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The Main Components Content, Rheology Properties and Lipid Profile of Wheat-Soybean Flour

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1. Introduction

Soybean (*Glycine max* (L.) Merr.) is a species of legume, native to a Eastern Asia and an important global crop, today. Soybean is rich in high quality proteins, contains essential amino acids, similar to those found in meat, minerals such as Fe, Zn, Cu, Mn, Ca and Mg as well as phytic acid. The bulk of seed soybean proteins contains albumins and globulins as major components, but there are minor, undesirable components such as inhibitors of trypsyn and chymotrypsin, and sugar-binding lecitins. The inhibitors and lecitins are generally inactivated by heat treatment. A new immunochemical methods can be used for quantitative detection of soybean proteins and production of healthful foods (Brandon & Friedman, 2002). The soybean lipid contains a significant amount of unsaturated acids: α-linolenic acid, known as omega-3 acid, linoleic, γ-linolenic and arachidonic acid, known as omega-6, and oleic acids known as omega-9 acid and are very important in human nutrition (Liu, 1997). The soybean lipid also contains saturated acids: palmitic and stearic acid (Bressani, 1972; Olguin et al., 2003; Bond et al., 2005), as well as tocopherols (Ortega-Garcia et.al., 2004, Yoshida et al., 2006). These soybean components make the products with soybean have higher nutritional value.

1.1 Soybean in bread making industry

In the bread making industry, the soybean is used with the aim to increase the bread protein value and decrease carbohydrate value. In ordinary white bread protein content ranges from 8 to 9% and by including soybean, the protein content can be made up to 16% (Ribotta et al. 2010) and at the same time, the dough and bread are richer with lipoxygenase enzymes preparation which can make the dough physical properties better. The fact is that bread made with soybean costs less and it is especially important in countries where wheat is not a major domestic crop. Bread and products with higher protein content and lower carbohydrate content are more suitable for use in some diets than bread and products formulations currently used (Mohamed et al., 2006). As the main protein component in white bread is gluten, a component which causes celiac disease, the usage of soy is useful for decreasing the gluten content in bread. A portion of 0.3 to 5% of soybean flour portion is usually added (Auerman, 1979), but rational addition for increasing bread protein value is 20-30% of soybean flour. Besides whole soybean flour, different soybean products can also be used: defatted soy flour (Mashayekh et al., 2008), physically modified soy flour (Maforimbo et al., 2008), soy flour and durum wheat flour mixture (Sabanis & Tzia, 2009), commercial soy protein isolate (Roccia et al., 2009), diffrerent kinds of soy protein powder (Qian et al., 2006) and part of soy seeds such as a hulls (Anjum et a., 2006). Based on these investigations results, different bread formulations are defined, and soy is used in portion up to 20%. When soy flour in wheat flour was to a level of 10% and in durum wheat flour up to 20%, the produced bread was without any negative effects in quality attributes such as colour, hardness and flavour, promising nutritious and healthy alternative to consumers (Sabanis & Tzia, 2009). By investigating the effect of defatted soy flour on sensory and rheological properties of wheat bread, Mashayekh et al. 2008, concluded that adding 3 and 7% defatted soy flour gives as good a loaf of bread as the 100% wheat bread and acceptable consumer attribute with rheological and sensory characteristics. Adding small quantity of soy protein powder of 3% to wheat flour did not change the sensory properties of bread and a large quantity of soy flour adding, exceeding 7%, can lead to stickiness and leguminous flavour (Roccia et al., 2009). The results of investigation (Anjum et al., 2006) of soy hulls usage showed the content of 4.5% soy hulls combined with wheat flour is acceptable and suitable level by the consumers.

1.2 Wheat-soybean bread manufacturing

The manufacture of bread from flour without gluten represents considerable technological difficulties (Jong et al., 1968; Schober et al., 2003) because gluten is the most important structure forming protein for making bread (Gujural et al., 2003; Moore et al,. 2004) and by using appropriate soy-wheat flour mixture these difficulties can be avoided. When soy was added to wheat, the soy globulins interact with wheat gluten proteins forming aggregates of high molecular weight. As reduction-reoxidation treatment facilitated the interaction of glutenin subunits and soy proteins (11S subunits), interaction probably occurs through the oxidation of SH groups (Maforimbo, 2008). Investigations of the changes in glutenin macro polymer content, protein composition and free sulfhydryl content showed that active soy flour decreased glutenin macro polymer content due to gluten depolymerization and glutenin macro polymer content increased by inactive soy flour because soy proteins became insoluble and precipitated together. Soy proteins were associated to wheat protein through physical interaction and covalent and non-covalent bonds during mixing and resting and these interactions produced large and medium-size polymers. This increased solubility of insoluble gluten proteins, producing a weakening of the gluten network (Perez et al., 2008) and decreasing availability of water to build up in gluten network (Roccia et all. 2009). Physicochemical status of soy protein in the product had a great influence on how wheat-soy proteins will interact (Perez et al., 2008). Incorporation of soy proteins changes the rheolocical and bread properties. The investigations showed that adding soy protein powder depresses loaf volume, gives poor crumb characteristics and decreases acceptability by consumers (Qian et al., 2006.) Ribotta et al. 2010. tested different additive combinations for improved bread quality obtained from soy-wheat flour in ratio of 90:10 w/w and found that the combination with transglutaminase showed a major improving effect on dough rheological properties and crumb uniformity.

1.3 Aims of investigations

Dough rheological properties have great relevance in predicting the mixing behaviour, sheeting and baking performance (Dobraszczyk & Morgenstain, 2003) and supplementation wheat flour with other changed these properties. Ribotta et al. 2005, presented data about effect of soybean addition on farinographic properties such as water absorption (WA), dough development time (DT), dough stability (Dst) and dough degree of softening (DSf). The present work has been undertaken with the objective to investigate the effect of whole soybean seed flour (»full-fat soy flour«) portion of 3 to 30% on dough farinographic, but extensographic and amylographic properties, too. Based on the composition of wheat and soybean flour lipids, the aim is to obtain lipid composition in wheat-soybean flour mixtures, and compare it to wheat flour only, with an emphasis on content of total saturated fatty acids (TS), total monounsaturated fatty acids (TMUS), total polyunsaturated fatty acids (TPUS) and total unsaturated fatty acids (TU). In order to value wheat-soybean flour mixtures, they were classified into groups by using statistical analysis and Euclidean distances and the correlations between content of some lipid components and rheological properties were found.

2. Experimental

2.1 Soybean seed

The whole soybean seeds (*Glycine max* L.) cultivars ZP Lana, grown in Serbia in summer of 2006 were used. The seeds were purchased in local store „Green Apple" (Leskovac, Serbia) and milled. The particle size was determined by method of insemination via riddles with gaps size from 0.315 to 3.15 mm. The overall particle size was determined using equations:

$$100/d_{sr} = \sum \Delta d_i / d_i \tag{1}$$

where Δd_i is weight of fraction with appropriate particle size in %, and

$$d_i = (d_{i-1} + d_{i+1})/2 \tag{2}$$

where d_{i-1} is bottom riddle gap size and d_{i+1} is upper riddle gap size.

2.2 Chemicals

Chemicals used for oil extraction were high quality chemicals (Centrohem, Serbia) and for HPLC and GC analysis they were analytical grade (Riedel-de Haën, Honeywell Specialty Chemicals, Germany).

2.3 Wheat and soybean flour and flour mixtures

The wheat flour, Kikinda Mill, Serbia (WF) was bought from the local market. The soybean flour (SF) as »full-fat soy flour« was obtained by soybean seeds milling (IKA Model M120), to an overall particle size (d_{sr}) of 0.4 mm. Quantities of 291, 285, 270, 240 and 210 g of wheat flour and 9, 15, 30, 60 and 90 g of soybean flour, respectively, were used to make flour mixture with 3, 5, 10, 20 and 30 % (w/w) soybean flour portion, without adding additives.

2.4 Flour analyses

Flour protein content was determined by the Kjeldahl method (Nx5.95). The moisture content was determined by Scaltec SMO 01 (Scaltec instruments, Germany) instruments: flour (5 g) was put into the disk plate analyzer, dried at 110^{o}C to a constant weight, and the moisture content was read out on the display. The ash content was determined by staking at 800°C during 5 h. For gluten content determination, the dough was prepared by adding a sodium chloride solution first; wet gluten was isolated by dough washing and weighed. The starch content was determined by polarimetry according to Grossfeld's method and the total carbohydrates content according to Luff-Schoorl's method (Trajković et al., 1983). The values for samples are from triplicate analysis and followed by standard deviation.

2.5 The wheat and soybean flour lipid content

The wheat or soybean flour (50g) were put into Erlenmeyer flask, 500 ml of trichloroethylene was added and extracted for 30 minutes, under reflux and by mixing (200 min^{-1}) at approximately 88°C. The extract was separated by using Buchner funnel under weak vacuum. The plant material was extracted three more times by the same method; the extracts were mixed together and eluted by water in the separation funnel (3 x 10 ml). The eluted extracts' volume was recorded and an aliquot (3 ml) was taken for the dry residue determination test. This test was performed by drying at 110°C to a constant weight and the dry residue content was read out on the analyzer display (Scaltec SMO 01, Scaltec instruments, Germany). The lipid content in wheat and soybean flour was calculated based on average value of three measurements. The remnant of lipid extracts, after dry residue determination test, is evaporated under vacuum and obtained residue was used for HPLC and GC analysis.

2.6 Rheology measurements

The Brabender farinograph (Brabender Model 8 10 101, Duisburg, Germany) according to ISO 5530-1 test procedure, was used for water absorption values (WA value in ml/100g), development time (DT in minutes), dough stability (DSt in minutes), degree of softening (DSf in BU) and farinograph quality number (QN) determination. For extensograph measurement, the Brabender extensograph (Brabender, Model 8600-01, Duisburg, Germany) and test procedure ISO 5530-2 were used. The samples were prepared from wheat flour and wheat-soybean flour mixtures, distilled water and salt, and data for energy (E in cm^2), resistance (R in EU), extensibility (Ex in mm) and ration number (R/Ex) were recorded on extensograph curve. To obtain amylograph data, such as gelatinization temperature (T_{max} in °C) and gelatization maximum (η_{max} in AU), the amylograph (Brabender Model PT 100, Duisburg, Germany) and ISO 7973 test procedure were used.

2.7 HPLC analysis

For HPLC analysis, Holčapek et al., (1999) modified HPLC method and the Agilent 1100 High Performance Liquid Chromatograph, a Zorbax Eclipse XDB-C18 column: 4.4 m x 150 mm x 5 µm, Agilent technologies, Wilmington, USA and an UV/ViS detector were used. The flow rate of binary solvent mixture (methanol, solvent A, and 2-propanol/*n*-hexane, 5:4 by volume, solvent B) was 1 ml/min, with a linear gradient (from 100% A to 40% A+ 60% B in 15 min). The column temperature was held constant at 40°C. The components were detected at 205 nm. The free fatty acids (FFA), methyl esters (ME), monoacylglycerols (MAG), diacylglycerols (DAG) and triacylglycerols (TAG) were identified by comparing the retention times of the lipid components with those of standards. The samples of the reaction mixture were dissolved into a mixture of 2-propanol:*n*-hexane, 5:4 v/v and filtered through 0.45 µm Millipore filters.

2.8 GC analysis

For GC analysis, fatty acids methyl esters were prepared. The lipid were alkaline hydrolyzed and methylated by methanol and BF_3 as catalysts. The final fatty acids methyl esters concentration was about 8 mg/ml in heptane. For obtaining a methyl esters GC spectra, the HP 5890 SERIES II GAS-CHROMATOGRAPH, HP with FID detector and 3396 A HP integrator was used. Column was ULTRA 2 (25m x 0.32mm x 0.52 μm) (Agilent Technologies, Wilington, USA), injector temperature of 320°C, and injector volume of 0.4 μl. The carrier gas was He at a constant flow rate of 1 ml/min. The flame ionization detector was at 350°C and split ratio was 1:20. Oven temperature was initially 120°C and was maintained at 120°C, for 1 min, then increased by 15°C/min until 200°C, increased by 3 °C/min until 240°C, increased by 8°C/min until 300°C and maintained at 300°C for 15 min. The fatty acids were identified by comparison of retention times of the lipid components with those of standards.

2.9 Energetic value

Based on total carbohydrates (CHC), protein (PC) and lipid content (LC), the energetic value (EV) of wheat flour and white-soybean flour mixtures was calculated as:

$$EV = (CHC + PC) \cdot 17 + LC \cdot 37 \tag{3}$$

2.9.1 Statistical analysis

STATISTICA, version 5.0 software was used to perform the statistical analysis: the means and standard deviations, the correlation coefficients and cluster analysis. The means and standard deviations were obtained by Descriptive Statistics, marking the Median & Quartiles and Confirm Limits for Means. In order to classify wheat flour and wheat-soybean mixtures, the cluster analysis and the Euclidean method with the complete linkage was used.

3. Results and discussion

3.1 The main components content

The results of wheat and soybean flour moisture, starch, protein, ash, lipid, gluten and carbohydrates content are showed in Table 1. Data are presented as means of three determinations with standard deviation. Based on these data, considering the soybean flour portion in mixtures and compared to wheat flour, it is evident the moisture (from 12.8 to 11.6%), the starch (from 76.6 to 56.8%), gluten (23.9 to 16.7%) and carbohydrates content (from 78.8 to 62.8%) decreased, while the protein (from 8.6 to 20.0%), ash (from 0.48 to 2.08%) and lipid (1.2 to 7.2%) content increased with increasing soybean flour portion in mixtures.

Taking into account the protein, carbohydrates and lipid content, based on formula (3) for energetic value determination, it was obtained that the soybean flour increased energetic value in flour mixtures (from 1530 obtained for wheat flour, to 1544, 1554, 1579, 1625, 1674 kJ/100g, when soy flour portion was 3, 5 10, 20 and 30%, respectively) and the maximal increasing of 9.4% was in mixture with soybean flour portion of 30%.

Content (g/100g)	Moisture	Starch	Protein	Ash	Lipid	Gluten	Carbo-hydrates
Wheat flour	12.8±0.6	76.6±1.2	8.6±0.34	0.48±0.04	1.2±0.05	23.9±0.4	78.8±2.2
Soybean flour	8.7±0.6	10.7±0.6	46.7±0.6	5.80±0.6	21.2±0.6	-	25.4±0.6
3%	12.7	74.6	9.7	0.64	1.8	23.2	77.2
5%	12.6	73.3	10.5	0.75	2.2	22.7	76.1
10%	12.4	69.9	12.4	1.01	3.2	21.5	73.5
20%	11.9	63.4	16.2	1.54	5.2	19.1	68.1
30%	11.6	56.8	20.0	2.08	7.2	16.7	62.8

Table 1. The main components content in wheat and soybean flour and their mixtures

3.2 Rheology properties

Farinograph, extensograph and amylograph data of flours and flour mixtures with different portions of soybean flour are given in Table 2. The results showed the farinograph data depended on the soybean flour portion in mixtures. The water absorption increased from 53.4 to 64.2% with increasing soybean flour portion in mixtures. It is well known, that the main dough component in wheat flour responsible for water absorption is gluten. The soy flour is without gluten but had even more than 5 times higher protein content than wheat flour (46.7 to 8.6 g/100g). The higher absorption ability could be due to soy protein components, such as globulins, which interacted with gluten protein in the composite dough (Maforimbo et al., 2008). The same effect of soy flour on water absorption value was reported by Ribotta et al., 2005, when heat-treated full-fat flour, enzyme-active defatted flour and soy protein isolates were used for wheat flour substitution in portion from 3 to 12% and enzyme-active full-fat flour in portion from 5 to 12%. The differences in dough development time and dough stability among flour mixtures with different soybean flour portions, ranged from 1.3 to 7.3 min and 0.7 to 2.9 min, respectively and both values for mixtures were higher than for wheat flour where value for dough development time was 1 min and for dough stability was 0.1 min. The effect of delaying dough development time and dough stability by addition the mostly of investigated soy flours was obtained in Ribotta et al. (2005) experiments, too.

Degree of dough softening was increased with increasing soybean flour portion in mixtures, from 45 to 66 BU and all values were lower than value for wheat flour, i.e. than 90 BU. The same effect of soybean flour on this farinographic characteristic was obtained by Ribotta et al. (2005), for all investigated samples, except for soy protein isolate Samsory 90 HI and portion of 5, 10 and 12%, when dough softening value was higher. According to appropriate triangle area on farinograph curves, the quality number, known as Honkocy number could be in the range from 0 to100, and quality groups are A_1 (area of 0-1.4 cm^2), A_2 (area of 1.5-5.5 cm^2), B_1 (area of 5.6-12.1 cm^2), B_2 (area of 12.2-17.9 cm^2), C_1 (area of 18.0-27.4 cm^2) and C_2 (area of 27.5-50.0 cm^2) (Djaković, 1980). The soybean flour addition in all investigated portions had positive influence on quality number and quality group which was B1 instead B2, which was for wheat flour.

Data obtained on extensograph, showed that dough with soybean flour portion of 20 and 30% had lower values for energy and dough resistance in comparison to dough made of wheat flour only. The extensibility of dough with soybean flour ranged from 125 to 89 EU and those values were lower than extensibility of dough with wheat flour only. The ration number, R/Ex, varied depending on soybean flour portion and ranged from 2.76 to 3.42. Based on curve of volume versus ratio number (Djaković, 1980), obtained for round bread, the bread volume was predicted to be in range from 576 to 557 cm^3 and it was lower than bread volume obtained from wheat flour only which was 581 cm^3.

Farinograph data						
	Wheat flour	3%	5%	10%	20%	30%
WA (ml/100g)	53.4±1.4	53.3±1.4	53.8±1.3	53.9±1.6	61.9±1.6	64.2±1.7
DT (min)	1±0.1	1.3±0.1	1.3±0.1	6.5±0.3	6.8±0.3	7.3±0.4
DSt (min)	0.1±0.1	0.7±0.2	0.8±0.2	1.8±0.4	2.3±0.5	2.9±0.5
DSf (BU)	90±1.6	45±1.4	50±1.4	65±1.7	65±1.7	66±1.5
QN	52.8±1.4	57.7±1.4	66.7±1.4	61.7±1.4	64.2±1.4	68.8±1.4
Group	B2	B1	B1	B1	B1	B1
Extensograph data						
E (cm^2)	67.8±1.3	70.5±1.5	74.2±1.5	79.0±1.6	57.1±1.4	43.4±1.2
R (EU)	315±5.5	345±6.0	350±6.0	355±5.5	345±5.5	305±5.5
Ex (EU)	126±3.0	125±3.0	123±3.0	117±2.5	101±2.5	89±2.0
R/Ex	2.50±0.2	2.76±0.3	2.84±0.4	3.03±0.4	3.41±0.4	3.42±0.4
V (cm^3)	581±10	576±10	574±10	566±10	559±10	557±10
Amylograph data						
T_{max} (°C)	81.2±0.5	81.5±0.5	82.0±0.5	85.0±0.5	86.5±0.5	88.8±0.5
η_{max} (AU)	630±20	315±15	250±10	180±10	120±10	90±5

Table 2. Rheological properties of wheat flour (WF) and wheat–soy bean flour mixtures

By amylograph data, dough with soybean flour had higher gelatinization temperature (in range 81.5 to 88.8°C) than dough with wheat flour only (81.2°C), and this value was higher when soybean flour portion was higher. Based on Stevenson et al. (2006) results, gelatinization temperature for wheat-soybean flour mixtures value could be lower. They found the gelatinization temperature of soybean starches were lower compared to wheat starch, due to the short amylopectin branch-chains and positive relationship between gelatinization temperature and amylopectin branch-chains. The reason why our gelatinization temperature values were higher maybe a different behaviour of soy starch in combination with wheat starch, which was present in wheat-soybean flour mixtures. The maximal pasta viscosity decreased from 315 to 90 AU when soybean flour portion was increased. The lowest pasta viscosity was when the soybean flour portion was 30% (w/w) and it was seven times lower than maximal pasta viscosity value for wheat flour only. The low peak viscosity of soybean starch could be due to short amylopectin branch-chain which has been correlated in wheat starches (Sasaki & Matsuki, 1998; Shibananuma et al., 1996).

3.3 Lipid profile

The lipid profile of wheat flour and soybean flour obtained by HPLC analysis and based on these results the lipid profile of wheat-soybean flour mixtures, is presented in Table 3. The content of components was determined by measuring the peak area at 1.76 min for free fatty acids, peak area at 2.15 min for methyl esters, peaks area in the range of 3.44-4.58 min for monoacylglycerols, peaks area in the range of 5.28-8.68 min for diacylglycerols and peaks area in the range of 10.91-15.81 min for triacylglycerols (Holčapek et al., 1999).

Flour	FFA (g/100g)	ME (g/100g)	MAG (g/100g)	DAG (g/100g)	TAG (g/100g)
Wheat	11.9±0.4	23.2±1.3	2.5±0.2	12.2±0.9	50.2±1.5
Soybean	25.9±1.2	1.6±0.3	0.8±0.1	2.3±0.4	69.4±1.6
3%	12.3	22.5	2.4	11.9	50.8
5 %	12.6	22.1	2.4	11.7	51.2
10 %	13.3	21.0	2.3	11.2	52.1
20 %	14.7	18.9	2.2	10.2	54.0
30 %	16.1	16.7	2.0	9.2	55.9

Table 3. The lipid profile of wheat and soybean flour and their mixtures obtained by HPLC

Fatty Acid content in g/100g	Wheat flour	Soybean flour	3%	5%	10%	20%	30%
Myristic ($C_{14:0}$)	0	0.11±0.01	<0.035	<0.035	<0.035	<0.035	<0.035
Palmitic ($C_{16:0}$)	19.45±0.45	10.35±0.6	19.17	18.99	18.54	17.63	16.71
Linoleic ($C_{18:2}$)	57.91±0.72	55.23±1.2	57.83	57.77	57.63	57.37	57.11
Oleic ($C_{18:1}$)	20.23±0.21	13.07±0.8	19.20	19.87	19.52	18.79	18.08
Linolenic ($C_{18:3}$)	0	13.46±0.9	0.40	0.67	1.34	2.69	4.04
Stearic ($C_{16:0}$)	1.36±0.14	5.23±0.8	1.48	1.56	1.76	2.18	2.58
Nonadecanoic ($C_{19:0}$)	0	0.52±0.09	<0.053	<0.053	<0.053	0.10	0.16
Arachidic ($C_{20:0}$)	0	0.60±0.1	<0.061	<0.061	<0.061	0.12	0.18
Behenic ($C_{22:0}$)	0.26±0.06	0.48±0.06	0.27	0.27	0.28	0.30	0.33
ND RT 25.96	0	0.17±0.04	<0.052	<0.052	<0.052	<0.052	<0.052
Lignocerinic ($C_{24:0}$)	0	0.55±0.01	<0.056	<0.056	<0.056	0.11	0.17
Phthalic acid	0.68±0.08	0	<0.069	<0.069	<0.069	0.14	0.20
TU	78.14	81.46	77.43	78.31	78.49	78.85	79.23
TMU	20.23	13.07	19.20	19.87	19.52	18.79	18.08
TPU	57.91	68.39	58.23	58.44	58.97	60.06	61.15
TS	21.07	17.84	20.92	20.82	20.58	20.44	20.13
TU/TS	3.71	4.58	3.70	3.76	3.81	3.86	3.94

Table 4. Fatty acids composition of wheat and soybean flour and their mixtures obtained by GC

The lipid from soybean flour had higher free fatty acids content (25.9 g/100g lipid) and triacylglycerols content (69.4 g/100g lipid) than lipid from wheat flour (11.9 and 50.2 g/100g lipid, respectively), while the contents of methyl esters, monoacylglycerols and diacylglycerols were lower than appropriate contents in wheat flour, even 14.5, 3.1 and 5.3 times, respectively. The content of free fatty acids and triacylglycerols in mixtures had the same changing tendency as soybean flour portion: it increased when the soybean flour portion in flour mixtures increased, while the contents of monoacylglycerols and diacylglycerols had opposite changing tendency: they decreased when the soybean flour portion in flour mixture increased. The properties of dough such as water absorption, development time, dough stability and gelatinization temperature had the same dependency on soybean flour portion as the content of free fatty acids and triacylglycerols and these components seemed to have a proper influence on the mentioned dough properties. Further, the content of monoacylglycerols and diacylglycerols had a proper influence on dough energy, resistance, extensibility and gelatization maximum. Some of these dependences were confirmed by determination of correlations coefficients.

The fatty acids composition of flours obtained by GC analysis and based on these results, the fatty acids composition in flour mixtures is presented in Table 4. The lipid contained: linoleic ($C_{18:2}$), α-linolenic ($C_{18:3}$), oleic ($C_{18:1}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), arachidonic ($C_{20:4}$), behenic ($C_{22:0}$), nonadecanoic ($C_{19:0}$), γ-linolenic, lignocerinic, myristic ($C_{14:0}$) and several non-determined components (ND). GC analysis showed the lipid from wheat flour contained 78.14 g/100g of the total unsaturated fatty acids, consisting of linoleic (57.91 g/100g) and oleic acid (20.23 g/100g). The total polyunsaturated fatty acids content in lipid was 57.91 g/100g and it was from linoleic acid. The monounsaturated fatty acids content was 20.23 g/100g, from oleic acid, while the total saturated fatty acids content was 21.07 g/100g where the main fatty acids were palmitic and stearic acid with the content of 19.45 and 1.36 g/100g, respectively. The ratio of total unsaturated to total saturated fatty acids content was 3.7.

The lipid from soybean flour contained 81.46 g/100g of the total unsaturated fatty acids, composed of linoleic, oleic and linolenic acid with content of 55.23, 13.07 and 13.46 g/100g of lipid, respectively. The palmitic and stearic acid were the main saturated fatty acids with content of 10.35 and 5.23 g/100g, respectively, while the content of other detected saturated fatty acid was 0.69 g/100g. The myristic, linolenic, nonadecanoic, arachidic and lignocerinic fatty acids were detected only in soybean flour, while phthalic acid was detected only in wheat flour. Based on this composition and content of fatty acids, the ratio of total unsaturated to total saturated content in soybean flour was 4.58 and it was higher than ratio in wheat flour. Such fatty acids composition had influence on fatty acids composition in wheat-soybean flour mixtures: the content of total saturated fatty acids decreased when soybean flour portion in flour mixture increased, and all values were lower than the content of total saturated fatty acids in wheat flour; the content of total unsaturated fatty acids increased when soybean flour portion in flour mixture increased and all values (except in mixture with portion of soybean flour of 3%) were higher than the content of total saturated fatty acids in wheat flour. Finally, the ratio of total unsaturated to total saturated fatty acids content was higher in flour mixtures (except in mixture with portion of soybean flour of 3%) than in wheat flour.

3.4 Statistical analysis data

The correlation coefficients between the rheological properties (water absorption, dough stability, Ex, gelatinization temperature and gelatization maximum) and the content of some lipid components (free fatty acids, monoacylglycerols, diacylglycerols, triacylglycerols, palmitic (Pal), linoleic (Lin) and oleic (Ole) acid) in wheat flour and wheat-soybean flour mixtures are presented in Table 5.

	WA	DSt	Ex	T_{max}	μ_{max}	FFA	MAG	DAG	TAG	Pal	Lin
DSt	**0.88**										
Ex	**-0.94**	-0.84									
T_{max}	**0.91**	**0.98**	**-0.90**								
μ_{max}	-0.64	**-0.86**	0.67	-0.78							
FFA	**0.96**	**0.96**	**-0.93**	**0.98**	-0.77						
MAG	-0.75	-0.78	**0.91**	-0.83	0.71	-0.83					
DAG	-0.81	-0.81	**0.94**	**-0.86**	0.73	**-0.86**	**-0.97**				
TAG	**0.94**	**0.94**	**-0.85**	**0.94**	-0.72	**0.97**	-0.69	-0.72			
Pal	**-0.89**	**-0.89**	**0.97**	**-0.93**	0.78	**-0.94**	**0.95**	**0.98**	-0.84		
Lin	-0.45	-0.47	0.71	-0.53	0.51	-0.51	**0.85**	**0.88**	-0.30	0.77	
Ole	**-0.85**	**-0.85**	**0.90**	**-0.85**	0.78	**-0.88**	**0.88**	0.84	-0.81	**0.92**	0.65

Table 5. Correlation coefficients between rheological properties and lipid components content (correlations are significant at $p< 0.05$, N=12)

The sample size was twelve (N=12 (6x2): wheat flour and five wheat-soybean flour mixtures with minimal and maximal obtained value). As there were many correlations, only the one which had absolute value 0.85 and above 0.85 were taken into consideration. There were 54.5% of correlations, among which 30.3% were proper, and 24.2% were opposite correlations. The correlations can be divided into three groups: correlations between rheological properties, between rheological properties and lipid components content and between lipid components content. In the first group, there are the correlations where high water absorption value is associated with high dough stability and gelatinization temperature and low extensibility, high dough stability is associated with high gelatinization temperature and low gelatization maximum, and correlation between low gelatization maximum and high extensibility. In the second group of correlations, the high content of free fatty acids caused high water absorption, dough stability, and gelatinization temperature but low extensibility as well as the high monoacylglycerols and diacylglycerols content was proper correlated with extensibility. Also, when triacylglycerols content was higher, the water absorption, dough stability and gelatinization temperature were higher and extensibility was lower. The palmitic and oleic acid had opposite effect on the rheological properties, such as water absorption, dough stability and gelatinization temperature and proper effect on extensibility. The linolenic acid content was not associated with any rheological properties. Among lipid components content, there were proper correlations between free fatty acids and triacylglycerols content, between monoacylglycerols and diacylglycerols contents on one side and palmitic, linoleic and oleic acid content on the other side. It can mean that monoacylglycerols and diacylglycerols are mainly consists of palmitic, linoleic and oleic acids. The higher oleic acid content was associated with the higher palmitic acid content and it was only correlation among fatty acids.

By cluster analysis, based on multiple variables, wheat and wheat-soybean flour mixtures were classified into groups. Number of variables was six: wheat and five wheat-soybean flour mixtures (3, 5, 10, 20 and 30% w/w of soybean flour portions); number of cases i.e. parameters were eight: water absorption, dough stability, free fatty acids, diacylglycerols, triacylglycerols, palmitic, linolenic and oleic acid content. Linkage distances were obtained and presented by dendrogram in Fig. 1.

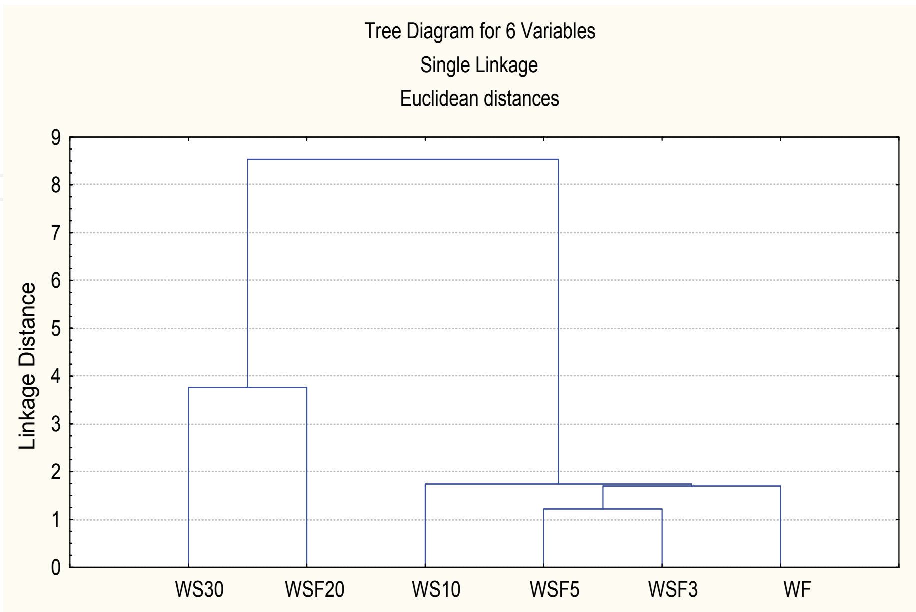

Fig. 1. Dendrogram obtained for wheat flour and wheat-soybean flour mixtures

The cluster analysis based on flour rheological and lipid characteristics, shows the linkage distance between wheat flour and flour mixtures increases when soybean flour portion in mixtures increases. The mixtures with soybean flour portion of 3 and 5% (w/w) are joined with wheat flour at the same distance level of 1.6 and make the first group. The mixtures with soybean flour portion of 10 % (w/w) are joined with wheat flour at the distance level of 1.7 and could be added to the first group. The mixture with soybean flour portion of 20 and 30% (w/w) is joined with wheat flour at distance level of 8.6 and make the second group. This means that soybean flour portion of 30% could be used to more enrich dough with soybean protein and the main rheological properties remain satisfactory as at portion of 20%. This provides a possibility of soybean flour being included in portions even higher than 30%, so future work could include this investigations as well as investigations to examine what happens with lipid components during dough mixing and baking: does their content and composition stay the same as in flour mixture or there occur changes.

4. Conclusion

The soybean flour addition increased the protein content up to 20.0%, the ash content up to 2.08% and lipid content up to 7.2%, while decreased starch, gluten and carbohydrates content for 19.8, 7.2 and 16 g/100 g flour mixture, respectively. Dough rheological properties and lipid profile depend on soybean flour portion. The soybean flour addition had positive influence on the quality number and group and extended duration of dough stability. The dough water absorption and the degree of softening increased with increasing soybean flour portion. The dough with soybean flour portion of 20 and 30% had lower values for energy in comparison to dough made of wheat flour only and it could be economically important. Values of gelatinization temperature for dough with soybean flour were higher than dough with wheat flour, maybe due to a specific behaviour of soy starch in combination with wheat starch. The maximal pasta viscosity decreased when soybean flour portion increased, even seven times when the soybean flour portion was 30%, probably due to short amylopectin branch-chain in soybean starch. All the wheat-soybean flour mixtures had higher free fatty acids and triacylglycerols content than wheat flour. Wheat-soybean flour mixtures had higher content of stearic and behenic acid compared to wheat flour, had almost the same content of linoleic acid as wheat flour and contained linolenic acid which was absent in wheat flour. The ratio of total unsaturated to total saturated fatty acids content was higher in flour mixtures than in wheat flour. The rheological properties of dough, such as water absorption and stability, had the same dependency on soybean flour portion in mixtures as the content of free fatty acids, while the content of triacylglycerols had the same dependency as water absorption and dough development time. In the same way, the content of monoacylglycerols and diacylglycerols had influence on dough extensibility and all these dependences were confirmed by statistical analysis with positive correlation coefficient value, higher than 0.85. The cluster analysis showed that the mixtures with soybean flour portions of 20 and 30% (w/w) were joined with wheat flour at the same distance level, so the soybean flour portion of 30% could be used to enrich even more dough with soybean protein and the main rheological properties remain satisfactory as with portion of 20%.

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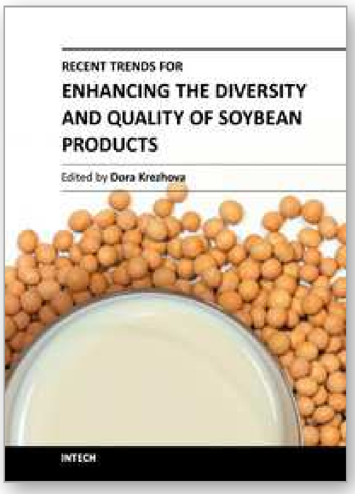

Recent Trends for Enhancing the Diversity and Quality of Soybean Products
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This book presents new aspects and technologies for the applicability of soybean and soybean products in industry (human food, livestock feed, oil and biodiesel production, textile, medicine) as well as for future uses of some soybean sub-products. The contributions are organized in two sections considering soybean in aspects of food, nutrition and health and modern processing technologies. Each of the sections covers a wide range of topics. The authors are from many countries all over the world and this clearly shows that the soybean research and applications are of global significance.