ISSN:
2217-5369 (print),
2217-5660 (online)

www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad

FINS

UDK 664.661/.663:577.164.2:664.1]:543.92

Original research paper

DOI 10.5937/ffr48-34944

EFFECTS OF ASCORBIC ACID AND SUGAR ON PHYSICAL, TEXTURAL AND SENSORY PROPERTIES OF COMPOSITE BREADS

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Abstract: The present study was carried out to demonstrate the combined effects of different maize flour, ascorbic acid and sugar on the physical, textural and sensory properties of composite breads. The composite flour was prepared using 70% of wheat flour and 30% of flour obtained from grain of differently colored maize - light blue, blue, red and yellow maize flour. Chemical characterization of composite flours made with four different types of maize was also assessed. Furthermore, the content of total phenolics, flavonoids, anthocyanins, phenolic acids and antioxidant capacity in composite flours was determined. A total of 12 breads were prepared, four of which were control composite breads, four breads with ascorbic acid, and four were breads with ascorbic acid and sugar. The content of total phenolic compounds showed clear differences among all composite flours. The anthocyanins content determined in composite flours was in the following descending order: blue>red>light blue, while in the yellow maize composite flour anthocyanins were not detected. The results showed that the addition of AsA (0.025%) and sugar (5%) negatively affected the volume as well as the specific volume of composite wheat-maize breads. The texture analysis showed that the addition of AsA in the amount of 0.025% had no impact on springiness, cohesiveness and resilience of bread crumb, while it increased crumb hardness. However, composite breads made with AsA and AsA/sugar showed a more compact structure, with a larger number of cells and smaller mean cell areas. AsA/sugar bread samples within the tested doses had the lowest springiness, which is indicative of brittleness and reflects the tendency of the bread to crumble when slicing. Results of the sensory evaluation revealed that the AsA and sugar addition had a generally positive effect on the investigated sensory attributes.

Key words: *colored maize, bread making, textural properties, sensory properties, ascorbic acid, sugar*

INTRODUCTION

Bread is one of the world's most widely consumed staple foods of immense importance. The utilisations of new raw materials, ingredients as well as bakery machinery and tools have resulted in ever improving bread making technology. Mandatory ingredients which involve in bread making are: flour, yeast and water, additional raw materials are: sugar,

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fat, milk, etc. and improvers such as: different types of additives and emulsifier (Demin et al., 2013; Umelo et al., 2014). In recent years, numerous studies were conducted to improve bread nutritional value (macronutrients: carbohydrates, proteins, fat and dietary fibres; micronutrients: minerals and vitamins), health supporting bioactive compounds, sensory acceptability, shelf life and to match its affordability. According to Menon, Swarnali & Usha (2015), the composite flours have considerably been used in making bread products to predominantly reveal functional roles of flour components and to analyze its sensory acceptability. Several authors reported negative effects of non-wheat flour (cassava, cocoyam, yam, sweet-potato, soy, maize, rye, rice) addition to wheat flour on sensory attributes in the scores for appearance, crust and crumb color, taste and flavor, which leading to a decreased overall acceptability of the composite bread (Almazan, 1990; Khalil, Mansour & Dawoud, 2000; Sabanis & Tzia, 2011; Mongi et al., 2011; Nindjin, Amani & Sindic, 2011; Rai, Kaur, Singh & Minhas, 2012; Demin et al., 2013; Bibiana, Grace & Julius, 2014; Trejo-Gonzalez, Loyo-González & Munguía-Mazariegos, 2014). Also, Kamoto, Kasapila & Tinna (2018) reported that mixing amaranth and wheat flour affects the rheological properties of dough, which, in turn, limits the baking characteristics and quality of the bread as a final product. Schoenlechner, Szatmari, Bagdi and Tömösközi (2013) noted that the incorporation of millet in wheat flour decreased bread volume and change crumb porosity and texture. In these studies, the reported levels of wheat substitution were in the range of 5-50%. Nevertheless, the use of composite flours with a high amount of non-wheat flour (≥ 10%) presents considerable technological difficulties due to the low levels or the absence of gluten (Cato, Gan, Rafael & Small, 2004). Changes in the everyday diet made the bioactive component-enriched foods become increasingly popular (Feng et al., 2020). Apart from its significant influence on the technological properties of bread, pigmented maize has gained newfound attention from a nutraceutical perspective due to its potential health benefits. In addition to the color that they impart, these maize genotypes contain considerable amounts of bioactive components which can promote a beneficial impact on human health. Pigmented maize contains many secondary metabolites, such as phenolic compounds and carotenoids. Anthocyanins and phenolic compounds are distributed in the aleurone and pericarp monolayer of the grain, which provides the characteristic blue, red, purple, and black color to the maize varieties (Espinosa et al., 2009). The anthocyanins, flavonoids, and phenolic acids act as antioxidants, antiinflammatory, anticarcinogenic, antimutagenic, antidiabetic, and ocular health enhancing agents, can promote estrogenic activities, inhibition of enzymes, and induction of detoxification enzymes (Adom & Liu, 2002; Abdel-Aal, Akhtar, Rabalski & Bryan, 2014). Some pigmented maize varieties rich in anthocyanins can be used for their commercial extraction, which can subsequently be used as a natural substitute to chemical food colorants, as components for some cosmetic products, as well as dietary supplements (Somavat, Li, de Mejia, Liu & Singh, 2016).

Due to its positive effects on dough properties, ascorbic acid (AsA) has been used as a flour improver for a long time. Ascorbic acid is a very popular and widely used flour improver in bread products to incentives oxidizing process (Joye, Lagrain & Delcour, 2009; Šimurina et al., 2014). The addition of ascorbic acid (AsA) has a well-studied strengthening effect on dough that leads to a higher rising of the dough (Koehler, 2003). This oxidizing agent is also used to improve the handling characteristics of the dough, specific volume and texture of the finished product (Umelo et al., 2014). Detailed studies have shown that in fact, L-threo-dehydroacorbic acid (DHAsA) is the actual dough improver, which is derived from AsA through oxidation by atmospheric oxygen or endogenous ascorbic acid oxidase during dough mixing (Faccio, Flander, Buchert, Saloheimo & Nordlund, 2012). However, an unsuitable AsA concentration (usually too high) can significantly increase dough resistance, as well as, significantly reduce its stretching (Tsen, 1965). It is typically used in doses of 50-70 mg/kg flour (Wieser, 2003), but wider ranges of 10-200 mg/kg are also proposed (Selomulyo & Zhou, 2007; Šimurina, Filipčev, Jovanov, Ikonić & Simović-Šoronja, 2013). Nevertheless, as for most dough con-ditioners, its effect is not only dose-dependent but dependent on the initial quality of flour and the type of bread-making method (Pečivová, Pavlínek & Hrabê, 2011; Šimurina et al., 2014).

In typical bread production, 2-3% sugar is adequate to sustain yeast activity. Due to the affinity with water, it has been reported that sugar exerts a limiting effect on forming of gluten network during the dough preparation stage. Sugar is used as a substrate for the yeast during the early stages of fermentation. Furthermore, sugar acts as antistaling ingredients inhibiting starch recrystallization (Levine & Slade, 1990). Nonetheless, sugar increases a product volume and a crust color if the oven temperature is adjusted (Brown, 1993). Sugar that remains unfermented by yeast appears as residual sugar in the finished products (Nip, 2006). Residual sugar takes part in caramelisation and the Maillard reaction (i.e., the reaction between reducing sugar and the proteins of flour promotes rapid color and taste formation).

The present study was carried out to demonstrate the combined effects of different maize flour, ascorbic acid and sugar on the physical, textural and sensory properties of composite breads. The physical and textural properties included bread loaf volumes and specific volumes, as well as the hardness, chewiness, cohesiveness, resilience and springiness of bread crumbs. The sensory properties included the crumb elasticity and appearance of crumb pores (structure and uniformity, Dallman pores), as well as the bread shape, odor, taste, aroma and chewiness. Since the various maize types (flint, popping, flour, dent and sweet) differ significantly in physicochemical characteristics and affects composite flour quality, chemical characterization of composite flours made with four different types of color maize was also assessed. Furthermore, the content of total phenolics, flavonoids, anthocyanins, phenolic acids and antioxidant capacity in composite flours was determined.

MATERIALS AND METHODS

Plant materials

The experimental material consisted of one bread wheat (*Triticum aestivum* L.) and four maize (*Zea mays* L.) genotypes recently developed at the Maize Research Institute, Zemun Polje, in the vicinity of Belgrade, Serbia. The maize genotypes were chosen on the basis of kernel color and kernel type (light blue maize - dent, blue and red maize - popping, yellow maize - semi-flint). All genotypes were grown in the field at the Maize Research Institute, Belgrade (44°52’ N, 20°19’ E, 82 m a.s.l.), Serbia, in the 2019 growing season. Standard cropping practices were used to provide adequate nutrition and to keep the disease- and weed-free plots.

Flour samples preparation

Wheat flour (particle size <180 µm) was produced in the experimental mill (Laboratory mill; Bühler MLU-202, Uzwil, Switzerland). The characteristics of the obtained flour correspond to those of flour with 0.45 to 0.55% of ash. In contrast to the debranned wheat flour, the whole grain maize flour was used. Maize samples were ground on a Perten 120 lab mill (Perten Instruments, Hägersten, Sweden) to fine powder (<500µm).

Bread-making procedure of composite bread

The composite flours were made of wheat flour (70%) and different maize flours (30%) (light blue, blue, red and yellow maize). The basic formulation of the composite breads included: 300g composite flour, 7.5g yeast, 6g salt, 3g vegetable fat and water according to farinograph absorption. The total of 12 breads were prepared in four replications out of which four were control breads (composite flour with light blue, blue, red and yellow maize), four were breads with ascorbic acid (AsA) and four were breads with ascorbic acid (AsA) and sugar. The ingredients were weighted according to the proportions listed in Table 1. All formulations of twelve bread samples were made according to the method described by Simić et al. (2018). Briefly, all ingredients were mixed in a laboratory mixer and the dough was left to rest in bulk. The dough was divided into 115±1 g portions, manually rounded, rolled and put into tin pans. The final fermentation lasted 35 min. The baking was carried out at 230 °C for 20 min in a deck type oven. Baked breads were cooled down and stored at 24 °C for 24 h and then their quality was evaluated.

Chemical procedure

Analysis of basic chemical compounds of composite flour

The standard chemical methods were applied to determine moisture, content of total proteins, fats, cellulose and ash (AOAC, 1990; AACC, 2000). Results were expressed as % of d.m.

Table 1.
The formulations of composite breads

Ingredient (%)	**Maize composite bread-Control**	**Maize composite bread with AsA**	**Maize composite bread with AsA and sugar**
Wheat flour	70	70	70
Whole grain maize flour	30	30	30
Baker's yeast	2.5	2.5	2.5
Salt	2	2	2
Vegetable fat	1	1	1
Ascorbic acid	-	0.025	0.025
Sugar	-	-	5

AsA- ascorbic acid

Extraction of phenolic compounds from composite flour

For the detection of the total phenolics, total flavonoids and phenolic acids extracts were prepared from 0.5 g of flour. All flour samples were stored in the dark at -18 °C until the analysis to protect bioactive components from degradation. After alkaline hydrolysis for 4 h at room temperature using 10mL of 4 M NaOH, extraction was done with ethyl acetate and diethylether (1:1, v/v) four times. Five mL of combined extracts were evaporated under the N_2 stream at 30 °C to dryness and final residues were re-dissolved in methanol. The extracts were kept at -70 °C until analyses.

Analysis of total phenolic content in composite flour (TPC)

The total phenolic content was determined according to the Folin-Ciocalteu procedure (Singleton, Orthofer & Lamuela-Raventos, 1999). The extract (270 μL) was transferred into a test tube and the volume adjusted to 500 mL with distilled water and oxidized with the addition of 250 μL Folin-Ciocalteu reagent. After 5 min, the mixture was neutralized with 1 mL of 20% aqueous Na_2CO_3 solution. After 40 min, the absorbance was measured at 725 nm. The total phenolic content was expressed as mg of gallic acid equivalent (GAE) per kg of dry matter (d.m.).

Analysis of total flavonoid content in composite flour (TFC)

The total flavonoid content was determined according to Eberhardt, Lee & Liu (2000). The 5% $NaNO_2$ solution (50 μL) was mixed with 200 μL of the extract. After 6 min, 500 μL of the 10% $AlCl_3$ solution was added, and after 7 min, 250 μL of 1 MNaOH was added. Absorbance was measured at 510 nm. The total flavonoid content was expressed as mg of catechin equivalent (CE) per kg of d.m.

Analysis of total anthocyanin content in composite flour (TAC)

Anthocyanins were extracted from 80 mg of maize flour and 250 mg of bread samples mixing with 5 mL of methanol acidified with 1 M HCl (85:15, v/v). After shaking, the absorbance was measured at 535 and 700 nm (Abdel-Aal & Hucl, 1999). The content was expressed as mg of cyanidin 3-glucoside equivalent (CGE) per kg of d.m.

Analysis of individual phenolic acids in composite flour

Chromatographic analyses were performed on the Thermo Scientific Ultimate 3000 HPLC with a photodiode array detector (Thermo Scientific, Waltham, Massachusetts, USA). Phenolic acids were separated on the Thermo Scientific Hypersil GOLD aQ C18 column (150 mm × 4.6 mm, i.d., 3 μm) using a linear gradient elution program with a mobile phase containing solvent A (formic acid/HO, 1:99, v/v) and solvent B (methanol) at a flow rate of 0.8 mL/min. The solvent gradient was programmed as described by Žilić et al. (2012). The chromatograms were recorded at 280 nm by monitoring spectra within the wavelength range of 190-400 nm. Identified phenolic acid peaks were confirmed and quantified using the Thermo Scientific Dionex Chromeleon 7.2. chromatographic software.

Analysis of the total antioxidant capacity of composite flour (AC)

The total antioxidant capacity was measured according to the direct or QUENCHER method described by Serpen, Gökmen, Pellegrini and Fogliano (2008) using the ABTS (2,2-azino-bis3-ethil-benothiazoline6-sulfonic acid) reagent. The total antioxidant capacity was expressed as mmol Trolox equivalents (Eq) per kg of d.m.

Composite bread evaluation

Physical and textural properties

Bread loaf volumes (mL) and specific volumes (mL/g) were determined by VolScan profiler (Stable Micro Systems, Surrey, UK). A bread loaf was cut into slices of 25 mm thickness. Slices from the middle of the loaf were analyzed for hardness, cohesiveness, adhesiveness, springiness, and chewiness. A texture analyzer, TA.XT plus (Stable Micro Systems, England, UK) was used to perform a texture profile analysis (TPA) on the crumb of baked bread. A test speed of 10 mm/s, return distance of 30 mm with contact force of 1.0 g and 80 % strain was used. An aluminium probe plate (35 mm diameter) was used for the test.

Sensory properties

The sensory properties included the crumb elasticity and appearance of crumb pores (structure and uniformity, Dallman pores), as well as, the bread shape, odor, taste, aroma and chewiness. Twenty four hours after baking, the breads were sliced and evaluated on the 5-point intensity scale by a 6-member panel (from the ages of 34-45) with necessary knowledge and experience in the sensory descriptive analysis, which included techniques and practice in attribute identification and terminology development (University of Novi Sad, Institute of Food Technology, Serbia). Sensory properties of bread samples were evaluated by using the 5-point category scale with end-points labeled from1 to 5 as described by Filipčev, Lević, Bodroža-Solarov, Mišljenović & Koprivica (2010).

Statistical analysis

The results were statistically analyzed using the Statistica software version 5.0 (StatSoft Co., Tulsa, OK, USA). Significance of differences between samples was analyzed by the Tukey's test. Differences at $p<0.05$ were considered as significant.

RESULTS AND DISCUSSION

Chemical composition of composite flours

The analysis of the chemical composition of composite flour is very important in order to determine its quality. The content of total protein, fat, cellulose and ash in composite flours with light blue, blue, red and yellow maize ranged from 9.69 to 10.93, 2.79 to 3.19, 0.94 to 1.29 and 0.89 to0.92, respectively (Table 2).

Table 2.
The chemical composition, content of phenolic compounds and antioxidant capacity of composite wheat-maize flours

Parameter	**Composite flour**			
	LBM	**BM**	**RM**	**YM**
Protein (% d.m.)	10.93±0.06^{a}	10.64±0.15^{a}	10.93±0.13^{a}	9.69±0.14^{b}
Fat (% d.m.)	3.19±0.07^{a}	2.79±0.04^{b}	2.87±0.08^{b}	2.82±0.08^{b}
Cellulose (% d.m.)	1.01±0.01^{ab}	1.29±0.02^{a}	1.25±0.11^{a}	0.94±0.06^{b}
Ash (% d.m.)	0.92±0.01^{a}	0.90±0.04^{a}	0.92±0.01^{a}	0.89±0.02^{a}
TPC (mg GAE/kg d.m.)	976.45±27.21^{c}	1380.07±35.01^{a}	1129.45±32.77^{b}	1037.28±85.04^{bc}
TFC (mg CE/kg d.m.)	129.91±0.03^{a}	194.54±31.61^{a}	190.64±30.19^{a}	108.39±4.36^{a}
TAC (mg CGE/kg d.m.)	88.16±4.47^{c}	286.79±2.60^{a}	179.63±1.27^{b}	n.d.
Individual phenolic acids				
Ferulic acid (mg/kg d.m.)	649.81±5.13^{c}	1010.70±13.79^{a}	740.43±7.48^{b}	466.36±6.25^{d}
p-coumaric acid (mg/kg d.m.)	70.17±0.61^{d}	94.06±0.52^{b}	102.10±0.86^{a}	75.95±0.95^{c}
AC (mmol Trolox Eq/kg d.m.)	18.96±0.37^{a}	20.09±1.31^{a}	19.89±1.43^{a}	17.56±0.05^{a}

Values are means of three determinations ± standard deviation
Values of the same row with the same letters are not significantly different ($p > 0.05$)
LBM-light blue maize; BM-blue maize; RM-red maize; YM-yellow maize; n.d. - not detected
TPC-total phenolic content; TFC-total flavonoid content; TAC-total anthocyanins content; AC-antioxidant capacity

The flour quality depend on interplay of its ingredients, such as proteins, starch, water, non-starch polysaccharides, in particular arabinoxylans and lipids, as well as phenolic compounds (Janković et al., 2015). Also, various maize types (flint, popping, flour, dent and sweet) differ significantly in physico-chemical characteristics and the horny to floury endosperm ratio (Hamilton, Hamilton, Johnson & Mitchell, 1951), affects composite flour quality. The yellow maize composite flour had the lowest protein content among tested composite flours. Composite flour made of light blue maize had the highest fat content, 3.19% of dry matter. According to the results presented in Table 2 the composite flours obtained from blue and red maize had a significantly higher ($p<0.05$) content of cellulose than remaining tested composite flours. The cellulose content in these samples was higher by about 22% and 27% than that in composite flours made of light blue and yellow maize, respectively. Our results indicate that differences in the genetic background of maize hybrids affected their chemical composition.

Content of total phenolic compounds, and the antioxidant activity

The content of total phenolic compounds showed clear differences among all composite flours (Table 2). Composite flour with blue maize had a higher total phenolic content (1380.07 mg GAE/kg d.m.) than those with yellow and red maize (1037.28 and 1129.45 mg GAE/kg d.m., respectively) as well as with light blue maize (976.45 mg GAE/kg d.m.). According to Hu and Xu (2011) blue maize is especially high in phenolic compounds as compared to light colored maize genotypes. Given that, phenolic compounds are primarily concentrated in the wheat bran (Liyana-Pathirana & Shahidi, 2006) that was lost during the process of wheat milling, it can probably be stated that the content of total phenolic compounds in composite flours originates from maize flour from whole grain. According to the research of Simić et al. (2018) total phenolic content in wheat white flour amounted only to 340 mg GAE/kg of d.m.

The total flavonoid content did not vary greatly among composite flour samples and it ranged from 108.39 to 194.54 mg CE/kg d.m. in yellow and blue maize composite flours, respectively. However, the data obtained points out to the significant difference in the content of anthocyanins between composite flour samples (Table 2). The anthocyanins content in composite flours was in the following descending order: blue>red>light blue, while in the yellow maize composite flour anthocyanins were not detected. This result was expected as having in mind that yellow colouration would indicated that carotenoids were the most abundant pigment types in light colored maize (Kurilich & Juvik, 1999). Our results demonstrated a maximum anthocyanin level in the blue maize composite flour (286.79 mg CGE/kg d.m.), which was higher by approximately 70% and 38% than the level measured in the light blue and red maize composite flours, respectively. Since the only white color is primary genetic characteristic of hexaploid wheat (Simić et al., 2018), the entire content of anthocyanins in composite flours could be attributed to the anthocyanin content of individual flour components specifically of light blue, blue and red maize that were incorporated in the composite flour formulation.

In composite flour samples two individual phenolic acids, namely, ferulic and p-coumaric acids, were detected. The ferulic acid content of tested composite flours, ranged from 466.36 mg/kg d.m. up to 1010.70 mg/kg d.m. (Table 2). The highest content of ferulic acid (1010.7 mg/kg d.m) was recorded in the blue composite flour, while the percentage contribution of ferulic acid of red, light blue and yellow composite flours to that in the blue composite flour was lower by about 27%, 36% and 54%, respectively. These results could be related to the differences in maize grain hardness among maize races. Findings reported by Chiremba, Taylor, Rooney & Beta (2012) showed that harder maize grains had higher ferulic acid contents than soft ones.

Most of the total ferulic acid in maize grain is attached to cell wall components by ester bonds (Saulnier & Thibault, 1999). The highest content of p-coumaric acid detected in the composite flour with red maize (102.10 mg/g d.m.) was significantly different from contents recorded in composite flours with blue (94.06 mg/g d.m.), yellow (75.95 mg/g d.m.) and light blue (70.17 mg/g d.m.) maize.

The antioxidant capacity did not significantly statistically vary among tested composite flours. The highest antioxidant capacity correlated with the highest content of total phenolic compounds in the blue maize composite flour (Table 2), which is in agreement with results reported by Simić et al. (2018).

Physical and textural properties of composite breads

The data for the physical and textural analysis of composite bread are presented in Figure 1. The results reveal that the loaf volume of the control maize composite bread samples ranged from 182.50 ml to 188.01 ml (Figure 1. a). In order to evaluate the AsA effect and the synergistic effect of AsA and sugar on physical properties of breads, all the results were compared with those no additive composite breads as a control samples. The control composite breads, those with AsA and AsA and sugar addition displayed differences in the crumb structure and texture parameters. The loaf volume parameter was greatly affected by the addition of AsA (0.025%) and sugar (5%) in wheat-maize composite breads. Addition of AsA and sugar in tested doses had a negative effect on the volume as well as the specific volume of maize composite breads. The volumes of loaves decreased with AsA and AsA and sugar addition and the differences were on average about 4%. The loaf volume of light blue maize composite bread was 182.50ml, while it was reduced by 6% and 5.6% with the addition of AsA and AsA and sugar, respectively. The same trend was observed in other samples of composite breads. The highest loaf volume reduction of 8.7% was detected in red maize composite bread with the AsA and sugar addition. These results are in agreement with results of studies previously carried out by Codina, Cretu and Paslaru (2007). In addition, the amount of added sugar (5%) in composite bread formulations is considered high and characteristic of sweet bakery goods (Schünemann & Treu, 2009). According to Barham and Johnson (1951) double sugar in bread formula increased the bread weight as expected but also greatly lowered the volume comparing with the bread with the same type of sugar at the regular amount (up to 2.5%). Higher amount of yeast is usually required when the sugar amount is high in the formula to avoid the lower volume issue. Obviously, its addition interfered fermentation processes by increasing the pressure in the liquid phase of the dough, which reduced the ability of the dough to retain gases and to obtain a larger volume of bread loaves. Similar results were reported for composite wheat breads with the increasing amount of maize and sweet potato flours (Bibiana et al., 2014) and for composite breads with added maize flour (Siddiq et al., 2009).

Also, ascorbic acid is expected to improve the bread volume and crumb structure. It is typically used in doses from 50 to 75 mg/kg flour (Wieser, 2003), but wider ranges of 10–200 mg/kg are also proposed (Selomulyo and Zhou, 2007). However, as for most dough conditioners, their effect are not only dose-dependent but dependent on the initial quality of flour (Šimurina et al. 2014), concentration of SH groups in dough (Maforimbo, Skurray G. R. & Nguyen, 2007) and the type of bread-making method (Pečivová et al., 2011). Maforimbo et al. (2007) showed that AsA has the effect on the reduction of gluten proteins and thus weakening of composite dough. The reason for this is the negative correlation between L-AA consumption and SH concentration for composite wheat-soy dough. It is assumed to be the same reason for the differences in specific loaf volumes, which were significant ($p<0.05$) between control composite bread samples and the bread samples with the AsA and AsA and sugar ad-dition (Fig 1b). The lowest specific volume of the bread loaf was detected in the light blue composite bread with AsA (1.77ml/g). Akanbi and Ikujenlola (2016) stated that a good indicator of the bread crumb is the loaf volume. It is also a good parameter of the lightness and airiness of the bread interiors. Smaller values point out to a denser and more compact crumb. In accordance with afore-mentioned investigation, red maize composite bread showed the lowest crumb hardness (906.86g) and the highest loaf volume (188.01ml) and specific volume (2.02g/ml) (Figure 1c, Figure 3g). These results coincide with the findings reported by Gallagher, Gormley & Arendt (2003), Sabanis, Lebesi & Tzia (2009), Komlenić et al. (2010) and Nkhabutlane, du Rand & de Kock (2014), which have shown an inverse relationship between bread volume/specific volume and hardness. Bread crumb hardness has maximum value for blue composite bread (1799.88g) (Figure 1c). The addition of AsA and both AsA and sugar had a variable effect on crumb hardness. The texture analysis showed that hardness was significantly higher

($p<0.05$) when AsA and sugar were added to the light blue composite bread. The AsA addition increased crumb hardness by 11.09% and 4.5% in light blue and yellow composite breads, respectively. This finding is in accordance with the research of Vukić et al. (2013).

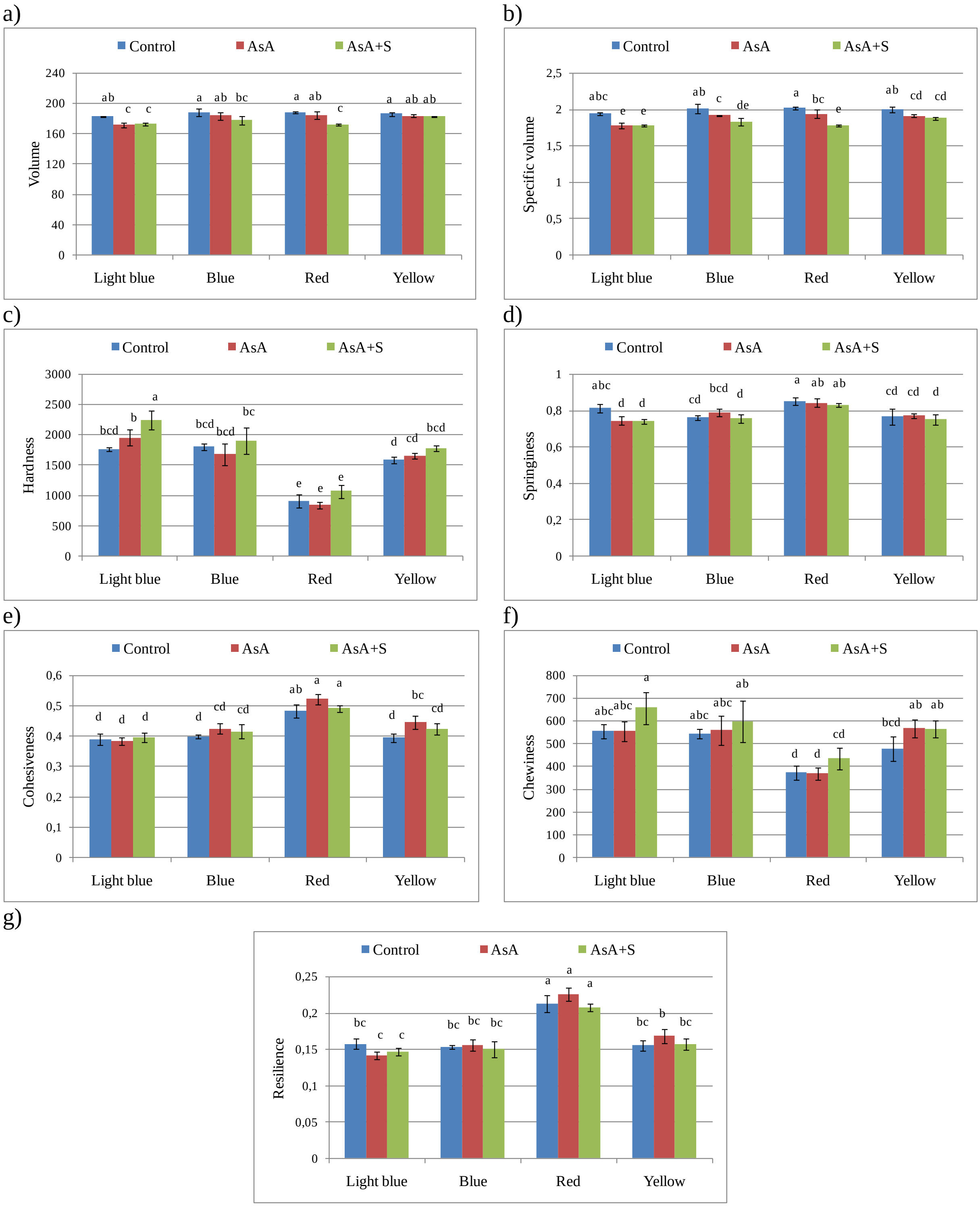


Figure 1. Physical and texture properties of composite breads a) volume; b) specific volume; c) hardness; d) springiness; e) cohesiveness; f) chewiness; g) resilience.
AsA - ascorbic acid, S - sugar. The vertical bars represent the standard deviation of each data point.
Bars with different letters are statistically significantly different, according to the Tukey's test ($p< 0.05$)

According to these authors the addition of ascorbic acid and glucose oxidase resulted in the increase of bread crumb hardness with significant changes compared to the control sample. It is obvious that lower gluten content in the maize composite bread causes the reduction of interchange SH/S-S reactions in gluten and the formation of a weak network of disulfide bonds, which are initiated by the oxidation of ascorbic acid to dehydroascorbic acid. The weak gluten matrix resulted in a discontinuous gluten structure and a low amount of gas generation in the dough. Therefore, the bread crumb could not keep sufficient gas during bread making, which results in the appearance of poorer texture properties of bread. As presented in Figure 1c, the composite bread made of blue maize flour exhibited a tendency towards the softest crumb whereas addition of AsA and sugar gave crumb firmer by 17.5%.

Gueven and Hicsasmaz (2013) stated that the relationship between the texture and the cell structure of porous foods depended not only on the percentage of pores, but also on their distribution and shape. Bread made of red maize flour had greater springiness, cohesiveness and resilience than light blue, blue and yellow composite breads (Fig. 1d, e, g). However, these results were not expected, since soft maize varieties have higher springiness than hard maize (Garzon, Rosell, Malvar & Revilla, 2017). Springiness is associated with a fresh and elastic product (McCarthy, Gallagher, Gormley, Schober & Arendt, 2005) therefore bread made of red maize flour with the highest springiness value could be rated as high quality bread. The texture analysis showed that the addition of AsA had no impact on springiness, cohesiveness and resilience of bread crumbs (Fig. 1 d, e, g). However, composite breads made with AsA and AsA and sugar showed a more compact structure, with a larger number of cells and smaller means cell area (Fig. 2).

This compact structure of the composite bread is explained by the fact that sugar plays an important role in the texture of bakery products. It hardens bakery products by competing with starch molecules and proteins for liquid components in the dough, which prevents overdevelopment of gluten and slows down gelanization (Varzakas, Labropoulos & Anestis, 2012).

Bread samples with AsA and sugar had the lowest springiness which is indicative of brittleness, according to Garzón et al. (2017), and this reflects the tendency of the bread to crumble when slicing. The cohesiveness characterizes the extent to which a material can be deformed before it ruptures, reflecting the internal cohesion of the material. Bread with high cohesiveness is desirable because it forms a bolus rather than disintegrates during mastication, whereas low cohesiveness indicates increased susceptibility of the bread to fracture or crumble (Onyango, Mutungi, Unbehend & Lindhauer, 2011). Bread crumb cohesiveness ranged from 0.389 to 0.483 in light blue and red composite bread samples, respectively. In breads with AsA cohesiveness was slightly higher than that in control samples, but no significant differences. There are no significant differences in cohesiveness between controls and samples with AsA and sugar.

Chewiness is the most indicative characteristic of bread. It is calculated by multiplying hardness, cohesiveness and springiness (Abdelghafor, Mustafa, Ibrahim & Krishnan, 2011). As expected, softer bread crumbs gave greater crumb chewiness. Bread crumb chewiness showed no statistical differences between the composite breads made of light blue, blue and yellow maize flours and those with AsA and sugar excluding maize composite breads with red flour (Fig. 1f).

Sensory properties of maize composite breads

Results of the sensory evaluation (Figure 3) revealed that the sensory properties (shape, crumb pore uniformity and structure, odor, taste, aroma and chewiness) varied greatly among the investigated maize composite bread samples. Nonetheless, the AsA and sugar addition were shown to have a quite various effect on sensory attributes.

The shape of tested maize composite bread loaf samples was almost regular (score 4.0) to regular (score 5.0), while the AsA addition has no impact on this sensory property. With respect to the crumb pore uniformity "control" samples of light blue, blue and red maize composite breads were evaluated with the 4.4, 3.5 and 4.0 values of scores, respectively. The addition of AsA and sugar had a great contribution and composite breads with light blue,

blue and red maize flours were evaluated with the highest scores (5.0). Also, a crumb pore structure was improved by the AsA and sugar addition. Nevertheless, Hruškova and Novotna (2003) stated that the addition of ascorbic acid at a concentration of over 0.025 % affected dough molding and may break open during proofing because of the lack of extensibility. Bread made from such dough has a small loaf volume with a rough crust and its crumb exhibits many ruptured cells and may have large holes.

Maize composite bread loaves had a typical and pleasant odor and taste, and the aroma was very intensive. According to Pereira, Bennett and Luckman (2005) the evaluation of the odor and flavor intensity involves the perception related to raw materials and the amount of ingredients added to the manufacture process, being also more or less related to other effects, such as toasted, vegetable fat, cereal aromas. Intense aroma of examined composite breads originates from wholegrain maize flour (Simić et al. 2018). It is known that wholegrain flours of colored maize contains a significant amount of phenolic compounds which is characterized by bitterness and astringency (Lesschaeve & Noble, 2005), which thus contributes to the intense aroma and typical taste of composite bread.

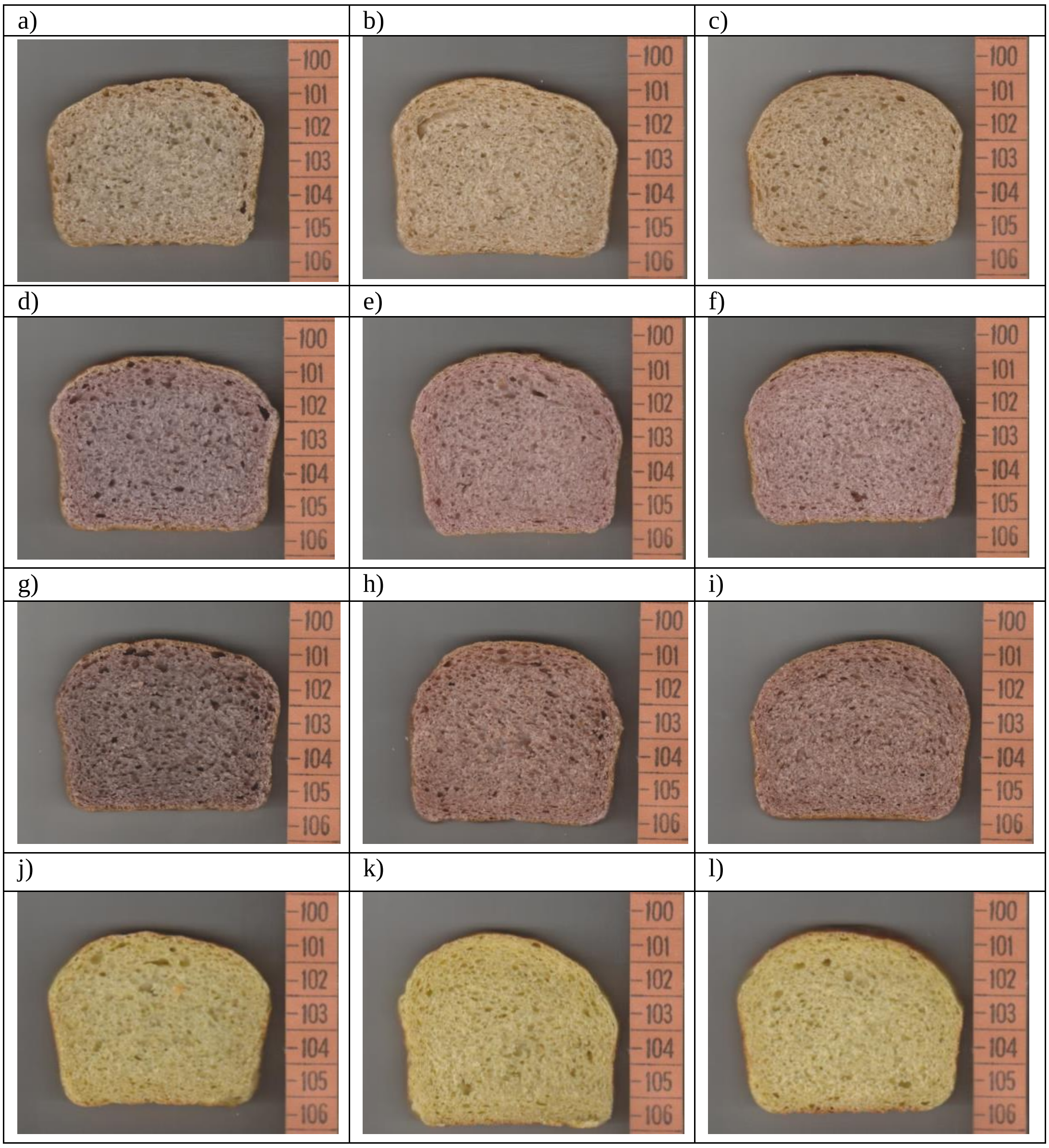
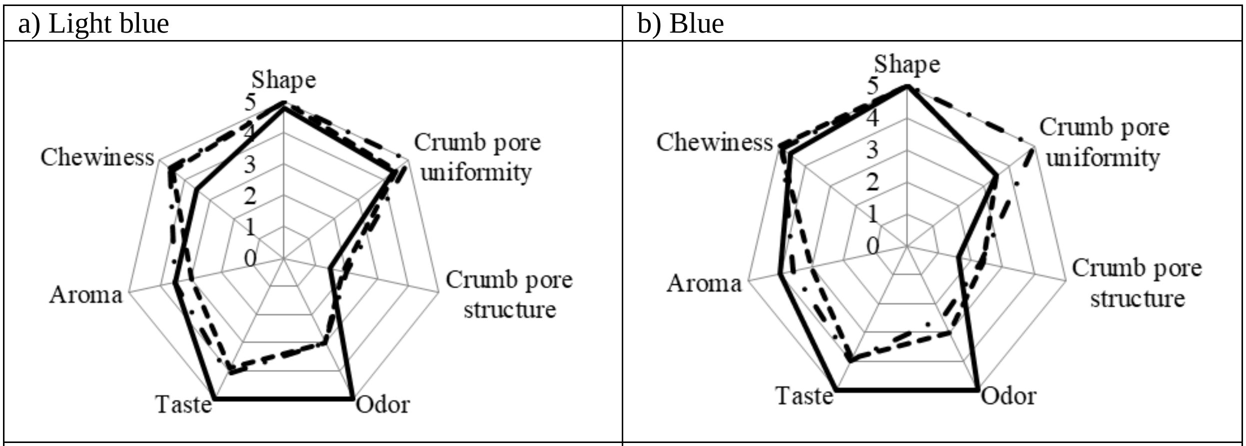
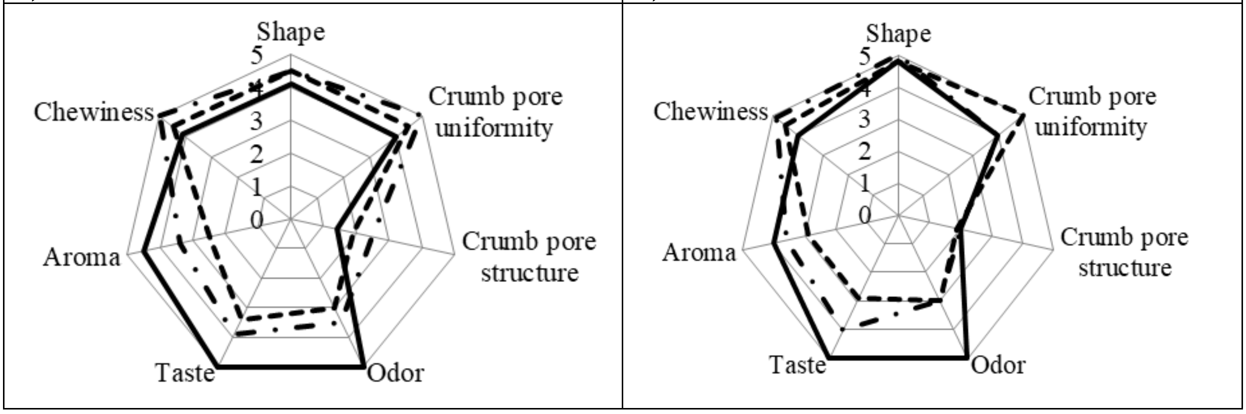


Figure 2. Cross-section of maize composite breads: a) Light blue control, b) Light blue with AsA, c) Light blue with AsA and S, d) Blue control, e) Blue with AsA, f) Blue with AsA and S, g) Red control, h) Red with AsA, i) Red with AsA and S, j) Yellow control, k) Yellow with AsA, l) Yellow with AsA and S.
AsA - ascorbic acid, S – sugar



a) Light blue

b) Blue



c) Red

d) Yellow

Figure 3. Spider web diagram of the sensory evaluation of control composite maize bread (▬), composite maize bread with ascorbic acid (▬ ▬) and composite maize bread with ascorbic acid and sugar (▬ • ▬)

In this research, the addition of AsA and sugar significantly deteriorated these attributes, with the greatest influence on light blue and yellow maize composite breads. Light blue and yellow composite breads with AsA and AsA and sugar were evaluated with partly atypical odor (score 3.0), partly typical taste (score 4.0) and satisfactorily aroma (scores 3.5 and 3.6) (Fig. 3 a,d). In food products, sugar plays an important and unique role in contributing to the flavor profile by interacting with other ingredients to enhance or lessen certain flavors. The addition of sugar enhances flavors by increasing the aroma of the flavor. A flavor aroma possesses no taste properties, but once combined with sugar, the sweetness of sugar and the flavor aroma work synergistically (Spillane 2006). It is assumed that the synergy of flavor of colored maize and sweetness sugar is added in the amount of 5% led to reduced scores for aroma, taste and odor.

The highest value for chewiness among maize composite breads was 4.6 and corresponding to the blue maize composite bread. The addition of AsA and AsA and sugar was shown to improve chewiness of composite bread samples.

CONCLUSIONS

According to the results from this study, the investigated ZP maize genotypes had different chemical compositions which diversify the possibilities of their use. The results showed that the addition of ascorbic acid in the amount of 0.025% and sugar in the amount of 5 % negatively affected the volume as well as the specific volume of maize composite breads, since the addition of sugar in high doses had a dominant influence on the bread loaf volume.

The texture analyses showed that the addition of ascorbic acid had no impact on springiness, cohesiveness and resilience of bread crumb, while it increased crumb hardness. However, composite breads made with ascorbic acid and ascorbic acid and sugar exhibited a more compact structure, with a larger number of cells and smaller mean cell areas. Bread samples with ascorbic acid and sugar had the lowest springiness, which is indicative of brittleness and reflects the tendency of the bread to crumble when slicing. Results of the sensory evaluation revealed that the ascorbic acid and sugar addition had a generally positive effect on sensory attributes. Bread made of only red maize flour with the highest springiness value could be rated as a high-quality bread. Results obtained might be beneficial to further study how ingredient proportion, improvers or processing conditions affect the textural and sensory attributes of the final product from composite wheat-maize flour.

ACKNOWLEDGEMENTS

These results are part of a project supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-9/2021-14/200040 and 451-03-9/2021-14/200222.

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UTICAJ ASKORBINSKE KISELINE I ŠEĆERA NA FIZIČKA, TEKSTURNA I SENZORNA SVOJSTVA MEŠANIH PŠENIČNO-KUKURUZNIH HLEBOVA

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Sažetak: U ovom radu, ispitivan je zajednički uticaj brašna kukuruza različite boje zrna, askorbinske kiseline i šećera na fizička, teksturna i senzorna svojstva mešanog pšenično-kukuruznog hleba. Smeša brašna je pripremljena od 70% pšeničnog brašna i 30% brašna dobijenog od različito obojenog kukuruznog zrna – svetloplavog, plavog, crvenog i žutog. Izvršena je i hemijska karakterizacija pšenično-kukuruznih smeša brašna. U pšenično-kukuruznim smešama brašna određen je sadržaj ukupnih fenola, flavonoida, antocijana, fenolnih kiselina i antioksidativni kapacitet. Ukupno je pripremljeno 12 hlebova, od kojih su četiri bila kontrolna, četiri sa askorbinskom kiselinom i četiri hleba sa askorbinskom kiselinom i šećerom. Sadržaj ukupnih fenolnih jedinjenja pokazao je jasne razlike između svih pšenično-kukuruznih smeša brašna. Sadržaj antocijana je u smešama pšenično-kukuruznog brašna imao sledeći opadajući redosled: smeša sa plavim kukuruzom>crvenim kukuruzom>svetlo plavim kukuruzom, dok u smeši pšeničnog brašna i brašna žutog kukuruza antocijani nisu detektovani. Rezultati su pokazali da je dodatak askorbinske kiseline (0,025%) i šećera (5%) negativno uticao na zapreminu kao i na specifičnu zapreminu mešanih pšenično-kukuruznih hlebova. Analiza teksture je pokazala da dodatak askorbinske kiseline u količini od 0,025% nije uticao na elastičnost, kohezivnost i elastičnost sredine hleba, ali je povećao tvrdoću sredine. Međutim, mešani pšenično-kukuruzni hlebovi sa dodatkom askorbinske kiseline i askorbinske kiseline i šećera, su pokazali kompaktniju strukturu sa većim brojem pora. Uzorci hleba sa askorbinskom kiselinom i šećerom u ispitivanim dozama imali su najmanju elastičnost, što ukazuje na krtost i odražava se na mrvljenje hleba prilikom sečenja. Rezultati senzorne analize pokazali su da je dodatak askorbinske kiseline i šećera generalno imao pozitivan uticaj na ispitivana senzorna svojstva.

Ključne reči: *obojeni kukuruz, pečenje hleba, teksturna svojstva, senzorna svojstva, askorbinska kiselina, šećer*

Received: *17 November 2021/* ***Received in revised form:*** *01 December 2021/* ***Accepted:*** *09 December 2021*

Available online: *December 2021*