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RAW AND COOKED BEETROOT (*BETA VULGARIS*) AS NATURAL PIGMENT SOURCES: EFFECTS ON COLOR AND SENSORY ATTRIBUTES OF SPONGE CAKES

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ABSTRACT

The growing concern over the potential health risks associated with synthetic food colorants has spurred the search for natural alternatives. This study evaluated the feasibility of using beetroot (*Beta vulgaris*) pigments—derived from both raw and cooked beets—as natural colorants in sponge cakes. The primary objective was to assess the impact of beetroot pigments on the sensory properties and overall consumer acceptance of the cakes. Two formulations were developed: one using raw beetroot pigment and another using cooked beetroot pigment, both compared to a control sample containing synthetic red colorant. For the sensory evaluation, 52 untrained panelists from the Culinary Arts and Tourism Administration programs at the Benemérita Universidad Autónoma de Puebla assessed the cakes' color, aroma, texture, flavor and overall acceptability using a structured 5-point hedonic scale. A blind-coded presentation ensured unbiased evaluations. Data were analyzed using one-way Analysis of variance (ANOVA), followed by Tukey's test at a 5% significance level, employing Minitab version 14. The results indicated that the use of beetroot as a natural colorant did not significantly affect the cakes' texture and flavor ($p > 0.05$), ensuring these key sensory attributes remained comparable to those of the control. However, significant differences were found in color, aroma and overall acceptability ($p < 0.05$). Cakes prepared with raw beetroot (M1) exhibited a more vibrant red color and stronger beetroot aroma compared to those made with cooked beetroot (M2), where thermal processing led to partial betalain degradation, resulting in a paler, pinkish hue. Despite these variations, both beetroot-colored cakes achieved high levels of consumer acceptance: 74.2% for raw beetroot and 75.6% for cooked beetroot. These findings underscore the potential of raw beetroot pigments as viable natural alternatives to synthetic colorants in bakery products, preserving color intensity and sensory quality. The study's novelty lies in its comparative analysis of raw versus cooked beetroot pigments, providing insights into how different processing methods affect sensory outcomes in baked goods. This contributes valuable knowledge to food manufacturers aiming to meet the rising demand for clean-label, health-oriented products. Future research should expand on these findings by incorporating instrumental analyses of color and texture, exploring different processing conditions, and testing broader consumer demographics to validate the application of beetroot pigments across diverse bakery formulations.

Key words: beetroot, bakery, betalain, sensory evaluation, food colorant, general acceptability

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INTRODUCTION

According to Godefroidt *et al*. [1], the primary ingredients of a sponge cake are: flour, egg and sugar. During its preparation, other ingredients can be added to provide different nuances and flavors, as well as artificial colorings to add tones to the cake. However, the effects of artificial colorings on human health can be negative, since according to the Federal Consumer Protection Agency [2], there are studies suggesting that constant consumption of these substances may be related to issues such as hyperactivity, allergies or asthma. According to Jiménez *et al.* [3], the food industry is adapting to the changing needs of consumers, this demand for products associated with health and well-being has led to a review of production practices, and thus companies seek to modify raw materials and reformulate products to make them healthier. In this regard, the inclusion of natural ingredients, such as beetroot, as an alternative to artificial colorings has become a key strategy. However, the production of healthier foods without reducing their sensory quality, poses a major challenge, as sensory quality directly influences product acceptance by consumers and therefore its market success.

Silva *et al.* [4] stated that color is one of the most relevant organoleptic attributes and directly affects the acceptance and selection of food. However, since food pigments are usually unstable and change during processing, to maintain or restore color uniformity, colorings are added to food products around the world. The Food and Drug Administration [5] divides approved colorings into those subject to certification (artificial or synthetic) and those exempt from certification (natural). "Synthetic colorings are widely used in the food industry for their stability and low cost. The most employed are azo dyes, accounting for 65% of the market. They offer bright colors and withstand various conditions of the food processing" Dey *et al*. [6]. However, the risks or harm they may cause to the consumer's body need to be considered [2].

According to Silvia *et al*. [4], synthetic colorings have been associated with a variety of side effects and toxicities, both short- and long-term. These include allergic reactions as well as behavioral and neurocognitive changes. The health risks posed by certain synthetic additives can be harmful at metabolic, physiological and toxicological levels, including potential genotoxic and carcinogenic effects, as well as symptoms such as hives, asthma, nausea, eczema, bronchitis, bronchospasm, headaches, reduced blood clotting and hyperactivity.

On the other hand, natural colorings used in various foods have health benefits, such as antioxidant properties and the reduction of chronic and degenerative diseases, including coronary diseases and hypertension, according to Vergara *et al.* [7]. According to Rodríguez-Mena *et al*. [8], natural pigments are usually obtained from fruits and vegetables, and it has been proven that they contribute positively to health by reducing the risk of diseases such as type 1 diabetes, obesity and heart disorders. "Natural resources can provide a wide variety of colors for the food industry. For example, carmine, bixin, anthocyanins, curcumin and betalains are natural colorings widely used in food products" [9]. Betalains, carotenoids, phycocyanins, and anthocyanins are important food colorings used in the food industry, with documented biological effects, particularly in the prevention and management of chronic diseases such as diabetes, obesity and cardiovascular diseases [10]. "Beetroot naturally has a color that ranges from red to deep purple, a coloration attributed to the pigments naturally found in its root" [11].

According to Flores *et al.* [12], the main source of concentrated or powdered betalains on the market comes from the beetroot. Betalains are nitrogen-containing and water-soluble pigments that can be divided into two groups: reddish-violet betacyanins and yellow betaxanthins [10]. In recent years, the health-promoting properties of these elements have been studied, including antioxidant activity, anti-diabetic effects, anti-inflammatory properties and anticancer potential [12]. These pigments can provide micronutrients like phosphorus, zinc, vitamin C, vitamin B6, magnesium and potassium, which improve the health of those who consume them [13]. The natural coloring properties of betalains and their lack of toxicity suggest extensive use of betalains as additives in the food industry. Therefore, the specific interest in beetroot has emerged due to its antioxidant compounds, making it one of the ten vegetables with the highest antioxidant capacity, due to its high betalain content [14].

Red beet is consumed mainly after processing, in the course of which the betalain structure and antioxidant properties of this raw material are changed [15]. Studies show that betalains can degrade at temperatures above 50–60°C, especially during prolonged treatments or with high oxygen exposure. However, this does not always indicate a loss of color, and their thermal behavior varies depending on the medium in which they are present [16]. This is why two samples were prepared: the first used beetroot without thermal treatment, while the second used previously cooked beetroot, in order to obtain different levels of pigmentation in the sponge cake, achieving two different tones in each sample.

On the other hand, according to Costell [17], the first stimuli received from food are visual; they provide information about size, shape and color. Usually, when consuming food, they enjoy a wide range of colors that immediately attract the consumer's attention and stimulate appetite. Because of this, colorings play an important role in improving the physical appearance of food. Acceptance is the result of the interaction between the food and the person at a given time. On one hand, the food's properties influence the willingness to accept or reject it, such as its chemical composition or physical attributes. On the other hand, each consumer's characteristics whether genetic, physiological or sociological play a role.

The use of a natural beet-based pigment in sponge cake preparation represents a change. Therefore, according to Ramírez-Navas [18], when developing or modifying a food product, it is important to consider what consumers like, dislike and their preferences. This increases the likelihood of a favorable response, especially for the benefit of producers, manufacturers and consumers. Preparing a sponge cake with beetroot as a coloring agent could alter its sensory characteristics—color, texture and even flavor—which is why it is important to measure the level of consumer acceptance by analyzing their reaction to the product through sensory evaluation. Stone and Sidel [19] define sensory evaluation as the scientific discipline used to evoke, measure, analyze and interpret reactions to products perceived through the senses of sight, smell, touch, taste and hearing.

Based on this, the objective of the study was to evaluate the impact of substituting artificial red colorants with beetroot on sensory and some physicochemical properties of the final product. Two sponge cake formulations were developed: one using raw beetroot and the other using cooked beetroot, to compare differences in color intensity, texture, flavor and overall consumer acceptance through a structured sensory evaluation, in order to analyze consumer responses and determine the feasibility of beetroot as a natural and healthy alternative to synthetic colorants. This study seeks to contribute to the development of healthier food products that maintain consumer appeal through the integration of natural ingredients.

MATERIALS AND METHODS

This mixed and cross-sectional study with exploratory and descriptive scope employed both documentary and field techniques. For the preparation of the cakes for sensory evaluation, the ingredients were based on the recipe proposed by Godefroidt *et al*. [1]: 150 g of flour, 150 g of granulated sugar, 120 g of eggs, 70 g of vegetable oil, milk, lemon juice, baking powder, plus the respective artificial and natural colorants: red colorant, peeled raw beetroot, peeled cooked beetroot and beetroot cooking water.

In Table 1, the variation of ingredients in the formulations of cakes made with natural pigment from raw and cooked beetroot compared to the control cake is shown.

Two methods were used to obtain the natural pigment from beetroot: Method 1 (M1): Obtaining natural pigment with raw beetroot. A 366 g beetroot purchased at the Emiliano Zapata market, Puebla, Mexico, was peeled using a kitchen peeler, yielding 298 g of drained mass, which was cut into small pieces. Subsequently, 68 mL of lemon juice purchased at the same market was added to 100 mL of milk to decrease the pH, and they were mixed. The beetroot and milk with lemon juice were blended in a blender for 5 minutes until a thick mixture was obtained. Finally, 401 g of pigment mixture was obtained with a yield of 75.1%.

Method 2 (M2): Obtaining natural pigment with cooked beetroot. A 490 g beetroot purchased at the Emiliano Zapata market, Puebla, Mexico, was peeled using a kitchen peeler, yielding 425 g of drained mass. It was cut into approximately 5 cm pieces to facilitate cooking, then cooked in 1 liter of water over high heat on a stove for 20 minutes. The cooked beetroot and 50 mL of beetroot cooking water were then transferred to a blender and blended for 5 minutes until a thick mixture was obtained. Finally, 453 g of colorant mixture was obtained with a yield of 83.8%.

Yield Calculation. The yield was calculated by weighing all the ingredients used for each natural colorant and the product obtained at the end of each extraction method, using the following equation

$$\%\,yield = \frac{Product\ mass\ obtained\ (g)}{Total\ product\ mass\ (g)}\, x\ 100 \qquad (1)$$

Preparation method of the control sponge cake made with artificial colorant

Egg yolks were separated and placed in the bowl of a mixer (Kitchenaid model KSM150PSWH, USA) along with the sugar and beaten for 5 minutes at medium-high speed until a creamy mixture was obtained. The oil was added in a thin stream while continuing to beat until incorporated. Then, the previously sifted flour and baking powder were added and mixed until unified. Subsequently, 100 g of milk and 5 g of artificial red colorant were added, beating until a homogeneous mixture was obtained. The egg whites were beaten in a mixer (Kitchenaid model KSM150PSWH, USA) until they turned white and fluffy, doubling in volume, and then added to the previous mixture by hand with a silicone spatula in an enveloping motion to avoid breaking the air bubbles. The final mixture was placed in an aluminum mold for 6 cupcakes (Ecko, Mexico) with liners and baked in an oven (Rational model ICOMBI PRO XS, Germany) for 20 minutes at 180 ºC.

Preparation method of the sponge cake made with cooked beetroot pigment
Same procedure as the control cake. Variations: When incorporating the flour into the mixture, baking powder was not included. Only 50 g of milk and 68 g of lemon juice were added. The artificial colorant was replaced with 250 g of natural pigment from cooked beetroot. Obtaining 6 pieces of cupcakes.

Preparation method of the sponge cake made with Raw Beetroot
Same procedure as the control cake. Variations: When incorporating the flour into the mixture, baking powder was not included, milk and artificial colorants were omitted from the preparation and 300 g of natural pigment from raw beetroot were incorporated. Obtaining 6 pieces of cupcakes.

Table 2 shows the ingredients of the three sponge cake preparation methods, where samples 1 and 2 had variations compared to sample 3 due to the incorporation of the obtained natural pigments. In Figure 1, the sizes and colors obtained in the different cake samples can be observed.

Figure 1: Sponge cakes samples
Note: M1 is sponge cake with natural pigment with raw beetroot; M2 is sponge cake with natural pigment with cooked beetroot and M3 is control sponge cake with artificial colorant

Sensory evaluation
A sensory evaluation of the cakes was conducted with 52 untrained panelists from the Bachelor of Gastronomy and Tourism Management programs at the Benemérita Universidad Autónoma de Puebla. The number of panelists was based on the recommendations of López and Ortiz [20].

The sensory evaluation was conducted using a blind-coded sample presentation method to eliminate potential biases from the panelists. Each sponge cake sample was assigned a random three-digit numerical code, which was generated using a random number table and placed on the serving containers to ensure anonymity of the formulations. The samples were presented in a balanced and randomized order to avoid positional effects.

Panelists (n = 52), who were untrained but familiar with baked products, evaluated the samples individually in a controlled environment with standardized lighting and minimal distractions. The evaluation was carried out using a structured 5-point hedonic scale, where 1 indicated "extremely dislike" and 5 indicated "extremely like." Attributes assessed included color, aroma, texture, flavor and overall acceptability. Between samples, participants were instructed to cleanse their palates with water to minimize carryover effects, cleanse their palates and prevent confusion during the evaluation. This blind coding protocol ensured that all evaluations were based solely on sensory perception without any influence from visual.

Prior to participation, all panelists received a clear explanation of the objectives and procedures of the sensory evaluation. Each participant voluntarily agreed to take part in the study and signed an informed consent form, in accordance with ethical guidelines for research involving human subjects. The consent form specified that the evaluation would involve tasting food samples under controlled conditions, that participation was anonymous, and that panelists could withdraw at any time without penalty. The study did not involve any health risks and adhered to the ethical standards established by the institution.

Statistical Analysis

The sensory evaluation tests were analyzed and interpreted using the one-way Analysis of variance (ANOVA) method. Following the ANOVA, when significant differences were detected, a Tukey's Honestly Significant Difference (HSD) post-hoc test was applied. All statistical analyses were performed using Minitab® software (version 14), and results were interpreted within a 95% confidence interval ($p<0.05$).

RESULTS AND DISCUSSION

Figure 2 shows the analysis of the color of the cakes. There was a statistically significant difference between sample M2 and the control sample M3. This difference is because in the formulation of the natural pigment used as colorant in M2, boiled beetroot was used, mixed with its cooking water. This method reduced the amount of natural pigment added to the mixture so as not to affect its consistency, resulting

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in a less intense color due to the degradation of betalains caused by direct thermal treatment. On the other hand, no notable differences were observed between samples M1 and M3, this suggests that the natural pigment used to color raw beets does not lose its color intensity with temperature. This is because using beetroot without prior thermal processing preserves the betalains, resulting in less degradation during baking compared to sample M2. Regarding the differences found between samples M1 and M2, a color degradation in M2 compared to M1 was observed, this was because in M2 the beetroot was used boiled, which resulted in a less intense color due to the degradation of betalains, leading to pinkish tones. In contrast, M1 used raw beetroot, thus preserving more pigmentation and resulting in a red color.

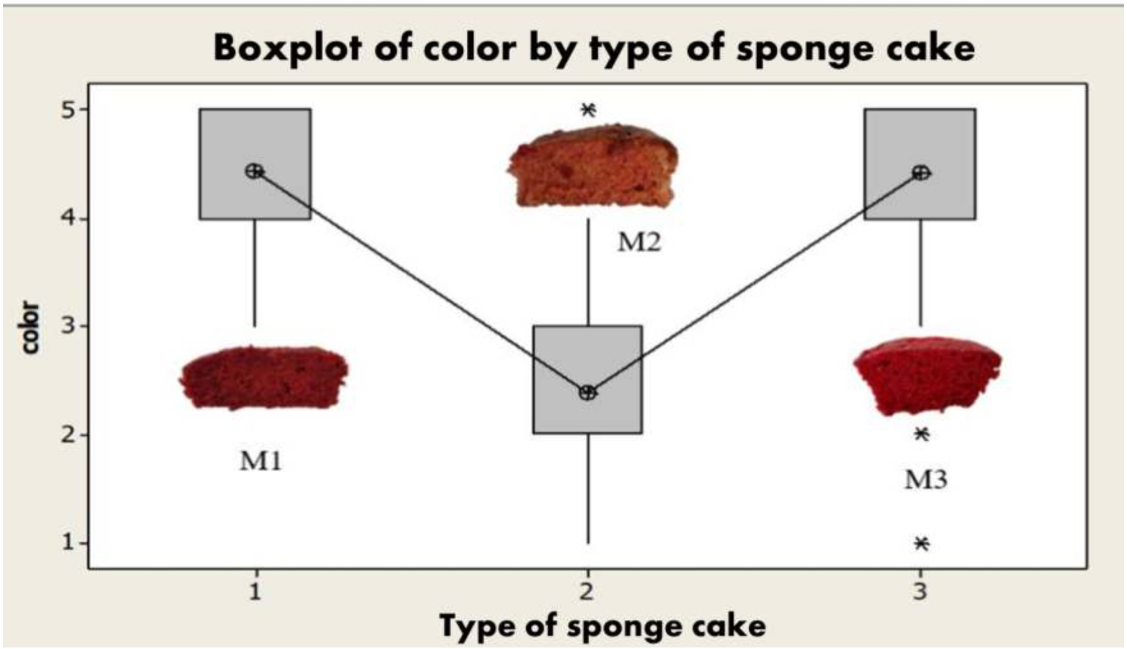

Figure 2: Color boxplot

Note: M1 is sponge cake with natural pigment with raw beetroot, M2 is sponge cake with natural pigment with cooked beetroot and M3 is control sponge cake with artificial colorant. The x-axis corresponds to the type of sponge cake, and the y-axis corresponds to the scores given by the judges in the sensory evaluation

Figure 3 shows the flavor analysis of the samples and shows a significant difference between all three, with a notable difference between M1 and M3. This is because the formulation of M1 used the natural pigment from the raw beetroot as a colorant, thus incorporating a higher percentage of beetroot in the mixture, which accentuated its aroma. On the other hand, M2 had less beetroot aroma due to its formulation, although the difference from M3 was still significant. When raw beetroot is used in bread preparation, a greater amount of betalains may remain intact, prolonging the

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persistence of its aroma. In addition, heat can potentially alter the chemical structure of betalains, which may also affect their longevity and persistence in bread aroma.

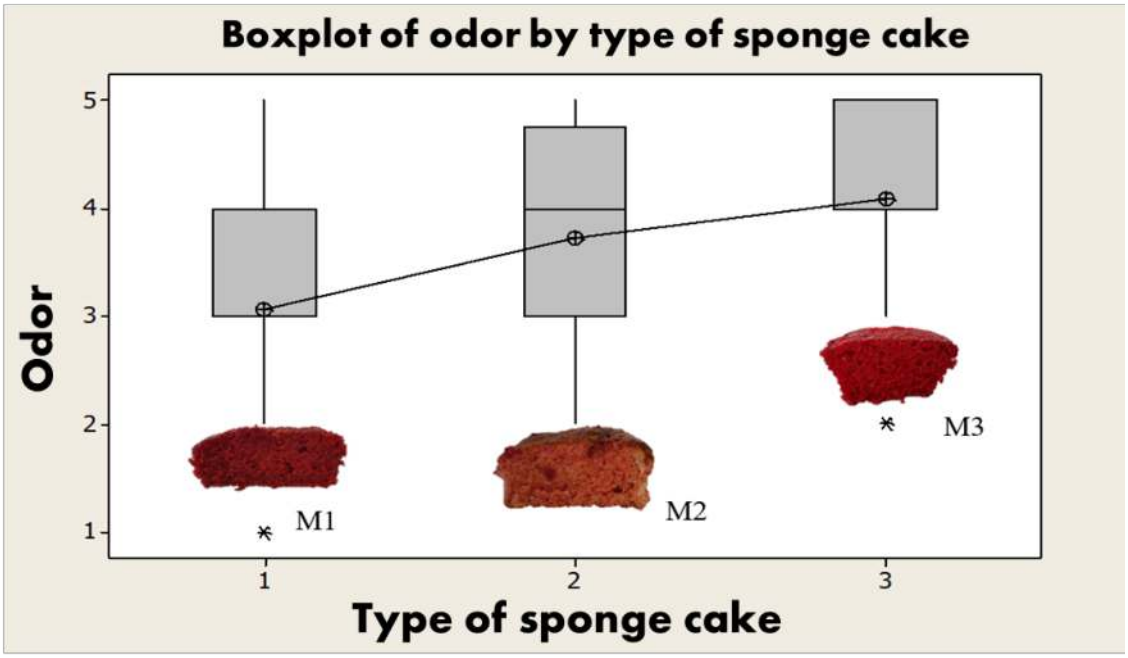

Figure 3: Odor boxplot

Note: M1 is sponge cake with natural pigment with raw beetroot, M2 is sponge cake with natural pigment with cooked beetroot and M3 is control sponge cake with artificial colorant

In Table 3, the ANOVA performed on the sensory attribute of texture of the cakes shows that no significant difference ($p > 0.05$) was observed between M1, M2 and M3. This indicates that the beetroot natural pigment formulations and the production process did not significantly alter the texture compared to the control. This is a positive result from the point of view of product production and acceptance.

In Table 4, the ANOVA performed on the taste of the cakes shows that no significant difference was found between samples M1, M2 and M3. This indicates that the use of beetroot as a colorant does not affect the taste in a way that is perceptible to the consumer, which is positive for the acceptance of the product. However, the difference between M1 and M2 using beetroot as a colorant is not statistically significant, indicating that the flavor between both samples does not change based on the method of obtaining the natural pigment used as a colorant. The overall acceptability of M1 was 74.2%, while that of M2 was 75.6%.

Studies conducted by authors such as Vickers *et al*. [21], Lucak *et al*. [22], and Amato [23] among others, recommend the use of water as a palate cleanser in these evaluations, as it is essential to select a cleanser that does not interfere with the sensory characteristics of the product being evaluated. The use of salty crackers or bread as palate cleansers in this type of product may introduce flavors or textures similar to those of the bread itself, which could affect the panelists' perception.

Figure 4 depicts the results of the overall acceptability analysis of the cakes, which revealed a statistically significant difference between M1 and M2 in comparison to M3.

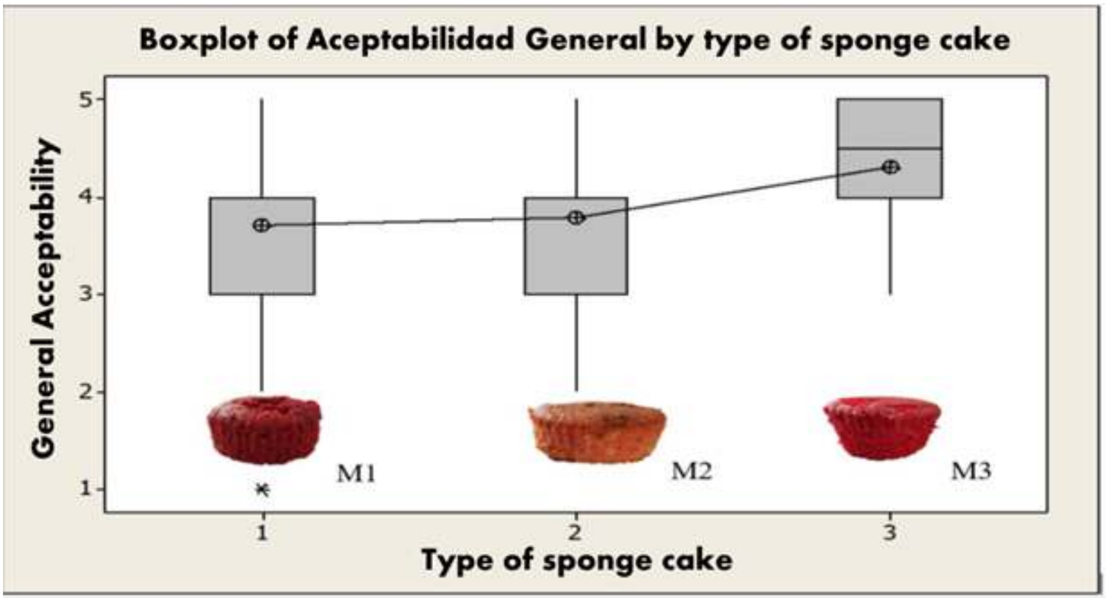

Figure 4: General acceptability Boxplot

Note: M1 is sponge cake with natural pigment with raw beetroot, M2 is sponge cake with natural pigment with cooked beetroot and M3 is control sponge cake with artificial colorant

The overall acceptability was calculated by computing the mean score assigned by the panelists for the attribute "overall acceptability" on a 5-point hedonic scale, where 1 = "extremely dislike" and 5 = "extremely like." Each of the 52 untrained panelists evaluated the three sponge cake samples (M1, M2 and M3), and their individual ratings were recorded. This approach allowed for the identification of the samples that were most preferred by the panel in terms of overall perception.

Similarly, an analysis of crumb size was conducted by cutting the samples transversely to obtain two halves of each. These halves were scanned using a Brother

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scanner (MFC-J4620DW, Mexico), connected to a MacBook Air PC (13-inch, 2017, United States). Images were saved as bitmap files with a resolution of 300 DPI. A field of view (FOV) of 236 x 236 pixels was selected and cut from the center, then processed using ImageJ software (National Institutes of Health, USA). Figure 5 displays a grayscale image of the final structure with different natural colorants and pigments used. It shows that M1 exhibited a more compact crumb (with fewer pores) compared to M3, while M2 also showed a more open crumb relative to M3.

As Rodríguez-Mena *et al*. [8] notes, natural dyes are derived from pigments obtained from natural resources such as vegetables. When incorporated into food or beverages, they provide an authentic or attractive hue and also offer health benefits. In this study, beetroot was employed as a natural dye in the preparation of cakes to assess its sensory attributes. It was observed that substituting the artificial red dye with the natural pigment derived from raw beetroot did not significantly affect the color of the cake. Accordingly, as posited by Bonilla [24], betalains may serve as a viable substitute for synthetic dyes in the food industry, given their extensive color spectrum.

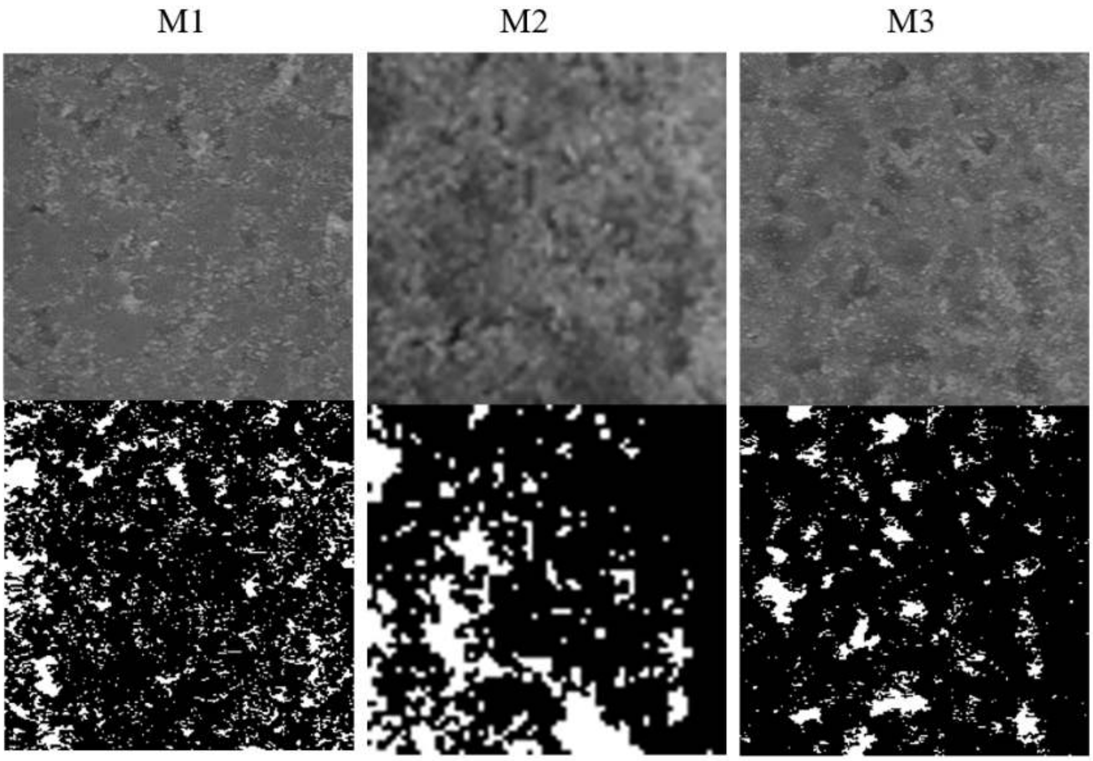

Figure 5: Grayscale of the final structure of the sponge cakes made with the different pigments

Note: The sponge cake samples were obtained from lateral cuts of each sample. The crumb images were scanned and analyzed using ImageJ software

The degradation of betalains depends on temperature and follows first-order reaction kinetics, with pH playing a significant role. Heating betacyanin solutions results in a gradual reduction of the characteristic red color of this pigment, accompanied by browning. In the case of whole or chopped baked beetroot, there are factors that modulate thermal degradation, such as the cellular structure, whose plant matrix can protect betalains by limiting their exposure to oxygen and direct heat. This creates a microenvironment that reduces the degradation rate [25]. On the other hand, a gradual reduction of the pigment due to heating can be observed in the color intensity of the coloring used in M2, where the beetroot was cooked, thus increasing its temperature and progressively reducing the red pigment. The color difference observed between M2 and the other samples was statistically significant.

Similarly, betalain pigments are stable within a pH range of 3.5 to 7.0, which is why lemon juice was added in the preparation of M2. Betalains show greater stability in foods or model systems with low moisture content and low water activity (aw), since water is less available for chemical reactions to occur [26]. Consequently, sample M1, prepared with natural pigment from raw beetroot, showed superior stability due to its formulation without additional moisture factors beyond milk. In contrast, M2, which included water in addition to milk, exhibited less stable pigmentation.

Another factor is the time and type of thermal treatment; baking, unlike boiling or frying, involves less direct contact with media that promote degradation, such as water or oil. On the other hand, a high initial concentration can still result in a noticeable coloration, even if part of the betalains degrades, due to the residual amount remaining. Additionally, sensory perception does not depend solely on the absolute concentration of betalains, but also on how they are visually distributed within the sample, their interaction with other molecules, and the lighting conditions of the environment [16].

Therefore, it can be stated that betalains do degrade with heat, but baked beetroot retains its color. It is important to recognize that the degradation is partial and medium-dependent, and that structural protection mechanisms exist. As outlined by Bonilla [24], among the colorants derived from beetroot is geosmin, which imparts a distinctive flavor when utilized in the food industry. This restricts its use in certain products. However, the results of the current research regarding the flavor of the cakes indicated that there was no significant difference between the samples prepared with beetroot and the control sample. Geosmin is a volatile organic compound found in soil and certain microorganisms. It is responsible for the earthy and fresh smell commonly associated with beetroot.

The evaluation of color, flavor and texture revealed that the use of beetroot as a natural pigment can be successfully incorporated into baked products without compromising sensory quality. In terms of color, cakes made with raw beetroot (M1) retained a vibrant red hue comparable to the control, while pre-cooked beetroot (M2) led to a noticeable degradation in color intensity due to the thermal sensitivity of betalains. Flavor analysis showed no significant differences among the samples, indicating that neither the earthy notes from geosmin nor the method of pigment extraction had a perceptible impact on taste. As for texture, although sensory evaluation did not detect changes, image analysis showed structural differences, with M1 exhibiting a more compact crumb and M2 showing increased porosity, likely due to variations in moisture content. Collectively, these results affirm that raw beetroot is more effective for color retention and crumb stability, while maintaining desirable flavor and texture properties.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The objective of the present study was to determine the overall acceptance and sensory properties of two cakes made with beetroot as a colorant through sensory evaluation. Based on the information gathered, the sensory characteristics that did not show significant differences were texture and flavor. However, significant differences were found in color, aroma and overall acceptability. The overall acceptability was 74.2% for the cake made with natural pigment from raw beetroot and 75.6% for the cake made with natural pigment from cooked beetroot.

Despite adding lemon juice to M2 (sponge cake with natural pigment with cooked beetroot), the color fixation in the cake was not achieved, which was a limiting factor for its acceptance in terms of color. Nonetheless, the rest of the attributes maintained a high level of consumer satisfaction.

The novelty of this study lies in the comparative sensory evaluation of sponge cakes using beetroot pigments derived from both raw and thermally processed beetroot, which has been rarely explored in prior literature. While several studies have investigated the color stability of beetroot pigments in general, few have focused specifically on how different beetroot preparations (raw vs. cooked) affect not only color but also overall consumer acceptability in baked goods.

This work contributes to the field by providing evidence that beetroot pigments can retain desirable sensory qualities even after baking, particularly when derived from raw beetroot. Furthermore, the rationale behind the study is grounded in the increasing consumer demand for clean-label products, free from synthetic additives. By offering a viable, health-oriented, and naturally sourced colorant, the study supports the development of bakery formulations that meet both regulatory and sensory quality expectations.

It is also important to emphasize that thermal degradation of betalains is a concern for food scientists and manufacturers; hence, comparing raw vs. cooked beetroot usage offers practical insights into color retention strategies during baking. This makes the findings not only academically relevant but also industrially applicable.
Practical relevance of the study to bakers consist of the use of beetroot as a natural colorant in food production in order to have a healthier alternative to synthetic colorants, ensuring the preservation of attributes such as texture and flavor for consumer acceptability, however, future lines of research should focus on the development of a natural colorant that preserves the rest of the sensory attributes with different kind of vegetables.

Future research should focus on expanding the sensory evaluation to a larger and more diverse panel, including trained assessors and consumer groups from different demographic backgrounds to validate the results more broadly. Additionally, instrumental color measurements (for example CIELAB system) and texture profile analysis (TPA) could be incorporated to complement the sensory data and provide a more comprehensive understanding of the physicochemical transformations associated with beetroot pigmentation during baking.

Further studies should also evaluate the stability of betalains under different processing conditions (for example varying time-temperature combinations, pH adjustments and packaging atmospheres) to optimize formulation strategies for bakery applications. Lastly, the development of natural colorant blends using beetroot combined with other plant-based pigments could be explored to enhance visual appeal and functional benefits in a broader range of food matrices.

Conflict of interest
The authors declare that they have no conflicts of interest.

Table 1: Ingredients for the control sponge cake and those made with beetroot (M1 and M2)

Ingredients	M1 (Sample with raw beetroot)	M2 (Sample with cooked beetroot)	M3 (Control sample with artificial red colorant)
Milk	100 g	50 g	100 g
Lemon juice	68 g	68 g	-
Red colorant	-	-	5 g
Baking powder	-	-	10 g
Peeled raw beetroot	298 g	-	-
Peeled cooked beetroot	-	410 g	-
Beetroot cooking water	-	50 g	-

Table 2: Ingredients used in the preparation of the sponge cakes

M3 (Control Cake)	M2 (Cake with Cooked Beetroot)	M1 (Cake with Raw Beetroot)
150 g of flour	150 g of flour	150 g of flour
150 g of granulated sugar	150 g of granulated sugar	150 g of granulated sugar
120 g of eggs	120 g of eggs	120 g of eggs
70 g of vegetable oil	70 g of vegetable oil	70 g of vegetable oil
10 g of baking powder	-	-
100 g of milk	50 g of milk	100 g of milk
5 g of red colorant	-	-
-	68 g of lemon juice	68 g of lemon juice
-	250 g of natural pigment from cooked beetroot	300 g of natural pigment from raw beetroot

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Table 3: One-way ANOVA: texture versus type of bread

Source	DF	SS	MS	F	P
Bread type	2	1.167	0.583	0.75	0.476
Error	153	119.519	0.781		
Total	155	120.686			
s=0.8838		R-Sq= 0.97%		R-Sq (adj) =0.00%	

Table 4: One-way ANOVA: flavor versus type of bread

Source	DF	SS	MS	F	P
Type of bread	2	2.782	1.391	1.50	0.227
Error	153	142.212	0.929		
Total	155	144.994			
s=0.9641		R-Sq= 1.92%		R-Sq (adj) =0.64%	

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