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PROPERTIES OF WHOLEMEAL SPELT DOUGH AND BREAD ENRICHED WITH PLANT PROTEINS

KARAKTERISTIKE TESTA I HLEBA OD PUNOG ZRNA SPELTA PŠENICE OBOGAĆENOG BILJNIM PROTEINIMA

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ABSTRACT

The aim of this study was to formulate high protein wholemeal spelt bread with addition of soy and pea protein preparations. Substituting wholemeal spelt flour with 11.5 % soy protein concentrate and 8 % pea protein isolates are an improvement for dough properties. Soy protein concentrate contains 65 % protein, and pea protein isolate contains 90 % protein so in order to achieve the same level of protein content of the bread it is necessary to enter a larger amount of soy protein concentrate than the pea protein isolates.

The stability of dough with the addition of soy protein concentrate was higher than dough with pea protein isolates, probably due to different protein behavior and difference in distribution of the protein classes in soybeans and peas. Furthermore, protein intake in the dough contributes to the increasing of its hardness. Addition of the above mentioned protein preparations leads to a significant decrease of bread volume. Decrease in specific volume is in the range from 24 % to 26 % for the soy protein or pea protein, respectively. The bigger flavor acceptability was registered in the case of a sample with pea protein isolates, than sample with soy protein concentrate. Results indicate that pea protein could be an alternative to soy protein concentrate.

Key words: *soy protein concentrate, pea protein isolates, high protein wholegrain spelt bread.*

REZIME

Cilj ovog istraživanja je bio formulisanje visokoproteinskog integralnog hleba od spelta pšenice. Supstitucijom integralnog speltinog brašna sa 11,5% sojinog proteinskog koncentrata i 8% proteinskog izolata od graška poboljšana su svojstva testa tokom mešanja. Sojin proteinski izolat sadrži 65% proteina, a proteinski izolat od graška sadrži 90% proteina, tako da se u cilju postizanja istog nivoa sadržaja proteina u hlebu potrebno uneti veću količinu sojinog proteinskog koncentrata u odnosu na proteinski izolat graška. Dodatakom biljnih proteinskih preparata postiže se poboljšanje koje se odnosi na povećanje moći upijanja vode i povećanje stabilnosti testa.

Stabilnost testa sa dodatkom koncentrata sojinih proteina je veća u odnosu na testo sa izolatom graška proteina, što je verovatno posledica različitog ponašanja proteina zbog razlike u distribuciji različitih klasa proteina u soji i grašku. Osim toga, primenjeni biljni proteini doprinose povećanju čvrstoće testa. Adhezivnost testa značajno je manja sa sojinim protenskim koncentratom u odnosu na primenu izolata graška, koja je na nivou kontrolnog uzorka. Primenom navedenih proteinskih preparata značajno se smanjenje zapreminu hleba. Smanjenje specifične zapremine hleba je u rasponu od 24% do 26% sa proteinima soje ili proteinima graška, respektivno. Dobijeni visokoproteinski hleb ima nižu prihvatljivost za ukus u odnosu na kontrolni hleb, ali ne značajno u slučaju primene proteina graška. Prihvatljiviji ukus je utvrđen kod hleba sa izolatom proteina graška u odnosu na hleb sa koncentratom sojinih proteina, zbog toga što je koncentrat kao sastojak dodat u većoj količini. Rezultati primenjenih ispitivanja koja se odnose na osobine testa i hleba od integralnog speltinog brašna ukazuju da izolati proteini graška mogu biti alternativa proteinima soje.

Ključne reči: *koncentrat sojinih proteina, izolat proteina graška, visokoproteinski integralni hleb od spelte.*

INTRODUCTION

The daily consumption of bread all over the world gives it an important position in human nutrition. Bread provides important amounts of nutrients, such as starch, dietary fibre, proteins, lipids, vitamins, minerals, etc. However, due to a growing consumer demand for healthier products as well as the increasing protein requirement caused by population growth, bread enrichment with protein becomes topical (*Paraskevopoulu et al., 2012*). Soybean (Glicinemax. L. Merr.) is considered as an important plant species both for human and animal consumption. Wide range of products obtained from soybean seed originates from the presence of protein (aprox. 40 %), and oil (approx. 20%) in seed *(Vujaković et al., 2012)*.

The development of new functional bakery products has been accelerated in recent years, especially with some raw materials which are based on proteins from plant sources, such as soybean and peas. Plant protein have the same quality as protein based meat and dairy substitutes while having lower costs (*Dijkstra, Linnemann and van Boekel, 2003; Linnemann and Dijkstra, 2002*). The incorporation of ingredients like legume flour, concentrate or isolates in cereal-based matrices can lead to the production of nutritionally enhanced products like bread with high protein content. Substitution with legume-based products allows to improve bread protein content and compensate wheat deficiencies in lysine and threonine, two essential amino acids (*Abdel-Aal et al., 2002; Des Marchais et al., 2011*). Lysine is an essential amino acid which cannot be synthesized by the body. Lysine is required for growth (*Tomé et al., 2007*). Wheat alone is low in lysine but high in methonine. Combining cereal and leguminose proteins helps to overcome the deficiencies of these crops, by balancing the amino acid profiles deficiency which can markedly improve protein quality (*Livingstone et al., 1993*).

Apart from being nutritious, pulse proteins are highly functional and exhibit properties like solubility, gelation and water binding playing a crucial role in structure formation and

mouthfeel of the finished products. The most important functional properties of protein in food include its solubility, water- and fat-binding capacities, gel forming and rheological behaviours, emulsifying capabilities, foaming and whipping abilities. Soy proteins have been studied most amongst the plant proteins for their emulsification properties (*Adachi et al., 2004; Keerati-u-rai and Corredig, 2009*). However, supplementing wheat flour with legume proteins in breadmaking applications in which gluten plays a critical role for dough viscoelastic properties has been challenging. The quality of the end-product is often compromised with negative impacts on loaf volume, texture and eating quality of bread (*Fenn et al., 2010*). These negative effects limit the levels of substitution of wheat flour with legume flour or protein isolates. In most cases, substitutions of wheat flour is up to 15 % of the flour of leguminous plants, or to 5 % with a protein concentrate (*Raidl and Klein, 1983*).

The incorporation of soy and pea protein isolates in spelt bread formulation represents an additional technological challenge. The most commonly cultivated cereals are wheat, maize, rice, barley, oats, rye, sorghum, millet, triticale, buckwheat and amaranth. Cereals have enormous importance for food. Man has been using cereals in his diet since ever. Daily consumption of whole-grain cereals is one of the fundamental principals of correct and healthy nour-*ishment (Radosavljević, 2010)*.

Spelt wheat is an ancient subspecies of common wheat which is suitable for cultivation by organic methods as it does not require high-level nitrogen fertilization or heavy chemical protection (*Filipčev et al., 2013a,b; Bodroža et al., 2010*). In contrast to the advantages regarding some agricultural and nutritional features, the dough quality and breadmaking ability of spelt has been described as poor compared to common wheat. Although having higher gluten quantities the quality of spelt gluten is inferior to that of modern wheat giving soft, less elastic, sticky and less dough stability (*Schober et al., 2006; Pruska-Kedzior et al., 2006*). Hence, the aim of this work was to estimate the possibility of incorporating soy protein concentrate and pea protein isolates in spelt bread formulation by elucidating their effect on dough properties and sensory quality of enriched bread.

MATERIAL AND METHOD

Material

Refined spelt wheat flour was obtained from the Jevtić organic farm (Bačko Gradište, Serbia).

This flour is originating from produced from varietie Nirvana (Serbia). The moisture content of the flours was 10.60, the ash content 1.52 % and the protein content 12.77 % in dry matter (d.m.). In addition the following high protein ingredients were used: TradKon SPC HC-200 soy protein concentrate - 65 % protein in d.m. (Sojaprotein, Serbia), pea protein isolates Nutralys F85M – 90 % protein in d.m. (Roquette, France). The L-ascorbic acid was purchased from BASF, Germany. Other raw materials (bakery's yeast and salt) were obtained from local supermarkets.

The Kjeldahl method (1883) was used for N determination. Protein content (%) was calculated as N x 5.7 for flour and N x 6.25 for pea protein isolates.

Baking Procedure

Ingredients for each recipe are listed in Table 1. The amount of protein source in each formulation was a djusted to obtain the equal protein content in bread (cca 14 %). The ingredients were mixed for 5 minutes and the dough was left for ripening (60 min) at 25 °C. Then, the dough was divided into 300 g portions, manually rounded and put into tin pans (24.5 cm×9 cm×6.5 cm). The final fermentation lasted 45 min and were baked at 230 °C for 20 min in a deck type oven. Bread quality characteristics were assessed 24 h after baking. Three loaves were analyzed per each sample. Cooled loaves were packed in polyethylene pouches and stored at ambient temperature.

Table 1. Ingredients used in wholemeal spelt bread formulation enriched with non-gluten proteins

*Ingredients	Wholemeal spelt bread Control	Wholemeal spelt bread with soy protein concentrate	Wholemeal spelt bread with pea protein isolates
Wholemeal spelt flour (g)	700	700	700
Baker's yeast (g)	17.5	17.5	17.5
Salt (g)	14.0	14.0	14.0
Ascorbic acid (mg/kg flour)	200	200	200
Water (ml)	448	525	560
Soy protein concentrte (g)	-	80.5	-
Pea protein isolates (g)	-	-	56.0

**Ingredients expressed in baker's percentage: wholemeal spelt flour + source of protein: 100 %; baker's yeast: 2.5 %; salt: 2.0 %; ascorbic acid: 0.02 %; water: 64 %, 75 %, 74 %, respectively.*

Dough analysis

Dough mixing properties

In the first test (control test) wholemeal spelt flour sample (4 g) and in the other tests mixture wholemeal spelt flour and protein preparation of soy and pea (4 g). At the same ratio as in Table 1. Mixtures were have mixed with water at a constant speed of 63 rpm using a Newport Micro-dough LAB mixer (Perten Instruments, Australia). The dough consistency was recorded using the DLW version 1.0.0.56 software. Mixing parameters such as water adsorption target (WAT), stability (S), arrival time (AT) and departure time (DT) were obtained from the software to assess the dough mixing properties.

Dough texture

Dough texture was evaluated using Texture Profile Analysis (TPA) on a TA.XT2 Texture Analyzer (version TA.XTPlus, Stable Micro Systems Ltd., Surrey, UK). For TPA test, dough were prepared as in Table 1 without yeast addition. The dough samples were cut with a cylindrical die to get the uniform size of 25 mm width and 25 mm height. A round disk probe of 36 mm diameter was used to exert the force in the middle of the each dough sample. The dough samples were tested in TPA mode consisting of two cycles with a recovery time of 10 seconds. The probe speed was 10 mm/sec and the distance of the probe was 80 % of the products height. The parameters such as hardness, cohesiveness and adhesiveness of dough were determined in five replications.

Bread analysis

Chemical and physical characteristics of bread

The volume of the loaves was assessed by means of laser measuring device Volscan profiler 600 (Stable Micro Systems,

England). On the bases of volume and mass of loaves, specific volume was calculated.

Its basic chemical composition was determined in the following way: protein (N × 6.25) content by Kjeldahl method (AAC, 2000), crude fat content by Soxhlet method and starch content by polarimetric method by Ewers.

Sensory evaluation of bread

Sensory analysis of bread was performed by a panel consisting of six trained persons. Analyses were done using the method of acceptance analysis, in which the pre-coded samples were given scores from a seven-point scale, where 1 means extremely dislike and 7 extremely like. The panelists considered the following quality attributes: appearance, structure and porosity, crumb color, smell and taste.

Statistical analysis

Analysis of variance (ANOVA) was performed using the one-way analysis of variance. Models procedure of Statistica 8.1 statistical software (StatSoft Inc., Tulsa, Oklahoma). Tukey's tests were used for comparison of sample data, and evaluations were based on a significance level of $P<0.05$. The results shown are mean values of at least three measurements.

RESULTS AND DISCUSSION

Effects of protein preparation on the dough mixing properties and texture

Table 2. Dough mixing propeties with and without protein preparations

Parameters	Dough without protein	Dough with soy protein concentrate	Dough with pea protein isolates
*Water absorption (%)	55.6[a]	66.8[b]	64.5[b]
**Departure Time (min)	2.8[a]	4.8[b]	3.2[a]
***Arrival Time (min)	1.0[a]	1.9[b]	1.3[a]
Stability (min)	1.8[a]	4.0[c]	2.9[b]

**Water absorption corrected for target peak resistance (500 FU).*
***Departure time-the required time for the top (maximum) curve to fall below the peak resistance.*
****Arrive time-the time required for the top (maximum) curve to reach the peak resistance.*
a,b,c – Different letters in a row denote significant difference at $p < 0.05$ (Tukey HSD)

The dough with the addition of protein preparations has better properties during kneading compared to the control dough, which can be seen from Table 2. The dough absorbs more water, this may been due to the water absorptive capacity of soy proteins (*Raidl and Klein, 1983*). Furthermore, *Sharma (2000)* observed that water absorption increases significantly with increased amount of cow-pea. All this leads to an increase in departure time. A longer departure time indicates a stronger mixture flour and other ingredients. The stability of dough with the addition of soy protein concentrate was higher than dough with pea protein isolates. This behavior of the dough can be explained by the difference in distribution of the different classes of proteins in soybeans and peas. The major protein fractions in seeds of soybeans and peas are albumins and globulins. Albumins are soluble in water and coagulatable by heat, whereas globulins are insoluble in water, but soluble in saline solutions. Unlike albumins, globulin proteins from plants contain relatively low levels of the sulphurcontaining amino acids, cysteine and methionine. Furthermore, plant globulins are the bearers of emulsifying properties due to their much larger molecular size and structural constraint by disulphide crosslinks (*Wong et al., 2012*).

On the basis of literature data soybean contains approximate 90 % of globulins, while peas 50-60 % of globulins and 15-25 % of albumins, although the concentration of globulins can vary depending on the different cultivars (*Day, 2013*). Stability of dough with the soy protein is higher than the stability of dough with pea proteins because of the higher concentration of the globulin in the soy protein structure.

Texture profile analysis (TPA) method is widely used for texture evaluation of food products. The TPA test performs two bites; every bite includes compression and decompression cycles. The results of texture profile analysis pertaining the analysis of instrumental texture parameters of spelt dough without and with protein preparations are shown in Figure 1.

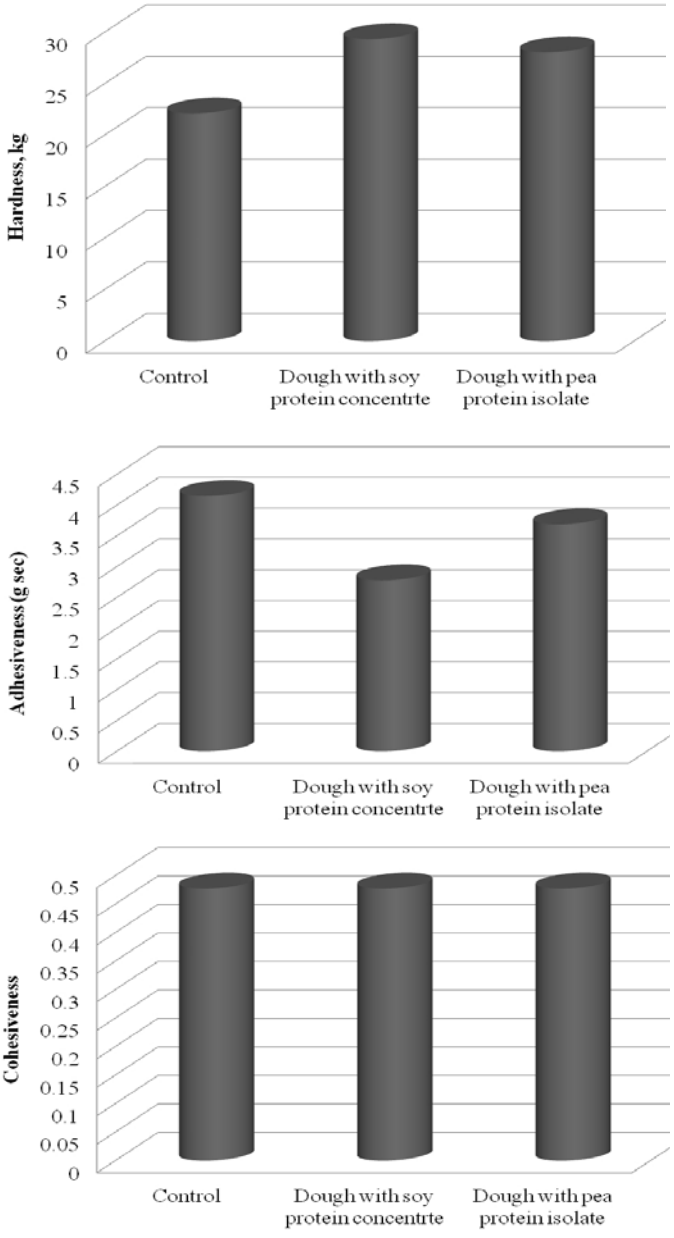

Fig 1. Texture Profile Analysis of dough without and with proteins preparation

Protein intake in the dough contributes to increasing its hardness. The cohesiveness analysis of the dough showed no significant difference in cohesiveness values of control samples and samples with proteins.

Adhesiveness of the sample dough with soy protein is significantly lower than the control sample, while the adhesiveness of the sample dough with pea protein at the level of the control.

Effects of protein preparation on the chemical and physical characteristics of bread

Basic parameters characterizing analyzed bread are collected in Table 3. Partial replacement of wholemeal spelt flour with soy and pea proteins caused a significant increase in the content of protein and a decrease in total carbohydrate content and fat in comparison with control bread. Application of the above protein preparations resulted in an increase in protein content by 50 %, and by national regulations this bread is one of the high protein bread, which is especially important for athletes, people in developing and obese adults on a protein diet. Bread with soy protein concentrate contents for 30 % less fat which can be declared as a high protein bread with reduced fat content. Decrease in specific volume is in the range from 24 % to 26 % for the soy protein or pea protein. The reduction in the volume of bread with soy protein and pea protein is the same level. This indicates that none of the protein preparations which are applied has an important advantage in relation to other.

Table 3. Chemical and physical characteristics of bread with and without protein preparations

Parameters	Bread without protein	Bread with soy protein concentrate	Bread with pea protein isolates
Protein (% d.m.)	9.39[a]	14.08[b]	14.1[b]
Fat (% d.m.)	1.90[c]	1.31[a]	1.76[b]
Carbohydrates (%d.m.)	46.00[c]	33.26[a]	37.82[b]
Volume (cm^3)	542[b]	409[a]	407[a]
Specific volume (cm^3g^{-1})	1.81[b]	1.37[a]	1.34[a]

a,b,c – Different letters in a row denote significant difference at $p < 0.05$ (Tukey HSD)

Effect of protein preparation on the sensory parameters

Results of sensory analysis of bread supplemented with various protein types are shown in Figure 2. Acceptability of overall appearance was the highest for control bread, without protein addition. The samples with soy protein received slightly lower acceptance of this parameter. Significantly lower scores were received by loaves with pea protein isolates. Low acceptance of bread with pea protein isolates appearance is probably due to its smallest specific volume (Table 3) accompanied with the most compact structure. However, significant differences in appearance among breads with proteins of soy and pea do not exist. Taking into account structure and porosity, the highest consumers acceptability was observed for control bread. Structure of loaves with the addition of soy protein concentrate and pea protein isolates was significant less acceptable compared to the control. By sensory evaluation panelists structure and porosity breads with soy and pea proteins did not significantly differ. In the case of color assessment, there are not any significantly visible differences among breads. This is because the basic matrix wholemeal spelt flour, bran covering the presence of differences in shading.

It is known that the addition of plant proteins can change the acceptability of sensory attributes of bread (*Raidl and Klein, 1983; Paraskevopoulou et al., 2012*). The control bread and bread with the addition of pea protein had the most acceptable smell. The lower flavor acceptability was registered at breads with proteins than it was at the control bread. The bread with pea protein isolates has greater flavor acceptability than bread with soy protein concentrate. It can be said that the reason for different level of flavor among samples is in the use of different preparations and even in their different amounts. Soy protein concentrate contains 65 % protein and the rest are the other components such as carbohydrates, fats, etc. which are the bearers of unpleasant odors. Pea protein isolate contains 90 % protein and less supporting components (carriers of unpleasant odor). Besides, in order to achieve the same level of protein content of the bread it is necessary to enter a larger amount of soy protein concentrate than the pea protein isolates.

Therefore, pea protein isolate has less impact on the smell and taste of the spelt bread.

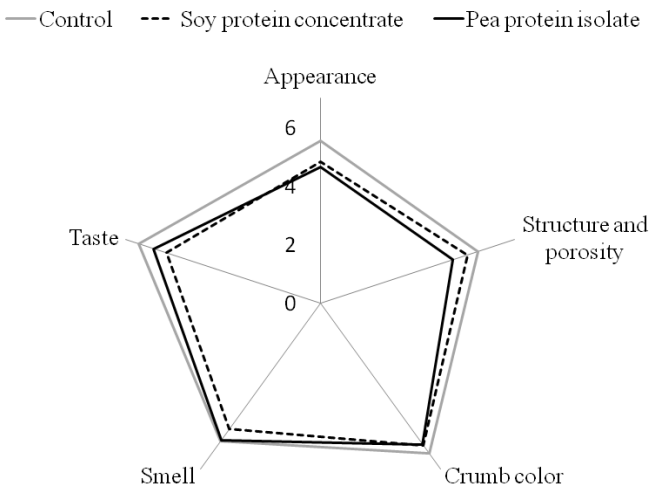

Fig 2. Results of sensory acceptance of control spelt bread and samples with proteins

CONCLUSION

Protein preparations derived from legumes contribute to better mixing properties of dough than wholemeal spelt flour. This improvement is primarily related to higher water absorption and greater stability. Textural properties of dough with soy and pea protein preparations were more favorable compared to the control sample of dough. Its hardness is increased but adhesiveness is decreased. Substituting of the wholemeal spelt flour with 11.5 % soy protein concentrate and 8 % pea protein isolates is obtained high protein bread with good physico-chemical characteristics and an adequate sensory profile. Wholemeal spelt bread with soy protein has a better appearance, crumb structure and porosity, while bread with pea protein isolates has better smell and taste. Results in all studied parameters indicate that pea protein could be a promising alternative to soy preparations. Further studies should be done by combining soy and pea protein in order to optimize protein bakery products.

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