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QUALITY CHARACTERISTICS AND ANTIOXIDANT PROPERTIES OF BREAD INCORPORATED BY BLACK CARROT (*DAUCUS CAROTA* SSP. *SATIVUS* VAR. *ATRORUBENS* ALEF) FIBER

Betül Bay Yılmaz, Hatice Pekmez[*]
University of Gaziantep, Naci Topçuoğlu Vocational School, Food Processing Department, Gaziantep, Turkey

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ABSTRACT

The objectives of this study were to determine the quality and antioxidant properties of bread fortified with black carrot fiber (BCF) at five levels (0, 1.0, 2.5, 5.0 and 7.5% (w/w)). In order to determine the effects of BCF fortification on the properties of bread, the total phenolic content (TPC), antioxidant activity (AA), color, physical and sensorial characteristics were examined. TPC and AA of the bread incorporated by BCF increased regularly with increasing percentages of BCF. The physical properties, diameter, thickness and spread ratio of bread samples supplemented with 1.0 to 7.5% BCF were not significantly different from the control sample (P>0.05). According to the sensory evaluation, the bread containing 1.0 and 2.5% BCF were found to be the most acceptable. The results showed that the BCF fortification of bread is an alternative way to provide more attractive apperance and increase the phenolic content for a healthy diet.
Keywords: Bread, phenolic content, antioxidant activity, quality characteristics

SİYAH HAVUÇ (*DAUCUS CAROTA* SSP. *SATIVUS* VAR. *ATRORUBENS* ALEF) LİFİ İLE ZENGİNLEŞTİRİLMİŞ EKMEĞİN KALİTE VE ANTİOKSİDAN ÖZELLİKLERİ

ÖZ

Bu çalışmanın amacı, beş farklı oranda siyah havuç lifi (BCF) (% 0.0, 1.0, 2.5, 5.0 ve 7.5 (m/m)) ile takviye edilmiş ekmeğin kalite ve antioksidan özelliklerini belirlemektir. BCF ilavesinin, ekmeğin özellikleri üzerine etkilerini belirlemek için toplam fenolik içerik (TPC), antioksidan aktivite (AA), renk, fiziksel ve duyusal özellikler incelenmiştir. BCF'nin dâhil olduğu ekmeğin TPC'si ve AA'sı, artan BCF oranı ile paralel olarak artmıştır. %1'den %7.5'a BCF eklenmiş ekmek örneklerinin fiziksel özellikleri, çapları, kalınlıkları ve yayılma oranları, kontrol örneklerinden önemli ölçüde farklı değildir (P>0.05). Duyusal değerlendirmeye göre, %1 ve 2.5 BCF içeren ekmekler, en kabul edilebilir bulunmuştur. Sonuçlar, BCF ile zenginleştirilmiş ekmeğin görsel olarak daha cazip ve sağlıklı beslenmek için fenolik içeriğini arttırmada alternatif bir yol olduğunu göstermiştir.
Anahtar kelimeler: Ekmek, fenolik içerik, antioksidan aktivite, kalite özellikleri

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[*] *Corresponding author* / Yazışmalardan sorumlu yazar
✉ pekmez@gantep.edu.tr ✆ (+90) 342 337 1137 🖷 (+90) 342 337 1138

Betül Bay Yılmaz; ORCID no: 0000-0002-3817-7886
Hatice Pekmez; ORCID no: 0000-0003-3903-469X

INTRODUCTION

Phenolic compounds are secondary metabolites and present in plant derived foods and beverages. They have a large variety of structures, either simple molecules (e.g. vanillin, gallic acid, caffeic acid, ferulic acid), and polyphenols such as stilbenes, flavonoids, and polymers derived from these various groups. Some phenolic compounds are extremely widespread while the others are specific of certain plants. For example, anthocyanins, water-soluble coloring pigments, are the most abundant pigment in nature and impart a characteristic color ranging from blue to red (Cheynier, 2012). Nowadays, there has been a great interest in anthocyanins not only their colorant abilities and aesthetic value but also their rich bioactive compounds, and their potential role in reducing the risk of coronary heart disease, cancer and stroke (Bay Yılmaz *et al.,* 2018). Due to strong legal restrictions on synthetic coloring agents and together with the consumer demand for natural food, coloring agents which originating from plants has become popular in recent years (Giusti and Wrolstad, 2003). There are many foods which are rich sources of anthocyanins include fruits, grains and vegetables such as berries, grapes, plums, cherries, black rice, black carrots, purple sweet potatoes, and red cabbages (Fernandes *et al.,* 2013; Wiczkowski *et al.,* 2014). Black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef), which are originated in Turkey, Afghanistan, Egypt, Pakistan, India, and the Far East (Kammerer *et al.,* 2003), have increasing popularity as a source of natural food colorants (Kammerer *et al.,* 2004). They also have a strong antioxidant activity because of high concentration of anthocyanin, i.e., 1750 mg/kg (fresh weight) especially acylated anthocyanins (Kırca and Özkan, 2006). According to the study of Zadernowski et al. (2010), 100 g fresh black carrot (11.35 g dry matter) has approximately 4.89 g reducing sugar, 7.95 g total sugar, 1.68 mg vitamin C, 0.19 g total acidity, 1.93 mg total carotenoids, 248.07 mg total phenolic compounds, and 44.25 mg anthocyanins, while 100 g dry black carrot has approximately 43.08 g reducing sugar, 70.04 g total sugar, 14.80 mg vitamin C, 1.67 g total acidity, 17.00 mg total carotenoids, 2185.72 mg total phenolic compounds, and 389.91 mg anthocyanins.

Nowadays, the food producers have focused on increasing the nutritional value, providing dietary fiber and other healthy compounds in food products. Cereal based food products such as bread, biscuit, cake and cookie are consumed daily by the majority of the population (Ktenioudaki and Gallagher, 2012), and there are numerous study on these products' formulations to enhance the nutrional value by combining the cereal-fruit and/or cereal-vegetable food system due to high antioxidant status of cereal based products. Cereal products are high in protein content with respect to fruits and vegetables, whereas fruits and vegetables are high in bioactive compounds such as carotenoids, ascorbic acid and phenolic acids (anthocyanins, tannins). Therefore, the finished product of combined food system can be used in daily life as a healty diet rich in proteins as well as phytochemicals (Francis and Phelps, 2003).

Bread which is the symbol of labor and abundance in all cuisines, is the most commonly consumed bakery product as the basic food stuff. It is cheap, filling, it is a source of energy and it meets a part of protein need (Pekmez and Bay Yılmaz, 2018). The nutritional quality of bread would be improved by adding additional components such as, mango, green tea, grape seed, turmeric powder etc. (Hayta *et al.,* 2014; Lim *et al.,* 2011; Lu *et al.,* 2010; Meral and Doğan 2013; Vergara-Valencia *et al.,* 2007). Moreover, in recent years, people show great interest into colored food products because of their having high charm. In this regard, there is a considerable interest to incorporate anthocyanin in food products such as jam, jellies, and dairy products like ice-cream and yoghurt. However, there are few investigations of bakery products fortified with anthocyanins (Bartl *et al.,* 2015; Sui *et al.,* 2015; Sui *et al.,* 2016; Song *et al.,* 2016; Turksoy *et al.,* 2011).

The aim of the present study is to evaluate the quality characteristics and antioxidant properties of bread fortified with BCF. In order to determine to what extent BCF fortification meets the properties of the bread, the changes of total phenolics content, antioxidant activity, color, physical and sensorial characteristics of bread were examined in terms of the effect of BCF addition from 0 to 7.5% (w/w).

MATERIALS and METHODS

Materials

The commercial wheat flour, salt, yeast and black carrots (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) were obtained from the local market. After the black carrot samples were washed in cold tap water, they were blended with 0.1 M citric acid (Merck) in a glass blender (8011ES MODEL HGB2WTS3, USA). The black carrot pomace which remains after the extraction of carrot was dried at 35°C in a forced-air oven for 24 h until 12% moisture content. Then, the dried sample was powdered using a laboratory mill (JXFM110 Lab Mill) to produce particles with a size 425 μm and they were stored in polyethylene bags and kept in fridge (+4°C) for further analysis. Blends of 0.0, 1.0, 2.5, 5.0 and 7.5% were prepared by addition of the black carrot fiber (BCF) into bread dough.

Preparation of bread

The bread making formula consisted of different percentages of wheat flour/BCF blends 100/0, 99/1, 97.5/2.5, 95/5 and 92.5/7.5 (w/w) were designated as the control, 1.0%, 2.5%, 5.0% and 7.5% respectively. Instant dry yeast (4.0%), salt (1.5%), and water (the amount of water required to reach 500 Brabender Unit (BU) of consistency) were added to the formulations. All ingredients were mixed by using a professional mixer at speed 2 for 3 min. After mixing, the dough was rested for 10 min and divided into 150 g loaves. The dough was kneaded and molded by hand to obtain a desirable shape as the loaf of bread. The dough was put on the tray in a fermentation unit at 35°C and 85% relative humidity for 30 min. Fermented dough was baked at 220°C for 20 min according to the modified method (Gómez *et al.,* 2008). The picture of control and BCF fortified bread samples were presented in Figure 1.

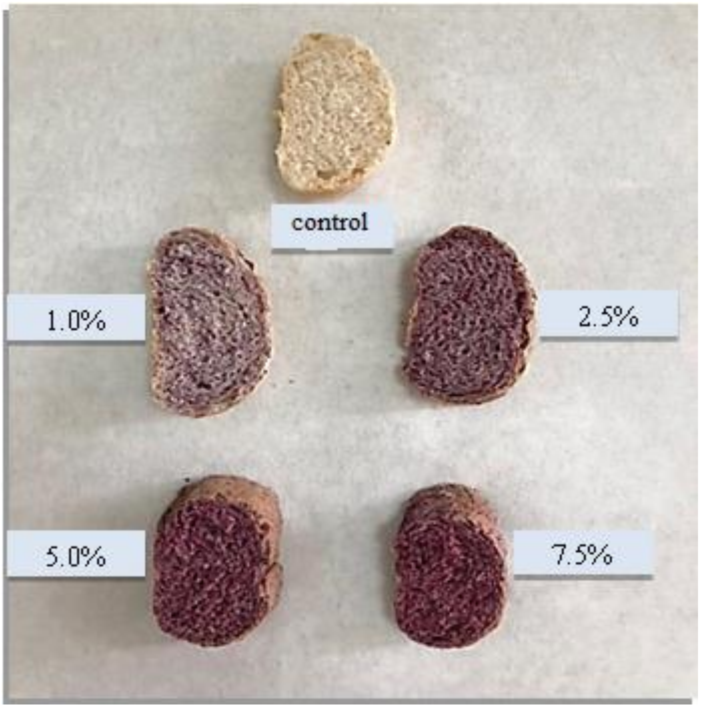

Fig. 1. Picture of control and different amounts of BCF fortified bread samples

Chemical analysis

The bread samples were extracted with acidified methanol (80%) solvent. 2 g of sample was mixed with 100 ml acidified methanol for 2 h at room temperature and then centrifuged. Supernatant was taken and used for the total phenolic content and antioxidant activity analysis.

Determination of total phenolic content

The TPC of control and different percentages of BCF added to bread samples were estimated by the Folin- Ciocalteau method by using gallic acid as standard according to the method of Al-Farsi and Lee, (2008). Briefly, 1 ml of extract was mixed with 1 ml of Folin–Ciocalteu's phenol reagent and allowed to react for 5 min. Then, 10 ml of 7% sodium carbonate solution (w/v) were added, and the final volume was made up to 25 ml with deionised water. After 1 h of reaction at room temperature, the absorbance at 750 nm was read using spectrophotometer. Measurements were calibrated to a standard curve of prepared gallic acid solution, and the TPC results were expressed as gallic acid equivalent (mg GAE/100 g).

Determination of antioxidant activity

The antioxidant activities were conducted according to the the ferric reducing antioxidant power (FRAP) (Thaipong *et al.,* 2006) and 2,2,-di-phenyl-2-picryl-hydrazyl (DPPH) (Brand-Williams *et al.,* 1995) methods. The FRAP reagent was prepared by mixing of acetate buffer (0.3 M, pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine) solution and ferric ($FeCl_3$) solution at a ratio of 10:1:1 (v:v:v). 200 μl of the extracted sample was added to 1.8 ml of the freshly prepared FRAP solution and mixed before incubation at 37°C water bath for 10 min. At the end of incubation the absorbance of the mixture was measured at 593 nm.

The DPPH stock solution was prepared by dissolving 25 mg DPPH with 100 ml of methanol. 150 μl bread extracts were added to 2850 μl of the DPPH solution and the mixture was waited for 3 h in a dark cabinet. Then the absorbance of the mixture was measured at 517 nm. The results were expressed as AA (%) which is the percentage inhibition of the DPPH radical and was determined by the following equation:

$$AA(\%) = \left[1 - \frac{Abs_{sample}}{Abs_{control}}\right] * 100 \qquad (1)$$

Quality characteristics

Determination of physical properties

Diameter (W) and thickness (T) of control and BCF added whole bread samples were measured. The spread ratio (W/T) was calculated as physical property.

Measurement of color characteristics

L (brightness), a (redness) and b (yellowness) values of control and BCF added bread samples were measured using a HunterLab Colorflex, A60-1010-615 model colormeter (Hunterlab, Reston, VA). The color at each point of samples was measured three times and the average of these measurement were calculated with standart deviations.

Evaluation of sensory properties

A 1-9 hedonic scale was used to evaluate the samples and panelists rated each sensory attribute between 1 point (dislike extremely), 5 point (neither like nor dislike) and 9 point (like extremely) (Lim *et al.,* 2011). After cooling of control and BCF added bread samples in room temperature, bread slices of 1.25 cm were prepared on white plates and defined with random three-digit number. The samples were evaluated in terms of crust color, crumb color, taste-flavor, odor, texture, appearance and overall acceptability.

Statistical analysis

Results were statistically analyzed with analyses of variance (One-Way ANOVA), followed by Duncan's test using SPSS, Version 16 (SPSS Inc., Chicago,IL). Statistical significance was indicated at a confidence level of 95%. The experiments were triplicated.

RESULTS AND DISCUSSION

Total phenolic content

Bread is one of the most popular food products all over the world. Food technologists and scientists do numerous studies in order to improve the functional properties of cereal-fruit and/or cereal-vegetable combining system. The effect of enrichment of the cereal-based food products by natural antioxidant sources have been investigated in the previous studies (Hayta et al., 2014; Lim et al., 2011; Lu et al., 2010; Meral and Doğan 2013; Vergara-Valencia et al., 2007). Total phenolic contents of bread samples are given in Table 1. The total phenolic content which was 55.78 mg GAE/100 g for crust and 54.25 mg GAE/100 for crumb in the control sample ($P \leq 0.05$) increased significantly depending on the BCF addition, and these values, respectively, increased to 446.76 mg GAE/100 g and 493.29 mg GAE/100 g in the sample with the 7.5% substitution with BCF. The total phenolic content for both crust and crumb was not affected significantly by the addition of the BCF of 1.0% ($P > 0.05$). These results were in aggrement with previous studies who found TPC of "black carrot range" between 350.5 mg GAE/100 g (Kaur and Kapoor, 2002) and 2536.25 mg FAE/100 g (Turksoy *et al.,* 2011).

Table 1. The total phenolic contents and antioxidant activity of bread samples fortified with different amounts of BCF

BC Fiber Level (%)	Bread portion	Total phenolics (mg GAE/100 g)	Ferric reducing/ antioxidant power (FRAP) (µmol TE/100 g)	Antioxidant activity (% Inhibition)
0 (control)	crust	55.78 ± 6.50^{d}	23.26 ± 3.95^{d}	$46.08\pm0,21^{c}$
1.0		152.62 ± 7.09^{d}	39.12 ± 16.25^{c}	$54.50\pm0,23^{b}$
2.5		250.44 ± 11.68^{c}	45.99 ± 8.03^{c}	$56.03\pm0,56^{b}$
5.0		313.32 ± 10.98^{b}	86.09 ± 2.70^{b}	$61.67\pm0,32^{a}$
7.5		446.76 ± 26.82^{a}	136.16 ± 24.42^{a}	68.96 ± 0.76^{a}
0 (control)	crumb	54.25 ± 3.12^{d}	24.57 ± 7.9^{d}	44.03 ± 0.15^{d}
1.0		184.44 ± 8.92^{d}	34.95 ± 8.3^{d}	57.09 ± 0.65^{c}
2.5		289.24 ± 10.29^{c}	53.81 ± 10.6^{c}	62.06 ± 0.13^{b}
5.0		319.48 ± 13.19^{b}	105.51 ± 6.03^{b}	68.92 ± 0.01^{a}
7.5		493.29 ± 42.78^{a}	183.02 ± 12.92^{d}	72.48 ± 0.26^{a}

All data are the mean ± SD of three replicates.
Mean in the same column with different letter differs significantly ($P \leq 0.05$)

In the literature there is no aggrement on the effect of thermal processing conditions, baking, on phenolic contents and antioxidant activity of breads (Gelinas and McKinnon 2006; Kim *et al.,* 2006; Leenhardt *et al.,* 2006; Menga *et al.,* 2010). Gelinas and McKinnon (2006) reported that baking increased the phenolic compounds in bread samples. Alternatively, Kim *et al.* (2006) demonstrated that thermal processing might cause phenolic compound to degrade, and thus may lead to reduced antioxidant activiy. It seems this variation was related to different extraction system, variety of the raw material used and geographic origin. There are also some investigations on a possible reduction in the phenolic content due to applied heat during baking (Ajila *et al.,* 2008; Meral and Doğan, 2013; Turkmen *et al.,* 2005). On the other hand, in some studies on fruits and vegetables, it was reported that the TPC of processed fruit was slightly lower than the fresh fruit (Turksoy *et al.,* 2011), but in some studies it was noted that a considerable decrease occurred in the phenolic content during cooking (Ajila *et al.,* 2008; Sui *et al.;* 2015, Turkmen *et al.;* 2005, Turksoy *et al.;* 2011). However, despite the loss of phenolic compounds after baking, the total phenolic contents of the bread portions incorporated by BCF were observed approximately 8 times for crust and 9 times for crumb, compared to the control samples.

Antioxidant activity

The antioxidant activities of bread samples prepared with different substitution levels of BCF were analyzed using the FRAP and DPPH radical scavenging activity assays, and the results are given in Table 1. The antioxidant activities of bread increased significantly with increased BCF level. Control bread had the lowest antioxidant activity for both crust and crumb portions. As the proportion of black carrot addition increased, the antioxidant activity toward the DPPH radical also increased significantly for both bread portions ($P \leq 0.05$). Estimation of antioxidant activity revealed that 7.5% BCF supplemented crust sample had highest antioxidant activity (68.96%) followed by 5.0, 2.5, 1.0% BCF added and control samples. Similarly, 7.5% BCF supplemented crumb portion of bread had highest antioxidant activity (72.48%) followed by decreasing BCF supplementation and control. Similar results were obtained by other studies for rocket used in bread (Jdir *et al.,* 2017), black carrot flour added sponge cake (Song *et al.,* 2016), jamun enriched flat bread (Kapoor *et al.,* 2015) and black carrot fiber incorporated cookies (Turksoy *et al.,* 2011). In the present study, the bread supplemented with BCF increased the antioxidant potential due to incorporation of phenolic compounds. The best antioxidant acitivity was observed when 7.5% BCF was added wtih radical inhibition up to 68.96% for crust and 72.48% for crumb.

Physical properties

The effect of the BCF addition at different ratios on the physical properties; diameter, thickness and spread ratio of bread are given in Table 2. The diameter (*W*), thickness (*T*) and spread ratio (*W/T*) values of the bread samples supplemented with 1 to 7.5% BCF were not significantly different from the control sample ($P > 0.05$). Although the high water absorption capacity of the BCF may have decreased the diameter and spread ratio of the bakery products due to increase the dough viscosity and limit the spread in other studies (Arshad *et al.,* 2007; Mc Watters *et al.,* 2003; Turksoy *et al.,* 2011), any significant change on the physical characteristics of bread was not observed in the present study.

Table 2. Effect of different amounts of BCF addition on the physical characteristics of bread samples

BC Fiber Level (%)	Diameter (W, mm)	Thickness (T, mm)	Spread ratio (W/T)
0 (control)	138.7±0.44^a	37.4±0.14^a	3.7±0.12^a
1.0	135.8±0.54^a	36.1±0.22^a	3.8±0.16^a
2.5	126.4±0.53^a	35.8±0.83^a	3.5±0.14^a
5.0	115.3±0.48^a	34.7±0.34^a	3.3±0.12^a
7.5	115.4±0.58^a	35.3±0.11^a	3.3±0.17^a

All data are the mean ± SD of three replicates.
Mean in the same column with different letter differs significantly ($P \leq 0.05$)

Color characteristics

The effect of the BCF on the color characteristics (L*, a* and b*) of bread portions were analyzed and the results are shown in Table 3. The color of the bread changed from pale to dark brown-purple by addition of BCF due to the high anthocyanin content of the BCF both for the crust and the crumb depending on the BCF level addition (Turksoy *et al.,* 2011). The BCF addition into dough considerably decreased the "L" values of both the crust and the crumb of bread samples ($P \leq 0.05$). This means that the bread containing high levels of BCF has a darker color compared to the control bread. The BCF addition decreased significantly the color parameter "b" for both bread portions. However, there is not a regular decrease when compared to the control samples ($P \leq 0.05$). Although the "a" values of the crust and crumb samples containing 1.0, 2.5, 5.0 and 7.5% of BCF are significantly high compared to the control sample. This phenomenon was attributable to the increasing of red color with increasing the percentage of BCF, leading to darker color. However, there is not a regular increase depending on the amount of the BCF. The most total color difference (ΔE) of crust was observed for 7.5% BCF level, while the most total color difference of crumb was observed for 2.5% BCF fortification. Although the crust color is associated with Maillard and caramelization reactions, there is a regular significant increase for total color difference of crust due to BCF level used (P≤0.05). However, irregular total color difference was observed for crumb portion of bread.

Table 3. Effect of different amounts of BCF addition on the color characteristics of bread samples

BC Fiber Level (%)	Bread portion	L value	a value	b value	ΔE
0 (control)	crust	69.52±0.95^{a}	1.97±0.12^{e}	17.86±0.13^{a}	12.93±0.74^{d}
1.0		50.58±0.15^{b}	7.19±0.41^{d}	10.29±0.59^{b}	21.74±0.13^{b}
2.5		44.56±0.13^{c}	9.47±0.03^{b}	8.04±0.49^{c}	13.98±0.30^{d}
5.0		42.17±0.34^{d}	8.92±0.13^{c}	9.06±0.015^{c}	18.88±0.07^{c}
7.5		40.58±0.36^{e}	10.36±0.06^{a}	11.27±0.29^{b}	27.84±0.54^{a}
0 (control)	crumb	70.96±0.39^{a}	0.21±0.07^{e}	13.42±0.36^{a}	18.57±0.17^{a}
1.0		56.39±0.62^{b}	7.26±0.13^{d}	3.85±0.26^{b}	23.40±0.27^{b}
2.5		49.16±0.87^{c}	11.78±0.45^{c}	1.34±0.08^{d}	49.47±0.68^{e}
5.0		44.13±0.12^{d}	17.18±0.04^{b}	1.42±0.06^{d}	42.62±0.78^{d}
7.5		38.55±0.31^{e}	18.86±0.10^{a}	1.90±0.03^{c}	34.31±0.29^{c}

All data are the mean ± SD of three replicates.
Mean in the same column with different letter differs significantly (P≤0.05)

Sensory properties

Data from the sensory evaluation of breads containing BCF are presented in Table 4. Sensory properties i.e. crust and crumb color, taste, odor, appearance and overall acceptability were affected positively by the fortification of BCF. Although the high-rate anthocyanins of BCF darkened the bread, crust color, crumb color, odor, texture and appearance are not significantly different from each other (P>0.05). Similarly, the results for overall acceptability of bread samples containing 1.0% to 7.5% BCF levels are not different from control sample statistically (P>0.05), for bread containing 7.5% the overall acceptability score was 6.4, on the other hand the score for bread containing 1.0% and 2.5% were considerably greater (7.5 and 7.4 respectively). However, taste-flavor characteristic of bread had significant low scores for 5.0% and 7.5% BCF levels (P≤0.05). The bread containing 7.5% BCF had the lowest firmness likability score (6.4), which could be due to high dietary fiber content of black carrot negatively affecting the firmness of bread. According to the sensory evaluation the bread containg 1.0% and 2.5% BCF was found the most acceptable by the panelists. Consequently, bread fortification with BCF could provide an important balance between nutritional value and sensory quality for consumer preferences towards cereal products.

Table 4. Effect of different amounts of BCF addition on the sensory characteristics of bread samples

BC Fiber Level (%)	Crust color	Crumb color	Taste-flavor	Odor	Firmness	Apperance	Overall acceptability
0(control)	7.3±0.41^{a}	7.4±0.30^{a}	7.3±0.40^{a}	7.6±0.40^{a}	7.00±0.30^{a}	7.9±0.99^{a}	7.0±0.97^{a}
1.0	7.4±0.57^{a}	7.5±0.62^{a}	7.2±0.42^{a}	7.1±0.18^{a}	7.20±1.04^{a}	7.5±1.18^{a}	7.5±1.14^{a}
2.5	7.6±0.81^{a}	7.4±0.65^{a}	7.4±0.67^{ab}	6.7±0.50^{a}	6.8±0.60^{a}	7.6±0.58^{a}	7.4±0.58^{a}
5.0	7.5±0.71^{a}	7.3±0.76^{a}	6.4±0.75^{b}	6.7±0.40^{a}	6.6±1.30^{a}	7.40±0.65^{a}	6.9±0.66^{a}
7.5	6.9±0.27^{a}	6.5±0.27^{a}	5.9±0.67^{b}	6.4±0.43^{a}	6.4±1.11^{a}	7.10±0.72^{a}	6.4±0.48^{a}

All data are the mean ± SD of ten panelists.
Mean in the same column with different letter differs significantly (P≤0.05)

CONCLUSION

The present study showed that the BCF fortification of bread is a simple method to increase antioxidant and polyphenolic contents. It can be concluded that acceptable products can be prepared by incorporation of BCF at 1.0, 2.5, 5.0, 7.5% levels for bread production without affecting their physical properties. BCF also provides more attractive apperance for bread especially for children due to its brown-purple color. Sensory analysis showed that bread containing 1.0% had the greatest overall acceptability score. Therefore, BCF fortification could be an alternative way to produce functional bread for healty diet. However, further analyses such as textural, rheological, dietary fiber content analyses and also flavor improvement studies are required for more acceptable bread samples.

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