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Investigating antioxidant and antibacterial activity of functional cookies enriched with beetroot during storage

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Abstract: Beetroot (*Beta vulgaris* L.) is one of the plants that contain biologically active compounds that have a function in the prevention and treatment of a wide variety of diseases. The study aims to design new cookies that will support certain groups, such as schoolchildren who may be anaemic. Also, to determine four cookie treatments that were planned to substitute white wheat flour with extraction rate of 72% as follows: T₀ (0%), T₁ (2.5%), T₂ (5.0%), T₃ (7.5%), and T₄ (10%) of beetroot powder to replace 100 g of flour; the cookies were baked at 180 °C for 30–35 min. The chemical composition was assessed, included total phenols, flavonoids, and minerals. Furthermore, during a three-week storage period, antioxidant activity and betalain pigments were evaluated, and sensory evaluation and microbiological assessment were done. Sensory evaluation revealed that the replacement ratio of 10% beetroot was acceptable to the cookies manufactured from white wheat flour with extraction rate of 72%. Compared to the control, a slight decrease was found in the total count of bacteria, fungi, and moulds. We recommend baking beetroot-enriched cookies since it enhances the organoleptic and microbiological characteristics.

Keywords: functional foods; HPLC; sensory evaluation; pigment; antimicrobial properties

Cookies are popular among adults and children alike. The main ingredients are wheat flour, sugar, butter, baking powder, and eggs. To make such biscuits more attractive, tasty, delicious and consumer-pleasing, additional ingredients such as cashew nuts, raisins, dried fruits, and vanilla essence are utilised (Atef et al. 2011).

Functional foods are helpful substances or foods containing microorganisms that have an important part in strengthening and enhancing health and well-being as well as suppressing some chronic diseases (Abdelazez

et al. 2018). As a result of the abundance of betalain pigments that exhibit considerable antioxidant and anti-inflammatory action, *Beta vulgaris* possesses powerful antioxidant and radical-scavenging properties and chemoprotective activity (Clifford et al. 2015).

Beetroot also has a high mineral concentration, including potassium (K), sodium (Na), phosphorus (P), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) (Mirmiran et al. 2020); in addition, fibre, folic acid, and carotenoid contents

are also high (Dias et al. 2009). Also, it has glycine, betalain, saponins, β -kinins, folate, betanin, polyphenols, and flavonoids. Hence, due to its nutritional value, it can prevent cancer (Váli et al. 2007), regulate diabetes, blood pressure and renal function, and serve as an antibacterial agent (Mirmiran et al. 2020).

The aim of our hypotheses is to produce functional cookies that should benefit certain groups, such as schoolchildren who may be suffering from anaemia. Because of the beet high nutritional content, the study evaluates the chemical composition, mineral concentration, and natural polyphenols found in dried beetroot powder and cookies.

MATERIAL AND METHODS

Beetroot powder preparation

Beetroot was purchased from the local market, Egypt. After washing, the fresh beets were boiled, peeled, and cut into 1–3 mm sizes using a special breadknife. Beetroot chips were dried in a tray desiccator (UM500; Memmert, Germany) at 60–65 °C for 7–8 h. Then they were ground and sieved through a 60-mesh sieve (laboratory test sieve D-42757; Retsch GmbH, Germany). Finally, the powder was sealed into plastic bags and kept refrigerated at 4 °C until needed (Refrigerator Combi Bottom Freezer; BOSCH, Germany).

Determination of chemical analysis of beetroot powder

Chemical composition was analysed according to Association of Official Agricultural Chemists (AOAC 2012) techniques as protein content, ether extract, ash, and crude fibre. The number of total carbohydrates in the sample was calculated according to the method described by Mathew et al. (2014).

Analysis of phenolic concentration

The total phenolic content of the samples was determined using the Folin-Ciocalteu phenol reagent (Merck, Germany) (Musa et al. 2011). A spectrophotometer (DU800; Beckman Coulter, US) was used to measure the absorbance at optical density (O.D.) 765 nm. The values were computed in mg 100 g⁻¹ of fresh weight in gallic acid equivalents (GAE) (Merck, Germany).

Evaluation of total flavonoid (TF) compounds

TF content in extracts was described as rutin equivalents (RE 100 g⁻¹) of sample fresh weight, and TF content was calculated as O.D. at 510 nm (Čanadanović-Brunet et al. 2011).

Evaluation of antioxidant activity of beetroot

Miller and Rice-Evans (1997) used a spectrophotometric scale to calculate antioxidant activity by adding 1 mL of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) reaction solution to 100 mL of sample extract and monitoring O.D. at 734 nm after 1 min of initial mixing.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assessment

The DPPH assay was evaluated as absorbance O.D. at 515 nm (Ravichandran et al. 2012).

Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was evaluated as absorbance O.D. at 593 nm (Benzie and Strain 1996).

Mineral content of beetroot

Beetroot powder has high concentrations of minerals such as K, Na, Ca, Mg, Fe, Zn, P, and Mn, which were measured using a flame photometer (EGA 330; Galienkamp, United Kingdom) and a Perkin Elmer atomic absorption spectrophotometer (DU 800, Beckman Coulter, US). Meanwhile, total phosphorus was measured using a spectrophotometer at 650 nm according to the method described by AOAC (2012).

Evaluation of beetroot betalain concentration

Ethanol (1 L, acidified with 2% citric acid) was agitated briskly for 15 min at room temperature with 200 g of beetroot and rested for 24 h. The extract was then filtered and concentrated under vacuum at 40.0 °C using a rotating vacuum evaporator (vacuum pump type 840.3FT.18; Lab Fort KnF, Germany) (Attia et al. 2013).

Identification of betalain pigments by high-performance liquid chromatography (HPLC)

Chemical reagents. The Milli-Q technology was used to doubly distil and purify water for phenolic extraction and the mobile phase (Millipore, US). HPLC-MS grade acetonitrile and formic acid were used as the mobile phases (Sigma, Germany).

Extraction. Dried beetroots were mashed to a fine powder (laboratory test sieve D-42757; Retsch GmbH, Germany), and 200 mg were homogenised for 1 min in 2.5 mL of demineralised water. The homogenate was centrifuged for 10 min at 10 000 rpm (Universal 320R; Hettich, Germany), and the clear supernatant was collected. In three processes, the insoluble component was re-extracted with 2.5 mL of demineralised water. The beetroot extracts were mixed and evaluated imme-

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diately in an HPLC system (Hewlett-Packard HP 1050; Thermo Scientific, US) (Nemzer et al. 2011).

Preparation of functional cookies enriched with beetroot

Functional cookies were prepared by replacing white wheat flour with extraction rate of 72% with beetroot powder. Then, it was expressed into five separate treatments. Other than the control T_0 (0%), four treatments of cookies were prepared with replacement rates of white wheat flour with extraction rate of 72% with T_1 (2.5%), T_2 (5.0%), T_3 (7.5%), and T_4 (10%) of beetroot powder according to the American Association of Cereal Chemists (AACC 2012) technique with slight modification.

The dry components were weighed using an analytical balance [Precision Electronic Balance (0.0001 g); Hogen-togler & Co., Inc., US]. Wheat flour 72% (250.0 g), sucrose (125.0 g), NaCl (3.50 g), skimmed milk (25.0 g), butter (53.50 g), egg (110.0 g), bicarbonate (12.50 g), and vanilla extract (2.0 g) were used to bake the cookies. All of the commercial-grade ingredients were purchased from the Egyptian local market.

Using a hand blender (MK-H4-W; Panasonic Co., Malaysia), all ingredients were gradually combined until they formed a homogeneous dough. After the dough had reached the desired texture, it was flattened out and cut using a round cutter with a diameter of 32 mm and a thickness of 5 mm, then baked for 30–35 min at 180 ± 5 °C in a Turbofan oven on a greased pan (Top Model E32; Bakbar Versatile Bench, Germany). The baked cookies were allowed to cool at room temperature before being put into aluminium foil bags and stored at 4 °C in the refrigerator for analysis (Refrigerator Combi Bottom Freezer; BOSCH, Germany).

Sensory evaluation of functional cookies

The sensory properties of cookies were determined by 20 highly experienced members of the Department of Food and Nutritional Sciences, College of Science, Taif University, Saudi Arabia, as described by AACC (2012) with minor modifications. After baking, the cookie samples were allowed to cool for one hour at room temperature before beginning sensory assessments based on the scores distributed as follows. Taste (20 points), smell (20 points), texture (15 points), shell colour (15 points), crumbs (15 points), overall appearance (15 points), and acceptance (100).

Physical properties of functional cookies

After the cookies had cooled to room temperature, the physical properties were investigated. A digital bal-

ance (Hogentogler & Co., Inc., US) was used to record the weight of the cookies. The rapeseed displacement technique AACC (2012) was used to determine the volume and specific volume.

Microbial evaluation of functional cookies

Total bacteria count (TBC) was determined by plating on a plate count agar medium Tryptone soy broth (TSB) (Oxoid, UK) at 37 °C for 48 h, whereas yeasts and moulds were assessed regularly on potato dextrose agar media for three weeks in various cookie treatments. Total moulds and yeasts were counted at 28 °C for 3–5 days, as indicated in the Difco manual (Power and Johnson 2009).

Statistical analysis

The trials were carried out in triplicate and assessed during a three-week storage period at room temperature. All values were expressed using mean \pm standard deviation (SD). The statistical significance of data comparisons was determined using a one-way analysis of variance (ANOVA). Statistical significance was defined as $P \leq 0.05$. To compute F -values and compare means using Duncan's multiple range test, statistical analysis was done using SAS system software 9.1 (SAS Institute, US).

RESULTS AND DISCUSSION

Chemical composition of beetroot powder. The chemical composition of beetroot powder is shown in Figure 1A as protein, ether extract, crude fibre, ash content and total carbohydrates: the respective values were 12.8, 1.36, 20.40, and 11.30 mg 100 g⁻¹ and 54.06%, respectively. Furthermore, the polyphenolic content (total phenolics, flavonoids) and antioxidant components (ABTS, DPPH, and FRAP) are presented in Figure 1B as 137, 190, and 181 mg 100 g⁻¹, respectively. It was 255.0 mg 100 g⁻¹ GAE of sample and 260 mg RE 100 g⁻¹ of sample for ABTS, DPPH, and FRAP. On the other hand, the antioxidant activity was 137, 190, and 181 mg Trolox equivalent antioxidant activity (TEAA) 100 g⁻¹, respectively.

The obtained results were consistent with Clifford et al. (2015), who indicated that the red beets may be exploited as natural antioxidants. Previous studies have revealed that beetroot juice has functional properties in the treatment and control of a wide range of diseases. Netzel et al. (2005) reported that ingesting a daily single dose of red beet juice may enhance an increase in human renal excretion of antioxidative

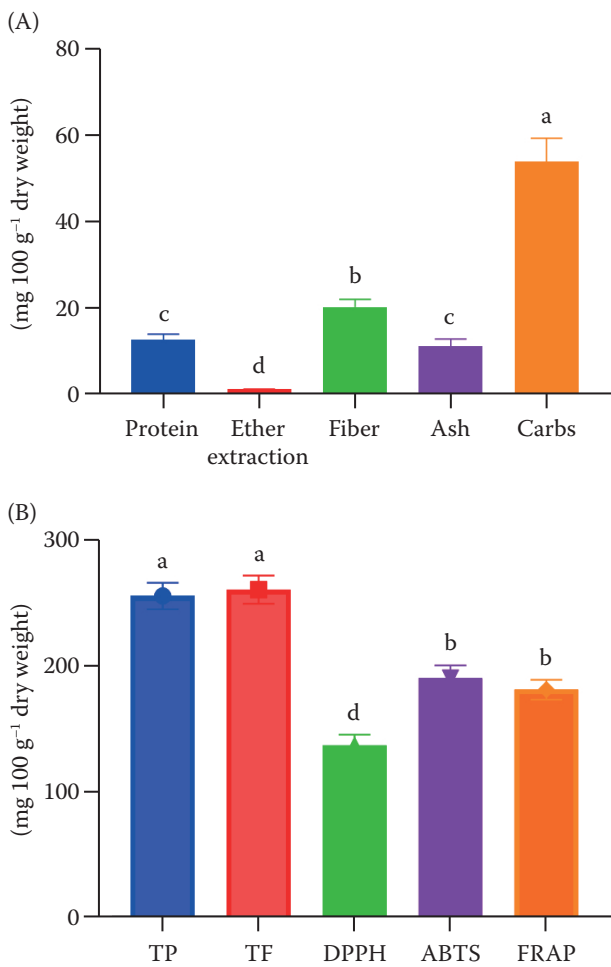


Figure 1. (A) Chemical composition and (B) antioxidant components of beetroots powder (mg 100 g⁻¹) (mean ± SD; n = 3)

^{a-d}According to Duncan's multiple $P \leq 0.05$ comparison test, different lowercase letters indicate significant differences; SD – standard deviation; TP – total phenolic; TF – total flavonoids; DPPH – 1,1-diphenyl-2-picrylhydrazyl; ABTS – 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP – ferric reducing antioxidant power

compounds containing betalain. Furthermore, several clinical studies have shown that both the natural pigments such as carotenoids, anthocyanins, and betalains and the phenolic components found in red beets might reduce oxidative damage to lipids and promote oxidation resistance. Also, they can help prevent cancer and cardiovascular diseases by eliminating free radicals (Mirmiran et al. 2020).

Mineral content of beetroot. The mineral composition of beetroot powder was presented as K, P, Ca, Mg, Mn, and Fe. The obtained results in Figure 2 show that K⁺¹ was the most abundant component in beetroot powder, with a concentration of 26.0 mg 100 g⁻¹ fol-

lowed by Na⁺¹ and P⁺⁵ at the level of 6.26 mg 100 g⁻¹ and 3.50 mg 100 g⁻¹, respectively. While the concentrations of Ca⁺², Mg⁺², and Mn⁺² were 2.28, 1.84, and 1.05 mg 100 g⁻¹, respectively. Meanwhile, Fe⁺³ and Zn⁺² showed 0.06 mg 100 g⁻¹ and 0.03 mg 100 g⁻¹, respectively.

Beetroot is a rich source of minerals such as Mn, Na, K, Mg, Fe, and Cu (Mirmiran et al. 2020). Our results are consistent with those of (Awasthi 2014), who determined that Fe⁺³, Ca⁺², and P⁺⁵ concentrations varied from 0.1 to 2.7, 32.0 to 64.0, and 310 to 532 mg 100 g⁻¹, respectively. Furthermore, the Zn⁺² content in beetroot powder was 0.35–0.96 mg 100 g⁻¹ (Borah et al. 2020).

Identification of betalain pigments from beetroot powder. Betalain pigments are nitrogen-containing water-soluble pigments that were discovered in large quantities in red beets. Betacyanins are red-violet pigments and betaxanthins (yellow-orange pigments) are two subclasses of betalains (Borah et al. 2020).

HPLC was used to determine the identification and separation of betalain pigments from red beetroot. Figure 3 indicates that vulgaxanthin had a relative abundance of 5.0% in the 9.70 min retention time, whereas betalain had a relative abundance of 62.6% in the 25.0 min retention time, and isobetalain had a relative abundance of 17.1% in the 31.0 min retention time. On the other hand, 15-dicarboxybetanene has a retention time of 46.9 min and a relative abundance of 25.3%.

Our obtained results were in agreement with those of Wybraniec (2005), who observed that betalain was the major component of pigments in red beetroot. While our data are in disagreement with Attia et al. (2013), who reported that vulgaxanthin had a relative abundance

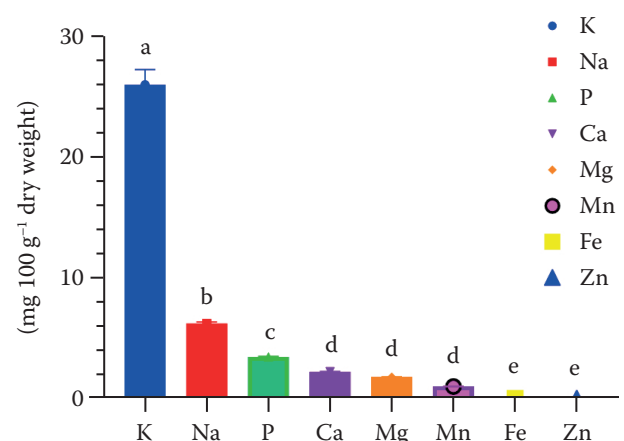


Figure 2. Minerals concentration of beetroot powder (mg 100 g⁻¹) (mean ± SD; n = 3)

^{a-e}According to Duncan's multiple $P \leq 0.05$ comparison test, different lowercase letters indicate significant differences; SD – standard deviation

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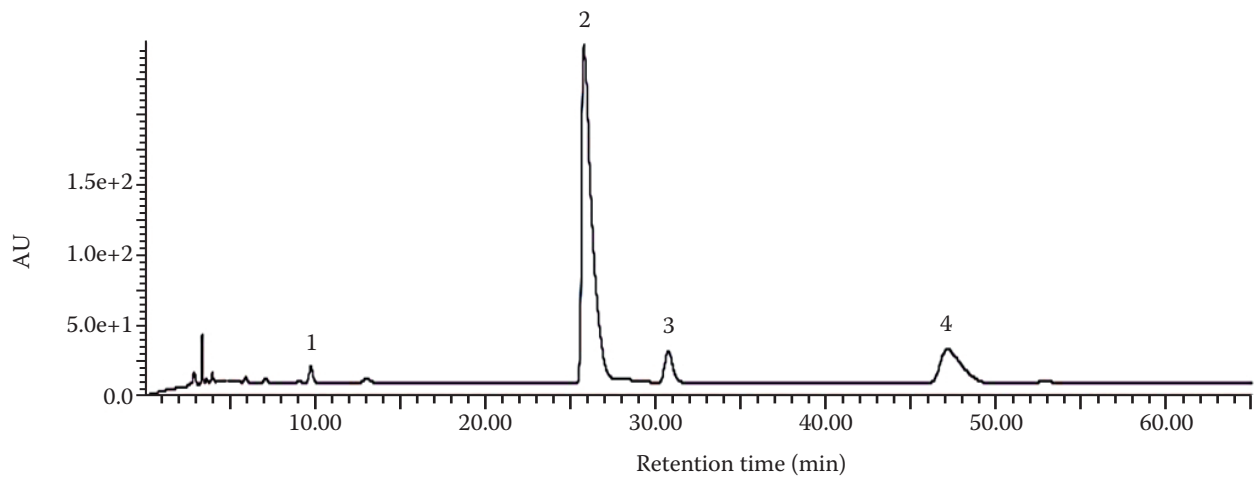


Figure 3. HPLC chromatogram of betalains extracted from beet powder

HPLC – high-performance liquid chromatography; AU – absorbance units

of 1.34% within 10.50 min and betalain had a relative abundance of 82.79% within 23.0 min. Also, isobetalain had a relative abundance of 11.40% within 25.7 min.

Sensory evaluation of functional cookies. As beetroot powder was added, the organoleptic characteristics of functional cookies swiftly dropped when compared to a control group, as seen in Figure 4. The crust of the cookies became semi-hard as a result of adding beets, which might be owing to the high beetroot crude fibre content of 20.4% dry matter (d.m.). Our findings were consistent with those of Attia et al. (2013), who stated that the overall acceptance rate of cookies would be reduced, particularly at 10% beetroot powder.

Physical characteristics of functional cookies. In comparison with the control group, the weight,

volume, and specific volume of the cookies fortified with beetroot were measured at 2.5, 5.0, 7.5, and 10.0%. The results in Figure 5 show an increase in weight when beetroot powder was increased from 51.0 g at 2.5% beetroot to 53.0 g at 10.0% beetroot. On the other hand, the volume and specific volume of the cookies shrank as the amount of beetroot increased, possibly due to the reduced quantity of gluten protein in the dough and the high quantity of raw fibre in the beetroot (Attia et al. 2013).

Evaluation of microbiological functional cookies. Microbial load is one of the most critical aspects defining the shelf life of baked products, which can have an economic impact on bakery projects. These hazards may be caused by the personnel, packaging, hygienic

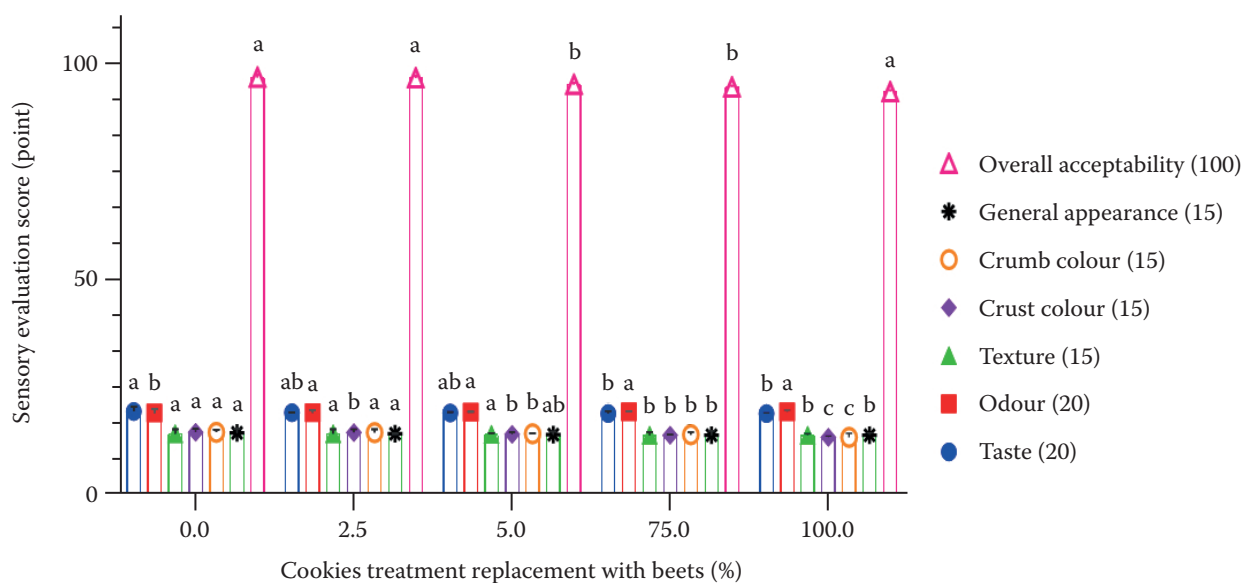


Figure 4. Sensory evaluation of functional cookies

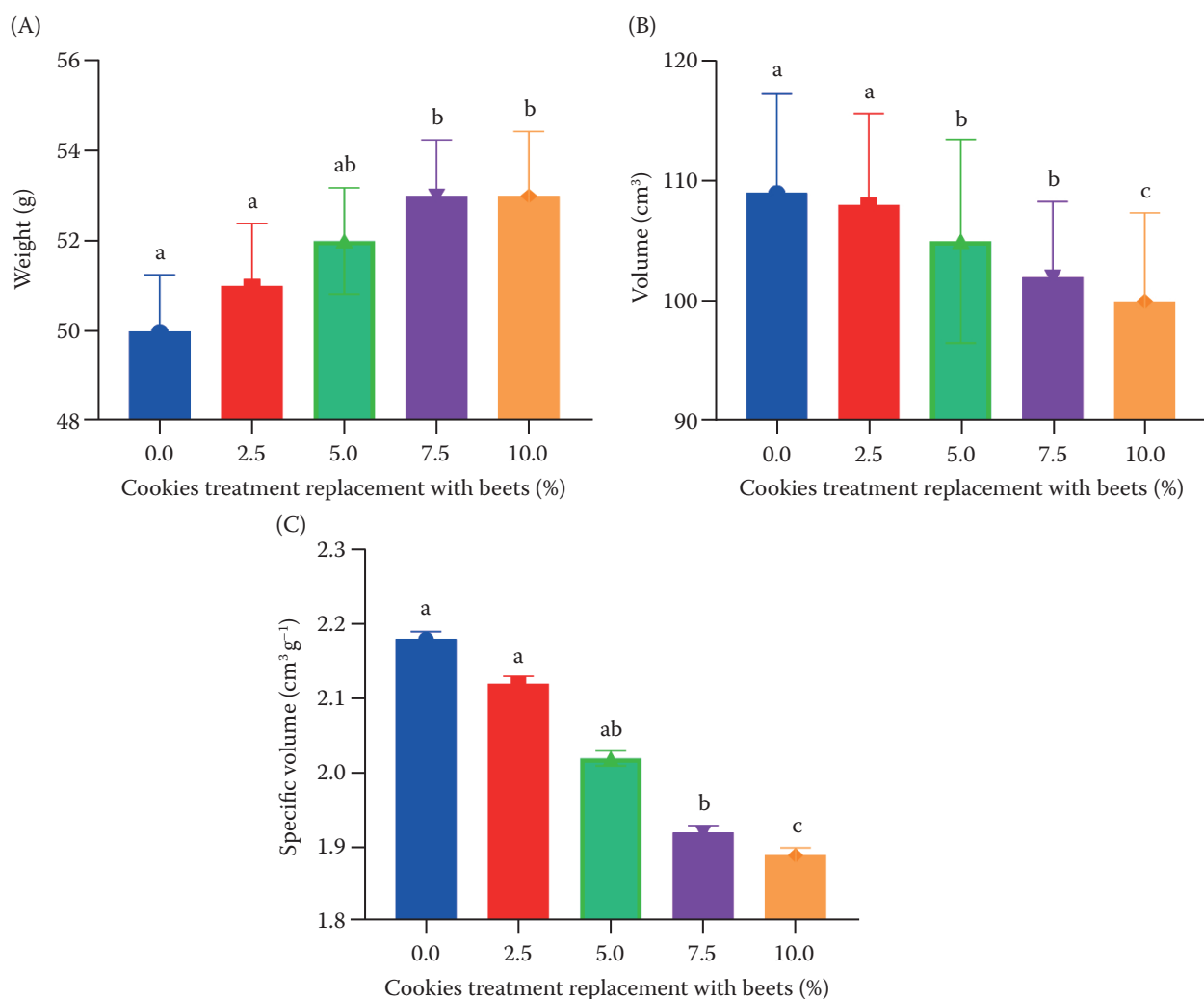


Figure 5. Physical characteristics of functional cookies: (A) weight (g), (B) volume (cm³), and (C) specific volume (cm³ g⁻¹)

manufacturing techniques, storage conditions, and market product turnover (Kumari et al. 2021).

Table 1 displays the biological activity when total bacterial and fungal counts were calculated in cookies throughout a three-week storage period. In comparison

with control cookies made from white wheat flour with extraction rate of 72%, the total count of bacteria and fungi was inhibited in cookies enriched with beetroot during the 21-day storage period at room temperature.

The total count of bacteria in T₄ treatment ranged from

Table 1. Evaluation of bacterial total count and yeast and molds of functional cookies (log CFU × 10⁶ week⁻¹) (mean ± SD; n = 3)

Treat-ments	Bacterial total count				Yeast and molds			
	day 0	week 1	week 2	week 3	day 0	week 1	week 2	week 3
T ₀	2.30 ± 0.13 ^a	3.50 ± 0.15 ^a	5.10 ± 0.32 ^a	7.80 ± 0.73 ^a	–	2.20 ± 0.11 ^a	2.90 ± 0.24 ^a	11.00 ± 1.42 ^a
T ₁	2.25 ± 0.11 ^a	3.10 ± 0.14 ^b	4.60 ± 0.29 ^{ab}	7.10 ± 0.54 ^{ab}	–	2.10 ± 0.12 ^a	2.80 ± 0.24 ^a	10.90 ± 1.28 ^{ab}
T ₂	2.20 ± 0.11 ^a	2.80 ± 0.16 ^{bc}	4.10 ± 0.21 ^b	6.60 ± 0.86 ^b	–	2.0 ± 0.14 ^a	2.70 ± 0.27 ^{ab}	10.80 ± 1.52 ^b
T ₃	2.10 ± 0.12 ^b	2.50 ± 0.12 ^{bc}	3.80 ± 0.25 ^{bc}	6.10 ± 0.62 ^{bc}	–	1.90 ± 0.13 ^b	2.60 ± 0.25 ^{ab}	10.70 ± 1.37 ^c
T ₄	2.10 ± 0.12 ^b	2.20 ± 0.11 ^c	3.10 ± 0.22 ^c	5.60 ± 0.43 ^c	–	1.95 ± 0.13 ^b	2.65 ± 0.25 ^b	10.75 ± 1.64 ^c

^{a-c}According to Duncan's multiple $P \leq 0.05$ comparison test, different superscript letters within columns indicate significant differences; CFU – colony-forming units; SD – standard deviation

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2.10×10^{-1} CFU at zero time to 5.60×10^{-1} CFU after three weeks, whereas T_0 ranged from 2.30×10^{-1} CFU to 7.80×10^{-1} CFU. The total count of bacteria dropped, as did the number of fungus and moulds, whether due to storage time or an increase in the proportion of beetroot based on the varied treatments. Cookies made with beetroot powder products are high in total dietary fibre and betalain pigment which acts as a natural antioxidant.

Mould growth is a crucial factor influencing the shelf life of baked products. Mould proliferation in bakery products is an issue that causes considerable economic harm estimated at 1–5% on a controlled basis based on product type and processing technique (Saranraj and Geetha 2012). Dried beetroot may be consumed in different ways, such as flakes as an alternative to typical snacks (Maurya 2020).

CONCLUSION

Beetroot powder is a fascinating addition to bakery products, particularly cookies, because of its mineral composition and natural antioxidant and pigment properties. Beetroot powder-enriched cookies offer great physical and sensory characteristics, such as flavour, texture, and bacterial and fungal suppression. As a result, developing new cookies that support certain populations, such as anaemic schoolchildren, is a smart idea. As a result, it is fair to expect that up to 10% of acceptable cookies may be substituted with beetroot red powder without compromising their quality.

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