

The effect of baking temperature and buckwheat flour addition on the selected properties of wheat bread

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Summary

Wholegrain buckwheat flour was used to substitute 15 %, 30 % and 40 % of wheat flour to make buckwheat enriched wheat bread. Proximate composition, sensory evaluation, total phenols content and antioxidant activity of buckwheat enriched wheat breads were analysed and compared with wheat bread. Wholegrain buckwheat flour contained higher total phenols than wheat flour. The incorporation of buckwheat flour from 15 % to 40 % in bread samples increased the total phenols content from 0.25 (mg GA/g d.m. sample) to 0.65 (mg GA/g d.m. sample), and antioxidant activity from 208.45 ($\mu\text{mol Fe}^{2+}/\text{L}$ extract) to 354.45 ($\mu\text{mol Fe}^{2+}/\text{L}$ extract). Total phenols content decreased during the baking process, while the antioxidant activity increased. Bread samples with 15 %, 30 % and 40 % of wholegrain buckwheat flour showed lower lightness (*L*) and whiteness index (*WI*) values of crust and crumb colour compared to the wheat bread. Addition of buckwheat flour increased redness (*a*) and yellowness (*b*) colour values for crumb. Sensory results indicating that three breads with buckwheat flour were moderately acceptable. No differences were found in overall sensory attributes between buckwheat flour enriched bread samples with 15 % and wheat bread (control sample).

Keywords: baking process, buckwheat bread, colour, total phenols, FRAP, sensory evaluation

Introduction

Bread and bakery products have an important role in human nutrition. Generally, wheat bread is a good source of irreplaceable nutrients and energy for the human body. The most common bakery products are obtained from white flour, but with increasing awareness of the importance of proper nutrition and healthy lifestyle there is a growing need for products that have improved nutritional composition with potentially preventive effects on health. Demand for products with improved functional properties is expressed in the bakery industry (Schönlechner, 2008). There are many special types of bread in the category of functional products that are rich in minerals, dietary fibre, vitamins, inulin, oligosaccharides, omega-3 fatty acids, β -glucans, flax seeds and others. Buckwheat is pseudocereal which does not contain gluten and is mainly used in the production of gluten-free products (Wronkowska et al., 2008). Buckwheat is a rich source of proteins, carbohydrates, minerals, fibre, flavonoids, and other compounds which are to participate in lowering blood pressure, reduce cholesterol levels, blood glucose control and prevention of cancer (Quettier-Deleu et al., 2000; Steadman et al., 2001; Holasova et al., 2002;

Gorinstein et al., 2004). Unlike wheat and other cereals, buckwheat proteins have a good balanced amino acids composition and contain all of essential amino acids (Wijngaard and Arendt, 2006), although their digestibility is relatively low (Kato et al., 2001; Tomotake et al., 2006). Buckwheat also contains phenolic compounds which show significant antioxidant activity (Dietrych-Szostak and Oleszek, 1999; Christa and Soral-Šmietana, 2008). Rutin and quercetin are the main antioxidants in buckwheat that prevent lipid peroxidation and activity of free radicals (Kreft et al., 1999; Zieliński and Kozłowska, 2000). Compared to antioxidant activity of frequently used cereals, buckwheat possesses higher antioxidant activity, mainly due to high rutin content (Zieliński and Kozłowska, 2000; Kreft et al., 2006). Wholegrain wheat and wholegrain buckwheat flours contain more phenols compounds and show greater antioxidant activity compared with refined wheat and buckwheat flour (Sedej et al., 2010). The aim of this work was to investigate influence of baking temperature and addition of wholegrain buckwheat flour on the total phenols content, antioxidant activity, colour and sensory evaluation of wheat bread.

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Materials and methods

Materials

The ingredients used in the bread making were wholegrain buckwheat flour (Rima Pak, BiH), wheat flour (KLAS Sarajevo, BiH), salt (Solana, Tuzla, BiH) dry bakery's yeast (Prigorje Brdovečko, Croatia) and improver ("Saturnus LC", Backaldrin, Austria). The improver has the following

components: wheat flour, enzymes, emulsifier E472e, L-ascorbic acid (manufacturer's declaration). All chemicals and reagents were purchased from: Fluka, Switzerland (2,4,6-tri[2-pyridyl]-s-triazine); Merck, Germany (gallic acid) and Semikem, Sarajevo (Folin-Ciocalteu's reagent, chloric acid 37 % p.a. ferrous sulphate heptahydrate; ferric chloride hexahydrate; sodium acetate trihydrate; acetic acid; sodium carbonate). The ingredients for bread making were dosed according to the formula in Table 1.

Table 1. The formulations of breads (% on the flour basis)

Ingredients	Bread samples			
	100	85	70	60
Wheat flour (%)	100	85	70	60
Wholegrain buckwheat flour (%)	-	15	30	40
Salt (%)	2	2	2	2
Yeast (%)	3.5	3.5	3.5	3.5
Improver (%)	0.3	0.3	0.3	0.3
*Water (%) (WA)	58	59	60	60.5

* according to water absorption (WA) (farinograph test)

Bread making

Bread making was conducted under the following conditions: mixing of ingredients (SMH-120N, Gostol-Gopan, N.Gorica, Slovenia) to form dough (11 minutes), dough resting (15 minutes, dough temperature 27 °C), manual remixing, dough dividing (manually) into dough pieces of equal weight (470 g) and rounding, intermediate fermentation (15 minutes), mechanically shaping the dough into loaves, final proofing (60 minutes, 35 ± 2 °C, 75 % relative humidity), baking at 240 °C (23 ± 1 minutes). After the baking, the bread samples were cooled to room temperature and packaged in plastic bags.

Chemical composition of flour

The composition of flours, including moisture content, ash, crude protein and total fat content, were determined according to the methods of ICC Standard Methods. All the measurements were taken in triplicate and expressed as mean value ± standard deviation (SD).

Sample preparation and extraction

In all samples of bread and flour, total phenol was extracted with water. Pieces of the crumb (50 g) and the crust (50 g) were taken from various parts of the bread, air-dried and they were chopped up together in a kitchen electric mill to a small granulation which is easy to homogenize. 5 g of chopped sample was taken for extraction and diluted in 100 mL distilled

water. The content was mixed from time to time for 60 minutes at room temperature (20 °C), and then filtered through filter paper. Extraction of phenol from 5 g flour sample was done according to the same procedure as for the chopped bread samples. The extract obtained by this procedure was used for determination of antioxidant activity and total phenols content.

Determination of total phenols (TP) content

Total phenols (TP) in water extracts of flour and bread were determined with Folin-Ciocalteu reagent using gallic acid as a standard (Singleton et al., 1999). The extract (200 µL) was mixed with 2 mL of Folin-Ciocalteu reagent (previously diluted 10 times with distilled water). After 5 minutes, 1.8 mL sodium bicarbonate solution (7.5 % w/v) was added to the mixture and after incubation for 120 min at room temperature, the absorbance was measured at 765 nm using a UV/VIS spectrophotometer (UV mini 1240, Shimadzu). The concentration of total phenols compounds in extracts was determined as gallic acid equivalent (GA) using an equation obtained from a standard gallic acid graph. Results are expressed as mg GA/g d.m. sample. All the measurements were taken in duplicate and expressed as mean value ± standard deviation (SD).

Determination of total antioxidant activity

The antioxidant activity of aqueous extracts of flour and bread was determined by FRAP (Ferric

Reducing Antioxidant Power) method (Benzie and Strain, 1996). FRAP reagent was prepared by mixing TPTZ (2, 4, 6-tripyridyl-s-triazine) solution (10 mM TPTZ solution was prepared in 10 mL of 40 mM HCl), 20 mM FeCl₃•6H₂O and acetate buffer (0.3 mol/L, pH 3.6) in the ratio 1:1:10. All solutions were used on the day of preparation. FRAP reagent was prepared and thermostated at 37 °C. A volume of 200 µL extract was mixed with 1.8 mL of FRAP reagent and the absorbance of the reaction mixture was measured at 593 nm (UV mini 1240, Shimadzu) after incubation at 37 °C for 10 min. The antioxidant activity was determined as µmol Fe²⁺/L of extract using an equation obtained from a standard curve. Standard curve was prepared using different concentrations (50-1000 µmol/L) of FeSO₄•7H₂O and absorbance were measured as sample solution. All the measurements were taken in duplicate and expressed as mean value ± standard deviation (SD).

Instrumental colour measurement of bread

The colour measurements were made with a colorimeter Croma Meter CR 300 (Konica Minolta, Japan) according to the method CIE 1976 - Lab Colour Space. A standard white plate was used to standardise the instrument. Colour of the crumb and crust of bread is expressed in the CIE-Lab parameters as *L* (white/black), *a* (red/green) and *b* (yellow/blue). Results are presented as the mean value of five measurements ± standard deviation (SD). Whiteness index (WI) was calculated based on the following equation (Lin et al., 2009):

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2} \quad (1)$$

Table 2. Chemical composition of flour samples

Flour samples	Water (%)	Ash (% d.m.)	Proteins (% d.m.)	Fats (% d.m.)
WF	13.40 ± 0.13	0.49 ± 0.03	11.69 ± 0.55	1.03 ± 0.16
WF + WBF (85 % + 15 %)	12.86 ± 0.03	0.82 ± 0.05	11.81 ± 0.12	1.23 ± 0.09
WF + WBF (70 % + 30 %)	12.09 ± 0.17	1.11 ± 0.09	11.9 ± 0.23	1.5 ± 0.04
WF + WBF (60 % + 40 %)	11.58 ± 0.10	1.28 ± 0.06	12.01 ± 0.11	1.62 ± 0.07
WBF	10.15 ± 0.04	2.4 ± 0.05	12.4 ± 0.22	2.5 ± 0.01

WF – wheat flour; WBF – wholegrain buckwheat flour

Sensory evaluation

The sensory evaluation was conducted by a panel of five experienced assessors. The following sensory properties were evaluated: external appearance, appearance of crumb, taste and flavour of crust and crumb. Each sensory property was rated by score 1-5 applying the factors of significance (FS). Factors of significance were: external appearance – 3; appearance of crumb – 5; flavour of crust and crumb – 3; taste of crust and crumb – 5. Total grade was obtained as the sum of individual properties. Weighted mean (WM) was calculated by dividing the total grade and sum of factors of significance.

Statistical analyses

One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's *post-hoc* test) were used to evaluate the significant difference of the data at $p < 0.05$. Pearson's correlation coefficient (*r*) was used for the analysis of linear correlation between certain parameters. Two-way t-test (2-tailed) was used to test the statistical significance of the correlation coefficient ($p < 0.05$). Data were analysed using the software package SPSS V.15.

Results and discussion

The analysis of wheat and buckwheat flour used for bread production showed that buckwheat flour has lower water content, while mineral and fat content is higher compared to wheat flour (Table 2). These results have been expected, having in mind that we used WBF for the experiment. This is why mixed bread flour containing 15 %, 30 % and 40 % WBF has a considerably increased mineral content (between 0.82 % and 1.28 %) and fats content (between 1.23 % and 1.62 %) compared to wheat flour (0.49 % and 1.03 %, respectively). The protein content was not significantly changed.

Increasing the proportion of buckwheat flour from 15 % to 40 %, yield of dough was increased for 5.5 % to 12.33 %, while the yield of bread increased for 4.56 % to 14.34 % as compared to wheat bread. Increasing of amount of water needed for mixing leads to increase of dough and bread yield.

The aroma and taste improvement can be achieved by acidity increase in the bread samples with 15 %, 30 % and 40 % of buckwheat flour. Height (h) and width (w) of cross-section were decreased proportionally by addition of buckwheat flour up to 30 % in comparison to wheat bread, while the addition of 40 % buckwheat flour significantly reduced height but increased bread width.

Table 3. Effect of buckwheat flour on dough yield, bread yield, losses during baking and cooling, acidity of bread samples and ratio of height and width (h/w)

Bread samples	Yield of dough (%)	Yield of bread (%)	Losses (%)	TTA	Ratio of height and width (h/w)
WF	158.33 ^a	136.09 ^a	14.04 ^a	2.2 ^a	0.64 ^a
WF + WBF (85 % + 15 %)	163.83 ^b	140.65 ^b	14.15 ^a	2.6 ^b	0.63 ^a
WF + WBF (70 % + 30 %)	169 ^c	150.05 ^c	11.2 ^b	3.6 ^c	0.62 ^a
WF + WBF (60 % + 40 %)	170.66 ^c	150.43 ^c	11.85 ^b	4.4 ^d	0.5 ^b

WF – wheat flour; WBF – wholegrain buckwheat flour

**TTA (total titratable acidity) – mL 0.1 M NaOH

***Values in the same column marked with different letters are statistically significantly different (Duncan test; $P < 0.05$)

By comparing the ratio h/w values (Table 3), it can be concluded that the only addition of the maximal tested buckwheat flour dose (40 %) significantly affected the reduction in the h/w value indicating a negative influence on the shape of bread. The results obtained by determination of TP content in water extract of flour samples (Table 4), show that

TP concentration in WBF (1.71 mg GA/g d.m. sample) is eight times higher than in wheat flour (0.21 mg GA/g d.m. sample). The samples of wheat flour with added buckwheat flour showed a considerably higher antioxidative activity compared to wheat flour alone (Table 4).

Table 4. Total phenols (TP) contents and antioxidant activity (FRAP) of the wheat and buckwheat flour and different wheat–buckwheat mixture before baking process

Flour	TP (mg GA/g d.m. sample)	FRAP ($\mu\text{mol Fe}^{2+}/\text{L extract}$)	PAC
WF	0.21 \pm 0.14	55.45 \pm 1.98	1.03
WF + WBF (85 % + 15 %)	0.37 \pm 0.63	110.95 \pm 3.57	2.04
WF + WBF (70 % + 30 %)	0.52 \pm 0.77	208.95 \pm 1.33	3.62
WF + WBF (60 % + 40 %)	0.71 \pm 0.59	249.95 \pm 4.92	3.92
WBF	1.71 \pm 0.81	461.95 \pm 2.87	4.39

WF – wheat flour; WBF – wholegrain buckwheat flour

**PAC- Phenol antioxidant coefficient, calculated as ratio FRAP ($\mu\text{mol/L}$) / total phenolic ($\mu\text{mol GA/L}$)

With the increase of the share of buckwheat flour from 15 % to 40 %, antioxidative activity increased 2 to 5 times, while antioxidative activity in water extract of buckwheat flour (461.95 $\mu\text{mol Fe}^{2+}/\text{L extract}$) was 8 times higher than in wheat flour (55.45 $\mu\text{mol Fe}^{2+}/\text{L extract}$). This increase in antioxidative activity can be explained with the fact that the increase of buckwheat flour share leads to

the increase in TP which are believed to have a considerable antioxidative activity. The correlation coefficient ($r = 0.978$; $p = 0.004$) shows that there is a statistically significant correlation between the concentration of TP and antioxidative activity in water extract of tested flour samples (Zieliński i Kozłowska, 2000; Sedej et al., 2010).

Table 5 shows the results of TP determination in wheat bread and bread samples with added buckwheat flour. Similar to flour samples, the increase of buckwheat flour from 15 % to 40 % leads to the increase in TP concentration in bread samples (0.25 – 0.65 mg GA/g d.m. sample) compared to

wheat bread (0.12 mg GA/g d.m. sample). TP concentration was lower in all bread samples, regardless of the share of buckwheat flour, when compared to the samples of the flour used for breadmaking. Reduction of TP concentration can be explained by negative effects of baking temperature.

Table 5. Total phenols (TP) contents and antioxidant activity (FRAP) of the bread samples

Bread	TP (mg GA/g d.m. sample)	FRAP ($\mu\text{mol Fe}^{2+}/\text{L extract}$)	PAC
WF	0.12 \pm 0.24	163.95 \pm 2.11	4.72
WF + WBF (85 % + 15 %)	0.25 \pm 0.31	208.95 \pm 2.25	4.75
WF + WBF (70 % + 30 %)	0.46 \pm 0.62	274.45 \pm 3.18	4.89
WF + WBF (60 % + 40 %)	0.65 \pm 0.35	354.95 \pm 3.43	5.66

WF – wheat flour; WBF – wholegrain buckwheat flour

*PAC- Phenol antioxidant coefficient, calculated as ratio FRAP ($\mu\text{mol/L}$) / total phenolic ($\mu\text{mol GA/L}$)

The highest loss of TP (40.92 %) was observed between wheat bread and wheat flour. In bread samples containing 15 %, 30 % and 40 % of buckwheat flour, the loss of TP compared to flour samples was 32.43 %, 13.46 % and 8.45 %. Since phenol determinations have been done in water extract, the results can be interpreted as baking temperature having more significant effect on the loss of TP (water-soluble) in wheat flour than in buckwheat flour. Antioxidative activity of wheat bread was 163.95 $\mu\text{mol Fe}^{2+}/\text{L extract}$, while addition of buckwheat flour significantly increased antioxidative activity. In bread samples with 15 %, 30 % and 40 % of buckwheat flour, there was an increase of antioxidative activity by 21.54 %, 40.26 % and 53.95 % compared to wheat bread. The correlation coefficients show that there is a statistically significant correlation between total TP concentration and antioxidative activity in water extract of the tested bread samples ($r = 0.998$; $p = 0.002$). All bread samples showed higher antioxidative activity compared to flour samples. Although baking temperature leads to reduced TP concentration, the increase of antioxidative activity in

bread samples can be explained by the formation of products of Maillard's reaction (Lindhauer, 2007; Holtekjølen et al., 2008; González-Mateo et al., 2009). The biggest difference between antioxidative activity of bread and flour samples was observed between wheat bread and wheat flour. The difference between antioxidative activity of samples of bread and flour with addition of buckwheat flour decreases with the increase of buckwheat flour share. Based on the information above, we can conclude that baking temperature had bigger influence on formation of products of Maillard's reaction in wheat bread than in breads with buckwheat flour. In our recent research (Selimović et al., 2013) we studied the effect of buckwheat flour on TP content and antioxidant activity of wheat bread without additives. The results obtained in this paper show that addition of additives does not have an influence on TP content and antioxidant activity.

Tables 6 and 7 shows the results of determination of L , a and b values of the colour of the bread crumb and crust. The lowest levels of red (a) and yellow (b) pigment in the colour of the crumb have been measured in wheat bread.

Table 6. The values of whiteness index (WI) and parameters L , a and b for colour of bread crumb

Bread	L	a	b	Whiteness index (WI)
Wheat bread	73.53 \pm 1.32 ^a	-1.04 \pm 0.06 ^d	9.27 \pm 0.67 ^c	71.93 ^a
Bread with 15% WBF	60.31 \pm 2.20 ^b	0.68 \pm 0.25 ^c	16.69 \pm 0.87 ^b	56.9 ^b
Bread with 30% WBF	53.26 \pm 0.73 ^c	2.30 \pm 0.05 ^b	17.58 \pm 0.53 ^{ab}	49.8 ^c
Bread with 40% WBF	47.97 \pm 0.43 ^d	3.03 \pm 0.07 ^a	18.96 \pm 0.36 ^a	45.19 ^d

*WBF – wholegrain buckwheat flour

**Values in the same column marked with different letters are statistically significantly different (Duncan test; $p < 0.05$)

With the increase of WBF share in bread from 15 % to 40 % the level of yellow pigment (*b*) considerably increases, while *L* value considerably decreases compared to *L* value of wheat bread. Based on whiteness index (WI), the darkest bread

crumb (WI = 45.19) was found in the bread sample with 40 % WBF, while wheat bread had the whitest crumb (WI = 71.93). All bread samples had a higher level of red (*a*) and yellow (*b*) pigment in the crust than in the crumb.

Table 7. The values of whiteness index (WI) and parameters *L*, *a* and *b* for colour of bread crust

Bread	<i>L</i>	<i>a</i>	<i>b</i>	Whiteness index (WI)
Wheat bread	53.53 ± 1.42 ^a	16.15 ± 0.22 ^a	33.14 ± 1.05 ^a	40.66 ^a
Bread with 15% WBF	46.37 ± 1.09 ^b	14.50 ± 0.14 ^b	29.54 ± 0.68 ^b	39.79 ^b
Bread with 30% WBF	44.98 ± 0.22 ^b	13.39 ± 0.15 ^c	27.26 ± 0.20 ^b	38.42 ^c
Bread with 40% WBF	42.05 ± 0.38 ^c	13.06 ± 0.02 ^c	22.17 ± 0.45 ^c	36.52 ^d

^aWBF – wholegrain buckwheat flour

^{**}Values in the same column marked with different letters are statistically significantly different (Duncan test; *p* < 0.05)

The crust of wheat bread had the highest level of yellow pigment (*b*). The lowest brightness of the crust was measured in samples with 40 % buckwheat flour (WI = 36.52). The increase of WBF share in bread from 15 % to 40 % significantly decreases the level of red pigment (*a*) in bread crust colour. The results of the statistic analysis (Table 8)

show that there is a correlation between certain parameters of colour (*L*, *a* and *b*) and total phenol concentration and antioxidant activity. The correlation was especially significant between *b* value for the colour of bread crust (yellow pigment level) and TP (*r* = 0.985; *p* = 0.015) and FRAP (*r* = 0.992; *p* = 0.008) (Table 9).

Table 8. The coefficients of correlation between TP, FRAP, WI and colour values (*L*, *a*, *b*) for the crumb and crust of bread

	Crumb				Crust			
	<i>L</i>	<i>a</i>	<i>b</i>	WI	<i>L</i>	<i>a</i>	<i>b</i>	WI
TP	-0.955* (<i>p</i> =0.045)	-0.973* (<i>p</i> =0.027)	0.846 (<i>p</i> =0.154)	-0.944 (<i>p</i> =0.056)	-0.915 (<i>p</i> =0.085)	-0.938 (<i>p</i> =0.062)	-0.985* (<i>p</i> =0.015)	-0.780 (<i>p</i> =0.22)
FRAP	-0.944 (<i>p</i> =0.056)	0.959* (<i>p</i> =0.041)	0.833 (<i>p</i> =0.167)	-0.941 (<i>p</i> =0.059)	-0.909 (<i>p</i> =0.091)	-0.921 (<i>p</i> =0.079)	-0.992** (<i>p</i> =0.008)	-0.769 (<i>p</i> =0.231)

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

***WI - whiteness index

Crumb of bread becomes less porous and less elastic while these changes are much less pronounced with the addition of 15 % and 30 % buckwheat flour. Sensory evaluation (Table 9) showed that the bread

made of wheat flour supplemented with 40 % of buckwheat flour is significantly different in the negative terms from the bread with 15 % buckwheat flour and wheat bread.

Table 9. Sensory evaluation of wheat bread made with buckwheat flour addition

Bread	External appearance	Appearance of crumb	Flavour of crust and crumb	Taste of crust and crumb	WM
Wheat bread	14.7 ^a	25 ^a	14.4 ^a	25 ^a	4.94 ^a
Bread with 15 % WBF	13.8 ^{ab}	24.5 ^a	14.7 ^a	22.5 ^b	4.71 ^{ab}
Bread with 30 % WBF	12.9 ^{ab}	24 ^a	14.7 ^a	22.5 ^b	4.63 ^{bc}
Bread with 40 % WBF	11.7 ^b	22 ^b	15 ^a	22 ^b	4.42 ^c

^aWBF – wholegrain buckwheat flour

^{**}Values in the same column marked with different letters are statistically significantly different (Duncan test; *p* < 0.05)

^{***}WM – Weighted mean was calculated by dividing the total grade and sum of factors of significance

Using 40 % of buckwheat flour, significantly affects the sensory properties of bread, especially appearance and taste of crust and crumb. According to our previous findings (Selimović et al., 2011), improver had significant influence on the sensory properties of bread with 40 % buckwheat flour.

Conclusions

Using buckwheat flour in the production of bread leads to increasing bread yield (4.56 % to 14.34 %) and acidity of bread crumb. Wholegrain buckwheat flour is a good source of phenols and possessed good antioxidant activity. The addition of buckwheat flour to wheat flour can increase total phenols concentration and improve antioxidant status of bread. Baking temperature influenced significantly more the loss of total phenols in wheat flour than in buckwheat flour and increase of antioxidative activity in bread samples by the formation of products of Maillard's reaction. Buckwheat enriched wheat bread samples showed less lightness and WI values and higher redness. Addition of buckwheat flour causes a darker colour of the bread crumb and crust. Substituting 15 % of wheat flour in the bread formula with buckwheat flour does not significantly affect bread properties and attributes such as external appearance, appearance of crumb, flavour and overall bread quality.

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