Effects of buckwheat and millet flour addition on the antioxidant potential of wheat flour and rheological properties of the dough

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Abstract

In this work, the antioxidant properties of three types of flour (wheat, buckwheat and millet flour) and rheological properties of three types of dough were investigated: wheat flour dough, dough obtained from a mixture of 50 wt.% wheat flour and 50 wt.% buckwheat flour and dough obtained from a mixture of 50 wt.% wheat flour and 50 wt.% millet flour. Polyphenol content and antioxidant tests have shown that buckwheat flour is superior to wheat and millet flour. The antioxidant activity expressed by the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity did not correlate with the content of polyphenols, which is a consequence of the presence of tannins and phytic acid in millet flour. Determination of rheological properties was performed by a universal dough characterizer. The substitution of wheat flour with buckwheat and millet flour leads to a weakening of the protein structure. Furthermore, the addition of buckwheat or millet flour reduces the rate of gelatinization and viscosity and has a positive effect on the reduction of retrogradation. The combination of these two types of flour with wheat flour can reduce the aging of bakery products.

Keywords: Mixolab; dough rheology; antioxidants; composite flour.

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1. INTRODUCTION

Bread and bakery products are the most represented group of products in our diet. The main raw material in these products is wheat flour, which is considered a nutritionally poor raw material in terms of essential amino acids, dietary fiber, polyphenols and minerals [1]. The amino acid profile of wheat flour protein is poor in essential amino acids such as lysine, threonine and methionine [2]. To overcome this deficiency in wheat bakery products, wheat is partially or completely replaced with other cereals or pseudocereals to create enriched functional products. The food industry is faced with a technological challenge, on one hand, how to enrich bakery products with missing nutritional ingredients and, on the other, not to damage the consistency of the dough, which is characteristic of wheat flour [3]. During dough kneading, wheat flour proteins (gliadin and glutenin) form the gluten matrix. Gliadin is responsible for the viscosity, and glutenin for elasticity and firmness of the dough [4]. Gluten helps in expansion and improves the ability to retain gases in the dough [5]. Enriching wheat flour with whole buckwheat or millet flour can yield bakery products with added value in the functional food category.

Buckwheat is classified as a nutritionally valuable raw material, primarily due to proteins rich in essential amino acids, vitamins, minerals and dietary fiber. The advantage of buckwheat is the lack of gluten in its composition. Hulled grain contains 55 % starch, 12 % protein, 7 % total dietary fiber, 4 % lipid, 2 % soluble carbohydrates, including sucrose and fagopyritols, 2 % ash and 18 % other components, such as vitamins, organic acids, polyphenolic compounds, tannins, nucleotides and nucleic acids. Depending on the type of buckwheat, the content of individual components may also vary [6]. Buckwheat contains large amounts of resistant starch (27 to 33.5 %), which can help control blood glucose and lipid levels, regulate intestinal microbiota and reduce obesity [7].

Buckwheat proteins have a high biological value due to a well-balanced composition of amino acids. In addition to the high content of lysine (6.1 %), buckwheat grain proteins also have a high content of arginine (9.1 %) and aspartic

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acid (11.3 %), compared to other grains. Buckwheat proteins were reported to lower cholesterol levels, have an antihypertensive effect and affect digestion [8,9].

Many studies have suggested a protective role of polyphenolic compounds in buckwheat, which help in prevention of coronary and cancer diseases. Buckwheat is a source of flavonoids, like rutin, quercetin, and kaempferol, which exhibit antioxidant and anti-inflammatory properties [10].

Millet is a grain that naturally does not contain gluten, which makes it an excellent food in the diet of people suffering from various forms of gluten and wheat intolerance. Millet has a high nutritional value, with a large amount of starch of about 60 to 70 %, protein up to 11 %, dietary fiber in the range 2 to 7 % [11,12], minerals: calcium, iron, potassium, magnesium, phosphorus, and zinc, and vitamins such as niacin, B-complex, and vitamin B6. It contains significant amounts of amino acids with sulfur (methionine and cysteine), but also other essential amino acids such as lysine and threonine. In addition, millet is rich in phenolic acids, melanoids, flavonoids and tannins, the content of which is several times higher than in other basic cereals [13].

Introducing millet into the diet, according to literature, can influence cholesterol metabolism, regulate blood pressure, liver function and slow down aging [14,15]. Millet flour is a promising source of micronutrients and proteins, but information on polyphenol compositions in flour is often limited. Nutrients and polyphenol compositions in cereals flours do not depend only on genotype but also on environmental factors. Introduction of buckwheat and millet flour into bakery and confectionery products improves functional properties of these products, while increasing the contents of proteins, dietary fibers, minerals, vitamins and antioxidants. It is undeniable that buckwheat and millet have great nutritional potential, so it is necessary to determine the influence of their addition on rheological properties of the resulting dough and antioxidant properties of the flour mixture.

2. MATERIALS AND METHODS

2. 1. Materials

Wheat flour, wholegrain buckwheat flour and millet flour were purchased in the local market (in Serbia). Chemical compositions of the three flour types (Table 1) were assessed regarding the contents of proteins, total fat, moisture and ash by using standard methods AOAC [16]. Then the carbohydrate content was determined as a balance to 100 %.

Table 1. *Chemical compositions of wheat, wholegrain buckwheat and millet flour*

	Flour samples		
	Wheat flour	Wholegrain buckwheat flour	Millet flour
	Content, wt.%		
Moisture	12.16±0.21^a	13.13±0.058^b	12.63±0.15^c
Proteins	11.03±0.12^a	10.33±0.15^b	8.81±0.10^c
Total fats	1.33±0.058^a	2.17±0.153^b	1.83±0.12^c
Carbohydrates	75.10±0.20^a	72.40±0.20^b	75.67±0.29^c
Ash	0.38±0.06^a	1.97±0.21^b	1.06±0.30^c

Values represent the means (n = 3) ± standard deviation. Values marked by different lower-case letters in the same row are significantly different from each other (p < 0.05)

Preparation of composite flours: WH - control sample (wheat flour), two types of mixture: BWH - wheat flour with wholegrain buckwheat flour (mass ratio 50 : 50) and MWH - wheat flour with millet flour (mass ratio 50 : 50)

2. 2. Determination of the total phenolic content and antioxidant activity

In this work, ethanol was used for extraction of antioxidant and phenolic compounds from flour. Extraction was performed by dissolving 1 g of flour in 20 cm^3 of 80 vol.% ethanol. The mixture was then shaken for 30 min at 25 °C and then centrifuged for 10 min at 10,000 rpm. The supernatant was used in further analysis.

2. 2. 1. Determination of the total phenolic content

The content of total phenolics in the flour was determined by the modified Folin/Ciocalteu (FC) method [17], 0.10 cm^3 of sample, 0.90 cm^3 of ethanol, 0.25 cm^3 of the FC reagent (Sigma-Aldrich, USA) and 0.75 cm^3 of sodium carbonate solution (7.5 %, m/v) were mixed. After 2 h, the absorbance was measured by using the UV/visible spectrophotometer (Ultraspec 3300 pro, Amersham Bioscience, Sweden) at 765 nm. The total phenolic content was assessed by plotting the gallic acid calibration curve and expressed as mg of GAE per g of dried sample.

2. 2. 2. Determination of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

Antioxidant activity of samples was measured based on scavenging activities of the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical [18]. The samples (0.2 cm^3) were mixed with 2 cm^3 of 0.15 mM DPPH solution (Merck, USA) in ethanol. After 30 min of incubation in the dark at room temperature, the absorbance was measured against a blank at 517 nm by using the UV/visible spectrophotometer (Ultraspec 3300 pro, Amersham Bioscience, Sweden). Inhibition of DPPH radical was calculated as a DPPH capacity, % by using the Equation (1):

$$\text{DPPH capacity} = \frac{A_b - A_s}{A_b} 100 \tag{1}$$

where A_b and A_s are the absorbance of the blank and of the sample, respectively.

2. 2. 3. Determination of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity of samples was measured by using a decolorization assay. The ABTS free radical solution (Sigma-Aldrich, USA) was prepared by reaction of a 7 mM aqueous ABTS solution and a 140 mM (2.45 mM final concentration) potassium-persulfate solution. After 16 h, the radical cation solution was diluted with 5 mM phosphate-buffered saline (PBS, pH 7.4) until the initial absorbance value of 0.7 ± 0.05 at 734 nm was reached. The solution with free radicals (1.98 mL) was mixed with 20 µL of each sample, absorbance was measured at 734 nm using a UV Visible spectrophotometer (Ultrospec 3300 pro, Amerischam Bioscienc), after 5 min. ABTS radical scavenging activity, % was calculated by using Equation (2) [19]:

$$\text{ABTS radical scavening activity} = \frac{A_b - A_s}{A_b} 100 \tag{2}$$

2. 3. Rheological characteristic of dough

The mixing and pasting behaviour of the composite flours were determined in a Mixolab 2 universal dough characterizer (Chopin Technologies, France) according to the ICC Standard Method 173 using the “Chopin+” protocol [20]. The procedure was divided into the following three stages:

- Constant temperature stage: temperature was maintained at 30 °C for 8 min;
- Heating stage: temperature was increased to 90 °C at the rate of 4 °C min^{-1} and maintained at 90 °C for 7 min;
- Cooling stage: temperature was reduced to 50 °C at the rate of 4 °C min^{-1} and maintained at 50 °C for 5 min.

The measurement stage required 45 min to complete. The evaluated parameters from the obtained curves were: water absorption (WA); dough development time (DDT); stability (mixing resistance of dough); C1 (maximum torque during mixing); Cs (torque at the end of the holding time at 30 °C); C2 (weakening of the protein, based on mechanical stress at increasing temperature); C3 (rate of starch gelatinization); C4 (minimum torque during the heating period); C5 (torque after cooling at 50 °C); C5-C4 (starch retrogradation at cooling stage, representing the shelf-life of the end products).

2. 4. Statistical analysis

All experiments were performed in triplicate. Mean values were analysed using one-way ANOVA. The Tukey *post hoc* test was performed for means comparison (OriginPro 8, www.originlab.com). Differences were considered as significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3. 1. Polyphenols and antioxidant capacity

Content of polyphenols and antioxidant activities expressed as DPPH and ABTS radical scavenging activities are shown in Table 2. Both methods measure free radical scavenging activity (radical-scavenging activity - RSA) (*i.e*. DPPH and ABTS). DPPH is one of free radicals which are relatively stable at room temperature and is commonly used for determination of the antioxidant capacity of different molecules. The scavenging capacity against DPPH radical is strongly influenced by the solvent and reaction pH. The sample is usually dissolved in methanol or ethanol so that only methanol /ethanol soluble antioxidants are detected (hydrophilic polar protic molecules). The ABTS radical is soluble in water and organic solvents, enabling determination of the antioxidant capacity of both hydrophilic and lipophilic compounds. The content of polyphenols is the highest in buckwheat flour, and the lowest in the millet flour. Buckwheat flour has 5.4 times more polyphenols than wheat flour, while the millet flour has a slightly lower content that is 22 % less polyphenols than the wheat flour.

Table 2. *Antioxidant activity indicators of flour samples*

Flour	Content of polyphenols, mg g^{-1}	Content of DPPH, %	Content of ABTS, %
Millet flour	0.46±0.01^{a}	19.17±0.78^{a}	1.00±0.21^{a}
Wholegrain buckwheat flour	3.20±0.27^{b}	90.76±3.14^{b}	78.90±8.52^{b}
Wheat flour	0.60±0.09^{ac}	14.02±0.82^{c}	16.73±1.82^{c}

Values represent the means (n = 3) ± standard deviation. Values followed by different lower-case letters in the same column are significantly from each other (p < 0.05)

Data reported in the literature [18] also indicate that the content of polyphenols in millet flour can be twice as low as in wheat flour. It has been also reported that polyphenolic compounds, particularly phenolic acids, negatively impact the formation of gluten networks and lead to the immediate disruption of dough structure [22,23]. The composition of polyphenols in various types of cereals is different, and therefore different antioxidant capacities should be expected [24,25]. There is a lack of correlation between the polyphenol content and antioxidant activity expressed as DPPH radical scavenging activity (Table 2). This can be explained by the presence of compounds such as tannins and phytic acid that act as antioxidants and are present in millet in greater quantity compared to wheat [25]. Antioxidant activities detected by ABTS radicals follow the content of polyphenols in the samples, and more significant differences are observed. They range from 1.0 % for millet flour to 78.9 % for wheat flour.

3. 2. Dough properties

Mixolab device offers the advantage of assessing various flour characteristics of cereals in a single test, determining the quality of proteins, starch, and associated enzymes. The influence of the addition of 50% whole buckwheat flour and 50% millet flour on the thermomechanical properties of wheat flour is shown in Figure 1, while the values of the main parameters derived from the Mixolab curve are summarized in Table 3.

The first part of the curve reveals the protein properties of the tested flour samples. Characteristic parameters for this stage of dough formation at a constant temperature of 30°C under mixing are: water absorption, dough development time, stability and mechanical weakening.

The Mixolab water absorption value signifies the quantity of water required to achieve a dough consistency of 1.1±0.05 N m and the dough development time refers to the moment of reaching the maximum torque of 1.1 N m and determines the strength of the flour. Addition of 50 % whole buckwheat flour to wheat flour led to an increase in water absorption by 9.4 %, which is due to naturally occurring hydrocolloids and buckwheat fibers [26] needing more time to absorb water. This resulted in an increase in the dough development time (by 30 %) and a decrease in the dough stability by 31.4 %. Addition of millet to wheat flour (MWH) led to a decrease in water absorption due to dilution of gluten, which is in agreement with professional literature [27,28]. The time needed to obtain 1.1 N m was higher when using BWF and MWH, even though WH contained more proteins. The dough made from wheat flour had the greatest stability, because it is also the strongest.

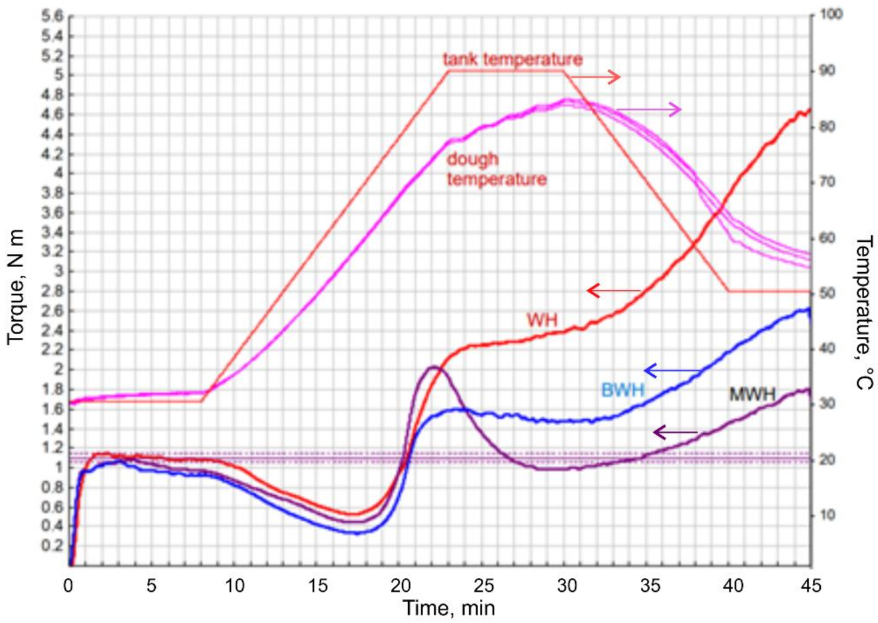

Figure 1. Typical *mixolab curves of tested flours (WH, BWH and MWH)*

Table 3. *Mixolab characteristics of tested flours*

Flour	WA, wt.%	DDT, min	Stability, min	Cs, N m	C2, N m	C3, N m	C4, N m	C5, N m
WH	53.21±0.23[a]	2.32±0.55[a]	9.33±0.09a	1.09±0.01[a]	0.53±0.01[a]	2.17±0.03[a]	2.34±0.03[a]	4.35±0.08[a]
BWH	57.22±0.11[b]	3.00±0.38[a]	6.41±0.21b	0.93±0.02[b]	0.33±0.01[b]	1.60±0.01[b]	1.46±0.02[b]	2.48±0.05[b]
MWH	45.00±0.12[c]	3.91±0.24[b]	8.41±0.18c	0.97±0.01[cb]	0.44±0.02[c]	2.02±0.01[c]	0.98±0.02[c]	1.69±0.03[c]

Values represent the means (n = 3) ± standard deviation. Values followed by different lower-case letters in the same column are significantly from each other (p < 0.05)

When the dough temperature is increased from 30°C at a rate of 4°C min^{-1} and mixing continues at a constant speed, the strength of the dough decreases as a result of protein attenuation. An increase in the temperature of the dough implies protein denaturation, which includes the release of water. A decrease in resistance occurs to the lowest point - C2 [29]. C2 point gauges the reduction in protein strength resulting from mechanical work and rising temperature. The lower the C2 value, the weaker the gluten quality, which weakens the ability to retain gas and rise the dough. The higher the C2 value, the better the gluten quality, but if the C2 value is too high, this means that the gluten is too strong and can therefore limit the rise of the dough. High-quality wheat flour exhibits a torque C2 value exceeding 0.4 N m. A range of 0.5 to 0.6 N m suggests superior protein quality, enhanced gluten resistance to heating, and a more robust gluten network.

The value of C2 depends on the characteristics and amount of protein and for the wheat flour it is 0.53 N m, while for the composite flour BWH and MWH it is 0.33 and 0.44 N m, respectively. The reduction of C2 in composite flours is the result of dilution of the gluten content, *i.e.* the addition of incompatible buckwheat and millet proteins, which leads to an increase in the rate of protein weakening due to heat [30,27]. Proteins of lower quality than gluten are less stable and exhibit lower C2. As heating continued, the second part of the curve follows the changes in the dough structure caused by the rise in temperature and mechanical forces of mixing. It depends on characteristics of the starch and enzymatic activity in the flour. The torque starts to increase as a consequence of the starch gelatinization process. In this process, consistency increases due to structural changes: loss of the crystal structure, breaking of glucosidic bonds and establishment of new molecular interactions. Starch grains in contact with water released by protein denaturation slowly swell and the crystal structure is lost. Swelling of starch originating from cereals is primarily a consequence of the presence of amylopectin, because the amylopectin shell breaks, and the contents are released into the surrounding. Amylose acts as a swelling inhibitor [31]. The poured grains slowly stick together forming a high viscosity mater. During heating, disruption of the crystalline regions, primarily composed of amylopectin, allows water absorption and granule expansion and amylose molecules leach out, resulting in an increase in the viscosity with a concomitant increase in the maximum peak torque C3 produced during this heating stage. The value of the maximum torque C3 can be influenced by the content and type of starch, amylolytic activity, damage to starch grains, wheat variety, agroecological conditions during cultivation, *etc.* [32]. Increasing the amount of additives (*i.e.* buckwheat and millet flour) in wheat flour yield lower values of the maximum torque C3, *i.e.* lower viscosity due to different nature of starch in gluten-free flours. The reduction compared to wheat flour is 26.3 % for BWH by and 6.9 % for MWH. The results obtained are consistent with data reported in the literature [33], according to which replacing wheat flour with millet flour leads to a decrease in viscosity. The maximum viscosity (C3) is positively correlated with the viscosity index of the Mixolab profiler, shown in Table 4 (wheat flour 8, mixture with whole buckwheat flour 4, and with millet flour 7). The Mixolab profiler converts the mixolab curve into 6 indexes rated 0-9. It profiles flour on the basic 6 fundamental criteria: absorption (reflects flour quality), mixing (assesses dough stability during kneading at 30°C), gluten (measures gluten behaviour under heat), viscosity, amylase (amylase activity) and retrogradation.

Table 4. *Mixolab profiler index for tested flours*

	Mixolab index rate					
Flour	Absorption	Mixing	Gluten+	Viscosity	Amylase	Retrogradation
WH	1	5	5	8	9	8
BWH	3	2	2	4	6	7
MWH	0	3	5	7	1	4

When the dough is kept at 90°C for 7 min, the torque of the dough decreases until the point C4 is reached, *i.e*. the resistance of the dough in the starch gel phase. The C4 torque reflects the stability of the hot starch paste which decreases slightly for the composite flours. The drop in hot consistency of starch (C3-C4) is greater if amylase activity is higher. Compared to wheat flour, amylase activity is higher in both MWH and BWH. Ninomiya *et al.* found that buckwheat albumin maintained high α-amylase inhibitory activity even after heating at 100 °C for 120 min [34].

During cooling to 50 °C, retrogradation of starch occurs and the consistency of the dough increases. The amylose molecules, which in the phase of swelling and destruction of the starch granule passed into the water phase, are oriented parallel to each other and connected by hydrogen bonds. The re-establishment of the crystalline structure leads to association of starch molecules, resulting in the formation of strong gels, increased viscosity (C5), and a loss of water retention capacity. Upon cooling, amylose reorganizes and forms a stable gel structure. Amylose, due to its linear structure, exhibits a greater tendency for retrogradation compared to amylopectin, which is more branched. This results in faster and more pronounced retrogradation in products that are rich in amylose. This process occurs spontaneously, as it results in a decrease in the free energy of the system, thereby maintaining thermodynamic stability. Wheat flour had the highest values of the degree of retrogradation (2.01 N m) expressed as the difference between C5 and C4 torque values than in the investigated composite flour types. For the mixture with buckwheat flour, the degree of retrogradation is 1.02 N m, and for the mixture with millet flour it is 0.71 N m. Thus, starch retrogradation is minimal in the samples with millet flour (MWH), which we consider to be a result of the highest amylose content. According to the literature [35], the amylose content in millet flour is approximately 33 wt.%, compared to 30 wt.% in wheat flour and around 25 wt.% in buckwheat flour. In a previous study [36], it was also observed that retrogradation is lower in millet flour, and it is suggested that the reduction in C5 as compared to wheat flour, is a consequence of a lower number of dextrins that can form following starch hydrolysis. Additionally, Bharati *et al.* [27] have noted the positive effect of incorporating millet flour on retrogradation.

. The retrogradation index of the Mixolab profiler shown in Table 3 is inversely proportional to the shelf life of the product, namely the higher the index, the faster the bakery product ages (WH flour 8, BWH flour 7, and MWH 4). So, reduction in retrogradation is significant due to the freshness of bakery products, so if we want the best freshness of those products, it is desired to add millet flour. The reason for reduction in retrogradation should be found in the difference in the structure and amount of amylose and amylopectin fraction in non-gluten flour starch compared to wheat starch.

4. CONCLUSION

Addition of whole buckwheat flour and millet flour to wheat flour led to an increase in lipids and mineral substances, and a weakening of the gluten structure of the dough. Prolonging the dough development time while simultaneously reducing dough stability is characteristic of the addition of buckwheat and millet to wheat dough. Also, both types of flour led to a decrease in the maximum torque of the hot starch suspension. Reduction in retrogradation is affected by buckwheat flour, but significantly more by millet flour. thus, if a bakery product with extended freshness is needed, wheat flour should be enriched with millet flour.

The polyphenol content, as well as the antioxidant activity expressed through DPPH and ABTS radicals, was highest in buckwheat flour. Thus, the polyphenol content was as much as 6.9 times higher than in millet flour and 5.4 times higher than in wheat flour. In terms of improving functionality of bakery and confectionery products made from wheat flour, a more significant contribution would be achieved by the addition of buckwheat flour compared to millet flour. Still, the contribution effects of both types of flour to wheat flour are evident. Further research can be directed to a blend of wheat, buckwheat and millet flour in proportions where the wheat flour content is lower than 50 wt.%.

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Uticaj dodatka heljde i prosa na antioksidativni potencijal i reološke karakteristike testa od pšeničnog brašna

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(Naučni rad)

Izvod

U ovom radu su ispitivana antioksidativna svojstva tri vrste brašna (pšeničnog, heljdinog I brašna od prosa) i reološka svojstva tri vrste testa: testa od pšeničnog brašna, testa od smeše 50 mas.% pšeničnog brašna/ 50 mas.% heljdinog brašna i 50 mas.% pšeničnog brašna/ 50 mas.% brašna od prosa. Sadržaj polifenola i antioksidativna ispitivanja su pokazala da je brašno od heljde značajno superiornije u odnosu i na brašno od pšenice i na brašno od prosa. Antioksidativna aktivnost izražena preko DPPH radikala nije bila u korelaciji sa sadržajem polifenola, što je posledica prisustva tanina i fitiske kiseline u brašnu od prosa. Reološka svojstva su ispitana pomoću univerzalnog uredjaja za karakterizaciju testa. Supstitucija dela pšeničnog brašna brašnom od heljde i prosa dovodi do slabljenja strukture proteina. Osim toga, dodavanje heljdinog ili brašna od prosa smanjuje brzinu želatinizacije i viskoznost, a pozitivno utiče na smanjenje retrogradacije. Kombinacija ove dve vrste brašna sa pšeničnim brašnom može dovesti do smanjivanja brzine starenja pekarskih proizvoda.

Ključne reči: Mixolab; reologija testa; antioksidansi; kompozitna brašna