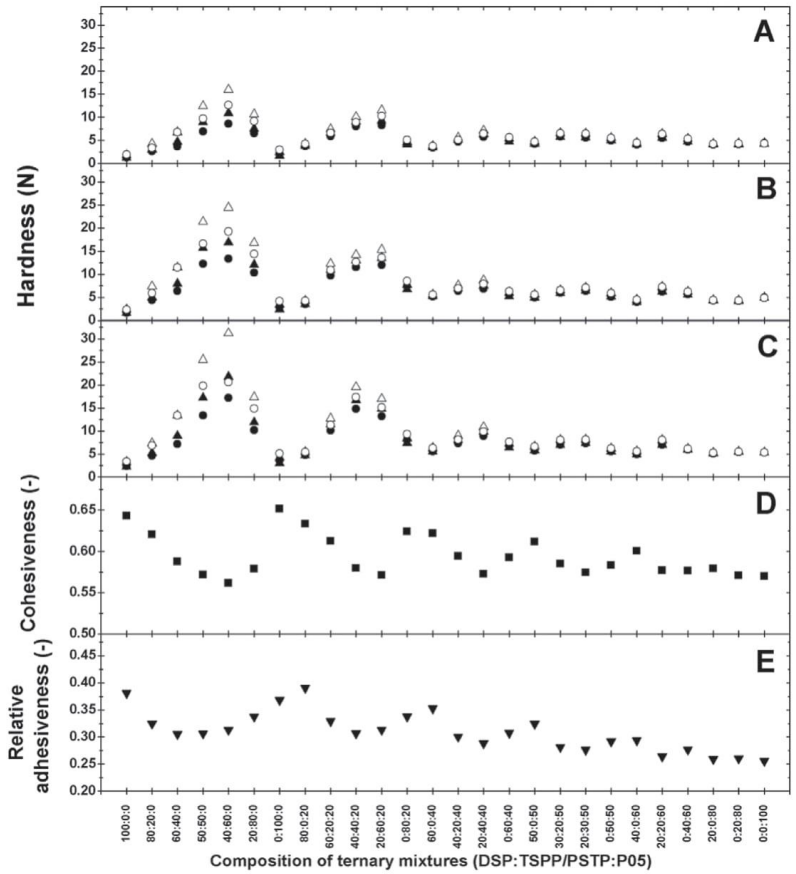

Figure 2. The dependence of processed cheese hardness (panels A to C), cohesiveness (unitless; panel D) and relative adhesiveness (unitless; panel E) on the relative amount (x-axis; %) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), or pentasodium triphosphate (PSTP), and sodium salt of polyphosphate (P05, sodium salt of polyphosphate with medium length, n ≈ 5) in a ternary mixture of emulsifying salts during 30 d of storage at 6°C (A: d 2; B: d 9; C: d 30). The solid symbols (A to C) represent the samples without adjustment of pH values, and the open symbols represent the samples whose pH values were adjusted to the optimal range of 5.65 to 5.81. The results of hardness in processed cheese containing TSPP and PSTP are depicted using triangles (▲, △) and circles (●, ○), respectively, in panels A to C. The results of cohesiveness (D; ■) and relative adhesiveness (E; ▼) are for the ternary mixtures containing TSPP on d 2 of storage.

The results of the dependence of cohesiveness and relative adhesiveness of the processed cheeses on the composition of ternary mixtures of DSP:TSPP:P05 for d 2 of storage at 6°C are illustrated in Figure 2D and E. With a constant content of P05 ≤60%, a rapid decrease in cohesiveness of the processed cheeses was observed at a ratio of DSP to TSPP of 1:1 to 1:2. Under the same conditions (a constant content of P05 ≤60%), a rapid decrease in relative adhesiveness of the samples was observed at a ratio of DSP to TSPP from 1:1 to 3:4. Outside this specific interval for the ratio of DSP to TSPP, both cohesiveness and relative adhesiveness of the samples increased significantly (compared with the samples with a constant content of P05). With an increasing proportion of P05 in the ternary mixtures of ES (up to 60%; Figure 2D and E), the influence of the specific ratio of DSP to TSPP on cohesiveness and relative adhesiveness of the samples decreased. With

a proportion of P05 >60%, only a gradual decrease in cohesiveness and relative adhesiveness was observed, regardless of the ratio of the other 2 ES (Figure 2D and E). This general trend corresponded to the description of dependence of cohesiveness and relative adhesiveness of the samples on the proportion of DSP, TSPP, and P05 in the ternary mixtures of ES, regardless of the storage period. After a 30-d storage period, a slight increase in the values of cohesiveness and a decrease in the values of relative adhesiveness occurred (comparing model processed cheeses made with a constant ratio of DSP:TSPP:P05 in the ternary mixture of ES; data not shown). However, most of these changes (depending on the storage period) were not significant ($P \geq 0.05$).

The above-mentioned dependence of the texture parameters of processed cheeses on the composition of ternary mixtures was presented on one type of ternary mixture (DSP:TSPP:P05) in the samples without pH adjustment. Different numbers of phosphorus atoms linearly bound in the polyphosphates added to the ternary mixtures with DSP and TSPP did not result in a significant change in the above-mentioned specific ratios (Figure 3). During application of all the polyphosphates tested (P05, P09, P13, P20, and P28), the interval for the value of the specific ratio of DSP:TSPP (which rapidly increased hardness and significantly decreased cohesiveness and relative adhesiveness of the processed cheeses) remained unchanged ($P \geq 0.05$). However, the absolute values of textural parameters were affected, especially those showing hardness of the samples (Figure 3). In the case of P05 and P09, the absolute values of hardness of the model cheeses were slightly higher with the application of ternary mixtures with 20 and 40% polyphosphate content compared with the products with the application of P20 and P28 polyphosphates ($P < 0.05$; Figure 3). The same trend for an increase in hardness of the model samples was observed in the ternary mixtures with 50% P05 and P09 (compared with the application of P20 and P28 polyphosphates) but these changes were not significant ($P \geq 0.05$; Figure 3).

With the application of polyphosphate reaching ≥60%, hardness of the processed cheeses decreased slowly as the number of phosphate units decreased (Figure 3). Significant differences were observed mainly between the samples with P05 and P09 compared with P20 and P28 ($P < 0.05$). Significant differences in hardness between the samples with P20 and P28 polyphosphates were not observed ($P \geq 0.05$); the same was true for samples containing P05 and P09 ($P \geq 0.05$). The absolute values of hardness of the processes cheeses with the application of P13 were close to that of samples containing P20 or P28 (Figure 3).

The application of PSTP in the ternary mixtures did not result in any changes in the general trends of dependence of the processed cheeses hardness on the composition of ternary mixtures of phosphates (compared with the application of TSPP; Figures 2 and 3). The replacement of TSPP with PSTP affected only the absolute values of hardness in some model samples. Significantly lower ($P < 0.05$; Figures 2 and 3) absolute values of hardness of the processed cheeses (with the exception of DSP and PSTP used on their own) were observed with a zero content of polyphosphate compared with the products containing TSPP. A similar conclusion concerning lower hardness of the model samples was reached for 20% and 40% polyphosphate content in the ternary mixtures ($P < 0.05$; compared with the same ternary mixtures containing TSPP; Figures 2 and 3). Changes in hardness of the processed cheeses containing ≥50% polyphosphate were not statistically significant ($P \geq 0.05$), regardless of the storage period. A longer storage period led to an increase in the values of hardness of the processed cheeses ($P < 0.05$) regardless of the ternary mixture used.

The values of cohesiveness of model samples ranged from 0.49 to 0.69. The values of relative adhesiveness of the individual samples of processed cheeses ranged from 0.20 to 0.41. Extending the length of the polyphosphate chain resulted in no clear trend in the changes of cohesiveness or relative adhesiveness of the model processed cheeses.

Adjusting the pH of the processed cheeses (to reach the optimal range of 5.65–5.81) significantly influenced ($P < 0.05$) the absolute values of hardness (Figures 2 and 3), cohesiveness, and relative adhesiveness (data not shown) of the samples (compared with the products without any pH adjustment). In products where pH was increased (by means of NaOH), the hardness and cohesiveness decreased, whereas relative adhesiveness increased slightly. When the pH of the processed cheeses was decreased (by means of HCl), hardness and cohesiveness of the samples increased and relative adhesiveness decreased. The greater the shift in pH, the more significant changes were observed (Figures 2 and 3). Adjusting the pH did not have a significant effect on the general trends of changes resulting from the application of sodium salts of polyphosphates with different lengths of chain (P5, P9, P13, P20, and P28) or from the replacement of TSPP with PSTP (Figures 2 and 3).

Results of the Optical Density Measurement of Milk Systems

The intensity of casein dispersion in 5% (wt/vol) milk system was evaluated by measuring the optical density

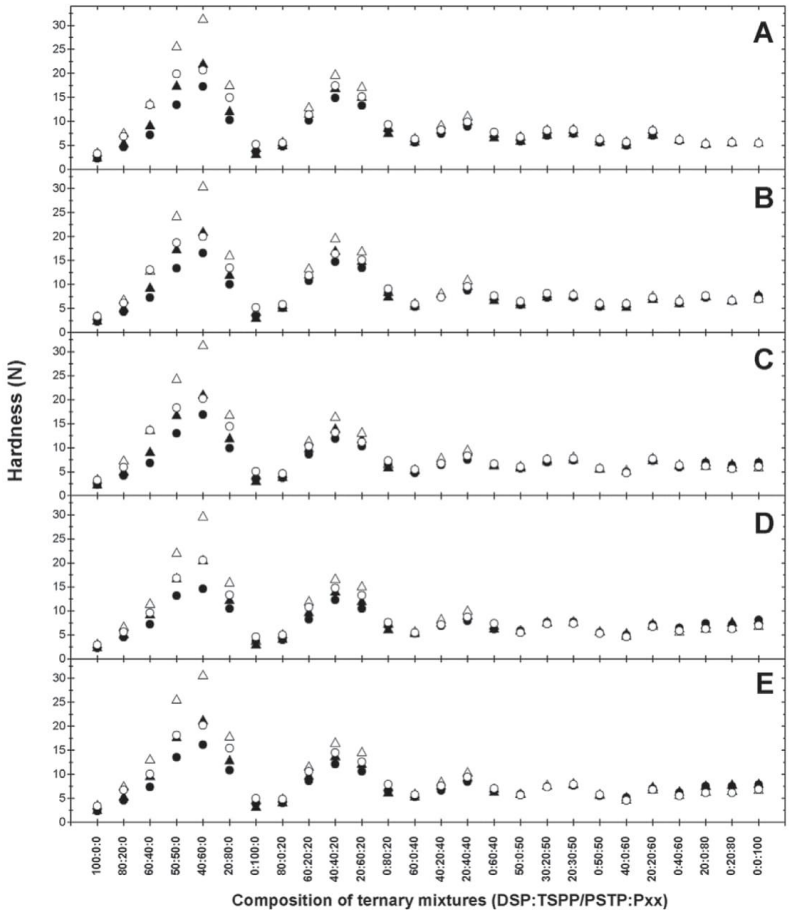

Figure 3. The dependence of processed cheese hardness on the relative amount (x-axis; %) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), or pentasodium triphosphate (PSTP) and sodium salt of polyphosphate (with different chain lengths): P05 (n ≈ 5, panel A), P09 (n ≈ 9, panel B), P13 (n ≈ 13, panel C), P20 (n ≈ 20, panel D), and P28 (n ≈ 28, panel E) in a ternary mixture of emulsifying salts after 30 d of storage at 6°C. The solid symbols (A to C) represent the samples without adjustment of pH values, and the open symbols represent the samples whose pH values were adjusted to the optimal range of 5.65 to 5.81. The results of processed cheese hardness containing TSPP and PSTP are depicted using triangles (▲, △) and circles (●, ○), respectively, in panels A to C.

at $\lambda = 700$ nm. The principle of this method is based on the assumption that the lower the value of optical density in the milk system, the more the casein is dispersed (Kaliappan and Lucey, 2011). The results of the optical density measurement of milk system containing the ternary mixtures of DSP, TSPP, and Pxx are shown in Figure 4 (the results were expressed as percentage with respect to the control sample, milk system without any phosphate addition). The addition of ternary mixtures of phosphates was set at 0.3% (wt/vol), which corresponds to the ratio of protein:phosphate in processed cheeses (Guinee et al., 2004).

When applying the phosphates individually, the intensity of casein dispersion increased with an increasing number of phosphorus atoms linearly bound in the phosphate molecule ($P < 0.05$; Figure 4; for PSTP used

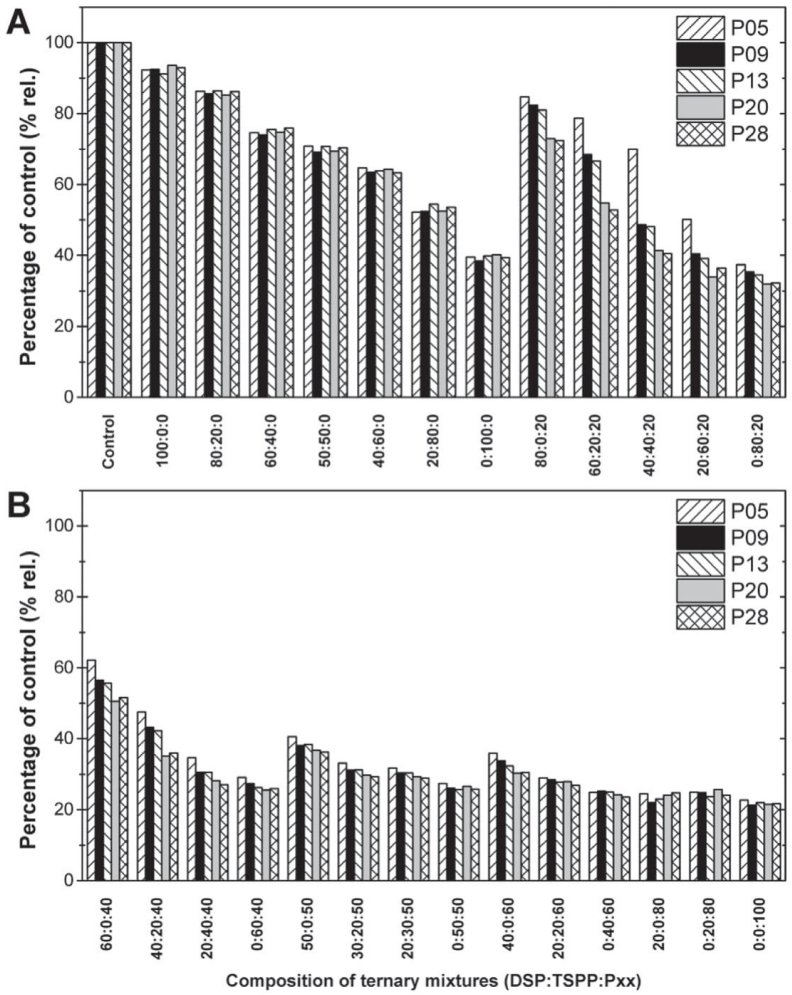

Figure 4. The dependence of percentage of absorbance of the model milk systems (% rel.; in relation to the model milk systems without phosphate addition = 100%) on the relative amount (x-axis; %) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), and sodium salt of polyphosphate (with different chain lengths): P05 (n ≈ 5), P09 (n ≈ 9), P13 (n ≈ 13), P20 (n ≈ 20), and P28 (n ≈ 28) in a ternary mixture of emulsifying salts. The results are expressed as means (n = 9).

individually, the values ranged from 31 to 33% with respect to the value of optical density in the control sample), which corresponds to several published studies (Mizuno and Lucey, 2005a; Kaliappan and Lucey, 2011). In the ternary mixtures of DSP, TSPP, and Pxx, the intensity of casein dispersion increased with an increasing proportion of longer-chain phosphates (Figure 4). Significant differences between casein dispersion were observed mainly in the milk systems containing 20 and 40% of the individual Pxx ($P < 0.05$; Figure 4). No significant differences ($P \geq 0.05$) between the milk systems containing different Pxx were observed

with 80 and 100% Pxx content. In the ternary mixtures containing DSP, PSTP, and Pxx, the general trends determined were similar to those of the ternary mixtures with TSPP (data not shown).

DISCUSSION

During the production of processed cheeses, phosphate ES play an important role in dispersion of the caseins present and subsequent formation of the final matrix of the processed cheeses; that is, the creaming process. Emulsifying salts also play a part in pH adjustment and stabilization (Marchesseau et al., 1997; Kawasaki, 2008). On the basis of our results, the pH of the processed cheeses decreases when the length of the linear phosphate chain is extended. This might be explained by the release of hydrogen cations (which are more numerous in long phosphates compared with shorter phosphates) into the melt, which decreases the pH. Therefore, the application of longer phosphates requires an adjustment of the pH of the processed cheeses by means of other ES to reach the optimal range for the given type of product.

The intensity of casein dispersion in the melt depends mainly on the type and concentration of the ES (Carić and Kaláb, 1997; Brickley et al., 2008; Dimitreli and Thomareis, 2009). From our results concerning the optical density measurement of milk systems with ternary mixtures of phosphates, more intensive casein dispersion is reached (1) with the application of a greater amount of polyphosphates (in ternary mixture); and (2) when using polyphosphates with a longer chain. This could be explained by the fact that the longer chain length of sodium salts of phosphates affects the intensity of ion exchange (of sodium ions for calcium ions). The addition of ES leads to casein dispersion, the intensity of which increases with the intensity of ion exchange (Mizuno and Lucey, 2007; Dimitreli and Thomareis, 2009). According to Dimitreli and Thomareis (2009), Shirashoji et al. (2010), and Cunha et al. (2013), casein dispersion is closely related to matrix formation of the final processed cheeses. More intensive casein dispersion enables these proteins to develop their emulsifying and hydration abilities and to stabilize the fat and water present in the mixture. Increasing intensity of protein hydration and fat emulsification leads to a higher intensity of interactions in the melt and thus a higher intensity of casein crosslinking. A greater number of cross linkages that are in the matrix of the product will result in a harder processed cheese (Mizuno and Lucey, 2005a; Shirashoji et al., 2010; Bayarri et al., 2012).

The above-mentioned processes can explain the phenomenon that was also observed in our study: the application of long-chain phosphates used individually resulted in greater hardness of the processed cheese ($P < 0.05$; Figures 2 and 3). Hardness increased with the application of phosphate ES used individually in the following order: DSP < TSPP < PSTP < P05 < P09 ≈ P13 < P20 ≈ P28. Similar trends for DSP, TSPP, PSTP, and P20 were published by Weiserová et al. (2011) and Buňka et al. (2012, 2013).

However, casein dispersion processes alone do not sufficiently explain the specific ratio of DSP:TSPP or DSP:PSTP (~1:1–3:4) that led to a rapid increase in hardness of the processed cheeses observed in our study. The influence of this specific ratio on texture parameters of the processed cheeses declined as the amount of polyphosphates increased (regardless of their chain length). With the relative amount of polyphosphates reaching ≥60%, the influence of the specific ratio on hardness of the processed cheeses became insignificant, in agreement with published studies (Buňka et al., 2012, 2013). The justification for the existence of the specific ratios of DSP:TSPP or DSP:PSTP (~1:1–3:4) was sought mainly in the ability of diphosphates and triphosphates to enhance gel formation of milk proteins. In all probability, another important aspect is the ability of monophosphates to permeate into cross-linked caseins and strongly bind water (Mizuno and Lucey, 2007; Shirashoji et al., 2010; Buňka et al., 2012). Kaliappan and Lucey (2011) observed a strong ability of the mixture of mono- and diphosphates to enhance gel formation in model milk systems, which was explained by the fact that monophosphates enhance the formation of bridges between diphosphate complexes with calcium ions and caseins. Mizuno and Lucey (2007) state that an optimal concentration of diphosphates exists for effective gel formation. Excessive or insufficient concentrations of diphosphates can lead to the formation of very weak gels.

The link between decreasing hardness of the processed cheeses with the ratio of DSP:TSPP or DSP:PSTP from 1:1 to 3:4 and an increasing concentration of polyphosphates can be found mainly in the ability of polyphosphates to give caseins multiple negative charges, which probably leads to a lower intensity of hydrophobic interactions of the dispersed caseins and thus decreased hardness of the final matrix (Mizuno and Lucey, 2007; Shirashoji et al., 2010; Buňka et al., 2012, 2013). However, confirmation of the above-mentioned hypotheses requires several further studies, which should focus mainly on the process of forming a 3-dimensional matrix of the processed cheeses.

Our results revealed that the usage of polyphosphates with different mean length of the linear chain (5 to 28 monomers) affects only the absolute values of the texture parameters measured, not the general trends of dependence of the texture parameters on the composi-

tion of ternary mixtures of ES such as monophosphate:diphosphate:polyphosphate described in Buňka et al. (2012, 2013). The values of the specific ratios of DSP:TSPP or DSP:PSTP leading to a rapid increase in hardness and a decrease in cohesiveness and relative adhesiveness remained practically unchanged, regardless of the length of the polyphosphate used. However, the type of polyphosphate used affected the absolute values of hardness in the processed cheeses, especially with the relative content of the polyphosphate in ternary mixtures reaching 20 to 50%. A decrease in the number of monomers linearly bound in the polyphosphate resulted in an increase in the absolute values of hardness in the processed cheeses with the polyphosphate content of 20 to 50% within the ternary mixture. The explanation of this phenomenon is not obvious. It might be based on the ability of polyphosphates to give caseins multiple negative charges, which might increase with the length of the linear polyphosphate (Shirashoji et al., 2010; Weiserová et al., 2011; Buňka et al., 2012, 2013). Shorter polyphosphates (i.e., P05 and P09) probably cause a lower intensity of the negative charge on caseins, which can lead to a higher intensity of hydrophobic interactions of the dispersed caseins and thus lower hardness of the final matrix (Shirashoji et al., 2010; Buňka et al., 2013). With a higher relative content of polyphosphates (>50%) in the ternary mixtures, the influences of the individual polyphosphates on casein dispersion seem to be predominant, as described above.

Pentasodium triphosphate is thought to enhance gel formation of caseins to a lesser extent than TSPP (Weiserová et al., 2011; Buňka et al., 2013), which could explain the lower values of hardness in the processed cheeses produced by means of ternary mixtures of DSP:PSTP:Pxx with Pxx content of 0 to 50% compared with the products made by means of ternary mixtures of DSP:TSPP:Pxx.

The adjustment of pH values to reach the optimal range (5.65–5.81 in our experiment) did not lead to a change in the general trend concerning the dependence of texture parameters on the composition of ternary mixtures of ES such as DSP:TSPP:Pxx or DSP:PSTP:Pxx. Only the absolute values of texture parameters of the samples were affected. An increase in pH led to a decrease in hardness of the processed cheeses and vice versa. The explanation could lie in the changed intensity of the negative charge on caseins as a result of the fluctuation in pH values. Increasing pH leads to a higher intensity of the negative charge on the dispersed caseins, the repulsion of which is stronger and thus the intensity of hydrophobic interactions in the system decreases. These processes result in lower hardness of the final matrix (Marchesseau et al., 1997; Lee and Klostermeyer, 2001).

During a 30-d storage period of the processed cheeses (at 6°C), a slight increase in hardness of the processed cheeses was observed, regardless of the composition of the ternary mixtures of ES applied or pH adjustment of the melt during production. A more significant change in the texture parameters was observed during the first 9 d. The change in texture parameters between d 9 and 30 of storage was much less significant compared with the change between d 2 and 9 of storage, which corresponds to several published studies (Carić and Kaláb, 1997; Weiserová et al., 2011; Buňka et al., 2012, 2013). The change in texture parameters of processed cheeses during their storage period can be explained by several factors: (1) a slight decrease in pH values of the processed cheeses (also observed in our study); (2) hydrolysis of the ES used (with more than 2 phosphorus atoms in a molecule); (3) possible changes in the forms of binding of the salts present and thus a change in their dissociative characteristics; and (4) polymorphism of dairy fat and a gradual change of its crystal form (Carić and Kaláb, 1997; Muslow et al., 2007; Shirashoji et al., 2010).

CONCLUSIONS

The aim of the study was to observe texture parameters of model samples of processed cheese with the addition of ternary mixtures containing DSP, TSPP, and polyphosphate with different mean lengths (n ≈ 5, 9, 13, 20, and 28). In the second part, TSPP was replaced with PSTP. The samples were made without and with pH adjustment (to achieve pH values typical of processed cheese spreads). The influence of the ternary mixture composition on the dispersion of casein micelles was also observed in the model milk system. With a low content of polyphosphate, hardness of the processed cheese increased and cohesiveness and relative adhesiveness decreased at a ratio of DSP to TSPP around 1:1 to 3:4. An increasing amount of polyphosphate (in the ternary mixture) led to a decrease in hardness of the processed cheese at this specific ratio. With the relative amount of polyphosphates reaching ≥60%, the influence of this specific ratio became insignificant. This trend was observed in all ternary mixtures; the only differences were found in the absolute values of texture parameters of the processed cheeses. Replacing TSPP with PSTP did not affect the general trend either. However, the absolute values of hardness of model samples with the addition of PSTP were lower compared with the usage of TSPP. Duration of the storage period increased hardness of the processed cheeses. In the samples where pH was intentionally increased, hardness and cohesiveness decreased and relative adhesiveness increased slightly. The reverse

trend was observed in samples in which pH was decreased. The more significant the pH adjustment, the more noticeable the changes observed. However, pH adjustment did not affect the value of the specific ratio of DSP:TSPP and DSP:PSTP and its general influence on texture parameters of the processed cheeses. The influence of the specific ratio of DSP:TSPP and DSP:PSTP and the general trend concerning the dependence of composition of the ternary mixtures of phosphate ES on the texture parameters of processed cheeses cannot be attributed only to the effect of phosphates on the dispersion of casein structures in the melt. During the creaming process, other interactions occur that affect the final form of the casein matrix.

ACKNOWLEDGMENTS

This study was supported by a project of the internal grants of Tomas Bata University (Zlín, Czech Republic; no. IGA/FT/2012/026 and IGA/FT/2013/010) funded from the resources for specific university research.

REFERENCES

Awad, R. A., L. B. Abdel-Hamid, S. A. El-Shabrawy, and R. K. Singh. 2002. Texture and microstructure of block type processed cheese with formulated emulsifying salt mixtures. Lebenson. Wiss. Technol. 35:54–61.

Bayarri, S., I. Carconell, and E. Costell. 2012. Viscoelasticity and texture of spreadable cheeses with different fat contents at refrigeration and room temperatures. J. Dairy Sci. 95:6926–6936.

Brickley, C. A., S. Govindasamy-Lucey, J. J. Jaegii, M. E. Johnson, P. L. H. McSweeney, and J. A. Lucey. 2008. Influence of emulsifying salts on the textural properties of nonfat process cheese made from direct acid cheese bases. J. Dairy Sci. 91:39–48.

Buňka, F., L. Doudová, E. Weiserová, D. Kuchař, J. Michálek, Š. Slavíková, and S. Kráčmar. 2012. The effect of different ternary mixtures of sodium phosphates on hardness of processed cheese spreads. Int. J. Food Sci. Technol. 47:2063–2071.

Buňka, F., L. Doudová, E. Weiserová, D. Kuchař, P. Ponížil, D. Začalová, G. Nagyová, V. Pachlová, and J. Michálek. 2013. The effect of ternary emulsifying salt composition and cheese maturity on the textural properties of processed cheese. Int. Dairy J. 29:1–7.

Carić, M., and M. Kaláb. 1997. Processed cheese products. Pages 467–505 in Cheese: Chemistry, Physics and Microbiology. Vol. 2. Major Cheese Groups. 2nd ed. P. F. Fox, ed. Chapman & Hall, London, UK.

Cunha, C. R., R. Grimaldi, M. R. Alcântara, and W. H. Viotto. 2013. Effect of the type of fat on rheology, functional properties and sensory acceptance of spreadable cheese analogue. Int. J. Dairy Technol. 66:54–62.

Cunha, C. R., and W. H. Viotto. 2010. Casein peptization, functional properties, and sensory acceptance of processed cheese spreads made with different emulsifying salts. J. Food Sci. 75:C113–C120.

Dimitreli, G., and A. S. Thomareis. 2009. Instrumental textural and viscoelastic properties of processed cheese as affected by emulsifying salts and in relation to its apparent viscosity. Int. J. Food Prop. 12:261–275.

El-Bakry, M., E. Duggan, E. D. O'Riordan, and M. O'Sullivan. 2011. Effect of chelating salt type on casein hydration and fat emulsification during manufacture and post-manufacture functionality of imitation cheese. J. Food Eng. 102:145–153.

Guinee, T. P., M. Carić, and M. Kaláb. 2004. Pasteurized processed cheese and substitute/imitation cheese products. Pages 349–394 in Cheese: Chemistry, Physics and Microbiology 2. 3rd ed. P. F. Fox, P. L. H. McSweeney, T. P. Cogan, and T. Guinee, ed. Elsevier Applied Science, London, UK.

Horne, D. S. 1998. Casein interactions: Casting light on the black boxes, the structure in dairy products. Int. Dairy J. 7:171–177.

ISO (International Organization for Standardization). 2004. ISO Standard No. 5534: Cheese and processed cheese—Determination of the total solids content (reference method). ISO, Geneva, Switzerland.

Kaliappan, S., and J. A. Lucey. 2011. Influence of mixtures of calcium-chelating salts on the physicochemical properties of casein micelles. J. Dairy Sci. 94:4255–4263.

Kapoor, R., L. E. Metzger, A. C. Biswan, and K. Muthukummarappan. 2007. Effect of natural cheese characteristics on process cheese properties. J. Dairy Sci. 90:1625–1634.

Kawasaki, Y. 2008. Influence of "creaming" on the properties of processed cheese and changes in the structure of casein during cheese making. Milchwissenschaft 63:149–153.

Lee, S. K., S. Anema, and H. Klostermeyer. 2004. The influence of moisture content on the rheological properties of processed cheese spreads. Int. J. Food Sci. Technol. 39:763–771.

Lee, S. K., and H. Klostermeyer. 2001. The effect of pH on the rheological properties of reduced-fat model processed cheese spreads. Lebenson. Wiss. Technol. 34:288–292.

Lu, Y., N. Shirashoji, and J. A. Lucey. 2008. Effects of pH on the textural properties and meltability of pasteurized process cheese made with different types of emulsifying salts. J. Food Sci. 73:E363–E369.

Macků, I., F. Buňka, V. Pavlínek, P. Leciánová, and J. Hrabě. 2008. The effect of pectin concentration on viscoelastic and sensory properties of processed cheese. Int. J. Food Sci. Technol. 43:1663–1670.

Marchesseau, S., E. Gastaldi, A. Laguade, and J.-L. Cuq. 1997. Influence of pH on protein interactions and microstructure of process cheese. J. Dairy Sci. 80:1483–1489.

Mizuno, R., and J. A. Lucey. 2005a. Effects of emulsifying salts on the turbidity and calcium-phosphate–protein interactions in casein micelles. J. Dairy Sci. 88:3070–3078.

Mizuno, R., and J. A. Lucey. 2005b. Effects of two types of emulsifying salts on the functionality of nonfat pasta filata cheese. J. Dairy Sci. 88:3411–3425.

Mizuno, R., and J. A. Lucey. 2007. Properties of milk protein gels formed by phosphates. J. Dairy Sci. 90:4524–4531.

Muslow, B. B., D. Jaros, and H. Rohm. 2007. Processed cheese and cheese analogues. Pages 210–235 in Structure of Dairy Products. Blackwell Publishing Ltd., Ames, IA.

Sádlíková, I., F. Buňka, P. Budínský, B. Voldánová, V. Pavlínek, and I. Hoza. 2010. The effect of selected phosphate emulsifying salts on viscoelastic properties of processed cheese. Lebenson. Wiss. Technol. 43:1220–1225.

Shirashoji, N., J. J. Jaeggi, and J. A. Lucey. 2006. Effect of trisodium citrate concentration and cooking time on the physicochemical properties of pasteurized process cheese. J. Dairy Sci. 89:15–28.

Shirashoji, N., J. J. Jaeggi, and J. A. Lucey. 2010. Effect of sodium hexametaphosphate concentration and cooking time on the physicochemical properties of pasteurized process cheese. J. Dairy Sci. 93:2827–2837.

Weiserová, E., L. Doudová, L. Galiová, L. Žák, J. Michálek, R. Janiš, and F. Buňka. 2011. The effect of combinations of sodium phosphates in binary mixtures on selected texture parameters of processed cheese spreads. Int. Dairy J. 21:979–986.

Research paper 2

A2

Salek, R. N., Černíková, M., Nagyová, G., Kuchař, D., Bačová, H., Minarčíková, L., Buňka, F.

The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese.

International Dairy Journal. 2015, 44, 37-43. ISSN: 09586946.

International Dairy Journal 44 (2015) 37–43

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese

Richardos Nikolaos Salek [a], Michaela Černíková [a], Gabriela Nagyová [a], Dalibor Kuchař [b], Helena Bačová [a], Lucie Minarčíková [a], František Buňka [a, *]

[a] Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, T.G. Masaryka 5555, 760 01, Zlín, Czech Republic
[b] Department of Research and Development, Fosfa, Hraniční 268, 691 41, Břeclav, Poštorná, Czech Republic

ARTICLE INFO

Article history:
Received 30 March 2014
Received in revised form
5 December 2014
Accepted 8 December 2014
Available online 27 December 2014

Keywords:
Processed cheese
Emulsifying salts
Phosphates
Citrates
Textural properties

ABSTRACT

Ternary mixtures consisting of phosphate and citrate emulsifying salts were studied for their impact on selected textural properties (especially hardness) of processed cheese spreads over a 30 day storage period at 6 ± 2 °C. Two different groups of samples were manufactured, one with pH adjustment (target values within the interval of 5.60–5.80) and one without pH adjustment. When binary mixtures with trisodium citrate (TSC) and tetrasodium diphosphate (TSPP) were used (with zero content of the other salts tested in the ternary mixture), the products consisting of TSC and TSPP at a ratio of approximately 1:1 were the hardest. Increasing the content of TSC, TSPP and/or sodium salt of polyphosphate and decreasing that of disodium hydrogen phosphate (DSP) in ternary mixtures resulted in the increasing of the hardness of processed cheese. The absolute values of processed cheese hardness significantly changed as a result of pH adjustment.

1. Introduction

The term "processed cheese" describes a dairy product made by heating a mixture of various cheese types of different degrees of maturity in the presence of appropriate emulsifying salts (mostly sodium phosphate, polyphosphates, citrates and/or their combinations), usually under reduced pressure (vacuum) with constant stirring, commonly in the temperature range of 90–100 °C, until a smooth and homogenous compact mass is formed with desired textural properties. Optional dairy (butter, anhydrous milk fat, skim milk powder, whey powder, coprecipitates, caseinates, etc.) and non-dairy (water, vegetables, spices, flavourings, colourings, salt, hydrocolloids, preservatives, etc.) ingredients can be added into the blend (Guinee, Carić, & Kaláb, 2004; Kapoor & Metzger, 2008).

Emulsifying salts (ES) have a key role during the manufacturing of processed cheese. They possess the ability to sequester calcium from the casein matrix by exchanging sodium ions, which results in the conversion of insoluble calcium paracaseinate into soluble sodium paracaseinate (Guinee et al., 2004; Kapoor & Metzger, 2008). Within the matrix sodium paracaseinate acts as an emulsifier at the oil-in-water interface. The control and stabilisation of the pH level and an influence on the formation of a final structure after cooling are some of the additional effects of ES (Dimitreli & Thomareis, 2009; El-Barky, Duggan, O'Riordan, & O'Sullivan, 2011; Guinee et al., 2004).

Not all emulsifying salts have the same calcium ion-exchange ability. The phosphate ion-exchange ability increases with increasing P_2O_5 content in the following order: monophosphate < diphosphate < triphosphate < polyphosphate (Buňka et al., 2013; Guinee et al., 2004; Shirashoji, Jaeggi, & Lucey, 2006). El-Bakry et al. (2011) and Mizuno and Lucey (2005) stated that trisodium citrate presents better calcium chelating ability and casein peptisation properties than do sodium mono- and diphosphates. The effect of the individual phosphates and composition of phosphate binary and ternary mixtures on textural properties of processed cheese has been previously studied (Buňka et al., 2013; Dimitreli & Thomareis, 2009; El-Bakry et al., 2011; Lu, Shirashoji, & Lucey, 2008; Sádlíková et al., 2010; Shirashoji et al., 2006; Weiserová et al., 2011).

* Corresponding author. Tel.: +420 576 033 011.
E-mail address: bunka@ft.utb.cz (F. Buňka).

http://dx.doi.org/10.1016/j.idairyj.2014.12.009
0958-6946/

Processed cheeses in which the content of DSP is predominant have softer consistency. On the other hand, when polyphosphates are dominant in the binary and ternary mixtures, the hardness of the product increases (Buňka et al., 2013; Weiserová et al., 2011). According to Buňka et al. (2013), a specific ratio of DSP to TSPP exists that significantly affects textural parameters of processed cheese. Buňka et al. (2013) found that when the polyphosphate content was at low levels (less than 60%) and the ratio of DSP to TSPP ranged from 1:1 to 3:4, the hardness of the processed cheeses increased, while their cohesiveness and relative adhesiveness decreased. However, there is no existing study delineating how ternary mixtures containing parallel phosphate and citrate ES influence textural properties of processed cheese.

The first aim of this study was to investigate the effect of the ternary mixtures of phosphate and citrate ES [consisting of disodium hydrogen phosphate (Na_2HPO_4; DSP), tetrasodium diphosphate ($Na_2P_2O_7$; TSPP), sodium salt of polyphosphate with the mean length n ≈ 20 (P20) and trisodium citrate ($C_2H_5Na_3O_7$; TSC)] on selected textural properties (hardness, relative adhesiveness, and cohesiveness) of processed cheese over a 30 day storage period. The effect was observed (i) in samples with non-modified pH arising from the interactions of the emulsifying salt mixtures, as well as (ii) in samples with modified pH (target values within the interval of 5.60–5.80), which correspond to the standard pH values of processed cheese spreads. The second aim was to investigate the effect of the above-mentioned mixtures on casein micelle dispersion in a model milk system.

2. Materials and methods

2.1. Processed cheese samples manufacture

The composition of the raw materials, comprising Edam cheese blocks (50%, w/w, dry matter content, 30%, w/w, fat on dry matter content, 7-week maturity), butter (82%, w/w, dry matter content) and water, for processed cheese production was designed to achieve final products with 40% (w/w) dry matter content and 50% (w/w) fat in dry matter content. Ternary mixtures of DSP, TSPP, P20 (all from Fosfa, Břeclav–Poštorná, Czech Republic) and TSC (Merck, Darmstadt, Germany) were applied as ES, resulting in four types of ternary mixtures comprising DSP:TSC:P20, DSP:TSPP:TSC, TSC:TSPP:P20 and DSP:TSPP:P20. All four types of ternary mixtures of ES were blended in staggered proportions in steps of 20% (the percentages of the components calculated on the basis of total weight of ES, where total weight = 100%) and with some selected 50:50 ratios (viz. 100:0:0; 80:20:0; 60:40:0; 50:50:0; 40:60:0; 20:80:0; 0:100:0; 80:0:20; 60:20:20; 40:40:20; 20:60:20; 0:80:20; 60:0:40; 40:20:40; 20:40:40; 0:60:40; 50:0:50; 30:20:50; 0:50:50; 40:0:60; 20:20:60; 0:40:60; 20:0:80; 0:20:80; 0:0:100). This gave 26 variants in total. Each combination was made in duplicate giving 208 lots in total (4 types of ternary mixtures × 26 variants × 2 productions). The total concentration of the applied ternary mixtures was 3% (w/w) of the total weight of the melt. For the manufacture of the model processed cheese samples a Thermomix TM 31-1 blender cooker (Vorkwerk & Co., GmbH, Wuppertal, Germany) with indirect heating was used. The same device was also used by Buňka et al. (2013), Černíková et al. (2010), Lee, Anema, and Klostermeyer (2004) and Weiserová et al. (2011). The manufacture protocol was described in Buňka et al. (2013) and Weiserová et al. (2011). The hot melt mass was set into cylindrical polypropylene pots (52 mm in diameter; 50 mm high) and wrapped with aluminium lids (at least 12 pots from each batch were obtained). The packed samples were cooled and stored at 6 ± 2 °C. Analyses were performed on the second, ninth and thirtieth day of storage (day 1 was the production day).

In addition, model samples of processed cheese were manufactured in which the pH was modified to approach a target value of 5.60–5.80, using acid or alkali (1 mol L^{-1} HCl or NaOH). The manufacturing technique was identical to that used for the manufacture of processed cheese samples without any pH value modification. The estimated (according to a pilot study – unpublished data) amount of acid or alkali was added at 85–86 °C, 30–50 s before reaching the melting point. Water addition was reduced by the estimated amount of acid or alkali to maintain constant values of dry matter and fat in dry matter contents, respectively. Thus, another 208 lots (4 types of ternary mixtures × 26 variants × 2 productions) were made in total.

2.2. Dry matter content and pH measurement

Dry matter content was gravimetrically analysed according to ISO (2005). Values of pH were determined at ambient temperature by inserting a glass tip electrode of a calibrated pH-meter (pH Sper, Eutech Instruments, Oakton, Malaysia) directly into the processed cheese at three randomly chosen locations (in each pot).

2.3. Texture profile analysis

Three textural parameters (i.e., hardness, cohesiveness, relative adhesiveness) of processed cheese samples were observed using a TA.XT plus texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK). Two sequential penetration events (cylindrical probe P20 with 20 mm diameter, penetration depth 10 mm, probe speed 2 mm s^{-1}, trigger force 5 g, strain of deformation 25%) were implemented during the measurements. The probe directly penetrated the samples in the polypropylene cups (after removing the aluminium foil). Results were recorded as force-displacement/time curves describing the force (N) needed to deform the sample proportionally with time (s) (Buňka et al., 2013; Piska & Štětina, 2004; Weiserová et al., 2011). Values of hardness, cohesiveness and relative adhesiveness were calculated according to Weiserová et al. (2011). Each cup was used for penetration only once. The measurement was carried out at 6 ± 2 °C (each cup was measured immediately after removing from a fridge where samples were stored). On each day of analysis, each variant of ternary mixtures was measured in triplicate; two batches were manufactured.

2.4. Model milk samples preparation and optical density measurements

The model milk samples were prepared according to Nagyová et al. (2014) using skim milk powder (protein content 35.91 ± 0.62%, w/w, analysed using Kjeldahl method and applying a multiplying coefficient of 6.38; Moravia Lacto PLC, Jihlava, Czech Republic) reconstituted (5%, w/v) in deionised water (YL Instrument Co., Ltd., Anyang, Korea) to maintain the ionic environment at constant level. Sodium azide (0.02%, w/v) was added to prevent potential microbial contamination. The pH of the models was adjusted (using 1 mol L^{-1} HCl) to approach the value of 5.80 ± 0.01. The total concentration of the applied ES was 0.3% (w/v). The optical density was measured using an UVmini-1240 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of 700 nm (using 10 mm cells made of optical glass; HellmaAnalytics, Müllheim, Germany) against deionised water. Ten minutes after ES addition the pH of systems were adjusted to the targeted pH-value (5.80 ± 0.01) and after additional 50 min stirring the samples were analysed. The control sample was prepared in the same manner but without the addition of emulsifying salt. The casein micelles dispersion evaluation was expressed in terms of optical density, according to the method of Kaliappan and Lucey (2011).

Table 1
The pH-values of emulsifying salt (ES) systems.[a]

ES	1.0% (w/v) ES in deionised water	0.3% (w/v) ES in model milk system	1.0% (w/v) ES in model milk system	Processed cheese with 3.0% (w/v) ES
DSP	9.46 ± 0.01	6.54 ± 0.01	7.19 ± 0.01	6.43 ± 0.02
TSPP	10.02 ± 0.01	6.66 ± 0.02	8.69 ± 0.02	6.48 ± 0.02
P20	5.95 ± 0.02	6.19 ± 0.02	6.22 ± 0.01	5.33 ± 0.02
TSC	8.36 ± 0.01	6.42 ± 0.01	6.81 ± 0.02	6.44 ± 0.02

[a] Abbreviations are: DSP, disodium hydrogen phosphate (E339); TSPP, tetrasodium diphosphate (E450); P20, sodium salt of polyphosphate (E452); TSC, trisodium citrate (E331). Values are means ± standard deviation (n = 18); for processed cheese with 3.0% (w/v) ES the values are after 2 days storage at 6 °C.

2.5. Statistical analysis

The results were analysed by non-parametrical analysis of variance of Kruskall-Wallis and Wilcoxon tests (Unistat® 5.5 software; Unistat, London, UK), where the significance level was 0.05.

3. Results

3.1. Basic chemical analysis

The dry matter content of the processed cheese samples ranged from 40.25 to 41.01% (w/w), a range is similar to that reported by Dimitreli and Thomareis (2009) and Lee et al. (2004).

The pH values of the model solutions of ES in deionised water and in milk systems are shown in Table 1. Results of samples with deionised water fluctuated in a wider pH range (5.92–10.07) compared with those in the milk system (6.23–8.66; comparing concentration of 0.3% and 1.0%, w/v, of ES). A possible explanation for the differences in the pH values is the buffering capacity of the milk system (Kaliappan & Lucey, 2011; Mizuno & Lucey, 2005).

The pH values of the processed cheeses with the addition of the individual phosphate or citrate ES are presented in Table 1. The pH values obtained for processed cheeses made with DSP, TSPP and TSC were similar and higher than for those made with P20 ($P < 0.05$).

Fig. 1 illustrates the dependence of pH values of the model processed cheese samples (stored for 2 days at 6 ± 2 °C) on the relative amount of ternary mixtures of DSP:TSPP:P20 (panel A), DSP:TSC:P20 (panel B), DSP:TSPP:TSC (panel C), TSC:TSPP:P20 (panel D). The inclusion of TSC into the processed cheese samples had an effect similar to that of DSP or TSPP. The pH values of the processed cheese samples significantly decreased ($P < 0.05$) with the increasing proportion of P20 in the ternary mixtures.

The actual pH values of the processed cheese samples with pH modification ranged from 5.65 up to 5.89. The interval (for samples with adjusted pH values) can be considered acceptable with respect to manufacture from actual ingredients such as cheese and butter. During the 30 day storage period at 6 ± 2 °C a minor decrease in the pH of the processed cheese samples occurred. The majority of the samples showed a decrease of pH-values between 0.1 and 0.2 ($P < 0.05$).

3.2. Effect of the composition of phosphate and citrate ternary mixtures on textural parameters

The results of hardness of the model processed cheeses are shown in Figs. 2–5. With respect to individual ES, the samples prepared with TSC were harder than those prepared with DSP ($P < 0.05$) and even slightly harder than that prepared with TSPP. The sample prepared with P20 was the hardest ($P < 0.05$).

Fig. 2 depicts the development of hardness of the processed cheese samples, depending on the composition of the ES ternary mixtures composed of DSP, TSC and P20. From the results it can be concluded that with the increasing proportion of TSC and P20 (with a reduction in DSP), the hardness of the processed cheese samples also increased ($P < 0.05$). A grater increase in hardness was particularly observed when the proportion of P20 increased in the ternary mixtures of ES ($P < 0.05$).

Fig. 3 shows values for hardness of the processed cheese samples of the ternary mixtures of TSC:TSPP:P20 with and without pH modification after 2, 9 and 30 days of storage at 6 ± 2 °C. When the proportion of P20 was at zero levels in the ternary mixtures of ES, firmer processed cheese samples were obtained with TSC and TSPP at a ratio approximately of 1:1 ($P < 0.05$). Any deviation from this

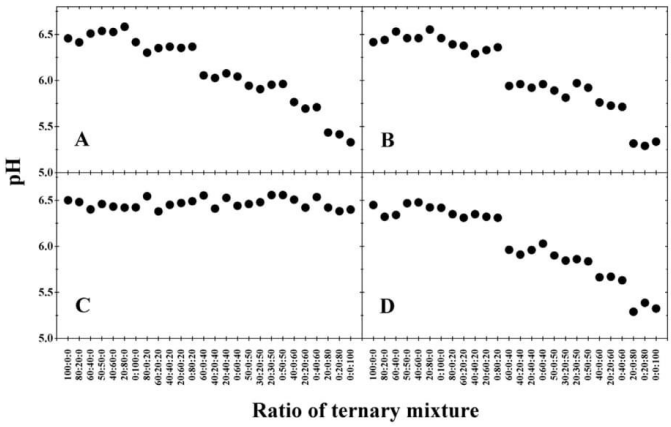

Fig. 1. The dependence of pH values of the model processed cheeses (stored 2 days at 6 °C) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of ternary mixtures of disodium hydrogen phosphate (DSP), tetrasodium diphosphate (TSPP), sodium salts of polyphosphate (P20) and trisodium citrate (TSC): panel A, DSP:TSPP:P20; panel B, DSP:TSC:P20; panel C, DSP:TSPP:TSC; panel D, TSC:TSPP:P20. The results are expressed as means (n = 18); standard deviations were in range of 0.01–0.03 and are not displayed.

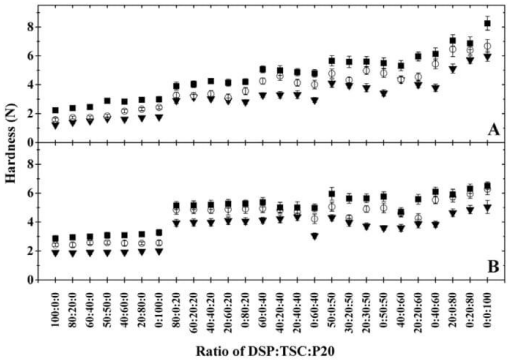

Fig. 2. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of disodium hydrogen phosphate (DSP), trisodium citrate (TSC) and sodium salt of polyphosphate P20 in a ternary mixture over 30 days storage at 6 °C (▼, day 2; ○, day 9; ■, day 30): panel A, samples without pH adjustment; panel B, samples with pH was adjustment to the optimal range of 5.65–5.89. The results are expressed as means and standard deviation (n = 6).

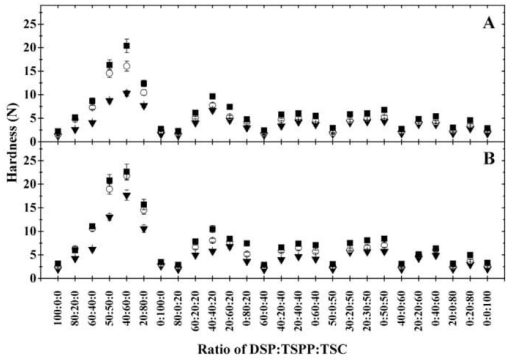

Fig. 4. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of disodium hydrogen phosphate (DSP), tetrasodium diphosphate (TSPP) and trisodium citrate (TSC) in a ternary mixture over 30 days storage at 6 °C (▼, day 2; ○, day 9; ■, day 30): panel A, samples without pH adjustment; panel B, samples with pH was adjustment to the optimal range of 5.65–5.89. The results are expressed as means and standard deviation (n = 6).

ratio resulted in a significant hardness decrease ($P < 0.05$). However, this phenomenon was only observed in the absence of P20 in the ternary mixtures of ES. With the increasing proportion of P20 the hardness of the model samples also increased ($P < 0.05$). On the other hand, at constant levels of P20 the hardness of the model samples was lightly reduced when TSC decreased and TSPP amount increased ($P \geq 0.05$).

In case of ES ternary mixtures composed of DSP, TSPP and TSC (Fig. 4) the specific ratio of DSP:TSPP (approximately 1:1–2:3) was identified, as leading to a significant increase in hardness of the samples ($P < 0.05$). However, the influence of the latter specific ratio rapidly decreased, with the increasing proportion of TSC ($P < 0.05$) in the ternary mixture. The influence of this specific ratio on hardness of the samples even at 40% levels of TSC was insignificant ($P \geq 0.05$). On the other hand, if the proportion of TSC was higher than 40% in the ternary mixture of ES, the hardness of the samples increased with the increasing proportion of TSPP and TSC and with the decreasing levels of DSP. Fig. 4 also shows the influence of the specific ratio of TSC to TSPP (at zero levels of DSP) on hardness of the processed cheese samples ($P < 0.05$), a phenomenon also observed in Fig. 3. Nevertheless, with an increasing proportion of DSP in the ternary mixtures of ES the impact effect of the specific ratio of TSC to TSPP on processed cheeses hardness was almost negligible.

Fig. 5 illustrates the values of hardness of the processed cheese samples of the ternary mixtures of DSP:TSPP:P20 with and without

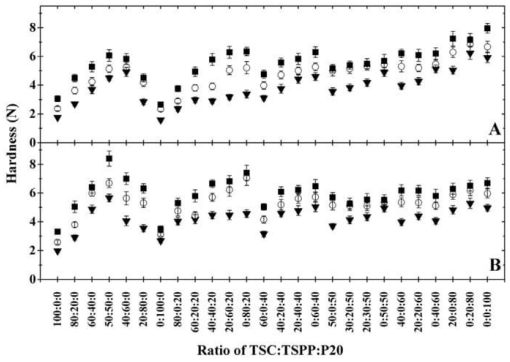

Fig. 3. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of trisodium citrate (TSC), tetrasodium diphosphate (TSPP) and sodium salt of polyphosphate (P20) in a ternary mixture over 30 days storage at 6 °C (▼, day 2; ○, day 9; ■, day 30): panel A, samples without pH adjustment; panel B, samples with pH was adjustment to the optimal range of 5.65–5.89. The results are expressed as means and standard deviation (n = 6).

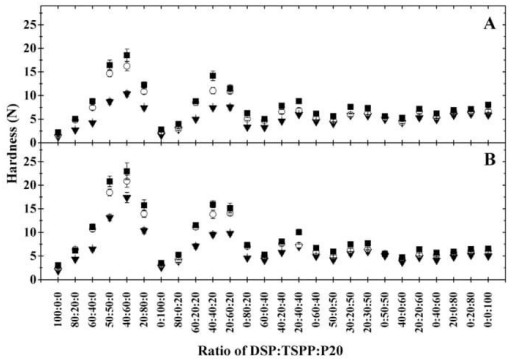

Fig. 5. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of disodium hydrogen phosphate (DSP), tetrasodium diphosphate (TSPP) and sodium salt of polyphosphate (P20) in a ternary mixture over 30 days storage at 6 °C (▼, day 2; ○, day 9; ■, day 30): panel A, samples without pH adjustment; panel B, samples with pH was adjustment to the optimal range of 5.65–5.89. The results are expressed as means and standard deviation (n = 6).

pH values adjustment after 2, 9 and 30 days of storage at 6 ± 2 °C. The effect of the specific ratio of DSP:TSPP (approximately 1:1–2:3) on hardness of the processed cheese samples were also noticed (the same phenomenon as in Fig. 4).

The pH values adjustment to the optimal range (actual achieved interval 5.65–5.89) resulted in significant changes in values of hardness of the samples ($P < 0.05$; Figs. 2–5). When the pH values were decreased (addition of acidic solution), the firmness of the samples increased. Conversely, when the pH values were increased (addition of alkaline solution), a decrease of the samples hardness was observed. The above mentioned trends of hardness of the processed cheese samples depending on the ternary mixtures of emulsifying salts composition, consisting of DSP, TSPP, TSC and P20 remained similar, only the absolute values of firmness of the samples changed significantly ($P < 0.05$).

During the 30 day storage period the hardness of the processed cheese samples significantly increased ($P < 0.05$) over the storage period (Figs. 2–5). For most of the compared samples with the same emulsifying salt composition during the storage period, this increase was statistically significant ($P < 0.05$).

The values of cohesiveness for the processed cheese samples were (in cases of individually used ES): DSP, 0.63–0.66; TSPP, 0.66–0.71; P20, 0.57–0.59; TSC, 0.65–0.67; adhesiveness values were: DSP, 0.35–0.37; TSPP, 0.31–0.34; P20, 0.24–0.27; TSC, 0.38–0.40. The development of cohesiveness and relative adhesiveness was in accord with the changes in proportion of DSP:TSC:P20 and TSC:TSPP:P20. Furthermore, with a constant content of P20 ≤60% and TSC ≤40%, a decrease in cohesiveness of the processed cheeses was observed at a ratio of DSP:TSPP (1:1–2:3). On the other hand, under the same conditions (a constant content of P20 ≤60% and TSC ≤40%), a decrease in relative adhesiveness of the samples was also observed at a ratio of DSP:TSPP (1:1–2:3). Moreover, outside this specific interval for the ratio of DSP:TSPP, both cohesiveness and relative adhesiveness of the model samples were significantly increased (comparing the samples with a constant content of P20 or TSC). At the end of the 30 day storage period, a slight increase in the values of cohesiveness and a decrease in the values of relative adhesiveness occurred (comparing the same types of model processed cheese samples).

3.3. Results of the optical density measurement of milk systems

Kaliappan and Lucey (2011) reported that the dispersion of casein is more extensive at lower values of optical density in the milk system. Fig. 6 illustrates the results of the optical density measurement of the milk system containing various ternary mixtures of DSP, TSPP, P20 and TSC (the results were expressed as percentage with respect to the control sample without emulsifying salt addition). The concentration of ternary mixtures of ES added was set at 0.3% (w/v), approximately corresponding to the ratio of protein to phosphate in processed cheeses (Guinee et al., 2004; Nagyová et al., 2014).

Application of individual phosphates resulted in a more intense casein dispersion with an increasing number of phosphorus atoms linearly bound in the molecule of a phosphate ($P < 0.05$; Fig. 6). In the ternary mixtures of DSP, TSPP, TSC and P20 it was observed that with an increasing proportion of longer-chain phosphates, the intensity of the casein dispersion rose ($P < 0.05$). The effect of addition of TSC and TSPP on the dispersion of casein was similar ($P \geq 0.05$). The individual application of TSC led to an increase of the casein dispersion compared with samples with individually applied DSP, but to less extensive casein dispersion than with mixtures where P20 was used alone. With an increasing proportion of DSP in the ternary mixtures of ES, the optical density increased (Fig. 6; $P < 0.05$) and therefore the casein dispersion intensity decreased.

4. Discussion

Emulsifying salts are characterised as 'key' ingredients during processed cheese production, participating in several important physicochemical phenomena such as, calcium sequestration, pH adjustment and stabilisation, casein dispersion, free fat emulsification and formation of final structure (Buňka et al., 2013; Kapoor & Metzger, 2008; Kawasaki, 2008). Based on our results, with

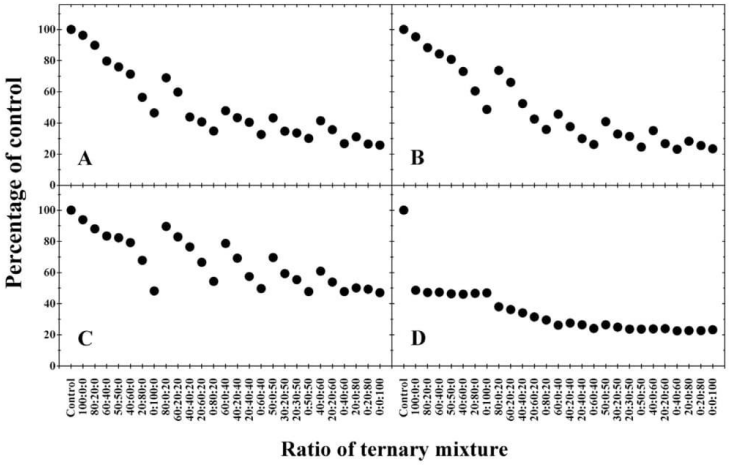

Fig. 6. The dependence optical density of the model milk systems (optical density expressed relative to that of model milk systems without emulsifying salts addition: control, 100%) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of disodium hydrogen phosphate (DSP), tetrasodium diphosphate (TSPP), sodium salts of polyphosphate (P20) and trisodium citrate (TSC) in a ternary mixture of emulsifying salts: panel A, DSP:TSPP:P20; panel B, DSP:TSC:P20; panel C, DSP:TSPP:TSC; panel D, TSC:TSPP:P20. The results are expressed as means (n = 8); standard deviations were in range of 0.42–1.59% and are not displayed.

increasing proportion of long-chain polyphosphate in the ternary mixture a decreasing trend of the pH of the processed cheese occurred. Buňka et al. (2013), Chen and Liu (2012), Lu et al. (2008) and Weiserová et al. (2011) reported analogous results; Buňka et al. (2013) and Weiserová et al. (2011) used the same phosphates including sodium salt of polyphosphate.

The sole application of phosphates with longer chains led to processed cheeses with higher values of hardness. When the phosphate and citrate ES were applied individually the hardness of the model processed cheeses rose in the following order: DSP < TSC ≈ TSPP < P20. Similar results for DSP, TSPP and P20 were reported by Buňka et al. (2013), Nagyová et al. (2014) and Weiserová et al. (2011) and for DSP and TSC by El-Bakry et al. (2011). The explanation may be that the ion-exchange intensity (of sodium ions for calcium ions) is affected by the chain-length of phosphates. Results of the optical density measurements of milk systems containing ternary mixtures of ES showed that (i) the application of a higher concentration of polyphosphate (in the ternary mixture) and (ii) the use of phosphates with longer chains can lead to a more intensive casein dispersion. The dispersing ability of TSC in the model milk system was comparable with that of TSPP. According to Cunha, Grimaldi, Alcântara, and Viotto (2013), Dimitreli and Thomareis (2009) and Shirashoji, Jaeggi, and Lucey (2010), the dispersion of casein is closely related to the processed cheese matrix formation. An intensive dispersion of casein allows caseins to develop their emulsifying and hydrating abilities and thus stabilise the fat and water present in the mixture. Increasing the range of protein hydration and fat emulsification results in higher intensity of casein crosslinking (Shirashoji et al., 2010). A harder processed cheese will occur with a greater number of cross linkages in its matrix (Buňka et al., 2013; Kaliappan & Lucey, 2011; Mizuno & Lucey, 2005, 2007; Nagyová et al., 2014; Shirashoji et al., 2010).

The dispersion of casein alone is not sufficient to fully explain the effect of the specific ratio of DSP to TSPP (approximately 1:1–2:3) on the processed cheese matrix resulting in a rapid increase in the hardness of the processed cheese samples. The impact of this ratio on processed cheese textural parameters weakened with the increasing proportion of polyphosphate and citrate (in the ternary mixtures DSP:TSPP:P20 and DSP:TSPP:TSC). When the relative concentration of P20 in the ternary mixture approached ≥60% or TSC ≥40% the effect of the above-mentioned ratio on the hardness of the processed cheese samples became insignificant.

The explanation of the existence of DSP:TSPP specific ratio (approximately 1:1–2:3) was proposed as the ability of diphosphates to enhanced milk protein gel formation, especially when diphosphates were in an optimal concentration (Buňka et al., 2013; Mizuno & Lucey, 2007). However, according to Mizuno and Lucey (2007), weak gels may be formed with an excessive or insufficient concentration of diphosphates. Kaliappan and Lucey (2011) reported that a mixture composed of mono- and diphosphates strongly promoted gel formation in model milk systems, which was explained by the ability of monophosphates to enhance the formation of bridges between diphosphate complexes with calcium ions and caseins.

The rising relative concentration of polyphosphate in the ternary mixture of emulsifying salts with DSP and TSPP in a range of 1:1–2:3 resulted in the hardness of processed cheese decreasing. The increasing amount of polyphosphate in the ternary mixture could lead to the increase of multiple negative charges of caseins and thus it could lead to hydrophobic interactions of the dispersed caseins with lower intensity and resulting in decreased hardness of the final matrix (Buňka et al., 2013; Mizuno & Lucey, 2007; Shirashoji et al., 2010).

The development of hardness of samples with the ternary mixtures containing TSC (excluding binary mixtures containing TSPP:TSC) could be explained by the ability of the mixture of emulsifying salts to disperse casein (Buňka et al., 2013; Mizuno & Lucey, 2007; Shirashoji et al., 2010). When the mixture of emulsifying salts with more intensive ability to disperse casein was used, a harder processed cheese was observed. This explanation of results of processed cheese hardness correlates with the impact of the applied ternary mixtures of ES on the intensity of casein dispersion in the model milk system in our study and also in previous published papers (e.g., Lu et al., 2008; Mizuno & Lucey, 2007; Shirashoji et al., 2010).

In the case of TSC and TSPP binary mixtures, a specific ratio was observed (approximately 1:1) that increased the hardness of the samples monitored. There is no clear explanation for this with respect to the interactions among TSC and TSPP and their involvement in the casein matrix. TSC does not participate in creating new networks, and therefore the effect of diphosphates on casein crosslinking would not be influenced by TSC (Kaliappan & Lucey, 2011; Lu et al., 2008; Mizuno & Lucey, 2005). A possible explanation may be that diphosphates are effective on enhancing casein proteins gel formation when their concentration is at optimum levels relatively to protein content (Mizuno & Lucey, 2007).

A slight increase in hardness of the processed cheese samples was observed during the 30 day storage period, regardless of the composition of the ternary mixtures of ES used or pH modification of the melt during manufacture. Several factors could attribute to the explanation of the change of the textural parameters, e.g., a slight decrease in pH values of the model processed cheese samples (which was also observed in our study), hydrolysis of ES, the dairy fat polymorphism and the gradual change of it into its crystalline form (Cunha et al., 2013; Guinee et al., 2004).

5. Conclusions

The impact of the ternary mixtures composition on textural parameters of processed cheese and on the casein micelle dispersion (in the model milk system) was studied. The impact of a specific ratio of DSP:TSPP and TSC:TSPP on processed cheese hardness was described. At constant content of P20 ≤60% or TSC ≤40% in the emulsifying salt ternary mixtures a rapid increase in hardness of the product was observed, especially at a specific ratio of DSP to TSPP of approximately 1:1 to 2:3. When the content of P20 or DSC was absent in the ternary mixture, the products consisting of TSC and TSPP in a range of approximately 1:1 were the hardest (among samples with the binary mixture of TSC and TSPP). The hardness of all processed cheese samples increased with the increasing storage period. The obtained results could be used in dairy industry during designing of emulsifying salts mixtures.

Acknowledgement

This study was supported by a project of the internal grants of Tomas Bata University in Zlin, Czech Republic no IGA/FT/2013/010 and IGA/FT/2014/001 funded from the resources for specific university research.

References

Buňka, F., Doudová, L., Weiserová, E., Kuchař, D., Ponížil, P., Začalová, D., et al. (2013). The effect of ternary emulsifying salt composition and cheese maturity on the textural properties of processed cheese. *International Dairy Journal, 29*, 1–7.

Černíková, M., Buňka, F., Pospiech, M., Tremlová, B., Hladká, K., Pavlínek, V., et al. (2010). Replacement of traditional emulsifying salts by selected hydrocolloids in processed cheese production. *International Dairy Journal, 20*, 336–343.

Chen, L., & Liu, H. (2012). Effect of emulsifying salts on the physicochemical properties of processed cheese made from Mozzarella. *Journal of Dairy Science, 95*, 4823–4830.

Cunha, C. R., Grimaldi, R., Alcântara, M. R., & Viotto, W. H. (2013). Effect of the type of fat on rheology, functional properties and sensory acceptance of spreadable cheese analogue. *International Journal of Dairy Technology, 66*, 54–62.
Dimitreli, G., & Thomareis, A. S. (2009). Instrumental textural and viscoelastic properties of processed cheese as affected by emulsifying salts and in relation to its apparent viscosity. *International Journal of Food Properties, 12*, 261–275.
El-Barky, M., Duggan, E., O'Riordan, E. D., & O'Sullivan, M. (2011). Effect of chelating salt type on casein hydration and fat emulsification during manufacture and post-manufacture functionality of imitation cheese. *Journal of Food Engineering, 102*, 145–153.
Guinee, T. P., Carić, M., & Kaláb, M. (2004). Pasteurized processed cheese and substitute/imitation cheese products. In P. F. Fox, P. L. H. McSweeney, T. P. Cogan, & T. P. Guinee (Eds.), *Cheese: Chemistry, physics and microbiology. Major cheese groups* (pp. 349–394). London, UK: Elsevier Applied Science.
ISO. (2005). *Cheese and processed cheese – Determination of the total solids content (Reference method)*. ISO Standard No. 5534. Geneva, Switzerland: International Standardisation Organisation.
Kaliappan, S., & Lucey, J. A. (2011). Influence of mixtures of calcium-chelating salts on the physicochemical properties of casein micelles. *Journal of Dairy Science, 94*, 4255–4263.
Kapoor, R., & Metzger, L. E. (2008). Process cheese: scientific and technological aspects - a review. *Comprehensive Reviews in Food Science and Food Safety, 7*, 194–214.
Kawasaki, Y. (2008). Influence of "creaming" on the properties of processed cheese and changes in the structure of casein during cheese making. *Milchwissenschaft, 63*, 149–153.
Lee, S. K., Anema, S., & Klostermeyer, H. (2004). The influence of moisture content on the rheological properties of processed cheese spreads. *International Journal of Food Science and Technology, 39*, 763–771.
Lu, Y., Shirashoji, N., & Lucey, J. A. (2008). Effects of pH on the textural properties and meltability of pasteurized process cheese made with different types of emulsifying salts. *Journal of Food Science, 73*, 363–369.
Mizuno, R., & Lucey, J. A. (2005). Effects of emulsifying salts on the turbidity and calcium-phosphate–protein interactions in casein micelles. *Journal of Dairy Science, 88*, 3070–3078.
Mizuno, R., & Lucey, J. A. (2007). Properties of milk protein gels formed by phosphates. *Journal of Dairy Science, 90*, 4524–4531.
Nagyová, G., Buňka, F., Salek, R. N., Černíková, M., Mančík, P., Grüber, T., et al. (2014). Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. *Journal of Dairy Science, 97*, 111–122.
Piska, I., & Štětina, J. (2004). Influence of cheese ripening and rate of cooling of the processed cheese mixture on rheological properties of processed cheese. *Journal of Food Engineering, 61*, 551–555.
Sádlíková, I., Buňka, F., Budinský, P., Voldánová, B., Pavlínek, V., & Hoza, I. (2010). The effect of selected phosphate emulsifying salts on viscoelastic properties of processed cheese. *LWT – Food Science and Technology, 43*, 1220–1225.
Shirashoji, N., Jaeggi, J. J., & Lucey, J. A. (2006). Effect of trisodium citrate concentration and cooking time on the physicochemical properties of pasteurized process cheese. *Journal of Dairy Science, 89*, 15–28.
Shirashoji, N., Jaeggi, J. J., & Lucey, J. A. (2010). Effect of sodium hexametaphosphate concentration and cooking time on the physicochemical properties of pasteurized process cheese. *Journal of Dairy Science, 93*, 2827–2837.
Weiserová, E., Doudová, L., Galiová, L., Žák, L., Michálek, J., Janiš, R., et al. (2011). The effect of combinations of sodium phosphates in binary mixtures on selected texture parameters of processed cheese spreads. *International Dairy Journal, 21*, 979–986.

Research paper 3

A3

Salek, R.N., Černíková, M., Maděrová, S., Lapčík, L., & Buňka, F.

The effect of different composition of ternary mixtures of emulsifying salts on the consistency of processed cheese spreads manufactured from Swiss-type cheese with different degrees of maturity.

Journal of Dairy Science. 2016, 99, 3274-3287. ISSN: 00220302.

J. Dairy Sci. 99:3274–3287
http://dx.doi.org/10.3168/jds.2015-10028

The effect of different composition of ternary mixtures of emulsifying salts on the consistency of processed cheese spreads manufactured from Swiss-type cheese with different degrees of maturity

R. N. Salek, M. Černíková, S. Maděrová, L. Lapčík, and F. Buňka[1]
Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, T. G. Masaryka 5555, 760 01, Zlín, Czech Republic

ABSTRACT

The scope of this work was to investigate the dependence of selected textural (texture profile analysis, TPA) and viscoelastic properties of processed cheese on the composition of ternary mixtures of emulsifying salts [disodium hydrogenphosphate, DSP; tetrasodium diphosphate, TSPP; sodium salt of polyphosphate (with mean length n ≈ 20), P20; and trisodium citrate, TSC] during a 60-d storage period (6 ± 2°C). The processed cheese samples [40% wt/wt dry matter (DM) content, 50% wt/wt fat in DM content] were manufactured using Swiss-type cheese (as the main raw material) with 4 different maturity degrees (4, 8, 12, and 16 wk of ripening). Moreover, the pH of the samples was adjusted (the target values within the range of 5.60–5.80), corresponding to the standard pH values of spreadable processed cheese. With respect to the individual application of emulsifying salts (regardless of the maturity degree of the Swiss-type cheese applied), the samples prepared with P20 were the hardest, followed by those prepared with TSPP, TSC, and DSP. Furthermore, a specific ratio of DSP:TSPP (1:1) led to a significant increase in the hardness of the samples. On the whole, the hardness of all processed cheese samples increased with the prolonging storage period, whereas their hardness significantly dropped with the rising ripening stage of the raw material utilized. In all of the cases, the trends of hardness development remained analogous, and only the absolute values differed significantly. Moreover, the findings of TPA were in accordance with those of the rheological analysis. In particular, the specific ratio of DSP:TSPP (1:1) resulted in the highest gel strength and interaction factor values, followed by P20, TSPP, TSC, and DSP (used individually), reporting the same trend which was demonstrated by TPA. The monitored values of the gel strength and interaction factor decreased with the increasing maturity degree of the Swiss-type cheese used. The intensity of the rigidity of the samples showed an analogous relationship to the intensity of the gel strength; the higher the gel strength of the sample, the more inflexible the product is expected to be.

Key words: Swiss-type cheese, processed cheese, sodium salt of phosphates, sodium salt of citrate, rheology

INTRODUCTION

Processed cheese (**PC**) is a multicomponent dairy complex system described as stable oil-in-water emulsion (Lee et al., 2003; Chen and Liu, 2012; Hanaei et al., 2015). The multilaterism of PC derives from the fact that it contains a wide variety of interacting components and a high water content (Marchesseau et al., 1997). Therefore, its matrix is formed by blending shredded cheese (of different types and maturity degrees) in the presence of emulsifying salts (**ES**; mainly sodium salts of phosphates, polyphosphates, citrates, or a combination of these), heated under partial vacuum and constant stirring, resulting in a homogeneous and smooth mass with desired properties (Guinee et al., 2004; Lee et al., 2004; Sádlíková et al., 2010; Chen and Liu, 2012).

Cheese ripening is the term describing a technological process during which biochemical and microbiological changes occur in cheese (raw material for PC manufacturing), resulting in the development of a specific flavor and consistency in the matured product (Pachlová et al., 2012; Ochi et al., 2013). The degree of casein proteolysis in the cheese applied during PC manufacture is a parameter that significantly influences its textural and viscoelastic properties (Piška and Štetina, 2004; Brickley et al., 2007; Buňka et al., 2013).

The consistency of PC can be affected by many factors, including the type, composition, and chemical profile of the cheese used (DM, fat, protein, and calcium ion content, and maturity degree), the type and concentration of ES, the presence and concentration of ions (especially calcium, sodium, and potassium), other optional dairy and nondairy ingredients, the pH of the mass to be melted, the processing and storage

Received June 29, 2015.
Accepted December 30, 2015.
[1]Corresponding author: bunka@ft.utb.cz

conditions (processing and storage temperature, stirring speed, time and temperature of the fusion, and cooling rate) and a possible use of some hydrocolloids (Shirashoji et al., 2006; Dimitreli and Thomareis, 2007; Gustaw and Mleko, 2007). Moreover, ES are ingredients of great importance in PC manufacture. Their ability to sequester calcium in the cheese matrix leads to the enhancement of casein emulsifying properties; the replacement of calcium from the insoluble calcium-paracaseinate (present in cheese) with sodium results in the formation of soluble sodium-paracaseinate, which can easily be dispersed and thus considerably influence the emulsification of fat (casein coats the surfaces of the dispersed free fat globules) and water stabilization within the matrix formed (Kawasaki, 2008; Chen and Liu, 2012; Buňka et al., 2014).

Furthermore, PC with diverse consistency and alternative functional properties may be manufactured as a result of the use of different types (phosphate, citrate, or both) and combinations of ES. In practice nowadays, the individual application of ES is very rare. In fact, ES are applied in ternary or even more componential mixtures (Guinee et al., 2004; Kapoor and Metzger, 2008; Salek et al., 2015). Generally, the effect of different composition of ternary mixtures of the individual sodium salts of phosphates (especially disodium hydrogenphosphate, tetrasodium diphosphate, and sodium salt of polyphosphate) has been described in the papers by Weiserová et al. (2011) and Buňka et al. (2012, 2013), but only for Dutch-type cheese as the raw material for the PC tested. Swiss-type cheese (**STC**) is a group of hard or semi-hard cheeses in texture, with desired propionic acid fermentation caused by propionic acid bacteria (especially *Propionibacterium freudenreichii* ssp. *freudenreichii* and *Propionibacterium freudenreichii* ssp. *shermanii*). Therefore, their flavor is characterized as sweet and nut-like. This is due to free fatty acids, peptides, AA, carbonyls, or their mutual interactions (Paulsen et al., 1980; Beuvier et al., 1997; Bouton et al., 2009). However, in the available literature, no existing study delineates PC manufacture using STC as the main raw ingredient. Swiss-type cheese is often used as part of the raw material for PC manufacture. On the other hand, the individual usage of STC in PC production has not been described. The influence of different maturity degrees of STC associated with different combinations of ES ternary mixtures affecting PC consistency has not been found in the literature.

The first aim of this study was to explore the dependence of selected textural properties (especially hardness, cohesiveness, and relative adhesiveness) and viscoelastic properties of PC on the composition of ternary mixtures of ES containing disodium hydrogenphosphate (Na_2HPO_4, **DSP**), tetrasodium diphosphate ($Na_2P_2O_7$, **TSPP**), sodium salt of polyphosphate with mean length n $\approx$ 20 (**P20**), and trisodium citrate ($C_2H_5Na_3O_7$, **TSC**) during a 60-d storage period. The above-mentioned dependence was observed in samples with adjusted pH values (target values within the interval of 5.60–5.80) corresponding to the standard pH values of PC spreads. The second aim was to investigate the effect of different maturity degrees of the STC (basic raw material) on the above-mentioned dependence.

MATERIALS AND METHODS

Manufacture of PC Samples

For the manufacture of the model PC samples with 40% (wt/wt) DM content and 50% (wt/wt) fat in DM, the following materials were used: STC block (60% wt/wt DM content, 30% wt/wt fat DM content; 4, 8, 12, and 16 wk of ripening; the same raw materials of STC were used in the whole experiment; MoraviaLacto, a.s., Jihlava, Czech Republic), butter (84% wt/wt DM content, 82% wt/wt fat content; Sachsenmilch Leppersdorf, GmbH, Wachau, Germany), water and ternary mixtures of DSP, TSPP, P20 (Fosfa PLC Company, Břeclav, Poštorna, Czech Republic), and TSC (SigmaAldrich Inc., St. Louis, MO). Moreover, the ES were applied into 4 types of ternary mixtures comprising DSP:TSC:P20, DSP:TSPP:TSC, TSC:TSPP:P20, and DSP:TSPP:P20. The total concentration of the ternary mixtures mentioned above was 3% (wt/wt) of the total weight of the melt. Each type of the ternary mixture was tested in 12 reciprocal percentage ratios (100:0:0; 50:50:0; 0:100:0; 40:40:20; 40:20:40; 20:40:40; 50:0:50; 0:50:50; 40:0:60; 20:20:60; 0:40:60; 0:0:100); the percentage of the components was estimated on the basis of the total weight of ES (total weight = 100%). Each combination of the ES formulation was made in duplicate resulting in 96 lots in total (4 types of ternary mixtures $\times$ 12 reciprocal percentage ratios $\times$ 2 repetitions). The scheme of the experiment design is shown in Figure 1. A Vorwerk Thermomix TM 31–1 blender cooker (Vorwerk & Co. Thermomix, GmbH, Wuppertal, Germany) with indirect heating was employed for the manufacture of the PC samples. The same apparatus was also used for a contiguous scope in the work by Lee et al. (2004, 2013) and Buňka et al. (2013). The manufacturing procedure was described in detail in the work by Buňka et al. (2013) and Salek et al. (2015). Briefly, a target temperature of 90°C was held for 1 min (the total melting time was 10–12 min) at approximately 2,750 $\times$ *g*. Therefore, the pH of the samples was adjusted (target values within the interval of 5.60–5.80) using acid or alkali (1 mol/L of HCl or NaOH). According to a pilot study (unpublished data) the calculated

amount of acid/alkali was added when the temperature approached 85 to 86°C, 30 to 50 s before reaching the melting point (Buňka et al., 2013; Salek et al., 2015). The hot melt was poured into polypropylene doses of cylindrical shape (52 mm in diameter and 50 mm high). Consequently, to maintain the values of DM and fat in DM without changes, the addition of water was reduced (by the calculated amount of acid/alkali). The PC samples were cooled and stored at 6 ± 2°C until the analyses were performed. The analyses were performed on d 2, 9, 30, and 60 of storage, with the exception of oscillation rheology, which was performed on d 30 (a typical period of PC storing). Each PC (manufactured from STC of certain maturity) was produced twice.

Basic Chemical Analysis of PC Samples

The DM content of the PC samples was gravimetrically determined according to ISO (2004). Moreover, the pH values were measured at ambient temperature using a glass tip electrode of a pH meter (pHSpear, Eutech Instruments, Oakton, Malaysia) by direct insertion of the spear into the PC samples at 3 randomly selected spots (in each pot).

Free Amino Acid Content of STC (Raw Material)

Before the analysis of free amino acid (**FAA**) content, the samples of the individual STC with different degrees of maturity (4, 8, 12 and 16 wk of ripening) were submitted to lyophilisation using the Christ Alpha 1–4 (Christ, Osterode, Germany) equipment and then stored at −80°C. All measurements were performed using the AAA 400 Amino Acid Analyzer (Ingos, Prague, Czech Republic) – ion-exchange chromatography apparatus according to the protocol described by Buňková et al. (2009) and Pachlová et al. (2011). The FAA content was calculated as a sum of 22 individual FAA and

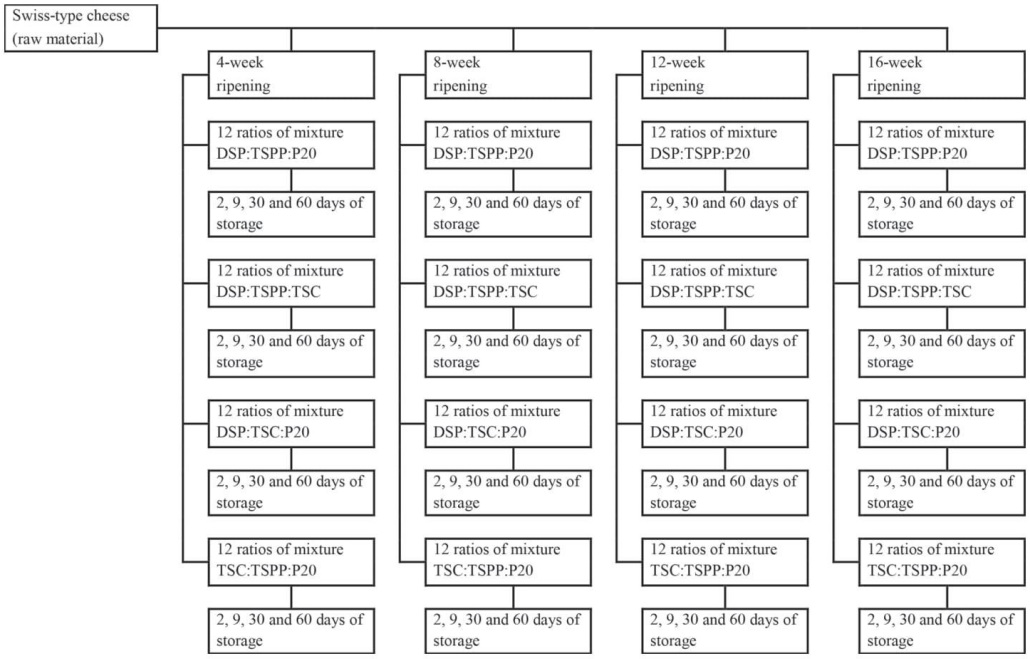

Figure 1. Scheme of the experimental design with model processed cheeses manufactured using Swiss-type cheese (in various time of ripening) and the different percentage ratios (100:0:0, 50:50:0, 0:100:0, 40:40:20, 40:20:40, 20:40:40, 50:0:50, 0:50:50, 40:0:60, 20:20:60, 0:40:60, 0:0:100) of the 4 types of ternary mixtures consisting of DSP:TSPP:P20, DSP:TSPP:TSC, DSP:TSC:P20, and TSC:TSPP:P20 (DSP, disodium hydrogenphosphate; TSPP, tetrasodium diphosphate; P20, sodium salt of polyphosphate with mean length n ≈ 20; and TSC, trisodium citrate). The model samples were tested after 2, 9, 30, and 60 d of storage.

the content of similar substances (γ-aminobutyric acid, alanine, aspartic acid, asparagine, arginine, citrulline, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, tyrosine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, valine). Each cheese (raw material) was lyophilisated twice, each lyophilisate was extracted twice and each extract was loaded on the column in triplicate (n = 12).

Texture Profile Analysis

The TPA method was performed for the evaluation of selected textural properties of the PC samples (hardness, cohesiveness, and relative adhesiveness) using a TA.XTplus texture analyzer (Stable Micro Systems Ltd., Godaming, UK). The tests were carried out at 6 ± 2°C (the sample measurement was performed immediately after removing them from a refrigerator where they were stored) after 2, 9, 30, and 60 d of storage according to the methodology described by Piska and Štětina (2004), Weiserová et al. (2011), and Sołowiej et al. (2014). During the measurements, 2 successive penetration events were implemented on the samples to ensure 25% deformation by a 20-mm cylindrical probe P20; the rate of the penetration was 2 mm·s^{-1} and the trigger force was 5 *g*. The results obtained were recorded as force-displacement/time curves describing the force (N) needed to deform the sample proportionally with time. On each day of the analysis, each variant of the ternary mixtures was measured in triplicate (n = 6).

Rheological Analysis

The rheological analysis of the PC samples on d 30 of storage (6 ± 2°C) was performed using a dynamic oscillatory shear rheometer (RheoStress 1, HAAKE, Bremen, Germany). Additionally, to describe the changes in the viscoelastic properties of the solidified melt mass, a plate-plate geometry (35 mm in diameter) was selected in dependence with frequency (ω; ranging from 0.01 to 100.00 Hz) at 20 ± 0.1°C. The selected monitored parameters, including elastic or storage (G′) and viscous or loss (G″) moduli (determined as a function of frequency), were used for complex modulus (G*) calculation according to equation [1]:

$$G^* = \sqrt{(G')^2 + (G'')^2}. \quad [1]$$

Winter's critical gel theory was implemented to evaluate the changes in the viscoelastic properties of the PC samples. According to the following equation [2], the complex modulus can be expressed as follows (Winter and Chambon, 1986; Gabriele et al., 2001; Macků et al., 2009):

$$G^*(\omega) = A_F \cdot \omega^{1/z}, \quad [2]$$

where A_F is the strength of the gel (Pa·s$^{1/z}$) and z is the interaction factor (defined as the number of structural units interacting with one another in a 3-dimensional network; unitless). The higher the interaction factor is, the more interactions occur in the matrix of the sample (Gabriele et al., 2001; Martínez-Ruvalcaba et al., 2007; Macků et al., 2009). The reported values were the mean of at least 4 replicates (n = 8).

Statistical Analysis

Nonparametrical analyses of variance of Kruskal-Wallis and Wilcoxon tests were used to evaluate the results obtained (Unistat 6.5 software, Unistat, London, UK; the significance level was 0.05). One-way tests were used, and therefore (1) the differences between the samples with a different ratio of ES (samples manufactured using raw material at a constant level of ripening at a constant time of storage) in the ternary mixture; (2) the differences between the samples with various times of STC ripening (samples with a constant ratio of ES in each ternary mixture type at a constant time of storage); and (3) the differences between the samples with various times of storage (samples with a constant ratio of ES in each ternary mixture type manufactured using raw material with a constant level of ripening) were evaluated independently. For the estimation of the gel strength and the interaction factor, nonlinear regression analysis (nonlinear least squares regression) was used for the following conditions: $A_F > 0$ and $z \geq 0$. The Marquardt-Levenburg method was applied (Unistat 6.5 software was also applied).

RESULTS

Basic Chemical Analysis of PC Samples and FAA Content of STC (Raw Material)

The DM levels of all samples were within the interval of 40.16 to 41.12%, depicting the stability of the DM content of the samples. Furthermore, another significant factor affecting the viscoelastic properties of PC is pH. The pH value adjustment resulted in PC samples with pH values ranging from 5.61 to 5.78, which can be characterized as acceptable for spreadable PC. The proteolytic changes occurring during cheese ripening were examined by the development of FAA content. The FAA concentrations of the STC (raw material)

after 4, 8, 12, and 16 wk of ripening were 17.49, 28.27, 38.48, and 44.75 g·kg^{-1}, respectively.

Texture Profile Analysis

The results of PC hardness are presented in Figures 1–4. With respect to the individual ES application and regardless of the maturity degree of the treated STC, the samples prepared with P20 were the hardest ($P < 0.05$). Moreover, the samples manufactured with TSC were harder than those manufactured with DSP and similarly hard to those manufactured with TSPP. The development of PC hardness, depending on the composition of ES ternary mixtures (composed of DSP, TSPP, and P20) and on the ripening stage (4, 8, 12, and 16 wk of maturity) of the STC applied after 2, 9, 30, and 60 d of storage at 6 ± 2°C is interpreted in Figure 2. Furthermore, a specific ratio of DSP:TSPP (1:1) was distinguished, leading to a considerable increase in hardness of the samples ($P < 0.05$). Nonetheless, with the increasing proportion of P20 in the ternary mixture, the effect of the above-mentioned ratio significantly decreased ($P < 0.05$). However, when P20 was present in the ES ternary mixture at levels ≥50%, the influence of the above-mentioned ratio on the hardness of the samples became insignificant ($P \geq 0.05$). Figure 3 illustrates the hardness values of the PC samples of the ternary mixture of DSP, TSPP, and TSC depending on the maturity degree of the raw material and on the storage period of the samples obtained (2, 9, 30, and 60 d at 6 ± 2°C). Additionally, the same phenomenon as in the previous case (Figure 2) was noticed due to the subsistence of the specific ratio of DSP:TSPP (1:1). Furthermore, firmer PC samples were obtained with TSPP and TSC at a ratio of 1:1 (Figure 3) when the proportion of DSP in the ternary mixtures of ES was at zero concentrations ($P < 0.05$). In addition, any deviation from the previously mentioned ratio (TSPP:TSC, 1:1) led to a significant decrease in hardness ($P < 0.05$). The hardness of the PC samples decreased with the increasing proportion of DSP in the ternary mixtures ($P < 0.05$).

Figure 4 shows the values of hardness in the PC samples of the ternary mixture consisting of DSP, TSC, and P20 depending up the maturity degree of the raw material and the storage period of the product samples. The rising proportion of TSC and P20 (with a reduction in DSP) resulted in the increasing hardness of the PC samples. Moreover, comparing the effect of TSC and P20, the effect of P20 in the ternary mixture evoked a greater increase in the hardness of the samples. In the case of the ternary mixtures of ES composed of TSC, TSPP, and P20 (Figure 5), the harder samples were detected when TSC and TSPP were applied at a ratio of 1:1 and in the absence of P20 in the ternary mixture ($P < 0.05$). Nevertheless, when the proportion of P20 was gradually increased in the mixture, the hardness of the PC samples presented a slightly rising trend.

The main results obtained can be summarized as follows: the hardness of all PC samples increased with the prolonging storage period, regardless of the ternary mixture applied and the maturity degree of the STC. By the same token, it can be depicted that the hardness of all samples decreased with the increasing ripening stage of the STC used, regardless of the ES ternary mixture applied. Likewise, in all cases, the trends of hardness of the PC samples remained analogous; only the absolute values of firmness differed significantly ($P < 0.05$). The more unripe the STC applied, the harder the samples were observed.

The development of cohesiveness and relative adhesiveness was insignificant in terms of the application of the ternary mixtures of ES ($P \geq 0.05$). Nevertheless, the adhesiveness values increased depending on the maturity degree of the cheese (raw material), whereas the values of cohesiveness decreased. The values of relative adhesiveness of the samples prepared using STC with 4, 8, 12, and 16 wk of ripening were 0.11 to 0.17, 0.22 to 0.24, 0.25 to 0.27, and 0.28 to 0.29, respectively (the values are shown as the interval of d 2 to 60 of the analysis; N). Moreover, the values of cohesiveness of the samples prepared using STC with 4, 8, 12, and 16 wk of ripening were 0.50 to 0.54, 0.46 to 0.47, 0.44 to 0.47, and 0.33 to 0.35, respectively (the values are shown as the interval of d 2 to 60 of the analysis).

Rheological Analysis

Figure 6 illustrates the dependence of the complex modulus (G*) on frequency (in range of 0.01–100.00 Hz) for selected PC samples (after 30 d of storage at 6 ± 2°C) manufactured by applying STC as raw material with different maturity degrees (4, 8, 12, and 16 wk of maturity). Moreover, for these measurements DSP, TSPP, TSC, P20, and DSP:TSPP (in a ratio of 1:1) were used individually as ES. According to Figure 6, in all of the cases the complex modulus (G*) increased in the whole frequency range tested. Correspondingly, these results indicate that each ES (phosphate, citrate, or their combination) has a unique effect on the rheological properties of PC. The above-mentioned results were also evaluated by the data obtained from Winter's critical gel theory, where the gel strength (A_F) and the interaction factor (z) were estimated from the complex modulus (G*) according to the equation 2 and are illustrated in Tables 1 and 2. The findings are in harmony

with the results of TPA mentioned above. Above all, the specific ratio of DSP:TSPP (1:1) was also identified, resulting in the highest gel strength and interaction factor values, followed by P20, TSPP, TSC, and DSP, respectively, reporting the same trend that was demonstrated by TPA. Similarly, the ratio of TSPP:TSC (1:1) was recognized leading to a significant rise in the data obtained from Winter's critical gel theory. Last but not least, the values of the gel strength and the interaction factor decreased with the increasing maturity degree of the STC used. Therefore, it could be assumed that the higher the gel strength of the sample, the more rigid product can be expected ($P < 0.05$).

DISCUSSION

The key factors influencing the PC properties are mainly ES (type and amount), the type and maturity degree of the cheese used (as raw material), and the processing and storage conditions. The DM content and pH values of the PC samples tested were in narrow intervals, which is crucial for maintaining the comparability of the PC studied (Marchesseau et al., 1997; Lee and Klostermeyer, 2001; Lee et al., 2004; Piska and Štětina, 2004). In addition, because the cheese maturity degree is a term closely related to the extent of proteolysis in cheese during ripening, it plays an important

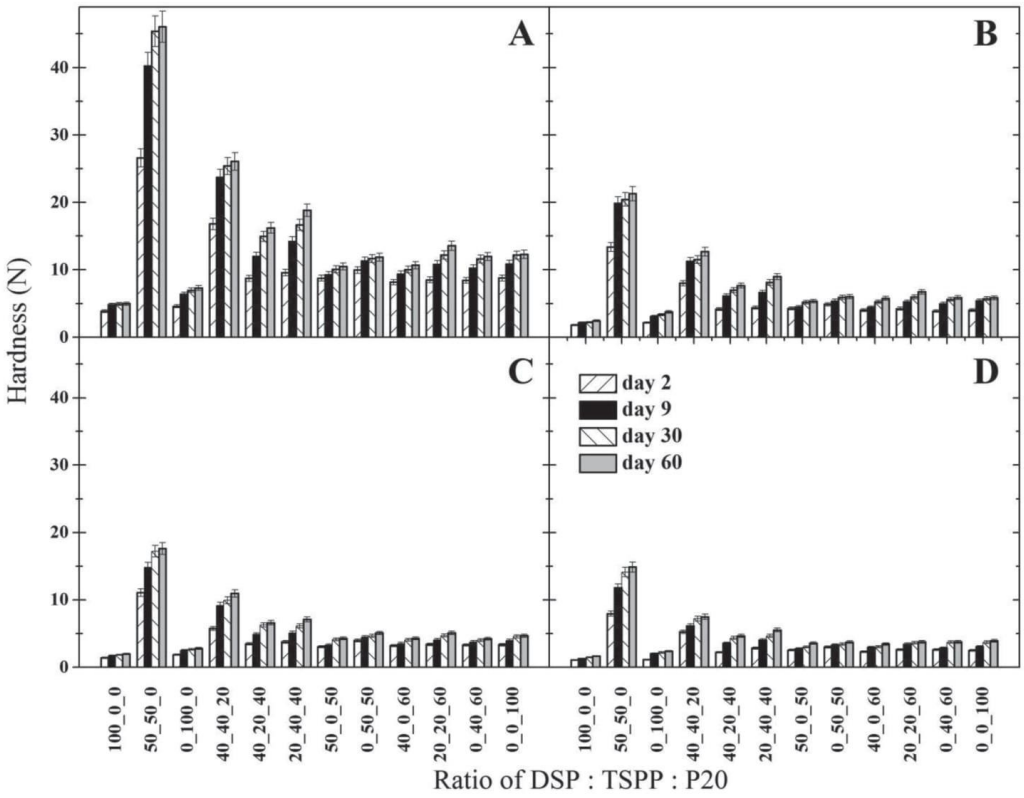

Figure 2. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%; axis x) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), and sodium salt of polyphosphate (P20) in a ternary mixture of emulsifying salts during 60 d of storage at 6°C [n = 6; the results were expressed as means (columns) ± standard deviations (bars); processed cheeses were sampled after 2, 9, 30, and 60 d of storage]. Processed cheeses were made from Swiss-type cheese after different times of ripening (part A, 4 wk; part B, 8 wk; part C, 12 wk; part D, 16 wk).

role in determining its textural and sensory properties. Thus, the concentrations of FAA (in the raw material) showed a clear tendency to increase with the ripening period as expected, because during proteolysis these compounds were released by the proteolytic agents, mainly by microbial enzymes, through the biochemical reactions evolving during cheese ripening (Hayaloglu et al., 2004; Ji et al., 2004; Poveda et al., 2004). These results agree with those of Vicente et al. (2001), Bustamante et al. (2003), and Pachlová et al. (2011), who reported a significant response between the FAA content and the ripening time. Based on our results, regardless of the maturity degree of the STC applied and the storage period of the samples obtained, the sole application of phosphate ES with longer length chains resulted in increasing hardness of the samples. However, the individual application of TSC led to similar results in hardness as that of TSPP. On the whole, it can be generalized that the sole application of ES resulted in rising hardness of the samples in the following order: DSP < TSC ≈ TSPP < P20. The same trend was reported by El-Bakry et al. (2011), Weiserová et al. (2011), and Nagyová et al. (2014). This could be explained by the fact that phosphates of longer chain length affect the ion-exchange intensity (of sodium ions for calcium ions). Moreover, this intensity (of ion-

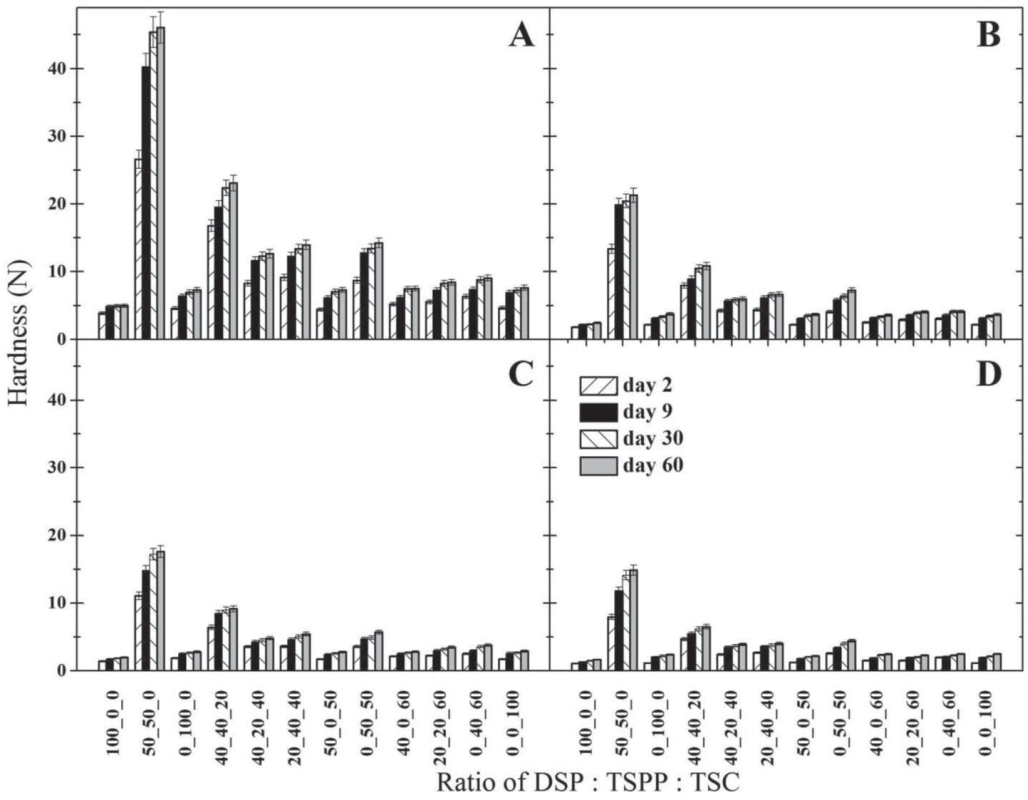

Figure 3. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%; axis x) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), and trisodium citrate (TSC) in a ternary mixture of emulsifying salts during 60 d of storage at 6°C [n = 6; the results were expressed as means (columns) ± standard deviations (bars); processed cheeses were sampled after 2, 9, 30, and 60 d of storage]. Processed cheeses were made from Swiss-type cheese after different times of ripening (part A, 4 wk; part B, 8 wk; part C, 12 wk; part D, 16 wk).

exchange) is increasing with the dispersion of casein intensity, resulting from the ES addition (Mizuno and Lucey, 2007; Dimitreli and Thomareis, 2009; Buňka et al., 2013). According to the previous studies, the PC matrix formation is in close dependence with casein dispersion in the system tested (Dimitreli and Thomareis, 2009; Cunha et al., 2013). Therefore, a higher degree of casein dispersion enhances casein developing their hydrating and emulsifying abilities; the latter abilities increase the intensity of interactions in the melt (El-Bakry et al., 2011; Kaliappan and Lucey, 2011). In general, it can be assumed that PC with higher values of hardness was obtained when the ternary mixture of ES applied was composed of salts with more intensive ability to disperse casein. The samples prepared with the addition of binary mixture of DSP:TSPP (in a ratio of 1:1) resulted in products with the highest values of hardness (regardless of the maturity degree of the STC tested). A possible explanation of this phenomenon, reported by Mizuno and Lucey (2007) and Buňka et al. (2013), could be the ability of diphosphates to enhance the gel formation ability of casein. However, insufficient concentrations of disphosphates may lead to the formation of very weak gels (Mizuno and Lucey, 2007). Moreover, another possible explanation of the significance of DSP:TSPP specific ratio (1:1) was reported by Kaliap-

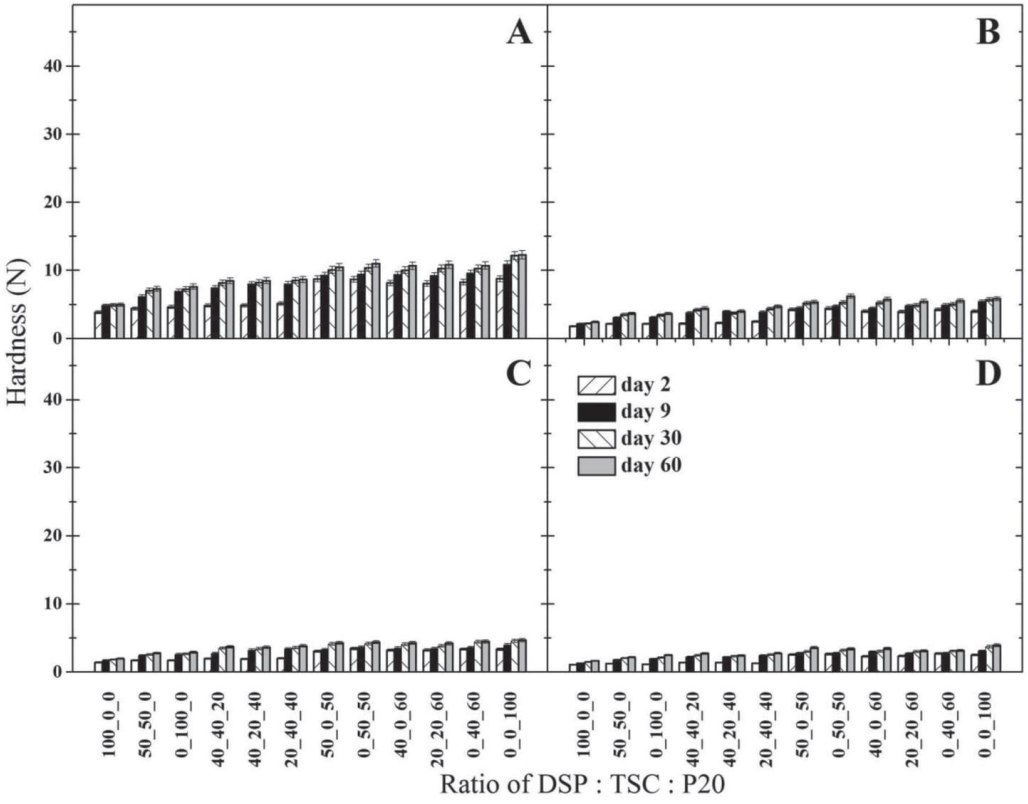

Figure 4. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%; axis x) of disodium phosphate (DSP), trisodium citrate (TSC), and sodium salt of polyphosphate (P20) in a ternary mixture of emulsifying salts during 60 d of storage at 6°C [n = 6; the results were expressed as means (columns) ± standard deviations (bars); processed cheeses were sampled after 2, 9, 30, and 60 d of storage]. Processed cheeses were made from Swiss-type cheese after different times of ripening (part A, 4 wk; part B, 8 wk; part C, 12 wk; part D, 16 wk).

pan and Lucey (2011). It is based on the ability of monophosphates to amplify the development of bridges among diphosphates, calcium ions, and casein. The observed decrease in PC hardness in the ternary mixture of DSP, TSPP, and P20 when the proportion of P20 was increased could be caused by the ability of polyphosphates to charge casein with multiple negative ions, which leads to lower-intensity hydrophobic interactions between the dispersed casein (Mizuno and Lucey, 2007; Buňka et al., 2013; Salek et al., 2015). Furthermore, the specific ratio of TSPP:TSC (1:1) resulted in increasing hardness of the samples (Figures 2 and 4). According to the available literature, a clear answer has still not been found elucidating this phenomenon with respect to the interactions occurring between TSPP and TSC and their influence on the development of casein matrix. In addition, an analogous phenomenon was reported in the work of Salek et al. (2015). However, TSC does not appear to have the ability to create new networks and thus the influence of TSPP on casein crosslinking would not be affected by the presence of TSC (Lu et al., 2007; Mizuno and Lucey, 2007; Kaliappan and Lucey, 2011). The PC samples were produced using STC with 4 different maturity degrees (4, 8, 12, and 16 wk of ripening). The hardness of the samples obtained decreased with the increasing maturity degree of the STC

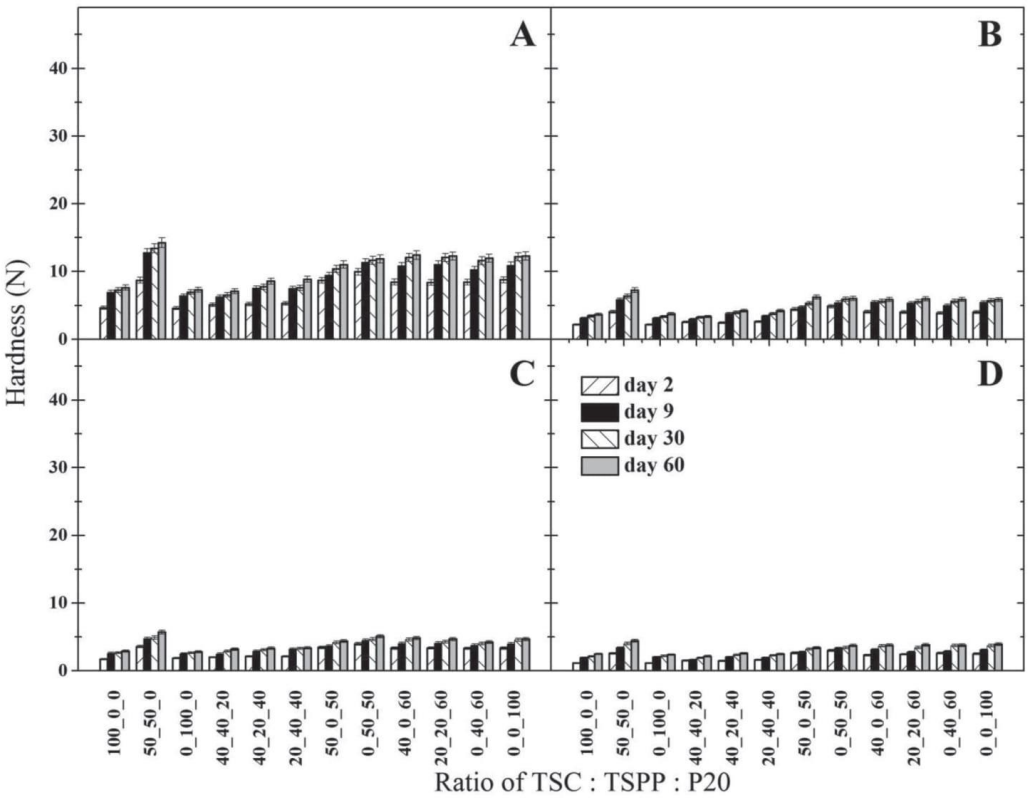

Figure 5. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%; axis x) of trisodium citrate (TSC), tetrasodium diphosphate (TSPP), and sodium salt of polyphosphate (P20) in a ternary mixture of emulsifying salts during 60 d of storage at 6°C [n = 6; the results were expressed as means (columns) ± standard deviations (bars); processed cheeses were sampled after 2, 9, 30, and 60 d of storage]. Processed cheeses were made from Swiss-type cheese after different time of ripening (part A, 4 wk; part B, 8 wk; part C, 12 wk; part D, 16 wk).

used, regardless of the ternary mixture of ES applied. Moreover, in all of the cases (different types of ternary mixtures of ES), the trends of hardness development were similar and only the absolute values were different. Additionally, a more extensive course of proteolysis occurs with an increasing ripening period. Therefore,

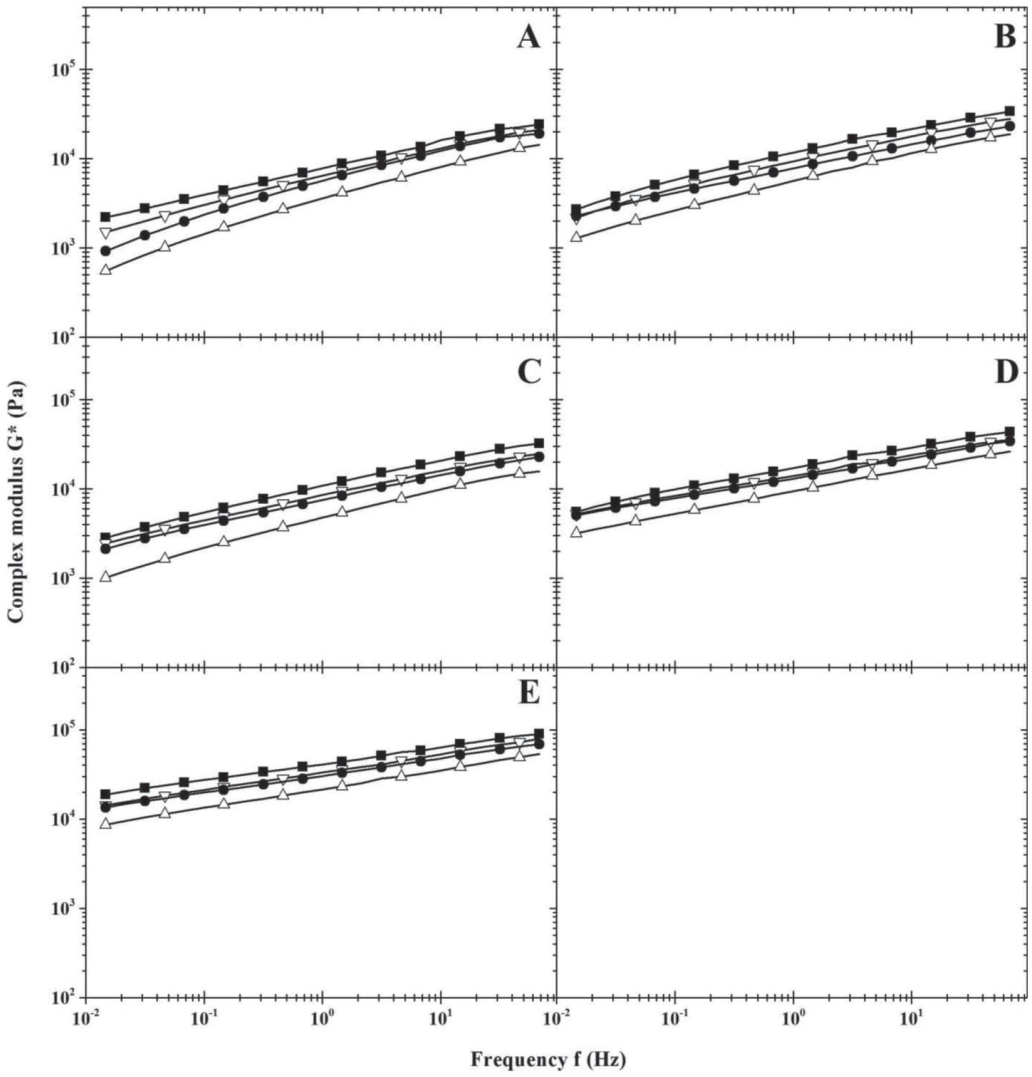

Figure 6. The dependence of complex modulus (G*) of processed cheese (after 30 d of storage) made from Swiss-type cheese after different time of ripening (■ 4 wk; ▽ 8 wk; ● 12 wk; Δ 16 wk) on frequency (f; in range of 0.01–100.00 Hz). Processed cheeses were manufactured using disodium phosphate (DSP; part A), tetrasodium diphosphate (TSPP; part B), trisodium citrate (TSC; part C), sodium salt of polyphosphate (P20; part D), or binary mixture of DSP and TSPP in ratio of 1:1 (part E).

the casein chains of shorter average length affect the properties of the final product [i.e., a final product with less compact casein matrix may be formed (Piska and Štětina, 2004; Brickley et al., 2007; Hladká et al., 2014)]. On the contrary, the hardness of all PC samples increased significantly with an increasing storage period (regardless of the ES ternary mixture applied and the maturity degree of STC). This current tendency of

Table 1. Values of gel strength (A_F; kPa·s$^{1/z}$) of processed cheese (after 30-d storage) made from Swiss-type cheese after different times of ripening (4, 8, 12, and 16 wk)[1]

Type of ternary mixture	Ratio of salts (%)	Time of ripening of raw material for processed cheese production			
		4 wk	8 wk	12 wk	16 wk
DSP:TSPP:P20	100:0:0	8.2 ± 0.6^{a,A}	6.6 ± 0.2^{a,B}	5.6 ± 0.4^{a,C}	3.5 ± 0.2^{a,D}
	50:50:0	41.1 ± 2.8^{i,A}	32.5 ± 1.9^{i,B}	30.6 ± 1.4^{i,B}	21.6 ± 1.2^{h,C}
	0:100:0	11.5 ± 0.7^{b,A}	9.2 ± 0.5^{b,B}	7.8 ± 0.3^{b,C}	5.6 ± 0.3^{b,D}
	40:40:20	27.9 ± 0.9^{h,A}	23.1 ± 1.1^{h,B}	18.1 ± 0.9^{h,C}	16.5 ± 1.2^{g,D}
	40:20:40	19.6 ± 0.8^{f,A}	17.1 ± 1.2^{f,B}	15.2 ± 0.6^{f,C}	14.1 ± 0.6^{f,C}
	20:40:40	21.5 ± 1.0^{g,A}	18.7 ± 0.2^{g,B}	16.9 ± 0.7^{g,C}	14.9 ± 0.8^{f,D}
	50:0:50	13.9 ± 0.6^{c,A}	11.4 ± 0.5^{c,B}	9.2 ± 0.5^{c,C}	7.5 ± 0.3^{c,D}
	0:50:50	15.0 ± 0.7^{d,A}	12.9 ± 0.4^{d,B}	9.9 ± 0.5^{cd,C}	8.4 ± 0.4^{d,D}
	40:0:60	13.9 ± 0.6^{c,A}	12.0 ± 0.8^{c,B}	9.5 ± 0.5^{c,C}	7.9 ± 0.5^{cd,D}
	20:20:60	17.3 ± 1.0^{e,A}	14.0 ± 0.6^{a,B}	13.0 ± 0.6^{e,C}	9.1 ± 0.6^{e,D}
	0:40:60	15.8 ± 1.0^{d,A}	13.0 ± 0.7^{d,B}	10.1 ± 0.3^{d,C}	8.0 ± 0.3^{d,D}
	0:0:100	17.0 ± 1.1^{e,A}	14.0 ± 0.5^{e,B}	13.1 ± 0.7^{e,C}	9.4 ± 0.5^{e,D}
DSP:TSPP:TSC	100:0:0	8.2 ± 0.6^{a,A}	6.6 ± 0.2^{a,B}	5.6 ± 0.4^{a,C}	3.5 ± 0.2^{a,D}
	50:50:0	41.1 ± 2.8^{g,A}	32.5 ± 1.9^{g,B}	30.6 ± 1.4^{f,B}	21.6 ± 1.2^{f,C}
	0:100:0	11.5 ± 0.7^{de,A}	9.2 ± 0.5^{d,B}	7.8 ± 0.3^{c,C}	5.6 ± 0.3^{c,D}
	40:40:20	19.8 ± 0.8^{f,A}	18.5 ± 0.9^{f,B}	15.9 ± 0.6^{e,C}	14.5 ± 0.8^{a,D}
	40:20:40	11.6 ± 0.7^{de,A}	11.0 ± 0.5^{e,B}	9.1 ± 0.5^{d,C}	7.4 ± 0.4^{d,D}
	20:40:40	12.6 ± 0.4^{e,A}	11.6 ± 0.5^{e,B}	9.3 ± 0.4^{d,C}	8.6 ± 0.5^{e,D}
	50:0:50	9.0 ± 0.5^{b,A}	7.5 ± 0.4^{b,B}	6.5 ± 0.3^{b,C}	4.5 ± 0.2^{b,D}
	0:50:50	13.1 ± 0.8^{e,A}	11.7 ± 0.7^{e,B}	9.4 ± 0.4^{d,C}	8.3 ± 0.4^{e,D}
	40:0:60	9.2 ± 0.5^{b,A}	7.7 ± 0.4^{b,B}	7.0 ± 0.3^{bc,C}	4.2 ± 0.1^{b,D}
	20:20:60	9.6 ± 0.6^{b,A}	8.0 ± 0.3^{bc,B}	7.4 ± 0.2^{c,C}	4.5 ± 0.3^{b,D}
	0:40:60	10.2 ± 0.6^{c,A}	8.2 ± 0.5^{c,B}	7.4 ± 0.5^{c,C}	4.6 ± 0.3^{b,D}
	0:0:100	10.9 ± 0.5^{cd,A}	8.5 ± 0.5^{c,B}	7.6 ± 0.4^{c,C}	4.8 ± 0.3^{b,D}
DSP:TSC:P20	100:0:0	8.2 ± 0.6^{a,A}	6.6 ± 0.2^{a,B}	5.6 ± 0.4^{a,C}	3.5 ± 0.2^{a,D}
	50:50:0	9.0 ± 0.5^{b,A}	7.5 ± 0.4^{b,B}	6.5 ± 0.3^{b,C}	4.5 ± 0.2^{b,D}
	0:100:0	10.9 ± 0.5^{c,A}	8.5 ± 0.5^{c,B}	7.6 ± 0.4^{c,C}	4.8 ± 0.3^{b,D}
	40:40:20	11.8 ± 0.6^{d,A}	9.7 ± 0.3^{d,B}	8.0 ± 0.4^{cd,C}	5.6 ± 0.4^{c,D}
	40:20:40	12.3 ± 0.5^{d,A}	10.4 ± 0.5^{d,B}	8.4 ± 0.4^{d,C}	6.5 ± 0.3^{d,D}
	20:40:40	12.4 ± 0.7^{d,A}	11.2 ± 0.6^{e,B}	8.8 ± 0.4^{d,C}	6.6 ± 0.3^{d,D}
	50:0:50	13.9 ± 0.6^{e,A}	11.4 ± 0.5^{e,B}	9.2 ± 0.5^{d,C}	7.5 ± 0.3^{e,D}
	0:50:50	14.4 ± 0.7^{e,A}	12.5 ± 0.8^{f,B}	10.4 ± 0.4^{e,C}	8.5 ± 0.5^{f,D}
	40:0:60	13.9 ± 0.6^{e,A}	12.0 ± 0.8^{ef,B}	9.5 ± 0.5^{d,C}	7.9 ± 0.5^{ef,D}
	20:20:60	14.2 ± 0.5^{e,A}	12.6 ± 0.5^{f,B}	9.9 ± 0.8^{de,C}	8.4 ± 0.5^{f,D}
	0:40:60	14.4 ± 0.7^{e,A}	12.7 ± 0.6^{f,B}	10.0 ± 0.4^{e,C}	8.4 ± 0.4^{f,D}
	0:0:100	17.0 ± 1.1^{f,A}	14.0 ± 0.5^{g,B}	13.1 ± 0.7^{f,C}	9.4 ± 0.5^{g,D}
TSC:TSPP:P20	100:0:0	10.9 ± 0.5^{a,A}	8.5 ± 0.5^{a,B}	7.6 ± 0.4^{a,C}	4.8 ± 0.3^{a,D}
	50:50:0	13.1 ± 0.8^{c,A}	11.7 ± 0.7^{d,B}	9.4 ± 0.4^{b,C}	8.3 ± 0.4^{d,D}
	0:100:0	11.5 ± 0.7^{b,A}	9.2 ± 0.5^{b,B}	7.8 ± 0.3^{a,C}	5.6 ± 0.3^{b,D}
	40:40:20	10.9 ± 0.6^{a,A}	9.0 ± 0.6^{ab,B}	7.9 ± 0.4^{a,C}	5.3 ± 0.2^{b,D}
	40:20:40	11.9 ± 0.5^{b,A}	10.8 ± 0.5^{c,B}	9.2 ± 0.5^{b,C}	6.6 ± 0.4^{c,D}
	20:40:40	12.7 ± 0.5^{c,A}	11.6 ± 0.6^{d,B}	9.6 ± 0.4^{b,C}	7.0 ± 0.4^{c,D}
	50:0:50	14.4 ± 0.7^{d,A}	12.5 ± 0.8^{e,B}	10.4 ± 0.4^{c,C}	8.5 ± 0.5^{d,D}
	0:50:50	15.0 ± 0.7^{d,A}	12.9 ± 0.4^{e,B}	9.9 ± 0.5^{b,C}	8.4 ± 0.4^{d,D}
	40:0:60	15.8 ± 0.8^{e,A}	13.0 ± 0.7^{e,B}	10.0 ± 0.4^{bc,C}	8.3 ± 0.4^{d,D}
	20:20:60	15.4 ± 0.7^{de,A}	12.8 ± 0.8^{e,B}	10.2 ± 0.5^{bc,C}	8.3 ± 0.5^{d,D}
	0:40:60	15.8 ± 1.0^{e,A}	13.0 ± 0.7^{e,B}	10.1 ± 0.3^{bc,C}	8.0 ± 0.3^{d,D}
	0:0:100	17.0 ± 1.1^{f,A}	14.0 ± 0.5^{f,B}	13.1 ± 0.7^{d,C}	9.4 ± 0.5^{e,D}

[a–i]The means within a column (the difference between samples with different ratio of emulsifying salts in the ternary mixture) followed by different superscript letters differ ($P < 0.05$); samples with each type of the ternary mixture (DSP:TSPP:P20, DSP:TSPP:TSC, DSP:TSC:P20, and TSC:TSPP:P20) were evaluated independently.

[A–D]The means within a row (the difference between samples with various times of Swiss-type cheese ripening) followed by different uppercase letters differ ($P < 0.05$); samples with each ratio of emulsifying salts in each ternary mixture were evaluated independently.

[1]Different ternary mixtures of emulsifying salts [disodium phosphate (DSP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate (P20), and trisodium citrate (TSC)] were used for manufacture of model samples. Amount of individual emulsifying salts in ternary mixture were expressed in percentage (percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%).

Table 2. Values of interaction factor (z) of processed cheese (after 30-d storage) made from Swiss-type cheese after different times of ripening (4, 8, 12, and 16 wk)[1]

Type of ternary mixture	Ratio of salts (%)	Time of ripening of raw material for processed cheese production			
		4 wk	8 wk	12 wk	16 wk
DSP:TSPP:P20	100:0:0	$3.60 \pm 0.18^{a,A}$	$3.44 \pm 0.11^{a,B}$	$3.12 \pm 0.10^{a,C}$	$2.95 \pm 0.13^{a,D}$
	50:50:0	$5.35 \pm 0.19^{g,A}$	$5.15 \pm 0.17^{g,B}$	$4.91 \pm 0.21^{e,C}$	$4.70 \pm 0.14^{g,C}$
	0:100:0	$3.81 \pm 0.17^{b,A}$	$3.80 \pm 0.11^{b,A}$	$3.70 \pm 0.15^{b,B}$	$3.38 \pm 0.14^{b,C}$
	40:40:20	$4.80 \pm 0.14^{f,A}$	$4.59 \pm 0.14^{f,B}$	$4.36 \pm 0.15^{d,C}$	$4.26 \pm 0.22^{f,C}$
	40:20:40	$4.18 \pm 0.13^{c,A}$	$4.01 \pm 0.18^{c,B}$	$3.89 \pm 0.15^{bc,C}$	$3.75 \pm 0.18^{c,D}$
	20:40:40	$4.50 \pm 0.18^{de,A}$	$4.26 \pm 0.19^{d,B}$	$4.09 \pm 0.14^{c,C}$	$3.88 \pm 0.11^{cd,D}$
	50:0:50	$4.12 \pm 0.13^{c,A}$	$3.92 \pm 0.12^{bc,B}$	$3.80 \pm 0.17^{bc,C}$	$3.65 \pm 0.12^{c,D}$
	0:50:50	$4.50 \pm 0.12^{de,A}$	$4.33 \pm 0.15^{e,B}$	$4.12 \pm 0.19^{c,C}$	$3.92 \pm 0.25^{d,D}$
	40:0:60	$4.18 \pm 0.15^{c,A}$	$4.11 \pm 0.15^{cd,B}$	$3.92 \pm 0.19^{c,C}$	$3.75 \pm 0.07^{c,D}$
	20:20:60	$4.40 \pm 0.20^{d,A}$	$4.17 \pm 0.14^{d,B}$	$4.00 \pm 0.20^{c,C}$	$3.84 \pm 0.20^{cd,C}$
	0:40:60	$4.35 \pm 0.16^{d,A}$	$4.21 \pm 0.17^{de,B}$	$4.03 \pm 0.14^{c,C}$	$3.95 \pm 0.16^{d,C}$
	0:0:100	$4.63 \pm 0.15^{e,A}$	$4.37 \pm 0.22^{e,B}$	$4.22 \pm 0.21^{d,C}$	$4.04 \pm 0.16^{e,C}$
DSP:TSPP:TSC	100:0:0	$3.60 \pm 0.18^{a,A}$	$3.44 \pm 0.11^{a,B}$	$3.12 \pm 0.10^{a,C}$	$2.95 \pm 0.13^{a,D}$
	50:50:0	$5.35 \pm 0.19^{f,A}$	$5.15 \pm 0.17^{e,B}$	$4.91 \pm 0.21^{e,C}$	$4.70 \pm 0.14^{f,C}$
	0:100:0	$3.81 \pm 0.17^{b,A}$	$3.80 \pm 0.11^{b,A}$	$3.70 \pm 0.15^{b,B}$	$3.38 \pm 0.14^{b,C}$
	40:40:20	$4.46 \pm 0.12^{e,A}$	$4.29 \pm 0.14^{d,B}$	$4.17 \pm 0.18^{d,C}$	$4.05 \pm 0.15^{c,D}$
	40:20:40	$4.16 \pm 0.24^{c,A}$	$4.07 \pm 0.15^{c,A}$	$3.98 \pm 0.19^{c,B}$	$3.96 \pm 0.12^{de,B}$
	20:40:40	$4.30 \pm 0.25^{d,A}$	$4.27 \pm 0.23^{d,A}$	$4.12 \pm 0.16^{d,B}$	$4.07 \pm 0.19^{e,B}$
	50:0:50	$4.20 \pm 0.21^{cd,A}$	$4.11 \pm 0.16^{c,A}$	$3.98 \pm 0.18^{c,B}$	$3.92 \pm 0.20^{d,B}$
	0:50:50	$4.21 \pm 0.20^{cd,A}$	$4.13 \pm 0.15^{c,B}$	$4.00 \pm 0.10^{c,C}$	$3.91 \pm 0.16^{d,C}$
	40:0:60	$3.88 \pm 0.16^{b,A}$	$3.77 \pm 0.13^{b,B}$	$3.69 \pm 0.14^{b,B}$	$3.48 \pm 0.15^{c,C}$
	20:20:60	$3.88 \pm 0.20^{b,A}$	$3.76 \pm 0.08^{b,B}$	$3.68 \pm 0.19^{b,C}$	$3.53 \pm 0.17^{c,D}$
	0:40:60	$3.94 \pm 0.24^{b,A}$	$3.76 \pm 0.09^{b,B}$	$3.67 \pm 0.16^{b,C}$	$3.51 \pm 0.16^{c,D}$
	0:0:100	$3.80 \pm 0.15^{b,A}$	$3.76 \pm 0.15^{b,A}$	$3.61 \pm 0.14^{b,B}$	$3.51 \pm 0.18^{c,B}$
DSP:TSC:P20	100:0:0	$3.60 \pm 0.18^{a,A}$	$3.44 \pm 0.11^{a,B}$	$3.12 \pm 0.10^{a,C}$	$2.95 \pm 0.13^{a,D}$
	50:50:0	$4.20 \pm 0.21^{c,A}$	$4.11 \pm 0.16^{d,A}$	$3.98 \pm 0.18^{d,B}$	$3.92 \pm 0.20^{d,B}$
	0:100:0	$3.80 \pm 0.15^{b,A}$	$3.76 \pm 0.15^{b,A}$	$3.61 \pm 0.14^{b,B}$	$3.51 \pm 0.18^{b,B}$
	40:40:20	$3.89 \pm 0.14^{b,A}$	$3.77 \pm 0.18^{b,B}$	$3.77 \pm 0.19^{c,B}$	$3.59 \pm 0.13^{bc,C}$
	40:20:40	$3.89 \pm 0.19^{b,A}$	$3.82 \pm 0.12^{bc,A}$	$3.65 \pm 0.10^{bc,B}$	$3.61 \pm 0.21^{bc,B}$
	20:40:40	$3.86 \pm 0.15^{b,A}$	$3.81 \pm 0.13^{bc,A}$	$3.67 \pm 0.16^{bc,B}$	$3.61 \pm 0.14^{bc,B}$
	50:0:50	$4.12 \pm 0.13^{c,A}$	$3.92 \pm 0.12^{c,B}$	$3.80 \pm 0.17^{c,C}$	$3.65 \pm 0.12^{c,D}$
	0:50:50	$4.19 \pm 0.21^{c,A}$	$4.10 \pm 0.14^{d,B}$	$3.93 \pm 0.17^{cd,C}$	$3.70 \pm 0.21^{c,D}$
	40:0:60	$4.18 \pm 0.15^{c,A}$	$4.11 \pm 0.15^{d,B}$	$3.92 \pm 0.19^{cd,C}$	$3.75 \pm 0.07^{c,D}$
	20:20:60	$4.43 \pm 0.22^{d,A}$	$4.16 \pm 0.09^{d,B}$	$4.05 \pm 0.08^{d,C}$	$3.92 \pm 0.12^{d,D}$
	0:40:60	$4.40 \pm 0.15^{d,A}$	$4.21 \pm 0.15^{d,B}$	$4.05 \pm 0.20^{d,C}$	$3.91 \pm 0.18^{d,D}$
	0:0:100	$4.63 \pm 0.15^{e,A}$	$4.37 \pm 0.22^{e,B}$	$4.22 \pm 0.21^{e,C}$	$4.04 \pm 0.16^{e,D}$
TSC:TSPP:P20	100:0:0	$3.80 \pm 0.15^{a,A}$	$3.76 \pm 0.15^{a,A}$	$3.61 \pm 0.14^{a,B}$	$3.51 \pm 0.18^{b,B}$
	50:50:0	$4.21 \pm 0.20^{c,A}$	$4.13 \pm 0.15^{b,B}$	$4.00 \pm 0.10^{b,C}$	$3.91 \pm 0.16^{d,C}$
	0:100:0	$3.81 \pm 0.17^{a,A}$	$3.80 \pm 0.11^{a,A}$	$3.70 \pm 0.15^{a,B}$	$3.38 \pm 0.14^{a,C}$
	40:40:20	$3.80 \pm 0.07^{a,A}$	$3.70 \pm 0.11^{a,A}$	$3.56 \pm 0.24^{a,B}$	$3.52 \pm 0.12^{b,B}$
	40:20:40	$3.93 \pm 0.12^{ab,A}$	$3.82 \pm 0.13^{a,A}$	$3.58 \pm 0.16^{a,B}$	$3.49 \pm 0.21^{b,B}$
	20:40:40	$3.96 \pm 0.12^{b,A}$	$3.78 \pm 0.16^{a,B}$	$3.58 \pm 0.12^{a,C}$	$3.47 \pm 0.15^{b,C}$
	50:0:50	$4.19 \pm 0.21^{c,A}$	$4.10 \pm 0.14^{b,B}$	$3.93 \pm 0.17^{b,C}$	$3.70 \pm 0.21^{c,D}$
	0:50:50	$4.50 \pm 0.12^{e,A}$	$4.33 \pm 0.15^{c,B}$	$4.12 \pm 0.19^{c,C}$	$3.92 \pm 0.25^{d,D}$
	40:0:60	$4.33 \pm 0.16^{d,A}$	$4.26 \pm 0.16^{bc,B}$	$4.02 \pm 0.19^{bc,C}$	$3.91 \pm 0.11^{d,D}$
	20:20:60	$4.28 \pm 0.14^{d,A}$	$4.24 \pm 0.25^{bc,A}$	$3.99 \pm 0.17^{b,B}$	$3.89 \pm 0.13^{d,B}$
	0:40:60	$4.35 \pm 0.16^{d,A}$	$4.21 \pm 0.17^{b,B}$	$4.03 \pm 0.14^{bc,C}$	$3.95 \pm 0.16^{d,C}$
	0:0:100	$4.63 \pm 0.15^{f,A}$	$4.37 \pm 0.22^{c,B}$	$4.22 \pm 0.21^{d,C}$	$4.04 \pm 0.16^{c,D}$

[a–g]The means within a column (the difference between samples with different ratio of emulsifying salts in the ternary mixture) followed by different lowercase letters differ ($P < 0.05$); samples with each type of the ternary mixture (DSP:TSPP:P20; DSP:TSPP:TSC; DSP:TSC:P20; TSC:TSPP:P20 were evaluated independently.

[A–D]The means within a line (the difference between samples with various times of Swiss-type cheese ripening) followed by different uppercase letters differ ($P < 0.05$); samples with each ratio of emulsifying salts in each ternary mixture were evaluated independently.

[1]Different ternary mixtures of emulsifying salts [disodium phosphate (DSP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate (P20), and trisodium citrate (TSC)] were used for manufacture of model samples. Amount of individual emulsifying salts in ternary mixture were expressed in percentage (percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%).

hardness development could mainly be elucidated in the hydrolysis of ES (with 2 or more phosphorus atoms in a molecule) and related to the protein matrix formation. Also, another possible explanation may lie in the changes in the binding forms of the ES present, leading to an adjustment to their characteristics of dissociation (Guinee et al., 2004; Shirashoji et al., 2006; Weiserová et al., 2011). The results of TPA were confirmed by

those of the rheological analysis. Likewise, higher values of the complex modulus (G*) indicate the increase in firmness of the PC samples. Furthermore, these data of the complex modulus (G*) of PC were also in accordance with those obtained from Winter's critical gel theory. The higher monitored values of the gel strength can probably be explained by more intensive interactions occurring in the PC samples (the values of the interaction factor are shown in Table 2) such as hydrogen bonds, hydrophobic interactions between caseins and fat or calcium-intervened electrostatic bonds among caseins, leading to the formation of a "denser" (more intensive) network structure. On the other hand, the reduced values of the gel strength observed during STC course of proteolysis could have been caused by a drop in the number of interactions in the PC matrix (Černíková et al., 2008; Kapoor and Metzger, 2008). Presumably, the novelty of this work lies in the fact that the textural and rheological properties of the PC manufactured from STC of different degrees of maturity and different composition of ES ternary mixtures have not been found in the literature.

CONCLUSIONS

The application of the binary mixture of DSP:TSPP (in a ratio of 1:1) resulted in products with the highest values of hardness (regardless of the maturity degree of the STC applied). Furthermore, the hardness of the samples obtained decreased with the rising maturity degree of the STC used (regardless of the ES mixture applied). However, on the contrary, the hardness of all PC samples increased with prolonging the storage period. Admittedly, the results of TPA corresponded to those of the rheological analysis. The highest overall rigidity (G*), gel strength, and interaction factor values were found in the samples prepared with DSP:TSPP (1:1), followed by the samples prepared with P20, TSPP, TSC, and DSP, respectively. The monitored values of the gel strength and interaction factor decreased with the increasing maturity degree of the STC used. The intensity of rigidity of the PC samples has an analogous relationship to the intensity of the gel strength; the higher the gel strength of the sample, the more inflexible the product that can be expected.

ACKNOWLEDGMENTS

This study was kindly supported by a project of the internal grants of Tomas Bata University in Zlin, Czech Republic, no. IGA/FT/2014/001 and IGA/FT/2015/004 funded from the resources of specific university research.

REFERENCES

Beuvier, E., K. Berthaud, S. Cegarra, A. Dasen, S. Pochet, S. Buchin, and G. Duboz. 1997. Ripening and quality of Swiss-type cheese made from raw, pasteurized or microfiltered milk. Int. Dairy J. 7:311–323.

Bouton, Y., S. Buchin, G. Duboz, S. Pochet, and E. Beuvier. 2009. Effect of mesophilic lactobacilli and enterococci adjunct cultures on the final characteristics of a microfiltered milk Swiss-type cheese. Food Microbiol. 26:183–191.

Brickley, C. A., M. A. E. Auty, P. Piraino, and P. L. H. McSweeney. 2007. The effect of natural Cheddar cheese ripening on the functional and textural properties of the processed cheese manufactured therefrom. J. Food Sci. 72:C483–C490.

Buňka, F., L. Doudová, E. Weiserová, D. Kuchař, J. Michálek, S. Slavíková, and S. Kráčmar. 2012. The effect of different ternary mixtures of sodium phosphates on hardness of processed cheese spreads. Int. J. Food Sci. Technol. 47:2063–2071.

Buňka, F., L. Doudová, E. Weiserová, D. Kuchař, P. Ponížil, D. Začalová, G. Nagyová, V. Pachlová, and J. Michálek. 2013. The effect of ternary emulsifying salt composition and cheese maturity on the textural properties of processed cheese. Int. Dairy J. 29:1–7.

Buňka, F., L., E. Doudová, M. Weiserová, D. Černíková, S. Kuchař, G. Slavíková, P. Nagyováa, T. Ponížile, Grůberd, and J. Michálek. 2014. The effect of concentration and composition of ternary emulsifying salts on the textural properties of processed cheese spreads. LWT Food Sci. Technol. 58:247–255.

Buňková, L., F. Buňka, M. Hlobilová, Z. Vaňatková, D. Nováková, and V. Dráb. 2009. Tyramine production of technological important strains of *Lactobacillus, Lactococcus* and *Streptococcus*. Eur. Food Res. Technol. 229:533–538.

Bustamante, M. A., M. Virto, M. Aramburu, L. J. R. Barron, F. J. Pérez-Elortondo, M. Albisu, and M. de Renobles. 2003. Lamb rennet paste in ovine cheese (Idiazabal) manufacture. Proteolysis and relationship between analytical and sensory parameters. Int. Dairy J. 13:547–557.

Černíková, M., F. Buňka, V. Pavlínek, P. Březina, J. Hrabě, and P. Valášek. 2008. Effect of carrageenan type on viscoelastic properties of processed cheese. Food Hydrocoll. 22:1054–1061.

Chen, L., and H. Liu. 2012. Effect of emulsifying salts on the physicochemical properties of processed cheese made from Mozzarella. J. Dairy Sci. 95:4823–4830.

Cunha, C. R., R. Grimaldi, M. R. Alcântara, and W. H. Viotto. 2013. Effect of the type of fat on rheology, functional properties and sensory acceptance of spreadable cheese analogue. Int. J. Dairy Technol. 66:54–62.

Dimitreli, G., and A. S. Thomareis. 2007. Texture evaluation of block-type processed cheese as a function of chemical composition and in relation to its apparent viscosity. J. Food Eng. 79:1364–1373.

Dimitreli, G., and A. S. Thomareis. 2009. Instrumental textural and viscoelastic properties of processed cheese as affected by emulsifying salts and in relation to its apparent viscosity. Int. J. Food Prop. 12:261–275.

El-Bakry, M., E. Duggan, E. D. O'Riordan, and M. O'Sullivan. 2011. Effect of chelating salt type on casein hydration and fat emulsification during manufacture and post-manufacture functionality of imitation cheese. J. Food Eng. 102:145–153.

Gabriele, D., B. de Cindio, and P. D'Antona. 2001. A weak gel model for foods. Rheol. Acta 40:120–127.

Guinee, T. P., M. Carić, and M. Kaláb. 2004. Pasteurized processed cheese and substitute/imitation cheese products. Pages 349–394 in Cheese: Chemistry, Physics and Microbiology. Major Cheese Groups. Vol. 2. P. F. Fox, P. L. H. McSweeney, and T. P. Cogan, ed. Elsevier Applied Science, London, UK.

Gustaw, W., and S. Mleko. 2007. The effect of polysaccharides and sodium chloride on physical properties of processed cheese analogs containing whey proteins. Milchwissenschaft 62:59–62.

Hanaei, F., G. Cuvelier, and J. M. Sieffermann. 2015. Consumer texture descriptions of a set of processed cheese. Food Qual. Prefer. 40:316–325.

Hayaloglu, A. A., M. Guven, P. F. Fox, J. A. Hannon, and P. L. H. McSweeney. 2004. Proteolysis in Turkish White-brined cheese made with defined strains of *Lactococcus*. Int. Dairy J. 14:599–610.

Hladká, K., Z. Randulová, B. Tremlová, P. Ponížil, P. Mančík, M. Černíková, and F. Buňka. 2014. The effect of cheese maturity on selected properties of processed cheese without traditional emulsifying agents. LWT Food Sci. Technol. 55:650–656.

ISO (International Organization for Standardization). 2004. ISO Standard No. 5534: Cheese and processed cheese—Determination of the total solid content (reference method). ISO, Geneva, Switzerland.

Ji, T., V. B. Alvarez, and W. J. Harper. 2004. Influence of starter culture ratios and warm room treatment on free fatty acid and amino acid in Swiss cheese. J. Dairy Sci. 87:1986–1992.

Kaliappan, S., and J. A. Lucey. 2011. Influence of mixtures of calcium-chelating salts on the physicochemical properties of casein micelles. J. Dairy Sci. 94:4255–4263.

Kapoor, R., and L. E. Metzger. 2008. Process cheese: Scientific and technological aspects—A review. Comp. Rev. Food Sci. Food Safety 7:194–214.

Kawasaki, Y. 2008. Influence of "creaming" on the properties of processed cheese and changes in the structure of casein during cheese making. Milchwissenschaft 63:149–153.

Lee, S. K., S. Anema, and H. Klostermeyer. 2004. The influence of moisture content on the rheological properties of processed cheese spreads. Int. J. Food Sci. Technol. 39:763–771.

Lee, S. K., R. J. Buwalda, S. R. Euston, E. A. Foegeding, and A. B. McKennan. 2003. Changes in the rheology and microstructure of processed cheese during cooking. LWT Food Sci. Technol. 36:339–345.

Lee, S. K., M. Huss, H. Klostermeyer, and S. G. Anema. 2013. The eefect of pre-denatured whey proteins on the textural and microstructural properties of model processed cheese spreads. Int. Dairy J. 32:79–88.

Lee, S. K., and H. Klostermeyer. 2001. The effect of pH on the rheological properties of reduced-fat model processed cheese spreads. LWT Food Sci. Technol. 34:288–292.

Lu, Y., N. Shirashoji, and J. A. Lucey. 2007. Rheological, textural and melting properties of commercial samples of some of the different types of pasteurized processed cheese. Int. J. Dairy Technol. 60:74–80.

Macků, I., F. Buňka, B. Voldánová, and V. Pavlínek. 2009. Effect of addition of selected cosolutes on viscoelastic properties of model processed cheese containing pectin. Food Hydrocoll. 23:2078–2084.

Marchesseau, S., E. Gastaldi, A. Lagaude, and J. L. Cuq. 1997. Influence of pH on protein interactions and microstructure of process cheese. J. Dairy Sci. 80:1483–1489.

Martínez-Ruvalcaba, A., E. Chornet, and D. Rodrigue. 2007. Viscoelastic properties of dispersed chitosan/xanthan hydrogels. Carbohydr. Polym. 67:586–595.

Mizuno, R., and J. A. Lucey. 2007. Properties of milk protein gels formed by phosphates. J. Dairy Sci. 90:4524–4531.

Nagyová, G., F. Buňka, R. N. Salek, M. Černíková, P. Mančík, T. Grůber, and D. Kuchař. 2014. Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. J. Dairy Sci. 97:111–122.

Ochi, H., Y. Sakai, H. Koisihara, F. Abe, T. Bamba, and E. Fukusaki. 2013. Monitoring the ripening process of Cheddar cheese based on hydrophilic component profiling using gas chromatography-mass spectrometry. J. Dairy Sci. 96:7427–7441.

Pachlová, V., F. Buňka, L. Buňková, E. Weiserová, P. Budinský, M. Žaludek, and S. Kráčmar. 2011. The effect of three different ripening/storage conditions on distribution of selected parameters in individual parts of Dutch-type cheese. Int. J. Food Sci. Technol. 46:101–108.

Pachlová, V., F. Buňka, R. Flasarová, P. Válková, and L. Buňková. 2012. The effect of elevated temperature on ripening of Dutch type cheese. Food Chem. 132:1846–1876.

Paulsen, P. V., J. Kowalewska, E. G. Hammond, and B. A. Glatz. 1980. Role of microflora in production of free fatty acids and flavor in Swiss cheese. J. Dairy Sci. 63:912–918.

Piska, I., and J. Štětina. 2004. Influence of cheese ripening and rate of cooling of the processed cheese mixture on rheological properties of processed cheese. J. Food Eng. 61:551–555.

Poveda, J. M., L. Cabezas, and P. L. H. McSweeney. 2004. Free amino acid content of Manchego cheese manufactured with different starter cultures and changes throughout ripening. Food Chem. 84:213–218.

Sádlíková, I., F. Buňka, P. Budinský, B. Voldánová, V. Pavlínek, and I. Hoza. 2010. The effect of selected phosphate emulsifying salts on viscoelastic properties of processed cheese. LWT Food Sci. Technol. 43:1220–1225.

Salek, R. N., M. Černíková, G. Nagyová, D. Kuchař, H. Bačová, L. Minarčíková, and F. Buňka. 2015. The effect of composition of ternary mistures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. Int. Dairy J. 44:37–43.

Shirashoji, N., J. J. Jaeggi, and J. A. Lucey. 2006. Effect of trisodium citrate concetration and cooking time on the physicochemical properties of pasteurized processed cheese. J. Dairy Sci. 89:15–28.

Sołowiej, B., I. W. Y. Cheung, and E. C. Y. Li-Chan. 2014. Texture, rheology and meltability of processed cheese analogues prepared using rennet or acid casein with or without added whey proteins. Int. Dairy J. 37:87–94.

Vicente, M. S., F. C. Ibáñez, Y. Barcina, and L. J. R. Barron. 2001. Changes in the free amino acid content during ripening of Idiazabal cheese: Influence of starter and rennet type. Food Chem. 72:309–317.

Weiserová, E., L., L. Doudová, L. Galiová, J. Žák, R. Michálek, Janiš, and F. Buňka. 2011. The effect of combinations of sodium phosphates in binary mixtures on selected texture parameters of processed cheese spreads. Int. Dairy J. 21:979–986.

Winter, H. H., and F. Chambon. 1986. Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. J. Rheol. (N.Y.N.Y.) 30:367–382.

Research paper 4

A4

Salek, R. N., Černíková, M., Pachlová, V., Bubelová, Z., Konečná, V., & Buňka, F.

Properties of spreadable processed Mozzarella cheese with divergent compositions of emulsifying salts in relation to the applied cheese storage period.

LWT – Food Science & Technology. 2017, 77, 30-38. ISSN: 00236438.

LWT - Food Science and Technology 77 (2017) 30–38

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Properties of spreadable processed Mozzarella cheese with divergent compositions of emulsifying salts in relation to the applied cheese storage period

Richardos Nikolaos Salek, Michaela Černíková, Vendula Pachlová, Zuzana Bubelová, Veronika Konečná, František Buňka*

Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, T.G. Masaryka 5555, 760 01, Zlín, Czechia

ARTICLE INFO

Article history:
Received 6 August 2016
Received in revised form
9 November 2016
Accepted 10 November 2016
Available online 12 November 2016

Keywords:
Processed cheese
Mozzarella
Emulsifying salts
Texture
Rheology

ABSTRACT

The study was focused on selected textural and viscoelastic characteristics of spreadable processed cheese (35 g/100 g dry matter; 50 g/100 g fat in dry matter) manufactured with different ternary mixtures of emulsifying salts (ES) and from Mozzarella-type cheese (MC) with different storage periods (0, 2 and 4 weeks) over the course of a 60-day storage period (6 ± 2 °C). The ES utilized consisted of disodium hydrogenphosphate (DSP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate with mean length n ≈ 20 (P20), and trisodium citrate (TSC). Furthermore, the hardest samples were those manufactured from DSP and TSPP in a ratio 1:1. This ratio resulted in processed cheese with the highest values of gel strength and interaction factor. When TSC was utilized in the mixtures, the hardness of the samples rose with the increase of P20 (≥50%). Additionally, when DSP, TSC, TSPP, and P20 were added as sole ingredients, hardness decreased in the following order: P20 > TSPP ≈ TSC > DSP. This trend was also observed with the values of gel strength and interaction factor. The hardness of all samples increased with increased storage periods. However, the hardness values dropped in relation to an increase in the storage period of the MC.

1. Introduction

Processed cheese (**PC**) is manufactured by mixing cheese, water, emulsifying salts (**ES**), and other dairy/non-dairy ingredients, commonly under vacuum, in the presence of heat and shear. Furthermore, the desired compact structure of PC is obtained by the addition of ES. Their ability to sequester calcium from the casein matrix (exchanging Na^{+} for Ca^{2+}) and the pH adjustment cause protein hydration and dispersion, and the casein present acts as the "true" emulsifier within the matrix (Awad, Abdel-Hamid, El-Shabrawy, & Singh, 2004; El-Bakry, Duggan, O'Riordan, & O'Sullivan, 2011; Kapoor & Metzger, 2008; Lee, Buwalda, Euston, Foegeding, & McKennan, 2003; Lee & Klostermeyer, 2001). The ion-exchange ability is not identical for all ES. Therefore, the phosphate ion-exchange ability increases with the increasing content of P_2O_5 (Buňka et al., 2014; Shirashoji, Jaeggi, & Lucey, 2006).

Traditional Mozzarella is a soft/semi-soft, unripened, pasta-filata cheese, originally manufactured from water buffalo (*Bubalus* sp.) milk, with high levels of moisture (50–60%) and a relatively high pH (>5.5), typically immersed in a hot liquid (mainly a combination of water, brine or whey) preserving the soft-springy texture, whereas the high amounts of expressible serum contribute to its flavor and physicochemical characteristics. Additionally, most Mozzarella cheese (**MC**) is manufactured from pasteurized, partly skimmed cow's milk. The immersion of the cheese-curd in the hot liquid is a specific process enhancing its plasticization and stretching properties. Mozzarella is packaged in a conditioning liquid and stored under refrigeration conditions (6 ± 2 °C). Moreover, MC is one of the most-consumed cheeses worldwide, is used as an ingredient in a series of food products (including PC), and is a high volume product supporting the food service industries (Francolino, Locci, Ghigliettti, Iezzi, & Mucchetti, 2010; Luo, Pan, Guo, & Ren, 2013; Segat et al., 2014; Zhu, Brown, Guo, & Ren, 2015).

During the storage of MC, complex biochemical events determine its final quality and acceptance. Proteolysis is the major phenomenon that occurs during cheese aging (besides glycolysis

* Corresponding author.
E-mail address: bunka@ft.utb.cz (F. Buňka).

http://dx.doi.org/10.1016/j.lwt.2016.11.019
0023-6438/

and lipolysis) that greatly affects the physical characteristics of nearly all cheeses. Generally, cheeses show similar proteolytic trends. On the other hand, differences in cheese nature and manufacturing processes influence the proteolytic pattern. In comparison to Cheddar and Dutch-type cheeses, pasta-filata cheeses represent a special case (in terms of proteolytic pattern). Particularly, the casein molecules ("fibres" or "strings") are arranged distinctly after the stretching process (Costabel, Pauletti, & Hynes, 2007; Sousa, Ardö, & McSweeney, 2001). In the case of PC, rheological and textural properties are influenced by the age of the applied cheese and/or also by specific technological operation during cheese manufacturing. Hence, more intensive proteolytic reactions result from an increasing cheese maturity level. However, the above-mentioned properties are also affected by factors such as: dry matter (**DM**), fat in DM content, pH value, type and amount of ES added, processing, and storage conditions (Brickley, Auty, Piraino, & McSweeney, 2007; Pachlová et al., 2011; Piska & Štětina, 2004).

To the best of our knowledge, there are only a few publications dealing with PC properties produced only from MC, particularly the works of Chavhan, Kanawjia, Khetra, and Puri (2015), Chen and Liu (2012), and Khetra, Chavhan, Kanawjia, and Puri (2015). Nevertheless, the combined effect of MC storage period and different ES (type and composition) on the textural and rheological characteristics of spread-type PC during its storage has not found in the literature.

The present work was undertaken with the primary objective of analyzing the dependence of selected textural properties (hardness) and viscoelastic properties of PC made from MC on the composition of ES ternary mixtures [composed of disodium hydrogenphosphate (**DSP**), tetrasodium diphosphate (**TSPP**), sodium salt of polyphosphate with mean length n ≈ 20 (**P20**), and trisodium citrate (**TSC**)] during a 60-day storage period. This dependence was observed in samples with adjusted pH (target values within the interval of 5.60–5.80, corresponding to the standard pH values of spreadable PC). A supplementary aim was to evaluate the effect of MC (basic raw material) age on the above-mentioned dependence.

2. Materials and methods

2.1. Materials

Mozzarella-type cheese [42 g/100 g DM content; 35 g/100 g fat in DM content; 0, 2, 4-weeks of maturity (storage at 6 ± 2 °C) – the same batch of cheese was applied during the whole experiment] was supplied by NET PLASY s.r.o. (Bystřice pod Hostýnem, Czech Republic). Butter (84 g/100 g, DM content; 82 g/100 g, fat content) was obtained from Sachsenmilch Leppensdorf, GmbH (Wachau, Germany). In addition, DSP (Na_2HPO_4), TSPP ($Na_4P_2O_7$), and P20 (sodium salt of polyphosphate with mean length n ≈ 20) were supplied by Fosfa PLC Company (Břeclav, Czech Republic); TSC ($C_6H_5Na_3O_7$), HCl, and NaOH were purchased from SigmaAldrich Inc. (Schnelldorf, Germany).

2.2. Preparation of the processed cheese samples

The production of the samples was designed in order to achieve end-products with 35 g/100 g DM content and 50 g/100 g fat in DM content. Furthermore, the ES were utilized in 4 types of ternary mixtures (TSC:TSPP:P20; DSP:TSC:P20; DSP:TSPP:TSC; DSP:TSPP:P20) and their total concentration was 3 g/100 g (calculated based on the total weight of the melt). In addition, 12 percentage ratios of each type of ternary mixture (100:0:0; 50:50:0; 0:100:0; 40:40:20; 40:20:40; 20:40:40; 50:0:50; 0:50:50; 40:0:60; 20:20:60; 0:40:60; 0:0:100 – the percentage of the substances was calculated on the basis of the total weight of the ES) were evaluated. Fig. 1 illustrates the schematic description of the experimental

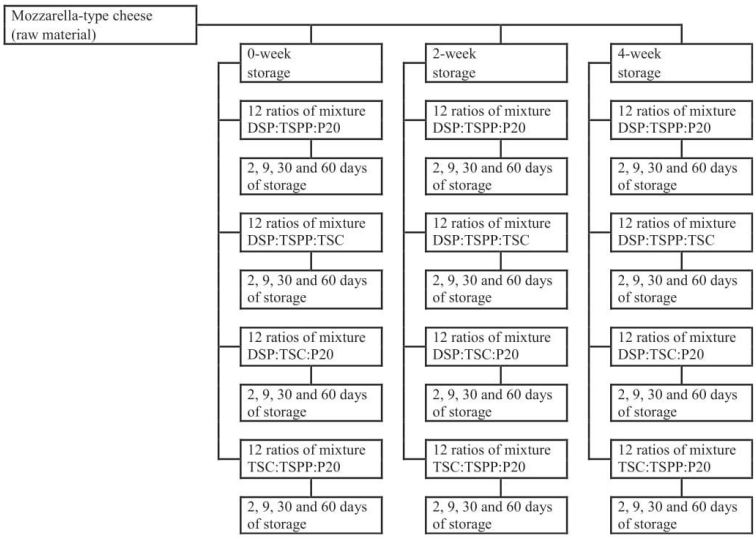

Fig. 1. Scheme of the experimental design with model processed cheeses manufactured using Mozzarella-type cheese (in various time of storage) and the different percentage ratios of the four types of ternary mixtures comprising DSP:TSPP:P20, DSP:TSPP:TSC, DSP:TSC:P20 and TSC:TSPP:P20 (DSP – Na_2HPO_4, TSPP – $Na_4P_2O_7$, P20 – sodium salt of polyphosphate with mean length n ≈ 20 (P20) and TSC – trisodium citrate). The model samples were tested after 2, 9, 30 and 60 days of storage.

design. All samples were prepared in a 2 L capacity Vorwerk Thermomix TM blender cooker (Vorkwerk & Co Thermomix GmbH, Wuppertal, Germany). The pH of the samples was modified (target values within 5.60–5.80) by the application of HCl/NaOH (1 mol/L). In order to maintain the DM content at a desirable level (50 g/100 g), the addition of water was decreased (based on the amount of the added acid/alcali). Finally, the hot molten mass was poured into cylindrical plastic containers (55 mm diameter, 50 mm height) and sealed. Thereafter, the samples were left to cool and were stored under refrigeration conditions (6 ± 2 °C) until the analyses were realized. All the subsisted analyses were performed on the 2nd, 9th, 30th, and 60th day after production, with the exception of the rheological tests which were performed on the 30th day. Each PC (manufactured from MC of certain storage time) was produced in duplicate.

2.3. Mozzarella cheese ripening index determination and basic chemical analysis of the processed cheese

One of the possible methods indicating the degree of proteolysis is the analysis of free amino acids (**FAA**) content (Innocente, 1997). The determination of the FAA content was performed according to the methodology previously described by Buňková et al. (2010) and Hladká et al. (2014). Moreover, for the calculation of the total FAA content of the MC, twenty nine FAA and their derivates were employed, and the results were expressed in g/1000 g. Each sample was lyophilizated twice, each lyophilisate was extracted twice and each extract was loaded on the column in triplicate (n = 12).

According to ISO 5534 (2004) the DM content of the samples was determined gravimetrically. Moreover, the pH of the samples was determined by means of a pH-meter equipped with a glass tip electrode (pHSpear, Eutech Instruments, Oakton, Malaysia) at 22 ± 2 °C. The spear was inserted into each PC sample at 3 randomly selected sites (in each container).

2.4. Determination of textural and rheological properties of processed cheese samples

The textural behaviour of PC samples was evaluated using a penetration by means of a TA.XT.plus texture analyser (Stable Micro Systems Ltd., Godalming, UK). The selected examined instrumental parameter was hardness and was calculated according to Szczesniak (2002). Furthermore, the obtained results were recorded as force-displacement/time curves, depicting the force needed (N) to deform the sample proportionally with time (s). Additionally, a cylindrical aluminum probe (20 mm diameter, penetration depth 10 mm, probe speed 2 mm/s, strain deformation 25%, trigger force 5 g) was implemented. The analyses were carried out immediately after removing the samples from the refrigerator where they were stored.

The RheoStress 1 (HAAKE, Bremen, Germany) dynamic oscillatory shear rheometer equipped with a plate-plate geometry (35 mm diameter) was used for the examination of the viscoelastic properties of the PC samples. An amplitude sweep test was applied for the determination of the linear viscoelastic region, whereas in order to evaluate the viscoelastic characteristics of the samples the frequency sweep mode was employed. The frequency (ω) range of 0.01–100.00 Hz was used for the determination of the storage (G′) and loss (G″) moduli. Hence, the complex modulus (G*) was calculated according to the following equation (Eq. (1)):

$$G^* = \sqrt{(G')^2 + (G'')^2} \quad (1)$$

Winter's critical "gel theory" for weak gels was implemented. Therefore, the complex modulus (G*) can be expressed according to the following equation (Eq. (2)):

$$G^*(\omega) = A_F \cdot \omega^{\frac{1}{z}} \quad (2)$$

where A_F is the strength number of rheological units correlated with one another within a three-dimensional network in which the droplet particles are linked by more or less strong interactions (Gabriele, De Cindio, & D'Antona, 2001; Winter & Chambon, 1986).

The recorded values were the mean of at least eight replicates (n = 8; 2 batches × 2 containers × 2 replicates) for studying of textural behaviour and also viscoelastic properties of samples.

2.5. Statistical analysis

The non-parametrical analyses of variance from the Kruskal-Wallis and Wilcoxon tests (Unistat® 6.5 software; Unistat, London, UK) were used in order to evaluate the obtained results (the significance level was 0.05). For an estimation of the gel strength and the interaction factor, non-linear regression analysis (non-linear least squares regression) was used under the following conditions: $A_F > 0$ and $z \geq 0$. The Marquardt-Levenburg method was applied (Unistat® 6.5; software Unistat, London, UK).

3. Results and discussion

3.1. Determination of the Mozzarella cheese ripening index and basic chemical analysis of the processed cheese

Proteolysis influences the final quality of the product and in the

Table 1
Development of free amino acid (FAA) content (g/1000 g) during the storage period (0.2. 4-weeks at 6 ± 2 °C) of Mozzarella-type cheese applied for the production of processed cheese samples. The values are expressed as means ± standard deviation (n = 12; each sample was lyophilisated twice, each lyophilisate was extracted twice and each extract was loaded on the column in triplicate).

Amino acid	Period of storage of Mozzarella-type cheese		
	0 weeks	2 weeks	4 weeks
Threonine	0.004 ± 0.000	0.008 ± 0.000	0.114 ± 0.001
Serine	0.005 ± 0.000	0.009 ± 0.000	0.110 ± 0.001
Aspartic acid	ND[a]	0.024 ± 0.000	0.057 ± 0.004
Asparagine	ND	ND	0.099 ± 0.001
Glutamic acid	0.024 ± 0.001	0.064 ± 0.002	0.485 ± 0.022
Glutamine	ND	0.070 ± 0.005	0.992 ± 0.020
Proline	0.023 ± 0.001	0.034 ± 0.001	0.199 ± 0.001
Glycine	ND	ND	0.013 ± 0.001
Alanine	0.017 ± 0.000	0.014 ± 0.000	0.106 ± 0.005
Citrulline	0.002 ± 0.000	ND	0.025 ± 0.000
Valine	0.002 ± 0.000	0.017 ± 0.001	0.231 ± 0.013
Cysteine	ND	ND	ND
Methionine	ND	0.005 ± 0.000	0.093 ± 0.009
Cystathionine	ND	0.003 ± 0.000	0.014 ± 0.000
Izoleucine	ND	0.006 ± 0.000	0.118 ± 0.001
Leucine	0.037 ± 0.001	0.096 ± 0.004	0.639 ± 0.061
Tyrosine	0.020 ± 0.001	0.010 ± 0.000	0.051 ± 0.001
Phenylalanine	0.012 ± 0.000	0.027 ± 0.000	0.213 ± 0.001
β-Alanine	ND	ND	ND
β-Aminobutyric acid	ND	ND	ND
γ-Aminobutyric acid	ND	0.023 ± 0.000	0.024 ± 0.002
Ethanolamine	ND	0.003 ± 0.000	ND
Ornithine	0.009 ± 0.001	0.023 ± 0.000	0.191 ± 0.004
Lysine	0.038 ± 0.000	0.016 ± 0.001	0.266 ± 0.000
Histidine	0.026 ± 0.001	0.018 ± 0.002	0.086 ± 0.002
1-Methyl-L-histidine	ND	ND	ND
Arginine	ND	ND	ND
α-Aminobutyric acid	ND	ND	ND
3-Methyl-L-histidine	ND	ND	ND
Total FAA	0.146 ± 0.013	0.470 ± 0.025	4.126 ± 0.239

[a] ND - not detected.

case of MC (Juan, Zamora, Quevedo, & Trujillo, 2016; Petrella et al., 2015). The development of FAA content (Table 1) was used as an "instrument" for the evaluation of the ripening process. From the obtained results it may be assumed that with the rising storage period of the MC, the total content of FAA increased (from 0.146 ± 0.013 to 4.126 ± 0.239 g/1000 g) Furthermore, the most abundant rise in individual FAA contents was observed in glutamine, leucine, and glutamic acid, respectively. Generally, the development rate of FAA during the initial 2 weeks of storage was slow, whereas after 4 weeks their releasing intensity accelerated ($P < 0.05$). Moreover, a relation between FAA development and the maturity period had been previously reported by Vicente, Ibáñez, Barcina, and Barron (2001) and Pachlová et al. (2011). The FAA are released during the proteolytic pathway by specific agents (enzymes from starter, secondary flora and non-starter microflora – in most cheese varieties) via biochemical reactions. However, in the case of MC, the enzymes of the residual coagulant are exposed to denaturation during the stretching process and thus weakly contribute to the proteolysis (Ji, Alvarez, & Harper, 2004; Poveda, Cabezas, & McSweeney et al., 2004; Sulejmani, Hayaloglu, & Rafajlovska, 2014).

Similar DM content values among the tested samples allows their comparison, as this factor could affect their viscoelastic properties (Marchesseau, Gastaldi, Lagaude, & Cuq, 1997). The DM content of the PC samples was within the interval of 35.18–35.77 (g/100 g). The obtained results depict the stability of the DM content of the samples. Additionally, the viscoelastic properties can also be influenced by the pH of the molten mass. The pH values of the samples, after adjustment, ranged from 5.63 to 5.81. A possible explanation for the "narrow" variability observed in the pH values could be found in the ES buffering capacity or in the fact that the production of the samples was undergone using "real" raw materials (natural cheese, butter) (Lee & Klostermeyer, 2001; Lu, Shirashoji, & Lucey, 2008).

3.2. Textural and rheological properties of the processed cheese samples

The textural properties of PC are of great importance for product characterization and for the consumers' hedonic reactions. The results of the development of PC hardness as a function of the storage period are presented in Figs. 2 and 3. On the whole, the applied ternary mixtures of ES influenced the hardness of the samples. From the results it can be reported that with an increasing storage period, the hardness of the samples rose (regardless of the applied ES or storage rate of the MC). Furthermore, this trend could be explained by: (i) the hydrolysis of ES (with ≥2 atoms of phosphorus in their molecule) into mono- and diphosphates, which are connected to the protein-fat network development, or (ii) some possible changes in the forms of bonds of the ES, resulting in the enhancement of their dissociation properties (Awad et al., 2004; Shirashoji et al., 2006; Weiserová et al., 2011). On the other hand, with the amplifying age of the utilized MC, the hardness of the samples decreased (regardless of the used ternary mixture of ES). The rising aging period probably resulted in a more thorough proteolytic pattern. In particular, casein fractions of a shorter length

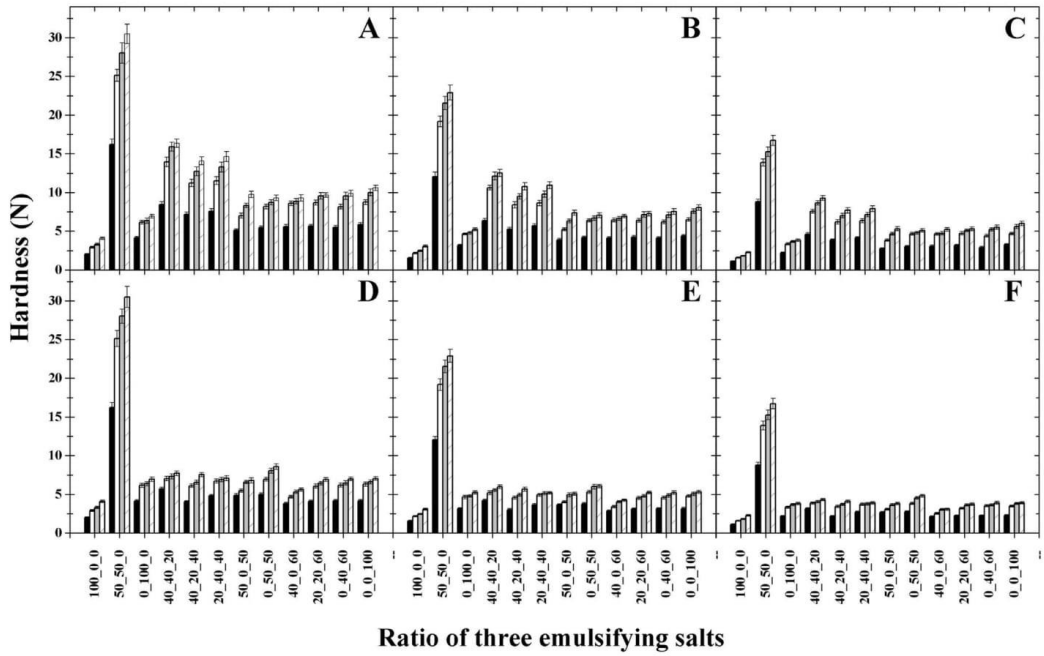

Fig. 2. The dependence of processed cheese hardness (N) on the relative amount (in percentage; axis x) of three emulsifying salts during 60-day storage at 6 °C (n = 6; the results were expressed as means (columns) and ± standard deviations (bars); processed cheese were sampled after 2 (black), 9 (white), 30 (grey) and 60 (section line) days of storage). Parts A–C: ternary mixtures contained Na_2HPO_4 (DSP), $Na_4P_2O_7$ (TSPP) and sodium salt of polyphosphate. Parts D–F: ternary mixtures contained DSP, TSPP and trisodium citrate. Processed cheeses were made from Mozzarella-type cheese after different time of storage (parts A and D – 0 weeks; parts B and E – 2 weeks; parts C and F – 4 weeks).

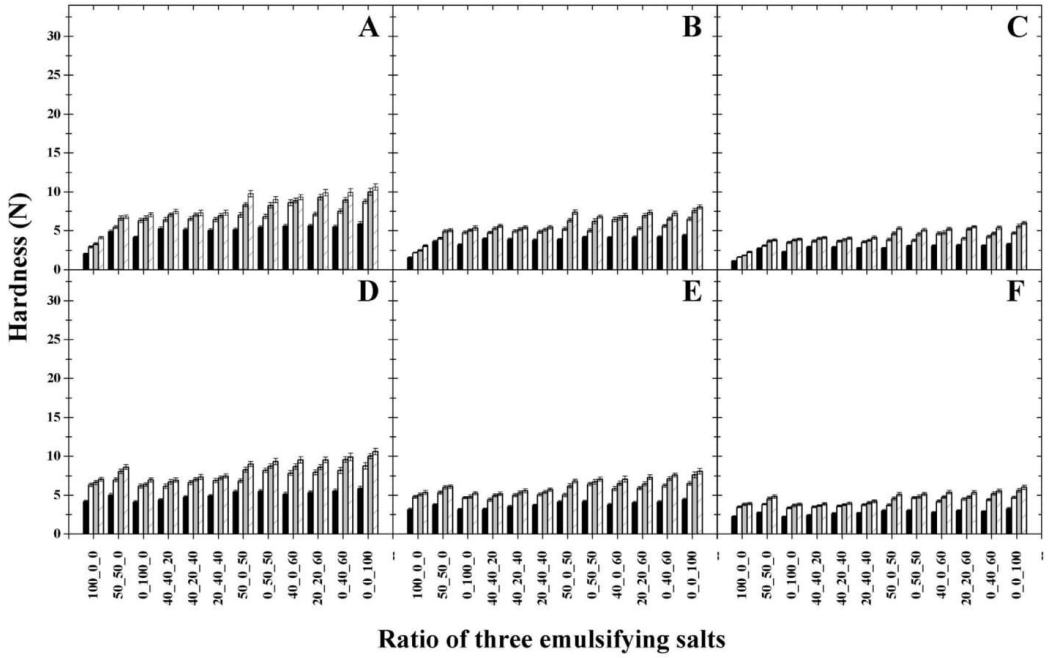

Fig. 3. The dependence of processed cheese hardness (N) on the relative amount (in percentage; axis x) of three emulsifying salts during 60-day storage at 6 °C (n = 6; the results were expressed as means (columns) and ±standard deviations (bars); processed cheese were sampled after 2 (black), 9 (white), 30 (grey) and 60 (section line) days of storage). Parts A–C: ternary mixtures contained Na_2HPO_4, trisodium citrate (TSC) and sodium salt of polyphosphate (P20). Parts D–F: ternary mixtures contained TSC, $Na_4P_2O_7$ and P20. Processed cheeses were made from Mozzarella-type cheese after different time of storage (parts A and D – 0 weeks; parts B and E – 2 weeks; parts C and F – 4 weeks).

were formed, leading to the modulation of a protein matrix of lesser massiveness (Brickley et al., 2007; Hladká et al., 2014; Piska & Štětina, 2004). Moreover, the hardest samples were those composed of DSP:TSPP (1:1), regardless of the age of the MC. This ratio was previously identified in the works of Buňka et al. (2014), Nagyová et al. (2014), Salek, Černíková, Maděrová, Lapčík, and Buňka (2016), and Weiserová et al. (2011), in which different types of natural cheeses were applied as the basic raw material. In the same token, it can probably be stated that the "action" of this ratio of developing samples to increased values of hardness appears to be independent of the variety of the natural cheese used. With the evolution of the storage period, the effect of this ratio was also recognized; thus the hardness of the samples followed the decreasing trend mentioned above. Mizuno and Lucey (2005) and Kaliappan and Lucey (2011) reported that: (i) disphosphates possess an efficient amelioration effect on the properties of casein gelation when used in sufficient concentrations, and (ii) monophosphates support the formation of bridges between caseins, calcium ions, and diphosphates. On the contrary, when DSP or TSPP were replaced in the mixture by TSC, the magnitude of this ratio was not observed. This may probably lead to the inference that TSC does not present the ability of creating new networks, and hence does not affect the crosslinking properties of DSP and TSPP (Kaliappan & Lucey, 2011; Lu et al., 2008; Mizuno & Lucey, 2005).

Furthermore, the results of the evolution of the samples' hardness during storage with ternary mixtures composed only of phosphate-ES are presented in Fig. 2 (parts A–C). The gradual rising amount of P20 in the mixture led to the samples' decreasing hardness. This decreasing tendency was observed up to a concentration of P20 $\geq$ 50%; above this "critical" concentration the trend was of minor significance. An analogous result was observed by

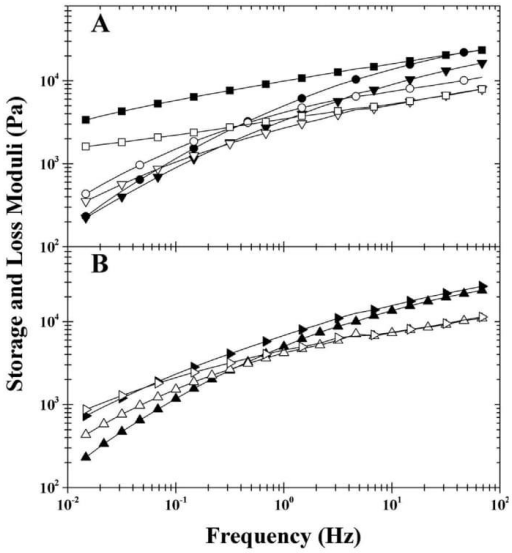

Fig. 4. The dependence of storage G′ (full symbols) and loss G″ (open symbols) moduli of processed cheese (after 30-day storage) made from Mozzarella-type cheese at the beginning of storage on frequency (in range of 0.01–100.00 Hz). Processed cheeses were manufactured using Na_2HPO_4 (▼ ▽; part A), $Na_4P_2O_7$ (● ○; part A), binary mixture of disodium phosphate and tetrasodium diphospate in ratio of 1:1 (■ □; part A), trisodium citrate (▲ △; part B) or sodium salt of polyphosphate (► ▷; part B).

Mizuno and Lucey (2005). According to the latter authors, the function of polyphosphates within the matrix may provide the caseins with manifold negative ions, resulting in the formation of hydrophobic interactions of lower intensity among the scattered proteins. Moreover, during the storage of the samples the trend remained, whereas only the absolute values of hardness decreased.

All in all, the utilization of TSC in the mixtures (Figs. 2 and 3) resulted in the increase of the samples' hardness in proportion to the growth of P20 concentration. The involvement of ES with higher casein dispersion ability (mainly longer-chain polyphosphates) resulted in products with greater values of hardness. Hence, the ES ion-exchange ability is enhanced by rising degrees of casein dispersion, resulting in the improved hydrating and emulsifying properties of the caseins present as more interactions thus occur within the matrix (Chen & Liu, 2012; Dimitreli & Thomareis, 2009; El-Bakry et al., 2011; Kaliappan & Lucey, 2011; Lu et al., 2008; Mizuno & Lucey, 2005; Shirashoji et al., 2006).

In addition, the evolution of the hardness of the PC samples comprised of ternary mixtures of DSP:TSPP:TSC is depicted in Fig. 2 (parts D–F). The specific ratio of DSP:TSPP (1:1) was once again recognized, resulting in samples with the highest values of hardness. Nevertheless, the gradual increase of TSC together with the simultaneous decrease of DSP and TSPP in the mixtures provided a noteworthy decrease in the samples' hardness.

Thereafter, when DSP, TSC, TSPP, and P20 were applied as sole ES during the manufacturing of the PC model samples, hardness levels

Table 2
Values of gel strength (A_F; kPa $s^{1/z}$) of processed cheese (after 30-day storage) made from Mozzarella-type cheese after different time of storage (0 weeks; 2 weeks; 4 weeks). Different ternary mixtures of emulsifying salts (disodium phosphate (DSP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate (P20) and trisodium citrate (TSC)) were used for manufacture of model samples. Amount of individual emulsifying salts in ternary mixture were expressed in percentage (percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%).

Type of ternary mixture	Ratio of salts (percentage)	Time of storage of raw material for processed cheese production		
		0 weeks	2 weeks	4 weeks
DSP:TSPP:P20	100:0:0	$4.50 \pm 0.26^{a}A$	$3.49 \pm 0.12^{a}B$	$2.54 \pm 0.12^{a}C$
	50:50:0	$12.42 \pm 0.75^{h}A$	$9.08 \pm 0.36^{h}B$	$6.72 \pm 0.24^{g}C$
	0:100:0	$6.84 \pm 0.23^{b}A$	$5.17 \pm 0.30^{b}B$	$3.63 \pm 0.16^{b}C$
	40:40:20	$9.48 \pm 0.31^{g}A$	$7.16 \pm 0.20^{g}B$	$5.21 \pm 0.26^{f}C$
	40:20:40	$8.68 \pm 0.31^{e,f}A$	$6.41 \pm 0.35^{e,f}B$	$4.71 \pm 0.16^{e}C$
	20:40:40	$9.05 \pm 0.67^{f,g}A$	$6.73 \pm 0.40^{f}B$	$4.87 \pm 0.22^{e}C$
	50:0:50	$7.22 \pm 0.37^{c}A$	$5.44 \pm 0.21^{c}B$	$3.89 \pm 0.28^{b,c}C$
	0:50:50	$7.72 \pm 0.30^{d}A$	$5.95 \pm 0.36^{d}B$	$4.25 \pm 0.25^{d}C$
	40:0:60	$7.22 \pm 0.31^{c}A$	$5.26 \pm 0.39^{b,c}B$	$3.98 \pm 0.15^{c,d}C$
	20:20:60	$8.28 \pm 0.20^{e}A$	$6.21 \pm 0.25^{e}B$	$4.67 \pm 0.25^{e}C$
	0:40:60	$7.86 \pm 0.36^{d}A$	$5.86 \pm 0.25^{d}B$	$4.13 \pm 0.18^{d}C$
	0:0:100	$8.27 \pm 0.43^{e}A$	$6.24 \pm 0.33^{e}B$	$4.55 \pm 0.19^{e}C$
DSP:TSPP:TSC	100:0:0	$4.50 \pm 0.26^{a}A$	$3.49 \pm 0.12^{a}B$	$2.54 \pm 0.12^{a}C$
	50:50:0	$12.42 \pm 0.75^{f}A$	$9.08 \pm 0.36^{f}B$	$6.72 \pm 0.24^{f}C$
	0:100:0	$6.84 \pm 0.23^{c}A$	$5.17 \pm 0.30^{d}B$	$3.63 \pm 0.16^{c}C$
	40:40:20	$8.28 \pm 0.24^{e}A$	$6.08 \pm 0.38^{e}B$	$4.54 \pm 0.21^{e}C$
	40:20:40	$6.83 \pm 0.33^{c}A$	$5.08 \pm 0.28^{d}B$	$3.67 \pm 0.15^{c}C$
	20:40:40	$6.92 \pm 0.30^{c}A$	$5.27 \pm 0.37^{d}B$	$3.85 \pm 0.17^{c}C$
	50:0:50	$5.79 \pm 0.29^{b}A$	$4.25 \pm 0.24^{b}B$	$3.12 \pm 0.13^{b}C$
	0:50:50	$7.19 \pm 0.44^{d}A$	$5.47 \pm 0.28^{d}B$	$3.99 \pm 0.23^{d}C$
	40:0:60	$5.86 \pm 0.42^{b}A$	$4.41 \pm 0.16^{b}B$	$3.33 \pm 0.16^{b}C$
	20:20:60	$5.97 \pm 0.32^{b}A$	$4.56 \pm 0.26^{b,c}B$	$3.28 \pm 0.17^{b}C$
	0:40:60	$6.60 \pm 0.26^{c}A$	$4.86 \pm 0.28^{c,d}B$	$3.51 \pm 0.21^{c}C$
	0:0:100	$6.73 \pm 0.32^{c}A$	$5.13 \pm 0.31^{d}B$	$3.67 \pm 0.13^{c}C$
DSP:TSC:P20	100:0:0	$4.50 \pm 0.26^{a}A$	$3.49 \pm 0.12^{a}B$	$2.54 \pm 0.12^{a}C$
	50:50:0	$5.79 \pm 0.29^{b}A$	$4.25 \pm 0.24^{b}B$	$3.12 \pm 0.13^{b}C$
	0:100:0	$6.73 \pm 0.32^{c}A$	$5.13 \pm 0.31^{c}B$	$3.67 \pm 0.13^{c}C$
	40:40:20	$6.94 \pm 0.31^{c}A$	$5.29 \pm 0.30^{c}B$	$3.79 \pm 0.15^{c}C$
	40:20:40	$7.06 \pm 0.30^{c,d}A$	$5.34 \pm 0.28^{c}B$	$3.90 \pm 0.19^{c}C$
	20:40:40	$7.17 \pm 0.33^{d}A$	$5.42 \pm 0.21^{c,d}B$	$3.95 \pm 0.21^{c}C$
	50:0:50	$7.22 \pm 0.37^{d,e}A$	$5.44 \pm 0.21^{c,d}B$	$3.89 \pm 0.28^{c}C$
	0:50:50	$7.53 \pm 0.30^{e}A$	$5.75 \pm 0.26^{d}B$	$4.18 \pm 0.26^{d,e}C$
	40:0:60	$7.22 \pm 0.31^{d,e}A$	$5.26 \pm 0.39^{c}B$	$3.98 \pm 0.15^{c,d}C$
	20:20:60	$7.74 \pm 0.28^{e,f}A$	$5.76 \pm 0.23^{d}B$	$4.30 \pm 0.20^{e,f}C$
	0:40:60	$7.98 \pm 0.32^{f,g}A$	$6.06 \pm 0.25^{e}B$	$4.53 \pm 0.32^{f}C$
	0:0:100	$8.27 \pm 0.43^{g}A$	$6.24 \pm 0.33^{e}B$	$4.55 \pm 0.19^{f}C$
TSC:TSPP:P20	100:0:0	$6.73 \pm 0.32^{a}A$	$5.13 \pm 0.31^{a}B$	$3.67 \pm 0.13^{a}C$
	50:50:0	$7.19 \pm 0.44^{b}A$	$5.47 \pm 0.28^{b}B$	$3.99 \pm 0.23^{b}C$
	0:100:0	$6.84 \pm 0.23^{a,b}A$	$5.17 \pm 0.30^{a}B$	$3.63 \pm 0.16^{a}C$
	40:40:20	$6.64 \pm 0.34^{a}A$	$4.88 \pm 0.17^{a}B$	$3.71 \pm 0.15^{a}C$
	40:20:40	$6.94 \pm 0.39^{a,b}A$	$5.21 \pm 0.24^{b}B$	$3.91 \pm 0.17^{b}C$
	20:40:40	$7.11 \pm 0.32^{b}A$	$5.36 \pm 0.27^{b}B$	$3.90 \pm 0.22^{b}C$
	50:0:50	$7.53 \pm 0.30^{c}A$	$5.75 \pm 0.26^{c}B$	$4.18 \pm 0.26^{c}C$
	0:50:50	$7.72 \pm 0.30^{c}A$	$5.95 \pm 0.36^{c}B$	$4.25 \pm 0.25^{c,d}C$
	40:0:60	$7.80 \pm 0.31^{c,d}A$	$5.75 \pm 0.24^{c}B$	$4.24 \pm 0.19^{c,d}C$
	20:20:60	$7.95 \pm 0.40^{d}A$	$5.95 \pm 0.17^{c}B$	$4.42 \pm 0.18^{a}C$
	0:40:60	$7.86 \pm 0.36^{c,d}A$	$5.86 \pm 0.25^{c}B$	$4.13 \pm 0.18^{c}C$
	0:0:100	$8.27 \pm 0.43^{e}A$	$6.24 \pm 0.33^{d}B$	$4.55 \pm 0.19^{e}C$

*The means within a column (the difference between samples with different ratio of emulsifying salts in the ternary mixture) followed by different superscript letters differ ($P < 0.05$); samples with each type of the ternary mixture (DSP:TSPP:P20; DSP:TSPP:TSC; DSP:TSC:P20; TSC:TSPP:P20 were evaluated independently. The means within a line (the difference between samples with various times of Mozzarella-type cheese storage) followed by different capital letters differ ($P < 0.05$); samples with each ratio of emulsifying salts in each ternary mixture were evaluated independently.

developed according to the following order: P20 > TSPP ≈ TSC > DSP. The results and the explanation therein were shared by Dimitreli and Thomareis (2009), El-Bakry et al. (2011), Nagyová et al. (2014), Salek et al. (2015, 2016), and Weiserová et al. (2011). The strong capability of polyphosphates with a longer than average chain-length to bind calcium into complexes, with a resultant improvement in casein dispersion, may serve as the explanation for the phenomenon described above (Mizuno & Lucey, 2005; Shirashoji et al., 2006).

The results of the rheological analysis are depicted in Fig. 4 and Tables 2 and 3. From the results obtained it may be assumed that samples with divergent viscoelastic characteristics were developed by means of the application of different types of ES. The PC samples comprised of DSP:TSPP (ratio 1:1) resulted in the highest values of storage and loss moduli. On the contrary, samples manufactured with TSC showed the lowest values of the moduli mentioned above. On the whole, when the tested ES were applied as sole ingredients the storage and loss moduli decreased in the following order: P20 > TSPP ≈ TSC > DSP. This specific trend was also confirmed by observing of the PC hardness. The samples presenting the lowest values of storage and loss moduli indicate a liquid-like behavior rather than a spreadable PC with a compact network (Sádlíková et al., 2010). Additionally, a decreasing trend denoted by the results of the hardness and the determination of storage and loss moduli was also identified after utilizing Winter's critical gel theory. Moreover, the specific function of the ratio of DSP:TSPP (1:1)

Table 3
Values of interaction factor (z) of processed cheese (after 30-day storage) made from Mozzarella-type cheese after different time of storage (0 weeks; 2 weeks; 4 weeks). Different ternary mixtures of emulsifying salts (disodium phosphate (DSP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate (P20) and trisodium citrate (TSC)) were used for manufacture of model samples. Amount of individual emulsifying salts in ternary mixture were expressed in percentage (percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%).

Type of ternary mixture	Ratio of salts (percentage)	Time of storage of raw material for processed cheese production		
		0 weeks	2 weeks	4 weeks
DSP:TSPP:P20	100:0:0	$3.07 \pm 0.04^{a}A$	$2.91 \pm 0.08^{a}B$	$2.79 \pm 0.07^{a}C$
	50:50:0	$4.88 \pm 0.22^{f}A$	$4.53 \pm 0.13^{f}B$	$4.36 \pm 0.17^{d}C$
	0:100:0	$3.10 \pm 0.12^{a}A$	$2.92 \pm 0.13^{a}B$	$2.74 \pm 0.07^{a}C$
	40:40:20	$4.63 \pm 0.17^{e}A$	$4.39 \pm 0.05^{e}B$	$4.18 \pm 0.16^{d}C$
	40:20:40	$3.99 \pm 0.10^{c}A$	$3.65 \pm 0.10^{c}B$	$3.55 \pm 0.11^{c}C$
	20:40:40	$4.15 \pm 0.17^{d}A$	$3.88 \pm 0.11^{d}B$	$3.69 \pm 0.10^{c}C$
	50:0:50	$3.24 \pm 0.07^{b}A$	$3.06 \pm 0.08^{b}B$	$2.86 \pm 0.12^{a,b}C$
	0:50:50	$3.26 \pm 0.14^{b}A$	$3.06 \pm 0.13^{b}B$	$2.93 \pm 0.11^{b}C$
	40:0:60	$3.29 \pm 0.06^{b}A$	$2.99 \pm 0.04^{a,b}B$	$2.99 \pm 0.11^{b}C$
	20:20:60	$3.85 \pm 0.12^{c}A$	$3.62 \pm 0.04^{c}B$	$3.51 \pm 0.07^{c}C$
	0:40:60	$3.27 \pm 0.07^{b}A$	$3.04 \pm 0.09^{b}B$	$2.86 \pm 0.07^{a,b}C$
	0:0:100	$3.37 \pm 0.07^{b}A$	$3.12 \pm 0.13^{b}B$	$3.01 \pm 0.12^{b}C$
DSP:TSPP:TSC	100:0:0	$3.07 \pm 0.04^{a}A$	$2.91 \pm 0.08^{a}B$	$2.79 \pm 0.07^{a}C$
	50:50:0	$4.88 \pm 0.22^{e}A$	$4.53 \pm 0.13^{e}B$	$4.36 \pm 0.17^{e}C$
	0:100:0	$3.10 \pm 0.12^{a}A$	$2.92 \pm 0.13^{a}B$	$2.74 \pm 0.07^{a}C$
	40:40:20	$4.34 \pm 0.19^{d}A$	$4.02 \pm 0.08^{d}B$	$3.93 \pm 0.07^{d}C$
	40:20:40	$3.98 \pm 0.19^{c}A$	$3.72 \pm 0.12^{c}B$	$3.59 \pm 0.11^{c}C$
	20:40:40	$4.12 \pm 0.13^{d}A$	$3.85 \pm 0.12^{c}B$	$3.75 \pm 0.10^{c}C$
	50:0:50	$3.14 \pm 0.09^{a}A$	$2.90 \pm 0.03^{a}B$	$2.80 \pm 0.07^{a}C$
	0:50:50	$3.53 \pm 0.12^{b}A$	$3.27 \pm 0.10^{b}B$	$3.16 \pm 0.07^{b}C$
	40:0:60	$3.39 \pm 0.13^{b}A$	$3.14 \pm 0.12^{b}B$	$3.10 \pm 0.08^{b}C$
	20:20:60	$3.42 \pm 0.11^{b}A$	$3.19 \pm 0.13^{b}B$	$3.06 \pm 0.10^{b}C$
	0:40:60	$3.17 \pm 0.06^{a}A$	$2.98 \pm 0.09^{a}B$	$2.85 \pm 0.04^{a}C$
	0:0:100	$3.08 \pm 0.09^{a}A$	$2.89 \pm 0.07^{a}B$	$2.77 \pm 0.06^{a}C$
DSP:TSC:P20	100:0:0	$3.07 \pm 0.04^{a}A$	$2.91 \pm 0.08^{a}B$	$2.79 \pm 0.07^{a}C$
	50:50:0	$3.14 \pm 0.09^{a}A$	$2.90 \pm 0.03^{a}B$	$2.80 \pm 0.07^{a}C$
	0:100:0	$3.08 \pm 0.09^{a}A$	$2.89 \pm 0.07^{a}B$	$2.77 \pm 0.06^{a}C$
	40:40:20	$3.12 \pm 0.05^{a,b}A$	$2.93 \pm 0.06^{a}B$	$2.83 \pm 0.09^{a}C$
	40:20:40	$3.22 \pm 0.12^{b,c}A$	$3.04 \pm 0.10^{b}B$	$2.94 \pm 0.06^{b}C$
	20:40:40	$3.28 \pm 0.10^{c}A$	$3.07 \pm 0.14^{b}B$	$2.91 \pm 0.09^{a,b}C$
	50:0:50	$3.24 \pm 0.07^{c}A$	$3.06 \pm 0.08^{b}B$	$2.86 \pm 0.12^{a,b}C$
	0:50:50	$3.30 \pm 0.13^{c}A$	$3.10 \pm 0.12^{b}B$	$2.98 \pm 0.10^{b}C$
	40:0:60	$3.29 \pm 0.06^{c}A$	$2.99 \pm 0.04^{a,b}B$	$2.99 \pm 0.11^{b}C$
	20:20:60	$3.32 \pm 0.14^{c,d}A$	$3.08 \pm 0.10^{b}B$	$3.00 \pm 0.11^{b}C$
	0:40:60	$3.33 \pm 0.03^{c,d}A$	$3.12 \pm 0.14^{b}B$	$2.99 \pm 0.09^{b}C$
	0:0:100	$3.37 \pm 0.07^{d}A$	$3.12 \pm 0.13^{b}B$	$3.01 \pm 0.12^{b}C$
TSC:TSPP:P20	100:0:0	$3.08 \pm 0.09^{a}A$	$2.89 \pm 0.07^{a}B$	$2.77 \pm 0.06^{a}C$
	50:50:0	$3.53 \pm 0.12^{e}A$	$3.27 \pm 0.10^{d}B$	$3.16 \pm 0.07^{e}C$
	0:100:0	$3.10 \pm 0.12^{a}A$	$2.92 \pm 0.13^{a}B$	$2.74 \pm 0.07^{a}C$
	40:40:20	$3.14 \pm 0.10^{a,b}A$	$2.90 \pm 0.04^{a}B$	$2.84 \pm 0.12^{a,b}C$
	40:20:40	$3.19 \pm 0.09^{b}A$	$2.95 \pm 0.06^{a,b}B$	$2.90 \pm 0.07^{b}B$
	20:40:40	$3.25 \pm 0.09^{b,c}A$	$3.01 \pm 0.14^{b}B$	$2.93 \pm 0.07^{b}C$
	50:0:50	$3.30 \pm 0.13^{c,d}A$	$3.10 \pm 0.12^{a}B$	$2.98 \pm 0.10^{c,d}C$
	0:50:50	$3.26 \pm 0.14^{b,c}A$	$3.06 \pm 0.13^{b,c}B$	$2.93 \pm 0.11^{b}C$
	40:0:60	$3.27 \pm 0.12^{b,c}A$	$2.96 \pm 0.11^{a,b}B$	$2.90 \pm 0.05^{b}B$
	20:20:60	$3.25 \pm 0.09^{b,c}A$	$3.06 \pm 0.08^{b,c}B$	$2.98 \pm 0.08^{c,d}C$
	0:40:60	$3.27 \pm 0.07^{b,c}A$	$3.04 \pm 0.09^{b,c}B$	$2.86 \pm 0.07^{a,b}C$
	0:0:100	$3.37 \pm 0.07^{d}A$	$3.12 \pm 0.13^{c}B$	$3.01 \pm 0.12^{d}C$

*The means within a column (the difference between samples with different ratio of emulsifying salts in the ternary mixture) followed by different superscript letters differ ($P < 0.05$); samples with each type of the ternary mixture (DSP:TSPP:P20; DSP:TSPP:TSC; DSP:TSC:P20; TSC:TSPP:P20 were evaluated independently. The means within a line (the difference between samples with various times of Mozzarella-type cheese storage) followed by different capital letters differ ($P < 0.05$); samples with each ratio of emulsifying salts in each ternary mixture were evaluated independently.

was recognized, resulting in samples with the highest values of gel strength and interaction factor. This result confirms the statement that disphosphates support, under specific conditions, the formation of a three-dimensional network and the emulsification of fat (Awad et al., 2004). Furthermore, with the increasing storage period of the PC samples the values of gel strength and interaction factor decreased. This decrease resulted in PC samples with lower values of hardness, probably due to the fewer number of interactions within the matrix occurring during storage (Kapoor & Metzger, 2008). Moreover, analogous results were reported by Sádlíková et al. (2010) and Salek et al. (2016); however, different cheeses were utilized as the main raw materials, signaling that the development of PC hardness during storage follows a contiguous model regardless of the natural cheese applied.

4. Conclusions

The combined impact of the MC age and the different ternary mixtures of ES on the textural and viscoelastic properties of PC was evaluated. The increasing storage period of the PC samples resulted in an increase in hardness. On the contrary, the hardness of the samples decreased with expanding MC storage time. Model samples with diverging properties were obtained by the application of different types of ternary mixtures of ES. The hardest samples were those comprised of DSP:TSPP (1:1). However, when DSP or TSPP were replaced by TSC, this ratio was not observed. The rising amount of P20 in the mixtures led to a decrease in the samples' hardness (up to ≥50%). The results obtained from the rheological analysis were in accordance to those of the hardness analysis. Hence, the ratio of DSP:TPSS resulted in PC with the highest values of gel strength and interaction factor. Moreover, with increasing MC storage periods the values of gel strength and interaction factor decreased. From the results obtained it may be reported that both the ES (type and composition) and MC storage period have an important effect on the textural and viscoelastic properties of spreadable PC.

Acknowledgement

This study was kindly supported by the internal grant agency of Tomas Bata University in Zlín, Czech Republic (IGA/FT/2015/004 and IGA/FT/2016/003) and funded by resources dedicated to specific university research.

References

Awad, R. A., Abde-Hamid, L. B., El-Shabrawy, S. A., & Singh, R. K. (2004). Physical and sensory properties of block processed cheese with formulated emulsifying salt mixtures. *International Journal of Food Properties, 7*, 429–448.

Brickley, C. A., Auty, M. A. E., Piraino, P., & McSweeney, P. L. H. (2007). The effect of natural Cheddar cheese ripening on the functional and textural properties of the processed cheese manufactured therefrom. *Journal of Food Science, 72*, 48–490.

Buňka, F., Doudová, L., Weiserová, E., Černíková, M., Kuchař, D., Slaviková, S., et al. (2014). The effect of concentration and composition of ternary emulsifying salts on the textural properties of processed cheese spreads. *LWT – Food Science and Technology, 58*, 247–255.

Buňková, L., Buňka, F., Mantlová, G., Čablová, A., Sedlaček, I., Švec, P., et al. (2010). The effect of ripening and storage conditions on the distribution of tyramine, putrescine and cadaverine in Edam-cheese. *Food Microbiology, 27*, 880–888.

Chavhan, G. B., Kanawjia, S. K., Khetra, Y., & Puri, R. (2015). Effect of potassium-based emulsifying salts on sensory, textural, and functional attributes of low-sodium proceese Mozzarella cheese. *Dairy Science & Technology, 95*, 265–278.

Chen, L., & Liu, H. (2012). Effect of emulsifying salts on the physicochemical properties of processed cheese made from Mozzarella. *Journal of Dairy Science, 95*, 4823–4830.

Costabel, L., Pauletti, M. S., & Hynes, E. (2007). Proteolysis in Mozzarella cheeses manufactured by different industrial processes. *Journal of Dairy Science, 90*, 2103–2112.

Dimitreli, G., & Thomareis, A. S. (2009). Instrumental textural and viscoelastic properties of processed cheese as affected by emulsifying salts and in relation to its apparent viscosity. *International Journal of Food Properties, 12*, 261–275.

El-Bakry, M., Duggan, E., O'Riordan, E. D., & O'Sullivan, M. (2011). Effect of chelating salt type on casein hydration and fat emulsification during manufacture and post-manufacture functionality of imitation cheese. *Journal of Food Engineering, 102*, 145–153.

Francolino, S., Locci, F., Ghigliettti, R., Iezzi, R., & Mucchetti, G. (2010). Use of milk protein concentrate to standardize milk composition in Italian citric Mozzarella cheese making. *LWT – Food Science and Technology, 43*, 310–314.

Gabriele, D., De Cindio, B., & D'Antona, P. A. (2001). Weak gel model for foods. *Rheologica Acta, 40*, 120–127.

Hladká, K., Randulová, Z., Tremlová, B., Ponížil, P., Mančík, P., Černíková, M., et al. (2014). The effect of cheese maturity on selected properties of processed cheese without traditional emulsifying agents. *LWT – Food Science and Technology, 55*, 650–656.

Innocente, N. (1997). Free amino acids and water-soluble nitrogen as ripening indices in Montasio cheese. *Le Lait, 77*, 359–369.

ISO Standard No 5534. (2004). *Cheese and processed cheese – determination of the total solids content (reference method)*. Geneva: International Organization for Standardization.

Ji, T., Alvarez, V. B., & Harper, W. J. (2004). Influence of starter culture ratios and warm room treatment on free fatty acid and amino acid in Swiss cheese. *Journal of Dairy Science, 87*, 1986–1992.

Juan, B., Zamora, A., Quevedo, J. M., & Trujillo, A. J. (2016). Proteolysis of cheese made from goat milk treated by ultra high pressure homogenisation. *LWT – Food Science and Technology, 69*, 1–23.

Kaliappan, S., & Lucey, J. A. (2011). Influence of mixtures of calcium-chelating salts on the physicochemical properties of casein micelles. *Journal of Dairy Science, 94*, 4255–4263.

Kapoor, R., & Metzger, L. E. (2008). Process cheese: Scientific and technological aspects – a review. *Comprehensive Reviews in Food Science and Food Safety, 7*, 194–214.

Khetra, Y., Chavhan, G. B., Kanawjia, S. K., & Puri, R. (2015). Storage changes in low sodium-processed Mozzarella cheese prepared using potassium-based emulsifying salts. *Dairy Science & Technology, 95*, 639–649.

Lee, S. K., & Klostermeyer, H. (2001). The effect of pH on the rheological properties of reduced-fat model processed cheese spreads. *LWT – Food Sciience and Technology, 34*, 288–292.

Lee, S. K., Buwalda, R. J., Euston, S. R., Foegeding, E. A., & McKennan, A. B. (2003). Changes in the rheology and microstructure of processed cheese during cooking. *LWT – Food Sciience and Technology, 36*, 339–345.

Luo, J., Pan, T., Guo, H. Y., & Ren, F. Z. (2013). Effect of calcium in brine on salt diffusion and water distribution of Mozzarella cheese during brining. *Journal of Dairy Science, 96*, 824–831.

Lu, Y., Shirashoji, N., & Lucey, J. A. (2008). Effects of pH on the textural properties and meltability of pasteurized process cheese made with different types of emulsifying salts. *Journal of Food Science, 73*, 1363–1369.

Marchesseau, S., Gastaldi, E., Lagaude, A., & Cuq, J. L. (1997). Influence of pH on protein interactions and microstructure of process cheese. *Journal of Dairy Science, 80*, 1483–1489.

Mizuno, R., & Lucey, J. A. (2005). Effects of emulsifying salts on the turbidity and calcium-phosphate–protein interactions in casein micelles. *Journal of Dairy Science, 88*, 3070–3078.

Nagyová, G., Buňka, F., Salek, R. N., Černíková, M., Mančík, P., Grüber, T., et al. (2014). Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. *Journal of Dairy Science, 97*, 111–122.

Pachlová, V., Buňka, F., Buňková, L., Weiserová, E., Budinský, P., Žaludek, M., et al. (2011). The effect of three different ripening/storage conditions on the distribution of selected parameters in individual parts of Dutch-type cheese: Cheese ripened/stored under three different conditions. *International Journal of Food Science & Technology, 46*, 101–108.

Petrella, G., Pati, S., Gagliardi, R., Rizzuti, A., Mastorilli, P., La Gatta, B., et al. (2015). Study of proteolysis in river buffalo Mozzarella cheese using a proteomics approach. *Journal of Dairy Science, 98*, 756–7572.

Piska, I., & Štětina, J. (2004). Influence of cheese ripening and rate of cooling of the processed cheese mixture on rheological properties of processed cheese. *Journal of Food Engineering, 61*, 551–555.

Poveda, J. M., Cabezas, L., & McSweeney, P. L. H. (2004). Free amino acid content of Manchego cheese manufactured with different starter cultures and changes throughout ripening. *Food Chemistry, 84*, 213–218.

Sádlíková, I., Buňka, F., Budinský, P., Voldánová, B., Pavlínek, V., & Hoza, I. (2010). The effect of selected phosphate emulsifying salts on viscoelastic properties of processed cheese. *LWT – Food Science and Technology, 43*, 1220–1225.

Salek, R. N., Černíková, M., Madĕrová, S., Lapčík, L., & Buňka, F. (2016). The effect of different composition of ternary mixtures of emulsifying salts on the consistency of processed cheese spreads manufactured from Swiss-type cheese with different degrees of maturity. *Journal of Dairy Science, 99*, 3274–3287.

Salek, R. N., Černíková, M., Nagyová, G., Kuchař, D., Bačová, H., Minarčíková, L., et al. (2015). The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. *International Dairy Journal, 44*, 37–43.

Segat, A., Biasutti, M., Iacumin, L., Comi, G., Baruzzi, F., Carboni, C., et al. (2014). Use of ozone in production chain of high moisture Mozzarella cheese. *LWT – Food Science and Technology, 55*, 513–520.

Shirashoji, N., Jaeggi, J. J., & Lucey, J. A. (2006). Effect of trisodium citrate

concentration and cooking time on physicochemical properties of pasteurized process cheese. *Journal of Dairy Science, 89*, 15–28.
Sousa, M. J., Ardö, Y., & McSweeney, P. L. H. (2001). Advances in the study of proteolysis during cheese ripening. *International Dairy Journal, 11*, 327–345.
Sulejmani, E., Hayaloglu, A. A., & Rafajlovska, V. (2014). Study of the chemical composition, proteolysis, volatile compounds, and textural properties of industrial and traditional beaten (Bieno Sirenje) ewe milk cheese. *Journal of Dairy Science, 97*, 1210–1224.
Szczesniak, A. S. (2002). Texture is a sensory property. *Food Quality and Preference, 13*, 215–225.
Vicente, M. S., Ibáñez, F. C., Barcina, Y., & Barron, L. J. R. (2001). Changes in the free amino acid content during ripening of Idiazabal cheese: Influence of starter and rennet type. *Food Chemistry, 72*, 309–317.
Weiserová, E., Doudová, L., Galiová, L., Žák, L., Michálek, J., Janiš, R., et al. (2011). The effect of combinations of sodium phosphates in binary mixtures on selected texture parameters of processed cheese spreads. *International Dairy Journal, 21*, 979–986.
Winter, H. H., & Chambon, F. (1986). Analysis of linear viscoelasticity of a cross-linking polymer at the gel point. *Journal of Rheology, 30*, 367–382.
Zhu, C. J., Brown, C., Gilles, G., Watkinson, P., & Bronlund, J. (2015). Characterizing the rheological properties of Mozzarella cheese at shear rate and temperature conditions relevant to pizza baking. *LWT – Food Science and Technology, 64*, 82–87.

Research paper 5

A5

Salek, R.N., Černíková, M., Lorencová, E., Pachlová, V., Kůrová, V., Šenkýřová, J., Buňka, F.

The impact of Cheddar or white brined cheese with various maturity degrees on the processed cheese consistency: A comparative study.

International Dairy Journal, *111*. 2020. ISSN: 09586946.

International Dairy Journal 111 (2020) 104816

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

The impact of Cheddar or white brined cheese with various maturity degrees on the processed cheese consistency: A comparative study

Richardos Nikolaos Salek [a], Michaela Černíková [a], Eva Lorencová [a], Vendula Pachlová [a], Vendula Kůrová [a], Jana Šenkýřová [a], František Buňka [a, b, *]

[a] Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, T.G. Masaryka 5555, 760 01, Zlín, Czech Republic
[b] Food Research Laboratory, Department of Logistics, Faculty of Military Leadership, University of Defence, Kounicova 65, 662 10, Brno, Czech Republic

ARTICLE INFO

Article history:
Received 22 May 2020
Received in revised form
7 July 2020
Accepted 8 July 2020
Available online 31 July 2020

ABSTRACT

This study focussed on the dependence on different emulsifying salt ternary mixture composition [disodium hydrogenphosphate (DSP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate (P20; number of phosphate units in the chain ≈20), trisodium citrate (TSC)] of hardness and gel strength of spreadable processed cheese (PC) manufactured from Cheddar and white brined cheeses. All PC samples were stored for 60 days (6 ± 2 °C). The hardest PC and samples with the highest gel strength were those produced from DSP and TSPP in a ratio 1:1. The hardness of all examined samples increased with the extending storage period, whilst their hardness and gel strength decreased with the rising maturity degree of the raw material utilised. Furthermore, higher values of gel strength were reported for the PC samples produced with Cheddar cheese in comparison with those made from white brined cheese.

1. Introduction

Processed cheese (PC) is a viscoelastic dairy-based gel described also as a stable oil-in-water emulsion (Chen & Liu, 2012; Hanaei, Cuvelier, & Sieffermann, 2015; Lee, Buwalda, Euston, Foegeding, & McKenna, 2003). In traditional PC production, the main raw material is natural cheese of various degrees of maturity. The types of cheese predominantly used in different world areas vary (including Cheddar, Dutch-type, Swiss-type, mozzarella and white brined cheeses). In the English-speaking countries (e.g., Britain, USA, Canada, Australia, New Zealand) the main raw material for PC production is usually Cheddar (CDC) and mozzarella cheeses (typically, e.g., for New Zealand). On the other hand, in countries around the Mediterranean area, Balkan, the Near and Middle East, white brined cheeses (WBC) represent the most consumed cheese varieties and are therefore widely used as the main raw material for the production of PC (Moatsou & Govaris, 2011; Černíková, Nebesářová, Salek, Řiháčková, & Buňka, 2017). Many dairy ingredients (e.g., anhydrous butterfat, butter, cream, milk powder, whey, buttermilk, caseinates, coprecipitates) or non-dairy components (e.g., stabilisers, preservatives, flavouring agents, hydrocolloids, acidifying agents) can be optionally added into the mixture of raw materials. Besides the ingredients in the formulation, physicochemical, technological, and microbiological factors could affect the properties of the final PC (Ferrão et al., 2016; Kapoor & Metzger, 2008).

The desired final smooth and homogeneous matrix of PC is formed by blending shredded natural cheese in the presence of emulsifying salts (ES; mainly, sodium salts of phosphates, polyphosphates, citrates or combinations of these), heated under partial vacuum and constant shear, commonly in a temperature range of 90–100 °C. ES are essential components in the formulation. The addition of ES results in ion exchange of calcium and sodium ions and, subsequently, casein dispersion. The dispersed proteins (sodium paracaseinates) can serve as effective surface active substances and emulsify the dispersed free fat globules. The control and stabilisation of the pH level and an influence on the formation of the final casein network over holding time at the melting temperature and/or over cooling are some additional roles of ES (Buňka et al., 2014; Chen & Liu, 2012; Dimitreli & Thomareis, 2009; Sádlíková et al., 2010; Salek, Černíková, Maděrová, Lapčík, & Buňka, 2016).

Furthermore, the consistency of PC can be affected by many factors, including, e.g.: (i) raw material composition – the type and

* Corresponding author. Tel.: +420 606 777 249.
E-mail address: frantisek.bunka@gmail.com (F. Buňka).

https://doi.org/10.1016/j.idairyj.2020.104816
0958-6946/

chemical profile of the natural cheese applied (dry-matter, fat, protein, and calcium ion contents, and maturity degree), composition and concentration of ES, addition of other optional dairy and non-dairy ingredients and also the pH of the mass to be melted; and (ii) processing and storage conditions – agitation speed, target melting temperature and holding time, cooling rate and also storage temperature. Nowadays, hydrocolloids, regularly used in the production of PC, are important components affecting the consistency of PC (Dimitreli & Thomareis, 2007; Shirashoji, Jaeggi, & Lucey, 2006).

CDC is a ripened hard cheese and its body has a near white or ivory through to light yellow or orange colour. In addition, its texture can be described as firm, smooth and waxy. Furthermore, gas-holes are absent, whereas some openings and splits are acceptable. The ripening period to develop typical flavour and body characteristics is normally from 5 weeks up to 2 years (at 7–15 °C), depending on the extend of maturity required (Codex standard 263-1966; Codex Alimentarius Commission, 2013). In contrast, WBC are produced from curds that are not subjected to any elevated heating (cooking) after coagulation (such as is the case for CDC, Dutch- or Swiss-type cheeses). Their flavour is slightly acid and salty that sometimes turns to rancid and piquant. Moreover, the cheese mass has no rind, no gas-holes or other openings, except for some small mechanical openings and its texture appears to be soft but sliceable. Thus, they are consumed after several days or up to some months of ripening in brine of various NaCl concentrations (10–18 g 100 g^{-1}) (Hayaloglu, 2016; Moatsou & Govaris, 2011).

The impact of ES composition on the consistency of PC produced from different natural cheeses (the main raw material), particularly Edam (Buňka et al., 2014; Salek et al., 2015), Cheddar (Brickley, Auty, Piraino, & McSweeney, 2007), mozzarella (Chavhan, Kanawjia, Khetra, & Puri, 2015; Chen & Liu, 2012; Khetra, Chavhan, Kanawjia, & Puri, 2015; Salek et al., 2017) and Swiss-type (Salek et al., 2016) cheeses has been previously reported. Nevertheless, research providing a direct comparison of the spread properties of PC produced under identical processing parameters and similar experimental design from two technologically very different varieties of natural cheese (CDC and WBC), and additionally with various levels of maturity, has not been performed to date. Therefore, the main aim of the present study was to compare the influence of two different varieties of natural cheese with varying levels of maturity in combination with the different composition of ES on the textural properties and the gel strength of spreadable PC during a 60-day storage period (6 ± 2 °C). Disodium hydrogenphosphate (DSP, Na_2HPO_4), tetrasodium diphosphate (TSPP, $Na_4P_2O_7$), sodium salt of polyphosphate with mean length n ≈ 20 (P20) and trisodium citrate (TSC, $Na_3C_6H_5O_7$) were used in four types of ternary mixtures of ES. The pH of the tested samples was adjusted to the target values within the interval of 5.60–5.80, corresponding to the standard pH values of PC spreads.

2. Materials and methods

2.1. Materials

Commercially available CDC blocks [dry-matter content 62 g 100 g^{-1}; fat in dry-matter content, 50 g 100 g^{-1}; 4, 8, 12 and 16 weeks of maturity (storage at 10 ± 2 °C); 1.8 g 100 g^{-1} NaCl content], WBC blocks [Akawi-type cheese; dry-matter content 48 g 100 g^{-1}; fat in dry-matter content 38 g 100 g^{-1}; 2, 4, 8, 16 and 24 weeks of maturity (storage at 10 ± 2 °C); 7.4 g 100 g^{-1} NaCl content] and butter (dry-matter content 84 g 100 g^{-1}, fat content 82 g 100 g^{-1}) were purchased wholesale in the Czech Republic. The same batch of the individual cheese varieties was used in the whole experiment.

When Cheddar is used as raw material in Central Europe (a minority, but some producers use it), the maturity is generally approximately 8 weeks. Therefore, the half of this period and twice this period (i.e., 4–16 weeks) were chosen as an appropriate storage interval for the experiment. According to the authors' knowledge, the selected maturity interval also covers usual storage times for raw material for PC in some other countries. In the case of white brine cheese, our practical experience from the Near and Middle East shows us that the storage period could be longer than the usual 2 or 3 months. This is valid also for raw material for PC. Therefore, approximately half year was chosen as the longest storage period. DSP, TSPP and P20 were obtained from Fosfa PLC Company (Břeclav, Czech Republic); TSC, HCl, and NaOH were purchased from Sigma Aldrich Inc. (Schnelldorf, Germany). Water was also added to adjusting to the required dry matter content of the model PC.

2.2. Manufacturing procedure of the processed cheese samples

The composition of raw materials of the PC samples was calculated to achieve final products with 40 g 100 g^{-1} dry matter content and 50 g 100 g^{-1} fat in dry matter content. Moreover, four ternary mixtures of ES (TSC:TSPP:P20, DSP:TSC:P20, DSP:TSPP:P20 and DSP:TSPP:TSC) were prepared. For all four ternary mixtures the ES were blended in 12 percentage ratios (100:0:0; 50:50:0; 0:100:0; 40:40:20; 40:20:40; 20:40:40; 50:0:50; 0:50:50; 40:0:60; 20:20:60; 0:40:60; 0:0:100 – the percentages of the components were calculated on the total weight of the ES; where total weight was 100%). The total concentration of the applied ES was 3 g 100 g^{-1} of the total weight of the melt. Fig. 1 illustrates the experimental design.

A Vorwerk Thermomix TM blender cooker (2 L capacity; Vorwerk & Co Thermomix GmbH, Wuppertal, Germany) with indirect heating was employed for the production of the model PC samples in laboratory scale (the same device was used previously by Lee et al., 2004) and Nagyová et al., 2014). The manufacturing procedure was described in detail in Salek et al. (2015, 2016, 2017). The processing conditions [melting temperature 90 °C held for 1 min (total melting time: 10–12 min) at approximately 2750 rpm] were the same for all the formulations. The pH of the samples was adjusted (target values within the interval of 5.60–5.80) using acid or alkali (1 mol L^{-1} HCl or NaOH). To maintain the dry-matter content at the desired target value (40 g 100 g^{-1}), the addition of water was decreased (based on the amount of the added acid/alkali). Finally, the hot molten PC mass was poured into cylindrical plastic containers (55 mm diameter, 50 mm height) and sealed. Thereafter, the samples were left to cool and were stored under refrigeration conditions (6 ± 2 °C) until further analysis.

Analyses were performed on the 2nd, 9th, 30th and 60th day of storage; rheological analysis was undertaken on the 30th day after the production. Each PC sample tested was produced in duplicate (CDC – 4 maturity levels × 4 types of ternary mixtures × 12 percentage ratios × 2 repetitions = 384 lots; WBC – 5 maturity levels × 4 types of ternary mixtures × 12 percentage ratios × 2 repetitions = 480 lots) resulting in 864 lots in total. The raw materials applied and processing parameters were arranged to imitate industrial conditions. The samples had the same target parameters (dry matter content and pH-value) and were produced under the same conditions (e.g., the equipment, the target melting temperature, the holding time, the same packaging, the cooling time, the storage time and temperature). Therefore, the model PCs manufactured were fully comparable.

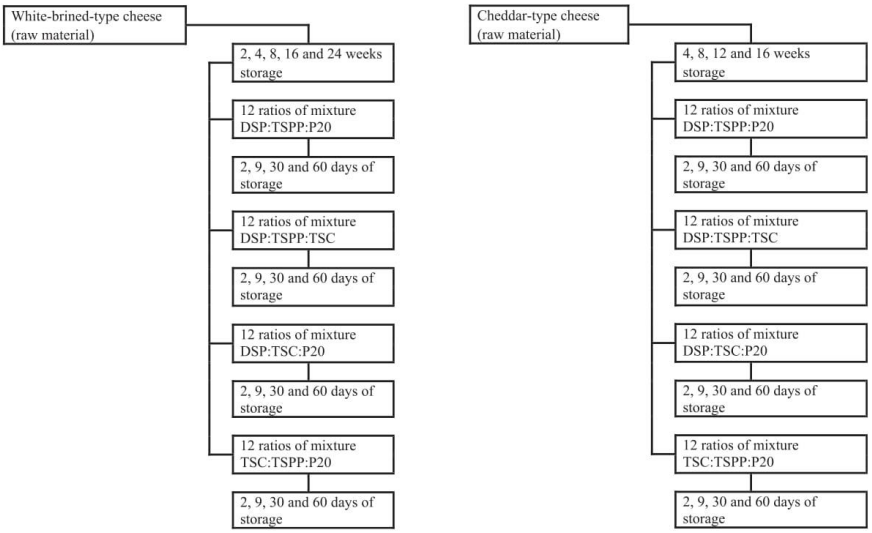

Fig. 1. Scheme of the experimental design with model processed cheeses manufactured using white-brined-type and Cheddar-type cheese in various time of storage and the different percentage ratios of the four types of ternary mixtures comprising DSP:TSPP:P20, DSP:TSPP:TSC, DSP:TSC:P20 and TSC:TSPP:P20 (abbreviations: DSP, Na_2HPO_4; TSPP, $Na_4P_2O_7$; P20, sodium salt of polyphosphate with mean length n ≈ 20; TSC, trisodium citrate). The model samples were tested after 2, 9, 30 and 60 days of storage.

2.3. Chemical analysis of the cheese and the processed cheese samples

In the processed cheese samples, the dry matter (DM) content and the pH-values were determined. DM content was gravimetrically analysed according to ISO 5534 (ISO, 2004) by drying the samples at 102 ± 2 °C to constant mass. The pH values were determined at ambient temperature by inserting a glass tip electrode of a calibrated pH-meter (pH Spear, Eutech Instruments, Oakton, Malaysia) directly into the cheese at three randomly chosen locations.

In the cheese (CDC and WBC), the determination of free amino acid (FAA) content was undertaken in accordance with the process previously performed by Buňková et al. (2009), Hladká et al. (2014) and Pachlová et al. (2011) using the AAA 400 amino acid analyser (Ingos, Prague, Czech Republic). The FAA content was calculated as a sum of 22 individual FAAs and the content of similar substances (γ-aminobutyric acid, alanine, aspartic acid, asparagine, arginine, citrulline, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, tyrosine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, valine; results were expressed in g kg^{-1}). Additionally, prior to the particular determination each natural cheese was lyophilised (Christ Alpha 1–4, Christ, Osterode, Germany) twice. Furthermore, each lyophilised sample was extracted twice and each extract was loaded on the column in triplicate (n = 12).

2.4. Hardness measurements of the processed cheese samples

The selected textural properties of the model PC samples were evaluated using a texture analyser TA.XTplus (Stable Micro Systems Ltd., Godaming, UK) equipped with a 20 mm in diameter cylindrical aluminium probe. The analysis was performed by penetration into the sample (depth 10 mm and trigger force 5 g; deformation rate was 2 mm s^{-1}) at 6 ± 2 °C (the measurement was carried out within the containers). From the force/time curves, the hardness value were obtain as the maximum force (N) observed during penetration (n = 6).

2.5. Rheological measurements of the processed cheese samples

A dynamic oscillatory shear rheometer (RheoStress 1, Haake, Bremen, Germany) equipped with a parallel plate–plate geometry having a 35 mm diameter was used for the determination of the PC viscoelastic properties. The rheological tests were carried out at 20.0 ± 0.1 °C and a gap of 1 mm was applied. Amplitude sweeps were performed to determine the linear viscoelastic regions at which the frequency sweep of the samples was obtained. During the tests, of the storage G' (elastic modulus) and G'' (viscous modulus) moduli were measured at frequencies between 0.01 and 100.00 Hz and subsequently the complex modulus (G^*) was calculated as the complex sum of G′ and G″.

The Winter and Chambon (1986) critical gel model was implemented for the changes evaluation in the samples viscoelastic properties as a function of frequency:

$$G^*(\omega) = A_F \cdot \omega^{\frac{1}{z}} \quad (1)$$

where A_F (Pa $s^{1/z}$) represents the gel strength, ω is the frequency (Hz) and z (dimensionless) corresponds to the interaction factor. The recorded values were the mean of at least eight replicates (n = 8).

2.6. Data analysis

The experimental data obtained were analysed using the Unistat® 6.5 (Unistat, London, UK) statistical software. Kruskal–Wallis and Wilcoxon tests were applied for the evaluation of the results. Significance was considered as $P < 0.05$. For the estimation of A_F the method of Marquardt–Levenberg, a nonlinear regression

analysis method ($A_F > 0$ and $z > 0$) was implemented. Correlation analysis was also carried out using Spearman correlation coefficient.

3. Results and discussion

3.1. Free amino acids content in Cheddar and white brined cheese

The total FAA concentrations of the CDC after 4, 8, 12 and 16 weeks were 2.75, 22.18, 41.72 and 48.66 g kg^{-1}, respectively. In comparison, in the WBC the total FAA concentrations after 2, 4, 8, 16 and 24 weeks of ripening were 8.47, 18.74, 37.96, 58.91 and 78.19 g kg^{-1}, respectively. With the prolonging of the ripening period (regardless of the natural cheese applied as the main raw material) more intensive proteolysis occurred ($P < 0.05$). Hydrolytic processing of caseins was faster in WBC in comparison with that in CDC ($P < 0.05$). A possible reason could lie in different cultures (microorganisms), which are used in the production of the above mentioned cheese. According to Fox, Guinee, Cogan, and McSweeney (2000) and Pachlová et al. (2011) it could be assumed that the amount of intact casein present in the natural cheese (main raw material) correlates with the concentration of FAA. The mean length of casein fragments after hydrolysis and so the amount of intact casein remaining could significantly influence the consistency of the PC (Diana, Rafecas, Arco, & Quílez, 2014; Petrella et al., 2015; Salek et al., 2017).

3.2. Chemical analysis of the processed cheese samples

The dry matter content (DM) of all PC samples tested (regardless of the type of cheese, the ripening period, the storage time and the composition of ES used) ranged in the interval 40.21–40.83 g 100 g^{-1} ($P \geq 0.05$). The composition of ES ternary mixture significantly influences the value of pH ($P < 0.05$) (Nagyová et al., 2014; Salek et al., 2015, 2016, 2017). Therefore, the pH-values were adjusted during manufacturing (see Subsection 2.2). At the beginning of the storage, the pH-values of all PC without regard to the studied factors were in the interval of 5.61–5.84. During the 60-day storage the pH-values slightly but significantly increased by 0.1–0.2 ($P < 0.05$). Similar DM content and the pH-value are essential for successful comparison of consistency of the final products (Lee & Klostermeyer, 2001; Marchesseau, Gastaldi, Lagaude, & Cuq, 1997; Piska & Štětina, 2004; Sádlíková et al., 2010).

3.3. Textural and rheological properties of the processed cheese samples

For the recent study, two approaches for evaluation of PC consistency were chosen. Firstly, hardness (N) was used as the parameter describing sample behaviour under large deformation. Subsequently, the testing was enhanced by using of rheological measurements (gel strength – A_F; Pa), for studying of sample changes under small shear deformation.

The effect of three factors influencing processed cheese consistency was observed: (i) the composition of ternary mixtures of four ES; (ii) the ripening period of the main raw material – natural cheese; and (iii) the storage time up to 60 days. All these factors were studied using samples manufactured using two very different varieties of cheese – CDC and WBC for a comparison of the effect of varying the main raw material. The results of the sample response on the large (hardness) and small (gel strength) deformations are shown in Figs. 2–7.

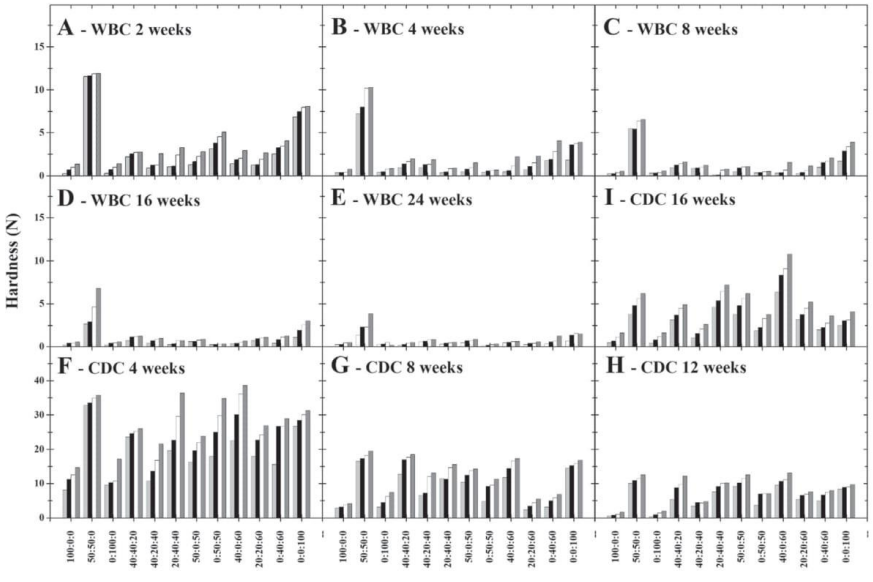

Fig. 2. The dependence of processed cheese hardness (N) on the relative amount (%) of three emulsifying salts (disodium phosphate, tetrasodium diphosphate and sodium salt of polyphosphate) during 60-day storage at 6 ± 2 °C [results expressed as means (n = 6); processed cheese were sampled after 2 (▒), 9 (■), 30 (□) and 60 (▩) days of storage]. Processed cheeses were made from white-brined-type (WBC) cheese after different time of storage (A, 2 weeks; B, 4 weeks; C, 8 weeks; D, 16 weeks; E, 24 weeks) and Cheddar-type cheese (CDC) after different time of storage (F, 4 weeks; G, 8 weeks; H, 12 weeks; I, 16 weeks). Please note the differences in y-axis values and hence the placement of panel I.

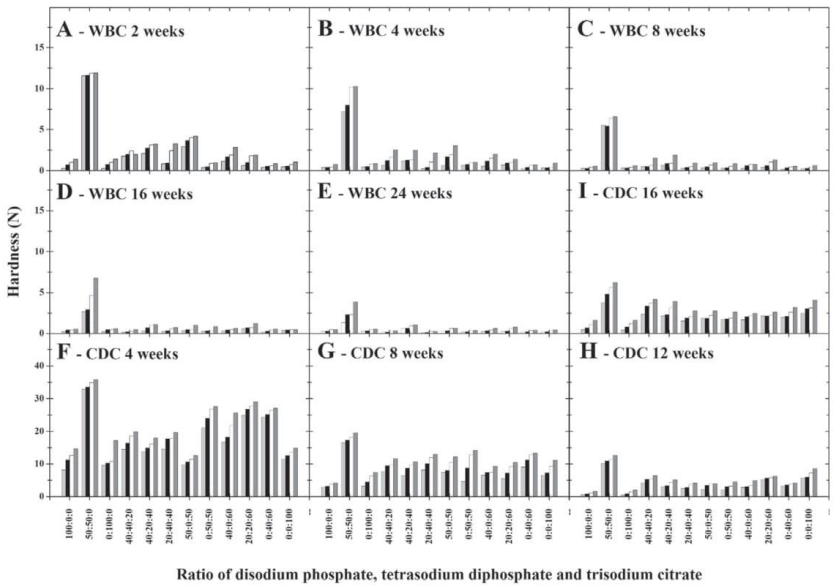

Fig. 3. The dependence of processed cheese hardness (N) on the relative amount (%) of three emulsifying salts (disodium phosphate, tetrasodium diphosphate and trisodium citrate) during 60-day storage at 6 ± 2 °C [results expressed as means (n = 6); processed cheese were sampled after 2 (▒), 9 (■), 30 (□) and 60 (▒) days of storage]. Processed cheeses were made from white-brined-type (WBC) cheese after different time of storage (A, 2 weeks; B, 4 weeks; C, 8 weeks; D, 16 weeks; E, 24 weeks) and Cheddar-type cheese (CDC) after different time of storage (F, 4 weeks; G, 8 weeks; H, 12 weeks; I, 16 weeks). Please note the differences in y-axis values and hence the placement of panel I.

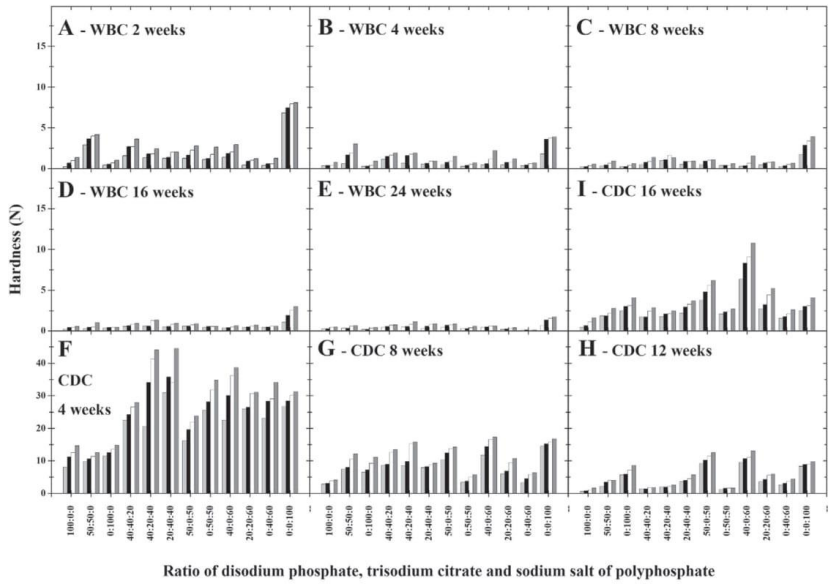

Fig. 4. The dependence of processed cheese hardness (N) on the relative amount (%) of three emulsifying salts (disodium phosphate, trisodium citrate and sodium salt of polyphosphate) during 60-day storage at 6 ± 2 °C [results expressed as means (n = 6); processed cheese were sampled after 2 (▒), 9 (■), 30 (□) and 60 (▒) days of storage]. Processed cheeses were made from white-brined-type (WBC) cheese after different time of storage (A, 2 weeks; B, 4 weeks; C, 8 weeks; D, 16 weeks; E, 24 weeks) and Cheddar-type cheese (CDC) after different time of storage (F, 4 weeks; G, 8 weeks; H, 12 weeks; I, 16 weeks). Please note the differences in y-axis values and hence the placement of panel I.

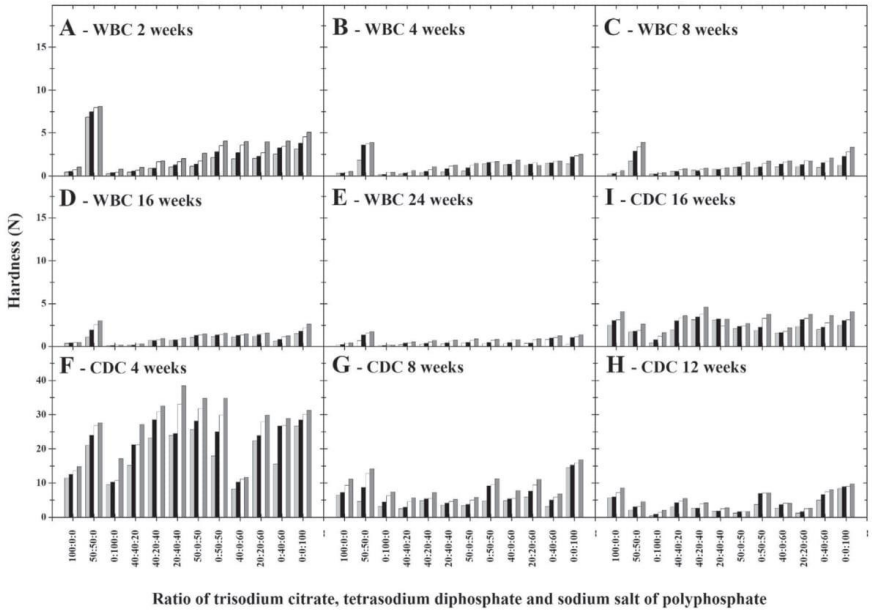

Fig. 5. The dependence of processed cheese hardness (N) on the relative amount (%) of three emulsifying salts (trisodium citrate, tetrasodium diphosphate and sodium salt of polyphosphate) during 60-day storage at 6 ± 2 °C [results expressed as means (n = 6); processed cheese were sampled after 2 (▒), 9 (■), 30 (□) and 60 (▩) days of storage]. Processed cheeses were made from white-brined-type cheese (WBC) after different time of storage (A, 2 weeks; B, 4 weeks; C, 8 weeks; D, 16 weeks; E, 24 weeks) and Cheddar-type cheese (CDC) after different time of storage (F, 4 weeks; G, 8 weeks; H, 12 weeks; I, 16 weeks). Please note the differences in y-axis values and hence the placement of panel I.

When ES were singly added (regardless the type of natural cheese used, the maturity level and the length of the storage period), increase of hardness and gel strength (Figs. 2–7) of PC with sodium salts of polyphosphate (P20) was observed in comparison with the samples with the other ES (DSP, TSPP and TSC; $P < 0.05$).

Sodium salt of polyphosphate probably can strongly bind calcium into complexes resulting in casein dispersion enhancement. Polyphosphates possess the strongest binding capability to bivalent ions in comparison with monophosphates, diphosphates and/or citrates (Buňka et al., 2012; Shirashoji et al., 2006). Additionally, the use of TSC resulted in PC samples with similar values of hardness to those made with TSPP ($P \geq 0.05$). Thus, TSC does not provide the ability to create new networks (Mizuno & Lucey, 2007). The results obtained are in accordance to those previously reported by El-Bakry, Duggan, O'Riordan, and O'Sullivan (2011), Nagyová et al. (2014), and Salek et al. (2015, 2016, 2017), who also used TSC.

Figs. 2, 6A and 7A illustrate the dependence of hardness and gel strength on the composition of the mixture of DSP, TSPP and P20. Regardless the other studied factors (the ripening time of raw material, the storage time and the type of cheese), there is a specific ratio of DSP and TSPP of approximately 1:1 under which the hardness and also the gel strength increased in comparison with the other mixtures of the tested ES ($P < 0.05$). This phenomenon was observed especially when the relative content of P20 was under 50%. However, this observation was not noticed when the relative ratio of P20 was over 50%. The above mentioned effect of the specific ratio of DSP:TSPP (including the dependence of the relative ratio of P20) was previously detected when Dutch-type, Swiss-type and mozzarella-type cheeses were applied (Salek et al., 2015, 2016, 2017). The explanation lies in (i) the high ability of TSPP to support forming casein gels and (ii) the small size of DSP and its capability to bind onto caseins and increase their hydration. When the relative amount of TSPP is too low, the gel is too weak due small number of interaction between caseins. On the other hand, when the relative concentration of TSPP is too high, the gel is also too weak because calcium ions are strongly bonded and are not possibly "used" during the protein network forming (Buňka et al., 2012; Kaliappan & Lucey, 2011; Mizuno & Lucey, 2007). The same effect ($P < 0.05$) was observed also when PC with the ternary mixtures of DSP:TSPP:TSC (Figs. 3, 6B and 7B). In the other samples, where DSP, TSPP and P20 were used, it was observed that with the rising relative amount of P20 in the mixture, the hardness and gel strength of the samples increased ($P < 0.05$; under the same levels of the other factors tested). The latter trend was noticed in all samples with this ternary mixture, without regard to the other tested factors (see above).

The next studied ternary mixture was the combination of DSP, TSPP and TSC. The results of consistency parameters tested are presented in Figs. 3, 6B and 7B. In some PC, the effect of TSPP on hardness and gel strength was slightly higher in comparison with the influence of TSC. In the remaining samples, the parameter tested was similar ($P \geq 0.05$) or the trend was opposite without any clear reason. The results obtained about the practical similarity of the effect of TSPP and TSC on the consistency of PC are in accordance with those previously reported by El-Bakry et al. (2011), Nagyová et al. (2014), and Salek et al. (2015, 2016, 2017). Beside that discussed above with respect to the specific 1:1 ratio of DSP and TSPP, the changes of hardness and gel strength were controlled by the relative amount of TSPP and/or TSC. When the relative amount of TSPP and/or TSC was increased in comparison with DSP, hardness

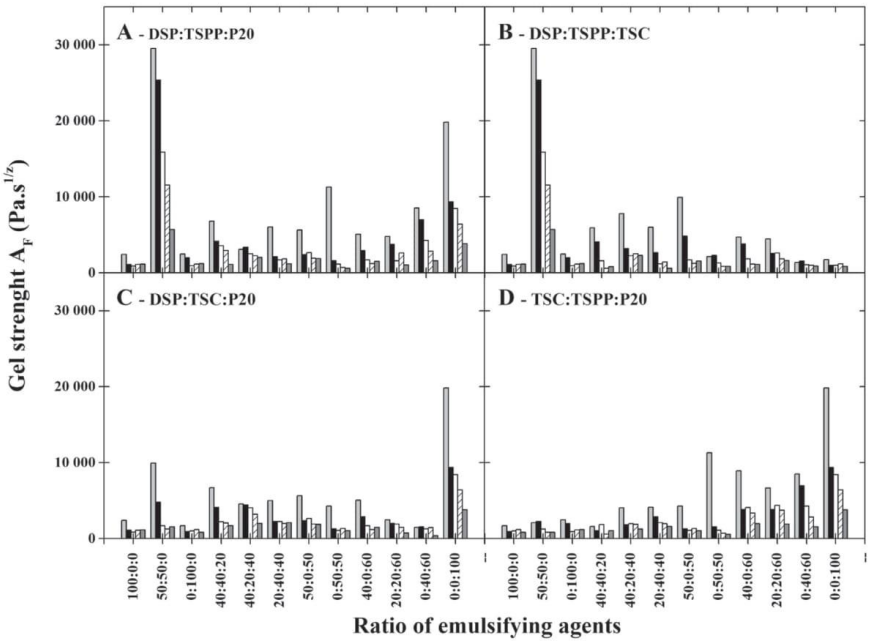

Fig. 6. The dependence of gel strength (Pa $s^{1/2}$) of processed cheese on the relative amount (%) of three emulsifying salts (A, DSP:TSPP:P20; B, DSP:TSPP:TSC; C, DSP:TSC:P20; D, TSC:TSPP:P20; where DSP, disodium phosphate; TSPP, tetrasodium diphosphate; P20, sodium salt of polyphosphate; TSC, trisodium citrate) after 30-day storage at 6 ± 2 °C [results expressed as means (n = 6)]. Processed cheeses were made from white-brined-type (WBC) cheese after different times of storage (▒, 2 weeks; ■, 4 weeks; □, 8 weeks; ▨, 16 weeks; ■, 24 weeks).

and gel strength increased and vice versa ($P < 0.05$). With regard to the fact that TSC does not provide the ability of creating new networks (Mizuno & Lucey, 2007), we could assume that the ability of ion exchange, and subsequently the intensity of caseins dispersion, are the main factors influencing hardness and gel strength in samples with this ternary mixture containing predominantly TSC. DSP possesses lower capacity of ion exchange in comparison with TSPP and/or TSC (Kapoor & Metzger, 2008; Molins, 1991). The effect of the specific ratio of DSP and TSPP 1:1 was decreasing when the relative concentration of TSC raised ($P < 0.05$). The latter mentioned trend was expected because the relative amount of the effectively and synergistically acting mixture (DSP and TSPP, 1:1) was decreasing. In most of the samples, where only TSPP and TSC were used in a ratio of 1:1, the increase of hardness and gel strength values of PC were observed ($P < 0.05$; compared with samples that also contained DSP, but excluding samples where DSP and TSPP were in ratio of approximately 1:1). The same situation was in the ternary mixture of TSC, TSPP and P20 (Figs. 5, 6D and 7D). The synergistic effect of the mixture of 50% TSPP and 50% TSC can also be expected; however, clear explanation was not found in the literature. The same phenomenon was also observed in the studies of Salek et al. (2015, 2016, 2017) where Dutch-type, Swiss-type and mozzarella type cheeses as the main raw material and the same ternary mixtures of ES were used.

Besides the effect of the specific ratios of DSP:TSPP and TSC:TSPP (described in detail above), the influence of the composition of the other ternary mixtures (DSP:TSC:P20 and TSC:TSPP:P20) on hardness (Figs. 4 and 5) and gel strength (Figs. 6C, D and 7C, D) of the samples was regulated by the ability of individual ES to ion exchange and therefore casein dispersion. The ion exchange capability of used ES was increasing in the following order: DSP > TSPP ≈ TSC > P20. The latter relationships between selected ES have been published in several articles, e.g., Dimitreli and Thomareis (2009), El-Bakry et al. (2011), Nagyová et al. (2014), Shirashoji et al. (2006), and are also in accordance with our results (Figs. 2–7). When the relative amount of ES with better ability to exchange ions was higher, the values of hardness and gel strength increased and vice versa ($P < 0.05$).

In all four times of storage (2, 9, 30 and 60 days) and in all four types of ternary mixtures, hardness and gel strength of PC (Figs. 2–7) decreased with the rising ripening period of the CDC and also the WBC ($P < 0.05$; samples with the same time of storage and the same composition of ES were compared). The explanation of these phenomena lies in proteolysis and "shortening" of caseins in cheeses during ripening. The results of FAA analysis unambiguously showed that in CDC, and also in WBC, intensive proteolytic reactions took place (see Section 3.1). When the mean length of proteins in cheese decreases, hardness and gel strength of PC is also lower and vice versa (Brickley et al., 2007; Buňka et al., 2013; Salek et al., 2015, 2016, 2017). Based on our results, we can assume that ripening period influenced the absolute values of hardness of the samples but the relations between four ES in the ternary mixtures and especially its effect on product consistency remained unchanged.

The hardness of all PC produced increased during the whole 60-day storage ($P < 0.05$) regardless of (i) the type and composition of ternary mixture; (ii) the ripening period of CDC and WBC; or (iii) the type of cheese – CDC or WBC. The absolute values of PC samples hardness (Figs. 2 and 3) increased, whereas the effect of ES was

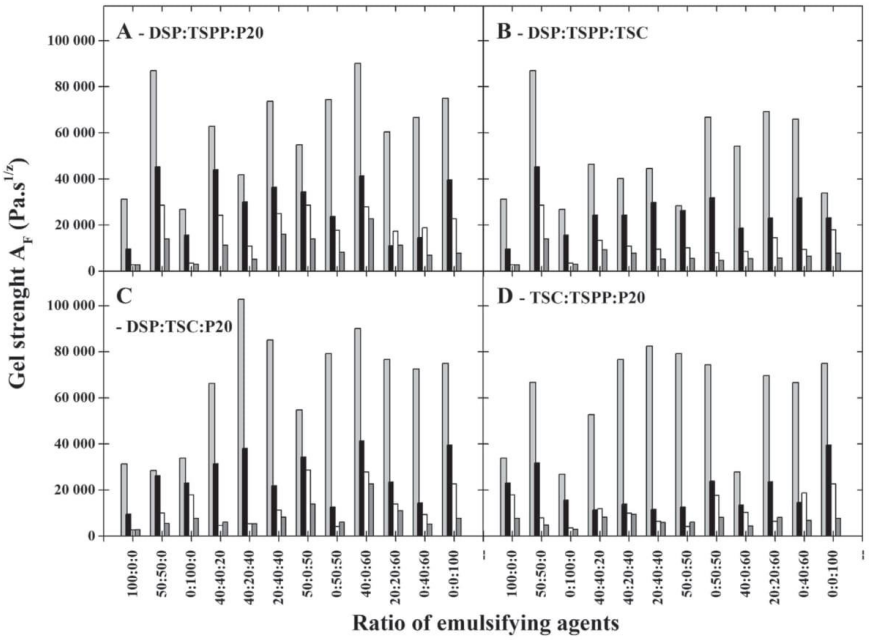

Fig. 7. The dependence of gel strength (Pa $s^{1/z}$) of processed cheese on the relative amount (%) of three emulsifying salts (A, DSP:TSPP:P20; B, DSP:TSPP:TSC; C, DSP:TSC:P20; D, TSC:TSPP:P20; where DSP, disodium phosphate; TSPP, tetrasodium diphosphate; P20, sodium salt of polyphosphate; TSC, trisodium citrate) after 30-day storage at 6 ± 2 °C [results expressed as means (n = 6)]. Processed cheeses were made from Cheddar-type cheese (CDC) after different time of storage (▒, 4 weeks; ■, 8 weeks; □, 12 weeks; ▒, 16 weeks).

practically the same. Hardness of PC could increase, e.g., due to hydrolysis of phosphates, possible changes in the forms of binding of the salts present and thus a change in their dissociative characteristics and also due to possible changes in the crystalline modifications of milk fat (Awad & Sato, 2002; Kapoor & Metzger, 2008; Molins, 1991).

In all four types of ternary mixtures and in all days when analyses were performed, hardness and gel strength (Fig. 7) of CDC were higher ($P < 0.05$) in comparison with WBC (Figs. 2–5). This difference in the hardness and gel strength values development could be due to different chemical composition (including pH, calcium content, NaCl content, residual lactose content, etc.) and cheese making process of CDC and WBC (Piska & Štětina, 2004; Purna, Pollard, & Metzger, 2006). The intensity of proteolysis played also important role.

At the end of our work, correlation analysis between the values of hardness and the values of gel strength were done. The Spearman correlation coefficients for the tested ternary mixtures (calculated individually for each ternary mixture type and CDC and WBC) ranged in the interval of 0.794–0.922 ($P < 0.05$). This finding confirmed again that the results obtained by the equipment using large uniaxial deformation and by the equipment using small shear deformation of material were in good accordance with each other.

With respect to our previous studies (Salek et al., 2015; 2016; 2017) and our recent work, we are able to infer that the general trend of the effect of the ternary mixtures composition is the same when different natural cheeses are used as raw material. Five types of natural cheese (Dutch-type, Swiss-type, mozzarella-type, Cheddar-type and white brined-type), which are used through the whole world, were tested. In all five cheese varieties the mechanisms of function of the above mentioned ternary mixtures were practically very similar regardless of the ripening period of the cheese utilised.

4. Conclusions

The impact of the CDC and WBC maturity and different compositions of ternary mixtures of ES on the hardness and gel strength of PC during 60-days of storage was investigated. With raising storage period of the PC samples, an increase in hardness was observed. On the other hand, the hardness and gel strength of the samples decreased with prolonging of cheese ripening period for both cheeses (CDC and WBC) used as the main raw material. The hardest samples were those composed of DSP:TSPP (1:1). However, when the relative amount of DSP and TSPP (in the ratio of 1:1) were replaced by TSC or P20, the influence of the latter mentioned ratio diminished. Furthermore, higher values of hardness and gel strength were reported for the PC samples produced with CDC in comparison with those made from WBC.

Credit author statement

Richardos Nikolaos Salek: Methodology; Investigation; Writing – Review & Editing; Visualization; Investigation; Writing – Original Draft; Revisions of the manuscript. Michaela Černíková: Methodology; Writing – Review & Editing. Eva Lorencová: Methodology; Writing – Review & Editing. Vendula Pachlová: Methodology; Writing – Review & Editing. Vendula Kůrová: Methodology; Writing – Review & Editing. Jana Šenkýřová: Methodology; Writing – Review & Editing. František Buňka: Conceptualization;

Supervision; Writing – Review & Editing; Project administration; Visualization; Revisions of the manuscript.

Acknowledgements

This study was kindly supported by the National Agency for Agriculture Research, project No. QK1710156 in the programme ZEMĚ and the internal grant agency of Tomas Bata University in Zlín, Czech Republic (IGA/FT/2020/003) and funded by resources dedicated to specific university research.

References

Awad, T. S., & Sato, K. (2002). Fat crystallization in O/W emulsions controlled by hydrophobic emulsifier additives. In A. G. Marangoni, & S. S. Narine (Eds.), *Physical properties of lipids* (pp. 37–62). Boca Raton, FL, USA: CRC Press.

Brickley, C. A., Auty, M. A. E., Piraino, P., & McSweeney, P. L. H. (2007). The effect of natural Cheddar cheese ripening on the functional and textural properties of the processed cheese manufactured therefrom. *Journal of Food Science, 72*, C483–C490.

Buňka, F., Doudová, L., Weiserová, E., Černíková, M., Kuchař, D., Slavíková, Š., et al. (2014). The effect of concentration and composition of ternary emulsifying salts on the textural properties of processed cheese spreads. *Lebensmittel-Wissenschaft und -Technologie – Food Science and Technology, 58*, 247–255.

Buňka, F., Doudová, L., Weiserová, E., Kuchař, D., Michálek, J., Slavíková, S., et al. (2012). The effect of different ternary mixtures of sodium phosphates on hardness of processed cheese spreads. *International Journal of Food Science and Technology, 47*, 2063–2071.

Buňka, F., Doudová, L., Weiserová, E., Kuchař, D., Ponížil, P., Začalová, D., et al. (2013). The effect of ternary emulsifying salt composition and cheese maturity on the textural properties of ptocessed cheese. *International Dairy Journal, 29*, 1–7.

Buňková, L., Buňka, F., Hlobilová, M., Vaňátková, Z., Nováková, D., & Dráb, V. (2009). Tyramine production of technological important strains of *Lactobacillus, Lactococcus* and *Streptococcus*. *European Food Research and Technology, 229*, 533–538.

Černíková, M., Nebesářová, J., Salek, R. N., Říhácková, L., & Buňka, F. (2017). Microstructure and textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content. *Journal of Dairy Science, 100*, 4300–4307.

Chavhan, G. B., Kanawjia, S. K., Khetra, Y., & Puri, R. (2015). Effect of potassium-based emulsifying salts on sensory, textural, and functional attributes of low-sodium processed Mozzarella cheese. *Dairy Science & Technology, 95*, 265–278.

Chen, L., & Liu, H. (2012). Effect of emulsifying salts on the physicochemical properties of processed cheese made from Mozzarella. *Journal of Dairy Science, 95*, 4823–4830.

Codex Alimentarius Commission. (2013). *Codex alimentarius: Standard for Cheddar*. Rome, Italy: World Health Organization/Food and Agriculture Organization of the United Nations.

Diana, M., Rafecas, M., Arco, C., & Quílez, J. (2014). Free amino acid profile of Spanish artisanal cheeses: Importance of gamma-aminobutyric acid (GABA) and ornithine content. *Journal of Food Composition and Analysis, 35*, 94–100.

Dimitreli, G., & Thomareis, A. S. (2007). Texture evaluation of block-type processed cheese as a function of chemical composition and in relation to its apparent viscosity. *Journal of Food Engineering, 79*, 1364–1373.

Dimitreli, G., & Thomareis, A. S. (2009). Instrumental textural and viscoelastic properties of processed cheese as affected by emulsifying salts and in relation to its apparent viscosity. *International Journal of Food Properties, 12*, 261–275.

El-Bakry, M., Duggan, E., O'Riordan, E. D., & O'Sullivan, M. (2011). Effect of chelating salt type on casein hydration and fat emulsification during manufacture and post-manufacture functionality of imitation cheese. *Journal of Food Engineering, 102*, 145–153.

Ferrão, L. L., Silva, E. B., Silva, H. L. A., Silva, R., Mollakhalili, N., Granato, D., et al. (2016). Strategies to develop healthier processed cheeses: Reduction of sodium and fat contents and use of prebiotics. *Food Research International, 86*, 93–102.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2000). *Fundamentals of cheese science* (1st ed.). Gaithersburg, MA, USA: Aspen Publishers.

Hanaei, F., Cuvelier, G., & Sieffermann, J. M. (2015). Consumer texture descriptions of a set of processed cheese. *Food Quality and Preference, 40*, 316–325.

Hayaloglu, A. A. (2016). *Cheese: Microbiology of cheese. Reference module in food science*. Amsterdam, The Netherlands: Elsevier.

Hladká, K., Randulová, Z., Tremlová, B., Ponížil, P., Mančík, P., Černíková, M., et al. (2014). The effect of cheese maturity on selected properties of processed cheese without traditional emulsifying agents. *Lebensmittel-Wissenschaft und -Technologie – Food Science and Technology, 55*, 650–656.

ISO. (2004). *ISO Standard No. 5534: Cheese and processed cheese – Determination of the total solid content (reference method)*. Geneva, Switzerland: International Organisation for Standardisation.

Kaliappan, S., & Lucey, J. A. (2011). Influence of mixtures of calcium-chelating salts on the physicochemical properties of casein micelles. *Journal of Dairy Science, 94*, 4255–4263.

Kapoor, R., & Metzger, L. E. (2008). Process cheese: Scientific and technological aspects—A review. *Comprehensive Reviews in Food Science and Food Safety, 7*, 194–214.

Khetra, Y., Chavhan, G. B., Kanawjia, S. K., & Puri, R. (2015). Storage changes in low sodium-processed Mozzarella cheese prepared using potassium-based emulsifying salts. *Dairy Science & Technology, 95*, 639–649.

Lee, S. K., Anema, S., & Klostermeyer, H. (2004). The influence of moisture content on the rheological properties of processed cheese spreads. *International Journal of Food Science and Technology, 39*, 763–771.

Lee, S. K., Buwalda, R. J., Euston, S. R., Foegeding, E. A., & McKenna, A. B. (2003). Changes in the rheology and microstructure of processed cheese during cooking. *Lebensmittel-Wissenschaft und -Technologie – Food Science and Technology, 36*, 339–345.

Lee, S. K., & Klostermeyer, H. (2001). The effect of pH on the rheological properties of reduced-fat model processed cheese spreads. *Lebensmittel-Wissenschaft und -Technologie – Food Science and Technology, 34*, 288–292.

Marchesseau, S., Gastaldi, E., Lagaude, A., & Cuq, J.-L. (1997). Influence of pH on protein interactions and microstructure of process cheese. *Journal of Dairy Science, 80*, 1483–1489.

Mizuno, R., & Lucey, J. A. (2007). Properties of milk protein gels formed by phosphates. *Journal of Dairy Science, 90*, 4524–4531.

Moatsou, G., & Govaris, A. (2011). White brined cheeses: A diachronic exploitation of small ruminants milk in Greece. *Small Ruminant Research, 101*, 113–121.

Molins, R. A. (1991). *Phosphates in food* (1st ed.). Boca Raton, FL, USA: CRC Press.

Nagyová, G., Buňka, F., Salek, R. N., Černíková, M., Mančík, P., Gruber, T., et al. (2014). Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. *Journal of Dairy Science, 97*, 111–122.

Pachlová, V., Buňka, F., Buňková, L., Weiserová, E., Budinský, P., Žaludek, M., et al. (2011). The effect of three different ripening/storage conditions on the distribution of selected parameters in individual parts of Dutch-type cheese. *International Journal of Food Science and Technology, 46*, 101–108.

Petrella, G., Pati, S., Gagliardi, R., Rizzuti, A., Mastrorilli, P., la Gatta, B., et al. (2015). Study of proteolysis in river buffalo mozzarella cheese using a proteomics approach. *Journal of Dairy Science, 98*, 7560–7572.

Piska, I., & Štětina, J. (2004). Influence of cheese ripening and rate of cooling of the processed cheese mixture on rheological properties of processed cheese. *Journal of Food Engineering, 61*, 551–555.

Purna, S. K. G., Pollard, A., & Metzger, L. E. (2006). Effect of formulation and manufacturing parameters on process cheese food functionality—I. Trisodium citrate. *Journal of Dairy Science, 89*, 2386–2396.

Sádlíková, I., Buňka, F., Budinský, P., Barbora, V., Pavlínek, V., & Hoza, I. (2010). The effect of selected phosphate emulsifying salts on viscoelastic properties of processed cheese. *Lebensmittel-Wissenschaft und -Technologie – Food Science and Technology, 43*, 1220–1225.

Salek, R. N., Černíková, M., Maděrová, S., Lapčík, L., & Buňka, F. (2016). The effect of different composition of ternary mixtures of emulsifying salts on the consistency of processed cheese spreads manufactured from Swiss-type cheese with different degrees of maturity. *Journal of Dairy Science, 99*, 3274–3287.

Salek, R. N., Černíková, M., Nagyová, G., Kuchař, D., Bačová, H., Minarčíková, L., et al. (2015). The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. *International Dairy Journal, 44*, 37–43.

Salek, R. N., Černíková, M., Pachlová, V., Bubelová, Z., Konečná, V., & Buňka, F. (2017). Properties of spreadable processed Mozzarella cheese with divergent compositions of emulsifying salts in relation to the applied cheese storage period. *Lebensmittel-Wissenschaft und -Technologie – Food Science and Technology, 77*, 30–38.

Shirashoji, N., Jaeggi, J. J., & Lucey, J. A. (2006). Effect of trisodium citrate concentration and cooking time on the physicochemical properties of pasteurized process cheese. *Journal of Dairy Science, 89*, 15–28.

Winter, H. H., & Chambon, F. (1986). Analysis of linear viscoelasticity of a cross-linking polymer at the gel point. *Journal of Rheology, 30*, 367–382.

Research paper 6

A6

Salek, R. N., Vašina, M., Lapčík, L., Černíková, M., Lorencová, E., Li, P., & Buňka, F.

Evaluation of various emulsifying salts addition on selected properties of processed cheese sauce with the use of mechanical vibration damping and rheological methods.

LWT – Food Science & Technology. 2019, 107, 178-184. ISSN: 00236438.

LWT - Food Science and Technology 107 (2019) 178–184

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Evaluation of various emulsifying salts addition on selected properties of processed cheese sauce with the use of mechanical vibration damping and rheological methods☆

Richardos Nikolaos Salek[a,*], Martin Vašina[b], Lubomír Lapčík[a], Michaela Černíková[a], Eva Lorencová[a], Peng Li[a], František Buňka[a]

[a] Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, T.G. Masaryka 5555, 760 01, Zlín, Czech Republic
[b] VŠB-Technical University of Ostrava, Department of Hydromechanics and Hydraulic Equipment, Faculty of Mechanical Engineering, 17. Listopadu 15/2172, 708 33 Ostrava-Poruba, Czech Republic

ARTICLE INFO

Keywords:
Processed cheese sauce
Emulsifying salts
Mechanical vibrations
Rheology
Storage

ABSTRACT

The scope of the study was the employment of an innovative method beneficial to evaluate the impact of various emulsifying salts (ES) addition on selected properties of processed cheese sauces (PCS). The ES applied were disodium hydrogenphosphate (DSP), tetrasodium disphosphate (TSPP), pentasodium triphosphate (PSTP), sodium salt of polyphosphate $n \tilde{}$ 20 (POLY) and trisodium citrate (TSC). Moreover, the examined effect was observed in two groups of samples, (i) PCS with non-adjusted pH and in (ii) PCS with adjusted pH. The hardness of the samples was influenced by the type of the applied ES, pH, and storage period. The PCS produced with DSP:TSPP (1:1; without pH adjustment) exhibited the higher hardness which was also confirmed by the increasing samples viscosity and storage modulus. The results obtained by the mechanical vibrations damping analysis were analogous to rheological analysis. Generally, it was found that more viscous PCS exhibited "better" vibration damping properties, which was reflected by a shift of the first resonance frequency peak position to lower frequencies. In case of all investigated samples it was verified that the increasing storage period caused increasing stiffness and led to a shift of the first resonance frequency peak position to higher frequencies.

1. Introduction

Processed cheese sauces (**PCS**) are novel cheese products and commercially can be found in many forms (frozen, semi-liquid, shelf-stable dry mixtures). PCS can serve as flavor enhancers, dipping sauces, act as the main attractiveness in many dishes or help to intensify or round out an appetizer flavor profile. However, at the moment there are no standards of identity or definitional legal for PCS. Thus, PCS can be produced by applying many ingredients such as natural cheese, cheese powder, processed cheese (**PC**) and other ingredients of dairy or non-dairy origin (Shalaby, Mohamed, & Bayoumi, 2017). PCS and PC could be described as stable oil in water emulsions. Hence, their manufacture (PCS and PC) could be realized by mixing natural cheese, milk fat, water, emulsifying salts (**ES**), other optional ingredients, commonly under vacuum in the presence of heat (temperature range of 85 up to 110 °C) and constant shear (Kapoor & Metzger, 2008; Lee, Buwalda, Euston, Foegeding, & McKennan, 2003; Mandala, Savvas, & Kostaropoulos, 2004; Přikryl et al., 2018; Salek, Černíková, Maděrová, Lapčík, & Buňka, 2016; Černíková, Nebesářová, Salek, Řiháčková, & Buňka, 2018). Moreover, the formation of the desired PCS-matrix (or PC-matrix) (with smooth and homogeneous consistency) can be affected by several factors, including: (**1**) composition of the applied raw materials (type and maturity degree of the natural cheeses, pH of the natural cheese to be melt, dry matter (**DM**) and fat in DM, type and concentration of the ES, possible presence of hydrocolloids), (**2**) processing parameters (stirring speed and duration, cooling time and rate) and (**3**) storage parameters (storage period and temperature, packaging material properties) (Kapoor & Metzger, 2008; Černíková et al., 2018).

Furthermore, during the production of PCS and PC ingredients of great importance are ES due to their ability of pH adjustment and their ability of sequestering calcium (exchanging Na^+ for Ca^{2+}) from the casein matrix. The latter "phenomena" can lead to hydration and dispertion of proteins, hence, the present casein can behave as the "true" emulsifier during the formation of the PC-matrix (Buňka et al., 2014;

☆ **Conflict of interest:** The authors declare no competing financial interest.

* Corresponding author.
E-mail address: rsalek@utb.cz (R.N. Salek).

https://doi.org/10.1016/j.lwt.2019.03.022
Received 7 December 2018; Received in revised form 4 March 2019; Accepted 6 March 2019
Available online 11 March 2019
0023-6438/

Dimitreli & Thomareis, 2009; Kapoor & Metzger, 2008; Salek et al., 2016; Weiserová et al., 2011).

Additionally, researchers continue to develop non-destructive mechanical methods (using vibration and acoustic characteristics) to evaluate the effect of their impact on agricultural products by applying high-tech methods. Mechanical vibration, which is given by oscillation of a mechanical or structural system about an equilibrium position, is usually expressed by time dependencies of displacement, velocity or acceleration of individual points during vibration. Furthermore, the mechanical vibration can be described by means of effective values of the above-mentioned basic kinematic quantities and by decibel levels (Kelly, 2011; Rao, 2005; Vašina, Hružík, & Bureček, 2016). Damping properties of a given commodity during cyclic oscillation are connected by converting the vibration energy into heat energy dissipated by the commodity (Ratna, Manoj, Chandrasekhar, & Chakraborty, 2004). The vibration damping can be affected by different factors (elasticity, porosity, composition, stalling process, excitation frequency, inertial mass) (Lapčík, Vašina, Lapčíková, & Valenta, 2016; Schaller, 2003). Moreover, the vibration properties of some commodities are correlated with product hardness and maturity Essex & Finney, 1972(Nourain, Ying, Wang, & Rao, 2005). For this reason the non-destructive mechanical vibration damping method could be applied as a potential technique to evaluate food quality. Nowadays, increasing attention has been placed on the functional properties of PC that can contribute to viscoelastic properties (mainly texture), enhancing versatility in the final use applications (e.g. 3D printing) (Fu et al., 2018; Le Tohic et al., 2018; Solowiej, Cheung, & Li-Chan, 2014).

However, there is no existing study focused on characterization of processed cheese sauce (**PCS**) viscoelastic properties using a technique of mechanical vibration damping. The objective of the study was to evaluate the effect of ES [consisting of disodium hydrogenphosphate (Na_2HPO_4; **DSP**), tertasodium diphosphate ($Na_2P_2O_7$; **TSPP**), pentasodium triphosphate ($Na_5O_{10}P_3$; **PSTP**), sodium salt of polyphosphate with the mean length $n \approx 20$ (**POLY**) and trisodium citrate ($C_2H_5Na_3O_7$; **TSC**)] addition on selected properties (physicochemical, rheological) of PCS using a novel experimental technique over a 60 day storage period (at 6 ± 2 °C). The examined effect was observed in two main groups of samples, (i) PCS with non-adjusted pH arising from the interactions of the applied ES as well as in (ii) PCS with adjusted pH (target values within the interval of 5.60–5.80). Furthermore, the current study probably will help to "increase" the optical view for the characterization of PC product properties suitable for quality control application in the certain field.

2. Materials and methods

2.1. Materials

Dutch-type cheese (50 g/100 g dry matter; 30 g/100 g fat in DM; 7-week maturity; the same batch of natural cheese was used in the whole experiment) was purchased from Kromilk, a.s. (Kroměříž, Czech Republic). Yoghurt (20 g/100 g dry matter; 10 g/100 g fat) was obtained from Kri, S.A., Milk Industry (Serres, Greece). Butter (82 g/100 g dry matter; 84 g/100 g fat) was supplied from Sachsenmilch Leppersdorf, GmbH (Wachau, Germany). In addition, DSP; TSPP; PSTP; POLY were purchased from Fosfa PLC Company, (Břeclav, Poštorná, Czech Republic); TSC, HCl and NaOH were supplied by SigmaAldrich Inc. (St. Louis, MO).

2.2. Preparation of the PCS samples

The Vorkwerk Thermomix TM blender cooker (Vorkwerk & Co, Thermomix GmbH, Wuppertal, Germany) was used to manufacture the model PCS samples. The same apparatus was also employed for the production of PC samples in the works of Lee et al. (2003) and Salek et al. (2016). Moreover, the production of the samples was designed to achieve end-products with 25 g/100 g dry matter and 40 g/100 g fat in DM. All ES were applied as sole ingredients and one binary mixture of ES consisting of DSP and TSPP (in a ratio 1:1) was also tested. The total concentration of the ES was 3 g/100 g of the total weight of the melt. The production protocol (total melting time 8–10 min) was described in detail in the work of Buňka et al. (2014) and Salek et al. (2016). The hot melt mass was transferred into cylindrical polypropylene pots (52 mm in diameter; 50 mm high) and sealed with aluminium lids. Additionaly, PCS in which the pH was adjusted [to achieve a target value within the interval of 5.60–5.80 using acid or alkali (1 mol/L of HCl or NaOH)] were manufactured. The calculated amount of acid/alkali was added to the production apparatus in the range of 85–86 °C. Hence, the water addition was decreased by the calculated amount of acid/alkali. The samples were cooled and stored (at 6 ± 2 °C). The analyses were realized on the 2nd, 9th, 16th, 30th and 60th day after the production day (day 1 was the production day).

2.3. Dry matter content and pH measurement of the PCS samples

The DM content of the PCS samples was gravimetrically determined according to ISO 5534 (2004). In addition, pH of the tested samples was measured (at a temperature of 25 ± 1 °C) by inserting a glass tip electrode of a calibrated pH-meter (pHSpear, Eutech Instruments, Oakton, Malaysia) directly into the sample at three randomly selected locations. Each PC sample was measured in triplicate (n = 9).

2.4. Determination of PCS samples textural and viscoelastic properties

The determined textural parameter of the PCS was hardness (defined as the force needed to attain a given deformation – maximum force during the first penetration cycle; N) (Chen & Opara, 2013) and was calculated according to Szczesniak (2002). The evaluation of hardness was performed using the TA.XTplus texture analyzer (Stable Micro Systems Ltd., Godalming, UK). The measurements were realized at 6 ± 2 °C (each pot was measured immediately after removing from the fridge). Moreover, the obtained results were recorded as force-displacement/time curves, describing the force (N) needed to deform the tested sample proportionally to time (s). A penetration event (cylindrical probe P20 with 20 mm diameter, penetration depth 10 mm, probe speed 2 mm/s, trigger force 5 g, strain deformation 25%) was employed during the measurement. On each day of analysis, each sample was measured in triplicate (n = 3).

A dynamic oscillatory shear rheometer (Rheostress 1, Haake, Bremen, Germany) equipped with a plate-plate geometry (35 mm diameter, 1 mm gap) was used for the determination of the PCS viscoelastic properties. Furthermore, all samples were measured in the control shear stress mode at a frequency ranging from 0.01 to 10.00 Hz (at 20.0 ± 0.1 °C). The amplitude of shear stress (20 Pa) was selected in the linear viscolelasticity region. Moreover, the storage (***G´***; Pa) and loss (***G´´***; Pa) moduli were determined as a function of the excitation frequency f. According to the following equation (1) was determined the complex modulus (***G****; Pa).

$$G^* = \sqrt{(G')^2 + (G'')^2} \quad (1)$$

The rheological analysis of the model PCS samples was performed on the 30th day of storage (6 ± 2 °C) and the reported values were the mean of at least 4 replicates (n = 4).

2.5. Determination of PCS samples mechanical vibration damping

The sample ability to damp mechanical vibration can be expressed by the transfer damping function (***D***; dB) (Lapčík et al., 2016) according to equation (2).

$$D = 20 \cdot log \frac{v_1}{v_2} \quad (2)$$

where v_1 (m/s) is the velocity amplitude on the input side of the tested sample and v_2 (m/s) is the velocity amplitude on the output side of the tested sample. Moreover, in case of the harmonic forced oscillation, it is also possible to express the transfer damping function by equation (3).

$$D = 20 \cdot log \frac{a_1}{a_2} \quad (3)$$

where a_1 (m/s^2) is the acceleration amplitude on the input side of the tested sample and a_2 (m/s^2) is the acceleration amplitude on the output side of the sample. Based on the transfer damping function value, there are three different types of mechanical vibration, i.e. damped ($D > 0$), undamped ($D = 0$) and resonance ($D < 0$) vibration (Vašina et al., 2016).

Harmonically excited systems with single degree of freedom are characterized by natural frequencies. In case of a spring-mass-system, consisting of a spring of stiffness k (N/m) and a connected mass m (kg) to the spring, the undamped natural frequency ω_n (rad/s) of the system (Rao, 2005) is given by equation (4).

$$\omega_n = \sqrt{\frac{k}{m}} \quad (4)$$

Real systems are characterized by an additional viscous damping under a harmonic motion. These spring-mass-damper systems have maximum displacement amplitudes, when are excited at the damped natural frequency ω_d (rad/s), which is expressed by the following equation (5).

$$\omega_d = 2\pi \cdot f_R = \omega_n \cdot \sqrt{1 - 2\xi^2} \quad (5)$$

where f_R (Hz) is the resonant frequency and ξ (−) is the damping ratio. The damped natural frequency is always less than the undamped natural frequency (Stephen, 2006).

The mechanical vibration damping testing of the PCS was performed by the forced oscillation method. In addition, the transfer damping function was experimentally measured using the BK 4810 vibrator device in combination with a BK 3560-B-030 three-channel signal pulse multi-analyzer and a BK 2706 power amplifier at the frequency range of 2–600 Hz. Sine waves were generated by the vibrator device. The acceleration amplitudes on the input and output sides of the investigated specimens were recorded by the BK 4393 accelerometers (Brüel & Kjær, Nærum, Denmark). The samples were located in a metal bowl. Furthermore, the measurements of the transfer damping function were performed for the mass load $m = 85$ g, which was located on the upper side of the periodically loaded tested samples. The sample dimensions were (60 × 60 × 20 mm; length × width × height; cuboid shape). Each measurement was repeated 5 times ($n = 5$) at 23 ± 1 °C.

2.6. Statistical analysis

The obtained results were analysed by non-parametrical analysis of variance of Kruskall-Wallis and Wilcoxon tests (Unistat® 5.5 software; Unistat, London, UK), where the significance level was 0.05.

3. Results and discussion

3.1. Basic chemical analysis of the PCS

Dry matter of all samples was within the interval 24.87–26.63 g/100 g. The results depict the stability in the DM content. The nessecity of dry matter similarity between the samples allows their comparison, as this factor could affect their properties (Lee et al., 2003; Marchesseau, Gastaldi, Lagaude, & Cuq, 1997; Weiserová et al., 2011).

Additionally, the properties of the PCS can be also influenced by the pH of the produced molten mass. The pH of samples without pH adjustment ranged from 5.23 to 6.51 (after 2 days of storage). Furthermore, the observed wide range in the pH could be explained by the buffering ability of ES (Dimitreli & Thomareis, 2009). The highest pH was reached when DSP:TSPP (1:1), DSP, TSPP and PSTP (in all cases pH ≥ 6.33) were used. Moreover, the pH of the samples prepared with TSC was lower (pH ≈ 5.91). On the other hand, the application of POLY resulted in an intensive pH decrease (pH ≈ 5.23). The current phenomenon might be explained by more intensive release of H^+ (from the molecules of polyphosphates) into the melt, resulting in a decrease of the pH. The pH of the samples, after pH adjustment, ranged from 5.62 to 5.87. Furthermore, a possible explanation for the "close" variability observed in the pH could be found in the ES buffering capacity or in the fact that the production of the samples was undergone using "real" raw materials (Lee & Klostermeyer, 2001; Lu, Shirashoji, & Lucey, 2008). According to Lee and Klostermeyer (2001) low pH processed cheese could be dry and crumbly whereas high pH processed cheese products could be moist and present an elastic behavior. Nevertheless, during the 60 d storage period the pH of all samples (with and without pH adjustment) showed a decreasing trend (data not shown). Thus, in most of the examined samples the observed decrease was in the range of 0.2–0.3 pH units. The results are in agreement with those of Shalaby et al. (2017).

3.2. Textural and rheological properties of the PCS samples

Fig. 1 shows the development of hardness of the PCS samples manufactured with different types of ES (with and without pH adjustment; Part A and B) during a 60 d storage period (at 6 ± 2 °C). From the obtained results it could be assumed that the type of applied ES and

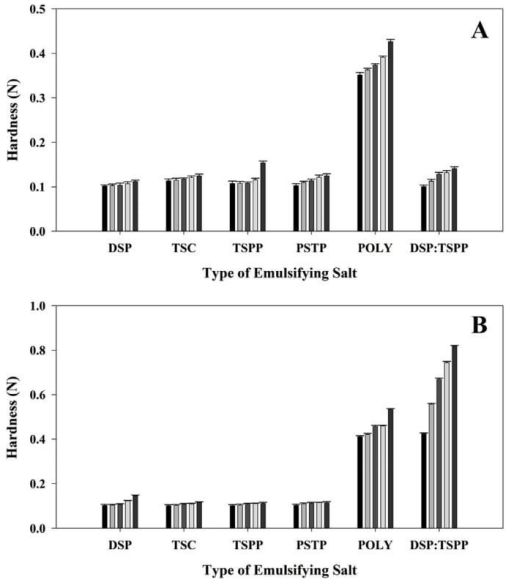

Fig. 1. The development of processed cheese sauce hardness (calculated as the maximum force during penetration, N) on the type of the applied emulsifying salt [disodium hydrogenphosphate (**DSP**), tertasodium diphosphate (**TSPP**), pentasodium triphosphate (**PSTP**), sodium salt of polyphosphate with the mean length n ≈ 20 (**POLY**), trisodium citrate (**TSC**) and binary mixture of disodium phosphate and tetrasodium diphospate in ratio of 1:1 (DSP:TSPP)] during a 60-day storage period at 6 ± 2 °C [$n = 3$; the results were expressed as means (columns) and standard deviations (bars); processed cheese sauces were sampled after 2 (black), 9 (silver), 16 (dark-gray), 30 (light-gray) and 60 (dim-gray) days of storage]. Two groups of processed cheese sauce were produced (part A, samples with pH adjustment; part B, samples without pH adjustment.

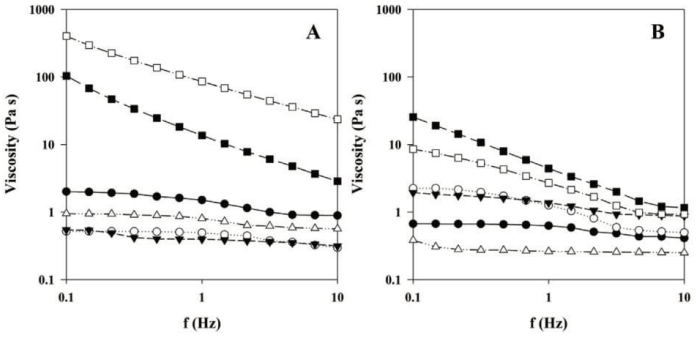

Fig. 2. Dependence of the viscosity of the processed cheese sauces (after 30 days of storage at 6 ± 2 °C) manufactured with different types of emulsifying salts [disodium hydrogenphosphate (Na_2HPO_4; DSP; *full circle*), tertasodium diphosphate ($Na_2P_2O_7$; TSPP; *full triangle*), pentasodium triphosphate ($Na_5O_{10}P_3$; PSTP; *open triangle*), sodium salt of polyphosphate with the mean length $n^{-}20$ (POLY; *full square*) and trisodium citrate ($C_2H_5Na_3O_7$; TSC; *open circle*) and binary mixture of disodium phosphate and tetrasodium diphospate in ratio of 1:1 (DSP:TSPP; *open square*)] on the frequency (range of 0.1–10.0). Two groups of processed cheese sauces were produced; samples without pH adjustment (part A) and samples with pH adjustment (part B).

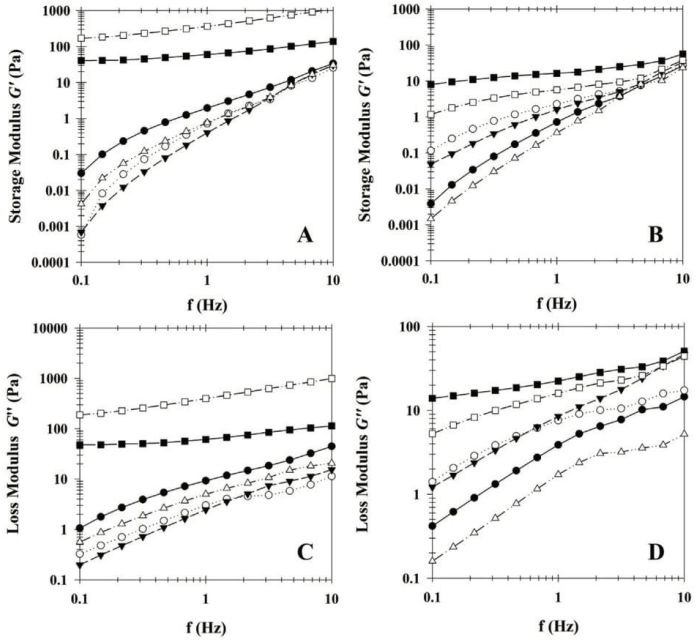

Fig. 3. Dependence of the storage (*G´*; part A and B) and loss (*G´´*; part C and D) moduli of processed cheese sauces (after 30 days of storage at 6 ± 2 °C) manufactured with different types of emulsifying salts [disodium hydrogenphosphate (Na_2HPO_4; DSP; *full circle*), tertasodium diphosphate ($Na_2P_2O_7$; TSPP; *full triangle*), pentasodium triphosphate ($Na_5O_{10}P_3$; PSTP; *open triangle*), sodium salt of polyphosphate with the mean length $n^{-}20$ (POLY; *full square*) and trisodium citrate ($C_2H_5Na_3O_7$; TSC; *open circle*) and binary mixture of disodium phosphate and tetrasodium diphospate in ratio of 1:1 (DSP:TSPP; *open square*)] on the frequency (range of 0.1–10.0). Two groups of processed cheese sauces were produced; samples with pH adjustment (part B and D) and samples without pH adjustment (part A and C).

pH of the melt significantly affected the hardness of the examined PC samples ($P < 0.05$). In the case of samples without pH adjustment, the PCS prepared with DSP, TSPP, PSTP and TSC presented lower hardness. On the contrary, higher hardness was observed in the samples manufactured with the binary mixture of DSP:TSPP, followed by those made with POLY ($P < 0.05$). The influence of the specific ratio of DSP:TSPP and POLY on PCS increasing hardness was explained by Dimitreli and Thomareis (2009), Kaliappan and Lucey (2011). Furthermore, in the case of PCS manufactured with pH adjustment, hardness followed a similar trend as in the above-mentioned case. However, a noteworthy difference observed it was that the higher hardness was detected in the samples prepared with POLY ($P < 0.05$). According to Mizuno and Lucey (2005) polyphosphates can charge the caseins with negative ions, resulting the development of "weak" hydrophobic interactions among the proteins within the cheese matrix. Nevertheless, the impact of the specific ratio of DSP:TSPP (1:1) on the PCS hardness was diminished ($P < 0.05$). The resulting hardness for the samples made with DSP:TSPP (1:1) was similar to those made with TSPP, PSTP and TSC. Additionally, the hardness of all examined samples increased with the rising of the storage period ($P < 0.05$). Analogous phenomena and their explanation can be found in the works of Awad, Abde-Hamid, El-Shabrawy, and Singh (2002) and Salek et al. (2016). With respect to our results, it could be stated that the "action" of the current ratio (DSP:TSPP; 1:1) developing samples of increasing hardness appears to be in close relation with the pH of the melt.

The rheological analysis results are presented in Fig. 2 and Fig. 3. From the obtained results it could be stated that the applied type of ES affected the viscoelastic characteristics of the PCS ($P < 0.05$). The ability of a PC product to spread and/or flow is described by viscosity (Kapoor & Metzger, 2008). Furthermore, the viscosity of the tested PCS was influenced by the applied type of ES (Fig. 2; $P < 0.05$). Since viscosity and hardness are correlated (Glibowski, Zarzycki, &

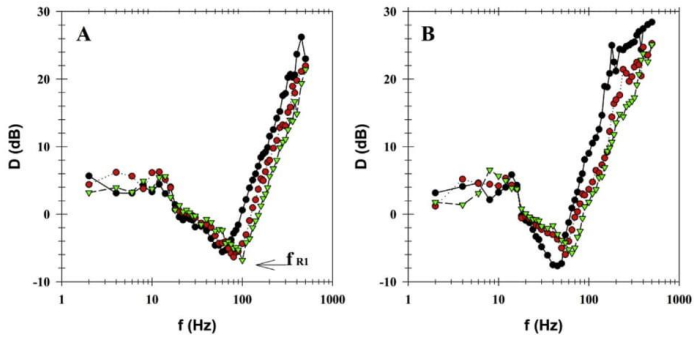

Fig. 4. Frequency dependence of the transfer damping function (*D*) of processed cheese sauce samples manufactured using disodium phosphate (Na_2HPO_4; DSP) without pH adjustment [part A; sampled after 2 (*black circle*), 30 (*red circle*) and 60 (*green triangle*) days of storage at 6 ± 2 °C] and sodium salt of polyphosphate with the mean length *n* ˜ 20 (POLY) with pH adjustment [part B; sampled after 2 (*black circle*), 16 (*red circle*) and 60 (*green triangle*) days of storage at 6 ± 2 °C] during storage. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

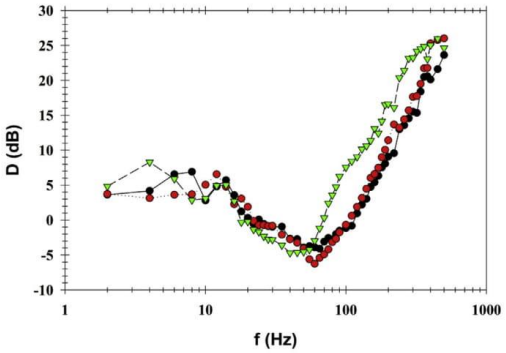

Fig. 5. Frequency dependence of the transfer damping function (*D*) of processed cheese sauce samples manufactured using different types of emulsifying salts [tetrasodium diphosphate ($Na_2P_2O_7$; TSPP; *black circle*), trisodium citrate ($C_2H_5Na_3O_7$; TSC; *red circle*), DSP:TSPP (ratio 1:1); *green triangle*] without pH adjustment. The processed cheese samples were evaluated after 2 days of storage at 6 ± 2 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

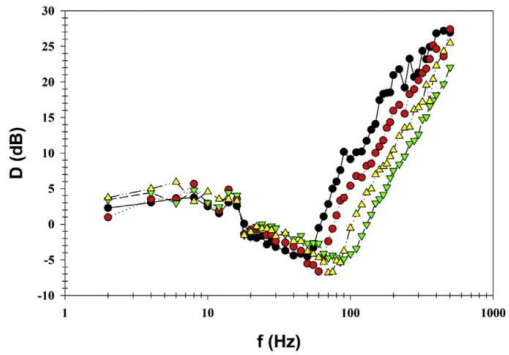

Fig. 6. Frequency dependence of the transfer damping function (*D*) of processed cheese sauce samples manufactured using the mixture of disodium hydrogen phosphate (Na_2HPO_4; DSP) and tetrasodium diphosphate ($Na_2P_2O_7$; TSPP) in a ratio 1:1 (DSP:TSPP; *black circle:* samples without pH adjustment, *red circle*: samples with pH adjustment), and tetrasodium diphosphate ($Na_2P_2O_7$; TSPP; *green triangle*: samples without pH adjustment, *yellow triangle*: samples with pH adjustment) with (WA). The processed cheese sauce samples were evaluated after 30 days of storage at 6 ± 2 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Krzepkowska, 2008), the viscosity of the analysed samples showed an analogous trend as was observed for hardness. Thus, the samples prepared with DSP, PSPT, TSPP, TSC and wihout pH adjustment could be characterized by more liquid-like behavior than that prepared with POLY and DSP:TSPP. The degrees of elastic and viscous behavior of viscoelastic materials are expressed by the storage (*G'*) and loss (*G''*) moduli (Fig. 3), respectively (Mleko & Lucey, 2003). According to Solowiej et al. (2015) when $G'' > G'$, PC has viscous properties. Moreover, it can be seen that all PCS samples presented a more viscous-like character (in all cases the $G'' > G'$). According to the literature, PC with low DM content are more viscous than products with higher level of DM content. On the same token, higher level of moisture could increase the rotational mobility of protein molecules and the „system" exhibits less shear-dependent character (Dimitreli & Thomareis, 2004; Solowiej et al., 2015). The PCS samples manufactured with POLY and DSP:TSPP without pH adjustment were harder and more elastic than the rest of the tested samples ($P < 0.05$). The latter observation could indicate a relationship between PCS hardness and storage modulus (*G'*). However, the adjustment of pH resulted in samples with lower viscosity, storage and loss moduli. The examined samples followed the same trend as was previously mentioned; the effect of the specific ratio of DSP:TSPP was weakened. Hence, the current samples showed similar viscosity, storage and loss moduli as those prepared with TSPP and TSC.

3.3. Mechanical vibration damping of the PCS samples

In general, from the obtained data (Figs. 4–6) it could be assumed that when *D* was lower than zero ($D < 0$) no damping properties occurred, indicating resonant mechanical vibration. On the other hand, when the values of the transfer damping function were higher than zero ($D > 0$) the mechanical vibrations were damped. Moreover, "better" vibration damping properties were observed at higher values of the transfer damping function in parallel examined samples (Figs. 4–6).

The effect of the storage period on the transfer damping function (*D*) for the PCS made with DSP without pH adjustment and the sample prepared with POLY with pH adjustment is shown in Fig. 4. It is evident, that the resonant mechanical vibration ($D < 0$) of the tested PCS was achieved at low excitation frequencies. Contrariwise, the mechanical vibration damping ($D > 0$; damped vibration) was achieved from certain frequencies, in this case from 95 to 150 Hz (DSP) and from 63 to 87 Hz (POLY) depending on the storage period (Fig. 4). Moreover, the mechanical damping properties of the investigated PCS were decreasing with the increasing storage period ($P < 0.05$). For this reason the increasing storage period contributed to lower transformation of

Table 1
First resonance frequency (f_{R1}; Hz) of the processed cheese sauce samples with thickness t = 20 mm and inertial mass m = 85 g.

Type of Emulsifying Salt	pH Adjustment[f]	Storage Time (days)				
		2	9	16	30	60
POLY	NA[g]	42 ± 1[a]	45 ± 2[b]	52 ± 2[c]	55 ± 2[d]	60 ± 3[e]
	WA[g]	44 ± 1[a]	48 ± 2[b]	55 ± 2[c]	59 ± 3[d]	66 ± 3[e]
DSP:TSPP	NA[g]	41 ± 2[a]	44 ± 1[b]	49 ± 2[c]	51 ± 2[d]	58 ± 2[e]
	WA[g]	49 ± 2[a]	52 ± 2[b]	55 ± 2[c]	62 ± 3[d]	70 ± 3[e]
TSC	NA[g]	62 ± 3[a]	71 ± 2[b]	79 ± 3[c]	91 ± 4[d]	109 ± 5[e]
	WA[g]	60 ± 2[a]	66 ± 2[b]	69 ± 3[c]	70 ± 3[d]	81 ± 3[e]
DSP	NA[g]	59 ± 3[a]	64 ± 2[b]	66 ± 3[c]	79 ± 3[d]	99 ± 4[e]
	WA[g]	63 ± 2[a]	65 ± 3[b]	77 ± 3[c]	83 ± 3[d]	100 ± 4[e]
PSTP	NA[g]	61 ± 3[a]	65 ± 2[b]	67 ± 3[c]	81 ± 4[d]	103 ± 5[e]
	WA[g]	63 ± 2[a]	66 ± 3[b]	70 ± 3[c]	85 ± 3[d]	106 ± 4[e]
TSPP	NA[g]	64 ± 3[a]	72 ± 3[b]	79 ± 3[c]	93 ± 4[d]	107 ± 5[e]
	WA[g]	57 ± 2[a]	67 ± 3[b]	68 ± 2[c]	69 ± 3[d]	88 ± 3[e]

[a-e]Means in the same row with different superscripts are significantly different ($P < 0.05$).
[f] NA = processed cheese sauce samples without pH adjustment; WA = processed cheese sauce samples with pH adjustment.
[g] Mean ± standard deviation.

input mechanical energy into heat during forced oscillations. This fact led to higher stiffness (k) and lower damping ratio (ξ) of the tested PCS, as shown in equations (4) and (5). The latter was in excellent agreement with the above-mentioned finding (Fig. 1) that the tested PCS hardness was increasing with the increasing storage period ($P < 0.05$). Therefore, the first resonance frequency ($f_{R1} \approx D_{min}$; peak minimum) (Fig. 4) of the tested samples was shifted to the right with the prolonging of the storage period (Fig. 4), i.e. from 63 Hz to 100 Hz (DSP) and from 44 Hz to 66 Hz (POLY). The latter shift of the first resonance frequency could indicate that the mechanical energy transfer in the PCS samples induced by the cyclic vibrator oscillations had a less dissipative character. Additionally, the frequency shift corresponded to formation of a viscoelastic three-dimensional network spreading within the matrix. Typically, more viscous behavior of a matrix is accompanied by higher mechanical energy dissipation and vice versa (Lapčík et al., 2017). These facts resulted in an increase of the damped and undamped natural frequencies (Rajoria & Jalili, 2005) as shown in equations (4) and (5). In addition, independently on the pH adjustment, the influence of the storage period on the $f_{R1}$was also found for the other tested PCS (Table 1). The results obtained from the mechanical vibration damping method were also in agreement with the measured viscoelastic properties. According to the available literature rheology might prove to be a very effective tool providing direct insight into the damping properties of a material such as material loss factor (tan δ), loss (G'') and storage (G') shear moduli. Moreover, the higher the viscous modulus and material loss factor, the better is the damping capacity of the material (Sharma et al., 2013). The loss factor (tan δ) can be applied as a parameter characterizing damping properties of a material. In general, the loss factor is defined as the ratio of energy dissipated in the material during vibrations to the maximum potential energy stored in the material, i. e. as the ratio of shear moduli ($\tan\delta = G''/G'$) measured by dynamic rheological properties. Furthermore, tan δ is correlated with the material internal friction (Zielonka & Dobkowski, 1998). The frequency dependencies of the transfer damping function for selected PCS samples without pH adjustment after two days of storage at 6 ± 2 °C are shown in Fig. 5. Thus, it is visible that "better" mechanical damping properties, which are characterized by a higher damping function D at higher excitation frequencies, were found for the samples manufactured with DSP:TSPP [(1:1); f_{R1} = 49 Hz] ($P < 0.05$). As in the previous case (Fig. 4), "better" mechanical damping properties led to a shift of the first resonance peak frequency to lower excitation frequencies (Fig. 5). Moreover, similar damping properties (f_{R1} = 44 Hz) were also obtained when POLY (Table 1) was applied in the production of the model PCS. A possible explanation could be found in a higher internal friction during the forced mechanical stress of these two PCS samples compared to the remaining samples (without pH adjustment; after 2 days of storage at 6 ± 2 °C), which embodied a higher first resonance frequency ($f_{R1} \approx 60$ Hz) (Table 1). A similar effect of the D on the f_{R1} was also found for the tested PCS with pH adjustment. Inner friction during the forced oscillations is in general increasing with the increasing viscosity and the loss moduli (G'') (Gojny & Schulte, 2004; Palou et al., 2011) (Figs. 2 and 3). For this reason, higher internal friction led to lower f_{R1} (Table 1) of the tested PCS. Thus, it is accompanied by "better" vibration damping properties and higher transformation of the mechanical energy into heat during the forced oscillations (Júnior, Júnior, Amico, & Amado, 2012). The effect of pH adjustment on vibration damping properties after 30 days of storage at 6 ± 2 °C is depicted in Fig. 6. The adjustment of pH had a "positive" influence on mechanical vibration damping for the TSPP sample, which was reflected in the decrease of the f_{R1} from 93 Hz to 69 Hz (Fig. 6) as well as in the case of the TSC sample (decrease of the f_{R1} from 91 Hz to 70 Hz) (Table 1). This fact was probably caused by a higher viscosity (Palou et al., 2011) of these samples (Fig. 2), resulting in higher damping ratio ξ during harmonic mechanical loading, lower stiffness k and subsequently in lower damped and undamped natural frequencies [equations (4) and (5)]. However, the opposite effect of the pH adjustment on the mechanical vibration damping was observed for the remaining PCS samples. In these cases, the pH adjustment caused a viscosity reduction, resulting in lower internal friction during harmonic loading of the PCS and higher f_{R1} (Table 1). For example, in the case of the DSP:TSPP (1:1) sample, the pH adjustment resulted in an increase of the f_{R1} from 51 Hz to 62 Hz [(Fig. 6); ($P < 0.05$)].

It was evident from the above-mentioned results that the novel mechanical vibration damping technique is in excellent agreement with the textural and rheological analyses of the investigated PCS. It could be also concluded that the vibration damping method is applicable in cases where there is a significant transformation of input mechanical energy into heat during forced oscillations. This phenomenon occurs especially in samples with higher damping ratio ξ and the viscosity. The vibration damping method could be applied to different types of more viscous materials, e.g. to sauces, honey, cheese, yoghurt, condensed milk/whey, concentrated solutions etc. Generally, viscoelastic materials (or food matrices) could be developed fairly efficiently for a specific application. Therefore, by proper manufacture process, complex food matrices (such as PCS) might be developed to possess a wide variety of desirable damping properties over selected frequency range to evaluate their quality characteristics (Hujare & Sahasrabudhe, 2014).

4. Conclusions

The aim of the current work was the application of a novel method to describe the effect of different type of ES on selected functional properties of PCS during a 60 day storage period. It could be stated that the hardness of the samples was affected by the applied type of ES, pH

of the melt and storage period. The results obtained by the rheological analysis were analogous to those of mechanical vibrations damping analysis. Furthermore, it was found that the samples prepared with DSP:TSPP (1:1) and POLY (without pH adjustment) presented the higher hardness. The latter phenomenon was also confirmed by the increasing samples viscosity and storage modulus. On the contrary, the remaining tested samples showed lower hardness, storage modulus, viscosity, however, they presented increased f_{R1}. The hardness of all tested samples increased with the increasing storage period. In general, it could be concluded that the hardness of the samples was affected by the storage modulus and additionally, the vibration damping properties were influenced by their viscosity and loss modulus. Finally, it could be stated that the novel mechanical vibration damping method is applicable in cases where there is a significant transformation of input mechanical energy into heat during forced oscillations. The latter phenomenon occurs especially in samples with higher damping ratio and viscosity.

Acknowledgements

This study was supported by the European Regional Development Fund in the Research Centre of Advanced Mechatronic Systems project, project number CZ.02.1.01/0.0/0.0/16_019/0000867 and by a project of the internal grants of Tomas Bata University in Zlín (Zlín, Czech Republic; no. IGA/FT/2019/006) funded from the resources for specific university research.

References

Awad, R. A., Abde-Hamid, L. B., El-Shabrawy, S. A., & Singh, R. K. (2002). Texture and microstructure of block type processed cheese with formulated emulsifying salt mixtures. *LWT–Food Science and Technology, 35*, 54–61.

Buňka, F., Doudová, L., Weiserová, E., Černíková, M., Kuchař, D., Slaviková, S., et al. (2014). The effect of concentration and composition of ternary emulsifying salts on the textural properties of processed cheese spreads. *LWT–Food Science and Technology, 58*, 247–255.

Černíková, M., Nebesářová, J., Salek, R. N., Řiháčková, L., & Buňka, F. (2018). Microstructure and textural and viscoelastic properties of model processed cheese spreads with different dry matter and fat in dry matter content. *Journal of Dairy Science, 100*, 4300–4307.

Chen, L., & Opara, U. L. (2013). Texture measurement approaches in fresh and processed foods–A review. *Food Research International, 51*, 823–835.

Dimitreli, G., & Thomareis, A. (2004). Effect of temperature and chemical composition on processed cheese apparent viscosity. *Journal of Food Engineering, 64*, 265–271.

Dimitreli, G., & Thomareis, A. S. (2009). Instrumental textural and viscoelastic properties of processed cheese as affected by emulsifying salts and in relation to its apparent viscosity. *International Journal of Food Properties, 12*, 261–275.

Fu, W., Watanabe, Y., Inoue, K., Moriguchi, N., Fusa, K., Yanagisawa, Y., et al. (2018). Effects of pre-cooked cheeses of different emulsifying conditions on mechanical properties and microstructure of processed cheese. *Food Chemistry, 245*, 47–52.

Glibowski, P., Zarzycki, P., & Krzepkowska, M. (2008). The rheological and instrumental textural properties of selected table fats. *International Journal of Food Properties, 11*, 678–686.

Gojny, F. H., & Schulte, K. (2004). Functionalisation effect on the thermo-mechanical behaviour of multi-wall carbon nanotube/epoxy-composites. *Composites Science and Technology, 64*, 2303–2308.

Hujare, P. P., & Sahasrabudhe, A. D. (2014). Experimental investigation of damping performance of viscoelastic material using constrained layer damping treatment. *Procedia Materials Science, 5*, 726–733.

ISO Standard No. 5534. (2004). *Cheese and processed cheese–Determination of the total solids content (reference method)*. Geneva: International Organization for Standardization.

Júnior, J. H. S. A., Júnior, H. L. O., Amico, S. C., & Amado, F. D. R. (2012). Study of hybrid intralaminate curaua/glass composites. *Materials & Design, 42*, 111–117.

Kaliappan, S., & Lucey, J. A. (2011). Influence of mixtures of calcium-chelating salts on the physicochemical properties of casein micelles. *Journal of Dairy Science, 94*, 4255–4263.

Kapoor, R., & Metzger, L. E. (2008). Process cheese: Scientific and technological aspects – a review. *Comprehensive Reviews in Food Science and Food Safety, 7*, 194–214.

Kelly, S. G. (2011). *Mechanical vibrations. Theory and applications* (1st). Akron: Cengage Learning (Chapter 1).

Lapčík, L., Vašina, M., Lapčíková, B., Plšková, M., Gál, R., & Brychtová, M. (2017). Application of a vibration damping technique in characterizing mechanical properties of chicken meat batters modified with amaranth. *Food Measure, 11*, 1987–1994.

Lapčík, L., Vašina, M., Lapčíková, B., & Valenta, T. (2016). Study of bread staling by means of vibro-acoustic, tensile and thermal analysis techniques. *Journal of Food Engineering, 178*, 31–38.

Le Tohic, C., O'Sullivan, J. J., Drapala, K. P., Chartrin, V., Chan, T., Morrison, A. P., et al. (2018). Effect of 3D printing on the structure and textural properties of processed cheese. *Journal of Food Engineering, 220*, 56–64.

Lee, S. K., Buwalda, R. J., Euston, S. R., Foegeding, E. A., & McKennan, A. B. (2003). Changes in the rheology and microstructure of processed cheese during cooking. *LWT–Food Science and Technology, 36*, 339–345.

Lee, S. K., & Klostermeyer, H. (2001). The effect of pH on the rheological properties of reduced-fat model processed cheese spreads. *LWT–Food Science and Technology, 34*, 288–292.

Lu, Y., Shirashoji, N., & Lucey, J. A. (2008). Effects of pH on the textural properties and meltability of pasteurized process cheese made with different types of emulsifying salts. *Journal of Food Science, 73*, 1363–1369.

Mandala, I. G., Savvas, A. E., & Kostaropoulos, A. E. (2004). Xanthan and locust bean gum influence on the rheology and structure of a white model-sauce. *Journal of Food Engineering, 64*, 335–342.

Marchesseau, S., Gastaldi, E., Lagaude, A., & Cuq, J. L. (1997). Influence of pH on protein interactions and microstructure of process cheese. *Journal of Dairy Science, 80*, 1483–1489.

Mizuno, R., & Lucey, J. A. (2005). Effects of emulsifying salts on the turbidity and calcium-phosphate–protein interactions in casein micelles. *Journal of Dairy Science, 88*, 3070–3078.

Mleko, S., & Lucey, J. A. (2003). Production and properties of processed cheese with reduced lactose whey. *Milchwissenschaft, 58*, 9–10.

Nourain, J., Ying, Y., Wang, J., & Rao, X. (2005). Finite element models of watermelon and their applications. *Journal of Zhejiang University–Science B, 6*, 483–490.

Palou, R. M., Mosqueria, M. L., Rendón, B. Z., Juárez, E. M., Huicochea, C. B., López, J. C. C., et al. (2011). Transportion of heavy and extra-heavy crude oil by pipeline: A review. *Journal of Petroleum Science and Engineering, 75*, 274–282.

Přikryl, J., Hájek, T., Švecová, B., Salek, R. N., Černíková, M., Červenka, L., et al. (2018). Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions. *LWT–Food Science and Technology, 87*, 266–271.

Rajoria, H., & Jalili, N. (2005). Passive vibration damping enhancement using carbon nanotube-epoxy reinforced composites. *Composites Science and Technology, 65*, 2079–2093.

Rao, S. S. (2005). *Mechanical vibrations* (5th ed.). Upper Saddle River: Pearson (Chapter 1).

Ratna, D., Manoj, N. R., Chandrasekhar, L., & Chakraborty, B. C. (2004). Novel epoxy compositions for vibration damping applications. *Polymers for Advanced Technologies, 15*, 583–586.

Salek, R. N., Černíková, M., Maděrová, S., Lapčík, L., & Buňka, F. (2016). The effect of different composition of ternary mixtures of emulsifying salts on the consistency of processed cheese spreads manufactured from Swiss-type cheese with different degrees of maturity. *Journal of Dairy Science, 99*, 3274–3287.

Schaller, R. (2003). Metal matrix composites, a smart choise for high damping materials. *Journal of Alloys and Compounds, 355*, 131–135.

Shalaby, S. M., Mohamed, A. G., & Bayoumi, H. M. (2017). Preparation of a novel processed cheese sauce flavored with essential oils. *International Journal of Dairy Science, 12*, 161–169.

Sharma, S. K., Gaur, H., Kulkarni, M., Patil, G., Bhattacharya, B., & Sharma, A. (2013). PZT-PDMS composite for active damping vibrations. *Composites Science and Technology, 77*, 42–51.

Sołowiej, B., Cheung, I. W. Y., & Li-Chan, E. C. Y. (2014). Texture, rheology and meltability of processed cheese analogues prepared using rennet or acid casein with or without added whey proteins. *International Dairy Journal, 37*, 87–94.

Sołowiej, B., Glibowski, P., Muszyński, S., Wydrych, J., Gawron, A., & Jeliński, T. (2015). The effect of fat replacement by inulin on the physicochemical properties and microstructure of acid casein processed cheese analogues with added whey protein polymers. *Food Hydrocolloids, 44*, 1–11.

Stephen, N. G. (2006). On energy harvesting from ambient vibration. *Journal of Sound and Vibration, 293*, 409–425.

Szczesniak, A. S. (2002). Texture is a sensory property. *Food Quality and Preference, 13*, 215–225.

Vašina, M., Hružík, L., & Bureček, A. (2016). Structural damping of mechanical vibration. *Manufacturing Technology, 16*, 1379–1382.

Weiserová, E., Doudová, L., Galiová, L., Žák, L., Michálek, J., Janiš, R., et al. (2011). The effect of combinations of sodium phosphates in binary mixtures on selected texture parameters of processed cheese spreads. *International Dairy Journal, 21*, 979–986.

Zielonka, M., & Dobkowski, Z. (1998). Rheological characterization of damping materials based on polysiloxanes. In I. Emri (Ed.). *Progress and trends in rheology V* (pp. 321). Heidelberg: Steinkopff.

Research paper 7

B1

Černíková, M., Nebesářová, J., Salek, R. N., Řiháčková, L., Buňka, F.

Microstructure and textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content.

Journal of Dairy Science. 2017, 100, 4300-4307. ISSN: 00220302.

J. Dairy Sci. 100:4300–4307
https://doi.org/10.3168/jds.2016-12120

Microstructure and textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content

Michaela Černíková,*[1] Jana Nebesářová,† Richardos Nikolaos Salek,* Lada Řiháčková,* and František Buňka*
*Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, T.G. Masaryka 5555, 760 01, Zlín, Czech Republic
†Laboratory of Electron Microscopy, Institute of Parasitology, Biology Centre ASCR, v.v.i., Branišovská 31, 370 05, České Budějovice, Czech Republic

ABSTRACT

The aim of this work was to examine the effect of a different dry matter (DM) contents (35 and 45% wt/wt) and fat in DM contents (40 and 50% wt/wt) on the textural and viscoelastic properties and microstructure of model processed cheeses made from real ingredients regularly used in the dairy industry. A constant DM content and constant fat in DM content were kept throughout the whole study. Apart from the basic chemical parameters, textural and viscoelastic properties of the model samples were measured and scanning electron microscopy was carried out. With increasing DM content, the rigidity of the products increased and the size of the fat globules in the model samples of the processed cheeses decreased. With increasing fat in DM content, the rigidity of the processed cheeses decreased and the size of the fat globules increased.
Key words: processed cheese, texture, rheology, scanning electron microscopy

INTRODUCTION

Spreadable processed cheese is defined by Codex Alimentarius (1978) as a product made by grinding, mixing, melting, and emulsifying with the aid of heat and emulsifying salts, one or more varieties of cheese with or without the addition of milk components or other foodstuffs in accordance with this standard. The rules for the relationship between the DM content and the fat in DM (**FDM**) content are also presented (Codex Alimentarius, 1978). In the region of Middle Europe, we could also find products in which the DM content is lower than the amount stated in the standard but that is still named "processed cheese."

Processed cheeses are traditionally manufactured from a mixture of natural cheeses and many other dairy (e.g., anhydrous butterfat, butter, cream, milk powder, whey, buttermilk) and nondairy (e.g., stabilizers, preservatives, flavor enhancers) ingredients. Important food additives during the production of processed cheeses are emulsifying salts (usually the sodium salts of phosphates, polyphosphates, and citrates or their mixtures), which help the casein proteins emulsify the fat present, hydrate the free water, and participate in developing the final matrix of the product. Processed cheeses are directly consumed and are used as raw material for further processing in the industry and catering (Lee et al., 2003; Kapoor and Metzger, 2008; Nagyová et al., 2014).

One of the most important and very critically evaluated parameters of processed cheeses is their consistency, which can be, according to the actual parameters, in the form of blocks, slices, spreads, or sauces (Kapoor and Metzger, 2008). The particular consistency of the product is affected by many factors, which can be divided into 4 main groups: (1) the final parameters of the processed cheese (especially DM, protein, fat and fat-free DM content, and pH value); (2) the composition of the raw material mixture (e.g., the type and degree of maturity of the natural cheese, the concentration and composition of the emulsifying salts, and the concentration and composition of the stabilizers), which to some extent determines the final parameters of the product quoted in (1); (3) processing parameters during production (especially the agitation speed, melting temperature, stirring time, and rate of cooling the melt); and (4) the storage conditions of processed cheeses (e.g., impermeability of the packaging, storage temperature, and length of the storage period; Lee and Klostermeyer, 2001; Dimitreli and Thomareis, 2004, 2007, 2008; Kapoor and Metzger, 2008; Bayarri et al., 2012; Buňka et al., 2013, 2014; Nagyová et al., 2014; Shirashoji et al., 2016). During the storage period, especially within the first 14 d of cold storage, a further change in consistency, along with an increase in the rigidity of the processed cheeses, is to be expected (Buňka et al., 2013; Nagyová et al., 2014).

Received October 7, 2016.
Accepted February 8, 2017.
[1]Corresponding author: cernikova@ft.utb.cz

In the industry, the consistency of processed cheeses regularly is assessed sensorially. However, the instrumental evaluation using small (e.g., dynamic oscillation rheometry) or large (e.g., texture profile analysis) deformations or their combinations is increasing (Lee et al., 2003; Kapoor and Metzger, 2008; Buňka et al., 2013, 2014; Nagyová et al., 2014). Rheological parameters measured in the area of small or large deformations are given mainly by the microstructure of processed cheeses and mutual bonds between the individual components (especially the properties of the protein network and its interactions with other components; Hosseini-Parvar et al., 2015; da Silva et al., 2016). To explain the nature of the current state of consistency, it is therefore useful to have the data about the mechanical properties of the processed cheese and also its microstructure. The microstructure of processed cheeses may be studied by several methods, the most common of which are optical microscopy (Hladká et al., 2014; da Silva et al., 2016), scanning electron microscopy (Kaláb and Modler, 1985; Noronha et al., 2008; Cunha et al., 2010), transmission electron microscopy (Lee et al., 2003; Zhang et al., 2011; Hoffmann and Schrader, 2015), and confocal laser scanning microscopy (Hosseini-Parvar et al., 2015; Lee et al., 2015).

Although the final parameters of processed cheeses (especially DM, FDM, and fat-free DM content) affect their consistency to a large extent, they have not been given sufficient attention in the literature over the past 10 yr. One of the few studies, by Lee et al. (2015), dealt with the effect of protein content (10–20% wt/wt) and fat content (0–40% wt/wt) on the viscoelastic properties of model samples of processed cheeses made from rennet casein (melting temperature = 85°C) and stored for 24 h. With the increasing protein content and decreasing fat content (constant protein-to-water content, variable DM content), the rigidity of the processed cheeses increased. A more significant effect of the protein content was observed compared with the fat content. The conclusions of the study were supported by the results of confocal laser scanning microscopy.

Guinee and O'Callaghan (2013) used processed cheeses made from cheddar and skim milk cheese (melting temperature = 80°C) with a fat content of 14 to 33% (wt/wt), protein content of 12 to 25% (wt/wt), and constant DM content of 46 to 47% (wt/wt) stored for a maximum of 4 d. With the increasing fat content (and decreasing protein-to-fat ratio), the rigidity of the samples declined. Dimitreli and Thomareis (2004, 2007, 2008) studied the viscosity and the textural and viscoelastic properties of processed cheeses made from Gouda (melting temperature = 80°C) with a different DM (38–62% wt/wt), protein (11–30% wt/wt), and fat (12–23% wt/wt) content stored for 24 h. With the decreasing DM and protein content and the increasing fat content, the rigidity of the model samples declined. Some of the other few studies dealing with the effect of the content components on the consistency of processed cheeses were published by Bayarri et al. (2012) and Chatziantoniou et al. (2015). In both of the studies (specific processed cheeses made from whey Myzithra-type cheese and the samples obtained from the retail network), the rigidity of the samples increased with the decreasing FDM content. None of the studies mentioned in this paragraph used any of the microscopic methods to explain the changes in consistency.

The aim of this work was to examine the effect of a different DM content (35 and 45% wt/wt) and FDM content (40 and 50% wt/wt) on the textural and viscoelastic properties and microstructure of model processed cheeses made from real ingredients regularly used in the dairy industry. A similar objective was already fulfilled in some of the previously mentioned studies. However, none of the published works took advantage of the rheological methods, using both large and small deformations in combination with scanning electron microscopy (which helps to explain the processes going on during the manufacture and storage of processed cheese), to describe the properties of the samples. A constant DM content and constant FDM content were kept throughout the whole study. These 2 parameters were variable in most previously published works. Most of the studies published in this area used model samples stored for only 24 h or for a maximum of 4 d at a cold storage temperature. This work uses processed cheeses stored for 14 d at 6 ± 2°C. Within this time, the most intensive changes in consistency occur during the storage period. In most producers, processed cheeses are dispatched after 10 to 14 d when the almost-final consistency of the product is already known. The samples with 40 and 50% (wt/wt) FDM content and 45% (wt/wt) DM content are "spreadable processed cheese" according to the standards of Codex Alimentarius (1978). The samples with lower DM content (35% wt/wt) and both FDM contents (40 and 50% wt/wt) correspond to products that are available in the region of Middle Europe.

MATERIALS AND METHODS

Preparation of the Samples

Dutch-type cheese (50% wt/wt DM content and 30% wt/wt FDM content), butter (84% wt/wt DM content and 82% wt/wt fat content), water, and emulsifying salts (the total concentration was 2.9% wt/wt of the total weight of the melt; the composition of the emulsifying salt mixture was 35% relative NaH_2PO_4, 20% rela-

Table 1. Formulation of the processed cheese samples with different DM content (% wt/wt) and fat in DM (FDM) content (% wt/wt)

	35% DM		45% DM	
Raw material	40% FDM	50% FDM	40% FDM	50% FDM
Dutch-type cheese (g)	785	636	1,050	847
Butter (g)	110	208	135	260
Emulsifying salts (g)	45	45	45	45
Water (g)	600	665	315	398
Total (g)	1,540	1,554	1,545	1,550

tive Na_2HPO_4, 25% relative $Na_4P_2O_7$, and 20% relative sodium salt of polyphosphate; total weight = 100%) were used for manufacturing 4 model processed cheeses with 35 and 45% (wt/wt) DM content and 40 and 50% (wt/wt) FDM content, respectively. The formulations for manufacturing the model samples are shown in Table 1. A Stephan UMC-5 (Stephan Machinery GmbH, Hameln, Germany; indirect heating tool) was used for the manufacture of the samples. A target temperature of 86°C was held for 1 min (agitation speed = 3,000 rpm). The hot melt was poured into polystyrene cups and closed with aluminum lids. For the analysis of the textural properties, polypropylene doses of cylindrical shape (52 mm in diameter and 50 mm high) were used. The processed cheeses were cooled and stored at 6 ± 2°C for 14 d. Each of the 4 model samples was produced 3 times.

Basic Chemical Analysis of the Model Processed Cheeses

The DM content, fat content, and protein content of the processed cheeses were determined according to methods 5534 (ISO, 2004b), 1735 (ISO, 2004a), and 8968–1 (ISO, 2014), respectively. The FDM content of the samples was calculated as fat content divided by DM. The ash content was obtained according to Černá and Mergl (1971): The sample was incinerated at 550 ± 5°C and the residue was weighed. Each method was applied 3 times on 2 samples from every batch (3 repetitions × 3 batches × 2 samples; n = 18). The pH values were measured at ambient temperature using a glass-tip electrode of a pH meter (pH Spear, Eutech Instruments, Oakton, Malaysia) by directly inserting the spear into the processed cheese (n = 18).

Textural and Viscoelastic Properties of the Model Processed Cheeses

The textural properties of the samples were evaluated using a TA.XTplus texture analyzer (Stable Micro Systems Ltd., Godalming, UK). Before measurement, the samples were tempered at 20°C. The texture analyses were carried out by 2 sequential penetration events (penetration depth = 10 mm, probe speed = 2 mm/s, trigger force = 5 g) using a 20-mm (diameter) P20 stainless steel cylinder probe. The following textural parameters were determined: hardness (the force needed to attain a given deformation minus the maximum force during the first penetration cycle; N), cohesiveness (the strength of the internal bonds of the cheese minus the ratio of the positive force area of the second peak to that of the first peak; unitless), and relative adhesiveness (the relative strength of adhesiveness between the cheese and the probe surface minus the ratio of the absolute value of the negative force area to the positive force area of the first peak; unitless; Fiszman and Damásio, 2000; Breuil and Meullenet, 2001; Weiserová et al., 2011). Each batch was analyzed 3 times (3 repetitions × 3 batches; n = 9; each dose was used for the textural analysis only once).

The viscoelastic properties of the model processed cheeses were analyzed using a dynamic oscillatory shear rheometer (RheoStress 1, Haake, Bremen, Germany) with a plate-plate geometry (diameter = 35 mm, gap = 1 mm) at a temperature of 20.0 ± 0.1°C. All samples were measured in the control shear stress mode at a frequency ranging from 0.01 to 100.00 Hz. The amplitude of shear stress (20 Pa) was chosen in the region of linear viscoelasticity. The exposed edge of the parallel-plates geometry was covered with a thin layer of silicone oil to prevent the samples from dehydrating. The selected monitored parameters (determined as a function of frequency) included the storage modulus ($\mathbf{G'}$) and loss modulus ($\mathbf{G''}$; n = 9; 3 repetitions × 3 batches). The loss tangent (tan δ) was calculated as tan δ = G″/G′. The complex modulus ($\mathbf{G^*}$) was obtained using the formula

$$G^* = \sqrt{\left(G'\right)^2 + \left(G''\right)^2}. \quad [1]$$

The frequency of 1 Hz was chosen as the reference for the presentation of tan δ and G*. Winter's critical gel

theory was also implemented using the following equation (Gabriele et al., 2001):

$$G^*(\omega) = A_F \cdot \omega^{1/z}, \quad [2]$$

where A_F is the gel strength (Pa·s$^{1/z}$), ω (Hz) is the frequency, and z is the interaction factor (defined as the number of structure units interacting with one another in a 3-dimensional network; unitless). The higher the interaction factor, the more interactions that occur in the matrix of the sample (Gabriele et al., 2001).

Scanning Electron Microscopy of the Model Processed Cheeses

A Jeol JSM-7401F scanning electron microscope (Jeol, Tokyo, Japan) was used to study the microstructure of the model processed cheeses. The preparation of the model processed cheese samples was as follows. Part of the processed cheese sample (size = approximately 5 × 2 × 2 mm) was put in a 3% (vol/vol) glutaraldehyde solution in 0.2 mol/L cacodylate buffer for 24 h. The samples were washed in cacodylate buffer 3 times for 15 min. The postfixation was performed for 48 h in 2% (wt/vol) OsO_4. The samples were washed again in cacodylate buffer 3 times for 15 min. The samples were dehydrated by solutions with an increased concentration of ethanol from 30 to 100%. Then the samples were frozen and fractured in liquid nitrogen and defatted by chloroform. The fragments of model processed cheeses were critical point dried using carbon dioxide (Leica EM CPD300, Leica Microsystems, Vienna, Austria). The samples were mounted on an aluminum stem and holder and sputter coated (Sputter Coater SCD 050, Bal-tec, Balzers, Liechtenstein) for 98 s (20-nm layer of gold; Kaláb and Modler, 1985). The samples were viewed using a Jeol JSM-7401F scanning electron microscope. Each image was analyzed using ImageJ software (National Institutes of Health, Bethesda, MD). The photograph of each model sample was analyzed to determine the fat globule diameter (μm). Each sample was analyzed twice (2 repetitions × 2 samples × 3 batches; n = 12), and the results were expressed as median ± standard error.

Statistical Analysis

Kruskal–Wallis and Wilcoxon tests were used to evaluate the results obtained (the significance level was 0.05) with the exception of the results of fat globule diameter. For this parameter, the Pearson test was applied. For the estimation of A_F and z, nonlinear regression analysis (the Marquardt–Levenberg method; $A_F > 0$ and $z \geq 0$) was used. Unistat 6.5 software (Unistat, London, UK) was applied for the statistical analysis.

RESULTS AND DISCUSSION

The results of the chemical, rheological, and microscopic analyses are summarized in Table 2. We managed to produce model processed cheeses with minimal deviations ($P \geq 0.05$) from the target values of DM (35 and 45% wt/wt) and FDM (40 and 50% wt/wt). As expected, the protein and ash content increased ($P < 0.05$) with the increasing DM. On the contrary, with the increasing FDM (at constant DM), the protein content decreased ($P < 0.05$). In the samples with the same FDM, a similar value of the ratio of protein to fat (~1:0.85 and ~1:1.30, respectively) was maintained (Table 2). In the samples with the same FDM, the ash content did not change significantly ($P \geq 0.05$). The pH value was higher in the samples with the higher FDM ($P < 0.05$), whereas it was similar in the samples with the same FDM ($P \geq 0.05$). The higher relative fat content and lower relative protein content may have led to the change in the acid dissociation constant (pK_a) of the chemicals present (Lu et al., 2008; Guinee and O'Callaghan, 2013), and thus to a slight increase (~0.3 unit) in pH level. According to Lee and Klostermeyer (2001) and Lu et al. (2008), a slight increase in the pH value leads to higher rigidity of processed cheeses. However, this shift (~0.3 unit) could be evaluated as not so significant.

The textural analysis of the samples using the area of large deformations of the material showed (Table 2) that with the increasing DM and decreasing FDM, hardness of the samples increases ($P < 0.05$) and the relative adhesiveness of the processed cheeses decreases ($P < 0.05$). The cohesiveness of the samples was not significantly affected by the DM and FDM levels used ($P \geq 0.05$).

The course of the storage (G′) and loss (G″) moduli depending on the frequency (0.01–100.00 Hz) is plotted in Figure 1. The G′ and G″ curves of the processed cheeses are, within the range of frequency observed, higher ($P < 0.05$; at constant FDM) in the products with higher DM (Figure 1, parts C and D) compared with the samples with a lower DM content (Figure 1, parts A and B). Furthermore, the G′ and G″ curves in processed cheeses with higher FDM (Figure 1, parts B and D) were significantly lower ($P < 0.05$; at constant DM) compared with the products with lower FDM (Figure 1, parts A and C). The storage modulus (G′) reached higher values ($P < 0.05$) than the loss modulus within the whole range of frequency tested in the samples with higher DM (regardless of FDM). This implies a significantly higher proportion of the storage

component G′ compared with the loss component G″, which is very typical of densely interlinked weak gels (Lee and Klostermeyer, 2001; Cunha et al., 2013; Lee et al., 2015). On the other hand, in the processed cheeses with a lower DM content, higher values at a lower frequency were reached by the loss modulus G″ ($P < 0.05$). It is assumed that if G″ > G′ (within a certain range of lower applied frequency), the intermolecular bonds in the protein matrix observed have sufficient time to weaken during the oscillation cycle. However, at higher frequency the system does not have sufficient time to weaken the intermolecular bonds and it starts to behave more like a solid (G″ < G′), which is typical of concentrated solutions and dispersions (Lee and Klostermeyer, 2001; Lee et al., 2015).

With the increasing frequency, intersection of both curves was observed in the samples with lower DM, and the values of G′ exceeded those of G″ (Figure 1, part A and B). The intersection point of the 2 curves (G′ and G″) was observed at higher frequency in the processed cheese with higher FDM. Within the range of frequency observed, the intersection of the storage and loss moduli curves did not occur in the samples with higher DM (Figure 1, part C and D). The intersection of G′ and G″ may have been expected at much lower frequency than observed in our case. The results of the development of G′ and G″ depending on frequency can be supported by the obtained values of the complex modulus (G*) and the tangent of the phase shift angle (tan δ) for the reference frequency f = 1 Hz (Table 2). With the increasing DM (at constant FDM) and decreasing FDM (at constant DM), G* was increasing and tan δ was decreasing ($P < 0.05$).

As it follows from these findings, a higher DM content and lower FDM content resulted in obtaining a stronger gel and thus a product with a tougher and less spreadable consistency. The stated information can also be supported by the calculated values of the A_F and z parameters (Table 2). With the increasing DM (at constant FDM) and the decreasing FDM (at constant DM), the gel strength (A_F; $P < 0.05$) of the model processed cheeses increased significantly ($P < 0.05$) along with the interaction factor (z; $P < 0.05$), which refers to the number of structural units mutually interacting in the protein network or the number of intermolecular bonds between proteins. The increasing gel strength was likely attributable to the increasing number of interactions in the 3-dimensional system studied (Cunaa et al., 2013; Guinee and O'Callaghan, 2013; Lee et al., 2015).

Apart from the values of the gel strength A_F and z factors, the results of dynamic oscillation rheometry can also be supported by the microstructure study of the model processed cheeses. The microstructure of the individual types of the model processed cheeses is presented in Figure 2, and the results of the calculation of the median diameter of the fat globules are shown in Table 2.

As it follows from the microphotographs (Figure 2) and the numerical calculation in Table 2, with the increasing FDM (at constant DM), the diameter of the fat globules increases ($P < 0.05$) and the ratio of protein to fat decreases. In the model processed cheeses studied, the ratio of proteins to fat was approximately 1:0.85 in the products with 40% (wt/wt) FDM and approximately 1:1.30 in the products with 50% (wt/wt) FDM. Proteins (caseins) are the main emulsifier in this

Table 2. Results of the analysis of the processed cheese samples with different DM content and fat in DM (FDM) content (% wt/wt)

	35% DM		45% DM	
Parameter	40% FDM	50% FDM	40% FDM	50% FDM
DM content[1] (% wt/wt)	35.18 ± 0.13[a]	35.09 ± 0.22[a]	45.16 ± 0.27[b]	45.21 ± 0.21[b]
Fat content[1] (% wt/wt)	14.2 ± 0.4[a]	17.5 ± 0.2[b]	18.3 ± 0.5[c]	22.5 ± 0.4[d]
FDM content, calculated (% wt/wt)	40.3	49.9	40.5	49.8
Protein content[1] (% wt/wt)	16.4 ± 0.5[b]	13.6 ± 0.2[a]	22.1 ± 0.6[d]	17.6 ± 0.3[c]
Ash content[1] (% wt/wt)	3.99 ± 0.15[a]	3.83 ± 0.26[a]	5.17 ± 0.21[b]	4.99 ± 0.19[b]
pH value[1]	5.77 ± 0.04[a]	6.05 ± 0.03[b]	5.74 ± 0.02[a]	5.97 ± 0.03[b]
Protein-to-fat ratio, calculated	1:0.87	1:1.29	1:0.82	1:1.28
Protein-to-moisture ratio, calculated	1:3.95	1:4.77	1:2.48	1:3.11
Hardness[1] (N)	6.04 ± 0.35[b]	1.53 ± 0.10[a]	66.98 ± 1.99[d]	28.25 ± 0.93[c]
Cohesiveness[1] (unitless)	0.60 ± 0.02[a]	0.61 ± 0.04[a]	0.62 ± 0.06[a]	0.60 ± 0.03[a]
Relative adhesiveness[1] (unitless)	0.42 ± 0.01[c]	0.54 ± 0.02[d]	0.01 ± 0.00[a]	0.07 ± 0.01[b]
Fat globule diameter[2] (μm)	1.53 ± 0.13[c]	2.16 ± 0.27[d]	0.44 ± 0.03[a]	0.65 ± 0.02[b]
Complex modulus in 1 Hz[1] (kPa)	4.8 ± 0.3[b]	1.2 ± 0.1[a]	55.9 ± 2.2[d]	27.8 ± 1.3[c]
Loss tangent in 1 Hz[1] (unitless)	0.66 ± 0.04[c]	1.14 ± 0.07[d]	0.29 ± 0.02[a]	0.37 ± 0.02[b]
Gel strength[1] (kPa·s$^{1/z}$)	4.7 ± 0.2[b]	1.3 ± 0.0[a]	55.6 ± 3.4[d]	26.9 ± 0.8[c]
Interaction factor[1] (unitless)	3.01 ± 0.16[b]	2.26 ± 0.06[a]	5.14 ± 0.22[d]	4.39 ± 0.19[c]

[a–d]Means within a row with different superscripts differ ($P < 0.05$).

[1]Mean ± standard deviation.

[2]Mean ± standard error.

network. Their lower relative amount causes a lower degree of emulsification, manifested as an increased diameter of the fat globules. The larger fat globules are then able to break the continuity of the protein matrix much more intensively (softer, more spreadable processed cheese) compared with a larger number of smaller fat globules (tougher, less spreadable processed cheese; Lee et al., 2003; Kapoor and Metzger, 2008; Noronha et al., 2008). At constant FDM and increasing DM (in this case, without the addition of ingredients other than the natural cheeses), the amount of the proteins that enhance the emulsifying ability of the system increases, which was manifested as a decreased diameter of the fat globules ($P < 0.05$; Table 2; Figure 2, parts C and D in comparison with parts A and B). Similarly, Lee et al. (2015) demonstrated the effect of the increasing concentration of protein on the decreasing size of the fat globules in processed cheeses. The higher relative protein content is able to cover a larger area of the fat globules, thus allowing the formation of smaller fat globules and vice versa (Dalgleish, 1997). As it follows from Figure 2B, the samples with the lowest protein content (and with the smallest ratio of protein to moisture at the same time; Table 2) showed partial coalescence of fat, which is evident in the formation of larger fat globules with a less regular spherical shape. Similar conclusions in the samples with the highest relative fat content were also reached in the study by Lee et al. (2015). Moreover, it can be assumed that a higher number of structural units of caseins will also form a denser protein network and that the number of interactions between the individual protein chains will increase (Lee et al., 2003). The latter is supported by a more compact network (which is particularly apparent in Figure 2C). This assumption is strongly supported by the values of the gel strength A_F and the interaction factor z observed in the processed cheeses studied. Additionally, higher viscosity of hot melt (in cheese with higher DM and protein content) could result in more shear (during manufacturing) and contribute to changes

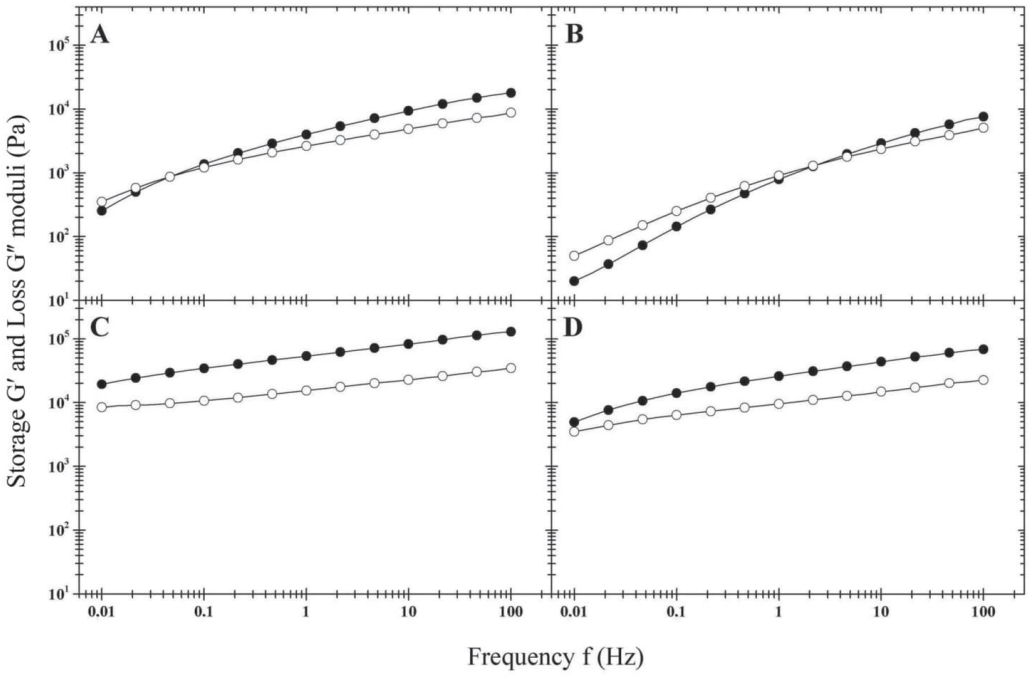

Figure 1. Dependence of the storage (G′; Pa; solid symbols) and loss (G″; Pa; open symbols) moduli of the model processed cheese on frequency (f; Hz). (A) Samples with 35% (wt/wt) DM content and 40% (wt/wt) fat in DM content. (B) Samples with 35% (wt/wt) DM content and 50% (wt/wt) fat in DM content. (C) Samples with 45% (wt/wt) DM content and 40% (wt/wt) fat in DM content. (D) Samples with 45% (wt/wt) DM content and 50% (wt/wt) fat in DM content.

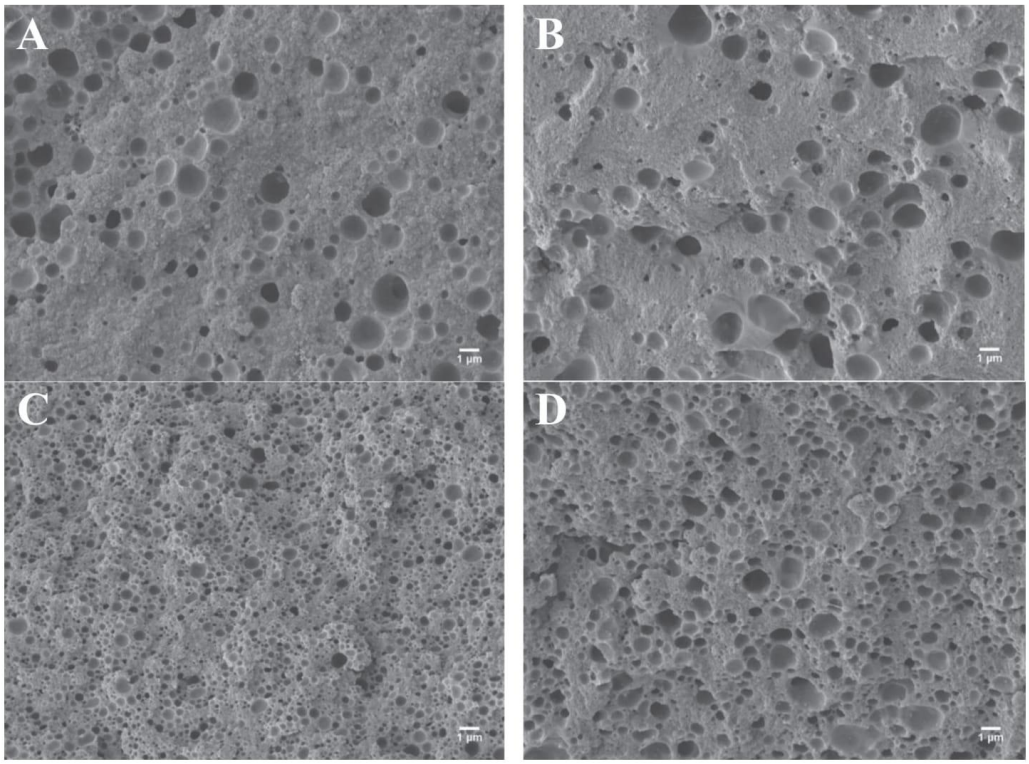

Figure 2. Scanning electron microscopy images of the model processed cheese (scale bars represent 1 µm; 5,000× magnification). (A) Samples with 35% (wt/wt) DM content and 40% (wt/wt) fat in DM content; (B) samples with 35% (wt/wt) DM content and 50% (wt/wt) fat in DM content; (C) samples with 45% (wt/wt) DM content and 40% (wt/wt) fat in DM content; (D) samples with 45% (wt/wt) DM content and 50% (wt/wt) fat in DM content.

in textural and viscoelastic properties of products and decreasing of fat droplets (Yoon and McCarthy, 2003; Zhu et al., 2015).

CONCLUSIONS

This work examined the effect of different DM contents (35 and 45% wt/wt) and FDM contents (40 and 50% wt/wt) on the textural and viscoelastic properties and microstructure of model processed cheeses made from real ingredients regularly used in the dairy industry. Apart from the basic chemical parameters, textural and viscoelastic properties of the model samples were measured and scanning electron microscopy was carried out. With increasing DM content, the rigidity of the products increased and thus the size of the fat globules in the model samples of the processed cheeses decreased. With increasing FDM content, the rigidity of the processed cheeses decreased and the size of the fat globules increased. Further studies will be necessary to obtain better insight into the effect of the protein content on the properties of processed cheeses.

ACKNOWLEDGMENTS

This study was kindly supported by the internal grant agency of Tomas Bata University in Zlín, Czech Republic (IGA/FT/2016/003 and IGA/FT/2017/004) and funded by resources dedicated to specific university research. We acknowledge the core facility Laboratory

of Electron Microscopy, Biology Centre of CAS (České Budějovice, Czech Republic), supported by the MEYS CR (LM2015062 Czech-BioImaging).

REFERENCES

Bayarri, S., I. Carobonell, and E. Costell. 2012. Viscoelasticity and texture of spreadable cheese with different fat contents at refrigeration and room temperatures. J. Dairy Sci. 95:6926–6936.

Breuil, P., and J. F. Meullenet. 2001. A comparison of three instrumental tests for predicting sensory texture profiles of cheese. J. Texture Stud. 32:41–55.

Buňka, F., L. Doudová, E. Weiserová, M. Černíková, D. Kuchař, Š. Slavíková, G. Nagyová, P. Ponížil, T. Grůber, and J. Michálek. 2014. The effect of concentration and composition of ternary emulsifying salts on the textural properties of processed cheese spreads. Lebensm. Wiss. Technol. 58:247–255.

Buňka, F., L. Doudová, E. Weiserová, D. Kuchař, P. Ponížil, D. Začalová, G. Nagyová, V. Pachlová, and J. Michálek. 2013. The effect of ternary emulsifying salt composition and cheese maturity on the textural properties of processed cheese. Int. Dairy J. 29:1–7.

Černá, E., and M. Mergl. 1971. Control Methods in Dairy Industry [in Czech]. 1st ed. State Technical Press, Prague, Czech Republic.

Chatziantoniou, S. E., A. S. Thomareis, and M. G. Kontominas. 2015. Effect of chemical composition on physico-chemical, rheological and sensory properties of spreadable processed whey cheese. Eur. Res. Food Technol. 241:737–748.

Codex Alimentarius. 1978. CODEX-STAN A-8(b)-1978: Codex general standard for process(ed) cheese and spreadable process(ed) cheese. World Health Organization, Geneva, Switzerland.

Cunha, C. R., A. I. Dias, and W. H. Viotto. 2010. Microstructure, texture, colour and sensory evaluation of a spreadable processed cheese analogue made with vegetable fat. Food Res. Int. 43:723–729.

Cunha, C. R., R. Grimaldi, M. R. Alcantara, and W. H. Viotto. 2013. Effect of the type of fat on rheology, functional properties and sensory acceptance of spreadable cheese analogue. Inter. J. Dairy Technol. 66:54–62.

da Silva, D., F. De Souza, S. B. Ferreira, M. L. Bruschi, M. Britten, and P. T. Matumoto-Pintro. 2016. Effect of commercial konjac glucomannan and konjac flours on textural, rheological and microstructural properties of low fat processed cheese. Food Hydrocoll. 60:308–316.

Dalgleish, D. G. 1997. Adsorption of protein and the stability of emulsions. Trends Food Sci. Technol. 8:1–6.

Dimitreli, G., and A. S. Thomareis. 2004. Effect of temperature and chemical composition on processed cheese apparent viscosity. J. Food Eng. 64:265–271.

Dimitreli, G., and A. S. Thomareis. 2007. Texture evaluation of block-type cheese as a function of chemical composition and in relation to its apparent viscosity. J. Food Eng. 79:1364–1373.

Dimitreli, G., and A. S. Thomareis. 2008. Effect of chemical composition on the linear viscoelastic properties of spreadable-type processed cheese. J. Food Eng. 84:368–374.

Fiszman, S. M., and M. H. Damásio. 2000. Instrumental measurement of adhesiveness in solid and semi-solid foods. A survey. J. Texture Stud. 31:69–91.

Gabriele, D., B. De Cindio, and P. D'Antona. 2001. A weak gel model for foods. Rheol. Acta 40:120–127.

Guinee, T. P., and D. J. O'Callaghan. 2013. Effect of increasing the protein-in-water ratio and reducing fat content on the chemical and physical properties of processed cheese product. J. Dairy Sci. 96:6830–6839.

Hladká, K., Z. Randulová, B. Tremlová, P. Ponížil, P. Mančík, M. Černíková, and F. Buňka. 2014. The effect of cheese maturity on selected properties of processed cheese without traditional emulsifying agents. Lebensm. Wiss. Technol. 55:650–656.

Hoffmann, W., and K. Schrader. 2015. Dispersion analysis of spreadable processed cheese with low content of emulsifying salts by photocentrifugation. Int. J. Food Sci. Technol. 50:950–957.

Hosseini-Parvar, S. H., L. Matia-Merino, and M. Golding. 2015. Effect of basil seed gum (BSG) on textural, rheological and microstructural properties of model processed cheese. Food Hydrocoll. 43:557–567.

ISO (International Organization for Standardization). 2004a. ISO standard no. 1735: Cheese and processed cheese—Determination of fat content—Gravimetric method. ISO, Geneva, Switzerland.

ISO (International Organization for Standardization). 2004b. ISO standard no. 5534: Cheese and processed cheese—Determination of the total solid content. ISO, Geneva, Switzerland.

ISO (International Organization for Standardization). 2014. ISO standard no. 8968–1: Milk and milk products—Determination of nitrogen content—Part 1: Kjeldahl principle and crude protein calculation. ISO, Geneva, Switzerland.

Kaláb, M., and W. H. Modler. 1985. Milk gel structure. XV. Electron microscopy of whey protein-based Cream cheese spread. Milchwissenschaft 40:193–196.

Kapoor, R., and L. E. Metzger. 2008. Process cheese: Scientific and technological aspects—A review. Compr. Rev. Food Sci. Food Safety 7:194–214.

Lee, S. K., R. J. Buwalda, S. R. Euston, E. A. Foegeding, and A. B. McKenna. 2003. Changes in the rheology and microstructure of processed cheese during cooking. Lebensm. Wiss. Technol. 36:339–345.

Lee, S. K., and H. Klostermeyer. 2001. The effect of pH on the rheological properties of reduced-fat model processed cheese spreads. Lebensm. Wiss. Technol. 34:288–292.

Lee, S. K., H. Klostermeyer, and S. G. Anema. 2015. Effect of fat and protein-in-water concentration on the properties of model processed cheese. Int. Dairy J. 50:15–23.

Lu, Y., N. Shirashoji, and J. A. Lucey. 2008. Effects of pH on the textural properties and meltability of pasteurized process cheese made with different type of emulsifying salts. J. Food Sci. 73:E363–E369.

Nagyová, G., F. Buňka, R. N. Salek, M. Černíková, P. Mančík, T. Grůber, and D. Kuchař. 2014. Usage of sodium polyphosphates with different linear length in the production of spreadable processed cheese. J. Dairy Sci. 97:111–122.

Noronha, N., E. D. O'Riordan, and M. O'Sullivan. 2008. Influence of processing parameters on the texture and microstructure of imitation cheese. Eur. Food Sci. Technol. 226:385–396.

Shirashoji, N., H. Aoyai, J. J. Jaeggi, and J. A. Lucey. 2016. Effect of tetrasodium pyrophosphate concentration and cooking time on the physicochemical properties of process cheese. J. Dairy Sci. 99:6983–6994.

Weiserová, E., L. Doudová, L. Galiová, L. Žák, J. Michálek, R. Janiš, and F. Buňka. 2011. The effect of combinations of sodium phosphates in binary mixtures on selected texture parameters of processed cheese spreads. Int. Dairy J. 21:979–986.

Yoon, W. B., and K. L. McCarthy. 2003. Flow behaviour of processed cheese melts. J. Food Process Eng. 26:559–576.

Zhang, X., Y. Yang, and D. Zhai. 2011. Effect of blueberry on spreadable processed cheese. J. Northeast Agric. Univ. 18:73–78. (English Edition).

Zhu, C., C. Brown, G. Gillies, P. Watkinson, and J. Bronlund. 2015. Charactering of rheological properties of mozzarella cheese at shear rate and temperature conditions relevant to pizza baking. Lebensm. Wiss. Technol. 64:82–87.

Research paper 8

B2

Černíková, M., Salek, R. N., Kozáčková, D., Buňka, F.

The effect of different agitations and temperature maintainings on viscoelastic properties of full-fat processed cheese spreads.

LWT – Food Science & Technology. 2018, 89, 244-247. ISSN: 00236438.

LWT - Food Science and Technology 89 (2018) 244–247

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Short communication

The effect of different agitations and temperature maintainings on viscoelastic properties of full-fat processed cheese spreads

Michaela Černíková, Richardos Nikolaos Salek, Dana Kozáčková, František Buňka*

Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, T.G. Masaryka 5555, 760 01, Zlín, Czech Republic

ARTICLE INFO

Keywords:
Processed cheese
Full-fat
Rheology
Speed of agitation
Holding time

ABSTRACT

The objective of this study was to determine the effect of different melt holding time (0–20 min) at a melting temperature of 90 °C on the viscoelastic properties of full-fat (fat content in dry matter 50 g/100 g, dry matter content 35 g/100 g) processed cheeses over the course of a sixty-day storage period (6 ± 2 °C). Three various agitation speeds (1000; 1500 and 3000 rounds per min) were used. For all three monitored agitation speeds, there was a significant decrease in the firmness of the processed cheeses in the first 3 min of the holding time. The subsequent extension of the melt holding time (up to 20 min) led to the significant increase the product firmness. Over the course of a sixty-day storage period, the firmness of all the monitored processed cheeses increased. Samples produced at 3000 rpm were significantly more solid in comparison with processed cheeses produced at lower agitation speeds (1000 and 1500 rpm).

1. Introduction

The consistency of processed cheeses can be affected by many factors, which can be divided into three main groups: (i) composition of the final product (dry matter, fat, protein and lactose content, use of hydrocolloids, emulsifiers, etc.); (ii) process parameters during production (in particular mixing speed, melting temperature, holding time at the melting temperature, cooling rate); and (iii) conditions during storage (in particular storage temperature and time) (Kapoor & Metzger, 2008). The effects of process parameters on the rheological properties of processed cheeses was examined in several works published in the last 20 years (e. g. Sutheerawattananonda, Fulcher, Martin, & Bastian, 1997; Bowland & Foegeding, 1999; Swenson, Wendorff, & Lindsay, 2000; Lee, Buwalda, Euston, Foegeding, & McKenna, 2003; Noronha, O´Riordan, & O´Sullivan, 2008). Unfortunately, the studies on which these works are based were conducted under varying conditions (e.g. different melting temperatures, holding times, agitation speeds, storage periods, etc.), which makes it very difficult to compare them. In addition, the conclusions reached by the authors were often contradictory.

In recent years, other works have been published which attempt to contribute to the clarification of, in particular, the role of agitation speed and holding time at a given melting temperature on the consistency of the resulting processed cheese (Shirashoji, Aoyai, Jaeggi, & Lucey, 2016; Černíková et al., 2017a). The conclusion drawn as a result of the studies conducted for both these works was the same, namely that when the holding time at the given melting temperature is extended, the firmness of the processed cheeses increases. From the article by Černíková et al. (2017a), it can be assumed that this trend is not linear. Furthermore, it was demonstrated that the effect of agitation speed on the consistency of the product is not clear, and depends significantly on the holding time. During short holding times (up to 3 min), processed cheeses produced at lower agitation speeds (1000 rpm) were more solid than those products produced at higher speeds (1500 and 3000 rpm). However, as the holding time was extended, this trend changed significantly. The study by Černíková et al. (2017a) was performed on model samples with 35 g/100 g dry matter content and 40 g/100 g fat in dry matter. However, full-fat products with a significantly higher fat content are also commonly produced. The question therefore remains whether the trends observed in Černíková et al. (2017a) also apply to full-fat processed cheeses.

The objective of this study was to determine the effect of changes in the holding time (0–20 min) at a melting temperature of 90 °C, and at various agitation speeds (1,000, 1500 and 3000 rpm), on the viscoelastic properties of full-fat (50 g/100 g fat in dry matter) model processed cheeses over the course of a sixty-day storage period. The second aim of this study was to compare the recent results with the work of Černíková et al. (2017a), in which samples with lower fat in dry matter content were used. Therefore, all samples types (with different holding times, agitatioin speeds and storage times) which were manufactured in publication of Černíková et al. (2017a) were repeated with the fat in dry matter content of 50 g/100 g.

* Corresponding author.
E-mail address: bunka@ft.utb.cz (F. Buňka).

http://dx.doi.org/10.1016/j.lwt.2017.10.054
Received 17 June 2017; Received in revised form 21 October 2017; Accepted 24 October 2017
Available online 05 November 2017

2. Material and methods

Processed cheese (PC) samples with 35 g/100 g dry matter content and 50 g/100 g fat in dry matter content (FDM) were produced under the conditions described in Černíková et al. (2017a), including raw materials, processing protocol, cooling and storage (6 ± 2 °C). A target temperature of 90 °C (the time for achieving the target temperature varied between 10 and 11 min) was held for 0, 1, 3, 5, 7, 9, 11, 13, 15, 17 and 20 min (11 holding times) and agitation was set at 1,000, 1500 and 3000 rpm (3 agitation speeds). Each PC was produced three times. In total, 99 lots were produced (11 holding times × 3 agitation speeds × 3 repetitions). The analyses were performed on the 1st d (24 h after production), and the 14th, 30th and 60th d of storage.

The dry matter content and the fat content of the PC samples was determined gravimetrically according to ISO 5534 (2004) and ISO 1735 (2004), respectively. The pH values were measured at ambient temperature using the glass tip electrode of a pH meter (pHSpear, Eutech Instruments, Oakton, Malaysia). The spear was directly inserted into the PC samples in three randomly selected spots (in each package). The pH-values were measured at laboratory temperature of 20–22 °C. The samples were tempered 4 h under laboratory temperature and the final temperature were controlled by the thermometer.

The rheological analysis of the PC samples was performed using a dynamic oscillatory shear rheometer (RheoStress 1, HAAKE, Bremen, Germany) with a plate-plate geometry (diameter 35 mm; gap 1 mm) at 20.0 ± 0.1 °C. Storage (G′) and loss (G″) moduli (determined as a frequency function ranging from 0.1 to 10.0 Hz; data not shown) were monitored (shear stress amplitude 1 Pa). For presentation, complex modulus (G*) at the reference frequency 1 Hz was calculated.

Kruskal-Wallis and Wilcoxon tests were used in order to evaluate the obtained results (the significance level was 0.05). Unistat® 6.5 software (Unistat, London, UK) was used for the statistical analysis.

3. Results and discussion

During storage, the dry matter content and fat content of the individual processed cheeses were within a range of 35.96–36.41 g/100 g ($P \geq 0.05$) and 17.90–18.24 g/100 g ($P \geq 0.05$), respectively. The aforementioned data indicates that comparable processed cheeses were produced, and the dry matter and fat content were under control. The pH value after 1 d storage was 6.12–6.19 ($P \geq 0.05$), decreasing slightly by 0.1–0.2 ($P < 0.05$) over the course of the sixty-day storage period. This phenomenon was also observed in a number of other works, for example Kapoor and Metzger (2008) and Černíková et al. (2017a). The pH values are slightly higher than in the work by Černíková et al. (2017a), in which the same materials, composition of melting salts for the products and dry matter content were used, but with a lower fat content. The explanation for this probably lies in the change in the dissociation constants (pK_a) of the substances present such as salts and/or proteins and shorter peptides (Lu, Shirashoji, & Lucey, 2008; Černíková, Nebesářová, Salek, Řiháčková & Buňka, 2017b).

For all three monitored agitation speeds (1,000, 1500 and 3000 rpm), it was determined (Fig. 1) that, on the first d of storage, in the first 3 min of the holding time at the melting temperature of 90 °C, there was a significant ($P < 0.05$) decrease in the G* values for the processed cheeses. The subsequent extension of the melt holding time (up to 20 min) at the melting temperature of 90 °C, resulted in a significant increase ($P < 0.05$) in the G* value. Fig. 1 clearly describes that, over the course of the sixty-day storage period, the G* values for the processed cheeses increased ($P < 0.05$). However, the trend described above with regards to the dependence of G* value for the processed cheeses on the melt holding time at the melting temperature of 90 °C, remained preserved.

The increase in the G* values of the viscoelastic materials is connected with the increasing firmness (gel strength and hardness observed as resistance to penetration) of the studied processed cheese (Černíková et al., 2017b). The identified decrease (in the first three minutes of agitation) in the firmness of the processed cheeses (with 50 g/100 g FDM) produced at agitation speeds of 1,000, 1500 and 3000 rpm, was also observed in the work of Černíková et al. (2017a) in the case of identically produced products with a lower FDM content (40 g/100 g). The explanation for this might be to the creation of a new protein network, where fat is emulsified and proteins and/or peptides is hydrated by a water layer, requires a certain minimum amount of time for the minimum desired interaction to take place (Lee et al., 2003; Kapoor & Metzger, 2008). The increase in the firmness of the processed cheeses with extended holding times at the melting temperature of 90 °C, was observed in this study for those samples with 50 g/100 g FDM, as well as for those products with an FDM content of 40 g/100 g (Černíková et al., 2017a). The explanation for this may be found both in the decreasing size of the fat droplets, whilst the holding time is extended at the melting temperature (Sutheerawattananonda et al., 1997), as well as in a complementary phenomenon, whereby greater mechanical stress results in a more intensive solubilization and hydratation of caseins (Bowland & Foegeding, 1999; Lee et al., 2003).

The process of the increasing firmness of processed cheeses during storage is usually connected with the continuation of the changes in the crystalline form of polymorphic milk fat, the decrease in pH of the products during storage, the hydrolysis of polyphosphate melting salts, or changes in the dissociations of the compounds present (Kapoor & Metzger, 2008; Černíková et al., 2017a).

From Fig. 1, it follows that the firmness of most of processed cheeses produced using an agitation speed of 1500 rpm was always the lowest at all holding and storage time conditions in comparison with samples, which were manufactured using an agitation speed 1000 or 3000 rpm ($P < 0.05$). The G* values of the processed cheeses produced at 3000 rpm were always higher ($P < 0.05$) than for matching products (same holding time and storage period) to which lower agitation speeds were applied. In this study (for products with 50 g/100 g fat in dry matter) and in the previous work (for products with 40 g/100 g fat in dry matter) (Černíková et al., 2017a), very similar trends of dependece of temperature maintainings on consistecy of model processed cheese were observed for all tested holding times. In the cases of holding times more than 9 min, Černíková et al. (2017a) described that firmness of model samples manufactured using 1500 rpm could be similar as processed cheeses produced using 1000 rpm. On the other hand, in the recent study, the most of samples made using 1000 rpm were firmer than processed cheeses manufactured using 1500 rpm.

The higher the number of revolutions, the higher the firmness of the final products. This also applied to the processed cheeses studied in the work of Noronha et al. (2008). However, it should be noted that products were used with a different raw material composition, and that the holding time was much shorter (approx. 2 min), and the agitation speeds lower. Due to the more intensive mechanical stress, higher agitation speeds, just like longer holding times at the melting temperature, can lead to the greater hydration proteins present and fat emulsification, thereby contributing to the increasing firmness of the processed cheeses (Bowland & Foegeding, 1999; Lee et al., 2003; Sutheerawattananonda et al., 1997). The aforementioned explanation is fully applicable for the comparison of processed cheese consistency of our products manufactured at 3000 rpm with consistency of samples produced at 1000 rpm and 1500 rpm. On the other hand, the suitable clarification of the firmness decrease of processed cheese produced using revolutions of 1500 rpm in comparison with samples manufactured using revolutions of 1000 rpm is missing.

4. Conclusion

This study looked at the effect of the holding time at a given melting temperature, and agitation speed, on the consistency of full-fat processed cheeses. It was determined that, after an initial decrease in the

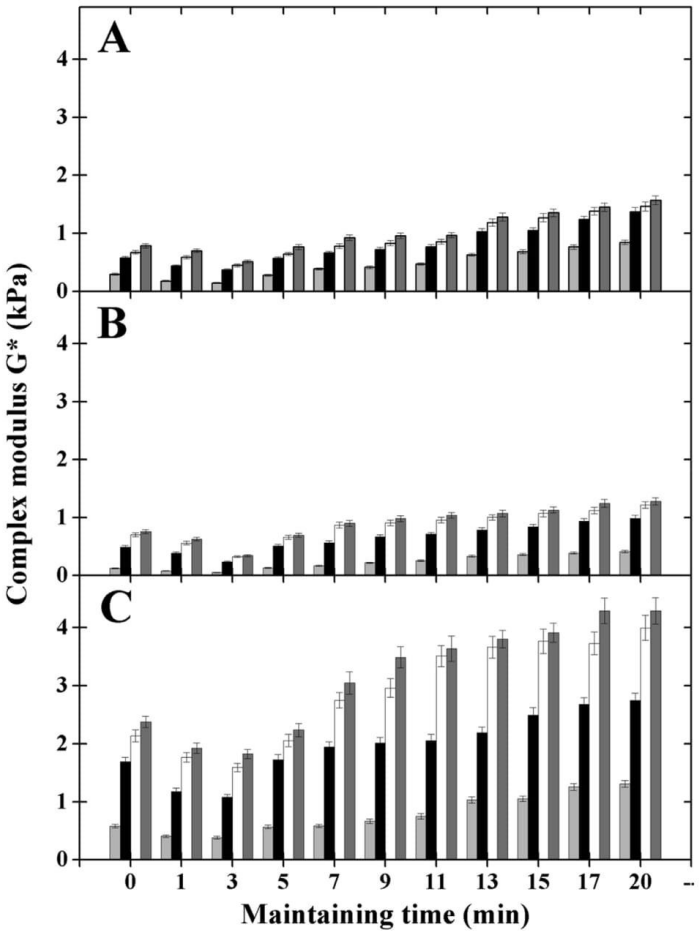

Fig. 1. Values of the complex modulus (G*; kPa) at the reference frequency of 1 Hz of the processed cheese spreads after 1 d (24 h; light grey), 14 d (black), 30 d (white) and 60 d (dark grey) of manufacturing using a different speed of Stephan kettle (1000 rounds per minute – part A; 1500 rounds per minute – part B and 3000 rounds per minute – part C) and different times (0–20 min) of temperature maintaining after holding a melting temperature of 90 °C. The values were expressed as mean (column) ± standard deviation (bar) (n = 12).

firmness of the processed cheeses (during holding times of up to 3 min), the firmness of the products increases significantly as the holding time is extended at the melting temperature. As the storage period extended to 60 d, the firmness of the processed cheeses also increased, regardless of either the applied holding time at the melting temperature, or the agitation speed.

Although the effect of extended holding times at the given melting temperature on the consistency of semi-fat and full-fat processed cheeses seems clear under the given conditions, this cannot be said of the effect of agitation speed, which remains unclear and requires further controlled studies and additional techniques (e. g. nuclear magnetic resonance spectroscopy, photocentrifugation, Rahman spectroscopy, microscopy techniques etc.).

Acknowledgement

This study was kindly support by a project of the internal grants of Tomas Bata University in Zlin, Czech Republic, No. IGA/FT/2016/003 and IGA/FT/2017/004 funded from the resources of specific university research.

References

Bowland, E. L., & Foegeding, E. A. (1999). Factors determining large-strain (fracture) rheological properties of model processed cheese. *Journal of Dairy Science, 82*, 1851–1859.

Černíková, M., Salek, R. N., Kozáčková, D., Běhalová, H., Luňáková, L., & Buňka, F. (2017a). The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads. *International Dairy Journal, 66*, 84–90.

Černíková, M., Nebesářová, J., Salek, R. N., Řihák̆ková, L., & Buňka, F. (2017b). Microstructure, textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content. *Journal of Dairy Science, 100*, 4300–4307.

ISO (International Organization for Standardization) (2004a). *ISO Standard No. 1735: Cheese and processed cheese—determination of fat content – gravimetric method (reference method)*. Geneva, Switzerland: ISO.

ISO (International Organization for Standardization) (2004b). *ISO Standard No. 5534: Cheese and processed cheese—determination of the total solid content (reference method)*. Geneva, Switzerland: ISO.

Kapoor, R., & Metzger, L. E. (2008). Process cheese: Scientific and technological aspects – a review. *Comprehensive Reviews in Food Science and Food Safety, 7*, 194–214.

Lee, S. K., Buwalda, R. J., Euston, S. R., Foegeding, E. A., & McKenna, A. B. (2003). Changes in the rheology and microstructure off processed cheese during cooking. *Lebensmittel-Wissenschaft und Technologie – Food Science and Technology, 36*, 339–345.

Lu, Y., Shirashoji, N., & Lucey, J. A. (2008). Effects of pH on the textural properties and meltability of pasteurized process cheese made with different type of emulsifying

salts. *Journal of Food Science, 73*, E363–E369.
Noronha, N., O´Riordan, E. D., & O´Sullivan, M. (2008). Influence of processing parameters on the texture and microstructure of iminitation cheese. *European Food Research and Technology, 226*, 385–393.
Shirashoji, N., Aoyai, H., Jaeggi, J. J., & Lucey, J. A. (2016). Effect of tetrasodium pyrophosphate concentration and cooking time on the physicochemical properties of process cheese. *Journal of Dairy Science, 99*, 6983–6994.
Sutheerawattananonda, M., Fulcher, R. G., Martin, F. B., & Bastian, E. D. (1997). Fluorescence image analysis of process cheese manufactured with trisodium citrate and sodium chloride. *Journal of Dairy Science, 80*, 620–627.
Swenson, B. J., Wendorff, W. L., & Lindsay, R. C. (2000). Effects of ingredients on the functioinality of fat-free process cheese spreads. *Journal of Food Science, 65*, 822–825.

Research paper 9

B3

Přikryl, J., Hájek, T., Švecová, B., Salek, R. N., Černíková, M., Červenka, L., Buňka, F.

Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions.

LWT – Food Science & Technology. 2018, 87, 266-271. ISSN: 00236438.

LWT - Food Science and Technology 87 (2018) 266–271

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions

Jakub Přikryl [a], Tomáš Hájek [a], Blanka Švecová [a], Richardos Nikolaos Salek [b], Michaela Černíková [b], Libor Červenka [a, *], František Buňka [b]

[a] Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, 53210, Pardubice, Studentská 573, Czech Republic
[b] Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, 76001, Zlín, nám. T. G. Masaryka, 5555, Czech Republic

ARTICLE INFO

Article history:
Received 12 June 2017
Received in revised form
31 August 2017
Accepted 31 August 2017
Available online 4 September 2017

Keywords:
Processed cheese
Flavonoids
Melting condition
Antioxidants

Chemical compounds studied in this article:
Quercetin (PubChem CID: 5280343)
Rutin (PubChem CID: 5280805)

ABSTRACT

Spreadable processed cheese (SPC) with addition of rutin or quercetin (0.5 g/100 g) were prepared at 80 °C and 90 °C for 1, 5 and 10 min. The effect of melting temperature and holding time of melting temperature on the quercetin/rutin retention, total phenolic content (TPC) and antioxidant capacity was studied. It was found that quercetin levels significantly decreased with the increase of holding time ($P < 0.01$) while rutin degradation was more affected by melting temperature ($P < 0.01$). An increase in TPC values and a decrease in antioxidant capacity measured by ABTS assay with the increase in melting temperature were observed in SPC with quercetin. The addition of rutin or quercetin significantly decreases the gel strength of the SPC samples.

1. Introduction

Spreadable processed cheese (SPC) is the multi-component system traditionally made from a mixture of cheeses, fat, water and emulsifying salts (sodium salts of phosphates, polyphosphates or citrates). The mixture of ingredients is stirred and then melted in temperatures ranged from 85 to 110 °C for a certain period of time (usually between 1 and 5 min). The resulted hot mixture is poured into the cups and cooled down below 8 °C (Kapoor & Metzger, 2008).

Processed cheeses are good source of proteins, fat, minerals and vitamins in the diet (Buňka, Hrabě, & Kráčmar, 2004). Although various cheese types have been identified as a good source of bioactive peptides (Korhonen, 2009), the fortification of cheeses with bioactive components has increased in the recent years. Incorporation of dried materials, extracts and essential oils of medicinal herbs into cheeses resulted in improvement of nutritional value and sensory attributes and decreased the deterioration process of quality parameters in various cheeses (Mohamed & Shalaby, 2016; Mohamed, Shalaby, & Gafour, 2016; Mehanna, Hassan, El-Messery, & Mohamed, 2017; Santos, Shetty, Cecchini, & da Silva Maglioranza, 2012). Polyphenols are the main compounds of interest among plant-based materials and they are the principal antioxidants in human diet. There are a limited number of studies regarding the evaluation of the effect of individual phenolic compounds on the antioxidant capacity of cheeses (Faion et al., 2015; Han et al., 2011; Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2014; Stratulat et al., 2014). To the best of our knowledge, SPC or their analogues were scarcely used as the basis for the incorporation of bioactive substances, probably due to the high temperature of processing. Carrot paste (Mohamed et al., 2016) and apricot pulp (Mohamed & Shalaby, 2016) were used for the preparation of processed cheese analogues. In a very recent study, the preparation of functional processed cheese with addition of tomato juice was described (Mehanna et al., 2017). However, authors usually studied the nutritional characteristics of cheese samples in

* Corresponding author.
E-mail address: libor.cervenka@upce.cz (L. Červenka).

http://dx.doi.org/10.1016/j.lwt.2017.08.093
0023-6438/

relation to the different amount of bioactive material. To the best of our knowledge, there is no published data that describe the effect of processing conditions on the functional characteristic of processed cheese spreads.

Addition of bioactive compounds could affect not only the taste but also the consistency of processed cheese (Kapoor & Metzger, 2008). On the other hand, the processing parameters such as the agitation speed, melting temperature and holding time of the melting temperature significantly affect the consistency of processed cheese. The latter mentioned factors influence especially intensity of fat emulsification and water hydration processes and therefore the microstructure of processed cheese (Swenson, Wendorff, & Lindsay, 2000; Černíková et al., 2017).

Quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-chromen-4-one) and rutin (quercetin-3-rutinoside) are the dietary flavonoids presented in plants. Both flavonoids are well-known for their therapeutic potential in various diseases like cancer, coronary artery, asthma and diabetes (D'Andrea, 2015). Due to the health-promoting effects of quercetin and rutin, an increased interest about their utilization in food systems has arisen (Cho & Lee, 2015; Rodriguez-Mateos, Cifuentes-Gomez, George, & Spencer, 2014).

The aim of the present study was to observe the effect of processing conditions (temperature and time) on the content of quercetin and rutin, as well as other functional characteristics of processed cheese spreads.

2. Materials and methods

2.1. Materials

All the solvents for extraction, LC-MS analysis and chemicals for antioxidant assays were purchased from Sigma-Aldrich (Prague, Czech Republic).

2.2. Processed cheese manufacturing

The composition of the raw materials is presented in Table 1 and was designed to achieve final products with 37 g/100 g dry matter content and 50 g/100 g fat in dry matter content. The total concentration of emulsifying salts was 2.3 g/100 g (the amount was calculated on the total weight of the melt). Two additions of flavonoids were applied for improving of functional properties of SPC/rutin (contains rutin hydrate, $\geq$ 94% purity) and SPC/quercetin (contains quercetin hydrate powders, $\geq$ 95% purity) at 0.5 g/100 g. The amount of butter and water applied were adjusted due to the above mentioned additions in order to maintain constant values of dry matter and fat in dry matter contents respectively. Control samples (without rutin or quercetin) were also produced.

For the laboratory manufacture of the model processed cheese samples, an equipment Stephan UMC-5 (Stephan Machinery GmbH, Halmen, Germany) with indirect heating was used. Firstly, Eidam block cheese and butter were cut into small pieces (approx. 2 × 2 × 2 cm) and put into the kettle and minced for 30 s (1400 × g). Subsequently, water, the mixture of emulsifying salts and butter, rutin and/or quercetin were added into the blend. The total amount of a batch was approximately 659–676 g. The mixture was heated up at 80 °C and 90 °C at a constant agitation (1500 min^{-1}) and kept for 1, 5 and 10 min at these temperatures. Finally, samples were poured into 80 g polystyrene doses with sealable lids. The packed samples were cooled down and stored (6 ± 2) °C until the analyses were performed. The addition of quercetin or rutin to the finished SPC sample (control) was also performed in our laboratory in order to assess the extraction efficiency. An appropriate amount of quercetin or rutin (0.5 g/100 g) was added to 1.0 g of processed cheese sample. The mixtures were vigorously stirred using stainless steel spatula and left in refrigerator overnight.

2.3. The preparation of the extracts

A glass vial with plastic cap containing 1.0 g of SPC sample and 10.0 mL of extraction solvent was put into the ultrasound bath Sonorex TK52 (Bandelin Electronic, Berlin, Germany) for 30 min. According to PubChem database, XLogP3 (a liphophility index) and TPSA (a polarity index) 1.5/128 and −1.3/266 for quercetin and rutin, respectively, indicate that rutin is more hydrophilic. Therefore, methanol and aqueous methanol (1:1) were used as the extraction solvents for SPC with quercetin or rutin, respectively. A clear supernatant was obtained after centrifugation at 1400 × g for 10 min (Vintrum NF400, Nüve, Ankara, Turkey) followed by the filtration using syringe polytetrafluoroethylene membrane filter (pore diameter 0.45 μm, Labicom, Olomouc, Czech Republic). Two extracts were prepared for each trial.

2.4. HPLC analysis of rutin and quercetin

Rutin and quercetin were analyzed using Agilent 1100 Series (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, a degasser, an autosampler, a thermostatted column compartment, a UV and MS detector Agilent 1100 Series LC/MSD Trap SL. A Gemini 5 μm C18 (150 × 3.0 mm) column was used (Phenomenex®, Torrance, CA, USA). Mixture of deionized water acidified with formic acid to pH 3.05 (0.21%, v/v) (solution A) and acetonitrile (solution B) was used as mobile phase at gradient flow rate 0.7 mL/min (formic acid: acetonitrile from 900: 100 mL: mL to 500: 500 mL: mL for 0–15 min). The analysis was performed at 40 °C and peaks of rutin and quercetin were detected at 360 nm. Quantification was based on the separation of standard solutions of quercetin and rutin dissolved in methanol at concentrations from 1 to 100 μg/mL. Peak area (Y) plotted against the concentration (c) of rutin and quercetin gave the calibration equation $Y = 2.26 \times c + 4.72$

Table 1
Formulation of the processed cheese samples with and without added antioxidants manufactured at different melting temperature and holding times.

Raw material	Producer	Dry matter content (g/100 g)	Fat in dry matter content (g/100 g)	Control	With rutin	With quercetin
Edam cheese*	Kromilk PLC, Kroměříž, Czech Republic	50	30	300.0	300.0	300.0
Butter	Madeta PLC, České Budějovice, Czech Republic	84	98	94.0	98.0	98.0
Water	–	–	–	250.0	260.0	260.0
Emulsifying salts**	Fosfa PLC, Břeclav-Poštorná, Czech Republic	>95	–	15.4	15.4	15.4
Rutin	TCI Chemicals, Tokio, Japan	>95	–	–	3.3	–
Quercetin	Sigma-Aldrich, Prague, Czech Republic	>95	–	–	–	3.3

* Dutch-type semihard cheese, 8-week maturity.
** Composition of the mixture of emulsifying salts: monosodium dihydrogenphosphate (19% rel.; the ratio calculated on the total amount of emulsifying salts = 100%), disodium hydrogenphosphate (37% rel.), tetrasodium diphosphate (22% rel.) and sodium salt of polyphosphate (22% rel.).

($R^2 = 0.998$) and Y = 4.79 × c-2.88 ($R^2 = 0.999$), respectively. An ion trap mass spectrometry detector with an ESI source was used to confirm the presence of both flavonoids. ESI mass spectra were measured in the range of m/z 200–1000 in negative-ion mode. The concentration of both flavonoids was expressed in μg per g of sample. Retention of flavonoids was calculated according to the following equation:

$$R\,(\%) = (\text{flavonoid found (mg/g)/flavonoid added (mg/g)}) \times 100 \quad (1)$$

2.5. Determination of antioxidant activity of spreadable processed cheese

The total phenolic assay (TPC) was adopted from Santos et al. (2012). A reagent mixture containing extraction solvent instead of the sample extract served as the blank. The results were expressed as the amount of gallic acid per ml of extract.

The DPPH (2,2-diphenyl-1-picrylhydrazil) and ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging activity assays were adopted from the experimental procedure of Mišan et al. (2011). Both DPPH• and $ABTS^{\bullet+}$ scavenging activities *I* were calculated using the formula:

$$I(\%) = (1 - A_1/A_0) \times 100 \quad (2)$$

where A_0 is the absorbance of blank solution; A_1 is the absorbance of radicals with sample extract. DPPH• and $ABTS^{\bullet+}$ scavenging activities *I* were then plotted against various concentration of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were expressed as Trolox equivalent antioxidant capacity ($TEAC_{DPPH}$ and $TEAC_{ABTS}$) in μg Trolox/ml of the extract. Each extract was examined in duplicate for its antioxidant activity.

2.6. Rheological properties

Rheological properties of model processed cheese were measured according to Černíková et al. (2017). Briefly, a dynamic oscillatory shear rheometer (RheoStress 1, Haake, Bremen, Germany) at 20.0 ± 0.1 °C with a plate-plate geometry (diameter 35 mm, gap 1 mm) were used. The complex modulus (G*) at reference frequency 1 Hz were calculated based on values of storage (G') and loss (G") moduli:

$$G^* = \sqrt{(G)^2 + (G)^2} \quad (3)$$

With increasing values of the complex moduli (G*) of processed cheese, the consistency become more rigid and the gel strength rises (Černíková et al., 2017).

2.7. Statistical analysis

The results represented the average means with standard deviation (SD) of repeated measurements ($N = 4$). Nonparametric statistical methods were used in this study. Two-factor Kruskal-Wallis analysis of variance (ANOVA) was applied in order to determine the effect of melting temperature (factor A) and holding time (factor B) on the content of flavonoids and antioxidant properties. Multiple comparison procedure among means was performed using the Tukey's method. Spearman correlation coefficients (r) were calculated to describe the mutual associations between variables. All the statistical methods were done at the probability level of $P = 0.05$ (Statistica CZ, StatSoft CR s.r.o., Prague).

3. Results and discussion

3.1. The effect of processing conditions on the content of quercetin and rutin in processed cheese spreads

As can be seen from Fig. 1, both quercetin and rutin were successfully extracted after manufacturing of processed cheese spreads using pure and aqueous methanol (water: methanol, 1:1), respectively. When quercetin or rutin were mixed with the finished SPC sample (control), the extraction efficiency and subsequent LC determination of quercetin and rutin exhibited 96.0 ± 4.0 and 91.0 ± 5.0% retention, respectively.

The concentration of quercetin ranged from 4.17 ± 0.15 to 2.39 ± 0.02 mg/g in SPC/quercetin samples. Significantly higher content of quercetin was determined in SPC/quercetin samples manufactured at 80 °C for 1 min ($P < 0.01$), and the lowest content was obtained after thermal treatment at 90 °C for 10 min ($P < 0.01$). Significant decrease of quercetin content with the increase of holding time was observed at both melting temperatures. Extraction of SPC/rutin samples to aqueous methanol and subsequent determination of rutin by LC-MS method resulted in its considerably lower amount. Rutin levels ranged from 1.92 ± 0.04 to 1.90 ± 0.02 mg/g after manufacturing of processed cheese spreads at 80 °C. A lower level of rutin was observed when SPC samples were prepared at 90 °C showing significant differences within the holding times. Both quercetin and rutin were considered as thermally unstable compounds particularly in alkali conditions and in the presence of oxygen in previous studies (Barnes, Foss, & Schug, 2013; Buchner, Krumbein, Rohn, & Kroh, 2006). After LC analysis, only peaks corresponded to quercetin ($t_R = 11.33$ min) and rutin ($t_R = 7.31$ min) were detected at 360 nm under the given experimental conditions for all the SPC samples (Suppl. 1A, 1B). No interference peaks occurred when control SPC sample was processed. The LC/MS spectrum of quercetin peak showed two fragment ions at m/z 300.9 and 600.3, the first corresponded to quercetin molecule, the latter can indicate the presence of a dimmer (Suppl. 2). Quercetin dimmer was identified as a product of the oxidation of quercetin molecule (Pham, Bortolazzo, & White, 2012). Rutin peak gives only one fragment ion at m/z 609.1 (not shown).

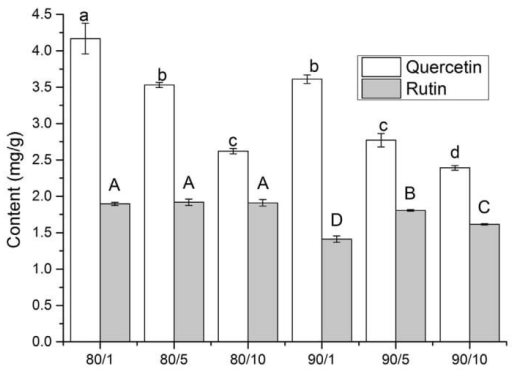

Fig. 1. The effect of processing temperature and time (80/1 means 80 °C for 1 min, 80/2 means 80 °C for 5 min, etc.) on the quercetin (white column) and rutin (grey column) levels extracted from processed cheese spread. Methanol and aqueous methanol (water: methanol, 1:1) were used for extraction of quercetin and rutin, respectively. Statistical differences in quercetin and rutin levels is indicated by different small and capital letters, respectively ($P < 0.05$). Average mean ± standard deviation ($N = 4$).

Thermal degradation of quercetin and rutin was extensively studied by Buchner, Krumbein, Rohn & Kroh (2006) and Barnes et al. (2013) using mass spectrometry techniques. In general, quercetin was more stable at acidic pH, and the degradation rate increased with the increase of pH and temperature. Barnes et al. (2013) identified degradation products of quercetin after heating its solution (pH 5.9) at 85 °C for >9.6 min. Their experimental conditions are close to those used in our experimental procedure. The pH of SPC samples have been measured in our study and ranged from 5.80 to 5.92. At the similar pH (5.0), rutin was found to be more stable than quercetin during heating of aqueous solutions at 100 °C for 300 min (Buchner, Krumbein, Rohn & Kroh, 2006). While the results of thermal degradation studies of quercetin and rutin in solution are consistent, contradictory conclusions have arisen from the experiments on foodstuffs. For instance, quercetin remained constant during the cooking of blueberry filling at 90 °C (Rodriguez-Mateos et al., 2014) whereas decrease after steaming of onion for > 10 min was reported (Harris, Brunton, Tiwari, & Cummins, 2015). In a study of Vallverdú-Queralt, Regueiro, de Alvarenga, Torrado, and Lamuela-Raventos (2014), quercetin decreased more abruptly during the cooking of tomato sauce than rutin. They explain the higher stability of rutin towards the oxidation by the presence of the sugar moiety in the 3-hydroxy-function at the C-ring, whereas in quercetin it remained unoccupied. Quercetin seemed to be more stable during manufacturing of processed cheese spread with clear pattern regarding the melting temperature and holding time (see Fig. 1). The higher stability of quercetin in our study is in agreement with the study of Vogrincic, Timoracka, Melichacova, Vollmannova, and Kreft (2010) who found quercetin more stable than rutin during the bread rising and baking process. The low retention of rutin after manufacturing of SPC sample can be explained by its interaction with L-amino acids, particularly with arginine or lysine via hydrophobic interactions. The stability of such a molecular complex has increased with the increase in temperature (Biçer & Özdemir, 2014). The complexation of rutin with protein was also described (Cui, Kong, Chen, Zhang, & Hua, 2014). Since processed cheese is rich source of protein and amino acids (Buňka et al., 2004), we may imply that such complexes were formed during manufacturing of processed cheese spreads and was not able to be extracted to aqueous methanol. In addition, rutin was found to be unstable in aqueous solutions when sonicated. This phenomenon is known as acoustic cavitation, and is attributed to the formation of highly reactive hydroxyl radicals during the passage of ultrasonic wave through the bubbles of water (Chua, 2014). The degradation rate of rutin by hydroxyl radicals was dependant on the temperature of extraction, liquid height, ultrasound intensity and pulse length. The sonication process during the extraction of rutin from SPC samples was found to be acceptable for our purposes since it gave high retention of rutin (91.0 ± 5.0%).

3.2. Antioxidant properties of processed cheese spread

Folin-Ciocalteaús assay was used to determine the total phenolic content (TPC) of SPC samples. Prior to analysis of SPC samples containing flavonoids, the control samples (without addition of quercetin and rutin) at each processing condition were screened for the TPC (Suppl. 3). As can be seen, TPC values ranged from 58.2 ± 0.5 to 65.7 ± 0.5 μg gallic acid/ml when extracted to methanol and from 27.2 ± 1.9 to 41.6 ± 1. μg gallic acid/ml, when extracted to aqueous methanol. FC assay was primarily designed for the determination of amino acid tyrosine containing phenol group (Apak, Özyörek, Güçlü, & Çapanoğlu, 2016). The amount of this amino acid in processed cheese spread was estimated in the range from 10.8 to 5.9 g/kg in the study of Buňka, Kříž, Veličková, Buňková, and Kráčmar (2009), therefore we may assume that it may also react with the FC reagent in our study. Antioxidant properties were measured in terms of DPPH and ABTS assays, however only latter gave positive results with the extracts of control SPC samples (Suppl. 3). The antioxidant properties of cheese was previously attributed to the content of bioactive peptides (Meira et al., 2012) and free amino acids, mainly tyrosine, methionine and tryptophan (Bottesini et al., 2013). The corresponded $TEAC_{ABTS}$ values for control SPC samples were in the range from 68.4 ± 3.0 to 78.0 ± 4.7 μg Trolox/ml of methanol extract and from 47.0 ± 0.4 to 76.4 ± 4.2 μg Trolox/ml when extracted to aqueous methanol. In order to evaluate the effect of quercetin or rutin content on the antioxidant activity of SPC samples, the results of TPC and ABTS assays were corrected for corresponded values obtained in control SPC samples.

The TPC values for the extract of SPC/quercetin were determined in the range from 251.7 ± 0.5 to 263.2 ± 0.5 μg gallic acid/ml at 80 °C and from 285.8 ± 0.2 to 318.6 ± 1.4 μg gallic acid/ml at 90 °C (Table 2). Antioxidant properties of SPC/quercetin extract measured in terms of DPPH assay showed increasing values of $TEAC_{DPPH}$ from 157.4 ± 19.7 to 263.5 ± 19.7 μg Trolox/ml at 80 °C with the increase in time. On the other hand, a decrease of $TEAC_{DPPH}$ values from 329.9 ± 30.7 to 216.0 ± 15.8 μg Trolox/ml with the increase of processing time at 90 °C was examined. Concerning the results of $ABTS^{\bullet+}$ assay, slight increase in $TEAC_{ABTS}$ values was observed with the increase of processing time from 5.0 to 10.0 min at each temperature.

TPC values for SPC/rutin extracted to aqueous methanol were shown in Table 2. The decrease from 88.2 ± 1.4 to 54.7 ± 2.9 μg gallic acid/ml with the increase of time was observed in samples manufactured at 80 °C and the increase has occurred when the processing time increased from 1.0 to 5.0 min at 90 °C (from 47.3 ± 1.9 to 70.6 ± 0.5 μg gallic acid/ml).

3.3. The results of Kruskal-Wallis ANOVA and correlation analysis

In order to determine the effect of melting temperature and holding time, Kruskal-Wallis ANOVA procedure was applied to all the variables. It is evident that both quercetin and rutin content decreased with the increase in temperature and time (Table 3). The degradation of quercetin was significantly enhanced by the increasing of processing time ($P < 0.01$) than by the temperature. On the contrary, temperature caused significant loss of rutin ($P < 0.01$) than the increasing time. For SPC/rutin sample extracts, both processing temperature and time did not significantly change TPC and antioxidant capacities. Processing temperature significantly affected the TPC ($P < 0.01$) and $TEAC_{ABTS}$ ($P < 0.05$) values in SPC/quercetin samples, however increasing trend in TPC and decreasing trend in $TEAC_{ABTS}$ values were obtained with the elevated melting temperature. The processing time was not significant factor. The Pearson's correlation coefficients as shown in Table 4 were performed to elucidate the trend of association between quercetin and rutin contents, TPC and antioxidant capacities. Quercetin content was weakly negative correlated with total phenolic content and antioxidant capacity (DPPH and ABTS). Weak positive correlation was observed between TPC and $TEAC_{DPPH}$, whereas negatively correlated with $TEAC_{ABTS}$ ($p = -0.813$; $P < 0.01$). The increase of total phenolic content associated with the decrease of antioxidant properties measured by $ABTS^{\bullet+}$ assay was explained by the hindrance of steric accessibility of phenolic groups to the $ABTS^{\bullet+}$ site, particularly in heterocyclic polymeric polyphenols (Apak et al., 2016). Similar results were obtained for medicinal plant extracts of *Saraca asoca* (Ghatak et al., 2015) and *Centella asiatica* (Chew et al., 2011). In addition, Buchner, Krumbein, Rohn & Kroh (2006) reported the increase of antioxidant activity even after the decrease of quercetin content in solution during

Table 2
The effect of melting temperature and holding time on the antioxidant properties of processed cheese spread with 0.5 g/100 g of quercetin or rutin.

	80 °C			90 °C		
	1 min	5 min	10 min	1 min	5 min	10 min
Extracted to methanol						
TPC	^{a}251.7 ± 0.5	^{b}263.2 ± 0.5	^{a}255.1 ± 1.4	^{e}318.6 ± 1.4	^{d}305.4 ± 1.0	^{c}285.8 ± 0.2
$TEAC_{DPPH}$	^{a}157.4 ± 19.7	^{bd}241.2 ± 4.0	^{bef}263.5 ± 19.7	^{cf}329.9 ± 30.7	^{ade}227.6 ± 1.0	^{ade}216.0 ± 15.8
$TEAC_{ABTS}$	^{b}1352.4 ± 23.1	^{b}1346.1 ± 3.4	^{c}1471.9 ± 58.5	^{a}1186.7 ± 57.3	^{a}1226.3 ± 14.3	^{b}1340.5 ± 29.2
Extracted to aqueous methanol (1:1)						
TPC	^{f}88.2 ± 1.4	^{d}76.4 ± 0.5	^{b}54.7 ± 2.9	^{a}47.3 ± 1.9	^{de}70.6 ± 0.5	^{ce}69.9 ± 0.5
$TEAC_{DPPH}$	^{d}106.2 ± 1.1	^{c}92.5 ± 5.0	^{cd}97.6 ± 3.3	^{b}74.7 ± 1.0	^{cd}99.8 ± 2.0	^{a}56.8 ± 3.0
$TEAC_{ABTS}$	^{a}756.1 ± 148.0	^{a}808.0 ± 139.0	^{a}780.0 ± 4.3	^{a}751.4 ± 9.2	^{a}854.5 ± 7.8	^{a}802.6 ± 18.0

Average mean ± standard deviation (N = 4); TPC, total phenolic content (μg gallic acid/ml); $TEAC_{DPPH}$, Trolox equivalent antioxidant capacity using 2,2-diphenyl-1-picrylhydrazil assay (μg Trolox/ml); $TEAC_{ABTS}$, Trolox equivalent antioxidant capacity using 2,2′- azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay (μg Trolox/ml); significant difference between means in row is indicated by different small letters in superscript ($P < 0.05$).

Table 3
The results of Kruskal-Wallis ANOVA on the effect of melting temperature (T) and holding time (t) on the quercetin and rutin contents, total phenolic content (TPC) and antioxidant capacity of processed cheese samples.

Parameter	T	t
Quercetin	n.s.	$\downarrow^{**}$
TPC	$\uparrow^{**}$	n.s.
$TEAC_{DPPH}$	n.s.	n.s.
$TEAC_{ABTS}$	$\downarrow^{*}$	n.s.
Rutin	$\downarrow^{**}$	n.s.
TPC	n.s.	n.s.
$TEAC_{DPPH}$	n.s.	n.s.
$TEAC_{ABTS}$	n.s.	n.s.

↑, increasing trend; ↓, decreasing trend; $^{*}P < 0.05$; $^{**}P < 0.01$; n.s., not significant ($P > 0.05$); TPC, total phenolic content (μg gallic acid/ml); $TEAC_{DPPH}$, Trolox equivalent antioxidant capacity using 2,2-diphenyl-1-picrylhydrazil assay (μg Trolox/ml); $TEAC_{ABTS}$, Trolox equivalent antioxidant capacity using 2,2′- azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay (μg Trolox/ml).

Table 4
Pearson's correlation coefficient between the content of quercetin, rutin, total phenolic content (TPC) and antioxidant capacities in processed cheese samples.

	TPC	$TEAC_{DPPH}$	$TEAC_{ABTS}$
Quercetin	−0.158	−0.030	−0.223
TPC		0.484	-0.813^{**}
$TEAC_{DPPH}$			−0.147
Rutin	0.807^{**}	0.747^{**}	0.123
TPC		0.622^{*}	0.266
$TEAC_{DPPH}$			0.014

$^{*}P < 0.05$; $^{**}P < 0.01$; TPC, total phenolic content (μg gallic acid/ml); $TEAC_{DPPH}$, Trolox equivalent antioxidant capacity using 2,2-diphenyl-1-picrylhydrazil assay (μg Trolox/ml); $TEAC_{ABTS}$, Trolox equivalent antioxidant capacity using 2,2′- azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay (μg Trolox/ml).

thermal treatment. They proved the formation of new substances (degradation products) with higher antioxidant activity. Based on our results and literature cited, we may hypothesize that new compounds were formed during the manufacturing of SPC/quercetin samples, which possessed antioxidant activity but were not detectable under our experimental conditions. The effect of processing time and temperature on both $TEAC_{DPPH}$ and $TEAC_{ABTS}$ values of SPC/rutin sample extracts was not confirmed by Kruskal-Wallis ANOVA (Table 3). Nevertheless, rutin content in SPC/rutin samples positively correlated with the TPC values ($r = 0.807$; $P < 0.01$) and $TEAC_{DPPH}$ values ($r = 0.747$; $P < 0.01$). TPC showed strong positive correlation with $TEAC_{DPPH}$ ($r = 0.622$; $P < 0.05$).These findings indicate that antioxidant properties of processed cheese spread was influenced by the presence of rutin molecule.

3.4. The results of rheological properties

The results of the complex modulus (G^*, the meaning was explained in part 2.6) of model processed cheeses manufactured under different agitation and melting temperature were displayed in Table 5. The values of G^* significantly increased ($P < 0.05$) with the increase of holding time. Elevated temperature of melting also caused the increase of G^* ($P < 0.05$). The higher levels of observed processing parameters led to development of denser net structure and therefore the model processed cheese became more rigid (Černíková et al., 2017).The addition of rutin or quercetin influenced the consistency of model processed cheeses ($P < 0.05$; Table 5). It could be hypothesed that the latter mentioned antioxidants could disrupt slightly the protein network. The effect of both of added substances on rheological properties of samples were practically similar ($P \geq 0.05$; Table 5).

Table 5
Values of the complex modulus at the reference frequency of 1 Hz (G^*; kPa) of the model processed cheese with and without added antioxidants manufactured at different melting temperature and holding times.

Samples with	Melting temperature (°C)	Holding time (min)		
		1	5	10
Control	80	3443 ± 138 ^{a}A	6748 ± 412 ^{a}B	9168 ± 494 ^{a}C
	90	4401 ± 190 ^{a}A	8499 ± 541 ^{a}B	12038 ± 601 ^{a}C
Rutin	80	2829 ± 126 ^{b}A	6481 ± 358 ^{a}B	8338 ± 330 ^{b}C
	90	3945 ± 216 ^{b}A	9014 ± 368 ^{a,b}B	11124 ± 536 ^{b}C
Quercetin	80	2814 ± 145 ^{b}A	6482 ± 358 ^{a}B	9288 ± 361 ^{a}C
	90	3677 ± 177 ^{b}A	9404 ± 521 ^{b}B	11055 ± 585 ^{b}C

The values were expressed as mean ± standard deviation (N = 4); significant difference between means in column is indicated by different superscript letters ($P < 0.05$); the means within a row followed by capital letters differ ($P < 0.05$).

4. Conclusions

Processed cheese spreads were not frequently used for the development of functional food probably due to the adverse conditions during manufacturing process. This paper describes the effect of melting temperature and holding time on the content of rutin and quercetin, and on the antioxidant properties of processed cheese spread. The results showed that both flavonoids decreased during the cheese processing. While quercetin content decreased with the increase of holding time, rutin degradation was pronounced at elevated processing temperature. Rutin content affected the antioxidant capacity of processed cheese samples showing strong positive correlation with total phenolic content and DPPH scavenging activity whereas quercetin content did not exhibit apparent association towards antioxidant capacity. Both rutin and quercetin significantly decreased the gel strength of the samples. We used chemical substances for the preparation of functionalized processed cheese spread in order to facilitate the experimental design and for subsequent interpretation of results. For practical purposes, the addition of plant extracts rich in quercetin/rutin or other polyphenolic substances should be further examined. Processed cheese spread fortified with rutin or quercetin has a potential to be a functional food and contribute to health when it is consumed.

Conflict of interest

None.

Acknowledgements

Financial support from Faculty of Chemical Technology, University of Pardubice (no. SGS_2017_001) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2017.08.093.

References

Apak, R., Özyörek, M., Güçlü, K., & Çapanoğlu, E. (2016). Antioxidant activity/capacity measurement. 2. Hydrogen atom transfer (HAT)-based, mixed-mode (electron transfer (ET)/HAT), and lipid peroxidation assays. *Journal of Agricultural and Food Chemistry, 64*, 1028–1045.

Barnes, J. S., Foss, F. W., & Schug, K. A. (2013). Thermally accelerated oxidative degradation of quercetin using continuous flow kinetic electrospray-ion trap-time of flight mass spectrometry. *Journal of the American Society for Mass Spectrometry, 24*, 1513–1522.

Biçer, E., & Özdemir, S. (2014). Interaction thermodynamics of rutin (vitamin P) with basic *L*-amino acids at physiological pH: The effect of temperature on the affinity order. *Russian Journal of Electrochemistry, 50*, 1058–1064.

Bottesini, C., Paolella, S., Lambertini, F., Galaverna, G., Tedeschi, T., Dossena, A., et al. (2013). Antioxidant capacity of water soluble extracts from Parmigiano-Reggiano cheese. *International Journal of Food Sciences and Nutrition, 64*, 953–958.

Buchner, N., Krumbein, A., Rohn, S., & Kroh, L. W. (2006). Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Communications in Mass Spectrometry, 20*(2006), 3229–3235.

Buňka, F., Hrabě, J., & Kráčmar, S. (2004). The effect of sterilisation on amino acid contents in processed cheese. *International Dairy Journal, 14*, 829–831.

Buňka, F., Kříž, O., Veličková, A., Buňková, L., & Kráčmar, S. (2009). Effect of acid hydrolysis time on amino acid determination in casein and processed cheese with different fat content. *Journal of Food Composition and Analysis, 22*, 224–232.

Černíková, M., Salek, R. N., Kozáčková, D., Běhalová, H., Luňáková, L., & Buňka, F. (2017). The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads. *International Dairy Journal, 66*, 84–90.

Chew, K. K., Ng, S. Y., Thoo, Y. Y., Khoo, M. Z., Wan Aida, W. M., & Ho, C. W. (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella asiatica* extracts. *Journal of Food Research International, 18*, 571–578.

Cho, Y. J., & Lee, S. (2015). Evaluation of rutin from Tartary buckwheat milling fractions and evaluation of its thermal stability in an instant fried noodle system. *Food Chemistry, 176*, 40–44.

Chua, L. S. (2014). A review on plant-based rutin extraction methods and its pharmacological activities. *Journal of Ethnopharmacology, 150*, 805–817.

Cui, Z., Kong, X., Chen, Y., Zhang, C., & Hua, Y. (2014). Effect of rutin incorporation on the physical and oxidative stability of soy protein-stabilized emulsions. *Food Hydrocolloids, 41*, 1–9.

D'Andrea, G. (2015). Quercetin: A flavonol with multifaceted therapeutic applications? *Fitoterapia, 106*, 256–271.

Faion, A. M., Beal, P., Ril, F. T., Cichoski, A. J., Cansian, R. L., Valduga, A. T., et al. (2015). Influence of the addition of natural antioxidant from mate leaves (*Ilex paraguariensis* St. Hill) on the chemical, microbiological and sensory characteristics of different formulations of Prato cheese. *Journal of Food Science and Technology, 52*, 1516–1524.

Ghatak, A., Nair, S., Vajpayee, A., Chaturvedi, P., Samant, S., Soley, K., et al. (2015). Evaluation of antioxidant activity, total phenolic content, total flavonoids, and LC-MS characterization of *Saraca asoca* (Roxb.) De. Wilde. *International Journal of Advanced Research, 3*, 318–327.

Han, J., Britten, M., St-Gelais, D., Champagne, C. P., Fustier, P., Salmieri, S., et al. (2011). Polyphenolic compounds as functional ingredients in cheese. *Food Chemistry, 124*, 1589–1594.

Harris, S., Brunton, N., Tiwari, U., & Cummins, E. (2015). Human exposure modelling of quercetin in onions (*Allium cepa* L.) following thermal processing. *Food Chemistry, 187*, 135–139.

Kapoor, R., & Metzger, L. E. (2008). Process cheese: Scientific and technological aspects – a review. *Comprehensive Reviews in Food Science and Food Safety, 7*, 194–214.

Korhonen, H. (2009). Milk-derived bioactive peptides: From science to applications. *Journal of Functional Foods, 1*, 177–187.

Mehanna, N. S., Hassan, F. A. M., El-Messery, T. M., & Mohamed, A. G. (2017). Production of functional processed cheese by using tomato juice. *International Journal of Dairy Science, 12*, 155–160.

Meira, S. M. M., Daroit, D. J., Helfer, V. E., Corrêa, A. P. F., Segalin, J., Carro, S., et al. (2012). Bioactive peptides in water-soluble extracts of ovine cheese from Southern Brazil and Uruguay. *Food Research International, 48*, 322–329.

Mišan, A., Mimica-Dukić, N., Sakač, M., Mandić, A., Sedej, I., Šimurina, O., et al. (2011). Antioxidant activity of medicinal plant extracts in cookies. *Journal of Food Science, 76*, 1239–1244.

Mohamed, A. G., & Shalaby, S. M. (2016). Texture, chemical properties and sensory evaluation of a spreadable processed cheese analogue made with apricot pulp (*Prunus armeniaca* L.). *International Journal of Dairy Science, 11*, 61–68.

Mohamed, A. G., Shalaby, S. M., & Gafour, W. A. (2016). Quality characteristics and acceptability of an analogue processed spreadable cheese made with carrot paste (*Daucus carota* L.). *International Journal of Dairy Science, 11*, 91–99.

Pham, A., Bortolazzo, A., & White, J. B. (2012). Rapid dimerization of quercetin through the oxidative mechanism in the presence of serum albumin decrease its ability to induce cytotoxicity in MDA-MB-231 cells. *Biochemical and Biophysical Research Communications, 427*, 415–420.

Rashidinejad, A., Birch, E. J., Sun-Waterhouse, D., & Everett, D. W. (2014). Delivery of green tea catechin and epogallocatechin gallate in liposomes incorporated into low-fat hard cheese. *Food Chemistry, 156*, 176–183.

Rodriguez-Mateos, A., Cifuentes-Gomez, T., George, T. W., & Spencer, J. P. E. (2014). Impact of cooking, proving, and baking on the (poly)phenol content of wild blueberry. *Journal of Agricultural and Food Chemistry, 62*, 3979–3986.

Santos, R. D., Shetty, K., Cecchini, A. L., & da Silva Maglioranza, L. H. (2012). Phenolic compounds and total antioxidant activity determination in rosemary and oregano extracts and its use in cheese spread. *Semina, 33*, 655–666.

Stratulat, I., Britten, M., Salmieri, S., Fustier, P., St-Gelais, D., Champagne, C. P., et al. (2014). Enrichment of cheese with bioactive lipophilic compounds. *Journal of Functional Foods, 6*, 48–59.

Swenson, B. J., Wendorff, W. L., & Lindsay, R. C. (2000). Effects of ingredients on the functionality of fat-free process cheese spreads. *Journal of Food Science, 65*, 822–825.

Vallverdú-Queralt, A., Regueiro, J., de Alvarenga, J. F. R., Torrado, X., & Lamuela-Raventos, R. M. (2014). Home cooking and phenolics: Effect of thermal treatment and addition of extra virgin olive oil on the phenolic profile of tomato sauces. *Journal of Agricultural and Food Chemistry, 62*, 3314–3320.

Vogrincic, M., Timoracka, M., Melichacova, S., Vollmannova, A., & Kreft, I. (2010). Degradation of rutin and polyphenols during the preparation of Tartary buckwheat bread. *Journal of Agricultural and Food Chemistry, 58*, 4883–4887.

Research paper 10

B4

Černíková, M., Salek, R. N., Kozáčková, D., Běhalová, H., Luňáková, L., Buňka, F.

The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads.

International Dairy Journal. 2017, 66, 84-90. ISSN: 09586946.

International Dairy Journal 66 (2017) 84–90

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads

Michaela Černíková [a], Richardos Nikolaos Salek [a], Dana Kozáčková [a], Hana Běhalová [b], Ludmila Luňáková [b], František Buňka [a, *]

[a] Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, T.G. Masaryka 5555, 760 01, Zlín, Czech Republic
[b] Department of Plant Origin Foodstuffs Hygiene and Behaviour, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42, Brno, Czech Republic

ARTICLE INFO

Article history:
Received 22 August 2016
Received in revised form
10 November 2016
Accepted 11 November 2016
Available online 20 November 2016

ABSTRACT

The effect of different agitation speeds, various holding times of the melt and the storage period on the consistency of model processed cheese spreads with 35% (w/w) dry matter content and 40% (w/w) fat in dry matter content was examined. Viscoelastic properties of the samples, especially the storage (G′) and loss (G″) moduli within the frequency of 0.1–100.0 Hz were measured. The complex modulus (G*) was calculated and Winter's critical gel theory was implemented to determine the values of the gel strength (A_F) and the interaction factor (z). Within the first three minutes of the holding time, a continuous decrease in firmness of the samples was observed. Subsequently, a steady increase in firmness of the samples was measured from the third to the twentieth minute of holding time regardless of the speed of agitation tested. All of the processed cheeses showed an increase in firmness over 60 days storage.

1. Introduction

The basic raw materials for the production of traditional processed cheeses (PC) are natural cheeses of Dutch- and Swiss-type; in Anglo-American countries it is usually Cheddar. In areas of the Near and Middle East "white cheeses" are also widely used. Other dairy raw materials (e.g., butter, cream, anhydrous milk fat, curd, milk powder, whey powder) and non-dairy raw materials (e.g., water, flavourings, hydrocolloids) as well as emulsifying salts (especially sodium salts of phosphates, polyphosphates and citrates) are added to this cheese base of different maturity stages. Ingredients are usually heated under constant stirring at a temperature of 90–110 °C until a homogenous mass of desired properties is formed (Kapoor & Metzger, 2008; Khetra, Chavhan, Kanawjia, & Puri, 2015; Muslow, Jaros, & Rohm, 2007).

Consistency is one of the most important properties of PC and can be affected by three groups of factors: (i) the composition of the raw material mixture, (ii) processing parameters during the production of PC (especially the speed of agitation, holding time, the temperature reached during the melting process and the rate of cooling), and (iii) the storage temperature and period (Bayarri, Carbonell, & Costell, 2012; Dimitreli & Thomareis, 2004; Kapoor & Metzger, 2008; Khetra et al., 2015; Muslow et al., 2007; Schatz, Hoffmann, Schrader, & Maurer, 2014; Subramanian, Muthukumarappan, & Gunasekaran, 2006).

The effect of the composition of the raw material mixture on the rheological properties of PC has recently been studied very intensively, e.g., (i) the effect of moisture/dry matter content, fat content and protein content (Chatziantoniou, Thomareis, & Kontominas, 2015; Guinee & O'Callaghan, 2013; Lee, Klostermeyer, & Anema, 2015), (ii) different composition of emulsifying salts and their concentrations (Buňka et al., 2013; Hoffmann, Gärtner, Lück, Johannsen, & Maurer, 2012; Salek et al., 2015), (iii) maturity stages of the basic raw material (Brickley, Auty, Piraino, & McSweeney, 2007; Buňka et al., 2013; Salek et al., 2015), (iv) the hydrocolloids used and their concentrations (Ciprysová, Buňka, Pavlínek, Hudečková, & Janiš, 2013; Černíková et al., 2008), and (v) the concentrations of calcium and phosphate ions (Biswas, Muthukumarappan, Marella, & Metzger, 2015).

However, in only a small number of studies have been devoted to the effects of processing parameters during the production of

* Corresponding author. Tel.: +420 576 033 11.
E-mail address: bunka@ft.utb.cz (F. Buňka).

http://dx.doi.org/10.1016/j.idairyj.2016.11.007
0958-6946/

processed cheeses. Moreover, the results were often contradictory. The reason for the discrepancies in the results may lie in the fact that the individual studies made use of different kinds of processed cheeses (blocks and/or spreads, cheese imitations, etc.), different raw materials and different composition of the ingredients (different dry matter/fat content, etc.).

Swenson, Wendorff, and Lindsay (2000) studied the effect of the holding time (0–20 min) of the melt at 75 °C and constant agitation speed (the exact value of rpm was not presented) on the final consistency of fat-free processed cheese spread. The authors found that by extending holding time, firmness of the processed cheeses was decreased significantly. Bowland and Foegeding (1999) observed the effect of processing time (10, 20 and 30 min) of the raw material at 80 °C and constant agitation speed on the consistency of processed cheese blocks; these authors noted that the strength of the gel in the final product increased as the processing time of the melt extends, unlike the results of Swenson et al. (2000). Sutheerawattananonda, Fulcher, Martin, and Bastian (1997), who studied the effect of the holding time (0–15 min) of the melt at 65.5 °C on the distribution of fat globules in processed cheese blocks, observed that within the first 5 min of the holding time the fat globules decreased in size. However, a further extension of the holding time did not have a significant effect on the size of the fat globules. Lee, Buwalda, Euston, Foegeding, and McKenna (2003) described the changes in viscosity of the hot melt (~80 °C) dosed into a rotational viscometer within a 50 min measurement. The composition of the raw materials for the preparation of the melt corresponded to processed cheese blocks. Within the first 25 min, an increase in viscosity of the hot melt was observed. After reaching the local maximum in viscosity (~25 min), the viscosity of the hot melt started to decrease gradually. However, the consistency of the final product (cold processed cheese) was not studied.

Noronha, O'Riordan, and O'Sullivan (2008) focused on the effect of different agitation speeds (100–1500 rpm) at a constant holding time (~2 min) at 80 °C on the consistency of processed cheese block imitations. Their results showed that with the increasing speed of agitation, the firmness of the final product, its cohesiveness and the storage modulus G′ increased while the size of the fat globules decreased.

It follows from the above-mentioned list of studies published in this area that a study focused on the effect of different agitation speeds and various holding times on the consistency of processed cheese spreads has not yet been published. Therefore, the purpose of this study was to examine the effect of various holding times (0–20 min) at a temperature of 90 °C, different agitation speeds (1000, 1500 and 3000 rpm) and the time of storage (up to 60 d) on the consistency of model processed cheese spreads with 35% (w/w) dry matter content and 40% (w/w) fat in dry matter content. The dependencies detected can be used to adjust the processing parameters in practical production in the processed cheese industry as the whole study was performed under the same conditions (the same raw material, the same production equipment and the same parameters for measuring the individual indicators of consistency) and thus the results are comparable.

2. Material and methods

2.1. Preparation of the samples

For the manufacture of the model PC samples with 35% (w/w) dry matter content and 40% (w/w) fat in dry matter content, the following materials were used: (i) Dutch-type cheese (50% (w/w), dry matter content; 30% (w/w), fat in dry matter content: Kromilk, a.s., Kroměříž, Czech Republic), butter (84%, w/w, dry matter content; 82%, w/w, fat content), water and emulsifying salts (the total concentration was 2.7%, w/w, of the total weight of the melt; the composition of the emulsifying salt mixture was: 18% NaH_2PO_4; 39% Na_2HPO_4; 21% $Na_4P_2O_7$, 22% sodium salt of polyphosphate; total weight, 100%).

A Stephan UMC-5 (Stephan Machinery GmbH, Halmen, Germany) with indirect heating was employed for the manufacture of the PC samples. The target temperature of 90 °C (the time for achieving the target temperature varied between 10 and 11 min) was held for 0, 1, 3, 5, 7, 9, 11, 13, 15, 17 and 20 min (11 holding times) and the agitation was set at 1000, 1500 and 3000 rpm (3 agitation speeds). The hot melt was poured into polypropylene containers of cylindrical shape (52 mm in diameter and 50 mm high). The PC samples were cooled and stored at 6 ± 2 °C until analyses were performed. Each PC was produced three times − 99 lots in total were made (11 holding times × 3 agitation speeds × 3 repetitions). The analyses were initially performed 24 h after manufacturing (1st day), then on the 14th, 30th and 60th days of storage.

2.2. Basic chemical analysis of the PC samples

The dry matter content and the fat content of the PC samples were determined according to ISO 5534 (ISO, 2005) and ISO 1735 (ISO, 2004), respectively. The pH values were measured at ambient temperature using a glass tip electrode of a pH-meter (pH Spear, Eutech Instruments, Oakton, Malaysia) by direct insertion of the spear into the PC samples at three randomly selected spots (in each packaging).

2.3. Rheological analysis

Rheological analysis of the PC samples was performed using a dynamic oscillatory shear rheometer (RheoStress 1, Haake, Bremen, Germany) at 20.0 ± 0.1 °C. Additionally, to describe the changes in the viscoelastic properties of the solidified melt mass, a plate–plate geometry (diameter 35 mm) was selected in dependence with frequency (in the range 0.1–100.0 Hz) at 20.0 ± 0.1 °C. The selected monitored parameters included the storage (G′) and loss (G″) moduli (determined as a function of frequency). The complex modulus (G*) was calculated using the formula:

$$G^* = \sqrt{(G')^2 + (G'')^2} \quad (1)$$

Winter's critical gel theory was implemented to evaluate the changes in the viscoelastic properties of the PC samples. According to the following Eq. (2) the complex modulus (Gabriele, de Cindio, & D'Antona, 2001; Sádlíková et al., 2010; Winter & Chambon, 1986) can be expressed as:

$$G^*(\omega) = A_F \cdot \omega^{\frac{1}{z}} \quad (2)$$

where A_F is the strength of the gel ($Pa \cdot s^{1/z}$) and z is the interaction factor (defined as the number of structure units interacting with one another in a three-dimensional network; unitless). The higher the interaction factor, the more interactions occur in the matrix of the sample (Gabriele et al., 2001; Sádlíková et al., 2010).

2.4. Statistical analysis

Kruskal–Wallis and Wilcoxon tests were used to evaluate the results obtained (the significance level was 0.05). For the

estimation of A_F and z, a non-linear regression analysis (the Marquardt–Levenburg method; $A_F > 0$ and $z \geq 0$) was applied. Unistat® 6.5 software (Unistat, London, UK) was used for the statistical analysis.

3. Results and discussion

3.1. Results of basic chemical analysis

Dry matter and fat content, which were determined in all the model processed cheeses, ranged within the interval of 36.05–36.48% and 14.62–14.91% (w/w; $P \geq 0.05$; data not shown), respectively, during a 60 d storage period. Similar values of dry matter and fat contents are crucial to maintaining constant conditions and objective monitoring of the factors studied (Chatziantoniou et al., 2015; Dimitreli & Thomareis, 2004; Lee et al., 2015; Muslow et al., 2007). Also, the pH values of the model samples were measured. On the first day of storage, the pH values ranged within the interval of 5.92–6.01 ($P \geq 0.05$; data not shown), which typically corresponds to the level of this parameter in processed cheese spreads (Kapoor & Metzger, 2008; Muslow et al., 2007; Salek et al., 2015). Within the 60 d storage period, the pH value slightly decreased ($P < 0.05$) by 0.1–0.2 (data not shown). This phenomenon could be explained by possible hydrolysis of diphosphates and polyphosphates during the storage period as well as by possible changes in dissociation of the salts or other compounds present (Awad, Abdel-Hamid, & El-Shabrawy, 2002; Dimitreli & Thomareis, 2009; Weiserová et al., 2011).

3.2. The effect of holding time on consistency of samples

Figs. 1A–F and 2A–E show the dependence of the storage (G′) and loss (G″) moduli of the model processed cheeses (manufactured using the agitation speed of 1000–3000 rpm with the holding time of 0–20 min and analysed after 1 d of storage at 6 ± 2 °C) on frequency (f). On the basis of the values of G′ and G″, the values of the complex modulus (G*; see Eq. (1)) for the frequency of 1 Hz (Piska & Štětina, 2004) were also calculated (shown in Table 1). Tables 2 and 3 show the results of the calculated (see Eq. (2)) gel strength (A_F) and interaction factor (z) of the model processed cheeses as quantities describing the development of the dependence of the complex modulus values (G*) on the range of the frequencies measured (f; 0.1–100.0 Hz). According to Gabriel et al. (2001), Piska and Štětina (2004), and Winter and Chambon (1986) the increase in the values of G′, G″, G* and A_F is related to the rising gel strength of the model samples of semi-solid materials including foodstuffs.

The curves of the storage and loss moduli (G′ and G″) of the processed cheeses made with the holding time of 1 min after 1 d of storage at 6 °C (within the range of the oscillation frequencies measured, i.e., 0.1–100.0 Hz) were lower ($P < 0.05$) in comparison with the curves of the model samples that were packaged and chilled immediately after reaching the melting temperature (90 °C) (holding time of 0 min). This trend was observed at all the agitation speeds tested (1000, 1500 and 3000 rpm; see Figs. 1 and 2). A reduction in G′ and G″ of the processed cheeses was accompanied with a decrease in G* for the reference frequency of 1 Hz (Table 1; the samples evaluated after 1 day of storage at 6 °C). The development of the above-mentioned parameters as a result of including the holding time of the melt of 1 min at 90 °C indicates weakening and further disintegration of the protein network of the model processed cheeses, which is supported by the detected decrease ($P < 0.05$) in the gel strength (A_F) and interaction factor (z) illustrated in Tables 2 and 3. When extending the holding time of the melt (at 90 °C) to 3 min (still evaluating the samples after 1 d storage at 6 °C) there was another slight weakening of the protein matrix of the model samples (evaluated according to the decrease in G′, G″ and G*; $P < 0.05$; Figs. 1 and 2, Table 1) only at 3000 rpm. This conclusion can also be supported by a decrease in the gel strength of the model products (A_F; $P < 0.05$; Table 2). When comparing the processed cheeses manufactured with the holding time of the melting temperature (90 °C) of 1 and 3 min, there was not a significant difference between the agitation speeds of 1000 and 1500 ($P \geq 0.05$).

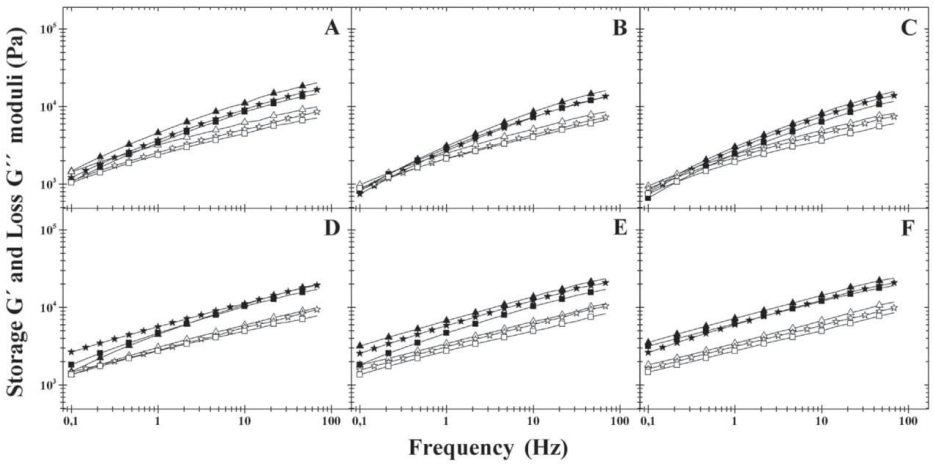

Fig. 1. Dependence of the storage (G′; Pa, closed symbols) and loss (G″; Pa, open symbols) moduli of the model processed cheeses manufactured using a speed of 1000 (▲,△), 1500 (★,☆) and 3000 (■,□) revolutions per minute (rpm) at different times at temperature in the Stephan kettle on frequency (f; in range of 0.1–100.0 Hz). Panels A, B, C, D, E and F are, respectively, maintaining for 0, 1, 3, 5, 7, and 9 min. The processed cheeses were measured 24 h after manufacturing at 6 ± 2 °C.

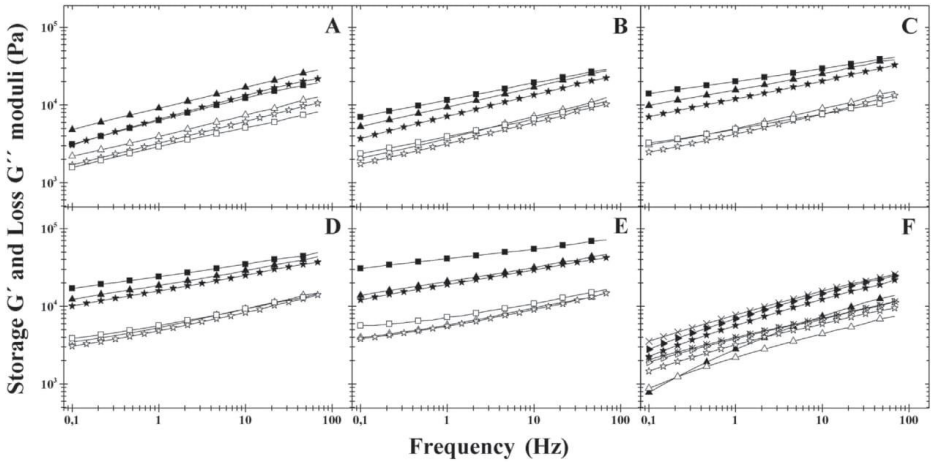

Fig. 2. Dependence of the storage (G′; Pa, closed symbols) and loss (G′; Pa, open symbols) moduli of the model processed cheeses manufactured using a speed of 1000 (▲,△), 1500 (★,☆) and 3000 (■,□) revolutions per minute (rpm) at different times at temperature in the Stephan kettle on frequency (f; in range of 0.1–100.0 Hz). Panels A, B, C, D, and E are, respectively, maintaining for 11, 13, 15, 17, and 20 min. The processed cheeses were measured 24 h after manufacturing at 6 ± 2 °C. Panel F shows the frequency dependence of the storage (G′; closed symbols) and loss (G′; Pa, open symbols) moduli of the model processed cheeses manufactured using a speed of 1500 rpm for 3 min after 1 d (▲,△), 14 d (★,☆), 30 d (▶,▷) and 60 d (×,✳) storage.

A further extension of the holding time of the melt at 90 °C (above 3 min) revealed that the values of the curve showing the dependence of G′ and G″ moduli of the model samples on the range of the oscillation frequencies measured (0.1–100.0 Hz) increased significantly ($P < 0.05$) at all the agitation speeds observed (1000, 1500 and 3000 rpm; the samples evaluated after 1 d storage at 6 °C; Figs. 1 and 2). At the same time, the values of G* of the processed cheeses (for the reference frequency f = 1 Hz) rose significantly with the extended holding time (within the range of 3–20 min; Table 1) ($P < 0.05$). The continuous strengthening of the protein matrix as a result of extending the holding time of the melt at a temperature of (90 °C) up to 20 min is also indicated by an increase in the gel strength (A_F; $P < 0.05$) and rising values of the interaction factor (z; $P < 0.05$) indicating more intensive internal bonds (Tables 2 and 3).

After reaching the melting temperature, the "creaming" stage of the processed cheeses occurs during which a new protein network, with emulsified fat and hydrated water, is formed (Kawasaki, 2008; Lee et al., 2003). A wide range of interactions is involved in the process of creaming, e.g., calcium bridges, the bridges of calcium-phosphate complexes (formed during the reactions of emulsifying salts and calcium ions), hydrogen bridges, hydrophobic interactions, disulfide bridges, etc. (Kapoor & Metzger, 2008; Muslow et al., 2007). According to Lee et al. (2003) the creaming is mainly dependent on the interactions of the proteins present and this process also occurs in matrices with the absence of fat. However, Kawasaki (2008) adds that the water and fat present (their absolute amounts and the size of the fat particles) significantly affect the strength of the final protein network.

Our study revealed that (except for the first minutes of the holding time) with the extending stirring time, the strength and coherence of the protein network rises and the final processed cheese is more rigid. A similar trend was also observed in the studies by Sutheerawattananonda et al. (1997) and Bowland and Foegeding (1999). In comparison with the latter studies, our results were obtained for a higher melting temperature (90 °C), which is closer to typical melting temperatures employed in the industry (Kapoor & Metzger, 2008; Muslow et al., 2007). Sutheerawattananonda et al. (1997) explained the above-mentioned phenomena by a decreasing size of the fat globules – a larger number of small fat globules break the continuity of the protein matrix less intensively in comparison with a smaller number of bigger fat globules. However, the above-mentioned authors also stated that the size reduction of the fat globules ceases within a few minutes. Therefore, apart from the effect of the size reduction of the fat globules, other interactions in the matrix can be expected with the extending holding time. These interactions could include more intensive solubilisation of caseins and hydration of the water present, i.e., processes which are supported by more extensive mechanical stress (Bowland & Foegeding, 1999; Lee et al., 2003).

3.3. The effect of storage period on consistency of samples

From Table 1, a significant ($P < 0.05$) increase in G* (for the reference frequency f = 1 Hz) was observed in all the samples during 60 d storage at 6 °C. The dependence of G′ and G″ of the processed cheeses on the range of the frequencies measured (0.1–100.0 Hz) for the model products manufactured at 1500 rpm with the holding time of 3 min is illustrated in Fig. 2F. An increase in the values of G′ and G″ with extended storage time (up to 60 d) is evident in the above-mentioned graph ($P < 0.05$). Slight strengthening of the protein matrix as a result of storing is also supported by the rising values of gel strength (A_F; $P < 0.05$) and interaction factor (z; $P < 0.05$) during the storage period (Tables 2 and 3). The explanation of this phenomenon may lie in the interactions and processes which could be occurring during the storage period of the processed cheeses, e.g., (i) polymorphism of milk fat and ongoing changes in its crystalline form, (ii) a slight decrease in pH values of the processed cheeses (also detected in our study – see above), (iii) possible hydrolysis of the emulsifying salts (the diphosphates and polyphosphates used),

Table 1
Values of the complex modulus (G*; kPa) at the reference frequency of 1 Hz of the model processed cheese after 1, 14 30 and 60 days of manufacturing using a different speed of Stephan kettle and different temperature maintaining times after holding a melting temperature of 90 °C.[a]

Maintaining time (d)	Days of storage			
	1	14	30	60
1000 rpm knife speed				
0	$5.6 \pm 0.2^{bA}{}_{b}$	$7.1 \pm 0.3^{aB}{}_{b}$	$8.6 \pm 0.4^{bcC}{}_{b}$	$8.9 \pm 0.4^{bcC}{}_{b}$
1	$4.0 \pm 0.2^{aA}{}_{b}$	$7.0 \pm 0.2^{aB}{}_{c}$	$7.0 \pm 0.2^{aB}{}_{c}$	$7.3 \pm 0.4^{aC}{}_{b}$
3	$3.9 \pm 0.2^{aA}{}_{b}$	$7.3 \pm 0.3^{aB}{}_{c}$	$7.9 \pm 0.3^{bC}{}_{b}$	$8.1 \pm 0.4^{bC}{}_{a}$
5	$5.4 \pm 0.3^{bA}{}_{a}$	$8.3 \pm 0.3^{bB}{}_{a}$	$8.9 \pm 0.3^{cC}{}_{a}$	$9.4 \pm 0.5^{cC}{}_{a}$
7	$7.6 \pm 0.4^{cA}{}_{c}$	$12.0 \pm 0.4^{cB}{}_{c}$	$12.6 \pm 0.5^{dB}{}_{c}$	$14.4 \pm 0.8^{dC}{}_{b}$
9	$8.0 \pm 0.4^{cA}{}_{b}$	$12.9 \pm 0.4^{cB}{}_{b}$	$13.3 \pm 0.5^{dB}{}_{b}$	$16.0 \pm 0.8^{eC}{}_{b}$
11	$9.9 \pm 0.5^{dA}{}_{b}$	$14.2 \pm 0.6^{dB}{}_{c}$	$14.5 \pm 0.6^{eB}{}_{b}$	$18.4 \pm 0.7^{fC}{}_{b}$
13	$10.1 \pm 0.4^{dA}{}_{b}$	$14.9 \pm 0.5^{dB}{}_{b}$	$19.1 \pm 0.7^{fC}{}_{b}$	$19.2 \pm 1.1^{fC}{}_{b}$
15	$16.4 \pm 0.5^{eA}{}_{b}$	$20.5 \pm 0.9^{eB}{}_{b}$	$20.8 \pm 0.6^{gB}{}_{b}$	$29.9 \pm 0.8^{gC}{}_{b}$
17	$19.3 \pm 0.7^{fA}{}_{b}$	$22.4 \pm 0.8^{fB}{}_{b}$	$26.7 \pm 0.8^{hC}{}_{b}$	$30.0 \pm 1.1^{gD}{}_{b}$
20	$21.6 \pm 0.6^{gA}{}_{b}$	$24.3 \pm 1.1^{gB}{}_{b}$	$25.5 \pm 1.0^{hB}{}_{b}$	$32.4 \pm 0.8^{hC}{}_{a}$
1500 rpm knife speed				
0	$4.5 \pm 0.2^{bA}{}_{a}$	$7.0 \pm 0.3^{cB}{}_{b}$	$7.9 \pm 0.3^{bC}{}_{a}$	$8.1 \pm 0.3^{bC}{}_{a}$
1	$3.5 \pm 0.1^{aA}{}_{a}$	$5.4 \pm 0.2^{aB}{}_{b}$	$5.6 \pm 0.2^{aB}{}_{b}$	$5.8 \pm 0.3^{aB}{}_{a}$
3	$3.5 \pm 0.2^{aA}{}_{b}$	$6.5 \pm 0.3^{bB}{}_{b}$	$7.9 \pm 0.4^{bC}{}_{b}$	$8.7 \pm 0.4^{bC}{}_{a}$
5	$6.3 \pm 0.2^{cA}{}_{b}$	$9.1 \pm 0.5^{dB}{}_{b}$	$11.8 \pm 0.6^{cC}{}_{c}$	$12.3 \pm 0.6^{cC}{}_{c}$
7	$6.6 \pm 0.2^{cA}{}_{b}$	$10.7 \pm 0.3^{eB}{}_{b}$	$11.7 \pm 0.6^{cC}{}_{b}$	$12.4 \pm 0.5^{cC}{}_{a}$
9	$6.8 \pm 0.3^{c,dA}{}_{a}$	$10.8 \pm 0.4^{eB}{}_{a}$	$11.8 \pm 0.5^{cB}{}_{a}$	$13.7 \pm 0.4^{dC}{}_{a}$
11	$7.3 \pm 0.3^{d,eA}{}_{a}$	$10.9 \pm 0.4^{eB}{}_{a}$	$13.2 \pm 0.7^{dC}{}_{a}$	$14.1 \pm 0.5^{dC}{}_{a}$
13	$7.8 \pm 0.3^{eA}{}_{a}$	$12.9 \pm 0.5^{fB}{}_{a}$	$15.1 \pm 0.6^{eC}{}_{a}$	$15.7 \pm 0.6^{eC}{}_{a}$
15	$12.7 \pm 0.6^{fA}{}_{a}$	$19.2 \pm 0.6^{gB}{}_{a}$	$19.5 \pm 0.7^{fB}{}_{a}$	$21.1 \pm 0.6^{fC}{}_{a}$
17	$16.5 \pm 0.4^{gA}{}_{a}$	$20.1 \pm 0.8^{gB}{}_{a}$	$21.0 \pm 0.8^{gB}{}_{a}$	$24.0 \pm 0.9^{gC}{}_{a}$
20	$19.9 \pm 0.5^{hA}{}_{a}$	$27.0 \pm 0.7^{hB}{}_{a}$	$28.8 \pm 1.0^{hB}{}_{a}$	$32.1 \pm 1.3^{hC}{}_{a}$
3000 rpm knife speed				
0	$4.1 \pm 0.2^{cA}{}_{a}$	$5.9 \pm 0.3^{bB}{}_{a}$	$7.8 \pm 0.3^{cC}{}_{a}$	$9.5 \pm 0.5^{cD}{}_{b}$
1	$3.6 \pm 0.2^{bA}{}_{a}$	$4.7 \pm 0.3^{aB}{}_{a}$	$4.9 \pm 0.2^{aB}{}_{a}$	$7.3 \pm 0.3^{aC}{}_{b}$
3	$3.1 \pm 0.1^{aA}{}_{a}$	$4.6 \pm 0.2^{aB}{}_{a}$	$6.5 \pm 0.2^{bC}{}_{a}$	$8.6 \pm 0.4^{bD}{}_{a}$
5	$5.4 \pm 0.3^{dA}{}_{a}$	$9.0 \pm 0.3^{cB}{}_{a}$	$10.7 \pm 0.5^{dC}{}_{b}$	$11.6 \pm 0.6^{dC}{}_{b}$
7	$5.4 \pm 0.2^{dA}{}_{a}$	$9.5 \pm 0.3^{cB}{}_{a}$	$11.0 \pm 0.6^{dC}{}_{a}$	$11.7 \pm 0.4^{dC}{}_{a}$
9	$6.8 \pm 0.3^{eA}{}_{a}$	$12.8 \pm 0.5^{dB}{}_{b}$	$13.9 \pm 0.7^{eB}{}_{b}$	$16.9 \pm 0.8^{eC}{}_{b}$
11	$7.0 \pm 0.4^{eA}{}_{a}$	$12.9 \pm 0.6^{dB}{}_{b}$	$15.7 \pm 0.9^{fC}{}_{b}$	$18.2 \pm 0.7^{fD}{}_{b}$
13	$12.2 \pm 0.5^{fA}{}_{c}$	$16.8 \pm 0.7^{eB}{}_{c}$	$21.9 \pm 0.9^{gC}{}_{c}$	$26.8 \pm 1.0^{gD}{}_{c}$
15	$20.7 \pm 1.0^{gA}{}_{c}$	$23.6 \pm 0.7^{fB}{}_{c}$	$24.6 \pm 0.7^{hB}{}_{c}$	$27.5 \pm 1.4^{gC}{}_{b}$
17	$24.7 \pm 0.9^{hA}{}_{c}$	$28.9 \pm 1.1^{gB}{}_{c}$	$29.1 \pm 1.3^{iB}{}_{c}$	$37.0 \pm 1.7^{hC}{}_{c}$
20	$41.8 \pm 1.6^{iA}{}_{c}$	$43.2 \pm 1.1^{hB}{}_{c}$	$51.0 \pm 1.3^{kC}{}_{c}$	$55.2 \pm 2.5^{iC}{}_{b}$

[a] Values are means ± standard deviation (n = 12). Within a set of data at the same knife speed, means within a column followed by different superscript letters differ significantly ($P < 0.05$). Means within a row followed by superscript uppercase letters differ significantly ($P < 0.05$). At different knife speeds but at the same maintaining time, means within a column followed by different subscript letters differ significantly ($P < 0.05$).

Table 2
Values of the gel strength (A_F; kPa $s^{1/z}$) of the model processed cheese after 1, 14, 30 and 60 days of manufacturing using a different speed of Stephan kettle and different times of temperature maintaining after holding a melting temperature of 90 °C.[a]

Maintaining time (d)	Days of storage			
	1	14	30	60
1000 rpm knife speed				
0	$5.8 \pm 0.2^{bA}{}_{b}$	$7.2 \pm 0.3^{aB}{}_{b}$	$8.6 \pm 0.4^{cC}{}_{b}$	$9.1 \pm 0.3^{cC}{}_{b}$
1	$4.2 \pm 0.0^{aA}{}_{b}$	$7.1 \pm 0.1^{aB}{}_{c}$	$7.1 \pm 0.1^{aB}{}_{c}$	$7.3 \pm 0.1^{aC}{}_{b}$
3	$4.0 \pm 0.1^{aA}{}_{b}$	$7.3 \pm 0.3^{aB}{}_{c}$	$8.0 \pm 0.3^{bC}{}_{b}$	$8.3 \pm 0.1^{bC}{}_{a}$
5	$5.5 \pm 0.2^{bA}{}_{a}$	$8.4 \pm 0.3^{bB}{}_{a}$	$9.1 \pm 0.4^{cC}{}_{a}$	$9.5 \pm 0.4^{cC}{}_{a}$
7	$7.6 \pm 0.3^{cA}{}_{c}$	$12.1 \pm 0.3^{cB}{}_{c}$	$12.6 \pm 0.6^{dB}{}_{c}$	$14.3 \pm 0.6^{dC}{}_{b}$
9	$8.1 \pm 0.3^{cA}{}_{b}$	$13.0 \pm 0.4^{dB}{}_{b}$	$13.3 \pm 0.7^{deB}{}_{b}$	$15.9 \pm 0.5^{eC}{}_{b}$
11	$9.9 \pm 0.6^{dA}{}_{b}$	$14.3 \pm 0.7^{eB}{}_{c}$	$14.6 \pm 0.7^{eB}{}_{b}$	$18.4 \pm 0.5^{fC}{}_{b}$
13	$10.1 \pm 0.3^{dA}{}_{b}$	$15.0 \pm 0.7^{eB}{}_{b}$	$19.1 \pm 0.6^{fC}{}_{b}$	$19.3 \pm 1.1^{fC}{}_{b}$
15	$16.4 \pm 0.8^{eA}{}_{b}$	$20.6 \pm 0.8^{fB}{}_{b}$	$20.8 \pm 0.8^{gB}{}_{b}$	$29.8 \pm 1.4^{gC}{}_{b}$
17	$19.4 \pm 0.6^{fA}{}_{b}$	$22.3 \pm 1.1^{gB}{}_{b}$	$26.6 \pm 0.9^{hC}{}_{b}$	$30.1 \pm 1.0^{ghD}{}_{b}$
20	$21.7 \pm 0.9^{gA}{}_{b}$	$24.3 \pm 1.1^{hB}{}_{b}$	$25.4 \pm 1.0^{hB}{}_{b}$	$32.1 \pm 1.3^{hC}{}_{a}$
1500 rpm knife speed				
0	$4.6 \pm 0.2^{bA}{}_{a}$	$7.1 \pm 0.3^{bB}{}_{b}$	$8.0 \pm 0.3^{bC}{}_{a}$	$8.2 \pm 0.3^{bC}{}_{a}$
1	$3.6 \pm 0.1^{aA}{}_{a}$	$5.6 \pm 0.2^{aB}{}_{b}$	$5.7 \pm 0.2^{aB}{}_{b}$	$5.9 \pm 0.1^{aB}{}_{a}$
3	$3.7 \pm 0.2^{aA}{}_{b}$	$6.6 \pm 0.3^{bB}{}_{b}$	$8.0 \pm 0.1^{bC}{}_{b}$	$8.8 \pm 0.3^{bC}{}_{a}$
5	$6.3 \pm 0.3^{cA}{}_{b}$	$9.1 \pm 0.3^{cB}{}_{b}$	$12.2 \pm 0.5^{cC}{}_{c}$	$12.2 \pm 0.4^{cC}{}_{c}$
7	$6.7 \pm 0.3^{cA}{}_{c}$	$10.7 \pm 0.5^{dB}{}_{b}$	$13.1 \pm 0.5^{cdC}{}_{b}$	$12.4 \pm 0.5^{cC}{}_{a}$
9	$6.8 \pm 0.3^{cdA}{}_{a}$	$10.8 \pm 0.4^{dB}{}_{a}$	$13.0 \pm 0.5^{cdC}{}_{a}$	$13.7 \pm 0.5^{cdC}{}_{a}$
11	$7.4 \pm 0.3^{deA}{}_{a}$	$10.9 \pm 0.5^{dB}{}_{a}$	$13.7 \pm 0.7^{dC}{}_{a}$	$14.1 \pm 0.5^{dC}{}_{a}$
13	$7.9 \pm 0.3^{eA}{}_{a}$	$12.8 \pm 0.5^{eB}{}_{a}$	$15.1 \pm 0.4^{eC}{}_{a}$	$15.7 \pm 0.6^{eC}{}_{a}$
15	$12.7 \pm 0.3^{fA}{}_{a}$	$19.3 \pm 0.8^{fB}{}_{a}$	$19.5 \pm 0.6^{fB}{}_{a}$	$21.1 \pm 1.0^{fC}{}_{a}$
17	$16.6 \pm 0.9^{gA}{}_{a}$	$20.1 \pm 0.7^{fB}{}_{a}$	$21.1 \pm 0.8^{gB}{}_{a}$	$24.0 \pm 0.8^{gC}{}_{a}$
20	$19.9 \pm 0.6^{hA}{}_{a}$	$26.8 \pm 1.1^{gB}{}_{a}$	$28.7 \pm 1.2^{hB}{}_{a}$	$32.0 \pm 1.3^{hC}{}_{a}$
3000 rpm knife speed				
0	$4.3 \pm 0.2^{cA}{}_{a}$	$5.9 \pm 0.2^{bB}{}_{a}$	$7.8 \pm 0.3^{cC}{}_{a}$	$10.4 \pm 0.4^{cD}{}_{b}$
1	$3.7 \pm 0.0^{bA}{}_{a}$	$4.8 \pm 0.1^{aB}{}_{a}$	$5.0 \pm 0.1^{aB}{}_{a}$	$7.4 \pm 0.3^{aC}{}_{b}$
3	$3.2 \pm 0.1^{aA}{}_{a}$	$4.7 \pm 0.2^{aB}{}_{a}$	$6.6 \pm 0.3^{bC}{}_{a}$	$8.7 \pm 0.2^{bD}{}_{a}$
5	$5.5 \pm 0.2^{dA}{}_{a}$	$9.1 \pm 0.3^{cB}{}_{a}$	$10.6 \pm 0.5^{dC}{}_{b}$	$11.7 \pm 0.6^{dC}{}_{b}$
7	$5.4 \pm 0.2^{dA}{}_{a}$	$9.6 \pm 0.4^{cB}{}_{a}$	$11.0 \pm 0.5^{dC}{}_{a}$	$12.0 \pm 0.4^{dC}{}_{a}$
9	$6.9 \pm 0.2^{eA}{}_{a}$	$12.8 \pm 0.5^{dB}{}_{b}$	$13.9 \pm 0.5^{eB}{}_{b}$	$16.9 \pm 0.6^{eC}{}_{b}$
11	$7.0 \pm 0.4^{eA}{}_{a}$	$13.0 \pm 0.6^{dB}{}_{b}$	$15.7 \pm 0.9^{fC}{}_{b}$	$18.2 \pm 0.8^{fD}{}_{b}$
13	$12.3 \pm 0.3^{fA}{}_{c}$	$16.8 \pm 0.5^{eB}{}_{c}$	$22.0 \pm 0.7^{gC}{}_{c}$	$26.9 \pm 1.1^{gD}{}_{c}$
15	$20.8 \pm 0.8^{gA}{}_{c}$	$23.6 \pm 1.1^{fB}{}_{c}$	$24.8 \pm 1.2^{hB}{}_{c}$	$27.5 \pm 1.0^{gC}{}_{b}$
17	$24.9 \pm 0.8^{hA}{}_{c}$	$29.1 \pm 0.9^{gB}{}_{c}$	$29.2 \pm 1.2^{iB}{}_{c}$	$37.1 \pm 1.1^{hC}{}_{c}$
20	$41.7 \pm 1.9^{iA}{}_{c}$	$43.1 \pm 1.8^{hB}{}_{c}$	$51.0 \pm 2.1^{kC}{}_{c}$	$55.4 \pm 2.3^{iC}{}_{b}$

[a] Values are expressed as means ± standard deviation (n = 12). Within a set of data at the same knife speed, means within a column followed by different superscript letters differ significantly ($P < 0.05$). Means within a row followed by superscript uppercase letters differ significantly ($P < 0.05$). At different knife speeds but at the same maintaining time, means within a column followed by different subscript letters differ significantly ($P < 0.05$).

and (iv) possible changes in dissociation of the salts or other compounds present (Awad et al., 2002; Dimitreli & Thomareis, 2009; Muslow et al., 2007; Nagyová et al., 2014; Shirashoji, Jaeggi, & Lucey, 2010; Weiserová et al., 2011). However, the general trends in the dependence of viscoelastic parameters of the model processed cheeses on the holding time of the melt at a melting temperature of 90 °C during 60 days storage were not affected in any way.

3.4. The effect of agitation speed on consistency of samples

The trend in the development of the observed viscoelastic parameters (Figs. 1 and 2 for the 1st day of storage and Tables 1–3 for the whole storage period) of the processed cheese spreads on the agitation speed (1000, 1500 and 3000 rpm) was changed depending on the holding time of the melt at the temperature of 90 °C. In the absence of the holding time of the melt (0 min) or at a short holding time (1 or 3 min), the processed cheeses manufactured using the agitation speed of 1000 rpm were firmer for most of the storage period (except for the 60th day) especially in comparison with the model samples produced at the agitation speed of 3000 rpm ($P < 0.05$). When the holding time of the melt was set for more than 9 min, the processed cheeses manufactured at 3000 rpm were significantly firmer ($P < 0.05$) in comparison with the products made at lower agitation speeds (1000 and 1500 rpm). On the other hand, at longer holding times (above 9 min) the products made at 1500 rpm showed the lowest firmness (for most of the storage period) in comparison with the processed cheeses manufactured at the agitation speed of 1000 and 3000 rpm ($P < 0.05$).

From the comparison of the results showing the viscoelastic properties of the processed cheeses manufactured at different agitation speeds, there is a combined effect of agitation speed and holding time at the melting temperature. A higher agitation speed, mainly when combined with a longer holding time, results in products with a firmer consistency. Noronha et al. (2008) reported that the increasing agitation speed in the device leads to more rigid final products. This conclusion only partially corresponds with our results. It may be primarily due to a lower melting temperature, the character of the cheese (our study was based on processed cheese spreads while the study by Noronha et al. (2008) dealt with

Table 3
Values of the interaction factor (z) of the model processed cheese after 1, 14, 30 and 60 days of manufacturing using a different speed of Stephan kettle and different times of temperature maintaining after holding a melting temperature of 90 °C.[a]

Maintaining time (d)	Days of storage			
	1	14	30	60
1000 rpm knife speed				
0	$3.00 \pm 0.16^{bA}{}_{a}$	$3.17 \pm 0.13^{aAB}{}_{a}$	$3.33 \pm 0.17^{abA}{}_{a}$	$3.34 \pm 0.13^{abB}{}_{a}$
1	$2.77 \pm 0.11^{aA}{}_{a}$	$3.12 \pm 0.13^{aB}{}_{a}$	$3.15 \pm 0.10^{aB}{}_{a}$	$3.16 \pm 0.12^{aB}{}_{a}$
3	$2.77 \pm 0.09^{aA}{}_{a}$	$3.18 \pm 0.13^{aB}{}_{a}$	$3.23 \pm 0.09^{abB}{}_{a}$	$3.27 \pm 0.11^{abB}{}_{a}$
5	$2.97 \pm 0.06^{bA}{}_{a}$	$3.33 \pm 0.15^{bB}{}_{a}$	$3.34 \pm 0.13^{bB}{}_{a}$	$3.37 \pm 0.07^{bB}{}_{a}$
7	$3.38 \pm 0.14^{cA}{}_{a}$	$3.77 \pm 0.15^{cB}{}_{a}$	$3.75 \pm 0.15^{cB}{}_{a}$	$3.92 \pm 0.17^{cB}{}_{a}$
9	$3.46 \pm 0.11^{cA}{}_{a}$	$3.92 \pm 0.14^{cB}{}_{a}$	$3.99 \pm 0.18^{cdBC}{}_{a}$	$4.17 \pm 0.13^{cdC}{}_{a}$
11	$3.70 \pm 0.18^{dA}{}_{a}$	$4.14 \pm 0.13^{dB}{}_{a}$	$4.19 \pm 0.17^{dB}{}_{a}$	$4.29 \pm 0.20^{deB}{}_{a}$
13	$3.85 \pm 0.18^{dA}{}_{a}$	$4.13 \pm 0.13^{dAB}{}_{a}$	$4.29 \pm 0.17^{dB}{}_{a}$	$4.41 \pm 0.22^{eB}{}_{a}$
15	$4.58 \pm 0.16^{eA}{}_{b}$	$4.83 \pm 0.21^{eB}{}_{a}$	$4.81 \pm 0.21^{eB}{}_{a}$	$5.20 \pm 0.18^{fC}{}_{a}$
17	$4.96 \pm 0.21^{e,fA}{}_{a}$	$5.12 \pm 0.25^{efAB}{}_{a}$	$5.18 \pm 0.20^{efAB}{}_{a}$	$5.29 \pm 0.23^{f,gB}{}_{a}$
20	$5.25 \pm 0.16^{fA}{}_{a}$	$5.34 \pm 0.18^{fAB}{}_{a}$	$5.54 \pm 0.28^{fB}{}_{a}$	$5.64 \pm 0.18^{gB}{}_{a}$
1500 rpm knife speed				
0	$2.95 \pm 0.13^{aA}{}_{a}$	$3.35 \pm 0.10^{aB}{}_{a}$	$3.35 \pm 0.18^{aB}{}_{a}$	$3.46 \pm 0.13^{bB}{}_{a}$
1	$2.82 \pm 0.10^{aA}{}_{a}$	$3.13 \pm 0.12^{aB}{}_{a}$	$3.18 \pm 0.13^{aB}{}_{a}$	$3.14 \pm 0.12^{aB}{}_{a}$
3	$2.82 \pm 0.10^{aA}{}_{a}$	$3.25 \pm 0.14^{aB}{}_{a}$	$3.35 \pm 0.11^{aB}{}_{a}$	$3.54 \pm 0.16^{bC}{}_{b}$
5	$3.39 \pm 0.13^{bA}{}_{b}$	$3.92 \pm 0.13^{bB}{}_{b}$	$3.83 \pm 0.07^{bB}{}_{b}$	$3.83 \pm 0.12^{cB}{}_{b}$
7	$3.31 \pm 0.12^{bA}{}_{a}$	$3.83 \pm 0.13^{bB}{}_{a}$	$3.96 \pm 0.17^{bcB}{}_{a}$	$3.96 \pm 0.13^{cdB}{}_{a}$
9	$3.39 \pm 0.18^{bA}{}_{a}$	$3.91 \pm 0.13^{bB}{}_{a}$	$4.03 \pm 0.18^{bcB}{}_{a}$	$4.11 \pm 0.16^{deB}{}_{a}$
11	$3.46 \pm 0.13^{bcA}{}_{a}$	$4.03 \pm 0.19^{bcB}{}_{a}$	$3.96 \pm 0.19^{bcB}{}_{a}$	$4.21 \pm 0.16^{deB}{}_{a}$
13	$3.67 \pm 0.22^{cA}{}_{a}$	$4.29 \pm 0.17^{cB}{}_{a}$	$4.26 \pm 0.20^{cB}{}_{a}$	$4.39 \pm 0.19^{eB}{}_{a}$
15	$4.19 \pm 0.25^{dA}{}_{a}$	$4.68 \pm 0.20^{deB}{}_{a}$	$4.77 \pm 0.22^{dB}{}_{a}$	$4.80 \pm 0.17^{fB}{}_{a}$
17	$4.83 \pm 0.25^{eA}{}_{a}$	$4.92 \pm 0.22^{efAB}{}_{a}$	$4.90 \pm 0.24^{dAB}{}_{a}$	$5.07 \pm 0.12^{fB}{}_{a}$
20	$5.14 \pm 0.15^{fA}{}_{a}$	$5.66 \pm 0.18^{fB}{}_{a}$	$6.17 \pm 0.24^{e}{}_{b}$	$6.36 \pm 0.28^{gC}{}_{b}$
3000 rpm knife speed				
0	$3.06 \pm 0.16^{a,bA}{}_{a}$	$3.37 \pm 0.18^{aAB}{}_{a}$	$3.55 \pm 0.13^{bBC}{}_{c}$	$3.79 \pm 0.16^{bC}{}_{b}$
1	$2.98 \pm 0.11^{aA}{}_{a}$	$3.20 \pm 0.12^{aB}{}_{a}$	$3.23 \pm 0.12^{aB}{}_{a}$	$3.52 \pm 0.15^{aC}{}_{b}$
3	$2.90 \pm 0.10^{aA}{}_{a}$	$3.17 \pm 0.15^{aB}{}_{a}$	$3.45 \pm 0.15^{b}{}_{a}$	$3.99 \pm 0.14^{bcD}{}_{b}$
5	$3.34 \pm 0.06^{bcA}{}_{b}$	$4.08 \pm 0.19^{bB}{}_{b}$	$3.80 \pm 0.07^{cB}{}_{b}$	$4.17 \pm 0.17^{cB}{}_{b}$
7	$3.54 \pm 0.15^{cA}{}_{b}$	$3.97 \pm 0.20^{bB}{}_{a}$	$4.01 \pm 0.17^{cB}{}_{a}$	$4.54 \pm 0.20^{cC}{}_{b}$
9	$3.79 \pm 0.12^{cA}{}_{b}$	$4.50 \pm 0.20^{cB}{}_{b}$	$4.50 \pm 0.14^{dB}{}_{b}$	$5.00 \pm 0.22^{dC}{}_{b}$
11	$3.78 \pm 0.18^{cA}{}_{b}$	$4.82 \pm 0.19^{cB}{}_{b}$	$4.83 \pm 0.24^{dB}{}_{b}$	$4.75 \pm 0.20^{eB}{}_{b}$
13	$4.60 \pm 0.21^{dA}{}_{b}$	$4.80 \pm 0.22^{cA}{}_{b}$	$5.44 \pm 0.24^{eB}{}_{b}$	$5.77 \pm 0.20^{fB}{}_{b}$
15	$5.92 \pm 0.20^{eA}{}_{c}$	$5.89 \pm 0.21^{dA}{}_{b}$	$5.84 \pm 0.21^{eA}{}_{b}$	$6.28 \pm 0.24^{gB}{}_{b}$
17	$6.15 \pm 0.26^{eA}{}_{c}$	$6.29 \pm 0.27^{dAB}{}_{b}$	$6.54 \pm 0.28^{fB}{}_{b}$	$7.47 \pm 0.25^{hC}{}_{b}$
20	$7.65 \pm 0.36^{fA}{}_{c}$	$7.04 \pm 0.22^{eA}{}_{b}$	$7.57 \pm 0.24^{gA}{}_{c}$	$8.54 \pm 0.36^{iB}{}_{c}$

[a] Values are expressed as mean ± standard deviation (n = 12). Within a set of data at the same knife speed, means within a column followed by different superscript letters differ significantly ($P < 0.05$). Means within a row followed by superscript uppercase letters differ significantly ($P < 0.05$). At different knife speeds but at the same maintaining time, means within a column followed by different subscript letters differ significantly ($P < 0.05$).

processed cheese blocks), a much shorter holding time (~2 min) and a lower agitation speeds (100–1500 rpm).

4. Conclusion

The aim of the work was to observe viscoelastic properties of model processed cheese spreads at various agitation speeds and holding times at the melting temperature over 60 days cold storage. Under the conditions of our experiment it was discovered that at all the agitation speeds tested (1000, 1500 and 3000 rpm) the firmness of the samples increased steadily from the 3rd to the 20th minute of the holding time. The most striking increase was observed in the model processed cheeses manufactured at 3000 rpm, especially from the 10th minute of the holding time onwards. However, a clear trend in the development of viscoelastic properties of the observed samples depending on the agitation speed could not be determined. This trend changed according to the particular holding time of the melt at the melting temperature. Other experiments will have to be conducted to get a clearer description of the combined effect of the holding time of the melt at the melting temperature and the agitation speed on the consistency of processed cheeses. During the 60 day storage period, the firmness of all the observed processed cheeses increased.

Acknowledgement

This study was kindly supported by a project of the internal grants of Tomas Bata University in Zlin, Czech Republic, No. IGA/FT/2016/003 and IGA/FT/2017/004 funded from the resources of specific university research.

References

Awad, R. A., Abdel-Hamid, L. B., & El-Shabrawy, S. A. (2002). Texture and microstructure of block type cheese with formulated emulsifying salt mixture. *Lebensmittel-Wissenschaft und Technologie – Food Science and Technology, 35*, 54–61.

Bayarri, S., Carbonell, I., & Costell, E. (2012). Viscoelasticity and texture of spreadable cheese with different fat contents at refrigeration and room temperatures. *Journal of Dairy Science, 95*, 6926–6936.

Biswas, A. C., Muthukumarappan, K., Marella, C., & Metzger, L. E. (2015). Understanding the role of natural cheese calcium and phosphorous content, residual lactose and salt-in-moisture content on block-type processed cheese functional properties: Cheese hardness and flowability/meltability. *International Journal of Dairy Technology, 68*, 44–52.

Bowland, E. L., & Foegeding, E. A. (1999). Factors determining large-strain (fracture) rheological properties of model processed cheese. *Journal of Dairy Science, 82*, 1851–1859.

Brickley, C. A., Auty, M. A. E., Piraino, P., & McSweeney, P. L. H. (2007). The effect of natural cheddar cheese ripening on the functional and textural properties of the processed cheese manufactured therefrom. *Journal of Food Science, 72*, 483–490.

Buňka, F., Doudová, L., Weiserová, E., Kuchař, D., Ponížil, P., Začalová, D., et al. (2013). The effect of ternary emulsifying salt composition and cheese maturity on the textural properties of processed cheese. *International Dairy Journal, 29*, 1–7.

Černíková, M., Buňka, F., Pavlínek, V., Březina, P., Hrabě, J., & Valášek, P. (2008). Effect of carrageenan type on viscoelastic properties of processed cheese. *Food Hydrocolloids, 22*, 1054–1061.

Chatziantoniou, S. E., Thomareis, A. S., & Kontominas, M. G. (2015). Effect of chemical composition on physico-chemical, rheological and sensory properties of spreadable processed whey cheese. *European Food Research and Technology, 241*, 737–748.

Ciprysová, Z., Buňka, F., Pavlínek, V., Hudečková, L., & Janiš, R. (2013). The effect of selected hydrocolloids on the rheological properties of processed cheese analogues made with vegetable fats during cooling. *International Journal of Dairy Technology, 66*, 484–489.

Dimitreli, G., & Thomareis, A. S. (2004). Effect of temperature and chemical composition on processed cheese apparent viscosity. *Journal of Food Engineering, 64*, 265–271.

Dimitreli, G., & Thomareis, A. S. (2009). Instrumental textural and viscoelastic properties of processed cheese as affected by emulsifying salts and in relation to its apparent viscosity. *International Journal of Food Properties, 12*, 261–275.

Gabriele, D., de Cindio, B., & D'Antona, P. (2001). A weak gel model for foods. *Rheologica Acta, 40*, 120–127.

Guinee, T. P., & ÓCallaghan, D. J. (2013). Effect of increasing the protein-to-fat ratio and reducing fat content on the chemical and physical properties of processed cheese product. *Journal of Dairy Science, 96*, 6830–6839.

Hoffmann, W., Gärtner, J., Lück, K., Johannsen, N., & Maurer, A. (2012). Effect of emulsifying salts containing potassium on the quality of block-type processed cheese. *International Dairy Journal, 25*, 66–72.

ISO. (2004). *ISO Standard No. 1735: Cheese and processed cheese—Determination of fat content – Gravimetric method (reference method)*. Geneva, Switzerland: International Organisation for Standardisation.

ISO. (2005). *ISO Standard No. 5534: Cheese and processed cheese—Determination of the total solid content (reference method)*. Geneva, Switzerland: International Organisation for Standardisation.

Kapoor, R., & Metzger, L. E. (2008). Process cheese: Scientific and technological aspects – A review. *Comprehensive Reviews in Food Science and Food Safety, 7*, 194–214.

Kawasaki, Y. (2008). Influence of "creaming" on the properties of processed cheese and changes in the structure of casein during cheese making. *Milchwissenschaft-Milk Science International, 63*, 149–152.

Khetra, Y., Chavhan, G. B., Kanawjia, S. K., & Puri, R. (2015). Storage changes in low sodium-processed Mozzarella cheese prepared using potassium-based emulsifying salts. *Dairy Science and Technology, 95*, 639–649.

Lee, S. K., Buwalda, R. J., Euston, S. R., Foegeding, E. A., & McKenna, A. B. (2003). Changes in the rheology and microstructure off processed cheese during cooking. *Lebensmittel-Wissenschaft und Technologie – Food Science and Technology, 36*, 339–345.

Lee, S. K., Klostermeyer, H., & Anema, S. G. (2015). Effect of fat and protein-in-water concentrations on the properties of model processed cheese. *International Dairy Journal, 50*, 15–23.

Muslow, B. B., Jaros, D., & Rohm, H. (2007). Processed cheese and cheese analogues. In A. Y. Tamime (Ed.), *Structure of dairy products* (pp. 210–235). Oxford, UK: Blackwell Publishing Ltd.

Nagyová, G., Buňka, F., Salek, R. N., Černíková, M., Mančík, P., Grüber, T., et al. (2014). Usage of sodium polyphosphates with different linear length in the production of spreadable processed cheese. *Journal of Dairy Science, 97*, 111–122.

Noronha, N., ÓRiordan, E. D., & ÓSullivan, M. (2008). Influence of processing parameters on the texture and microstructure of imitation cheese. *European Food Research and Technology, 226*, 385–393.
Piska, I., & Štětina, J. (2004). Influence of cheese ripening and rate of cooling of the processed cheese mixture on rheological properties of processed cheese. *Journal of Food Engineering, 61*, 551–555.
Sádlíková, I., Buňka, F., Budinský, P., Voldánová, B., Pavlínek, V., & Hoza, I. (2010). The effect of selected phosphate emulsifying salts on viscoelastic properties of processed cheese. *Lebensmittel-Wissenschaft und Technologie – Food Science and Technology, 43*, 1220–1225.
Salek, R. N., Černíková, M., Nagyová, G., Kuchař, D., Bačová, H., Minarčíková, L., et al. (2015). The effect of ternary mixtures composition containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. *International Dairy Journal, 44*, 37–43.
Schatz, K., Hoffmann, W., Schrader, K., & Maurer, A. (2014). Effect of emulsifying salts containing potassium on the melting properties of block-type dairy cheese analogues. *International Journal of Dairy Technology, 67*, 202–210.
Shirashoji, N., Jaeggi, J. J., & Lucey, J. A. (2010). Effect of sodium hexametaphosphate concentration and cooking time on the physicochemical properties of pasteurized process cheese. *Journal of Dairy Science, 93*, 2827–2837.
Subramanian, R., Muthukumarappan, K., & Gunasekaran, S. (2006). Linear viscoelastic properties of regular- and reduced-fat pasteurized processed cheese during heating and cooling. *International Journal of Food Properties, 9*, 377–393.
Sutheerawattananonda, M., Fulcher, R. G., Martin, F. B., & Bastian, E. D. (1997). Fluorescence image analysis of process cheese manufactured with trisodium citrate and sodium chloride. *Journal of Dairy Science, 80*, 620–627.
Swenson, B. J., Wendorff, W. L., & Lindsay, R. C. (2000). Effects of ingredients on the functionality of fat-free process cheese spreads. *Journal of Food Science, 65*, 822–825.
Weiserová, E., Doudová, L., Galiová, L., Žák, L., Michálek, J., Janiš, R., et al. (2011). The effect of combinations of sodium phosphates in binary mixtures on selected texture parameters of processed cheese spreads. *International Dairy Journal, 21*, 979–986.
Winter, H. H., & Chambon, F. (1986). Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. *Journal of Rheology, 30*, 367–382.

Research paper 11

B5

Pluta-Kubica, A., Černíková, M., Dimitreli, G., Nebesářová, J., Exarhopoulos, S., Thomareis, A. S., Salek, R. N., Buňka, F.

Influence of the melt holding time on fat droplet size and the viscoelastic properties of model spreadable processed cheeses with different compositions.

International Dairy Journal. 2021, 113. ISSN: 09586946.

International Dairy Journal 113 (2021) 104880

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Influence of the melt holding time on fat droplet size and the viscoelastic properties of model spreadable processed cheeses with different compositions

Agnieszka Pluta-Kubica [a, *], Michaela Černíková [b], Georgia Dimitreli [c], Jana Nebesářová [d], Stylianos Exarhopoulos [c], Apostolos S. Thomareis [c], Richardos N. Salek [b], František Buňka [b, e]

[a] Department of Animal Product Processing, Faculty of Food Technology, University of Agriculture in Krakow, Balicka 122, 30-149, Krakow, Poland
[b] Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, nám. T. G. Masaryka 5555, 760 01, Zlín, Czech Republic
[c] Department of Food Science and Technology, International Hellenic University, P.O. Box 141, GR 57400, Thessaloniki, Greece
[d] Laboratory of Electron Microscopy, Institute of Parasitology, Biology Centre ASCR, v.v.i., Branišovská 31, 370 05, České Budějovice, Czech Republic
[e] Food Research Laboratory, Department of Logistics, Faculty of Military Leadership, University of Defence, Kounicova 65, 662 10, Brno, Czech Republic

ARTICLE INFO

Article history:
Received 5 April 2020
Received in revised form
11 September 2020
Accepted 14 September 2020
Available online 1 October 2020

ABSTRACT

Spreadable processed cheese (SPC) samples, with 30 and 40% (w/w) dry matter (DM) and 30, 40 and 50% (w/w) fat in dry matter (FDM), were produced with nine individual melt holding times (between 0 and 10 min) and stored for 30 days. Milk fat droplet size and viscoelastic properties were determined. In general, longer holding times resulted in decreased diameter of the milk fat droplets in all tested SPC samples. Furthermore, the size of the milk fat droplets decreased with increasing DM content and decreasing FDM content. Furthermore, for most of the produced SPCs, with the progress of the storage time, the G* values decreased over the first 2 or 3 min (of the applied holding time). In addition, prolonging the holding time and storage period resulted in an increase of the samples G* values. Increased DM content and decreased FDM content in SPC samples resulted in increased G* values.

1. Introduction

According to Codex Alimentarius Commission (2000), processed cheese (PC) is manufactured from one or more varieties of natural cheese. Moreover, other optional dairy ingredients (e.g., anhydrous butterfat, butter, cream, milk powder, whey, buttermilk, caseinates, coprecipitates) or non-dairy ingredients (preservatives, stabilisers, flavouring agents) can be added into the processed cheese blend to improve functional properties or modify composition (Codex Alimentarius Commission, 2000; Černíková, Nebesářová, Salek, Řiháčková, & Buňka, 2017). Thereafter, the applied raw materials are shredded, blended, melted and emulsified at elevated temperatures in the presence of appropriate emulsifying salts (ES; e.g., sodium, potassium and/or ammonium salts of the citric, lactic, mono-, di- and/or polyphosphoric acids) (Codex Alimentarius Commission, 2000; El-Bakry, Duggan, O'Riordan, & O'Sullivan, 2010). In addition, the relationship between a minimum level of dry matter (DM) and a minimum level of fat in DM (FDM) in PC is also specified by the Codex Alimentarius Commission (2000). However, Codex standards are not legislation. Therefore, on the markets of the European Union (EU), there exist products with DM content lower than the amount required by the standard, whereas they still appear to be named as "processed cheese" (Černíková, Nebesářová, et al., 2017). In particular, the above-mentioned products must comply with the internal legal regulations of the individual member EU countries. In general, according to Hickey (2011), legislation on PC and related products varies a lot around the world.

One of the most important stages of PC manufacture is the continuous heating and stirring of the ingredients for a period of time allowing the formation of a homogenous and smooth mass (Fu & Nakamura, 2020). In addition, during blending and melting, ES partially solubilise caseins due to the ion-exchange (calcium to sodium or potassium) phenomenon (Fu et al., 2018b). In particular, the fat present is emulsified and the proteins are hydrated. Both the solubilisation and the hydration of casein, resulting in a temporary loosening of the protein network and a decrease in the viscosity of the melt. However, because of the swelling of the protein units, the

* Corresponding author. Tel.: +48126624805.
E-mail address: agnieszka.pluta-kubica@urk.edu.pl (A. Pluta-Kubica).

https://doi.org/10.1016/j.idairyj.2020.104880
0958-6946/

protein–protein interactions intensify as the degree of peptisation increases.

The solubilised protein molecules may also associate with lipids. The proteins present in the formed gel network could form hydrogen and disulphide bonds, as well as electrostatic and hydrophobic interactions may occur. Furthermore, denatured β-lactoglobulin can interact with other proteins in the network such as κ-casein and other whey proteins by forming disulphide bridges. These interactions can cause an increase in the firmness of PC and decrease its meltability (Bowland & Foegeding, 2001; Nogueira de Oliveira, Ustunol, & Tamime, 2011). Calcium bridges and calcium–phosphate complexes may also be involved during the processing (Buňka et al., 2014). The re-association of the proteins results in an increase in viscosity.

The creation of the final network of the PC matrix is called creaming. The latter phenomenon is realised during heating, cooling and storage (Dimitreli, Thomareis, & Smith, 2005; Kawasaki, 2008; Lee, Buwalda, Euston, Foegeding, & McKenna, 2003; Mozuraityte, Berget, Mahdalova, Grønsberg, Øye, & Greiff, 2019).

Furthermore, consistency is one of the most important properties of PC and many factors can influence this. The latter factors can be categorised into three main groups: (i) composition of the raw materials applied (type and degree of maturity of natural cheeses, their chemical composition, type and quantity of ES, additional ingredients, etc.), (ii) processing parameters during manufacturing (temperature during melting, speed of agitation, holding time under melting temperature, rate of cooling), and (iii) storage conditions (temperature, time and permeability of the packaging material used) (Fu et al., 2018a; Fu & Nakamura, 2018; Černíková, Pachlová, et al., 2018).

The effect of processing parameters, such as holding time of the melt, on the consistency of PC spreads has been studied extensively. Swenson, Wendorff, and Lindsay (2000) investigated fat-free PC (with 40%, w/w, DM content) and stated that, the longer the holding time, the lower the firmness of the product. However, Bowland and Foegeding (2001) examined the effect of processing time (10, 20 and 30 min) on the viscoelastic properties of model PC (49.5–52.5%, w/w, DM; 51.4–54.5%, w/w, FDM) over a decreasing temperature regime from 25 °C to 80 °C (to determine sample solidification). The authors concluded that there was no relationship when the small strain analyses (G', G'', G^* and δ) were performed at temperatures lower than 80 °C. Moreover, Lee et al. (2003) found that the apparent viscosity of spreadable processed cheese (SPC) melt containing 50% (w/w) DM and 50% (w/w) FDM rose until 25 mins of processing at 80 °C and then decreased. Furthermore, Černíková et al. (2017) and Černíková, Salek, Kozáčková, and Buňka (2018) investigated the effect of holding time of the melt in a selected temperature on the viscoelastic properties of PC with 35% (w/w) DM and 40% (w/w) and 50% (w/w) FDM content. These authors concluded that the firmness of PC decreased up to the 3rd minute of holding time but then increased significantly (the maximum holding time applied was 20 min). Přikryl et al. (2018) also examined the consistency of PC spreads (37%, w/w, DM and 50%, w/w, FDM) after holding times of 1, 5 and 10 min, they stated that, the longer the melt is maintained at the melting temperature, the more rigid the product becomes.

Nevertheless, the above-mentioned results are contradictory and the effect of holding time on the consistency of PC spreads with different DM and FDM contents remains unclear. Especially, the effect of holding times below 10 min (in close gaps within the holding time range) on SPC samples (with different DM and FDM contents; produced under identical processing protocol) viscoelastic properties described by the complex modulus and phase shift up to now is missing from the existing scientific literature. In general, it is accepted that the short duration of the holding time is economically advantageous. In the present study, model SPCs, manufactured with identical raw materials and under constant processing parameters (temperature, agitation speed) as well as using the same laboratory equipment, were examined. The aim of the research was to determine the effect of the holding time (0, 1, 2, 3, 4, 5, 6, 8 and 10 min) of the SPC melt (at 90 °C) on the size of milk fat droplets and selected viscoelastic properties (complex modulus and phase shift) of model SPC samples with different DM (30 and 40%, w/w) and FDM (30, 40 and 50%, w/w) contents during storage.

2. Materials and methods

2.1. Manufacture of the samples

SPC samples [6 different PC formulations (2 DM × 3 FDM = 6)] × 9 (holding times) = 54 samples in total] were manufactured according to the protocol previously described by Černíková et al. (2017b). The formulation of the PC samples is presented in Table 1. The total weight of the produced SPC samples ranged within the interval of 1105.6–1166.4 g per batch. The composition of the ES used was as in the research of Černíková et al. (2017b). However, their total amount was calculated as a constant ratio of ES to protein (0.15). The relative amount of ES applied is given in Table 1. Total masses of ingredients prepared for the manufacture of the SPC samples were calculated to be similar so as to provide comparable heat transfer.

The model SPCs were manufactured under laboratory conditions using a Stephan UMC-5 (Stephan Machinery GmbH, Halmen, Germany) equipped with indirect heating. The target temperature was 90 °C (reached after approximately 12 min of processing) and the mixture was heated under partial vacuum with an agitation speed of 1500 rpm. The applied holding times at 90 °C were: 0, 1, 2, 3, 4, 5, 6, 8 and 10 min (a separate batch of PC for each holding time was prepared). Furthermore, the hot melt (immediately after production) was packed in polypropylene containers (cuboid shape; length: 95 mm, width: 75 mm, height: 30 mm). The weight of the sample in one container was approximately 85 ± 5 g. Containers were sealed with aluminium lids, left to cool down at ambient temperature (target temperature 25 ± 1 °C; approximately 5 h) and then the samples were transferred into a refrigerator (6 ± 2 °C) where they were stored over the whole experiment. The samples were analysed 24 h after the manufacturing and at the 14th and 30th day of storage.

2.2. Basic chemical composition analysis of the samples

The DM and fat contents were determined according to ISO (2004a) and ISO (2004b), respectively. The FDM content of the PC samples was calculated as fat content divided by DM. The pH was measured using a pH-meter equipped with a glass tip electrode (pH Spear, Eutech Instruments, Oakton, Malaysia) into the samples at three randomly chosen locations. Analyses were performed in triplicate.

2.3. Rheological analysis of the samples

A dynamic oscillatory shear rheometer (Rheostress 1, Haake, Bremen, Germany) equipped with a plate–plate geometry (35 mm diameter, 1 mm gap) was used for the determination of the SPC viscoelastic properties. Furthermore, all tested samples were measured in the control shear stress mode at a frequency ranging from 0.05 to 100.00 Hz (at 20.0 ± 0.1 °C). The amplitude of shear stress (20 Pa) was selected in the linear region of viscoelasticity. Additionally, the exposed edge of the parallel-plates geometry was

Table 1
Formulation of the processed cheese samples with different dry matter content (DM) and fat in dry matter content (FDM).[a]

Raw materials (%)	Type of processed cheese (%, w/w)					
	30% DM			40% DM		
	30% FDM	40% FDM	50% FDM	30% FDM	40% FDM	50% FDM
Dutch-type cheese	53.5	45.4	37.6	71.2	61.0	50.2
Butter	1.1	6.4	11.4	1.6	8.4	15.2
Emulsifying salt components						
Na_2HPO_4	1.0	0.8	0.6	1.3	1.1	0.9
NaH_2PO_4	0.4	0.4	0.3	0.6	0.5	0.4
$Na_4P_2O_7$	0.5	0.4	0.4	0.6	0.5	0.5
Sodium salt of polyphosphate	0.5	0.4	0.4	0.7	0.6	0.5
Water	43.0	46.2	49.3	24.0	28.2	32.3
Emulsifying salts-to-protein ratio	0.15	0.15	0.15	0.15	0.15	0.15
Relative amount of emulsifying salts	2.4	2.0	1.7	3.2	2.7	2.3

[a] The total weight of the melt (g) ranged from 1105.6 to 1166.4; the percentages of emulsifying salts applied were: Na_2HPO_4, 39%; NaH_2PO_4, 18%; Na_4P_2O, 21%; sodium salt of polyphosphate, 22%.

covered with a thin layer of silicone oil to prevent sample dehydration. In oscillatory shear tests, the overall response of the sample may be characterised by the complex modulus $G^* = [(G')^2 + (G'')^2]^{1/2}$, where G' is the elastic modulus (kPa) and G'' is the viscous modulus (kPa). The G^* describes the total resistance to deformation of a material (considered as an elastic solid) and is therefore, a measure of its consistency (Dimitreli & Thomareis, 2008). Moreover, phase shift is the phase angle between stress and strain. In particular, if $\delta < 45^o$ or $\tan\delta$ $(G''/G') < 1$ or $G' > G''$, the material is more elastic than viscous (solid-like behaviour). On the contrary, if $\delta > 45^o$ or $\tan\delta > 1$ or $G'' > G'$, the material is more viscous than elastic (liquid-like behaviour) (Dimitreli & Thomareis, 2008; Sołowiej, Cheung, & Li-Chan, 2014).

2.4. Scanning electron microscopy analysis of milk fat droplet size

The analysis of the size of milk fat droplets was performed using a scanning electron microscope JEOL JSM-7401F (Jeol, Japan) and ImageJ software (Wayne Rasband, Maryland, USA). Before viewing the samples were prepared by chemical fixation, dried using Leica EM CPD300 (Leica Microsystems, Austria) (Černíková, Nebesářová, et al., 2017) and gold-plated in Sputter Coater SCD 050 (Bal-tec, Liechtenstein). The microphotograph of each sample was analysed to determine the fat droplet diameter (expressed in μm). Each sample was analysed twice (2 repetitions × 3 batches; n = 6), and the results were expressed as median ± standard error. The analysis of the size of milk fat droplets of the SPC samples was performed after 30 days of storage.

2.5. Statistical analysis

The results obtained were evaluated using Kruskal-Wallis and Wilcoxon tests (the significance level was 0.05). The chi-square test was applied for the comparison of the fat droplet size of model SPC. Unistat® 6.5 software (Unistat, London, UK) and Microsoft Excel (Microsoft Corporation, Santa Rosa, CA, USA) were used for the statistical analysis.

3. Results and discussion

3.1. Basic chemical composition of the samples

The chemical composition of the model SPC is presented in Table 2. The values of DM were comparable during the 30-day storage time and ranged from 31.11 to 31.39% (w/w) for 30% DM SPC and from 41.09 to 41.49% (w/w) for 40% DM SPC. The calculated FDM levels were also in agreement with the target values (Table 2; $P > 0.05$). Therefore, these samples can be used to determine the effect of the holding time on the size of milk fat droplets and the viscoelastic properties.

Regardless of the combination of DM, FDM and storage time, prolonging the holding time did not significantly affect the pH of samples ($P > 0.05$). Hence, the samples with 40% (w/w) DM and 50%, w/w, FDM stored for 1 day showed pH values in the range 5.66–5.76 and 5.62 to 5.72 after 0 and 10 min of holding time, respectively. The ES applied can stabilise the pH of the PC due to high buffering capacity (Fox, Guinee, Cogan, & McSweeney, 2017). Moreover, regardless of the combination of DM, FDM and holding time applied, storage for 30 days resulted in a slight but statistically significant ($P < 0.05$) decrease in sample pH. In addition, the pH of the SPC samples ranged from 5.68 to 5.78 after 1 day and from 5.56 to 5.64 after 30 days of storage in the samples with 30% (w/w) DM and 30% (w/w) FDM produced with 0 min of holding time.

These results are in agreement to those previously reported by Černíková, Nebesářová, Salek, Popková, and Buňka (2018), Černíková et al. (2017b, 2018c) and Salek et al. (2015). A possible explanation could be hydrolysis of polyphosphate salts, which are more susceptible to the nucleophilic attack of water at pH 5.6 than at pH 6.0 (Barth, Tormena, & Viotto, 2017). The pH values depended on the DM and the FDM content ($P < 0.05$). The lowest values were determined for the samples with 40% (w/w) DM and 30% (w/w) FDM (5.50–5.68 and 5.38–5.57 after 1 and 30 days of storage, respectively), with the highest values for those with 30% (w/w) DM and 50% (w/w) FDM (5.83–6.03 and 5.72–5.91 after 1 and 30 days of storage, respectively) ($P < 0.05$). The current observation could be attributed to higher concentration of lactic acid (from the applied natural cheese – Edam) and ES. Furthermore, the addition of ES promotes an increase in electrostatic repulsion, and greater casein dispersion (or peptisation) might occur (Lu, Shirashoji, & Lucey, 2008). The pH of PC is affected by a few main factors regarding the applied ingredients and ES: the proportions and types of different raw materials, their acidity and buffering capacity as well as the level, type and buffering capacity of the ES (Fox et al., 2017). The model SPCs examined in this study were manufactured using the same ingredients and types of ES, although in different proportions.

3.2. Viscoelastic properties of the samples

The inner structure of the PC samples was evaluated by the complex modulus and loss angle δ. Hence, the loss angle is related to PC melting properties and provides information about its

Table 2
Basic chemical analysis of the processed cheese samples with different dry matter content (DM; % w/w) and fat in dry matter content (FDM; % w/w) during 30-day storage.[a]

Parameters	Type of processed cheese (% w/w)					
	30% DM			40% DM		
	30% FDM	40% FDM	50% FDM	30% FDM	40% FDM	50% FDM
Dry matter content (%, w/w)	31.27 ± 0.33^{a}	31.39 ± 0.27^{a}	31.11 ± 0.26^{a}	41.49 ± 0.25^{b}	41.09 ± 0.33^{b}	41.36 ± 0.31^{b}
Fat content (%, w/w)	9.19 ± 0.30^{a}	12.75 ± 0.36^{b}	15.83 ± 0.38^{c}	12.43 ± 0.32^{b}	16.58 ± 0.34^{d}	20.70 ± 0.48^{e}
Fat in dry matter content (%, w/w)	29.40 ± 1.10^{a}	40.63 ± 0.71^{b}	50.88 ± 0.68^{c}	29.95 ± 0.78^{a}	40.35 ± 0.97^{b}	50.04 ± 0.65^{c}

[a] For dry matter and fat content expressed as 95% confidence interval for mean of samples manufactured with different holding times and stored 30 days; fat in dry matter content calculated from means of dry matter and fat contents and expressed only as mean values. Means within a row followed by different superscript letters differ significantly ($P < 0.05$).

viscoelastic properties. In addition, higher loss angle values indicate higher degree of flowability (Schädle, Eisner & Bader-Mittermaier, 2020).

The results of the complex modulus (G*) and the phase shift (δ) of the model SPCs are shown in Figs. 1 and 2, respectively. These parameters were not determined for the samples with 30% (w/w) DM and 50% (w/w) FDM at 24 h after manufacturing because they presented very liquid-like behaviour. Furthermore, for most of the SPC samples produced, it was demonstrated that, with longer storage times, the G* value significantly decreased in the first 2 or 3 mins of the holding time ($P < 0.05$). Nevertheless, a different pattern was observed in the sample with 30% (w/w) DM and 50% (w/w) FDM contents, the G* of which was constant up to the 5th min of holding time ($P > 0.05$). In all tested samples, prolonging the holding time (up to 10 min) resulted in an increase of the G* values ($P < 0.05$). Moreover, a similar trend was previously reported by Černíková et al. (2017b, 2018c) in PC with 35% (w/w) DM and 40 or 50% (w/w) FDM contents, respectively. However, the current decreasing trend was identified only in the first three mins of processing ($P < 0.05$). Fu et al. (2018a) found that stirring at 1500 rpm at 90 °C could increase the viscosity of PC after approximately 4–6 min. However, those PCs had pH from 5.8 to 5.9 and higher DM levels (54–55%, w/w) than those which were investigated in the present study.

In addition, during the continuous heating and stirring of the ingredients for SPC manufacture, the formation of a homogenous mass occurred. Firstly, ES solubilise para-casein molecules by breaking calcium phosphate bridges. Then, the fat becomes emulsified and the proteins are hydrated (Mozuraityte et al., 2019). Therefore, casein peptisation could occur during the initial holding time (2–3 min) leading to a decrease in the G* values of the examined SPC samples. Furthermore, a new protein network needs some time to be created. Probably the presence of milk fat droplets with higher values of diameter could extends this time. In addition, the complex modulus in the samples with 30% (w/w) DM and 50% (w/w) FDM started to increase after the 6th min of holding time and these SPCs were characterised by milk fat droplets of the largest diameter (Table 3, Figs. 1 and 3). Hence, due to swelling of protein units, the interactions between proteins increased and association with lipids may have occurred. Thereafter, the re-association of the proteins during the creation of a new protein network resulted in increasing firmness. The continuous increase in G* values of the

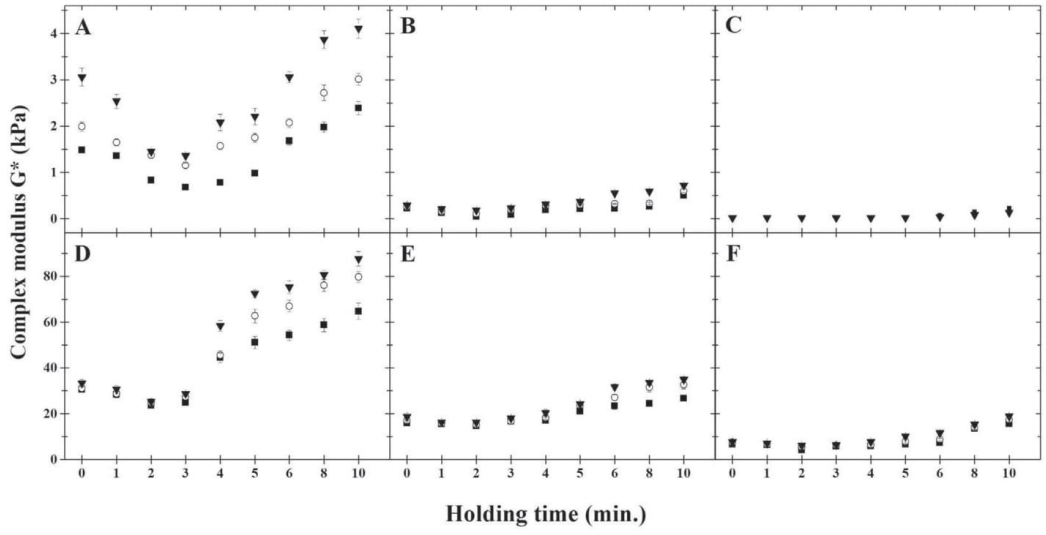

Fig. 1. The dependence of the complex modulus (G*; kPa) of the model processed cheese (PC) 1 day (24 h; ■), 14 days (○) and 30 days (▼) after manufacture using different holding times (0–10 min) at a melting temperature of 90 °C. Panels A, B and C: samples with 30% (w/w) dry matter content. Panels D, E and F: samples with 40% (w/w) dry matter content. Panels A and D, B and E, and C and D: PCs with 30%, 40% and 50% (w/w) fat in dry matter content, respectively. Values are expressed as mean ± standard deviation (n = 8).

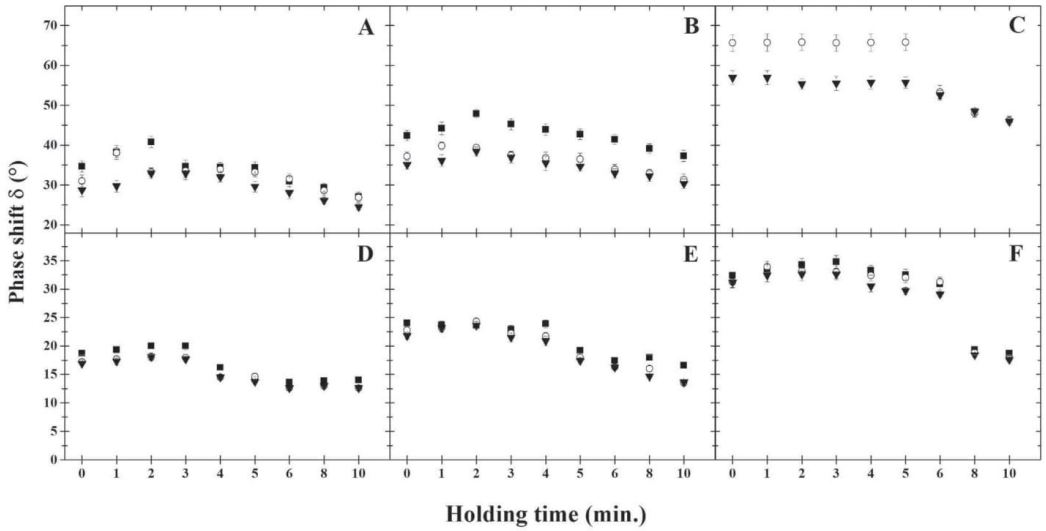

Fig. 2. The dependence of the phase shift (δ; °) of the model processed cheese (PC) 1 day (24 h; ■), 14 days (○) and 30 days (▼) after manufacture using different holding times (0–10 min) at a melting temperature of 90 °C. Panels A, B and C: samples with 30% (w/w) dry matter content. Panels D, E and F: samples with 40% (w/w) dry matter content. Panels A and D, B and E, and C and D: PCs with 30%, 40% and 50% (w/w) fat in dry matter content, respectively. Values are expressed as mean ± standard deviation (n = 8).

SPC samples, observed during the holding time up to 10 min, corresponds to the progressive evolution of the creaming action and may be due to the following reasons. Firstly, the size of milk fat droplets decreases when the holding time is prolonged (Table 3 and Figs. 3 and 4). Moreover, the agitation process causes mechanical stress, which accelerates solubilisation and hydration of the present proteins and peptides (Bowland & Foegeding, 2001; Buňka et al., 2014; Lee et al., 2003; Černíková, Salek, et al., 2018). Furthermore, interactions between proteins can be enhanced by calcium ions, which may neutralise the charge repulsion between caseins. On the other hand, interactions may occur by cross-linking or bridging between proteins. The strengthening of interactions between proteins can cause a more rigid structure of PC (Sharma, Munro, Dessev, & Wiles, 2016). In parallel with the increase in G* values, the observed decrease in δ values ($P < 0.05$), during the holding time up to 10 min, showed that SPC samples became more elastic.

Regardless of the combination of DM and FDM applied, the values of the complex modulus increased during the 30-day storage period ($P < 0.05$). A more pronounced increase in G* was observed in the samples with 30% (w/w) DM, as the difference between the 1st and the 30th day of storage was often over 100%. The increase in the G* values could be caused by a decrease of the pH which was most likely a result of hydrolysis of the applied ES or dissociation of other compounds present in the PC (Černíková, Nebesářová, et al., 2018). Increasing G* can also be triggered by changes in the crystalline form of polymorphic milk fat (Černíková, Salek, et al., 2018). A decrease of pH can cause an increase of hardness of PC when disodium orthophosphate is used as ES, probably be due to the decreased electrostatic repulsion (Lu et al., 2008). In contrast, phase shift values decreased over the 30-day storage period ($P < 0.05$), with SPC samples becoming increasingly elastic.

The complex modulus was also dependent on the DM and the FDM contents. The lowest G* was determined in the samples with

Table 3
Size of milk fat droplets of model processed cheese samples after 30 days of storage.[a]

Holding time (min)	Size of milk fat droplets (μm)					
	30% DM			40% DM		
	30% FDM	40% FDM	50% FDM	30% FDM	40% FDM	50% FDM
0	0.675 ± 0.013^{Fd}	1.027 ± 0.037^{Ee}	1.366 ± 0.064^{Gf}	0.207 ± 0.009^{Da}	0.285 ± 0.007^{Eb}	0.589 ± 0.017^{Fc}
1	0.668 ± 0.025^{Fd}	1.024 ± 0.017^{Ee}	$1.355 \pm 0.086^{F,Gf}$	0.204 ± 0.007^{Da}	0.283 ± 0.009^{Eb}	0.581 ± 0.015^{Fc}
2	0.656 ± 0.029^{Ed}	$1.018 \pm 0.062^{D,Ee}$	1.338 ± 0.042^{Ff}	$0.198 \pm 0.009^{C,Da}$	$0.279 \pm 0.010^{D,Eb}$	0.566 ± 0.014^{Ec}
3	0.613 ± 0.023^{Dd}	1.015 ± 0.037^{De}	1.238 ± 0.079^{Ef}	0.197 ± 0.006^{Ca}	0.272 ± 0.008^{Db}	0.544 ± 0.014^{Dc}
4	0.519 ± 0.024^{Dd}	1.010 ± 0.026^{De}	1.077 ± 0.094^{Df}	0.196 ± 0.006^{Ca}	0.255 ± 0.009^{Cb}	0.504 ± 0.009^{Cc}
5	0.511 ± 0.019^{Cc}	$0.991 \pm 0.036^{C,Dd}$	$1.064 \pm 0.068^{C,De}$	$0.195 \pm 0.006^{B,Ca}$	0.247 ± 0.007^{Cb}	0.500 ± 0.013^{Cc}
6	0.496 ± 0.016^{Cc}	0.951 ± 0.030^{Cd}	$1.036 \pm 0.048^{B,Ce}$	$0.193 \pm 0.005^{B,Ca}$	0.234 ± 0.007^{Bb}	0.492 ± 0.012^{Cc}
8	0.462 ± 0.015^{Bd}	0.916 ± 0.028^{Be}	$1.009 \pm 0.044^{A,Bf}$	$0.186 \pm 0.006^{A,Ba}$	$0.230 \pm 0.006^{A,Bb}$	0.439 ± 0.005^{Bc}
10	0.449 ± 0.021^{Ad}	0.844 ± 0.026^{Ae}	0.993 ± 0.052^{Af}	0.182 ± 0.004^{Aa}	0.224 ± 0.007^{Ab}	0.403 ± 0.020^{Ac}

[a] Abbreviations are DM, dry matter content (%, w/w); FDM, fat in dry matter content (%, w/w). Values are expressed as mean ± standard error (n = 6); means within a column (the difference between the different holding times) and within a row (the difference between the dry matter content and the fat in dry matter content) followed by different superscript uppercase and lowercase letters differ significantly ($P < 0.05$).

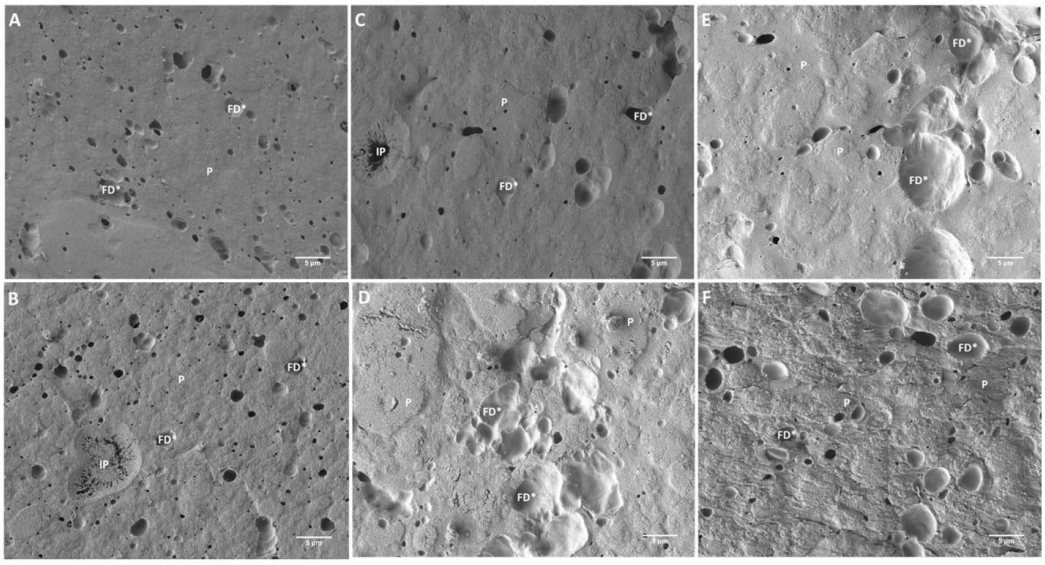

Fig. 3. Scanning electron microscopy images of processed cheeses (PCs) with 30% (w/w) dry matter content (scale bar 5 µm; magnification 2500×). Panels A and B, C and D, E and F: show PCs with 30%, 40% and 50% (w/w) fat in dry matter content, respectively. Panels A, C, E: PCs produced with holding time 0 min. Panels B, D, F: PCs produced with 10 min holding time. FD*, place after milk fat droplets removed; P, protein; IP, insoluble phosphate.

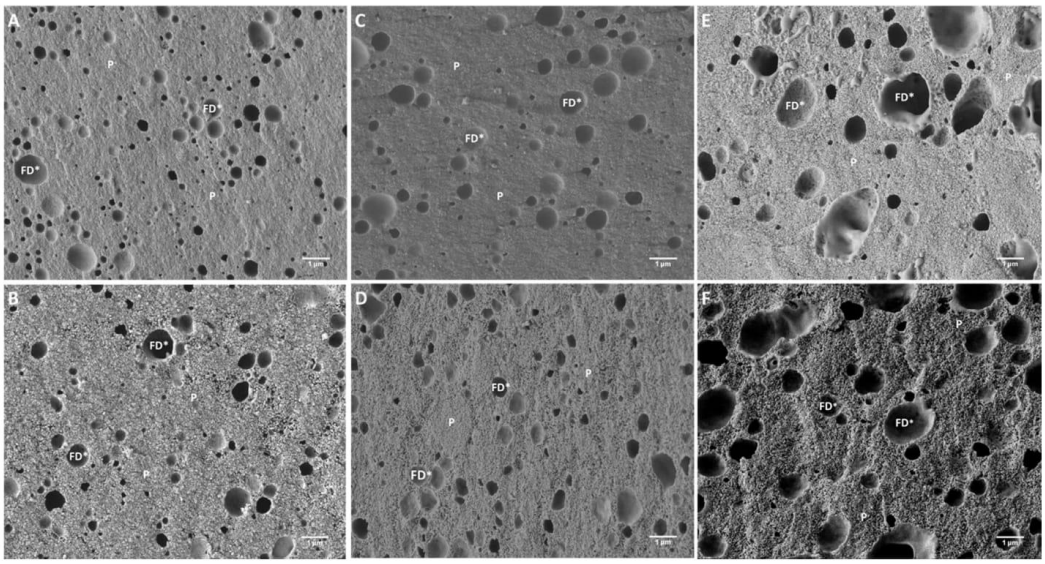

Fig. 4. Scanning electron microscopy images of processed cheeses (PCs) with 40% (w/w) dry matter content (scale bar 1 µm; magnification 10,000×). Panels A and B, C and D, E and F: show PCs with 30%, 40% and 50% (w/w) fat in dry matter content, respectively. Panels A, C, E: PCs produced with holding time 0 min. Panels B, D, F: PCs produced with 10 min holding time. FD*, place after milk fat droplets removed; P, protein.

30% (w/w) DM and 50% (w/w) FDM ($P < 0.05$). The G* increased as the DM increased and the FDM decreased ($P < 0.05$). In particular, the highest values of G* were reported in the samples with 40% (w/w) DM and 30% (w/w) FDM. Generally, we could assume that, the higher DM and the lower FDM contents, the more rigid the SPC became and a tougher and less spreadable consistency was seen.

This could be probably attributed to the increase in NFS and protein contents and, hence, to the strengthening of the protein network of the samples. Similar findings were demonstrated by Černíková, Nebesářová, Salek, Říháčková, and Buňka (2017), Dimitreli and Thomareis (2008) and Guinee and O'Callaghan (2013). Moreover, analysis of the phase shift showed that all SPC samples with 40% (w/w) DM and the those with 30% (w/w) DM and 30% (w/w) FDM, independently of the holding time applied and the time of storage, exhibited more elastic than viscous consistency (solid-like behaviour; phase shift less than 45°). Most of the samples with 30% (w/w) DM and 40% (w/w) FDM also had this feature, except one, the sample manufactured with 2 min of holding time. However, its consistency changed into more elastic after 14 days of storage. The samples with 30% (w/w) DM and 50% (w/w) FDM were found to be more viscous than elastic (liquid-like behaviour; $\delta > 45°$), regardless of the holding time applied and the storage period.

It could be concluded that the DM, FDM contents, holding time and length of the storage time affected the rheological and thus, the sensory properties of the PC samples. In particular, the increasing fat content reduced the values of complex modulus, resulting in softer PC products. Moreover, the PCs with low DM content were more viscous than samples with higher level of DM content. In general, some sensory properties (hardness, gumminess, chewiness and meltability) can be affected similarly such as rheological properties with the prolonging of the storage time. Furthermore, increasing holding time resulted in higher values of the G* modulus.

Furthermore, from an economic point of view, shorter holding times could be evaluated as more advantageous for the producers of PC. However, the production cost for PC manufacture can vary significantly and could be affected by multiple factors (raw materials, energy costs, operation costs, location, inflation, taxes, etc.) which could differ between countries. With respect to the applied ingredients (natural cheese − Edam; butter; water and ES) cost implemented in the current study the estimated production cost (€ kg^{-1}; prices are for year 2019) of 1 kg of final product could be as follows: €1.70, 30% (w/w) DM, 30% (w/w) FDM; €1.57, 30% (w/w) DM, 40% (w/w) FDM; €1.43, 30% (w/w) DM, 50% (w/w) FDM; €2.26, 40% (w/w) DM, 30% (w/w) FDM; €2.09, 40% (w/w) DM, 40% (w/w) FDM; €1.91, 40% (w/w) DM, 50% (w/w) FDM. In general, PC cheese formulation can have an impact on final product price. Hence, higher DM content can result in higher PC price. However, in the case of FDM content (comparing PCs with the same DM content) the higher the FDM content, the lower the price of the final product.

3.3. Scanning electron microscopy of the samples and size of milk fat droplets

The development of the size of milk fat droplets of the model SPCs after 30 days of storage in relation to the duration of the holding time is shown in Table 3 and Figs. 3 and 4. In general, most of the samples presented diameter values lower than 1 μm. Furthermore, similar findings were previously reported by Gliguem, Lopez, Michon, Lesieur, and Ollivon (2011). Regardless of the combination of DM and FDM applied, prolonging the holding time up to 10 min resulted in decreased diameter of the milk fat droplets ($P < 0.05$), probably due to the extended rate of shear. Moreover, a significant difference was observed between 0 and 2 or 3 min of the holding time ($P < 0.05$). According to Sutheerawattananonda, Fulcher, Martin and Bastian (1997) prolonging the holding time can result in a reduction in the diameter of the milk fat droplets over the first 5 min. However, in the aforementioned study, trisodium citrate was used as ES, which strongly chelates micellar calcium, forms soluble complexes, and causes the dispersion of the proteins present, leading to sufficient emulsification of the fat present within the PC matrix (Fu et al., 2018b; Sutheerawattananonda et al., 1997). In addition, according to Fu et al. (2018a,b), longer stirring times result in decreasing size of the milk fat droplets.

The size of the milk fat droplets depended on the DM and FDM contents and also on the processing parameters. In particular, the diameter of the milk fat droplets decreased as the DM content increased and the FDM content decreased ($P < 0.05$). Thus, the smallest diameter of milk fat droplets was observed in the samples with 40% (w/w) DM and 30% (w/w) FDM contents (Figs. 3 and 4). The largest fat droplets were determined in processed cheese samples produced with 0 min (Figs. 3 and 4, panels A, C, E) of holding time and the smallest fat droplets were present in processed cheeses with the holding time 10 min (Figs. 3 and 4, panels B, D, F). This could be attributed to the viscosity of the melt, which, as it increases, impedes the movement of the fat droplets and contributes to their shearing during stirring. In fact, the more the DM increases and the FDM decreases, the more the non-fat solids (NFS) and the protein contents increase.

The increase in firmness of processed cheeses was explained by Sutheerawattananonda et al. (1997) by reducing the size of fat droplets, where a larger number of small fat droplets disrupt the continuity of the protein matrix less intensely compared with the presence of a smaller number of order of magnitude larger fat droplets. Simultaneously with the decrease in the size of fat beads, those authors found that the stiffness of the monitored samples also increases with increasing holding time. However, the abovementioned authors also stated that the reduction in the size of the fat droplets stops after about 5 mins of holding. Dimitreli and Thomareis (2004) found that increasing the protein content resulted in higher viscosity values of PC melt. Moreover, the samples with 30% (w/w) DM and 30% (w/w) FDM and those with 40% (w/w) DM and 50% (w/w) FDM did not differ in fat droplet size ($P > 0.05$), as they had similar NFS contents (20.50−22.10%, w/w). Černíková, Nebesářová, et al. (2017) reported that also for PC with 35% (w/w) and 45% (w/w) DM and 40% (w/w) and 50% (w/w) FDM the diameter of the milk fat droplets increased with the increasing level of FDM. In addition, Lee, Klostermeyer and Anema (2015) also observed that the milk fat droplet diameter decreased as the DM increased.

4. Conclusions

The study of six different types of model SPCs prepared and stored for 30 days showed that the viscoelastic properties depend on the holding time, time of storage and DM and FDM contents. For most of the produced SPCs, it was demonstrated that, on the 1st, 14th and 30th day of storage, G* (a measure of consistency) decreased in the first 2 or 3 min of the holding time and gradually increased afterwards. In the most cases of DM and FDM contents, prolonging the holding time from the 3rd min up to the 10th min and storage for 30 days increased the G* in all samples examined. Also, G* increased with increasing DM content at constant FDM and also with decreasing FDM. The same DM content and increasing FDM content caused decreasing value of G*. Nevertheless, inverse relationships were observed in the case of the phase shift evaluation. In addition, most of the SPCs produced exhibited more elastic than viscous consistency (solid-like behaviour).

It could be concluded that DM and FDM contents, holding time and length of the storage time affected the rheological properties of the PC samples. In particular, increasing fat content reduced the values of complex modulus, resulting in more soft PC final products. Moreover, the PCs with low DM content were more viscous than the samples with higher level of DM content. This information may be relevant to industry practice. Moreover, longer holding times of

the melt can result in smaller diameter of milk fat droplets in the final product. However, a significant decrease in size was observed after 2 or 3 min. Furthermore, the size of milk fat droplets decreased as the DM content increased and the FDM content decreased. In general, from an economic point of view, shorter holding times could be evaluated as more advantageous for producers of PC. In addition, PC cheese formulation can have an impact on final product price, as higher DM content can result in higher PC price. Comparing PCs with the same DM content, the higher the FDM content, the lower the price of the final product.

Acknowledgements

This research was supported by Own Scholarship Fund of the University of Agriculture funded by the Rector of University of Agriculture in Cracow. We acknowledge the core facility HR SEM JEOL7401F, Institution Laboratory of Electron Microscopy, Biology Centre CAS, České Budějovice, supported by the MEYS CR (LM2015062 Czech-BioImaging) and ERDF (No. CZ.02.1.01/0.0/0.0/16_013/0001775).

References

Barth, A. P., Tormena, C. F., & Viotto, W. H. (2017). pH influences hydrolysis of sodium polyphosphate in dairy matrices and the structure of processed cheese. *Journal of Dairy Science, 100*, 8735–8743.

Bowland, E. L., & Foegeding, E. A. (2001). Small strain oscillatory shear and microstructural analyses of a model processed cheese. *Journal of Dairy Science, 84*, 2372–2380.

Buňka, F., Doudová, L., Weiserová, E., Černíková, M., Kuchař, D., Slavíková, Š., et al. (2014). The effect of concentration and composition of ternary emulsifying salts on the textural properties of processed cheese spreads. *LWT - Food Science and Technology, 58*, 247–255.

Černíková, M., Nebesářová, J., Salek, R. N., Popková, R., & Buňka, F. (2018a). The effect of rework content addition on the microstructure and viscoelastic properties of processed cheese. *Journal of Dairy Science, 101*, 2956–2962.

Černíková, M., Nebesářová, J., Salek, R. N., Řihačková, L., & Buňka, F. (2017a). Microstructure and textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content. *Journal of Dairy Science, 100*, 4300–4307.

Černíková, M., Pachlová, V., Holas, O., Moudrá, K., Slintáková, K., & Buňka, F. (2018b). The effect of dairy fat source on viscoelastic properties of full-fat processed cheese spreads. *European Journal of Lipid Science and Technology, 120*, 4–7.

Černíková, M., Salek, R. N., Kozáčková, D., & Buňka, F. (2018c). The effect of different agitations and temperature maintainings on viscoelastic properties of full-fat processed cheese spreads. *LWT - Food Science and Technology, 89*, 244–247.

Černíková, M., Salek, R. N., Kozáčková, D., Běhalová, H., Luňáková, L., & Buňka, F. (2017b). The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads. *International Dairy Journal, 66*, 84–90.

Codex Alimentarius Commission. (2000). Codex general standard for process. In Cheese and Spreadable Process (Ed.), *Cheese. CODEX STAN A-8(b)-1978* (Vol. 12). Rome, Italy: FAO/WHO Codex Alimentarius Commission.

Dimitreli, G., & Thomareis, A. S. (2004). Effect of temperature and chemical composition on processed cheese apparent viscosity. *Journal of Food Engineering, 64*, 265–271.

Dimitreli, G., & Thomareis, A. S. (2008). Effect of chemical composition on the linear viscoelastic properties of spreadable-type processed cheese. *Journal of Food Engineering, 84*, 368–374.

Dimitreli, G., Thomareis, A. S., & Smith, P. G. (2005). Effect of emulsifying salts on casein peptization and apparent viscosity of processed cheese. *International Journal of Food Engineering, 1*(2), 1–17.

El-Bakry, M., Duggan, E., O'Riordan, E. D., & O'Sullivan, M. (2010). Effects of emulsifying salts reduction on imitation cheese manufacture and functional properties. *Journal of Food Engineering, 100*, 596–603.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2017). *Fundamentals of cheese science* (2nd ed., *Chapt. 17*. New York, NY, USA: Springer.

Fu, W., & Nakamura, T. (2018). Effects of starches on the mechanical properties and microstructure of processed cheeses with different types of casein network structures. *Food Hydrocolloids, 79*, 587–595.

Fu, W., & Nakamura, T. (2020). Moisture content impact creaming effect and microstructure of processed cheese containing different textural starches. *International Dairy Journal, 105*, 1–6.

Fu, W., Watanabe, Y., Inoue, K., Moriguchi, N., Fusa, K., Yanagisawa, Y., et al. (2018a). Effects of pre-cooked cheeses of different emulsifying conditions on mechanical properties and microstructure of processed cheese. *Food Chemistry, 245*, 47–52.

Fu, W., Watanabe, Y., Satoh, H., Inoue, K., Moriguchi, N., Fusa, K., et al. (2018b). Effects of emulsifying conditions on creaming effect, mechanical properties and microstructure of processed cheese using a rapid visco-analyzer. *Bioscience, Biotechnology and Biochemistry, 82*, 476–483.

Gliguem, H., Lopez, C., Michon, C., Lesieur, P., & Ollivon, M. (2011). The viscoelastic properties of processed cheeses depend on their thermal history and fat polymorphism. *Journal of Agricultural and Food Chemistry, 59*, 3125–3134.

Guinee, T. P., & O'Callaghan, D. J. (2013). Effect of increasing the protein-to-fat ratio and reducing fat content on the chemical and physical properties of processed cheese product. *Journal of Dairy Science, 96*, 6830–6839.

Hickey, M. (2011). Current legislation on processed cheese and related products. In A. Y. Tamine (Ed.), *Processed cheese and analogues* (pp. 25–80). Oxford, UK: Wiley-Blackwell.

ISO. (2004a). *Cheese and processed cheese – determination of the total solids content (Reference method). International standard ISO 5534|IDF 4* (2nd ed.). Geneva, Switzerland: International Organisation for Standardisation.

ISO. (2004b). *Cheese and processed cheese products – determination of fat content – gravimetric method (Reference method). International standard ISO 1735|IDF 5* (3rd ed.). Geneva, Switzerland: International Organisation for Standardisation.

Kawasaki, Y. (2008). Influence of 'creaming' on the properties of processed cheese and changes in the structure of casein during cheese making. *Milchwissenschaft, 63*, 149–152.

Lee, S. K., Buwalda, R. J., Euston, S. R., Foegeding, E. A., & McKenna, A. B. (2003). Changes in the rheology and microstructure of processed cheese during cooking. *LWT - Food Science and Technology, 36*, 339–345.

Lee, S. K., Klostermeyer, H., & Anema, S. G. (2015). Effect of fat and protein-in-water concentrations on the properties of model processed cheese. *International Dairy Journal, 50*, 15–23.

Lu, Y., Shirashoji, N., & Lucey, J. A. (2008). Effects of pH on the textural properties and meltability of pasteurized process cheese made with different types of emulsifying salts. *Journal of Food Science, 73*, E363–E369.

Mozuraityte, R., Berget, I., Mahdalova, M., Grønsberg, A., Øye, E. R., & Greiff, K. (2019). Sodium reduction in processed cheese spreads and the effect on physicochemical properties. *International Dairy Journal, 90*, 45–55.

Nogueira de Oliveira, M., Ustunol, Z., & Tamime, A. Y. (2011). Manufacturing practices of processed cheese. In A. Y. Tamime (Ed.), *Processed cheese and analogues* (pp. 148–178). Oxford, UK: Wiley-Blackwell.

Přikryl, J., Hájek, T., Švecová, B., Salek, R. N., Černíková, M., Červenka, L., et al. (2018). Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions. *LWT - Food Science and Technology, 87*, 266–271.

Salek, R. N., Černíková, M., Nagyová, G., Kuchař, D., Bačová, H., Minarčíková, L., et al. (2015). The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. *International Dairy Journal, 44*, 37–43.

Schädle, C. N., Eisner, P., & Bader-Mittermaier, S. (2020). The combined effects of different fat replacers and rennet casein on the properties of reduced-fat processed cheese. *Journal of Dairy Science, 103*, 3980–3993.

Sharma, P., Munro, P. A., Dessev, T. T., & Wiles, P. G. (2016). Shear work induced changes in the viscoelastic properties of model mozzarella cheese. *International Dairy Journal, 56*, 108–118.

Sołowiej, B., Cheung, I. W. Y., & Li-Chan, E. C. Y. (2014). Texture, rheology and meltability of processed cheese analogues prepared using rennet or acid casein with or without added whey proteins. *International Dairy Journal, 37*, 87–94.

Sutheerawattananonda, M., Fulcher, R. G., Martin, F. B., & Bastian, E. D. (1997). Fluorescence image analysis of process cheese manufactured with trisodium citrate and sodium chloride. *Journal of Dairy Science, 80*, 620–627.

Swenson, B. J., Wendorff, W. L., & Lindsay, R. C. (2000). Effects of ingredients on the functionality of fat-free process cheese spreads. *Journal of Food Science, 65*, 822–825.

Research paper 12

B6

Černíková, M., Nebesářová, J., Salek, R. N., Popková, R., Buňka, F.

The effect of rework content addition on the microstructure and viscoelastic properties of processed cheese.

Journal of Dairy Science. 2018, 101, 2956-2962. ISSN: 00220302.

J. Dairy Sci. 101:2956–2962
https://doi.org/10.3168/jds.2017-13742

The effect of rework content addition on the microstructure and viscoelastic properties of processed cheese

Michaela Černíková,*[1] Jana Nebesářová,† Richardos Nikolaos Salek,* Romana Popková,* and František Buňka*
*Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, T.G. Masaryka 5555, 760 01, Zlín, Czech Republic
†Laboratory of Electron Microscopy, Institute of Parasitology, Biology Centre, Czech Academy of Science, v.v.i., Branišovská 31, 370 05, České Budějovice, Czech Republic

ABSTRACT

The aim of this work was to add various amounts of rework (0.0 to 20.0% wt/wt) to processed cheeses with a dry matter content of 36% (wt/wt) and fat with a dry matter content of 45% (wt/wt). The effect of the rework addition on the viscoelastic properties and microstructure of the processed cheeses was observed. The addition of rework (in this case, to processed cheese with a spreadable consistency) in the amounts of 2.5, 5.0, and 10.0% (wt/wt) increased the firmness of the processed cheese. With the further addition of rework, the consistency of the processed cheeses no longer differed significantly. The conclusions obtained by the measurement of viscoelastic properties were supported by cryo-scanning electron microscopy, where fat droplets in samples with added rework of over 10.0% (wt/wt) were smaller than fat droplets in processed cheeses with lower additions of rework.
Key words: processed cheese, rework, rheology, cryo-scanning electron microscopy

INTRODUCTION

Processed cheeses (**PC**) are produced at an increased temperature and under moderate underpressure, from basic raw materials (cheeses, butter, water, emulsifying salts) to which other ingredients of a dairy or nondairy origin can be added. The consistency of PC is affected by several different factors, which we can divide into the following groups: (1) raw material composition (content of cheeses of various types and maturity, DM and fat in DM contents, concentration and composition of emulsifying salts, presence of hydrocolloids, rework content, and so on), (2) technological production parameters (agitation speed, agitation duration, cooling time and rate, and so on), (3) storage conditions (storage length and temperature, packaging characteristics; Kapoor and Metzger, 2008; Salek et al., 2015; Černíková et al., 2017).

Rework is a PC that has already been processed once and in which creaming has already occurred; it is used as a raw material for the production of PC. Therefore, its consistency is affected by all of the aforementioned factors. Rework is created in the industry either (1) intentionally (production of PC for rework or residue of PC in production equipment) or (2) unintentionally (production of PC originally intended for the market network but ultimately not released for market; for example, due to unsuitable packaging or incorrect DM or fat in DM content). Rework is usually used fresh (PC residue in production equipment) or 3 to 14 d old (PC unsuitable for the market network due to unsuitable fat or DM content, or an incorrect packaging weight). Meyer (1973) and Guinee et al. (2004) defined 3 types of rework: (1) from young cheese with long protein chains (unripened raw material used for processing), (2) regular PC with a creamy structure, and (3) overcreamed product with a delicate structure. In Kaláb et al. (1987), 3 types of rework were also used: (1) fresh rework (produced and rapidly frozen immediately after production), (2) regular rework from previous processes, and (3) the so-called hot melt, which simulates PC subjected to extreme stress (cooled from 82 to 4°C in 5 h), which Meyer (1973) refers to as overcreamed. All of the aforementioned types of reworks are processed under industrial conditions to prevent economic losses (Kaláb et al., 1987). The reasons for using rework can be economic (see above), but it is also used to increase viscosity (with the increasing age of the rework and with its increasing concentration) after production, increase firmness, improve meltability, or reduce the emulsifying salt content, as rework already contains emulsifying salt (Lauck, 1972; Meyer, 1973; Kaláb et al., 1987; Pluta et al., 2000; Kapoor and Metzger, 2008). However, individual types of reworks are added in different quantities for various reasons.

Received August 25, 2017.
Accepted December 2, 2017.
[1]Corresponding author: cernikova@utb.cz

If we use the division as per Meyer (1973), then fresh rework is used in a concentration of 1 to 2% (wt/wt) to increase the creaming of PC spreads, which contain a high proportion of ripened or very ripened cheeses in their raw material composition with intact casein content approximately 70%, or more precisely, less than 65%. The second type of rework (regular PC with a creamy structure) is used for block-type PC, to increase firmness and elasticity, in a quantity of 2 to 30% (wt/wt). Meyer (1973) recommended that the third type of rework (over-creamed product with a delicate structure) be used only in a quantity of less than 1%, as it has a very strong creaming effect and could easily lead to over-creaming. Lauck (1972) wrote about the addition of rework, usually in a quantity of 2 to 15% (wt/wt). Explanations of the applications of individual types of reworks are listed by Guinee et al. (2004), who claims that the additional heating of preprepared PC can cause a higher degree of temperature-induced dehydration and paracasein aggregation, particularly in the third type of rework (over-creamed product with a delicate structure), which increases the elasticity of the resulting product. The more effective dispersion of the emulsifying salts in rework (due to a longer contact period) leads to the faster hydration of paracasein and the high concentration of proteins, thanks to the high degree of emulsification and in the third type of rework (over-creamed product with a delicate structure), which leads to increased viscosity, the effective dispersion of fat, and the emulsification of the fresh melting mixture.

The literature has not described the effects of various concentrations of rework on the consistency of PC in detail, and in practice rework is added exclusively on the basis of empiricism. The aim of this study was to observe the effect of adding various quantities of rework to the raw material composition on the resulting consistency of the PC. Model samples with a DM content of 36% and fat in DM content of 45% were produced with an addition of 0.0, 2.5, 5.0, 10.0, 15.0, and 20.0% (wt/wt) rework under industrial conditions. The consistency was examined using dynamic oscillatory rheometry over a 60-d storage period. Individual analyses were performed on d 1, 7, 14, 30, and 60 after production.

MATERIALS AND METHODS

The model samples of PC were produced under industrial conditions, from raw materials manufactured by the PC producer. The following were used: Dutch-type cheese [50% (wt/wt) DM content, 30% (wt/wt) fat in DM content, 8-wk maturity, Lacrum PLC, Velké Meziříčí, Czech Republic], unsalted butter [84% (wt/wt) DM content and 82% (wt/wt) fat content, Lacrum PLC], water, emulsifying salts [3% (wt/wt) calculated per total weight, composition of emulsifying salts (% relative)]: 34% Na_2HPO_4, 26% NaH_2PO_4, 20% $Na_4P_2O_7$, and 20% sodium polyphosphate; Fosfa PLC, Břeclav, Czech Republic), and rework. The effect of individual emulsifying salts on pH shift in a model environment of deionized water, liquid dairy system, and PC was presented in Nagyová et al. (2014) and Salek et al. (2015). The rework added to the raw material composition was produced from the same raw material composition as the control sample (without the addition of rework) and added after 3 d of storage at a temperature of 6 ± 2°C (72 h), in quantities of 0.0, 2.5, 5.0, 10.0, 15.0, and 20.0% (wt/wt). The samples were produced using Stephan UM 130 equipment (Stephan Machinery GmbH, Hameln, Germany), at a melting temperature of 90°C (the holding time was 1 min and direct heating was used) and a blade revolution speed of 1,500 rpm. The total producing time was about 9 to 10 min. Before the manufacture, slight underpressure was generated in the kettle (using a vacuum pump). Each PC with the certain rework concentration was manufactured 3 times (3 repetitions × 6 concentrations of rework = 18 manufactured batches). After production, the samples were packaged in plastic jars closed with lids, cooled to 6 ± 2°C, and stored for a period of 2 mo. The samples of each manufactured batch were analyzed after 1, 7, 14, 30, and 60 d of storage.

The DM content and the fat content of the PC samples were gravimetrically determined according to ISO 5534 (ISO, 2004a) and ISO 1735 (ISO, 2004b), respectively. The pH values were measured at ambient temperature using the glass tip electrode of a pH meter (pH Spear, Eutech Instruments Europe B.V., Landsmeer, the Netherlands), by directly inserting the spear into the PC samples in 3 randomly selected spots (in each packaging).

The rheological analysis of the PC samples was performed using a dynamic oscillatory shear rheometer (RheoStress 1, Haake, Bremen, Germany) with a plate-plate geometry (diameter 35 mm, gap 1.0 mm) at 20.0 ± 0.1°C. Storage (**G′**) and loss (**G″**) moduli [determined as functions of frequency (ω) ranging from 0.1 to 100.0 Hz] were monitored (shear stress amplitude 20 Pa). The complex modulus (**G***) was calculated using the following formula:

$$G^* = \sqrt{\left(G'\right)^2 + \left(G''\right)^2}. \quad [1]$$

Increasing G′, G″, and G* indicated the growing gel strength and firmness of the PC (Lee et al., 2004; Černíková et al., 2017). A frequency (ω) of 1 Hz was chosen as the reference for the presentation of G*. Win-

ter's critical gel theory was also implemented using the following equation (Gabriele et al., 2001):

$$G^*(\omega) = A_F \cdot \omega^{\frac{1}{z}}, \quad [2]$$

where A_F is the gel strength (Pa·s$^{1/z}$) and z is the interaction factor (defined as the number of structure units interacting with one another in a 3-dimensional network; unitless). The higher the interaction factor is, the more interactions occur in the matrix of the sample (Gabriele et al., 2001). Each sample was analyzed 3 times (3 repetitions × 3 samples; n = 9) and the results were expressed as median ± standard deviation.

A Jeol JSM-7401F scanning electron microscope (Jeol, Tokyo, Japan) equipped with cryo-system ALTO 2500 (Gatan, Abingdon, UK) was used to study the microstructure of the model PC and distribution of fat globule size. Model PC samples were inserted in the sample holder, frozen rapidly with slush nitrogen, and transferred under vacuum to the cryo chamber of the Alto 2500 system, which was connected to the microscope. Here the sample was fractured at a temperature of −135°C and a high vacuum (less than 10^{-4} Pa). Then, the sample was moved into the scanning electron microscope cold stage and examined at −135°C. Each image was analyzed using ImageJ software (National Institutes of Health, Bethesda, MD). The photograph of each model sample was analyzed to determine the fat globule diameter (μm). Each sample was analyzed twice (3 repetitions × 2 samples; n = 6) and the results were expressed as median ± standard error.

Kruskal-Wallis and Wilcoxon tests were used to evaluate the obtained results (the significance level was 0.05). For the estimation of A_F and z, nonlinear regression analysis (the Marquardt-Levenburg method; $A_F > 0$ and $z \geq 0$) was used. For the comparison of the fat globule distribution of samples with different rework concentration, chi-squared test was used. Unistat 6.5 software (Unistat, London, UK) and Microsoft Excel (Microsoft Corporation, Redmond, WA) were used for the statistical analysis.

RESULTS AND DISCUSSION

The DM content of individual processes cheeses fluctuated within a range of 36.31 to 36.68% (wt/wt; $P \geq 0.05$), whereas the fat content was 16.3 to 16.5% (wt/wt; $P \geq 0.05$). The basic chemical parameters of the model samples are comparable; therefore, the samples can be evaluated in terms of the effect of the additions of various quantities of rework. The results of the pH measurements for all the samples during the 60-d storage period are listed in Table 1. Within the scope of individual storage days, the pH values of samples with various quantities of added rework were comparable ($P \geq 0.05$). During the 60-d storage period, there was a slight decrease in pH ($P < 0.05$), which was observed for these types of model samples in several works (Dimitreli and Thomareis, 2009; Weiserová et al., 2011; Salek et al., 2015). The explanation could lie in hydrolysis of phosphate salts or change of bonds of the compounds present and in the change of their dissociation (Dimitreli and Thomareis, 2009; Weiserová et al., 2011; Salek et al., 2015).

The results of the elastic (G′) and loss (G″) moduli for individual model samples are listed in Figure 1 for d 1 and 60 of storage, at 6°C, contingent on oscillation frequency (0.1–100 Hz). The rework is a PC produced in the usual manner (i.e., with a completed emulsification process) and already contains emulsifying salt; it has the ability to influence the functional properties of the resulting PC to which it is added during production. According to Kaláb et al. (1987), the addition of

Table 1. Results of pH measurement of model processed cheese manufactured with addition of rework after 1 d (24 h), 7 d, 14 d, 30 d, and 60 d of storage and size of fat globules of model processed cheese after 60 d of storage[1]

Rework amount (% wt/wt)	pH					Size of fat globules (μm)
	1 d	7 d	14 d	30 d	60 d	
0.0	$5.73 \pm 0.03^{a,A}$	$5.61 \pm 0.03^{a,B}$	$5.60 \pm 0.04^{a,B}$	$5.56 \pm 0.02^{a,B}$	$5.50 \pm 0.03^{a,C}$	0.92 ± 0.09^{a}
2.5	$5.71 \pm 0.03^{a,A}$	$5.60 \pm 0.03^{a,B}$	$5.60 \pm 0.02^{a,B}$	$5.51 \pm 0.03^{a,C}$	$5.49 \pm 0.03^{a,C}$	0.84 ± 0.06^{b}
5.0	$5.75 \pm 0.03^{a,A}$	$5.62 \pm 0.02^{a,B}$	$5.56 \pm 0.03^{a,B}$	$5.54 \pm 0.02^{a,B}$	$5.48 \pm 0.02^{a,C}$	0.77 ± 0.08^{c}
10.0	$5.69 \pm 0.03^{a,A}$	$5.62 \pm 0.03^{a,B}$	$5.58 \pm 0.02^{a,B}$	$5.51 \pm 0.03^{a,C}$	$5.51 \pm 0.02^{a,C}$	0.65 ± 0.06^{d}
15.0	$5.74 \pm 0.02^{a,A}$	$5.64 \pm 0.02^{a,B}$	$5.61 \pm 0.03^{a,B}$	$5.52 \pm 0.02^{a,C}$	$5.52 \pm 0.03^{a,C}$	0.54 ± 0.05^{d}
20.0	$5.75 \pm 0.02^{a,A}$	$5.62 \pm 0.03^{a,B}$	$5.61 \pm 0.05^{a,B}$	$5.53 \pm 0.02^{a,C}$	$5.52 \pm 0.02^{a,C}$	0.56 ± 0.04^{d}

[a–d]Means within a column (the difference between the amount of rework added) followed by different lowercase letters differ ($P < 0.05$); samples stored during different times were evaluated independently.

[A–C]Means within a row (the difference between the storage time) followed by different uppercase letters differ ($P < 0.05$); samples manufactured using a different amount of rework were evaluated independently.

[1]Values were expressed as mean ± SD (for pH values; n = 9) and mean ± SE (for size of fat globules; n = 6).

rework at a maximum quantity of 4% (wt/wt) does not affect the change in consistency of the final product. On the basis of the results presented in Figure 1 and the complex module (G*) values and the gel strength values (A_F) listed in Tables 2 and 3, we can state that the addition of only 2.5% (wt/wt) rework has a significant ($P < 0.05$) effect on the change in consistency of the resulting PC, whose firmness increases as a result of the added rework. Furthermore, we can state that a significant ($P < 0.05$) increase in firmness was observed until the addition of 10.0% (wt/wt) rework. With the addition of rework over 10.0% (wt/wt), the firmness of the produced PC no longer increased significantly ($P \geq 0.05$). Through a more detailed comparison of individual model samples in Figure 1, we can say that, at lower frequencies and on the first day after production, PC produced without any addition of rework showed higher values ($P < 0.05$) in the loss module (G″) than in the elastic module (G′). It is assumed that if the values of G″ > G′, the intermolecular bonds in the melt (protein network) have sufficient time to weaken during the oscillation cycle. As the measurement frequency increased, the curves of the elastic and loss flexibility modules for a sample without added rework intersected. That is because, at higher frequencies, the intermolecular bonds no longer have sufficient time to weaken, so the PC behaves more like a solid (Cunha et al., 2013). A similar trend was also observed in PC with 2.5 and 5.0% (wt/wt) rework contents (Figure 1, A–C). However, we must point out that the intersection of both curves (G′ and G″) moved to lower frequencies ($P < 0.05$) as the rework content increased. Samples with added rework of 10.0% (wt/wt) and more (Figure 1, D–F) behaved more like solids at lower frequency measurements, as G′ > G″, which is characteristic of densely interconnected biopolymeric networks (Cunha et al., 2013). As the storage time lengthened, changes occurred in the matrix of the PC even without added rework. G′ and G″ for d 60 of storage are also listed in Figure 1. In the sample without added rework, the loss flexibility module (G″) once again prevailed at lower frequencies ($P < 0.05$), and the intersection of both curves (G′ and G″) occurred at lower frequencies than the first day after production (Figure 1, A). Therefore, at lower frequencies, the samples behaved more like a fluid than a solid. All samples with added rework from 2.5 to 20.0% (wt/wt) behaved like solids after d 60 of storage, as their G′ showed higher values than G″ for the entire period of measured frequencies (Figure 1, B–F). The aforementioned results are supported by the gel firmness values (A_F) and the interaction factor (z) set forth in Tables 3 and 4. Gel firmness increased significantly even when only 2.5% (wt/wt) of rework was added ($P < 0.05$). At the same time, the value of the interaction factor increased ($P < 0.05$), which is evidence of the number of structural units interacting with one another in the protein network, or more precisely of the number of intermolecular bonds among the proteins. Thus, the increasing firmness of the gel was given by the increasing number of interactions in the 3-dimensional system studied (Macků et al., 2008, 2009; Cunha et al., 2013). Generally, the increase in the firmness of PC after the addition of rework was also described by Kaláb et al. (1987), who also stated that the firmest samples were ones with a "hot melt" content (heated for a longer period without mixing), whereas the softest samples were those without added rework. The work of Kaláb et al. (1987) also involved electron microscopy and stated that the greatest coalescence of fat occurred in hot melt samples. On the basis of the aforementioned results, we can state that regular PC with a creamy structure used as rework has a different effect on consistency and microstructure than rework in the form of an over-creamed product

Table 2. Values of complex modulus at reference frequency of 1 Hz (G*; kPa) of model processed cheese after 1 d (24 h), 7 d, 14 d, 30 d, and 60 d of storage using different amounts of rework[1]

Rework amount (% wt/wt)	G*				
	1 d	7 d	14 d	30 d	60 d
0.0	$0.49 \pm 0.02^{a,A}$	$0.58 \pm 0.02^{a,B}$	$0.63 \pm 0.04^{a,C}$	$0,70 \pm 0.04^{a,D}$	$0.79 \pm 0.04^{a,E}$
2.5	$0.73 \pm 0.04^{b,A}$	$0.91 \pm 0.03^{b,B}$	$1.02 \pm 0.05^{b,C}$	$1.07 \pm 0.06^{b,C}$	$1.19 \pm 0.05^{b,D}$
5.0	$0.88 \pm 0.05^{c,A}$	$1.21 \pm 0.05^{c,B}$	$1.32 \pm 0.08^{c,C}$	$1.37 \pm 0.05^{c,C}$	$1.38 \pm 0.05^{c,C}$
10.0	$1.09 \pm 0.04^{d,A}$	$1.32 \pm 0.06^{d,B}$	$1.50 \pm 0.09^{d,C}$	$1.63 \pm 0.10^{d,D}$	$1.71 \pm 0.07^{d,E}$
15.0	$1.09 \pm 0.05^{d,A}$	$1.30 \pm 0.04^{d,B}$	$1.54 \pm 0.10^{d,C}$	$1.61 \pm 0.06^{d,C}$	$1.79 \pm 0.07^{d,D}$
20.0	$1.07 \pm 0.05^{d,A}$	$1.36 \pm 0.06^{d,B}$	$1.57 \pm 0.07^{d,C}$	$1.73 \pm 0.12^{d,D}$	$1.78 \pm 0.07^{d,D}$

[a–d]Means within a column (the difference between the amount of rework added) followed by different lowercase letters differ ($P < 0.05$); samples stored during different times were evaluated independently.

[A–E]Means within a row (the difference between the storage time) followed by different uppercase letters differ ($P < 0.05$); samples manufactured using a different amount of rework were evaluated independently.

[1]Values were expressed as mean ± SD (n = 9).

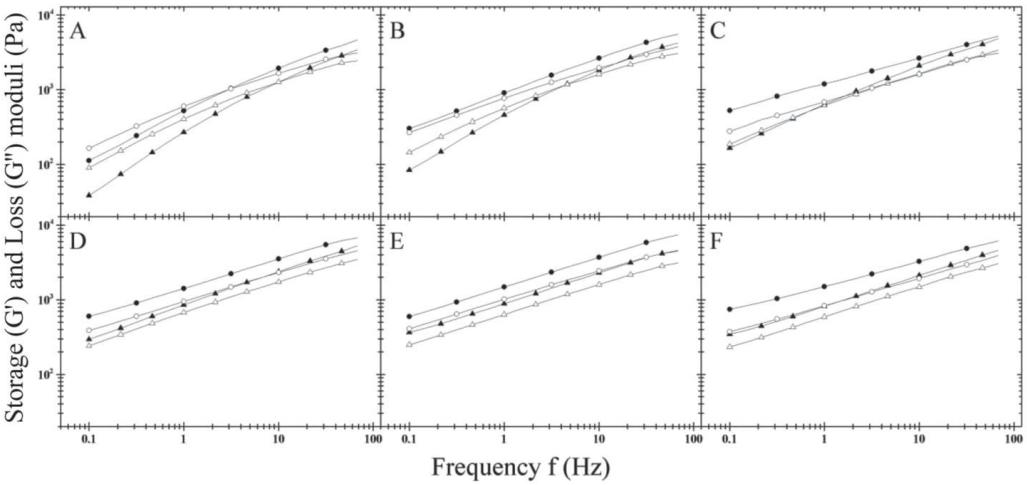

Figure 1. Dependence of the elastic (G′; full symbols; Pa) and the loss (G″; open symbols; Pa) moduli of the processed cheese spreads (36% wt/wt in DM content; 45% wt/wt fat in DM content) after 1 d (▲△) and 60 d (●○) of storage at 6 ± 2°C on frequency (range of 0.1–100.0 Hz). (A) 0.0% (wt/wt) rework, (B) 2.5% (wt/wt) rework, (C) 5.0% (wt/wt) rework, (D) 10.0% (wt/wt) rework, (E) 15.0% (wt/wt) rework, and (F) 20.0% (wt/wt) rework.

with a delicate structure. The adding or replacing part of the raw material with rework increases the firmness and elasticity of the resulting product (Guinee et al., 2004).

Apart from the interconnectedness of the protein network evaluated by the interaction factor and gel firmness, the different consistencies of the PC with the addition of 2.5 to 10.0% (wt/wt) rework can also be explained by the varying sizes of the fat droplets (Figure 2 and Table 1). Statistically, the median value of fat droplets (Table 1) in a sample without added rework differed significantly from samples with added rework ($P < 0.05$). Statistically, the addition of rework in a concentration of 10.0 to 20.0% (wt/wt; during mutual comparison) no longer significantly affected the size of the fat droplets ($P \geq 0.05$). We could observe possible correlation between compactness of protein network and the gel strength (A_F) and also the interaction factor (z). With higher number of interaction factor we find out more compact protein matrix. The addition of rework affected the intensity of the emulsification of the present fat, which was demonstrated by the reduction in the size of the fat droplets. As the degree of fat emulsification grew, the firmness of the PC increased.

Table 3. Values of gel strength (A_F; kPa·s$^{1/z}$, where z is the interaction factor) of model processed cheese after 1 d (24 h), 7 d, 14 d, 30 d, and 60 d of storage using different amounts of rework[1]

Rework amount (% wt/wt)	A_F				
	1 d	7 d	14 d	30 d	60 d
0.0	$0.56 \pm 0.03^{a,A}$	$0.63 \pm 0.04^{a,B}$	$0.70 \pm 0.03^{a,C}$	$0.76 \pm 0.03^{a,C}$	$0.87 \pm 0.04^{a,D}$
2.5	$0.83 \pm 0.03^{b,A}$	$1.00 \pm 0.06^{b,B}$	$1.09 \pm 0.05^{b,C}$	$1.14 \pm 0.04^{b,D}$	$1.26 \pm 0.05^{b,E}$
5.0	$0.94 \pm 0.04^{c,A}$	$1.28 \pm 0.07^{c,B}$	$1.35 \pm 0.09^{c,B}$	$1.44 \pm 0.07^{c,C}$	$1.38 \pm 0.06^{c,BC}$
10.0	$1.12 \pm 0.07^{d,A}$	$1.39 \pm 0.06^{d,B}$	$1.50 \pm 0.07^{d,C}$	$1.60 \pm 0.09^{d,C}$	$1.77 \pm 0.10^{d,D}$
15.0	$1.15 \pm 0.05^{d,A}$	$1.30 \pm 0.06^{cd,B}$	$1.55 \pm 0.11^{d,C}$	$1.58 \pm 0.08^{d,C}$	$1.85 \pm 0.09^{d,D}$
20.0	$1.03 \pm 0.05^{d,A}$	$1.33 \pm 0.07^{cd,B}$	$1.50 \pm 0.05^{d,C}$	$1.62 \pm 0.09^{d,D}$	$1.73 \pm 0.09^{d,E}$

[a–d]Means within a column (the difference between the amount of rework added) followed by different lowercase letters differ ($P < 0.05$); samples stored during different times were evaluated independently.

[A–E]Means within a row (the difference between the storage time) followed by different uppercase letters differ ($P < 0.05$); samples manufactured using a different amount of rework were evaluated independently.

[1]Values were expressed as mean ± SD (n = 9).

Table 4. Values of interaction factor (z) of model processed cheese after 1 d (24 h), 7 d, 14 d, 30 d, and 60 d of storage using different amounts of rework[1]

Rework amount (% wt/wt)	z				
	1 d of storage	7 d of storage	14 d of storage	30 d of storage	60 d of storage
0.0	$2.05 \pm 0.15^{a,A}$	$2.09 \pm 0.11^{a,A}$	$2.16 \pm 0.09^{a,B}$	$2.20 \pm 0.13^{a,B}$	$2.23 \pm 0.07^{a,BC}$
2.5	$2.23 \pm 0.12^{b,A}$	$2.31 \pm 0.11^{b,B}$	$2.34 \pm 0.09^{b,B}$	$2.42 \pm 0.13^{b,C}$	$2.47 \pm 0.12^{b,C}$
5.0	$2.27 \pm 0.16^{b,A}$	$2.43 \pm 0.14^{bc,B}$	$2.53 \pm 0.14^{c,C}$	$2.61 \pm 0.11^{c,D}$	$2.83 \pm 0.18^{c,E}$
10.0	$2.42 \pm 0.09^{d,A}$	$2.48 \pm 0.15^{c,A}$	$2.86 \pm 0.10^{d,B}$	$2.93 \pm 0.15^{d,B}$	$2.71 \pm 0.18^{c,B}$
15.0	$2.64 \pm 0.13^{e,A}$	$2.45 \pm 0.12^{c,A}$	$2.86 \pm 0.10^{d,B}$	$2.93 \pm 0.12^{d,B}$	$2.68 \pm 0.13^{c,A}$
20.0	$2.50 \pm 0.14^{de,A}$	$2.56 \pm 0.13^{d,A}$	$2.86 \pm 0.14^{d,B}$	$2.93 \pm 0.14^{d,C}$	$2.92 \pm 0.18^{d,C}$

[a–e]Means within a column (the difference between the amount of rework added) followed by different lowercase letters differ ($P < 0.05$); samples stored during different times were evaluated independently.

[A–E]Means within a row (the difference between the storage time) followed by different uppercase letters differ ($P < 0.05$); samples manufactured using a different amount of rework were evaluated independently.

[1]Values were expressed as mean ± SD (n = 9).

This phenomenon corresponds to the results of the works of Kapoor and Metzger (2008), Lee et al. (2015), and Černíková et al. (2017). With higher additions of rework (above 10.0% wt/wt), no further changes in the fat droplets or viscoelastic properties were observed in our work. On the other hand, Kaláb et al. (1987), who used block-type PC with a higher DM content (55% wt/wt) for their experiment, observed structure and consistency changes in products manufactured with the rework content higher than 10.0% (wt/wt).

CONCLUSIONS

This work studied the effect of rework content at an age of 72 h on the consistency of PC. It was discovered that a lower quantity of added rework, up to 10.0% (wt/wt), caused a gradual increase in the firmness of the PC; simultaneously, the size of the fat droplets gradually decreased, which results primarily from the improvement of the emulsification properties as the amount of rework added increases. As the rework concentration in

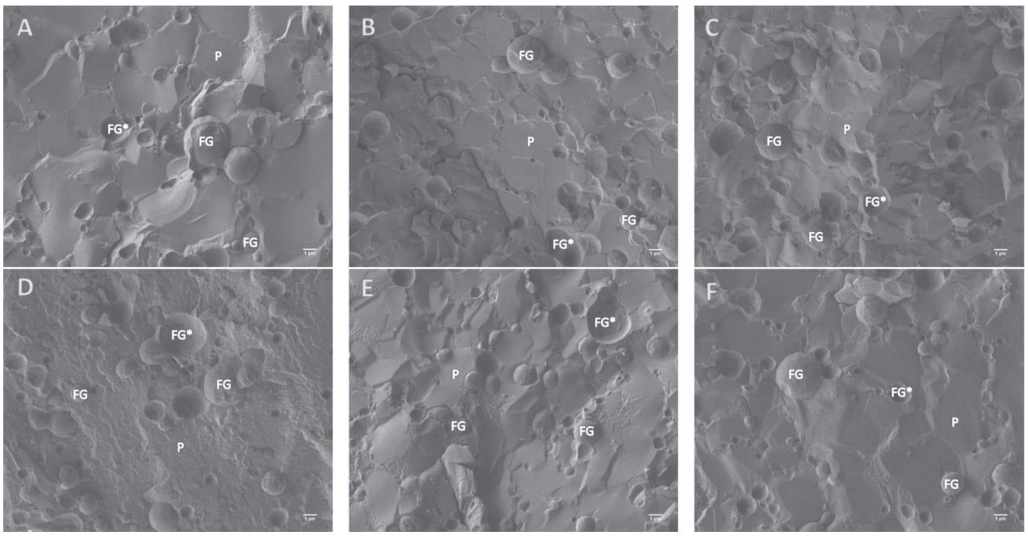

Figure 2. Scanning electron microscopy images of model processed cheese with different rework content. (A) 0.0% (wt/wt) rework, (B) 2.5% (wt/wt) rework, (C) 5.0% (wt/wt) rework, (D) 10.0% (wt/wt) rework, (E) 15.0% (wt/wt) rework, and (F) 20.0% (wt/wt) rework. FG = fat globule; FG* = removed fat globule; P = protein phase. Scale bar = 1 μm.

the raw material composition increased further (from 10.0–20.0% wt/wt), the firmness of the PC no longer increased, and the median size value of the fat droplets, which was smaller than that in samples with less added rework, also no longer differed significantly.

ACKNOWLEDGMENTS

This study was kindly supported by a project of the internal grants of Tomas Bata University in Zlin, Czech Republic, no. IGA/FT/2018/003, funded from the resources of specific university research. We acknowledge the core facility Laboratory of Electron Microscopy, Biology Centre of Czech Academy of Science, České Budejovice, supported by the Ministry of Education, Youth and Sports of the Czech Republic (LM2015062 Czech-BioImaging, Prague, Czech Republic).

REFERENCES

Černíková, M., J. Nebesářová, R. N. Salek, L. Řiháčková, and F. Buňka. 2017. Microstructure, textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content. J. Dairy Sci. 100:4300–4307. https://doi.org/10.3168/jds.2016-12120.

Cunha, C. R., R. Grimaldi, M. R. Alcantara, and W. H. Viotto. 2013. Effect of the type of fat on rheology, functional properties and sensory acceptance of spreadable cheese analogue. Int. J. Dairy Technol. 66:54–62. https://doi.org/10.1111/j.1471-0307.2012.00876.x.

Dimitreli, G., and A. S. Thomareis. 2009. Instrumental textural and viscoelastic properties of processed cheese as affected by emulsifying salts and in relation to its apparent viscosity. Int. J. Food Prop. 12:261–275. https://doi.org/10.1080/10942910802256164.

Gabriele, D., B. de Cindio, and P. D'Antona. 2001. A weak gel model for foods. Rheol. Acta 40:120–127. https://doi.org/10.1007/s003970000139.

Guinee, T. P., M. Carić, and M. Kaláb. 2004. Cheese: Pasteurized processed cheese and substitute/imitation cheese products. Pages 349–393 in Cheese: Chemistry, Physics and Microbiology, Volume 2: Major Cheese Group. 3rd ed. P. F. Fox, P. L. H. McSweeney, and T. P. Cogan, ed. Elsevier Applied Science, London, UK.

ISO (International Organization for Standardization). 2004a. ISO Standard No. 5534: Cheese and processed cheese—Determination of the total solid content (reference method). ISO, Geneva, Switzerland.

ISO (International Organization for Standardization). 2004b. ISO Standard No. 1735: Cheese and processed cheese products—Determination of fat content—Gravimetric method (reference method). ISO, Geneva, Switzerland.

Kaláb, M., J. Yun, and S. H. Yiu. 1987. Textural properties and microstructure of process cheese food rework. Food Microstruct. 6:181–192.

Kapoor, R., and L. E. Metzger. 2008. Process cheese: Scientific and technological aspects–A review. Comp. Rev. Food Sci. Saf. 7:194–214. https://doi.org/10.1111/j.1541-4337.2008.00040.x.

Lauck, R. M., inventor; Stauffer Chemical Co., assignee. 1972. Using salvage cheese in preparing pasteurized process cheese. New York. US patent 3,697,292.

Lee, S. K., S. Anema, and H. Klostermeyer. 2004. The influence of moisture content on the rheological properties of processed cheese spreads. Int. J. Food Sci. Technol. 39:763–771. https://doi.org/10.1111/j.1365-2621.2004.00842.x.

Lee, S. K., H. Klostermeyer, and S. G. Anema. 2015. Effect of fat and protein-in-water concentration on the properties of model processed cheese. Int. Dairy J. 50:15–23. https://doi.org/10.1016/j.idairyj.2015.06.001.

Macků, I., F. Buňka, V. Pavlínek, P. Leciánová, and J. Hrabě. 2008. The effect of pectin concentration on viscoelastic and sensory properties of processed cheese. Int. J. Food Sci. Technol. 43:1663–1670. https://doi.org/10.1111/j.1365-2621.2008.01734.x.

Macků, I., F. Buňka, B. Voldánová, and V. Pavlínek. 2009. Effect of addition of selected solid cosolutes and pectin on viscoelastic properties of model processed cheese. Food Hydrocoll. 23:2078–2084. https://doi.org/10.1016/j.foodhyd.2009.03.020.

Meyer, A. (1973). Processed cheese Manufacture, Food Trade Press ltd., London.

Nagyová, G., F. Buňka, R. N. Salek, M. Černíková, P. Mančík, T. Grůber, and D. Kuchař. 2014. Usage of sodium polyphosphates with different linear length in the production of spreadable processed cheese. J. Dairy Sci. 97:111–122. https://doi.org/10.3168/jds.2013-7210.

Pluta, A., M. Ziarno, and A. Smolińska. 2000. Możliwości zastosowania hydrokoloidów w produkcji serów topionych. Premysl Spożywczy. 5:42–44.

Salek, R. N., M. Černíková, G. Nagyová, D. Kuchař, H. Bačová, L. Minarčíková, and F. Buňka. 2015. The effect of ternary mixtures composition containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. Int. Dairy J. 44:37–43. https://doi.org/10.1016/j.idairyj.2014.12.009.

Weiserová, E., L. Doudová, L. Galiová, L. Žák, J. Michálek, R. Janiš, and F. Buňka. 2011. The effect of combinations of sodium phosphates in binary mixtures on selected texture parameters of processed cheese spreads. Int. Dairy J. 21:979–986. https://doi.org/10.1016/j.idairyj.2011.06.006.

Richardos Nikolaos Salek

Faktory ovlivňující funkční vlastnosti tavených sýrů

Factors affecting the functional properties of processed cheeses

Habilitační práce

Vydala Univerzita Tomáše Bati ve Zlíně,

nám. T. G. Masaryka 5555, 760 01 Zlín.

Publikace neprošla jazykovou ani redakční úpravou.

Rok vydání 2021