

Effect of specific hydrocolloids and hydrocolloid blends on gluten-free bread quality

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Doctoral Thesis Summary



Tomas Bata University in Zlín

Faculty of Technology

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Effect of specific hydrocolloids and hydrocolloid blends on gluten-free bread quality

Vliv vybraných hydrokoloidů a směsí hydrokoloidů na kvalitu bezlepkového pečiva

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ABSTRACT

Increasing demand of gluten-free breads leads to widespread researches to offer quality goods. Gluten-free flours (amaranth, buckwheat, chickpea, millet, quinoa and rice) themselves, in two-component blend (50% rice flour and 50% amaranth, buckwheat, chickpea, millet or quinoa flour) and in three-component blend (60% rice flour, 20% amaranth flour and 20% buckwheat flour etc.) were submitted to the baking test. Satisfactory results presented the combination of buckwheat and rice flour in portion of 50% buckwheat and 50% rice flour, thus baking test of the blends from buckwheat 10% and rice 90% to buckwheat 90% and rice 10% was conducted and the sample buckwheat 40% and rice 60% evaluated as the best sample with $1.30 \text{ cm}^3 \text{ g}^{-1}$ specific volume, hardness of 17.1 N and any negative effect on sensory properties. To improve the overall bread quality, eight hydrocolloids (agar, carob bean gum, gelatine, κ -carrageenan, sodium alginate, sodium carboxymethyl cellulose, tragacanth and xanthan gum) themselves and in two-component blend were applied to the rice flour in 0.5 and 1.0% portion to flour weight and submitted to the baking test including hardness and moisture content 24 and 72 hours after baking. The best results reached the rice samples in combination with agar-cellulose 0.5%, alginate-cellulose 0.5%, alginate-xanthan gum 1.0%, carob gum-cellulose 0.5%, carrageenan-gelatine 0.5%, cellulose-gelatine 1.0% and gelatine-tragacanth 0.5%. The blends were then applied into the sample of 40% buckwheat flour and 60% rice flour (BR 4060) and baking test evaluated. The hydrocolloid blends improved loaf specific volume from $1.30 \text{ cm}^3 \text{ g}^{-1}$ to $1.85 \text{ cm}^3 \text{ g}^{-1}$ (BR 4060-agar-cellulose 0.5%), improved dough and bread yield, did not significantly affect baking loss and moisture content 24 and 72 h after baking but deteriorated hardness 24 and 72 h after baking (except for BR-alginate-cellulose 0.5%) compared to the rice and BR 4060 samples.

ABSTRAKT

Zvyšující se poptávka po bezpečném pečivu vede k rozšiřující se snaze o zlepšení kvality těchto výrobků. Bezlepkové mouky (amarantová, pohanková, cizrnová, jáhlová, merlíková a rýžová) samostatně, ve dvousložkové směsi (50 % rýžové mouky a 50 % amarantové, pohankové, cizrnové, jáhlové nebo merlíkové mouky) a třísložkové směsi (60 % rýžové mouky, 20 % amarantové mouky a 20 % pohankové mouky atd.) byly podrobeny pekařskému pokusu. Uspokojivého výsledku dosáhla kombinace rýžové a pohankové mouky v poměru 50 % rýžové mouky a 50 % pohankové mouky, proto byly dále testovány kombinace od 10 % pohankové mouky s 90 % rýžové mouky, po vzorek s 90 % pohankové mouky a 10 % rýžové mouky. Z těchto vzorků dosáhla nejlepších výsledků kombinace se 40 % pohankové a 60 % rýžové mouky se specifickým objemem bochníku $1,30 \text{ cm}^3 \text{ g}^{-1}$, tvrdostí 17,1 N a žádným negativním vlivem na senzorní vlastnosti vzorku. Ke zlepšení pekařských vlastností bezlepkového pečiva bylo vybráno osm hydrokoloidů (agar, karubin, želatina, κ -karagenan, alginát sodný, sodná sůl karboxymetyl celulózy, tragakant a xantanová guma), které byly aplikovány do rýžové mouky samostatně a ve dvousložkové směsi v množství 0,5 a 1,0 % (vztaženo na hmotnost mouky). U všech vzorků byl proveden pekařský pokus včetně ověření tvrdosti a vlhkosti střídky 24 a 72 hodin po upečení. Nejlepších výsledků dosáhly bochníky s kombinacemi agar-celulóza 0,5 %, alginát-celulóza 0,5 %, alginát-xantanová guma 1,0 %, karubin-celulóza 0,5 %, karagenan-želatina 0,5 %, celulóza-želatina 1,0 % a želatina-tragakant 0,5 %. Tyto kombinace byly následně testovány ve vzorku se 40 % pohankové mouky a 60 % rýžové mouky (BR 4060), u kterého došlo ke zlepšení specifického objemu bochníku z $1,30 \text{ cm}^3 \text{ g}^{-1}$ na $1,85 \text{ cm}^3 \text{ g}^{-1}$ (BR 4060-agar-celulóza 0,5 %) a zvýšení výtěžnosti těsta i pečiva. Ztráty pečením a vlhkost 24 a 72 h po upečení nebyly statisticky významně ovlivněny, ale došlo ke statisticky významnému zhoršení tvrdosti 24 i 72 h po upečení (s výjimkou vzorku s kombinací alginátu a celulózy v množství 0,5 %) ve srovnání s čistým rýžovým vzorkem a vzorkem BR 4060.

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1. INTRODUCTION

Wheat (*Triticum aestivum* L.) flour is functional in many applications. Its unique characteristics absolutely differ from other cereals and can be ascribed to the viscoelastic properties of gluten proteins. Gluten proteins represent about 80 to 85% of total wheat proteins and consist of monomeric gluten units (gliadin) which cause viscous behaviour while polymeric gluten units (glutenin) are elastic. When kneading and/or mixing wheat flour with water facilitate a formation of cohesive viscoelastic dough able to retain gas produced during fermentation. That results in typical foam structure of bread. Although the role of other flour components is important too, it is evident that gluten protein functionality is crucial [1–6].

Other cereal flours do not contain these key gluten proteins thus they are worse treatable in comparison with wheat flour. Different studies claim, that the baking quality of other cereal flours is much lower which is related to the lower gas holding capacity of the dough [7–9]. Nevertheless, fermented pastry has been produced not only from wheat, but the loaf formation mechanism is different. Baking performance of, i.e. rye (*Secale cereale* L.) has been ascribed to the pentosans (arabinoxylans and arabinogalactans). These polysaccharides are thought to stabilize foams by decreasing the gas diffusion however rye pastry will never give such volume and shape typical of the wheat bread. On the other hand, it can improve an intake of dietary fibre and antioxidants which is far below the recommendations [10–17]. However, in cases of celiac disease gluten must be absolutely eliminated from nutrition because its ingestion causes serious intestinal damage. The gluten proteins are classified as storage proteins and even if rye does not contain gluten proteins its storage proteins (secalins) are able to cause the allergic reaction too [18]. The intolerance is called celiac disease and it is a chronic enteropathy characterised by an inflammation of small intestinal mucosa that results from a genetically based immunological intolerance to gluten [19–22]. The inadequate immunological response to gluten proteins may lead to nutrient malabsorption. General symptoms include diarrhoea, weight loss and fatigue and the only therapy for celiac patients is based on a lifelong gluten-free diet [23–25].

2. CURRENT STATE OF SOLVED ISSUES

The most used material for gluten-free bread production is rice (*Oryza sativa*), buckwheat (*Fagopyrum esculentum* Moench) and maize (*Zea mays*) flour. Other flours such as amaranth (*Amaranthus hypochondriacus* L.), chickpea (*Cicer arietinum*), quinoa (*Chenopodium quinoa*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*), soya (*Glycine max*), tapioca (*Manihot esculenta*), teff (*Eragrostis tef*) have been used recently [26–42].

These products with lack of gluten matrix are typical of worse technological quality, low specific volume, high crumb hardness and short staling time [7, 43–51]. The shelf life is influenced by moisture loss, staling conditions, and microbial deterioration and this process involves crumb firming which is caused by amylopectin crystallization and water redistribution [52–54].

Worse machine workability of gluten-free dough and lower final bread quality is usually improved using various processes and natural substances which are partly able to substitute the missing gluten network. The results published by Gänzle et al. [55], Katina et al. [56], Moore et al. [57], Moroni et al. [8] showed the possibility of sourdough use for improving the gluten-free bread quality. The studies of Gallagher et al. [58] and Nunes et al. [59] described the effect of dairy powder. Other experiments conducted by Aguilar et al. [60] Anton and Artfield [61], Collar et al. [62], Gallagher et al. [63], Guarda et al. [64], Lazaridou et al. [65], Peressini et al. [66], Ronda et al. [67] Rosell et al. [68], Sciarini et al. [9], showed the effect of different types of hydrocolloids.

To overcome the questionable viscoelastic properties of gluten-free doughs and to obtain quality bread products, various gluten-free formulations involving diverse approaches, such as use of maize and sorghum flour [69–71], legume flours (soya, chickpea, pea) [60], starches (corn, potato, cassava) [64, 72], and ingredients such as previously mentioned hydrocolloids, emulsifiers, shortenings or combinations thereof as alternatives to gluten, to improve their technological, sensory and nutritional properties, and also the shelf-life [73].

Studying these experiments' conclusions, amaranth, buckwheat, chickpea, millet, quinoa and rice flour were selected as primary material on the contrast to previous mentioned studies that predominantly worked with starch isolates (cassava corn, potato), and hydrocolloids agar, carob bean gum, xanthan gum, gelatine, κ -carrageenan, sodium alginate, sodium carboxymethyl cellulose and tragacanth were chosen for this work.

3. AIMS OF THE THESIS

The aim of the dissertation was to study the quality of gluten-free breads and verify the correctness of the hypotheses about:

- 1 The effect of specific flour on final bread quality.
- 2 The effect of flour mixtures and different ratio of flours in the mixture on final bread quality.
- 3 The effect of specific hydrocolloids on final bread quality.
- 4 The effect of hydrocolloid blends on final bread quality.

4. LITERATURE REVIEW

4.1 Celiac disease

Celiac disease is becoming an increasingly recognized autoimmune enteropathy of approximately 1% of population in regions such as Europe, North and South America, north Africa and the Indian subcontinent, thus is an important public health issue [74]. It is a chronic enteropathy characterised by an inflammation of small intestinal mucosa that results from a genetically based immunological intolerance to gluten [75, 76]. The inflammation occurring in celiac disease usually results in malabsorption of nutrients, vitamins and minerals with diarrhoea, weight loss and failure to thrive. The most important environmental factor in celiac disease is gluten. The harmful proteins are cereal storage proteins such as gliadins (wheat), secalins (rye), hordeins (barley), and avenins (oats). These grain plants containing risk proteins share a common taxonomy: all are grasses, although oats are less related and may not be injurious in moderate doses. These storage proteins share some repetitive sequences, but the exact peptide sequences involved have not been identified precisely, although peptides rich in glutamines and prolines are potent activators of the immune response in celiac disease [19, 77, 78]. Early diagnosis and treatment, together with regular visits with a dietician are necessary to ensure nutritional adequacy and to prevent malnutrition while adhering to the gluten-free diet for life. All foods and medications containing gluten from wheat, rye and barley (in some cases oats) and their derivatives are eliminated as even small quantities of gluten may be harmful and must be absolutely excluded from the patient nutrition [79, 80]. The aim of the gluten-free diet is to achieve healing and maintain health through the adaptation of a well-balanced, varied diet that avoids gluten [81].

4.1.1 Gluten-free diet

When a patient begins to consume gluten-free food, there is much more concern and confusion as to which foods are allowed and which are not. Many foods are naturally gluten-free, such as milk, butter and cheese, fruits and vegetables, meats, corn, and rice [79]. But even if the demand for gluten-free products is still rising, the most of gluten-free products available at the market are usually of a very poor quality because gluten is predominantly present in breads, cereals, and pastas as the main structure-forming protein of wheat flour. In bread making it is often termed “structural” protein. It is responsible for the elastic characteristic of dough and contributes to the appearance and crumb structure of baked products [19]. The gluten proteins in wheat flour are embedded into other flour particles mainly starch granules – the structure of gluten is a big complex stabilised by intermolecular disulphide, hydrogen and hydrophobic bonds. The properties of gluten express after hydrating flour – giving extensibility, holding gas, providing good texture and crumb structure of baked bread [82]. Specifically,

gluten fraction called glutenins form rough, rubbery mass when fully hydrated, while gliadins give a viscous, fluid mass on hydration. The result of both is cohesive, elastic and viscous properties of wheat dough characterized by variety extensibility, resistance to stretch, mixing tolerance, gas-holding ability. Gluten removal results in major problems for bakers which is the reason why baking gluten-free breads has become focused recently and its replacement is one of the biggest challenges in developing gluten-free cereal products. The absence of gluten results in a liquid batter and after baking in a crumbling texture and for example poor colour [79].

According to the Codex Standard for gluten-free foods which was adapted by the Codex Alimentarius Commission of the World Health Organization (WHO) and by the Food and Agriculture Organization (FAO) in 1976, amended in 1983 and revised in 2008 the gluten-free foods are described as: (a) consisting of, or made only from ingredients that do not contain any prolamins from wheat or all *Triticum* species such as spelt, kamut or durum wheat, rye, barley, oats or their crossbred varieties, with a gluten level not exceeding 0.2 g kg^{-1} , or (b) consisting of ingredients from wheat, rye, barley, oats, spelt or their crossbred varieties, which have been rendered gluten-free, with a gluten level not exceeding 0.02 g kg^{-1} ; or (c) any mixture of two ingredients as mentioned in (a) and (b) with gluten level not exceeding 0.02 g kg^{-1} [83].

Recently there have been numbers of researches and development on gluten-free products, including different approaches with the use of dairy products, starches, gums, other non-gluten proteins, prebiotics, hydrocolloids and their combinations to improve the texture, mouthfeel, acceptability and shelf-life of gluten-free bakery products as gluten-free breads are usually characterised by deficient quality characteristics in comparison with wheat breads [84]. Several studies were conducted [7, 30, 57, 58, 63] using novel ingredients – dairy powder, pseudocereals, sorghum, rice, starches combined with hydrocolloids to replace gluten. All these studies showed that gluten-free bread production needs different approach and technology. The gluten network absence results in fluid dough, very similar to cake batters [57, 85]. Furthermore, in these batters the gas holding is very problematic, thus the use of gums, stabilisers and starch have been used to provide gas occlusion and stabilising mechanism [85].

4.2 Ingredients suitable for gluten-free bread production

Currently different gluten-free flours and ingredients are under investigation for their suitability to produce gluten-free bread of a good quality. Generally, there are two major subclasses of plants: (a) monocotyledonous (one seed leaf) and (b) dicotyledonous (two seed leaves). Wheat, rye, barley and oats are monocotyledonous, while amaranth, buckwheat and quinoa are dicotyledonous and very distantly related to grains of the monocotyledonous subclass). They are classified as pseudocereals for their unique chemical structures [86] and their nutritional value is closely connected to their protein content. Amaranth has

a higher protein content than buckwheat or quinoa and about 65% of the proteins are located in the germ and seed coat, the rest is in the endosperm. Common raw materials in gluten-free breads and baking mixes are corn starch, potato flour/starch, tapioca flour/starch, and rice flour. Flours from wheat, rye and barley are fortified with vitamins, minerals, such as B vitamins and the same situation occurs with gluten-free flours. Thompson [87, 88] found that many gluten-free cereal products contain inadequate amounts of thiamine, riboflavin, niacin, folate, iron and fibre due to the fortification and fact that for example amaranth, quinoa and buckwheat are all good sources of fibre and iron. In addition, the riboflavin content of quinoa and the niacin content of buckwheat flour compare favourably with those of enriched wheat flour. The addition of amaranth, buckwheat and amaranth adds value to the diet not only to patients with celiac disease [88].

The machine workability and final gluten-free bread quality is insufficient as gluten is the main structure-forming protein in flour and contributes to the appearance of crumb structure. Thus, the replacement of gluten network is a major challenge to food scientists and technologists that leads to application of hydrocolloids, starches, fibre, dairy products into gluten-free bread formulations as believed to be a promising alternative for developing the high-quality food for celiac patients [61].

4.3 Improving gluten-free breads

In the respect of the fact, that gluten is responsible for the viscoelastic properties of bread, its replacement has become one of the biggest challenges when developing gluten-free cereal products. The absence of gluten network usually results in a liquid batter that leads to crumbling texture, poor colour and other quality defects post-baking. In recent years, there has been much research and development on gluten-free products and testing the use of different starches, dairy products, gums, and hydrocolloids, other non-gluten proteins, prebiotics and different combinations of thereof. The intention is to improve the structure, mouthfeel, acceptability and shelf-life of gluten-free bakery products [89]. Problems related to volume and crumb texture are associated with gluten-free bread even if rice flour is used and seems to be the best raw material [84]. The use of additives has recently become common practice. They are applied to improve dough handling properties, enhance the quality of fresh bread and extend the shelf-life of stored bread. All hydrocolloids interact with water, reducing its diffusion and stabilizing its presence. Xanthan, guar gum and sodium carboxymethylcellulose (CMC) are soluble in cold water but κ -carrageenan, carob bean gum and many alginates require hot water for complete hydration. Some hydrocolloids, such as carob bean gum and xanthan gum, may form strong gels. As hydrocolloids can dramatically affect the flow behaviour when present at low concentrations, most of them are used to increase viscosity, which improves dough stabilization and bread quality [90].

5. METHODS

5.1 Material

In this thesis six commercial flours available in the health food store were used: amaranth flour (*Amaranthus hypochondriacus* L.), buckwheat flour (*Fagopyrum esculentum* Moench), chickpea flour (*Cicer arietinum*), millet flour (*Panicum miliaceum*), quinoa flour (*Chenopodium quinoa*) and rice flour (*Oryza sativa*). All flours were used either separately or in the mixtures of at least two flours.

Eight types of hydrocolloids (Sigma-Aldriche, Merck) were used to improve the gluten-free bread quality. These were agar, carob bean gum (hereinafter carob gum), gelatine, κ -carrageenan (hereinafter carrageenan), sodium alginate (hereinafter alginate), sodium carboxymethyl cellulose (hereinafter cellulose), tragacanth and xanthan gum. Each of them was used separately in two different portions (0.5 and 1.0% to flour weight) and in the two-component blend (0.5 and 1.0 % portion) with rice flour. Table 1 shows the samples of gluten-free flours and their mixtures and Table 2 presents samples with hydrocolloids and hydrocolloid blends.

Table 1: Gluten-free flours and flour mixtures

Sample	Proportion (%)					
	Rice	Amaranth	Buckwheat	Chickpea	Millet	Quinoa
1	100					
2		100				
3			100			
4				100		
5					100	
6						100
7	50	50				
8	50		50			
9	50			50		
10	50				50	
11	50					50
12	60	20	20			
13	60	20		20		
14	60	20			20	
15	60	20				20
16	60		20	20		
17	60		20		20	
18	60		20			20
19	60			20	20	
20	60			20		20
21	60				20	20

Table 2: Rice flour with hydrocolloids

Rice	Proportion (%)						
	Agar	Alginate	Carob gum	Carrageena	Cellulose	Tragacanth	Xanthan gum
1	0.5						
2	1.0						
3		0.5					
4		1.0					
5			0.5				
6			1.0				
7				0.5			
8				1.0			
9					0.5		
10					1.0		
11						0.5	
12						1.0	
13							0.5
14							1.0
15	0.25	0.25					
16	0.25		0.25				
17	0.25			0.25			
18	0.25				0.25		
19	0.25					0.25	
20	0.25						0.25
21		0.25	0.25				
22		0.25		0.25			
23		0.25			0.25		
24		0.25				0.25	
25		0.25					0.25
26			0.25	0.25			
27			0.25		0.25		
28			0.25			0.25	
29			0.25				0.25
30				0.25	0.25		
31				0.25		0.25	
32				0.25			0.25
33					0.25	0.25	
34					0.25		0.25
35						0.25	0.25
36	0.5	0.5					
37	0.5		0.5				
38	0.5			0.5			
39	0.5				0.5		
40	0.5					0.5	
41	0.5						0.5
42		0.5	0.5				
43		0.5		0.5			
44		0.5			0.5		
45		0.5				0.5	
46		0.5					0.5
47			0.5	0.5			
48			0.5		0.5		
49			0.5			0.5	
50			0.5				0.5
51				0.5	0.5		
52				0.5		0.5	
53				0.5			0.5
54					0.5	0.5	
55					0.5		0.5
56						0.5	0.5

5.2 Methods

5.2.1 Water absorption

This standard ISO 5530-1 [91] specifies a method, using the Brabender farinograph, or the determination of the water absorption of flours and the mixing behaviour of the dough made from them. In this standard the word "flour" also means "meal". Water absorption is an appropriate volume of water required to produce a dough with a maximum consistency of 500 farinographic units (FU), under the operating conditions and is expressed in millilitres per 100 g of flour at 14% (m/m) moisture content. The maximum consistency of the dough is adjusted to a fixed value by adapting the quantity of water added. The correct water addition, which is called the water absorption, is used to obtain a complete mixing curve, the various features of which are a guide to the rheological properties of the flour.

5.2.2 Baking test

Baking test was conducted on 300 g flour samples using a straight-dough baking formula and short fermentation time in accordance with ICC standard No. 131 [92]. High speed dough mixing and a short fermentation time are typical of this method. Bread loaves were evaluated in relation to yield (dough and bread), baking loss, specific volume (ratio of bread volume and weight in $\text{cm}^3 \text{g}^{-1}$). Dough was prepared from flour (100%), 1.8 dry yeast, 1.5 salt, 1.86 sugar, 0.005% ascorbic acid, respectively, related to flour weight, and water according to farinographic parameters.

5.2.3 Bread texture parameters

Texture analysis of bread crumb was performed with cylinder of 2.5 cm diameter and 2 cm thickness using Texture Analyser TA.XT Plus (Stable Micro Systems, Surrey, UK) which was equipped with a compression cell of 30 kg and a matrix of 50 mm in diameter. The speed of matrix was set at 1 mm s^{-1} . This analysis was performed 24 and 72 hours after baking.

The texture analyses were carried out by two sequential compression events (compression depth 40%, probe speed 2 mm s^{-1} , trigger force 5 g). The test was performed using a 50 mm stainless steel cylinder and the force-deformation curve was recorded. Hardness (force needed to attain a given deformation – maximum force during the first deformation cycle; N) was evaluated using EsxponentLite software.

5.2.4 Moisture content

Moisture content was determined using a drying method at $130 \text{ }^\circ\text{C}$ for 90 min according to CSN 56 0116-3 [93]. The samples were prepared from the inside part of bread crumb (1.5 cm from bread crust). The crumb was crumbled, divided

into three 5 g samples, put into aluminium bowl and dried. All samples were dried 24 and 72 h after baking. The sample cooled in an exsicator and then weighted. The moisture content was calculated from the weight change:

$$x = \frac{m_a}{m_b} \cdot 100[\%]$$

Where m_a is the sample weight after drying [g];
 m_b is the sample weight before drying [g].

5.2.5 Statistical analysis

Results were analysed using one-way and analysis of variance (ANOVA) and the appropriate test of significant difference at a significance level of $p < 0.05$. These tests were realized in Statistica 9 software (StatSoft, Inc.). The purpose of analysis of variance is to test for significant differences between means [94].

The differences were tested on $\alpha = 0.05$ significance level using Fisher LSD test. Statistical analysis was performed using Statistica CZ9.1 software (StatSoft, CR, Ltd).

5.3 Experiment

The experimental part of the dissertation was divided into several phases. The first phase was focused on selecting the convenient material for gluten-free products (buckwheat, rice, amaranth, quinoa, millet, chickpea), determination of the water absorption, preparing the flour mixtures with specific ratio of gluten-free flours, next, performing the baking test and evaluating the final bread quality. Other phase consisted of blending the rice flour with selected hydrocolloids and hydrocolloid blends in two concentrations, performing and evaluating the baking test, hardness and moisture content 24 and 72 hours after baking.

Based on the results, hydrocolloid blends with the best results of baking test were chosen, put into the mixture of 40% buckwheat and 60% rice flour and samples evaluated.

6. RESULTS

6.1 Quality of gluten-free breads from chosen flours

Gluten-free bread samples were prepared from amaranth, buckwheat, chickpea, millet, quinoa and rice flour using baking test. As the rice is a very important grain among gluten-free products, it was determined as a check sample. It has many unique attributes as easy digestion, bland taste and hypoallergenic properties. However, rice has relatively low amounts of proteins and most of them are hydrophobic therefore resist swelling in water at neutral pH. Rice proteins are also devoid of the elastic plastic properties that are key factors in wheat bread production. The low protein contents and absence of gliadin make rice ideal for gluten-free products, but their quality is questionable thus challenge for improving [29, 95]. The quality parameters of gluten-free breads are summarized in Table 3.

Table 3: Average values of gluten-free bread characteristics*

Sample	Loaf specific volume (cm ³ g ⁻¹)	Dough yield (%)	Bread yield (%)	Baking loss (%)	Hardness (N)
Amaranth	1.748 ± 0.017 ^a	198.1 ± 0.4 ^e	157.2 ± 0.4 ^a	20.6 ± 0.6 ^c	43.9 ± 0.4 ^c
Buckwheat	1.671 ± 0.015 ^a	193.1 ± 1.8 ^d	157.6 ± 0.4 ^a	18.4 ± 2.5 ^a	30.9 ± 1.1 ^b
Chickpea	1.706 ± 0.020 ^a	177.9 ± 1.2 ^c	149.4 ± 0.6 ^c	16.0 ± 0.2 ^b	48.9 ± 4.4 ^d
Millet	1.436 ± 0.002 ^b	162.2 ± 1.1 ^b	133.4 ± 0.3 ^b	17.7 ± 0.7 ^a	32.5 ± 1.7 ^b
Quinoa	1.479 ± 0.018 ^c	202.2 ± 0.9 ^a	168.9 ± 1.1 ^c	16.4 ± 1.5 ^b	31.0 ± 1.9 ^b
Rice	1.716 ± 0.003 ^a	204.0 ± 0.1 ^a	166.1 ± 0.1 ^d	18.5 ± 0.4 ^a	13.9 ± 1.2 ^a

*Values in one column with different letters are significantly different $p < 0.05$

To verify the influence of chosen flours on the quality of bread, loaf specific volume, dough and bread yield, baking loss and crumb hardness 24 h after baking were evaluated. The rice flour was chosen as a check sample and the results revealed the same specific volume for amaranth, buckwheat and chickpea flour; millet and quinoa had significantly lower loaf specific volume. As presented Moore [30] and Sciarini et al. 2010 [54], all gluten-free breads showed lower volume than wheat bread that is valid also for our results where wheat bread reached 3.1 cm³ g⁻¹ (results from previous research, data not shown). The quinoa bread reached very similar dough (202.2%) and bread (168.9%) yield as the rice check sample (204.0 and 168.9%). Other flours had significantly worsening influence. Almost all samples presented very similar baking loss – in the range from 16.0% (chickpea) to 18.5% (rice). The highest significantly different baking loss was found for amaranth bread (20.6%). The results were published in Journal of Cereal Science: The relationship between rheological characteristics of gluten-free dough and the quality of biologically leavened bread, Burešová et al. [96].

Crumb hardness was tested 24 h after baking and rice check sample had very similar value (13.9 N) as previously tested common wheat flour (15.3 N). The samples of gluten-free flours presented significantly higher hardness compared to the rice check sample. These results are in agreement with Burešová et al. [97] (except for amaranth flour) who studied the effect of amaranth, buckwheat, chickpea, corn, millet and quinoa flour on rice bread. In our study, the hardness varied from 30.9 N (buckwheat) to 48.9 N (chickpea).

6.2 Quality of two-component flour gluten-free bread

In comparison with rice sample, only the combination of rice and buckwheat flour had significant improving effect on the loaf specific volume and it is in agreement with Krupa-Kozak et al [40], Alvarez-Jubete et al. [32] and Wronkowska et al. [39] who studied the effect of addition of buckwheat flour to gluten-free formula. The rice-buckwheat sample also showed acceptable values of dough and bread yield and had acceptable baking loss (23.2%) compared to the rice check sample (18.5%). Though amaranth and quinoa are recommended for gluten-free bread production for their nutritional and functional properties [33], their combinations with rice flour were negatively affected by typical amaranth aroma and taste but as indicated by Turkut et al. [98] 25% of quinoa flour could be successfully incorporated to the commercial gluten-free bread without any negative effect on sensory properties. The sample of rice and millet got very dry consistency but still able to be subjected to the analyses. This combination probably required amended baking technology. Other flours had significantly deteriorating effect on both dough and bread yield and baking loss. Miñaro et al. [99] studied the effect of legume flours on soya flour-based gluten-free bread and proved that adding of chickpea flour into the formula did not negatively affect the technological parameters and even reached the best results of loaf specific volume and hardness among used legume flours.

6.3 Quality of three-component gluten-free bread

The crumb structure of gluten-free bread was rather wet after baking and next day became rough and crumbly that was also reported by Torbica et al. [37]. As bread is prepared for couple of days, it is necessary to keep the sensory quality within the staling. We decided to mix selected gluten-free flour in a specific ratio to support the technological quality. The main part of blend was rice mixed with two other flour in proportions of 60% rice flour and 40% remaining two flours (20:20).

Statistical analysis showed various significant effect on bread parameters. The biggest loaf specific volume was measured for the combination of rice with buckwheat and quinoa ($2.399 \text{ cm}^3 \text{ g}^{-1}$). The increasing trend can be observed at all samples containing buckwheat which was finally the key for the following research. The only significantly deteriorating effect on the loaf specific volume

had samples with quinoa flour. Concerning dough and bread yield, the significantly highest value reached rice check sample (204.0 and 166.2%) and mixing the flours did not prove any positive effect on these parameters. The baking loss varied from 17.4 to 22% and not many significant differences were found among these values.

6.4 Quality of buckwheat-rice bread

Since the rice-buckwheat combination gave very satisfactory results of baking quality, hardness 24 after baking and good subjective sensory evaluation, the samples of proportion 10% buckwheat and 90% rice flour to 90% buckwheat and 10% rice flour were baked and tested. It is reported that rice flour is very popular as a substitute of wheat flour in the preparation of products consumed by wheat-intolerant celiac patients, and for its bland taste, white colour, digestibility and hypoallergenic properties, it is the most suitable cereal grain flour [2] together with buckwheat flour with its well-balanced amino acid composition, high vitamin content, good source of microelements and as a potential improver of the gluten-free nutritional and technological quality [100] could reach satisfactory results. Buckwheat-rice blend is abbreviated as BR.

In case of loaf specific volume, the best result presented the samples of clear buckwheat and rice flour (1.671 and 1.716 cm³ g⁻¹). Comparing the increasing amount of buckwheat in the blend, the loaf specific volume increased with higher portion of buckwheat in the blend; from 1.100 cm³ g⁻¹ (BR 1090) to 1.467 cm³ g⁻¹ (BR 7030). Very similar conclusion presented Wronkowska et al. [100] who tested the inclusion of buckwheat flour into the starch-based bread and presented that the water-binding capacity and the buckwheat proteins are the reasons of growing loaf bread volume.

The best results of dough and bread yield (204.0 and 166.2%) presented the rice sample together with clear buckwheat sample. Both samples were of a satisfactory baking quality but were not acceptable for their sensory aspects and dry crust. Adding of buckwheat flour to rice flour had first negative effect on baking quality – decreasing specific volume, dough and bread yield, but positive effect on baking loss in comparison to the rice and buckwheat sample; from 18.6 (rice) and 18.4% (buckwheat) to 10.3% (BR 2080). But among the buckwheat-rice blends, increasing portion of buckwheat flour caused volume, yield and baking loss improvement.

6.5 Effect of chosen hydrocolloids on quality of rice bread

Aging of gluten-free breads leads to the loss of acceptable quality characteristics and flavour due to loss of moisture, crumb firming, recrystallization of amylopectin and water redistribution. Although this has been studied for a long time, gluten-free bread staling is still not clear and it is responsible for economic losses both – baking industry

and the customers' [45, 54]. As the starch is the main part of the gluten-free breads and is fully responsible for the aging we decided to verify the ability of selected hydrocolloids to slow the aging by extending the water-binding capacity and enhancing the technological parameters. The rice bread samples were prepared with agar, alginate, carob gum, carrageenan, cellulose, gelatine, tragacanth and xanthan gum. Each hydrocolloid was tested in the amount of 0.5 and 1.0% to flour weight.

The hydrocolloids in two specific portions added to rice flour gave variable and significantly different results. Hydrocolloids' effect on loaf specific volume is not easy to generally describe as it highly depends on the formulation of the gluten-free bread, the level of hydrocolloid incorporation, the origin and source of the gum, interactions with other ingredients and the parameters of the process [73]. Sciarini et al. [9] who worked with, alginate, carrageenan, carboxy methyl cellulose, gelatine and xanthan gum (in the portion of 0.5% to flour basis) found that addition of xanthan gum reached the highest loaf specific volume same as Anton and Artfield 2008 [61], but contrarywise our measurements proved that the best result of loaf specific volume (approximately $1.8 \text{ cm}^3 \text{ g}^{-1}$) presented the samples with 0.5% alginate, carob gum and sodium hydroxypropyl methyl cellulose. In his next study [101], carrageenan addition 0.5% led to the highest loaf specific volume, followed by carboxymethyl cellulose and breads with xanthan gum and alginate did not affect this parameter which is not in agreement with our study where alginate reached the biggest loaf specific volume. In our research, significantly worst results showed samples with portion of 1.0% hydrocolloid in the formula, specifically carob gum and xanthan gum (1.396 and $1.400 \text{ cm}^3 \text{ g}^{-1}$) which decreased the specific volume by 17.6% and the same negative effect of xanthan gum in higher concentrations proved the study of Hager and Arendt [102] and Peressini et al [66]. The results of satisfactory loaf specific volume in the sample with added cellulose are in agreement with Lazaridou et al. [65] who worked with carboxymethyl cellulose, xanthan gum and other hydrocolloids.

6.6 Effect of hydrocolloid blends on quality of rice bread (0.5 and 1.0% w/w)

To prove the effect of hydrocolloids in synergy, the hydrocolloid blends (two-component) were prepared and applied to the rice sample in the proportion of 0.5 and 1.0% on flour weight and the results compared with the clear rice sample as the check. The hypothesis worked with the possibility of hydrocolloid reaction with both, flour structures and the other hydrocolloid used in the blend. The results were summarized and divided into 8 groups (alphabetical order). Bread quality parameters, hardness 24 and 72 hours after baking and moisture content 24 and 72 hours after baking were evaluated.

The current researches present results of the effect of only single hydrocolloid in the sample, thus the results were discussed only in the context of single hydrocolloids. The only effect of hydrocolloid mixtures published Gambus et al. [103] who studied breads prepared from potato starch, corn starch, corn flour and pectin with guar gum in 1:1 mixture. And that was the basic idea of this research, to verify the possibility of hydrocolloid interaction and their integration to the gluten-free structures that could lead to higher improving effect on the technological parameters of the gluten-free breads.

Based on the results of bread quality, seven specific hydrocolloid combinations in specific portions were selected, applied to the best flour combination (buckwheat 40% and rice 60%) and evaluated.

6.7 Final samples

Evaluation of the bread technology parameter enabled to choose the best samples among the hydrocolloid blends that improved the results of the rice bread. The loaf specific volume as the parameter most important for the customers was the primary criterion, but the remaining results of the bread making quality must have been satisfactory too, thus agar-cellulose 0.5%, alginate-cellulose 0.5%, alginate-xanthan gum 1.0%, carob gum-cellulose 0.5%, carrageenan-gelatine 0.5%, cellulose-gelatine 1.0% and gelatine-tragacanth 0.5% were selected. These hydrocolloid blends significantly improved the rice loaf specific volume from 1.716 up to 1.896 cm³ g⁻¹, but any of the blends was able to positively affect the rice dough yield (204.0%), the values varied from 177.0 to 193.6%. Two hydrocolloid blends (alginate-cellulose 0.5% and alginate-xanthan gum 1.0%) improved the bread yield from 166.2 to 167.8 and 166.9%, but these differences were not significant; remaining samples diminished the bread yield, but none of them fell under 154.0%. All samples reached very satisfactory results of baking loss. The results of blends' baking loss were significantly lower than the rice check sample. Another criterion – hardness 24 and 72 h showed acceptable values for all samples (except for agar-gelatine 0.5%) – significantly lower than the rice check sample (24 h) and 4 samples of 7 measured 72 h after baking and staling. The blends' moisture content reached more than 50% (24 h after baking) and did not fall under 49% (72 h after baking).

As the rice-buckwheat sample in the portion of 40% buckwheat and 60% rice flour (BR 4060) was evaluated as the best of flours and flour combinations based on the bread technological quality and subjective sensory evaluation, the selected hydrocolloid blends in their specific portions were applied to this sample to obtain the sample with remarkable technological parameters. The statistic evaluation of the technological parameters is summarized in the Table 4. For the results to be complete and for imagination, the rice check sample was added.

Table 4: Average values of buckwheat-rice bread characteristics prepared with selected hydrocolloid blends in specific portions (w/w, flour basis)*

Sample	Loaf specific volume (cm ³ g ⁻¹)	Dough yield (%)	Bread yield (%)	Baking loss (%)
BR 4060	1.300 ± 0.082 ^a	171.4 ± 0.5 ^c	151.5 ± 0.4 ^b	11.6 ± 0.2 ^f
BR-Agar-Cel 0.5%	1.718 ± 0.003 ^{bc}	184.4 ± 0.3 ^e	152.2 ± 0.3 ^c	17.5 ± 0.3 ^e
BR-Alg-Cel 0.5%	1.850 ± 0.005 ^d	195.3 ± 0.2 ^a	158.5 ± 0.2 ^e	18.9 ± 0.2 ^{bcd}
BR-Alg-XG 1.0%	1.788 ± 0.003 ^c	195.8 ± 0.2 ^a	161.2 ± 0.3 ^a	17.7 ± 0.6 ^e
BR-CG-Cel 0.5%	1.870 ± 0.006 ^d	174.3 ± 0.3 ^d	142.6 ± 0.3 ^d	18.2 ± 0.3 ^a
BR-Carrag-Gel 0.5%	1.767 ± 0.002 ^c	186.0 ± 0.4 ^f	151.8 ± 0.2 ^{bc}	18.4 ± 0.2 ^{ab}
BR-Cel-Gel 1.0%	1.828 ± 0.007 ^c	199.4 ± 0.3 ^b	161.4 ± 0.5 ^a	19.1 ± 0.4 ^d
BR-Gel-Trag 0.5%	1.811 ± 0.001 ^c	198.8 ± 0.1 ^b	161.0 ± 0.2 ^a	19.0 ± 0.2 ^{cd}
Rice	1.716 ± 0.003 ^{bc}	204.0 ± 0.1 ^g	166.2 ± 0.1 ^f	18.6 ± 0.1 ^{abc}

*Values in one column with different letters are significantly different $p < 0.05$

B: buckwheat. R: rice. Alg: alginate. CG: carob gum. Carrag: carrageenan. Cel: cellulose. Gel: gelatine. Trag: tragacanth. XG: xanthan gum.

The buckwheat-rice (BR) sample reached the loaf specific volume only $1.300 \text{ cm}^3 \text{ g}^{-1}$ that was significantly the lowest value. All hydrocolloid blends improved the loaf specific volume to $1.718 \text{ cm}^3 \text{ g}^{-1}$ (agar-cellulose 0.5%), $1.788 \text{ cm}^3 \text{ g}^{-1}$ (alginate-xanthan gum 1.0%), $1.767 \text{ cm}^3 \text{ g}^{-1}$ (carrageenan-gelatine 0.5%), $1.828 \text{ cm}^3 \text{ g}^{-1}$ (cellulose-gelatine 1.0%), $1.811 \text{ cm}^3 \text{ g}^{-1}$ (gelatine-tragacanth 0.5%) and $1.850 \text{ cm}^3 \text{ g}^{-1}$ (alginate-cellulose 0.5%) and $1.870 \text{ cm}^3 \text{ g}^{-1}$ (carob gum-cellulose 0.5%).

The lowest value of dough bread yield presented the BR sample itself (171.4%) and all hydrocolloid blends significantly improved this result. The highest dough yield was calculated for the BR sample with cellulose and gelatine 1.0% (199.4%) together with gelatine-tragacanth 0.5% (198.8%). Other remarkable results presented the combinations of alginate-xanthan gum 1.0% (195.8%) and alginate-cellulose 0.5% (195.3%). Remaining hydrocolloid blends either significantly improved the BR dough yield but varied only from 174.3% (carob gum-cellulose 0.5%) to 186.0% (carrageenan-gelatine 0.5%).

The BR sample presented 151.5% of bread yield. Five of the selected hydrocolloid combinations significantly improved the BR bread yield. The highest values were calculated for cellulose-gelatine 1.0% (161.4%), alginate-xanthan gum 1.0% (161.2%) and gelatine-tragacanth 0.5% (161.0%). The sample with carob gum-cellulose 0.5% presented only 142.6% bread yield that was significantly lowest value.

The only technological parameter that was not improved by the hydrocolloid blends was the baking loss. The BR sample presented 11.6% baking loss and all hydrocolloid blends significantly diminished this result. The values ranged from 17.5% (agar-cellulose 0.5%), 17.7% (alginate-xanthan gum 1.0%) through 18.2% (carob gum-cellulose 0.5%), 18.4% (carrageenan-gelatine 1.0%), 18.9% (alginate-cellulose 0.5%) to 19.0% (gelatine-tragacanth 0.5%) and 19.1% (cellulose-gelatine 1.0%).

7. CONTRIBUTION TO THE SCIENCE AND PRACTICE

Many researches have been conducted to improve the gluten-free bread overall quality with the use of specific hydrocolloids. Though only few of them study the possibility of supporting effect in two-component blends. One of the major contributions of this thesis is the evaluation of the overall effect on baking quality, hardness and moisture content 24 and 72 h after baking of prepared rice bread. Even if there are many researches describing the effect of hydrocolloids themselves, the samples differ in formula. Most of them deal with starch-based gluten-free breads containing emulsifiers and shortenings. These extensive formulae in majority of the studies led us to the basics and use of gluten-free flours, not only isolates and starches. That means improved nutritional value but complicated processing. It was proved that the hydrocolloid blends are able to influence the gluten-free bread quality, but not all observed parameters were improved. The blends were enhanced the customers' most important value – volume of the bread, but on the other hand, deteriorated the crumb hardness.

Concerning the practice, as the effect of hydrocolloid blends was not only positive, their use is at least economically questionable. Understanding the dough viscoelastic properties, using specific gluten-free flour blends with an appropriate water amount in formula could lead to satisfactory product.

8. CONCLUSION

The aim of the thesis was to prove the effect of blending gluten-free flours on baking quality of gluten-free breads and suggest the possibilities of substitution the gluten-gliadin complex in such bread. The experiments were conducted to verify four hypotheses. For each hypothesis following conclusions can be drawn:

Hypothesis 1: The type of flour affects the final bread quality.

Specific gluten-free flour affects the final bread quality and specifically amaranth, buckwheat, chickpea and rice flour reached very good loaf specific volume, additionally, the rice bread showed the lowest hardness among all samples. It was proved that none of the flours can be used itself for its dry crust and crumb, unsatisfactory crumb porosity and sensory properties, but the rice sample, for its neutral smell and taste, was selected as a check sample for following experiments.

Hypothesis 2: The mixtures and different ratio of flours in the mixture affect the final bread quality.

Two-component flour blends affect the final bread quality and the best loaf specific volume was recorded for the rice-buckwheat bread. Compared to the rice check sample, all gluten-free blends reached higher hardness. Among three-component flour blends, it was proved that most blends positively affected the loaf bread volume and the best result showed the combination of rice, buckwheat and quinoa flour. Except for the blend of rice, amaranth and buckwheat flour, all remaining samples deteriorated crumb hardness compared to the rice check sample and the highest hardness reached all samples with millet. The samples containing amaranth and quinoa flour proved specific smell and taste and were excluded from remaining experiment.

Based on the results, the combination of rice and buckwheat flour was evaluated as the best, thus blends of 10% buckwheat and 90% rice flour to 90% buckwheat and 10% rice flour were prepared and evaluated. It can be concluded that with increasing amount of buckwheat flour, the loaf specific volume was increasing too up to 80% of buckwheat flour in the blend. Compared to the clear buckwheat sample, all buckwheat-rice blends reached lower crumb hardness but higher crumb hardness than clear rice sample. The sample of 40% buckwheat and 60% rice flour for its satisfactory baking and sensory quality was evaluated as the best and appropriate for following experiments.

Hypothesis 3: The specific hydrocolloid affects final bread quality.

It was proved that the specific hydrocolloid affects final bread quality, but there is no evidence that higher portion of hydrocolloid in formula gives better baking quality, additionally, 1.0% portion of hydrocolloid in formula deteriorated the loaf specific volume compared to the samples with 0.5% of hydrocolloid. But it can be concluded that the presence of hydrocolloid improves crumb hardness 72 h after baking and it is able to keep satisfactory moisture content 72 h after baking.

Hypothesis 4: The hydrocolloid blends affect final bread quality.

Hydrocolloid blends partly improved final baking quality and there is no important evidence of increasing improving effect with increasing amount of hydrocolloid blend in formula (0.5 and 1.0%), except for alginate-xanthan gum blend and cellulose-gelatine blend. The most improving effect showed the combinations with cellulose in the portion of 0.5%, specifically agar, alginate, carob gum-cellulose blend.

The hydrocolloid blends mentioned above together with carrageenan-gelatine and gelatine-tragacanth blends improved the final quality of buckwheat-rice (40:60) sample, specifically loaf specific volume of agar-cellulose and carob gum-cellulose blends, both in the portion of 0.5% reached almost $1.9 \text{ cm}^3 \text{ g}^{-1}$ but only agar-cellulose sample showed satisfactory hardness.

The functionality of rice-buckwheat flours combination in terms of breadmaking performance, nutritional and sensory quality is evident and can be successfully used for gluten-free bread production. The final bread quality can be more or less influenced by the application of hydrocolloids even in very low addition levels. The type of hydrocolloid, hydrocolloid combination and its portion in formula, however, are the key factors also from the economic point of view. The effect is strongly dependent on the material used for the bread production as the gluten-free flours vary in chemical composition and different components may interact with hydrocolloids in a different extent. As hydrocolloids are very expensive material it should be used at the lowest level that promises positive effect.

SHRNUTÍ

Úvod

Pšenice se svými výjimečnými vlastnostmi zastává v potravinářském průmyslu mnoho funkcí a jsou to visko-elastické vlastnosti, za které odpovídají lepkové bílkoviny a které se zcela odlišují od ostatních cereálií. Lepkové bílkoviny představují zhruba 80 až 85 % ze všech bílkovin pšenice a sestávají z monomerních jednotek (gliadin), které způsobují viskózní chování, zatímco polymerní jednotky (glutenin) zodpovídají za elasticitu. Během zpracování těsta podporují vznik soudružného visko-elastického těsta, které je schopné zadržovat plyn produkovaný během kynutí, což vede k typické pěnovité struktuře pšeničného pečiva. I když v tomto procesu hrají roli i další složky, je evidentní, že funkce lepkových bílkovin je zásadní [1–6].

Mouky jiných obilovin tyto klíčové bílkoviny neobsahují, takže se hůře zpracovávají a jejich pekárenská kvalita je mnohem nižší, což je úzce spojeno se zhoršenou schopností těsta zadržovat plyn a výslednou neuspokojivou kvalitou pečiva [7–9].

Nicméně, lepkové bílkoviny musí být v případě pacientů s nesnášenlivostí lepku úplně vyloučeny z výživy, protože jejich příjem ve stravě způsobuje závažné poškození střeva. Proto se pro výživu těchto pacientů využívají bezlepkové obiloviny jako rýže, pohanka, kukuřice, případně méně častý amarant, cizrna, merlík, proso a škrob z čiroku, sóji, tapioky a teffu [26–42].

Bezlepkové pečivo je typické svou horší technologickou kvalitou, nízkým specifickým objemem, větší tvrdostí střídky i kůrky a krátkou skladovatelností [7, 43–51]. Skladovatelnost je navíc ovlivněna ztrátou vlhkosti, podmínkami skladování a celý proces vyústí ve ztvrdnutí kůrky a střídky, způsobené krystalizací amylopektinu [52–54].

Zhoršená zpracovatelnost bezlepkových těst a nižší technologická kvalita bezlepkového pečiva je obvykle zlepšována pozměněním technologických procesů a aplikací přírodních látek, které jsou schopny alespoň částečně nahradit lepkovou strukturu. Tím zvýší technologickou kvalitu konečného produktu, podpoří senzory a výživou hodnotu a prodlouží jeho životnost. To zahrnuje například využití škrobů různého původu, luštěninových mouk a zařazení emulgátorů, tuků, hydrokoloidů a jejich kombinací [69–73].

Cíle práce

Cílem dizertační práce bylo určení kvality bezlepkového pečiva a ověření správnosti stanovených hypotéz o:

- 1 Vlivu vybraných mouk na výslednou kvalitu pečiva.
- 2 Vlivu vybraných směsí mouk a různého poměru mouk ve směsi na výslednou kvalitu pečiva.
- 3 Vlivu vybraných hydrokoloidů na výslednou kvalitu pečiva.
- 4 Vlivu směsí hydrokoloidů na výslednou kvalitu pečiva.

Metody

Materiál

Na základě prostudované literatury a publikovaných výsledků byly vybrány běžně dostupné mouky: amarantová, pohanková, cizrnová, jáhlová, merlíková a rýžová mouka jako materiály, které by v různých kombinacích mohly zlepšit jak celkovou kvalitu konečného produktu, tak jeho nutriční kvalitu. Dále bylo vybráno osm hydrokoloidů: agar, alginát sodný, karubin, κ -karagenan, sodná sůl karboxymetyl celulózy, tragakant a xantanová guma, které měly částečně nahradit lepkovou strukturu, a tím podpořit jednak specifický objem produktu a jeho dobu skladovatelnosti.

Vaznost vody

Vaznost vody byla stanovena podle normy ISO 5530-1 [91], která specifikuje využití Brabenderova farinografu pro stanovení vaznosti vody mouky a chování během hnětení těsta připraveného z příslušného druhu mouky. Vaznost vody se stanoví jako přesný objem vody potřebný k vytvoření těsta s maximální konzistencí 500 farinografických jednotek (FU) a je vyjádřena v ml na 100 g mouky o vlhkosti 14 %.

Pekařský pokus

Pekařský pokus byl proveden na 300 g vzorku mouky metodou přímého vedení těsta a zkrácené doby zrání v souladu s ICC standardem č. 131 [92], které jsou typické právě pro tuto metodu. Těsto bylo připraveno z příslušné mouky nebo směsi mouk (100 %); 1,8 % sušeného droždí; 1,5 % soli; 1,86 % cukru; 0,005 % kyseliny citronové – vztaženo na hmotnost mouky, a vody podle farinografických měření. Bochníky pečiva byly hodnoceny 24 h po upečení a byly sledovány následující parametry: výtěžnost těsta a pečiva (%), ztráty pečením (%), specifický objem bochníku (poměr mezi objemem bochníku a jeho hmotností v $\text{cm}^3 \text{g}^{-1}$).

Texturní parametry

Texturní analýza střídky byla provedena na vzorku o průměru 2,5 cm a tloušťce 2,0 cm na Texturním Analyzátoru TA.XT Plus (Stable Micro Systems, Surrey, UK) s využitím kompresního zařízení a matrice o průměru 5,0 cm. Rychlost matrice byla nastavena na 1 mm s⁻¹ a tvrdost střídky byla měřena 24 a 72 h po upečení a vyhodnocena softwarem ExponentLite.

Vlhkost

Vlhkost vzorků byla stanovena podle metody ČSN 56 0116-3 [93] při 130 °C po dobu 90 min. Vzorky byly připraveny z vnitřní části střídky (ze vzdálenosti nejméně 1,5 cm od kůrky). Střídka byla rozdrobena, rozdělena na 5 g vzorky a vysušena v hliníkových kelímcích. Všechny vzorky byly testovány 24 a 72 h po upečení. Po vysušení vzorky vychladly v exsikátoru, pak byly zváženy a vlhkost byla vypočítána z rozdílu hmotností.

Statistická analýza

Výsledky byly vyhodnoceny jednofaktorovou analýzou variance (ANOVA) na hladině pravděpodobnosti $p < 0,05$ za použití software Statistica 9.1 (StatSoft, Inc.). Účelem statistického vyhodnocení bylo zjistit statisticky významné rozdíly mezi vzorky. Statisticky významné rozdíly byly testovány Fisherovým LSD testem.

Experiment

Mouky byly testovány jak samostatně, tak ve dvou a tříložkových směsích. Z výsledků testování samostatných mouk vyplynulo, že nejvhodnější základním materiálem pro další experimenty bude rýžová mouka. Tedy dvousložkové vzorky byly míchány v poměru 50 % rýžové mouky a 50 % amarantové, pohankové, cizrnové, jáhlové nebo merlíkové mouky. Tříložkové vzorky byly míchány vždy tak, že 60 % tvořila rýžová mouka, 20 % pak amarantová a 20 % pohanková mouka. Další vzorek byl opět 60 % rýžová mouka, 20 % amarantová a 20 % cizrnová mouka atd., viz Table 1.

Pro dobré výsledky kombinace rýžové a pohankové mouky byla otestována kompletní řada pohankovo-rýžové směsi. Od vzorku s 10 % pohankové a 90 % rýžové mouky, po vzorek s 90 % pohankové a 10 % rýžové mouky.

Hydrokoloidy byly nejdříve testovány na vzorku rýžové mouky, a to v množství 0,5 a 1,0 % vztaženo na hmotnost mouky. Dále byly smíchány do dvousložkové směsi v poměru 50:50 a tato směs opět testována na vzorku rýžové mouky v množství 0,5 a 1,0 %; viz Table 2.

Z výsledků bylo vybráno 7 směsí hydrokolodů ve specifickém množství, které byly aplikovány do vzorku se 40 % pohankové a 60 % rýžové a mouky.

U všech vzorků byla vyhodnocena technologická kvalita výsledného pečiva, tvrdost a u vzorků obsahujících hydrokoloidy i vlhkost 24 a 72 h po upečení.

Výsledky a závěr

Cílem dizertační práce bylo ověřit vliv bezlepkových mouk a jejich míchání na kvalitu bezlepkového pečiva. Dále navrhnout možnosti alespoň částečné náhrady glutenin-gliadinového komplexu v tomto pečivu. Experimenty měly prověřit správnost 4 hypotéz a pro každou z nich je možné stanovit následující závěry:

Hypotéza 1: Druh mouky ovlivňuje výslednou kvalitu pečiva.

Druh mouky má vliv na výslednou kvalitu pečiva a konkrétně vzorky amarantové, pohankové, cizrnové a rýžové mouky dosáhly velmi dobrého specifického objemu bochníku. Navíc, rýžový vzorek dosáhl nejnižší tvrdosti mezi všemi testovanými moukami. Bylo prokázáno že žádná z vybraných mouk není vhodná pro použití samostatně kvůli suché kůrce, nedostatečné pórovitosti střídky a sensorickým vlastnostem. Vzorek rýžové mouky byl pro svoji neutrální chuť a vůni zvolen jako kontrolní vzorek pro další experimenty.

Hypotéza 2: Směsi a mouk a specifický poměr mouk ve směsi ovlivňuje výslednou kvalitu pečiva.

Dvousložkové směsi mouk ovlivňují výslednou kvalitu pečiva a nejlepšího specifického objemu bochníku dosáhl vzorek kombinace rýžové a pohankové mouky (50:50). Ve srovnání s kontrolním rýžovým vzorkem, všechny bezlepkové směsi měly tvrdší střídku.

Bylo prokázáno, že téměř všechny třísložkové směsi měly větší specifický objem bochníku než rýžový vzorek a nejlepšího výsledku dosáhla kombinace se 60 % rýžové, 20 % pohankové a 20 % merlíkové mouky. S výjimkou směsi rýžové (60 %), amarantové (20 %) a pohankové mouky (20 %), všechny zbývající směsi měly horší tvrdost střídky ve srovnání s kontrolním rýžovým vzorkem a nejvyšší tvrdosti dosáhly vzorky s obsahem jáhlové mouky. Vzorky s amarantovou a merlíkovou byly kvůli své specifické vůni a chuti vyloučeny z dalších experimentů.

Kombinace rýžové a pohankové mouky byla na základě výsledků vyhodnocena jako nejlepší, tudíž byly dále testovány všechny kombinace – od 10 % pohankové a 90 % rýžové mouky ve směsi, po 90 % pohankové a 10 % rýžové mouky. Se zvyšujícím se podílem pohankové mouky ve směsi se zvětšoval i specifický objem bochníku, až po 80% podíl pohankové mouky. Ve srovnání s pohankovým vzorkem měly všechny pohankovo-rýžové kombinace měkčí střídku, ale tvrdší ve srovnání s rýžovým vzorkem. Vzorek s podílem 40 % pohankové a 60 %

rýžové mouky dosáhl uspokojivé pekařské a sensorické kvality a byl tak vyhodnocen jako nejvhodnější kombinace pro konečné experimenty.

Hypotéza 3: Vybraný druh hydrokoloidu ovlivňuje výslednou kvalitu pečiva.

Bylo prokázáno, že vybraný druh hydrokoloidu ovlivňuje výslednou kvalitu pečiva, ale neexistuje žádný důkaz o zvyšujícím se pozitivním efektu se zvyšujícím se podílem hydrokoloidu v receptuře. Navíc, 1,0 % hydrokoloidu (vztaženo na hmotnost mouky) v receptuře prokazatelně zhoršilo specifický objem bochníku ve srovnání se vzorky s množstvím 0,5 % hydrokoloidu. Obecně lze shrnout, že přítomnost hydrokoloidu zlepšuje tvrdost střídky 72 hodin po upečení a udrží uspokojivou vlhkost vzorku taktéž, 72 h po upečení.

Hypotéza 4: Směs hydrokoloidů ovlivňuje výslednou kvalitu pečiva.

Směsi hydrokoloidů zlepšily výslednou kvalitu pečiva, ale nebylo prokázáno, že se zvyšujícím se množstvím směsi v receptuře se zvyšuje i výsledný pozitivní efekt, s výjimkou směsí alginátu-xantanové gumy a celulózy-želatiny, kde množství 1,0 % v receptuře prokázalo lepší výsledky. Nejvíce zlepšující efekt prokázala celulóza, a to ve směsi s agarem, alginátem a karubinem v množství 0,5 %.

Výše zmíněné kombinace hydrokoloidů společně se směsí karagenanu-želatiny a želatiny-tragakantu (obojí v množství 0,5 %) zlepšily i výslednou kvalitu pohankovo-rýžového (40:60) pečiva. Konkrétně vzorky s kombinacemi agaru-celulózy a karubinu-celulózy, obojí v množství 0,5 % dosáhly specifického objemu bochníku téměř $1,9 \text{ cm}^3 \text{ g}^{-1}$, ale pouze vzorek s agarem-celulózou (0,5 %) dosáhl uspokojivé tvrdosti střídky.

Význam pohankovo-rýžových směsí ve smyslu jejich zpracovatelnosti, nutriční a sensorické kvality je nezanedbatelný a mohou tak být velmi uspokojivě využívány pro výrobu bezlepkových produktů. Kvalita výsledného pečiva může být více či méně ovlivňována použitím hydrokoloidů už ve velmi malém množství. Nicméně, druh hydrokoloidu, jejich kombinace a množství v receptuře jsou klíčovými faktory také z ekonomického hlediska. Jejich výsledný vliv totiž velmi závisí na materiálech použitých pro výrobu bezlepkových produktů a jejich chemickém složení, protože každá složka může s použitým hydrokoloidem reagovat jinak a v různém rozsahu.

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**Effect of specific hydrocolloids and hydrocolloid blends
on gluten-free bread quality**

Vliv vybraných hydrokoloidů a směsí hydrokoloidů na kvalitu bezlepkového
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