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Research article

Development and quality evaluation of polyphenols enriched black carrot (Daucus carota L.) powder incorporated bread

Pragya Pandey[a,b,*], Kiran Grover[b], Tarsem Singh Dhillon[c], Neena Chawla[c], Amarjeet Kaur[d]

[a] *Department of Food and Nutrition, Acharya Narendra Deva University of Agriculture and Technology, Ayodhya, 224229, India*
[b] *Department of Food and Nutrition, Punjab Agricultural University, Ludhiana, 141004, India*
[c] *Department of Vegetable Science, Punjab Agricultural University, Ludhiana, 141004, India*
[d] *Department of Food Science and Technology, Punjab Agricultural University, Ludhiana, 141004, India*

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ABSTRACT

Black carrot is a prominent source of polyphenols and the cheapest source of anthocyanins in India. In this study, an attempt has been made to examine the feasibility of black carrot powder as an ingredient in bread. Black carrot bread was prepared by incorporating different concentrations of black carrot powder (BCP) at 2.5, 5.0, 7.5 and 10 %. The developed bread samples were analyzed for physical and textural quality, proximate composition, bioactive compounds, antioxidant properties, sensory characteristics, mineral content and storage quality. The results revealed that loaf volume and specific volume decreased (1995–1254 mL, 5.25–3.28 mL/g) with the incorporation of BCP into bread. Textural analysis revealed that the addition of BCP led to increased hardness in the bread (0.110–12 0.151 N), whereas the resilience (43.64–35.10 %), cohesion and springiness (89.930–13 82.146 %) decreased significantly. The content of bioactive compounds such as total phenols, anthocyanins (29.63–112.68 mg/100 g) and flavonoids increased to exceptionally high levels in BCP-incorporated bread and showed high antioxidant activity. Incorporation of BCP up to 7.5 % showed the most acceptable sensory analysis score (7.85) with a significant increase in dietary fiber (40 %) and total mineral content (50 %), which revealed that black carrot powder could be used up to 7.5 % as an ingredient into bread with high acceptability. The present study revealed significant enhancement in bioactive compounds and mineral content of bread after the incorporation of black carrot powder, which supports its immense potential in preventing hunger and oxidative stress-induced disorders in developing countries.

1. Introduction

The tenet “Let food be thy medicine and medicine be thy food”, quoted by Hippocrates nearly 2500 years ago, is lately attracting renewed interest from the scientific community, which has sparked a surge of interest in the study of bioactive compounds and functional foods (Hasler, 1998). Epidemiological studies suggest that a high intake of fruits and vegetables is linked to a lower risk of chronic diseases, which can be attributed to high availability of bioactive compounds, particularly polyphenols. Polyphenols are

* Corresponding author. Acharya Narendra Deva University Of Agriculture And Technology, Ayodhya, India.
E-mail addresses: pragya1pandey@gmail.com, pragyapandeyfn@nduat.org (P. Pandey).

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secondary metabolites found in plants that play a significant role in determining the sensory and nutritional quality of fruits and vegetables [1]; Aguilera et al., 2016).

Black carrots, a unique carrot cultivar, have drawn attention in recent research due to their high polyphenol content, especially anthocyanins. Black carrots contain four primary anthocyanins, with the majority (41 %) being acylated. Among these, cyanidin 3-feruloyl-xylosyl-glucosyl-galactoside (13.5 %) and cyanidin 3-sinapoyl-xylosyl-glucosyl-galactoside (27.5 %) are the most common anthocyanins [2,3]. The acylated anthocyanins exhibit a high degree of stability due to their inherent capacity for intramolecular co-pigmentation, which inhibits the nucleophilic attack of water and the hydrolytic cleavage of the aromatic system, consequently preventing the pigment loss (Kammerer et al., 2004a). Under acidic conditions, these anthocyanins produce a brilliant strawberry red hue [4]. This attribute makes it an ideal pigment for water-based, low-pH food systems.

The inclusion of black carrot pigments in food products does not necessitate the declaration of an E-number on food labels, as it is classified as an ingredient. The application of black carrot extract pigments is widespread in the food industry for imparting vibrant purple hues to various acidic food items, including dairy products, preserves, and confectionery [5–7]. Black carrot anthocyanins emerge as a promising alternative to synthetic dyes, exhibiting an appealing reddish-purple hue resembling FD&C Red 40 (Allura Red). This property of black carrot highlights their increasing significance in response to consumer demand for natural colours and regulatory constraints on synthetic dyes (Ekici et al., 2015).

In India, the primary causes of disability and mortality are oxidative stress-induced chronic diseases such as diabetes, cancer, cardiovascular disease, and mental illness. It is anticipated that their contribution to the disease burden will increase over the next 25 years [8]. Polyphenols have been demonstrated to have a vital role in lowering oxidative stress levels in both the intestinal contents and the cells of the intestinal epithelium because of their high antioxidant potential. Black carrots possess notable characteristics, such as high antioxidant capacity, polyphenolic content, anthocyanin pigment, and a considerable mineral profile. Black carrot roots have the potential to be a low-cost but high-quality source of polyphenols (particularly anthocyanin) for the development of functional foods. However, despite their potential to be seamlessly incorporated into various food products without compromising taste or functionality, they have not received adequate recognition in the food industry. Historically, the use of black carrots in rural northern India was restricted to the production of a traditional fermented beverage known as *Kanji*. To date, only a limited number of black carrot-based products, such as dairy products, preserves, marmalades, and cakes, have been developed [5]; Kamiloglu et al., 2015; [9]. Few studies have been conducted to develop anthocyanins-enriched bread by incorporating black rice and black corn [10]; (Blanch et al., 2021). The potential of black carrots as a source of polyphenols in bread is still underexplored. As a dietary staple, bread presents an opportunity for the incorporation of black carrots, which could result in a polyphenol-rich delivery system within a staple food context, thereby providing a viable strategy for mitigating oxidative stress-related disorders and the dual burden of malnutrition. In light of these considerations, this study was conducted to investigate the feasibility of fortifying black carrot powder (BCP) in the development of bread. Bread containing BCP may provide health benefits to consumers seeking a healthier alternative to conventional bread. The findings of this study may serve as a guideline for bread manufacturers regarding the optimal level of BCP to develop polyphenol-enriched functional bread without compromising its quality attributes.

2. Materials and methods

2.1. Materials

The fresh black carrot variety 'Punjab Black Beauty' was harvested from the vegetable farm of the Punjab Agricultural University, Ludhiana. Freshly harvested black carrots were washed, peeled, sliced and kept overnight (10 h) for drying in the hot air cabinet drier at 50 °C. Dehydrated carrot slices were ground to powder into a particle size of 100 μm as that of wheat flour used in this study, and stored in polythene bags at room temperature [11]. The raw ingredients, including refined wheat flour, sugar, salt, yeast, and refined oil (Fortune Sunlite Oil) used in the product formulation were procured from the local market of Ludhiana, Punjab. The preliminary analysis of black carrot powder was done to evaluate the content of bioactive compounds and antioxidant activity (Table 1).

Table 1
Bioactive compounds content and antioxidant activity of black carrot powder.

Bioactive compounds	Black carrot powder
Total Phenols (mg GAE/100 g)	2337.10 ± 18.11
Total Flavonoids (mg CE/100 g)	756.12 ± 18.32
Anthocyanin (mg/100 g)	1600.30 ± 26.87
TDF (g/100 g)	22.32 ± 0.01
IDF (g/100 g)	16.80 ± 0.00
SDF (g/100 g)	5.52 ± 0.00
Antioxidant Activity	
DPPH (%)	84.30 ± 1.31
ABTS (μmolTE/g)	31.43 ± 0.67
FRAP (μmolTE/g)	17.67 ± 1.61

TDF = Total Dietary Fiber, IDF= Insoluble Dietary Fiber, SDF= Soluble Dietary Fiber.

2.2. Bread making

The bread was developed using the straight dough method [12]. Bread formulations were developed by substituting refined wheat flour with different concentrations of BCP (0, 2.5, 5.0, 7.5 %, and 10 %) (Fig. 1). The bread was prepared with refined wheat flour: BCP premix (100 g), 4 g of sugar, 2 g of salt, 1.75 g of yeast, 2 g of Fortune Refined Sunflower Oil, and 60 mL of water, without the addition of any additives or bread enhancers. First, yeast and sugar were dissolved in lukewarm water, then all the ingredients were mixed together and blended in an Electric Kent dough mixer for 2 min of low-speed blending followed by 6 min of high-speed blending. The developed dough was transferred to an incubator for fermentation at 30 °C and 85 % relative humidity. After an initial fermentation period of 70 min, the dough was taken out, punched, and placed back in the incubator for an additional 35 min for the first proofing. The dough was then given a second punch and divided into pieces of equal weight, which were then shaped and placed in the same incubation conditions for the last 20 min. The breads were then baked at 220 °C for 20 min in a preheated electric oven (Electric Deck baking oven by Arise Equipment, India). The bread was removed from the bread can and permitted to cool for 1 h at room temperature. After aseptically separating the crust from the crumb, the crumb was freeze-dried, pulverized, and screened through a 0.50 mm sieve. The pulverized bread's moisture content ranged from 36.71 to 37.56 %, and it was analyzed further.

2.3. Determination of physical characteristics

The bread's moisture content was determined using the [13] method. The rapeseed displacement method was used to estimate bread volume [14]. The weights were recorded using a digital 2-decimal weighing balance. The specific volume of the bread was calculated by dividing the volume of the loaf by the weight of the loaf [15]. To determine the textural properties of bread, texture profile analysis (TPA) was conducted using a TA-XT texture analyzer (Stable Micro Systems, Model TA-HDi, UK).

2.4. Bioactive analysis

2.4.1. Determination of total anthocyanins

The total anthocyanin content of Black Carrot Powder (BCP) and bread samples was determined according to the method of [16]. One gram of finely pulverized fresh sample was taken, and 10 mL of 1 % methanolic HCl (w/w) was added to it, followed by overnight storage at 4 °C. The following day, sample extracts were filtered, and absorbance measurements were recorded at 530 and 657 nm using a spectrophotometer. The total anthocyanin content was calculated using the formula A530–0.33 A657. Cyanidin 3-glucoside (Cyd 3-glu) was used as standard and the results were expressed as expressed in mg/100 g of fresh sample weight.

2.4.2. Determination of total flavonoids content

The total flavonoid content was determined using the method. 1 mL of the extract was added to 4 mL ddH_2O in a volumetric flask, followed by the addition of 0.3 mL 5 % $NaNO_2$, rested for 5 min; then, 3 mL 10 % $AlCl_3$ was added and rested for 6 min. Subsequently, 2 mL 1 N NaOH was added, and the volume was made to 10 mL with distilled water, with the absorbance at 510 nm immediately measured. The results were expressed as catechin equivalents (CE) mg/100 g of fresh sample.

2.4.3. Determination of total phenols

The method established by Ref. [17] was used to determine the total phenols content. Gallic acid equivalent (mg GAE/100 g) of the fresh sample was used to express the results. In a test tube, 0.5 mL of the extract, 5 mL of distil water, and 5 mL of the Folin-Ciocalteu reagent were added and vortexed. After 5 min of reaction time, 1 mL of saturated Na_2CO_3 solution was added and thoroughly mixed. The solution was incubated for 1 h in the dark at room temperature (27 $\pm 1^{O}$C). The absorbance was measured at 725 nm using a spectrophotometer (Spectronic- 20, USA), and the results were expressed in gallic acid equivalents (GAE; mg/100 g fresh mass). Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

2.4.4. Determination of dietary fiber content

Determination of total dietary fiber, soluble dietary fiber and insoluble dietary fiber was done by using [13] method.

Fig. 1. Cross-sections of breads prepared by incorporating black carrot powder. Control, BB2.5, BB5, BB7.5 and BB10 are breads prepared with 0 %, 2.5 %, 5.0 %, 7.5 % and 10 % replacement of refined wheat flour with black carrot powder, respectively.

2.5. Determination of total antioxidant activity

In this study, total antioxidant activity was determined using three methods: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant power (FRAP), and 2,2-azinobis (3-ethylbenzothiazole)-6-sulfonic acid (ABTS) for better accuracy and predictability of the antioxidant activity of experimental samples. Aliquots (1 mL) of extract solutions were added to 3 mL of DPPH solution (0.2 mM), and the absorbance was measured at 517 nm after 90 min for the DPPH assay. For the control, the same procedure was followed, but methanol was used in place of the sample solution. DPPH scavenging capacity was measured as a decrease in absorbance and calculated as follows [18].

$$\text{Scavenging capacity }\% = (\text{Control absorbance} - \text{extract absorbance}) \times 100/\ \text{Control absorbance}$$

The FRAP assay complied with [19] method, while the ABTS assay followed [20] protocol. Using Trolox as a standard, both assay results were expressed as mol Trolox Equivalent (TE) per gram of fresh sample weight.

2.6. Determination of sensory quality

The developed bread samples were subjected to a sensory evaluation using a nine-point hedonic scale in order to evaluate several sensory aspects such as appearance, colour, flavour, texture, and overall acceptability. Thirty-five semi-trained panelists (28 Female, 7 Male, ages ranging between 24 and 56 years) were recruited by using a simple taste recognition test from the Department of Food and Nutrition and Department of Food Science and Technology, PAU, Ludhiana [21]. Ten panelists were chosen based on their ability to perceive intensity and accurately identify the parameters they had given in the tests, and the rest were discontinued because of irregularities in the sensory test. Bread samples were coded and presented to panelists at room temperature on plastic plates under daylight room conditions. Plain water was given to them to rinse their mouths between the evaluation of their respective samples. Sensory evaluation was done at 30 ± 2 °C. The index of acceptability (IA) was calculated using the following formula [22].

$$\text{Index of acceptability (IA) (\%)} = (\text{Overall acceptability score} \times 100)/9$$

2.7. Proximate analysis

Proximate analysis of the control and most acceptable experimental bread was performed by standard procedure [13].

2.8. Determination of minerals

The mineral composition of the developed bread, specifically its calcium, phosphorus, magnesium, iron, and zinc content, was evaluated using the methodology outlined by Ref. [23]. The standard solutions of minerals were prepared in distilled water. These standard and sample solutions were evaluated for mineral content with an atomic absorption spectrophotometer (Model: AA-6300, Shimadzu, ASIA Japan-Korea). The standard curves for absorption against concentration were drawn to calculate the results.

2.9. Determination of storage stability

The effect of storage conditions on the stability and quality characteristics of the most acceptable experimental bread (7.5 % BCP) was analyzed. The bread was evaluated every two days during its six-day storage at 28–30 °C in hermetic HDPE packaging. The physicochemical parameters of the samples, including Total solids, Free Fatty Acid, and Peroxide Value, were assessed using the standard procedure AOAC (2020). The determination of Total Plate Count (TPC) and Yeast and Mold Count (YMC) was done using the method proposed by Ref. [24]. Additionally, sensory analysis was carried out as per the procedure outlined in Section 2.6.

2.10. Statistical analysis

The data were expressed as mean ± SD of three replicates. The results were subjected to ANOVA followed by Tukey's test with $P < 0.05$ significance level on SPSS 18.0 statistical software (SPSS Inc.) to study the effect of treatment variation. Kruskal Wallis H-test was used for sensory analysis of samples, and the student's t-test was applied to understand the difference between the control and most

Table 2
Physical properties of bread fortified with different levels black carrot powder.

Physical Characteristics	Black carrot incorporation (BCP) level in experimental bread				
	Control	2.5 %	5.0 %	7.5 %	10 %
Moisture Content (%)	37.56 ± 1.89[a]	37.35 ± 0.73[a]	37.15 ± 0.57[a]	36.93 ± 0.97[a]	36.71 ± 0.63[a]
Loaf weight (g)	380.3 ± 1.50[a]	380.5 ± 2.10[a]	381.3 ± 1.80[a]	381.9 ± 2.20[a]	382.6 ± 1.80[a]
Loaf Volume (ml)	1995.0 ± 20.30[a]	1806.7 ± 23.51[b]	1700.0 ± 21.47[c]	1536.7 ± 18.30[d]	1254.7 ± 24.01[e]
Specific Volume (ml/g)	5.25 ± 0.03[a]	4.75 ± 0.036[b]	4.46 ± 0.035[c]	4.02 ± 0.025[d]	3.28 ± 0.047[e]

[a] Each value is expressed as mean ± SD (n = 3) and means having different letter superscripts within a same row are significantly different ($P < 0.05$).

acceptable experimental bread sample (7.5 % BCP). Correlation (r) analysis between the variables was carried out to study the correlation between the total anthocyanins content and physical properties of bread by using the R Studio version (2023.06.1 + 524).

3. Results and discussion

3.1. Effect of BCP incorporation on physical properties of breads

The physical parameters of the developed bread are presented in Table 2. There were no significant differences seen in the moisture content and weight of the bread loaf across the various treatment groups. However, the incorporation of BCP resulted in a significant decrease in the volume of the bread. The control bread exhibited a mean loaf volume of 1995 mL, which consistently decreased in each experimental bread sample as the level of BCP incorporation increased. Similarly, as the level of BCP substitution with refined wheat flour increased, the specific volume of the developed bread decreased significantly from 5.25 to 3.28 mL/g. The substitution of refined wheat flour with BCP led to a reduction in the quantity of gluten protein, thereby leading to a diminished network structure in the bread. Previous research conducted by Ref. [25] has demonstrated that the inclusion of non-endosperm constituents of wheat caryopsis in bread formulation results in a decreased specific volume. The use of alternative ingredients, such as black rice and turmeric, has been found to have a negative impact on the volume of bread loaves [10,26]. However, the impact of these additives cannot be solely attributed to the dilution of gluten-forming proteins, and the precise mechanism behind the decrease in loaf volume resulting from their incorporation remains unidentified [27,28].

The bread samples exhibited an increased amount of dietary fiber content in correlation with the higher level of BCP. Water-absorbing properties of dietary fiber inhibit gluten formation, resulting in a thicker texture and lower fermentation rise. The incorporation of higher amounts of dietary fiber into bread could potentially lead to a decrease in the loaf volume of the bread. In order to improve bread volume when using low-gluten and high-fiber ingredients, it might become necessary to employ a variety of treatments and dough-handling modifications. Bread improvers are frequently added to uphold the desired volume of the loaf. Studies indicate that the inclusion of milk proteins in bread formulation yields positive effects in terms of bread colouration and volume [29]. produced a protein-rich gluten-free bread by adding 15 % whey protein concentrate and 3 % HPMC. According to Ref. [30]; buckwheat flour can improve the volume, porosity, and crumb texture of bread [31]. reported that loaf volumes increased when a 15 % concentration of Plant-Based High-Protein constituents such as gluten, zein, and potato was added to bread. Notably, at concentrations below 15 %, most protein hydrolysates can, in most studies, enhance bread volume [32–34]; and [35].

3.2. Effect of BCP incorporation on texture analysis of breads

Table 3 presents the effect of different concentrations of BCP on the qualitative characteristics of bread crumbs. Breadcrumb hardness was found to increase significantly with increasing levels of BCP, and significant declines were observed in resilience, cohesion, and springiness. There were no significant differences between the control and BCP-incorporated breads in terms of chewiness. The study revealed a significant increase in bread firmness, with the control group measuring 0.110 N and the sample with 10 % BCP measuring 0.151 N. This increase in firmness can be due to the negative impact of higher concentrations of BCP on gluten networks, which subsequently restricts the retention of gas, resulting in a tight structure of bread crumb, which led to the lower loaf volume and increase in loaf density [36]. The introduction of Black Carrot Powder (BCP) into the formulation of black carrot bread resulted in changes to the gluten content, which subsequently impacted the overall texture of the bread. These modifications in texture were found to be consistent with the observations made by Ref. [37] about the influence of ingredient modifications on sensory qualities, specifically firmness. Our research is consistent with the findings of [26,38]; and [10]; which suggest that the replacement of wheat flour with non-wheat elements in bread formulations leads to an increase in hardness. Several studies have reported alterations to the crumb appearance, texture profile, and flavour profile of bread enriched with non-wheat constituents such as tenebrio molitor powder, flaxseed, and green tea extracts [39]; Mirzaei & Mirzaei, 2013, [38]; and [10]. The observed reduction in resilience, cohesiveness, and springiness in bread containing BCP may be attributed to the weakened strength and elasticity of the gluten matrix caused by the presence of anthocyanins. The elevated levels of anthocyanins reduce the number of S–S bonds and consequently weaken the matrix structure of the bread.

Table 3
Texture Analysis of bread fortified with different levels black carrot powder.

Texture Profile Parameter	Black carrot incorporation (BCP) level in experimental bread				
	Control	2.5 %	5.0 %	7.5 %	10 %
Hardness N	0.110 ± 0.001^{a}	0.120 ± 0.000^{b}	0.129 ± 0.004^{c}	0.136 ± 0.002^{c}	0.151 ± 0.005^{d}
Resilence %	43.64 ± 0.930^{d}	41.36 ± 0.560^{c}	40.92 ± 0.430^{c}	38.01 ± 0.81^{b}	35.10 ± 0.660^{a}
Cohesion	0.701 ± 0.020^{a}	0.616 ± 0.010^{b}	0.630 ± 0.020^{b}	0.593 ± 0.030^{bc}	0.553 ± 0.01^{c}
Springiness %	89.930 ± 0.630^{a}	88.980 ± 0.950^{ab}	87.532 ± 0.670^{bc}	86.526 ± 0.580^{c}	82.146 ± 0.71^{d}
Chewiness	6.937 ± 0.309^{a}	6.580 ± 0.18^{a}	7.121 ± 0.501^{a}	6.984 ± 0.502^{a}	6.864 ± 0.411^{a}

[a] Each value is expressed as mean ± SD (n = 3) and means having different letter superscripts within a same row are significantly different ($P < 0.05$).

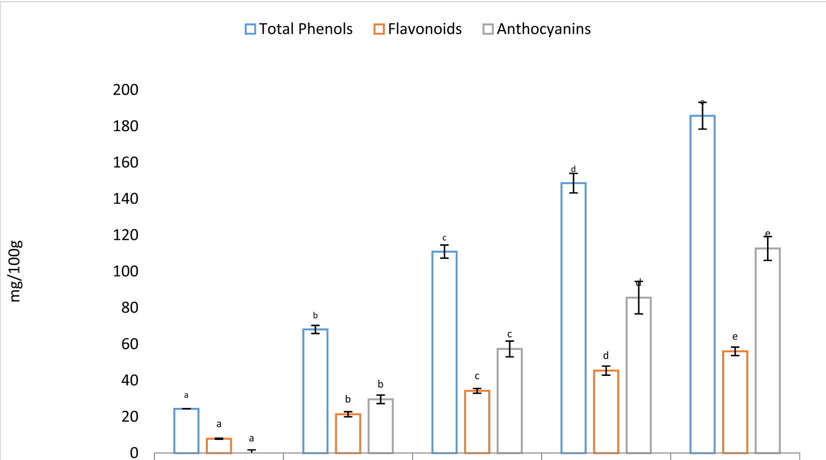
Further, it has been observed that under oxidizing conditions, the amino groups in the side chains of peptides endure reactions with polyphenols, resulting in the formation of cross-links within proteins [40]. state that the mechanical strength and swelling properties of these cross-linked protein polymers are greatly improved. Our findings are consistent with those of [10]; who found that the addition of bread enriched with black rice extract decreased the bread's resilience and increased its density. The significantly lower resilience, cohesiveness, and springiness of bread containing BCP may be attributed to the weaker and less elastic gluten structure of the bread produced by the anthocyanins in BCP, which led to fewer S–S bonds, thereby weakening the matrix structure of bread. Our results were in accordance with [10], who reported that the incorporation of black rice extract-enriched bread caused less resilience and higher density of bread.

3.3. Effect of BCP incorporation on bioactive compounds profile and antioxidant capacity of breads

It was revealed that 24.36 mg GAE/100 g of total phenols were present in control bread with 0 % BCP (Fig. 2) [41]. reported that wheat had a total phenolic concentration of 29.1 mg GAE/100 g, which may account for the total phenolic content of the control bread. In the experimental bread samples, the total phenols ranged from 68.11 to 185.78 mg GAE/100 g, which indicates a 65–80 % increase in comparison to the control. The analysis of BCP revealed a total phenol content of 2337 mg GAE/100 g, as presented in Table 1. A comparison between the total phenol content of black carrot bread and the expected values revealed a deterioration of 21 %. The decrease in polyphenol retention observed may be attributed to thermal degradation during the baking process, as phenolics are heat-sensitive and chemically reactive [42]. Despite the decrease in total phenol content observed during the baking process, all experimental bread treatments contained significantly higher phenolic compounds than the control.

The total flavonoid content of the bread followed a similar pattern to that of phenolic compounds, exhibiting a substantial increase with increasing levels of BCP concentration within the bread formulations (Fig. 2). Polyphenols have been positively linked with antioxidant and radical scavenging capabilities [43]; Olszowy, 2019) [44]. summarized the findings of various studies by Refs. [45,46]; Butalla et al. (2009), [47–50]; and [51]; determining that black carrot polyphenols play a significant role in conferring health-promoting effects, particularly against cancers, oxidative stress, obesity, and diabetes.

The total anthocyanin content of the black carrot powder used in this study was 1600 mg/100 g, and as expected, anthocyanins were only detected in bread loaves containing BCP (Fig. 2). The concentration of anthocyanin in bread exhibited a significant increase as the quantity of BCP was increased. The highest recorded concentration (112.68 ± 7.36 mg/100 g) was observed in bread containing 10 % BCP incorporation. In this experiment, it was observed that there was a retention of 65–75 % of anthocyanins during the baking process. Anthocyanins in black carrots are reported to be more stable, and heating has a mild effect on their concentration [52]. As a result of this property, black carrot is considered one of the best sources of polyphenols and anthocyanins. In similar studies, anthocyanin-enriched bread developed from purple and blue wheat reported 45–55 % anthocyanin loss during baking [53,54]. [55] observed a 60 % loss of cyanidin-3-O-glucoside in black corn (Zea mays L.) bread when baked at 150 °C or higher temperatures, which exhibited a reduced digestion rate and additional health-enhancing properties. The degradation mechanism of anthocyanins comprises the hydrolysis of sugar moieties, which generally occur prior to their thermal degradation. This breakdown sequence leads to the deglycosylation of anthocyanins, resulting in the formation of anthocyanidins, which then degrade into chalcones. The subsequent



	Control	BB2.5	BB5	BB7.5	BB10
Total Phenols	24.36	68.11	110.97	148.67	185.78
Flavonoids	7.93	21.41	34.29	45.43	56.07
Anthocyanins	0	29.63	57.37	85.63	112.68

Fig. 2. Total Phenols, Flavonoids and Anthocyanins content of breads prepared by incorporating black carrot powder. Control, BB2.5, BB5, BB7.5 and BB10 are breads prepared with 0 %, 2.5 %, 5.0 %, 7.5 % and 10 % replacement of refined wheat flour with black carrot powder, respectively. Bars represent standard deviation of means (n = 3) and means with different letters are significantly different ($P < 0.05$).

degradation of chalcones results in the formation of phenolic acids and carboxyaldehydes. Notably, the degradation products of anthocyanins retain their antioxidant properties even after heat-induced degradation. Therefore, despite heat-induced degradation, the antioxidant capacity of these degradation products might not be significantly diminished [56,57].

The analysis of dietary fiber revealed that the incorporation of BCP in bread resulted in a significant increase in the dietary fiber content in the experimental bread, as depicted in Fig. 3. The initial high levels of dietary fiber in black carrots may account for the higher dietary fiber content of the experimental bread (Table 1).

The study revealed a significant increase in the antioxidant activity of the bread as the levels of BCP incorporation increased, as indicated in Table 4. It was found that the antioxidant activity of experimental breads corresponded with their total phenols, flavonoids, and anthocyanins content. The observed high antioxidant activity of black carrot bread can be attributed to the presence of total phenols, flavonoids, and anthocyanins, as well as the generation of additional phenolic compounds resulting from the thermal degradation of anthocyanin during baking [57,58]. The results suggest that the incorporation of BCP into bread could result in the development of bread with added health benefits, which might be effective against oxidative stress-induced disorders [59].

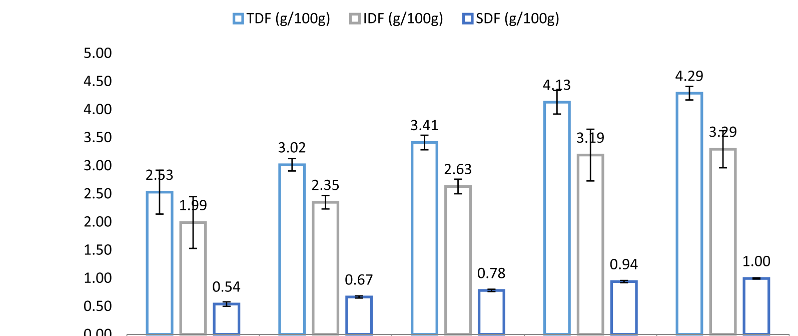
3.4. Correlation matrix of anthocyanins and bread quality parameters of developed bread

To determine the impact of anthocyanin levels on the quality parameters of bread, a correlation matrix was used, as illustrated in Fig. 4. The levels of anthocyanin in bread demonstrated a strong negative correlation with moisture content ($r = -1$), loaf volume ($r = -0.99$), and specific volume ($r = -0.99$) while displaying a strong positive correlation ($r = 0.99$) with loaf weight. A similar trend was observed in the study of [60] where a reduction in loaf volume was observed with increased incorporation of anthocyanin-rich popping maize flour into the bread samples.

3.5. Effect of BCP incorporation on sensory characteristics of breads

Fig. 5a depicts the sensory analysis of bread produced with the incorporation of BCP. Overall sensory results showed that panelists found BCP incorporation up to the 7.5 % level to be acceptable in bread, but scores dropped significantly at the 10 % level. The scores for appearance and colour ranged from 7.7 to 8.53 up to a 7.5 % BCP incorporation level, which significantly declined at a 10 % level (6.3–7.5). This result could be attributed to the fact that upon the addition of BCP to the bread up at a concentration of 7.5 %, a distinctly pleasing light purple hue developed. However, an increased concentration of 10 % led to a significantly darker colour, which was perceived as less desirable by the panelists. The sensory scores for texture quality of BCP breads depicted a non-significant difference up to 7.5 % BCP incorporation (7.7–8.1), which dropped dramatically to 5.20 at the 10 % level. Increasing levels of BCP incorporation led to increased hardness, resulting in a harder crumb and a grainy texture in bread. A similar pattern was observed with respect to sensory scores obtained for flavour and taste among BCP-incorporated bread. The overall acceptability of the bread was significantly influenced by appearance, colour, texture, flavour and taste scores, which showed non-significant differences up to 7.5 % BCP incorporation (7.83–8.05) and declined significantly at 10 % concentration of BCP in breads (6.2).

A non-significant difference was observed in the Index of Acceptability (IA) scores of experimental breads up to the 7.5 % level of BCP incorporation in breads (87.00–89.44); however, a significant decline was observed at the 10 % level (68.89), as shown in Fig. 5b. As the mean scores and IA for sensory characteristics were similar for concentrations of 2.5, 5.0, and 7.5 %, the maximum concentration of BCP (7.50 %) incorporated bread was selected for further nutritional and storage analysis [61]. reported that bread fortified



	Control	BB2.5	BB5	BB7.5	BB10
TDF (g/100g)	2.53	3.02	3.41	4.13	4.29
IDF (g/100g)	1.99	2.35	2.63	3.19	3.29
SDF (g/100g)	0.54	0.67	0.78	0.94	1.00

Fig. 3. TDF, IDF and SDF content of breads prepared by incorporating black carrot powder. Control, BB2.5, BB5, BB7.5 and BB10 are breads prepared with 0 %, 2.5 %, 5.0 %, 7.5 % and 10 % replacement of refined wheat flour with black carrot powder, respectively. Bars represent standard deviation of means ($n = 3$) and means with different letters are significantly different ($P < 0.05$).
TDF = Total Dietary Fiber, IDF= Insoluble Dietary Fiber, SDF= Soluble Dietary Fiber.

Table 4
Antioxidant Activity of bread fortified with different levels black carrot powder (Fresh Weight Basis).

BCP (%)	DPPH	ABTS (μmolTE/g)	FRAP (μmol TE/g)
0.0	27.65 ± 1.88^{a}	2.53 ± 0.03^{a}	0.93 ± 0.01^{a}
2.5	33.63 ± 1.06^{b}	5.41 ± 0.02^{b}	1.53 ± 0.02^{b}
5.0	50.23 ± 1.73^{c}	9.53 ± 0.02^{c}	3.78 ± 0.02^{c}
7.5	66.67 ± 2.01^{d}	14.23 ± 0.10^{d}	7.31 ± 0.14^{d}
10.0	70.01 ± 2.11^{d}	20.62 ± 0.49^{e}	11.21 ± 0.04^{e}

[a] Each value is expressed as mean ± SD (n = 3) and means having different letter superscripts within a same row are significantly different (P < 0.05).

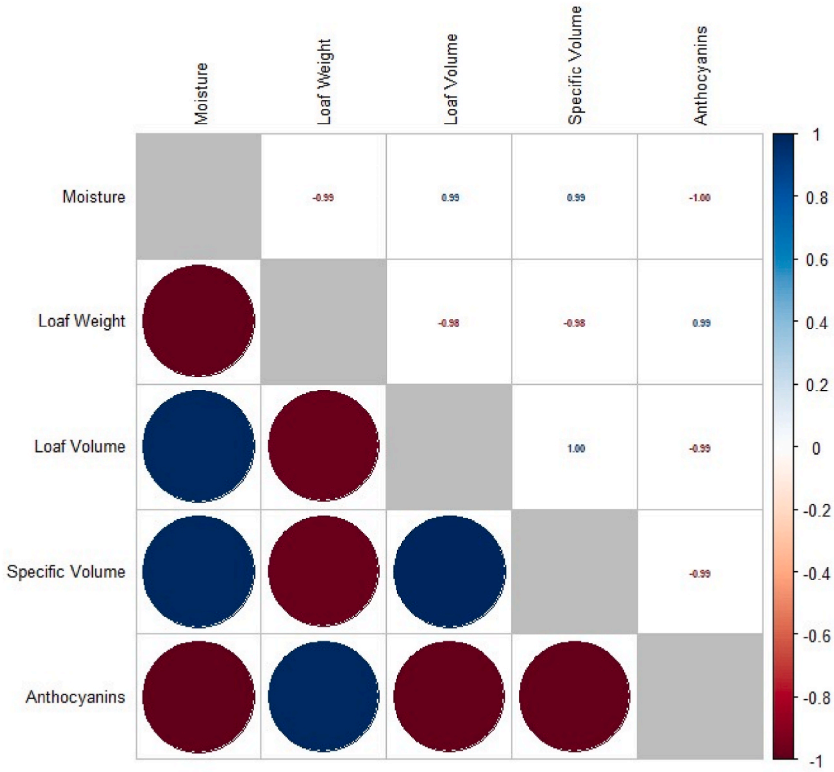


Fig. 4. Correlation matrix of 5 treatments (control, 2.5 %, 5 %, 7.5 % and 10 %) of BCP Bread for five variables (Anthocyanins, Moisture content, Loaf weight, Loaf Volume and Specific Volume.

with up to 5 % carrot pomace had the greatest organoleptic scores and was generally accepted by consumers. Similar results have been reported in previous studies wherein a high substitution level of wheat flour with turmeric powder [26] and onion powder [62] significantly influenced consumer acceptability. The results of this study showed that a wheat-BCP blend containing 7.5 % incorporation could be used to produce nutritionally improved bread with positive sensory attributes.

3.6. Effect of BCP incorporation on proximate composition

Table 5 describes the proximate composition of the experimental and control bread. A non-significant difference was observed for moisture, protein, crude fat and carbohydrate content by incorporation of black carrot powder at 7.5 % in bread samples. This is due to the fact that black carrot powder has a similar protein and fat content to that of wheat flour. Thus, these two nutrients were compensated with the supplementation of black carrot powder into the bread [63]. developed bread with carrot pomace incorporated at 2.5, 5.0, and 7.5 % and reported similar results for moisture, protein, and fat content. On the other hand, the crude fiber and total ash content of the bread samples increased significantly after being incorporated with black carrot powder. The crude fiber and total ash increased by 3.5 times and 2 times in the experimental sample (1.09 and 1.60 %) as compared to control (0.31 and 0.79 %). This suggests that the incorporation of black carrot powder into food products could be advantageous as a dietary supplement, particularly in cases where the enhancement of fiber and mineral content is highly desirable.

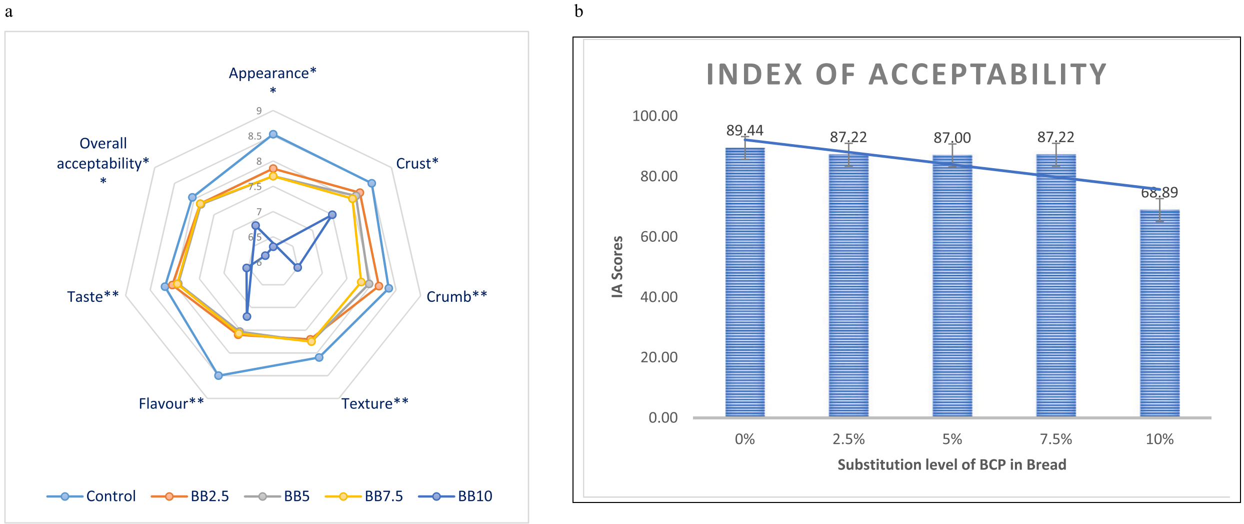


Fig. 5. a). Sensory analysis of breads prepared by incorporating black carrot powder. Control, BB2.5, BB5, BB7.5 and BB10 are breads prepared with 0 %, 2.5 %, 5.0 %, 7.5 % and 10 % replacement of refined wheat flour with black carrot powder, respectively. ** Significant at 1 % level of significance ($p < 0.01$), *Significant at 5 % level of significance ($p < 0.05$).
Fig. 5b: 0 %, 2.5 %, 5.0 %, 7.5 % and 10 % represent bread with respective % level of BCP. Bars represent standard deviation of means.

Table 5
Proximate composition of bread fortified with black carrot powder (Fresh Weight Basis).

Product	Parameters	Moisture	Protein	Crude fat	Crude fiber	Total ash	Carbohydrate
Bread	Control	37.56 ± 1.89	7.13 ± 0.55	3.73 ± 0.83	0.31 ± 0.01	0.79 ± 0.04	50.48 ± 3.32
	Experimental[a]	36.93 ± 0.97	7.07 ± 0.85	3.39 ± 0.53	0.96 ± 0.02	1.60 ± 0.02	49.92 ± 2.39
	t-value	0.51[NS]	0.10 [NS]	0.59 [NS]	50.34**	31.37**	0.24 [NS]

** Significant at 1 % level of significance ($p < 0.01$), *Significant at 5 % level of significance ($p < 0.05$), NS - Non-significant.
Control – Product developed with standard recipe, Experimental-product developed by incorporating black carrot powder at 1 % level in standard recipe.
[a] Experimental-bread developed by incorporating black carrot powder at 7.5 % level in standard recipe.

3.7. *Effect of BCP incorporation on mineral content of breads*

Table 6 illustrates the variability of the mineral content of control and experimental bread. The calcium content of black carrot bread was significantly higher ($p \leq 0.01$) than that of the control (19.01 mg/100 g). However, phosphorus content showed no significant difference. It may be attributable to the high phosphorus content of refined wheat flour (148 mg/100 g), as reported by Ref. [64]; which was unaffected by the substitution of wheat flour with 7.5 % BCP in experimental bread. The experimental bread exhibited a statistically significant increase ($p \leq 0.01$) in magnesium content compared to the control bread. The magnesium content of the experimental bread was 35.32 mg/100 g, whereas the control bread had a magnesium content of 28.38 mg/100 g. The study revealed that the iron content in black carrot bread containing 7.5 % black carrot powder (BCP) was approximately 30 % higher compared to the control. Additionally, the zinc content in the black carrot bread was found to be approximately 25 % higher than the control bread. The elevated levels of estimated minerals found in black carrot bread can be attributed to the notably higher mineral

Table 6
Mineral content of bread fortified with black carrot powder (mg/100 g Dry weight basis).

Parameters	Bread		
	Control	Experimental[a]	t-value
Calcium	19.01 ± 0.89	42.67 ± 2.28	16.74**
Phosphorus	98.58 ± 5.83	105.83 ± 6.12	1.49[NS]
Magnesium	28.38 ± 1.26	35.32 ± 1.95	5.18**
Iron	1.65 ± 0.03	2.14 ± 0.04	16.97**
Zinc	0.81 ± 0.01	0.86 ± 0.01	6.12**

** Significant at 1 % level of significance ($p < 0.01$), [a]Significant at 5 % level of significance ($p < 0.05$), NS - Non-significant.
Control – Product developed with standard recipe.
[a] Experimental-bread developed by incorporating black carrot powder at 7.5 % level in standard recipe.

content present in black carrots, which became nearly 88 % more concentrated in black carrot powder. The results are consistent with the findings reported by Ref. [65]; which indicated a significant increase in the iron and calcium levels of bread when dry curry leaves were added at a concentration of 5 %.

3.8. Effect of BCP incorporation on shelf-life evaluation of breads

The analysis of the storage quality characteristics was conducted on the experimental bread containing 7.5 % BCP, which was found to be the most acceptable as per sensory analysis (Table 7). The bread was sealed in aluminum laminate pouches and stored away from direct sunlight under ambient conditions. The storage quality of the experimental bread was evaluated by monitoring changes in moisture, free fatty acid value (FFA), peroxide value (PV), microbial properties, and overall acceptability over the course of storage. One major factor influencing the ability of the product to preserve its quality throughout storage is its moisture content. A decrease in moisture content from 36.93 % to 34.33 % was found in the bread sample as the storage duration increased [61,66]. also reported a decrease in moisture content with the increased storage time of bread. No significant difference was observed in the levels of free fatty acid content (FFA) and peroxide value (PV) after a 6-day storage period. Initially, FFA was estimated to be 0.043 % in bread, which was found to be 0.040 % at the end of the storage period. The initial estimated peroxide value (PV) of bread at day 0 was 0.023 Meq. O2/Kg, which subsequently increased to 0.025 Meq. O2/Kg at the end of the storage period.

The Yeast and Mold Count (YMC) indicated a consistent increasing trend in bacterial populations (TPC) on the bread by the sixth day, with a peak of 3.02 log10 CFU/g. However, the Colony Forming Units (CFU) remained below the safe thresholds, thereby confirming the bread's suitability for human consumption. Baking can deactivate a significant number of microorganisms that proliferate on the bread surface [67]. Due to the lower contact temperature in the interior of the crumb, many microorganisms manage to survive. Furthermore, the food products can be re-contaminated with microorganisms after baking, during cooling, and packaging [67,68]. Water activity has a significant impact on microbial development since higher moisture levels in food products are conducive to microbial proliferation [69]. The presence of sufficient moisture and water activity in food products typically leads to the development of Bacillus bacteria and several species of moulds, such as Rhizopus sp [68]. The data obtained from the Total Plate Count (TPC) and Yeast and Mold Count (YMC) analyses demonstrated that the bread samples remained within acceptable safety limits during the storage time.

The results of the sensory study indicated a substantial decline in the overall acceptability scores of black carrot bread as the storage duration increased, with scores decreasing from 7.85 to 6.36. A correlation was observed between the overall acceptability scores and the reduction in moisture loss. During the process of storage, bread undergoes a loss of moisture, leading to an increase in hardness and a decrease in freshness, ultimately resulting in a decline in overall acceptability. It can be inferred that bread maintained in appropriate packaging and stored at room temperature retains its sensory qualities for a duration of three days.

4. Conclusion

Black carrot is a highly nutritious and significant reservoir of bioactive compounds, specifically polyphenols and anthocyanins, which are widely recognized for their health-promoting and antioxidant properties. The incorporation of black carrot powder (BCP) into bread has exhibited considerable promise in enhancing the nutritional and functional qualities of the product. The desirable physical, functional, and sensory properties of bread were preserved when up to 7.5 % BCP was incorporated; however, higher concentrations resulted in a decrease in sensory scores, specifically for appearance, colour, and texture. The concentration of total phenols and flavonoids increased by over fivefold, with an exceptionally high anthocyanin content of 85.63 mg/100 g. The addition of BCP to the bread resulted in a significant increase in its mineral content, including zinc, calcium, phosphorus, magnesium, and iron. The storage study suggested that BCP bread can maintain its quality for a maximum of three days when stored at ambient temperature.

The findings of the storage study suggest that when BCP bread is stored in ambient conditions, its optimal quality can be maintained for a maximum duration of three days. The incorporation of black carrot powder as an ingredient in bread offers a promising approach to enhance its mineral composition, antioxidant potential, and overall nutritional profile. However, further research is required in order to fully capitalize on the potential of black carrots in food products through the exploration of innovative processing techniques. The effective incorporation of black carrot powder into bread has the potential to enhance the concentration of bioactive compounds, improve antioxidant characteristics, and increase the mineral composition. The implementation of large-scale production of black carrot-enriched bread has the potential to contribute to the mitigation of the dual burden of malnutrition in the country.

Data availability statement

All data contained within this article.

CRediT authorship contribution statement

Pragya Pandey: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kiran Grover:** Validation, Supervision, Conceptualization. **Tarsem Singh Dhillon:** Validation, Resources, Funding acquisition. **Neena Chawla:** Resources, Methodology, Formal analysis. **Amarjeet Kaur:** Validation, Supervision, Investigation.

Table 7
Shelf life stability and sensory quality profile of bread fortified with black carrot powder at 7.5 % level.

Storage days	Moisture (%)	FFA (% oleic acid)	Peroxide value (Meq.O2/Kg)	TPC (log10 cfu/g)	Y&MC log10 cfu/g)	Overall acceptability
0	36.93 ± 0.97[a]	0.043 ± 0.002[a]	0.023 ± 0.001[a]	2.16 ± 0.40[a]	1.73 ± 0.12[a]	7.85 ± 0.20[a]
3	35.35 ± 0.63[ab]	0.041 ± 0.001[a]	0.022 ± 0.002[a]	2.59 ± 0.30[a]	2.44 ± 0.15[a]	7.06±±0.23[b]
6	34.53 ± 0.53[b]	0.040 ± 0.001[a]	0.025 ± 0.001[a]	3.02 ± 0.05[b]	2.61 ± 0.15[b]	6.36±±0.29[c]

[a] Each value is expressed as mean ± SD (n = 3) and means having different letter superscripts within a same row are significantly different (P < 0.05).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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