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Evaluation of the physicochemical and sensory properties of functional sponge cake with stevia and chlorella vulgaris microalgae.

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ABSTRACT

As consumers now pay moreattention to the nutritional value of food, the food industry is focusing on re-designing traditional foods. Cake is one of the most commonly consumed foods that nutritionists recommend reducing its consumption in the diet due to its high sugar and fat content. Chlorella algae have great potential for use in human nutrition due to its high-quality protein content and its nutritional and functional properties. One of the reasons is that chlorella algae contain essential amino acids that are not found in wheat, as well as the use of the stevia plant, a compound of natural origin that has no caloric content and can be a good substitute for sucrose. In this research work, wheat flour was replaced with 2, 4 and 6% chlorella algae powder and sucrose were replaced with 25% stevia plant powder. In this study, the percentage of fat, protein, moisture, fiber, and ash of wheat flour, stevia plant powder, and chlorella algae powder were measured. Then, the properties of the cake, such as the percentage of chemical compounds, volume, antioxidant activity, total mold and yeasts counts, color indexes, Hardness, springiness, and chewiness using texture analyzer and sensory properties were evaluated. Increasing the percentage of chlorella algae and adding stevia plant powder in the cake formulation, increased the amount of ash, fat, protein, moisture, aw, antioxidant activity and volume of the cake texture, but decreased the amount of carbohydrate, the chewiness and Hardness of the cake, b* (yellow), a* (red), and L* (lightness). Springiness of the cake increased at first and did not change significantly on the 7th and 15th day. In terms of overall acceptability, the sample with 2% chlorella algae and 25% stevia plant had the highest score close to the control sample from the consumers' point of.

1- Introduction

At present, increasing attention is being paid to food safety and quality, alongside the growing consumer demand for healthier products capable of meeting nutritional requirements through the consumption of appropriate and wholesome foods. Furthermore, due to the widespread prevalence of micronutrient deficiencies in human populations, particularly during specific stages of life, the production, importation, and consumption of fortified foods have been steadily increasing. From the perspective of nutritional science, one of the most effective approaches for improving micronutrient intake and enhancing the consumption of essential nutrients on a population-wide scale, while minimizing adverse side effects, is the fortification of foods and beverages [1]. Fortification refers to the addition of one or more nutrients to the habitual diet of a target population and may take various forms, including the incorporation of vitamins, iron, folic acid, and calcium. Among the fortification strategies that have recently attracted considerable attention is algal fortification [2].

In most of these products, sugar is included as part of the formulation. However, sugar consumption is now regarded as a limiting factor for certain groups, particularly individuals with diabetes. Consequently, the development of sugar-free or reduced-sugar products has become a major focus for food manufacturers seeking to address consumer needs. Therefore, there is a clear demand for sugar substitutes that, on the one hand, enable the production of foods with sweetness comparable to that of conventional sugar-containing products and, on the other hand, impose no consumption restrictions on these groups. Most sweeteners commonly used in food products are artificial, and each has specific limitations within food formulations. Hence, the use of natural sweeteners that do not possess such adverse effects and can effectively replace sugar has become increasingly important in modern societies [3].

Today, the use of stevia as a natural-origin sweetener has attracted substantial attention from researchers and food industry specialists in many countries. Stevia is a disaccharide with a (2–1) glycosidic bond. Research findings indicate that diterpene glycosides are the principal compounds responsible for the intensely sweet taste of stevia plant extracts, with a sweetness estimated to be approximately 250–300 times greater than that of sucrose [4], although a slight bitter aftertaste has also been reported. Since stevia sugars, unlike sugar alcohols, remain stable at elevated temperatures and under baking conditions without losing their sweetening properties, they may be considered suitable substitutes for sugar in cake formulations. Moreover, this compound is non-caloric and may serve as an appropriate alternative to artificial sweeteners such as aspartame, saccharin, and cyclamate, without producing the adverse effects associated with the excessive consumption of these sweeteners [5].

Several studies have indicated that stevia sweetener compounds possess functional properties that can influence the stability of food products. Microalgae are microscopic algae found in both freshwater and marine environments. They are unicellular species that exist individually, in chains, or in colonies. Microalgae are capable of synthesizing and accumulating macromolecules, such as proteins, carbohydrates, and lipids, utilizing solar energy, carbon dioxide, and nutrients. Furthermore, due to their capacity to produce high-value compounds such as carotenoids, long-chain fatty acids, sugars, essential and non-essential amino acids, enzymes, vitamins, and minerals beneficial to human health, microalgae represent suitable candidates for use as nutraceuticals or functional foods.

The green microalga *Chlorella vulgaris* is one of the most widely recognized green

microalgal species cultivated in freshwater environments. *Chlorella vulgaris* is a unicellular alga classified within the kingdom Protista. This alga is spherical, featuring a relatively thin cell wall, a large cup-shaped chloroplast (with or without a pyrenoid), and a colorless cytoplasm containing a relatively large nucleus positioned above the chloroplast. Based on prior studies, *Chlorella vulgaris* has been reported as a microalga rich in bioactive compounds with potential antioxidant, anti-inflammatory, and immunomodulatory activities [6]. This alga has also been utilized as a natural additive with antimicrobial and antioxidant properties in food products, and studies have demonstrated that it can effectively enhance the microbial safety and shelf-life stability of products such as cakes [7].

In addition to being a complete food source, *Chlorella vulgaris* can serve as a dietary supplement for preventing or treating nutritional deficiencies. The compounds present in *Chlorella vulgaris* can satisfy a substantial portion of daily human nutritional requirements. Depending on age and physiological status, individuals require daily intakes of proteins, lipids, carbohydrates, vitamins, and minerals, many of which can be supplied by *Chlorella vulgaris* alone. The presence of a diverse array of nutrients in *Chlorella vulgaris* has attracted considerable scientific interest regarding its nutritional applications. Currently, Chlorella biomass is experimentally utilized in livestock, poultry, and aquaculture feeds, as well as in food formulations for color enhancement. In the food sector, this alga can be employed as a dietary supplement and a functional food ingredient.

Previous studies have investigated the application of stevia as a sugar substitute in a variety of food products, including biscuits [8], cakes [9], cookies [10], jam [11], candies [12], and doughnuts [13]. Chlorella has also been utilized as a dietary supplement for the fortification of bread [14], cakes [15, 7], pasta [16], and snacks [17]. However, to date, no study has simultaneously examined the use of stevia and the microalga *Chlorella vulgaris* in the formulation of sponge cake. Therefore, the aim of the present study was to incorporate stevia and the microalga *Chlorella vulgaris* into sponge cake formulation and to evaluate the physicochemical, microbiological, antioxidant, and sensory properties of the produced cakes.

2-Materials and Methods

2.1. Materials

The raw materials required for cake preparation, including cake flour, sugar, baking powder, whey powder, vanilla, milk powder, eggs (Talavang brand), and oil (Ladan brand), were purchased from a confectionery supply store in Tehran, Iran. *Chlorella vulgaris* powder (100 g package) was obtained from Gohar Sabz Company (Isfahan, Iran). Dried leaves of the stevia plant were purchased from a herbal store in Tehran. The leaves were ground and passed through a 40-mesh sieve, then packaged and stored in a dry and cool environment until use. Other chemicals used in this study were purchased from reputable suppliers.

2.2. Cake preparation method

A multi-stage method was used to prepare the sponge cake batter. In this method, ingredients were mixed separately before being incorporated into the main batter (Table 1). The cake batter was prepared using the sugar–batter creaming method. Initially, oil and sugar were creamed for 10 minutes until a light-colored mixture was obtained. Eggs were then added gradually in 4–5 portions and mixed with a high-speed electric mixer (model Worlostar-WH502) for 3–5 minutes .

Subsequently, all powdered ingredients, including flour, baking powder, vanilla, whey powder, milk powder, stevia powder (used as a 25% replacement for sugar), and *Chlorella*

vulgaris powder (used as a replacement for flour at levels of 2%, 4%, and 6%), were combined and sieved together before being added to the mixture. These dry ingredients were incorporated into the batter in two stages along with water and mixed at low speed using the electric mixer for 1–3 minutes until a smooth and homogeneous batter was obtained. The prepared batter was then poured into molds and baked in an oven at 175 °C for 20 minutes. After baking, the cakes were removed from the oven and allowed to cool at room temperature (25 °C) for 20 minutes, then packaged in polyethylene zip-lock bags [18].

The relevant analyses were conducted on the samples immediately after production and after 7, 15, and 30 days of storage at room temperature.

Table 1. Sponge cake formula (Percentage based on the weight of flour)

Ingredients	Treatment 1 (Control)	Treatment 2 (A)	Treatment 3 (B)	Treatment 4 (C)	Treatment5 (D)
Wheat flour	100	100	98	96	94
egg	72	72	72	72	72
water	25	25	25	25	25
Sucrose	72	54	54	54	54
oil	57	57	57	57	57
Dry milk	2	2	2	2	2
Baking powder	1.34	1.34	1.34	1.34	1.34
Vanilla	0.5	0.5	0.5	0.5	0.5
Whey powder	4	4	4	4	4
Stevia powder (percentage of sugar)	-	0.9	0.9	0.9	0.9
Chlorella vulgaris (percentage of flour)	-	-	2	4	6

2.3. Material and Method

2.3.1. Determination of moisture content

Approximately 2–2.5 g of each sample was weighed with a precision of 0.001 g into plates that had previously been dried to constant weight. The plates were then placed in an oven (Memmert, Germany) at 130 °C for 90 minutes. After drying and reaching constant weight, the plates were cooled in a desiccator and weighed. Moisture content was calculated using Equation (1) [19].

$$\text{Moisture } (\%) = 100 \times (w_1 - w_2) / m \quad \text{(Eq.1)}$$

In this equation, w_1 represents the initial weight of the plate plus the sample (g), w_2 represents the final weight of the plate plus the dried sample (g), and m represents the weight of the sample (g).

2.3.2. Determination of ash content (wet weight basis)

Approximately 2 g of the sample was weighed into porcelain crucibles that had previously reached constant weight. For preliminary charring, the crucibles containing the samples were placed over a flame under a fume hood and heated until smoke emission ceased and the organic matter was completely burned. Subsequently, the crucibles were transferred to a muffle furnace (Exciton, Iran) at 550 °C. Heating was continued until the samples turned completely white. After cooling, the crucibles were placed in a desiccator and weighed, and the ash content was calculated using Equation (2) [20].

$$\text{Ash (\%)} = 100 \times (w_1 - w_2) / m \quad \text{(Eq. 2)}$$

In this equation, w_1 represents the final weight of the crucible plus ash (g), w_2 represents the weight of the empty crucible (g), and m represents the weight of the sample (g)

2.3.3. Determination of protein

The protein content of the flour was determined using the AACC 46-12 (1999) method. For this purpose, the total nitrogen content was measured, and the protein content was calculated using the conversion factor for cereal proteins (5.7) [21]. Briefly, 1 g of finely ground flour was weighed into a digestion flask, and 25 mL of 98% sulfuric acid was added for digestion. The digestion process continued until a clear green solution was obtained .

Following complete digestion, nitrogen distillation was performed. To do this, 250 mL of distilled water and 50 mL of NaOH, along with boiling chips for uniform boiling, were added to the digested solution. An Erlenmeyer flask containing 50 mL of boric acid and a few drops of methyl red indicator was placed at the end of the distillation apparatus, ensuring the tip of the condenser was fully submerged. Distillation proceeded until a minimum volume of 250 mL was collected. The resulting solution was titrated with 0.1 N sulfuric acid until a pink color was achieved. The total protein percentage was calculated using Equation (3):

$$\textbf{Nitrogen (\%)} = \frac{\text{Volume of acid consumed (mL)} \times \text{Normality of acid} \times 14}{\text{Sample weight (g)} \times 1000} \times 100 \quad \text{(Eq. 3)}$$

Protein (%) = Nitrogen (%) × Protein factor

2.3.4. Determination of fat

The fat content of the samples was determined using the Soxhlet method (Bakhshi, Iran). One gram of each sample was weighed into filter paper, and the filter paper was placed in the extractor section of the Soxhlet apparatus. After attaching the flask, two-thirds of its volume was filled with petroleum ether, and the apparatus temperature was set to 60 °C. The fat extraction process was carried out for 6 hours. Subsequently, the flasks were placed in an oven at 80 °C for 30 minutes, followed by one hour at 100 °C. The difference between the initial and final weights of the flask represents the amount of extracted fat. The percentage of fat was calculated using Equation (4) .

$$\text{Fat (\%)} = (w_2 - w_1)/m \times 100$$

In this equation, $w_2$is the weight of the flask with the extracted fat (g), $w_1$is the weight of the empty flask (g) and m is the weight of the sample (g) [22].

2.3.5. Determination of carbohydrates

The carbohydrate percentage was calculated by subtracting the sum of moisture, ash, protein, and fat contents from 100 [23].

2.3.6. Water activity (aw)

Water activity was measured using a water activity meter (Novasina, Germany) [24].

2.3.7. Color measurement

Color analysis of the cake crust was performed 2 hours after baking using a HunterLab colorimeter. The color parameters were determined based on three indices: L* (lightness), a* (redness–greenness), and b* (yellowness–blueness) [25].

2.3.8. Cake volume

Cake volume was measured using the rapeseed displacement method. In this method, the weight of a known volume of rapeseeds was first determined. Based on the weight and volume of the seeds, the bulk density of rapeseeds was calculated. The cake sample and rapeseeds were then placed together in a container of known dimensions and weighed, and the cake volume was subsequently determined [26].

2.3.9. Texture analysis

A cylindrical probe (TA5) with a diameter of 12.7 mm was used for texture profile analysis (TPA) to evaluate the crumb characteristics of the prepared cakes. The test conditions included a speed of 0.1 mm/s, a strain of 50%, and a time interval of 0 seconds between the two compression cycles. The forward and return speeds of the probe were set at 0.2 mm/s. In this test, hardness, springiness, and chewiness of the cakes were measured and reported on days 1, 7, and 15 of storage [27].

2.3.10. Total mold and yeast count

This test was carried out according to Iranian National Standard No. 10899-2. To prepare the initial dilution, 10 g of cake from each package was transferred into an Erlenmeyer flask containing 90 mL of Ringer solution, yielding a 10^{-1} dilution (1% w/v). Then, 1 mL of this suspension was added to a tube containing 9 mL of diluent to prepare the 10^{-2} dilution, and subsequent serial dilutions were prepared in the same manner .

Mold and yeast counts were determined by the surface plating method using YGC culture medium. Approximately 15–20 mL of the culture medium was poured into each sterile plate. After solidification of the medium, 0.1 mL of the dilutions ranging from 10^{-1} to 10^{-6} was spread onto the surface of the medium using a sampler and a spreader. After drying, the plates were incubated at 25 °C for 3–5 days. Mold and yeast enumeration was performed on the produced cake samples on days 1, 7, and 15 of storage (from production until the end of shelf life) [28].

2.3.11. Free radical scavenging activity by the DPPH method

Antioxidant activity was evaluated using the DPPH radical scavenging assay. Briefly, 1.5 mL of methanolic DPPH solution (60 µM) was added to 500 µL of the extract solution. The resulting mixture was allowed to stand for 30 minutes, after which the absorbance was measured at 515 nm using a spectrophotometer. The absorbance at time zero, as well as the absorbance of the pure DPPH solution without sample, was also recorded .

Antioxidant activity was expressed as percentage inhibition according to Equation (5). Finally, by plotting the inhibition curve and calculating the IC50 value (the concentration of sample extract required to achieve 50% antioxidant activity), the antioxidant activity of the sample was determined [29].

$$\text{Inhibition (\%)} = (A_0 - A_1)/ A_0) \times 100$$

In this equation, A_0 is the absorbance of the control sample and A_1 is the absorbance of the extracted sample.

2.3.12. Sensory evaluation

Sensory attributes were evaluated by ten trained panelists. A 5-point hedonic scale was used (1 = excellent, 2 = very good, 3 = good, 4 = fair, 5 = poor). The cake samples were randomly coded, and sensory properties, including texture, flavor and taste, aroma, and perceived sweetness were assessed on the first day after baking at room temperature. Fresh water was provided to the panelists between samples for palate cleansing [30].

2.4. Statistical analysis

The study was conducted using a completely randomized design (CRD). One-way analysis of variance (ANOVA) was used to determine statistical significance, and Duncan's multiple range test was employed for mean comparisons at a 95% confidence level. All tests were performed in triplicate. SPSS 26 and Microsoft Excel 2019 software were used for statistical analysis and plotting the graphs.

3- Results and Discussion

3.1. Chemical composition of wheat flour, stevia powder, and *Chlorella vulgaris* algae

The chemical characteristics of wheat flour, stevia powder, and *Chlorella vulgaris* powder are presented in Table 2.

Table 2. Chemical composition of flour, dried Stevia leaves and *Chlorella vulgaris* powder

Components (%)	Flour	Stevia leaves	*Chlorella vulgaris* powder
Moisture	13.00±0.01	9.55±1.86	5.00±0.05
Protein	9.68±0.17	10.35±0.06	59.30±1.55
Ash	0.53±0.03	3.00±0.35	5.10±0.16
Fat	1.20±0.05	3.47±0.55	12.18±0.73

3.2. Protein

According to Figure 1, the results for protein content determination indicate that the protein levels in the produced cake samples differed significantly from the control sample ($P < 0.05$). Based on Duncan's multiple range test, the control treatment exhibited the lowest protein content at 7.87%, while Treatment D (6% Chlorella) recorded the highest level at 13.13%. According to the Iranian National Standard No. 2553, the protein content in sponge cake should exceed 8% .

As the concentration of *Chlorella vulgaris* increased, the protein percentage of the cake samples increased significantly compared to the control. This enhancement is attributed to the higher protein content of *Chlorella vulgaris* (59.3 ± 1.55%) and stevia (10.35 ± 0.06%) compared to wheat flour (9.68 ± 0.17%). The findings of this study are consistent with the research conducted by Hadisi and Atazadeh (2018), who investigated the production of low-fat cream enriched with Chlorella; they reported that protein content increased from 2.6% in the control sample to 3.86% in the treatment containing 12% Chlorella [31]. Similar results were reported by Elsebaie and Mostafa (2018) in their study on the production of low-calorie cakes using stevia [32]. Furthermore, Abdel-Moatamed et al. (2025) reported a dose-dependent increase in protein by adding 0.5, 1, and 2% *Chlorella vulgaris* to sponge cake. In their study, the protein content significantly ($P < 0.05$) increased from 13.5 ± 0.20% in the control sample to 15.3 ± 0.14% in the sample containing 2% *Chlorella vulgaris* [7].

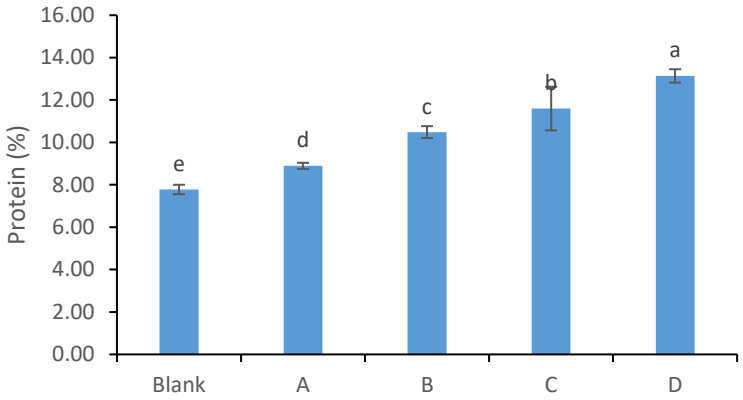


Fig. 1. Protein content of sponge cake with stevia and different concentration of *Chlorella vulgaris* powder

Means with different letter within columns are significantly different (P<0.05).

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.3. Fat

According to Figure 2, the results of fat content determination showed that the fat levels of the cake samples were significantly different from that of the control sample (*P< 0.05*). With increasing levels of *Chlorella vulgaris*, the fat content of the produced cakes increased compared with the control. This result was expected based on the measured fat contents of wheat flour (1.20±0.05%), stevia (3.47±0.55%), and Chlorella vulgaris (12.18±0.73%).

The results of fat evaluation were consistent with those reported by Alaei et al. (2017), who used chickpea flour in the production of sponge and oily cakes [33], and by Arab et al. (2010), who applied chickpea flour to improve the nutritional and functional properties of spaghetti [34]. However, Abdel-Moatamed et al. (2025) reported that the addition of *Chlorella vulgaris* to sponge cake did not result in a significant change in fat and moisture content [7].

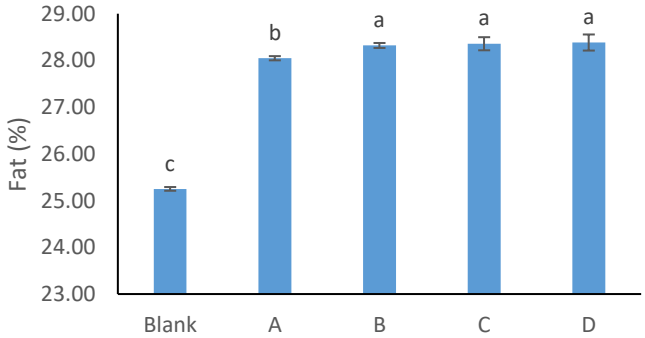


Fig 2. Fat content of sponge cake with stevia and different concentration of *Chlorella vulgaris* powder

Means with different letter within columns are significantly different (P<0.05).

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.4. Ash

According to Figure 3, the results of ash content determination showed that the ash content of the samples increased significantly compared with the control (*P< 0.05*). This increase can be attributed to the presence of mineral elements and fibrous compounds in stevia and *Chlorella vulgaris*, including potassium, magnesium, calcium, and iron .

Similarly, Arab Sorkhi et al. (2017) conducted a study and reported that the ash content increased in gummy candy samples enriched with Chlorella, which is consistent with the findings of the present study [35]. Khormaee Pour et al. (2019) investigated the fortification of sponge cake with lemon peel powder and stevia as a sugar substitute and observed that increasing the levels of lemon powder and stevia led to an increase in ash content [36].

Abdel-Moatamed et al. (2025) also reported an increase in ash content from 1.13±0.13%1.13±0.13% in the control sample to 1.95±0.14%1.95±0.14% in the sample containing 2% Chlorella vulgaris, attributing this increase to the higher ash content of the algae compared with flour (*P< 0.05*) [7].

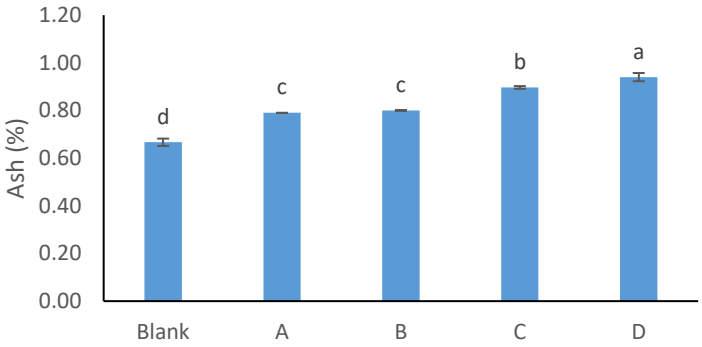


Fig 3. Ash content of sponge cake with stevia and different concentration of *Chlorella vulgaris* powder

Means with different letter within columns are significantly different (P<0.05).

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.5. Carbohydrates

The results related to the carbohydrate content of the produced treatments are presented in Figure 4. These findings indicate that the carbohydrate content of the cake samples decreased significantly with increasing levels of *Chlorella vulgaris* (*P< 0.05*) .

Similar results regarding the reduction of carbohydrate content with increasing levels of plant-based ingredients have been reported in previous studies, including the incorporation of spinach puree (Gala et al., 2017) and carrot pomace powder (SY et al., 2014) [37, 38].

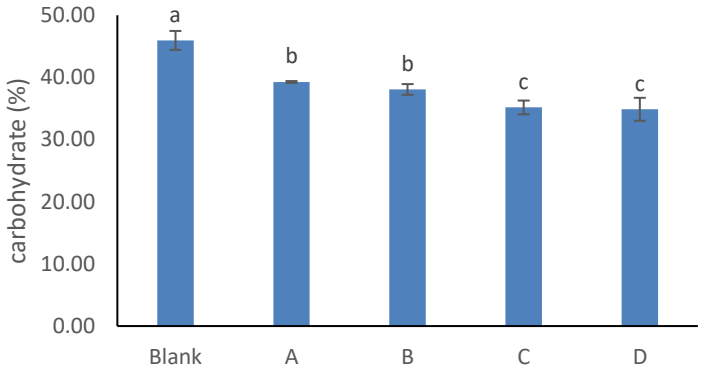


Fig 4. Carbohydrate content of sponge cake with stevia and different concentration of *Chlorella vulgaris* powder

Means with different letter within columns are significantly different (P<0.05).

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.6. Moisture

Based on the moisture content analysis conducted over the storage period (Figure 5), the results of the analysis of variance showed that the moisture content of the samples significantly decreased over time ($P< 0.05$). The results also demonstrated that replacing wheat flour with *Chlorella vulgaris* had a significant effect on moisture content ($P< 0.05$). The highest moisture content was observed in samples containing 4% *Chlorella vulgaris*, while the control samples showed the lowest.

In treatment A, substituting a portion of sugar with stevia led to an increase in moisture compared to the control. This change can be attributed to the fact that sugar increases the gelatinization temperature of starch and the denaturation temperature of proteins; this higher gelatinization temperature facilitates the loss of moisture from the product. Consequently, by reducing the sugar content in the formulation, the moisture content increases. It was also observed that increasing the percentage of *Chlorella vulgaris* in the cake samples increased moisture relative to the control. Since *Chlorella vulgaris* powder contains 6.9% fiber and 55.2% protein, it is highly likely that these components increase the water-holding capacity in the cake, thereby increasing the final product's moisture. Increased moisture enhances water retention in the cake, which contributes to a softer texture and delays staling [39].

Cho and Kim (2016) showed in their research that adding 1%, 2%, and 3% Chlorella to Yukwa snacks increased the moisture content, which is consistent with the results of the present study [40]. Research by Vatankhah et al. (2014) on the effects of using stevioside sweetener in diet biscuits showed that increasing the percentage of stevioside led to an increase in the moisture percentage of the treatments, which is also in agreement with the current findings [41]. Similarly, the research of Sedaghat and Movahed (2014) on the production of diet cake using stevia and sucralose indicated that increasing the sucrose percentage (and decreasing the stevia percentage) in the formulation resulted in decreased moisture and increased hardness, consistent with our results [42]. In a study conducted by Jeon in 2006, it was concluded that increasing the percentage of Chlorella had no significant effect on the moisture content of

cheese samples compared to the control [43]. Furthermore, Abdel-Moatamed et al. (2025) observed no significant change ($P< 0.05$) in moisture content when adding *Chlorella vulgaris* to sponge cake [7].

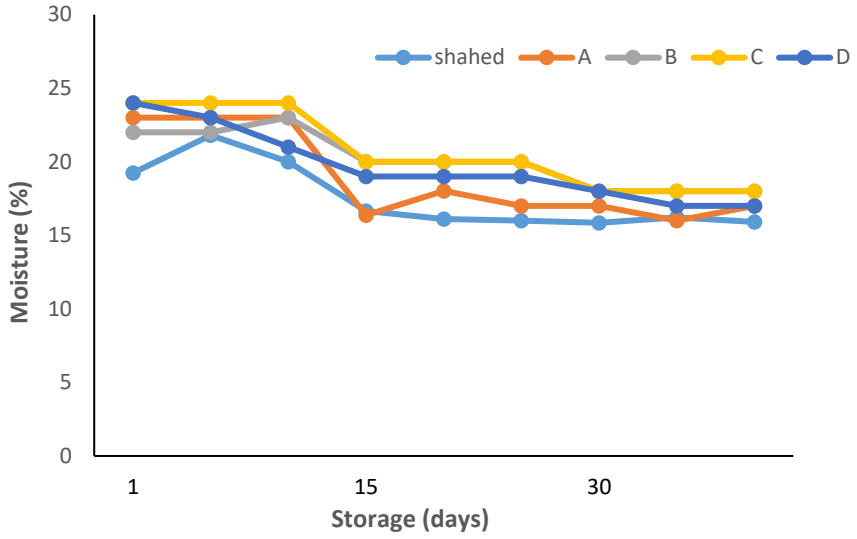


Fig 5. Moisture content of sponge cake with stevia and different concentration of *Chlorella vulgaris* powder

Means with different letter are significantly different (P<0.05).

(Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella)

3.7. Water Activity

Water activity (a_w) is considered a key indicator for assessing the shelf life and microbiological stability of food products. It is a critical parameter that influences the growth of microorganisms and is of particular importance in food preservation. The analysis of variance for the data presented in Figure 6 showed that, similar to moisture content, water activity decreased significantly over time ($P< 0.05$). Therefore, it appears that there is a direct relationship between the water activity and moisture content of the cake samples.

The findings from the evaluation of water activity in treatment A, which contains 25% stevia, showed that replacing a portion of sugar with stevia in the cake formulation causes a significant increase in water activity compared to the control. The major effect of sucrose in baked goods arises from its affinity for water and the bonds formed between them; therefore, sucrose reduces the amount of free water. Furthermore, increasing the percentage of Chlorella algae resulted in a significant increase ($P< 0.05$) in the water activity of the produced cake samples compared to the control. The highest water activity levels were observed in samples containing 2% and 4% algae. It can thus be concluded that the protein molecules of this algae likely have an anti-staling effect, extending the product's shelf life up to the 4% level; however, beyond 4%, the water activity shows a significant decrease compared to other treatments, although it remains higher than the control. The reason for the lower water activity in the control sample may be attributed to the hygroscopic property of sucrose.

Zoulias et al. (2000), after investigating the replacement of sugar with sugar alcohols in

cookies, stated that this substitution led to a significant increase in water activity [44]. In other studies, conducted by Akesowan (2009) on replacing sugar with sucralose-erythritol in cakes, it was observed that reducing the sugar percentage in the formulation leads to an increasing trend in the product's water activity [45]. Similarly, research by Vatankhah et al. (2014) on the possibility of producing diet biscuits using stevioside sweetener reported consistent results. They found that the use of stevia in the biscuit formulation significantly increases water activity compared to the control, which is in agreement with the results of the present study [41].

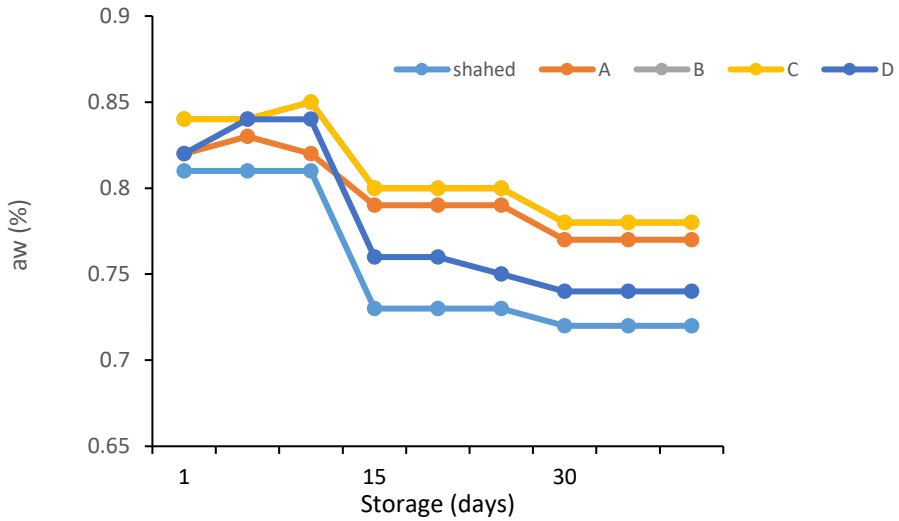


Fig 6. aw of sponge cake with stevia and different concentration of *Chlorella vulgaris* powder

Means with different letter are significantly different (P<0.05).

(Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella)

3.8. Color

The color of the final product may depend on many factors, including interactions among ingredients, compositional changes, and color changes occurring during processing [46]. The crust color of cake is developed during baking through Maillard reactions and caramelization, whereas the crumb color is influenced mainly by the compounds used in the formulation [47].

As shown in Figure 7, the addition of stevia powder, as well as increasing the percentage of *Chlorella vulgaris*, led to a significant decrease in the L*, b*, and a* color indices ($P< 0.05$).

In addition to the presence of the green pigment in the algae, *Chlorella vulgaris* and stevia contain 59.3±1.55% and 10.35±0.06% protein, respectively. Due to their relatively high protein content, they may promote more intense browning reactions (Maillard reaction), resulting in the formation of more brown pigments; this can explain the darker color observed in the samples [48].

Chang and Choi (2005) reported results similar to those of the present study, showing that increasing the percentage of Chlorella in pancake samples reduced the L*, b*, and a* values [49]. Likewise, the study by Kim and Chang (2010) demonstrated that increasing

Chlorella concentration decreased the L*, b*, and a* values in yellow layer cake [50].

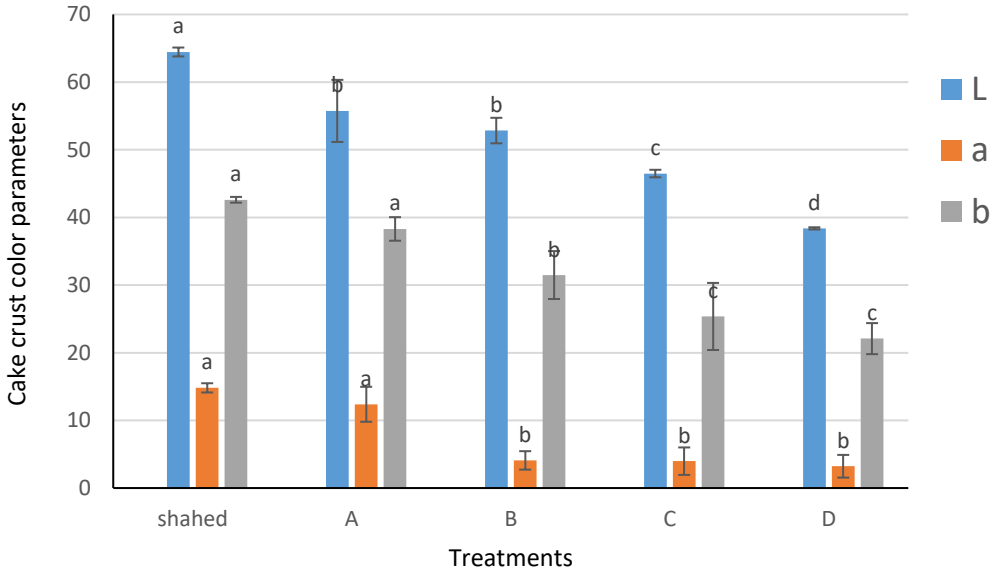


Fig 7. Effect of stevia and *Chlorella vulgaris* powder on the crust color indexes (L* a* b*) of sponge cake.

Means with different letter within columns are significantly different (P<0.05).

reatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

According to the results presented in Figure 8, increasing the percentage of *Chlorella vulgaris* and adding stevia powder to the produced cakes led to a significant decrease in the L*, b*, and a* indices (*P< 0.05*). The brightest crumb color was observed in the control sample, while the darkest was recorded in treatment D. This is likely due to the dark color of Chlorella powder, which produces a darker cake crumb.

Moreover, higher levels of *Chlorella vulgaris* powder resulted in lower a* values in the cake crumb. The b* value of the crumb also decreased significantly with increasing levels of *Chlorella vulgaris* powder (*P< 0.05*). The reduction in b* values in the batter, crust, and crumb of the cake samples with the addition of *Chlorella vulgaris* powder indicates a decrease in yellowness. This change may be attributed to the pigments present in *Chlorella vulgaris* [51].

Hesarinejad et al. (2016) reported that replacing egg white with *Chlorella vulgaris* powder resulted in a significant decrease in the L*, b*, and a* indices of cake batter, which is consistent with the findings of the present study [52].

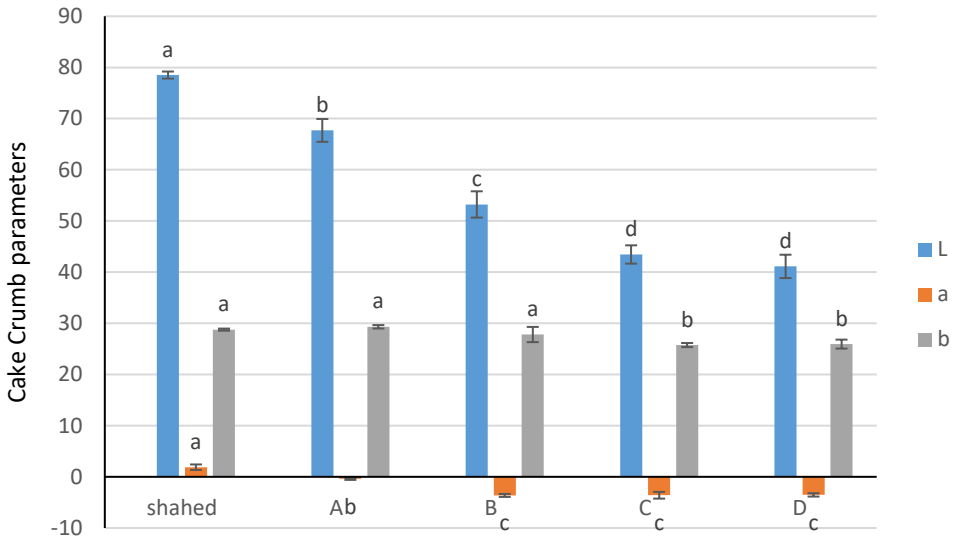


Fig 8. Effect of stevia and *Chlorella vulgaris* powder on the crumb color indexes (L* a* b*) of sponge cake.

Means with different letter within columns are significantly different (P<0.05).

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.19. Mold and Yeast Count

Table 3 shows the results of the analysis of variance regarding the effect of storage time and the interaction between *Chlorella vulgaris* powder and stevia leaf powder on the total mold and yeast count. The results indicated that over the 15-day storage period, the total mold and yeast count increased significantly in the control sample and the sample containing only 25% stevia; however, no significant effect was observed in the treatments containing both stevia and algae ($P< 0.05$) .

Evaluating the effect of replacing wheat flour with *Chlorella vulgaris* on the total mold and yeast count showed no significant impact on the first and fifteenth days; however, this effect was significant on the seventh day of storage, where the addition of stevia and an increase in the percentage of *Chlorella vulgaris* reduced mold and yeast growth ($P< 0.05$). Furthermore, no mold or yeast was observed at the time of production. The total mold and yeast count decreased as the replacement percentage of *Chlorella vulgaris* increased, which may indicate the effectiveness of this alga in controlling the number and proliferation of microorganisms. Stevia possesses antimicrobial effects against food-spoiling bacteria. The increase in the percentage of Chlorella algae led to a decrease in mold and yeast counts, which can be attributed to the medicinal properties of Chlorella, including its antibacterial, antifungal, and anti-cancer properties [53].

Preethi et al. (2011) identified various compounds in different extracts of the stevia plant and determined that flavonoids, alkaloids, steroids, tannins, and terpenes are present in this plant [54]. In that study, the antimicrobial effect of stevia extract was attributed to the presence of these compounds. The antibacterial properties of Chlorella can be attributed to the carotenoids, proteins, vitamins, pigments, and phenolic compounds present in the algae [55]. Meanwhile,

Greengard et al. (1994) attributed the antimicrobial property of Chlorella to a mixture of 18-carbon fatty acids, which possess not only antibacterial properties but also allelopathic effects [56]. Research by Hashemi et al. (2014) on the effects of substituting sugar with stevia on the shelf life of saffron syrup also found that the stevioside present in stevia has antimicrobial effects on the bacterial population in the syrup, which is consistent with the results of the present study [57].

Table 3. Mold and yeast growth rate in different treatments prepared from stevia leaf powder and *Chlorella vulgaris* (log CFU.g)

Treatments	Storgae (days)		
	1	7	15
Control	0	0.92±0.80 ^{ABa}	1.28±0.48 ^{Aa}
A	0	0.33±0.57 ^{Aab}	0.92±0.80 ^{Aa}
B	0	0	0.66±0.57 ^{Aa}
C	0	0	0.43±0.75 ^{Aa}
D	0	0	0.33±0.57 ^{Aa}

($p<0.05$) *Different capital letters in each row indicate statistically significant difference

*Different small letters in each column indicate statistically significant differences ($p<0.05$)

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.10. Antioxidant Activity Measurement

The results regarding the measurement of the antioxidant activity of *Chlorella vulgaris* and stevia in the sponge cake samples are shown in Figure 9. With an increase in the percentage of Chlorella algae, the IC50 value decreased. Since IC50 is inversely proportional to antioxidant activity, an increase in the percentage of *Chlorella vulgaris* resulted in a significant increase in the antioxidant activity of the extract ($P < 0.05$). The reason for this increase in antioxidant properties can be attributed to the presence of phenolic and flavonoid compounds in Chlorella algae and the stevia plant, which possess antioxidant, anti-inflammatory, and anti-cancer properties.

In a study conducted by Arab Sorkhi et al. (2017), the reported results are consistent with the present study; as the percentage of Chlorella algae powder increased, the amount of phenolic compounds and, consequently, the level of antioxidant activity increased [35]. These results are also in agreement with the study by Abdel-Moatamed et al. (2025). These researchers demonstrated that the addition of *Chlorella vulgaris* to cake not only significantly increased antioxidant activity but also significantly reduced the microbial load [7].

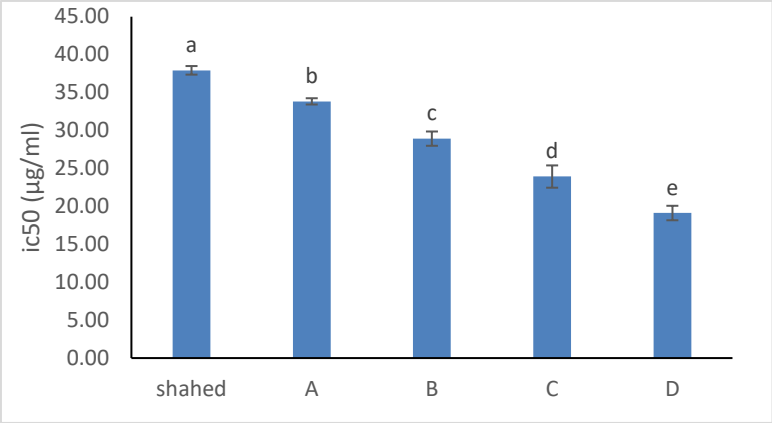


Fig 9. The effect of *Chlorella vulgaris* powder and stevia on IC50 compared to the control

Means with different letter within columns are significantly different (P<0.05).

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.11. Texture Firmness

After baking, products of the baking industry undergo physicochemical changes that are generally referred to as staling. The term staling denotes a reduction in consumer acceptability of bakery products and is mainly associated with changes occurring in the crumb. Staling can be recognized through many physical and chemical phenomena, such as changes in texture, water migration, starch crystallization (retrogradation), and interactions among the constituent components. In other words, this process alters both external and internal characteristics, including flavor, taste, aroma, and chewability, thereby leading to product aging. The occurrence of staling in bakery products such as cake is closely related to the moisture content of the product. Higher cake moisture contributes to a softer crumb texture [52].

As shown in Table 4, in all samples, texture hardness increased significantly with increasing storage time ($P< 0.05$). It was also observed that increasing the level of *Chlorella vulgaris* substitution significantly reduced the hardness of cake texture. These results indicated that increasing the amount of *Chlorella vulgaris* powder up to 4%, as well as replacing part of the sugar with stevia in the cake formulation, reduced texture hardness and produced a softer texture. Moisture content has an inverse relationship with cake hardness [58].

The stevioside present in stevia has high solubility in water and greater moisture-retention capacity at high temperatures than sucrose; therefore, it has a greater tendency to bind water. This characteristic reduces the number of bonds between the gluten network

and water molecules, resulting in a softer texture as the level of sucrose replacement with stevia increases compared to the control sample. In the control sample, because of the greater presence of sucrose, the batter had higher viscosity, which consequently produced greater texture hardness. When sucrose content was reduced, this viscosity-building effect decreased sharply, and as a result, texture hardness also decreased.

The reduction in texture hardness under these conditions may occur for two reasons. First, it may be due to the limited development of the gluten network. For proper development, the gluten network must bind water molecules; however, sugar has a greater affinity for water and more rapidly forms hydrogen bonds with water molecules, thereby preventing adequate gluten network development and the stiffening of the batter [59]. In addition, due to the higher moisture-retention property of stevia and its prevention of moisture loss, the stevia-containing treatment increased cake moisture and ultimately reduced product staling.

The increase in texture hardness again at the 6% treatment may be attributed to the higher protein content of this treatment. At the 2% and 4% levels, the structural groups may have maintained their condition more effectively; however, since the protein fraction in *Chlorella vulgaris* differs from glutenin and gliadin, which are the main proteins present in flour, this difference becomes more pronounced at higher algae levels and may interfere with proper cake structure formation.

In a study by Pareyt et al. (2009) on the role of sugar in the structural and textural properties of cookies, similar results were obtained [60]. The findings of Mushtaq et al. (2010) showed that increasing the level of xylitol substitution for sugar in cookie formulations reduced hardness, which is in agreement with the results of the present study. They also attributed the higher hardness of the control sample to the lack of proper gluten network development due to interference with water absorption, as well as to sugar crystallization after cooling [61]. Walter and Soliah (2010), after evaluating cookie texture during sugar replacement with stevia, identified the sample containing 50% stevia and 50% sugar as the softest sample [62]. Taghdiri et al. (2024) also evaluated the effect of adding *Chlorella vulgaris* protein hydrolysate on the quality characteristics of cake during storage. These researchers likewise reported a significant reduction in hardness in samples containing the protein hydrolysate compared with the control cake sample [63].

Table 4 Hardness in different treatments prepared from stevia leaf and *Chlorella vulgaris* powder (N)

Treatments	Storage (days)		
	1	7	15
Cotrol	4.49 ± 0.07^{Ca}	7.74 ± 0.36^{Ba}	11.27 ± 0.95^{Aa}
A	3.34 ± 0.23^{Cb}	7.05 ± 1.19^{Ba}	8.90 ± 0.63^{Ab}
B	4.19 ± 0.12^{Ba}	6.67 ± 0.17^{Aa}	7.05 ± 1.19^{Ac}
C	3.26 ± 0.34^{Bb}	7.02 ± 0.95^{Aa}	8.02 ± 0.23^{Abc}
D	3.16 ± 0.07^{Cb}	7.17 ± 1.06^{Ba}	8.62 ± 0.34^{Ab}

*Different capital letters in each row indicate statistically significant differences($P<0.05$).

*Different small letters in each column indicate statistically significant differences($P<0.05$).

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.12. Springiness of Texture

Springiness (elasticity) represents the ability of a sample to return to its original shape after deformation during the first compression. As shown in Table 5, the springiness of the cake texture decreased significantly during the storage period (*P< 0.05*). The control sample exhibited lower springiness compared to the other samples. Increasing the percentage of *Chlorella vulgaris* and stevia significantly increased the springiness of the cake texture on the first day, which may be attributed to the improved and softer texture produced by stevia and *Chlorella vulgaris* in the cakes. However, on days 7 and 15 after production, no significant change in cake springiness was observed (*P< 0.05*).

Furthermore, there is a direct relationship between cake sample volume and springiness, and an inverse relationship between texture hardness and springiness. The results obtained in this study are consistent with the findings reported by Jeon (2006), Park (2003), and Abdel-Moatamed et al. (2025), who investigated the effects of Chlorella in cheese, bread dough, and sponge cake, respectively [64, 43, 7].

Table 5 Springiness of samples during storage (mm)

Treatments	Storage (days)		
	1	7	15
Control	8.16 ± 0.15^{Ab}	7.95 ± 0.11^{Aa}	6.71 ± 0.22^{Ba}
A	9.05 ± 0.45^{Aab}	8.03 ± 0.17^{ABa}	7.37 ± 1.08^{Ba}
B	9.17 ± 0.40^{Aa}	8.06 ± 0.52^{Ba}	7.56 ± 0.59^{Ba}
C	9.87 ± 0.34^{Aa}	8.48 ± 0.35^{Ba}	7.59 ± 0.54^{Ca}
D	9.53 ± 0.82^{Aa}	8.58 ± 0.39^{Aa}	7.26 ± 0.31^{Ba}

*Different capital letters in each row indicate statistically significant differences(P<0.05).

*Different small letters in each column indicate statistically significant differences(P<0.05).

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.13. Chewiness

Changes in chewiness during the storage period showed a significant difference, as presented in Table 6 (*P< 0.05*). The control sample exhibited higher chewiness compared to the other samples, while increasing the percentage of *Chlorella vulgaris* led to a significant reduction in chewiness. Since this parameter is closely related to hardness, the decrease in sample hardness resulted in a corresponding reduction in chewiness (*P< 0.05*). The results obtained in the present study are consistent with the findings reported by Jeong et al. (2006), who investigated the addition of Chlorella in white bread [43].

Table 6 Chewiness of samples during storage (mj)

Treatments	Storage (days)		
	1	7	1
Cotrol	22.13 ± 0.41^{Ba}	30.66 ± 13.81^{Aa}	20 ± 5.18^{Ba}
A	12.73 ± 1.60^{Abb}	14.70 ± 2.20^{Ab}	13.20 ± 3.25^{ABab}
B	15.33 ± 0.45^{Ab}	14.40 ± 1.30^{Bb}	13.36 ± 4.10^{Bab}
C	14.86 ± 2.20^{Bb}	17.03 ± 3.32^{ABb}	19.80 ± 2.78^{Aa}
D	14.80 ± 1.20^{Bb}	20.26 ± 0.94^{Aab}	12.43 ± 2.22^{ABb}

*Different capital letters in each row indicate statistically significant differences(P<0.05).

*Different small letters in each column indicate statistically significant differences(P<0.05).

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

3.14. Sensory Evaluation

Sensory evaluation assesses the acceptability of a product from the consumers' perspective. Whether a product is accepted by consumers depends on their perception of taste, texture, aroma, and overall product quality. The results of the sensory evaluation of cake samples after baking are presented as a spider chart in Figure 10.

The obtained results showed that there were no significant differences among the samples in terms of aroma, taste, and sweetness scores ($P< 0.05$). The texture score of treatment A, which contained only 25% stevia as a sugar substitute, was the lowest among the samples due to the formation of a non-uniform texture. However, with increasing percentages of *Chlorella vulgaris* added along with stevia, the panelists reported improved and more cohesive texture characteristics. Treatment D, containing 25% stevia and 6% *Chlorella vulgaris*, obtained the closest texture score to the control sample.

In terms of overall acceptability, the scores of the control, A, B, C, and D sponge cake samples were 4.8, 4.2, 4.2, 3.7, and 3.5 out of 5, respectively. In other words, the addition of stevia alone as a sugar substitute, as well as its combination with *Chlorella vulgaris* powder at a level of 2% as a partial replacement for wheat flour, did not negatively affect the sensory properties of sponge cake.

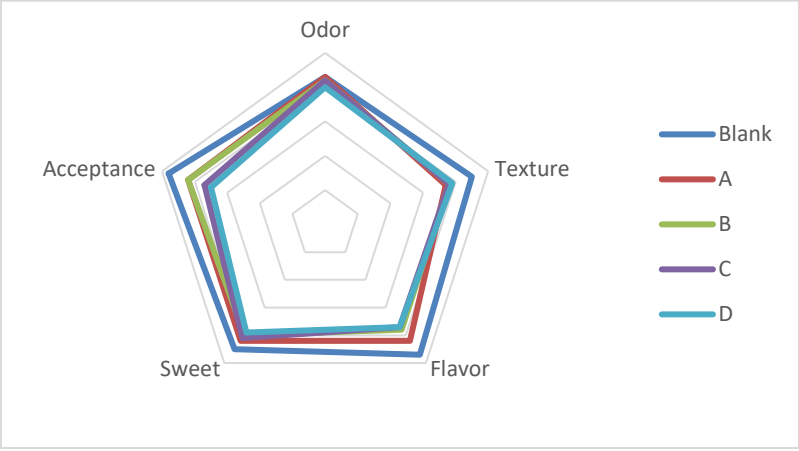


Fig 10. Sensory properties of sponge cake with different concentrations of stevia and *Chlorella vulgaris* powder

Treatment A: The sample contains 25% stevia, Treatment B: The sample contains 25% stevia and 2% chlorella, Treatment C: The sample contains 25% stevia and 4% chlorella, Treatment D: The sample contains 25% stevia and 6% chlorella

4- Conclusion

At present, population growth and concerns regarding the negative effects of excessive consumption of certain food ingredients, such as sugar, have created a demand for the gradual replacement of processed substances with natural alternatives, as well as for the production of foods with enhanced nutritional value. Today, the use of stevia sweetener and the microalga *Chlorella vulgaris*, both of

natural origin, has been well received in many countries. Due to their rich nutrient profile and antioxidant compounds, stevia and *Chlorella vulgaris* are considered high-value nutritional ingredients. Their incorporation into cake formulation not only improves the nutritional properties of the product but also delays staling.

In addition, because stevia and *Chlorella vulgaris* contain antifungal and antibacterial compounds, they significantly increase the microbial shelf life of cake ($P< 0.05$). In the present study, the addition of stevia and *Chlorella vulgaris* led to increases in ash, protein, fat, volume, antioxidant activity, moisture content, and water activity, while reducing carbohydrate content, texture hardness, chewiness, and the color indices L*, a*, and b*. Texture springiness increased significantly on the first day, but no significant changes were observed on days 7 and 15. The texture evaluation score of treatment A, which contained only stevia, showed a significant and noticeable decrease; however, with increasing levels of *Chlorella vulgaris* added to this formulation, the texture score improved and became closer to that of the control treatment ($P< 0.05$). With stevia substitution and increasing percentages of *Chlorella vulgaris*, no significant differences were observed among the treatments in terms of taste, aroma, and sweetness acceptability ($P< 0.05$). In terms of overall acceptability, the treatment containing 25% stevia alone and the treatment containing 25% stevia plus 2% *Chlorella vulgaris* were similar to the control treatment ($P< 0.05$).

Overall, if one treatment is to be selected between these two, the treatment containing 25% stevia and 2% *Chlorella vulgaris* would be the preferred option because of its better texture and overall acceptability close to that of the control.

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Author Contributions

All activities were performed by the author.

Conflict of Interest

The author declares that there are no financial or competing conflicts of interest regarding this study.

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مقاله علمی_پژوهشی

تولید کیک اسفنجی فراسودمند حاوی گیاه استویا (*Stevia rebaudiana*) و ریز جلبک *کلرلا ولگاریس* و ارزیابی خواص فیزیکوشیمیایی و حسی آن

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چکیده

امروزه به واسطه توجه مصرف‌کنندگان به ویژگی‌های تغذیه‌ای و سلامتی‌بخشی مواد غذایی، صنعت غذا به طراحی مجدد مواد غذایی سنتی متمایل شده است. کیک یکی از فراورده‌های غذایی پرمصرف است که متخصصین تغذیه کاهش مصرف آن را به دلیل قند و چربی بالا، در رژیم غذایی توصیه می‌کنند. در این تحقیق کیک اسفنجی با جایگزینی آرد گندم با ۲، ٤ و ٦ درصد پودر جلبک *کلرلا ولگاریس* و جایگزینی کامل ساکارز با ۲۵ درصد پودر گیاه استویا (*Stevia rebaudiana*)، تولید شد. درصد چربی، پروتئین، رطوبت، فیبر و خاکستر آرد گندم، پودر گیاه استویا و پودر جلبک *کلرلا ولگاریس* اندازه‌گیری شد. خصوصیات کیفی و ماندگاری کیک طی ۳۰ روز نگهداری در ۲۵ درجه سلسیوس شامل درصد ترکیبات، حجم، فعالیت آنتی‌اکسیدانی، شمارش کلی کپک و مخمر، رنگ، ویژگی‌های بافتی (میزان سفتی، فنریت، قابلیت جویدن در آزمون بافت سنجی دو مرحله‌ای) و ارزیابی حسی بررسی شد. نتایج نشان داد با افزایش درصد جلبک *کلرلا ولگاریس* و افزودن پودر گیاه استویا در فرمولاسیون کیک، میزان خاکستر، چربی، پروتئین، رطوبت، فعالیت آبی، فعالیت آنتی‌اکسیدانی، حجم کیک و فنریت بافت کیک افزایش، ولی میزان کربوهیدرات، سفتی بافت، قابلیت جویدن کیک و شاخص‌های *b (آبی- زردی)، *a (سبزی- قرمزی) و *L (روشنایی) کاهش معنی‌دار ($p<0.05$) یافت. بیشترین مقدار پروتئین ۱۳/۱۳ درصد در مقایسه با نمونه شاهد (۷/۷۸ درصد) متعلق به کیک حاوی ٤ درصد جلبک و ۲۵ درصد استویا بود. کاربرد استویا و *کلرلا ولگاریس*، ماندگاری کیک را در برابر عوامل میکروبی به طور معنی‌داری افزایش داد. با گذشت زمان تا ۷ روز نگهداری، تعداد کلی کپک و مخمر در نمونه شاهد و نمونه دارای استویا (فاقد جلبک *کلرلا ولگاریس*) به طور معنی‌داری ($p<0.05$) افزایش یافت. در نمونه‌های حاوی استویا و جلبک، صرف‌نظر از مقدار افزودن جلبک، کپک و مخمر مشاهده نشد اما پس از ۱۵ روز، مقدار کپک و مخمر در تمامی نمونه‌ها یکسان بود. میزان فنریت بافت در روز اول افزایش معنی‌داری پیدا کرد ولی در روز ۷ و ۱۵ تغییر معنی‌داری نداشت. با گذشت زمان مقدار رطوبت و میزان فعالیت آبی به طور معنی‌داری کاهش پیدا کرد. بیشترین میزان فعالیت آبی مربوط به کیک‌های حاوی ۲ و ٤ درصد جلبک بود. ($P<0.05$). از لحاظ پذیرش کلی امتیاز تیمار حاوی ۲۵ درصد استویا و همچنین تیمار حاوی ۲۵ درصد استویا و ۲ درصد جلبک کلرلا ولگاریس شبیه به تیمار شاهد بود ($p<0.05$). در این پژوهش، تیمار حاوی ۲۵ درصد استویا و ۲ درصد جلبک *کلرلا ولگاریس* به دلیل بافت بهتر و پذیرش کلی نزدیک به شاهد، توصیه می‌شود.