Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

Defatted wheat germ application: Influence on cookies' properties with regard to its particle size and dough moisture content

Jovana Petrović[1], Dušan Rakić[1], Aleksandar Fišteš[1], Biljana Pajin[1], Ivana Lončarević[1], Vladimir Tomović[1] and Danica Zarić[2]

Abstract

The introduction of agro-food industry by-products rich in bioactive compounds represents major challenge in food industry sector. The influence of wheat germ particle size (<150 μm, 150–1000 μm, and 800–2000 μm), wheat germ content (5, 10, and 15%), and dough moisture content (20, 22, and 24%) on chemical, textural, and sensory characteristics of cookies was investigated using the Box–Behnken experimental design. The substitution of wheat flour with wheat germ increased the protein, fat, mineral, and fiber content of the cookies. The particle size of wheat germ affected the textural properties of cookies. As the particle size of wheat germ increased, the hardness of cookies decreased. The color of the cookie was most influenced by the interaction of dough moisture content and wheat germ particle size. Wheat germ level up to 15% had no significant effect on the sensory characteristics of cookies. A suitable combination of defatted wheat germ level, its particle size, and dough moisture content can improve the nutritional value of cookies, without causing a negative effect on the cookies' sensory characteristics.

Keywords

Wheat germ, cookies, Box–Behnken design, by-product, sensory characteristics

Date received: 1 February 2017; accepted: 2 May 2017

INTRODUCTION

The fast-growing population which is estimated to reach 9 billion by 2050 requires a 60–70% increase in food production (Foresight, 2011). According to Hodges et al. (2010), 70% increase in food production in order to feed 9 billion people will be very hard to achieve. A third of the total weight of edible parts of food produced for human consumption is lost or wasted (FAO, 2014). By-products formed during food processing that may not be directly considered as a useful resource by its producer, could still contain highly complex components that can be used to produce new, useful products (Chandrasekaran, 2012). Concurrently, there is a growing interest toward the consumption of food products with added health benefits, not only of foods that meet basic nutritional needs.

Wheat germ is rich and cheap source of bioactive components, produced as a by-product during the milling process of wheat grains. The world annual deposit of wheat germ is evaluated to be ca. 25,000,000 tons (FAOSTAT, 2008). Wheat germ contains important bioactive compounds such as antioxidants (tocoferols, tocotrienols, phenolics, and carotenoids) vitamins of group B, minerals, dietary

Food Science and Technology International 23(7) 597–607

DOI: 10.1177/1082013217713101
journals.sagepub.com/home/fst
SAGE

[1]Faculty of Technology, University of Novi Sad, Novi Sad, Serbia
[2]Ihis Techno Experts doo, Research Development Center, Belgrade, Serbia

Corresponding author:
Jovana Petrović, Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia.
Email: jovana@tf.uns.ac.rs

fiber, and sterols (Amadò and Arrigoni, 1992). It has 3 times more protein, 7 times as much as fat, 15 times as much sugar, and 6 times more than the mineral content of wheat flour (Rao et al., 1980). Wheat germ shows stronger antioxidant activities than those of vitamin E, vitamin C, and synthetic antioxidants (Yener, 2015). Also, Zalatnai et al. (2001) showed that the processed wheat germ has the potential in the prevention and therapy of carcinogenesis.

Wheat germ contains some factors, which mainly limited human consumption: raffinose, phytic acid, wheat germ agglutinin, and high lipase and lipoxygenase activities which favor sensitivity to oxidation and determine the poor and unstable sensory properties of baked goods. Stabilization of wheat germ may be conducted by cooking treatments, removing the oil fraction from wheat germ, and using antioxidants (Rizzello et al., 2010). Defatted wheat germ (DWG), the main by-product of the wheat germ in the oil extraction process, is a high-nutritive value protein material containing about 300 g kg^{-1} protein, with well-balanced amino acid profile especially rich in albumin (345 g kg^{-1} of total protein) and globulin (156 g kg^{-1}), whose ratio is close to the model value issued by the FAO/WHO with good amino acid equilibrium. Its principal mineral constituents are potassium, magnesium, calcium, zinc, and manganese in decreasing order. Total flavonoid content is about 0.35 g rutin equivalent/100 g dry matter. The wheat germ is therefore a unique source of concentrated nutrients, highly valued as food supplement (Brandolini and Hidalgo, 2012).

Cookies represent good foundation for nutritional improvements due to their long shelf life and are world-spread consumption, especially among children. In order to increase nutritional value of cookies, legume flours, cereal brans, fruit pomace, banana flour, β-glycan, wheat, and corn germ have been used (Agama-Acevedo et al., 2012; Arshad et al, 2007; Kerckhoffs et al., 2003; Nasir et al., 2010; Wang and Thomas, 1989; Zucco et al., 2011).

Arshad et al. (2007) showed that cookies containing DWG had significantly higher protein, fiber, calcium, iron, and potassium content compared to the control cookie. FAO/WHO (1973) recommended daily requirements for protein at about 25–30 g/day for humans aged between 5 years and 19 years and consumption of about 100 g of these cookies would provide more than half of recommended protein intake. Majzoobi et al. (2012) concluded that the germ level and its particle size, affected crumb color and texture of cake, while other sensory parameters remained unaffected. The same authors reported 15% as the highest level of germ applied and particle size of 280 µm as the most suitable for the production of an appropriate germ cake. Bansal and Sudha (2011) showed that the by-product of milling industry could be processed into value-added products. Biscuits prepared by replacing 40% of wheat flour with steamed wheat germ had higher protein and dietary fibers content and also had considerable amount of vitamin E.

In the presented investigation, the authors evaluated the influence of DWG, its particle size, and cookies dough moisture on color, chemical, textural, and sensory characteristics of cookies using a three-factor on three-level Box–Behnken design. The corresponding experimental design changes the variables simultaneously, conducting a limited number of experiments and represent useful method for studying the influence of several variables on the responses.

MATERIAL AND METHODS

Wheat flour for cookies and biscuits were obtained from milling company "Ratar," Pančevo, Serbia. DWGs were supplied by Hochdorf Nutrifood AG (Hochdorf, Switzerland): VIOGERM1055 (microfine, granulation <150 µm)—fraction 1; VIOGERM1080 (fine, granulation 150–1000 µm)—fraction 2; and VIOGERM1115 (coarse granules, granulation 800–2000 µm)—fraction 3. Other ingredients for cookie making—vegetable fat, salt, sodium bicarbonate, ammonium bicarbonate, and powdered sugar were purchased in a local food store.

Experimental design

As used in the study by Petrović et al. (2015), for the analysis of the influence of input factors and regression, response surface methodology and the Box–Behnken experimental design were used (Myers and Montgomery, 2008). Response surface methodology is able to predict how the inputs affect the outputs in a complex process where different factors can interact among themselves. The input factors were seated on three levels—low (−1), medium (0), and high (1): factor A (wheat germ fraction)—low: fraction 1, medium: fraction 2, and high: fraction 3; factor B (percentage of wheat flour substitution with wheat germ)—low: 5%, medium:10%, and high: 15%, and factor C (moisture content of cookie dough)—low: 20%, medium: 22%, and high: 24%. For factor combination recommended by chosen design dependent responses were measured: Cookies chemical composition (g kg^{-1}) (Y1: moisture, Y2: protein, Y3: fat, Y4: ash, Y5: fiber, and Y6: total non-fiber carbohydrates); physical characteristics (X1: cookie hardness (N), color: X2: L* (lightness), X3: a* (redness-greenness), X4: b* (yellowness-blueness)); sensory characteristics: R1: color, R2: surface appearance, R3: hardness, R4: grittiness, R5: air cell, and R6: beany flavor)). Box–Behnken design reduces the number of runs from 27 (full factorial design) to 15, where three central points are included (which gives sufficient information for testing the lack of fit).

Experimental data are described by the second-order response surface model:

$$R = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad (1)$$

where R is a measured response; β_0 is an intercept; β_i to $\beta_{ij,\ i,j\in\{1,2,3\}}$ are regression coefficients; A, B, and C are the coded levels of input factors. The terms AB, AC, and BC represent interactions of input factors, while A^2, B^2, and C^2 are quadratic terms. For each of responses, a response surface regression analysis was performed using a fitted full quadratic model given in equation (1) . In the observed model, significance of influence of input factors and their interactions on each response is studied by statistical method of analysis of variance (ANOVA) using 5% level of significance ($p < 0.05$). Values of sum of squares determined in ANOVA calculations are used to obtain the contributions of input factors and their interactions. The statistical analysis was performed using Statistica 12 and Design-Expert 10 (trial version).

Cookie preparation

The dough for cookies was prepared according to Petrović et al. (2015). Flour-DWG blends (blends of wheat flour and DWG of different particle sizes containing 5, 10, and 15% DWG on a replacement basis) were mixed using the F-6-RVC agitator (Forberg International AS, Oslo, Norway). Control cookie samples were prepared using wheat flour without DWG. Total amount of flour blend (200 g) was mixed in a mixer for 0.5 min. Mixing was maintained for 5.5 min with addition of vegetable fat (42 g) and sugar (70 g). The total amount of water required to obtain dough samples with 20, 22, and 24% moisture content was calculated in relation to the moisture of flour. At the end, the remaining powdered material (NaCl 1.1 g, $NaHCO_3$ 0.6 g and NH_4HCO_3 0.4 g) previously dissolved in calculated amount of distilled water was added and mixing was extended for 15 min. After mixing of all ingredients and dough rest period of 30 min, the dough samples were sheeted between two cylinders of laminator (Marchand LA4-500, Materiel modern marchand, Rueil—Malmaison, France). The gap settings between the cylinders were 14 mm, 10 mm, 7 mm, and 5 mm, with 15-s resting period between each passage. Consequently, the dough was cut using a round stainless mould and prepared cookie dough was baked for 15 min at 230°C in a laboratory oven followed by 120 min of cooling and packaging.

Determination of chemical composition

Moisture, protein, fat, fiber, and ash contents of wheat, wheat germ, and cookies were determined 24 h after baking according to the methods described in the A.O.A.C (2000). The factors, n = 5.70 (for wheat flour) and n = 6.25 (for cookies) were used for conversion of nitrogen to crude protein (Arshad et al., 2007). Total non-fiber carbohydrates were calculated by difference.

Physical characteristics

Cookie hardness. Cookie hardness was measured by a texture analyser TA.HD Plus (Stable Micro systems, UK). A method specified by the manufacturer (hardness measurement of biscuit by cutting, BIS2/KB, Texture Exponent 32 software version 4.0.11.0 Stable Micro Systems, Godalming, Surrey, UK) was used. The cookies were analyzed using a knife edge with slotted insert (HDP/BS) and 25 kg load cell at a 1.5 mm/s pre-test speed, test speed of 2 mm/s, 10 mm/s post-test speed, and 5 mm distance. Sample is placed centrally under the knife edge and the samples were fractured into two major pieces. The resulting force-deformation curve was analyzed by stable micro systems software and values of hardness (mean maximum force values) were obtained automatically. The analysis was carried out in triplicates.

Cookie color. Cookies' upper surface color was measured in triplicates, 24 h after baking. The CIE*Lab* color coordinates (L*—lightness, a*—redness to greenness and b*—yellowness to blueness) (CIE, 1976) were determined using MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan) using D-65 lighting, a 2° standard observer angle and an 8-mm aperture in the measuring head. The chroma meter was calibrated using a Minolta calibration plate (no. 11333090; Y = 92.9, x = 0.3159; y = 0.3322). Total color difference (ΔE^*) between the cookies containing DWG and control cookies with the same moisture content was calculated as follows:

$$\Delta E^* = \left[\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2\right]^{1/2}$$

where subscript 2 is the control and the subscript 1 is the sample containing DWG.

Table 1. Chemical composition of wheat flour and wheat germs (g kg^{-1}).

	Moisture	Protein	Fat	Ash	Fiber	Total non-fiber carbohydrates
Wheat flour	108.3	100.4	9.5	5.4	45.7	730.7
viogerm1055	40.2	289.9	45.7	42.3	178.9	363.0
viogerm1080	51.3	281.6	49.2	47.5	233.1	337.3
viogerm1115	46.5	319.9	54.4	49.7	199.8	329.7

The value used to determine whether the total color difference was visually obvious was $\Delta E^* > 3$: color differences are obvious to the human eye (Francis and Clydesdale, 1975).

Sensory analysis

Cookie samples were evaluated by a panel of 10 trained members, baking technologists. The panelists were trained according to ISO 8586 (2012). Each training session took 2 h. Initially, assessors were provided with the terms which may be used for cookies evaluation (ISO 5492, 1992), also, they were familiar with extremely defective sensory attributes of the examined cookies. On the last training session, panelists developed the final list of six meaningful sensory attributes to be use in the study. Intensity of each attribute was specified on 7-point scale (1 = the least intensity and 7 = the most intensity) (ISO 4121, 2002). Evaluated attributes were color (lightness of color on the surface – 1 for light to 7 for dark); surface appearance (number of cracks on cookie surface – 1 for none to 7 for very cracked); hardness (the easiness with which cookies can be broken in two parts – 1 for crumbly to 7 for rigid); grittiness (amount of small, hard particles between teeth during chew – 1 for none to 7 for many); air cells (size of air cells in cookies – 1 for small to 7 for large), and bean flavor (intensity of strange flavor untypical for wheat flour cookies, associated with bean – 1 for not conspicuous to 7 for very conspicuous). The cookies evaluation was carried out 24 h after baking at the sensory laboratory of the Faculty of Technology, University of Novi Sad, Serbia. Cookie samples were served to the panelists on white plastic plates labeled with three-digit codes from a random number table and each subject assessed five samples per session. Panelists were asked to swallow samples and to rinse their mouths with water between samples.

RESULTS AND DISCUSSION

Chemical composition

Chemical composition of wheat flour and wheat germs is presented in Table 1. Wheat flour has lower protein (100.4 g kg^{-1}), fiber (47.5 g kg^{-1}), and mineral content (5.4 g kg^{-1}) compared to the milled wheat germ (319.9 g kg^{-1}, 233.1 g kg^{-1}, and 49.5 g kg^{-1}, respectively).

These results confirm potential application of wheat germ as favorable nutritious food supplement and are in accordance with results of (previous researchers) Aktaş et al. (2015) and Bilgiçli et al. (2006) concluded that wheat germ also has significantly higher content of Fe, Ca, Mg, K, P, and Zn compared to the wheat flour. On the other side, the content of total non-fiber carbohydrates in wheat germ is lower (329.7 g kg^{-1}) compared with wheat flour (730.7 g kg^{-1}).

The Box–Behnken experimental design and obtained responses for chemical characteristics of cookies are shown in Table 2. Coefficients of regression equation (equation (1)) are listed in Table 3, where *** indicates strong ($p < 0.001$), ** indicates moderate ($0.001 < p < 0.01$), and * represents slight ($0.01 < p < 0.05$) significance of corresponding terms on responses. Our model had a highly significant coefficient of determination (R^2) indicating that the model was sufficient for estimation of the responses Y1-Y6.

Contributions of main factors and factor interactions including squared terms on responses Y1-Y6 (chemical characteristics of cookies) are shown in Figure 1. The percentage of wheat flour substitution (factor B) had the biggest influence on the chemical composition of cookies.

As expected, the substitution of wheat flour with wheat germ increased the protein, fat, mineral, and fiber content of the cookies (factor B had a strong, significant, and positive influence on responses Y2, Y3, Y4, and Y5, Table 3, Figure 1(a)), while the total non-fiber carbohydrate content is decreased. Arshad et al. (2007) also concluded that wheat germ addition increased protein, fiber, and mineral content of cookies but also significantly increased the levels of calcium, iron, and potassium, and therefore the cookies supplemented with DWG were found to be nutritious.

Cookie hardness

The textural properties are one of the most important quality parameters of cookies. Table 4 shows the

Table 2. The Box–Behnken experimental design and obtained responses for chemical characteristics of cookies (g kg^{-1}).

Run	Factor A	Factor B	Factor C	Y1	Y2	Y3	Y4	Y5	Y6
1	3	10	20	42.1	79.5	137.3	9.0	41.8	690.3
2	1	10	20	43.4	78.6	136.4	7.9	41.1	692.6
3	3	15	22	43.3	84.9	138.0	9.8	43.7	680.3
4	2	10	22	45.8	81.7	135.9	8.9	39.1	688.6
5	1	10	24	53.0	79.4	136.3	8.2	34.6	688.5
6	2	10	22	45.0	82.2	135.5	8.5	38.7	690.1
7	1	5	22	55.1	70.7	131.5	6.7	29.1	706.9
8	2	15	24	49.7	87.7	137.9	10.1	45.5	669.1
9	2	10	22	45.5	80.7	135.7	9.0	38.8	690.3
10	3	10	24	47.3	80.2	139.2	8.4	42.8	682.1
11	2	5	24	48.7	72.4	131.2	8.0	25.9	713.8
12	1	15	22	47.9	82.2	136.8	9.2	41.1	682.8
13	2	5	20	45.3	73.6	131.3	7.1	22.3	720.4
14	2	15	20	44.6	84.3	137.6	10.0	43.7	679.8
15	3	5	22	37.6	74.5	131.8	7.5	35.6	713.0

Y1:Moisture; Y2:Protein; Y3:Fat; Y4:Ash; Y5:Fiber; Y6:Non-fiber carbohydrates.

Table 3. Regression equation coefficients for response Y1-Y6.

	Y1	Y2	Y3	Y4	Y5	Y6
Intercept						
β_0	4.543	8.152	13.570	0.880	3.820	69.001
Linear						
β_1	−0.364**	0.103*	0.066**	0.034**	0.225	−0.064
β_2	−0.015	0.599***	0.306***	0.123***	0.764***	−1.776***
β_3	0.291**	0.046	0.025	0.009	−0.001	−0.370*
Interaction						
β_{12}	0.323*	−0.028	0.023	−0.005	−0.098	−0.215
β_{13}	−0.110	−0.003	0.050	−0.023	0.188	−0.103
β_{23}	0.043	0.115*	0.010	−0.020	−0.045	−0.103
Quadratic						
β_{11}	−0.004	−0.176**	0.081*	−0.046**	0.245	−0.083
β_{22}	0.058	−0.169**	−0.199***	−0.004	−0.328	0.657**
β_{33}	0.106	−0.034	0.079*	0.004	−0.058	−0.081
R^2	0.8687	0.9810	0.9869	0.9754	0.8912	0.9570
Lack of Fit	0.0232	0.4371	0.1214	0.7495	0.1098	0.0909

Y1: moisture; Y2: protein; Y3: fat; Y4: ash; Y5: fiber; Y6:non-fiber carbohydrates
*$0.01 < p < 0.05$; **$0.001 < p < 0.01$; ***$p < 0.001$.

experimentally obtained values according to the Box–Behnken experimental design for responses X1 (cookie hardness), while Table 5 represents regression equation coefficients. Generally, high moisture content would give a soft product. Nevertheless, it can be noticed that moisture content (factor C) affected cookie hardness with contribution of only 4.85% (Figure 2(a)). In some cases, the texture profile analysis showed different results for samples with the same moisture content (Table 4).

On the other hand, cookie hardness values of samples with moisture content of 24% did not differ significantly compared to samples with moisture content of 20%. In this case, the other two parameters have a much greater impact on cookie hardness (factor A and factor B) than moisture content. Chuang and

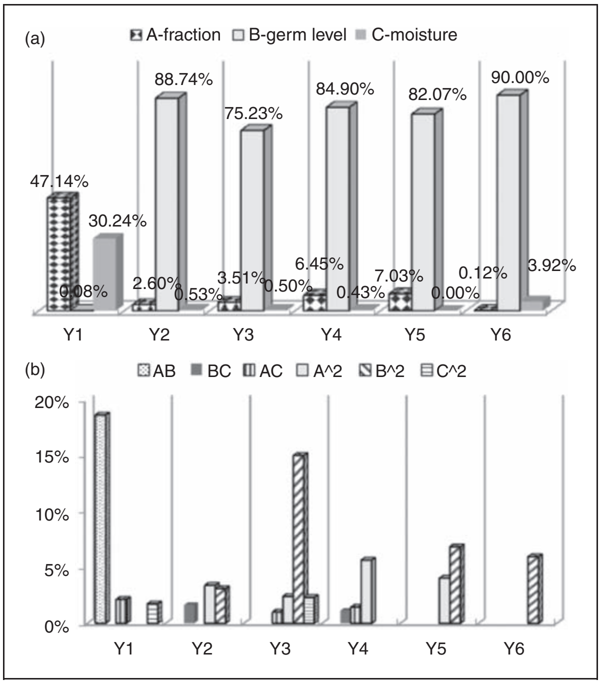

Figure 1. Contributions of main effect (a) and factor interactions and squared terms (b) on chemical characteristics of cookies: Y1: moisture; Y2: protein; Y3: fat; Y4: ash, Y5: fiber; Y6: non-fiber carbohydrates.

Yeh (2006) also indicated that the texture of the final product is not affected only by water.

Change of the wheat germ fraction (particle size) had significant impact on cookies hardness. Contributions of factor A on cookies hardness were 48.55% (Figure 2(a)), i.e. as wheat germ particle size increased, cookies hardness decreased (Figure 3(a)). Greater hardness of cookies with smaller wheat germ particles is the result of denser structure and more compact cells. Samples with larger wheat germ particles (fraction 3) in his structure had larger spaces filled with air causing crumbly structure and lower hardness compared to the samples with smaller wheat germ particles.

Percentage of wheat substitution had the square influence on the cookie hardness (quadratic factor B^2 had high contribution for response X1 as can be seen at Figure 2(b)). As level of wheat germ in the dough increased from 5% to 10%, cookie hardness decreased, while the increase of wheat germ content from 10% to 15% increased cookie hardness to values similar to samples with 5% wheat germ content as can be seen on Figure 3(b). These results are in accordance with Arshad et al. (2007), who did not find significant difference in hardness of cookies supplemented with 5–15% of DWG flour. Although wheat germ contains significant content of proteins and fibers which absorb free water molecules causing cookie hardening,

Table 4. The Box–Behnken experimental design and obtained responses X1–X4.

Run	Factor A	Factor B	Factor C	X1 (N)	X2	X3	X4	ΔE*
1	2	15	24	137.88	72.98	1.34	26.55	15.22
2	3	5	22	88.55	74.38	4.32	29.67	11.55
3	3	10	20	87.47	76.20	0.21	26.40	5.11
4	1	10	20	145.82	66.90	7.67	32.23	17.57
5	1	5	22	245.16	73.88	0.38	26.07	8.48
6	3	15	22	135.72	74.91	0.94	26.29	7.88
7	2	15	20	105.32	71.29	3.68	29.27	11.18
8	1	10	24	117.38	75.83	0.44	26.19	12.71
9	1	15	22	231.24	75.34	1.08	27.89	8.73
10	2	10	22	108.46	74.92	0.47	27.37	8.55
11	2	10	22	100.51	75.77	0.55	25.19	7.27
12	2	10	22	90.51	75.67	0.96	26.21	6.47
13	2	5	20	118.85	72.85	2.21	28.67	9.28
14	3	10	24	105.61	75.02	0.39	25.68	12.89
15	2	5	24	198.48	76.34	0.86	25.59	12.09

X1: hardness; X2: lightness; X3: redness-greenness; X4: yellowness-blueness; ΔE*: total color difference between the cookies containing DWG and control cookies with the same moisture content

Table 5. Regression equation coefficients for response X1-X4.

	X1	X2	Ln (X3)	X4
Intercept				
β_0	10.179	75.453	−0.467	26.256
Linear				
β_1	−4.109**	1.070	−0.177	−0.542
β_2	−0.523	−0.367	0.060	0.001
β_3	1.299	1.615*	−0.522	−1.569**
Interaction				
β_{12}	1.560	−0.232	−0.643	−1.301
β_{13}	1.186	−2.528**	0.875	1.330
β_{23}	−1.200	−0.450	−0.018	0.090
Quadratic				
β_{11}	2.512	−0.352	−0.148	0.663
β_{22}	5.170*	−0.475	0.740	0.563
β_{33}	−1.058	−1.614	0.287	0.703
R^2	0.7892	0.7815	0.6247	0.6856
Lack of Fit	0.0549	0.0799	0.1165	0.4221

X1: hardness; X2: lightness; X3: redness-greenness; X4: yellowness-blueness.
*$0.01 < p < 0.05$; **$0.001 < p < 0.01$; ***$p < 0.001$.

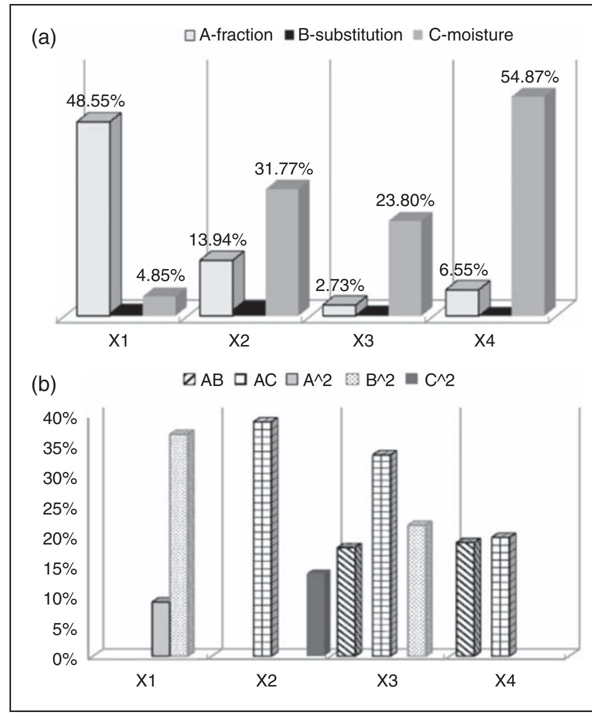

Figure 2. Contributions of main effect (a) and factor interactions and squared terms (b) on hardness (X1) and color (X2: Lightness; X3: redness-greenness; X4: yellowness-blueness) of cookies.

simultaneously, higher content of fat in wheat germ affects cookie softening and compensate for hardness.

Cookie color

Color is strictly linked to food quality and safety of thermally treated foods. Cookie surface color is a very important characteristic for consumers' acceptance. Non-enzymatic browning of baked products is influenced by Millard reaction along with caramelization and ascorbic acid oxidation (Troisea and Foglianob, 2013).

As can be seen in Table 5 and Figure 2, the L* value was significantly affected by moisture content (with contribution of 31.77%) and interaction of factors A and C (contribution is 38.91%), while percentage of wheat flour substitution had no effect on L* value of cookies surface color. Bruce et al. (1994) concluded that browning rate is a strong function of moisture content. As was expected, L* values increased as moisture content increased, i.e. cookies surface color became lighter. Also, L* values of cookies increased as germ fraction (wheat germ particle size) increased (Table 5). These results are in accordance with the results of Majzoobi et al. (2012) who also reported that L* value of the cookie crumb increased slightly with increasing the particle size of wheat germ.

Redness (a* values) and yellowness (b* values) of cookies surface decreased with increasing moisture content in the cookie dough (Table 5). Factor C had significant contributions in both cases (Figure 2(a)). Increase in a* and b* values and decrease in L* values indicate that the cookies are darker. L* values slightly decreased and a* and b* values increased with increasing wheat germ level due to presence of yellow pigments in the germ, but this influence is not very pronounced for germ level up to 15%. The highest a* and b* values had sample which has the smallest L* value, i.e. the sample which contain wheat germ fraction with the smallest particle size and lowest moisture content (Table 4). These results are also in accordance with the results of Majzoobi et al. (2012). They also reported that a* and b* values decreased as wheat germ particle size increased and as wheat germ level decreased. Values of total color difference (ΔE^*) between the cookies containing DWG and control cookies with the same moisture content increased as wheat germ fraction decreased and as wheat germ level increased. All ΔE^* values were higher than 3, which means that color differences were obvious to the human eye (Francis and Clydesdale, 1975).

Sensory analysis

The obtained values for responses R1-R6, according to Box–Behnken experimental design are presented in

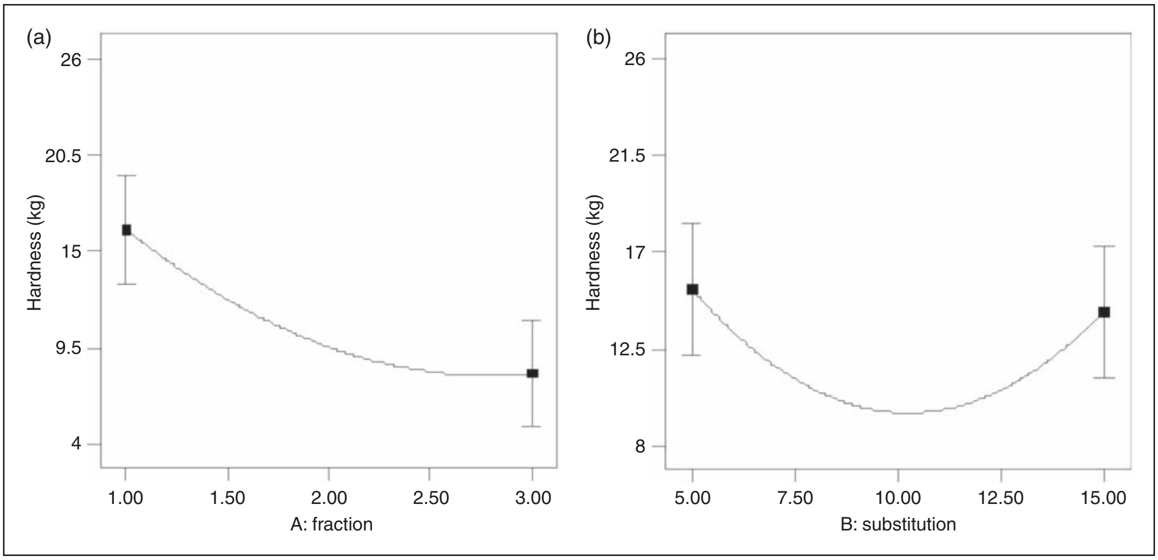

Figure 3. Impact on cookie hardness: (a) wheat germ fraction and (b) level of wheat germ.

Table 6. The Box–Behnken experimental design and obtained responses for sensory characteristics of cookies.

Run	Factor A	Factor B	Factor C	R1	R2	R3	R4	R5	R6
1	2	5	20	5.5	4.6	4.3	4.3	4.7	4.5
2	2	15	24	4.5	4.1	5.8	5.0	4.8	5.0
3	3	15	22	4.4	4.3	3.6	6.2	5.6	5.2
4	2	10	22	5.7	5.0	3.7	5.0	5.2	3.5
5	2	10	22	5.1	4.4	4.0	5.1	4.8	4.2
6	1	5	22	5.7	2.7	3.2	1.8	3.9	4.3
7	1	10	24	4.5	2.8	5.1	4.6	4.5	5.3
8	3	10	24	4.3	3.8	2.7	6.0	5.6	5.2
9	2	15	20	5.5	5.9	3.5	5.2	5.0	4.2
10	2	5	24	3.9	3.4	3.0	4.2	4.8	3.7
11	3	10	20	3.3	5.0	1.8	5.3	5.3	4.2
12	1	10	20	4.8	3.3	4.4	3.5	3.7	4.9
13	2	10	22	4.7	5.0	3.6	4.6	5.4	4.2
14	3	5	22	5.2	4.6	3.7	4.8	5.0	3.8
15	1	15	22	5.3	3.4	3.9	4.5	4.4	4.2

R1: Color; R2: Surface appearance; R3: Hardness; R4: Grittiness; R5: Air cell; R6: Beany flavor.

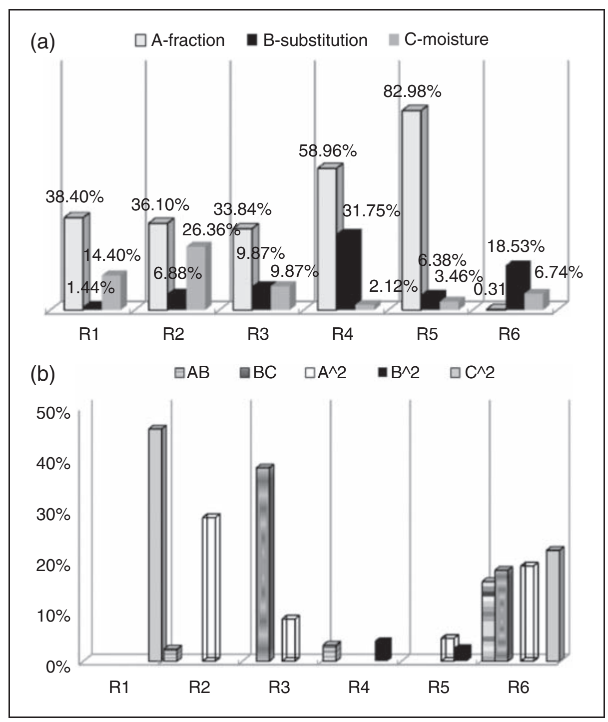

Figure 4. Contributions of main effect (a) and factor interactions and squared terms (b) on sensory characteristics of cookies: R1: color; R2: surface appearance; R3: hardness; R4: grittiness; R5: air cell; R6: beany flavor.

Table 6. Contributions of main factors and factor interactions and squared terms on sensory characteristics of cookies are presented in Figure 4. Scores for cookie color were in accordance with instrumental measurements. Cookie color was affected by wheat germ particle size (factor A) and moisture content (factor C) (Figure 4(a)). Increasing particle size of wheat germ and higher amount of water during making cookies caused lower scores for cookie color, i.e. cookies became lighter (negative sign of regression coefficients β_1 and β_3 for response R1 in Table 7). Wheat germ level up to 15% did not significantly influence this attribute (as can be seen at Figure 4(a), contribution of factor B for response R1 is only 1.44%).

Table 7. Regression equation coefficients for response R1-R6.

	R1	R2	R3	R4	R5	R6
Intercept						
β_0	5.133	4.800	3.767	4.908	5.125	3.967
Linear						
β_1	0.388***	0.688***	−0.600*	0.988***	0.625***	−0.038
β_2	0.025	0.300*	0.325	0.725**	0.175	0.288
β_3	0.363***	−0.588**	0.325	0.188	0.125	0.175
Interaction						
β_{12}	−0.125	−0.250	−0.200	−0.325	0.025	0.375
β_{13}	0.050	−0.175	0.050	−0.100	−0.125	0.150
β_{23}	−0.025	−0.150	0.900*	−0.025	−0.075	0.400
Quadratic						
β_{11}	0.033	−0.913***	−0.408	−0.204	−0.225	0.479*
β_{22}	−0.042	−0.138	0.242	−0.379	−0.175	−0.071*
β_{33}	0.133	−0.163	0.142	0.146	−0.125	0.454
R^2	0.9241	0.9018	0.6699	0.8704	0.8623	0.8024
Lack of Fit	0.6105	0.5626	0.0704	0.2055	0.6986	0.6747

***$p < 0.001$; **$0.001 < p < 0.01$; *$0.01 < p < 0.05$
R1: color; R2: surface appearance; R3: hardness; R4: grittiness; R5: air cell; R6: beany flavour.

Wheat germ particle size and moisture content had significant impact on cookies surface appearance, too. Panelist scores for cookie surface appearance increased as cookie contained larger particles of wheat germ (positive sign of regression equation coefficient of factor A for response R2 in Table 7).

As the particle size of wheat germ increased, the number of cracks on the cookie surface was higher. The increasing moisture content contributed to the reduction of surface cracks and the scores for surface appearance decreased. Doescher and Hoseney (1985) reported that as the moisture content increased, the number of islands on the cookie surface decreased.

Taking into account the scores for cookie hardness evaluated by panelists, it can be concluded that cookie hardness decreased with increasing wheat germ particle size and increased slightly with increasing moisture content (Table 7 and Figure 4). These results are in agreement with instrumental measurements of cookie hardness using Texture Analyzer. Interaction between moisture content and wheat germ level had great impact on cookie hardness (contribution 38.07%). The increase in wheat germ particle size, as expected, contributed to the increase of cookie grittiness. Contribution of factor A on the cookie grittiness was 82.98% (Figure 4(a)). The samples with highest wheat germ particle size (fraction 3) had the higher amount of small, hard particles between teeth during chew and acquired the highest scores for grittiness. Majzoobi et al. (2012) also showed that the panelist indicated that the cookies became slightly grainy and course when higher particle sizes of wheat germ were added.

Air cells were also influenced by wheat germ particle size (contribution of factor A was 58.96%) and by wheat germ level (contribution of factor B was 31.75%) (Figure 4(a)). Size of air cells in cookies increased as these two factors increased (positive sign of regression coefficients β_1 and β_2 for response R5 in table 7). Cookies air cells increased slightly with increasing moisture content, but that influence was not significant. Wheat germ level slightly increases the intensity of beany flavor in samples but it can be observed that there was no significant difference in flavor between samples.

CONCLUSION

DWG is a nutrition-valued by-product rich in bioactive compounds. The wheat germ content of up to 15% increased protein, fibers, and mineral content of cookies, however, did not significantly influenced hardness and sensory characteristics of cookies. Wheat germ particle size had significant influence on cookie hardness, cookie color, and cookie grittiness (as the wheat germ size increased, the cookie hardness decreased). Cookie surface color became lighter (decrease in L* value) as the wheat germ particle size decreased. Moisture content in the cookie dough had an impact on the cookie color and the number of cracks on the cookie surface (as the water content increased, the cookie color became lighter and the number of cracks on the cookie surface decreased). It can be concluded that DWG can be used for increased nutritional value of cookies, and with an appropriate combination of wheat germ content, its particle size, and dough moisture content we can get nutritional valuable cookies with fully acceptable sensory characteristics.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors highly appreciate financial support of the Provincial Secretariat for High Education and Scientific Research of the Government of Autonomous Province of Vojvodina, Republic of Serbia, project 142-451-2483/2017-01 and the Croatian Science Foundation under the Project 1321.

REFERENCES

Agama-Acevedo E, Islas-Hernández JJ, Pacheco-Vargas G, Osorio-Díaz P and Bello-Pérez LA. (2012). Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour. *LWT - Food Sci Technol* 46(1): 177–182.

Aktaş K, Bilgiçli N and Hacer L. (2015). Influence of wheat germ and beta glucan on some chemical and sensory properties of noodle. *Journal of Food Science and Technology* 52(9): 6055–6060.

Amadò R and Arrigoni E. (1992). Nutritive and functional properties of wheat germ. *International Food Ingredient* 4: 30–34.

AOAC. (2000). *Official methods of analysis of the association of official analytical chemists international*. Vols. I and II. 17th ed. Arlington: Association of Official Analytical Chemists Inc.

Arshad MU, Anjum FM and Zahoor T. (2007). Nutritional assessment of cookies supplemented with defatted wheat germ. *Food Chemistry* 102(1): 123–128.

Bansal S and Sudha ML. (2011). Nutritional, microstructural, rheological and quality characteristics of biscuits using processed wheat germ. *International Journal of Food Science and Nutrition* 62(5): 474–479.

Bilgiçli N, Elgün A, Herken EN, Türker S, Ertaş N and Ibanoğlu Ş. (2006). Effect of wheat germ/bran addition on the chemical, nutritional and sensory quality of tarhana, a fermented wheat flour-yoghurt product. *Journal of Food Engineering* 77: 680–686.

Brandolini A and Hidalgo A. (2012). Wheat germ: not only a by-product. *International Journal of Food Science and Nutrition* 63(1): 71–74.

Bruce PI, Chao-Hsiang T, Chi-Tang H and Bruce WA. (1994). Effect of moisture content on Millard browning kinetics of a model system during microwave heating. *Journal of Agricultural and Food Chemistry* 42: 1884–1887.

Chandrasekaran M. (2012). *Valorization of Food Processing by-Products*. Boca Raton, FL: CRC Press(2012).

CIE International Commission on Illumination. (1976). *Colorimetry: Official Recommendation of the International Commission on Illumination*. Publication CIE No. (E-1.31), Paris, France: Bureau Central de la CIE.

Chuang GCC and Yeh AI. (2006). Rheological characteristics and texture attributes of glutinous rice cakes (mochi). *Journal of Food Engineering* 74(3): 314–323.

Doescher LC and Hoseney RC. (1985). Effect of sugar type and flour moisture on surface cracking of sugar-snap cookies. *Cereal chemistry* 62(4): 263–266.

FAO. (2014). Save food – global initiative on food loss and waste reduction. Available at http://www.fao.org/ (accessed 16 May 2017).

FAO/WHO. (1973). *Energy and protein requirement*. Report of a Joint FAO/WHO *ad hoc* Expert Committee. Vol. 52. Geneva: FAO/WHO.

FAOSTAT. (2008). *FAO agricultural statistics*. Rome, Italy: Food and Agriculture Organization of the United Nations. Available at: http://faostat.fao.org.

Foresight Project. (2011). *The Future of Food and Farming: Challenges and Choices for Global Sustainability*. London, United Kingdom: The Government Office for Science.

Francis FJ and Clydesdale FM. (1975). *Food colorimetry: theory and applications*. Westport, CT: AVI Publishing Co. Inc.

Hodges RJ, Buzby JC and Bennett B. (2011). Postharvest losses and waste in developed and less developed countries: opportunities to improve resource use. *Journal of Agricultural Science* 149(S1): 37–45.

ISO 5492. (1992). Sensory analysis – vocabulary. Geneva, Switzerland: ISO.

ISO 4121. (2002). Sensory analysis – methodology – evaluation of food product by methods of using scales. Geneva, Switzerland: ISO.

ISO 8586. (2012). Sensory analysis – general guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. Geneva, Switzerland: ISO.

Kerckhoffs DA, Hornstra G and Mensink RP. (2003). Cholesterol-lowering effect of beta-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when beta-glucan is incorporated into bread and cookies. *American Journal of Clinical Nutrition* 78: 221–227.

Majzoobi M, Darabzadeh N and Farahnaky A. (2012). Effects of percentage and particle size of wheat germ on some properties of batter and cake. *Journal of Agricultural Science and Technology* 14(4): 827–836.

Myers RH and Montgomery DC. (2008). *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, 2nd ed. Hoboken, NJ: John Wiley & Sons Inc.

Nasir M, Siddiq M, Ravi R, Harte JB, Dolan KD and Butt MS. (2010). Physical quality characteristics and sensory evaluation of cookies made with added defatted maize germ flour. *Journal of Food Quality* 33(1): 72–84.

Petrović J, Fišteš A, Rakić D, Pajin B, Lončarević I and Šubarić D. (2015). Effect of defatted wheat germ content and its particle size on the rheological and textural properties of the cookie dough. *Journal of Texture Studies* 46(5): 374–384.

Rao HP, Kumar GV, Rao RG and Shurpaleker SR. (1980). Studies on stabilisation of wheat germ. *LWT – Food Science and Technology* 13: 302–307.

Rizzello CG, Nionelli L, Coda R, De Angelis M and Gobbetti M. (2010). Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. *Food Chemistry* 119(3): 1079–1089.

Troise AD and Fogliano V. (2013). Reactants encapsulation and Maillard reaction. *Trend Food Science and Technology* 33(1): 63–74.

Wang HJ and Thomas RL. (1989). Direct use of apple pomace in bakery products. *Journal of Food Science* 54(3): 618–620.

Yener ME. (2015). Supercritical fluid processing for the recovery of bioactive compounds from food industry by-products. In: Fornari Tiziana and Stateva Roumiana P (eds) *High Pressure Fluid Technology for Green Food Processing*. New York, NY: Springer International Publishing, pp. 305–355.

Zalatnai A, Lapis K, Szende B, Rásó E, Telekes A, ResetárÁ, et al. (2001). Wheat germ extract inhibits experimental colon carcinogenesis in F-344 rats. *Carcinogenesis* 22(10): 1649–1652.

Zucco F, Borsuk Y and Arntfield S. (2011). Physical and nutritional evaluation of wheat cookies supplemented with pulse flours of different particle sizes. *LWT – Food Science and Technology* 44(10): 2070–2076.