Tomas Bata University in Zlín
Faculty of Technology

Doctoral Thesis

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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Zlín, August 2018

Published by **Tomas Bata University in Zlín** in **Doctoral Thesis Summary.**
The publication was released in 2018.

Key words: *gluten-free flour*, *baking quality*, *hydrocolloid*, *hydrocolloid blends*

Klíčová slova: *bezlepková mouka, pekárenská kvalita, hydrokoloid, směs hydrokoloidů*

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ACKNOWLEDGEMENTS

I would like to thank my supervisor prof. Ing. Stanislav Kráčmar, DrSc. and my consultant doc. RNDr. Iva Burešová, Ph.D., whose wise expertise and support have been invaluable throughout my doctoral studies.

I wish to thank my husband and children for their understanding, encouragement and patience during writing the thesis.

Experimental part of the thesis was supported by project of the internal grant of Tomas Bata University in Zlín No. IGA/FT/2012/034/D funded from the resources for specific university research.

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 cm^3 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 to the sample of 40% buckwheat and 60% rice flour (BR 4060) and baking test evaluated. The hydrocolloid blends improved loaf specific volume from 1.30 cm^3 g^{-1} to 1.85 cm^3 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 4060-alginate-cellulose 0.5%) compared to the rice and BR 4060 samples.

ABSTRAKT

Zvyšující se poptávka po bezlepkovém 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, 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 (BR 4060) se specifickým objemem bochníku 1,30 cm^3 g^{-1}, tvrdostí 17,1 N a žádným negativním vlivem na senzorické vlastnosti vzorku. Ke zlepšení 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 a 60 % rýžové mouky (BR 4060), kde došlo ke zlepšení specifického objemu bochníku z 1,30 cm^3 g^{-1} na 1,85 cm^3 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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LIST OF ABBREVIATIONS

Alg	alginate
ANOVA	analysis of variance
BR	buckwheat-rice
Carrag	carrageenan
CD	celiac disease
Cel	cellulose
CG	carob gum
CMC	sodium carboxymethylcellulose
FU	farinographic unit
Gel	gelatine
HPC	hydroxypropyl cellulose
HPMC	hydroxypropyl methyl cellulose
MC	methyl cellulose
MEC	methyl ethyl cellulose
NPU	net protein utilization
RS	resistant starch
Trag	tragacanth
XG	xanthan gum

1. CURRENT STATE OF SOLVED ISSUES

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 entheropaty 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].

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 bicolour*), 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 isolate es (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.

1.1 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 adaption of a well-balanced, varied diet that avoids gluten [81].

1.1.1 The diagnosis history

Celiac disease has probably existed for over 2000 years. It is very common in European population with prevalence 1:100–300. The disease was firstly described in an article called "on the Coeliac Affection" by Samuel Gee in 1888 based on the inconsistency of the child size and age (those children were much older, than their appearance would suggest). The celiac disease treatment was described in 1924 by Sidney Haas whose treatment was based on anorexia nervosa treatment. The diet excluded bread, crackers, potatoes, and cereals and included bananas which were gradually added to the diet. During the Second World War there was a shortage of cereals and bread that led to the decrease of celiac sprue among children. The paediatrician W. K. Dicke observed that following re-introduction of gluten into the children nutrition with celiac diagnosis caused the previous difficulties. Dicke and his co-workers pursued to prove that wheat flour and especially gluten fraction was the reason [81].

Celiac disease was considered very rare and connected with childhood long time ago [82] with uniform clinical presentation of weight loss and diarrhoea. But recent data have shown that it is more common than supposed [83]. In Europe an estimated 1% of adults and children have the disease. The prevalence varies widely; for ages 30–64 years, it is eight times higher in Finland (2.4%) than in Germany (0.3%), perhaps relating to both genetic and environmental factors. In Finland, the prevalence has doubled over 20 years which cannot be explained by better detection rates [84].

In the Czech Republic it is estimated that there are about 0.5% of population suffering from the celiac disease (every 200–250th person, which is 40–50 000 people). Other resources suggest even 1% of population, but due to many different symptoms the disease has currently been diagnosed and treated only among about 4000 of celiac in the Czech Republic [85].

1.1.2 Pathology and body response

Celiac disease means that mucosa of usually the proximal small intestine is affected by consuming food containing gluten. In severe cases the damage

progresses to the distal small intestine, ileum and colon [81], concretely described by crypt hyperplasia, jejunal mucosa villous atrophy and inflammatory infiltrate in lamina propria associated with an increased number of intraepithelial lymphoyces [86]. The ingestion of gluten induces an inflammatory response that leads into the destruction of villous structure of the small intestine, which ends in a flat jejunal mucosa [87]. Figure 1 shows the characteristic appearance of healthy jejuna mucosa and the mucosa of untreated celiac disease.

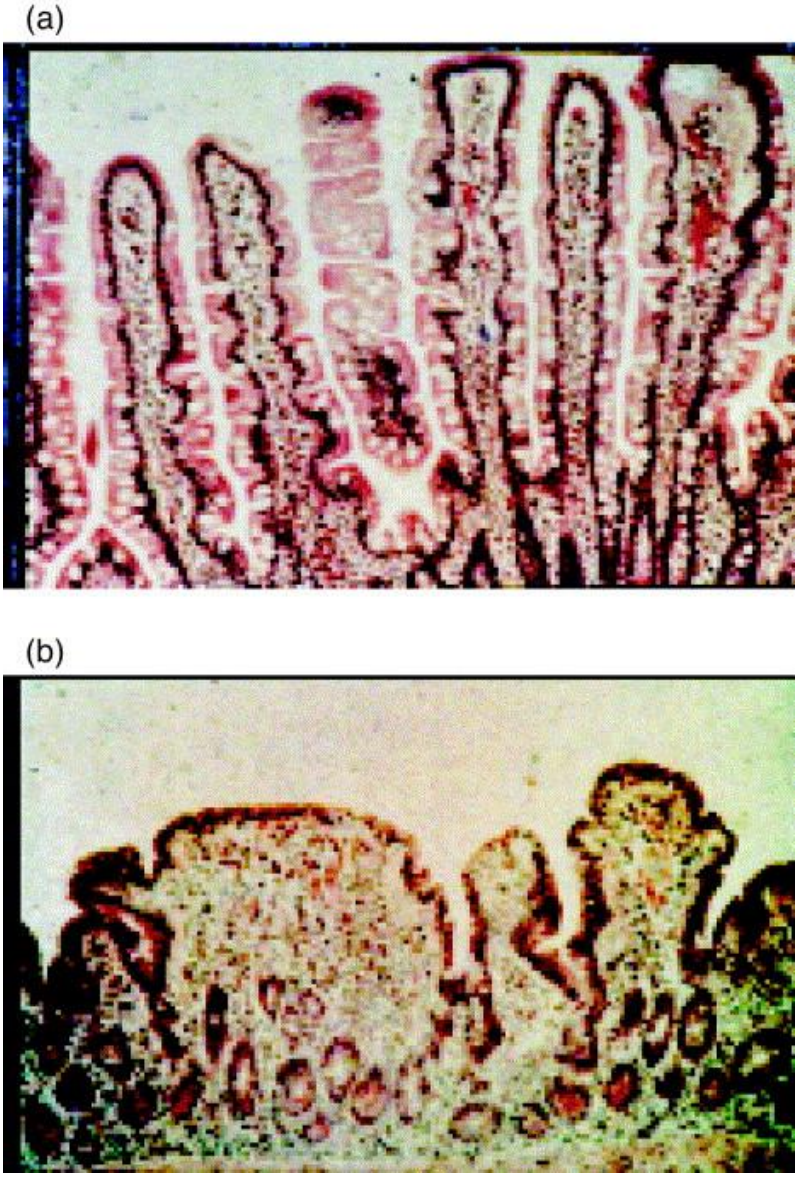


Figure 1. Histological appearance of normal small intestinal mucosa (a) and mucosa in untreated celiac disease (b) [81].

Generally, it is a flattening of mucosa that can vary from mild through partial villous atrophy to a total absence of villi or reduction of the villous height/depth ratio from 5:1 to 3:1 [81].

As mentioned above, celiac disease is a body response to the cereal proteins, especially gliadin and glutenin peptides of wheat gluten and then similar

alcohol-soluble proteins (prolamins) of rye (secalins), barley (hordeins), and oats (avenins) [19]. Some available clinical data show that great majority of celiac patients tolerate oats, and the risk is particularly based on the contamination with wheat gluten from the mill. Thus, oats currently remain on the Codex alimentarius list of the gluten-containing cereals. The symptoms of celiac disease include serious symptoms of malabsorption such as stools characterised by pale and bulky passage, abdominal discomfort, weight loss or gain, tiredness, anaemia and severe diarrhoea [88]. Table 1 outlines the most common reactions to gluten according to [89].

Table 1: Symptoms and related signs of celiac disease

Infancy (0−2 years)	**Childhood**	**Adulthood**
Diarrhoea (miserable, pale) Abdominal distension (enlarged abdomen)	Diarrhoea or constipation. Anaemia	Diarrhoea or constipation. Anaemia.
Failure to thrive (low weight, lack of fat, hair thinning)	Loss of appetite (short stature, osteoporosis)	Aphthous ulcers, sore tongue and mouth (mouth ulcers, glossitis, stomatitis)
Anorexia, vomiting		Dyspepsia, abdominal pain, bloating (weight loss)
Psychomotor impairment (muscle wasting)		Fatigue, infertility, neuropsychiatric symptoms (anxiety, depression). Bone pain (osteoporosis). Weakness (myopathy, neuropathy)

The European Society for Paediatric Gastroeterology, Hepatology and Nutrition defined the criteria for celiac disease as detected flat mucosa by biopsy and disappearance of symptoms after following gluten-free diet [90]. Murray [19] claims that celiac disease is the result of genetic predisposition, immunologically based inflammation and environmental factors. The longer consuming gluten, the higher increase of internal and external consequences appear among celiac – the internal effects are constant presence of a flat intestinal mucosa very often followed by a reduction in enzyme activity and lack of absorption of vitamins, minerals leading to different deficiencies; the external effects are dermatitis, pale skin, dry hair, abdominal pain, pale and foul-smelling stools, bloating and poor growth especially among children younger than 2 year old. Other usually observed external symptoms among infants are failure to thrive, vomiting, muscle wasting, signs of hypoproteinaemia, general

irritability and unhappiness; at older ages may include anaemia or failure to grow normally – therefore measurements of height and weight are very valuable showing a slowing of the weight gain or weight loss. If the disease was present for a long time there is also slowing growth connected with micronutrient deficiencies (iron, folic acid, calcium, vitamin D, vitamin B_{12}). Women with untreated celiac disease are at increased risk of having low birth weight children or in an extreme miscarriage [81]. The symptoms usually develop few weeks after cereals are introduced into the diet. Babies nowadays represent only 5% of newly diagnosed celiac, however 90% are diagnosed over 16 years old. The Coeliac Society believes the average prevalence could be as high as one in 300 people in Europe. The only possible treatment is to return the intestine to normal by means of a strict and whole life gluten-free diet, that was established by Codex Standard for gluten-free foods even though the Codex Alimentarius tolerates 0.200 g kg^{-1} of gluten per food. The speed of response to a gluten-free diet is variable – about 70% of patients noticeably improved in two weeks. Using series of biopsies and absorption tests has shown intestinal permeability improvement in two months from starting a gluten-free diet, but a measurable improvement usually requires a gluten-free diet for at least 3–6 months [83].

1.1.3 The chemical cause of celiac disease

Wheat grain has three major constituents that are separated by milling: the outer bran, the germ and the endosperm, usually called white flour. The endosperm contains storage cereal proteins that are divided into two major groups: the ethanol soluble fraction (prolamins – gliadins in wheat) and the glutenins. Prolamins are present in rye, barley and oats too. The gliadin fraction of wheat proteins is known to be toxin in celiac disease and consists of subfractions – α, β, γ and ω. Peptides from α-gliadin were determined to characterize the toxic epitopes – antigenic determinant, the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells [81].

1.2 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 [91]. 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} [92].

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 [93]. 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, 94]. 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 [94].

1.2.1 Labelling gluten-free products and foodstuffs

According the Regulation (EU) No 609/2013 [95] labelling, advertising, and presentation of the products and foodstuffs for people intolerant to gluten, consisting of or containing one or more ingredients made from wheat, rye, barley, oats or their crossbred varieties which have been especially processed to reduce gluten, shall not contain a level of gluten exceeding 0.1 g kg^{-1} in the food as sold to the final consumer shall bear the term *'very low gluten'*. They may bear the term *'gluten-free'* if the gluten content does not exceed 0.02 g kg^{-1} in the food as sold to the final consumer. Oats contained in foodstuffs for people intolerant to gluten must have been specially produced, prepared and/or processed in a way to avoid contamination by wheat, rye, barley, or their crossbred varieties and the gluten content of such oats must not exceed 0.02 g kg^{-1}.

1.3 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 [96] 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 [97, 98] 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 [98].

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].

1.3.1 Pseudocereals

Amaranth and quinoa were the major crops for the Pre-Colombian cultures. Since it has been revealed both grains are of good nutritional properties, the interest has risen. The production of quinoa was 79 269 tonnes in Peru, 65 548 tonnes in Bolivia and 3 903 tonnes in Ecuador in 2016 [97]. The production of amaranth is still very low thus it is not listed in the FAO statistics. Although an appreciable commercial cultivation of amaranth for human nutrition does take place – it is produced in Latin American countries, USA, China and Europe (98). Buckwheat originates from Central Asia and was transferred to Central and Eastern Europe. It was in a great interest in Germany, Austria and Italy in the thirteenth century, but it declined for the cultivation of other cereals. Nowadays, buckwheat has come to an interest again due to the demand for gluten-free diets. In 2016 Russian Federation produced the most amount of buckwheat – 1 186 333 tonnes, followed by China (404 259 tonnes), Ukraine (179 020 tonnes), France (122 206 tonnes) and Poland (118 562 tonnes) [97].

As all the pseudocereals are valued for their chemical composition and positive effect on the human health, the chemical composition of amaranth, quinoa, and buckwheat is shown in the Table 2.

Table 2. Chemical composition of pseudocereals [98]

Chemical composition of amaranth, buckwheat and quinoa			
	Composition (average value in %, range in brackets)		
Component	Amaranth.	Buckwheat	Quinoa
Water	11.1 (9.1−12.5)	14.1 (13.4−19.4)	12.8
Protein	14.6 (14.5−14.8)	10.9 (10.4−11.0)	13.8 (12.2−13.8)
Fat	8.81 (6.56−10.3)	2.71 (2.40−2.80)	5.04 (5.01−5.94)
Starch	55.1	67.2	67.4
Dietary fibre	11.14	8.62	12.88
Minerals	3.25	1.59 (1.37−1.67)	3.33 (2.46−3.36)

Amaranth

Over 60 species of amaranth are known worldwide. The main grain amaranth species used today are *Amaranthus caudatus* L., *Amaranthus cruentus* L., and *Amaranthus hypochodriacus*. Amaranth seeds are lentil-shaped and measure about 1 mm in diameter. The 1000 kernel weight is only 0.5–1.4 g. Analysis of amaranth carbohydrates, specifically starch revealed two main differences

in comparison to cereals: (a) starch is the main component of carbohydrates in amaranth, but in lower amounts than in cereals; (b) amaranth starch is not situated in endosperm, but in perisperm, where the typical starch particles of approximately 50–90 μm in diameter are generated. Suspended in water, small single starch granules of 1–3 μm were extracted. Starch consisting of small granules is typical of most starch materials and these particles aggregate together to minimize the surface thus form the characteristic compound and properties. Using scanning electron microscopy (SEM), starch granules appeared polygonal with a diameter of 0.8–1.0 μm [99].

Resistant starch (RS) is naturally presented in food and formed during processing. Similar to fibre, resistant starch is not susceptible to human digestive enzymes and reaches the colon, where it is fermented by the bacteria. Resistant starch has beneficial physiological effects – lowering blood lipids or the risk of colon cancer. The RS content depends on many factors (type of granule, amylose/amylopectin ratio and crystallinity of starch). Additionally, the food processing may also influence the content of RS. Gonzales [100] found that the content of RS in amaranth is 0.65%. The extrusion cooking increased while cooking and popping decreased the amount of RS. In addition, the studies of showed the efficiency and utilization of amaranth starch may be very high. Also, the content of dietary fibres (soluble and insoluble) which have beneficial effects on human health is appreciable – the fraction of soluble dietary fibre varies between 19.5–49.5% due to analysed specie [101].

The amount of mono- and disaccharides is unlikely very low. According to Gamel [102] the sugar content ranges from 18.4–21.7 g kg^{-1} with dominant sucrose (5.8–7.5 g kg^{-1}), then in descending order galactose, glucose, fructose, maltose, raffinose, stachyose, and inositol.

The storage proteins in amaranth are predominantly albumins and globulins Specifically, 40% albumins, 20% globulins, 25–30% glutelins, and only 2–3% prolamins. The protein proportions of amaranth are similar to those of rice; thermal treatment decreased both the water-soluble and alcohol-soluble protein fractions. And it can be concluded that the amaranth proteins are similar to seed proteins in other dicotyledonous crops such as legumes [103].

The amino acid composition of generally pseudocereals is outstanding – high content of essential amino acids – particularly methionine, lysine, arginine, tryptophan and sulphur-containing amino acids can be found here at higher levels than in other cereals. Amaranth contains 476.5 g kg^{-1} essential amino acids in the protein. In comparison with for example soy bean, the amaranth has higher portions of glutamine, glycine, and methionine, unlikely tyrosine, and cystein were significantly lower. Protein quality depends first on amino acid composition and second on the bioavailability or digestibility; net protein utilization NPU is widely used as an indicator of the nutritional quality of proteins. In this regard, the values for pseudocereal proteins are higher than those for cereals. For example, Gamel [102] measured the average protein

digestibility of raw amaranth wholemeal flour as 81–86% which was even increased after heating (opening the carbohydrate-protein complex and inactivation of anti-nutritional factors such as trypsin inhibitors or polyphenols – tannic acid) [104].

The fat content of amaranth is about two or three times higher than other cereals have. Amaranth oil contains more than 75% unsaturated fatty acids and is particularly rich in linoleic acid (35–55%). Palmitic acid accounts for 20–23%, palmitoleic acid 16%, stearic acid 3–4%, and oleic acid 18–38%. Amaranth contains high levels of squalene 2–8%, which lowers the levels of cholesterol. The content of minerals in amaranth is about twice as high as in other cereals. Particularly calcium, magnesium, iron, potassium, phosphorus and zinc. On the other hand, does not contain an important source of vitamins, but it can be a good source of riboflavin, vitamin C, folic acid and vitamin E [36, 102].

Due to the very small size of the amaranth seeds, specific adaptations of the milling procedures are required. The production of wholemeal flour is not very complicated but specific demands occur during grinding and separation when producing flour fractions with different chemical composition and chemical properties. Thus, the mill and technology play a key role in determining the quality [98].

Quinoa

Among quinoa, sweet and bitter varieties exist – dependent on the saponins (when the saponin content is below 0.11% the variety is considered a sweet variety). Quinoa seeds are a little bit larger than amaranth seeds, the 1000 kernel weight is approximately 1.9–4.3 g. In contrast to cereals the embryo is surrounded by starch-based tissue (perisperm) in the form of a ring and makes about 25% of the total seed weight [105].

The main component of carbohydrates in quinoa is the starch, however the content is much lower in comparison with other cereals. The starch is situated mainly in the perisperm and small amount in the seed coat and embryo. Quinoa starch consists of polygonal granules with size ranging from 0.63–1.8 μm. The complexes (spheroidal or oblong) of starch granules is formed by up to 14 000 single granules bounded together surrounded by protein matrix. Quinoa starch has higher gelatinization temperatures and higher pasting viscosities than other cereals and the values increase with cooling. Furthermore, the quinoa starch has high water-binding capacity, high swelling power and retrogradation stabilities due to lower content of amylose [106].

The values of resistant starch (RS) were measured as 12.6 ± 1.29 g kg^{-1} seeds which is much lower than for other cereals like wheat (39.0 ± 5.7 g kg^{-1}) or rye (49.0 ± 7.3 g kg^{-1}). The reason of the lower portions of RS in quinoa is the low content of amylose thus low formation of RS. The content of mono-

and disaccharides is rather low: glucose 0.019%, fructose 0.019%, galactose 0.06%, ribose 0.07%, and maltose 0.1%. The content of dietary fibre (12.88%) is comparable to that of other cereals with the embryo containing higher amounts than the perisperm. The soluble fibre amount is only 13.5% of total dietary fibre and decreases with cooking and autoclaving [107].

The quinoa protein content and quality are higher than that of other cereals and consists mainly of albumins and globulins. The seed protein consists of (regarding to solubility) 31% water, 37% saline, 0.8% alcohol, 11.5% alkali soluble and 19.7% insoluble protein fractions with a balanced content of essential amino acid with high level of lysine (4.5–7.0%) [33]. Generally, the amino acid is present in a concentration of 387.1 g kg^{-1} protein which is only 16% lower than that of whole egg protein thus quinoa protein is very close to the FAO recommended pattern in essential amino acids. The lysine level (6.3%) is comparable to the soybean level, but methionine is deficient. The digestibility of quinoa protein is 84.3% and the NPU value 75.2% [108].

The content of fat in quinoa is higher than in cereals – ranges between 5–6% and depends on varieties. The fat content is higher in the germ and seed coat than in perisperm. The fat is typical of high content of unsaturated fatty acids with linoleic acid of more than 50%. Palmitic acid accounts for around 20%, followed by oleic acid with about 8% and linolenic acid with more than 6%. The degree of unsaturation is over 87% The quinoa fats are relatively stable during storage due to high vitamin E content [109].

The content of minerals in quinoa is approximately twice as high as in cereals and is affected by growing conditions. The highest contents were measured for calcium, magnesium, iron, potassium and zinc. The content of vitamins in quinoa is almost equal to wheat and is a good source of thiamine, folic acid, vitamin C, riboflavin, and is particularly good source of vitamin E [110].

Due to the small size, quinoa is usually milled to wholemeal flour followed by removing of the saponins by washing, or abrasive milling and as the saponins are concentrated in the hulls, their content can be minimized by dehulling of the seed. The protein content falls from 12.5% in the wholemeal to 3.55% in the flour [109].

Buckwheat

Two varieties of buckwheat are commonly cultivated: common buckwheat (*Fagopyrum esculentum* Moench) and tatary buckwheat (*Fagopyrum tataricum*). The buckwheat seed is a three-angled achene, 6–9 mm long. The fruit of *F. tataricum* is smaller (4–5 mm) and more rounded at the edges. The 1000 kernel weight (10–20 g) depends mainly on the hull thickness. Structurally and chemically, the endosperm resembles that of a cereal grain consisting of a non-starchy aleurone layer and large cells packed with starch granules constituting most of the endosperm [98].

Buckwheat is a dicotyledonous plant and from 1975 has been suggested by for the best cure of celiac disease, as it does not contain gluten-like proteins and therefore can be used for production of gluten-free products. The unique protein structure and amino acid composition suppose the buckwheat might be a very valuable resource and could help to treat some chronic diseases such as diabetes mellitus II, hypertension and other cardiovascular diseases. Buckwheat is usually used as a basic component of gluten-free blend to improve the quality of gluten-free bread by hydrocolloids and other improving components. The addition of buckwheat was reported to increase the water absorption of the bread formulation, however, the volume of bread decreased. The authors concluded that buckwheat-containing bread was firmer in texture, the staling time was lower in comparison to the starch-based commercial gluten-free bread indicating that buckwheat is suitable to produce high quality gluten-free bread [111].

Buckwheat has a total carbohydrate content of 67–70% [112] and 54.5% of it is starch. Buckwheat starch granules have a polygonal shape and are very often aggregated. The starch granules size is rather smaller (2–14 μm) and a mean diameter of 6.5 μm. The ratio between amylose and amylopectin is 1:1 thus the buckwheat starch visibly differs from cereal starch and is similar to high amylose maize. Amylose content of buckwheat starch can be up to 46%, other studies revealed the amount of amylose content of 16–18% due to high iodine affinity of buckwheat long-chain amylopectin. Buckwheat starch exhibits a higher gelatinization temperature, peak and set back viscosities than cereal starches. High viscosity values can be explained by supermolecular glucan structures and higher granules swelling of the buckwheat starch [113]. The water binding capacity of buckwheat starch is 109.9% – higher than wheat and maize starch and it is explained by small size of buckwheat starch granules [114].

Raw buckwheat groats contain 73.5–76.0% of starch and 33.5–37.8% of this is resistant starch which predicts the buckwheat an interesting material for designing low glycemic index foods. Thermal treatment (cooking, dry heating) decreases the RS to 7.4%. Buckwheat bran consists of coat and embryo tissues and the milling fraction is rich in proteins (35%), lipids (11%) and dietary fibres (15%); the dietary fibre fraction forms 27.38% of buckwheat seeds. Soluble fraction is especially in the bran at levels of 1% where D-*chiro*-inositol useful in the treatment of non-insulin dependent diabetes mellitus can be found [115].

The major components of buckwheat seed proteins are albumins (about 43.8%) of total seed proteins, then glutelins (14.6%), prolamins (10.5%) and globulins (7.82%) but it is very dependent on extraction methods and cultivars used in reported studies. The amino acid compositions vary among parts of the investigated seed Buckwheat proteins have higher or similar content of all amino acids in comparison with wheat proteins (with exception

to glutamine and proline). Specifically, the content of the limiting amino acid lysine is 2.5 times higher than that found in wheat flour. Most represented amino acids are glutamic acid, aspartic acid, arginine and lysine. The less represented are cysteine and methionine. It can be concluded that the amino acid composition of buckwheat is well balanced and nutritionally higher to that of cereal grains in terms of biological value and net protein utilization, however, the digestibility is lower for buckwheat than for wheat (GF). Due to the lower digestibility buckwheat helps to reduce serum cholesterol and retard mammary carcinogenesis by lowering serum estradiol; suppress colon carcinogenesis by cell proliferation [116].

Lipids are concentrated in the buckwheat embryo and thus the bran is the most lipid-rich milling fraction. The total lipid amount in buckwheat grains is about 2.48%. Linoleic acid, oleic acid and palmitic acid account for 88% of the total fatty acids with 80% unsaturated fatty acids and 40% of the polyunsaturated essential linoleic acid buckwheat is nutritionally superior to cereal grains and comparable with amaranth and cotton seed oil [117].

The content of minerals in buckwheat seeds is lower than in wheat, however, except from calcium, buckwheat is a richer source of nutritionally important minerals than many cereals such as rice, sorghum, millet and maize. The concentration of potassium, phosphorus, and magnesium increases after removal of the hulls, while calcium and zinc are probably accumulated in the hulls [118].

The buckwheat groats have higher content of total folate (300 μg kg^{-1}) than rye flour (290 μg kg^{-1}), barley groats (210 μg kg^{-1}), wheat flour (19 μg kg^{-1}). The vitamin B_2 and B_6 are present in buckwheat seeds. In addition, buckwheat contains about 6% of the daily therapeutic dose of pyridoxine which reduces blood plasma homocysteine levels which contributes to coronary angioplasy [119].

When producing flours, usually roller milling is used. Fine flour contains mostly endosperm and is rich in starch, while bran composed of seed coat and embryo has low amounts of starch Buckwheat bran is very valuable fraction in terms of nutritional components – proteins (350 g kg^{-1}), lipids (110 g kg^{-1}), dietary fibre (150 g kg^{-1}), and fagopyritols (26 g kg^{-1}). Beside starch, proteins are the most important fraction affecting textural characteristic of buckwheat products, thus choosing the appropriate ratio between starch protein content is an important aspect when making buckwheat products [120].

1.3.2 Other appropriate gluten-free cereals

Rice

Rice has been one of the most important foods in the human diet and one of the most extended cereal crops and sustains two-thirds of the world's population. Rice is usually consumed as white grain but many rice products

on the food market can be found. Two main species of rice are cultivated: *Oryza sativa* and *Oryza glaberrima*. *Oryza sativa* originated in the wet tropic of Asia, but is cultivated around the world, whereas *Oryza glaberrima* has been cultivated in West Africa for the last 3500 years [98]. Cultivation of rice is concentrated in the developing countries, especially in Asia where 90.9% of the total world production is located. The largest five producers of rice are: China with production of 211 090 813 tonnes, India 158 756 871 tonnes, Indonesia 77 297 509 tonnes, Bangladesh 52 590 000 tonnes and Viet Nam 43 437 229 tonnes in 2016 [97]. Rice provides 27% of the total energy intake in the developing countries, and only 4% in developed countries. It is a cheap source of protein and in developing countries supplies 20% of the dietary protein intake [121].

The content of carbohydrates together with starch is approximately 80% of the whole grain. Rice starch is composed of amylose and amylopectin in different ratio according to the rice variety. The content of starch rises from the surface to the core thus that milled rice is rich in starch which is considered non-allergenic as it contains hypo allergenic proteins. Amylopectin is the branched polymer and is more abundant however amylose is the linear polymer and is considered an indicator of cooking quality [122].

Protein is the second most represented component of rice. In the milled rice ranges between 5–7%. Unlike starch, protein content decreases from the surface to the centre of kernel and is very deficient in the essential amino acid lysine. The protein composition is unique among all cereals with a high concentration of glutelins and low concentration of prolamins [123]. The most abundant essential amino acids are glutamic acid, aspartic acid, leucine, and arginine, followed by alanine, valine, phenylalanine, and serine.

In rice, lipids are minor components, but contribute to the nutritional, sensory, and functional characteristics as they form many complexes with the amylose chains. The most of lipids are non-starchy lipids located in the aleurone layer and germ [124].

The rice grain is rich in complex carbohydrates, and is a good source of proteins, minerals and vitamins, mainly B vitamins. The most important minerals in the rice grain are iron, phosphorus, potassium, and magnesium. The chemical composition changes during milling, outer bran removal causes a loss of proteins, fats, and a large percentage of the fibre, vitamins and minerals [121].

In developed countries rice milling has become a very sophisticated process. Milled rice is obtained after series of cleaning and removing the bran and germ from brown rice which is due to its bland taste, white colour, digestibility, and hypoallergenic properties, low protein and sodium content the most suitable cereal grain flour for celiac patients [125].

Proso millet

The study of millet literature is problematical because different common names are used for the same species. The flour commonly used in the Czech Republic is a common millet or proso millet (*Panicum miliaceum* L.).

Generally, the production of millets is the highest in India 10 280 000 tonnes, Niger 3 886 079 tonnes, China 1 996 378 tonnes, Mali 1 806 559 tonnes in 2016 [97].

Proso millet is widely grown in temperature climates across the world with major importance in China, India and Eastern Europe, USA, and Australia. It is well adapted to many soil and climatic conditions cultivated up to 3500 m altitudes. The grain colours vary from white cream, yellow, orange, red, brown and black, and have a spherical to oval shape, about 3 mm long and 2 mm diameter [126]. The 1000 kernel weight is about 7.1 g. The starchy granules in proso millet endosperm are mostly small and spherical rather than large and polygonal, and range between 1.3–8.0 μm. The endosperm protein bodies are globular in shape with 2.5 μm diameter and the main part forms the prolamins which account for 80% of the total proteins. The amount of starch can vary between 62–68% and the amylose content is about 17% of the grain dry basis. Concerning the nutritive value of protein, proso millet has an *in vitro* digestibility of about 80% and compared to casein, proto millet protein has beneficial effect by suppressing liver injury induced by D-galactosamine [127].

Proso millet lipids contain linoleic acid (60%) followed by oleic acid (14%) and has been found to increase the level of the desirable high-density lipoprotein in the blood plasma. The total polyphenolic and carotenoid contents of proso millet have been reported as 290, 740 μg kg^{-1}, respectively, with good antioxidant properties [128].

There are many traditional millet foods categorized as wholegrain foods, foods made from meal/flour and beverages. From the bread making point of view, probably the most common and known unfermented flatbread is chapatti, 12–25 cm diameter pancake with a soft, flexible puffed texture. Gluten-free bread making requires 100–150% water addition to weight flour and all the process is likely cake making [98].

Chickpea

Chickpea is a mild-flavoured bean of *Cicer arietinum*; also known as garbanzo beans in Spanish speaking countries and Bengal gram in India [129]. And it is an important plant in many regions including the Middle East, Mediterranean and Latin America. The main five world producers are India 7 818 984 tonnes, Australia 874 593 tonnes, Pakistan 517 107 tonnes, Turkey 455 000 tonnes, Iran 177 493 tonnes in 2016 [97].

Chickpea can be divided into two major types: Desi – relatively small and dark in colour and Kabuli – Mediterranean and Middle Eastern origin.

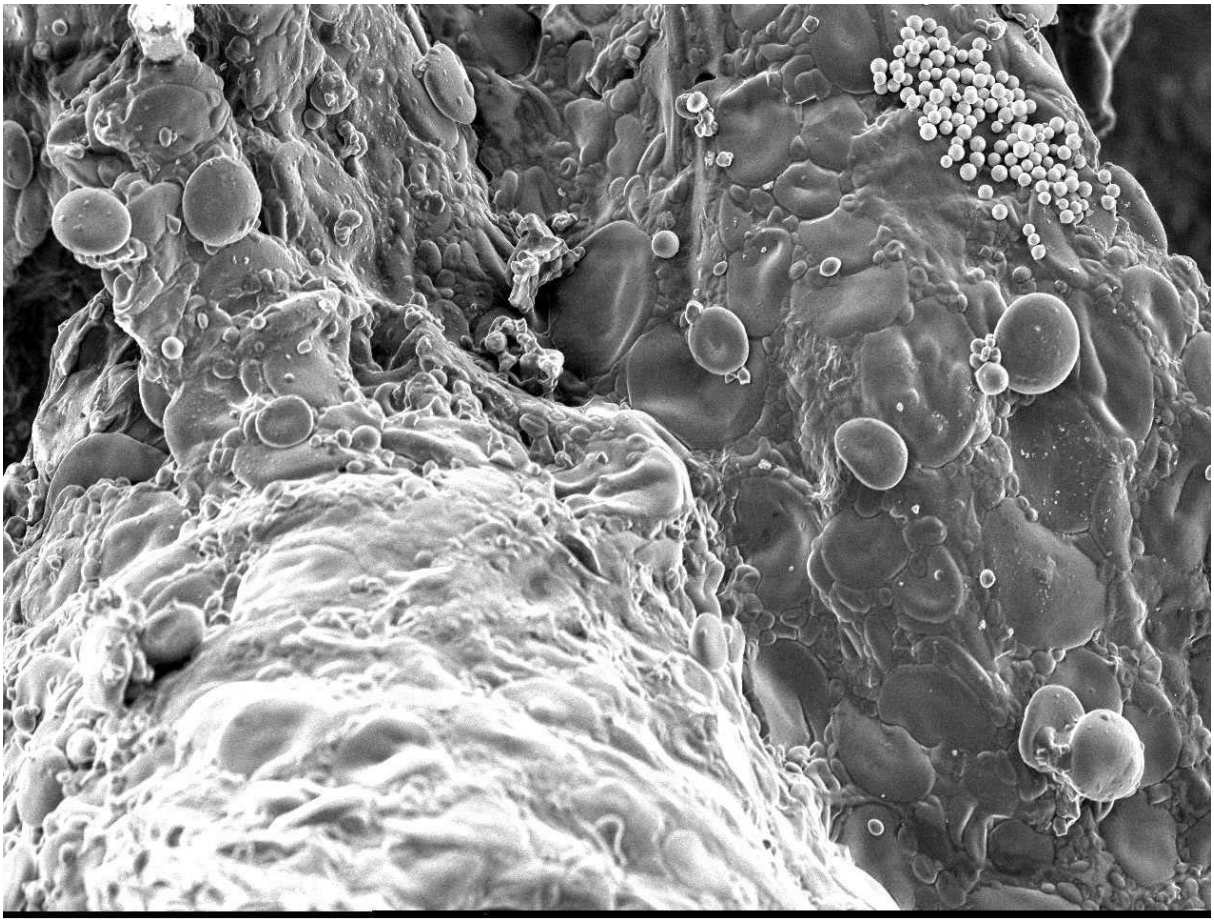
Chickpea contains high amounts of good-quality protein and it is also a good source of folates and other B vitamins and is used in many foods including salads, pasta, dips, and it is the basis of humus and [130].

Initially, the chickpea flour was used to improve the nutritional value of wheat bread, as known that generally cereal flours are poor protein quality in the respect of essential amino acids (particularly lysine, threonine, and tryptophane) [131]. Legume flours are relatively cheap protein source, chickpea contains 23% of proteins and can be used as ingredients of a great variety of foods for human consumption (high content of lysine and tryptophane) however legume flours are generally poor in sulphur amino acids methionine and cysteine [132].

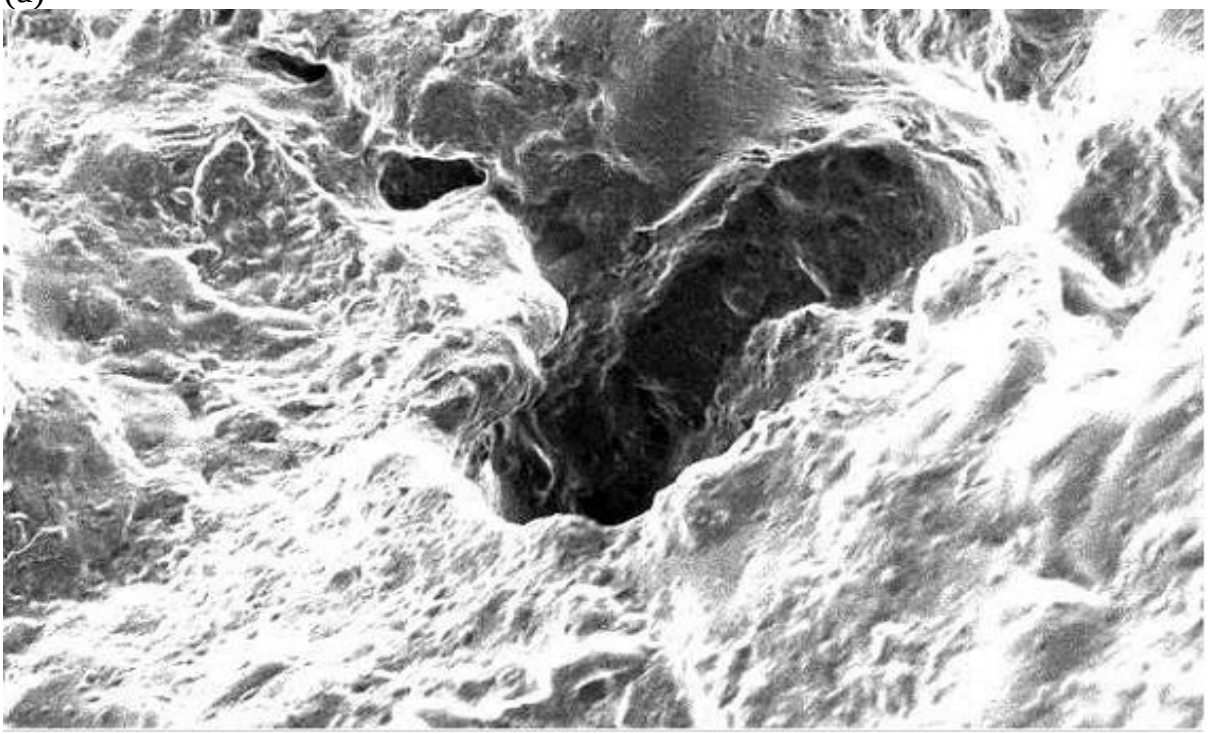
The chickpea flour consists primarily of carbohydrates – which constitutes of sugars (10%), and starch (48%), dietary fibre (10%), proteins (23%) and lipids (7%). The lipids are composed of 10% fatty acids and 22.4% of polyunsaturated fatty acids (linoleic and linolenic acid; monounsaturated fatty acids – elaidic acid). In addition, chickpea is a rich source of minerals – calcium, iron, magnesium, phosphorus, potassium and selenium. The content of vitamins is also significant – vitamin A, B vitamins, vitamin E, folate, and thiamine [133].

1.4 Improving gluten-free bread quality

In the respect of the fact, that gluten is responsible for the viscoelastic properties of bred, 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. Figure 2 shows the different structure of wheat and gluten-free bread.



(a)



(b)

Figure 2. Scanning electron micrographs of wheat bread (a) and gluten-free bread (b). Magnification ×430 [134].

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 [134]. 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 [93]. All studies solving the gluten-free bread quality shows that a different producing technology is required.

The use of additives has recently become common practice in the bakery industry. 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 [135].

The use of hydrocolloids has been increasing in the bakery industry for diverse purposes. Guar gum has been employed for improving the bread volume and texture of frozen dough [136, 137], while the employment of hydroxypropyl methyl cellulose (HPMC) has resulted in soft bread crumb loaves with higher specific bread volume, better sensory characteristics and an extended shelf-life. Similar behaviour has been reported for HPMC when it was studied in the performance of bread stored at sub-zero temperatures [62]. Xanthan gum, HPMC and other hydrocolloids have been tested for their potential as bread improvers and anti/staling agents [64]. It was concluded that all of these hydrocolloids were able to decrease the loss of moisture content during storage and to reduce the dehydration rate, consequently retarding the crumb hardening [68].

The addition of hydrocolloids as binding agents and gluten substitutes in bread made from maize starch has been reported by Acs et al. [138]. In this study, the bread volume and firmness were evaluated to investigate the technological effect of xanthan, guar gum, carob bean gum and tragacanth. The authors showed these agents could be efficiently assigned in substituting the technological effect of gluten in gluten-free systems, resulting in a highly significant increase in bread volume and loosening of the crumb. Regarding the effects of the individual gums, the difference among them was significant, where the highest quality bread was the one containing xanthan gum. Also, in 1997, the use of HPMC was reported to be the most appropriate for best rice bread volume expansion among several gums [139]. This study verified

the feasibility of the application of HPMC, carob bean gum, guar gum, κ-carrageenan, xanthan gum and agar on the improvement of rice bread. Based on these studies' conclusions, eight hydrocolloids with potential to replace gluten network functionality in gluten-free breads were selected.

1.4.1 Agar

Agar is a polysaccharide that accumulates in the cell walls of agarophyte algae (*Gelidium amansii*). It is embedded in a structure of fibres of crystallised cellulose, constituting its polysaccharide reserve. Agar is defined as a strong gelling hydrocolloid from marine algae. Its main structure is chemically characterised by repetitive units of D-galactose and 3,6-anhydro-L-galactose, with few variations, and a low content of sulfate esters. The extraordinary gelling power of agar is based exclusively in the hydrogen bonds formed among its linear galactan chains. Agar is tasteless and cannot be detected in foodstuffs with delicate flavours. In contrast, those gelling agents that need the presence of cations (alginates, calcium or carrageenans, potassium) to gel should be blended with foodstuffs with strong flavours to mask the characteristic flavour [140].

Agar applications are fundamentally based in the enormous gelling power and perfect gel. Although agar has multiple applications, the traditional one is as a food ingredient that accounts for 80% of its consumption [141].

1.4.2 Alginate

Alginates are quite abundant in nature because they are structural components of marine brown algae (*Phaeophyceae*) and capsular polysaccharides in soil bacteria. The sources for industrial production of alginate may be regarded as unlimited even for a steadily growing industry since macroalgae may also be cultivated and since production by fermentation is technically possible. The biological function of alginate in brown algae is as a structure-forming component. The intercellular alginate gel matrix gives the plants both mechanical strength and flexibility [142]. This relation between structure and function is reflected in the compositional difference of alginates in different algae or even between different tissues from the same plant. Alginate is located in the intercellular matrix as a gel containing sodium, calcium, magnesium, strontium and barium ions and it is widely used in industry because of its ability to retain water, and its gelling, viscosifying and stabilising properties.

Commercial alginates are produced mainly from *Laminaria hyperborea*, *Macrocystis pyrifera, Laminaria digitata, Ascophyllum nodosum, Laminaria japonica*, *Eclonia maxima*, *Lessonia nigrescens*, *Durvillea antarctica* and *Sargassum* spp. The ion-binding characteristics of alginates represent the basis for their gelling properties. Dry sodium alginate powder may have a shelf-life of several months provided it is stored in a dry, cool place without exposure

to light. Alginate gels are more or less independent of temperature. The kinetics of the gelling process may, however, be strongly modified by a change in temperature. The properties of the final gel will also change if gelling occurs at different temperatures [143].

Their ability to improve, modify and stabilise the texture of foods represents the basis for applying alginates as food additives, e.g. as a viscosity enhancer, gel former and in the stabilisation of aqueous mixtures, dispersions and emulsions in general. Alginates are also used to control the melting behaviour of ice cream. Most applications are based on the physical properties of alginates themselves but may also result from interactions with other components of the food product, e.g. proteins or fibres [144].

1.4.3 Carob bean gum

Carob bean gum is a type of galactomannans which are multifunctional macromolecular carbohydrates found in various albuminous or endospermic seeds. The seed galactomannans from the carob tree (*Ceratonia siliqua* L.) is widely used. These polysaccharides are strongly hydrophilic, enabling the endosperm to absorb water to protect the embryo against subsequent drought, during and before germination and they become metabolized after germination. The evergreen carob tree can be planted in semi-arid or subtropical zones and grows in calcareous soils. The carob tree can grow as high as 10–15 m and its roots can reach a depth of 25 m. It can live for more than 100 years. The carob tree normally yields fruits after 8–10 years and the fruits, i.e. the pods, can be harvested once a year. The pods are 10–30 cm long, 1.5–3.5 cm wide and 1.0 cm thick. They are dark brown in colour, and straight or curved in shape. The pods contain 8–12 seeds or kernels, but exceptionally up to 15 kernels. The fruits are collected when they have a moisture content of 12–18% [145].

The carob bean gum is a thickening and gelling agent widely used as additive in food products to improve shelf-life by binding water, control the texture, influence crystallization and prevent the retrogradation of starch products. These food additives find applications mainly in convenience food, dairy products, including frozen products (ice cream), soft drinks and fruit juices, bread and pastry, fruit preserves, baby food, and as household gelling agents in puddings, flans and pudding powder, as dietary fibres, and in pet foods [146].

1.4.4 Cellulose

Cellulose is probably the most abundant organic substance existing in nature and it is the major constituent of most land plants. It is the starting material for a wide range of modifications with use in the food industry. Cellulose covers the range of modified celluloses generally approved as food additives. These are methyl cellulose (MC), hydroxypropyl cellulose (HPC),

hydroxypropyl methyl cellulose (HPMC), methyl ethyl cellulose (MEC) and sodium carboxymethyl cellulose (CMC). The common feature of all of these additives is that they are hydrocolloids derived from cellulose raw material by chemical modification.

The raw material for modified celluloses is cellulose tissue, which in turn is produced from wood pulp from specified species. The polymer chain length of cellulose varies with the different raw materials and hence the polymer length and the resultant viscosity required in the final product will govern the selection of the raw material. In general terms, cellulose pulp is dispersed in alkali solution to form alkali cellulose and is then treated with appropriate reagents, under tightly controlled conditions, to substitute the anhydroglucose monomers of the cellulose chain [147].

There are three main factors, which influence the properties of modified celluloses. These are first, and most importantly, the type of substitution of the cellulose, secondly, the average chain length or degree of polymerisation of the cellulose molecules and thirdly, the degree of substitution of the chain. In general, the modified celluloses give neutral-flavoured, odourless and colourless clear solutions. Celluloses are used reformed in vegetable products such as potato croquettes and waffles, onion rings and the whole range of shaped soya protein and similar vegetarian products and in gluten-free bread production [148].

1.4.5 Gelatine

Gelatine is one of the most versatile biopolymers and has numerous applications in food, confectionery, pharmaceutical/medical, cosmetic, and technical products. Gelatine has been investigated and studied by scientists at least since the early twentieth century but was used in foods even before this. Gelatines are derived from the protein collagen and the origin of the parent collagen and the severity of the extraction procedures determine the properties of the final gelatine. Today gelatines are mainly produced from bovine and porcine sources, but gelatine may also be extracted from fish and poultry. Collagen is the major constituent of all white fibrous connective tissues occurring in animal bodies surrounding muscles and muscle fibres, skin and ossein (the protein matrix of bone). While collagen is insoluble in water, gelatine is easily dissolved in water upon heating to temperatures above the denaturing temperature of the native collagen [149].

For gelatine production the raw material may be any collagen-containing tissue. Hides, skins and bones from mammalian sources such as porcine and bovine are preferred, but gelatines are also produced from the skins of cold and warm water fish species. The manufacturing process involves cleaning of the source tissues followed by pre-treatment, extraction of gelatine, filtration, purification, sterilization, concentration, drying and finally milling.

The food industry is still one of the major consumers of gelatines. Gelatine desserts, all types of jellies, are examples of food products that take advantage of the thermo-reversible gel formation [150].

1.4.6 κ-Carrageenan

Red seaweeds contain naturally occurring polysaccharides which fill the pores within the cellulose structure of the plant. This family of polysaccharides includes carrageenan and furcellaran. Gels are produced by heating and cooling solutions of these polysaccharides to give soft, elastic gels with iota-carrageenan and firm, brittle gels with kappa-carrageenan and furcellaran. Carrageenan is utilised for water dessert gels and glazes. In addition, carrageenan interacts with protein to stabilise, thicken and gel. As a consequence of the regular helical conformation adopted when kappa-carrageenan solutions are cooled, the chains are able to interact synergistically with other gums, such as carob bean gum and konjac mannan, to further modify the gel texture [151].

The main species of *Rhodophyceae* used in the commercial production of carrageenan include *Eucheuma cottonii* and *E. spinosum*, now reclassified as *Kappaphycus alverezii* and *Eucheuma denticulatum*. These are spiny bushy plants, about 50 cm high, which grow on reefs and in shallow lagoons around the Philippines and Indonesia and other island coasts in the Far East. *K. alverezii* yields kappa-carrageenan and *E. denticulatum* contains iota-carrageenan.

All carrageenans are soluble in hot water but only the sodium salts of kappa and iota are soluble in cold water. Hot solutions of kappa and iota-carrageenans set to give a range of gel textures when cooled to 40 to 70 °C. Carrageenans able to hydrate at low temperatures present problems for their efficient use. Any lumps which are produced when the carrageenan is dispersed in water greatly reduce the rate of hydration and may limit the development of full viscosity or gel strength [152].

1.4.7 Tragacanth

Although gum arabic is by far the most important plant exudate hydrocolloid, there are other related gums that have retained their economic and technological importance for centuries despite the availability of several new alternative industrial hydrocolloids. In fact, natural plant gums are the most widely used and traded non-wood forest products other than items consumed directly as food. Gums are secreted by the bark of trees in the form of tear-like, striated nodules or amorphous lumps, which are vitrified upon drying, thus forming hard, glassy lumps (gum karaya and mesquite gum) or tough thin ribbons (gum tragacanth) of different colours. In general, the gums are produced by the stem under conditions of heat and drought stress, partly as a natural phenomenon (as part of the normal metabolism of plants) and partly as a result of injury to the bark or stem (due to fungal or bacterial attack) by a process

known as gummosis. Chemically, these materials are known to be comprised to varying extents either by arabinogalactan (AG) hetero polysaccharides or mixtures of galacturonan regions and type II AG as gum tragacanth [153]. The gum is obtained from small shrubs of the *Astragalus* genus, comprising up to 2000 species indigenous to mountain areas of south west Asia from Pakistan to Greece. *Astragalus gummifer* was considered the main tragacanth yielding species, but a field survey established that *A. microcephalus* was the principal source of the gum. The plants are small, low bushy perennial shrubs having a large tap root along with branches. The root and lower stem are tapped for gum. The gum is obtained in two basic physical forms, namely ribbons (superior quality) and flakes (inferior quality). These two forms are obtained from different sub-species of the shrub.

It has many industrial uses (arts, foods, pharmacy) because of its bland flavour and mucilaginous qualities and stability to heat and acids. Another important characteristic of tragacanth is its bifunctional action as an emulsifier that increases the viscosity of the aqueous phase and lowers the interfacial tension between oil-water emulsions [154].

1.4.8 Xanthan gum

Xanthan gum is an extracellular polysaccharide secreted by the micro-organism *Xanthomonas campestris*. Xanthan gum is soluble in cold water and solutions exhibit highly pseudoplastic flow. Its viscosity has excellent stability over a wide pH and temperature range and the polysaccharide is resistant to enzymatic degradation. Xanthan gum exhibits a synergistic interaction with galactomannans such as carob bean gum. This results in enhanced viscosity with guar gum and soft, elastic thermally reversible gel.

The bacterium *Xanthomonas campestris* produces the polysaccharide at the cell wall surface during its normal life cycle by a complex enzymatic process. The bacteria are found naturally on the leaves of the *Brasica* vegetables such as cabbage. Commercially, xanthan is produced from a pure culture of the bacterium by an aerobic, submerged fermentation process. When the final fermentation has finished, the broth is pasteurised to kill the bacteria and the xanthan gum is recovered by precipitation with isopropyl alcohol or ethanol. Finally, the product is dried, milled and packaged [155].

To obtain the optimum functionality, xanthan gum must be properly hydrated before use. Hydration depends on dispersion, agitation rate of the solvent, composition of the solvent, particle size. To hydrate properly the gum particles must be well dispersed. Poor dispersion leads to clumping of particles during mixing which results in formation of partially swollen lumps of gum [156].

Xanthan gum contributes to the smoothness, air incorporation and retention of batters for cakes, muffins, biscuits and bread mixes. In wet prepared batters, xanthan gum reduces flour sedimentation and improves gas retention. Baked

goods have increased volume and moisture, higher crumb strength, less crumbling and greater resistance to transport damage. Xanthan gum improves volume, texture and moisture retention in gluten-free breads [157].

2. 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.

3. METHODS

3.1 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 with rice flour. Table 3 shows the samples of gluten-free flours and their mixtures and Table 4 presents samples with hydrocolloids and hydrocolloid blends.

Table 3: Gluten-free flours and flour mixtures

	Proportion (%)					
Sample	**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 4: Rice flour with hydrocolloids

	Proportion (%)						
Rice	**Agar**	**Alginate**	**Carob gum**	**Carrageenan**	**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

3.2 Methods

3.2.1 Phases of the dissertation

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.

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.

3.2.2 Water absorption

This standard ISO 5530-1 [158] 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. Further mixings as necessary were made, until two mixings were available with the maximum consistencies between 480 and 520 FU. Then the correct volume V_c in millilitres, of water corresponding to a maximum consistency of 500 FU was calculated:

$$V_c = V + 0.096(C - 500)$$

where v is the volume, in millilitres, of water added;
c is the maximum consistency, in FU, given by

$$C = \frac{c_1 + c_2}{2}$$

where c_1 is the maximum height of the upper contour of the curve, in F;
c_2 is the maximum height of the lower contour of the curve, in FU.

The farinograph water absorption, expressed in millilitres per 100 g of flour at 14% (m/m) moisture content, is equal to:

$$(V_c + m - 300) \times \frac{1}{3}$$

where V_c is the mean value of the duplicate determinations of the corrected volume, in millilitres, of water corresponding to a maximum consistency of 500 FU;
m is the mass, in grams, of the test portion.

3.2.3 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 [159]. High speed dough mixing and a short fermentation time are typical of this method. 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. Bread loaves were evaluated in relation to yield (dough and bread), baking loss, specific volume (ratio of bread volume and weight in $cm^3\ g^{-1}$).

Solution preparation

Solution A (saccharose, salt, and ascorbic acid solution): 15 ± 0.5 g salt, 15 ± 0.5 g saccharose, and 0.05 ± 0.002 g ascorbic acid dissolved in 400 ± 5 g of water. This amount got along 3 tests and was fresh prepared.

Solution B (saccharose solution to yeast reactivation): 5.0 ± 1.0 g dissolved in 95.0 ± 10 g of water. This amount got along 3 tests and was fresh prepared.

Yeast reactivation

Adjusted solution B to 35 ± 1 °C. 5.4 g of dried yeast was added to 21.6 g of the solution B. With a slight hand movement motion, the solution dewed all the yeast. It was not mixed. The suspension stayed for 10 ± 1 minute, then blended properly until a homogenous suspension was formed. The suspension was used in max. 5 minutes.

The flour, solutions, and water temperature were adjusted in order to the final dough temperature (after kneading) was 27 ± 1 °C. If the flour was room temperature it was necessary to cool the liquid components under 10 °C.
From prepared 129 ± 5 g of the solution A, according to the farinographic water absorption the water necessary to prepare the dough of an appropriate consistency was reckoned:

$$D = W - 140.5$$

where *D* is the volume, in millilitres, of water needed;
W is the volume, in millilitres, of water added according to farinographic water absorption;
140.5 is the water volume added in solutions A and B. The yeast suspension contained 20.5 ml and the solution B was added in amount 129 g and contained 120 ml of water, that was 140.5 ml of water in total.

The flour was put into the kneader, kneaded separately for 2 minutes, then the liquid components added and after 30 s the dough parts cleaned of the mixing vessel and mixed for an appropriate time. The dough temperature was measure and if it was higher than 28 °C, the experiment was repeated until the temperature was between 26–28 °C. The dough was divided in three uniform parts:

$$d = Q/3$$

where *d* is the weight of each part;
Q is the overall weight of the dough.

The parts were put into the shapes and the shapes inserted in a proofer (30 °C) for 30 ± 2 min. The loaves were baked until it was done. Immediately after baking the loaves were put out of the shapes and let to cool down at the room temperature.

Evaluating the baking test:

The brad loaves were evaluated in terms of specific volume, dough yield, bread yield, and baking loss 20 ± 4 h after baking.

Loaf specific volume:

The loaves were weighted and the loaves volume (in cm^3) determined using raduated vessel filled with the rape seeds. Then the specific volume (in $cm^3\ g^{-1}$) was calculated as:

$$V_s = V / m$$

where *V* is volume, in cm^3, of the bread loaves;
m is weight, in grams, of the bread loaves.

Dough yield:

Dough yield was determined by the dough and flour weight:

$$x = \frac{m_d}{m_f} \cdot 100[\%]$$

where m_d is the dough weight, in grams;
m_f is the flour weight, in grams.

Bread yield:

Bread yield was determined by the bread and flour weight:

$$x = \frac{m_p}{m_f} \cdot 100[\%]$$

where m_p is the bread weight, in grams;
m_f is the flour weight, in grams.

Baking loss:

Baking loss was determined by the dough and bread weight:

$$x = \frac{m_d - m_p}{m_d} \cdot 100[\%]$$

where m_d is the dough weight, in grams;
m_p is the bread weight, in grams.

3.2.4 Bread texture parameters

Texture analysis of bread crumb was performed with cylinder of 2.5 cm diameter and 2.0 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 5 cm 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 ExponentLite software.

3.2.5 Moisture content

Moisture content was determined using a drying method at 130 °C for 90 min according to CSN 56 0116-3 [160]. 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 was 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].

3.2.6 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 9.1 software (StatSoft, CR, Ltd). The purpose of analysis of variance is to test for significant differences between means [161]. The differences were tested on $\alpha = 0.05$ significance level using Fisher LSD test.

4. RESULTS AND DISCUSSION

4.1 Water absorption

The farinograph measures a dough consistency during its formation. The gluten-free flours provided various curves in several repetitions as it was difficult to reach the consistency of 500 FU. The water absorptions values and development time of selected gluten-free flours are summarized in the Table 5.

Table 5: Water absorption and development time of gluten-free flours

Sample	Water absorption [%]	Development time [min]
Amaranth	62.3	2.0
Buckwheat	58.8	14.0
Chickpea	50.3	9.9
Millet	did not reach 500 FU	20.0
Quinoa	64.1	1.7
Rice	57.4	13.7

When compared to the wheat water absorption (58%) and development time (2 min) published by Sivaramakrishnan et al. [43] the gluten-free flours reached similar results but the development time was very long for buckwheat and rice. This long development time was later confirmed by Lazaridou et al. [65] who also studied the rheological characteristics of rice flour compared to gluten-free formulations.

4.2 Quality of gluten-free bread 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, 162].

The quality parameters of gluten-free breads are summarized in Table 6.

Table 6: 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^e	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 $p < 0.05$

4.2.1 Baking test

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 $cm^3 g^{-1}$ (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. [163] and Figure 3 presented in the article shows the digital images of bread crumbs presented in. To imagine the differences in the porosity, the most frequented material for gluten-free bread production were recorded and compared to wheat bread (w). The digital images reveal that the only satisfactory porosity had amaranth, buckwheat and rice sample compared to the wheat sample. It is evident that remaining samples, especially quinoa bread was almost without pores, very dry and dense.

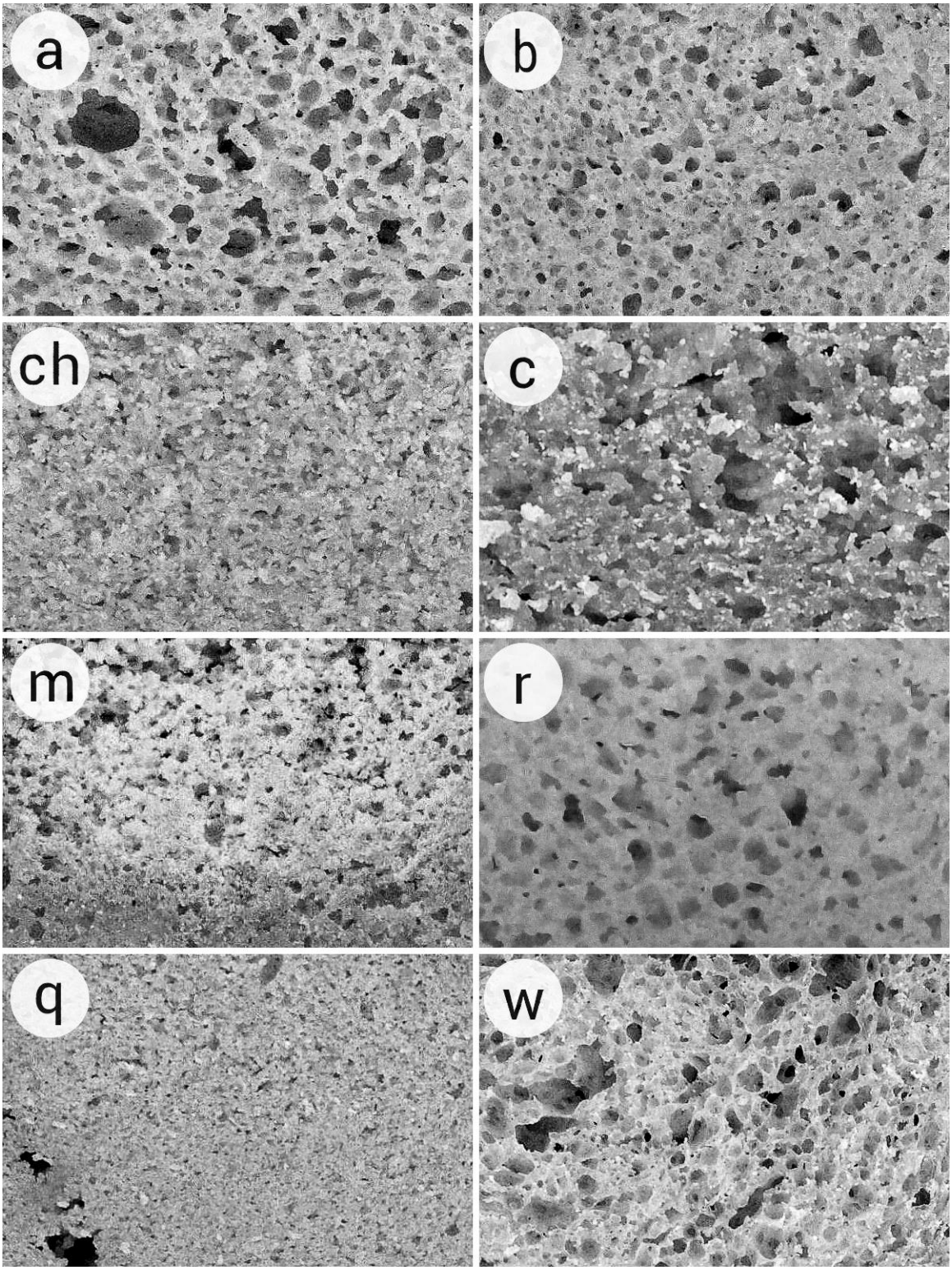


Figure 3: Differences in crumb porosity of gluten-free breads
a: amaranth. b: buckwheat. c: corn. ch: chickpea. q: quinoa. m: millet. r: rice. w: wheat.

4.2.2 Hardness

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. [164] (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).

4.3 Quality of two-component flour gluten-free bread

All two-component flour blends included 50% of rice flour and 50% of amaranth, buckwheat, chickpea, millet and quinoa flour. The rice bread was selected as a check sample. Table 7 reveals significant differences among results of baking test and hardness measured 24 h after baking.

Table 7: Average values of two-component gluten-free bread characteristics*

Sample	Loaf specific volume ($cm^3 g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)	Hardness (N)
R-A	1.680 ± 0.013^{a}	184.6 ± 0.7^{a}	148.8 ± 0.2^{a}	19.4 ± 1.3^{a}	14.2 ± 0.8^{a}
R-B	1.996 ± 0.025^{b}	196.9 ± 1.5^{b}	151.1 ± 0.7^{c}	23.2 ± 0.2^{b}	18.0 ± 1.5^{b}
R-Ch	1.687 ± 0.014^{a}	185.8 ± 0.8^{a}	142.3 ± 0.4^{b}	23.4 ± 0.5^{b}	47.0 ± 1.8^{d}
R-M	1.709 ± 0.010^{a}	198.2 ± 0.4^{bc}	147.2 ± 0.1^{a}	25.7 ± 1.3^{c}	28.8 ± 2.4^{c}
R-Q	1.699 ± 0.007^{a}	200.0 ± 0.5^{c}	161.7 ± 0.5^{d}	19.1 ± 0.3^{a}	15.7 ± 1.0^{ab}
Rice	1.716 ± 0.003^{a}	204.0 ± 0.1^{d}	166.2 ± 0.2^{e}	18.5 ± 0.4^{a}	13.9 ± 1.2^{a}

*Values in one column with different letters are significantly different $p < 0.05$
R: rice. A: amaranth. B: buckwheat. Ch: chickpea. M: millet. Q: quinoa.

4.3.1 Baking test

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. [165] 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 balking loss. Miñaro et al. [166] 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.

4.3.2 Hardness

The combinations of rice with amaranth, buckwheat and quinoa gave almost identical values of crumb hardness (from 13.9 N for rice to 18.0 N for rice-buckwheat bread). Elgeti et al. [167] studied the effect of quinoa addition to the gluten-free formula and found the improving effect with increasing portion of quinoa in the bread. Unlike rice-millet and rice-chickpea samples in our research which significantly worsened this parameter reaching 28 N and 47 N. Alvarez-Jubete et al. [32] tested the technological quality of gluten-free breads with 50% addition of amaranth, quinoa and buckwheat into the formula based on rice flour but he found all samples had softer crumb than the check sample. These differences between our results can probably be caused by different formula and technology.

It can be concluded that the best two-component combination was evaluated rice with buckwheat flour with no specific aroma, satisfactory crumb porosity and acceptable technological parameters and the same concluded Burešová [164].

4.4 Quality of three-component flour 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).

Table 8 shows statistically evaluated results of baking test and hardness 24 h after baking.

Table 8: Average values of three-component gluten-free bread characteristics*

Sample	Loaf specific volume ($cm^3 g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)	Hardness (N)
R-A-B	1.772 ± 0.012^{c}	193.3 ± 0.7^{be}	158.0 ± 0.5^{c}	18.3 ± 1.4^{a}	11.7 ± 0.9^{a}
R-A-Ch	1.785 ± 0.011^{c}	186.9 ± 1.6^{cd}	147.9 ± 1.2^{ab}	20.9 ± 0.9^{b}	24.0 ± 2.0^{d}
R-A-M	1.744 ± 0.009^{b}	190.3 ± 0.6^{ab}	155.8 ± 0.6^{cd}	18.1 ± 1.9^{a}	19.1 ± 0.9^{c}
R-A-Q	1.621 ± 0.027^{a}	199.3 ± 1.5^{f}	164.6 ± 0.8^{e}	17.4 ± 1.4^{a}	15.9 ± 1.0^{b}
R-B-Ch	1.827 ± 0.013^{c}	190.2 ± 0.8^{ab}	148.7 ± 1.0^{ab}	21.8 ± 2.8^{b}	30.4 ± 1.2^{e}
R-B-M	1.783 ± 0.013^{c}	191.3 ± 0.4^{ab}	149.5 ± 0.4^{a}	21.9 ± 1.0^{b}	34.7 ± 2.5^{fg}
R-B-Q	2.399 ± 0.035^{d}	194.9 ± 0.7^{e}	154.2 ± 0.2^{d}	20.9 ± 0.4^{b}	32.5 ± 2.5^{ef}
R-Ch-M	1.787 ± 0.029^{c}	184.9 ± 2.5^{c}	150.1 ± 0.9^{a}	18.8 ± 1.3^{a}	37.1 ± 1.5^{g}
R-Ch-Q	1.735 ± 0.009^{b}	190.9 ± 1.2^{ab}	157.5 ± 0.8^{c}	17.5 ± 0.9^{a}	26.1 ± 1.1^{d}
R-M-Q	1.641 ± 0.015^{a}	188.1 ± 0.8^{ad}	146.3 ± 0.6^{b}	22.2 ± 2.4^{b}	45.0 ± 2.1^{h}
Rice	1.716 ± 0.003^{b}	204.0 ± 0.1^{g}	166.2 ± 0.1^{e}	18.6 ± 0.4^{a}	13.9 ± 1.2^{ab}

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

R: rice. A: amaranth. B: buckwheat. Ch: chickpea. M: millet. Q: quinoa.

4.4.1 Baking test

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 cm^3 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.

4.4.2 Hardness

Unlike the loaf specific volume, the values of hardness 24 h after baking at the samples containing buckwheat flour showed rather significant worsening effect (values approximately 30 N and more) in comparison with rice check sample (13.9 N). The addition of amaranth and quinoa flour in the mixture gave significantly softer samples compared to the other mixtures. The same trend described Alencar et al. [168] in her study of addition of quinoa and amaranth in gluten-free breads. These variable effects were probably caused by the component interactions of used flours.

4.5 Quality of buckwheat-rice gluten-free bread

Since the buckwheat-rice 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 [169] could reach satisfactory results summarized in Table 9, where buckwheat-rice blend is abbreviated as BR.

Table 9: Average values of buckwheat-rice gluten-free bread characteristics*

Sample	Loaf specific volume ($cm^3 g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)	Hardness 24 h (N)	Hardness 72 h (N)
Rice	1.716 ± 0.003^{d}	204.0 ± 0.1^{f}	166.2 ± 0.1^{g}	18.6 ± 0.4^{e}	13.9 ± 1.2^{a}	27.0 ± 2.3^{a}
BR 1090	1.100 ± 0.081^{c}	170.6 ± 0.2^{a}	152.1 ± 0.5^{ab}	10.8 ± 0.8^{bc}	14.2 ± 0.3^{ab}	28.1 ± 0.4^{ab}
BR 2080	1.101 ± 0.011^{c}	170.3 ± 0.5^{a}	152.8 ± 0.8^{a}	10.3 ± 1.2^{b}	16.5 ± 0.5^{c}	29.9 ± 1.0^{bcd}
BR 3070	1.302 ± 0.082^{a}	170.5 ± 0.4^{a}	152.3 ± 0.2^{ab}	10.7 ± 0.1^{bc}	15.8 ± 1.3^{bc}	31.2 ± 0.8^{bcde}
BR 4060	1.300 ± 0.071^{a}	171.4 ± 0.4^{ac}	151.5 ± 0.2^{b}	11.6 ± 0.5^{d}	17.1 ± 0.2^{cd}	28.9 ± 0.2^{abc}
BR 5050	1.376 ± 0.047^{b}	173.2 ± 0.2^{cd}	150.2 ± 0.1^{e}	13.3 ± 0.3^{a}	18.5 ± 0.5^{de}	32.4 ± 0.8^{de}
BR 6040	1.400 ± 0.012^{b}	175.3 ± 0.1^{b}	153.2 ± 0.3^{ac}	12.6 ± 0.7^{a}	20.9 ± 1.2^{ef}	31.5 ± 1.7^{e}
BR 7030	1.467 ± 0.094^{e}	175.2 ± 0.4^{b}	152.1 ± 0.2^{ab}	13.2 ± 0.1^{a}	27.3 ± 0.4^{g}	41.7 ± 1.0^{f}
BR 8020	1.401 ± 0.062^{b}	176.5 ± 0.2^{b}	154.1 ± 0.2^{cd}	12.7 ± 0.4^{a}	28.7 ± 1.1^{g}	42.2 ± 1.7^{fg}
BR 9010	1.367 ± 0.094^{b}	175.1 ± 0.4^{bd}	155.2 ± 0.1^{d}	11.3 ± 0.2^{cd}	30.9 ± 0.6^{h}	44.8 ± 0.9^{gh}
Buckwheat	1.671 ± 0.015^{d}	193.2 ± 1.8^{e}	157.6 ± 0.4^{f}	18.4 ± 2.6^{e}	30.9 ± 1.1^{h}	45.2 ± 1.7^{h}

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

B: buckwheat. R: rice. BR 1090: 10% buckwheat flour and 90% rice flour *etc*.

4.5.1 Baking test

In case of loaf specific volume, the best result presented the samples of clear buckwheat and rice flour (1.671 and 1.716 $cm^3 g^{-1}$). 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 $cm^3 g^{-1}$ (BR 1090) to 1.467 $cm^3 g^{-1}$ (BR 7030). Very similar conclusion presented Wronkovska et al. [169] 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.

4.5.2 Hardness

Even if the buckwheat sample showed the best volume, it currently had significantly worst impact on crumb hardness 24 and 72 hours after baking (30.9 and 45.2 N). The best results of crumb hardness (13.9 and 27.0 N) presented the clear rice sample. The significantly different results with increasing trend of both, hardness 24 and 72 h after baking can be observed among the buckwheat and rice blends. The bigger portion of buckwheat flour, the higher value of hardness ranging from 14.2 and 28.7 N for BR 1090 to 30.9 and 44.8 N for BR 9010. It can be concluded that increasing amount of buckwheat flour in the blend linearly increased the samples hardness which confirmed Torbica et al. [37] who studied the effect of increasing amount of buckwheat flour up to 30%. Unlike Wronkovska et al. [169] who found the opposite effect, but in the starch-based breads.

Finally, the sample with 40% buckwheat and 60% rice flour was evaluated as the best. It reached acceptable baking quality and hardness and the portion of buckwheat flour did not negatively affect consumer preferences, on the contrary enhanced the taste in comparison with the rice sample. These results were published and presented in Nitra [170].

4.6 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.

Baking quality, hardness 24 and 72 h, moisture content 24 and 72 h after baking and staling at the constant conditions were tested and the results are presented in the Tables 10 and 11.

4.6.1 Baking Test

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 than 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 cm^3 g^{-1}) presented the samples with 0.5% alginate, carob gum and sodium hydroxypropyl methyl cellulose. In his next study [171], 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 cm^3 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 [172] 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.

Table 10: Average values of rice bread characteristics prepared with specific hydrocolloids 0.5 and 1.0% (w/w, flour basis)*

Sample	Loaf specific volume ($cm^3 g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)
R-Agar 0.5%	1.639 ± 0.014^{cd}	185.4 ± 1.2^{de}	158.6 ± 0.6^{d}	14.4 ± 0.4^{g}
R-Agar 1.0%	1.532 ± 0.005^{bc}	175.1 ± 1.3^{b}	152.3 ± 0.3^{c}	13.1 ± 1.5^{bc}
R-Alg 0.5%	1.782 ± 0.002^{e}	193.2 ± 2.0^{hi}	164.6 ± 0.1^{fg}	14.8 ± 2.0^{g}
R-Alg 1.0%	1.656 ± 0.008^{d}	192.9 ± 1.5^{hi}	165.0 ± 0.1^{fgh}	14.4 ± 2.0^{efg}
R-CG 0.5%	1.762 ± 0.004^{e}	173.0 ± 1.5^{b}	149.4 ± 0.3^{b}	13.6 ± 1.1^{cdef}
R-CG 1.0%	1.396 ± 0.010^{ab}	168.4 ± 0.4^{a}	145.7 ± 0.4^{a}	13.5 ± 1.3^{cde}
R-Carrag 0.5%	1.523 ± 0.015^{bc}	183.9 ± 2.3^{cd}	159.8 ± 1.7^{de}	13.1 ± 1.0^{bc}
R-Carrag 1.0%	1.479 ± 0.018^{bc}	182.5 ± 0.9^{c}	160.1 ± 0.5^{de}	12.3 ± 0.4^{ab}
R-Cel 0.5%	1.764 ± 0.003^{e}	187.5 ± 0.8^{ef}	159.8 ± 0.2^{de}	14.8 ± 0.7^{g}
R-Cel 1.0%	1.710 ± 0.007^{d}	191.7 ± 1.1^{gh}	164.3 ± 0.4^{fg}	14.3 ± 1.2^{defg}
R-Gel 0.5%	1.621 ± 0.016^{cd}	193.4 ± 1.1^{hi}	167.6 ± 0.1^{h}	13.3 ± 1.1^{cd}
R-Gel 1.0%	1.550 ± 0.005^{cd}	192.0 ± 1.0^{ghi}	167.7 ± 0.1^{h}	12.6 ± 0.9^{abc}
R-Trag 0.5%	1.611 ± 0.009^{cd}	194.5 ± 1.1^{i}	166.3 ± 0.01^{gh}	14.5 ± 1.3^{fg}
R-Trag 1.0%	1.579 ± 0.001^{cd}	193.1 ± 2.0^{hi}	164.7 ± 0.1^{fg}	14.7 ± 2.5^{g}
R-XG 0.5%	1.589 ± 0.004^{cd}	184.5 ± 0.4^{cd}	162.5 ± 0.3^{ef}	11.9 ± 0.2^{a}
R-XG 1.0%	1.400 ± 0.018^{ab}	189.7 ± 2.0^{fg}	165.5 ± 0.1^{gh}	12.8 ± 2.1^{abc}
Rice	1.716 ± 0.003^{d}	204.0 ± 0.1^{j}	166.2 ± 0.1^{gh}	18.6 ± 0.4^{h}

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

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

Table 11: Average values of rice bread hardness and moisture content prepared with specific hydrocolloids 0.5 and 1.0% (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
R-Agar 0.5%	13.9 ± 0.5^{ab}	35.8 ± 4.1^{d}	53.1 ± 0.4^{uvwxy}	51.9 ± 0.4^{mno}
R-Agar 1.0%	33.4 ± 1.6^{e}	95.7 ± 6.2^{g}	50.4 ± 0.3^{defgh}	49.6 ± 0.1^{bcdefgh}
R-Alg 0.5%	15.9 ± 0.8^{abc}	18.7 ± 1.2^{abc}	$54.3 \pm 0.1^{b'c'd'e'f'}$	53.8 ± 0.4^{pqr}
R-Alg 1.0%	18.6 ± 0.9^{cd}	18.6 ± 0.9^{abc}	$54.3 \pm 0.1^{a'b'c'd'e'f'}$	54.8 ± 0.3^{r}
R-CG 0.5%	22.1 ± 1.8^{d}	74.9 ± 1.7^{ef}	49.3 ± 0.3^{abc}	48.3 ± 0.2^{ab}
R-CG 1.0%	36.9 ± 1.8^{e}	68.7 ± 2.6^{e}	50.6 ± 0.4^{efghi}	$49.7 \pm 0.1^{bcdefghijk}$
R-Carrag 0.5%	33.2 ± 5.8^{e}	79.7 ± 8.2^{f}	$51.8 \pm 0.2^{jklmnopqrst}$	50.9 ± 0.4^{ghijklm}
R-Carrag 1.0%	46.4 ± 0.4^{f}	97.8 ± 9.9^{g}	$51.8 \pm 0.1^{jklmnopqrs}$	50.6 ± 0.2^{fgh}
R-Cel 0.5%	13.0 ± 0.8^{a}	16.4 ± 1.6^{a}	$54.3 \pm 0.1^{a'b'c'd'e'f''}$	54.2 ± 0.5^{pqr}
R-Cel 1.0%	14.4 ± 0.5^{ab}	18.7 ± 1.7^{abc}	$53.7 \pm 0.1^{yza'b'c'}$	54.2 ± 0.1^{pqr}
R-Gel 0.5%	15.7 ± 1.5^{abc}	23.6 ± 2.6^{abc}	$52.2 \pm 0.1^{lmnopqrstuv}$	48.8 ± 4.2^{abcd}
R-Gel 1.0%	14.8 ± 1.4^{ab}	25.2 ± 1.8^{abc}	$52.2 \pm 0.1^{mnopqrstuv}$	52.8 ± 0.1^{nop}
R-Trag 0.5%	14.9 ± 0.5^{ab}	17.8 ± 1.0^{ab}	$54.2 \pm 0.1^{yza'b'c'd'e'f'}$	54.3 ± 0.5^{pqr}
R-Trag 1.0%	13.3 ± 1.2^{ab}	16.5 ± 0.3^{a}	$54.7 \pm 0.2^{c'-d'e'f'g'}$	54.6 ± 0.1^{qr}
R-XG 0.5%	16.7 ± 1.0^{bc}	23.4 ± 0.3^{abc}	$52.2 \pm 0.4^{klmnopqrstu}$	53.2 ± 0.3^{opq}
R-XG 1.0%	19.1 ± 1.1^{cd}	27.8 ± 2.1^{cd}	53.2 ± 0.2^{vwxyz}	54.0 ± 0.1^{pqr}
Rice	13.9 ± 1.2^{ab}	27.0 ± 2.3^{bcd}	$54.8 \pm 0.2^{c'd'e'f'g'}$	54.8 ± 0.1^{r}

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

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

He also presented the decrease of loaf specific volume with increased portion of xanthan gum in the sample that is in agreement with our results – we did not find any evidence of higher improvement of the loaf specific volume with higher portion of hydrocolloid in the sample. On contrary Demirkesen et al. [18] in his study with xanthan gum, guar gum and hydroxypropyl methyl cellulose found that all used hydrocolloids increased the loaf bread volume. The loaf specific volume of the samples with remaining hydrocolloids varied from 1.523 to 1.710 $cm^3 g^{-1}$.

The differences among our results and the results referred in the literature were probably caused by the different formulations and technological process used in our research and other studies.

Any of the samples did not approach the dough yield of the rice check sample (204.0%) and the closest results were measured for the sample with tragacanth 0.5% (194.5%), alginate 0.5% (193.2%) and tragacanth 1.0% (193.1%), contrarywise, the worst dough yield showed the sample with 1.0% carob gum (168.4%) that also proved the worst bread yield (145.7%). The best result, even better then rice check sample was observed for gelatine 1.0% (167.7%) and gelatine 0.5% (167.6%), but these differences were not statistically significant in comparison with the rice sample (166.2%). Remaining bread yield ranged from 149.4 to 166.3%. Contrary to our results, Mohammadi et al. [173] found the increasing dough and bread yield using xanthan gum and cellulose compared to check sample without hydrocolloids.

All hydrocolloids in all portions in the sample enhanced the baking loss. Statistically significant best result was found for xanthan gum 0.5% that decreased the baking loss by 36% (from 18.6% for rice flour to 11.9% for rice flour with xanthan gum). None of the hydrocolloids reached as high baking loss as the rice check sample and all the results were significantly different.

4.6.2 Hardness 24 and 72 hours after baking

Hydrocolloids are applied to gluten-free products to improve their shelf life by keeping the moisture content and slowing the staling that is closely related to the samples' hardness. In our research it was found that the only better hardness results (24 h after baking), but not significantly different, compared to the rice check sample presented cellulose 0.5% (13.0 N) and tragacanth 1.0% (13.3 N). This improving effect of cellulose proved the research of Sciarini et al. [9] and Sciarini et al. [171] who also referred that alginate, carrageenan and gelatine had rather deteriorating effect. The worst influence on the hardness 24 h was observed for the sample with carrageenan 0.5% (33.2 N), agar 1.0% (33.4 N), carob gum 1.0% (39.9 N) and 1.0% of carrageenan (46.4 N). Confirmed in the study by Hager and Arendt [172] the addition of xanthan gum 0.5% did not significantly affect the hardness (16.7 N) compared to the rice check sample (13.9 N). Other samples' hardness varied from 13.9 to 22.1 N

that is relatively closed to the rice check sample. Softer crumbs were related to the higher loaf specific volume.

The smallest crumb hardness 72 h after baking was measured for cellulose 0.5% (16.4 N) which is nearly 40% less in comparison with the rice check sample. The samples with alginate (0.5 and 1.0%), cellulose (0.5 and 1.0%), gelatine (0.5 and 1.0%), tragacanth (0.5 and 1.0%) and xanthan gum (0.5 and 1.0%) also achieved very satisfactory hardness results 72 h after baking as the deterioration of the hardness (comparing with hardness 24 h) was less than 50%. Other samples presented worsening more than 55% within staling.

The reason for keeping satisfactory softness after certain staling time is the water retention which causes higher moisture content thus retarding the starch retrogradation and firming. Another effect of hydrocolloids is their possibility to weaken the starch structure, leading to better water distribution and a decrease in the crumb stiffness. Hydrocolloids affect the retrogradation by limiting both the diffusion and loss of water from bread crumb, indicating that the control of water content and its mobility may be the key factors controlling crumb firmness [173].

4.6.3 Moisture content 24 and 72 hours after baking

Bread staling is a very complex process that cannot be explained by a single effect (starch retrogradation, polymers reorganization, water distribution, crumb structure *etc*.), but the moisture content and moisture transfer between bread components is believed to be important factor contributing to bread staling [174].

The presence of hydrocolloid in all samples decreased the moisture content in comparison with the rice check sample, but the results were so close that the statistical evaluation and significant differences were not very clear. Surprisingly the best result was measured for the rice check sample without hydrocolloid and this value 54.8% stayed the same also 72 h hours after baking unlike the samples with hydrocolloid that registered slight deterioration 72 h after baking. Brennan et al. [175] reported that xanthan gum is able to stabilise starch gels thus reduces the starch retrogradations that is probably valid for the hydrocolloids generally and it is the reason for keeping the samples' moisture content. The same results presented Mohammadi et al. [173] who studied the effect of xanthan gum and carboxymethyl cellulose on gluten-free flat bread. He confirmed that both hydrocolloids are good anti-staling agents already in the amount of 0.5%.

Texture and moisture analysis showed that breads, which retained more moisture, had lower hardness value which is in agreement with study of Demirkesen [176] who also tested the starch retrogradation and concluded that these results indicate that migration of water has dominant role on crumb firmness increase as the role of starch.

4.7 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. [177] 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.

4.7.1 Agar blends

Tables 12 and 13 show average values of rice bread quality characteristics prepared with agar blends. Agar was mixed with the rest seven hydrocolloids.

Baking Test

The best result of the loaf specific volume (1.896 cm^3 g^{-1}) was measured for the combination agar-cellulose in proportion of 0.5% to flour weight and this was with agar-tragacanth 0.5% (1.784 cm^3 g^{-1}) the only better result comparing the rice check sample (1.716 cm^3 g^{-1}). The worst sample – agar-xanthan gum 1.0% reached 1.278 cm^3 g^{-1} and significant differences among the samples were found. Even if the agar-cellulose 0.5% combination proved worse dough yield (177.9%; rice – 204.0%), it had acceptable bread yield (160.0%; rice 166.2%) and best baking loss (10.0%; rice 18.6%). Very similar satisfactory results presented the agar-tragacanth combination in the proportion of 0.5% – 177.9% for dough yield, 160.7% for bread yield and 11.8% for baking loss. Remaining samples did not have satisfactory loaf bread volume – ranging from 1.3 to max. 1.6 cm^3 g^{-1} which is very important parameter for the customers. So even if the samples reached acceptable results of dough yield (185.9% for agar-carrageenan 0.5%), bread yield (161.6% for agar-carrageenan 1.0%) or baking loss (10.8% for agar-gelatine 1.0%) they were not considered as satisfactory samples if they did not reach at least the loaf bread volume of the check sample. But all the hydrocolloid combinations, except for agar-alginate 0.5% significantly decreased the baking loss.

Hardness 24 and 72 hours after baking

The combination of agar-cellulose 0.5% gave the least crumb hardness (11.7 N; rice – 13.9 N), but this difference was not significant. Most of remaining samples of the both proportions were harder than the rice check sample and the worst result presented the combination of agar-alginate 0.5%. Only the samples of agar-gelatine 0.5% (12.6 N) and agar-tragacanth 0.5% (13.4 N) had better results than the check sample. The measurement 72 hours after baking showed deterioration by more than 50%, but the sample of agar-cellulose 0.5% reached the hardness of only 37.7 N that could be considered acceptable. More than a half of the samples proved hardness higher than 50.0 N (e.g. agar-alginate 0.5% – 172.3 N; agar-gelatine 1.0% – 126.6 N; agar-xanthan gum 1.0% – 110.7 N) which is significantly different value in comparison with the rice check sample (27.0 N).

Moisture content 24 and 72 hours after baking

The moisture content 24 and 72 hours after baking revealed very close results in the range of 47.27% (agar-alginate 0.5%) to 54.84% (rice) 24 h after baking and 46.38% (agar-alginate 0.5%) to 54.80% (rice) 72 h after baking. Any remarkable deterioration within staling time was observed, the maximum deterioration presented the sample agar-carrageenan 0.5% from 52.22% (24 h) to 49.34% (72 h). All the samples' moisture content varied from 46.38 to 54.84%. The statistic evaluation showed many significant differences which made the result description very unclear.

Table 12: Average values of rice bread characteristics prepared with agar blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Loaf specific volume ($cm^3\ g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)
R-Agar-Alg 0.5%	1.384 ± 0.006^{b}	184.8 ± 2.8^{e}	148.2 ± 0.1^{a}	19.9 ± 1.2^{g}
R-Agar-Alg 1.0%	1.472 ± 0.019^{bc}	184.8 ± 0.1^{e}	160.7 ± 1.3^{e}	13.0 ± 0.7^{cde}
R-Agar-CG 0.5%	1.576 ± 0.019^{c}	172.3 ± 1.8^{a}	152.3 ± 0.6^{b}	11.6 ± 0.7^{abcd}
R-Agar-CG 1.0%	1.272 ± 0.023^{ab}	179.0 ± 3.0^{bc}	158.6 ± 0.4^{cde}	11.4 ± 2.4^{abc}
R-Agar-Carrag 0.5%	1.549 ± 0.007^{bc}	185.9 ± 1.0^{e}	162.5 ± 0.5^{ef}	12.6 ± 2.2^{bcde}
R-Agar-Carrag 1.0%	1.341 ± 0.004^{ab}	184.3 ± 2.6^{de}	161.6 ± 0.9^{e}	12.3 ± 1.0^{bcde}
R-Agar-Cel 0.5%	1.896 ± 0.020^{e}	177.9 ± 3.0^{b}	160.0 ± 1.6^{cde}	10.0 ± 2.8^{a}
R-Agar-Cel 1.0%	1.588 ± 0.017^{c}	180.9 ± 0.8^{bcd}	156.3 ± 1.5^{bc}	13.6 ± 2.5^{e}
R-Agar-Gel 0.5%	1.703 ± 0.017^{cd}	185.2 ± 0.6^{e}	156.5 ± 0.5^{bcd}	15.5 ± 1.8^{f}
R-Agar-Gel 1.0%	1.253 ± 0.013^{ab}	171.6 ± 3.9^{a}	153.0 ± 1.6^{b}	10.8 ± 1.3^{ab}
R-Agar-Trag 0.5%	1.784 ± 0.014^{d}	182.2 ± 0.7^{cde}	160.7 ± 0.5^{de}	$11.8 \pm 1.8^{a-e}$
R-Agar-Trag 1.0%	1.479 ± 0.004^{bc}	184.8 ± 0.3^{de}	160.1 ± 0.3^{cde}	13.4 ± 0.4^{de}
R-Agar-XG 0.5%	1.596 ± 0.009^{c}	182.1 ± 3.5^{cde}	159.8 ± 0.4^{cde}	12.3 ± 3.2^{bcde}
R-Agar-XG 1.0%	1.278 ± 0.006^{ab}	177.7 ± 2.0^{b}	156.5 ± 0.9^{bcd}	11.9 ± 0.5^{bcde}
Rice	1.716 ± 0.003^{cd}	204.0 ± 0.1^{f}	166.2 ± 0.1^{f}	18.6 ± 0.1^{g}

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

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

Table 13: Average values of rice bread hardness and moisture content prepared with agar blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
R-Agar-Alg 0.5%	50.4 ± 2.6^{f}	172.3 ± 7.1^{g}	47.27 ± 0.07^{a}	46.38 ± 0.06^{a}
R-Agar-Alg 1.0%	18.5 ± 0.6^{bc}	42.6 ± 6.7^{abc}	$51.56 \pm 0.29^{jklmnopq}$	51.47 ± 0.10^{nop}
R-Agar-CG 0.5%	35.3 ± 3.2^{d}	96.0 ± 10.7^{e}	50.00 ± 0.82^{cdefg}	48.77 ± 0.17^{cdef}
R-Agar-CG 1.0%	33.2 ± 6.3^{d}	76.6 ± 6.7^{d}	49.83 ± 0.04^{cdef}	49.28 ± 0.23^{cdefgh}
R-Agar-Carrag 0.5%	22.5 ± 1.8^{c}	56.5 ± 4.2^{c}	$52.22 \pm 0.02^{opqrstuv}$	49.34 ± 1.15^{cdefghi}
R-Agar-Carrag 1.0%	36.7 ± 2.1^{de}	58.0 ± 5.0^{c}	51.13 ± 0.03^{hijkl}	50.85 ± 0.17^{ijklmno}
R-Agar-Cel 0.5%	11.7 ± 0.5^{a}	37.7 ± 7.4^{ab}	51.72 ± 0.02^{klmopqr}	51.25 ± 0.32^{lmnop}
R-Agar-Cel 1.0%	17.3 ± 0.8^{abc}	55.9 ± 5.2^{c}	50.30 ± 0.12^{defgh}	$49.94 \pm 0.09^{efghijklmn}$
R-Agar-Gel 0.5%	12.6 ± 1.0^{ab}	31.8 ± 2.3^{a}	52.26 ± 0.01^{pqrstuw}	51.31 ± 0.02^{mnop}
R-Agar-Gel 1.0%	47.9 ± 6.3^{f}	126.6 ± 10.1^{f}	48.25 ± 0.14^{ab}	47.87 ± 0.04^{bc}
R-Agar-Trag 0.5%	13.4 ± 0.6^{ab}	36.3 ± 3.9^{ab}	$51.84 \pm 0.07^{klmnopqrst}$	51.14 ± 0.20^{klmno}
R-Agar-Trag 1.0%	17.3 ± 0.8^{abc}	41.8 ± 2.4^{abc}	$52.14 \pm 0.09^{lmnopqrstu}$	51.45 ± 0.24^{nop}
R-Agar-XG 0.5%	17.5 ± 2.0^{abc}	49.3 ± 5.0^{bc}	51.15 ± 0.09^{hijklm}	$50.58 \pm 0.16^{hijklmno}$
R-Agar-XG 1.0%	42.1 ± 2.4^{e}	110.7 ± 9.6^{ef}	48.44 ± 0.11^{b}	48.51 ± 0.05^{cde}
Rice	13.9 ± 1.2^{ab}	27.0 ± 2.3^{a}	54.84 ± 0.21^{xyz}	54.80 ± 0.11^{q}

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

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

4.7.2 Alginate blends

All the following tables of hydrocolloid blends contain the results in sum to get a comprehensive idea of their effect to technological parameters. That means also agar-alginate blend commented above. The mean values of all agar blends put into the rice flour are shown in Tables 14 and 15.

Baking test

Alginate in the blend with other hydrocolloid positively affected the loaf specific volume and unlike the agar, even the portion of 1.0% hydrocolloid on flour weight increased or did not worsen the bread volume in comparison to the rice check sample. Specifically, the combination of alginate and cellulose 0.5% significantly improved the volume from 1.716 $cm^3\ g^{-1}$ to 1.784 $cm^3\ g^{-1}$ and 1.924 $cm^3\ g^{-1}$ (alginate-gelatine 0.5 and 1.0%). Very similar result (1.809 $cm^3\ g^{-1}$) was measured for alginate-xanthan gum 1.0% and the combinations of alginate with carob gum (0.5%), carrageenan (0.5 and 1.0%) and cellulose (1.0%) presented very similar value as the rice check sample. Other combinations significantly decreased this parameter; from 1.582 $cm^3\ g^{-1}$ (alginate-xanthan gum 0.5%) to 1.311 $cm^3\ g^{-1}$ (alginate-carob gum 1.0%). It cannot be concluded that higher portion of hydrocolloid increased the loaf specific volume. Satisfactory results for dough and bread yield were measured again for alginate with cellulose 0.5% (193.2 and 167.8%) and xanthan gum 1.0% (193.6 and 166.9%) – almost the best results among all samples and in case of the bread yield even better results than rice check sample (166.2%). The only better result of the dough yield presented the combination of gelatine in both 0.5 and 1.0% to flour weight, but the differences were not statistically significant. Statistically significant dough yield deterioration showed the combinations of alginate and tragacanth (0.5 and 1.0%), gelatine (0.5 and 1.0%), carrageenan (0.5 and 1.0%), carob gum (0.5 and 1.0%) and agar (0.5 and 1.0%). The baking loss varied from 11.8% (agar-cellulose 1.0%) to 19.9% (alginate-agar 0.5%). The same worsening effect can be observed for the bread yield. The lower dough yield, the lower bread yield, so the best result proved the sample of alginate-cellulose 1.0% (168.4%) and worst result alginate-agar 0.5% (148.1%).

All the hydrocolloid blends, except for alginate-agar 0.5%, were able to significantly decrease the baking loss in comparison with the rice check sample (18.6%) and the best results presented the alginate-cellulose 1.0% and alginate-carob gum 0.5% (11.8 and 12.3%) that decreased the baking loss by more than 30%. Other samples' baking loss varied from 12.9 to 19.9%.

Table 14: Average values of rice bread characteristics prepared with alginate blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Loaf specific volume ($cm^3\ g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)
R-Alg-Agar 0.5%	1.384 ± 0.006^{bc}	184.8 ± 2.8^{d}	148.2 ± 0.1^{a}	19.9 ± 1.2^{d}
R-Alg-Agar 1.0%	1.472 ± 0.019^{c}	184.8 ± 0.1^{d}	160.7 ± 1.3^{cd}	13.0 ± 0.7^{abc}
R-Alg-CG 0.5%	1.738 ± 0.025^{e}	170.5 ± 1.7^{a}	149.6 ± 2.1^{a}	12.3 ± 1.4^{ab}
R-Alg-CG 1.0%	1.311 ± 0.032^{a}	179.5 ± 2.0^{bc}	156.4 ± 2.3^{bc}	12.9 ± 1.4^{abc}
R-Alg-Carrag 0.5%	1.743 ± 0.009^{e}	183.5 ± 1.3^{cd}	156.8 ± 1.5^{bc}	14.5 ± 0.3^{bc}
R-Alg-Carrag 1.0%	1.680 ± 0.037^{e}	176.1 ± 2.7^{b}	152.8 ± 3.9^{ab}	13.2 ± 2.2^{abc}
R-Alg-Cel 0.5%	1.778 ± 0.027^{fg}	193.2 ± 2.0^{f}	167.8 ± 3.2^{e}	13.2 ± 0.8^{abc}
R-Alg-Cel 1.0%	1.723 ± 0.038^{e}	191.0 ± 1.0^{ef}	168.4 ± 4.1^{e}	11.8 ± 1.7^{a}
R-Alg-Gel 0.5%	1.784 ± 0.033^{fg}	194.2 ± 3.0^{f}	164.5 ± 3.1^{de}	15.3 ± 0.4^{c}
R-Alg-Gel 1.0%	1.924 ± 0.036^{g}	193.3 ± 2.8^{f}	163.8 ± 3.7^{de}	15.3 ± 0.7^{c}
R-Alg-Trag 0.5%	1.482 ± 0.027^{c}	187.2 ± 0.9^{de}	160.8 ± 0.4^{cd}	14.1 ± 0.3^{abc}
R-Alg-Trag 1.0%	1.437 ± 0.013^{bc}	191.0 ± 0.8^{ef}	165.9 ± 1.0^{de}	13.1 ± 0.9^{abc}
R-Alg-XG 0.5%	1.582 ± 0.054^{de}	191.7 ± 2.9^{f}	165.5 ± 2.8^{de}	13.6 ± 2.8^{abc}
R-Alg-XG 1.0%	1.809 ± 0.049^{fg}	193.6 ± 3.4^{f}	166.9 ± 4.0^{e}	13.8 ± 1.5^{abc}
Rice	1.716 ± 0.003^{d}	204.0 ± 0.1^{j}	166.2 ± 0.1^{gh}	18.6 ± 0.1^{h}

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

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

Table 15: Average values of rice bread hardness and moisture content prepared with alginate blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
R-Alg-Agar 0.5%	50.4 ± 2.6^{f}	172.3 ± 7.1^{f}	47.337 ± 0.065^{a}	46.382 ± 0.058^{a}
R-Alg-Agar 1.0%	18.5 ± 0.6^{d}	42.6 ± 6.7^{c}	$51.567 \pm 0.297^{jklmnopq}$	51.478 ± 0.100^{lmn}
R-Alg-CG 0.5%	15.2 ± 2.4^{bcd}	47.3 ± 7.9^{c}	$50.232 \pm 0.193^{cdefgh}$	$49.622 \pm 0.693^{bcdefghi}$
R-Alg-CG 1.0%	42.6 ± 4.6^{e}	57.6 ± 4.5^{d}	51.046 ± 0.010^{ghijk}	$50.037 \pm 0.141^{bcdefghi}$
R-Alg-Carrag 0.5%	16.3 ± 0.9^{cd}	47.2 ± 8.4^{c}	$51.863 \pm 0.041^{klmnopqrst}$	$49.982 \pm 0.596^{cdefghijkl}$
R-Alg-Carrag 1.0%	15.3 ± 1.1^{bcd}	109.4 ± 16.5^{e}	49.241 ± 1.821^{bc}	$49.550 \pm 0.324^{cdefghijkl}$
R-Alg-Cel 0.5%	6.7 ± 0.3^{a}	14.6 ± 1.2^{a}	$52.584 \pm 0.520^{qrstuvwx}$	$54.179 \pm 0.002^{bcdefgh}$
R-Alg-Cel 1.0%	7.1 ± 0.5^{a}	17.1 ± 1.7^{ab}	$53.564 \pm 0.212^{xyza'b'}$	53.995 ± 0.132^{opq}
R-Alg-Gel 0.5%	7.1 ± 0.3^{a}	17.7 ± 2.1^{ab}	$54.385 \pm 0.053^{b'c'd'e'f'}$	54.380 ± 0.135^{opq}
R-Alg-Gel 1.0%	7.6 ± 0.6^{a}	18.2 ± 1.0^{ab}	$54.261 \pm 0.019^{a'b'c'd'e'f'}$	54.586 ± 0.021^{pq}
R-Alg-Trag 0.5%	8.5 ± 0.8^{a}	17.3 ± 2.3^{ab}	$53.176 \pm 0.593^{uvwxyz}$	54.814 ± 0.075^{q}
R-Alg-Trag 1.0%	12.4 ± 1.5^{b}	20.9 ± 1.8^{ab}	$53.743 \pm 0.126^{yza'b'c'd'}$	54.328 ± 0.052^{opq}
R-Alg-XG 0.5%	8.0 ± 0.4^{a}	23.9 ± 2.8^{ab}	$54.772 \pm 0.003^{d'e'f'g'}$	54.318 ± 0.172^{opq}
R-Alg-XG 1.0%	6.8 ± 0.4^{a}	21.2 ± 3.6^{ab}	$54.731 \pm 0.006^{c'd'e'f'g'}$	54.216 ± 0.072^{opq}
Rice	13.9 ± 1.2^{ab}	27.0 ± 2.3^{bcd}	$54.841 \pm 0.210^{c'd'e'f'g}$	54.801 ± 0.113^{r}

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

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

Hardness 24 and 72 hours after baking

The crumb hardness 24 h after baking showed satisfactory decreasing results at the half of the samples, where the best result and improvement by 51.7% in comparison to the rice check sample was measured for the blend of alginate-cellulose 0.5% (from 18.6 to 6.7 N). Also, the samples with cellulose 1.0%, gelatine 0.5 and 1.0%, tragacanth 0.5% and xanthan gum 0.5 and 1.0% reached very similar statistically different values (from 6.8 to 8.5 N) compared to the check sample and other samples proved higher (50.4 N for alginate-agar 0.5%) or lower (15.3 N for alginate-carrageenan 1.0%) deterioration compared with the check sample. But all the values were significantly different.

The worsening of the hardness 72 h after baking was not as high to be significantly different from the check sample. Specifically, the samples with cellulose, gelatine, tragacanth and xanthan gum in both portions 0.5 and 1.0%. Unlike the rest of the samples – agar, carob gum and carrageenan in 0.5 and 1.0% portions that significantly differed from the rice check sample. The best result was registered for alginate-cellulose 0.5% (14.6 N), the worst result for alginate-agar 0.5%.

Moisture content 24 and 72 hours after baking

The presence of hydrocolloid blends in the rice flour did not prove any remarkable changes in the moisture content 24 and 72 hours too. The rice check sample reached 54.841% in both measurements and the lowest value in comparison had the sample of alginate blended with agar 0.5% (46.382%). The hydrocolloids proved the ability to hold the moisture 72 h after baking, because the moisture content declined only by several percentage.

4.7.3 Carob gum blends

The carob gum was blended with the rest seven hydrocolloids and applied to the rice flour in the portion of 0.5 and 1.0% to flour weight. For the results to be complete, the blends of agar and alginate are commented above are summarized in the Tables 16 and 17 that shows the significant differences of the bread technological parameters, hardness and moisture content 24 and 72 hours after baking.

Table 16: Average values of rice bread characteristics prepared with carob gum blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Loaf specific volume ($cm^3 g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)
R-CG-Agar 0.5%	1.576 ± 0.019^{d}	172.3 ± 0.7^{a}	152.3 ± 1.8^{ab}	11.4 ± 0.6^{a}
R-CG-Agar 1.0%	1.272 ± 0.023^{a}	179.0 ± 2.4^{bcd}	158.6 ± 3.0^{cd}	11.6 ± 0.4^{a}
R-CG-Alg 0.5%	1.738 ± 0.025^{de}	170.5 ± 1.7^{a}	149.6 ± 2.1^{a}	12.3 ± 1.4^{ab}
R-CG-Alg 1.0%	1.311 ± 0.032^{a}	179.5 ± 2.0^{bcde}	156.4 ± 2.3^{bcd}	12.9 ± 1.4^{abc}
R-CG-Carrag 0.5%	1.673 ± 0.007^{de}	183.3 ± 1.1^{efg}	156.4 ± 0.5^{bcd}	12.4 ± 0.8^{bc}
R-CG-Carrag 1.0%	1.429 ± 0.043^{bc}	181.5 ± 3.9^{cdef}	156.4 ± 5.2^{bcd}	12.9 ± 1.1^{bcd}
R-CG-Cel 0.5%	1.807 ± 0.003^{f}	181.9 ± 1.6^{cdef}	154.1 ± 0.7^{abc}	13.0 ± 0.9^{de}
R-CG-Cel 1.0%	1.581 ± 0.038^{d}	186.1 ± 2.4^{g}	151.8 ± 1.3^{ab}	13.2 ± 0.4^{e}
R-CG-Gel 0.5%	1.693 ± 0.029^{de}	178.7 ± 1.9^{bcd}	156.6 ± 0.5^{bcd}	13.7 ± 1.2^{ef}
R-CG-Gel 1.0%	1.712 ± 0.019^{de}	178.2 ± 3.6^{bc}	153.9 ± 3.9^{abc}	13.9 ± 1.1^{ef}
R-CG-Trag 0.5%	1.522 ± 0.033^{cd}	176.7 ± 0.9^{b}	153.4 ± 2.3^{ab}	14.7 ± 1.7^{fg}
R-CG-Trag 1.0%	1.641 ± 0.017^{d}	185.1 ± 0.3^{fg}	153.4 ± 0.2^{ab}	15.3 ± 0.1^{fg}
R-CG-XG 0.5%	1.618 ± 0.007^{d}	182.7 ± 1.3^{defg}	160.7 ± 1.4^{d}	17.1 ± 0.5^{h}
R-CG-XG 1.0%	1.379 ± 0.032^{bc}	177.9 ± 2.0^{bc}	154.7 ± 3.0^{bc}	18.4 ± 0.7^{i}
Rice	1.716 ± 0.003^{de}	204.0 ± 0.1^{h}	166.2 ± 0.1^{e}	18.6 ± 0.1^{i}

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

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

Table 17: Average values of rice bread hardness and moisture content prepared with carob gum blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
R-CG-Agar 0.5%	35.3 ± 3.2^{e}	96.0 ± 10.7^{g}	50.003 ± 0.822^{abcde}	48.776 ± 0.171^{abcd}
R-CG-Agar 1.0%	33.2 ± 6.3^{e}	76.6 ± 6.7^{ef}	49.832 ± 0.046^{abcd}	$49.284 \pm 0.232^{abcdef}$
R-CG-Alg 0.5%	15.2 ± 2.4^{abc}	47.3 ± 7.9^{bc}	$50.232 \pm 0.194^{abcdef}$	$49.622 \pm 0.693^{bcdefghi}$
R-CG-Alg 1.0%	42.6 ± 4.6^{f}	57.6 ± 4.5^{cd}	51.046 ± 0.010^{efghi}	$50.037 \pm 0.141^{cdefghijkl}$
R-CG-Carrag 0.5%	18.2 ± 1.2^{bcd}	55.2 ± 2.6^{bcd}	$52.282 \pm 0.034^{nopqrstu}$	$50.891 \pm 0.096^{ghijklm}$
R-CG-Carrag 1.0%	31.7 ± 0.8^{e}	67.7 ± 2.0^{de}	51.146 ± 0.003^{fghij}	$50.438 \pm 0.004^{efghijklm}$
R-CG-Cel 0.5%	10.9 ± 1.1^{a}	38.6 ± 1.9^{ab}	$51.755 \pm 0.036^{ijklmnopq}$	$50.421 \pm 0.001^{efghijklm}$
R-CG-Cel 1.0%	19.3 ± 1.4^{cd}	52.4 ± 5.7^{bc}	50.482 ± 0.020^{cdefg}	$49.546 \pm 0.034^{bcdefgh}$
R-CG-Gel 0.5%	13.5 ± 1.3^{ab}	48.7 ± 8.1^{bc}	$51.411 \pm 0.034^{ghijklmn}$	$49.283 \pm 0.615^{abcdef}$
R-CG-Gel 1.0%	16.9 ± 1.5^{bcd}	43.8 ± 1.0^{bc}	50.940 ± 0.075^{efghi}	$49.715 \pm 0.174^{bcdefghij}$
R-CG-Trag 0.5%	22.2 ± 3.8^{d}	90.5 ± 10.6^{fg}	49.580 ± 0.067^{abc}	48.721 ± 0.271^{abcd}
R-CG-Trag 1.0%	14.5 ± 1.2^{abc}	48.5 ± 9.8^{bc}	$51.715 \pm 0.111^{ijklmnop}$	$50.921 \pm 0.211^{hijklm}$
R-CG-XG 0.5%	17.0 ± 1.8^{bcd}	43.6 ± 7.7^{b}	$51.889 \pm 0.052^{ijklmnopqr}$	48.999 ± 0.604^{abcde}
R-CG-XG 1.0%	50.1 ± 1.7^{f}	80.3 ± 13.6^{ef}	50.627 ± 0.089^{defgh}	$49.927 \pm 0.408^{cdefghijkl}$
Rice	13.9 ± 1.2^{ab}	27.0 ± 2.3^{a}	54.841 ± 0.210^{vwx}	54.801 ± 0.113^{n}

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

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

Baking test

Baking test was evaluated 24 hours after baking. The loaf specific volume revealed that only the combination of carob gum with cellulose 0.5% compared to the rice check sample (1.716 cm^3 g^{-1}) reached significantly better result 1.807 cm^3 g^{-1}. Other samples got very similar (alginate 0.5% – 1.738 cm^3 g^{-1}, gelatine 0.5% – 1.693 cm^3 g^{-1} and gelatine 1.0% – 1.712 cm^3 g^{-1}) or lower (agar 0.5 and 1.0%, alginate, carrageenan and cellulose 1.0%, tragacanth 0.5 and 1.0%, xanthan gum 0.5 and 1.0%). The results 1.429 cm^3 g^{-1} and lower were significantly different from the rice check sample.

The dough yield ranged from 172.3 % (carob gum-agar 0.5%) to 204% (rice check sample), and all the blends results were significantly different from the check sample. The best results exceeded 180%, specifically carob gum blends with carrageenan 0.5 and 1.0% (183.3 and 181.5%), cellulose 0.5 and 1.0% (181.9 and 186.1%), tragacanth 1.0% (185.1%) and xanthan gum 0.5% (182.7%). Other samples' dough yield ranged from 172.3% to 179.5%.

The best result of bread yield reached the rice check sample (166.2%) and the result of hydrocolloid blends samples significantly differed. No enhancement was observed with the higher portion of hydrocolloid blend in the sample and the higher portion of hydrocolloid blend in the rice sample significantly decreased the bread yield. Only the increased portion of carob gum-agar combination improved the bread yield by 6.3% and the result was significant.

It was proved that all hydrocolloid combinations in both portions were able to decrease the baking loss and except for the carob gum-xanthan gum 1.0% all the results were significantly different. The best result presented the blend with agar 0.5% (11.4%) and on the contrary, the worst result was calculated for the carob gum-xanthan gum 1.0% (187.4%) compared to the rice check sample (18.6%).

Hardness 24 and 72 hours after baking

The lowest hardness 24 h after baking was measured for the carob gum-cellulose 0.5% (10.9 N) which is by 21.5% lower than the rice check sample (13.9 N). More than a half of the results, specifically the carob gum blends with alginate 0.5%, carrageenan 0.5%, gelatine 0.5% and 1.0%, tragacanth 0.5 and 1.0% reached very similar result as the rice check sample and these results were not significantly different. Remaining samples, mostly the combinations of 1.0% blend to flour weight presented significantly higher hardness compared to the rice check sample. Measurements 72 h after baking proved the significant deterioration within the staling time. All samples were significantly different from the rice check sample and the check sample kept the lowest hardness value (27.0 N) 72 h after baking. The next best result was

measured for the combination carob gum-cellulose 0.5% (38.6 N). The rest samples reached values of 43.6 up to 96.0 N for carob gum-agar 0.5%.

Moisture content 24 and 72 hours after baking

The moisture content deteriorated with the staling time, but even the worst value reached 48.7% which can be considered an acceptable result. As the best result reached the rice check sample (54.8%) other results were significantly different, and many differences were found among the samples. The presence of higher blend portion in the sample did not prove higher ability to retain moisture.

4.7.4 Carrageenan blends

Baking test

The blends of agar, alginate and carob gum with carrageenan were described above, but it was necessary to compare the results with other blends and compare them in context of other carrageenan blends. The results of baking test, hardness and moisture content 24 and 72 h after baking are summarized in the Tables 18 and 19.

Comparing the loaf bread volume, the combinations of carrageenan with cellulose 0.5% and gelatine 0.5% reached better result (1.806 and 1.841 $cm^3 g^{-1}$) than the rice check sample (1.716 $cm^3 g^{-1}$). And it can be concluded, that the results except for the blends with agar 1.0% (1.341 $cm^3 g^{-1}$) and carob gum 1.0% (1.429 $cm^3 g^{-1}$) reached acceptable values from 1.479 to 1.743 $cm^3 g^{-1}$ as the check sample.

The highest dough yield was calculated for the rice check sample (204.0%), but most samples, except for blends with alginate 1.0%, gelatine 0.5% and xanthan gum 0.5% presented the dough yield more than 180% (from 181.5% for carrageenan-carob gum 1.0% to 189.9% for carrageenan gelatine 1.0%) which is significantly lower than the check sample, but still an acceptable result. The similar situation can be observed for the parameter bread yield. Any sample reached the value of the rice check sample (166.2%), but the blends of carrageenan with agar 0.5% (162.5%) and 1.0% (161.6%), gelatine 1.0% (165.0%), tragacanth 1.0% (160.7%) got very close. Other samples proved significant deterioration of the bread yield while they reached the values from 152.8% (carrageenan-alginate 1.0%) to 160.0% (carrageenan-xanthan gum 1.0%).

The carrageenan blends with agar, alginate, carob gum, cellulose and gelatine proved that the higher portion of hydrocolloid blend in the sample decreased the baking loss, but the difference between 0.5 and 1.0% was not significant. The lowest baking loss was found for the sample with carrageenan-agar 1.0% (12.3%) and it was verified that the hydrocolloid blends positively affect this parameter.

Table 18: Average values of rice bread characteristics prepared with carrageenan blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Loaf specific volume ($cm^3\ g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)
R-Carrag-Agar 0.5%	1.549 ± 0.007^{cd}	185.9 ± 2.2^{efg}	162.5 ± 1.0^{cde}	12.6 ± 0.5^{ab}
R-Carrag-Agar 1.0%	1.341 ± 0.004^{a}	184.3 ± 1.0^{def}	161.6 ± 2.6^{cde}	12.3 ± 0.9^{a}
R-Carrag-Alg 0.5%	1.743 ± 0.008^{e}	183.5 ± 1.3^{cdef}	156.8 ± 1.5^{ab}	14.5 ± 0.3^{ab}
R-Carrag-Alg 1.0%	1.680 ± 0.037^{e}	176.1 ± 2.7^{a}	152.8 ± 3.9^{a}	13.2 ± 2.2^{ab}
R-Carrag-CG 0.5%	1.673 ± 0.007^{e}	183.3 ± 1.1^{cdef}	156.4 ± 0.5^{ab}	14.7 ± 0.8^{ab}
R-Carrag-CG 1.0%	1.429 ± 0.043^{bc}	181.5 ± 3.9^{bcde}	156.4 ± 5.2^{ab}	13.9 ± 1.1^{ab}
R-Carrag-Cel 0.5%	1.806 ± 0.022^{f}	180.3 ± 1.8^{abcd}	153.2 ± 3.2^{a}	15.0 ± 0.9^{b}
R-Carrag-Cel 1.0%	1.674 ± 0.019^{e}	184.1 ± 1.6^{cdef}	157.3 ± 1.1^{abc}	14.5 ± 0.4^{ab}
R-Carrag-Gel 0.5%	1.841 ± 0.023^{f}	177.2 ± 2.6^{ab}	154.2 ± 2.0^{a}	13.0 ± 0.1^{ab}
R-Carrag-Gel 1.0%	1.607 ± 0.017^{d}	189.9 ± 3.9^{g}	165.0 ± 2.7^{de}	13.1 ± 1.8^{ab}
R-Carrag-Trag 0.5%	1.582 ± 0.051^{d}	180.9 ± 1.2^{bcd}	156.9 ± 4.2^{ab}	13.3 ± 2.9^{ab}
R-Carrag-Trag 1.0%	1.515 ± 0.021^{cd}	187.7 ± 1.6^{fg}	160.7 ± 3.0^{bcde}	14.4 ± 0.9^{ab}
R-Carrag-XG 0.5%	1.522 ± 0.015^{cd}	179.4 ± 2.2^{abc}	156.6 ± 2.0^{ab}	12.7 ± 0.1^{ab}
R-Carrag-XG 1.0%	1.479 ± 0.014^{cd}	184.1 ± 4.0^{cdef}	160.0 ± 2.0^{bcd}	13.1 ± 1.0^{ab}
Rice	1.716 ± 0.003^{e}	204.0 ± 0.1^{h}	166.2 ± 0.1^{e}	18.6 ± 0.1^{c}

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

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

Table 19: Average values of rice bread hardness and moisture content prepared with carrageenan blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
R-Carrag-Agar 0.5%	22.5 ± 1.8^{def}	56.5 ± 4.2^{defg}	$52.221 \pm 0.021^{klmnopqr}$	$49.347 \pm 1.156^{bcdefgh}$
R-Carrag-Agar 1.0%	36.7 ± 2.1^{h}	58.0 ± 5.0^{defg}	51.133 ± 0.003^{defgh}	$50.852 \pm 0.173^{hijklmn}$
R-Carrag-Alg 0.5%	16.3 ± 0.9^{ab}	47.2 ± 8.4^{bcd}	$51.863 \pm 0.041^{ghijklmnop}$	$49.982 \pm 0.596^{defghijklm}$
R-Carrag-Alg 1.0%	15.3 ± 1.1^{ab}	109.4 ± 16.5^{i}	49.204 ± 1.820^{ab}	$49.550 \pm 0.324^{cdefghi}$
R-Carrag-CG 0.5%	18.2 ± 1.2^{bc}	55.2 ± 2.6^{def}	$52.282 \pm 0.034^{lmnopqrs}$	$50.890 \pm 0.096^{hijklmn}$
R-Carrag-CG 1.0%	31.7 ± 0.8^{g}	67.7 ± 2.0^{gh}	51.146 ± 0.003^{defgh}	$50.438 \pm 0.004^{fghijklmn}$
R-Carrag-Cel 0.5%	16.1 ± 1.9^{ab}	54.2 ± 2.5^{cdef}	50.943 ± 0.129^{cdefg}	$50.072 \pm 0.096^{efghijklm}$
R-Carrag-Cel 1.0%	19.8 ± 0.7^{cd}	39.2 ± 2.3^{ab}	$52.316 \pm 0.009^{lmnopqrs}$	51.252 ± 0.431^{klmno}
R-Carrag-Gel 0.5%	21.9 ± 0.4^{de}	71.1 ± 6.5^{h}	$51.170 \pm 0.067^{defghij}$	$49.355 \pm 0.605^{bcdefgh}$
R-Carrag-Gel 1.0%	15.3 ± 1.0^{ab}	42.3 ± 2.3^{bc}	$52.780 \pm 0.011^{opqrstu}$	51.729 ± 0.206^{nop}
R-Carrag-Trag 0.5%	21.1 ± 1.1^{cde}	61.9 ± 11.8^{efgh}	50.958 ± 0.172^{cdefg}	$50.428 \pm 0.124^{fghijklmn}$
R-Carrag-Trag 1.0%	24.1 ± 0.5^{ef}	53.3 ± 6.3^{cde}	52.999 ± 0.526^{qrstu}	51.894 ± 0.010^{nop}
R-Carrag-XG 0.5%	25.4 ± 4.0^{f}	59.8 ± 5.4^{efgh}	$51.198 \pm 0.044^{defghijk}$	47.889 ± 0.235^{ab}
R-Carrag-XG 1.0%	32.0 ± 1.1^{g}	67.1 ± 3.8^{fgh}	$51.946 \pm 0.285^{ghijklmnop}$	$50.916 \pm 0.052^{ijklmn}$
Rice	13.9 ± 1.2^{a}	27.0 ± 2.3^{a}	54.841 ± 0.210^{vwx}	54.801 ± 0.113^{q}

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

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

Hardness 24 and 72 hours after baking

No significant difference in the values of hardness 24 h after baking was found among the rice check sample (13.9 N) and carrageenan blends with alginate 0.5 and 1.0% (16.3 and 15.3 N), cellulose 0.5% (16.1 N) and gelatine 1.0% (15.3 N). No sample exceeded 40.0 N but at the same tame did not give lower hardness than the rice check sample (13.9 N). All samples' hardness values worsen during the staling time and the measurements 72 hour after baking showed deterioration by more than 50% at all the samples containing the hydrocolloid blends. The only result close to the rice check sample (27.0 N) was observed for the sample of carrageenan-cellulose 1.0% (39.2 N). Remaining samples presented significant hardening.

Moisture content 24 and 72 hours after baking

The moisture content remained very similar during the staling, but none of the samples reached the values of the rice check sample in both measurements 24 and 72 h after baking. All results presented significantly worse moisture content compared to the check sample (54.8%), but the closest value 24 h was measured for carrageenan-tragacanth 1.0% (53.0%). The deterioration of the moisture content within the staling is evident, but all samples kept the values about approximately 50%.

4.7.5 Cellulose blends

Through previous results, the blends with cellulose very often presented remarkable results of bread technology parameters and hardness and moisture content 24 and 72 h after baking. The results of all cellulose blends are summarized in the Tables 20 and 21.

Baking test

As was presented above, cellulose in the blends with agar, alginate, carob gum and carrageenan gave satisfactory results with the rice flour in portion of 0.5% to flour weight. Cellulose in combination with gelatine 0.5% presented very similar result as the rice check sample and the higher portion of this blend resulted in statistically significant improvement (1.800 cm^3 g^{-1}) together with previously mentioned blends with agar 0.5%, alginate 0.5%, carrageenan 0.5% compared to the rice check sample (1.716 cm^3 g^{-1}). The combinations with tragacnath 0.5 and 1.0% presented significantly smaller specific volume (1.394 and 1.490 cm^3 g^{-1}) compared to the rice check sample. The cellulose-xanthan gum blends both 0.5 and 1.0% did not give significantly improved results but reached acceptable results of 1.616 and 1.600 cm^3 g^{-1}.

Table 20: Average values of rice bread characteristics prepared with cellulose blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Loaf specific volume ($cm^3\ g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)
R-Cel-Agar 0.5%	1.896 ± 0.020^{f}	177.9 ± 2.8^{a}	160.0 ± 3.0^{cd}	10.0 ± 1.6^{a}
R-Cel-Agar 1.0%	1.658 ± 0.017^{cd}	180.9 ± 2.5^{ab}	156.3 ± 0.8^{abc}	13.6 ± 1.4^{bc}
R-Cel-Alg 0.5%	1.778 ± 0.027^{e}	193.2 ± 2.0^{fg}	167.8 ± 3.2^{f}	13.2 ± 0.6^{bc}
R-Cel-Alg 1.0%	1.723 ± 0.038^{d}	191.0 ± 1.0^{efg}	168.4 ± 4.1^{f}	11.8 ± 1.7^{ab}
R-Cel-CG 0.5%	1.807 ± 0.003^{e}	181.9 ± 1.6^{ab}	154.1 ± 0.7^{ab}	15.3 ± 0.8^{c}
R-Cel-CG 1.0%	1.581 ± 0.038^{cd}	186.1 ± 2.4^{cd}	151.8 ± 1.3^{a}	18.4 ± 0.4^{d}
R-Cel-Carrag 0.5%	1.806 ± 0.022^{e}	180.3 ± 1.8^{ab}	153.2 ± 3.2^{ab}	15.0 ± 0.9^{c}
R-Cel-Carrag 1.0%	1.674 ± 0.019^{d}	184.1 ± 1.6^{bc}	157.3 ± 1.1^{bc}	14.5 ± 0.4^{c}
R-Cel-Gel 0.5%	1.715 ± 0.017^{d}	194.6 ± 3.6^{g}	165.2 ± 1.7^{ef}	15.1 ± 0.7^{c}
R-Cel-Gel 1.0%	1.800 ± 0.024^{e}	193.1 ± 1.6^{fg}	163.9 ± 0.9^{def}	15.1 ± 1.2^{c}
R-Cel-Trag 0.5%	1.394 ± 0.009^{ab}	189.5 ± 2.0^{def}	163.8 ± 1.7^{def}	13.5 ± 1.6^{bc}
R-Cel-Trag 1.0%	1.490 ± 0.019^{bc}	187.7 ± 2.0^{cde}	161.1 ± 2.6^{cde}	14.2 ± 0.5^{bc}
R-Cel-XG 0.5%	1.616 ± 0.007^{cd}	194.6 ± 0.7^{g}	167.1 ± 0.8^{f}	14.1 ± 0.2^{bc}
R-Cel-XG 1.0%	1.600 ± 0.039^{cd}	194.3 ± 2.0^{g}	167.8 ± 5.3^{f}	13.7 ± 2.7^{bc}
Rice	1.716 ± 0.003^{d}	204.0 ± 0.1^{h}	166.2 ± 0.1^{ef}	18.6 ± 0.1^{d}

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

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

Table 21: Average values of rice bread hardness and moisture content prepared with cellulose blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
R-Cel-Agar 0.5%	11.7 ± 0.5^{d}	37.7 ± 7.4^{d}	$51.726 \pm 0.018^{hijklmno}$	51.253 ± 0.320^{ijklm}
R-Cel-Agar 1.0%	17.3 ± 0.8^{fg}	55.9 ± 5.2^{e}	50.304 ± 0.123^{abcde}	$49.937 \pm 0.097^{bcdefghijk}$
R-Cel-Alg 0.5%	6.7 ± 0.3^{a}	14.6 ± 1.2^{a}	$52.584 \pm 0.520^{nopqrstu}$	54.179 ± 0.002^{nop}
R-Cel-Alg 1.0%	7.1 ± 0.5^{ab}	17.1 ± 1.6^{ab}	53.564 ± 0.211^{uvwxy}	53.995 ± 0.132^{nop}
R-Cel-CG 0.5%	10.9 ± 1.1^{cd}	38.6 ± 1.9^{d}	$51.756 \pm 0.037^{hijklmnop}$	$50.421 \pm 0.001^{defghijkl}$
R-Cel-CG 1.0%	19.3 ± 1.4^{g}	52.4 ± 5.7^{e}	50.482 ± 0.020^{bcdef}	$49.512 \pm 0.034^{abcdefg}$
R-Cel-Carrag 0.5%	16.1 ± 1.9^{f}	54.2 ± 2.5^{e}	50.944 ± 0.129^{defgh}	$50.072 \pm 0.096^{cdefghijk}$
R-Cel-Carrag 1.0%	19.8 ± 0.7^{h}	39.2 ± 2.3^{d}	$52.316 \pm 0.009^{mnopqrst}$	51.252 ± 0.431^{ijklm}
R-Cel-Gel 0.5%	6.5 ± 0.2^{a}	18.8 ± 3.1^{ab}	$54.601 \pm 0.034^{yza'b'c'd'}$	54.473 ± 0.300^{op}
R-Cel-Gel 1.0%	6.6 ± 0.1^{a}	16.2 ± 1.3^{ab}	$54.638 \pm 0.151^{za'b'c'd'}$	54.492 ± 0.007^{op}
R-Cel-Trag 0.5%	11.9 ± 2.4^{de}	20.4 ± 1.6^{b}	$54.098 \pm 0.428^{wxyza'b'}$	54.417 ± 0.073^{op}
R-Cel-Trag 1.0%	10.7 ± 1.3^{cd}	19.4 ± 2.1^{ab}	53.280 ± 0.019^{tuvwx}	54.523 ± 0.050^{op}
R-Cel-XG 0.5%	9.2 ± 0.2^{bc}	19.7 ± 1.5^{ab}	$54.709 \pm 0.101^{za'b'c'd'}$	54.557 ± 0.085^{op}
R-Cel-XG 1.0%	8.7 ± 0.5^{abc}	19.9 ± 0.9^{ab}	$54.615 \pm 0.038^{za'b'c'd'}$	54.304 ± 0.140^{nop}
Rice	13.9 ± 1.2^{e}	27.0 ± 2.3^{c}	$54.801 \pm 0.210^{b'c'd'}$	54.801 ± 0.113^{p}

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

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

The cellulose blends with gelatine (0.5 and 1.0%) and xanthan gum (0.5 and 1.0%) reached remarkable results of the dough yield. Compared to the rice check sample that achieved 204.0% all these samples gave either significantly lower values, but more than 190%, together with alginate blend.

The combination of cellulose and xanthan gum as the first sample presented even higher bread yield in comparison with the rice check sample (166.2%), specifically 167.1% for cellulose-xanthan gum 0.5% and 167.8% for cellulose-xanthan gum 1.0%. The combinations with other hydrocolloids – gelatine 0.5 and 1.0%, tragacanth 0.5 and 1.0% showed lower values (from 161.1 to 165.2%) which were not significantly different from the rice check sample. Other blends caused significant bread yield deterioration, except for alginate 0.5 and 1.0%.

It was confirmed before, that the presence of hydrocolloid blends improved significantly the baking loss. Among these cellulose blends, the best result showed cellulose-agar 0.5% and all the rest samples had significantly lower baking loss than the rice bread sample (18.6%). The samples with gelatine, tragacanth and xanthan gum all 0.5 and 1.0% presented statistically lower values ranging from 13.5% (cellulose-tragacanth 0.5%) to 15.1% (cellulose-gelatine 0.5 and 1.0%).

Hardness 24 and 72 hours after baking

The samples with blends of alginate, gelatine, tragacanth, xanthan gum (0.5 and 1.0%) had lower hardness than the rice check sample. These values were significantly different from the check sample and varied from 6.5 N (gelatine 0.5%) to 10.7 N (tragacanth 1.0%). And, also the samples described before reached acceptable and significantly lower values, for example, cellulose-alginate 0.5% (6.7 N) or cellulose-carob gum 0.5% (10.9 N).

Comparing the blends of cellulose with gelatine, tragacanth, xanthan gum (0.5 and 1.0%), all the results of hardness 72 h after baking proved significantly lower values than the rice check sample (27.0 N). The lowest value was measured for cellulose-gelatine 1.0% (16.2 N) that is 40% improvement compared to the rice sample, contrarywise the highest value presented the sample cellulose-agar 1.0%.

Moisture content 24 and 72 hours after baking

The samples containing gelatine, tragacanth, xanthan gum (0.5 and 1.0%) did not significantly affect the moisture content neither 24 h nor 72 h after baking but reached very close results to the rice check sample. All these values achieved 54% except for cellulose-tragacanth 1.0% (53.3%). The moisture content measured 72 h after baking and staling did not reveal any remarkable deterioration; the moisture content decreased but almost all the values exceeded 50%.

Mohammadi et al. [172] in his study of effect of carboxymethyl cellulose in a blend with xanthan gum also proved the improving effect on technological parameters, hardness and moisture content in fresh a and stored bread.

4.7.6 Gelatine blends

The results of samples with gelatine blends are summarized in the Tables 22 and 23. The mean values of bread making quality, hardness and moisture content 24 and 72 h after baking were statistically evaluated and the statistically significant differences are quantified.

Baking test

Among the samples which have not been commented yet, the best result presented the sample gelatine-tragacanth 0.5% with 1.832 $cm^3\ g^{-1}$, this blend significantly improved the loaf bread volume of the rice check sample (1.716 $cm^3\ g^{-1}$). Gelatine-tragacanth 1.0% (1.612 $cm^3\ g^{-1}$), gelatine-xanthan gum 0.5 and 1.0% (1.433 and 1.367 $cm^3\ g^{-1}$) significantly deteriorated the rice bread volume. The best result of the loaf bread volume was measured for gelatine-alginate sample 1.0% (1.924 $cm^3\ g^{-1}$) and the significantly biggest deterioration presented the sample of gelatine-agar 1.0% (1.253 $cm^3\ g^{-1}$).

The blends of gelatine with tragacanth and xanthan gum (0.5 and 1.0%) had significantly negative effect on the dough yield, but the results of gelatine-tragacanth blends can be still considered satisfactory as they reached 189.3% and 194.2%. The presence of the xanthan gum in the blend deteriorated the dough bread yield by more than 15%. The same result was observed for the bread yield. The samples containing gelatine-tragacanth blends 0.5 and 1.0% reached 163.5 and 168.6%, while the combination of gelatine and xanthan gum significantly worsen this parameter compared to the rice check sample (166.2%), both achieved 150.6% of the bread yield. Among other samples, any of them fell under 150.0%, but the decrease was statistically significant.

It was proved that almost all the gelatine hydrocolloid blends (0.5 and 1.0%) significantly improved the baking loss. The gelatine blended with tragacanth presented 13.6% (for 0.5% portion) and 13.2% (for 1.0% portion). The combination with xanthan gum also showed lower baking loss (14.5 and 16.6%), but these results were not significantly different from the rice check sample (18.6%).

Table 22: Average values of rice bread characteristics prepared with gelatine blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Loaf specific volume ($cm^3\ g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)
R-Gel-Agar 0.5%	1.704 ± 0.017^{de}	185.2 ± 1.8^{d}	156.5 ± 0.6^{b}	15.5 ± 0.5^{ef}
R-Gel-Agar 1.0%	1.253 ± 0.013^{a}	171.6 ± 1.3^{a}	153.0 ± 3.9^{ab}	10.8 ± 1.6^{a}
R-Gel-Alg 0.5%	1.784 ± 0.033^{f}	194.2 ± 3.0^{ef}	164.5 ± 3.1^{cd}	15.3 ± 0.4^{ef}
R-Gel-Alg 1.0%	1.924 ± 0.036^{g}	193.3 ± 2.8^{ef}	163.8 ± 3.7^{c}	15.3 ± 0.7^{ef}
R-Gel-CG 0.5%	1.693 ± 0.029^{de}	178.7 ± 1.9^{c}	156.6 ± 0.5^{b}	12.4 ± 1.2^{ab}
R-Gel-CG 1.0%	1.712 ± 0.020^{de}	178.2 ± 3.6^{c}	153.9 ± 4.0^{ab}	13.7 ± 1.1^{bcde}
R-Gel-Carrag 0.5%	1.841 ± 0.023^{f}	177.2 ± 2.5^{bc}	154.2 ± 2.0^{ab}	13.0 ± 0.1^{bc}
R-Gel-Carrag 1.0%	1.607 ± 0.017^{c}	189.9 ± 3.9^{def}	165.0 ± 2.7^{cd}	13.1 ± 1.8^{bcd}
R-Gel-Cel 0.5%	1.715 ± 0.017^{de}	194.6 ± 3.6^{f}	165.2 ± 1.7^{cd}	15.1 ± 0.7^{def}
R-Gel-Cel 1.0%	1.800 ± 0.024^{f}	193.1 ± 1.6^{ef}	163.9 ± 0.9^{c}	15.1 ± 1.2^{def}
R-Gel-Trag 0.5%	1.832 ± 0.005^{f}	189.3 ± 1.7^{de}	163.5 ± 0.8^{c}	13.6 ± 0.4^{bcde}
R-Gel-Trag 1.0%	1.612 ± 0.040^{c}	194.2 ± 2.7^{ef}	168.6 ± 1.3^{d}	13.2 ± 1.6^{bcd}
R-Gel-XG 0.5%	1.433 ± 0.047^{a}	171.5 ± 0.8^{a}	150.6 ± 0.4^{a}	14.5 ± 2.9^{cdef}
R-Gel-XG 1.0%	1.367 ± 0.125^{a}	173.2 ± 1.1^{ab}	150.6 ± 0.6^{a}	16.6 ± 4.8^{fg}
Rice	1.716 ± 0.003^{de}	204.0 ± 0.1^{g}	166.2 ± 0.1^{cd}	18.6 ± 0.1^{g}

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

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

Table 23: Average values of rice bread hardness and moisture content prepared with gelatine blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
R-Gel-Agar 0.5%	12.6 ± 1.0^{bc}	31.8 ± 2.3^{bc}	$52.567 \pm 0.019^{lmnopqrs}$	51.306 ± 0.024^{lmno}
R-Gel-Agar 1.0%	47.9 ± 6.4^{f}	126.6 ± 10.1^{g}	48.256 ± 0.140^{ab}	47.872 ± 0.044^{ab}
R-Gel-Alg 0.5%	7.1 ± 0.3^{a}	17.7 ± 2.1^{a}	$54.385 \pm 0.053^{wxyza'}$	54.380 ± 0.135^{rs}
R-Gel-Alg 1.0%	7.6 ± 0.6^{a}	18.2 ± 1.0^{a}	$54.262 \pm 0.019^{vwxyza'}$	54.586 ± 0.021^{rs}
R-Gel-CG 0.5%	13.5 ± 1.3^{cd}	48.7 ± 8.1^{d}	$51.411 \pm 0.034^{efghijkl}$	$49.283 \pm 0.615^{bcdefg}$
R-Gel-CG 1.0%	16.9 ± 1.5^{d}	43.8 ± 1.0^{cd}	50.940 ± 0.075^{cdefg}	$49.715 \pm 0.174^{cdefghijk}$
R-Gel-Carrag 0.5%	21.9 ± 0.4^{e}	71.1 ± 6.6^{ef}	$51.170 \pm 0.067^{defghij}$	$49.355 \pm 0.605^{bcdefgh}$
R-Gel-Carrag 1.0%	15.3 ± 1.0^{cd}	42.3 ± 2.9^{cd}	$52.781 \pm 0.011^{opqrstu}$	51.729 ± 0.206^{nop}
R-Gel-Cel 0.5%	6.5 ± 0.2^{a}	18.8 ± 3.1^{ab}	$54.601 \pm 0.034^{wxyza'b'}$	54.473 ± 0.300^{rs}
R-Gel-Cel 1.0%	6.6 ± 0.2^{a}	16.2 ± 1.3^{a}	$54.638 \pm 0.151^{xyza'b'}$	54.492 ± 0.007^{rs}
R-Gel-Trag 0.5%	7.1 ± 0.6^{a}	15.0 ± 0.5^{a}	$52.858 \pm 0.109^{pqrstu}$	54.331 ± 0.010^{qrs}
R-Gel-Trag 1.0%	8.4 ± 0.1^{ab}	14.3 ± 1.8^{a}	53.029 ± 0.302^{qrstu}	54.308 ± 0.147^{qrs}
R-Gel-XG 0.5%	53.0 ± 1.3^{g}	84.5 ± 2.1^{f}	$51.203 \pm 0.122^{defghijk}$	54.623 ± 0.011^{rs}
R-Gel-XG 1.0%	44.3 ± 0.9^{f}	62.6 ± 1.2^{e}	$52.312 \pm 0.400^{lmnopqrs}$	54.820 ± 0.231^{s}
Rice	13.9 ± 1.2^{cd}	27.0 ± 2.3^{ab}	$54.841 \pm 0.210^{za'b'}$	54.801 ± 0.113^{s}

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

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

Hardness 24 and 72 hours after baking

In the context of all gelatine blends results, the lowest hardness 24 h after baking presented the gelatine blend with cellulose 0.5% (6.5 N) that together with gelatine-alginate 0.5% (7.1 N), gelatine-alginate 1.0% (7.6 N), gelatine-cellulose 1.0% (6.6 N), gelatine-tragacanth 0.5% (7.1 N) and alginate-tragacanth 1.0% (8.4 N) present the samples that significantly improved the rice bread hardness (27.0 N) 24 h after baking. The samples blended with gelatine and xanthan gum were significantly the hardest; they reached 53.0 N (0.5%) and 44.3 N (1.0%).

The rice check sample 72 h after baking and staling worsen its value by 48.5% from 13.9 to 27 N and any combination of hydrocolloid blends was able to significantly enhance the hardness 72 h after baking. Lower, but not significantly different values were measured for gelatine-alginate 1.0% (18.2 N), gelatine-cellulose 0.5 and 1.0% (18.8 and 16.2 N), gelatine-tragacanth 0.5 and 1.0% (15.0 and 14.3 N). Other samples presented significant deterioration.

Moisture content 24 and 72 hours after baking

Too many statistically significant differences were found among the results of both moisture content 24 and 72 h after baking, so this complicated the statistic description. All the samples with hydrocolloid blends kept their moisture content very similar even after 72 h after baking. The values rarely fell under 50% and ranged from 48.3% for gelatine-agar 1.0% to 54.8% for the rice check sample 24 h after baking and from 47.9% for gelatine-agar 1.0% to 54.8% for gelatine-xanthan gum 1.0% 72 h after baking and staling.

4.7.7 Tragacanth blends

Tragacant is the other frequented hydrocolloid among gluten-free bread studies. Tragacnath was blended with the hydrocolloids and 15 samples with proportion of 0.5 and 1.0% were prepared and evaluated. The mean values of the results are presented in the Tables 24 and 25.

Baking test

The most important value among costumers is the bread volume. The blends containing tregacanth revealed that only the combination with agar and gelatine both 0.5% were able to significantly improve the volume of rice bread from 1.716 $cm^3 g^{-1}$ to 1.784 and 1.832 $cm^3 g^{-1}$. Other combination did not prove any enhancing effect and the values ranged from 1.360 $cm^3 g^{-1}$ (tragacanth-xanthan gum 1.0%) to 1.641 $cm^3 g^{-1}$ (tragacanth-carob gum 1.0%); these values were significantly different from the rice check sample (1.716 $cm^3 g^{-1}$).

All samples with flour blends significantly deteriorated the dough yield except the tragacanth-xanthan blend, all the remaining hydrocolloids have

already been commented in the context of the specific hydrocolloid they were blended with. The presence of xanthan gum in the blend (0.5 and 1.0%) significantly deteriorated the dough yield from 204.0 to 188.7 and 190.1%. Among other samples any of them was able to improve this parameter and only blends with alginate, gelatine and tragacanth exceeded at least 190%. The worst results presented the blend with agar – 182.2% (0.5%) and 184.8% (1.0%).

The tragacanth-xanthan blend 1.0% reached better result (166.8%) than the rice check sample (166.2%), but this improvement was not significant. The same blend in the 0.5% portion deteriorated the bread yield (164.1%) compared with the rice check sample, but the difference was not significant either. The values of remaining samples varied from 153.4 to 163.5% that means significantly worsening effect. The only exception was the tragacanth-gelatine 1.0% sample which improved the bread yield to 168.6%.

It was proved that the occurrence of the hydrocolloid blends significantly improved the baking loss. All the blends, except for tragacanth-carob gum 1.0%, reached better results ranging from 11.8% (tragacanth-agar 0.5%) to maximum 14.4% (tragacanth-carrageenan 1.0%). But there is no remarkable evidence of higher improvement with increasing portion of hydrocolloid blend in the sample.

Hardness 24 and 72 hours after baking

The combination of traganth and xanthan gum gave lower hardness 24 h compared to the rice check sample (13.9 N), but only the 0.5% portion (8.5 N) was significant. Tragacanth-xanthan gum 1.0% improved the value of hardness 24 h to 12.3 N. Both samples with xanthan gum in the blend presented satisfactory value for the hardness 72 h after baking; the results were not significantly different (17.6 and 23.3 N) but reached lower hardness than the rice check sample (27.0 N). The lowest hardness 72 h after baking presented the tragacanth blended with gelatine and applied to the rice sample in the portion of 1.0% (14.3 N).

Moisture content 24 and 72 hours after baking

All samples reached lower values of moisture content compared to the rice check sample both 24 and 72 h. The statistical analysis showed so many statistical differences that the interpretation is not very clear. It can be concluded that the addition of the hydrocolloid blends was able to keep the moisture content close to the rice check sample or decrease to maximum 49.5 and 48.7% (tragacanth-carob gum 0.5% 24 and 72 h after baking).

Table 24: Average values of rice bread characteristics prepared with tragacanth blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Loaf specific volume ($cm^3\ g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)
R-Trag-Agar 0.5%	1.784 ± 0.014^{f}	182.2 ± 1.8^{bc}	160.7 ± 0.7^{bc}	11.8 ± 0.5^{a}
R-Trag-Agar 1.0%	1.479 ± 0.004^{bc}	184.8 ± 0.4^{cd}	160.1 ± 0.3^{bc}	13.4 ± 0.3^{a}
R-Trag-Alg 0.5%	1.483 ± 0.027^{bc}	187.2 ± 0.9^{def}	160.8 ± 0.4^{bc}	14.1 ± 0.3^{a}
R-Trag-Alg 1.0%	1.437 ± 0.014^{ab}	191.0 ± 0.8^{hi}	165.9 ± 1.0^{de}	13.1 ± 0.9^{a}
R-Trag-CG 0.5%	1.522 ± 0.033^{bc}	176.7 ± 0.9^{a}	153.4 ± 2.3^{a}	13.2 ± 1.6^{a}
R-Trag-CG 1.0%	1.641 ± 0.018^{c}	185.1 ± 0.3^{cde}	153.4 ± 0.2^{a}	17.1 ± 0.1^{b}
R-Trag-Carrag 0.5%	1.582 ± 0.051^{c}	180.9 ± 1.2^{b}	156.9 ± 4.2^{ab}	13.3 ± 2.9^{a}
R-Trag-Carrag 1.0%	1.515 ± 0.021^{bc}	187.7 ± 2.0^{def}	160.7 ± 3.0^{bc}	14.4 ± 0.9^{a}
R-Trag-Cel 0.5%	1.394 ± 0.009^{ab}	189.5 ± 2.0^{g}	163.8 ± 1.7^{cd}	13.5 ± 1.6^{a}
R-Trag-Cel 1.0%	1.490 ± 0.019^{bc}	187.7 ± 2.0^{def}	161.1 ± 2.6^{bc}	14.2 ± 0.5^{a}
R-Trag-Gel 0.5%	1.832 ± 0.005^{f}	189.3 ± 1.7^{f}	163.5 ± 0.8^{cd}	13.6 ± 0.4^{a}
R-Trag-Gel 1.0%	1.612 ± 0.040^{c}	194.2 ± 2.7^{i}	168.6 ± 1.2^{e}	13.2 ± 1.6^{a}
R-Trag-XG 0.5%	1.544 ± 0.002^{bc}	188.7 ± 4.5^{ef}	164.1 ± 1.7^{cde}	13.0 ± 1.2^{a}
R-Trag-XG 1.0%	1.360 ± 0.030^{ab}	190.1 ± 2.0^{h}	166.8 ± 5.1^{de}	12.3 ± 2.3^{a}
Rice	1.716 ± 0.003^{de}	204.0 ± 0.1^{j}	166.2 ± 0.1^{de}	18.6 ± 0.1^{b}

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

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

Table 25: Average values of rice bread hardness and moisture content prepared with tragacanth blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
R-Trag-Agar 0.5%	13.4 ± 0.6^{cd}	36.3 ± 3.9^{cd}	$51.848 \pm 0.074^{hijklmnopq}$	51.145 ± 0.202^{ijklm}
R-Trag-Agar 1.0%	17.3 ± 0.8^{e}	41.8 ± 2.5^{de}	$52.142 \pm 0.097^{ijklmnopqr}$	51.453 ± 0.238^{lmn}
R-Trag-Alg 0.5%	8.5 ± 0.8^{ab}	17.3 ± 2.3^{ab}	$53.176 \pm 0.593^{rstuvw}$	54.814 ± 0.075^{r}
R-Trag-Alg 1.0%	12.4 ± 1.5^{cd}	20.9 ± 1.8^{ab}	$53.743 \pm 0.126^{vwxyza'}$	54.328 ± 0.052^{pqr}
R-Trag-CG 0.5%	22.2 ± 3.8^{f}	90.5 ± 10.6^{h}	49.523 ± 0.067^{abc}	48.722 ± 0.271^{abcd}
R-Trag-CG 1.0%	14.5 ± 1.2^{de}	48.5 ± 9.9^{ef}	$51.716 \pm 0.111^{hijklmno}$	$50.921 \pm 0.211^{hijklm}$
R-Trag-Carrag 0.5%	21.1 ± 1.1^{f}	61.9 ± 11.8^{g}	50.958 ± 0.172^{defgh}	$50.428 \pm 0.124^{efghijklm}$
R-Trag-Carrag 1.0%	24.1 ± 0.5^{f}	53.3 ± 6.3^{fg}	52.999 ± 0.526^{rstuv}	51.894 ± 0.010^{mno}
R-Trag-Cel 0.5%	11.9 ± 2.4^{cd}	20.4 ± 1.6^{ab}	$54.098 \pm 0.428^{wxyza'b'}$	54.417 ± 0.073^{qr}
R-Trag-Cel 1.0%	10.7 ± 1.3^{bc}	19.4 ± 2.1^{ab}	53.280 ± 0.019^{tuvwx}	54.523 ± 0.050^{qr}
R-Trag-Gel 0.5%	7.1 ± 0.6^{a}	15.0 ± 0.5^{a}	$52.858 \pm 0.109^{qrstuv}$	54.331 ± 0.010^{qr}
R-Trag-Gel 1.0%	8.4 ± 0.1^{ab}	14.3 ± 1.8^{a}	53.029 ± 0.302^{rstuv}	54.308 ± 0.147^{pqr}
R-Trag-XG 0.5%	8.5 ± 0.9^{ab}	17.6 ± 1.7^{ab}	$51.784 \pm 1.873^{hijklmnop}$	54.449 ± 0.272^{qr}
R-Trag-XG 1.0%	12.3 ± 3.4^{cd}	23.3 ± 5.7^{ab}	$52.339 \pm 1.108^{mnopqrst}$	54.404 ± 0.320^{qr}
Rice	13.9 ± 1.2^{cd}	27.0 ± 2.3^{bc}	$54.841 \pm 0.210^{b'c'd'}$	54.801 ± 0.113^{r}

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

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

4.7.8 Xanthan gum blends

Tables 26 and 27 summarize the results of xanthan gum and rest seven hydrocolloids in the portion of 0.5 and 1.0% in the rice sample. All blends have already been discussed above, but this table is necessary to evaluate the effect of the blends in the context of xanthan gum.

Baking test

The only remarkable result among these blends presented xanthan gum in combination with alginate and applied into the rice sample in 1.0% portion. This blend was able to significantly improve the loaf specific volume from 1.716 $cm^3 g^{-1}$ (rice) to 1.809 $cm^3 g^{-1}$. Other blends significantly deteriorated loaf bread volume and the lowest value fell to 1.278 $cm^3 g^{-1}$ (xanthan gum-agar 1.0%) and there is no evidence that higher portion of hydrocolloid blend in the sample improves the loaf specific volume more than the lower portion (except for xanthan-alginate).

Satisfactory results of dough yield proved the sample which exceeded 190% (xanthan gum-alginate 0.5 and 1.0%, xanthan gum-cellulose 0.5 and 1.0% and xanthan gum-tragacanth 1.0%). Other samples reached values between 171.5 (xanthan gum-gelatine 0.5%) and 118.7% (xanthan gum-tragacanth 0.5%). All values were significantly lower than the rice check sample (204.0%).

Any xanthan gum blend significantly improved the bread yield, but almost half of them reached very similar value ranging from 160.7 to 166.8% as the rice check sample (166.2%). Remaining blends had significantly lower bread yield and the lowest value was calculated fort the xanthan gum blend with gelatine (0.5 and 1.0%).

The biggest baking loss presented clear rice check sample (18.6%). All xanthan gum blends significantly improved this parameter. It was proved that the higher portion of xanthan gum blended with agar, cellulose and tragacanth had lower baking loss compared to their 0.5% portion, but the differences were not significant. The lowest value was found for xanthan gum-agar 1.0% (11.9%); it was an improvement by 36% compared to the rice check sample (18.6%).

Table 26: Average values of rice bread characteristics prepared with xanthan gum blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Loaf specific volume ($cm^3\ g^{-1}$)	Dough yield (%)	Bread yield (%)	Baking loss (%)
R-XG-Agar 0.5%	1.569 ± 0.010^{d}	182.1 ± 3.2^{cde}	159.8 ± 3.5^{bcd}	12.3 ± 3.2^{a}
R-XG-Agar 1.0%	1.278 ± 0.006^{ab}	177.7 ± 0.5^{bc}	156.5 ± 2.0^{bc}	11.9 ± 0.5^{a}
R-XG-Alg 0.5%	1.582 ± 0.054^{d}	191.7 ± 2.9^{gh}	165.5 ± 2.8^{def}	13.6 ± 2.8^{ab}
R-XG-Alg 1.0%	1.809 ± 0.049^{f}	193.6 ± 3.4^{gh}	166.9 ± 4.0^{f}	13.8 ± 1.5^{ab}
R-XG-CG 0.5%	1.618 ± 0.007^{d}	182.7 ± 1.3^{de}	160.7 ± 1.4^{cde}	17.1 ± 0.5^{a}
R-XG-CG 1.0%	1.379 ± 0.032^{bc}	177.9 ± 2.0^{bcd}	154.7 ± 3.4^{ab}	18.4 ± 0.7^{a}
R-XG-Carrag 0.5%	1.522 ± 0.015^{cd}	179.4 ± 2.2^{cde}	156.6 ± 2.0^{bc}	12.7 ± 0.1^{a}
R-XG-Carrag 1.0%	1.479 ± 0.014^{cd}	184.1 ± 4.0^{ef}	160.0 ± 2.0^{bcd}	13.1 ± 1.0^{a}
R-XG-Cel 0.5%	1.616 ± 0.007^{d}	194.6 ± 0.7^{h}	167.1 ± 0.8^{f}	14.1 ± 0.2^{abc}
R-XG-Cel 1.0%	1.600 ± 0.039^{d}	194.3 ± 2.0^{h}	167.8 ± 5.3^{f}	13.7 ± 2.7^{ab}
R-XG-Gel 0.5%	1.433 ± 0.047^{bc}	171.5 ± 0.8^{a}	150.6 ± 0.4^{a}	14.5 ± 2.9^{bcd}
R-XG-Gel 1.0%	1.367 ± 0.125^{bc}	173.2 ± 1.1^{ab}	150.6 ± 0.6^{a}	16.9 ± 4.8^{cd}
R-XG-Trag 0.5%	1.544 ± 0.002^{cd}	188.7 ± 4.5^{fg}	164.1 ± 1.7^{def}	13.0 ± 1.2^{a}
R-XG-Trag 1.0%	1.360 ± 0.030^{bc}	190.1 ± 2.0^{gh}	166.8 ± 5.1^{f}	12.3 ± 2.3^{a}
Rice	1.716 ± 0.003^{e}	204.0 ± 0.1^{i}	166.2 ± 0.1^{ef}	18.6 ± 0.1^{d}

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

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

Table 27: Average values of rice bread hardness and moisture content prepared with xanthan gum blends 0.5 and 1.0% (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
R-XG-Agar 0.5%	17.5 ± 2.0^{e}	49.3 ± 5.0^{c}	$51.156 \pm 0.094^{defghi}$	$50.577 \pm 0.165^{fghijklm}$
R-XG-Agar 1.0%	42.1 ± 2.4^{h}	110.7 ± 9.6^{f}	48.445 ± 0.105^{a}	48.511 ± 0.046^{bcd}
R-XG-Alg 0.5%	8.0 ± 0.4^{a}	23.9 ± 2.8^{ab}	54.772 ± 0.003^{tuvw}	54.318 ± 0.172^{nop}
R-XG-Alg 1.0%	6.8 ± 0.4^{a}	21.2 ± 3.6^{ab}	54.713 ± 0.006^{stuvw}	54.216 ± 0.072^{nop}
R-XG-CG 0.5%	17.0 ± 1.8^{de}	43.6 ± 7.7^{c}	$51.889 \pm 0.052^{ghijklmnop}$	48.998 ± 0.604^{bcde}
R-XG-CG 1.0%	50.1 ± 1.7^{i}	80.3 ± 13.6^{e}	50.627 ± 0.089^{bcdef}	$49.927 \pm 0.408^{cdefghijkl}$
R-XG-Carrag 0.5%	25.4 ± 4.0^{f}	59.8 ± 5.4^{d}	$51.198 \pm 0.044^{defghijk}$	47.889 ± 0.235^{ab}
R-XG-Carrag 1.0%	32.0 ± 1.1^{g}	67.1 ± 3.8^{d}	$51.946 \pm 0.285^{ghijklmnop}$	$50.916 \pm 0.052^{hijklm}$
R-XG-Cel 0.5%	9.2 ± 0.3^{ab}	19.7 ± 1.5^{ab}	54.709 ± 0.101^{stuvw}	54.557 ± 0.085^{op}
R-XG-Cel 1.0%	8.7 ± 0.5^{ab}	19.9 ± 0.9^{ab}	54.615 ± 0.038^{stuvw}	54.304 ± 0.140^{nop}
R-XG-Gel 0.5%	53.0 ± 1.3^{i}	84.5 ± 2.1^{e}	$51.203 \pm 0.122^{defghijk}$	54.623 ± 0.011^{op}
R-XG-Gel 1.0%	44.3 ± 0.8^{h}	62.6 ± 1.2^{d}	$52.312 \pm 0.400^{lmnopqr}$	54.820 ± 0.231^{p}
R-XG-Trag 0.5%	8.5 ± 0.9^{a}	17.6 ± 1.7^{a}	$51.784 \pm 1.873^{ghijklmno}$	54.449 ± 0.272^{op}
R-XG-Trag 1.0%	12.3 ± 3.4^{bc}	23.3 ± 5.7^{ab}	$52.339 \pm 1.108^{lmnopqr}$	54.403 ± 0.320^{op}
Rice	13.9 ± 1.2^{cd}	27.0 ± 2.3^{b}	54.841 ± 0.210^{tuvw}	54.801 ± 0.113^{p}

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

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

Hardness 24 and 72 hours after baking

Not many samples presented better values of hardness 24 h after baking. The lowest and significantly different results showed xanthan gum blends with alginate 0.5 and 1.0% (8.0 and 6.8 N), cellulose 0.5 and 1.0% (9.2 and 8.7 N) and tragacanth 0.5% (8.5 N). On the other hand, the hardest sample was xanthan gum-gelatine 0.5%.

As presented above, the samples with hydrocolloid blends proved the higher or lower worsening of the hardness 72 h after baking. Compared to the rice check sample that deteriorated from 13.9 N (24h) to 27 N (72 h) therefore by 48.5% only 4 (xanthan gum blends with carob gum 1.0%, gelatine 0.5 and 1.0% and tragacanth 1.0%) from 14 remaining samples kept the deterioration under 50%.

Moisture content 24 and 72 hours after baking

The xanthan gum blends kept the moisture content 24 h after baking and staling between 48.4% (agar 1.0%) and 54.8% (rice check sample) and got very similar results 72 h after baking (48.5 to 54.8%).

4.8 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 $cm^3 g^{-1}$, 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 buckwheat-rice 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 Tables 28 and 29. For the results to be complete and for imagination, the rice check sample was added.

4.8.1 Baking Test

The buckwheat-rice (BR) sample reached the loaf specific volume only 1.300 $cm^3 g^{-1}$ that was significantly the lowest value. All hydrocolloid blends improved the loaf specific volume to 1.718 $cm^3 g^{-1}$ (agar-cellulose 0.5%), 1.788 $cm^3 g^{-1}$ (alginate-xanthan gum 1.0%). 1.767 $cm^3 g^{-1}$ (carrageenan-gelatine 0.5%), 1. 828 $cm^3 g^{-1}$ (cellulose-gelatine 1.0%), 1.811 $cm^3 g^{-1}$ (gelatine-tragacanth 0.5%) and 1.850 $cm^3 g^{-1}$ (alginate-cellulose 0.5%) and 1.870 $cm^3 g^{-1}$ (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%).

Figure 4 shows digital images of the bread crumb and Figure 5 presents differences in bread crust. All final samples displayed satisfactory porosity compared to the rice sample. It can be concluded, that BR sample (ch) and BR sample with the combination of Carrageenan and Cellulose 0.5% w/w gave either the best porosity, but not very regular. Other samples' crumbs revealed smaller pores, thus bigger density. Figure 5 shows that samples' crust probably did not reach solid crust. All crusts were cracked but crispy.

Table 28: 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.

Table 29: Average values of buckwheat-rice bread hardness and moisture content prepared with selected hydrocolloid blends in specific portions (w/w, flour basis)*

Sample	Hardness 24 h (N)	Hardness 72 h (N)	Moisture content 24 h (%)	Moisture content 72 h (%)
BR 4060	17.1 ± 0.3^{c}	28.9 ± 0.2^{de}	55.44 ± 0.10^{bc}	54.63 ± 0.13^{ab}
BR-Agar-Cel 0.5%	26.4 ± 2.0^{ab}	47.2 ± 2.2^{f}	52.46 ± 0.11^{d}	51.33 ± 0.13^{c}
BR-Alg-Cel 0.5%	18.6 ± 2.1^{cd}	33.2 ± 4.2^{ae}	55.06 ± 0.16^{ab}	54.51 ± 0.13^{ab}
BR-Alg-XG 1.0%	26.2 ± 0.2^{a}	41.7 ± 2.5^{c}	55.18 ± 0.18^{abc}	54.93 ± 0.03^{a}
BR-CG-Cel 0.5%	28.9 ± 0.6^{b}	50.2 ± 0.6^{f}	49.76 ± 0.18^{e}	48.96 ± 0.15^{d}
BR-Carrag-Gel 0.5%	27.3 ± 1.2^{ab}	37.2 ± 4.6^{abc}	52.68 ± 0.11^{d}	51.83 ± 0.24^{c}
BR-Cel-Gel 1.0%	21.4 ± 0.8^{e}	37.9 ± 1.7^{bc}	55.20 ± 0.13^{abc}	54.84 ± 0.30^{a}
BR-Gel-Trag 0.5%	20.0 ± 0.7^{de}	35.7 ± 2.5^{ab}	55.59 ± 0.15^{c}	54.24 ± 0.22^{b}
Rice	13.9 ± 1.2^{f}	27.0 ± 2.3^{d}	54.84 ± 0.19^{a}	54.80 ± 0.11^{ab}

*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.

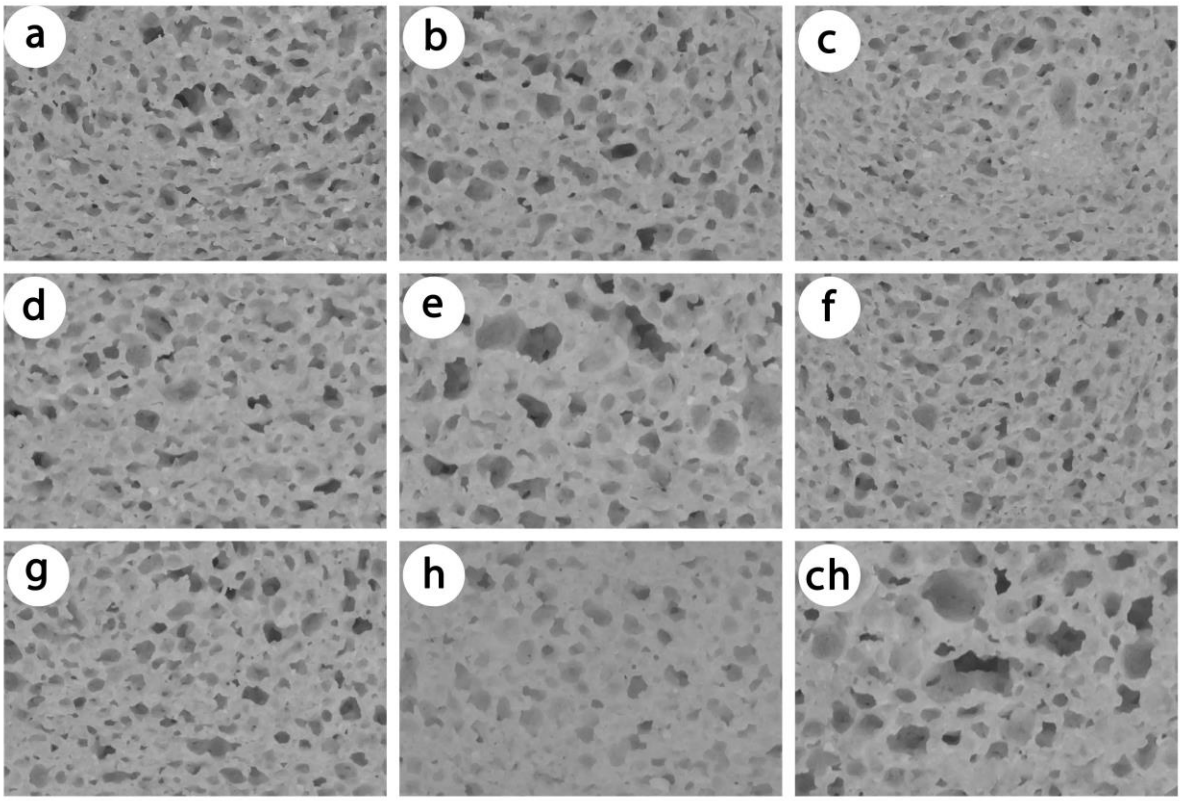


Figure 4: Differences in crumb porosity of buckwheat-rice bread with hydrocolloid blends
a: RB-Agar-Cel 0.5%. b: RB-Alg-Cel 0.5%. c: RB-Alg-XG 1.0%. d: RB-CG-Cel 0.5%. e: RB-Carrag-Gel 0.5%. f: RB-Cel-Gel 1.0%. g: RB-Gel-Trag 0.5%. h: rice. ch: RB 6040

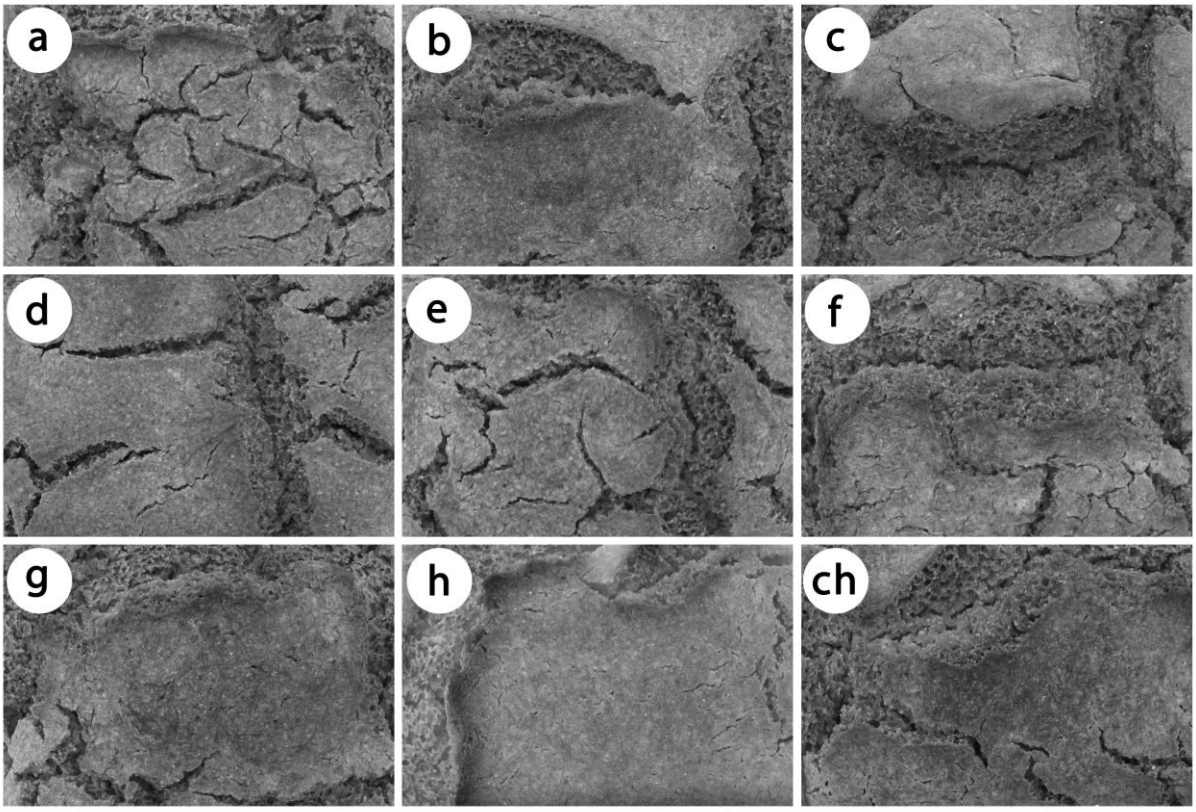


Figure 5: Differences in crust of buckwheat-rice bread with hydrocolloid blends a: RB-Agar-Cel 0.5%. b: RB-Alg-Cel 0.5%. c: RB-Alg-XG 1.0%. d: RB-CG-Cel 0.5%. e: RB-Carrag-Gel 0.5%. f: RB-Cel-Gel 1.0%. g: RB-Gel-Trag 0.5%. h: rice. ch: RB 6040

4.8.2 Hardness 24 and 72 hours after baking

The lowest hardness 24 h after baking was measured for alginate-cellulose 0.5% (18.6 N) and this was the closest value compared to the BR sample (17.1 N); this result was not significantly different. Remining hydrocolloid combinations significantly hardened the hardness 24 h after baking. Very similar trend was observed for the hardness 72 h where the lowest hardness among the hydrocolloid blends was measured again for alginate-cellulose 0.5% (33.2 N) but compared to the BR sample (28.9 N), the result was not significantly different. Other samples presented hardness 72 h between 35.7 N (gelatine-tragacanth 0.5%) and 50.2 N (carob gum-cellulose 0.5%) which was significant deterioration.

4.8.3 Moisture content 24 and 72 hours after baking

All samples (except for carob gum-cellulose 0.5%) presented very similar results of moisture content 24 and 72 h after baking, exceeding 50%. Compared to the BR sample none of the hydrocolloid blends was able to significantly improve the moisture content 24h. Three significant differences were found but proving the worsening effect (carob gum-cellulose 0.5% – 49.8%,

agar-cellulose 0.5% – 52.5% and carrageenan-gelatine 0.5% – 52.7%). All samples' moisture content 24 h deteriorated in time but by 1.4% maximum (gelatine-tregacanth 0.5%). The best result 72 h after baking (54.8%) was found for the sample of cellulose-gelatine 1.0%, but not significantly different from the BR (54.6%). Remaining samples did not reach the BR value and carrageenan-gelatine 0.5% (51.8%), agar-cellulose 0.5% (51.3%) and carob gum-cellulose 0.5% (49.0%) had significantly worse moisture content 72 h compared to the BR sample.

5. 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 enhanced the customers' most important value – bread volume, 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.

6. 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 buckwheat-rice 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 and the highest hardness reached the 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 cm^3 g^{-1} but only agar-cellulose sample showed satisfactory hardness.

The functionality of buckwheat-rice 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, hydrocolloids 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.

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

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Doctoral Thesis

Published by Tomas Bata University in Zlín

nám. T. G. Masaryka 5555, 760 01 Zlín.

This publication has not been edited

Year of publishing 2018