Tomas Bata University in Zlín
Faculty of Technology

Doctoral Thesis

Selected properties of dairy model systems containing ternary mixtures of phosphate and citrate salts

Vybrané vlastnosti modelových systémů mléka obsahujících ternární směsi fosforečnanových a citronanových solí

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Study subject:	Food Technology 2901V013
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Zlín, February 2017

Vydala **Univerzita Tomáše Bati ve Zlíně** v edici **Doctoral Thesis.**
Publikace byla vydána v roce 2017.

Klíčová slova: *přírodní sýr; tavený sýr; ternární směsi; fosforečnanové a citrátové soli; stupeň zralosti; skladování*.

Key words: *natural cheese; processed cheese; ternary mixtures; phosphate and citrate salts; maturity degree; storage*.

ABSTRACT

The main aim of the current thesis was to evaluate the combined effect of the composition of ternary mixtures of emulsifying salts [consisting of disodium hydrogen phosphate (Na_2HPO_4; DSP), tetrasodium diphosphate ($Na_4P_2O_7$; TSPP), sodium salt of polyphosphate with a mean length $n \approx 20$ (P20) and trisodium citrate ($C_2H_5Na_3O_7$; TSC)] on selected functional properties (textural and rheological) of model processed cheese samples manufactured from different cheese matrices and with different degrees of maturity during storage. The above-mentioned emulsifying salts were applied into four types of ternary mixtures (DSP:TSC:P20, DSP:TSPP:TSC, TSC:TSPP:P20 and DSP:TSPP:P20). The total concentration of the ternary mixtures was 3 % (w/w) of the total weight of the melt. Moreover, the selected cheese matrices (applied as the basic raw material) with/without different degrees of maturity were: Edam (7 weeks of ripening at 10 ± 2 °C), Swiss-type (4, 8, 12, 15 weeks ripening of at 10 ± 2 °C), Mozzarella-type (0, 2, 4 weeks of storage at 6 ± 2 °C) cheeses, respectively. Furthermore, the production of the samples was designed to achieve final products with 40 % (w/w) dry matter content and 50 % (w/w) fat in dry matter content (in cases of Edam, Swiss-type cheeses, respectively). In case of Mozzarella cheese, the production of the samples was designed to achieve end-products with 35 % (w/w) dry matter content and 50 % (w/w) fat in dry matter contnet. Moreover, a secondary objective of the study was the evaluation of the casein micelles dispersion in model milk samples, composed of skimmed milk powder and teranry mixtures of phosphate and citrates salts. The study was subdivided into four experimental stages. During the first experimental stage, model milk samples (consisting skimmed milk powder and ternary mixtures of phosphate/citrate emulsifying salts) were developed and their optical density was evaluated. Furthermore, during the second stage, model processed cheese samples were manufactured from Edam cheese with adjusted (target values within the interval of 5.60 – 5.80) and non-adjusted pH values. The developer samples were evaluated in terms of texture profile analysis (hardness, relative adhesiveness, cohesiveness) during a 30-day storage period. Each combination of ternary mixtures resulted in 26 variants in straggered proportions in steps of 20 % and with some selected 50:50 ratios (26 variants × 4 types of ternary mixtures of emulsifying salts × 2 batches = 208 lots). In addition, during the third stage, Swiss-type cheese was applied for the production of the samples. From the results obtained from the second stage, 12 reciprocal percentage ratios of ternary mixtures were selected (12 ratios × 4 types of ternary mixtures emuslifying salts × 2 batches = 96 lots). The pH of the samples was adjusted (target values within the interval of 5.60 – 5.80) and the samples were evaluated (textural and rheological analyses) during a 60-day storage period. Moreover, during the fourth experimental stage, model processed cheese samples from Mozzarella-type cheese were manufactured and analysed in simiral way as the samples manufactured from Swiss-type cheese. Finally, for all the processed cheese samples basic chemical analysis (dry matter content and pH value determination), texture profile analysis and rheological measurments (except samples made from Edam cheese) were performed (after 2, 9, 30 days after the production day – Edam cheese; after 2, 9, 30, 60 days after the production day – Swiss-type, Mozzarella-type cheeses, respectively).

ABSTRAKT

Hlavním cílem této dizertační práce bylo vyhodnotit kombinovaný účinek ternárních směsí tavicích solí [skládajících se z hydrogenfosforečnanu disodného (Na_2HPO_4; DSP), pyrofosforečnanu sodného ($Na_4P_2O_7$; TSPP), sodných polyfosforečnanů s délkou řetězce $n \approx 20$ (P20) a citronanu trisodného ($C_2H_5Na_3O_7$; TSC)] na vybrané funkční vlastnosti (texturní a reologické) modelových vzorků tavených sýrů, které byly vyrobeny z odlišných druhů matric v různém stupni zralosti. Výše zmíněné tavicí soli byly aplikovány ve specifických ternárních směsích (DSP:TSC:P20, DSP:TSPP:TSC, TSC:TSPP:P20 a DSP:TSPP:P20). Konečná koncentrace ternárních směsí byla 3 % (w/w) z celkové hmotnosti tavené hmoty. Vybrané sýrové matrice, nezralé nebo v určitém stupni zralosti, byly: Eidamská cihla (zralost 7 týdnů při 10 ± 2 °C), sýr švýcarského typu (po 4, 8, 12, 16 týdnech zrání při 10 ± 2 °C), Mozzarella (0, 2, 4 týdnů skladování při 6 ± 2 °C). Postup výroby byl navržen tak, aby konečné modelové vzorky vykazovaly 40 % (w/w) sušiny a 50 % (w/w) tuku v sušině (v případě vzroků sýru Edam a sýru švýcarského typu). V případě sýru Mozzarella byla sušina výrobku nastavena na 35 % (w/w) a obsah tuku v sušině na 50 % (w/w). Dalším předmětem zkoumání bylo vyhodnocení stupně disperze kaseinových micel v modelových vzorcích mléka, které byly tvořeny sušeným odstředěným mlékem a ternárními směsmi fosforečnanových a citronanových solí. Experimentální část práce byla rozdělena do čtyř fází. Během první fáze byly připraveny modelové vzorky mléka (sušené odstředěné mléko, ternární směsi fosforečnanů a citrátových solí), u kterých byla vyhodnocena optická hustota. V druhé fázi byly vyrobeny modelové vzorky tavených sýrů z Eidamské cihly s úpravou pH (cílová hodnota 5,60-5,80) a bez úpravy, které byly následně vyhodnoceny texturní profilovou analýzou (tvrdost, relativní lepivost, kohezivnost) během třicetidenní skladovací doby. Každá kombinace ternárních směsí vytvořila 26 variant v rámci různých procentuálních zastoupení (26 variant × 4 typy ternárních směsí tavicích solí × 2 výroby = 208 vzorků). Během třetí fáze experimentu, byl jako matrice pro výrobu modelových vzorků využit sýr švýcarského typu. Z výsledků získaných z třetí fáze experimentální části bylo vybráno dvanáct poměrů ternárních směsí (12 poměrů × 4 typy ternárních směsí tavicích solí × 2 výroby = 96 vzorků). Hodnota pH vzorků byla upravena na cílovou hodnotu 5,60 – 5,80 a vzorky byly vyhodnoceny (texturní a reologická analýza) během 60 denní doby skladování. Během čtvrté fáze experimentální části práce byly vyrobeny modelové vzorky tavených sýrů ze sýru Mozzarella, u kterých proběhla výroba stejným způsobem jako u vzorků ze sýru švýcarského typu. U všech modelových vzorků byla v odběrových časech prováděna základní chemická analýza (stanovení sušiny a hodnoty pH), texturní profilová analýza a reologická měření (kromě vzorků vyrobených z Eidamské cihly), které byly u modelových vzorků tavených sýrů ze sýru Edam zjišťovány po 2, 9, 30 dnech od výroby; u vzorků ze sýru švýcarského typu a ze sýru Mozzarella po 2, 9, 30, 60 dnech od výroby.

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

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincerest gratitude to my supervisor, doc. Ing. František Buňka, Ph.D., for the continuous support during my Ph.D. study and related research, for his guidance, motivation, encouragement and neverending patience. Moreover, wholeheartedly I appreciate all his contributions of time, ideas and funding to make my Ph.D. experience productive and stimulating.

Besides my superivisor, my great thanks also belong to both my consultants, prof. Lubomir Lapčík and Mgr. Martina Bučková, Ph.D., for their support, encouragement and valuable reccomendations.

Furthermore, I would like to extend my thanks to my friends, colleagues and the technicians of the Department of Food Technology at Tomas Bata University in Zlín, for their help and technical support.

I would also like to express my deepest gratitude to my family for their motivation, support and understanding through this „path“ I chose.

Last but not least, many thanks goes to the Tomas Bata University in Zlín, Faculty of Technology, for giving me the opportunity of doctoral studies. The current thesis was kidnly supported by the internal grants of Tomas Bata University in Zlín (IGA/FT/2013/010, IGA/FT/2014/010, IGA/FT/2015/004 and IGA/FT/2016/003).

CONTENTS

1. THEORITICAL BACKGROUND

1.1 Current state of the examined topic

Nowadays, the application of different natural cheeses as food ingredients has increased due to the elevated interest of consumers. Furthermore, for the past few decades in the Czech Republic, processed cheese is an important sector of the local dairy industry. Hence, this category of dairy products is very popular among the Czech consumers, which can be undoughtely confirmed by the high per capita consumption. Specifically, in 2014 the per capita consumption of processed cheese in the Czech Republic was 2.1 kg. Moreover, the total per capita cheese consumption was 12.8 kg per head (in year 2014), and thus it can be concluded that processed cheese consumption represented a noteworthy amount. However, this phenomenon is considered to be something very rare, since in most of the European countries is not observed in such emphasis (exception is Ireland). In addition, factors that could explain the continued growing popularity of processed cheese in the Czech Republic could be:

- their versatility as food, offering a wide range in flavor, texture, size and shape of the end product;
- their convinience of application in the food service sector and the home, combined with prolonged shelf-life;
- their nutritive value, mainly as calcium and protein source;
- their relatively low cost in comparison to most of natural cheeses [1 – 5].

1.2 Historical background

Natural cheese varieties are known and appreciated as a valuable food component since ancient Roman times (more than 2,000 years ago), processed cheese could be characterised as a "new-born" dairy product, originating in the 20th century, though based on natural cheese [6]. The production of processed cheese started in Europe and might be dated near to the early 1890s. Moreover, it may be recommended, that the concept of processed cheese originated from the search of new strategies for a sophisticated manufacture technique of cheese, in order to extend the shelf-life of natural cheese, bypassing traditional preservation methods; such as air-drying or smoking. Likewise, another possible reason could be the desire to develop a new dairy product characterized of milder taste and more stable [6, 7]. According to Carić & Kaláb [1] the idea of processed cheese stemmed from a typical Swiss traditional dish called "*Fondue*", for which natural cheese is melted (together with application of heat) in the presence of wine, in which tartrate from the wine had an emulsifying effect. However, Jan Hendrikzoon was the first who established a thermal treatment for canned Gouda cheese in 1899 [8]. 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 to achieve a stable but modified final product [6]. 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 attempt, natural Emmentaler cheese was applied to produce a heat-treated cheese (known as "*Schachtelkäse*") by the addition of sodium citrate as an emulsifying salt. About the same period in the USA (1917), independently, James Lewis Kraft, an enterpreuer, 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 [6, 7, 9 – 11].

1.3 Processed cheese clasification

Processed cheese is a multicomponent dairy complex system; described as stable oil-in-water emulsion [12 – 14]. In physico-chemical way, processed cheese could be characterized as a dispersion of fat droplets in a concentrated, gelled protein network [15]. Therefore, the multilateralism of the system derives from that it contains plethora of interacting components and high water content [16]. Hence, its matrix is formed by blending shredded cheese (of different types and maturity degrees) in the presence of emulsifying salts (mainly sodium salts of phosphates, polyphosphates and/or citrates), under partial vacuum, constant stirring and upon heating; resulting in the constitution of a homogeneous and smooth mass with desired properties [4, 11 – 13, 17, 18]. Nevertheless, the application of heat during processing inactivates the starter culture microorganisms and other bacteria, including also enzymes, all present in natural cheese; resulting in the shelf-life extension of the final product [6, 7]. According to the decree 77/2003 Collection of Laws [20], edited by the Czech Ministry of Agriculture, laying down 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 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 lactose, must be designated as processed cheese product (*tavený sýrový výrobek* – in Czech).

The decree mentioned above categorizes processed cheese into two main groups:

- High-fat processed cheese (fat in dry matter contetn is at least 60 % w/w)
- Low-fat processed cheese (fat in dry matter content not exceeding 30 % w/w)

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, processed cheese spreads. Moreover, additional subcategories are processed cheese slices and smoked processed cheese [7].

International standards for proceesed cheese are defined by the Codex Alimentarius Commission; these and some selected characteristics of the three main processed cheese categories are summarized in Table 1.

Tab. 1: Selected characteristics of processed cheese types [7].

Type of product	Cooking temperature (°C)	Composition	pH
Processed cheese block	71 - 80	Moisture and fat contents to the legal limit for natural cheese	5.6 - 5.8
	80 - 85		5.4 - 5.6
	74 - 85		5.4 - 5.6
Processed cheese food	79 - 85	≤ 44 % moisture, < 23 % fat	5.2 - 5.6
Processed cheese spread	88 - 91	40 – 60 % moisture, ≥ 20 % fat	5.2
	85 - 98		
	90 - 95		

1.4 The role of emulsifying salts

Emulsifying salts (so called emulsifying agents) are more commonly known in the dairy industry as "melting" salts and are of crucial importance in processed cheese manufacture. These salts are ionic compounds made up of monovalent cations (sodium, potassium) and polyvalent anions (phosphates, polyphosphates and/or citrates) [13, 20]. The most commonly applied emulsifying salts are sodium citrates, sodium hydrogen monophosphates, diphosphates and polyphosphates. Nowadays, 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) [13, 15]. However, emulsifying salts cannot be characterized as true emulsifiers (low molecular-weight surfactants) as they are not surface-active. 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 (caseins). [6, 7, 15, 21, 22, 23]. In addition, caseins have the ability of binding calcium, which has the effect of reducing their solubility and thus their emulsifying properties. Emulsifying salts have a 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 „weak“ 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 soft 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 cheese with smaller droplets of fat [15]. According to the European Union legislation (Commission Regulation No. 1129/2011) the maximum permitted level of emulsifying salts in processed cheese production is 20,000 mg/kg (or mg/L), as appropriate. In general, depending on the nature of the applied salt, are added in an amount from 1 – 3 % (w/w) [6, 7]. 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 [9, 10, 24]. Moreover, pH adjustment and calcium sequestering are among the main functions of emulsifying salts during processed cheese production. They possess the ability to sequester calcium from the casein matrix; by exchanging sodium ions, resulting in the conversion of insoluble calcium paracaseinate into soluble sodium paracaseinate [1, 13, 25, 26]. Within the matrix sodium paracaseinate acts as an emulsifier stabilizing the oil-in-water interface. The control and stabilization (upward adjustment) of the pH level and an influence on the formation of the final product structure after cooling are some of the additional properties of emulsifying salts [1, 27, 28]. The application of the correct blend of emulsifying salts increases (due to their buffering capacity) the pH from typical values of ~4.6 – 5.5 in the natural cheese to values ranging from 5.6 to 6.2 in the spread-type 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. Additionally, the high water-binding capacity of the paracaseinate enhances the formed emulsion stability as it leads to high viscosity of the aqueous phase and thus a reduction in the collision frequency of the emulsified particles is observed [4]. However, not all emulsifying salts have the same calcium ion-exchange ability (affinity). The phosphate ion-exchange ability increases with increasing P_2O_5 content in the following order: citrates ≈ monophosphate < diphosphate < triphosphate < polyphosphate [1, 29, 30]. El-Bakry et al. [28] and Mizuno and Lucey [31] stated that trisodium citrate presents better calcium chelating ability and casein peptisation properties than do sodium monophosphates and diphosphates. Moreover, on a mole for mole basis, phosphates have a higher calcium chelating ability than citrates [27] The ion-exchange ability increases with the extending length of the polyphosphate chain; it results in better casein dispersion and better fat emulsification and water stabilisation, which leads to better crosslinking of the matrix network in the final product [13, 28, 32 – 34]. The 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 [1, 20]. Concretely, by the heating (to temperatures applied in processed cheese production) and stirring of the used natural cheese together with the other ingredients of the formula (in the absence of emulsifying salts); would 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 (a defect known as oiling-off) [1]. Furthermore, another important property of emulsifying salts is their bacteriostatic effects. Manufacture of processed cheese normally involves processing temperatures (70 – 95 °C) which are lower than that used for sterilization. Hence, processed cheese might contain viable microbial spores (especially of *Clostridium* genus) originating mainly from the applied raw materials. Nevertheless, spores germination during storage often can lead to serious technological problems (or defects) such as blowing of the packaging material (when cans are used), protein putrefaction and off-flavors. The bacterial spoilage, in general, can be eliminated by preservatives addition. Hence, some emulsifying salts possess noteworthy bacteriostatic properties. Concretely, polyphosphates inhibit many microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium sporogenes* and various *Salmonella* spp.). Moreover, monophosphates and diphosphates have been found to inhibit the growth of dangerous for public health *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 [11, 35, 36, 37, 64].

1.5 Selected factors affecting the consistency of processed cheese

The consumer's criteria for processed cheese selections are primarily based on its sensory characteristics. In addition to the organoleptic profile of processed cheese another important factor is its consistency. Processed cheese products can be found in the global market in various forms; from blocks and slices to spreadable or even liquid products [38, 39]. The consistency of processed cheese can be affected by many factors; including type, composition and chemical profile of the used cheese (dry matter, fat, protein, calcium ions contents and maturity degree); type and concentration of emulsifying salts; presence and ion concentration (especially calcium, sodium and potassium); other optional dairy and non-dairy ingredients; pH of the mass to be melted; processing and storage conditions (processing and storage temperature, stirring speed, time and temperature of fusion, cooling rate) and the possible use of some hydrocolloids [4, 12, 13, 16, 30, 40 – 47].

1.5.1 Effect of the natural cheese

The main raw material on processed cheese production is a natural cheese (or natural cheeses) of different types and various degrees of maturity, significantly affecting the final product's desired organoleptic and rheological properties [12, 36, 48, 49]. Furthermore, natural cheeses with visual defects can be used during the processed cheese production (mainly mechanical defects) or that which do not meet the requirements for fat and dry matter contents. However, on the other hand, natural cheeses with microbial defects should be never used, specially, cheeses containing spore-forming microorganisms or molds, because of possible presence of mycotoxins [12]. The propert selection of natural cheese can affect not only textural, but also chemical and functional properties of the final processed cheese [1, 30, 50 – 52]. In addition, among the factors (of the used natural cheese) influencing the quality of the final product are; mainly pH value, calcium content, fat content and the amount of intact casein [40, 53]. In general, if a natural cheese of high pH values has been applied in the production of processed cheese, the resultant product will have higher values of hardness and will be less spreadable, compared to processed cheese samples made from cheese of lower pH values [50]. With the increasing maturity degree of the natural cheese the content of intact casein and hardness of the final product are decreasing, resulting in a more spreadable product [50, 54]. Hence, for processed cheese products characterised of accepted spreadabilty and of fine consistency, more mature natural cheese (level of intact casein 60 – 75 %) is preferred. Therefore, natural cheeses with high degree of maturity "carry" significant amounts of sensory active substances and the flavor of the final processed cheese product is more intense [1, 46, 50]. Nevertheless, the application of very mature natural cheese can lead to important consistency defects of the final product (graininess, incomplete emulsification of the present fat). In real industrial conditions, the manufacturers are using mixtures of cheeses with various degrees of maturity [41].

1.5.2 Effect of water addition, dry matter content and fat in dry matter content

The main reason of water addition during processed cheese manuafacture is to achieve the desired level of dry matter content. The amount of the added water can affect the consistency (smoothiness, spreadability) of the end product and ultimately can reduce the cost of its production. Moreover, the presence of water facilitates the release of calcium (followed by the emulsifying salts addition), hydration and dispertion of proteins present in the cheese matrix [12]. In addition, processed cheese with increased levels of moisture depict a more liquid-like „character" and thus, moisture acts as plasticizer, resulting in weaker protein matrix formation [55]. During processing the unfolded protein molecules are hydrated and bind water. The more the quantity of the added water, the more the swelling of the proteins occurs and the greater their expansion is because of lessened attractive forces. The increased solubility of protein molecules results in increased sequestering ability of emulsifying salts. Moreover, during cooling, water-molecules are immobilized within the protein matrix and weaken the structure of the final network [55]. The decrease in dry-matter content has a similar effect on the final product as the decrease in temperature and pH levels, respectively. However, a serious disadvantage of processed cheese containing high amount of water content is its lower shelf-life [56]. Another important factor affecting the consistency of processed cheese is the relation between dry matter and fat in dry matter contents, respectively. Moreover, stiffer consistency of the product could be achieved by maintaining constant the fat content and by increasing the dry matter content. Conversely, in order to achieve a more spreadable final product the fat content should be increased whereas the dry matter content should be maintained at constant levels [1, 6, 7, 57]. According to the findings of Guinee & O'Callaghan [5], the reduce of fat content in processed cheese can lead in product's hardness increase and decrease in flowability. Moreover, similar findings confirming the statement above was previously reported in the work by Dimitreli & Thomareis [55], processed cheese with increased levels of fat exhibit a more liquid-like behavior. Furthermore, the amount and the type of the added fat can significantly influence the flavor, structure and textural properties of processed cheese. In addition, during the production of processed cheese, particularly during stirring and heating, a breakdown of the fat globules can occur, resulting in the formation of fat globules of various sizes, which are subsequently emulsified by the combined action of the added emulsifying salts and present proteins (caseins) [58]. Moreover, the emulsification degree plays a very important role in the proper structure formation of processed cheese. The increasing degree of emulsification process leads to fat globules diameter reduction and to their increasing amount. Hence, resulting in higher amount of bindings between the proteins, thus the resultant processed cheese could be characterised of greater hardness and gumminess, but lower relative adhesiveness. Additionally, the size of the fat globules may also affect the color of processed cheese. Concretely, the more smaller the fat globules are the more light can be dispersed and the products color is whiter. The added fat is usually in the form of butter, cream or anhydrous milk-fat [1, 58].

1.5.3 Effect of the pH value of the processed cheese

Generally, the pH value of the processed cheese is influenced by the pH and the type (variety) of the utilized natural cheese during the manufacture proces, the concentration and type of the added emulsifying salts. According to Brickley et al. [40], Piska & Štětina [47] the more mature natural chees is used (in the production of processed cheese) a final product with higher pH value can be expected. Moreover, emulsifying salts also have the ability of pH adjustment (mainly upward trend). A typical, pH range for natural cheese is 5.0 – 5.5 and after the addition of emulsifying salts the pH is shifted to 5.6 – 6.1 [4, 16, 30, 41, 59]. The pH level adjustment of the processed cheese will increase the negative charges of the present casein and thus the electrostatic repulsion of the casein matrix, resulting in a more "open" and "free" network, higher water binding capacity and better emulsifying properties of the proteins. Additionally, the emulsifying salts ability to isolate calcium i salso increased, leading to more effective dispersion of casein. Finally, the pH increase leads to the reduce of hydrophobic interactions between the individual casein molecules and their electrostatic interactions are increased [31, 41, 59, 60]. However, processed cheese with low pH value (< 5.4) a stiffer consistency could be expected [56, 61]. In other words, when the pH of the processed cheese approaches the isoelectric point of caseins (pI ≈ 4.6), the higher are the interactions between proteins and a harder final product could be formed [12, 46, 56]. On the contrary, the higher the pH of the processed cheese (> 6.1) a decrese in electrostatic interactions and an increase of the proteins charge will occur, resulting in protein subsequent repulsion. Hence, the end product is ultimately reflected in excessively soft consistency [41, 56].

1.5.4 Effect of processing conditions

Between the factors which can influence the consistency and the functional properties of processed cheee during processing are; temperature, time and stirring speed [12, 24, 25, 30, 42]. Therefore, with the increasing processing temperature the hardness of the processed cheese increases. However, if the processing temperature exceeds the limiting tempertaure of 95 °C, the hardness of the product will decrease, due to insuficient casein hydration or due to increased hydrolysis of the added emulsifying salts and increased aggregation of the present proteins [1]. On the other hand, Swenson et al.[62] reported that with the decreasing processing temperature the more stiffer processed cheese could be expected. Hence, a probable explanation between the diversities among the results of the studies mentioned before could be the differences between; the applied emulsifying salts, duraration of the processing and profile of the designated end-product. Furtermore, the duaration of the processing can also influence the textural properties of processed cheese. Additional, processed cheese with stiffer consistency was observed with the prolonging duration of the processing period [42]. The stirring speed during processed cheese production may also affect its textural characteristics. With the increasing stirring speed the viscosity of the melt is rising while the meltability and flowability are decreasing. According to Kapoor & Metzger [25] a probable explanation could bet the fat globules decreasing size affected by greater stirring intensity. The manufacture protokol of processed cheese also includes the cooling of the hot molten mass, thus this last step can affect the properties of the end product. Moreover, according to previous scientific studies it has been found that slow cooling rate could result in processed cheese characterised of higher levels of hardness and vice versa [25, 47].

1.5.5 Effect of storage period and temperature

The consistency of processed cheese i salso influenced during storage (usualy, at 4 – 8 °C). Moreover, during storage a gradual soldification of processed cheese occurs [17, 20, 26, 34, 63]. The polyphosphates are gradually hydrolysed into simple phosphates. The decrease of the amount of monomers in the linear chain of polyphosphates results in a decrease in the afinity for calcium ions, which in turn leads to a release of these ions from the emulsifying salts and to their subsequent involvment in the protein matrix. However, as a result of the phenomenon described above is an increase in hardness of processed cheese during storage [1, 57, 61]. Moreover, higher storage temperatures (25 – 30 °C) could also lead to hardness rising [32, 63]. On the other hand, storage at low temperatures (< 4 °C) could lead to the crystallization of emulsifying salts, certain amino acids or lactose. Nevertheless, long-term storage of processed cheese can also affect its microbiological quality and thus its shelf-life. The shelf-life, in general, of stiffer processed cheese is higher than that of more spreadable (or softer) cosnistency, since the latter contain higher amount of water [47, 63 – 66].

2. MANUFACTURE TECHNOLOGY OF NATURAL CHEESE

2.1 Natural cheese – an overiew

Cheese is the generic name for a group of fermented milk-based food products, produced throughout the world (about 500 varieties) in a great diversity of flavors, textures and shapes. Furthermore, cheese could be characterized as a complex dairy product; a concentrated protein gel, which occludes fat and moisture, that has been pro-duced since prehistoric times (estimated approx. 5000 – 6000 B.C.) [1, 6, 7, 9, 11, 67]. Generally, it is accepted that milk belongs among food of high importance for the human kind. Probably the concept of cheese rooted from the desire to prolong the shelf-life of milk. Hence, transforming milk into a stable product with extended shelf-life and thus, maintaining the biological value of milk [11]. Moreover, manufacture of cheese is a typical example of food preservation. Concretely, preservation of the most important constituents of milk (fat, proteins) as cheese exploits two of the classical principles of food preservation; lactic acid fermentation and reduction of water activity by water removal and NaCl addition. The establishment of a low redox potential and antibiotics secretion by starter microorganisms contributes to the storage stability of cheese. Additionally, cheese is the most diverse group of dairy products and is, arguably, the most academically interesting and challenging. While many dairy products (if are properly manufactured and stored) are biologically, biochemically and chemically stable, in contrast, cheeses are biologically and biochemically active; and consequently undergo changes in flavor, texture and functionality during storage [9].

2.2 Cheesemaking technology

Cheesemaking is a rather simple process in itself, but it involves complex chemical and physical phenomena. It is essentially a concentration process, beginning with the coagulation of the main milk protein, casein and then proceeding with manufacturing steps designed to control the chemistry of the casein molecules. The physical or rheological characteristics of cheese are governed by interactions between casein molecules [68]. Factors that influence these interactions are the following: pH; dissolution of colloidal calcium phosphate; proteolysis; temperature; cheese composition (in particular, casein content and distribution of moisture and fat). Whilst each factor can be considered independently, it must also be considered in context with all the other factors. In turn, these physical and chemical parameters dictate the manufacturing process. The key elements in producing the desired cheese are: (a) milk composition (because this, in part, determines cheese composition) and (b) the rate and extent of acid development during manufacture (because this influences the loss of moisture, the extent of dissolution of colloidal calcium phosphate and the lowest pH obtainable in the cheese, all key factors in deciding the texture of the finished cheese) [6, 7].

2.2.1 Milk selection

The chemical composition of milk (especially; the content of fat, protein, calcium and pH value), that will be applied in cheesemaking process has a strong impact on the composition of the final product. Moreover, the milk's chemical composition is influenced by several factors; including the species, breed, individuality, nutritional status, health and lactasion stage of the producing mammal (animal). The milk should be free of chemical taints and free fatty acids, which could lead to undesirable off-flavors in cheese, and free of antibiotics, which could also cause bacterial cultures inhibition. Furthermore, the milk should be of good microbiological quality, as contaminating bacteria would concentrate in cheese curd and may cause potential quality defects, or even worse, public health problems [1, 9].

2.2.2 Standardization of milk

In order to produce a cheese of consistent composition, the first condition is necessary to start with milk of consistent composition. However, to avoid the problem of producing a cheese of different characteristics is critical to maintain milk in consistent composition. Most countries have laws governing two aspects of the composition of cheese; minimum fat in dry matter and maximum moisture levels. Nevertheless, not all cheese varieties have legislation standards. The latter serve to ensure a certain degree of composition continuity for a given variety, even though produced by different manufacturers, and to protect both the consumer and the manufacturing process. These setting standards also can vary from country to country. The composition of milk which will be subjected to cheese production can be modified (standardized) by adding milk solids (condensed milk, skim milk, milk powder, ultrafiltration retentate). The ratio of casein to fat determines cheese compositon in terms of the amount of fat in the total solids portion of cheese. The total amount of casein and fat determines the yield potential of milk. In addition, during the coagulation of milk by rennet and the subsequent processing of the formed coagulum, calcium plays major role. A common practise in cheesemaking is the addition of calcium in the form of $CaCl_2$ to cheese milk. Another critical factor affecting the whole process of cheese production is the pH of the milk. The pH is inadretently adjusted by the addition of starter cultures , which decreases the pH value of the milk immediately by about 0.1 units [6, 7, 9, 11].

2.2.3 Heat treatment of milk

The reason that selected thermal treatment is applied to cheese milk is mainly to ensure the microbiological quality and hence, to avoid public health problems and inconstant end-products. The fermentation of the milk itself will often inhibit the growth of undesirable bacteria (pathogenic/spoilage), whereas in order to avoid disadvantageous bacterial enzymatic activities, it is preferred that the contaminating flora should be removed. The inactivation is commonly performed by heat treatment (pasteurization) of milk (at a minimum temperature of 72 °C for at least 15 s, or any combination of time and temperature in order to achieve an equivalent effect) using plate heat-exchangers. Pasteurization of milk is based on a 9-log destruction of *Coxiella burnetti*. Nevertheless, some spores in particular may survive the thermal treatment and might cause problems during subsequent ripening of cheese, particularly spores of *Clostridium tyrobutyricum*. The named spores during cheese maturation grow and produce H_2 and CO_2, which may cause „blowing“ of the cheese as well as butyric acid and other fermentation products resulting in unpleasant flavor or defects [19, 67, 69]. During traditional cheesemaking all cheese was produced from raw milk, until the 1940s, when the pasteurization process of milk became widespread. However, even nowadays, significant amounts of unique varieties of cheese are made via using raw milk (Swiss Emmental, Gruyère, Parmigiano-Reggiano). Cheeses made from unpasteurized milk shall be held for 60 days at a temperature not less than 1.7 °C [91]. It is though that pathogens will die out during this storage period because of acidic conditions in cheese and due to the growth of non-starter lactic acid bacteria [69]. The need of milk with longer shelf-life resulted from the development of the dairy industry, as the production of cheese significantly grew and larger amounts of milk were stored for longer periods. In addition, mainly 4 alternatives technological operations for lessening the number of microorganisms in milk exist; treatment with H_2O_2, activation of the lactoperoxidase-H_2O_2-thiocyanate system, bactofugation and microfiltration [9]. After heat treatment milk is cooled to the temperature conductive for optimal starter activity into specially designed vessels, named vats.

2.2.4 Clotting (gelation) of milk – formation of the cheese curd

At this particular stage of cheesemaking the milk is converted into cheese curd; a process involving three principal operations; acifidication, coagulation (precipitation) and dehydration. After the standardization, thermal treatment or other proper applied treatment of milk have been successfully performed, milk is transferred into tanks (of various shapes, constructions and volumes), where the clotting will take place. Acidification is achieved by the addition of lactic acid bacteria (biochemical acidification) or by the direct acidification (chemical) using an acid (usually lactic or hydrochloric) or an acidogen (usually gluconic acid-δ-lactone). When biological acidification is applied the production of lactic acid is a product of lactose fermentation by lactic acid bacteria. Moreover, direct acidification method is commercially applied in the manufacture of Cottage cheese and Feta-type cheese from ultrafiltered milk and Mozzarella cheese. Furtermore, direct acidification has the advantage of a more controllable proces than biochemical acidification. However, the addition of bacteria has key functions during cheese ripening. The ultimate pH of the curd for most hard cheese varieties lies in the range of 5.0 to 5.8, while for soft cheese varieties it is within the range of 4.3 – 5.0 [70]. Coagulation is the essential step in the manufacture of all cheese varieties and is in close relation to caseins of milk in order to form a gel which will enclose the present fat. Three ways exist by which the caseins can be coagulated and the cheese variety to be produced dictates the method employed. Concretely, coagulation may be achieved by: (i) limited proteolysis using selected proteinases (rennet), (ii) acidification to pH 4.6 and (iii) acidification to a pH level higher than 4.6 (≈ 5.2) in combination with heating to 90 °C, (iiiiv) precipitation of the caseins by 40 % (v/v) ethanol solution at pH 6.7 or at lower concentrations if the pH value is reduced. The majority of cheese varieties (representing about 75 % of the total production) are rennet-coagulated. In this case the added coagulating enzyme, destabilizes the casein micelles colloidal suspension and thus makes them aggregate to form a gel. Moreover, the first coagulants were derived from calf stomachs (called rennets), whereas today there are many sources for coagulants, including plants and fungi. Additionally, the characteristic rennet enzymes are chymosine and bovine pepsin, which can be added either as purified standardized enzyme or as an extract of the fourth stomach of calves. The optimal temperature for rennet activity is within 29 – 33 °C; normaly renneting takes 20 – 30 min. The direct acidification method is mainly used for Cottage, Cream cheese, Fromage frais and some varieties of Quesco blanco, when low pH is used to cause the caseins to form a clot. In this case, the destabilization process is purely physical. The lessen pH value reduces the repulsive chargé between the casein micelles to a point at which they aggregate and start forming a gel or clot. The third coagulation method is a combination of acid and high temperature to cause precipitation of both casein and whey proteins to form a clot. These cheese varieties are called acid-heat-coagulated cheeses and are of relatively minor importance. They are produced from whey or a blend of whey and skim milk. In this cheese category the most known members are Riccota and related varieties, Queso Blanco, Anari and Manouri cheeses [9, 67, 71]. Regardless of the coagulation method applied, milk fat is surrounded by the present casein as the coagulum forms and is trpped together with serum (whey). The serum contains water-soluble components (lactose, whey proteins, minerals). The subsequent processing steps are used to remove the serum coagulum. However, these steps differ, and are based on the method that the casein was coagulated [71].

2.2.5 Cutting of the formed cheese-curd

Renneting process is followed by the cutting of the curd. This is a very important and effective operation for whey release from the coagulated casein. By the moment that the coagulum will reach the required level of firmness, it is then very carefully cut and separated into small pieces (called cheese grains), by special equipment supplied with knife blades or wires. This cutting tools can be designed in different ways. Internally, the casein molecules are rearranging and „tightening“, and thus the curd immediately begins to shrink and expel whey. This process is known as „syneresis“ and results in the squeezing of the whey from the casein network [11].

2.2.6 Stirring, heating (cooking or scalding) and whey draining (dewheying)

After the cutting is performed, the curd must be handled gently, so that the formed cheese grains are not broken apart, leading to fat loss in the whey. Stirring is applied to asist the proces of „syneresis“, in a way that the mechanical effect (of stirring) will cause the collision of the grains, and the resulted pressure will cause the whey to be pressed out of the grains. The current process proceeds for 1 to 2 h. The main purpose of this phase is to regulate the pH and the moisture content of the final cheese. The increase of the final temperature inhibits the growth of the added starter culture, resulting in a higher pH of the cheese. Heating to temperature above 40 °C, is typically known as cooking and heating to temperatures beyond 44 °C, is called scalding. Moreover, the velocity of the heating influences whey expel. The cooking of semihard cheeses made with mesophilic starter cultures (Cheddar, Gouda, Edam) is usually performed at a temperature range 34 – 40 °C; depending on the fat content.

Furthermore, scalding for hard cheese varieties made with thermophilic cultures (Emmental, Gruyère, Comté, Parmigiano Reggiano, Grana Padano) is performed at 50 – 56 °C. The final temperature also has important effect on the later forming curd. The lower the temperature at which the cheese is formed, the more difficult it is to achieve proper pressing and get the cheese grains to stick together. The proces can take about 1 h (for many cheese varieties), although it can vary from 30 to 90 min [9, 67, 71]. The texture, color and flavor of the cheese are affected by the method applied for the separation of the whey from the curd formed. The curd can be placed directly in the molds (in the manufacture of soft cheese) or it can be given further treatment before final molding (in the manufacture of most semihard or hard cheese); depending on the cheese variety to be produced. Whey may be withdrawn directly from the cheese vat. Another possible way to withdraw whey is; by pumping the curd/whey mixture across vibrating or sloping sieves, so that the whey passes through the sieve and the curd grains are separated from the whey [67, 71, 72].

2.2.7 Salting of cheese

Salting is a vital part of the cheesemaking process. Salt (NaCl) has very important roles in flavor enhancement and development, in the control of microbiology, the final cheese pH; giving a balanced, pleasant taste, with desired consistency product. Salt increases the osmotic pressure of the aqueous phase, leading to the dehydration of bacterial cells and killing them or inactivating their growth and thus extending the shelf-life of the product. Moreover, for many cheese varieties, salt also affects the moisture content decrease. Concretely, salt affects the syneresis of the curd, resulting in whey expulsion and thus in reduction of the moisture of cheese, which also affects the activity of microorganisms and enzymes. There are two components to the salting process; the application of salt (salting) and the sunsequent mixing uptake, associated with moisture loss (mellowing). The amount of salt required will vary depending on the type of cheese being manufactured. In general, salt levels in cheese range from ~1.0 % in Swiss type cheese, 1.8 % in Cheddar, to 3 % or more in Feta type cheeses. For most semihard and semisoft varieties, a NaCl concentration of 1.5 – 2.0 % is suitable. On the other hand, for Emmental or other large-eyed cheeses, the salt content is lower (e.g. 0.8 –1.4 %), in order to enhance the growth of propionic acid bacteria. Salting can be proceeded, mainly by 4 ways; by adding salt into the curd before molding, by diffusion of salt into cheese after molding, by immersion in brine and by dry salting [9, 11, 71, 72].

2.2.8 Ripening/maturation of cheese

Cheese can be described as an essential matrix principally, formed by protein, fat and carbohydrates, containing a wide range of enzymes and microorganisms. Their activities are allowing the changes that convert the young (*fresh*) cheese into an end-product with desired properties, mainly via proteolysis, lipolysis and glycolysis. Furthermore, ripening is a highly complex of several phenomena involving changes to the microflora of the cheese, including the death an lysis of the starter cells, development of the adventitious non-starter microflora and in many cheeses growth of a secondary microflora [1]. Moreover, cheese ripening can be a slow process ranging from about 3 weeks (Mozzarella) to 2 or more years (Parmessan and extra-mature Cheddar) [9]. During this period, in general, a very complex group of biological, biochemical and chemical reactions occur; resulting in the development of the characteristic flavor compounds and texture modification. Moreover, the rate of ripening is directly related to the moisture content of the cheese; low-moisture cheeses hav slow ripening rate [9]. Cocretelly, the reactions occuring in the cheese matric during ripening are categorized into 4 major groups: (1) glycolysis of the residual lactose and lactate catabolism, (2) catabolism of citrate, (3) lipolysis and free fatty acids catabolism and (4) proteolysis and amino acids catabolism. The metabolism of lactose to lactate is essential in the production of all cheese varieties. Since cheese curd contains a low level of residual lactose (most of lactose is lost in the whey – draining process), the latter is rapidly metabolized to lactate at the early phases of ripening. The final step in the glycolysis process is the convertion of pyruvate to lactate, which is catalyzed by lactate dehydrogenase. Lactate is the end product of glycolysis which converts 1 mol of lactose to 4 mol of lacate with the production of 4 mol of ATP. Lactate is an important substrate for a range of reactions, with positive or negative impact on the final cheese product. Furthermore, in Cheddar and Dutch-type cheeses, L-lactate is produced by *Lactococcus* strains, which can be racemised to DL-lactate by non-starter lacticic bacteria. The DL-lactate is however, less soluble than L-lactate, resulting in the formation of Ca-D-lactate crystals, which appear as white specks on the surface of the matued cheese. Nevertheless, catabolism of lactate is very important in the production of Swiss-type and mould-ripened cheeses. During the production of Swiss-type cheeses, lactate is catabolized by e. g. *Propionibacterium freundenreichii* subsp. *shermanii* to propionate, acetate, H_2O, and CO_2. Propionate and acetate contribute to the formation of Swiss-type cheese typical flavor. Hence, the CO_2 formed migrates through the curd to points of "weakness", where it collects and forms the large eyes, characteristic for this type of cheeses [1]. Milk fat is essential in the development of flavor of all ripened cheese varieties. Lipids in cheese can undergo hydrolytic or oxidative degradation. However, in most of varieties, lipolysis is rather limited. Major exceptions are Blue and some Italian cheese varieties (Romano and Provolone); in which the fatty acids and/or their degradation products contribute in flavor. Enzymatic activities of lipases of various sources (raw milk, rennet, starter bacteria) contribute to lipolysis during ripening. Concretely, by the lipase enzymes the triacylglycerides of milk can by hydrolyzed into free fatty acids and glycerol. The short chain fatty acids (C_4 – C_{10}) have a sharp, pungent flavor [9, 67]. Levels of fatty acids vary considerably between varieties. Many internal bacterially ripened varieties (Edam, Swiss-type, and Cheddar) contain low levels of free fatty acids (200 – 2,000 mg/kg), on the other hand, Blue cheeses have high levels of fatty acids (30,000 mg/kg). Additionally, fatty acids are precursors for the production of other volatile compounds (fatty acid esters; ethyl esters are more common in cheese, thioesters, fatty acid lactones and methyl ketones) during ripening [1]. Proteolysis is the most complex, in most cheese varieties, also the most significant event occurring during ripening of cheese. Additionally, is very important affecting cheese texture; by hydrolyzing the para-casein matrix, which gives cheese its structure (body) and by increasing the water-binding ability of the curd [1]. The caseins are gradually by rennet proteinase enzymes and by plasmin (an indigenous milk protease), which yields a number of polypeptides. The latter does not affect the flavor of the cheese, however, some hydrophobic peptides may be astringent or may cause bitter taste [67]. These first stages of proteolysis are of crucial importance for the transformation of the structure of cheeses; from a rubber-like to a sliceable consistency. Furthermore, the large polypeptides generated by chymosin (which is trapped in the curd) are degraded further by enzymes from the starter bacteria and adventitious populations of non-starter lactobacilii in cheese. Lactobacilii have proteinases that can degrade α_{s1}- and β-caseins. The above mentioned microorganisms also possess intramolecular aminopeptidases, dipeptidases, carboxypeptidases and endopeptidases, playing a vital role in the production of free amino acids. The latter is characterized as precursors for a range of catabolic reactions which produce many volatile flavor compounds [1, 73 – 77].

2.3 Edam cheese manufacture

Edam cheese (*Edammer kaas* – in Dutch) together with Gouda cheese (*Goudse kaas* – in Dutch) are traditionally the two predominant Dutch-type cheeses, which are mainly produced in the Netherlands [1, 11, 78]. Furthermore, Edam cheese originates from the homonymous area in the province of North Holland and derive its name from a city (Edam, Netherlands) which is famous market for this kind of cheese [11]. According to the Codex standard dor Edam [91], developed by the Codex Alimentarius, Edam cheese is defined as it follows: *Edam is a ripened firm/semi-hard cheese in conformity with the General Standard for Cheese. The body has a near white or ivory through to light yellow or yellow colour and a firm-textured (when pressed by thumb) texture, suitable for cutting, with few more or less round rice to pea sized (or mostly up to 10 mm in diameter) gas holes, distributed in a reasonable regular manner throughout the interior of the cheese, but few openings and splits are acceptable. The shape is spherical, of a flat block or of a loaf. The cheese is manufactured and sold with dry rind, which may be coated. Edam of fl at block or loaf shape is also sold without rind.* Edam cheese is characterised of a semi-hard to hard consistency, and a smooth texture having small eyes (about the size of a pea – diameter between 2 and 10 mm) with a flavor intensity that ranges greatly [9, 11, 78 – 81]. According to the FDA standard of identity for Edam cheese, the latter may be classified as a food prepared by any procedure which produces an end-product (cheese) with a minimum milk fat content of 40 % by weight of the solids, and a maximum moisture content of 45 % by weight [11, 78]. Edam is a mild cheese with a rubbery texture. Traditionally, it is manufactured in a spherical shape and is coated in a red/yellow wax, or plastic coating, however, other shapes may also be found (rectangular blocks, loaf). Moreover, mature Edam should have a fat in dry-matter content of 42 % (w/w) and consequently the milk for cheese production is standardized to 2.5 % (w/w) fat. For the production of Edam cheese a mixture of skimmed evening milk and fresh morning milk is used during the traditional cheese-making procedure. The standardized milk (for its fat:protein ratio in order to control the fat content in the dry matter of the cheese) is pasteurized and mixed-strained with mesophilic starter culture (containing citrate fermenting lactococci and *Leuconostoc* spp.) is added at a level 0.3 – 0.5 % w/v. Frequently used mesophilic dairy cultures during the production of natural Dutch-type cheese include the representatives of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* and *Lactococcus lactis* subsp. *cremoris* [78, 81, 82]. Together with the starter culture addition, the milk coagulation process (approximately at 30 °C) occurs and is enhanced by the action of renneting enzymes (25 – 30 ml/100 l of milk), usually chymosin preparation (calf rennet). Additionally, the milk is often bactofugated to reduce the number of spores of butyric acid bacteria. However, the bactofugation technique may be applied when cheese is made from raw milk. Following starter addition, calcium chloride (0.015 %) is added in order to optimize renneting of the low acid milk. In addition, at the same time, sodium or potassium nitrate (0.015 %) or lysozyme can be added to prevent early blowing of the cheese through gas production by coliforms or *Clostridium tyrobutyricum*. Moreover, annatto or β-carotene can be added at low levels as coloring agents. Thereafter, the formed curd is cut approximately 30 min after the rennet addition. The curd is then gently stirred and 50 % of the whey is removed. Water of 55 – 60 °C is added into the vat to bring the temperature of the curd-whey mixture to 36 °C (mild scalding). Washing of the curd reduces the lactose level and prevents the development of excess acidity during cheese ripening. Once the final scald temperature has been achieved, approximately 30 % of the whey is removed and the curd is pressed mechanically in the vat to encourage it to fuse together. The curd then is transferred into moulds and pressed lightly. Moreover, the pressed curd is immersed in saturated brine (14 – 20 % NaCl content w/w, at 12 – 15 °C) for 2 – 3 days, to obtain a salt content of 2.5 %. The cheeses then are stored in ripening cellars (14 -15 °C at a relative humidity of 85 – 90 %) for the rind to dry off and harden. Edam cheese must be sold after undergoing ripening of 4 weeks at minimum. The ripening period of Dutch type cheeses can range from some weeks or longer, to sometimes for more than a year. Nevertheless, a typical ripening period for Edam cheese lies within the interval of 6 up to 8 weeks [9, 11, 75, 79, 82 – 85].

2.4 Swiss-type cheese manuafcture

Swiss-type cheeses are hard or semihard brine-salted cheeses, of nutty flavor, containing characteristic eyes, resulting from the metabolism of various substances [86 – 89]. This cheese category was originally manufactured in the Swiss German: Emmental (Emmen valley) in Switzerland; their precursors were mountain cheesed. However, there is no general internationally recognized definition of Swiss-type cheeses that could differentiate them among other cheese varieties and there is a lack of consensus regarding the cheese varieties to be included in this category [88, 90]. Emmental cheese is probably the best-known representative of all Swiss-type cheeses around the world, and is frequently referred to simply as Swiss cheese (it comprises cheese with the desired propionic acid fermentation [11, 88]. According to Zerfiridis [11], the general term Swiss cheese (referring to Emmental) probably dominated due to the fact that Emmental presents the largest size (or shape) and the greatest eyes and in Switzerland is considered at the "king" of all cheeses. The characteristic eyes, a typical sign of the Swiss-type cheeses are formed due to the production of CO_2 which is product of the propionic acid fermentation. Hence, these cheeses are also called "propionic acid fermentation cheeses" [11, 88]. Nevertheless, in 2007, the Codex Alimentarius Commission of the Food and Agriculture Organisation (FAO) of the United Nations developed an internationally recognized Codex standard for Emmental as follows: *Emmental is a ripened hard cheese, usually manufactured as wheels and blocks of weights from 40 kg or more, adapted to various use conditions. The body of the cheese has an elastic texture, with regular cherry to walnut sized gas holes, or eyes. The activity of propionic acid bacteria is essential to eye formation* [92 – 94]. Swiss-type cheeses are manufactured by techniques differing from traditional Swiss procedures. Thus, the milk treatment, the starters used, the weight, shape, ripening period and shelf-life are some of the main differences [11, 88, 93].

2.5 Mozzarella cheese manufacture

Mozzarella cheese originates from the southern regions of Italy and is typical of *pasta filata* or stretched-curd variety (together with Provolone the most important cheeses of this category). The term *pasta filata* is derived from an Italian phrase, that literally means "spun paste" or "stretched curd", which refers to a unique plastificization and stretching process; that is shared by all cheeses in this category, giving them their common identity. *Pasta filata* cheeses enclose a wide range of cheese varieties (Mozzarella, Provolone, Scarmorza, Caciocavallo, Kashkaval), which originated primarily in the northern Mediterranean region (Italy, Greece, the Balkans, Romania and Turkey [10, 11, 71, 96 – 98]. The term Mozzarella stemed from the Italian word "*mozzare*", meaning to tear, indicative to the stretching process applied during its manufacturing [10]. Furthermore, over the last decades, this cheese variety has experienced substantial growth, mainly because of its use as ingredient in a range of foods (including pizzas) and is a high volume cheese supporting food service industries. Mozzarella exhibits desirable functional properties, such as stretching, melting and browning (due to Maillard reaction) [10, 99]. Moreover, according to the Codex Standard for Mozzarella cheese [100]: *Mozzarella is an unripened cheese in conformity with the General Standard for Cheese and the Standard for Unripened Cheese Including Fresh Cheese. It is a smooth elastic cheese with a long stranded parallel-orientated fibrous protein structure without evidence of curd granules. The cheese is rindless and may be formed into various shapes.* Nowadays, Mozzarella is typically manufactured from cow's milk, whereas during traditional manufacture is made mainly from buffalo (*Bubalus bubalis*) milk [in 1996 gained a Protected Designation of Origin (PDO) recognition under European Union disciplinary], or mixtures of cow and buffalo milk [10, 96]. This cheese is considered a fresh cheese and is characteristic for its typical mild flavor and recognizable rubbery texture. However, Mozzarella cheese presents high values of moisture up to 60 % (generally 45 – 52 % w/w), elevated pH (in the range of 5.1 – 5.3), lack of ripening and maximum of 2 % of salt. The latter "microenviromental" cheese conditions provide adequate surroundings for the survival and growth of pathogenic microorganisms that may pose risks to public health. Hence, due to the above mentioned reason, Mozzarella is made from pasteurized milk [10, 101 – 104]. Typical pasteurization treatments are in the range of 73 – 74 °C for 17 – 20 s. Such thermal treatments are considered sufficient (assuming of good quality raw milk), to minimize pathogens and lessen spoilage and competitive microflora, as well as inactivating several enzyme systems [75]. Milk that is used for Mozzarella manufacture is almost always standardized to a specific protein-to-fat or casein-to-fat ratio to produce a cheese of desired fat in dry-matter content [71]. The traditional manufacture of Mozzarella is similar to Cheddar cheese manufacture (in the initial production-phases) as far as the milling stage. However, higher temperatures are used in processing (up to 42 °C), thus consequently a thermophilic starter culture (usually of 1.5 – 2.0 %) is more essential. This normally consists of a mixture of *Streptococcus thermophilus* alone or in combination with *Streptococcus delbrueckii* subsp. *bulgaricus* or *Lactobacillus heleveticus* [10, 71, 105, 106]. Aditionally, Mozzarella can be manufactured also by using mesophilic (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*) lactic acid bacteria, typically used in the manufacture of Cheddar. However, the final product has a blander flavor than the typical acetaldehyde flavor obtained when thermophilic starters are applied. The main role of the added starter culture is to produce lactic acid (in adequate level) to transform the curd into that will plasticize and stretch in hot water. Therefore, the starter culture selection is dependant on the desired rate of acid production during the subsequent steps of cheesemaking [10, 71]. These bacteria convert lactose to lactic acid, lowering the pH, promoting whey expulsion from the curd and altering the protein structure. In addition, an alternative method to destabilize the casein micelles and thus cause the curd formation (also used in Mozzarella manufacture), is the direct application of a food-grade acidulant (e.g. acetic, lactic or citrc acid) [10, 106, 107]. Thereafter, in the inoculated milk rennet is added. The resultant coagulum is cut gently, approx. into 1.0 – 1.5 cm cubes. Immediately the curd begins to expel whey and shrink. Subsequently, the curd-mass is left to rest (or to "heal") for 5 min and then is gently stirred for clumping prevention. If cultures have been applied the temperature of the curd is increased to 41 °C. The curd is separated from the whey, at the moment when proper level of acidity of the curd is reached (typically pH of 5.9). At this stage, the curd is allowed to mat together or is gently stirred, until is ready for stretching (optimal curd pH within the interval of 5.0 – 5.3). Aditionally, at the pH range mentioned previously, calcium phosphate is becoming more dissociated and a decrease in the net charge on proteins facilitates an increasing degree of hydrophobic interaction between casein molecules. Curd stretched at pH 5.3 is more structured, as indicated by a higher apparent viscosity immediately after manufacture that continues during the ripening process, compared to Mozzarella curd made from curd stretched at pH 5.0 [108 – 111]. Before mixing takes place, the curd is usually pre-salted at a rate of up to 2 % (salt to curd ratio), significantly reducing the future brining period [10]. Furthermore, the subsequent step is the heating and stretching process of the curd-mass, resulting in a visible alignment of protein fibres within the curd. Concretely during this stage, fat globules form channels between the protein strands and allow protein fibers to align and separate [112, 113]. In reality, the curd is immersed in hot water (68 – 74 °C), salt brine or whey, where is warms gradually to 60 °C. The heated curd is kneaded by a auger system. Protein fibres align and entrap coalesced fat and moisture, resultin in a plasticizing effect on the curd [10]. Thereafter, the hot plasticized curd is forced under pressure into chilled moulds, giving the cheese the desired shape. The cheeses then may undergo further cooling and salting (by immersion in cold brine or by direct salting in some cases). However, in practice many variations of the basic Mozzarella manufacture protocol exist, but the underlying principles are common to all procedures [10, 71].

3 MANUFACTURE TECHNOLOGY OF PROCESSED CHEESE

In general, the processed cheese manufacture is characterized by complexity and thus it is primarily affected by the chemical interactions occurring between the dairy ingredients and the emulsifying salts utilized. On the contrary, the principal technological operations of processed cheese manufacture are rather simple; however, they require a skillful professional in order to control all the applied ingredients, their concentrations in the blend and processing parameters. The basic theory of processed cheese manufacture lies on the change of the state of casein from coarsely dispersed calcium-paracaseinate present in natural cheese, due to application of heat, agitation and presence of particular salts (as emulsifying/peptizing agents), into a homogeneous free flowing condition (the sol state) with desired properties. The general technological processes for processed cheese production consist of the following operations: (i) natural cheese/cheeses selection, (ii) formulation of the blend, (iii) blending, (iv) shredding, (v) emulsifying salts addition, (vi) processing (thermal treatment), (vii) packaging, (viii) cooling and (ix) storage of the final product [6, 9, 10].

3.1 Natural cheese selection, formulation of the blend and blending

The unconditional importance for a prosperous production of processed cheese is mainly the proper quality and selection of natural cheese. Cheeses of one or more different varieties and degrees of maturity are possible to be used, resulting in easier processing and a advantageous flavour balance. Nevertheless, from the processed cheese production from only one variety of natural cheese is not excluded. The latter processed cheese products are very popular in some countries, e.g. Cheddar (in the USA, UK, Australia), Emmental (in the Western Europe), Mozzarella and Gruyère (in the USA, Canada). Furthermore, among the most paramount criteria for natural cheese selection are included: type, flavour, maturity degree, consistency, texture and level of acidity (pH value). Degraded natural cheese (off-flavour – rancid, or with microbial defects) should not be applied in processed cheese-making, as the quality of the final product will be diminshed or unaaceptable. Moreover, natural cheese with microbial defects; containing spore-forming, gas-producing and pathogenic bacteria are particularly hazardous for human health. On the other hand, cheese with mechanical defects could be used, since i tis possible to correct some physical properties by skilful blending. The proper selection of a high-quality natural cheese is not a quarantee that the developer processed cheese will be of the desired quality. Finally, when the natural cheese selection has been realized, the products are removed from the wrapper, de-ringed, cleaned and ground before thermal processing takes place. Hence, this physical treatment facilitates an easier melt, ensures proper belnding of the ingredinets added and enhances better contact between the emulsifying salts and cheese compnents. Concretely, increasing the surface area of the cheese (by size reducing), increases also the homogenity of the formulated blend. Moreover, the maximizing of the cheese's surface area facilitates the heat traansfer to the blend during the subsequent processing [6, 9, 10]. The main components of natural cheeses are fat, solids-not-fat (proteins, minerals and sodium chloride) and moisture. Generelly, computation of the ingredients is conducted on the basis of established fat and dry matter contents of the natural cheese components. In order to yield a final product with desired composition and properties is strongly dependant to the correct formulation of the materiál balance of fat and dry matter (including all blend ingredients, added water and condesate from the live steam applied during processing) [4, 9, 10]. The blending operation has a significant impact on the desired characteristics of the finished processed cheese product. However, maturity degree (and hence, level of proteolysis) appears to have the major influence on the properties of the final developer product. During manufacture of rennet-curd cheeses (Cheddar, Gouda) the paracasein is increasingly hydrolysed into peptides and free amino acids by numerous enzymatic activities, including residual coagulant and the proteinase and/or peptidases systems of milk, starter culture lactic acid bacteria, non-starter lactic acid bacteria, secondary cultures and/or exogeneous enzyme preparations. The level of intact protein, measured by the insoluble nitrogen at pH 4.6 (casein isoelectric point), decreases concomitantly with the hydrolysis of paracasein. Moreover, the intact protein level (the fraction of non-degraded protein expressed as percentage of the total protein) in the natural cheese has a major effect on the properties of the subsequent processed cheese. As the level of the intact casein in the cheese used for processing decreases, a significant decrease in the firmness of the product and increase in meltability of the product is observed [40]. Block-type processed cheese with elasticity and sliceability require predominantly young cheese (70 – 90 % intact-unhydrolysed casein). A general formulation exists: 70 – 75 % of mild cheese and 25 – 30 % semi-mature or mature cheese. On the other hand, predominantly medium ripe cheese (60 – 75 % intact casein) is used for the production of spreadable processed cheese [9, 10, 75]. For the production of processed cheese in slices, where a high content of elastic, intact casein is required, the ratio proposed above is modified to 30 – 40 % young cheese, 50 – 60 % mild cheese and only 10 % mature cheese. Furthermore, a similar ratio was also proposed by Kosilowski, in order to obtain a product with optimal firmness and slicing properties. The basic raw materiál for the production of processed cheese spreads is semi-mature cheese of shorter structure (approximately, 50 % semi-mature cheese, 20 % mature cheese and 30 % of young cheese. Some advantages and disadvantages of a high content of young cheese, as well as of a high content of extra-mature cheese, in blends for processed cheese production exist. The major advantages if a high content of young cheese is applied are: reduction of raw materiál costs, possibility of using cheeses with poor curing properties, formation of a stable emulsion with high water binding capacity and production of a firm product with appropriate slicing properties. In contrast, the basic disadvantages include: production of a processed cheese poor in taste, exessive swelling, tendency to harden during storage and the presence of small air-openings; formed due to the high viscosity of the blend. Likewise, a high content of extra-mature cheese in the blend has certain advantages: full-flavour development, good flow, high melting properties. Nevertheless, some disadvantages also exist, including sharp flavour development, low emulsion stability and soft consistency [10]. Plethora of other dairy and non-dairy ingredients can be used in the manufacture of different processed cheese types. The most frequently used dairy ingredinets are; skim mik powder, casein, caseinates, whey products and milkfat products. The last step in preparing the blend for processing is the addition of emulsifying salts. The type and role of emulsifying salts addition is of significant importance in processed cheese manufacture [11]. Therefore, other components, such as meat products, hydrocolloids, spices, fruits, or vegetables may be added at the beginning, or towards the end of the processing. An advantage of the inclusion addition at the beginning is bacteriological safety, although addition at the end prevents of susceptible fragile components.

3.2 Processing

The term processing describes the thermal treatment of the previously prepared blend, with direct (food-grade steam; up to 550 kPa; steam injector stainless-steel cookers) or indirect (steam-jacketed kettles) steam, under partial vacuum. Moreover, the product is subjected to constant agitation (50 – 3000 rpm), when either continuous or discontinuous (batch) method is applied [4, 9, 10]. The processing has two principal functions. The first one and most important (from consumption safety point of view) is to kill/destroy any potential pathogenic and spoilage microorganisms and hence extend the shelf-life of the final processed cheese product. Likewise, the second function is to facilitate the physico-chemical and microstructural changes, transforming the blend into a product with desired properties and stability. In addition, the application of partial vacuum is optional, but might be used for moisture regulation, when direct steam injection is applied. Moreover, is beneficial in removing air and thus preventing the presence of air-openings in the finished processed cheese product. Subsequently, the blend is transferred into the cooker, where the processing is realized. The order of ingredient addition varies with the plant practices, cooker type, overall plant design and duration of the processing. A typical order of ingredient addition is: ground cheese, a dry blend of emulsifying salts and other optional dairy ingredients, water and flavors [4, 9, 10]. The level of the heating applied during the production of processed cheese ranges between 72 and 145 °C; these products are categorized as "pasteurized" or "sterilized". Generally, products that are strelised are higher in water activity, requiring additional security of high temperature processing conditions, in order to minimize the threat of hazardous clostridial spores, which could germinate and thus grow during storage; if the product would not be refrigereated. Moreover, sterilized products are not usually produced by aseptic conditions, due to the complexicity of packaging. Nevertheless, the pasteurized products are those heated to 72 – 95 °C by means of direct steam injection under vacuum. The thermal treatment is ususally realized in batch cookers. However, the important point is to maintain a minimum filling temperature of 68 °C, ensuring adequate pasteurization effect [4, 9, 10, 11] Generally, high-temperature treatment is suitable for the production of spreadable processed cheese, whereas lower temperatures are applied in block-type and slices of processed cheese manufacture [8]. Therefore, in the case of sterilized products with prolonged shelf-life, amino acid degradation and browning (result of Maillard reaction complex) may likely occur [20, 41]. Processed cheese should not be heated for not less that 30 s at a temperature of not less that 65.6 °C [9, 10, 11].

The time-temperature regime in batch processing varies (70 – 95 °C for 4 – 15 min), depending on the formulation, the extend of the agitation, the desired product texture, the body (consistency) and the shelf-life characteristics [1, 114]. Moreover, the latter treatment provides a pasteurization effect. Additionally, it is generally admitted that these heat-treatments are sufficient to kill vegetative cells, however, are not adequate to eliminate microbial spores. Temperatures of 70 – 75 °C are needed for appropriate melting, and creaming is optional between 80 and 90 °C. The batch processing, however, appears a serious disadvantage, which is the longer processing times. A temperature higher of 130 °C may be required to kill some spores [1, 4, 9, 10, 11]. On the other hand, when continuous processing devices are used, the blend is, typically heated and held at 140 °C for 5 – 20 s and subsequently cooled to 70 – 95 °C, by flash evaporation of moisture by pressure drop, or by passing through external coolers (e.g. scrape surface tubular coolers) or by addition of a defined amount of water. Therefore, the product is held at this temperature for 4 – 15 min, allowing adequate time for interaction occurrence of the various blend ingredients, the desired physico-chemical changes to occur, the development ot the desired texture and for the removal of undesired odours. Moreover, at this temperature the blend thickens progressively and viscosity increases by several magnitudes [1, 4, 44, 55]. However, continuous devices (cookers) are not commonly used in the processed cheese industry. A possible explantion could stand on the processed cheese products capacious versatility; therefore, changing over to a different product in the continuous processing line, requiring intermediate cleaning, which could not be considered as economically beneficial [10].

3.3 Packaging, cooling and storage

Nowadays, numerous packaging formats and materials are available for the processed cheese industry. Processed cheese is typically packed and wrapped in laminated foil (tubes, cups, cans, cardboard) or plastic containers (in sausage form) and however, rarely in glass jars. Moreover, the method and rate of cooling strongly depends on the type of the developed processed cheese. Processed cheese blocks are cooled slowly, whereas the cooling of spreadable processed cheese should be as fast as possible because cooling softens the product. However, slow cooling may intensify the Maillard reaction and promote growth of spores. A usual temperature range for processed cheese storage lies within 5 to 10 °C, which is cool enough to prevent further creaming. However, sometimes calcium diphosphate or calcium pyrophosphate crystals may be formed on the surface of the product, at such temperature range, leading to a gritty texture, which is considered as a defect [1, 4, 9, 10, 11]. Modern processing technologies and careful selection of the utilized ingredients implement the production of a bacteriologically stable processed cheese. Hence, processed cheese is often expected to be a product with a shelf-life more than 1 year. Nevertheless, even products of high bacteriological stability in good packaging usually maintain their quality only for 6 to 12 months at room temperature [66]. Processed cheese is not a preserved food, but a "semi-preserved" food, with limited shelf-life. Moreover, premium grade proceesed cheese should be given a shelf-life quarantee not exceeding 3 to 4 months. Additionally, the shelf-life of processed cheese in slices is estimated to 8 weeks, for small portions 20 weeks and more than 1 year for products packed in tubes or cans (all stored at room temperature) [66]. According to Buňka et al. [57] is possible to prolong the shelf-life of processed cheese products to a minimal period of 24 months, if the melted product is packaged is suitable material which could undergo sterilization (115 – 125 °C for 5 – 20 min).

4. AIMS OF THE DOCTORAL STUDY

The aim of the current study was to observe the effect of selected ternary mixtures (of emulsifying salts) with different concentrations of phosphate and citrate salts on specific functional properties of processed cheese model samples manufactured from different cheese matrixes (Edam, Swiss-type, Mozzarella-type cheeses, respectively). Hence, in order to achieve the main objective of the thesis, it was necessary to establish the following sub-objectives:

- monitoring the dispersion intensity of the caseim micelles in model milk samples composed of skimmed milk powder and ternary mixtures of emulsifying salts [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)];
- the manufacture (under laboratory conditions) of model samples of processed cheese containing ternary mixtures of phosphate and citrate salts with different concentrations;
- the manufacture of processed cheese model samples was performed using different types of natural cheeses (Edam, Swiss-type, Mozzarella-type);
- the manufacture of the model samples was performed using different types of natural cheeses (Swiss-type, Mozzarella-type) with different degrees of maturity;
- in case of Edam cheese the manufacture of the model samples was performed using natural cheese of 7-week maturity and samples with modified (target values within the interval of 5.60 – 5.80) and non-modified pH values (arising from the interactions of the emulsifying salt mixtures) were produced;
- in case of Swiss-type cheese the manufacture of the model samples was performed using natural cheese with different maturity degree (4, 8, 12, 16-weeks of maturity);
- in case of Mozzarella-type cheese the manufacture of the model samples was performed using natural cheese with different maturity degree (0, 2, 4, 6-weeks of maturity);
- for all the model processed cheeses samples basic chemical analysis (dry matter content and pH value determination) and texture profile analysis were performed (after 2, 9, 30 days after the production day – Edam cheese; after 2, 9, 30, 60 days after the production day – Swiss-type, Mozzarella-type cheeses, respectively);
- performing rheological analysis of the model processed cheese samples on the 30th day of their storage (30 day after the production);
- to statistically evaluate all the obtained results (mehod of least squares, using the polynomials 1 to 4 degrees; Kruskal-Wallis and Wilcox tests) and to formulate the outcomes;

5. EXPERIMENTAL PART

5.1 Description of the experimental part of the dissertation thesis

The practical part of the dissertation thesis was divided into four experimental stages. During the first stage, model milk samples were prepared by stirring skimmed milk powder in deionised water. The added amount of the skimmed milk corresponded to 5 % (w/v) in order to develop a dispersion (model milk system). Furthermore, after careful agitation, dissolving (~1 h of stirring at ambient temperature of 22 ± 1 °C) and addition of sodium azide (0.2 % w/v), the pH of the system was modified onto 5.80 ± 0.01 (HCl at 1 and 0.1 mol/l was applied). Therefore, the system was allowed to stand overnight (~18 h at ambient temperature of 22 ± 1 °C) for the stabilization of the formed enviroment. Moreover, ternary mixtures consisting disodium hydrogen phosphate (Na_2HPO_4; DSP), tetrasodium diphosphate ($Na_2P_2O_7$; TSPP) and five sodium salts of polyphosphate (Pxx) with different mean length (*n≈5, 9, 13, 20, 28*; designated as P05, P09, P13, P20, P28, respectively) were applied, which resulted in five ternary mixtures of DSP:TSPP:Pxx (for P05, P09, P13, P20, P28). The ternary mixtures of emulsifying salts were aplied in percentage proportions with an increasing step of 20 % and some selected ratios with 50 %. Moreover, the ternary mixtures of emulsifying salts were added at a total concentration of 0.3 % (w/v). Following the addition of the emulsifying salts, the formed mixtures were agitated for 10 min and the pH of the system adjusted again to 5.80 ± 0.01 (HCl/NaOH at 1 and 0.1 mol/l was applied). The formed dispersions were stirred at 22 ± 1 °C for another 50 min. Finally, the optical density was determined at λ=700 nm.

During the second stage, processed cheese model samples were manufactured using Edam cheese (7-week maturity) as the main raw material. During this stage, the impact of the ternary mixtures of phosphate and citrate emulsifying salts [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 specific textural properties (hardness, relative adhesiveness, and cohesiveness) of processed cheese over a 30 day storage period was evaluated. The above-mentioned 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). Moreover, a secondary objective of this experimental phase was to investigate the effect of the above-mentioned mixtures on casein micelle dispersion in a simplified model milk system. The above mentioned emulsifying salts were applied into four types of ternary mixtures (DSP:TSC:P20, DSP:TSPP:TSC, TSC:TSPP:P20 and DSP:TSPP:P20). The total concentration of the ternary mixtures mentioned above was 3 % (w/w) of the total weight of the melt. Particularly, all four types of ternary mixtures of ES were blended in staggered proportions in steps of 20 % (the percentages of the components were calculated on the basis of the total weight of ES, where total weight = 100 %) and with some selected 50:50 ratios (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). Moreover, the selected maturity degree of the raw materiál (Edam cheese; 50 % w/w, dry matter content; 30 % w/w, fat in dry matter content) was 7 weeks (at 10 ± 2 °C).

Furthermore, based on the results obtained from the second experimental phase, during the third stage, processed cheese samples were developed using Swiss-type cheese (as the main raw material) with different degrees of maturity (4, 8, 12, 16-weeks of ripening; at 10 ± 2 °C – the same batch of cheese was used during the whole experiment). The apllied natural cheese (Swiss-type cheese), at this experimental stage was characterised of 60 % w/w dry matter content and 30 % fat in dry matter content, respectively. Moreover, on the current processed cheese samples, the dependence of selected textural properties (hardness, cohesiveness, and relative adhesiveness) and viscoelastic properties on the composition of ternary mixtures of emulsifying salts, composed of 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 day storage period was evaluated. In addition, each type of ternary mixture was tested in 12 selected 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 total weight of ES (total weight=100 %). The above-mentioned phenomenon 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 processed cheese spreads. A supplementary aim at this stage was to investigate the effect of different maturity degrees of the Swiss-type cheese on the above-mentioned dependence.

Moreover, based on the results obtained from the third experimental phase, during the fourth and final experimental part, the dependencies mentioned above (in the third experimental stage) were investigated in samples manufactured from Mozzarella-type cheese (ingredient appied as the main raw material) with different degrees of maturity during a 60 day storage period. In particular, the selected maturity degrees for Mozzarella cheese (35 % w/w dry-matter content and 50 % w/w fat in dry matter content) were 0, 2, 4 weeks (ripening at 6 ± 2 °C – the same batch of cheese was used during the whole experiment). Nevertheless, the remainder parameters of the current experiments were identical to those of mentioned in the third experimental stage.

5.2 Model milk samples preparation and optical density measurements

The model milk samples were prepared using skimmed milk powder reconstituted (5 %, w/v) in deionised water, in order to maintain the ionic environment at constant level. Moreover, sodium azide (0.02 %, w/v) was added to prevent potential microbial contamination. The pH of the formed dispersion was adjusted (HCl at 1 and 0.1 mol/l was applied) to approach the value of 5.80 ± 0.01. The total concentration of the applied emulsifying salts 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 the emulsifying salts addition the pH of the system was adjusted to the targeted pH-value (5.80 ± 0.01) and after additional 50 min of agitation the samples were analysed. The control sample was prepared in the same manner but without the addition of emulsifying salts. The casein micelles dispersion evaluation was expressed in terms of optical density, according to the method presented in the work of Kaliappan and Lucey (2011). Ternary mixtures consisting disodium hydrogen phosphate (Na_2HPO_4; DSP), tetrasodium diphosphate ($Na_2P_2O_7$; TSPP) and five sodium salts of polyphosphate (Pxx) with different mean length (n≈5, 9, 13, 20, 28; designated as P05, P09, P13, P20, P28, respectively) were applied, which resulted in five ternary mixtures of DSP:TSPP:Pxx (for P05, P09, P13, P20, P28). The ternary mixtures of emulsifying salts were aplied in percentage proportions with an increasing step of 20 % and some selected ratios with 50 % (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 390 lots (26 variants × 5 types of ternary mixtures × 3 productions). The ternary mixtures of emulsifying salts were added at a total concentration of 0.3 % (w/v). Furthermore, in the case of ternary mixtures composed of disodium hydrogenphosphate (Na_2HPO_4; DSP), tetrasodium diphosphate ($Na_2P_2O_7$; TSPP), sodium salt of polyphosphate with mean length n≈20 (P20), and trisodium citrate ($C_2H_5Na_3O_7$; TSC), each type was tested in 12 selected 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 – 12 variants × 4 types of ternary mixtures × 2 repetitions = 96 lots). The results were expressed with respect to the optical density of the milk system without the addition of emulsifying salts, the pH of which was adjusted to reach 5.80 ± 0.01 (100%; after ~18 h). Each variant (including also the control sample) was measured 9 times.

5.3 Processed cheese model samples manufacture

The composition of the main raw materials, comprising Edam cheese blocks (50 % w/w, dry matter content, 30 % w/w, fat in dry matter content, 7-week maturity), Swiss-type cheese (60 % w/w, dry matter content, 30 % w/w, fat in dry matter content, 4, 8, 12, 16-week maturity – the same batch of cheese was used in the whole experiment), Mozzarella-type block cheese (42 % w/w, dry-matter content, 17 % w/w, fat in dry-matter content, 0, 2, 4 week maturity – the same batch of cheese was used in the whole experiment) were used for the production of the model processed cheese samples. Furthermore, the production of the samples was designed to achieve final products with 40 % (w/w) dry matter content and 50 % (w/w) fat in dry matter content (in case of Edam and Swiss-type cheeses, respectively). In the case of Mozzarella cheese, the production of the samples was desighned to achieve samples with 35 % (w/w) dry matter content and 50 % (w/w) fat in dry matter content. Among the other ingredients applied in processed cheese manufacture were: butter (84 % w/w, dry matter content and 82 %, w/w fat), water and ternary mixtures of disodium hydrogenphosphate (Na_2HPO_4; DSP), tetrasodium diphosphate ($Na_2P_2O_7$; TSPP), sodium salt of polyphosphate with mean chain-length n≈20 (P20) and trisodium citrate ($C_2H_5Na_3O_7$; TSC). The above mentioned emulsifying salts were applied into four types of ternary mixtures (DSP:TSC:P20, DSP:TSPP:TSC, TSC:TSPP:P20 and DSP:TSPP:P20). The total concentration of the ternary mixtures mentioned above was 3 % (w/w) of the total weight of the melt. Particularly, in the case of Edam cheese, all four types of ternary mixtures of emulsifying salts were blended in staggered proportions in steps of 20 % (the percentages of the components were calculated on the basis of the total weight of emulsifying salts, where total weight = 100 %) and with some selected 50:50 ratios (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 resulted in 26 variants in total. Each combination was made in duplicate giving 208 lots in total (4 types of ternary mixtures × 26 variants × 2 productions). Moreover, in cases of Swiss-type and Mozzarella cheeses, respectively, each type of 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 total weight of ES (total weight=100 %). Each combination was made in duplicate resulting in 96 lots in total (4 types of ternary mixtures × 12 reciprocal percentage ratios × 2 repetitions). The Vorkwerk Thermomix TM 31-1 blender cooker (Vorkwerk & Co. Thermomix, GmbH, Wuppertal, Germany) with indirect heating was employed for the manufacture of the processed cheese samples. The same apparatus was also used far a contiguous scope in the work by Buňka et al. [29], Černíková et al. [43], Lee et al. [12], Macků et al. [115] and Sádlíková et al. [17]. The manufacture protocol was as follows: natural cheese and butter were cut in pieces (cca 2 × 2 × 2 cm), placed into the above mentioned device and were minced for 60 s (approximately 5,000 rpm). Ternary mixtures of emulsifying salts (3% w/w) and water were added into the blend (approximately at 2,000 rpm). Briefly, a melting temperature of 90 ˚C was held for 1 min (the total melting time was 10 – 12 min) at approximately 4000 rpm. Hence, 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). According to a pilot study (unpublished data) the calculated amount of acid/alkali was added when the temperature approached 85 – 86 ˚C, 30 – 50 s before reaching the melting point. Consequently, in order to maintain the values of dry matter and fat on dry matter stable, respectively, the addition of water was reduced (by the calculated amount of acid/alkali). Thereafter, the hot melt mass was set into cylindrical polypropylene pots (52 mm in diameter; 50 mm high) and wrapped with aluminium lids. The packed samples were cooled and stored to 6 ± 2 °C until the analyses were performed. The processing parameters and the raw materials were selected to simulate the industrial conditions.

5.4 Basic chemical analyis

5.4.1 Determination of dry matter content and pH measurement

The dry matter content was gravimetrically analysed according to ISO 5534 [116] for both types of samples (natural cheese samples – raw material and processed cheese samples), by drying the samples at 102 ± 2 °C until constant mass. Moreover, 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. Both analyses were realized at the 2nd, 9th, 30th and 60th day after processed cheese manufacture and were performed in triplicate.

5.4.2 Determination of free amino acids of the applied natural cheeses (basic raw material)

Before the analysis of free amino acids content, the samples of the individual natural cheeses with different degrees of maturity (depending on the used natural cheese; Edam – 7 week maturity; Swiss-type and Cheddar – 4, 8, 12, 16-week maturity; Mozzarella – 2, 4, 6, 8-week maturity) 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 Analyser (Ingos, Prague, Czech Republic) ion-exchange chromatography apparatus according to the method protocol described by Buňková et al. [117] and Pachlová et al. [84]. Therefore, the free amino acids content was calculated as a sum of 22 individual free amino acids and similar substances contents (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.

5.5 Texture profile analysis of the processed cheese samples

Texture profile analysis (TPA) is a well-established method of analysis and frequently referred as a standard method for texture characterization [118]. The principle of the current method stands on the fact, that two successive penetrations onto a test-sample are applied, using mechanical testing equipment (in imitation of the chewing process). Three textural parameters (hardness, cohesiveness, relative adhesiveness) of processed cheese samples were observed using a TA.XT plus texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK). Definitions of the above textural properties (hardness, cohesiveness and relative adhesiveness) are given by Civille & Szczesniak, [120], Weiserová et al. [119].

Furthermore, two sequential penetration events (penetration depth 10 mm, probe speed 2 mm s^{-1}, trigger force 5 g, strain of deformation 25 %) were implemented during the measurements. A stainless steel cylindrical probe with 20 mm diameter was directly penetrated to the cylindrical cup (after removing the aluminium foil). The results were recorded as force-displacement/time curves describing the force (N) needed to deform the sample proportionally with time (s). Piška and Štětina [47], Cunha and Viotto [122], Weiserová et al. [119], Buňka et al., [29] and Sołowiej et al. [121] have applied a corresponding penetration method for processed cheese textural properties measurement. The texture profile analysis (for all obtained samples) was performed after 2, 9, 30 and 60 days of storage at 6 °C. On each day of analysis, each variant of ternary mixtures was measured in triplicate; 2 batches were manufactured.

5.6 Rheological analysis of the processed cheese samples

The rheological analysis of the processed cheese samples was performed only on the 30th day of storage (at 6 ± 2 ˚C) using a dynamic oscillatory shear rheometer (RheoStress 1, HAAKE, Bremen, Germany). Additionally, in order to describe the changes of the soldified melt mass (processed cheese) viscoelastic properties, a plate-plate geometry (diameter 35 mm) was selected in dependence to frequency (ranging from 0.01 to 100.00 Hz) at 20.0±0.1 ˚C, with applied shear stress amplitude of 20 Pa. The selected monitored parameters included elastic or storage (G′) and viscous or loss (G″) moduli (determined as function of frequency).

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

Furthermore, the Winter's critical gel theory was implemented in order to evaluate the changes of the viscoelastic properties of the processed cheese samples. According to the following equation the complex modulus can be expressed as [17, 115, 123, 124]:

$$G^{*}(\omega) = A_{F} \cdot \omega^{\frac{1}{z}} \tag{2}$$

Where (A_F) is the strength of the gel (Pa $s^{1/z}$) and (z) is the interaction factor (defined as number of structure units intracting with one another in a three-dimensional network; unitless). The higher the interaction factor is, the more interactions occur in the matrix of the sample [17, 115, 123, 125]. The reported values were the mean of at least four replicates.

5.7 Statistical evaluation of the obtained data

Non-parametrical variance analyses of 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 estimation of the gel strength and the interaction factor, non-linear regression analysis (non-linear least squares regression) for the following conditions: AF > 0 and $z \geq 0$. The Marquardt-Levenburg method was applied (Unistat® 6.5 software was also applied).

6. LIST OF REFERENCES

[1] GUINEE, T.P; CARIĆ, M; KALÁB, M. Pasteurized Processed Cheese and Substitute/imitation Cheese Products. Cheese: Chemistry, Physics and Microbiology. 2004, vol. 2 s. 349-394. ISSN:1874-558X.

[2] ANONYM. Spotřeba potravin a nealkoholických nápojů (na obyvatele a rok) v ČR v letech 2001 – 2014. In: *Český statistický úřad České republiky* [online]. [2016-05-21]. Available from: https://www.czso.cz/documents/10180/20562003/2701391501.pdf/1547f1b0-eeac-482f-8ea0-2289d3b4ed3e?version=1.1

[3] FERRAO, L.L; SILVA, E.B; SILVA, H.L.A; SILVA, R; MOLLAKHALILI, N; GRANATO, Daniel; FREITAS, M.Q; SILVA, M.C; RAICES, R.S.L; PADILHA, M.C; ZACARCHENCO, P.B; BARBOSA, M.I.M.J; MORTAZAVIAN, A.M; CRUZ, A.G. Strategies to Develop Healthier Processed Cheeses: Reduction of Sodium and Fat Contents and Use of Prebiotics. *Food Research International*. 2016. ISSN:0963-9969.

[4] GUINEE, T.P. Pasteurized Processed Cheese Products. *Encyclopedia of Dairy Sciences*. 2011, s. 805-813. ISBN: 978-0-1237-4407-4.

[5] GUINEE, T.P. and O‘ CALLAGHAN, D.J. The effect of increasing the protein-to-fat ration and reducing fat content on the chemical and physical properties of processed cheese product. *Journal of Dairy Science*. 2013, 96, 11, 6830 – 6839.

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

[7] TAMIME, A.Y. Structure of Dairy Products. 2007, s. 1-288. ISBN: 978-1-4051-2975-6.

[8] KAMMERLEHNER, J. Käsetechnologie. 2012, Verlug Reisinger, Kuenstlerpresse W Bode, Frising. ISBN 978-00-037724-2.

[9] FOX, P. *Fundamentals of cheese science*. Gaithersburg, MD: Aspen Publishing, 2000. ISBN 978-0-8342-1260-2

[10] HUI, Y.H. Handbook of Food Science, Technology, and Engineering. 2005, vol. 148. ISBN: 9781466507876.

[11] ΖΕΡΦΙΡΙΔΗΣ, Γ.Κ. Τεχνολογία προιόντων γάλακτος. Θεσσαλονίκη: Εκδόσεις Γιαχούδη, 2. Εκδ., 2001, 55 – 58.

[12] LEE, S.K; ANEMA, S; KLOSTERMEYER, H. The Influence of Moisture Content on the Rheological Properties of Processed Cheese Spreads. International *Journal of Food Science & Technology*. 2004, vol. 39, no. 7 s. 763-771. ISSN:0950-5423.

[13] CHEN, L; LIU, H. Effect of Emulsifying Salts on the Physicochemical Properties of Processed Cheese Made from Mozzarella. *Journal of Dairy Science*. 2012, vol. 95, no. 9 s. 4823-4830. ISSN:0022-0302.

[14] HANAEI, F; CUVELIER, G; SIEFFERMANN, JM. Consumer Texture Descriptions of a Set of Processed Cheese. *Food Quality and Preference*. 2015, vol. 40 s. 316-325. ISSN:0950-3293.

[15] HASENHUETTL, G.L; HARTEL, R.W. Food Emulsifiers and Their Applications: Second Edition. 2008, s. 1-426. ISBN: 9780387752839.

[16] MARCHESSEAU, S; GASTALDI, E; LAGAUDE, A; CUQ, J.-L. Influence of PH on Protein Interactions and Microstructure of Process Cheese. *Journal of Dairy Science*. 1997, vol. 80, no. 8 s. 1483-1489. ISSN:0022-0302.

[17] SÁDLÍKOVÁ, I; BUŇKA, F; BUDINSKÝ, P; BARBORA, V; PAVLÍNEK, V; HOZA, I. The Effect of Selected Phosphate Emulsifying Salts on Viscoelastic Properties of Processed Cheese. *LWT - Food Science and Technology*. 2010, vol. 43, no. 8 s. 1220-1225. ISSN:0023-6438.

[18] BUŇKA, F; KŘÍŽ, O; VELIČKOVÁ, A; BUŇKOVÁ, L; KRÁČMAR, S. Effect of Acid Hydrolysis Time on Amino Acid Determination in Casein and Processed Cheeses with Different Fat Content. *Journal of Food Composition and Analysis*. 2009, vol. 22, no. 3 s. 224-232. ISSN:0889-1575.

[19] ČESKO.Vyhláška Ministerstva zemědělství č. 77/2003 Sb., kterou se stanoví *Požadavky pro mléko a mléčné výrobky, mražené krémy a jedlé tuky aoleje*, vplatném znění.2003. Dostupné z: http://www.esipa.cz/sbirka/sbsrv.dll/sb?DR=SB&CP=2003s77

[20] BUŇKA, F; DOUDOVÁ, L; WEISEROVÁ, E; ČERNÍKOVÁ, M; KUCHAŘ, D; SLAVIKOVÁ, S; NAGYOVÁ, G; PONÍŽIL, P; GRUBER, T; MICHÁLEK, J. The Effect of Concentration and Composition of Ternary Emulsifying Salts on the Textural Properties of Processed Cheese Spreads. *LWT-Food Science and Technology*. 2014, vol. 58, no. 1 s. 247-255. ISSN:0023-6438.

[21] CARIC, M.; GANTAR, M.; and KALAB, M. 1985. Effects of emulsifying agents on the microstructure and other characteristics of process cheese — A review. *Food Microstructure*. 4:297–312.

[22] ZEHREN, V.L; and NUSBAUM, D.D. *Process cheese*. 2nd ed. Madison, Wisconsin, USA: Cheese Reporter Publishing, Co, Inc., 2000.

[23] LUCEY, J.A. Formation and Physical Properties of Milk Protein Gels. *Journal of Dairy Science*. 2002, vol. 85, no. 2 s. 281-294. ISSN:0022-0302.

[24] BERGER, W; KLOSTERMEYER, H; MERKENICH, K; and UHLMANN, G. *Processed cheese manufacture.* A Joha Guide, Ladenburg: BK Giulini Chemie GmbH & Co. OHG, 2002.

[25] KAPOOR, R; METZGER, L.E; BISWAS, A.C; MUTHUKUMMARAPPAN, K. Effect of Natural Cheese Characteristics on Process Cheese Properties. *Journal of Dairy Science*. 2007, vol. 90, no. 4 s. 1625-1634. ISSN:0022-0302.

[26] SALEK, RN; 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, vol. 44 s. 37-43. ISSN:0958-6946.

[27] DIMITRELI, G.; THOMAREIS, A.S. 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*. 2009, vol. 12, no. 1 s. 261-275. ISSN:1094-2912.

[28] EL-BAKRY, M; DUGGAN, E; O'RIORDAN, E.D; O'SULLIVAN, M. Effect of Chelating Salt Type on Casein Hydration and Fat Emulsification During Manufacture and Post-manufacture Functionality of Imitation Cheese. *Journal of Food Engineering*. 2011, vol. 102, no. 2 s. 145-153. ISSN:0260-8774.

[29] BUŇKA, F; DOUDOVÁ, L; WEISEROVÁ, E; KUCHAŘ, D; PONÍŽIL, P; ZAČALOVÁ, D; NAGYOVÁ, G; PACHLOVÁ, V; MICHÁLEK, J. The Effect of Ternary Emulsifying Salt Composition and Cheese Maturity on the Textural Properties of Processed Cheese. *International Dairy Journal*. 2013, vol. 29, no. 1 s. 1-7. ISSN:0958-6946.

[30] SHIRASHOJI, N., JAEGGI, J.J., a LUCEY, J.A. Effect of trisodium citrate concentrationand cooking time on physicochemical properties of pasteurized process cheese. *Journal of Dairy Science*, 2006, 89, 15–28. ISSN 0022-0302.

[31] MIZUNO, R; LUCEY, J.A. Effects of Emulsifying Salts on the Turbidity and Calcium-Phosphate–Protein Interactions in Casein Micelles. *Journal of Dairy Science*. 2005, vol. 88, no. 9 s. 3070-3078. ISSN:0022-0302.

[32] SHIRASHOJI, N; JAEGGI, J.J; LUCEY, J.A. Effect of Sodium Hexametaphosphate Concentration and Cooking Time on the Physicochemical Properties of Pasteurized Process Cheese. *Journal of Dairy Science*. 2010, vol. 93, no. 7 s. 2827-2837. ISSN:0022-0302.

[33] KALIAPPAN, S; LUCEY, J.A. Influence of Mixtures of Calcium-chelating Salts on the Physicochemical Properties of Casein Micelles. *Journal of Dairy Science*. 2011, vol. 94, no. 9 s. 4255-4263. ISSN:0022-0302.

[34] MOLINS, R.A. *Phosphates in food*. Boca Raton: CRC Press. 1991. 261 s. ISBN 0-8493-4588-X.

[35] FOX, P.F.; GUINEE, T.P.; COGAN, T.M.; MCSWEENEY, P.L.H. *Fundamentals of Cheese Science*. Gaithersburg, USA: Aspen Publication, 2000.

[36] CARIĆ, M. and 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

[37] TANAKA, Y; TAKEI, T; AIBA, T; MASUDA, K; KIUCHI, A; FUJIWARA, T. Development of Synthetic Lung Surfactants. *Journal of Lipid Research*. 1986, vol. 27, no. 5 s. 475-485. ISSN:0022-2275.

[38] BAYARRI, S; CARBONELL, I; COSTELL, E. Viscoelasticity and Texture of Spreadable Cheeses with Different Fat Contents at Refrigeration and Room Temperatures. *Journal of Dairy Science*. 2012, vol. 95, no. 12 s. 6926-6936. ISSN:0022-0302.

[39] FAGAN, C.C.; EVERARD, C.; O´DONNELL, C.P.; DOWNEY, G.; SHEENAN, E.M.; DELAHUNTY, C.M.; O´CALLAGHAN, D.J.; HOWARD, V. Prediction of processed cheese instrumental texture and meltability by mid-infared spectroscopy coupled with chemometric tools. *Journal of Food Engineering,* 2007, 80, 1068–1077. ISSN 0260-8774.

[40] BRICKLEY, C.A; AUTY, M.A.E; PIRAINO, P; MCSWEENEY, P.L.H. The Effect of Natural Cheddar Cheese Ripening on the Functional and Textural Properties of the Processed Cheese Manufactured Therefrom. *Journal of Food Science*. 2007, vol. 72, no. 9 s. C483-C490. ISSN:0022-1147..

[41] BUŇKA, F. and HRABĚ, J. Tavené sýry. *Potravinářská revue*. 2006, 4, 13-16.

[42] BOWLAND, E.L; FOEGEDING, E.A. Small Strain Oscillatory Shear and Microstructural Analyses of a Model Processed Cheese. *Journal of Dairy Science*. 2001, vol. 84, no. 11 s. 2372-2380. ISSN:0022-0302.

[43] ČERNÍKOVÁ, M; BUŇKA, F; PAVLÍNEK, V; BŘEZINA, P; HRABĚ, J; VALÁŠEK, P. Effect of Carrageenan Type on Viscoelastic Properties of Processed Cheese. *Food Hydrocolloids*. 2008, vol. 22, no. 6 s. 1054-1061. ISSN:0268-005X.

[44] DIMITRELI, G; THOMAREIS, A.S. Texture Evaluation of Block-type Processed Cheese as a Function of Chemical Composition and in Relation to Its Apparent Viscosity. *Journal of Food Engineering*. 2007, vol. 79, no. 4 s. 1364-1373. ISSN:0260-8774.

[45] GUSTAW, W; MLEKO, S. The Effect of Polysaccharides and Sodium Chloride on Physical Properties of Processed Cheese Analogs Containing Whey Proteins. *Milchwissenschaft*. 2007, vol. 62, no. 1 s. 59-62. ISSN:0026-3788.

[46] LU, Y; SHIRASHOJI, N; LUCEY, J.A. Effects of PH on the Textural Properties and Meltability of Pasteurized Process Cheese Made with Different Types of Emulsifying Salts. *Journal of Food Science*. 2008, vol. 73, no. 8 s. E363-E369. ISSN:0022-1147.

[47] PISKA, I; ŠTĚTINA, J. Influence of Cheese Ripening and Rate of Cooling of the Processed Cheese Mixture on Rheological Properties of Processed Cheese. *Journal of Food Engineering*. 2004, vol. 61, no. 4 s. 551-555. ISSN:0260-8774.

[48] BUŇKA, F. and HRABĚ, J. Tavené sýry. *Potravinářská revue*. 2006, 4, 13-16.

[50] KAPOOR, R; METZGER, L.E. Process Cheese: Scientific and Technological Aspects - a Review. *Comprehensive Reviews in Food Science and Food Safety*. 2008, vol. 7, no. 1 s. 194-214. ISSN:1541-4337.

[51] JOHNSON, M.E; KAPOOR, R; MCMAHON, D.J; McCOY, D.R; NARASIMMON, R.G. Reduction of Sodium and Fat Levels in Natural and Processed Cheeses: Scientific and Technological Aspects. *Comprehensive Reviews in Food Science and Food Safety*. 2009, vol. 8, no. 3 s. 252-268. ISSN:1541-4337.

[53] UPRETI, P; METZGER, L.E. Influence of Calcium and Phosphorus, Lactose, and Salt-to-Moisture Ratio on Cheddar Cheese Quality: Manufacture and Composition. *Journal of Dairy Science*. 2006, vol. 89, no. 2 s. 420-428. ISSN:0022-0302.

[54] PURNA, S.K.G; POLLARD, A; METZGER, L.E. Effect of Formulation and Manufacturing Parameters on Process Cheese Food Functionality—I. Trisodium Citrate. *Journal of Dairy Science*. 2006, vol. 89, no. 7 s. 2386-2396. ISSN:0022-0302.

[55] DIMITRELI, G; THOMAREIS, A.S. Effect of Chemical Composition on the Linear Viscoelastic Properties of Spreadable-type Processed Cheese. *Journal of Food Engineering*. 2008, vol. 84, no. 3 s. 368-374. ISSN:0260-8774.

[56] LEE, S.K; KLOSTERMEYER, H. The Effect of PH on the Rheological Properties of Reduced-fat Model Processed Cheese Spreads. *LWT - Food Science and Technology*. 2001, vol. 34, no. 5 s. 288-292. ISSN:0023-6438.

[57] BUŇKA, F., BUŇKOVÁ, L. a KRÁČMAR, S. Základní principy výroby tavených sýrů. *Folia Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* Brno: MZLU, 2009. 70 s. ISSN 1803-2109.

[58] CUNHA, C.R; GRIMALDI, R; ALCANTARA, M.R; VIOTTO, W.H. Effect of the Type of Fat on Rheology, Functional Properties and Sensory Acceptance of Spreadable Cheese Analogue. International *Journal of Dairy Technology*. 2013, vol. 66, no. 1 s. 54-62. ISSN:1364-727X.

[59] MIZUNO, R; LUCEY, J.A. Properties of Milk Protein Gels Formed by Phosphates. *Journal of Dairy Science*. 2007, vol. 90, no. 10 s. 4524-4531. ISSN:0022-0302.

[60] MIZUNO, R; LUCEY, J.A. Effects of Two Types of Emulsifying Salts on the Functionality of Nonfat Pasta Filata Cheese. *Journal of Dairy Science*. 2005, vol. 88, no. 10 s. 3411-3425. ISSN:0022-0302.

[61] MULSOW, B.B; JAROS, D; ROHM, H. Processed Cheese and Cheese Analogues. *Structure of Dairy Products*, Oxford: Blackwell Publishing Ltd. 2007, 210-235.

[62] SWENSON, B.J; WENDORFF, W.L; LINDSAY, R.C. Effects of Ingredients on the Functionality of Fat-free Process Cheese Spreads. *Journal of Food Science*. 2000, vol. 65, no. 5 s. 822-825. ISSN:0022-1147.

[63] AWAD, R.A; ABDEL-HAMID, L.B; EL-SHABRAWY, S.A; SINGH, R.K. Physical and Sensory Properties of Block Processed Cheese with Formulated Emulsifying Salt Mixtures. *International Journal of Food Properties*. 2004, vol. 7, no. 3 s. 429-448. ISSN:1094-2912.7.

[64] BUŇKOVÁ, L; PLEVA, P; BUŇKA, F; VALÁŠEK, P; KRÁČMAR, S. Antibacterial Effects of Commercially Available Phosphates on Selected Microorganisms. *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis*. 2008, vol. 56, no. 5 s. 19-24. ISSN:1211-8516.

[65] ABDEL-HAMID, L.B; EL-SHABRAWY, S.A; AWAD, R.A; SINGH, R.K. Chemical Properties of Processed Ras Cheese Spreads as Affected by Emulsifying Salt Mixtures. *Journal of Food Processing and Preservation*. 2000, vol. 24, no. 3 s. 191-208. ISSN:0145-8892.

[66] SCHÄR, W; and BOSSET, J.O. Chemical and Physicochemical Changes in Processed Cheese and Ready-made Fondue during Storage. A Review. *LWT-Food Science and Technology,* 2002, 35, 15-20. ISSN 0023-6438.

[67] HUI, Y.H; EVRANUZ, E.Ö; CHANDAN, R.C. Handbook of Animal-based Fermented Food and Beverage Technology. 2012. ISBN: 9781439850237.

[68] JOHNSON, M.E; LUCEY, J.A. Major Technological Advances and Trends in Cheese. *Journal of Dairy Science*. 2006, vol. 89, no. 4 s. 1174-1178. ISSN:0022-0302.

[69] MARTH, E.H; and STEELE, J.L. Applied Dairy Microbiology. 2001. Boca Raton: CRC Press, 2., ed. ISBN 978-0824705367.

[70] AGUILERA, J.M; and STANLEY, D.W. Microstructural Principles of food processing and engineering. 2nd Ed. SciTech Book News. 2000, vol. 24143. ISSN:0196-6006.

[71] LAW, B.A; TAMIME, A.Y. Technology of Cheesemaking. 2010, . ISBN: 9781444323740.

[72] CHANDAN, R.C; KILARA, A; SHAH, N. Dairy Processing and Quality Assurance. 2009, . ISBN: 9780813827568.

[73] FOX, P.F; MCSWEENEY, P.L.H. Proteolysis in Cheese During Ripening. *Food Reviews International*. 1996, vol. 12, no. 4 s. 457-509. ISSN:8755-9129.

[74] MCSWEENEY, P.L.H. Biochemistry of Cheese Ripening. International *Journal of Dairy Technology*. 2004, vol. 57, no. 2-3 s. 127-144. ISSN:1364-727X.

[75] FOX, P.F; UNIACKE-LOWE, T; MCSWEENEY, P.L.H; O'MAHONY, J.A. Dairy Chemistry and Biochemistry, Second Edition. . 2015, . ISBN: 3319148915.

[76] MCSWEENEY, P.L.H; SOUSA, M.J. Biochemical Pathways for the Production of Flavour Compounds in Cheeses During Ripening: A Review. *Lait*. 2000, vol. 80, no. 3 s. 293-324. ISSN:0023-7302.

[77] 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.

[78] WEIMER, B.C. Improving the Flavour of Cheese. . 2007. ISBN 978-1-84569-007-6.

[79] MCSWEENEY, P.L.H. Cheese Problems Solved. 2007. ISBN 978-1-84569-060-1.

[80] COMMISSION, The Codex Alimentarius. CXS 265 - Codex Standard for Edam. Codex Alimentarius. 2009, 1.

[81] DÖÜSTERHÖFT, E.-M; VAN DEN BERG, G. Dutch-type Cheeses. Cheese Problems Solved. 2010, s. 230-245. ISBN: 9781845690601

[82] FLASAROVA, R; PACHLOVA, V; BUNKOVA, L; MENSIKOVA, A; GEORGOVA, N; DRAB, V; BUNKA, F. Biogenic Amine Production by *Lactococcus Lactis* Subsp. *Cremoris* Strains in the Model System of Dutch-type Cheese. *Food Chemistry*. 2016, vol. 194 s. 68-75. ISSN:0308-8146.

[83] PACHLOVÁ, V; BUŇKA, F; FLASAROVÁ, R; VÁLKOVÁ, P; BUŇKOVÁ, L. The Effect of Elevated Temperature on Ripening of Dutch Type Cheese. *Food Chemistry*. 2012, vol. 132, no. 4 s. 1846-1854. ISSN:0308-8146.

[84] PACHLOVÁ, V; BUŇKA, F; BUŇKOVÁ, L; WEISEROVÁ, E; BUDINSKÝ, P; ŽALUDEK, M; KRÁČMAR, S. 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*. 2011, vol. 46, no. 1 s. 101-108. ISSN:0950-5423.

[85] ALJEWICZ, M; CICHOSZ, G. Protective Effects of Lactobacillus Cultures in Dutch-type Cheese-like Products. *LWT-Food Science and Technology*. 2015, vol. 63, no. 1 s. 52-56. ISSN:0023-6438.

[86] Interpretive Summaries, April 2016. *Journal of Dairy Science*. 2016, vol. 99, no. 4 s. ix-xvii. ISSN:0022-0302.

[87] REINBOLD, R.S; ERNSTROM, C.A; HANSEN, C.L. Effect of Container Materials on Temperature Profiles of Large Blocks of Swiss and Cheddar Cheese1. *Journal of Dairy Science*. 1992, vol. 75, no. 10 s. 2637-2647. ISSN:0022-0302.

[88] BISIG, W; FROHLICH-WYDER, MT; JAKOB, E; WECHSLER, D. Comparison Between Emmentaler PDO and Generic Emmental Cheese Production in Europe. *Australian Journal of Dairy Technology*. 2010, vol. 65, no. 3 s. 206-213. ISSN:0004-9433.

[89] O'SULLIVAN, D.J; MCSWEENEY, P.L.H; COTTER, P.D; GIBLIN, L; SHEEHAN, J.J. Compromised. *Journal of Dairy Science*. 2016, vol. 99, no. 4 s. 2625-2640. ISSN:0022-0302.

[90] FOX, P.F. Cheese and Fermented Milk Foods (3 Rd Edition). Volume 1: Origins and Principles (728 Pp.) Volume 2: Procedures and Analyses (330 Pp.). *International Dairy Journal*. 1997, vol. 7, no. 5 s. 360-361. ISSN:0958-6946.

[91] COMMISSION, The Codex Alimentarius. CXS 283 - Codex General Standard for Cheese. Codex Alimentarius. 2009, 1.

[92] COMMISSION, The Codex Alimentarius. CXS 269 - Codex Standard for Emmental. Codex Alimentarius. 2009, 1.

[93] ISOLINI, D; BACHMANN, H-P; BÜTIKOFER, U; JAKOB, E; FRÖHLICH-WYDER, M-T. Swiss-Type Cheeses. *Encyclopedia of Dairy Sciences*. 2011, s. 712-720.

[94] PEPPLER, H. J; PERLMAN, D. Microbial Technology: Fermentation Technology. 2014, vol. 2.

[95] ROBINSON, R.K. Dairy Microbiology Handbook: Microbiology of Milk and Milk Products. . 2002, .

[96] PISANO, MB; SCANO, P; MURGIA, A; COSENTINO, S; CABONI, P. Metabolomics and Microbiological Profile of Italian Mozzarella Cheese Produced with Buffalo and Cow Milk. *Food Chemistry*. 2016, vol. 192 s. 618-624. ISSN:0308-8146.

[97] FERRARI, E; GAMBERI, M; MANZINI, R; PARESCHI, A; PERSONA, A; REGATTIERI, A. Redesign of the Mozzarella Cheese Production Process Through Development of a Micro-forming and Stretching Extruder System. *Journal of Food Engineering*. 2003, vol. 59, no. 1 s. 13-23. ISSN:0260-8774.

[98] GERNIGON, G; SCHUCK, P; JEANTET, R. Processing of Mozzarella Cheese Wheys and Stretchwaters: A Preliminary Review. *Dairy Science & Technology*. 2010, vol. 90, no. 1 s. 27-46. ISSN:1958-5586.

[99] ZHU, CJ; BROWN, C; GILLIES, G; WATKINSON, P; BRONLUND, J. Characterizing the Rheological Properties of Mozzarella Cheese at Shear Rate and Temperature Conditions Relevant to Pizza Baking. *LWT-Food Science and Technology*. 2015, vol. 64, no. 1 s. 82-87. ISSN:0023-6438.

[100] COMMISSION, The Codex Alimentarius. CXS 262 - Codex Standard for Mozzarella. Codex Alimentarius. 2009, 1.

[101] AMAGLIANI, G; GIAMMARINI, C; OMICCIOLI, E; BRANDI, G; MAGNANI, M. Detection of Listeria Monocytogenes Using a Commercial PCR Kit and Different DNA Extraction Methods. *Food Control*. 2007, vol. 18, no. 9 s. 1137-1142. ISSN:0956-7135.

[102] RUBIO-TEXEIRA, M. Endless Versatility in the Biotechnological Applications of *Kluyveromyces* LAC Genes. *Biotechnology Advances*. 2006, vol. 24, no. 2 s. 212-225. ISSN:0734-9750.

[103] COSTA DIAS, M.A; SANT'ANA, A.S; CRUZ, A.G; FARIA, J.d.A.F; FERNANDES DE OLIVEIRA, C.A; BONA, E. On the Implementation of Good Manufacturing Practices in a Small Processing Unity of Mozzarella Cheese in Brazil. *Food Control*. 2012, vol. 24, no. 1-2 s. 199-205. ISSN:0956-7135.

[104] GORRASI, G; BUGATTI, V; TAMMARO, L; VERTUCCIO, L; VIGLIOTTA, G; VITTORIA, V. Active Coating for Storage of Mozzarella Cheese Packaged Under Thermal Abuse. *Food Control*. 2016, vol. 64 s. 10-16. ISSN:0956-7135.

[105] DE ANGELIS, M; GOBBETTI, M. Environmental Stress Responses in Lactobacillus: A Review. *Proteomics*. 2004, vol. 4, no. 1 s. 106-122. ISSN:1615-9853.

[106] GUIDONE, A; ZOTTA, T; MATERA, A; RICCIARDI, A; DE FILIPPIS, F; ERCOLINI, D; PARENTE, E. The Microbiota of High-moisture Mozzarella Cheese Produced with Different Acidification Methods. International Journal of *Food Microbiology*. 2016, vol. 216 s. 9-17. ISSN:0168-1605.

[107] FACCIA, M; TRANI, A; DI LUCCIA, A. Short Communication: Relationships Between Milk Quality and Acidification in the Production of Table Mozzarella Without Starters. *Journal of Dairy Science*. 2009, vol. 92, no. 9 s. 4211-4217. ISSN:0022-0302.

[108] YUN, J.J; BARBANO, D.M; LAROSE, K.L; KINDSTEDT, P.S. Mozzarella Cheese: Impact of Nonfat Dry Milk Fortification on Composition, Proteolysis, and Functional Properties. *Journal of Dairy Science*. 1998, vol. 81, no. 1 s. 1-8. ISSN:0022-0302.

[109] YUN, J.J; KIELY, L.J; KINDSTEDT, P.S; BARBANO, D.M. Mozzarella Cheese: Impact of Coagulant Type on Functional Properties. *Journal of Dairy Science*. 1993, vol. 76, no. 12 s. 3657-3663. ISSN:0022-0302.

[110] ROWNEY, M.K; ROUPAS, P; HICKEY, M.W; EVERETT, D.W. The Effect of Compression, Stretching, and Cooking Temperature on Free Oil Formation in Mozzarella Curd. *Journal of Dairy Science*. 2003, vol. 86, no. 2 s. 449-456. ISSN:0022-0302.

[111] ROWNEY, M.K; HICKEY, M.W; ROUPAS, P; EVERETT, D.W. The Effect of Homogenization and Milk Fat Fractions on the Functionality of Mozzarella Cheese. *Journal of Dairy Science*. 2003, vol. 86, no. 3 s. 712-718. ISSN:0022-0302.

[112] OBERG, E.N; OBERG, C.J; MOTAWEE, M.M; MARTINI, S; MCMAHON, D.J. Increasing Stringiness of Low-fat Mozzarella String Cheese Using Polysaccharides. *Journal of Dairy Science*. 2015, vol. 98, no. 7 s. 4243-4254. ISSN:0022-0302.

[113] MCMAHON, D; OBERG, J. Pasta-Filata Cheeses: Low-Moisture Part-Skim Mozzarella (Pizza Cheese). *Encyclopedia of Dairy Sciences*. 2011, s. 737-744. ISSN:-.

[114] KOSIKOWSKI, F.V; MISTRY, V.V. Microfiltration, Ultrafiltration, and Centrifugation Separation and Sterilization Processes for Improving Milk and Cheese Quality. *Journal of Dairy Science*. 1990, vol. 73, no. 6 s. 1411-1419. ISSN:0022-0302.

[115] MACKŮ, I; BUŇKA, F; VOLDÁNOVÁ, B; PAVLÍNEK, V. Effect of Addition of Selected Solid Cosolutes on Viscoelastic Properties of Model Processed Cheese Containing Pectin. *Food Hydrocolloids*. 2009, vol. 23, no. 8 s. 2078-2084. ISSN:0268-005.

[116] ČSN EN ISO 5534. *Sýry a tavené sýry –Stanovení obsahu celkové sušiny (Referenční metoda)*. Praha: Český normalizační institut, 2008.

[117] BUŇKOVÁ, L; BUŇKA, F; MANTLOVÁ, G; ČABLOVÁ, A; SEDLÁČEK, I; ŠVEC, P; PACHLOVÁ, V; KRÁČMAR, S. The Effect of Ripening and Storage Conditions on the Distribution of Tyramine, Putrescine and Cadaverine in Edam-cheese. *Food Microbiology*. 2010, vol. 27, no. 7 s. 880-888. ISSN:0740-0020.

[118] BOURNE, M.C. Food Texture and Viscosity: Concept and Measurement. . 2002.

[119] WEISEROVÁ, E; DOUDOVÁ, L; GALIOVÁ, L; ŽÁK, L; MICHÁLEK, J; JANIŠ, R; BUŇKA, F. The Effect of Combinations of Sodium Phosphates in Binary Mixtures on Selected Texture Parameters of Processed Cheese Spreads. *International Dairy Journal*. 2011, vol. 21, no. 12 s. 979-986. ISSN:0958-6946.

[120] SZCZESNIAK, A.S. Texture is a Sensory Property. *Food Quality and Preference*. 2002, vol. 13, no. 4 s. 215-225. ISSN:0950-3293.

[121] SOLOWIEJ, B; CHEUNG, IWY; LI-CHAN, ECY. Texture, Rheology and Meltability of Processed Cheese Analogues Prepared Using Rennet or Acid Casein with or Without Added Whey Proteins. *International Dairy Journal*. 2014, vol. 37, no. 2 s. 87-94. ISSN:0958-6946.

[122] CUNHA, C.R.; DIAS, A.I.; VIOTTO, W.,H. Microstructure, texture, colour and sensory evaluation of a spreadable processed cheese analogue made with vegetable fat. *Food Research International*, 2010, 43, 723–729. ISSN 0963-9969.

[123] GABRIELE, D; DE CINDIO, B; D'ANTONA, P. A Weak Gel Model for Foods. *Rheologica Acta*. 2001, vol. 40, no. 2 s. 120-127. ISSN:0035-4511.

[124] WINTER, H. H.; and F. CHAMBON. 1986. Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. Journal of Rheology. 1986, 30, 367–382.

[125] MARTÍNEZ-RUVALCABA, A; CHORNET, E; RODRIGUE, D. Viscoelastic Properties of Dispersed Chitosan/xanthan Hydrogels. *Carbohydrate Polymers*. 2007, vol. 67, no. 4 s. 586-595. ISSN:0144-8617.

7. THE THESIS CONTRIBUTION TO SCIENCE AND PRACTICE

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

- Were characterised possible interactions of different emulsifying salts (phosphate and/ or citrate salts), applied as sole ingredients or in the form of binary or ternary mixtures with casein micelles dispersion intensity in model milk samples;
- Was described the development of pH in dairy systems after the application of different phosphate and/or citrate emulsifying salts, utilized as sole ingredients or in the form of binary or ternary mixtures;
- Were compared the textural properties of processed cheese samples in which the pH values were and were not adjusted;
- Were described the changes of textural and viscolelastic properties occuring in real dairy samples arising from the application of different emulsifying salts during storage;
- Were described the changes of textural and viscoelastic characteristics of processed cheese samples manufactured from different natural cheeses (main raw material) with various degrees of maturity during storage;
- Were manufactured wide series of samples providing a comprehensive study allowing the comparability of the obtained results;
- The obtained results could be used as models describing the dependance of processed cheese functional properties on the composition of mixtures of emulsifying salts for the production of end products of different consistency;
- Under real industrial praxis the obtained model could be applied as a description of the samples‘ textural and viscolelastic properties arising from the type and maturity degree of the used natural cheese in combination with the utilized type and concentration of emulsifying salts;
- From the obtained data it 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 with desirable consistency and organoleptic properties.

8. LIST OF RESEARCH PAPERS

Research paper I

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, 111-122. IF = 3.29 (for year 2014).

Research paper II

SALEK, R.N., ČERNÍKOVÁ, M., NAGYOVÁ, G., KUCHAŘ, D., BAČOVÁ, H., MINARČIKOVÁ, 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. IF = 2.27 (for year 2015).

Research paper III

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. IF = 3.02 (for year 2016).

Research paper IV

SALEK, R.N., ČERNÍKOVÁ, M., PACHLOVÁ, V. BUBELOVÁ, Z., KONEČNÁ, V., BUŇKA, F. 2016. Properties of spreadable processed Mozzarella cheese with divergent composition of emulsifying salts in relation to the applied cheese storage period. *LWT-Food Science and Technology*, 77, 30 – 38. IF = 2.711 (for year 2015).

9. SUMMARY OF THE RESEARCH PAPERS

The present doctoral thesis is focused on the effect of the composition of ternary mixtures of emulsifying salts (consisted of phosphate and/or citrate salts) on selected functional characteristics of model dairy systems. The key-findings are highlighted as summaries of each of the four scientific reaserch papers presented below.

Research paper I was focused on the description of the dependance of selected textural properties (hardness, relative adhesiveness and cohesiveness) of spreadable type processed cheese (40 % w/w dry matter content, 50 % fat in dry matter conent) on the proportion of phosphate emulsifying salts in the form of ternary mixtures. The applied emulsifying salts were disodium phosphate (DSP), tertasodium diphosphate (TSPP) and sodium salt of polyphosphate with different mean lengths (n ≈ 5, 9, 13, 20, and 28). Moreover, in the second part of the study pentasodium triphosphate (PSTP) was utilized instead of TSPP. Final processed cheese samples with and without pH adjustment were examined (the target values were within the range of 5.60 – 5.80). In addition, the link between the development of the above mentioned textural characteristics of processed cheese samples related to the casein micelles dispersion intensity in model milk samples (containing skimmed milk powder and ternary mixtures of phosphate emulsifying salts) was examined. 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; however, 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 emulsifying salts 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. From our results concerning the optical density measurement of milk systems with ternary mixtures of phosphates, more intensive casein dispersion is reached with the application of a greater amount of polyphosphates (in the ternary mixtures); and when using polyphosphates with a longer chain length. 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.

Research paper II was concentrated on the impact of the ternary mixtures consisting of phosphate and/or citrate emulsifying salts [disodium hydrogenphosphate, DSP; tetrasodium diphosphate, TSPP; sodium salt of polyphosphate (with mean length n ≈ 20), P20; and trisodium citrate, TSC] composition on textural parameters of spread-type processed cheese and on the casein micelle dispersion (in the model milk system). The processed cheese samples [40 % w/w dry matter content, 50% w/w fat in dry matter content] were produced using Edam cheese, characterised of 7 weeks maturity level. Two different groups of processed cheese samples were manufactured, one with pH adjustment (target values within the interval of 5.60 – 5.80) and one without pH adjustment. The sole application of phosphates with longer chains led to processed cheeses with higher values of hardness. When the phosphate and citrate emulsifying salts were applied individually the hardness of the model processed cheeses rose in the following order: DSP < TSC < TSPP < P20. 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. The hardness of all processed cheese samples increased with the increasing storage period. 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 emulsifying salts on the intensity of casein dispersion in the model milk system.

Research paper III was aimed at the examination of the dependence of selected textural 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-day storage period (6 ± 2°C). The processed cheese samples [40% w/w dry matter (DM) content, 50% w/w 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 weeks 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. 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.

Research paper IV was focused on selected textural and viscoelastic characteristics of spreadable processed cheese (35 % w/w dry matter; 50 % w/w fat in dry matter) manufactured with different ternary mixtures of emulsifying salts 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 emulsifying salts utilized consisted of disodium hydrogenphosphate (DSP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate with mean length n≈20 (P20), and trisodium citrate (TSC). 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 TPA. Hence, the ratio of DSP:TPSS resulted in processed cheese 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 processed cheese.

Research paper I

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

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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

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Received July 1, 2013.
Accepted September 22, 2013.
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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 $\lambda = 700$ 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.

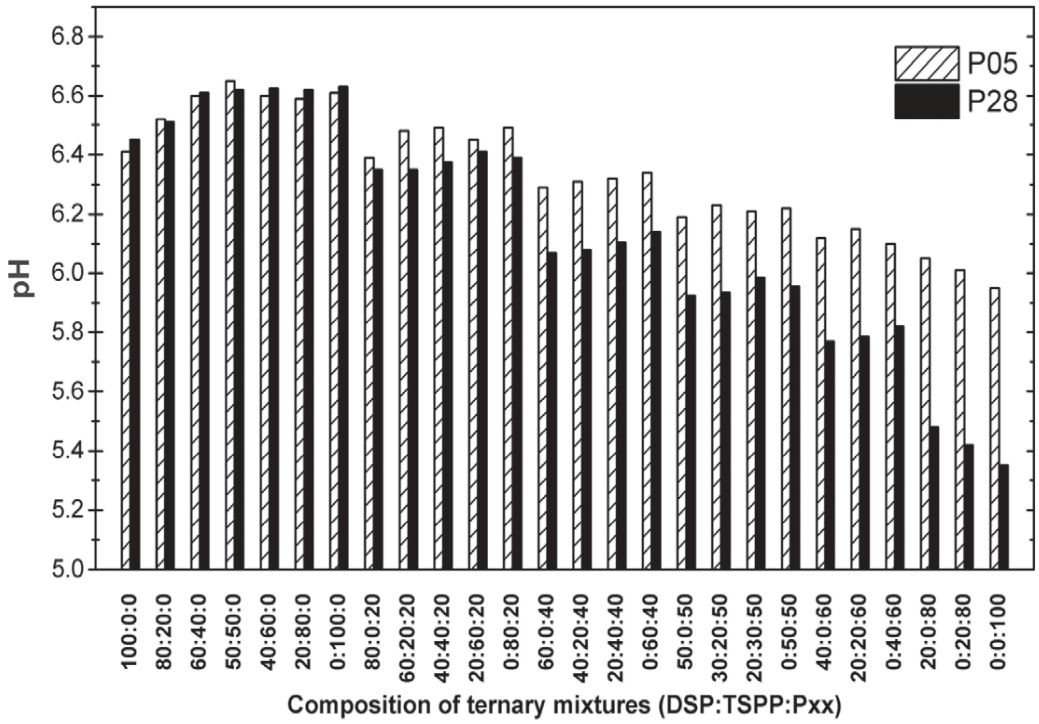

Figure 1. The dependence of pH values of the model samples (stored for 2 d at 6°C) on the relative amount (x-axis; %) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), and sodium salts of polyphosphate (P05, sodium salts of polyphosphate with medium length, n ≈ 5; P28 = sodium salts of polyphosphate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

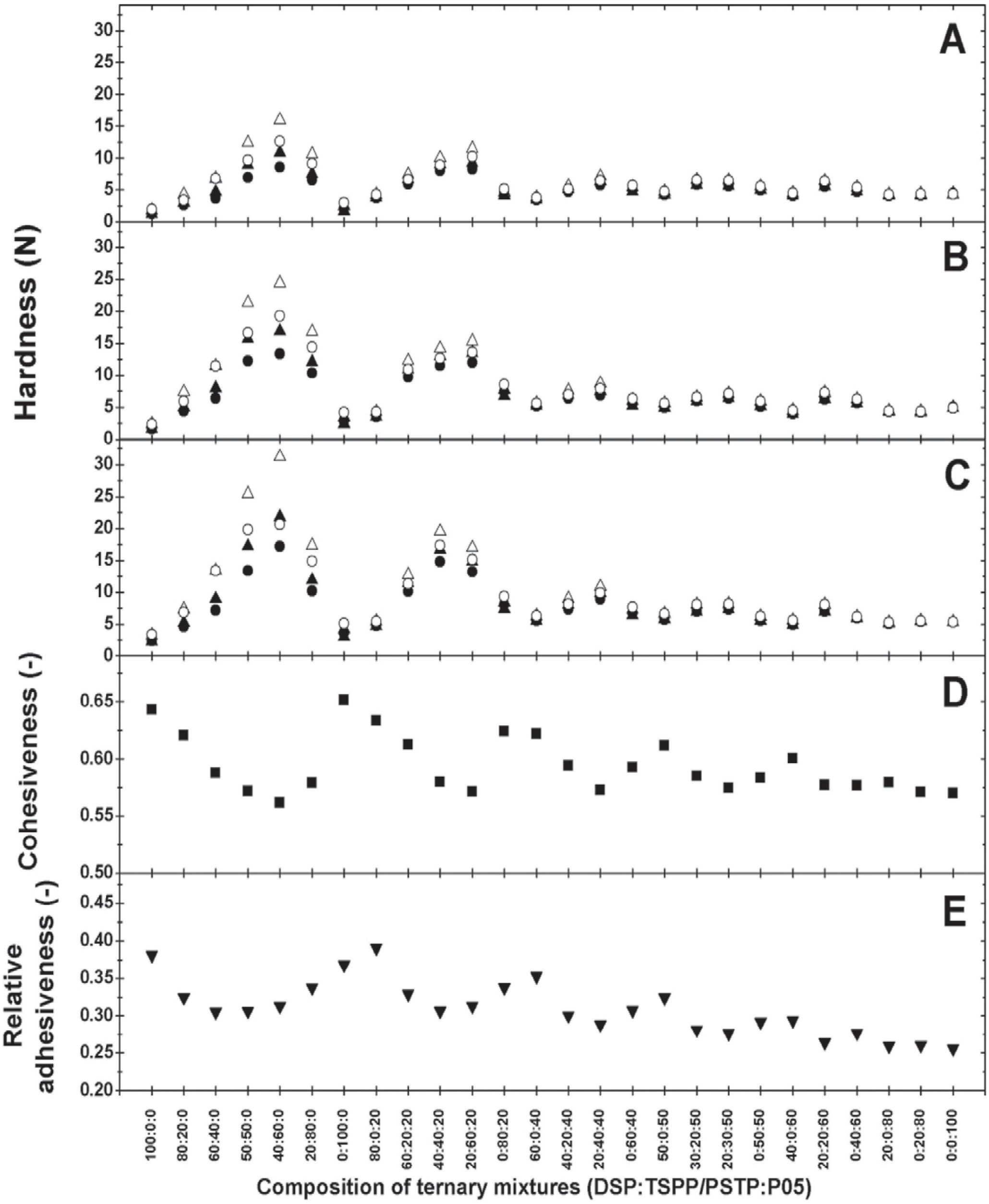

Figure 2. The dependence of processed cheese hardness (panels A to C), cohesiveness (unitless; panel D) and relative adhesiveness (unitless; panel E) on the relative amount (x-axis; %) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), or pentasodium triphosphate (PSTP), and sodium salt of 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 $\leq 60\%$, 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 $\leq 60\%$), 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

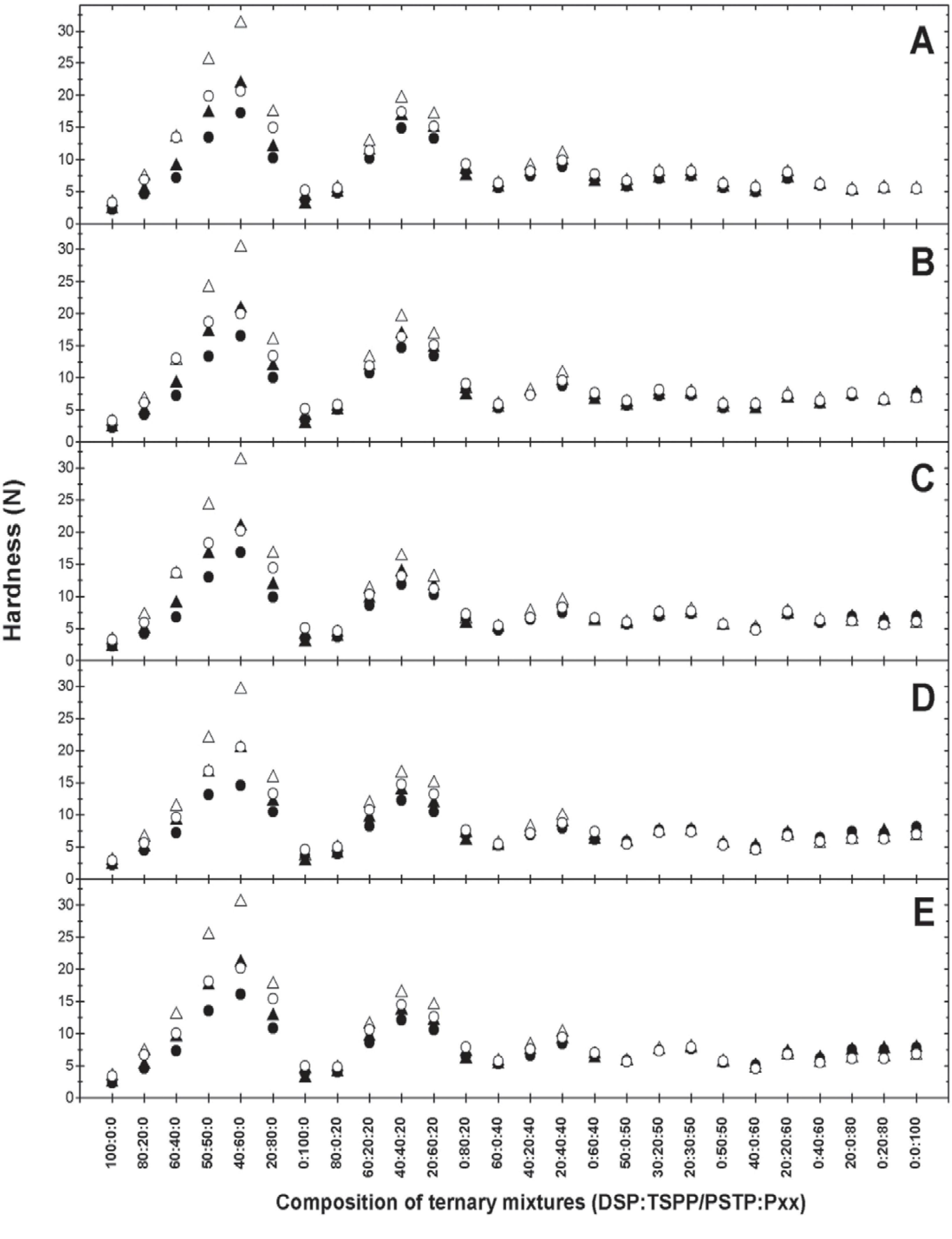

Figure 3. The dependence of processed cheese hardness on the relative amount (x-axis; %) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), or pentasodium triphosphate (PSTP) and sodium salt of polyphosphate (with different chain lengths): P05 (n ≈ 5, panel A), P09 (n ≈ 9, panel B), P13 (n ≈ 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

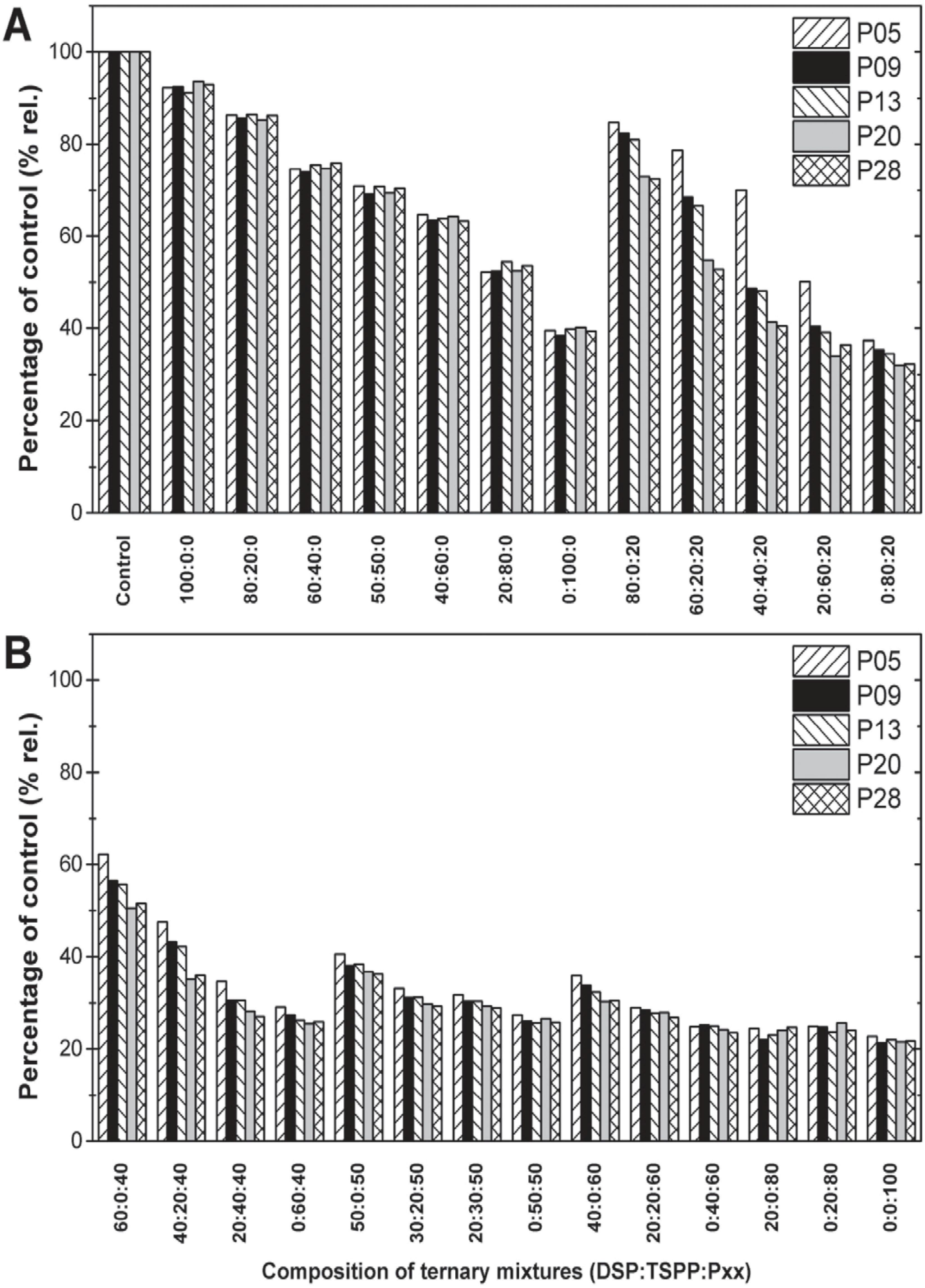

Figure 4. The dependence of percentage of absorbance of the model milk systems (% rel.; in relation to the model milk systems without phosphate addition = 100%) on the relative amount (x-axis; %) of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), and sodium salt of polyphosphate (with different chain lengths): P05 ($n \approx 5$), P09 ($n \approx 9$), P13 ($n \approx 13$), P20 ($n \approx 20$), and P28 ($n \approx 28$) 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 compositrend 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 II

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

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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).

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http://dx.doi.org/10.1016/j.idairyj.2014.12.009
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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

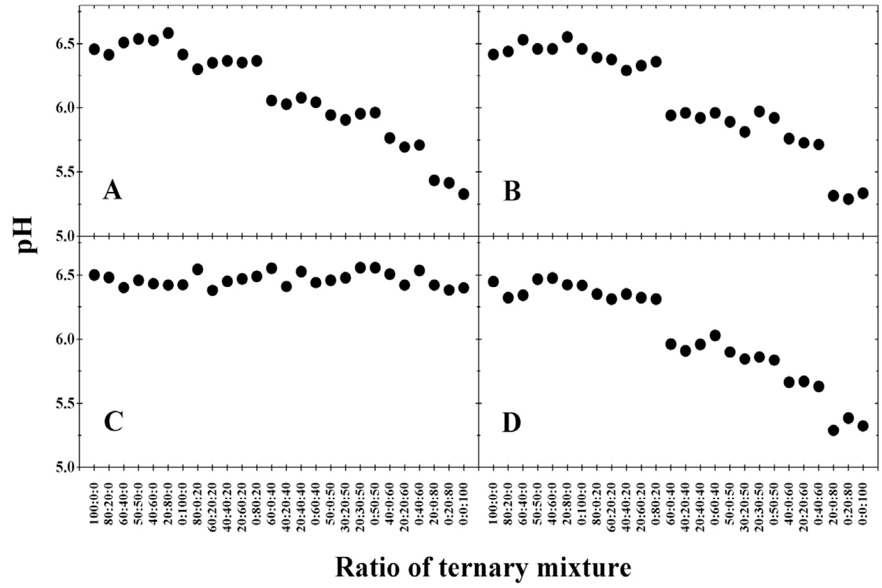

Fig. 1. The dependence of pH values of the model processed cheeses (stored 2 days at 6 °C) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of ternary mixtures of disodium hydrogen phosphate (DSP), tetrasodium diphosphate (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.

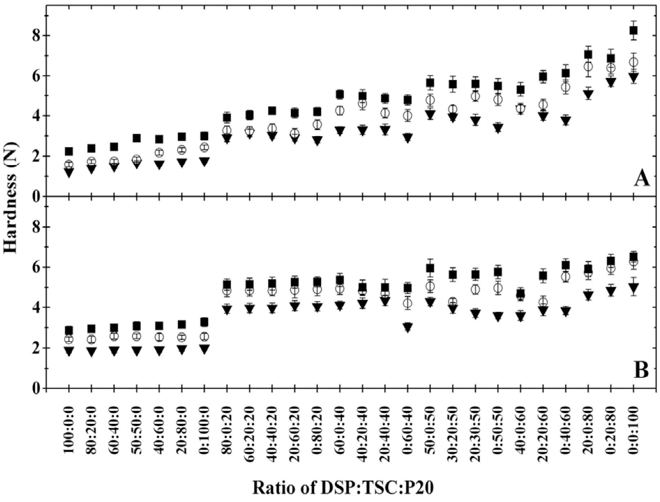

Fig. 2. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of disodium hydrogen phosphate (DSP), trisodium 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).

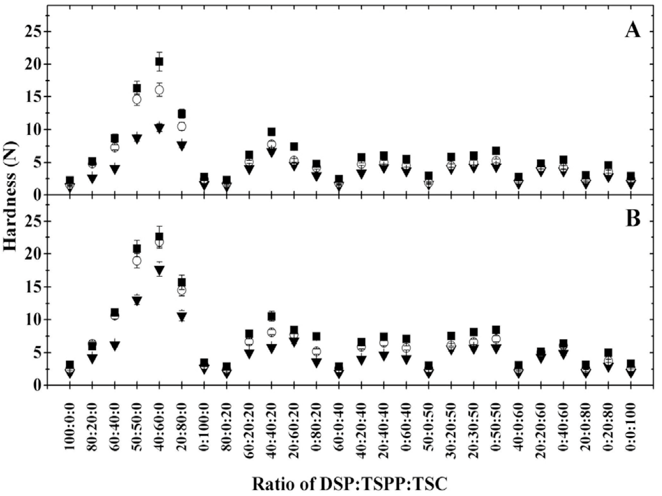

Fig. 4. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of disodium hydrogen phosphate (DSP), tetrasodium 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

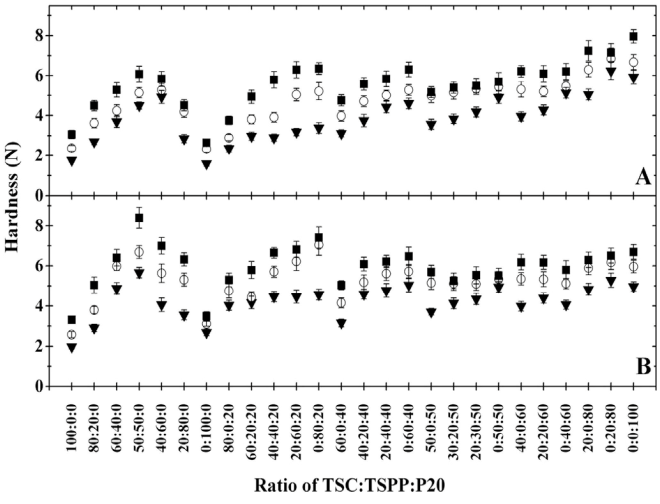

Fig. 3. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of trisodium citrate (TSC), tetrasodium diphosphate (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).

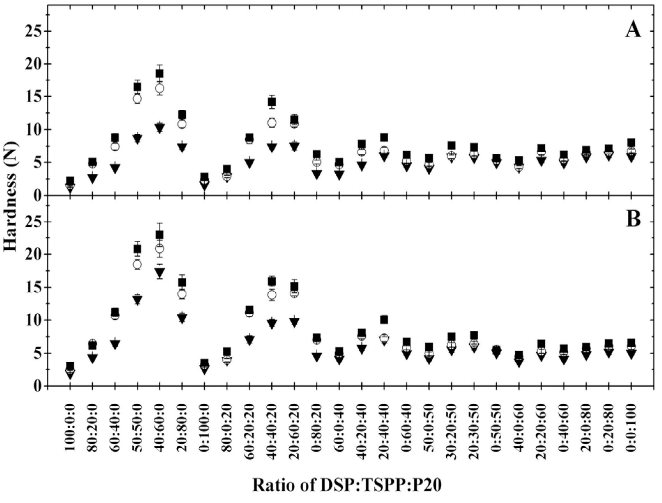

Fig. 5. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%) of disodium hydrogen phosphate (DSP), tetrasodium 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 $\leq$60% and TSC $\leq$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 $\leq$60% and TSC $\leq$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

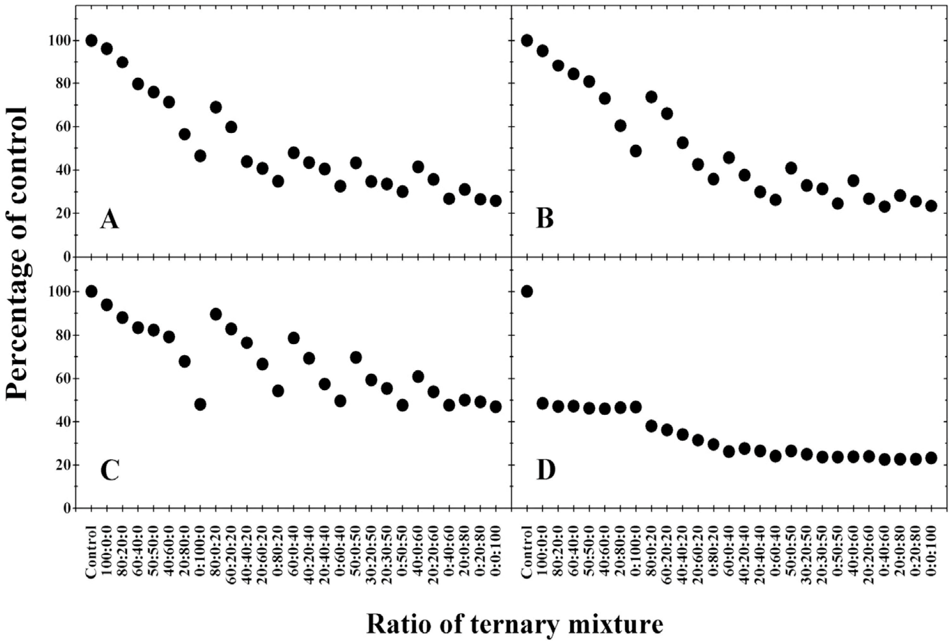

Fig. 6. The dependence optical density of the model milk systems (optical density expressed relative to that of model milk systems without emulsifying salts addition: control, 100%) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying 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., Grůber, 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 III

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

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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

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Received June 29, 2015.
Accepted December 30, 2015.
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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 ≈ 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 × 12 reciprocal percentage ratios × 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 × *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

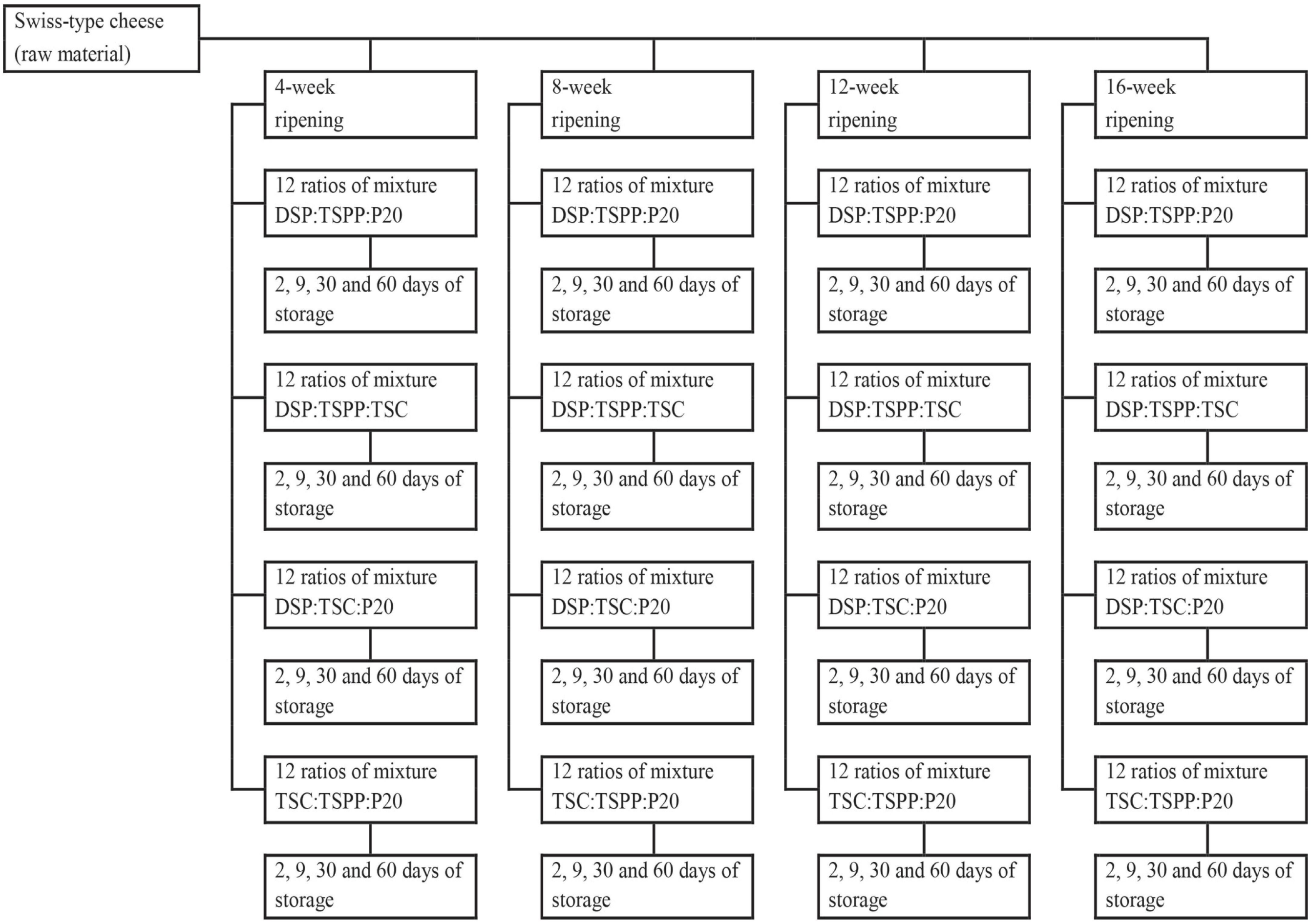

Figure 1. Scheme of the experimental design with model processed cheeses manufactured using Swiss-type cheese (in various time of ripening) and the different percentage ratios (100:0:0, 50:50:0, 0:100:0, 40:40:20, 40:20:40, 20:40:40, 50:0:50, 0:50:50, 40:0:60, 20:20:60, 0:40:60, 0:0:100) of the 4 types 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 $\text{mm}\cdot\text{s}^{-1}$ 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]:

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

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):

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

where $\mathrm{A_F}$ is the strength of the gel ($\text{Pa}\cdot\text{s}^{1/\text{z}}$) 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: $\mathrm{A_F} > 0$ and $\mathrm{z} \geq 0$. 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 $g \cdot kg^{-1}$, 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

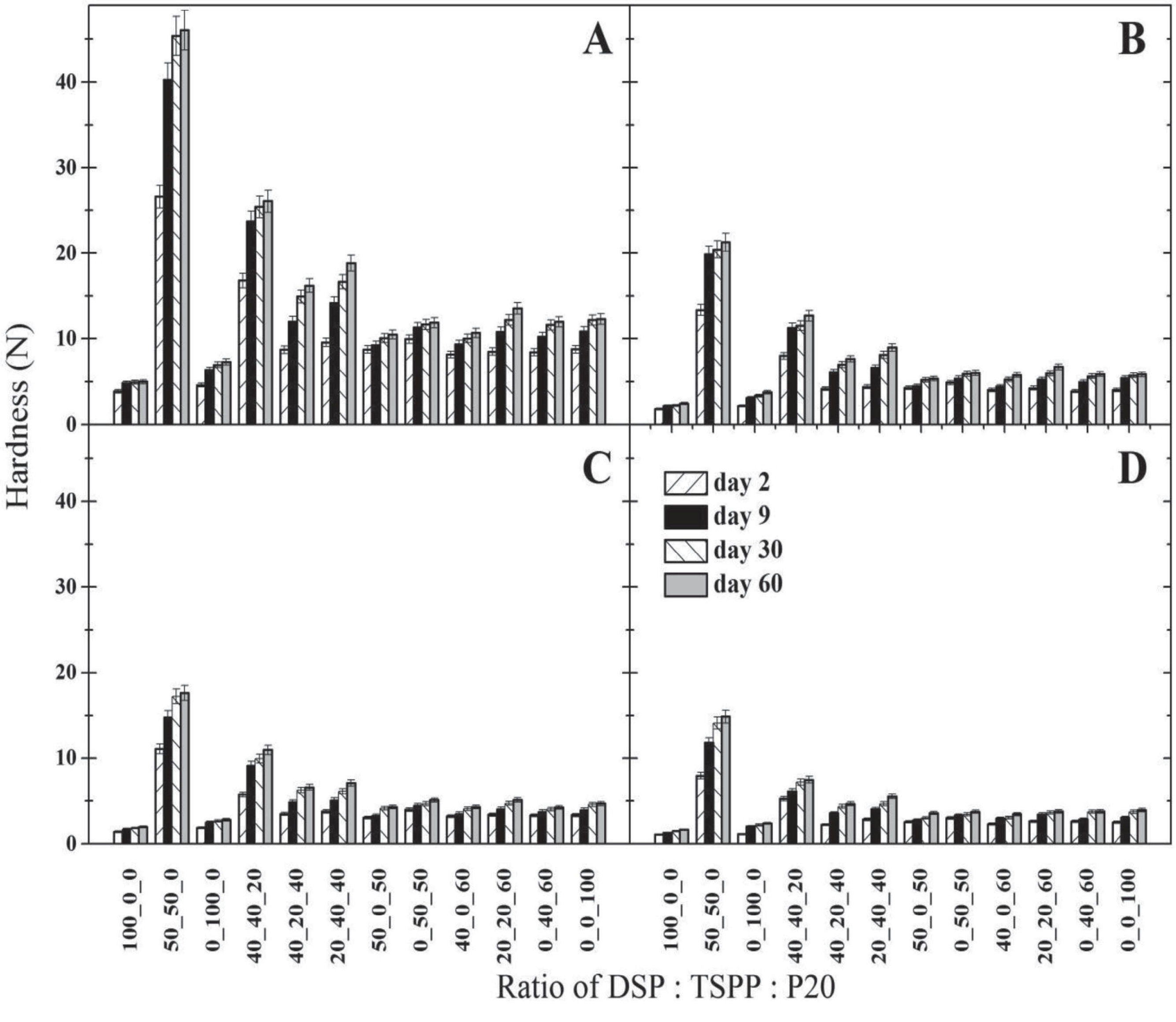

Figure 2. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%; axis x) of disodium phosphate (DSP), tetrasodium 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-

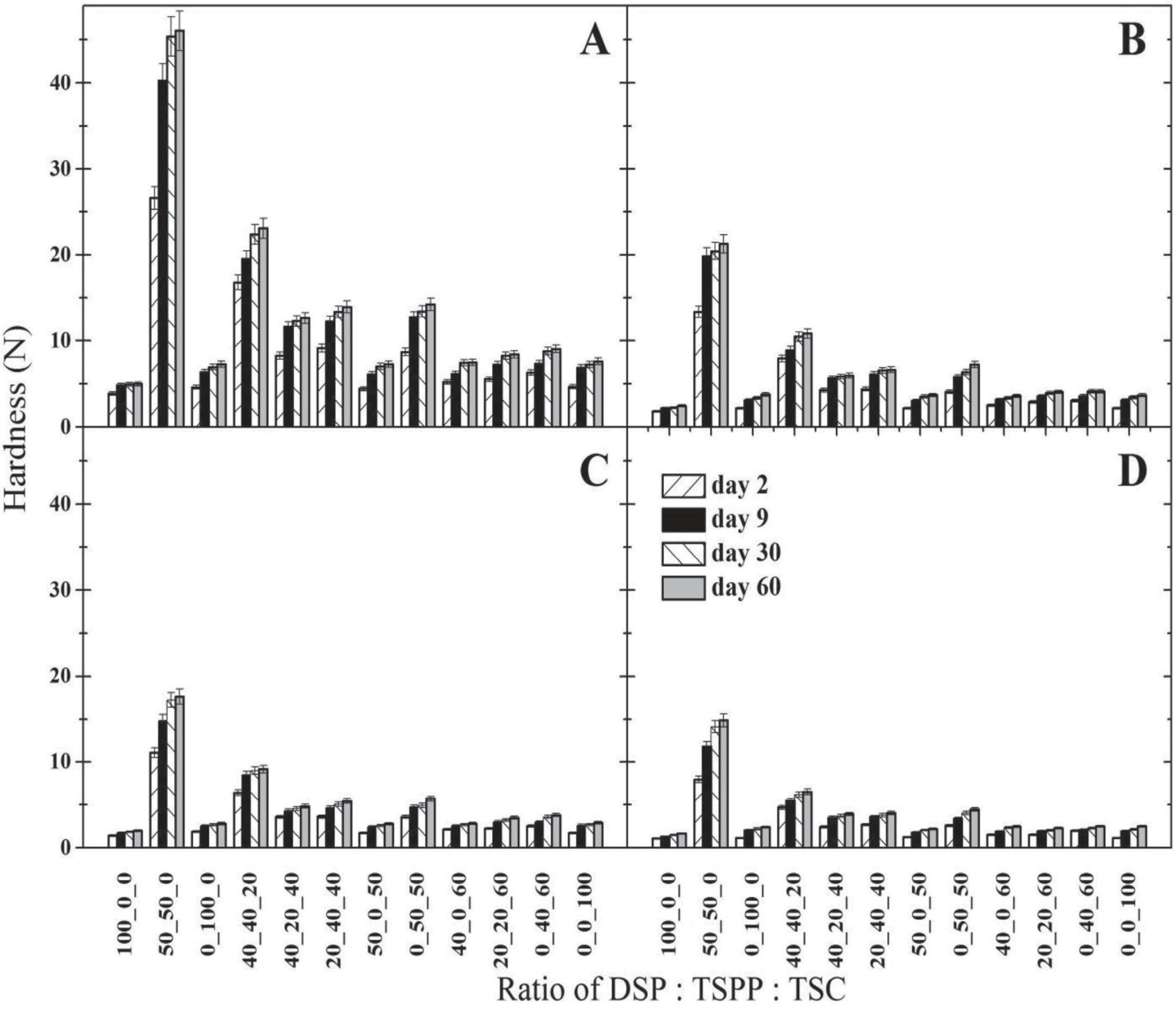

Figure 3. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%; axis x) of disodium phosphate (DSP), tetrasodium 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-

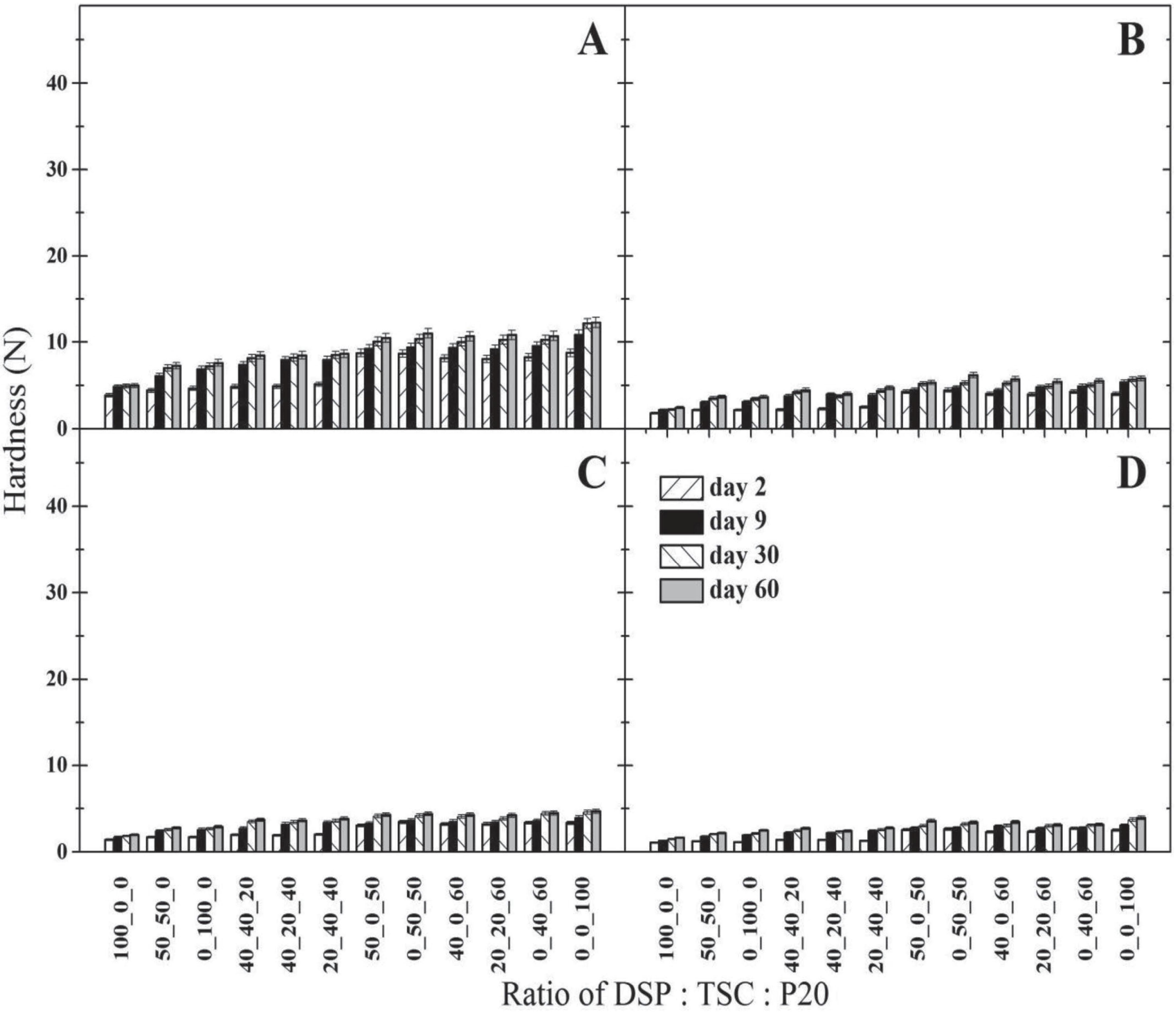

Figure 4. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%; axis x) of disodium phosphate (DSP), trisodium 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

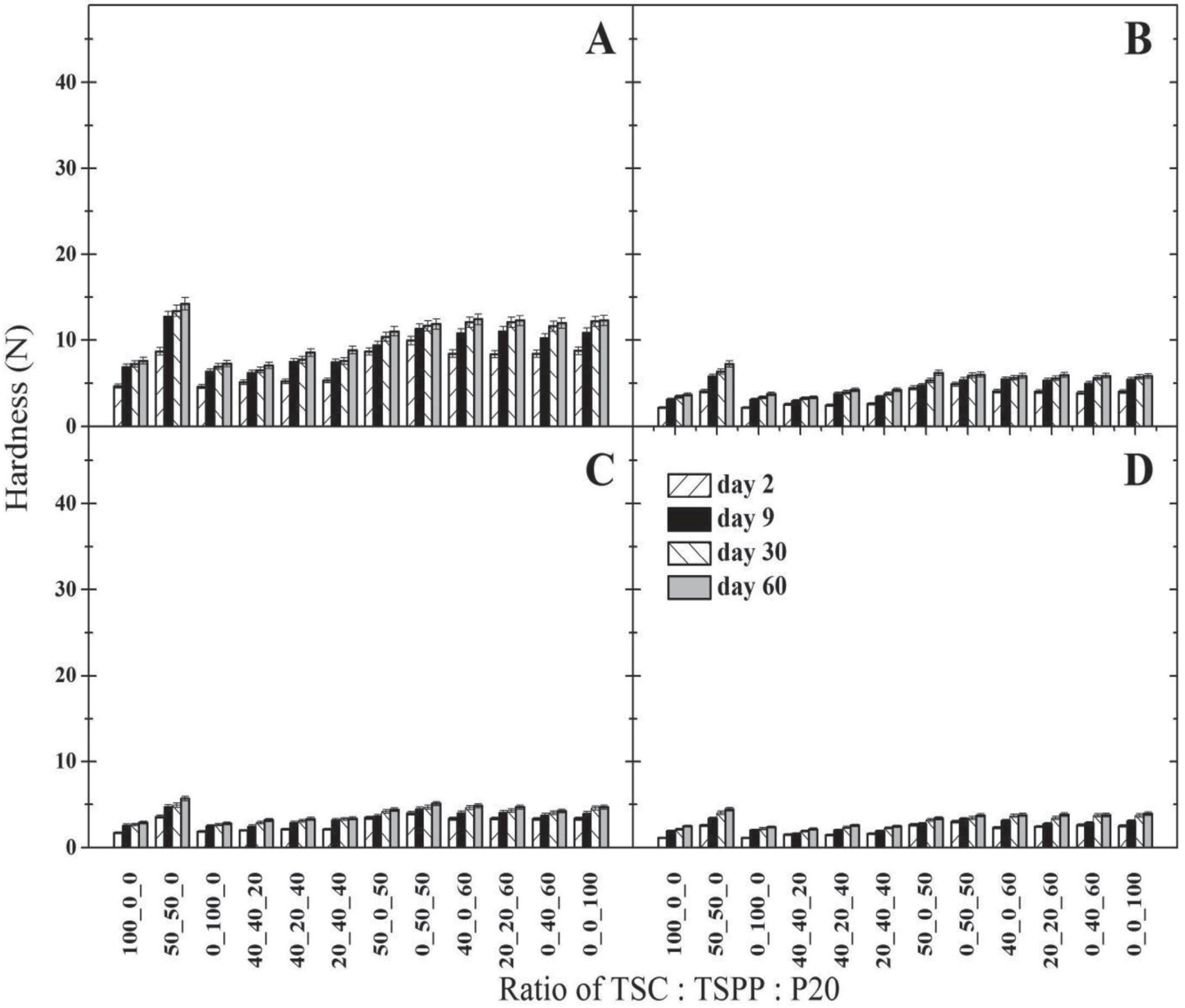

Figure 5. The dependence of processed cheese hardness (calculated as maximum force during the first penetration cycle; N) on the relative amount (in percentage; percentage of ternary mixtures was calculated on the total weight of emulsifying salts = 100%; axis x) of trisodium citrate (TSC), tetrasodium 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,

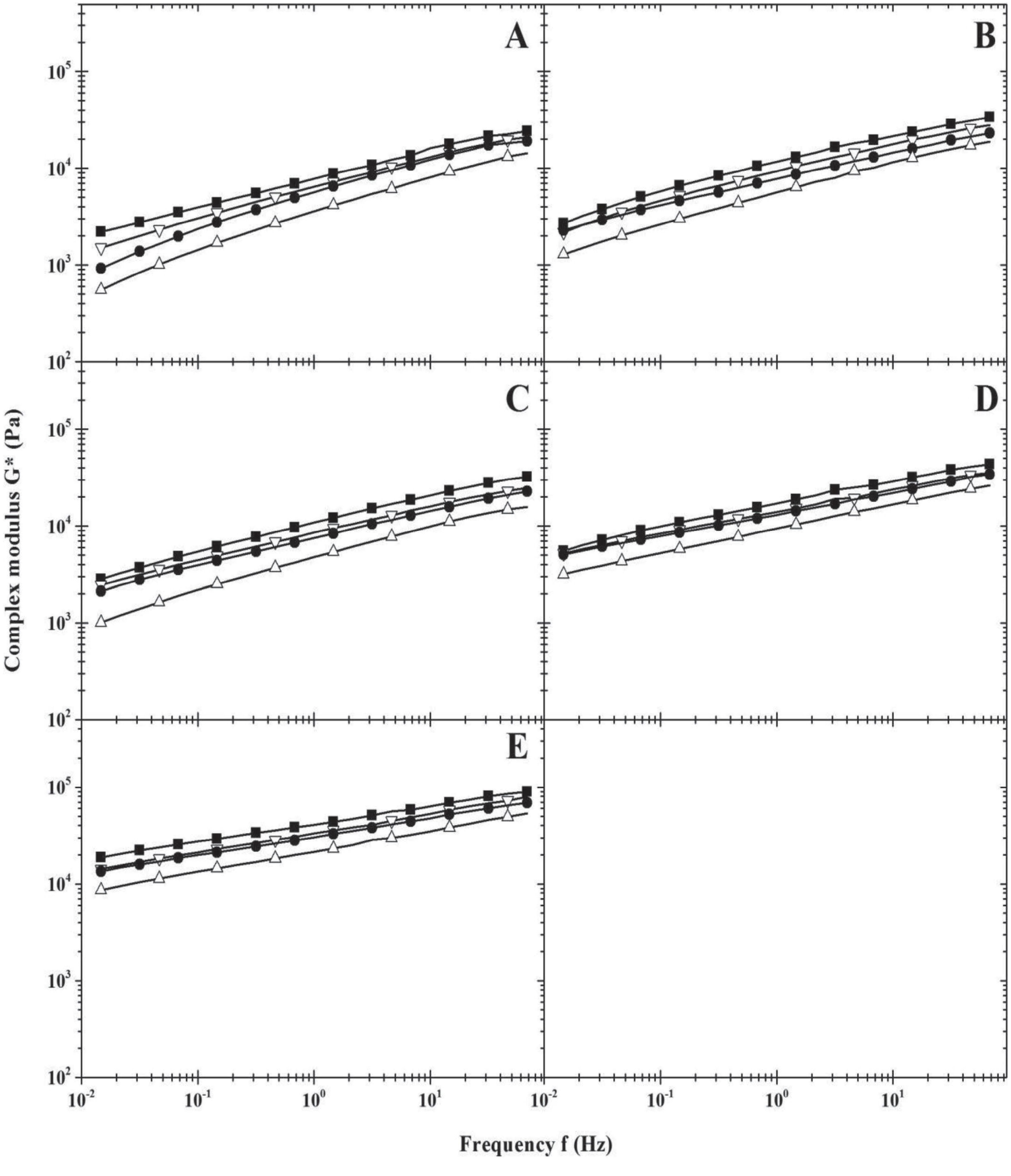

Figure 6. The dependence of complex modulus (G*) of processed cheese (after 30 d of storage) made from Swiss-type cheese after different time of ripening (■ 4 wk; ▽ 8 wk; ● 12 wk; Δ 16 wk) on frequency (f; in range of 0.01–100.00 Hz). Processed cheeses were manufactured using disodium phosphate (DSP; 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	$8.2 \pm 0.6^{a,A}$	$6.6 \pm 0.2^{a,B}$	$5.6 \pm 0.4^{a,C}$	$3.5 \pm 0.2^{a,D}$
	50:50:0	$41.1 \pm 2.8^{i,A}$	$32.5 \pm 1.9^{i,B}$	$30.6 \pm 1.4^{i,B}$	$21.6 \pm 1.2^{h,C}$
	0:100:0	$11.5 \pm 0.7^{b,A}$	$9.2 \pm 0.5^{b,B}$	$7.8 \pm 0.3^{b,C}$	$5.6 \pm 0.3^{b,D}$
	40:40:20	$27.9 \pm 0.9^{h,A}$	$23.1 \pm 1.1^{h,B}$	$18.1 \pm 0.9^{h,C}$	$16.5 \pm 1.2^{g,D}$
	40:20:40	$19.6 \pm 0.8^{f,A}$	$17.1 \pm 1.2^{f,B}$	$15.2 \pm 0.6^{f,C}$	$14.1 \pm 0.6^{f,C}$
	20:40:40	$21.5 \pm 1.0^{g,A}$	$18.7 \pm 0.2^{g,B}$	$16.9 \pm 0.7^{g,C}$	$14.9 \pm 0.8^{f,D}$
	50:0:50	$13.9 \pm 0.6^{c,A}$	$11.4 \pm 0.5^{c,B}$	$9.2 \pm 0.5^{c,C}$	$7.5 \pm 0.3^{c,D}$
	0:50:50	$15.0 \pm 0.7^{d,A}$	$12.9 \pm 0.4^{d,B}$	$9.9 \pm 0.5^{cd,C}$	$8.4 \pm 0.4^{d,D}$
	40:0:60	$13.9 \pm 0.6^{c,A}$	$12.0 \pm 0.8^{c,B}$	$9.5 \pm 0.5^{c,C}$	$7.9 \pm 0.5^{cd,D}$
	20:20:60	$17.3 \pm 1.0^{e,A}$	$14.0 \pm 0.6^{a,B}$	$13.0 \pm 0.6^{e,C}$	$9.1 \pm 0.6^{e,D}$
	0:40:60	$15.8 \pm 1.0^{d,A}$	$13.0 \pm 0.7^{d,B}$	$10.1 \pm 0.3^{d,C}$	$8.0 \pm 0.3^{d,D}$
	0:0:100	$17.0 \pm 1.1^{e,A}$	$14.0 \pm 0.5^{e,B}$	$13.1 \pm 0.7^{e,C}$	$9.4 \pm 0.5^{e,D}$
DSP:TSPP:TSC	100:0:0	$8.2 \pm 0.6^{a,A}$	$6.6 \pm 0.2^{a,B}$	$5.6 \pm 0.4^{a,C}$	$3.5 \pm 0.2^{a,D}$
	50:50:0	$41.1 \pm 2.8^{g,A}$	$32.5 \pm 1.9^{g,B}$	$30.6 \pm 1.4^{f,B}$	$21.6 \pm 1.2^{f,C}$
	0:100:0	$11.5 \pm 0.7^{de,A}$	$9.2 \pm 0.5^{d,B}$	$7.8 \pm 0.3^{c,C}$	$5.6 \pm 0.3^{c,D}$
	40:40:20	$19.8 \pm 0.8^{f,A}$	$18.5 \pm 0.9^{f,B}$	$15.9 \pm 0.6^{e,C}$	$14.5 \pm 0.8^{a,D}$
	40:20:40	$11.6 \pm 0.7^{de,A}$	$11.0 \pm 0.5^{e,B}$	$9.1 \pm 0.5^{d,C}$	$7.4 \pm 0.4^{d,D}$
	20:40:40	$12.6 \pm 0.4^{e,A}$	$11.6 \pm 0.5^{e,B}$	$9.3 \pm 0.4^{d,C}$	$8.6 \pm 0.5^{e,D}$
	50:0:50	$9.0 \pm 0.5^{b,A}$	$7.5 \pm 0.4^{b,B}$	$6.5 \pm 0.3^{b,C}$	$4.5 \pm 0.2^{b,D}$
	0:50:50	$13.1 \pm 0.8^{e,A}$	$11.7 \pm 0.7^{e,B}$	$9.4 \pm 0.4^{d,C}$	$8.3 \pm 0.4^{e,D}$
	40:0:60	$9.2 \pm 0.5^{b,A}$	$7.7 \pm 0.4^{b,B}$	$7.0 \pm 0.3^{bc,C}$	$4.2 \pm 0.1^{b,D}$
	20:20:60	$9.6 \pm 0.6^{b,A}$	$8.0 \pm 0.3^{bc,B}$	$7.4 \pm 0.2^{c,C}$	$4.5 \pm 0.3^{b,D}$
	0:40:60	$10.2 \pm 0.6^{c,A}$	$8.2 \pm 0.5^{c,B}$	$7.4 \pm 0.5^{c,C}$	$4.6 \pm 0.3^{b,D}$
	0:0:100	$10.9 \pm 0.5^{cd,A}$	$8.5 \pm 0.5^{c,B}$	$7.6 \pm 0.4^{c,C}$	$4.8 \pm 0.3^{b,D}$
DSP:TSC:P20	100:0:0	$8.2 \pm 0.6^{a,A}$	$6.6 \pm 0.2^{a,B}$	$5.6 \pm 0.4^{a,C}$	$3.5 \pm 0.2^{a,D}$
	50:50:0	$9.0 \pm 0.5^{b,A}$	$7.5 \pm 0.4^{b,B}$	$6.5 \pm 0.3^{b,C}$	$4.5 \pm 0.2^{b,D}$
	0:100:0	$10.9 \pm 0.5^{c,A}$	$8.5 \pm 0.5^{c,B}$	$7.6 \pm 0.4^{c,C}$	$4.8 \pm 0.3^{b,D}$
	40:40:20	$11.8 \pm 0.6^{d,A}$	$9.7 \pm 0.3^{d,B}$	$8.0 \pm 0.4^{cd,C}$	$5.6 \pm 0.4^{c,D}$
	40:20:40	$12.3 \pm 0.5^{d,A}$	$10.4 \pm 0.5^{d,B}$	$8.4 \pm 0.4^{d,C}$	$6.5 \pm 0.3^{d,D}$
	20:40:40	$12.4 \pm 0.7^{d,A}$	$11.2 \pm 0.6^{e,B}$	$8.8 \pm 0.4^{d,C}$	$6.6 \pm 0.3^{d,D}$
	50:0:50	$13.9 \pm 0.6^{e,A}$	$11.4 \pm 0.5^{e,B}$	$9.2 \pm 0.5^{d,C}$	$7.5 \pm 0.3^{e,D}$
	0:50:50	$14.4 \pm 0.7^{e,A}$	$12.5 \pm 0.8^{f,B}$	$10.4 \pm 0.4^{e,C}$	$8.5 \pm 0.5^{f,D}$
	40:0:60	$13.9 \pm 0.6^{e,A}$	$12.0 \pm 0.8^{ef,B}$	$9.5 \pm 0.5^{d,C}$	$7.9 \pm 0.5^{ef,D}$
	20:20:60	$14.2 \pm 0.5^{e,A}$	$12.6 \pm 0.5^{f,B}$	$9.9 \pm 0.8^{de,C}$	$8.4 \pm 0.5^{f,D}$
	0:40:60	$14.4 \pm 0.7^{e,A}$	$12.7 \pm 0.6^{f,B}$	$10.0 \pm 0.4^{e,C}$	$8.4 \pm 0.4^{f,D}$
	0:0:100	$17.0 \pm 1.1^{f,A}$	$14.0 \pm 0.5^{g,B}$	$13.1 \pm 0.7^{f,C}$	$9.4 \pm 0.5^{g,D}$
TSC:TSPP:P20	100:0:0	$10.9 \pm 0.5^{a,A}$	$8.5 \pm 0.5^{a,B}$	$7.6 \pm 0.4^{a,C}$	$4.8 \pm 0.3^{a,D}$
	50:50:0	$13.1 \pm 0.8^{c,A}$	$11.7 \pm 0.7^{d,B}$	$9.4 \pm 0.4^{b,C}$	$8.3 \pm 0.4^{d,D}$
	0:100:0	$11.5 \pm 0.7^{b,A}$	$9.2 \pm 0.5^{b,B}$	$7.8 \pm 0.3^{a,C}$	$5.6 \pm 0.3^{b,D}$
	40:40:20	$10.9 \pm 0.6^{a,A}$	$9.0 \pm 0.6^{ab,B}$	$7.9 \pm 0.4^{a,C}$	$5.3 \pm 0.2^{b,D}$
	40:20:40	$11.9 \pm 0.5^{b,A}$	$10.8 \pm 0.5^{c,B}$	$9.2 \pm 0.5^{b,C}$	$6.6 \pm 0.4^{c,D}$
	20:40:40	$12.7 \pm 0.5^{c,A}$	$11.6 \pm 0.6^{d,B}$	$9.6 \pm 0.4^{b,C}$	$7.0 \pm 0.4^{c,D}$
	50:0:50	$14.4 \pm 0.7^{d,A}$	$12.5 \pm 0.8^{e,B}$	$10.4 \pm 0.4^{c,C}$	$8.5 \pm 0.5^{d,D}$
	0:50:50	$15.0 \pm 0.7^{d,A}$	$12.9 \pm 0.4^{e,B}$	$9.9 \pm 0.5^{b,C}$	$8.4 \pm 0.4^{d,D}$
	40:0:60	$15.8 \pm 0.8^{e,A}$	$13.0 \pm 0.7^{e,B}$	$10.0 \pm 0.4^{bc,C}$	$8.3 \pm 0.4^{d,D}$
	20:20:60	$15.4 \pm 0.7^{de,A}$	$12.8 \pm 0.8^{e,B}$	$10.2 \pm 0.5^{bc,C}$	$8.3 \pm 0.5^{d,D}$
	0:40:60	$15.8 \pm 1.0^{e,A}$	$13.0 \pm 0.7^{e,B}$	$10.1 \pm 0.3^{bc,C}$	$8.0 \pm 0.3^{d,D}$
	0:0:100	$17.0 \pm 1.1^{f,A}$	$14.0 \pm 0.5^{f,B}$	$13.1 \pm 0.7^{d,C}$	$9.4 \pm 0.5^{e,D}$

[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	$4.46 \pm 0.12^{e,A}$	$4.29 \pm 0.14^{d,B}$	$4.17 \pm 0.18^{d,C}$	$4.05 \pm 0.15^{e,D}$
	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	$4.63 \pm 0.15^{f,A}$	$4.37 \pm 0.22^{c,B}$	$4.22 \pm 0.21^{d,C}$	$4.04 \pm 0.16^{e,D}$

[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 IV

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

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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 ($\geq$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, Ghiglietti, 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

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http://dx.doi.org/10.1016/j.lwt.2016.11.019
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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 $n \approx 20$ (**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 $n \approx 20$) 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

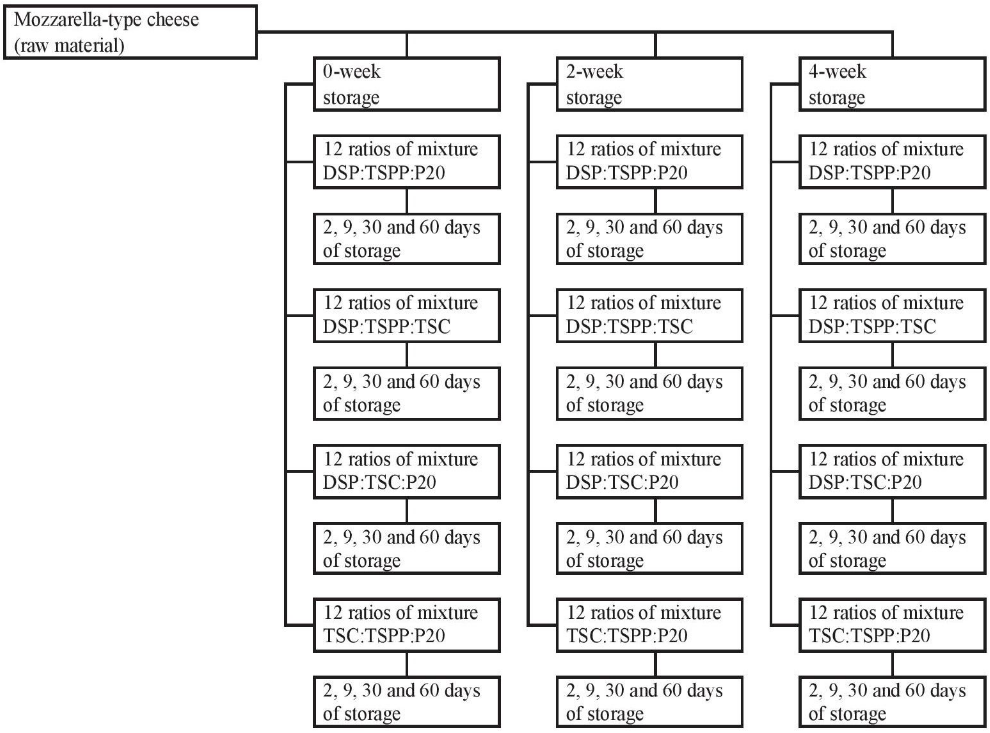

Fig. 1. Scheme of the experimental design with model processed cheeses manufactured using Mozzarella-type cheese (in various time of storage) and the different percentage ratios of the four types of ternary mixtures comprising DSP:TSPP:P20, DSP:TSPP:TSC, DSP:TSC:P20 and TSC:TSPP:P20 (DSP – Na_2HPO_4, TSPP – $Na_4P_2O_7$, P20 – sodium salt of polyphosphate with mean length $n \approx 20$ (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)):

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

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)):

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

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 lyophilised 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

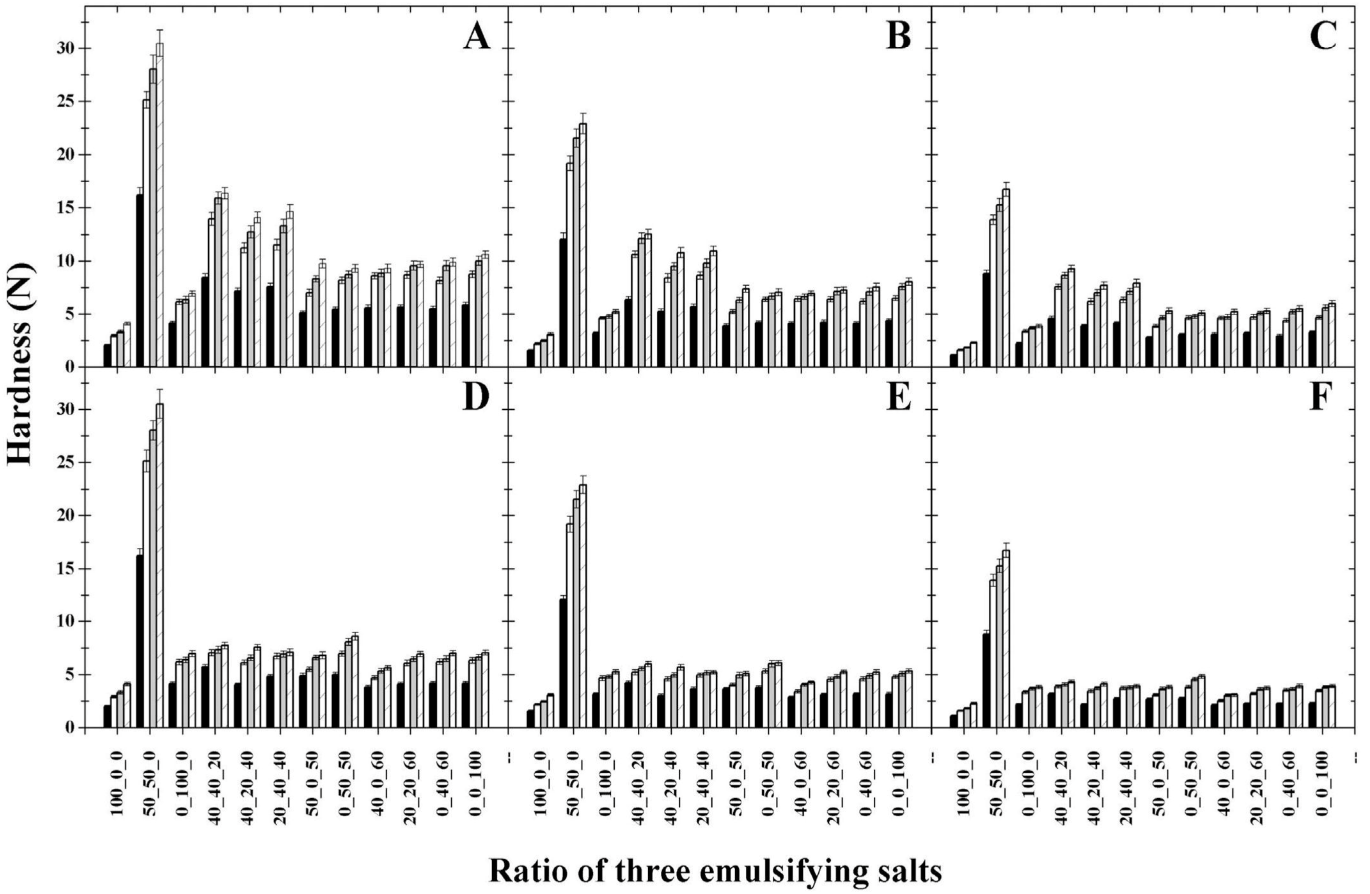

Fig. 2. The dependence of processed cheese hardness (N) on the relative amount (in percentage; axis x) of three emulsifying salts during 60-day storage at 6 °C ($n = 6$; the results were expressed as means (columns) and ± standard deviations (bars); processed cheese were sampled after 2 (black), 9 (white), 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).

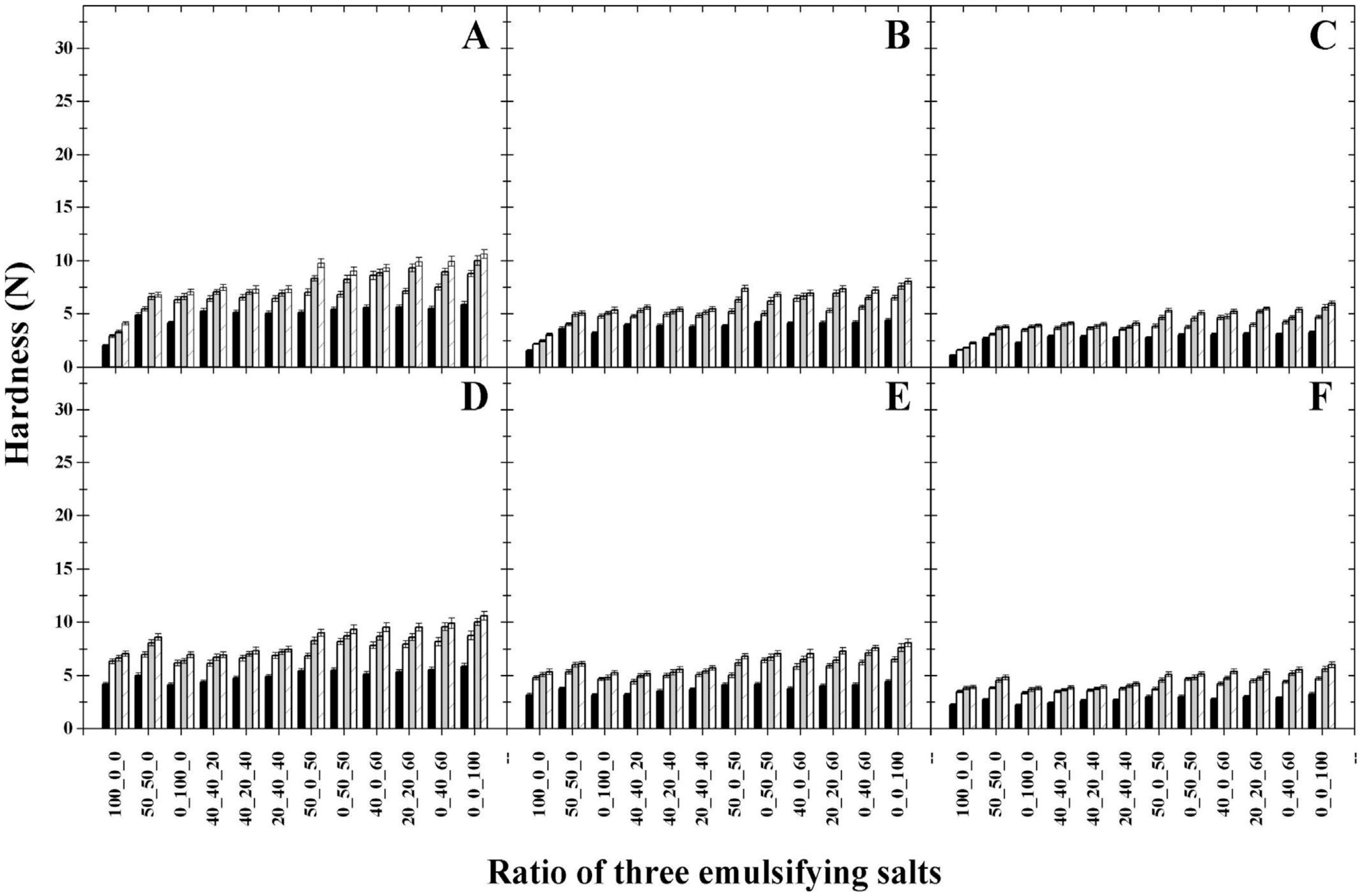

Fig. 3. The dependence of processed cheese hardness (N) on the relative amount (in percentage; axis x) of three emulsifying salts during 60-day storage at 6 °C (n = 6; the results were expressed as means (columns) and ±standard deviations (bars); processed cheese were sampled after 2 (black), 9 (white), 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 ≥ 50%; above this "critical" concentration the trend was of minor significance. An analogous result was observed by

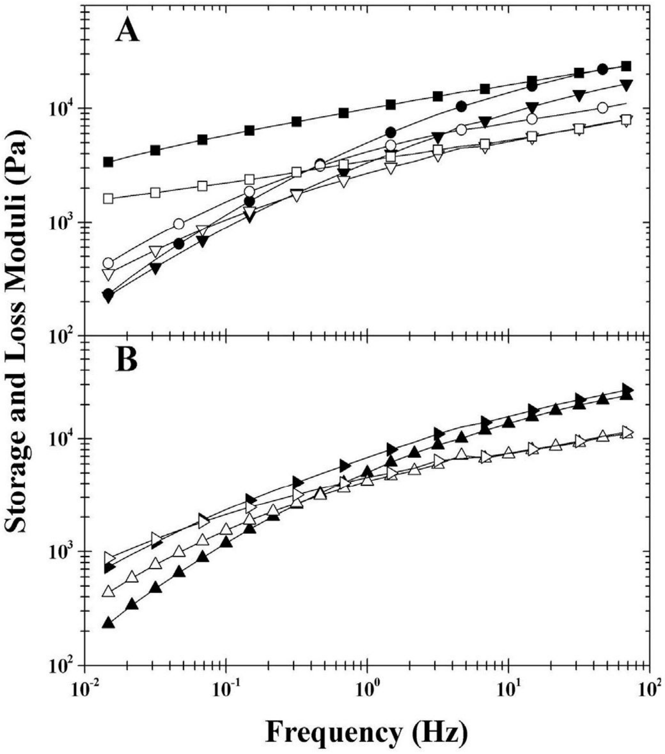

Fig. 4. The dependence of storage G′ (full symbols) and loss G″ (open symbols) moduli of processed cheese (after 30-day storage) made from Mozzarella-type cheese at the beginning of storage on frequency (in range of 0.01–100.00 Hz). Processed cheeses were manufactured using Na_2HPO_4 (▼ ▽; part A), $Na_4P_2O_7$ (● ○; 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 ± 0.26^{a}A	3.49 ± 0.12^{a}B	2.54 ± 0.12^{a}C
	50:50:0	12.42 ± 0.75^{h}A	9.08 ± 0.36^{h}B	6.72 ± 0.24^{g}C
	0:100:0	6.84 ± 0.23^{b}A	5.17 ± 0.30^{b}B	3.63 ± 0.16^{b}C
	40:40:20	9.48 ± 0.31^{g}A	7.16 ± 0.20^{g}B	5.21 ± 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 ± 0.16^{e}C
	20:40:40	$9.05 \pm 0.67^{f,g}$A	6.73 ± 0.40^{f}B	4.87 ± 0.22^{e}C
	50:0:50	7.22 ± 0.37^{c}A	5.44 ± 0.21^{c}B	$3.89 \pm 0.28^{b,c}$C
	0:50:50	7.72 ± 0.30^{d}A	5.95 ± 0.36^{d}B	4.25 ± 0.25^{d}C
	40:0:60	7.22 ± 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 ± 0.20^{e}A	6.21 ± 0.25^{e}B	4.67 ± 0.25^{e}C
	0:40:60	7.86 ± 0.36^{d}A	5.86 ± 0.25^{d}B	4.13 ± 0.18^{d}C
	0:0:100	8.27 ± 0.43^{e}A	6.24 ± 0.33^{e}B	4.55 ± 0.19^{e}C
DSP:TSPP:TSC	100:0:0	4.50 ± 0.26^{a}A	3.49 ± 0.12^{a}B	2.54 ± 0.12^{a}C
	50:50:0	12.42 ± 0.75^{f}A	9.08 ± 0.36^{f}B	6.72 ± 0.24^{f}C
	0:100:0	6.84 ± 0.23^{c}A	5.17 ± 0.30^{d}B	3.63 ± 0.16^{c}C
	40:40:20	8.28 ± 0.24^{e}A	6.08 ± 0.38^{e}B	4.54 ± 0.21^{e}C
	40:20:40	6.83 ± 0.33^{c}A	5.08 ± 0.28^{d}B	3.67 ± 0.15^{c}C
	20:40:40	6.92 ± 0.30^{c}A	5.27 ± 0.37^{d}B	3.85 ± 0.17^{c}C
	50:0:50	5.79 ± 0.29^{b}A	4.25 ± 0.24^{b}B	3.12 ± 0.13^{b}C
	0:50:50	7.19 ± 0.44^{d}A	5.47 ± 0.28^{d}B	3.99 ± 0.23^{d}C
	40:0:60	5.86 ± 0.42^{b}A	4.41 ± 0.16^{b}B	3.33 ± 0.16^{b}C
	20:20:60	5.97 ± 0.32^{b}A	$4.56 \pm 0.26^{b,c}$B	3.28 ± 0.17^{b}C
	0:40:60	6.60 ± 0.26^{c}A	$4.86 \pm 0.28^{c,d}$B	3.51 ± 0.21^{c}C
	0:0:100	6.73 ± 0.32^{c}A	5.13 ± 0.31^{d}B	3.67 ± 0.13^{c}C
DSP:TSC:P20	100:0:0	4.50 ± 0.26^{a}A	3.49 ± 0.12^{a}B	2.54 ± 0.12^{a}C
	50:50:0	5.79 ± 0.29^{b}A	4.25 ± 0.24^{b}B	3.12 ± 0.13^{b}C
	0:100:0	6.73 ± 0.32^{c}A	5.13 ± 0.31^{c}B	3.67 ± 0.13^{c}C
	40:40:20	6.94 ± 0.31^{c}A	5.29 ± 0.30^{c}B	3.79 ± 0.15^{c}C
	40:20:40	$7.06 \pm 0.30^{c,d}$A	5.34 ± 0.28^{c}B	3.90 ± 0.19^{c}C
	20:40:40	7.17 ± 0.33^{d}A	$5.42 \pm 0.21^{c,d}$B	3.95 ± 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 ± 0.28^{c}C
	0:50:50	7.53 ± 0.30^{e}A	5.75 ± 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 ± 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 ± 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 ± 0.25^{e}B	4.53 ± 0.32^{f}C
	0:0:100	8.27 ± 0.43^{g}A	6.24 ± 0.33^{e}B	4.55 ± 0.19^{f}C
TSC:TSPP:P20	100:0:0	6.73 ± 0.32^{a}A	5.13 ± 0.31^{a}B	3.67 ± 0.13^{a}C
	50:50:0	7.19 ± 0.44^{b}A	5.47 ± 0.28^{b}B	3.99 ± 0.23^{b}C
	0:100:0	$6.84 \pm 0.23^{a,b}$A	5.17 ± 0.30^{a}B	3.63 ± 0.16^{a}C
	40:40:20	6.64 ± 0.34^{a}A	4.88 ± 0.17^{a}B	3.71 ± 0.15^{a}C
	40:20:40	$6.94 \pm 0.39^{a,b}$A	5.21 ± 0.24^{b}B	3.91 ± 0.17^{b}C
	20:40:40	7.11 ± 0.32^{b}A	5.36 ± 0.27^{b}B	3.90 ± 0.22^{b}C
	50:0:50	7.53 ± 0.30^{c}A	5.75 ± 0.26^{c}B	4.18 ± 0.26^{c}C
	0:50:50	7.72 ± 0.30^{c}A	5.95 ± 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 ± 0.24^{c}B	$4.24 \pm 0.19^{c,d}$C
	20:20:60	7.95 ± 0.40^{d}A	5.95 ± 0.17^{c}B	4.42 ± 0.18^{a}C
	0:40:60	$7.86 \pm 0.36^{c,d}$A	5.86 ± 0.25^{c}B	4.13 ± 0.18^{c}C
	0:0:100	8.27 ± 0.43^{e}A	6.24 ± 0.33^{d}B	4.55 ± 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 Mozzarrella-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 ± 0.04^{a}A	2.91 ± 0.08^{a}B	2.79 ± 0.07^{a}C
	50:50:0	4.88 ± 0.22^{f}A	4.53 ± 0.13^{f}B	4.36 ± 0.17^{d}C
	0:100:0	3.10 ± 0.12^{a}A	2.92 ± 0.13^{a}B	2.74 ± 0.07^{a}C
	40:40:20	4.63 ± 0.17^{e}A	4.39 ± 0.05^{e}B	4.18 ± 0.16^{d}C
	40:20:40	3.99 ± 0.10^{c}A	3.65 ± 0.10^{c}B	3.55 ± 0.11^{c}C
	20:40:40	4.15 ± 0.17^{d}A	3.88 ± 0.11^{d}B	3.69 ± 0.10^{c}C
	50:0:50	3.24 ± 0.07^{b}A	3.06 ± 0.08^{b}B	$2.86 \pm 0.12^{a,b}$C
	0:50:50	3.26 ± 0.14^{b}A	3.06 ± 0.13^{b}B	2.93 ± 0.11^{b}C
	40:0:60	3.29 ± 0.06^{b}A	$2.99 \pm 0.04^{a,b}$B	2.99 ± 0.11^{b}C
	20:20:60	3.85 ± 0.12^{c}A	3.62 ± 0.04^{c}B	3.51 ± 0.07^{c}C
	0:40:60	3.27 ± 0.07^{b}A	3.04 ± 0.09^{b}B	$2.86 \pm 0.07^{a,b}$C
	0:0:100	3.37 ± 0.07^{b}A	3.12 ± 0.13^{b}B	3.01 ± 0.12^{b}C
DSP:TSPP:TSC	100:0:0	3.07 ± 0.04^{a}A	2.91 ± 0.08^{a}B	2.79 ± 0.07^{a}C
	50:50:0	4.88 ± 0.22^{e}A	4.53 ± 0.13^{e}B	4.36 ± 0.17^{e}C
	0:100:0	3.10 ± 0.12^{a}A	2.92 ± 0.13^{a}B	2.74 ± 0.07^{a}C
	40:40:20	4.34 ± 0.19^{d}A	4.02 ± 0.08^{d}B	3.93 ± 0.07^{d}C
	40:20:40	3.98 ± 0.19^{c}A	3.72 ± 0.12^{c}B	3.59 ± 0.11^{c}C
	20:40:40	4.12 ± 0.13^{d}A	3.85 ± 0.12^{c}B	3.75 ± 0.10^{c}C
	50:0:50	3.14 ± 0.09^{a}A	2.90 ± 0.03^{a}B	2.80 ± 0.07^{a}C
	0:50:50	3.53 ± 0.12^{b}A	3.27 ± 0.10^{b}B	3.16 ± 0.07^{b}C
	40:0:60	3.39 ± 0.13^{b}A	3.14 ± 0.12^{b}B	3.10 ± 0.08^{b}C
	20:20:60	3.42 ± 0.11^{b}A	3.19 ± 0.13^{b}B	3.06 ± 0.10^{b}C
	0:40:60	3.17 ± 0.06^{a}A	2.98 ± 0.09^{a}B	2.85 ± 0.04^{a}C
	0:0:100	3.08 ± 0.09^{a}A	2.89 ± 0.07^{a}B	2.77 ± 0.06^{a}C
DSP:TSC:P20	100:0:0	3.07 ± 0.04^{a}A	2.91 ± 0.08^{a}B	2.79 ± 0.07^{a}C
	50:50:0	3.14 ± 0.09^{a}A	2.90 ± 0.03^{a}B	2.80 ± 0.07^{a}C
	0:100:0	3.08 ± 0.09^{a}A	2.89 ± 0.07^{a}B	2.77 ± 0.06^{a}C
	40:40:20	$3.12 \pm 0.05^{a,b}$A	2.93 ± 0.06^{a}B	2.83 ± 0.09^{a}C
	40:20:40	$3.22 \pm 0.12^{b,c}$A	3.04 ± 0.10^{b}B	2.94 ± 0.06^{b}C
	20:40:40	3.28 ± 0.10^{c}A	3.07 ± 0.14^{b}B	$2.91 \pm 0.09^{a,b}$C
	50:0:50	3.24 ± 0.07^{c}A	3.06 ± 0.08^{b}B	$2.86 \pm 0.12^{a,b}$C
	0:50:50	3.30 ± 0.13^{c}A	3.10 ± 0.12^{b}B	2.98 ± 0.10^{b}C
	40:0:60	3.29 ± 0.06^{c}A	$2.99 \pm 0.04^{a,b}$B	2.99 ± 0.11^{b}C
	20:20:60	$3.32 \pm 0.14^{c,d}$A	3.08 ± 0.10^{b}B	3.00 ± 0.11^{b}C
	0:40:60	$3.33 \pm 0.03^{c,d}$A	3.12 ± 0.14^{b}B	2.99 ± 0.09^{b}C
	0:0:100	3.37 ± 0.07^{d}A	3.12 ± 0.13^{b}B	3.01 ± 0.12^{b}C
TSC:TSPP:P20	100:0:0	3.08 ± 0.09^{a}A	2.89 ± 0.07^{a}B	2.77 ± 0.06^{a}C
	50:50:0	3.53 ± 0.12^{e}A	3.27 ± 0.10^{d}B	3.16 ± 0.07^{e}C
	0:100:0	3.10 ± 0.12^{a}A	2.92 ± 0.13^{a}B	2.74 ± 0.07^{a}C
	40:40:20	$3.14 \pm 0.10^{a,b}$A	2.90 ± 0.04^{a}B	$2.84 \pm 0.12^{a,b}$C
	40:20:40	3.19 ± 0.09^{b}A	$2.95 \pm 0.06^{a,b}$B	2.90 ± 0.07^{b}B
	20:40:40	$3.25 \pm 0.09^{b,c}$A	3.01 ± 0.14^{b}B	2.93 ± 0.07^{b}C
	50:0:50	$3.30 \pm 0.13^{c,d}$A	3.10 ± 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 ± 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 ± 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 ± 0.07^{d}A	3.12 ± 0.13^{c}B	3.01 ± 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 $\geq$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., Mančik, 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., Grůber, 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., 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.

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 crosslinking 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.

10. AUTHORS‘ LIST OF PUBLICATIONS

Researh papers in international journals with impact factor

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., MINARČIKOVÁ, 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.

LAPČÍK, L., LAPČÍKOVÁ, B., OTYEPKOVÁ, E., OTYEPKA, M., VLČEK, J., BUŇKA, F., **SALEK, R.N.** 2015. Surface energy analysis (SEA) and rheology of powder milk dairy products. *Food Chemistry*, 174, 25-30.

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, 111-122.

Research papers without impact factor

NAGYOVÁ, G., BUŇKA, F., **SALEK, R.N.**, ČERNÍKOVÁ, M., BAČOVÁ, H., KRÁČMAR. S. 2013. The effect of individual phosphate emulsifying salts and their selected binary mixtures on hardness of processed cheese spreads. *Potravinárstvo*, 7, 191-196.

PACHLOVÁ, V., **SALEK, R.N.**, BUŇKA, F., BUŇKOVÁ, L. 2013. Changes of biogenic amine content and other selected parameters in white cheese model matrix. *Journal of Microbiology, Biotechnology and Food Sciences*, 2, 1175-1184.

Research papers in conference proceedings

SALEK, R.N., ČERNÍKOVÁ, M., NAGYOVÁ, G., BAČOVÁ, H., MINARČIKOVÁ, L., BUŇKA, F. The impact of ternary mixtures composed of sodium phosphate and citrate salts on processed cheese spreads hardness. XVI Konference mladých vědeckých pracovníku s mezinárodní účastí, Brno (28.5.2014), ISBN 978-80-7305-670-4.

SALEK, R.N., BUŇKA, F., ČERNÍKOVÁ, M., NAGYOVÁ, G., KUCHAŘ, D., BAČOVÁ, H., MINARČIKOVÁ, L. Ternární směsi tavicích solí obsahující citronan sodný a jejich vliv na tvrdost modelových tavených sýrů. Celostátní přehlídka sýrů a konference mléko a sýry, Praha; VŠCHT (22.1.2014), ISBN 978-80-7080-909-9.

11. Curriculum vitae

PERSONAL INFORMATION

Name	**RICHARDOS NIKOLAOS SALEK**
Address	PRINOS, THASSOS, T.K. 64010, GREECE/HELLAS
Telephone	+420 777 009 241
E-mail	rsalek@ft.utb.cz
Nationalities	Greek, Czech
Date of birth	06.07.1985

WORK EXPERIENCE

• Dates (from – to)	2014 – to present
• Name and address of employer	Tomas Bata University in Zlín, Faculty of Technology
•Occupation or position held	Assistant

EDUCATION

• Dates (from – to)	2012 – present
• Name and type of organization providing education and training	Tomas Bata University in Zlín, Faculty of Technology, Czech Republic
• Principal subjects	Food Technology
• Title of qualification awarded	Planned finish in 12.2016 and receiving the title Ph.D.

• Dates (from – to)	2010 – 2012
• Name and type of organization providing education and training	Tomas Bata University in Zlín, Faculty of Technology, Czech Republic
• Principal subjects	Technology, Hygiene and Economics of Food Production
• Title of qualification awarded	Master of science (M.Sc.) degree

• Dates (from – to)	2006 – 2010
• Name and type of organization providing education and training	Alexander Technological Educational Institute of Thessaloniki, Department of Food Technology, Greece/Hellas
• Principal subjects	Food Technology
• Title of qualification awarded	Bachelor’s (Bc.) degree

PERSONAL SKILLS AND COMPETENCES

MOTHER TONGUE	**GREEK**
OTHER LANGUAGES	
	Czech, English, Italian
• Reading skills	Czech: excellent, English: excellent (C1 level), Italian: basic
• Writing skills	Czech: good, English: excellent (C1 level), Italian: basic
• Verbal skills	Czech: good, English: excellent (C1 level), Italian: basic

WORK ON PROJECTS

2013

Grant IGA/FT/2013/010

Ternary mixtures of phosphate and citrate emulsifying salts in processed cheese manufacture.

Research team member

2014

Grant IGA/FT/2014/010

Application of phosphates and hydrocolloids in selected foods.

Research team member

2015

Grant IGA/FT/2015/004

The study of selected additives and biologically active substances in foods.

Research team member

2016

Grant IGA/FT/2016/003 Application of additives and other functional substances in the manufacture of selected foods.

Research team member