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Hydroxymethylfurfural content and colour parameters of cookies with defatted wheat germ

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Abstract: Defatted wheat germ was used to substitute wheat flour by 5–15% to produce cookies enriched with protein, fat, sugar and minerals. The effects of different level of substitution of wheat flour by defatted wheat germ and dough moisture content (20–24%) on hydroxymethylfurfural (HMF) formation in cookies were investigated. Colour parameters (L^*, a^*, and b^*) were also measured, total colour difference (ΔE) and browning index (BI) were calculated and their correlations with HMF content were studied. The substitution of wheat flour by defatted wheat germ led to a higher HMF content compared to control cookies ($P < 0.05$). However, its formation was more strongly influenced by the dough moisture content than by the level of substitution. HMF content is positively correlated to the a^* ($r = 0.890$; $P < 0.01$), b^* ($r = 0.605$; $P < 0.01$) and BI ($r = 0.710$; $P < 0.01$) values, and negatively correlated to the L^* ($r = -0.624$; $P < 0.01$). Results of the HMF formation and the correlation between HMF content and colour parameters were confirmed by the PCA analysis.

Keywords: browning marker; colour changes; cookies

Defatted wheat germ is rich in nutrients, such as proteins, dietary fiber, B vitamins, pigments, minerals, and certain functional micro components, *e.g.* polyphenols (Zhu *et al.* 2006). Therefore, some studies suggested the use of defatted wheat germ for the preparation of cereal-based products with an added nutritional value. Replacement of wheat flour by defatted wheat germ by 0–25% increased functional and nutritional properties of cookies (Arshad *et al.* 2007) and improved nutritional profile of bread (Sun *et al.* 2015). Pasta produced of semolina blended with 15% of wheat germ showed significant increase in nutritional value (Pinarli *et al.* 2005).

Some chemical reactions, such as Maillard reaction and caramelisation take place during baking, resulting in the development of compounds responsible for the final sensory attributes of the baked products, like colour, texture and flavour. Both reactions lead to the formation of various products. One group of the products, furfurals, is considered undesirable, because of an evidence that some of them, namely hydroxymethylfurfural (HMF), can be converted into cytotoxic and mutagenic compound 5-sulfoxymethyfurfural (Capuano & Fogliano 2011).

Since HMF forms during baking of carbohydrate-containing products and during their storage

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(Ait Ameur *et al.* 2006; Capuano *et al.* 2009), it can be used as a marker of quality deterioration that results from excessive thermal treatment or prolonged storage period (Ramírez-Jiménez *et al.* 2000). The amount of HMF depends on the product composition, moisture content, pH, temperature and duration of the heat treatment (Gökmen *et al.* 2007). Ait Ameur *et al.* (2006) found that HMF amount in cookies varies between 0.5 and 75 mg/kg, while Rufián-Henares & De La Cueva (2008) reported the range from 3.1 to 182.5 mg/kg in biscuits.

Visual changes of colour occur during baking. They can be measured by the CIE L^*, a^*, b^* colour system, describing the formation of the caramelisation and Maillard reaction products. Purlis (2010) described the usual values of lightness (L^*) as well as HMF contents in bakery products during various baking conditions, while Ait Ameur *et al.* (2006; 2007) showed that formation of HMF in biscuits was followed by the colour development.

Having in mind all the facts mentioned above, this study aimed to investigate the effects of the substitution of wheat flour by defatted wheat germ by 5–15% and different dough moisture content (20–24%) on the HMF formation in cookies and to determine the correlation between the HMF content and the colour parameters in different composition of cookies.

MATERIAL AND METHODS

Material. Wheat flour (type T-500) was obtained from Ratar (Serbia). Defatted wheat germ (IO-GERMR1080, fine granulation 150–1000 μm) was supplied by Hochdorf Nutrifood AG (Switzerland). Vegetable fat 'Vitalina' was obtained from Dijamant (Serbia). Salt, sodium bicarbonate, ammonium bicarbonate and powdered sugar were purchased at a local grocery store.

Preparation of cookies. Mixtures of wheat flour and defatted wheat germ were prepared, with the ratio of wheat flour to defatted wheat germ 95:5, 90:10, and 85:15 using an F-6-131 RVC agitator (Forberg International AS, Norway). Wheat flour alone was used for the preparation of the control cookies. Laboratory-scale equipment was used for dough mixing, processing and baking, as described by Petrović *et al.* (2015).

Composition. Composition of flour, defatted wheat germ and cookies including protein (method No. 950.36), fat (method No. 935.38), total sugar (method No. 975.14), total dietary fibre (method No. 958.29), ash (method No. 930.22) and moisture contents (method No. 926.5) were determined by AOAC (2000). Starch content was determined by the hydrochloric acid dissolution according to the ICC Standard No. 123/1 (1994).

Hydroxymethylfurfural (HMF) analysis. An extraction procedure was performed according to the method described by Rufián-Henares *et al.* (2006) with the modifications which were done by Petisca *et al.* (2014). A sample (10 g) was diluted in 5 ml water:methanol (70:30). The mixture was thoroughly stirred for 1 min and 2.0 ml Carrez I and Carrez II solution was added. Solution was centrifuged at 5000 rpm (4°C) for 15 min. The supernatant was pipetted to a 15-ml test tube. Two more extractions were carried out in 2 ml of water:methanol (70:30) until 10 ml of supernatant was collected. Two mililiters of this solution was centrifuged at 8000 rpm for 15 min before being analysed.

The chromatographic separation and quantification of HMF was performed using the HPLC methods described by Ariffin *et al.* (2014) and Tomasini *et al.* (2012) with some modifications. Liquid chromatograph (Agilent 1200 series; Agilent Technologies, USA), equipped with a DAD detector and an Eclipse XDB-C18 (1.8 μm, 4.6 × 50 mm column) (Agilent Technologies, USA) was used for quantification of hydroxymethylfurfural in the extracts obtained. Separation of the analyte was performed using a column temperature 30°C and sample injection volume 2 μl. The mobile phase consisted of two eluents, H_2O (0.1% HCOOH) (A) and methanol (B), delivered at a flow rate 0.75 ml/min. The isocratic elution was performed with the ratio A:B (90:10, v/v). The DAD wavelength was set at 284 nm. The total time of the analysis was 5 minutes.

Colour. The colour of cookies was measured on cookie surface using a Minolta Chromameter (Model CR-400; Minolta Co., Japan) to obtain CIE $L^*a^*b^*$ coordinates. The browning index (*BI*) was calculated by using the Equation (1) defined by Buera *et al.* (1986):

$$BI = (x - 0.31)/0.172 \times 100 \qquad (1)$$

The x variable represents the chromaticity coordinate calculated from the $L^*a^*b^*$ values using the following Equation (2):

$$x = (a^* + 1.75\, L^*)/(5.645\, L^* + a^* - 3.012\, b^*) \qquad (2)$$

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The total colour difference between the control cookies and the substituted cookies, ΔE, was calculated using the Equation (3):

$$\Delta E = \sqrt{(L_{init} - L^*)^2 + (a_{init} - a^*)^2 + (b_{init} - b^*)^2} \qquad (3)$$

Statistical analysis. Results were expressed as a mean value ± standard deviation of three analyses for each measurement, except the colour determination of the samples which was repeated 25-times. An analysis of variance and Tukey's HSD test were used to compare mean values at 5% significance level by using Statistica (StatSoft Inc., 2010).

RESULTS AND DISCUSSION

The composition of the main ingredients used in cookie preparation (wheat flour and defatted wheat germ) is presented in Table 1.

Wheat germ was richer in protein, fat, sugar and ash content compared to type 500 wheat flour. Similar nutrient content of wheat germ was registered by Ma *et al.* (2014) and Zhu *et al.* (2011). Therefore, the cookies containing 5–15% of defatted wheat germ as a substitute of wheat flour had significantly higher ($P < 0.05$) protein, fat, sugar and ash content in comparison to the control cookies (Table 2).

During the preparation and baking of the dough, many complex reactions occur, such as evaporation of water, protein denaturation, starch gelatinisation, volume expansion and browning. Browning results from sugar degradation during baking. One of the key intermediates in browning process, HMF, was determined during the production of cookies (Ait Ameur *et al.* 2006, 2007).

Table 1. Composition of wheat flour type 500 and defatted wheat germ (n = 3)

Parameter (% in DM)	Wheat flour type 500	Defatted wheat germ
Moisture (%)	10.5 ± 0.34[b]	4.52 ± 0.45[a]
Protein	10.5 ± 0.38[a]	29.1 ± 0.92[b]
Fat	0.98 ± 0.12[a]	5.31 ± 0.34[b]
Sugar	1.72 ± 0.11[a]	14.4 ± 0.88[b]
Starch	69.2 ± 0.89[b]	8.59 ± 0.43[a]
Fibre	2.57 ± 0.21[a]	18.4 ± 1.40[b]
Total carbohydrates	77.5 ± 0.32[b]	56.4 ± 1.75[a]
Ash	0.51 ± 0.03[a]	4.65 ± 0.38[b]

Values are presented as a mean ± s.d.; values in the same row with a different index are statistically different ($P < 0.05$)

As shown in Table 3, HMF was formed in defatted wheat germ-containing cookies during baking as well as in the control cookies. HMF content varied from 1.08 ± 0.06 mg/kg in DM to 6.49 ± 0.21 mg/kg in DM in the analysed cookies, which corresponds to previously published HMF contents – 7.8 mg/kg in biscuits (Petisca *et al.* 2014), 0.5–74.6 mg/kg in cookies (Ait Ameur *et al.* 2006) and 1.7–1.9 mg/kg in fresh biscuits (Yang *et al.* 2013).

All of the prepared cookies can be considered low in HMF, being below the maximum of 25 mg/kg defined by the European Food Safety Authority (EFSA). The measured relatively low HMF contents in our cookies can be the result of low baking temperature (230°C) and short baking time (15 min). Ait Ameur *et al.* (2008) found that baking time longer than 8 min is followed by remarkable decreases in HMF, evaporated from cookies and it can be detected in baking vapours.

The lowest HMF content was formed in all types of the control cookies. The addition of defatted wheat germ to cookies resulted in increased HMF content, which depended on the level of substitution (5–15%), being higher with increasing amount of defatted wheat germ. However, HMF formation was more strongly influenced by the dough moisture content than by the level of substitution (Table 4), because HMF is a dehydration product and its formation is inhibited by water, *e.g.* its production is supported under drier conditions (Purlis 2010). The dough with the lowest moisture content (20%) first reached the water activity level that causes a significant increase in HMF formation during baking (> 0.4) (Ait Ameur *et al.* 2006) compared to other types of dough. Therefore, the cookies made of that dough had the highest HMF content (Table 3).

Surface browning of cookies is a parameter important for their sensory acceptance and its functions as an indicator for the development of caramelisation and Maillard reaction during baking. In order to evaluate the differences in colour changes resulting from the substitution of wheat flour by defatted wheat germ in cookies as well as from the differences in the dough moisture content, colour analysis on cookie surface was performed to determine colour parameters (L^*, a^* and b^*). Furthermore, total colour difference, ΔE, and browning index, BI, were calculated (Table 3).

The obtained colour parameters show that control cookies had the lightest surface, especially when

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Table 2. Composition of cookies

Dough moisture (%)	Moisture (%)	Protein	Fat	Sugar	Starch	Ash
		(% in DM)				
Control cookies						
20	5.02 ± 0.07^{ef}	7.07 ± 0.12^{g}	14.4 ± 0.11^{ac}	20.0 ± 0.07^{a}	48.2 ± 0.06^{b}	0.53 ± 0.03^{e}
22	5.11 ± 0.04^{fg}	5.78 ± 0.09^{e}	14.4 ± 0.12^{ac}	20.1 ± 0.06^{a}	47.8 ± 0.02^{b}	0.61 ± 0.04^{ef}
24	5.24 ± 0.04^{g}	6.12 ± 0.03^{f}	14.3 ± 0.14^{abc}	20.7 ± 0.08^{d}	47.9 ± 0.02^{b}	0.67 ± 0.03^{fg}
Cookies with 5% defatted wheat germ						
20	4.53 ± 0.03^{d}	7.41 ± 0.02^{a}	14.0 ± 0.05^{b}	19.8 ± 0.06^{a}	45.7 ± 0.10^{a}	0.75 ± 0.03^{ag}
22	4.70 ± 0.05^{ac}	7.61 ± 0.05^{a}	14.1 ± 0.31^{ab}	25.3 ± 0.19^{bc}	45.9 ± 0.10^{a}	0.79 ± 0.04^{ab}
24	4.87 ± 0.05^{ae}	7.61 ± 0.02^{a}	14.2 ± 0.07^{ab}	25.4 ± 0.11^{c}	46.1 ± 0.11^{a}	0.84 ± 0.04^{a}
Cookies with 10% defatted wheat germ						
20	4.30 ± 0.04^{b}	8.27 ± 0.01^{c}	14.1 ± 0.09^{ab}	21.5 ± 0.10^{e}	43.3 ± 0.08^{d}	0.83 ± 0.03^{g}
22	4.58 ± 0.03^{cd}	8.36 ± 0.15^{cd}	14.2 ± 0.20^{ab}	23.2 ± 0.15^{g}	43.9 ± 0.07^{f}	0.88 ± 0.03^{be}
24	4.73 ± 0.06^{ac}	8.62 ± 0.09^{bd}	14.7 ± 0.10^{c}	25.0 ± 0.19^{b}	42.9 ± 0.47^{d}	0.92 ± 0.05^{bd}
Cookies with 15% defatted wheat germ						
20	4.26 ± 0.07^{b}	8.81 ± 0.07^{b}	14.2 ± 0.08^{ab}	21.9 ± 0.09^{f}	40.3 ± 0.13^{e}	1.04 ± 0.03^{h}
22	4.33 ± 0.04^{b}	8.88 ± 0.21^{b}	14.5 ± 0.18^{ac}	24.0 ± 0.15^{h}	40.9 ± 0.07^{c}	1.05 ± 0.04^{ce}
24	4.77 ± 0.11^{a}	9.20 ± 0.14^{h}	14.7 ± 0.10^{c}	25.3 ± 0.10^{bc}	41.3 ± 0.03^{c}	1.06 ± 0.01^{c}

Values are presented as a mean of three repetitions ± s.d.; values in the same column with a different index are statistically different ($P < 0.05$)

Table 3. HMF content ($n = 3$), browning index (*BI*) and top colour parameters ($n = 25$)

Dough moisture (%)	HMF (mg/kg in DM)	*BI*	L^*	a^*	b^*	*DE*
Control cookies						
20	1.88 ± 0.04^{c}	32.0 ± 0.16^{d}	79.3 ± 0.10^{k}	0.10 ± 0.01^{g}	22.3 ± 0.06^{e}	–
22	1.47 ± 0.02^{b}	29.2 ± 0.08^{c}	80.8 ± 0.06^{i}	–0.21 ± 0.02^{f}	21.2 ± 0.04^{d}	–
24	1.08 ± 0.06^{a}	21.3 ± 0.113^{h}	84.2 ± 0.10^{j}	–0.49 ± 0.01^{e}	17.0 ± 0.06^{c}	–
Cookies with 5% defatted wheat germ						
20	3.90 ± 0.09^{f}	50.2 ± 0.23^{i}	73.0 ± 0.09^{a}	2.20 ± 0.08^{i}	28.7 ± 0.08^{j}	9.19 ± 0.14^{c}
22	1.15 ± 0.02^{ad}	42.9 ± 0.27^{g}	74.1 ± 0.11^{e}	1.22 ± 0.08^{cd}	26.1 ± 0.06^{a}	8.37 ± 0.09^{b}
24	1.05 ± 0.03^{a}	40.1 ± 0.09^{e}	76.4 ± 0.14^{g}	0.86 ± 0.05^{a}	25.6 ± 0.07^{f}	11.7 ± 0.14^{e}
Cookies with 10% defatted wheat germ						
20	4.83 ± 0.07^{g}	53.3 ± 0.10^{j}	71.6 ± 0.12^{d}	2.67 ± 0.06^{j}	29.3 ± 0.06^{b}	10.7 ± 0.11^{d}
22	1.55 ± 0.05^{be}	43.5 ± 0.09^{h}	74.9 ± 0.10^{b}	0.96 ± 0.04^{ab}	26.8 ± 0.03^{h}	8.22 ± 0.14^{a}
24	1.39 ± 0.08^{bd}	41.4 ± 0.19^{f}	75.5 ± 0.05^{f}	0.42 ± 0.09^{h}	26.2 ± 0.05^{a}	12.7 ± 0.17^{f}
Cookies with 15% defatted wheat germ						
20	6.49 ± 0.21^{h}	54.6 ± 0.11^{k}	71.3 ± 0.06^{c}	3.68 ± 0.04^{k}	29.3 ± 0.06^{b}	11.2 ± 0.14^{e}
22	1.76 ± 0.04^{ce}	44.3 ± 0.09^{a}	75.2 ± 0.13^{b}	1.34 ± 0.06^{d}	27.1 ± 0.04^{i}	8.29 ± 0.14^{ab}
24	1.98 ± 0.08^{c}	44.6 ± 0.14^{a}	73.0 ± 0.07^{a}	1.07 ± 0.07^{bc}	26.6 ± 0.03^{g}	14.8 ± 0.16^{g}

Values are presented as a mean ± s.d.; values in the same column with a different index are statistically different ($P < 0.05$)

they had the highest moisture content (24%). The L^* parameter decreased with increasing amounts of defatted wheat germ in cookies and decreasing moisture content of the dough, while a^* and b^* showed the opposite trend. In addition, browning index was significantly higher ($P < 0.05$) in defatted wheat germ-containing cookies. ΔE for all the analysed cookies was > 3, which means that the colour as a result

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of incorporation of defatted wheat germ could be perceived by the human eye (Francis & Clydesdale 1975).

Some studies describe high correlation between HMF content and browning development in foods. Capuano *et al.* (2009) and Capuano & Fogliano (2011) found correlation in bread.

According to our results, HMF content is positively correlated to a^* colour coordinate ($r = 0.890$; $P < 0.01$), b^* ($r = 0.605$; $P < 0.01$) and *BI* ($r = 0.710$; $P < 0.01$), and negatively correlated to L^* ($r = -0.624$; $P < 0.01$).

PCA analysis shows the good differentiation of cookie samples in the PCA chart (Figure 1). The first principal component describes the differentiation resulting from the level of substitution of wheat flour with defatted wheat germ, while the second principal component describes the variation resulting from the dough moisture content. The map of PCA also confirms the HMF formation and the correlation between HMF content and colour parameters.

The ANOVA analysis (Table 4) showed that the HMF formation was more strongly influenced by the dough moisture content than by the level of substitution ($P < 0.01$), while the linear term of substitution was superior for *BI* compared to the linear term of dough moisture content ($P < 0.01$).

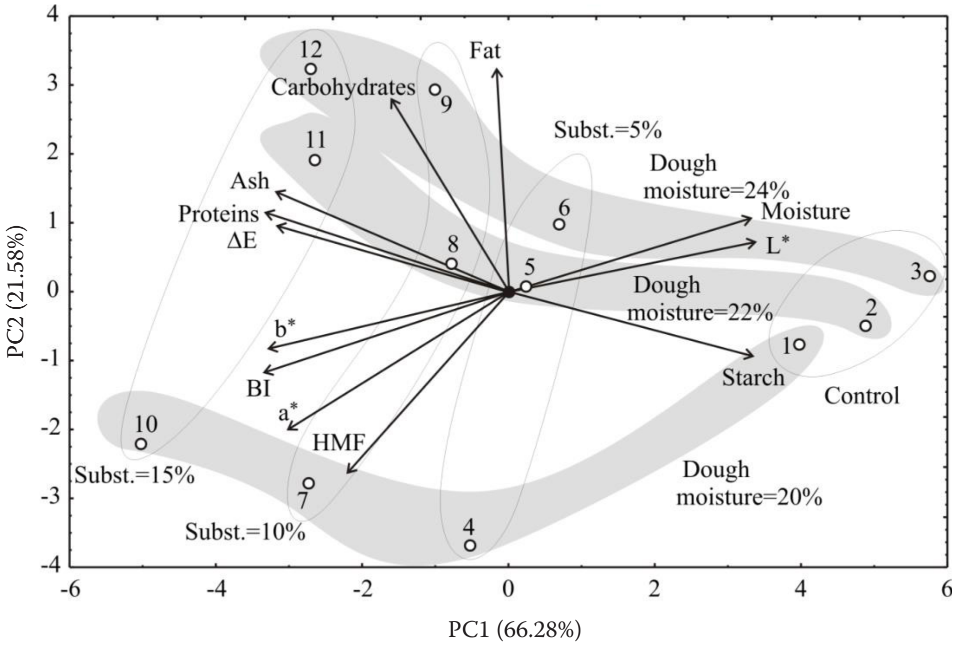

Figure 1. PCA of analysed parameters

1, 2 and 3 – control cookies (dough moisture 20%, 22% and 24%, respectively); 4, 5 and 6 – cookies with 5% defatted wheat germ (dough moisture 20%, 22% and 24%, respectively); 7, 8 and 9 – cookies with 10% defatted wheat germ (dough moisture 20%, 22% and 24%, respectively); 10, 11 and 12 – cookies with 15% defatted wheat germ (dough moisture 20%, 22% and 24%, respectively); Subst. – level of substitution

Table 4. ANOVA table of principal chemical parameters, HMF, and colour parameters evaluation (sum of squares)

	df	Moisture	Proteins	Fat	Sugar	Starch	Ash	HMF	L^*	a^*	b^*	*BI*
Subst.	1	0.710*	11.584*	0.035	14.873**	84.974*	0.301*	6.085*	96.064*	6.547*	79.680*	604.134*
Subst.^{2}	1	0.088*	0.342***	0.126**	5.545	0.186	0.000	0.053	27.847**	0.680	30.731*	177.245*
Moist.	1	0.283*	0.000	0.213**	21.652**	0.085	0.014*	16.822*	23.909**	5.764*	25.407*	233.178*
Moist.^{2}	1	0.003	0.141	0.001	1.336	0.100	0.000	4.803**	1.452	0.633	0.271	13.291
Subst. × Moist.	1	0.023***	0.441***	0.104**	0.848	0.312	0.003	3.427**	2.048	1.228***	1.502	0.002
Error	6	0.027	0.499	0.099	11.592	0.830	0.006	2.311	13.956	1.320	8.342	60.799
r^2		0.976	0.962	0.829	0.792	0.990	0.982	0.931	0.916	0.918	0.943	0.944

*$P < 0.01$; **$P < 0.05$; ***0.10; *df* – degrees of freedom; subst. – level of substitution; moist. – dough moisture content

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CONCLUSIONS

The substitution of wheat flour with defatted wheat germ by 5–15% in cookies resulted in obtaining nutrient-enriched products.

Although the substitution of wheat flour by defatted wheat germ led to higher accumulation of HMF in cookies compared to the controls, all determined HMF contents were below the maximum of 25 mg per kg defined by the European Food Safety Authority (EFSA), and the safety of the enriched products was confirmed.

The HMF formation was more strongly influenced by the dough moisture content than by the level of substitution.

HMF content is positively correlated to a^* colour coordinate ($r = 0.890$; $P < 0.01$), b^* ($r = 0.605$; $P < 0.01$) and BI ($r = 0.710$; $P < 0.01$), and negatively correlated to L^* ($r = -0.624$; $P < 0.01$).

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