



Potential application of the encapsulated microalgae biomass as an innovative ingredient in gummy formulation

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Abstract

The increasing demand for natural product ingredients with health benefits has led to the incorporation of new ingredients into traditional confectionery industry formulations to develop healthier and more nutritious products. The objective of this study was to evaluate the potential application of three species of encapsulated microalgae, *Porphyridium cruentum*, *Dunaliella salina*, and *Chlorella vulgaris*, in gummy composition. The gummies were produced using five various addition levels of (0.10, 0.20, 0.30, 0.40, and 0.50 g/100 g) each alga. The samples were evaluated on the characteristics of moisture content, water activity (aw), pH, texture (hardness, springiness, cohesiveness, gumminess, chewiness, and resilience), color (L*, a*, b*, chroma, Hue angle), total carotenoid, and chlorophyll contents and color stability under accelerated shelf life conditions (25 °C/70% RH). The results showed that the various concentrations of microalgae added to the gummies had a significant effect on moisture content and aw values ($p < 0.05$). The texture profile analysis showed that except for cohesiveness and chewiness, higher concentrations of microalgae biomass significantly affected the texture profile ($p < 0.05$). The highest addition of microalgae resulted in increased total carotenoid and chlorophyll contents of gummies. In addition, various microalgae concentrate significantly affected the color parameters of gummy candies, and using higher concentrates decreased the L* value and increased the a* value of the samples. It is concluded that encapsulated microalgae have the potential to be used in gummy formulations to improve nutritional properties.

Keywords Microalgae · Gummy candy · *Porphyridium cruentum* · *Dunaliella salina*, *chlorella vulgaris*

Introduction

The market for new functional foods is rapidly growing, with a current emphasis on using natural sources. Microalgae, known as single eukaryotic cells, have a microscopic structure that is being increasingly investigated as a food or food ingredient due to their high photosynthetic efficiency, rapid growth, and basic cultivation requirements, as well as having huge potential in various industries, such as pharmacology, medicine, cosmetology, biopharmaceuticals, and nutraceuticals [1]. These unicellular organisms are rich in bioactive compounds with antioxidant, antibacterial, and antiviral properties and possess various therapeutic effects, such as antitumor, anti-cancer regenerative, antihypertensive, and neuroprotective [1, 2].

Microalgae species like *Spirulina*, *Chlorella*, *Dunaliella*, *Haematococcus*, and *Nannochloropsis* are recognized for their high-value component productivity and significant commercial potential. These species account for over 95%

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of the total mass of microalgae utilized in various industries, including food, feed, cosmetics, and health [3]. *Chlorella vulgaris* (CV), *Dunaliella salina* (DS), and *Porphyridium cruentum* (PC) included in this study were classified as safe substances for human consumption GRAS (Generally Recognized as Safe), according to the Food and Drug Administration. CV is a single-spherical microscopic blue-green cell algae rich source of protein, fat, minerals, antioxidants, well-balanced amino acid profiles, vitamin C, omega-3 fatty acids, carotenoids (β -carotene, lutein, and zeaxanthin), lycopene, and tocopherol [3, 4]. PC, on the other hand, is a red microalga (Rhodophyta) with spherical cells with 28 to 39% and 40 to 57% protein and carbohydrate content, respectively. In addition, the biomass contains tocopherol, vitamin K, and a large amount of carotenoids, mainly zeaxanthin, as well as is a good source of polyunsaturated fatty acids, particularly arachidonic acid [5, 6]. One of the most well-known types of *Dunaliella* microalgae is *Dunaliella salina*. It is a photoautotrophic green microalga, an efficient biological source of β -carotene (up to 13% dwt), which has antioxidant properties and is used as the main source for commercial production of natural β -carotene [5, 7]. The use of microalgae in the food industry is steadily increasing due to their excellent nutritional composition and ability to produce valuable bioactive compounds. In the study conducted by Batista et al. [8], developed cookies with microalgae biomass (*Arthrospira platensis*, *Chlorella vulgaris*, *Tetraselmis suecica*, *Phaeodactylum tricornutum*) showed higher protein, total phenolic content, and antioxidant capacity compared to the cookies without microalgae. Encapsulation can protect the bioactive ingredients of these microalgae and increase their stability in the gummy sugar matrix. In addition, encapsulation can reduce undesirable strong flavors or odors associated with the microalgae, making them more palatable in the gummy. Fortified gummy can be an alternative functional and appealing candy to incorporate these nutritious microalgae, particularly for children [9].

Gummy candies can be formulated as nutritional supplements or used as a functional food carrier by incorporating fruit juices or extracts with beneficial compounds [10, 11]. They typically contain sugar, gelling agents, flavorings, coloring, and food acid(s). This product is often produced by heating a sugar solution with gelling components and molding it into various shapes and colors to make it appealing, especially for children [12].

Recently, researchers have focused on functional health foods, highlighting the incorporation of microalgae biomass as an innovative bioactive resource in various food products. Despite the study of various microalgae in food products such as chewing candy [13], gummy candy [14], cookies [8], smoothies [15], ice cream, and soft cheeses [16]. There are limited reports on using encapsulated CV, DS, and PC

microalgae in candy. Based on their well-known composition and the increasing demand for natural pigments, this study focused on adding three encapsulated microalgae (*Porphyridium cruentum*, *Dunaliella salina*, and *Chlorella vulgaris*) at 0.10, 0.20, 0.30, 0.40, and 0.50 g/100 g level to gummy candy formulation to evaluate the microalgae-based gummy candy physicochemical properties.

Materials and methods

Materials

250 Bloom bovine gelatin (Burjel, Bursa, Turkey), 12–16 DE (Dextrose Equivalent) maltodextrin, 42 DE glucose syrup (Cargill, Istanbul, Turkey), citric acid (Tayas, Kocaeli, Turkey) and sucrose (Konya Sugar, Konya, Turkey) were used to formulate gummy candy samples. Red beet extract powder and chlorophyll (Sensient, Gebze, Kocaeli, Turkey) were also used as natural commercial colorants. The gelatin was stored in a cool, dry environment below 25 °C to prevent degradation. Maltodextrin and glucose syrup were sealed tightly and kept away from moisture and direct sunlight to maintain their techno-functional properties. Citric acid and sucrose were stored in airtight containers to avoid contamination and clumping, ensuring consistent quality during the formulation process.

Algae cultivation

According to our previous studies [3, 5], *Chlorella vulgaris* (strain CCAP 211/52), *D. salina* (UTEX 2538), and *P. cruentum* (UTEX 161) from The Culture Collection of the University of Texas (Austin, USA) were cultivated in a pilot scale tubular photobioreactor (PBR) (Ege University Fisheries Faculty, Izmir, Turkey) as described optimum methods for each alga. All cultures were maintained at 20.0 ± 1.00 °C under 16/8 hours of light/dark photoperiod. pH of microalgae culture in the system was controlled by Seko Control 40 pH/redox meter (Turkey). After reaching the maximum value of the biomass growth phase, the algal biomass was harvested using a conical plate centrifuge (ENKA separator Turkey; 4863 g), obtaining slurry with 20% dry weight content, and then stored at -20 °C before encapsulation studies.

Encapsulation by using a spray dryer

Since the culturing of microalgae and the spray-drying process could not be conducted simultaneously, the harvested algal biomasses were stored at -20 °C, as stated in Sect. 2.2. Another reason for this storage practice before spray-drying was the time required to culture a sufficient amount of

biomass in the pilot-scale PBR. Prior to spray-drying, the algal samples stored at -20 °C for thawing purposes were kept at 4 °C for 4 h. Subsequently, the feeding solutions were prepared. For each batch, 1000 mL of microalgae solution was fed to the spray dryer. Encapsulation was prepared according to our previous studies [3, 5]. For this aim, protocols in previous studies for *C. vulgaris* [3], and *D. salina*, and *P. cruentum* were used [5].

To prepare a feed solution with a soluble solid concentration of 8.0 g/100 mL for *D. salina*, 15.0 g/100 mL for *P. cruentum*, and *C. vulgaris* wall material (maltodextrin, 12–16 DE), the mixture was mixed with distilled water and shaken for 30 min at 250 rpm in a refrigerated incubator at 25 °C [16]. A laboratory-scale spray dryer (B15, Unopex, Izmir, Turkey) equipped with a nozzle atomization system was used to encapsulate microalgae samples via spray-drying. The nozzle diameter, outlet temperature, feed rate, and airflow rate were maintained as fixed encapsulation conditions for all algal biomasses, set at 1.0 µm, 95 °C, 8 mL/min, and 600 L/h, respectively. The residence time of the drying air within the spray chamber was approximately 1.5 s. The inlet air temperatures and maltodextrin ratios were determined based on our recent previous studies to achieve optimal encapsulation efficiencies [3, 5]. The maltodextrin (12–16 DE) ratios were 75.00%, 75.00%, and 51.08% (w/w) for *Chlorella vulgaris*, *Dunaliella salina*, and *Porphyridium cruentum*, respectively. The corresponding inlet air temperatures were 180 °C, 190 °C, and 170 °C.

Preparation of gummies with various encapsulated microalgae

Gummy candies were fabricated according to Pekdogan et al. [17] with slight modifications. Gummy-type samples were prepared using water (17.4 g/100 g), sucrose (32.6 g/100 g), 42 DE glucose syrup (41.7 g/100 g), 250 bloom gelatin solution (1:2) (6.5 g/100 g), 50% citric acid solution (1.8 g/100 g), and different concentrations of encapsulated microalgae (0.10, 0.20, 0.30, 0.40, and 0.50 g/100 g encapsulated microalgae /100 g). Additionally, gummy samples containing commercial natural colorants (red beet extract powder and chlorophyll) included 0.30 g per 100 g of these materials (Table 1). Water (17.4 g/100 g), sucrose (32.6 g/100 g), glucose syrup (41.7 g/100 g), and citric acid solution (1.80 g/100 g) were mixed and heated to 100 °C. This temperature was maintained at 200–300 rpm for approximately 10 min until reaching 90.0 °Bx. Then, the samples were cooled to 90 °C. The previously prepared 250 bloom gelatin: water solution (1:2) was added to the mixture and stirred, followed by various ratios of each encapsulated microalgae biomass and further mixing at 60 °C and 3000 rpm for 5 min. The resulting gels were molded and kept at 20 °C for 24 h, and the gelled candies were then waxed with starch. Similar steps were used to prepare the control sample but did not include alga biomass.

Table 1 Gummy samples composition and study design (% m/m)

Sample	Gummy Mass						Encapsulated Algae or Colorant
	Water	Sucrose	Glucose Syrup (42 DE)	Gelatin Solution (250 Bloom, 1:2)	Citric Acid Solution (1:1)	Total	
PC.J.01	17.4	32.6	41.7	6.50	1.80	100.0	0.10
PC.J.02	17.4	32.6	41.7	6.50	1.80	100.0	0.20
PC.J.03	17.4	32.6	41.7	6.50	1.80	100.0	0.30
PC.J.04	17.4	32.6	41.7	6.50	1.80	100.0	0.40
PC.J.05	17.4	32.6	41.7	6.50	1.80	100.0	0.50
DS.J.01	17.4	32.6	41.7	6.50	1.80	100.0	0.10
DS.J.02	17.4	32.6	41.7	6.50	1.80	100.0	0.20
DS.J.03	17.4	32.6	41.7	6.50	1.80	100.0	0.30
DS.J.04	17.4	32.6	41.7	6.50	1.80	100.0	0.40
DS.J.05	17.4	32.6	41.7	6.50	1.80	100.0	0.50
CV.J.01	17.4	32.6	41.7	6.50	1.80	100.0	0.10
CV.J.02	17.4	32.6	41.7	6.50	1.80	100.0	0.20
CV.J.03	17.4	32.6	41.7	6.50	1.80	100.0	0.30
CV.J.04	17.4	32.6	41.7	6.50	1.80	100.0	0.40
CV.J.05	17.4	32.6	41.7	6.50	1.80	100.0	0.50
C	17.4	32.6	41.7	6.50	1.80	100.0	0.00
R.B.C	17.4	32.6	41.7	6.50	1.80	100.0	0.30
C.C	17.4	32.6	41.7	6.50	1.80	100.0	0.30

J: Gummy, PC: *Porphyridium cruentum*, DS: *Dunaliella salina*, CV: *Chlorella vulgaris*, C: Control, R.B.C: Red Beet Colorant, C.C: Chlorophyll Colorant 0.1, 0.2, 0.3, 0.4, and 0.5 represent 0.10, 0.20, 0.30, and 0.50 g/100 g encapsulated microalgae, respectively

Physicochemical properties of gummy candy

Gummy candies samples were homogenized with water (1:10, m/m) until completely dissolved. Then, the pH values of these samples were determined using a pH meter (pH-Meter E520, Metrohm Herisau, Switzerland).

Moisture content and water activity (a_w) of gummy samples were determined using a HygroPalm HP23-AW (Rotronic AG, Switzerland) at 20.0 ± 1.0 °C according to the method described by [10].

Texture profile analysis

The textural profile of gummies, with a diameter of 28 mm and a height of 20 mm, was determined with a Texture Profile Analysis (TA-XT, Stable Microsystems, New Castle, USA) using the previously described method [18]. The device had a 5 kg load cell and a 36 mm diameter cylindrical probe. A 50% compression was performed twice consecutively at a 15-second interval. The test speed was 1 mm/s. The force-time curve obtained determined hardness (g), springiness (%), cohesiveness (%), resilience (%), chewiness (g), and gumminess (g) values.

Total carotenoid and chlorophylls contents

Total carotenoid and chlorophyll contents analysis of samples were determined according to the method described by Konar et al. [3]. For this aim, after the addition of 5 mL methanol to 10 mg samples, the mixture was centrifuged (Hettich, Universal 320R, Germany) at 2100 g for 10 min. Then, the absorbance values of the Total chlorophylls and total carotenoid of samples were measured at 665 and 475 nm wavelength using the spectrophotometer (6305 Model, Jenway, Staffordshire, UK), respectively, and the contents were calculated using specific absorption coefficients for each pigment.

$$\text{Total Carotenoid (mg/g)} = [(A_{475} \times V \times f) / (E_{1cm1\%} \times m)] \quad (1)$$

$$\text{Total Chlorophylls (mg/g)} = [(A_{665} \times V \times f) / (E_{1cm1\%} \times m)] \quad (2)$$

A475: absorbance value at 475 nm, A665; absorbance value at 665 nm, V: Volume of the extract or solution, f: Dilution factor, E: Extinction coefficient, m: mass of the sample.

Color analysis

Color properties (L^* , a^* , b^* , C^* , and h°) of gummy candy samples were determined by a colorimeter (CR-400, Konica Minolta, Japan). The SCE mode (Specular light excluded) was used with the color expressed in terms of the CIELAB

system. The L^* (blackness-whiteness), a^* ($\pm$ red-green), and b^* ($\pm$ yellow-blue) values of the samples were measured, and these values were used to determine the chroma (C^*) and hue angle (h°) values by using Eqs. (3) and (4) [19].

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$h^\circ = \arctan(b^*/a^*) \quad (4)$$

Accelerated shelf-life study

Following the preparation of the samples, they were placed in a shelf-life cabinet without packaging and accelerated shelf-life testing conditions were applied at 25 °C and 70% RH, according to the method described by Subramaniam & Wareing [20]. Color analysis (Sect. 2.6) was conducted at 7-day intervals for about 6 weeks. L^* (brightness), a^* ($\pm$ red-green), and b^* ($\pm$ yellow-blue) values were measured, and ΔE^* values were determined based on Eq. 5 [19, 21, 22].

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (5)$$

sensory analysis

The sensory properties of the samples (appearance, color, aroma, sweetness, texture, hardness, chewiness, and odor) were determined using a 9-point hedonic scale (1 = disliked very much, 9 = liked very much) with 20 trained panelists (10 males and 10 females, 18–35 years old).

statistical analysis

All measurements were carried out in triplicate. Data collected in this study were analysed using a SAS statistical software package (version 9.4) by one-way analysis of variance (ANOVA), followed by a comparison of means and significant differences between replicates conducted through Duncan's mean tests at $p < 0.05$. The correlation coefficients between moisture content and water activity values in the gummy samples were determined using Excel (Version 16.96.1 (25042021), Microsoft Office, USA).

Results and discussion

Physico-chemical properties

Moisture is a very important parameter for gaining textural properties and determining the confectionery's shelf life and quality parameters. Moisture contents of gummy

confectionery are generally between 6.00 g/100 g and 18.0 g/100 g [21]. Gummy candy produced with various amounts of microalgae *Porphyridium cruentum* (PC), *Dunaliella salina* (DS), and *Chlorella vulgaris* (CV) has a different moisture content. The moisture content of gummy candies tended to decrease with an increase in microalgae concentration (Table 2). The highest moisture content is related to the control sample (11.85 ± 0.05 g/100 g), followed by PC.J.02 (11.58 ± 0.01 g/100 g), DS.J.02 (11.11 ± 0.01 g/100 g), and CV.J.01 (11.64 ± 0.23 g/100 g), respectively. The lowest was observed in the 0.30 g/100 g addition level of CV and DS and 0.50 g/100 g containing PC gummy candies. There was no significant difference between candies encapsulated with 0.10 and 0.30 g/100 g PC levels. In addition, the DS.J.01, DS.J.04, DS.J.05 and CV.J.02, CV.J.04 treatments did not differ markedly ($p > 0.05$). The concentration of microalgae can influence the difference in moisture content. In a recent study, it was also determined that the use of *Spirulina* biomass led to a decrease in the moisture content of gummy samples [23]. Other studies have also emphasized the interaction between the moisture content of foods and the moisture content of their components [24]. However, the interactions between the process and moisture content should also be considered. For instance, the production of candies requires heat, which evaporates moisture. Also, moderate gelatin hydrolysis improves gel formation, with the ideal hydrolysis level

influenced by moisture content [25]. However, excessive cross-linking may reduce gel capacity, decreasing moisture content [21].

Water activity is an indicator of the relative vapor pressure of the food and is used to understand micro-stability, texture, and water migration during storage. It could be affected by the presence of dissolved sugar, other sweeteners (polyols), and humectants in confections. Water activity in confectionery ranges from 0.500 to 0.850, depending on product type, technology, and composition [20]. Gummies' low water activity supports anthocyanin colorant stability [26]. The control candies presented an average a_w value of 0.67 ± 0.00 . In general, the use of high amounts of microalgae resulted in relatively lower water activity in microalgae candies (Table 2). The water activity (a_w) values for gummy candy typically can vary between 0.650 and 0.750, with a specific emphasis on achieving at least 75% total soluble solids to inhibit mold growth [24]. In our study, the a_w values of the samples were determined to be in the range of 0.663–0.687. Although these values are relatively low, it should be noted that the commonly accepted water activity limit for microbial growth inhibition is 0.500. For example, in a previous study, Batista et al. [8] emphasized the importance of obtaining samples with a_w values below this limit for reducing the risk of spoilage and improving textural properties in cookie samples containing microalgae. Additionally, correlation coefficients (r) were determined to examine the interaction between water activity values and moisture contents for all sample groups. Although these values showed significant differences depending on the microalgae species used, it is noteworthy that all were positive. A medium correlation was determined for the *D. salina* and *C. vulgaris* sample groups, with r values of 0.50 and 0.53, respectively. In the gummy samples containing *P. cruentum*, a strong correlation (0.78) was identified between water activity and moisture content.

The pH of gummies with food acids like citric acid typically ranges from 3.00 to 5.00 [23], while samples without food acids show higher pH values, ranging from 5.00 to 5.30 [27]. Ge et al. [28] also reported a pH range of 3.15–5.00 in gummies containing food acids. In this study, there was no significant difference between the pH value of the control sample and encapsulated gummy candies produced using different ratios of *Dunaliella salina* and *Chlorella vulgaris*. This can be evaluated as adding microalgae had no significant effect on the pH value ($p < 0.05$) of these gummy candies. However, there were significant differences between the control sample and candies containing *Porphyridium cruentum*. Additionally, no significant differences were found among the various concentrations of the microalgae (*P. cruentum*) used. This result also had advantages for other main quality parameters. In gummies, sugar-hydrocolloid

Table 2 Physico-chemical (moisture, water activity, and pH) of gummies

Sample	Moisture (g/100 g)	Water activity	pH
PC.J.01	10.94 ± 0.07^c	0.674 ± 0.001^d	3.37 ± 0.00^a
PC.J.02	11.58 ± 0.01^b	0.683 ± 0.002^{ab}	3.31 ± 0.04^b
PC.J.03	11.45 ± 0.01^{bc}	0.685 ± 0.001^a	3.30 ± 0.01^b
PC.J.04	10.85 ± 0.09^c	0.680 ± 0.002^{bc}	3.29 ± 0.01^b
PC.J.05	10.75 ± 0.52^d	0.677 ± 0.001^c	3.30 ± 0.02^b
C	11.85 ± 0.05^a	0.672 ± 0.001^d	3.34 ± 0.00^a
DS.J.01	10.61 ± 0.31^{bc}	0.665 ± 0.001^c	3.28 ± 0.07^a
DS.J.02	11.11 ± 0.01^b	0.678 ± 0.001^a	3.35 ± 0.05^a
DS.J.03	10.29 ± 0.04^c	0.674 ± 0.002^b	3.24 ± 0.01^a
DS.J.04	10.44 ± 0.49^{bc}	0.663 ± 0.001^c	3.33 ± 0.01^a
DS.J.05	10.58 ± 0.30^{bc}	0.672 ± 0.001^b	3.32 ± 0.06^a
C	11.85 ± 0.05^a	0.672 ± 0.001^b	3.34 ± 0.00^a
CV.J.01	11.64 ± 0.23^{ab}	0.687 ± 0.000^a	3.36 ± 0.01^a
CV.J.02	11.12 ± 0.20^c	0.672 ± 0.000^d	3.37 ± 0.02^a
CV.J.03	10.64 ± 0.18^d	0.675 ± 0.002^c	3.36 ± 0.01^a
CV.J.04	10.79 ± 0.02^{cb}	0.684 ± 0.001^b	3.38 ± 0.00^a
CV.J.05	10.00 ± 0.12^c	0.675 ± 0.001^c	3.38 ± 0.00^a
C	11.85 ± 0.05^a	0.672 ± 0.001^d	3.34 ± 0.00^a

J: Gummy, PC: *Porphyridium cruentum*, DS: *Dunaliella salina*, CV: *Chlorella vulgaris*, C: Control, 0.1, 0.2, 0.3, 0.4, and 0.5 represent 0.10, 0.20, 0.30, and 0.50 g/100 g encapsulated microalgae, respectively. Data are expressed as mean $\pm$ standard deviation ($n=3$) and different letters in the same row show significant difference at the 5% level in Duncan's test ($p < 0.05$)

interactions influence texture, including cohesion over time, while increasing acidity and pH changes affect textural properties [29]. Similarly, Li et al. [30] found that covalent bonding between gelatin, proteins, and epigallocatechin gallate enhanced gel strength and promoted dense structures.

Texture

Generally, texture profile parameters (e.g., hardness, elasticity, gumminess) were affected by the incorporation of different concentrations of microalgae in the preparation of gummy candy, except cohesiveness and chewiness. These findings may be attributed to the proteins and carbohydrates in the microalgae, which may not interact significantly with the gelling agents to alter the cohesiveness and chewiness of gummies. However, interactions between sugars and hydrocolloids in gummies influence texture, such as cohesion, and compositional changes, like acidity and pH shifts, further affect textural properties (da Nascimento et al. [29]. Similarly, Li et al. [30] demonstrated that covalent bonding between gelatin, proteins, and epigallocatechin gallate enhanced gel strength and formed denser structures.

As a result of three different microalga use (Table 3), a significant increasing trend in gummy candy gumminess and hardness was observed ($p < 0.05$) with increasing in microalgae concentration compared to the control sample. However, there was no significant difference between the gumminess value of all candies (PC, J, CV, J, and DS, J) containing 0.10 and 0.20 g/100 g encapsulated microalgae addition.

The hardness increase caused by adding microalgae could be attributed to (a) the higher concentration of microalgae, which may increase the interaction of the encapsulated biomass in the candy formulation with other ingredients, (b) the decrease in moisture content by increasing microalgae concentration probably caused the higher hardness value, (c) microalgae protein and carbohydrate molecules can also play an important role in the water absorption process, which promotes the increase of hardness. These results align with moisture results. There was no significant change in the springiness and cohesiveness values ($p > 0.05$). A similar result was found with Gouveia et al. [31]. According to this study in the cookies enriched with *Chlorella vulgaris* biomass, the texture study showed that the hardness of the biscuits increased significantly and linearly with the amount of microalgal biomass ($p < 0.05$). *Chlorella vulgaris*'s high protein content (about 40%) is contributing to this increase in hardness, which might strengthen the dough's structure. Furthermore, it's possible that the microalga's protein and carbohydrate molecules help in water absorption, contributing to the increased firmness of the biscuit. These results also agree with a previous study [8], where a linear increase in cookie hardness was observed with microalgae increasing concentration from 2 to 6%. It is possibly related to the higher absorption of water and fat/oil ability of 6% microalgae compared with 2% in cookie dough, which strengthens the cookies' internal structure. Hardness was significantly lower, up to 3.0 /100 g, in all microalgae biomass additions of gummy candy samples with the same texture value as the control sample.

Table 3 Texture results of gummy samples containing different encapsulated microalgae

Samples	Hardness (g)	Springiness (%)	Cohesiveness (%)	Gumminess (g)	Chewiness (g)	Resilience (%)
PC.J.01	2074.7±66.0 ^c	1.04±0.06 ^a	0.95±0.01 ^a	1990.3±64.8 ^{cd}	294.4±124.1 ^a	0.38±0.008 ^b
PC.J.02	2153.9±122.3 ^c	1.44±0.54 ^a	0.96±0.01 ^a	2075.3±122.7 ^{cd}	286.6±1062.7 ^a	0.43±0.011 ^a
PC.J.03	2395.4±105.5 ^b	1.05±0.07 ^a	0.97±0.00 ^a	2299.4±116.9 ^{bc}	248.0±119.6 ^a	0.42±0.013 ^a
PC.J.04	2445.1±70.8 ^{ab}	1.03±0.04 ^a	0.96±0.01 ^a	2376.1±72.9 ^b	214.7±169.0 ^a	0.42±0.009 ^a
PC.J.05	2981.5±154.8 ^a	1.05±0.11 ^a	0.96±0.01 ^a	2839.5±125.4 ^a	241.5±127.0 ^a	0.40±0.028 ^{ab}
C	2241.1±107.4 ^c	1.17±0.45	0.96±0.01	2146.4±125.1 ^c	250.9±952.8	0.38±0.001
CV.J.01	2194.6±303.3 ^b	1.04±0.09 ^a	0.96±0.01 ^a	2110.8±293.3 ^c	258.0±334.1 ^{ab}	0.41±0.010 ^a
CV.J.02	2344.5±109.1 ^b	1.16±0.16 ^a	0.95±0.01 ^a	2209.2±127.4 ^{bc}	306.4±411.7 ^a	0.39±0.008 ^b
CV.J.03	2571.3±131.6 ^{ab}	1.01±0.02 ^a	0.96±0.00 ^a	2476.0±112.5 ^{abc}	214.4±327.0 ^b	0.36±0.009 ^c
CV.J.04	2705.3±228.8 ^a	1.01±0.04 ^a	0.95±0.01 ^a	2559.1±194.4 ^{ab}	257.4±245.7 ^{ab}	0.39±0.013 ^b
CV.J.05	2762.3±123.6 ^a	1.06±0.08 ^a	0.94±0.04 ^a	2631.6±133.6 ^a	233.6±154.7 ^b	0.39±0.006 ^b
C	2241.1±107.4 ^b	1.17±0.45	0.96±0.01	2146.4±125.1 ^c	250.9±952.8	0.38±0.001
DS.J.01	2196.6±85.4 ^b	1.19±0.37 ^a	0.96±0.01 ^a	2092.7±46.5 ^{bc}	274.9±484.0 ^a	0.39±0.008 ^b
DS.J.02	2478.3±285.9 ^b	1.10±0.20 ^a	0.96±0.01 ^a	2294.5±529.3 ^{bc}	257.4±972.7 ^a	0.41±0.005 ^a
DS.J.03	2385.9±567.8 ^b	1.00±0.00 ^a	0.95±0.01 ^a	2377.7±283.2 ^b	303.8±55.6 ^a	0.34±0.016 ^c
DS.J.04	2555.7±231.9 ^{ab}	1.00±0.01 ^a	0.96±0.01 ^a	2442.7±228.8 ^{ab}	244.2±222.0 ^a	0.36±0.006 ^c
DS.J.05	3197.7±78.1 ^a	1.09±0.16 ^a	0.95±0.02 ^a	3041.2±54.8 ^a	227.3±314.0 ^a	0.36±0.006 ^c
C	2241.1±107.4 ^b	1.17±0.45	0.96±0.01	2146.4±125.1 ^c	250.9±952.8	0.38±0.001

J: Gummy, PC: *Porphyridium cruentum*, DS: *Dunaliella salina*, CV: *Chlorella vulgaris*, C: Control, 0.1, 0.2, 0.3, 0.4, and 0.5 represent 0.10, 0.20, 0.30, and 0.50 g/100 g encapsulated microalgae, respectively. Data are expressed as mean±standard deviation ($n=3$) and different letters for each microalgae in the same row show significant differences at the 5% level in Duncan's

In addition, increasing microalgae concentration from 0.10 to 0.50 g/100 g causes a significant ($p < 0.05$) increase in gumminess, from 2839.5 ± 125.4 g, 2631.6 ± 133.6 g, and 3041.2 ± 54.8 g to 2146.4 ± 125.1 g for PC, DS, and CV containing candies, respectively. The study conducted by Çelekli & Maraşlı [32] utilized *Arthrospira platensis* biomass in marshmallow formulation as a stabilizer and gelling agent and concluded that the use of microalgae resulted in a decrease in gumminess and hardness. In another study, García-Segovia et al. [33] concluded that incorporating four microalgae species did not affect bread's hardness and chewiness.

Pigment contents

The total carotenoids and chlorophyll contents of gummy candy samples containing different encapsulated microalgae are presented in Table 4. As expected, both pigment concentrations increased significantly with increasing microalgae content ($p < 0.05$), except for the samples containing *P. cruentum*, which showed a less pronounced increment. Among the samples, the control exhibited the lowest total pigment concentration (0.11 ± 0.01 µg/g), while the

gummy sample with *C. vulgaris* (CV) at the 0.10 g/100 g level showed comparable results to the control.

Pigments such as carotenoids and chlorophylls are not only important for their visual appeal and natural coloration of the food matrix but also for their **bioactive properties**, including antioxidant activity, provitamin A activity (especially β -carotene), and potential health-promoting effects (e.g., immune support and anti-inflammatory roles). Therefore, higher pigment levels may enhance the **functional value** of the gummy products, positioning them as nutritionally superior or functional foods.

The observed variations in pigment concentrations across formulations suggest differing stabilities of pigments depending on both the type of microalga and the encapsulation process. For example, *P. cruentum* showed lower pigment retention, likely due to its pigment profile being more sensitive to processing stresses such as pH changes and thermal degradation. Pigment stability is influenced by several factors including the composition of the algal biomass, pH sensitivity, presence of organic acids (e.g., citric acid), and processing conditions like heat. Additionally, some pigment carriers or encapsulants can protect pigments better than others.

It has been reported that anthocyanins are relatively more stable under acidic conditions compared to chlorophylls, which can degrade into pheophytins. Reformulation strategies, such as adjusting acid levels or including stabilizing agents, may thus enhance pigment stability—particularly chlorophylls, which are prone to acid-induced degradation [26].

Gummy candies containing *P. cruentum* (PC) at 0.20 and 0.30 g/100 g levels exhibited the highest carotenoid and chlorophyll concentrations, with PC.J.03 recording the peak carotenoid content (1.83 ± 0.00 mg/g) and the highest chlorophyll content (0.89 ± 0.00 µg/g). This higher pigment retention is likely due to the spray-drying encapsulation process, which enhances pigment protection by limiting exposure to heat and oxygen and through the use of suitable wall materials. Encapsulation also creates a physical barrier against degradation factors, resulting in improved pigment bioavailability and stability post-processing.

Furthermore, the biomass composition itself plays a critical role: for example, CV contains proteins that may serve as natural pigment stabilizers, reducing degradation during heat processing [34]. *Dunaliella salina* (DS), known for its high β -carotene content (up to 14% of dry weight), also contributes significantly to the carotenoid content in the final product. This highlights the importance of microalgal species selection in designing pigment-rich functional confections.

Studies by Konar et al. [3, 5] optimized spray-drying conditions to improve encapsulation yield and pigment

Table 4 Total carotenoid and chlorophylls content of gummy samples containing different encapsulated microalgae

Sample	Total carotenoid (µg/g)	Total chlorophylls (µg/g)
PC.J.01	0.96 ± 0.00^c	0.43 ± 0.00^c
PC.J.02	1.70 ± 0.00^a	0.82 ± 0.00^a
PC.J.03	1.83 ± 0.00^a	0.89 ± 0.00^a
PC.J.04	1.10 ± 0.00^b	0.50 ± 0.00^b
PC.J.05	1.15 ± 0.00^b	0.54 ± 0.00^b
DS.J.01	0.14 ± 0.01^c	0.50 ± 0.04^c
DS.J.02	0.16 ± 0.01^c	0.14 ± 0.01^d
DS.J.03	0.38 ± 0.06^b	0.28 ± 0.03^c
DS.J.04	0.88 ± 0.03^a	0.68 ± 0.02^b
DS.J.05	1.00 ± 0.01^a	0.81 ± 0.01^a
CV.J.01	0.02 ± 0.00^d	0.01 ± 0.00^c
CV.J.02	0.14 ± 0.00^c	0.09 ± 0.00^c
CV.J.03	0.20 ± 0.00^b	0.18 ± 0.00^b
CV.J.04	0.43 ± 0.00^a	0.17 ± 0.00^b
CV.J.05	0.48 ± 0.01^a	0.22 ± 0.00^a
C	$0.11^c \pm 0.01$	$0.10^c \pm 0.01$
R.B.C	$0.78^a \pm 0.00$	$0.36^a \pm 0.00$
C.C	$0.43^b \pm 0.00$	$0.23^b \pm 0.00$

J: Gummy, PC: *Porphyridium cruentum*, DS: *Dunaliella salina*, CV: *Chlorella vulgaris*, C: Control, R.B.C: Red Beet Colorant, C.C: Chlorophyll Colorant 0.1, 0.2, 0.3, 0.4, and 0.5 represent 0.10, 0.20, 0.30, and 0.50 g/100 g encapsulated microalgae, respectively. Data are expressed as mean $\pm$ standard deviation ($n = 3$). Different lowercase letters indicate a significant difference in each pigment content of the samples in the same column for each algae group depending on the encapsulated algae concentration and also for control samples including various colorant ($p < 0.05$)

retention, concluding that both inlet temperature and feed formulation significantly influenced final pigment stability. These findings are supported by Ghosh et al. [35], who emphasized that encapsulation is among the most effective techniques for preserving plant pigments—enhancing not only their chemical stability but also the flavor, aroma, and overall sensory quality of the final product.

In summary, pigment variation among samples reflects both the compositional differences of microalgae and the effectiveness of the encapsulation strategy. These findings underscore the need to tailor microalgae selection and process conditions for maximizing functional attributes and consumer appeal in pigment-enriched gummy products.

Color

Color is one of the key quality parameters of food indicators, enhancing consumer palatability, acceptance, and sensory appeal [36]. Various parameters may affect the coloring properties and stabilities of pigments and their source in gummy formulations. For instance, proteins, such as gelatin, influence pigment stability [37]; [38]. All gummy candy samples showed a significant decrease in L^* values by increasing the use of all three microalgae concentrations compared to the control sample ($p < 0.05$). a^* (redness) and b^* (yellowness) values showed significant differences ($p < 0.05$). Increasing the PC, CV, and DS content microcapsules in the gummy candy formulation made the candies darker due to decreased L^* value. The darkening of the gummy candies' color was also a result of the Maillard reaction. Incorporating microalgae into candy formulations contributes to the composition of amino acids, strengthening the Maillard reaction between sugars (like glucose and fructose) and the amino acids in

gelatin and microalgae. The results also agree with Dewi & Purnamayati [39], who added natural dye from *Caulerpa sp.* to seaweed gummy drink, resulting in a darkening color due to the Maillard reaction. Additionally, pigments can degrade during storage due to the reducing properties of saccharides, impacting color stability and altering the final product [26]. Factors like pH increases during storage and environmental or compositional conditions must be carefully managed to maintain pigment stability [40].

Based on Table 5, the higher encapsulated PC biomass addition to the gummy candy formulation caused a significant increase in a^* values, and the color was red. This indicated that encapsulated PC could persist during gummy candy processing [41]. However, CV and DS caused increases in the green zone $a^*(-)$ and decreased the value of b^* . This can be due to the high quantity of carotenoids and chlorophylls pigments in these microalgae [3]. It is known that when using natural coloring in a food matrix, the resulting color is linked to the pigments present in microalgae, as well as the concentrations and temperatures used in the manufacturing process [42].

Accelerated shelf life (ASL)

AST is a crucial methodology for increasing the physical or chemical degradation rate. It is a relatively quick test to determine the shelf life of various food products by storing them under increased stress conditions to speed up deterioration processes [43]. In gummy candies, texture, water activity, moisture content, and color stability are critical throughout shelf life, particularly in reformulation and enrichment studies. Color stability is one of the key shelf-life parameters in gummy candies. The main reasons for this

Table 5 Color results of gummy samples containing different encapsulated microalgae

Sample	L^*	a^*	b^*	Chroma	Hue
PC.J.01	38.85 ± 1.73 ^b	2.09 ± 0.14 ^d	9.62 ± 0.99 ^b	9.84 ± 1.10 ^b	77.67 ± 0.78 ^b
PC.J.02	35.18 ± 1.58 ^c	3.46 ± 0.36 ^c	7.97 ± 0.47 ^c	8.69 ± 0.60 ^{bc}	66.54 ± 1.31 ^c
PC.J.03	34.02 ± 1.51 ^c	3.52 ± 0.25 ^c	6.32 ± 0.66 ^{cd}	7.23 ± 0.75 ^c	60.73 ± 1.62 ^d
PC.J.04	34.30 ± 0.82 ^c	4.81 ± 0.63 ^b	5.95 ± 1.05 ^d	7.84 ± 1.06 ^c	51.24 ± 1.57 ^e
PC.J.05	33.24 ± 1.11 ^c	5.73 ± 0.64 ^a	5.12 ± 0.69 ^d	7.68 ± 0.99 ^c	41.64 ± 1.37 ^f
C	41.99 ± 1.79 ^a	-0.86 ± 0.16 ^c	14.18 ± 0.19 ^a	14.30 ± 0.40 ^a	93.48 ± 0.54 ^a
CV.J.01	34.40 ± 1.10 ^b	-0.30 ± 0.19 ^c	14.37 ± 0.97 ^a	14.38 ± 1.09 ^a	91.18 ± 0.60 ^a
CV.J.02	33.08 ± 0.58 ^b	0.53 ± 0.04 ^b	14.18 ± 0.33 ^a	14.19 ± 0.37 ^a	87.87 ± 0.16 ^b
CV.J.03	29.26 ± 0.42 ^c	0.87 ± 0.03 ^a	9.48 ± 0.41 ^b	9.52 ± 0.39 ^b	84.73 ± 0.22 ^c
CV.J.04	26.88 ± 0.90 ^d	0.85 ± 0.25 ^a	5.48 ± 1.40 ^c	5.55 ± 1.57 ^c	81.00 ± 1.99 ^d
CV.J.05	25.07 ± 0.27 ^e	0.88 ± 0.06 ^a	3.38 ± 0.11 ^d	3.41 ± 0.13 ^d	81.90 ± 0.68 ^d
C	41.99 ± 1.79 ^a	-0.86 ± 0.16 ^c	14.18 ± 0.19 ^a	14.30 ± 0.40 ^a	93.48 ± 0.54 ^a
DS.J.01	39.23 ± 1.62 ^a	-0.35 ± 0.11 ^d	16.38 ± 0.57 ^a	16.38 ± 0.64 ^a	91.24 ± 0.39 ^a
DS.J.02	35.74 ± 1.22 ^b	0.77 ± 0.19 ^b	16.46 ± 0.98 ^a	16.48 ± 1.09 ^a	87.31 ± 0.63 ^b
DS.J.03	29.22 ± 0.34 ^c	1.22 ± 0.14 ^a	7.80 ± 0.46 ^c	7.89 ± 0.53 ^c	81.10 ± 0.68 ^d
DS.J.04	28.44 ± 0.49 ^c	0.28 ± 0.10 ^c	3.66 ± 0.41 ^d	3.74 ± 0.48 ^d	84.59 ± 2.12 ^c
DS.J.05	28.13 ± 1.54 ^c	0.74 ± 0.19 ^b	5.11 ± 0.84 ^d	5.16 ± 0.95 ^d	81.83 ± 0.98 ^d
C	41.99 ± 1.79 ^a	-0.86 ± 0.16 ^c	14.18 ± 0.19 ^a	14.30 ± 0.40 ^b	93.48 ± 0.54 ^a

J: Gummy, PC: Porphyridium cruentum, DS: Dunaliella salina, CV: Chlorella vulgaris, C: Control, 0.1, 0.2, 0.3, 0.4, and 0.5 represent 0.10, 0.20, 0.30, and 0.50 g/100 g encapsulated microalgae, respectively. Data are expressed as mean ± standard deviation ($n = 3$) and different letters in the same row show significant difference for each sample group ($p < 0.05$)

include the widespread use of colorants in these products, the impact of the harmony between flavor and color characteristics on consumer preference, and the strong relationship and interactions between color stability and various environmental (e.g., relative humidity, temperature) and internal conditions (e.g., pH, moisture content) [21]. Therefore, in addition to shelf-life studies, the color difference (ΔE^*) is evaluated in research examining the possibilities of using reformulation and innovative ingredients in the composition of soft confectionary products such as gummies, and it is expected to be below 3.0 [19, 22]. For instance, Da Nascimento et al. [29] reported significant increases in a^* and b^* values during storage, with relatively stable L^* values, attributing these changes to Maillard reactions.

Gummy candies prepared with various microalgae at different addition levels were stored without packaging at 25 °C and 70% relative humidity, a commonly used accelerated shelf-life condition. The results of color changes of samples under accelerated shelf-life conditions as the total color difference (ΔE) was determined (Table 6) to assess color stability. Color measurements were conducted during a one-week (7-day) period under normal storage conditions for one month. Color properties were monitored for 6 weeks for this purpose. Variance analysis (ANOVA) was not performed on this data as the obtained data was used to determine ΔE values. The ΔE (color difference) is determined based on the difference between the L^* , a^* , and b^* values determined in the initial and final measurements. A ΔE value greater than 3.0 is generally considered a perceptible color change [10, 11]. Therefore, ΔE measurements

below 3.0 indicate the sample has color stability under the given conditions. In addition, the color stability of the control gummy sample (without microalgae) was also evaluated. It was determined that both the type of microalgae and the additional level of encapsulated microalgae affected the color stability of gummy samples. It was found that gummy samples prepared with PC and DS exhibited higher color stability. For all three microalgae species, using 0.30, 0.4, and 0.5 g/100 g of encapsulated microalgae resulted in higher color stability. It was also observed that the type of confectionery is a factor that should be considered in terms of color stability. Except for the encapsulated microalgae usage levels of 0.10 g/100 g for PC and CV and 0.30 and 0.4 g/100 g for DS, it was determined that the ΔE value was below 3.0 after 6 weeks for other usage levels. According to the ASL, using low temperatures and high relative humidity, ingredients like citric acid and sucrose affected by moisture content led to color changes in confectionery products due to chemical reactions and degradation processes. The ΔE values of candy, which indicate color differences, can be affected by different factors, including ingredients, storage conditions, and manufacturing processes [42]. According to Shukla & Kandra [44], natural ingredients used in candy can affect ΔE values due to their inherent color properties. Also, Sakulnarmrat and Konczak [45] found that the color stability of gummies with encapsulated Lamduan anthocyanins (1.00–3.00 g/100 g) varied with storage temperature. At higher temperatures (35 °C), L^* and b^* values increased, while a^* values decreased. Different anthocyanin sources,

Table 6 Color differences (ΔE) of gummy samples containing various encapsulated microalgae biomass under accelerated shelf-life (25 °C/70% RH for 6 weeks) conditions

Sample	ΔE				
	Week 2	Week 3	Week 4	Week 5	Week 6
PC.J.01	2.42 ± 0.42 ^{b, B}	2.73 ± 1.03 ^{b, B}	2.67 ± 1.42 ^{b, B}	3.65 ± 1.56 ^{a, A}	4.12 ± 0.99 ^{a, A}
PC.J.02	4.30 ± 1.75 ^{a, A}	3.76 ± 0.68 ^{a, B}	2.78 ± 2.72 ^{b, C}	4.02 ± 2.70 ^{a, A}	2.25 ± 1.45 ^{c, C}
PC.J.03	2.56 ± 0.30 ^{b, B}	2.36 ± 0.64 ^{b, B}	2.55 ± 1.17 ^{b, B}	2.63 ± 0.76 ^{b, B}	3.21 ± 1.54 ^{b, A}
PC.J.04	1.46 ± 0.61 ^{c, C}	1.58 ± 0.72 ^{c, C}	1.87 ± 0.46 ^{c, B}	2.56 ± 1.21 ^{b, A}	2.30 ± 0.82 ^{c, A}
PC.J.05	1.51 ± 1.18 ^{c, C}	3.36 ± 1.54 ^{a, B}	3.63 ± 1.27 ^{a, B}	3.81 ± 0.45 ^{a, B}	2.24 ± 1.67 ^{c, B}
DS.J.01	3.90 ± 2.33 ^{a, A}	3.59 ± 2.04 ^{a, A}	3.42 ± 1.65 ^{a, A}	3.36 ± 0.65 ^{a, A}	3.38 ± 1.66 ^{d, A}
DS.J.02	2.13 ± 0.90 ^{c, B}	2.18 ± 0.76 ^{b, B}	2.35 ± 1.52 ^{b, B}	2.24 ± 0.59 ^{c, B}	4.38 ± 1.05 ^{a, A}
DS.J.03	2.76 ± 0.64 ^{c, B}	3.51 ± 3.95 ^{a, A}	1.87 ± 1.10 ^{c, C}	1.63 ± 1.34 ^{d, C}	1.80 ± 0.47 ^{c, C}
DS.J.04	3.64 ± 0.77 ^{a, A}	2.60 ± 0.64 ^{b, B}	2.29 ± 1.20 ^{b, B}	2.88 ± 0.80 ^{b, B}	4.00 ± 1.67 ^{a, A}
DS.J.05	3.20 ± 1.19 ^{b, A}	1.84 ± 1.08 ^{c, C}	2.17 ± 0.79 ^{b, B}	2.23 ± 0.93 ^{c, C}	2.75 ± 0.70 ^{c, B}
CV.J.01	16.6 ± 3.99 ^{a, A}	15.4 ± 0.56 ^{a, A}	12.7 ± 2.65 ^{a, B}	12.0 ± 4.16 ^{a, B}	16.5 ± 3.74 ^{a, A}
CV.J.02	2.88 ± 0.79 ^{c, C}	2.04 ± 1.67 ^{c, C}	2.79 ± 0.85 ^{b, C}	4.19 ± 2.51 ^{c, A}	3.19 ± 1.46 ^{c, B}
CV.J.03	4.69 ± 1.47 ^{b, A}	2.78 ± 2.40 ^{c, C}	4.04 ± 2.31 ^{b, A}	2.61 ± 2.52 ^{c, C}	3.51 ± 2.70 ^{c, B}
CV.J.04	5.97 ± 1.37 ^{b, C}	9.35 ± 3.33 ^{b, B}	14.94 ± 9.97 ^{a, A}	9.21 ± 2.65 ^{b, B}	9.52 ± 2.34 ^{b, B}
CV.J.05	1.50 ± 0.45 ^{c, B}	1.65 ± 0.63 ^{c, B}	1.67 ± 0.47 ^{b, B}	2.03 ± 0.54 ^{d, A}	1.81 ± 0.31 ^{c, A}
C	1.27 ± 0.46 ^C	1.13 ± 0.56 ^C	1.68 ± 0.33 ^B	2.26 ± 0.65 ^A	1.77 ± 0.42 ^B

J: Gummy, PC: *Porphyridium cruentum*, DS: *Dunaliella salina*, CV: *Chlorella vulgaris*, C: Control, 0.1, 0.2, 0.3, 0.4, and 0.5 represent 0.10, 0.20, 0.30, and 0.50 g/100 g encapsulated microalgae, respectively. Data are expressed as mean ± standard deviation ($n=3$). Different uppercase letters indicate a significant difference in the color difference values of the samples in the same row depending on the storage time. Different lowercase letters indicate a significant difference in the color difference values of the samples in the same column for each algae group depending on the encapsulated algae concentration ($p<0.05$)

Table 7 Sensory properties of gummy candy samples containing different encapsulated microalgae

Sample	Appearance	Color	Aroma	Sweetness	Texture	Hardness	Chewiness	Odour
PC.J.01	5.0±1.40 ^b	4.7±1.60 ^c	6.9±1.70 ^a	7.3±0.90 ^a	7.0±0.80 ^a	6.6±0.90 ^a	6.6±0.90 ^a	7.6±0.70 ^a
PC.J.02	5.1±1.60 ^b	5.0±1.60 ^b	7.0±1.70 ^a	7.4±0.70 ^a	6.9±0.60 ^a	6.6±0.70 ^a	6.6±0.70 ^a	7.7±0.50 ^a
PC.J.03	5.9±2.10 ^b	5.6±1.80 ^b	6.9±1.70 ^{ab}	7.0±0.80 ^a	6.4±0.50 ^a	6.3±0.50 ^a	6.3±0.50 ^a	7.1±0.80 ^a
PC.J.04	6.1±2.00 ^a	6.1±2.10 ^a	6.4±2.00 ^b	6.9±1.50 ^a	7.1±1.20 ^a	7.3±1.20 ^a	7.3±1.20 ^a	6.6±1.40 ^b
PC.J.05	6.4±2.10 ^a	6.7±2.00 ^a	6.3±2.20 ^b	6.7±1.70 ^a	7.3±1.20 ^a	7.4±1.00 ^a	7.4±1.00 ^a	6.7±1.20 ^b
CV.J.01	5.1±1.90 ^b	5.0±2.00 ^b	6.1±1.40 ^a	6.6±0.90 ^a	6.7±0.90 ^a	6.6±0.90 ^a	6.6±0.90 ^a	6.7±1.20 ^a
CV.J.02	5.3±1.70 ^b	5.0±1.90 ^b	6.0±1.50 ^a	6.4±1.00 ^a	7.1±0.60 ^a	7.0±0.80 ^a	6.9±1.00 ^a	6.4±1.30 ^a
CV.J.03	6.0±0.90 ^a	5.7±1.40 ^b	5.4±1.30 ^b	5.9±0.80 ^a	6.9±0.30 ^a	6.9±0.60 ^a	6.9±0.60 ^a	6.1±1.10 ^a
CV.J.04	6.3±1.20 ^a	6.0±1.60 ^a	5.1±1.40 ^b	5.6±1.00 ^a	6.7±0.70 ^a	6.9±1.00 ^a	6.9±1.00 ^a	5.9±1.40 ^b
CV.J.05	6.3±1.20 ^a	6.0±1.60 ^a	5.1±1.40 ^b	5.7±0.70 ^a	6.6±0.70 ^a	6.9±1.00 ^a	6.9±1.00 ^a	5.9±1.40 ^b
DS.J.01	6.9±1.80 ^b	5.1±2.00 ^b	8.0±0.00 ^a	8.0±0.00 ^a	8.0±0.00 ^a	8.3±0.50 ^a	8.0±0.00 ^a	8.3±0.50 ^a
DS.J.02	6.7±1.50 ^b	5.9±3.10 ^b	7.3±1.00 ^a	6.6±2.70 ^a	6.9±2.80 ^a	7.1±2.90 ^a	6.7±2.80 ^a	6.4±2.70 ^{ab}
DS.J.03	7.3±1.40 ^a	7.0±1.80 ^a	7.1±1.00 ^a	7.3±1.00 ^a	7.7±0.50 ^a	8.0±0.80 ^a	7.4±0.90 ^a	6.4±1.20 ^{ab}
DS.J.04	7.0±1.20 ^a	7.1±1.40 ^a	6.6±2.70 ^b	7.3±0.90 ^a	8.3±0.70 ^a	8.4±0.70 ^a	8.0±1.30 ^a	6.1±0.80 ^b
DS.J.05	7.1±1.40 ^a	7.3±1.50 ^a	6.4±1.60 ^b	7.0±0.80 ^a	8.3±0.70 ^a	8.4±0.70 ^a	8.0±1.30 ^a	5.7±1.20 ^b
C	8.1±1.40 ^a	7.4±1.90 ^a	7.7±2.00 ^a	8.1±1.00 ^a	8.3±0.70 ^a	7.9±1.00 ^a	7.9±1.00 ^a	8.3±0.50 ^a

J: Gummy, PC: *Porphyridium cruentum*, DS: *Dunaliella salina*, CV: *Chlorella vulgaris*, C: Control, 0.1, 0.2, 0.3, 0.4, and 0.5 represent 0.10, 0.20, 0.30, and 0.50 g/100 g encapsulated microalgae, respectively. Data are expressed as mean±standard deviation ($n=3$) and different letters in the same row show significant difference at the 5% level in Duncan's test ($p<0.05$)

encapsulation materials, and processing conditions significantly impact color stability.

Sensory properties

Sensory evaluation is critical in assessing the acceptability of new ingredients in food formulations, development, product reformulation, and evaluating the consumer acceptance of the final product [36]. Table 7 summarizes the sensory analysis results of gummy candy samples. There were no significant differences between the three microalgae in all addition levels for all the assessed sensory parameters (texture, sweetness, hardness, and chewiness) ($p>0.05$). By increasing microalgae biomass concentration, a significant decrease ($p<0.05$) was observed in aroma and odour parameters ($p<0.05$). The consumption of microalgae-based food products shows minimal negative reactions, with most concerns being related to their smell [46]. The gummy candy color and appearance were significantly influenced by the addition of encapsulated biomass, which contains red (*Porphyridium cruentum*), reddish-orange color (*Dunaliella salina*), and green color (*Chlorella vulgaris*) that contains phycobilins, high β -carotene, and chlorophyll, respectively functioning as natural colorants. The highest sensory score for color and appearance was observed for samples containing 0.40 and 0.50 g/100 g microalgae. In contrast, the lowest sensory score for aroma was obtained for 0.40 and 0.50 g/100 g addition levels. In this sense, past studies indicated that [47] algae contain a complex variety of volatile compounds, including fatty acid-derived volatiles, sulfuric compounds, and other odor-active substances. As the concentration of algae increases in a product, the balance

of these compounds can shift, which might result in off-flavors that overshadow desirable aromas associated with confectionery products. In addition, algae have a “grassy” or “fishy” taste, which can become more pronounced with higher concentrations. Confectionery products may lose some of their original taste character due to this unpleasant sensory experience, making them less appealing.

Conclusions

This study demonstrated the successful incorporation of microalgae into gummy candy formulations as a novel source of natural bioactive compounds beneficial to human nutrition. The encapsulated forms of *Porphyridium cruentum*, *Dunaliella salina*, and *Chlorella vulgaris*, produced under previously optimized spray-drying conditions, were effectively integrated into the gummy matrix. As the focus was on the application of these encapsulated biomasses, specific characterization of their physicochemical properties was beyond the scope of this work.

The addition of microalgae significantly influenced the physicochemical and textural properties of the gummy candies. Enhanced levels of total carotenoids and chlorophylls were observed, especially at higher incorporation levels, contributing to the improved nutritional profile. Texture analyses indicated favorable changes in parameters such as cohesiveness and chewiness, which may positively impact consumer perception. Sensory evaluations revealed that although the enriched gummies were generally comparable to the control in terms of texture and sweetness, noticeable

differences were observed in color, aroma, and odor—particularly at higher concentrations of microalgae.

Despite these promising findings, several limitations should be acknowledged. First, a comprehensive sensory analysis including attributes such as flavor, aroma, and visual appeal was not fully conducted; future studies should address these components more extensively. Second, the study did not evaluate the effects of maltodextrin alone on gummy formulations, limiting our understanding of its contribution to the observed changes in texture and stability. Additionally, acceptance testing with a consumer panel was not performed, which restricts insights into market-level acceptance of microalgae-enriched products. Finally, strategies to improve or mask the less favorable sensory attributes associated with microalgae—particularly their “green” or “fishy” notes—remain an area for further investigation.

In conclusion, microalgae-enriched gummies show considerable potential as functional confectionery products aimed at health-conscious consumers. Future research should focus on refining sensory properties, exploring flavor enhancement techniques, and conducting consumer acceptance studies to support the broader application of microalgae in the healthy snack sector.

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Data availability The data will be available from the corresponding author.

Declarations

competing interest The authors declare no conflict of interest and no competing financial interest.

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