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EFFECT OF REPLACEMENT OF WHEAT FLOUR WITH BLACK LENTIL FLOUR ON BREAD QUALITY

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Abstract: The aim of this study was to investigate the effect of black lentil flour (BLF) on the quality of wheat bread. The control bread with wheat flour and BLF-supplemented breads (2, 6 and 10 %, respectively) were prepared and analyzed in terms of proximate composition, physicochemical and sensorial properties, total phenolic content (TPC), and antioxidant activity. The BLF-supplemented breads provided significantly higher protein compared to the control bread ($p < 0.05$). Moreover, it improved significantly TPC and antioxidant capacity ($p < 0.05$). However, the addition of BLF reduced significantly the specific volume of the bread, L^* and b^* values of both crumb and crust ($p < 0.05$). The BLF had no influence overall acceptability. Our findings revealed that the BLF may a have potential use to enhance the quality of wheat bread.

Keywords: *antioxidants, lentil, nutritional quality, phenolic compounds, wheat bread*

INTRODUCTION

Bread, a popular staple food, is a source of energy and carbohydrates. The main ingredient of bread is cereal flours and wheat flour is widely used in bread making. Wheat contains carbohydrates (starch and dietary fiber), proteins (gliadin and glutelin), vitamins (B vitamins), and minerals (potassium, calcium, and magnesium, etc). Wheat proteins have lower levels of essential amino acids such as lysine and threonine. Moreover, the refinement process reduces the dietary fiber and micronutrients contents of wheat flour [1]. Therefore, studies on the enrichment of white flour with other flours have been conducted to improve the nutritional quality of white bread. Legume flours, one of the flours, are utilized to improve the nutritional values of white bread since they are rich in protein, dietary fiber, and minerals. Legumes proteins include arginine and leucine as essential amino acids, and glutamic and aspartic acids as non-essential amino acids. The blend of legume with cereal provides well balanced essential amino acid profile [2]. The use of carob flour [3], chickpea flour [4 – 6], lentil flour [7], lentil and carob flours [8], lupine flour [9, 10], and soy flour [11, 12] have been investigated to improve the quality of wheat bread.

Lentils are one of the most consumed legumes throughout world. They are a good source of phenolic compounds including phenolic acids, flavan-3-ols, proanthocyanidins, anthocyanidins, flavonols, flavones, and flavanones [13, 14]. Phenolic compounds may exert protective effects against chronic diseases such as cancer and cardiovascular diseases due to their antioxidant activity [15].

The aim of this study was to investigate the usability of black lentil flour (BLF) in the production of wheat bread. To our knowledge, the black lentil is the less utilized lentil and there is no available study on the use of BLF in the production of bread. The control (0 %) and BLF-supplemented breads (2, 6 and 10 %, respectively) were prepared with the wheat flour (WF) and BLF. Their proximate composition, physicochemical and sensorial properties, TPC, and antioxidant activity were determined to evaluate the effects of the replacement of wheat flour with the BLF on the bread quality.

MATERIALS AND METHODS

Materials

Commercial wheat flour (Safmill Food Inc./Tekirdağ, Turkey), black lentil (Yayla Agro Food Industry and Transport Inc./Mersin, Turkey), dry yeast, and salt were provided from the local market. The black lentils were ground by using a laboratory mill (IKA 20, IKA-Werke, GmbH&Co.KG, Staufen, Germany) and passed through a 200-micron sieve. The wheat flour had contents of ash 0.70 - 80 % (dry basis), protein 10.50 % (dry basis), fat 1.7 % (dry basis), and total dietary fiber 2.6 % (dry basis) according to its producer.

Chemicals

The solvents (diethyl ether, petroleum benzine, acetone, and methanol etc) and reagents (acetic acid, hydrochloric acid sulphuric acid, boric acid, sodium hydroxide, sodium acetate, Folin Ciocalteu reagent, iron (III) chloride, TPTZ and DPPH etc) were analytical grade and were purchased from Merck KGaA (Darmstadt, Germany).

Bread baking

Four bread formulations were prepared by the replacement of wheat flour with the BLF (0, 2, 6 and 10 % respectively). The maximum replacement level of the BLF was selected as 10 % according to the farinograph and extensongraph analysis results (data not shown). Bread formulations were presented in Table 1. Bread-making machine (Arçelik K2715, Arçelik Inc., İstanbul, Turkey) was used to make breads. The program included 6 steps (knead 1 : 14 min, rise 1 : 32 min, knead 2 : 8 min, rise 2 : 31 min, rise 3 : 50 min, baking at 180 °C for 62 min). Bread production was carried out in three replicates.

Table 1. *Bread formulations*

Component [g]	CB	2LB	6LB	10LB
WF	300	294	282	270
BLF	0	6	18	30
Yeast	3	3	3	3
Salt	4.5	4.5	4.5	4.5
Water	180	180	180	180

WF: wheat flour; BLF: black lentil flour
CB: control bread; 2LB: 2 % black lentil flour-supplemented bread; 6LB: 6 % black lentil flour-supplemented bread 10LB: 10 % black lentil flour-supplemented bread

Proximate composition

The moisture content of the breads was determined according to TS 5000 [16]. The contents of ash (AOAC 923.03), protein (AOAC 920.87), and fat (AOAC 922.06) were determined according to the AOAC methods [17]. The total carbohydrates were calculated following formula (1):

$$\text{Total carbohydrates } \% = 100 - (\text{moisture } \% + \text{ash } \% + \text{protein } \% + \text{fat } \%) \quad (1)$$

Weight and specific volume

The weight of the bread sample was measured using a balance (Radwag WTC 600, Poland) after it was cooled at room temperature. Its volume was measured using the rapeseed displacement method. The ratio of its mass to volume was calculated as the specific volume ($g \cdot cm^{-3}$).

Color properties

The color properties (L^*, a^*, and b^* values) of the crumb and crust were measured with chromometer (Konica Minolta, CR-400, Japan).

Sensorial properties

The overall acceptability of the crumb and crust were evaluated by the university staffs as the panelists. Only 25 of panelists (men: 13 and women: 12) were reached because of the pandemic. The loaves (20 - 25 g) were coded randomly with three-digit random number and served to the panelists in booths. A nine-point scale (1: dislike extremely, 9: like extremely) was used for the evaluation. Water was given to the panelists between the samples.

Authorization for research with human subjects was provided from Ethical Commission of Gümüşhane University (Date: 26/10/2020- Number: 2020/10). The informed consent was obtained from the panelists.

Preparation of extracts

The extraction procedure was adopted from Xu *et al.* [18] with slight modification.
A 1 g of the sample was weighed, and 5 mL of 80 % methanol was added. The test tubes were stirred at 37 °C for 2 hours and then sonicated for 30 min. The test tubes were centrifuged at 5000 rpm for 5 min. The supernatant was collected, and the extraction procedure was repeated. The combined extract was stored at -18 °C until analysis.

Total phenolics content

The TPC was determined using Folin Ciocalteu method. The extract (0.5 mL) was mixed with Folin Ciocalteu reagent (0.5 mL) and stored at dark for 5 min. The sodium carbonate (1 M, 0.5 mL) and water (3.5 mL) were added. After mixing, they were stored at dark for 2 hours. The absorbance (760 nm) was measured. The calibration curve was prepared with gallic acid (10 - 100 $mg \cdot L^{-1}$). The TPC was expressed as mg gallic acid equivalents per 100 g sample (dry basis)

DPPH radical scavenging activity

The extract (1 mL) and DPPH radical solution (60 µM, 1 mL) were mixed, and stored at dark for 1 h. The absorbance (515 nm) was measured. The DPPH radical scavenging activity was expressed as µmole Trolox equivalents per 100 g sample (dry basis).

FRAP

The extract (0.5 mL) and FRAP reagent (1.5 mL) were mixed, and stored at dark for 20 min. The absorbance (595 nm) was measured. The FRAP was expressed as µmole Trolox equivalents per 100 g sample (dry basis).

Statistical analysis

At least two analysis was performed in three replicates of breads. Mean values and standard deviations of the bread samples were calculated. One-way ANOVA and

Duncan multiple range test were applied to investigate the differences between bread samples. SPSS 17.0 software (SPSS Inc., IL, USA) were used for data processing.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of the bread samples is presented in Table 2. The moisture content of the bread samples ranged from 35.16 to 35.67 %. The addition of black lentil flour (BLF) into the bread formulation had no significant effect on the moisture content ($p > 0.05$). The protein contents of the bread samples increased significantly with the addition of the BLF ($p < 0.05$). The 10LB had the highest value (12.83 %), whereas the control sample presented the lowest value (11.32 %). The fat contents of the bread samples ranged from 1.59 to 1.68 %, that showed no significant difference ($p > 0.05$). The total carbohydrate contents of the bread samples decreased from 86.06 to 84.40 %. Although the ash contents of the bread samples increased from 1.03 to 1.16 % with the addition of the BLF, no significant difference was observed among the bread samples ($p > 0.05$). Similar findings were found in the literature. A significant increase in the protein content and non-significant increase in the ash content were reported for the bread supplemented with legume flours such as soy flour at 10 % level [11] and lupine flour at 20 % level [9]. It can be concluded that the addition of the BLF enhanced the nutritional quality of the wheat bread.

Table 2. *Proximate composition of bread samples*

Component [%]	CB	2LB	6LB	10LB
Moisture	35.67±1.00^{a}	35.62±1.65^{a}	35.33±0.66^{a}	35.16±0.90^{a}
Protein[1]	11.32±0.30^{c}	12.05±0.35^{b}	12.50±0.25^{ab}	12.83±0.24^{a}
Fat[1]	1.59±0.15^{a}	1.68±0.09^{a}	1.65±0.01^{a}	1.59±0.14^{a}
Total carbohydrate[1]	86.06±0.36^{a}	85.22±0.29^{b}	84.77±0.25^{bc}	84.40±0.18^{c}
Ash[1]	1.03±0.05^{a}	1.05±0.12^{a}	1.08±0.04^{a}	1.16±0.06^{a}

[1] expressed as dry basis.
Data are expressed as means ± SD
Different letter in row present significant difference ($p<0.05$)

Weight and specific volume

The weight and specific volumes of the bread samples are presented in Table 3. The addition of the BLF had no significant effect on the weight of the bread samples ($p > 0.05$). However, it reduced significantly the specific volumes of the bread samples ($p < 0.05$). The control sample had the highest value (3.02 $g \cdot cm^{-3}$), whereas the 10 LB had the lowest value (2.10 $g \cdot cm^{-3}$). Similar findings were reported for the wheat bread supplemented with the legume flours. The specific volume of the bread was determined to decrease significantly when the wheat flour was replaced with the chickpea flour at 5 % level [6] and at 10 % level [19], the soy flour at 5 % level [20], and the lupine flour at 15 % level [10]. Previous studies stated that this reduction could be related to the dilution of gluten in the flour blends by the addition of the legume flours. The dilution of gluten and interaction with fibers and non-gluten proteins could weaken the gluten network, which reduces the gas-retention ability.

Table 3. Weight and specific volumes of bread samples

Properties	CB	2LB	6LB	10LB
Weight [g]	415.22±7.20^{a}	414.48±5.30^{a}	418.25±4.00^{a}	417.58±5.42^{a}
Specific volume [g·cm^{-3}]	3.02±0.42^{a}	2.67±0.16^{ab}	2.40±0.21^{bc}	2.10±0.04^{c}

Data are expressed as means ± SD
Different letter in row present significant difference ($p < 0.05$)

Color properties

The color values of the bread samples are presented in Table 4. It can be seen from Table 4, the addition of the BLF reduced significantly the L^* and b^* values of the crumb and crust ($p < 0.05$). However, it increased significantly the a* value ($p<0.05$). The control sample had the highest L^* (L^*_{crumb}:69.02, L^*_{crust}:72.23) and b^* (b^*_{crumb}:18.91, $b^*_{crust:}$ 22.81) values and the lowest the a^* value (a^*_{crumb}:-0.90, a^*_{crust}:1.17), whereas the 10LB had the lowest L^* (L^*_{crumb}:55.68, L^*_{crust}:62.50) and b^* (b^*_{crumb}:11.56, $b^*_{crust:}$ 18.83) values and the highest a^* value (a^*_{crumb}: 1.43, a^*_{crust}: 2.75). The addition of the BLF could reduce the lightness and yellowness of the crumb since black lentil has dark color due to its anthocyanin content. Takeoka *et al.* [21] detected the delphidin 3-O-(2-O-β-D-Glucopyranosyl-α-L-arabinopyranoside) in the black lentil.

Our findings were consistent with the literature. The lightness of the crumb was found to decrease with the addition of carob flour [22], chickpea flour [6 – 19], and lupine flour [10]. This finding was previously attributed to partially alteration of white color with the legume flour substitution [6]. Both lightness and yellowness of the crust also decreased with the addition of the BLF. Maillard and caramelization reactions contribute to the crust color [6]. The darkening effect of the BLF could be associated with an increase in the Maillard reaction during the baking due to its higher protein content. Similar darkening effects of legume flours such as chickpea [6 – 19] and lupine [10] were reported in the literature.

Table 4. Color properties of bread samples

Properties	CB	2LB	6LB	10LB
Crust				
L^*	72.23±0.66^{a}	70.50±0.45^{a}	66.42±0.83^{b}	62.50±1.94^{c}
a^*	1.17±0.23^{c}	1.72±0.25^{b}	1.97±0.25^{b}	2.75±0.26^{a}
b^*	22.88±1.29^{a}	20.60±2.66^{ab}	18.27±0.90^{b}	18.83±1.34^{b}
Crumb				
L^*	69.02±1.86^{a}	64.85±0.98^{b}	59.02±2.12^{c}	55.68±2.70^{c}
a^*	-0.90±0.06^{d}	-0.34±0.03^{c}	0.90±0.10^{b}	1.43±0.12^{a}
b^*	18.91±0.07^{a}	14.49±0.31^{b}	12.64±0.82^{c}	11.56±0.03^{d}

Data are expressed as means ± SD
Different letter in row present significant difference ($p<0.05$)

Sensorial properties

The sensorial properties of the bread samples are presented in Table 5. It can be seen from Table 5, the addition of the BLF had no significant effect on the sensorial properties ($p > 0.05$). The 6LB showed the highest overall acceptability values (crust: 6.92 and, crumb: 7.08). It could be interpreted that the addition of the BLF at 2 - 10 %

level had no adverse impact on the acceptability of the bread by consumers. Similar findings were reported in the literature. The breads up to 10 % level of legume flours such as soy flour [11] and commercial lentil flour [7] were reported to show similar overall acceptability values to the control bread. However, the level of legume flours more than 10 % level was found to reduce the acceptability of the bread [11, 19 – 23]. It can be concluded that the bread supplemented with the BLF at 2 – 10 % can be consumed with pleasure by consumers as wheat bread.

Table 5. *Sensorial properties of bread samples*

Properties	CB	2LB	6LB	10LB
Overall acceptability (crust)	6.84±1.59^{a}	6.64±1.25^{a}	6.92±1.22^{a}	6.80±1.35^{a}
Overall acceptability (crumb)	6.80±1.35^{a}	6.92±0.99^{a}	7.08±1.11^{a}	6.80±1.55^{a}

Data are expressed as means ± SD
Same letter in row presents no significant difference ($p > 0.05$)

TPC and antioxidant activity

The TPC and antioxidant capacity of the bread samples are presented in Table 6. The addition of the BLF into the bread formulation significantly increased the TPC, DPPH radical scavenging activity and FRAP ($p < 0.05$). The 10LB had the highest values (TPC: 13.22 mg / 100 g and FRAP: 54.12 µmol / 100 g). No significant difference was found between the control and 2LB samples. Black lentil was determined to include phenolic acids (gallic, caffeic, ferulic, p-coumaric, and sinapic acids etc), flavanols (catechin and epicatechin), proanthocyanidins, luteiolin-3-7-glucoside, kaempferol dirutinoside [13], and anthocyanidins [21], which could explain an increase in the TPC with the addition of BLF into the bread formulation. The previous studies supported our findings. Both TPC and antioxidant activity of the breads were found to significantly increase when the wheat flour was replaced by the legume flours including chickpea flour [6], green lentil and carob flours [8], and lupine flour [9].

The addition of the BLF at 6 - 10 % level, the TPC, DPPH radical scavenging activity, and FRAP of the breads increased approximately 1.25-1.36 folds, 1.52 folds, and 1.10 - 1.31 folds, respectively. It could be concluded that the addition of the BLF at 6 - 10 % level may enhance the content of antioxidant compounds of wheat bread. Antioxidant compounds such as phenolic compounds are considered to provide some health benefits [15].

Table 6. *TPC and antioxidant activity of bread samples*

Properties	CB	2LB	6LB	10LB
TPC [mg·100 g^{-1}][1]	9.69±1.65^{b}	9.59±1.25^{b}	12.09±1.28^{ab}	13.22±1.54^{a}
DPPH [µmol·100 g^{-1}][1]	10.34±2.12^{b}	9.11±0.34^{b}	15.75±0.84^{a}	15.69±2.46^{a}
FRAP [µmol·100 g^{-1}][1]	41.26±5.73^{b}	34.38±7.56^{b}	45.25±5.59^{ab}	54.12±5.91^{a}

[1] expressed as dry basis
Data are expressed as means ± SD; Different letter in row present significant difference ($p < 0.05$)

CONCLUSION

Our findings revealed that the replacement of wheat flour with the black lentil flour improved the nutritional quality and antioxidant capacity of the wheat bread. Moreover, the replacement of wheat flour with the BLF had no adverse impact on the acceptability of bread. It can be concluded that consumers could be willing to consume the BLF-supplemented bread. Therefore, the BLF may be proposed to develop functional bread at 6 - 10 % level. It can be easily produced in home or bakery.

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