BEAN STARCH AS INGREDIENT FOR GLUTEN FREE BREAD

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ABSTRACT

Commercially available gluten free breads are of low quality and have a rapid staling during storage, therefore attempts were made to obtain gluten free bread of improved structure properties and extended shelf-life. For this purpose laboratory obtained bean starch, both native or hydrothermally modified, was added to a gluten free formulation. Texture results revealed differences between the bottom -harder, and upper -softer part of fresh bread containing native bean starch. Modified starch reduced the hardness and diminish the differences between the upper and the bottom part of bread slice. Independently of storage duration breads were crumbly. Considerable decrease of the peak and final viscosity was observed with the increase of the storage time in sample with native bean starch, whereas the presence of modified starch induced the opposite tendency. The addition of native starch increased the tendency of amylopectine to retrograde during storage, whereas the presence of modified starch decreased the retrogradation enthalpy by 16%.

PRACTICAL APPLICATION

Gluten free breads exhibite dry crumbling crumb, poor mouthfeel and flavour. To gain approval, gluten free bread need to resemble wheat flour bread. This research provided the preliminary guidance to apply bean starch as the ingredient of gluten free bread and determine its influnce on the structure and texture behaviour of crumb during storage.

Key words: gluten free bread, bean starch, storage, texture, RVA, DSC

INTRODUCTION

Epidemiological studies have shown that the prevalence of coeliac disease remains significantly underestimated (Fasano and Catassi 2001). The reason for that state arose from the problem with diagnosis, as in the most cases that intestine condition has no symptoms, or the symptoms have not been linked to coeliac disease. To accurately evaluate the true prevalence of coeliac disease, the modern and more sensitive methods of screening must be applied. Coeliac disease, first considered to be a gastrointestinal disease, is a gluten-sensitive entheropathy with a genetic, immunologic and environmental background. The factors responsible for that primary life-long intolerance in genetically predisposed individuals are wheat gliadins and prolamins of rye, barley and possibly oats (Murray 1999). Those peptides, released during digestion, are toxic for coeliac patients, whereas corn, buckwheat and rice proteins are considered to be safe. The reaction to gluten ingestion by patients suffering from that chronic disease is inflammation of the small intestine leading to the malabsorption of several important nutrients including minerals, folic acid and fat-soluble vitamins (Kelly *et al.* 1999). Although the numerous cases of “silent” or “latent” coeliac disease remain undiagnosed, recent studies suggest an increasing prevalance of that disease of up to 1 in 200-300 (Holmes 2001), likely due to the development of new serological tests. Therefore, the apparent or real increase in coeliac disease, gluten-sensitive enteropathy (GSE) or other allergic reactions/intolerances to gluten parallels the rising demands for gluten free products. The only effective treatment for coeliac disease is a strict adherence to a gluten-free diet throughout the patient’s life, which, in time results in clinical and mucosal recovery.

At present, the majority of commercially available gluten free breads are of low quality, exhibiting dry crumbling crumb, resulting in poor mouthfeel and a poor flavour. To gain approval the quality of gluten free breads and its sensory characteristics must be similar to those obtained from wheat flour bread. Gluten is the main structure-forming protein in flour, and is responsible for the elastic characteristics of dough, and contributes to the appearance and crumb structure of many baked products. Bread is a composite solid comprised, at a microscopic level, of phases- a fluid (air) and a solid (cell wall material) (Scanlon *et al*. 2000). That lack of homogeneity causes even greater differences in the mechanical properties of bread crumb within a single loaf than between loaves of different treatments (Ponte and Faubion 1987). The solid part of bread contains a continous phase composed of an elastic network of cross-linked gluten molecule and leached starch polymer molecules, primary amylose, and a discontinuous phase of entrapped, partially gelatinized, swollen, deformed starch granules (Gray and BeMiller 2003). The lack of gluten proteins makes it very difficult to obtain an acceptable yeast-leavened bread because of the absence of a proper network necessary to hold the carbon dioxide produced during proofing. Furthermore, gluten-free breads elaborated with different starches age very fast (Defloor *et al*. 1993). Bread staling manifests as the physico-chemical and structural changes observed both, in crust and in crumb. During storage the crust loss its crunchiness, whereas the increase of the crumb firmness is observed. Starches and hydrocolloids are widely used in the bakery industry to impart texture and appearance properties to cereal-based foods (Ward and Andon 2002). Different approches have been developed to extend the freshness of gluten free bread. A number of additives have been used in order to retard its crumb firming, among them a range of starches with several emulsifiers, enzymes (α-amylases, hemicellulases, lipases), and hydrocolloids (carboxymethylocellulose, guar gum, alginate, xantan) (Gujral *et al*. 2003; Gujral *et al*. 2004).

The aim of the current study was to investigate the effects of bean starch from non-processed and autoclaved seeds incorporated into a gluten free formulation on the baking characteristics, crumb properties and the shelf-life of the gluten free bread.

MATERIALS AND METHODS

Materials

The raw materials used for the gluten free formulation were: corn starch (AGROTRADE, Warsaw, Poland) and potato starch (Niechlów, Poland). A sunflower oil (Bartek from ZPT Warsaw, Poland) and fresh yeast were used. The premix ingredients, salt, sugar, guar gum (IGGUARFG-33, E 412 from HORTIMEX, Konin, Poland) and pectin (E 440(i) from ZPOW PEKTOWIN, Jasło, Poland) were commercially available.

Bean starch was obtained in the laboratory conditions from non-processed and autoclaved bean seeds (101 kPa, 121˚C, 16 min, seeds:water w/w, 1:3) according to Soral-Śmietana method (1993) in a triple extraction with distilled water (1:6 w/v), then double extraction with 0.1% NaOH (1:6 w/v). Starch samples were recovered by 10 min centrifugation (1800 x g) and freeze-dried. Dry starch was further ground and sieved through a 160-µm sieve. Bean starch was added to the experimental formulation, and its amount constituted the equivalent of ten percent of amount of corn starch.

Bread was made according to modified prescription in Polish Standard PN-A-74123:1997 for gluten free bread. Our modification concerned the utilisation of guar gum and pectin altogether. Water (460 mL) was added to a mix of solid ingredients (Table 1) and mixed at speed 4 for 12 min. A KitchenAid Professional K45SS (USA) mixer with stainless steel bowl and flat beater was used.

A sample of the resulting batter was placed in a greased bread pan and proofed at 35-40˚C and 70% humidity for 40 min. Bread was then baked at 210˚C for 40 min in the laboratory oven with electric heating and temperature control.

Bread storage at low temperature

After baking all breads were cooled at room temperature for 2 h. Each loaf of bread was packed in a clip-on polyethylene bag and were stored for 1, 3, 6 and 10 days in a temperature controlled cabinet (POL-EKO-APARATURA, Wodzisław Śląski, Poland) at 4˚C.

Chemical composition

The contents of total starch (AOAC 1975) and ash (AOAC 1990) were estimated in both, starch samples and freeze dried crumbs of gluten free breads. Besides, total proteins content was determined using the standard method estimated by calculating total nitrogen using Kjeldahl method (AOAC 1990) and multiplied by factor 6.25 or 5.70, for starch and bread crumb respectively. Analysis of resistant starch (RS) content was carried out according to Champ *et al.* (1999).

Loaf weight, volume and specific volume

Loaves were evaluated 2 h after baking. The weight of bread samples was determined using a digital balance (0.01g accuracy) and loaf volume was determined by a bread volumeter using millet seeds displacement method. The specific volume of each loaf was then calculated as:

$$\text{Specific volume (cm}^3\text{/g)} = \text{loaf volume/loaf mass}$$

Characteristic of bread texture

Texture properties of crumbs were measured using compression device of Instron 1011 (Instron Ltd, High Wycombe, England). The crumb samples of fresh bread (20 × 20 × 20 mm) were twice compressed until 70% strain at crosshead speed 20 mm/min. Hardness expressed as maximum force during first compression, F_1 (kPa), elasticity and cohesiveness expressed as ratio of maximum forces, F_2/F_1 (-), and energies, E_2/E_1 (-), determined in both compressions, and gumminess characterized by expression, E_2 x F_1/E_1 (kPa), were calculated according to Mohan and Skiner (1986). Additionally, crumb springiness was described by volume recovery expressed as ratio of sample volumes before second and first compression, V_1/V_2 (-) according to Sadowska *et al*. (1999). Eight replicates were made for each loaf. Fracture stress F_f (kPa) and strain (%) were the parameters describing the resistance for compression of bread crumb stored for 1, 3, 6, and 10 days.

Viscosity analysis (RVA)

Gluten free bread crumbs were dried at 30˚C overnight in a laboratory oven. The dried crumbs were carefully pulverized using a grinder. To determine the RVA viscosities of the gluten free bread crumbs with addition of bean starch, the Rapid Visco TM Analysed (RVA) (Newport Scientific Pty LTD., Australia) was used as previously described (Collar 2003). Pasting properties were determined following the Newport Scientific Corn Starch Method. The heating cycle was 50 to 95˚C in 282 s and holding at 95˚C for 150 s and then cooling to 50˚C. Each cycle was initiated by a 10 s mixing at 960 rpm paddle speed, and 160 rpm rpm paddle speed was used for the rest of the test. To evaluate the RVA viscosities, 3.5 g of grounded crumb were weighed into RVA aluminium canister; 25 mL of distilled water was added to make slurry prior to viscosity analysis. The parameters recorded were final viscosity (CPV), peak viscosity (PV), holding (HPV), breakdown (PV-HPV) and setback (CPV-HPV) as well as the time and temperature to reach peak viscosity.

Differential scanning calorimetry (DSC)

Analysis was performed in a DSC-7 (Perkin-Elmer, USA), using stainless steel pans. Briefly, ten mg of pulverized bread crumbs were precisely weighed and loaded into the stainless steel pans (Perkin-Elmer) and distilled water was added (20 μL). Samples were hermetically sealed by using a press. An empty pan was used as a reference. The endotherms were analysed by the system programme (Pyris Toolbars Application, version 3.01). A constant scan rate from 30 to 110˚C was used for determination of the onset (T_o), peak (T_p), and conclusion (T_c) temperatures (˚C), as well as the gelatinisation enthalpy (ΔH), expressed as J/g of dry matter.

Statistical analysis

The statistical analysis of the results was carried out with a Statistica ver.5 (StatSoft, USA) software using variance analysis Anova (General Convention and Statistics (1995) In: Statistica for Windows. vol I-III, 2nd Edition Statsoft Inc. (ed), Tulsa, USA).

RESULTS AND DISCUSSION

Chemical characteristic

The chemical characteristic of starch obtained from non-processed or autoclaved bean seeds and gluten free breads containing bean starch (A – bread with starch of non-processed seeds; B - bread with starch of autoclaved seeds) is presented in Table 2. Negligible amounts of proteins and mineral compounds in starch sample of non-processed bean seeds indicated its high purity, whereas starch obtained from bean seeds subjected to autoclave processing was recognized as a starch-protein complex, with constituent's ratio of 3:1. Our previous study (Krupa *et al.* 2007) establihed that starch obtained from microwaved bean seeds was also a starch-protein complex. The comparison of control gluten free bread with breads A and B did not show the meaningful differences in ash content, while the content of starch was higher in control bread. Bread B, with addition of starch preparation rich in proteins, was characterised with the highest proteins content. High resistant starch (RS) content in native bean starch did not increase its level in bread A. RS, a component of dietary fibre (Soral-Śmietana 2000), plays important physiological and biological functions (Soral-Śmietana and Wronkowska 2004; Soral-Śmietana *et al.* 2005). It is not digested in the small intestine and as a component of carbohydrates passes into the large intestine where it is a valuable source of carbon and energy for intestine bacteria (Wronkowska *et al.* 2006).

Size-related characteristic

Results of size-related parameters analysis of gluten free breads are shown in Table 3. Higher loaf weight and volume affect in a positive way the retail of bread and is perceived as an evidence of its high quality. Therefore, the reduction of loaf size during baking process is undesirable. Shittu *et al.* (2007) indicated that baking time and temperature affect the physical properties of bread of cassave-wheat flour. The volume of assessed gluten free breads, the control bread and bread containing native bean starch (A), were similar. Loaf volume is affected by the quantity and quality of proteins in the flour (Ragaee and Abdel-Aal 2006) as well as proofing time (Zghal *et al.* 2002), therefore the presence of starch of autoclaved bean seeds in bread B did not influence significantly its volume value (Table 3). The mass of all evaluated bread loaves was similar and reached about 250 g. That parameter is basically determined by the quantity of dough baked, and the amount of moisture and carbon dioxide diffused out of the loaf during baking. The specific volume, which is the ratio of volume and mass, stays in direct proportion to volume. Many studies have been performed to improve the specific volume of gluten free bread from rice flour (Ylimaki *et al.* 1988; Kang *et al.* 1997). Gujral *et al.* (2003) stated that increasing level of HPMC, oil, and the type of α-amylase incorporated into bread from rice flour increased the specific volume. Generally, the value of specific volume of analysed gluten free breads was higher than states Polish Standard for gluten free bread (Polish Standard PN-A-74108) and was close to the values for wheat bread (Polish Standard PN-A-74123), however, incorporation of modified bean starch into a gluten free bread diminished its value, although not significantly.

Texture profile analysis

The evaluation of the texture properties of bread crumb is necessary for its quality assurance and consumers acceptibility. Besides, it is important for assessing the effects of addition of various dough ingredients and processing conditions. The texture profile analysis of fresh experimental gluten free bread crumbs included hardness, gumminess, elasticity, cohesiveness and recovery of volume (Figure 1 and 2). Significant differences were observed between the upper and the bottom part of the bread slice. The analysis of hardness indicated that the bottom part of bread slice was harder in comparison with its upper part. Those diversity were observed especially in the case of bread containing native bean starch (A), where the bottom part was five-times harder than the upper part (Figure 1). The early research of Short and Roberts (1971) ascertained that bread crumb firmness varied across bread slice with the highest values in the center. Bread containing starch of autoclaved bean seeds was less hard than control bread and bread A (Figure 1). Besides, the presence of modified starch reduced the differences in hardness between the upper and the bottom part of that bread. The results of gumminess evaluation of experimental bread crumbs indicated only slight differences between the upper and the bottom part of bread slices (Figure 1). The presence of bean starch, native or modified, did not affect maeningful changes in crumb gumminess. The crumb elasticity of the upper and the bottom part of bread slices were generelly similar (Figure 2). In the case of bread crumb containing starch of autoclaved bean seeds the elasticity values exceeded the remain results. Bread B, especially its upper part, was distinguished by the highest cohesiveness of crumb. After compression all analysed bread crumbs recovered their primary volume (Figure 2). The results of texture measurements stated that crumb of fresh gluten free bread contained modified bean starch generally demonstrated a great similarity to wheat bread, though was slightly softer and more elastic than crumb of wheat bread (Soral-Śmietana *et al*. 1998).

Unexpectedly, the shape of stress-strain curves for fresh and stored breads appeared different (Figure 3 and 4). Compression curves of all fresh breads were linear although a slight inflection above 50 - 60% strain, which can be related to the beginning of single cell microcracks, appeared sometimes. In case of experimental gluten free bread stored at low temperature it was not possible to examine the same texture parameters as in fresh bread because its structure was destroyed at very low strain (about 20%). Thus, the parameters of fracture point i.e. fracture stress and strain were accepted as a measure of structure changes of crumb examined during storage (Table 4). Independently of storage duration all examined stored breads were very crumbly. The absence of gluten proteins resulted in the lack of springiness and the elasticity of that bread crumb. The significant differences of texture parameters between upper and bottom layers of breads did not appeared during storage. Thus, statistical estimation of differences between breads stored were performed for both layers separately (Table 4.). Within upper layer the fracture force of all stored breads increased statistically significant between 1 and 3 days of storage. Results of variance analysis showed that the changes of fracture force of control bread stored 3, 6, and 10 days were small creating homogenous Duncan group. The fracture force of bread A (with native bean starch) and B (with starch of autoclaved bean seeds) increased regularly during storage (Table 4). Within bottom layer the fracture force of control and A breads slightly increased during storage but the differences were not significant. Fracture force of bread B increased significantly during storage and statistical calculation led to discriminate two homogenous subsets (1 and 3) and (6 and 10) days of storage. Then, the rheological behavior of control and A bread during storage was similar but the presence of modified bean starch influenced considerably on the texture properties of crumb. Such similarity was confirmed by results of statistical calculation within beginning days of storage. Within upper and bottom layers the fracture force for control and A breads (stored 1 and 3 days) belonged to one Duncan subset and bread B was classified to other one (Table 4). This clear difference disappeared partly after 6 day of storage but bread B showed the lowest fracture force at the beginning and the highest at the end of storage for upper and bottom levels.

Pasting properties

In order to assess the effect of addition of modified bean starch on crumb viscosity behaviour, the pasting properties of gluten free breads were studied. Analysed bean starch, was initially modyfied during bread baking process, therefore it should be treated as gelatinised and retrograded starch. It was expected that its viscosity behaviour would differ from unmodified starch granules. Native starch granules are generally insoluble in water below 50˚C. When they are heated in water beyond a critical temperature, the granules absorb a large amount of water and swell, resulting in an increase in viscosity. Hydrothermally modified starch gels are usually shear thinning, showing lower viscosities. The differences in the viscosity between analysed fresh bread crumbs during heating-cooling cycle are shown in the RVA pasting curves (Figure 5). The pasting curves of crumb of control bread and bread containing native starch of bean seeds shown similar tendency, however, during the heating hold period an increase in the peak viscosity appeared in bread A. It resulted probably from the presence of the native bean starch, which might be not completely gelatinized during the bread baking process. The values of viscosity of crumb B sample, containing hydrothermally modified bean starch, were significantly lower (Figure 5). The presence of starch from autoclaved bean seeds (bread B) was strongly connected with the presence of proteins, which could also affect the pasting properties. Ragaee and Abdel-Aal (2006), studing the pasting properties of selected cereals, found a significant correlation between starch and protein peak viscosity. Results may indicate degree of starch gelatinization, level of proteins denaturation and their interactions. The viscosity parameters of stored gluten free crumbs containing bean starch are showed in Table 5. The differences found between the value of peak viscosity of control 0 and sample A0 were little, however in both cases a considerable decrease of the peak viscosity was observed within the storage time. In the case of crumb from bread B the opposite tendency was observed. The initial peak viscosity (B0) was low, and during storage the increase of its value was observed. That phenomenon could be explained by the presence of hydrothermally modified bean starch within bread crumb, which constituted the additional source of resistant starch in that bread. The debranching and autoclaving processes increased the resistant starch level in banana starch (Gonzalez-Sato *et al.* 2007). Takahashi *et al.* (2002) analysing the effect of heat treatment on physicochemical properties of rice flour ascertained that the peak viscosity in the RVA viscograms decreased with increasing temperature of heat-dry and heat-moisture treatments. The breakdown in viscosity occurred during the holding period when the sample is subjected to a period of constant high temperature (95˚C) and mechanical shear stress. The rate of reduction depends on the temperature and degree of mixing, or shear stress, and the nature of the material itself. High values of breakdown are associated with high peak viscosities, which in turn, are related to the degree of swelling of starch granules during heating. The ability of starches to withstand heating at high temperature and shear stress is an important factor of many processes. Generally, all analysed crumbs showed low values of breakdown (Table 5). However, in contrast with the control bread, in breads A and B the significant reduction of breakdown values during storage followed. During cooling from 95˚C to 50˚C, re-association between starch molecules resulted in the formation of a gel structure, therefore the viscosity increased to the final value. Final viscosity describes sample's quality, as it indicates the ability of the material to form a viscous paste or gel after cooking and cooling. The final viscosity of the sample A did not differed from control, thereas in the case of sample B its value was significantly lower and increased during storage (Table 5). Brennan *et al.* (2004), evaluating the pasting properties of natural maize starch pasted in the presence of resistant starch, indicated that the final viscosity of the paste was unaffected by the addition of resistant starch, however addition of mixtures of combinations of resistant starch and xanthan gum increased its peak viscosity. The difference measured between final viscosity and holding strength is commonly described as the setback and is related to retrogradation and reordering of starch molecules. The low setback values may indicate lower rate of starch retrogradation and syneresis. There were significant differences in setback values between the control bread and bread containing thermally-modified bean starch. Moreover, during storage the pasting "behaviour" of bread B was counter to the control sample and a significant increase of the setback value was observed thus the retrogradation tendency of the amylose increased.

Starch retrogradation

Starch retrogradation is one of the processes involved in bread staling. The thermal parameters of retrogradation endotherms of analysed crumbs are shown in Table 6. In the case of crumb sample containing native starch (A), similary to control, the onset (T_o), peak (T_p) and conclusion (T_c) temperatures decreased significantly after ten days of storage. Whereas, the retrogradation temperatures of the crumb sample B were not modified during storage. Regarding retrogradation enthalpy, initially the sample containing native bean starch (A) showed very low enthalpy value, similary as in the case of the control sample. After ten days of storage its value increased and reached 0.352 J/g d.m. that corresponded with the widen range of starch retrogradation. Rice bread crumb analysed by Gujeal *et al.* (2003) showed much faster amylopectin retrogradation, whereas the addition of starch hydrolyzing enzymes influence a reduction of the retrogradation enthalpy. The hydrocolloids themselves, depending on the type and supplementation level, and/or in combination with α-amylase lowered retrogradation after storage in rice flour chapati (Gujral *et al*. 2004). The stabilizing effects of the hydrocolloids on starch retrogrdation result from the interactions of them cooperatively with water as well as with starch chains in the mixture (Lee *et al*. 2002). Hydrothermal treatment of bean seeds caused the starch retrogradation. The addition of that modified starch into the gluten free formulation resulted in the high value of the initial retrogradation enthalpy (Table 6). The value of retrogradation enthalpy of the sample B after storage did not change meanigly.

CONCLUSIONS

The addition of modified starch obtained from autoclaved bean seeds to the gluten free formulation increases the protein content in bread. The presence of modified starch reduces crumb hardness and increases its elasticity, besides, diminishes the differences between the upper and the bottom part of bread slice, what makes bread crumb more homogeneous, althougt the bread specific volume lowers. The use of hydrothermally modified bean starch in gluten free bread improved the chemical composition and quality of fresh bread, however, did not extend its freshness during storage.

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FIGURE LEGENDS:

FIGURE 1.
HARDNESS AND GUMMINESS OF FRESH GLUTEN FREE BREAD CRUMBS:
A – with native bean starch; B - with bean starch from autoclaved seeds.

FIGURE 2.
ELASTICITY, COHESIVENESS AND RECOVERY OF VOLUME OF FRESH GLUTEN FREE BREAD CRUMBS: A – with native bean starch; B - with bean starch from autoclaved seeds.

FIGURE 3.
THE EXAMPLE OF DOUBLE COMPRESSION CURVES (UP TO D = 70%) OF FRESH GLUTEN FREE BREAD.

FIGURE4.
THE EXAMPLE OF SINGLE COMPRESSION CURVES FOR STORED GLUTEN FREE BREAD
(arrows - fracture point)

FIGURE 5.
RVA PASTING CURVE SHOWING THE EFFECT OF BEAN STARCH ADDITION ON THE PASTING PROPERTIES OF FRESH GLUTEN FREE BREAD.

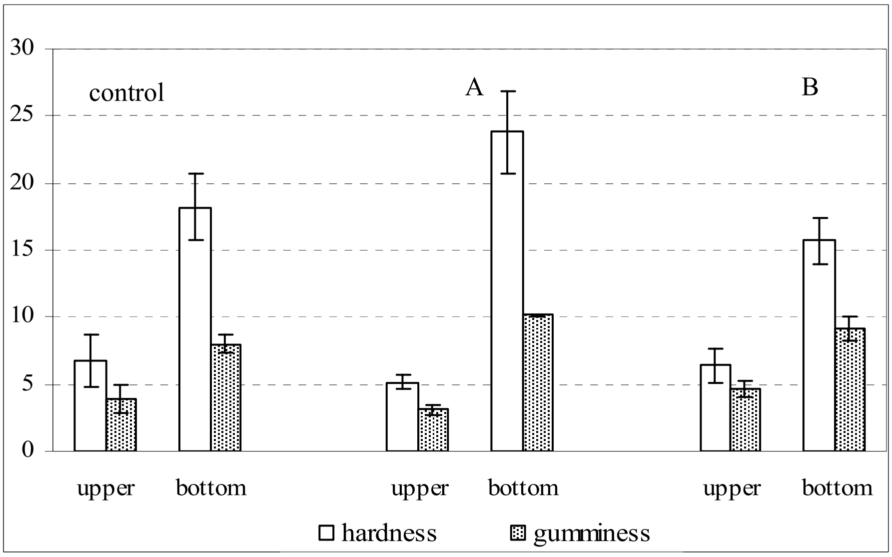

FIGURE 1. HARDNESS AND GUMMINESS OF FRESH GLUTEN FREE BREAD CRUMBS: A – with native bean starch; B - with bean starch from autoclaved seeds.

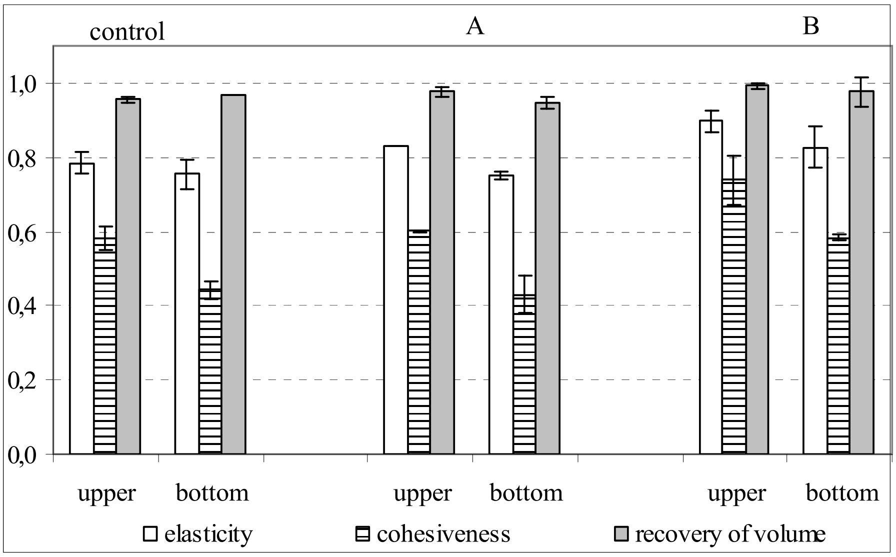

FIGURE 2. ELASTICITY, COHESIVENESS AND RECOVERY OF VOLUME OF FRESH GLUTEN FREE BREAD CRUMBS: A – with native bean starch; B - with bean starch from autoclaved seeds.

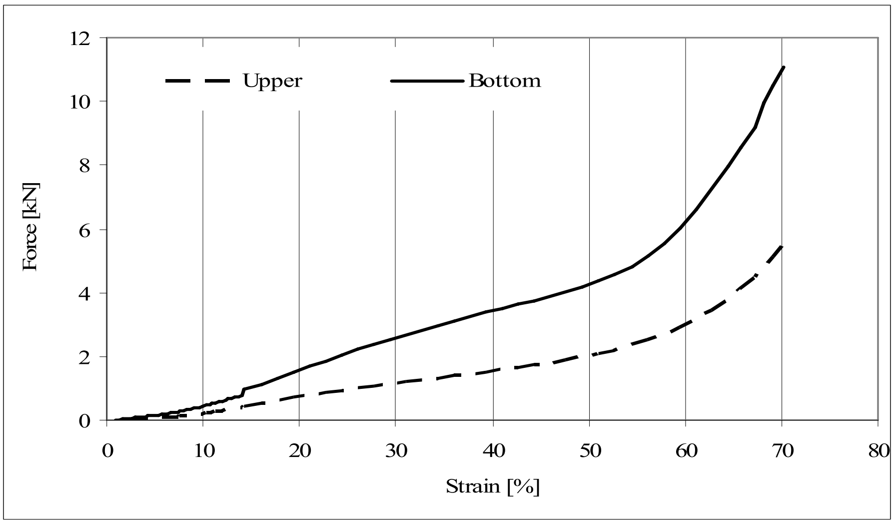

FIGURE 3. THE EXAMPLE OF THE DOUBLE COMPRESSION CURVES (UP TO D = 70%) FOR FRESH GLUTEN FREE BREAD.

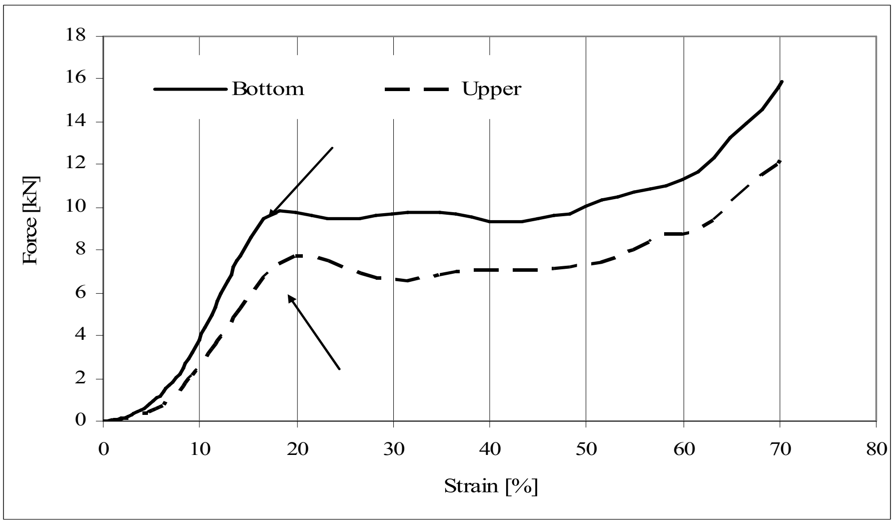

FIGURE 4. THE EXAMPLE OF THE SINGLE COMPRESSION CURVES FOR STORED GLUTEN FREE BREAD (arrows - fracture point)

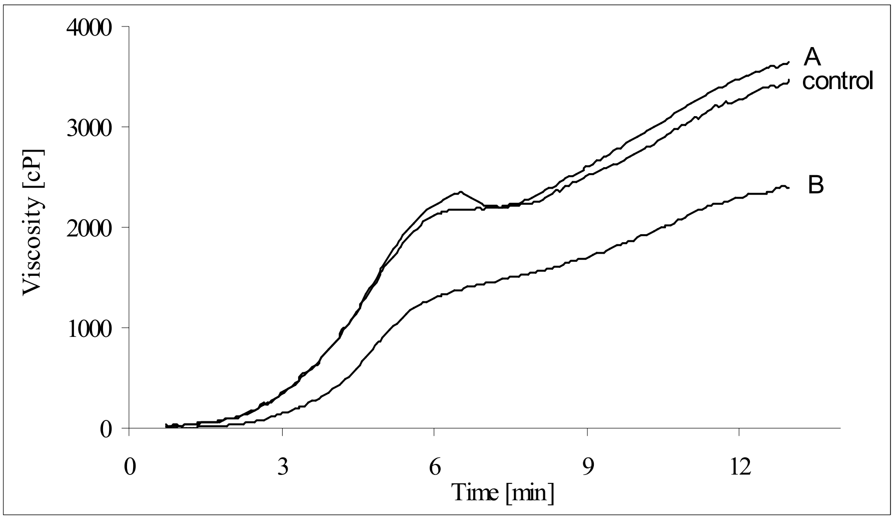

FIGURE 5. RVA PASTING CURVE SHOWING THE EFFECT OF BEAN STARCH ADDITION ON THE PASTING PROPERTIES OF FRESH GLUTEN FREE BREAD.

TABLE 1.
COMPOSITION OF BATTERS

Formulation	Corn starch [g] (1)	Potato starch [g] (2)	Bean starch [g] (3)	Premix [g] (4)	Weight of all solid components*** [g]
Control bread	370	93	-	40	540
Bread A	333	93	37*	40	540
Bread B	333	93	37**	40	540

* Native bean starch.
** Bean starch from autoclaved seeds.
*** Components in column (1) + (2) + (3) + 23 g yeast + 14 g fat.

TABLE 2.
CHEMICAL COMPOSITION OF BEAN STARCH AND GLUTEN FREE BREADS.

Sample	Protein [% d.m.]	Ash [% d.m.]	Starch [% d.m.]	RS [% d.m.]
Starch of non-processed seeds	2.63 ± 0.01	0.40 ± 0.02	95.95 ± 1.55	16.54 ± 1.05
Starch of autoclaved seeds	26.52 ± 0.60	0.84 ± 0.01	70.17 ± 0.95	6.10 ± 0.85
Control bread	1.65 ± 0.16	1.17 ± 0.22	91.99 ± 0.71	4.15 ± 0.23
Bread A	1.40 ± 0.08	1.05 ± 0.11	88.77 ± 0.94	4.34 ± 0.28
Bread B	3.11 ± 0.17	1.03 ± 0.05	85.16 ± 0.69	5.52 ± 0.07

TABLE 3.
SIZE-RELATED PARAMETERS OF GLUTEN FREE BREADS.

Sample	Volume [cm^3]	Weight [g]	Specific volume [cm^3/g]
control	737 ± 70	247 ± 24	2.99
Bread A	724 ± 44	249 ± 13	2.91
Bread B	692 ± 68	250 ± 25	2.77

TABLE 4.
FRACTURE STRESS FOR CRUMB OF GLUTEN FREE BREAD STORED FOR TEN DAYS AT 4˚C.

Sample	Days of storage			
	1	3	6	10
	Upper layer			
Control	13.64 $_{aB}$	15.96 $_{bB}$	16.05 $_{b}$	16.44 $_{bA}$
Bread A	12.13 $_{aB}$	14.61 $_{bB}$	15.32 $_{b}$	19.67 $_{cB}$
Bread B	7.96 $_{aA}$	12.27 $_{bA}$	14.45 $_{c}$	23.92 $_{dC}$
	Bottom layer			
Control	26.92 $_{B}$	27.77 $_{B}$	27.33	31.65
Bread A	29.29 $_{B}$	30.54 $_{B}$	30.53	32.67
Bread B	14.32 $_{aA}$	17.20 $_{aA}$	26.63 $_{b}$	33.59 $_{b}$

The same following letters in the rows denote Duncan’s homogenous subsets for particular breads and the same following capital letters in the columns denote Duncan’s homogenous subsets in particular days. Lack of letters showed lack of statistically significant differences.

TABLE 5.
EFFECT OF BEAN STARCH ADDITION ON THE PASTING PROPERTIES OF GLUTEN FREE CRUMBS STORED FOR TEN DAYS AT 4˚C.

Sample	Peak time [min]	Pasting temperature [˚C]	Peak viscosity [cP]	Holding strengh [cP]	Breakdown [cP]	Final viscosity [cP]	Setback [cP]
Control 0	6.9	73.4	2189	2138	51	3461	1323
Control 10	7.0	88.8	1362	1264	98	2126	862
Bread A0	6.5	68.5	2347	2204	143	3638	1434
Bread A10	6.9	88.8	1307	1240	67	2149	909
Bread B0	6.9	90.3	1441	1308	133	2391	1083
Bread B10	6.2	85.5	1845	1798	47	3215	1417

TABLE 6.
EFFECT OF BEAN STARCH ADDITION ON THE THERMAL PARAMETERS OF ENDOTHERMS OF GLUTEN FREE CRUMBS STORED FOR TEN DAYS AT 4˚C.

Sample	T_o [˚C]	T_p [˚C]	T_c [˚C]	ΔH [J/g d.m.]
Control 0	77.55	84.45	89.50	0.015
Control 10	49.22	56.76	67.48	0.285
Bread A0	77.59	81.72	89.31	0.017
Bread A10	46.65	55.78	69.43	0.352
Bread B0	47.88	55.98	65.54	0.287
Bread B10	50.14	57.34	64.55	0.242

T_o, onset temperature, T_p, peak temperature, T_c, conclusion temperature, ΔH, enthalpy.