

## Effects of Anthocyanin-Rich Popping Maize Flour on the Phenolic Profile and the Antioxidant Capacity of Mix-Bread and Its Physical and Sensory Properties

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**Key words:** anthocyanin-rich popping maize flour, bread, phenolic profile, anthocyanins, antioxidant capacity, physical and sensory properties

In this study, the effect of anthocyanin-rich popping maize flour on phenolic profile, antioxidant capacity and color of bread were investigated. Texture and sensory properties of maize mix-breads were evaluated also. Replacing 30% of wheat flour with blue- and dark-red-seeded maize flours increased the content of total phenolic compounds, ferulic and *p*-coumaric acids of mix-breads compared to the control bread. In addition, anthocyanins from maize flours improved functional profile of breads. Despite thermal degradation, the content of anthocyanins in crumb of blue and dark-red maize mix-breads was high and amounted to 142.3 mg CGE/kg and 84.4 mg CGE/kg, respectively. In its acylated and non-acylated forms, cyanidin 3-glucoside was the most prevalent anthocyanins in maize mix-breads. However, given to the synergistic and antagonistic interaction that arises from the co-existence of numerous antioxidant compounds in food, anthocyanin-rich maize flour had no effect on the increase in the bread antioxidant capacity.

Anthocyanin-rich maize flour changed the bread crumb color to a red range. Although maize mix-breads had worse textural properties compared to the wheat bread, their sensory quality was improved.

### INTRODUCTION

There is a growing recognition that the bioactive compounds present in food have beneficial effects on human health through their antioxidant actions [Dey *et al.*, 2016]. Due to the putative role in the prevention of various diseases associated with oxidative stress, phenolic compounds have become especially interesting for researchers, food manufacturers and consumers. A current estimated dietary intake of total phenolic compounds is >1 g/day [Scalbert & Williamson, 2000]. This intake is about 10 times higher than that of vitamin C, more than 100 times higher than that of vitamin E and about 500 times higher than that of  $\beta$ -carotene [Hooper & Cassidy, 2006]. Fruits and beverages are the main dietary sources of phenolic compounds. Cereals also contribute to the phenolics intake, particularly of insoluble bound phenolic forms. However, due to the removal of phenolic-rich bran and the aleurone layer during whole grains milling, cereal products, such as wheat bread, are scarce in these phytochemicals. Given that bread is a major staple wheat-based food product widely accepted among consumers in the world and that it is predominantly rich in carbohydrates, it is the food suitable for fortification with bioactive compounds, essential micro- and macronutrients, as well as an appropriate vehicle for their delivery. There have been many

studies concerning the enrichment of wheat bread with different small grain cereals, cereal by-products and pseudo-cereals, as well as spices, fruits and herbs in the order to produce bread with improved antioxidant properties [Yousif *et al.*, 2012; El-Megeid *et al.*, 2009]. However, studies of blue and dark-red maize flour used for this purpose are rare.

Anthocyanins are the main antioxidants in colored maize grains. Studies have shown that the main anthocyanins present in colored maize were identified as cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside, along with their corresponding malonyl derivatives [Žilić *et al.*, 2012]. Recent results of Mora-Rochín *et al.* [2016] have shown that the acyl type anthocyanins, such as cyanidin-3-(6-succinyl glucoside) and cyanidin-3-(6-disuccinyl glucoside) were the most abundant compounds in blue colored maize, accounting for 52.1% and 15.6% the total anthocyanins, respectively. It should be noted that monomeric anthocyanins possess limited stability against hydration, pH changes, temperature and light exposure [Dangles *et al.*, 1993] while recent studies have shown that the anthocyanins with acylating substituents were more stable during processing and storage [Bąkowska-Barczak, 2005]. A partial substitution of white wheat flour with wholegrain anthocyanin-rich maize flour can greatly improve functional properties, as well as the nutritional value of bread. Nevertheless, it poses considerable technological difficulties because maize proteins lack the ability to form the necessary gluten network for holding the gas produced during dough fermentation.

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The objective of this study was to investigate the effect of blue- and dark-red-seeded popping maize flour on the phenolic profile and the antioxidant capacity of mix-breads, as well as their physical characteristics and sensory properties.

## MATERIAL AND METHODS

### Plant materials

The wheat (*Triticum aestivum* L.) genotype ZP 87/I and two popping maize (*Zea mays* spp. *everta*) genotypes recently developed at the Maize Research Institute, Zemun Polje (MRIZP), in the vicinity of Belgrade, Serbia, were used. One blue- and one dark-red-seeded maize genotype were used. 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 2016 growing season. Standard cropping practices were applied to provide adequate nutrition and to keep the disease- and weed-free plots.

### Flour samples preparation

Wheat flour (<180  $\mu\text{m}$ ) was produced in a milling company (Žitoprodukt d.o.o. Belgrade-Batajnica, Serbia) 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 wheat flour that was debranned, the wholegrain maize flour was used. Grains of blue and dark-red-seeded popping maize were ground on a Perten 120 lab mill (Perten Instruments, Hägersten, Sweden) to powder (<500 $\mu\text{m}$ ). Flour samples were prepared immediately prior to bread-making.

### Bread-making procedure

Three types of bread samples were prepared in this study based on different wheat/maize flour ratios and maize genotype used: wheat flour bread (100:0 w/w), wheat and blue popping maize bread (70:30), and wheat and dark-red popping maize bread (70:30 w/w). A basic bread formula, based on flour weight, was used as follows: flour (300 g), yeast (7.5 g), salt (6.0 g), vegetable fat (3.0 g), and water according to farinograph absorption. The ingredients were mixed for 5 min in a laboratory Diosna mixer and the dough was left for ripening (40 min) at 85% relative humidity and 30 $\pm$ 1°C. Then, the dough was divided into 115 $\pm$ 1 g portions, manually rounded, rolled and put into tin pans (9.5  $\times$  7.5 cm  $\times$  5.5 cm). The final fermentation lasted 43 min for wheat bread and 35 min for bread with added blue and red maize flour. The breads were baked at 230°C for 20 min in a deck type oven (Miwe condo, D-97450, Arnstein, Germany). All baking experiments were performed in four repetitions. Baked breads were chilled and stored at 24°C and relative humidity of 34% for 24 h and then bread quality was evaluated. Bread crumb samples and manually separated upper crusts were lyophilized and used for analyses of phenolic compounds.

### Analytical procedures

#### Extraction of phenolic compounds from flour and bread

For the detection of the total phenolics, total flavonoids and phenolic acids extracts were prepared from 0.5 g of flour

or bread. All flour and lyophilized bread samples were stored in dark at -18°C before analysis to protect bioactive components from degradation. The extraction of phenolic compounds was performed within 10 days. After alkaline hydrolysis for 4 h at room temperature using 10 mL of 4 M NaOH, extraction was done with ethyl acetate and diethyl ether (1:1, v/v) four times. Five mL of combined extracts were evaporated under N<sub>2</sub> stream at 30°C to dryness and final residues were redissolved in methanol. The extracts were kept at -70°C until analyses. All extractions were performed in duplicate per each replications of the baking experiment.

#### Analysis of total phenolic content (TPC)

The total phenolic content was determined according to the Folin-Ciocalteu procedure [Singleton *et al.*, 1999]. Briefly, the extract (270  $\mu\text{L}$ ) was transferred into a test tube and the volume adjusted to 500 mL with distilled water and oxidized with the addition of 250  $\mu\text{L}$  Folin-Ciocalteu reagent. After 5 min, the mixture was neutralized with 1 mL of 20% aqueous Na<sub>2</sub>CO<sub>3</sub> 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 (TF)

The total flavonoid content was determined according to Eberhardt *et al.* [2000]. The solution of 5% NaNO<sub>2</sub> (50  $\mu\text{L}$ ) was mixed with 200  $\mu\text{L}$  of extract. After 6 min, 500  $\mu\text{L}$  of a 10% AlCl<sub>3</sub> solution was added, and after 7 min, 250  $\mu\text{L}$  of 1 M NaOH 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 (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

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  $\times$  4.6 mm, i.d., 3  $\mu\text{m}$ ) using a linear gradient elution program with a mobile phase containing solvent A (formic acid/H<sub>2</sub>O, 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 individual anthocyanins

Depending on the sample, 80 to 500 mg were mixed with 1.5 mL of methanol acidified with 1 M HCl (85:15, v/v) in three subsequent extractions. After centrifugation (Dina-

myca Pty Ltd, Clayton Victoria, Australia) at 10,000 rpm for 3 min the supernatants were collected. In order to confirm the presence of acylated forms of anthocyanin, the alkaline hydrolysis of the anthocyanin extracts was carried out with 10% KOH. The hydrolysates were kept in darkness for 10 min and then 6 M HCl was added to the solution until stable red color appeared. Both, anthocyanin extracts and their hydrolysates were filtrated through nylon syringe filter of 0.45  $\mu\text{m}$  and used for the chromatographic analysis. The HPLC analysis was carried out with Thermo Scientific UltiMate 3000 HPLC system. Chromatograms were obtained at 530 nm after injection of 10  $\mu\text{L}$  of sample. Separation was performed on a Thermo Scientific Hypersil GOLD aQ C18 column (150 mm  $\times$  4.6 mm, i.d., 3  $\mu\text{m}$ ) using a linear gradient elution program with a mobile phase containing solvent A (formic acid/H<sub>2</sub>O, 1:99, v/v), and solvent B (formic acid/acetonitrile, 1:99, v/v) at a flow rate of 0.7 mL/min and the column oven temperature of 30°C. The following gradient was established: linear gradient elution from 10% B to 30% B, 0–30 min; linear gradient elution from 30% B to 100% B, 30–35 min; isocratic elution of 100% B, 35–38 min; linear gradient elution from 100% B to 10% B, 38–40 min; isocratic elution of 10% B, 40–45 min. Pure anthocyanin compounds such as delphinidin 3-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-glucoside, petunidin 3-glucoside, pelargonidin 3-glucoside, and malvidin 3-glucoside produced by Sigma-Aldrich (Taufkirchen, Germany) were used as standards. Identified anthocyanin peaks were confirmed and quantified using the Thermo Scientific Dionex Chromeleon 7.2. chromatographic software.

#### *Analysis of the total antioxidant capacity (AC)*

The total antioxidant capacity was measured according to the direct or QUENCHER method described by Serpen *et al.* [2008] using the ABTS (2,2-azino-bis 3-ethyl-benzothiazoline-6-sulfonic acid) reagent. The total antioxidant capacity was expressed as mmol Trolox equivalents (Eq) per kg of d.m.

#### *Measurement of color*

Color properties of flour and bread samples were measured with a chroma meter (CR-400/410, Konica, Minolta, Japan), which was calibrated against white calibration standard (CM –A70). The surface color of samples were given as average  $L^*$  (lightness),  $a^*$  (redness),  $b^*$  (yellowness) values of four measurements per samples.

#### *Analysis of physical properties of bread*

Bread loaf volumes (mL) and specific volumes (mL/g) were determined by VolScan profiler (Stable Microsystem, Surrey, UK). The hardness, chewiness, cohesiveness, resilience and springiness of bread crumbs were instrumentally recorded on a texture analyzer TA.XT plus (Stable Micro Systems, England, UK) equipped with a 36 mm cylindrical probe according to AACC method 7410A modified as described by Filipčev *et al.* [2010].

#### *Sensory analysis of bread*

The sensory properties included the crumb elasticity and appearance of crumb pores (structure and uniformity, Dallman pores), as well as bread shape, smell, taste, aroma

and chewiness. After 24 h baking, the breads were sliced and evaluated on the 5-point intensity scale by a 6-member panel whose members had been trained in the sensory evaluation. Sensory properties of bread samples were evaluated by the 5-point category scale with end-points labelled from 1 to 5 as described by Filipčev *et al.* [2010].

#### **Statistical analysis**

The data were reported as mean  $\pm$  standard deviation of at least four independent repetitions. 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 (HSD). Differences at  $p < 0.05$  were considered significant.

## **RESULTS AND DISCUSSION**

### **Phenolic compounds content, the antioxidant capacity and the color of wheat and maize flour**

The content of total phenolic compounds, flavonoids, total anthocyanins, as well as phenolic acids and individual anthocyanins in both wheat and maize flour is given in Table 1. On average, in wheat flour the content of total phenolic compounds and flavonoids was about 8-fold and 3.3-fold lower than that in blue and dark-red popping maize flour, respectively (Table 1). Since the only white color is primary genetic characteristic of hexaploid wheat, it is quite understandable that anthocyanins were not found in wheat flour (Table 1). In complete contrast to wheat flour, the wholegrain maize flour was rich in anthocyanins. Although dark-red popping maize flour had a considerably higher content of total phenolic compounds than those from blue popping maize, higher total anthocyanins content was measured in blue popping maize flour (824 mg CGE/kg d.m.). The observed differences in total anthocyanins content could be attributed primarily to the location of anthocyanins in the maize grain, and depending on that to their varying availability. The chromatographic profile for the anthocyanins indicated the presence of six anthocyanins in maize flours (Figure 1a). However, comparing retention times and UV–visible spectral data with known available standards, the presence of cyanidin 3-glucoside and pelargonidin 3-glucoside were confirmed. Peaks 3–6 did not match any of the anthocyanin standards. The most common quantified anthocyanin in blue and dark-red maize flour was cyanidin 3-glucoside (137.5 and 95.8 mg/kg, respectively) (Table 1), which is in agreement with results reported by Žilić *et al.* [2012]. The content of second major defined anthocyanin pelargonidin 3-glucoside was on average a 6.5-fold lower than that of cyanidin 3-glucoside glucoside in the both maize flour samples. Previous investigations [dePascual-Teresa *et al.*, 2002; Pedreschi & Cisneros-Zevallos, 2007] showed that the retention times for the acylated and aglycone forms of anthocyanins was longer than the retention time for the anthocyanin glycosides found. The comparison of our chromatographic profiles and retention times (Figure 1a) with the same of aforementioned authors, shows that they were quite similar. Further, the presence of acylated forms was confirmed by the basic hydrolysis of the methanolic extract (data not shown). According to Pedreschi &

TABLE 1. The content of phenolic compounds and antioxidant capacity of cereal flours, wheat bread and maize mix-breads.

Samples	TPC (mg GAE/kg)	TF (mg CE/kg)	TAC (mg CGE/ kg)	Phenolic acids		Anthocyanins		AC (mmol Trolox Eq/kg)
				Ferulic acid (mg/kg)	<i>p</i> -Coumaric acid (mg/kg)	C3G (mg/kg)	Pg3G (mg/kg)	
Flour*								
Wheat	340±17 <sup>c</sup>	91.9±1.2 <sup>c</sup>	n.d.	48.6±0.8 <sup>c</sup>	n.d.	n.d.	n.d.	19.9±0.3 <sup>c</sup>
Blue popping maize	2564±59 <sup>b</sup>	344±18 <sup>a</sup>	824±1 <sup>a</sup>	4096±30 <sup>a</sup>	333±11 <sup>b</sup>	137.5±7.1 <sup>a</sup>	20.6±1.7 <sup>a</sup>	21.7±0.2 <sup>b</sup>
Red popping maize	2882±41 <sup>a</sup>	284±19 <sup>b</sup>	574±2 <sup>b</sup>	3417±29 <sup>b</sup>	389±21 <sup>a</sup>	95.8±4.2 <sup>b</sup>	14.4±1.4 <sup>b</sup>	36.8±0.3 <sup>a</sup>
Bread**								
W (crumb)	327±16 <sup>c</sup>	64.2±0.1 <sup>a</sup>	n.d.	43.1±3.0 <sup>c</sup>	n.d.	n.d.	n.d.	18.7±0.8 <sup>ab</sup>
W (crust)	469±27 <sup>c</sup>	22.6±0.4 <sup>f</sup>	n.d.	41.1±2.1 <sup>c</sup>	n.d.	n.d.	n.d.	21.4±2.1 <sup>a</sup>
70%W+30%B.P.M. (crumb)	1291±12 <sup>b</sup>	51.4±1.0 <sup>b</sup>	142.3±0.5 <sup>a</sup>	1051±46 <sup>a</sup>	82.3±1.6 <sup>b</sup>	42.7±0.1 <sup>a</sup>	6.89±0.07 <sup>a</sup>	17.9±1.0 <sup>b</sup>
70%W+30%B.P.M. (crust)	1639±13 <sup>a</sup>	38.3±1.2 <sup>d</sup>	81.9±1.6 <sup>b</sup>	1027±57 <sup>a</sup>	81.4±0.4 <sup>b</sup>	32.6±0.2 <sup>b</sup>	2.99±0.48 <sup>b</sup>	21.3±0.5 <sup>a</sup>
70%W+30%R.P.M. (crumb)	1350±8 <sup>b</sup>	45.9±0.4 <sup>c</sup>	84.4±0.6 <sup>b</sup>	993±59 <sup>a</sup>	106.9±7.4 <sup>a</sup>	29.8±0.2 <sup>c</sup>	3.61±0.06 <sup>b</sup>	17.9±1.0 <sup>b</sup>
70%W+30%R.P.M. (crust)	1323±15 <sup>b</sup>	35.3±0.7 <sup>c</sup>	54.1±0.6 <sup>c</sup>	900±26 <sup>b</sup>	99.6±0.8 <sup>a</sup>	22.8±0.1 <sup>d</sup>	1.70±0.03 <sup>c</sup>	21.3±0.9 <sup>a</sup>

\*content is expressed per kg of dry matter of flour; \*\*content is expressed per kg of dry matter of bread. TPC – total phenolic compounds, TF – total flavonoids, TAC – total anthocyanins, C3G – cyanidin 3-glucoside, Pg3G – pelargonidin 3-glucoside, AC – antioxidant capacity, W - wheat, B.P.M. – blue popping maize, R.P.M. – dark-red popping maize, n.d.- not detected. The content of TPC, TF and phenolic acids was determined after base hydrolysis of crude samples. Means followed by the same letter (lower case) within the same column are not significantly different ( $p > 0.05$ ). Means followed by the same letter (upper case) within the same column are not significantly different ( $p > 0.05$ ). Letters correspond to ranking of groups after Tukey's test.

Cisneros-Zevallos [2007], the six anthocyanin glucosides and the respective acylated forms were detected in Andean purple maize. In our study, a relative area of the peak with the retention time at 14.36 min (peak 4) was about 3-fold higher than that of cyanidin 3-glucoside in both pigmented maize flours. These compounds, which corresponded to acylated cyanidin 3-glucoside, represented the major derivate of anthocyanin in blue and dark-red maize flours accounting for about 48–52% of the total peaks area of anthocyanins. It was followed by the non-acylated cyanidin 3-glucoside that represented about 17% of the total peaks area of anthocyanins. These results are consistent with those of Mora-Rochín *et al.* [2016]. Compared to acylated anthocyanins, the color contribution of monomeric non-acylated forms is lesser [Stintzing *et al.*, 2002]. The results of these authors show that the color impact of cyanidin 3-glucoside, as the major compound of blackberry extract, was only about 50%. According to our results, anthocyanins of blue and dark-red popping maize at pH of about 5.4 provided bright blue and pale red color of flour (Figure 2), respectively.

Although ferulic and *p*-coumaric acids are the primary and most abundant phenolic acids in cereals [Irmak *et al.*, 2008; Montilla *et al.*, 2011; Žilić *et al.*, 2012], *p*-coumaric acid was not detected in wheat flour (Table 1). The content of ferulic acid in wheat flour amounted to 48.6 mg/kg that was 84-fold and 70-fold lower than that found in blue and dark-red popping maize flour, respectively. It is noticeable that the total content of identified phenolic acids in maize flour samples was high in comparison with their content of total phenolic compounds measured by the Folin-Ciocalteu as-

say. Use of gallic acid for the expression of the total phenolic compounds content is one of the possible reasons for discrepancies that have arisen. In addition to the flavonoids and phenolic acids, it was evident that some other bioactive compounds contributed to the antioxidant capacity of wheat flour. The antioxidant capacity of phenolic-poor wheat flour was 19.9 mmol Trolox Eq/kg, while the anthocyanin-rich blue popping maize flour had the antioxidant capacity of 21.7 mmol Trolox Eq/kg. The highest antioxidant capacity correlated with the highest content of total phenolic compounds in dark-red popping maize flour (Table 1).

#### Phenolic compounds content, the antioxidant capacity and the color of bread crumb and crust

Having in mind thermal energy transfer through bread, and effects of temperature, as a dominating factor on the baking mechanisms including gelatinization, enzymatic activity, yeast growth and browning reaction, the content of phenolic compounds and the antioxidant capacity was measured in the crumb and crust of breads. In order to evaluate functional advantages of enriched maize mix-bread, all the results were compared with those of wheat bread as a control. As expected, replacing 30% of wheat flour with wholegrain blue and dark-red maize flour had a positive impact on phenolic compounds content in bread and its color (Table 1, Figure 2). On average, the crumb and crust of both enriched breads had about 4-fold and 3-fold higher contents of total phenolic compounds in comparison to that in the control sample, respectively. The content of ferulic acid was by 22 to 25-fold higher in maize mix-bread samples than that in the wheat bread



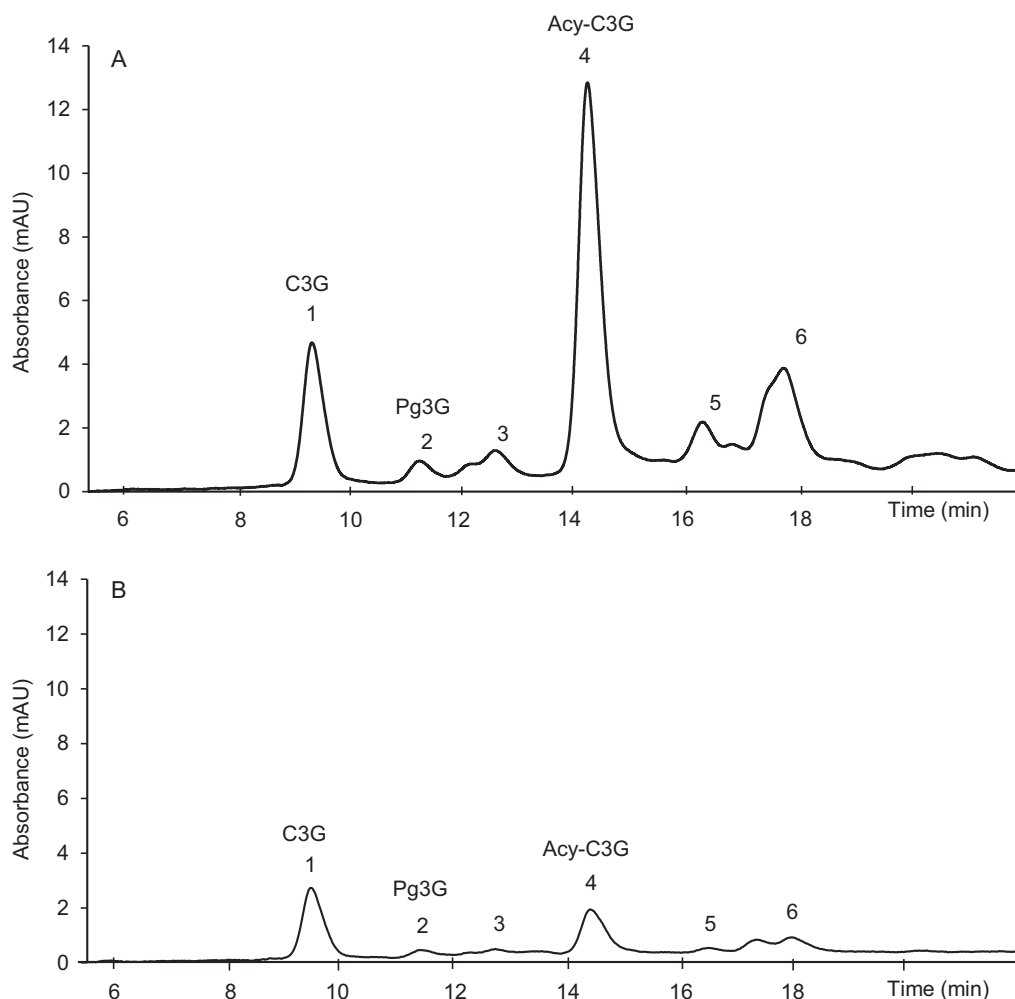


FIGURE 1. HPLC chromatograms of anthocyanins from: A) dark-red maize flour; B) dark-red maize mix-bread crumb. Peaks are noted as follows: 1 – cyanidin 3-glucoside (C3G); 2 – pelargonidin 3-glucoside (Pg3G); 3 – glycosidic form of anthocyanin; 4 – acylated cyanidin 3-glucoside (Acy-C3G); and 5 and 6 – acylated anthocyanins.

samples, while *p*-coumaric acid was not detected in the wheat bread, and its entire content in the enriched bread came from maize (Table 1). Menga *et al.* [2010] reported that the content of ferulic acid was about 2-fold and 3-fold higher in bread samples obtained by wheat flour enriched with 6 and 10% of bran than that in control sample, respectively. Similarly, Świeca *et al.* [2017] have found that the bread enriched with 5% of green coffee had by 2-fold higher content of ferulic acid than common wheat bread after *in vitro* digestion. However, similar to our results, the authors state that the entire content of *p*-coumaric acid in enriched bread originated from coffee.

According to our results, the baking process increased the content of total phenolic compounds in bread samples in comparison to those in composite flours having in mind their percentage share in bread. This is most easily observed in the case of wheat bread. The content of total phenolic compounds in wheat bread crust was by 30% higher than that in the initial raw material. These increases can be, to some extent, a consequence of bound phenolic acid release during baking. However, it should be mentioned that the Folin-Ciocalteu reagent, used for the analysis of total phenols, may react with any reducing substance. Žilić *et al.* [2016] stated that the Maillard reaction products which have reductone struc-

tures contributed to the increase in total phenolic compounds in cookies measured by the Folin-Ciocalteu assay. Unlike the phenolic acids, flavonoids primarily occur in the conjugated form that makes them less thermally stable. During baking, temperature increases from 150 to 205°C in bread crust, while in the bread crumb does not exceed 100°C. Therefore, a significant reduction in the content of total flavonoids was detected especially in bread crusts in the comparison to composite flours. This reduction in wheat bread crust amounted to 55.6%. Despite the degradation, the content of total anthocyanins in crumb of blue and dark-red maize mix-breads was relatively high and amounted to 142.3 mg CGE/kg and 84.4 mg CGE/kg, respectively, which is a considerable enrichment of wheat bread in terms of its functionality and health effects. The thermal degradation of anthocyanins was more intensive in crusts of mix-bread samples than in their crumbs (Table 1). Cyanidin 3-glucoside was the most prevalent anthocyanin in maize mix-bread samples. However, the percentage contribution of its acylated form to the total peaks area of anthocyanins was significantly reduced in the samples of blue and dark-red maize mix-breads compared to that in the flours amounting to 43 and 37% in bread crumbs and 30 and 25% in bread crusts, respectively (Figure 1b). Although the con-

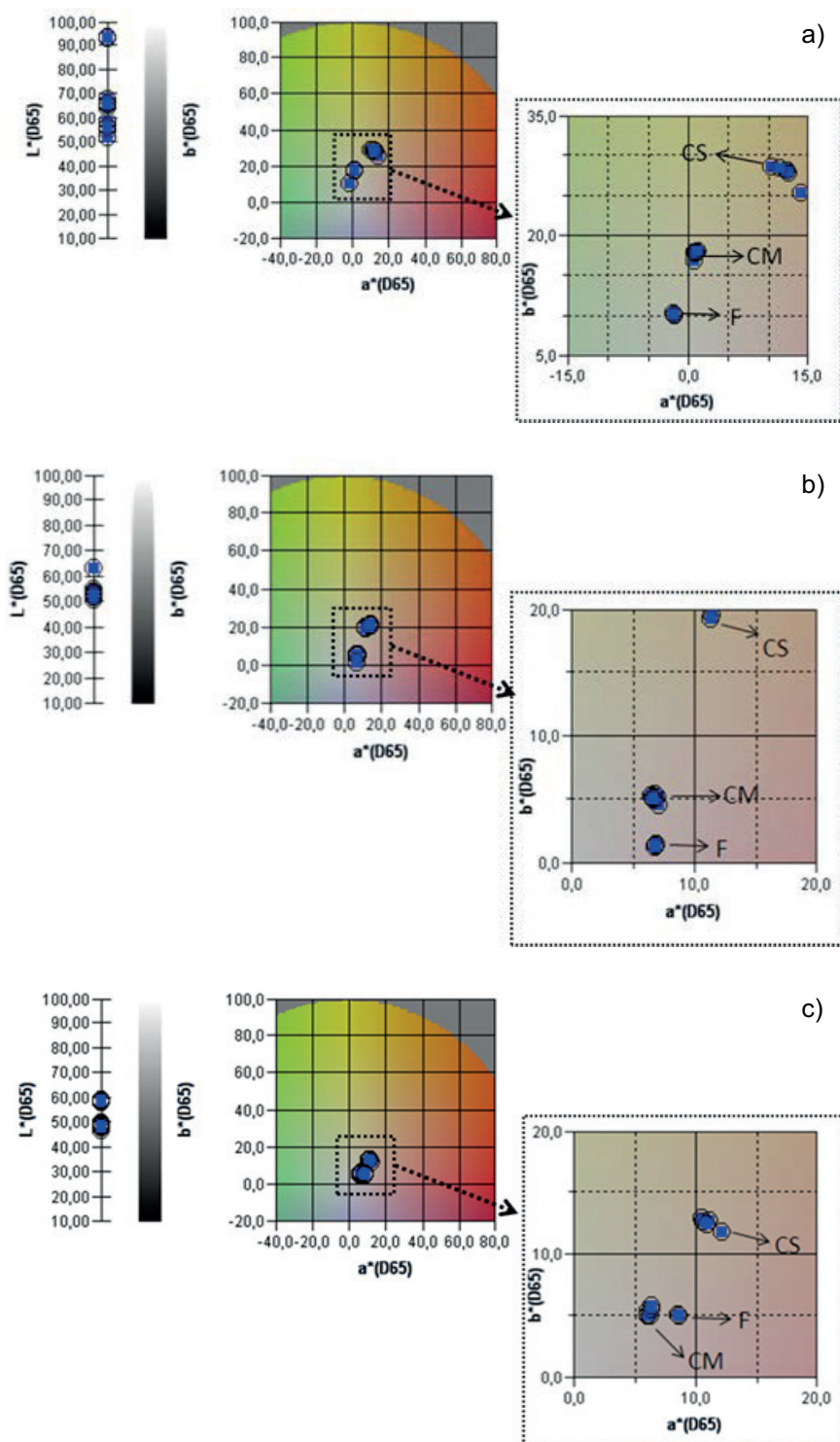


FIGURE 2. CIE  $a^*$ ,  $b^*$  and  $L^*$  chromaticity diagram for: a) wheat flour, wheat bread crust and wheat bread crumb; b) blue maize flour, blue maize mix-bread crust and blue maize mix-bread crumb; c) dark-red maize mix-bread crust and dark-red maize mix-bread crumb. F-flour; CS-crust; CM-crumb.

tent of non-acylated glucoside of cyanidin was significantly reduced in maize mix-bread samples, its percentage contribution to the total peaks area of anthocyanins of bread crumbs and crusts was about 2 to 2.5-fold higher in comparison with those in maize flours (Figure 1a and 1b). West & Mauer [2013] noted that the results for cyanidin 3-glucoside in the presence of an acylated derivative, as occurs in certain purple maize extracts, could be erroneously high. Namely, according to

the study of these authors about 68% of the purified cyanidin 3-(6''-malonyl) glucoside was lost during storage at 40°C and converted primarily to cyanidin 3-glucoside. Xu [2013] reported that mono-acylated anthocyanins showed a higher resistance against heat than di- and non-acylated. Anthocyanins mono-acylated with *p*-hydroxybenzoic acid were best retained after heating, followed by ferulic acid and caffeic acid. According to our results, the replacement of 30% of wheat

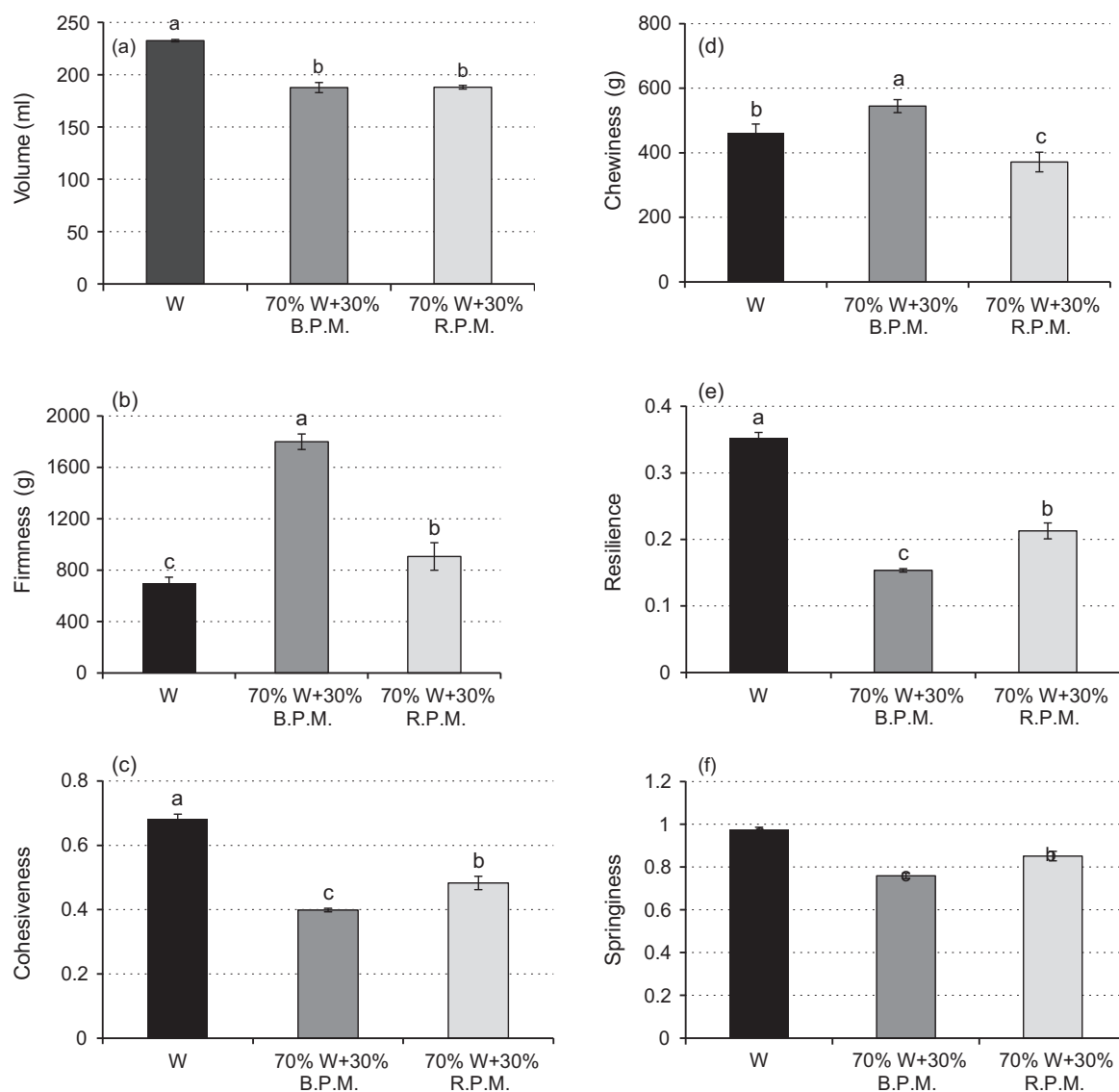


FIGURE 3. Texture properties of wheat bread and maize mix-breads. a) volume; b) firmness; c) cohesiveness; d) chewiness; e) resilience; f) springiness. W- wheat; B.P.M. – blue popping maize; R.P.M. – dark-red popping maize. The vertical bars represent the standard deviation of each data point. Bars with different letters are statistically significantly different, according to Tukey's test ( $p < 0.05$ ).

flour with anthocyanin-rich popping maize flour had no effect on the mix-bread antioxidant capacity (Table 1). Although dark-red maize had almost two times higher antioxidant capacity compared to wheat flour used, antioxidant capacity of wheat bread and maize mix-bread crumbs, as well as crusts was quite uniform. This could be explained by the synergistic and antagonistic interaction that arises from the coexistence of numerous antioxidant compounds in food. On the other hand, the baking process had a great effect on the antioxidant capacity of bread samples. A higher temperature in bread crusts than that in bread crumbs during baking contributed to the breads surface antioxidant capacity that was by 13% higher compared to the one in the inner part of breads. This study confirms previous results of Žilić *et al.* [2016], who showed that the Maillard reaction had a crucial influence on the increment of antioxidant capacity in cookies.

The addition of anthocyanin-rich maize flour changed the bread crumb color to a red range. Crumbs of blue

and dark-red maize mix-bread had CIE  $a^*$  values more than 6-fold higher than wheat bread crumb (Figure 2). In addition, wheat bread crumb had yellow color values (CIE  $b^*$ ) higher by 71.5% and 70.5% than crumbs of blue and dark-red bread samples, respectively. The basic difference in the crumbs color of two breads enriched with blue and dark-red maize flour was in CIE  $L^*$  values. The dark-red maize mix-bread had darker crumb. Generally, bread samples enriched with maize flours had a darker crust than wheat bread. The brown color can be result of thermal degradation of anthocyanins, as well as the formation of brown polymers as the end-product of the Maillard reaction. A high CIE  $b^*$  value of control wheat bread crust indicated its intense yellow color, which may be due to the intensity of the intermediate stages of the Maillard reaction [Hofmann, 1998], and may partly be due to the thermal oxidation of shortening. According to Kocadağlı *et al.* [2016],  $\alpha$ -dicarbonyl compound trapping ability of phenolic compounds was attributed to the reduction of some

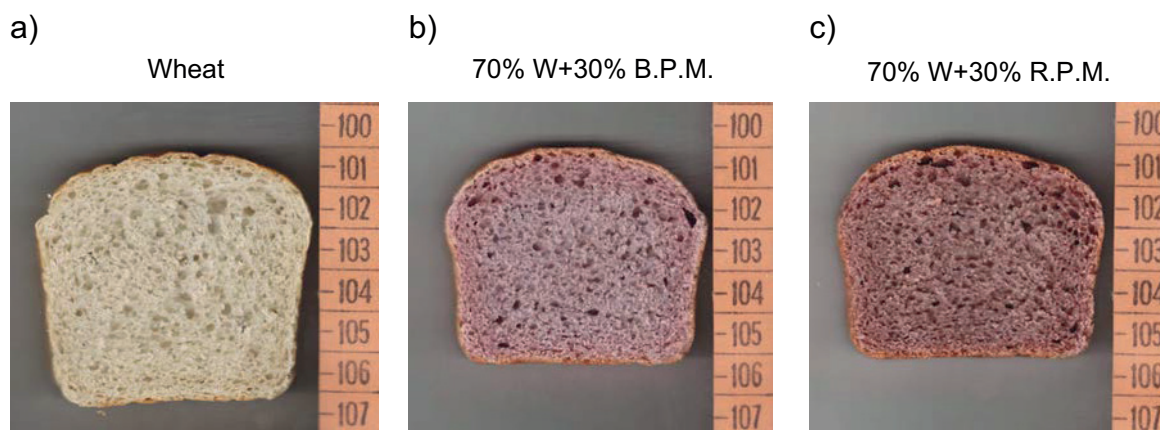


FIGURE 4. Cross-section of breads: a) wheat bread; b) blue maize mix-bread; c) dark-red maize mix-bread. W- wheat; B.P.M. – blue popping maize; R.P.M. – dark-red popping maize.

reactive Maillard reaction intermediates during baking of cookies, that, in our case, could be reflected in significantly lower CIE  $b^*$  values of maize mix-bread crusts compared with the value of wheat bread crust.

#### Physical and sensory properties of bread samples

The results of the texture analysis of wheat and maize mix-breads are presented in Figures 3 and 4. These results indicate that the substitution of wheat flour with maize flour reduced the baking potential in terms of the loaf volume. As a consequence of the addition of gluten-free flour, samples with 30% of blue and dark-red popping maize flour showed a volume reduction of 20% compared to wheat bread (Figure 3a). According to Eduardo *et al.* [2013], bread volume directly influences the firmness of bread. The texture analysis showed a significantly higher firmness of mix-breads when compared to control bread ( $p < 0.05$ ) with maximum values for blue maize mix-bread (Figure 3b). Firmness of this mix-bread was 2.6 and 2-fold higher than that of wheat bread and dark-red maize mix-bread, respectively. Considering that wheat and maize flour have a quite different water absorption capacity, the addition of maize flour influenced the crumb structure by retaining more moisture, thus increasing the hardness values [Siddiq *et al.*, 2009]. High crumb firmness caused the crumb structure of mix-breads to have some few big pores of an irregular shape, non-uniformly distributed pores and the rough crumb (Figure 4). Blue maize mix-bread had the highest chewiness and the lowest volume, which is in accordance with the above mentioned investigation [Eduardo *et al.*, 2013]. On the contrary, the wheat bread had the softest crumb (696 g), the highest loaf volume (232 mL) and the chewiness of 461 g (Figures 3a, 3b and 3c). The texture profile analysis showed that the addition of blue and dark-red popping maize flour to wheat flour significantly decreased springiness, cohesiveness and resilience of bread crumbs. Cohesiveness, resilience and springiness were decreased by 42, 53 and 20% for blue maize mix-bread and by 30, 40 and 16% for dark-red maize mix-bread compared with the properties of wheat bread, respectively (Figures 3d, 3e and 3f).

The sensory evaluation showed differences between wheat bread and maize mix-breads (Figure 5). Although numer-

ous phenolic compounds with important health benefits are characterized by bitterness and astringency [Lesschaev & Noble, 2005], the obtained results indicated that the wholegrain maize flour affected bread flavor providing a pleasant smell and taste and very intensive aroma. Mix-bread made by replacement of 30% of wheat flour with phenolic-rich dark-red maize flour had the best sensory quality in terms of the aforementioned properties. In contrast, results of Holtekjølen *et al.* [2008] indicated that mix-bread, made with barley flour contained the highest amount of phenolic compounds, had a lower acceptance among consumers than the other barley mix-breads and showed the highest score for bitterness, off-odor and off-flavor. The comparison of the results gained by the texture analysis (Figure 3) with the results obtained by sensory analysis (Figure 5) shows that the sensory analysis did not correlate with the instrumental parameters. Namely, blue maize mix-bread had the highest chewiness (544.7 g) and the lowest volume (187.7 mL) (Figure 3), while the sensory evaluation showed the best chewiness (4.6) (Figure 5). This shows that the sensory evaluation of bread

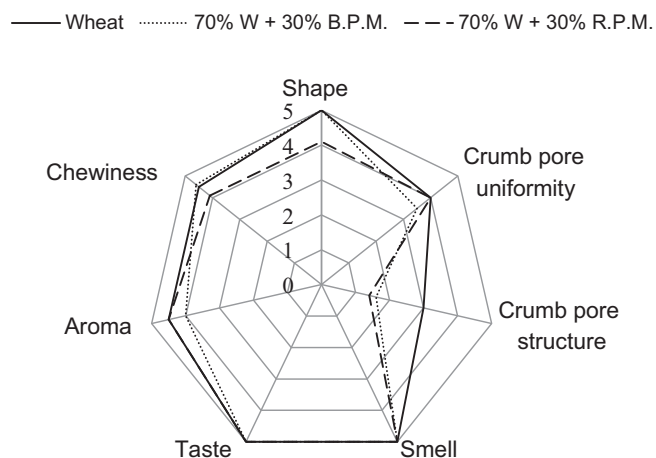


FIGURE 5. Spider web diagram of the sensory evaluation of wheat bread and maize mix-breads. W- wheat; B.P.M. – blue popping maize; R.P.M. – dark-red popping maize.



is a very sensitive task, often giving contradictory results, even with the highly experienced and trained sensory panel.

## CONCLUSION

The replacement of 30% of wheat flour with wholegrain blue and dark-red popping maize flour, rich in phenolic compounds with biological activity, improved the functional profiles of end products increasing the content of total phenolics compounds, anthocyanins, and phenolic acids in maize mix-bread. Despite thermal degradation during baking, anthocyanins from maize flour could significantly contribute to the health benefits of bread, as well as its attractive color. Given to the synergistic and antagonistic interaction that arises from the coexistence of numerous antioxidant compounds in food, anthocyanin-rich maize flour had no effect on the increase in the bread antioxidant capacity. The Maillard reaction had a crucial influence on the increment of antioxidant capacity in wheat bread, as well as maize mix-breads.

The maize flour, 30% which was added, had an unfavorable effect on all texture properties of breads, while their sensory quality was improved. The obtained results indicated that the wholegrain maize flour affected bread flavor providing a pleasant taste and very intensive aroma.

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## CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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