



EVALUATION OF NATURAL PIGMENTS AS ALTERNATIVES TO SYNTHETIC FOOD COLORANTS IN RED-COLORED CAKE LAYERS

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Received 19 February 2026, accepted 30 September 2026

Abstract: *This study investigated the replacement of the synthetic colorant Ponceau 4R (E124) with beetroot powder at levels of 5%, 8%, 11%, and 14% in cake layer formulations, focusing on the evaluation of technological, sensory, and antioxidant (betalain content, total phenolic content, and antioxidant activity) properties. Incorporation of beetroot powder increased weight loss during baking and moisture content in the final product. Textural analysis revealed that higher beetroot levels led to increased hardness, gumminess, and chewiness, accompanied by decreased adhesiveness and stickiness, producing a denser and more cohesive crumb structure. Color assessment indicated that the cake layer containing 11% beetroot powder most closely matched the redness of the control sample, providing a suitable visual alternative to the synthetic colorant. Antioxidant activity rose proportionally with beetroot powder concentration, reflecting the contribution of betalains and phenolic compounds. Sensory evaluation demonstrated that formulations containing up to 11% beetroot powder were well accepted, maintaining desirable flavor, texture, and appearance. These findings suggest that beetroot powder can effectively replace synthetic colorants at appropriate levels, offering a clean-label, health-oriented bakery product with enhanced functional properties, while preserving sensory quality and visual appeal.*

Keywords: *beetroot powder; natural colorant; cake layer; antioxidant activity; sensory properties*

1. Introduction

Bakery products, also referred to as bakery wares as defined by the Codex Alimentarius, constitute a diverse and complex category of food items that include breads, cakes, biscuits, and related baked goods [1]. Wheat flour is typically the principal ingredient, serving a critical functional role by contributing to the volume and structural integrity of the final product. In the production of bakery items, baking is predominantly performed using hot air ovens. As staple foods, bakery products are widely consumed across the globe, often in substantial quantities and with high frequency, thereby contributing significantly to the dietary intake of both macronutrients and micronutrients [2]. Cakes are distinguished from other sweet bakery products, such as biscuits, primarily by their

formulation, notably the inclusion of eggs. Additionally, cakes generally exhibit higher moisture content than biscuits in the final product [3]. There are two principal categories of cakes: (a) shortened or high-ratio cakes and (b) foam cakes, which differ markedly in their preparation methods. Shortened cakes incorporate a fat-based component known as shortening, which may include butter or margarine and is characterized by a network of fat crystals that stabilize the structure. During the creaming process, this shortening is mixed with sugar to entrap air, contributing to the cake's volume and texture [3,4]. In contrast, foam cakes do not contain a fat phase; instead, aeration is achieved through the formation of egg white foam, which imparts the characteristic light structure of these cakes. Cake formulations frequently include various

functional additives designed to enhance batter stability, flavor profile, product volume, and shelf life. Common examples of such additives are emulsifiers, colorants, flavoring agents, and enzymes. Food colorants serve both aesthetic purposes and functional roles, such as masking natural color inconsistencies or compensating for color degradation during storage [5]. Both natural and synthetic food colorants are employed, with gel and liquid-gel formulations being particularly favored due to their high pigment concentration and superior stability. Within the European Union, the use of food colorants, along with other food additives, is subject to stringent regulatory frameworks. These regulations specify the categories of food products in which colorants may be used, the maximum permissible levels, and the required chemical characterization and purity standards [6]. Certain synthetic color additives have been associated with potential health risks, including allergenic reactions, and in some cases, adverse effects such as hyperactivity in children, as well as mutagenic and/or carcinogenic outcomes [6]. As a result of findings from food safety assessments, several synthetic colorants have been prohibited. Notably, E128 (Red 2G) and E154 (Brown FK) have been banned due to their identified health hazards [7,8]. In light of growing health concerns regarding

synthetic food additives, this research explores the feasibility of using beetroot powder as a natural pigment source to replace synthetic red dyes (E124, Ponceau 4R) in red-colored cake layers. The impact of this substitution was evaluated with respect to technological parameters (including weight loss after baking, moisture content, and texture), sensory attributes, and antioxidant properties of the cake, assessed through betalain content, total phenolic content, and overall antioxidant activity.

2. Materials and methods

2.1. Materials

The ingredients used to prepare cake layers, including almond flour, powdered sugar, granulated sugar, cocoa powder, eggs, and butter (82% fat), were purchased from a local market in Suceava, Romania. Beetroot powder was also purchased from the market, while the food colorant E124 (Ponceau 4R, gel form) was provided by a local bakery chain. All chemicals used in this study were of analytical grade and were obtained from Merck KGaA (Germany).

2.2. Cake batter preparation and baking

The quantities of ingredients required to produce a single 400 g cake layer are presented in Table 1. Beetroot powder was incorporated into the formulation at levels of 5%, 8%, 11%, and 14% (w/w), relative to the weight of almond flour.

Table 1.

Formulation of cake layer samples					
Ingredient	CL-control	CL-5%BP	CL-8%BP	CL-11%BP	CL-14%BP
Almond flour (g)	54	54	54	54	54
Powdered sugar (g)	38	38	38	38	38
Cacao powder (g)	6	6	6	6	6
Egg whites (g)	142	142	142	142	142
Granulated sugar (g)	78	78	78	78	78
Egg yolk (g)	113	113	113	113	113
Butter (g)	27	27	27	27	27
Water (g)	2	2	2	2	2
Colorant/ Beetroot powder (g)	0.6	2.7	4.3	5.9	7.5

CL – cake layer, BP – beetroot powder

The preparation of the cake batter was conducted with the objective of ensuring uniform distribution of all ingredients and the incorporation of a high number of finely dispersed gas cells [4]. Initially, the dry ingredients – almond flour, powdered sugar, and cocoa powder – were mixed together. Separately, egg whites were whipped with granulated sugar to form a foam, into which the egg yolks were gradually incorporated. The dry mixture was then slowly folded into the egg foam, followed by the addition of melted butter. Finally, E124 (Ponceau 4R) or beetroot powder was dissolved in water and added to the batter. The resulting mixture was evenly spread in a circular pan and baked at 170 °C for 18 minutes.

2.3. Physicochemical measurements

The characteristics of cakes layers from two independent batches were evaluated 24 hours after baking. Weight loss after baking was calculated as described by Paesani et al. [9] as the difference between the weight of the batter prior to baking and the final weight of the baked cake, expressed as a percentage of the initial batter weight. Moisture content was determined using a previously described method [10]. Each cake layer sample (~5 g) was weighed in a covered dish, with the net sample weight calculated by subtracting the weight of the empty dish from the total. The uncovered samples were then placed in a hot air oven at 103 ± 1 °C for 72 hours. Upon completion of the drying period, the dishes were immediately covered and transferred to a desiccator to cool. Final weighing was performed once the samples reached ambient temperature. Moisture content was calculated using Equation (1):

$$\text{Moisture}(\%) = \frac{A}{B} \times 100 \quad (1)$$

where: A – moisture loss (g), calculated as the difference between the original weight of the

sample and its weight after drying; B – original weight of sample (g).

Texture profile analysis of the cake layer samples was conducted one hour after baking, following the procedure described by Principato et al. [11]. Each sample was cut into rectangular pieces measuring 3×3 cm with a height of 1.5 cm. Textural parameters (hardness, adhesiveness, springiness, stickiness, cohesiveness, chewiness, and gumminess) were determined using a double compression test performed with a TVT 6700 texture analyzer (Perten Instruments, Hägersten, Sweden) equipped with a 75 mm cylindrical probe. Samples were compressed to 30% of their original height at a test speed of 2 mm/s under ambient conditions. A holding time of 15 seconds was applied between the first and second compression. Five replicates were performed for each sample.

The color of the cake layer samples was analyzed in triplicate at 25 °C using a CR-400 chromameter (Konica Minolta, Japan), calibrated with a standard white reference plate. The CIE L^* , a^* , and b^* color coordinates, along with the derived parameters hue angle (h^*_{ab}) and chroma (C^*_{ab}), were obtained from the reflection spectra of the samples using illuminant D65 and a 2° standard observer.

To evaluate the natural pigment content in the cake layer samples, the total phenolic content and antioxidant activity, an extract was prepared following the procedure described by Ravichandran et al. [12], with minor modifications.

The extract was obtained by mixing 1 g of sample with 10 mL of 50% (v/v) ethanol. The mixture was agitated for 10 seconds and subsequently centrifuged at 5000 rpm for 15 minutes.

The concentrations of betacyanins and betaxanthins in the extracts were determined at 538 nm and 480 nm, respectively, using a UV-Vis-NIR spectrophotometer (Shimadzu

Corporation, Japan), according to previously described methods [12,13]. The absorbance values obtained were used to calculate the betalain concentration for each sample. Betalain content was calculated using Equation (2):

$$\text{Betalain content (mg/L)} = (A \times DF \times MW \times 1000) / (\varepsilon \times l) \quad (2)$$

where: A – absorbance, DF – dilution factor, MW – molecular weight, ε – molar extinction coefficient, l – pathlength of the cuvette (1 cm).

For quantification, the following values were used: for betacyanins, $MW = 550$ g/mol and $\varepsilon = 60,000$ L/mol·cm; for betaxanthins, $MW = 308$ g/mol and $\varepsilon = 48,000$ L/mol·cm (in water). The results were expressed as total betalain content, in mg/ 100 g.

Total phenolic content was determined using the Folin-Ciocalteu method, following the procedure described by Spinei & Oroian [14]. Briefly, 0.2 mL of extract was mixed with 2 mL of 1:10 diluted Folin-Ciocalteu reagent and 1.8 mL of 7.5% (w/v) sodium carbonate solution in a test tube. The mixture was allowed to stand at room temperature for 30 minutes and then the absorbance was measured at 750 nm. A calibration curve was generated using gallic acid as the standard, at concentrations of 1, 2, 3, 4, and 5 mg/L, yielding a regression coefficient (R^2) of 0.98. All measurements were performed in triplicate. The results were expressed in mg GAE/ 100 g.

The antioxidant activity was evaluated using the DPPH radical scavenging assay, as described by Spinei & Oroian [14]. Briefly, 20 μ L of extract was mixed with 750 μ L of DPPH solution and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 515 nm against a blank. The percentage of DPPH radical scavenging activity (RSA) was calculated using Equation (3):

$$\text{RSA (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

where: A_0 – absorbance of the control sample (nm), A_1 – absorbance of the test sample (nm).

Antioxidant activity was measured in triplicate for each sample.

2.4. Sensory analysis

Sensory evaluation of the cake layer samples was conducted to assess consumer acceptance based on color, taste, flavor, texture, mouthfeel, appearance, and overall acceptability, following the method described by Paesani et al. [9]. A 9-point hedonic scale was employed, ranging from 1 ("dislike very much") to 9 ("like very much"). The panel consisted of 30 untrained volunteers (12 males and 18 females) aged between 18 and 55 years, all of whom were regular consumers of cake. The evaluation was carried out at 25 °C under uniformly distributed white lighting. Cake samples were cut into 2 cm-wide portions, placed on white plastic plates coded with numbers, and presented to participants in a randomized order.

2.5. Statistical analysis

The data obtained from the analysis of the physicochemical properties were subjected to analysis of variance (ANOVA) using Statgraphics Centurion XVI software (Statgraphics Technologies, Inc., The Plains, VA, USA). Differences between means were evaluated using Fisher's Least Significant Difference (LSD) test at a 95% confidence level. Principal component analysis (PCA) was performed using OriginPro 2025b software, trial version (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

The cake layer samples were evaluated in terms of technological properties (including weight loss during baking, moisture content, texture parameters, and color), betalains content, total phenolic content, antioxidant

activity, and sensory attributes. Although Ponceau 4R (E124), the synthetic colorant used in the control sample, has not been associated with carcinogenicity, genotoxicity, neurotoxicity, or reproductive and developmental toxicity at authorized dietary exposure levels, the widespread consumption of sweet bakery products containing this additive, such as red velvet cake and similar cake pops, muffins, and macarons, raises concerns regarding potential intake above the established regulatory limits. Studies have indicated that exceeding the acceptable daily intake of such azo dyes may trigger allergic reactions, particularly in individuals with acetylsalicylic acid intolerance, a condition also linked to hyperkinesias [6]. Moreover, Ponceau 4R may contain trace levels of unsulfonated aromatic amines, which are recognized as

potentially carcinogenic. Additional research has reported DNA damage, tumor formation in animal studies, and adverse respiratory effects in asthmatic individuals [15]. Given these concerns, this study investigates the potential of beetroot powder, which is rich in natural pigments such as betalains, as a safer alternative to Ponceau 4R (E124) in the formulation of cake layers. The results of the physicochemical and sensory analyses are presented below.

3.1. Technological characteristics

Table 2 presents the weight loss during baking and the moisture content of cake layer samples formulated with the synthetic colorant Ponceau 4R (E124), as well as those in which this colorant was replaced with beetroot powder, a natural pigment source, at substitution levels of 5%, 8%, 11%, and 14% (w/w).

Table 2.

Technological characteristics of cake layer samples. Mean values $\pm$ standard deviation.

Sample	Weight loss during baking (%)	Moisture content (%)
CL-control	13.16 $\pm$ 0.05 ^d	22.03 $\pm$ 0.50 ^c
CL-5%BP	13.48 $\pm$ 0.07 ^c	27.97 $\pm$ 0.60 ^d
CL-8%BP	13.61 $\pm$ 0.03 ^c	28.35 $\pm$ 0.30 ^c
CL-11%BP	13.93 $\pm$ 0.05 ^b	28.99 $\pm$ 0.20 ^b
CL-14%BP	14.22 $\pm$ 0.06 ^a	29.48 $\pm$ 0.40 ^a

^{a-c} Different letters in the same column indicate significant differences among samples ($p < 0.05$)

CL – cake layer, BP – beetroot powder

Weight loss during baking primarily results from the evaporation of moisture and the volatilization of certain compounds from the cake batter [16]. This phenomenon is influenced by the internal structure of the batter and the physicochemical properties of its components, which can, in turn, affect key quality attributes of the final product, including crumb softness, moisture retention, and cohesiveness [16,17]. As shown in Table 2, cake layer samples in which the synthetic colorant Ponceau 4R (E124) was replaced with beetroot powder exhibited significantly higher weight loss values compared to the control ($p < 0.05$). Moreover, weight loss during baking increased progressively with

higher levels of beetroot powder substitution, ranging from 13.84% at 5% addition to 14.22% at 14%, indicating a 0.38% increase across the tested levels. This increase may be attributed to the additional moisture content introduced by the beetroot powder, which likely contributed to a denser batter and an increased specific gravity. These changes can alter air cell distribution within the batter, ultimately impacting crumb structure and sensory characteristics, as similarly observed in muffins enriched with blueberry and cranberry powders [18].

Regarding moisture content, cake layer samples with increasing levels of beetroot powder exhibited significantly higher values compared to the control ($p < 0.05$), which

may be attributed to the intrinsic composition of beetroot powder, including its inherent moisture content. Similar trends have been reported in studies evaluating the incorporation of beetroot in muffins [19] and beetroot skin powder in nougat formulations [20]. Beetroot powder is rich in dietary fiber (18.8 g/100 g, as reported in the specification sheet of the product), which contributes to its high water-holding capacity and reduces moisture loss during baking. Additionally, its substantial sugar content (44.59 g/100 g, as reported in the specification sheet of the product) imparts humectant properties, enhancing water retention within the cake matrix. These characteristics likely promote increased interaction with water, resulting in elevated moisture content in the final

product. This higher moisture content may influence the textural attributes of the cake layers by contributing to a softer crumb and enhancing perceived freshness and moistness [18]. Elevated moisture levels may negatively impact product stability by facilitating microbial growth and accelerating staling, thereby reducing shelf life. Nonetheless, this was not the case for the cake layer samples produced in this study, which exhibited moisture contents within the acceptable threshold of 30% [21].

The textural properties evaluated for the cake layer samples included hardness, adhesiveness, springiness, stickiness, cohesiveness, chewiness, and gumminess. The corresponding values obtained for each parameter are presented in Table 3.

Table 3.

Textural parameters of cake layer samples. Mean values $\pm$ standard deviation

Sample	Hardness (g)	Adhesiveness (J)	Springiness	Stickiness (g)	Cohesiveness	Gumminess (g)	Chewiness (g)
CL-control	473.87 $\pm$ 15.07 ^c	-0.18 $\pm$ 0.05 ^a	0.99 $\pm$ 0.002 ^a	-0.96 $\pm$ 0.06 ^a	0.93 $\pm$ 0.008 ^b	441.74 $\pm$ 15.99 ^c	441.73 $\pm$ 16.66 ^c
CL-5%BP	1029.08 $\pm$ 23.71 ^d	-29.87 $\pm$ 1.45 ^b	0.99 $\pm$ 0.001 ^a	-2.45 $\pm$ 0.08 ^b	0.97 $\pm$ 0.006 ^a	1003.28 $\pm$ 23.62 ^d	1002.47 $\pm$ 22.82 ^d
CL-8%BP	1122.85 $\pm$ 19.26 ^c	-37.78 $\pm$ 1.37 ^c	0.99 $\pm$ 0.001 ^a	-3.65 $\pm$ 0.09 ^c	0.97 $\pm$ 0.011 ^a	1095.23 $\pm$ 7.07 ^c	1094.28 $\pm$ 6.81 ^c
CL-11%BP	1322.57 $\pm$ 21.86 ^b	-46.24 $\pm$ 1.43 ^d	1.00 $\pm$ 0.001 ^a	-5.75 $\pm$ 0.09 ^d	0.98 $\pm$ 0.009 ^a	1291.76 $\pm$ 28.96 ^b	1292.87 $\pm$ 20.81 ^b
CL-14%BP	1513.34 $\pm$ 16.86 ^a	-51.67 $\pm$ 0.32 ^e	1.00 $\pm$ 0.001 ^a	-7.76 $\pm$ 0.08 ^e	0.98 $\pm$ 0.01 ^a	1480.65 $\pm$ 7.79 ^a	1483.41 $\pm$ 6.59 ^a

^{a-e} Different letters in the same column indicate significant differences among samples ($p < 0.05$)

CL – cake layer, BP – beetroot powder

Hardness reflects the force required to deform a product and is an important indicator of its structural integrity. The cake layer samples formulated with beetroot powder exhibited significantly higher hardness values compared to the control sample ($p < 0.05$). This increase in hardness is likely attributable to the high moisture content of these samples, as previous studies have reported a strong correlation between water content and crumb hardness [22]. Similar increases in hardness have also been

documented in other investigations of sweet baked goods [23]. In contrast, the parameters of springiness and cohesiveness were not significantly affected by the addition of beetroot powder ($p > 0.05$), suggesting that the elastic and structural properties of the crumb remained relatively stable despite formulation changes. Interestingly, the evolution of hardness, springiness, and cohesiveness in the present study diverges from the findings of Jaiswal et al. [19], who reported different trends in muffins enriched

with beetroot.

Two additional textural attributes, namely adhesiveness and stickiness, exhibited a significant decrease ($p < 0.05$) with increasing levels of beetroot powder. These properties are critical in determining the perception of the consumer regarding the product, as they describe the extent to which the cake adheres to the surfaces of the mouth during mastication [24].

While adhesiveness is a mechanical parameter that quantifies the tensile force required to overcome the attraction between the sample and a contacting surface, stickiness is a general and more qualitative descriptor of the overall tendency of a substance to cling [25]. Notably, the control sample containing the synthetic colorant Ponceau 4R displayed the lowest levels of both adhesiveness and stickiness. In contrast, the formulation containing 14% beetroot powder demonstrated markedly higher negative values for these parameters, indicating potential interaction with the surfaces of the mouth.

This increase may be associated with the hygroscopic nature of beetroot constituents, including its dietary fiber and sugar content, which could alter water distribution and binding within the cake matrix. Gumminess

and chewiness both exhibited statistically significant increases ($p < 0.05$) in response to the progressive addition of beetroot powder. Gumminess represents the amount of energy required to disintegrate a semisolid food until it is suitable for swallowing and is calculated as the product of hardness and cohesiveness. In contrast, chewiness refers to the energy needed to chew a solid food until it is ready for swallowing and is derived from the product of gumminess and springiness. Among all samples, the control formulation (containing the synthetic colorant) recorded the lowest values for both gumminess and chewiness, whereas the cake layer sample with 14% beetroot powder exhibited the highest values.

This indicates that higher levels of beetroot powder incorporation result in firmer cake structures that require greater masticatory effort. A similar trend was observed by Holovko et al. [26], who reported an increase in chewiness in sponge cakes as the beetroot powder content increased from 5% to 15%. Changes in color parameters determined by the addition of beetroot powder in the composition of the cake layer are shown in Table 4.

Color variation can also be observed in Figure 1.

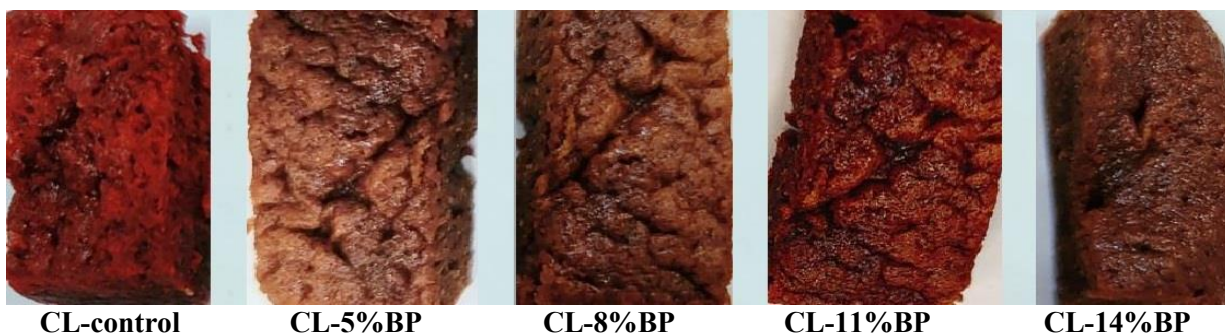


Fig. 1. Color variation of the cake layer samples

Table 4.

Color parameters of cake layer samples. Mean values $\pm$ standard deviation.

Sample	L*	a*	b*	h _{ab} *	C _{ab} *
CL-control	31.99 $\pm$ 0.04 ^b	12.66 $\pm$ 0.09 ^a	4.89 $\pm$ 0.04 ^d	21.12 $\pm$ 0.01 ^c	13.56 $\pm$ 0.09 ^b
CL-5%BP	33.12 $\pm$ 1.46 ^a	8.92 $\pm$ 0.47 ^d	5.99 $\pm$ 0.61 ^c	32.83 $\pm$ 0.11 ^d	10.75 $\pm$ 0.73 ^d
CL-8%BP	31.02 $\pm$ 0.32 ^c	9.83 $\pm$ 0.44 ^c	6.51 $\pm$ 0.49 ^b	35.10 $\pm$ 0.90 ^c	11.58 $\pm$ 0.64 ^c
CL-11%BP	29.82 $\pm$ 1.02 ^c	11.41 $\pm$ 0.28 ^b	8.93 $\pm$ 0.01 ^a	38.06 $\pm$ 0.63 ^b	14.49 $\pm$ 0.23 ^a
CL-14%BP	30.34 $\pm$ 0.04 ^d	8.79 $\pm$ 0.02 ^d	8.10 $\pm$ 0.06 ^a	42.67 $\pm$ 0.13 ^a	11.95 $\pm$ 0.04 ^c

^{a-c} Different letters in the same column indicate significant differences among samples ($p < 0.05$)

CL – cake layer, BP – beetroot powder

Among all samples, the control cake layer sample formulation exhibited the highest values for both redness (a^*) and yellowness (b^*). In comparison, cake layer samples incorporating beetroot powder demonstrated decreases in lightness (L^*), and increases in redness (a^*) and yellowness (b^*) with rising levels of beetroot powder up to 11% (w/w). Beyond this concentration, a slight increase in lightness was noted, accompanied by a decrease in redness and yellowness. This trend, particularly the enhancement in redness and yellowness, aligns with the findings reported by Jaiswal et al. [19]. Based

on the values of the color parameters, the sample containing 11% beetroot powder most closely approximated the redness of the control sample formulated with the synthetic colorant Ponceau 4R (E124). Statistically significant differences ($p < 0.05$) were observed across all samples.

3.2. Betalain content, total phenolic content, and antioxidant activity

The total betalain content (TBC), total phenolic content (TPC) and the antioxidant activity (RSA – radical scavenging activity) determined for the cake layer samples in shown in Table 5.

Table 5.

Total betalain content, total phenolic content, and antioxidant activity of cake layer samples. Mean values $\pm$ standard deviation.

Sample	TBC (mg/100 g)	TPC (mg/100 g)	RSA (%)
CL-control	0	8.83 $\pm$ 0.04 ^c	34.89 $\pm$ 0.07 ^c
CL-5%BP	29.30 $\pm$ 0.08 ^d	53.64 $\pm$ 0.06 ^d	63.55 $\pm$ 0.14 ^d
CL-8%BP	37.60 $\pm$ 0.03 ^c	91.29 $\pm$ 0.10 ^c	71.03 $\pm$ 0.64 ^c
CL-11%BP	41.58 $\pm$ 0.51 ^b	122.90 $\pm$ 0.08 ^b	77.01 $\pm$ 1.14 ^b
CL-14%BP	62.33 $\pm$ 1.21 ^a	207.34 $\pm$ 0.14 ^a	83.38 $\pm$ 0.36 ^a

^{a-c} Different letters in the same column indicate significant differences among samples ($p < 0.05$)

TBC – total betalain content, TPC – total phenolic content, RSA – radical scavenging activity; CL – cake layer, BP – beetroot powder

Betalains are water-soluble, nitrogen-containing pigments predominantly found in beetroot, where they occur in substantial concentrations. These pigments are classified into two main subclasses: betacyanins, which impart red to violet hues, and betaxanthins, responsible for yellow to orange coloration [27]. In the control cake layer sample, which

did not contain beetroot powder, the total betalain content was recorded as 0 mg/100 g. However, the extract from this sample still exhibited absorbance at the characteristic wavelengths used for betalain quantification. This apparent discrepancy is attributed to the spectral overlap between betalains and the synthetic colorant Ponceau 4R (E124), which

shares similar absorption spectra, thus potentially interfering with spectrophotometric determination [28]. In contrast, the cake layer samples formulated with increasing levels of beetroot powder exhibited progressively higher total betalain content, with statistically significant differences observed among all samples ($p < 0.05$). This dose-dependent increase confirms the effective incorporation of natural pigments through beetroot powder addition. Furthermore, the total betalain concentrations obtained in this study align with previously reported values for other sweet bakery products, such as muffins enriched with beetroot-derived pigments [19], thereby supporting the potential of beetroot powder as a natural colorant and functional ingredient in cake formulations.

In addition to betalains, beetroot powder serves as a valuable source of phenolic compounds, which are well known for their antioxidant properties. The predominant phenolic constituents in beetroot powder include flavonoids, phenolic amides, and ferulic acid conjugates, which contribute significantly to its bioactive profile [29,30]. In the present study, the total phenolic content (TPC) was found to be lowest in the control sample, which contained no beetroot powder, with a value of 8.83 mg GAE/100 g. As the proportion of beetroot powder incorporated into the cake layer formulations increased, a corresponding and statistically significant ($p < 0.05$) increase in TPC was observed.

The highest TPC value, 207.37 mg GAE/100 g, was recorded in the cake layer sample containing 14% beetroot powder. Although the control sample lacked beetroot-derived phenolics, the minor amount of total phenolics detected is likely attributable to the presence of cocoa powder, a known source of antioxidant compounds, included in the formulation [31]. The phenolic contents observed in the beetroot-enriched samples

were consistent with values previously reported in the literature for similar bakery products containing beetroot powder [29]. These findings further support the functional potential of beetroot powder not only as a natural colorant but also as an ingredient capable of enhancing the antioxidant capacity of cake layer formulations.

Alongside the observed increase in phenolic content, the addition of beetroot powder also markedly improved the antioxidant capacity of the cake layer samples, as determined by the DPPH radical scavenging activity (RSA) assay. The RSA values increased proportionally with the level of beetroot powder added to the formulations, indicating a strong correlation between beetroot enrichment and antioxidant activity. The control sample, which contained no beetroot powder, exhibited the lowest RSA value of 34.89%.

This baseline antioxidant activity is likely attributable to other minor antioxidant components present in the formulation, such as cocoa powder. Upon the addition of beetroot powder, a substantial increase in RSA was observed: the sample containing 5% beetroot powder demonstrated an RSA of 63.55%, while the sample formulated with 14% beetroot powder achieved the highest antioxidant activity, with an RSA of 83.38%. These differences were statistically significant ($p < 0.05$), confirming the impact of beetroot powder on the antioxidant potential of the cake layers. This marked enhancement in radical scavenging activity can be attributed to the presence of bioactive compounds in beetroot, particularly betalains and various phenolics, which are recognized for their strong antioxidant properties [30]. These results are in agreement with findings from previous studies that reported increased antioxidant activity in bakery products enriched with beetroot powder [29]. The progressive increase in RSA with beetroot concentration underscores the potential of

beetroot powder not only as a natural colorant but also as a functional ingredient that contributes to the nutritional and oxidative stability of bakery products.

3.3. Sensory properties

The results of the sensory evaluation, which assessed the color, taste, flavor, texture, mouthfeel, appearance, and overall acceptability of the cake layer samples, are presented in Figure 2. The values shown represent the mean scores obtained from evaluations by 30 untrained panelists. Notably, the cake layer sample formulated with 11% beetroot powder received a color score comparable to that of the control sample containing synthetic colorant, which aligns with the findings of instrumental color measurements. In terms of appearance, the control sample and those containing 11% and 14% beetroot powder were rated more favorably than the samples with lower beetroot powder levels (5% and 8%).

The control sample was the only one that received a high overall acceptability score. Attributes such as texture, flavor, and mouthfeel were more positively perceived in the samples containing less than 10% beetroot powder. These findings are consistent with the observations of Alshehry [27], who reported lower consumer ratings for taste and texture in muffins supplemented with beetroot powder at concentrations ranging from 2.5% to 10%. In the present study, the lower scores for texture and mouthfeel in samples with higher beetroot powder concentrations may be attributed to increased moisture content and stickiness, which can negatively influence the structural integrity and sensory experience of the cake. Furthermore, the decrease in taste scores with higher beetroot content is likely due to the intensification of the characteristic earthy aroma associated with beetroot. This aroma, often perceived as undesirable by consumers, is primarily caused by the presence of volatile

compounds such as geosmin and pyrazine derivatives. Similar sensory impacts of these compounds have been documented in previous studies [29,32].

Overall, while moderate levels of beetroot powder can maintain or enhance certain sensory attributes, higher concentrations may compromise consumer acceptability due to textural and flavor-related changes.

3.4. Principal component analysis

Principal component analysis (PCA) was conducted to assess the impact of incorporating beetroot powder at varying levels on the physicochemical characteristics and sensory attributes of cake layer samples. Figure 3 presents the bi-plot (PCA scores and loading plots), illustrating the distribution of the samples and the contribution of the physicochemical and sensory variables, respectively. The first principal component (PC1) accounted for 77.71% of the total variance, while the second principal component (PC2) explained an additional 15.92%, together accounting 93.63% of the overall variability in the dataset. The PCA score plot revealed a clear separation between the control sample and those containing beetroot powder, primarily due to differences in redness and selected sensory attributes, including color, adhesiveness, taste, and overall acceptability. The sample formulated with 5% beetroot powder also exhibited a distinct positioning, ascribed to its high lightness values. Samples containing increasing concentrations of beetroot powder were clustered within the same quadrant, with their separation from other samples explained by specific loading vectors. These distinguishing variables included weight loss during baking, moisture content, total betalain content, total phenolic content, antioxidant activity (RSA), as well as several textural parameters (hardness, cohesiveness, chewiness, and gumminess), and selected color parameters (b^* and h^*_{ab}).

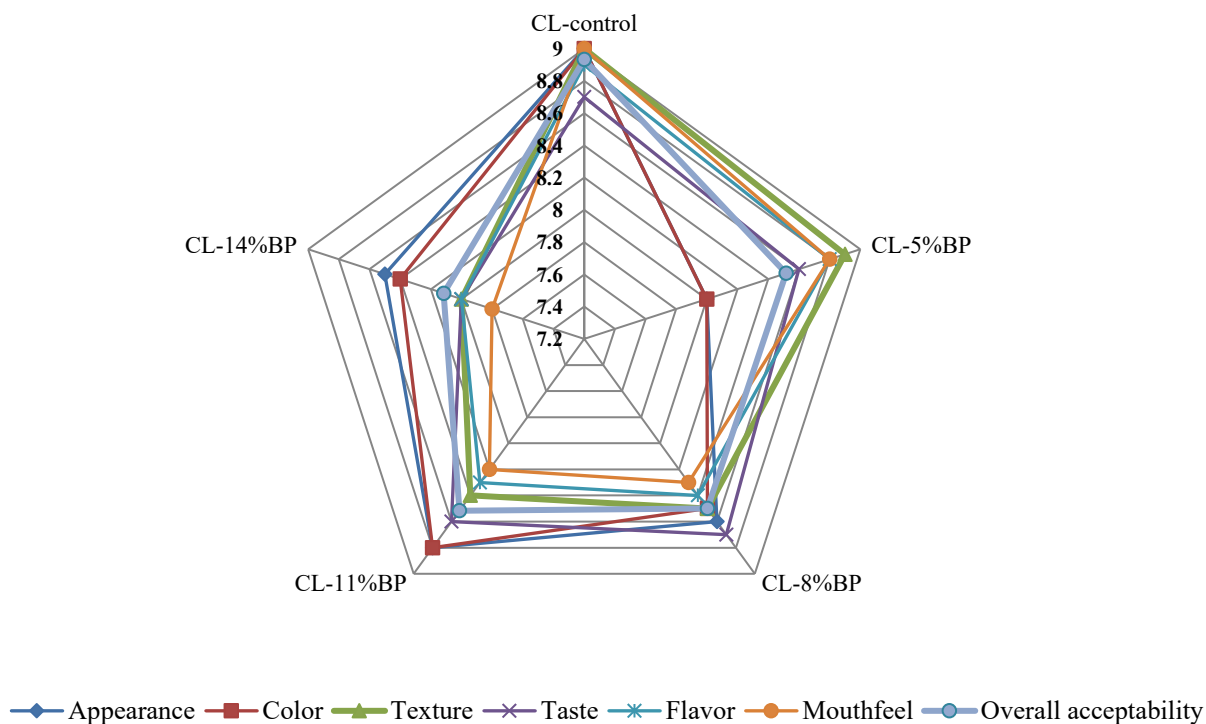


Fig. 2. Sensory evaluation of cake layer samples

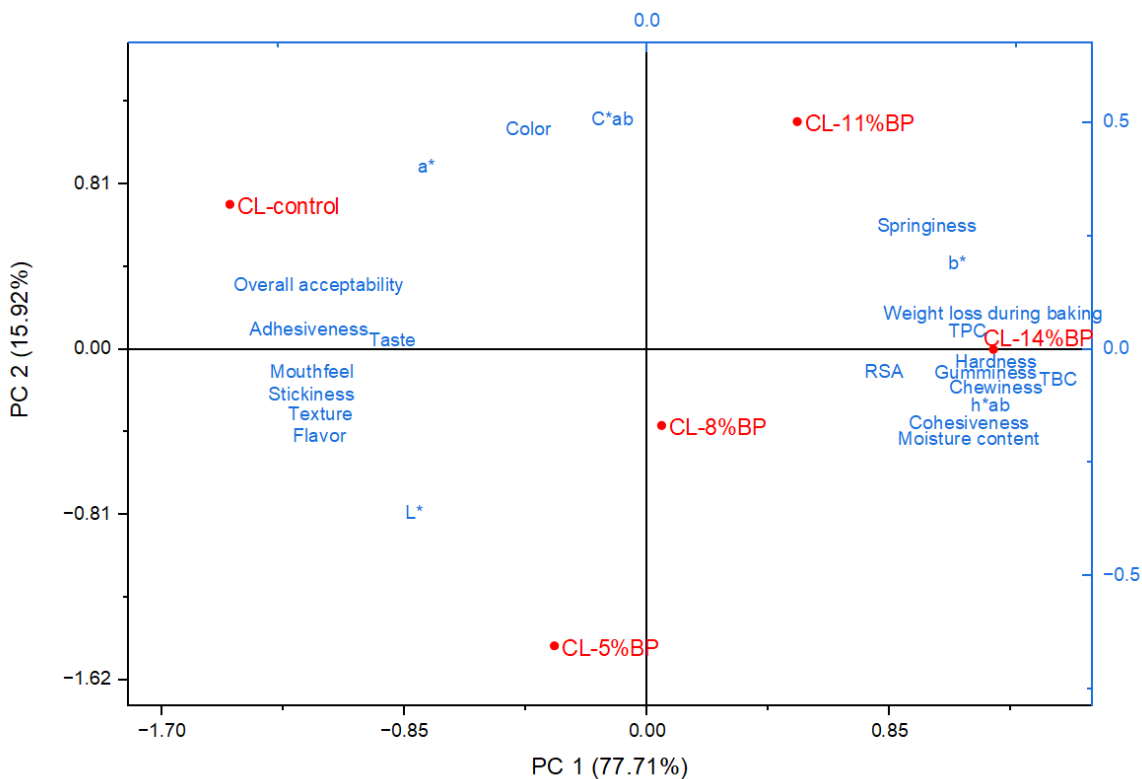


Fig. 3. Principal component bi-plot showing scores of cake layer samples (in red) and loadings of physicochemical and sensory parameters (in blue)

Notably, the sample containing 11% beetroot powder was positioned closer to the control sample, likely due to the influence of the a^* and C^*_{ab} color parameters.

4. Conclusions

This study evaluated the effects of substituting the synthetic colorant Ponceau 4R (E124) with beetroot powder, incorporated at levels ranging from 5% to 14%, on the technological characteristics, sensory attributes, and antioxidant properties of cake layer samples. Antioxidant potential was assessed through the quantification of total betalain content, total phenolic content, and overall antioxidant activity. The inclusion of beetroot powder in the cake formulations resulted in increased weight loss during baking and higher moisture content in the final product. Increasing levels of beetroot powder led to elevated values for hardness, gumminess, and chewiness, while simultaneously reducing the values for adhesiveness and stickiness. These changes resulted in a denser, more cohesive crumb structure that required greater masticatory effort. Among all samples, the cake layer containing 11% beetroot powder exhibited a redness value most comparable to the control sample, suggesting a suitable visual match to the synthetic colorant. Antioxidant activity increased proportionally with beetroot powder concentration, an effect attributed to the elevated levels of betalains and phenolic compounds introduced through its incorporation. Based on sensory evaluation results, formulations containing up to 11% beetroot powder were favorably received, maintaining acceptable sensory quality while enhancing functional properties. Therefore, the use of beetroot powder at this level is recommended for consumers seeking clean-label or health-oriented bakery products with improved antioxidant potential.

5. References

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