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Using spray-dried microalgae as a natural coloring agent in chewing gum: effects on color, sensory, and textural properties

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Abstract Chlorophyll-*a* and total carotenoid quantities of *Isochrysis galbana* and *Nannochloropsis oculata* biomass dried at different inlet temperatures (150–200 °C) using the spray dryer technique were investigated. The amounts of chlorophyll-*a* in *I. galbana* and *N. oculata* biomass were determined in the range of 7.775–11.377 mg g^{-1} and 1.141–1.836 mg g^{-1}, respectively. Total carotenoids were found as 1.984–3.373 mg g^{-1} (dry biomass) for *I. galbana* and 0.378–0.077 mg g^{-1} (dry biomass) for *N. oculata*. Then, these biomasses were used at levels of 0.5% and 1% as natural green colorants in chewing gum, and color, texture, and sensory properties of chewing gums were investigated. The samples were identified as khaki or light khaki. *L** value of the samples containing *I. galbana* species ranged from 42 to 53, *a** value ranged from − 1.3 to − 2.88, *b** value ranged from 11.5 to 15, and *C** value ranged from 9.4 to 15.2, while *L** values ranged from 55 to 65, *a** value varied from − 0.03 to − 1.7, *b** value varied from 15 to 18.2, and the *C** value varied from 15 to 18.2 for *N. oculata* biomass. The highest cohesiveness value was found as 0.26 in the samples containing 1% *I. galbana* species dried at 180 °C. There were no alga tastes except for chewing gum containing 1% *N. oculata* dried at 150 °C.

Keywords Confectionery · Chewing gum · Natural additive · Coloring agent · Drying · *Nannaochloropsis oculata* · *Isochrysis galbana* · Microalgae

Introduction

Chewing gum is a different foodstuff due to its long duration in the mouth. It is inedible and various components release during chewing. Bioactive compounds present in the chewing gum product can be released from the bulk into the saliva and absorbed by the oral mucosa or metabolized by reaching the stomach for gastrointestinal absorption. For this reason, released bioactive compounds might be absorbed by two different ways (Konar et al. 2016).

General regulations in many countries and consumer trends around the world are driving food producers to color foods with natural ingredients or substances. There is a fairly limited number of studies on the use and properties of colorants in chewing gum matrix. Charanioti et al. (2015) examined the use of saffron and red beet extracts as colorants in chewing gum after encapsulation with gum arabic and modified starch, and the coloring performances of these encapsulated extracts were evaluated positively. Another potential natural coloring source is microalgae. Microalgae also contain bioactive components such as polyunsaturated fatty acids (PUFA) in their composition, which can be mentioned as another element that promotes consumer health as well as the coloring effect.

The microalgae are valuable natural products used as animal and human food sources and for pharmaceuticals and medicines. In some countries, microalgal biomass is added

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to food products such as noodles to improve the nutrient profile (Huo et al. 1997; Fuentes et al. 2000; Gizberg et al. 2000). The main source of ω3 fatty acids is marine algae and humans are unable to synthesize ω3 and ω6 fatty acids (Gizberg et al. 2000). Furthermore, microalgae-derived PUFA is added to artificial baby food (Cohen 1991). Eicosapentaenoic acid (EPA, 20:5(n–3)) and docosahexaenoic acid (DHA, 22:6(n–3)) have also been reported to have beneficial effects such as reducing coronary heart disease risk and blood cholesterol, thus reducing the risk of arteriosclerosis, inflammation and several carcinomas (Gizberg et al. 2000; Guil-Guerrero et al. 2001; Otles and Pire 2001; Pulz and Gross 2004).

Nannochloropsis species (Eustigmatophyta) are an important food source for aquatic organisms. The nutritional value of the microalgae is related to their biochemical composition and especially to its lipid and fatty acid composition (Sukenik et al. 1993; Durmaz 2007). They are also a source of EPA that is an indispensable food chain component for organisms cultured in hatcheries (Lubzens et al. 1995).

The golden-brown flagellate *Isochrysis galbana* is a rich source of PUFA, mainly EPA (Molina et al. 1994; Fidalgo et al. 1998). Its richness in PUFA makes it promising as an animal and/or human nutraceutical food (Otles and Pire 2001). *Isochrysis galbana* synthesizes important bioactive metabolites such as sterols (Volkman et al. 1981), tocopherols (Fábregas and Herrero 1990), carotenoids (Flynn et al. 1993), and pharmaceuticals (Fábregas et al. 1985).

In the European Union, 46% of consumers prefer mint, while 24% prefer chewing gum with mint and fruit flavor (Hearty et al. 2014). Among the colors, most associated with these aromas is the green color. Therefore, in this study, it was aimed to investigate pigment (total carotenoid and chlorophyll-*a*) quantity of *I. galbana* and *Nannochloropsis oculata* microalgae biomass dried at different inlet spray dryer temperatures, and the use of these dried algae as a food colorant in chewing gum and effects on color, texture, and sensory properties of colored chewing gums.

Materials and methods

Microalgae cultures and culture conditions

Nannochloropsis oculata (Droop) Hibberd (CCAP 849/1) and *Isochrysis galbana* Parke (CCAP 927/1) were obtained from The Culture Collection of Algae and Protozoa (CCAP), Scotland. Culture was kept illuminated with halogen lamp (Philips Halogen lamp 400W E40 38 × 215 mm) in this study. Irradiance was measured on the surface of tube as 200 μmol photons m^{-2} s^{-1}. Culture medium (F/2 medium) (Guillard and Ryther 1962) was added 1 mL per liter daily. All cultures were maintained at 35‰ salinity and 20 ± 1 °C under 24 h light regime. The tubular photobioreactor was inoculated and operated in semi-continuous mode for 11 days.

Growing and harvesting of microalgae

The experiments were performed in a tubular photobioreactor. The tubular photobioreactor was wound on a rigid vertical structure, 2 m in length, 0.5 m width, and 1.6 m height and was divided into two parts; a tubular illumination receiver with a degasser and a cooler tank. Tubular tube system was positioned in a fence-like structure made of transparent plexiglass tubes and consisted of 125 m total length with an internal diameter of 4.6 cm and 0.2 cm wall thickness. The degasser and cooler tank were used for mixing, degassing, and heat exchange of culture medium. The pH control unit was set at 7.5 and automatic injection of pure industrial-grade CO_2 gas at 5 L min^{-1}. The tubular photobioreactor was disinfected by using sodium hypochlorite overnight and neutralized for 2 h with sodium thiosulfate. While preparing the tubular photobioreactor for microalgae culture, marine water was sterilized by passing through 0.02 μm filtration system, and also, sterilized marine water was used during the harvest period of the system. Daily culture volume was taken from the culture of tubular photobioreactor system according to dilution ratio. The biomass was harvested and concentrated with disc separator (GEA Westfalia Separator, Germany).

Spray drying

Spray drying process was performed in a laboratory scale spray dryer (Buchi B290, England), with a nozzle atomization system with 1.0 μm diameter nozzle. The separated algal biomasses were fed into the main chamber by a peristaltic pump and the feed flow rate was controlled by the pump rotation speed. The main parameters important for the spray drying phase are as follows: inlet air temperature and outlet air temperature. Compressor air pressure was 0.04 MPa. Inlet air temperatures were 150, 160, 170, 180, 190, and 200 °C, and outlet air temperature was 95 °C. Feed flow rate was 12 ± 2 mL min^{-1}. Each experiment was performed in triplicate.

Drying efficiency was determined by using the following Eq. (1):

Drying efficiency = 100 × [dried alga mass (g) / dry matter content of alga mass (g)].

Determination of pigment contents of dried microalgae biomasses

Analysis of pigments of samples was performed according to Gouveia et al. (1997). Total carotenoid and chlorophyll-*a* content of the samples were determined spectrophotometrically after extraction with methanol. Ten milligram spray-dried microalgae sample passed through the

Table 1 Pigment content, drying efficiency, and color properties of spray-dried *Nannochloropsis oculata* and *Isochrysis galbana*

Microalgae	Drying temperature (°C)	Total carotenoid (mg g^{-1}, dry weight)	Chlorophyll-*a* (mg g^{-1}, dry weight)	Drying efficiency (%)	*L**	*a**	*b**	*C**
N. oculata	150	0.378 ± 0.123^{b}	1.142 ± 0.253^{b}	42.2 ± 1.35^{c}	54.50 ± 0.40^{c}	-11.01 ± 0.31^{ab}	44.34 ± 0.22^{d}	45.69 ± 0.14^{d}
	160	0.546 ± 0.047^{ab}	1.616 ± 0.172^{ab}	45.3 ± 2.70^{bc}	56.95 ± 0.59^{a}	-11.22 ± 0.13^{ab}	44.54 ± 0.25^{d}	45.93 ± 0.21^{d}
	170	0.645 ± 0.091^{ab}	1.738 ± 0.157^{ab}	49.4 ± 3.84^{abc}	56.38 ± 1.04^{ab}	-11.32 ± 0.05^{ab}	44.40 ± 0.34^{d}	45.82 ± 0.32^{d}
	180	0.779 ± 0.144^{a}	1.836 ± 0.401^{a}	53.5 ± 1.95^{ab}	56.52 ± 0.34^{ab}	-11.67 ± 0.18^{b}	46.25 ± 0.45^{c}	47.70 ± 0.39^{c}
	190	0.668 ± 0.101^{a}	1.656 ± 0.197^{ab}	51.0 ± 5.20^{abc}	55.13 ± 0.42^{bc}	-10.98 ± 0.49^{ab}	47.28 ± 0.35^{b}	48.54 ± 0.23^{b}
	200	0.537 ± 0.049^{ab}	1.494 ± 0.173^{ab}	56.5 ± 4.70^{a}	56.57 ± 0.28^{ab}	-9.42 ± 1.75^{a}	48.43 ± 0.52^{a}	49.36 ± 0.18^{a}
I. galbana	150	2.800 ± 0.216^{AB}	9.291 ± 0.657^{A}	59.0 ± 1.74^{C}	40.99 ± 0.25^{A}	-3.74 ± 0.11^{A}	49.54 ± 0.12^{A}	49.68 ± 0.11^{A}
	160	3.075 ± 0.793^{AB}	11.377 ± 0.283^{A}	65.6 ± 0.99^{B}	40.02 ± 0.16^{AB}	-4.08 ± 0.23^{AB}	41.12 ± 0.49^{D}	41.32 ± 0.46^{D}
	170	3.373 ± 0.469^{A}	10.807 ± 0.189^{A}	65.7 ± 0.47^{B}	39.47 ± 0.60^{ABC}	-4.58 ± 0.50^{ABC}	42.21 ± 0.62^{CD}	42.46 ± 0.56^{CD}
	180	1.984 ± 0.355^{B}	7.775 ± 0.258^{A}	67.5 ± 2.24^{B}	38.35 ± 0.82^{C}	-4.97 ± 0.55^{BC}	42.55 ± 0.13^{C}	42.84 ± 0.07^{C}
	190	3.174 ± 0.573^{AB}	10.893 ± 0.235^{A}	75.2 ± 1.76^{A}	39.75 ± 0.70^{ABC}	-5.40 ± 0.11^{C}	44.16 ± 0.91^{B}	44.49 ± 0.89^{B}
	200	2.727 ± 0.788^{AB}	9.626 ± 0.246^{A}	75.8 ± 3.71^{A}	38.81 ± 0.59^{BC}	-5.41 ± 0.38^{C}	44.19 ± 0.14^{B}	44.52 ± 0.09^{B}

* dry bases, Different superscript lowercase and uppercase letters show the significant differences between the spray-dried *N. oculata* and *I. galbana* samples, respectively, ($p < 0.05$). *L**, brightness; *a**, ± red-green; *b**, ± yellow-blue; *C**, chroma; *h*°, hue angle. Data are the means ± SD of three replicates

homogenization procedure with the addition of 5 mL methanol; they passed through the centrifuge procedure for 10 min at 2100×*g*. After these samples were read in 475 and 665 nm wavelength on the spectrophotometer (Jenway 6305 model), a calibration curve was made using the absorbance values in 5 mL methanol solution which had 0.16, 1.63, 2.04, 3.27, and 4.09 mg g^{-1} β-carotene to determine the quantity of total carotenoid. Total chlorophyll concentration was measured at 665 nm and its quantity calculated using a specific absorption coefficient of 13.9 (Hu and Richmond 1994).

Determination of color properties of dried microalgal biomasses and chewing gum samples

Color parameters of spray-dried microalgae biomasses and chewing gum samples were determined using colorimeter (Chroma Meter CR-400, Konica Minolta, Japan). Chroma (*C**) values were calculated using the following equation.

$$C^{*} = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

Preparation of chewing gum samples

Chewing gum base from Maykim, Turkey, was heated to 70 °C in an oven and taken out from the oven for the addition of ingredients. Firstly, microalgae were mixed with glucose syrup. Then, microalgae (0.5 or 1%) and glucose syrup (20%), powdered sugar (53 or 52.5%), glycerin (1%), lecithin (0.25%), and sorbitol (0.25%) were added to gum base (25%) and mixed for 5 min. In order to ease mixing, the blend was put in the oven at 70 °C for 5 min again, taken out, and mixed 10 min to obtain homogeneous mixture. One gram of samples was formed from the mixture, molded, and stored at room temperature in cap tight containers prior to analysis.

Texture analysis of chewing gum samples

Textural properties of the samples were determined using texture analyzer (TA.HD Plus, Stable Micro systems, UK) equipped with 5 kg load cell. TPA test was conducted to determine textural properties of chewing gums. P/2 probe (2 mm diameter) was used for the analysis. Pre-test, test, and post-test speeds were adjusted to 1, 5, and 5 mm s^{-1}, respectively. The samples were compressed twice 1 cm inside the samples to calculate textural parameters.

Sensory properties of chewing gum samples

Sensory parameters (appearance, chewiness, adhesiveness, algal taste, overall acceptability) of the samples were evaluated by ten panelists. Trained panelists evaluated the effects of addition of

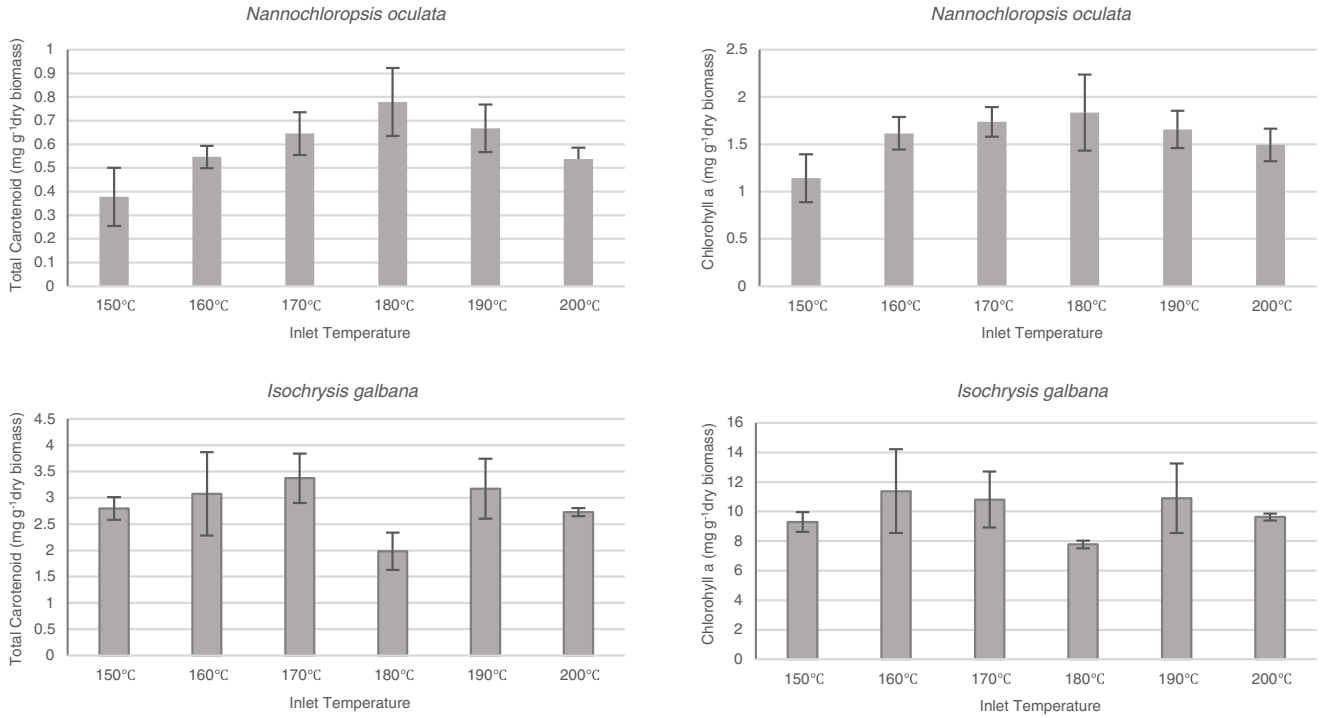

Fig. 1 Pigment contents of spray-dried microalgae biomasses. Data are the means ± SD of three replicates

different alga sources dried at different temperatures on sensory characteristics of chewing gum samples and consumed water and crackers between assessments. Responses were recorded using a hedonic scale where the trained panelists scored from 1 to 5 for the corresponding attributes.

Statistical analyses

ANOVA was conducted using MINITAB-16 to determine if the spray-drying inlet temperatures and using different concentrations of spray-dried biomasses on sensory, texture, and color properties of chewing gum samples were significant or not ($p < 0.05$). Significant differences were determined by using Tukey test.

Results

Effect of spray-dried inlet temperature on pigment content, color, and drying efficiency of microalgae

The effect of inlet temperatures on chlorophyll-*a* and total carotenoid amounts of *I. galbana* and *N. oculata* species is shown in Table 1 and Fig. 1. Chlorophyll-*a* amounts of *I. galbana* and *N. oculata* biomasses were determined as 7.775–11.377 mg g^{-1} and 1.141–1.836 mg g^{-1}, respectively, in the dry biomass. Total carotenoids were found as 1.984–3.373 mg g^{-1} (dry biomass) for *I. galbana* and 0.3780–0.077 mg g^{-1} (dry biomass) for *N. oculata*.

Different spray dryer inlet temperatures (150–200 °C) were found to influence chlorophyll-*a* and carotenoid amounts in both species ($p < 0.05$). Taking into account the amounts of chlorophyll-*a* and carotenoids, 170 °C for *I. galbana* and 180 °C for *N. oculata* were found to be advantageous for higher pigment quantities.

The color qualities of microalgae obtained at different temperatures were determined by examining the values of *L** (brightness), *a** (red-green), *b** (yellow-blue), and *C** (chroma) (Table 1). In this study, microalgae were used to provide especially green color. All species and temperature differences were found to be effective on *L**, *a**, *b**, and *C** parameters ($p < 0.05$). For *N. oculata*, increase in temperature decreased *a** value, indicating the loss of green color. However, color saturation (*C**) increased with temperature increase ($p < 0.05$). When the color characteristics of *I. galbana* biomass are examined, *a** value dropped with temperature increase which was advantageous for the green color.

Quality parameters of chewing gum

Photographs of chewed and non-chewed chewing gums colored with spray-dried *I. galbana* and *N. oculata* are shown in Fig. 2.

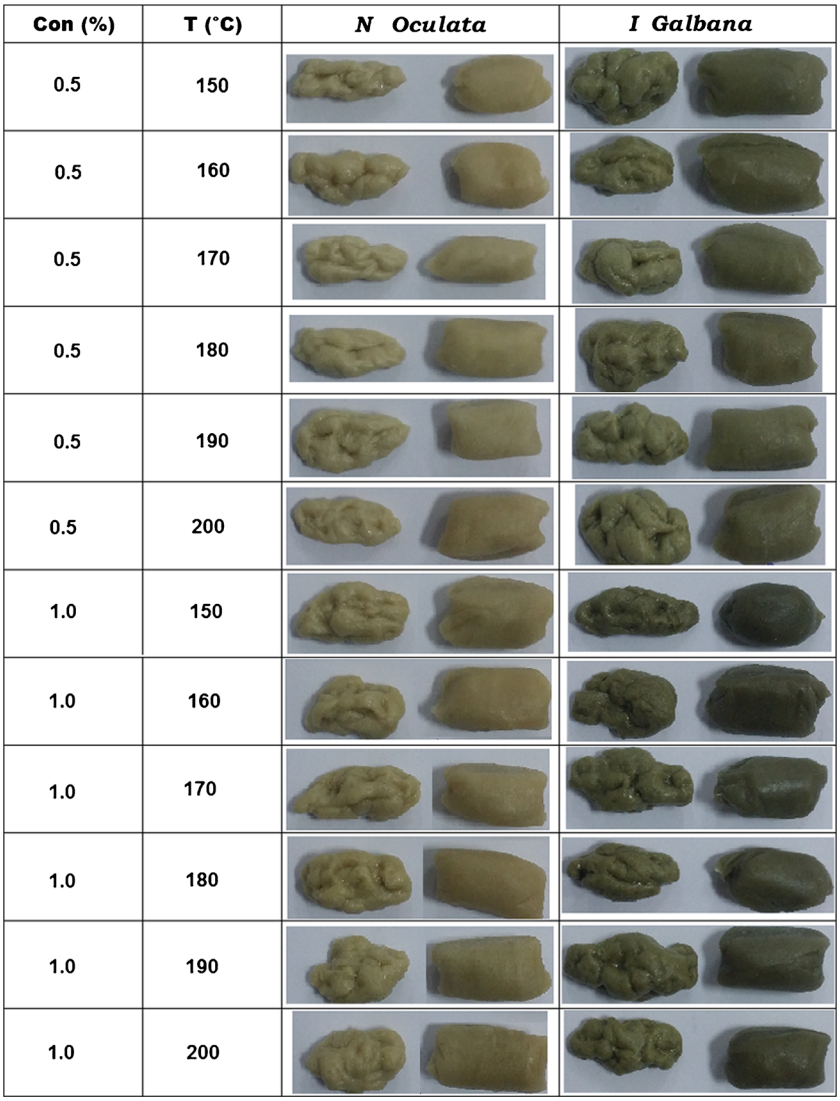

Fig. 2 Photographs of chewing gums colored with microalgae dried at different conditions

Texture of chewing gum samples

After spray-dried microalgal biomass was obtained at different inlet temperatures, microalgae were added to chewing gum formulation as a coloring agent at ratios of 0.5 and 1%. Since texture is one of the most important quality parameters of chewing gums, texture analysis was conducted, and hardness, adhesiveness, springiness, cohesiveness, chewiness, and resilience parameters were determined (Table 2).

The spray dryer inlet temperature and microalgae usage level did not cause significant difference in hardness, springiness, gumminess, and chewiness properties in the gum samples containing *I. galbana* ($p < 0.05$). Although there is a difference between the samples for the resilience property ($p < 0.05$), it can be considered to be negligible for the reason that it changed in a fairly narrow range (0.014–0.015).

In the samples containing *N. oculata*, only a significant difference was determined between cohesiveness values ($p < 0.05$). Similarly, resilience values varied slightly in a narrow range (0.012–0.015). Generally, it was not possible to correlate significantly changed textural parameters with microalgae concentration, type, and the spray dryer inlet temperature.

Color properties of chewing gum samples

Color is one of the most important characteristics of foods, being considered as a quality indicator that determines their acceptance (Charanioti et al. 2015). Chemical food additives such as colorants have been widely applied for coloring purposes of food

Table 2 Textural properties of chewing gum containing microalgae dried under various spray dryer inlet temperatures

Microalga concentration	Drying temperature (°C)	Hardness (g)	Adhesiveness (J)	Springiness	Cohesiveness	Chewiness (J)	Resilience
N. oculata 0.5 g/100 g	150	879 ± 259^{ab}	-20.8 ± 1.6^{abc}	0.794 ± 0.042^{a}	0.199 ± 0.032^{ab}	135.1 ± 11.4^{bc}	0.015 ± 0.000^{a}
	160	951 ± 82^{ab}	-18.3 ± 7.1^{abc}	0.770 ± 0.035^{a}	0.169 ± 0.002^{ab}	123.4 ± 3.6^{bc}	0.013 ± 0.000^{bc}
	170	925 ± 626^{ab}	-15.5 ± 1.1^{ab}	0.750 ± 0.021^{a}	0.153 ± 0.002^{b}	106.0 ± 11.5^{c}	0.014 ± 0.00^{ab}
	180	908 ± 3^{ab}	-32.7 ± 1.3^{d}	0.819 ± 0.014^{a}	0.234 ± 0.019^{a}	174.0 ± 16.3^{ab}	0.013 ± 0.000^{bc}
	190	1120 ± 138^{a}	-26.9 ± 0.9^{cd}	0.821 ± 0.017^{a}	0.217 ± 0.016^{ab}	198.3 ± 5.4^{a}	0.014 ± 0.000^{ab}
	200	660 ± 76^{b}	-18.0 ± 5.7^{abc}	0.760 ± 0.097^{a}	0.187 ± 0.008^{ab}	94.9 ± 26.9^{c}	0.014 ± 0.000^{ab}
N. oculata 1.0 g/100 g	150	632.0 ± 133.1^{b}	-13.9 ± 1.2^{a}	0.718 ± 0.003^{a}	0.183 ± 0.046^{ab}	85.5 ± 38.6^{c}	0.014 ± 0.001^{ab}
	160	781.4 ± 47.4^{ab}	-22.1 ± 3.8^{abc}	0.740 ± 0.049^{a}	0.199 ± 0.034^{ab}	114.7 ± 20.3^{bc}	0.014 ± 0.000^{ab}
	170	857.2 ± 13.2^{ab}	-24.3 ± 2.8^{bcd}	0.787 ± 0.073^{a}	0.217 ± 0.013^{ab}	147.1 ± 24.8^{abc}	0.013 ± 0.001^{bc}
	180	934.5 ± 118.6^{ab}	-33.0 ± 0.6^{d}	0.770 ± 0.069^{a}	0.205 ± 0.033^{ab}	146.3 ± 18.4^{abc}	0.012 ± 0.000^{c}
	190	902.0 ± 151.4^{ab}	-25.1 ± 1.4^{bcd}	0.765 ± 0.000^{a}	0.202 ± 0.004^{ab}	139.5 ± 26.2^{abc}	0.013 ± 0.001^{bc}
	200	813.7 ± 71.1^{ab}	-14.1 ± 4.0^{a}	0.777 ± 0.066^{a}	0.162 ± 0.040^{ab}	101.9 ± 24.6^{c}	0.013 ± 0.001^{bc}
I. galbana 0.5 g/100 g	150	660.7 ± 69.6^{A}	-12.7 ± 1.7^{AB}	0.762 ± 0.052^{A}	0.220 ± 0.005^{ABCD}	111.2 ± 17.0^{A}	0.014 ± 0.000^{AB}
	160	800.5 ± 188.0^{A}	-28.0 ± 4.9^{BC}	0.745 ± 0.007^{A}	0.201 ± 0.030^{ABCD}	118.0 ± 11.4^{A}	0.014 ± 0.000^{AB}
	170	913.9 ± 58.6^{A}	-21.6 ± 3.0^{ABC}	0.804 ± 0.035^{A}	0.184 ± 0.003^{BCD}	135.1 ± 16.3^{A}	0.014 ± 0.000^{AB}
	180	728.3 ± 85.4^{A}	-19.0 ± 3.1^{ABC}	0.831 ± 0.073^{A}	0.250 ± 0.050^{ABC}	154.9 ± 61.1^{A}	0.015 ± 0.001^{A}
	190	893.6 ± 29.6^{A}	-15.3 ± 2.5^{ABC}	0.745 ± 0.000^{A}	0.174 ± 0.006^{CD}	115.6 ± 6.1^{A}	0.014 ± 0.00^{AB}
	200	629.2 ± 140.3^{A}	-8.0 ± 1.3^{A}	0.789 ± 0.028^{A}	0.206 ± 0.022^{ABCD}	104.2 ± 37.3^{A}	0.014 ± 0.001^{AB}
I. galbana 1.0 g/100 g	150	619.9 ± 120.6^{A}	-16.9 ± 5.9^{ABC}	0.730 ± 0.007^{A}	0.164 ± 0.009^{D}	74.7 ± 19.4^{A}	0.014 ± 0.000^{AB}
	160	659.7 ± 146.8^{A}	-17.9 ± 4.9^{ABC}	0.757 ± 0.045^{A}	0.193 ± 0.032^{BCD}	99.3 ± 43.4^{A}	0.013 ± 0.000^{B}
	170	679.3 ± 91.1^{A}	-23.7 ± 5.8^{ABC}	0.755 ± 0.049^{A}	0.219 ± 0.031^{ABCD}	114.6 ± 38.3^{A}	0.015 ± 0.001^{A}
	180	682.0 ± 92.6^{A}	-32.0 ± 15.0^{C}	0.789 ± 0.000^{A}	0.262 ± 0.033^{AB}	139.8 ± 1.3^{A}	0.014 ± 0.000^{AB}
	190	834.6 ± 151.3^{A}	-13.5 ± 4.3^{AB}	0.757 ± 0.094^{A}	0.183 ± 0.055^{BCD}	122.4 ± 70.2^{A}	0.014 ± 0.000^{AB}
	200	726.6 ± 108.8^{A}	-15.1 ± 3.9^{ABC}	0.828 ± 0.000^{A}	0.222 ± 0.006^{ABCD}	133.7 ± 23.4^{A}	0.015 ± 0.001^{A}

Different superscript lowercase and uppercase letters show the significant differences between the samples colored with spray-dried *N. oculata* and *I. galbana*, respectively, ($p < 0.05$). Data are the means ± SD of three replicates

products, but their use is a controversial issue in the food industry due to their toxicological potential on human health (Mizutani 2009).

Color parameters of L^* (brightness), a^* (red-green), b^* (yellow-blue), and C^* (chroma) were determined in the chewing gum samples containing both microalgae obtained from spray dryer under different inlet temperature conditions (Table 3). In all samples prepared using *N. oculata* and *I. galbana*, brightness decreased with increasing microalgae concentration ($p < 0.05$). This could affect the perception of consumers negatively. Spray dryer inlet temperature values could not change this trend ($p < 0.05$).

For both microalgae, the increase in concentration decreased green color intensity as a^* values decreased significantly ($p < 0.05$). This implied that there was an optimum concentration level of algal biomass and concentration increase in chewing gum did not necessarily give more green color to the product. However, increase in b^* value ($p < 0.05$) by increasing the concentrations could be stated as an advantage in terms of green color, except for microalgae obtained using 200 °C spray dryer inlet temperature.

In chewing gum samples, concentration increased color saturation (C^*) with the use of *I. galbana*, but there was a negative correlation in the use of *N. oculata* ($p < 0.05$). For this reason, it can be stated that biochemical composition which changes for each microalgae species has different effects on the saturation of color in the chewing gum samples.

Sensory properties of chewing gum samples

The sensorial properties of appearance, chewiness, adhesiveness, alga taste, and overall acceptability of the chewing gums containing *I. galbana* and *N. oculata* biomass dried at different spray dryer inlet temperatures were examined (Table 4). No significant difference was found between sensory properties in all samples prepared using *I. galbana* ($p < 0.05$).

In samples prepared using *N. oculata*, increase in concentration increased algal taste which caused decrease in the

Table 3 Color properties of chewing gum containing microalgae dried under various spray dryer inlet temperatures

Microalga concentration	Drying temp. (°C)	*L**	*a**	*b**	*C**
N. oculata 0.5 g/100 g	150	65.43 ± 2.32^{a}	-0.60 ± 0.05^{bc}	14.99 ± 0.21^{e}	15.00 ± 0.21^{d}
	160	64.08 ± 1.17^{a}	-0.61 ± 0.17^{bc}	16.51 ± 0.22^{bcde}	16.53 ± 0.22^{abcd}
	170	63.73 ± 0.77^{ab}	-0.71 ± 0.16^{bcd}	16.31 ± 0.27^{cde}	16.32 ± 0.28^{bcd}
	180	63.53 ± 1.81^{ab}	-1.12 ± 0.19^{de}	18.07 ± 0.95^{ab}	18.10 ± 0.94^{a}
	190	63.20 ± 2.36^{ab}	-1.70 ± 0.29^{f}	16.67 ± 0.91^{abcd}	16.76 ± 0.93^{abc}
	200	61.09 ± 1.10^{abc}	-1.64 ± 0.14^{f}	16.49 ± 0.39^{bcde}	16.58 ± 0.40^{abcd}
N. oculata 1.0 g/100 g	150	58.80 ± 1.20^{cd}	-0.06 ± 0.00^{a}	16.78 ± 0.28^{abcd}	16.78 ± 0.29^{abc}
	160	55.87 ± 0.82^{d}	-0.03 ± 0.00^{a}	18.15 ± 0.28^{a}	18.15 ± 0.28^{a}
	170	59.38 ± 0.87^{bcd}	-0.12 ± 0.15^{a}	17.48 ± 0.48^{abc}	17.48 ± 0.48^{ab}
	180	57.31 ± 0.98^{cd}	-0.38 ± 0.04^{ab}	17.77 ± 0.78^{abc}	17.78 ± 0.78^{ab}
	190	56.63 ± 2.01^{d}	-1.37 ± 0.16^{ef}	17.33 ± 0.64^{abc}	17.38 ± 0.65^{ab}
	200	58.24 ± 1.16^{cd}	-0.92 ± 0.07^{cde}	15.64 ± 0.48^{de}	15.67 ± 0.48^{cd}
I. galbana 0.5 g/100 g	150	50.43 ± 0.31^{ABC}	-2.49 ± 0.13^{DEF}	13.59 ± 0.51^{ABCD}	13.81 ± 0.52^{ABCD}
	160	49.43 ± 1.11^{C}	-2.44 ± 0.15^{DEF}	13.91 ± 0.38^{ABC}	14.12 ± 0.39^{ABC}
	170	53.19 ± 0.36^{A}	-2.88 ± 0.05^{F}	14.76 ± 0.27^{AB}	15.04 ± 0.27^{AB}
	180	52.36 ± 1.13^{ABC}	-2.49 ± 0.18^{DEF}	14.03 ± 0.87^{ABC}	14.25 ± 0.89^{ABC}
	190	52.95 ± 1.38^{AB}	-2.53 ± 0.20^{EF}	15.00 ± 0.68^{A}	15.21 ± 0.70^{A}
	200	49.97 ± 1.15^{BC}	-2.04 ± 0.18^{BCD}	12.20 ± 0.84^{CDE}	12.37 ± 0.86^{CDE}
I. galbana 1.0 g/100 g	150	43.23 ± 0.75^{D}	-1.42 ± 0.16^{A}	9.32 ± 0.71^{G}	9.43 ± 0.73^{G}
	160	44.64 ± 0.94^{D}	-1.31 ± 0.09^{A}	9.79 ± 0.47^{FG}	9.88 ± 0.48^{FG}
	170	44.34 ± 1.03^{D}	-1.68 ± 0.08^{ABC}	11.63 ± 0.37^{DEF}	11.75 ± 0.37^{DEF}
	180	44.53 ± 1.38^{D}	-1.96 ± 0.17^{BC}	12.86 ± 1.01^{BCDE}	13.01 ± 1.02^{BCDE}
	190	42.89 ± 1.04^{D}	-1.57 ± 0.11^{AB}	11.51 ± 0.47^{EF}	11.62 ± 0.48^{EF}
	200	45.10 ± 1.32^{D}	-2.09 ± 0.30^{CDE}	13.66 ± 1.08^{ABC}	13.82 ± 1.11^{ABC}

Different superscript lowercase and uppercase letters show the significant differences between the samples colored with spray-dried *N. oculata* and *I. galbana*, respectively, ($p < 0.05$). *L**, brightness; *a**, ± red-green; *b**, ± yellow-blue; *C**, chroma; *nd*, not determined. Data are the means ± SD of three replicates

appreciation level of chewing gums ($p < 0.05$). The least preferred chewing gum samples included *N. oculata* biomass dried at inlet temperature of 150 °C. This can be result of the presence of microalga's volatile compounds and higher temperatures than 150 °C were needed to remove this strange odor. However, this effect depended on species used in chewing gum samples.

Discussion

Total carotenoid amounts were consistent with the previous studies concerning untreated microalgae samples, whereas chlorophyll-*a* amounts of *I. galbana* were determined at a higher level (Durmaz 2007; Durmaz et al. 2008). A remarkable result was that for both types and pigments, an increase in the pigment values occurred after the stated temperature values were reached, and then a fall occurred. However, with the application of other spray dryer process conditions, alternative techniques (e.g., encapsulation) or performing process optimization, it could be possible to achieve an advantage in pigment concentrations. The optimum conditions for each type should be determined for the microalgae drying by spray dryer application, because the inlet temperature influenced on the color characteristics of the obtained powdered biomasses.

Hardness values of all samples were found to be lower than those previously determined with TPA and chewing gum samples (Santos et al. 2014). This could be advantageous in terms of consumer acceptability and quality (McGowan et al. 2005). Likewise, chewiness values are low in all samples (74.7–174.7 g). Springiness values of the gum samples were determined at low levels consistent with the chewiness values (0.831–0.740). These textural properties might also be regarded as an advantageous feature for the process of masticating gums containing dried microalgae in their composition.

Table 4 Sensory properties of chewing gum containing microalgae dried under various spray dryer inlet temperatures

Microalga concentration	Drying temperature (°C)	Appearance	Chewiness	Adhesiveness	Alga taste	Overall acceptability
N. oculata 0.5 g/100 g	150	4.25 ± 0.43^{a}	4.25 ± 0.43^{a}	4.00 ± 0.71^{a}	3.00 ± 1.00^{b}	3.75 ± 0.43^{a}
	160	4.50 ± 0.87^{a}	4.75 ± 0.43^{a}	4.25 ± 0.43^{a}	5.00 ± 0.00^{a}	4.75 ± 0.43^{a}
	170	4.75 ± 0.43^{a}	4.25 ± 0.43^{a}	4.00 ± 0.00^{a}	4.50 ± 0.43^{a}	4.00 ± 0.71^{a}
	180	4.50 ± 0.43^{a}	4.50 ± 0.50^{a}	4.25 ± 0.43^{a}	5.00 ± 0.00^{a}	4.25 ± 0.83^{a}
	190	4.50 ± 0.87^{a}	4.50 ± 0.50^{a}	4.50 ± 0.50^{a}	4.50 ± 0.43^{a}	4.50 ± 0.50^{a}
	200	4.50 ± 0.50^{a}	4.00 ± 0.71^{a}	4.50 ± 0.50^{a}	5.00 ± 0.00^{a}	3.75 ± 0.43^{a}
N. oculata 1.0 g/100 g	150	4.00 ± 1.23^{a}	4.00 ± 0.71^{a}	3.75 ± 0.43^{a}	3.75 ± 0.43^{ab}	4.00 ± 0.71^{a}
	160	4.00 ± 0.71^{a}	3.75 ± 0.43^{a}	4.25 ± 0.43^{a}	4.25 ± 0.43^{ab}	4.00 ± 0.71^{a}
	170	4.00 ± 0.71^{a}	3.75 ± 0.83^{a}	4.50 ± 0.50^{a}	4.50 ± 0.50^{a}	3.75 ± 0.83^{a}
	180	4.50 ± 0.50^{a}	4.25 ± 0.43^{a}	4.25 ± 0.43^{a}	4.25 ± 0.43^{ab}	3.75 ± 0.43^{a}
	190	4.00 ± 0.71^{a}	4.00 ± 0.71^{a}	4.50 ± 0.50^{a}	4.50 ± 0.50^{a}	4.00 ± 1.00^{a}
	200	4.00 ± 0.71^{a}	4.50 ± 0.50^{a}	4.25 ± 0.43^{a}	4.25 ± 0.43^{ab}	4.25 ± 0.43^{a}
I. galbana 0.5 g/100 g	150	4.25 ± 0.83^{A}	4.25 ± 0.43^{A}	3.75 ± 0.43^{A}	5.00 ± 0.00^{A}	4.25 ± 0.43^{A}
	160	4.75 ± 0.43^{A}	3.75 ± 0.43^{A}	4.50 ± 0.50^{A}	3.50 ± 0.83^{A}	4.00 ± 1.00^{A}
	170	4.25 ± 0.43^{A}	4.00 ± 0.00^{A}	4.25 ± 0.43^{A}	4.50 ± 0.43^{A}	4.25 ± 0.43^{A}
	180	4.50 ± 0.50^{A}	4.25 ± 0.83^{A}	4.25 ± 0.43^{A}	4.00 ± 0.50^{A}	4.50 ± 0.50^{A}
	190	4.25 ± 0.43^{A}	4.50 ± 0.50^{A}	4.25 ± 0.43^{A}	4.00 ± 0.87^{A}	4.50 ± 0.50^{A}
	200	4.50 ± 0.50^{A}	3.75 ± 0.43^{A}	4.50 ± 0.50^{A}	4.50 ± 0.43^{A}	4.50 ± 0.50^{A}
I. galbana 1.0 g/100 g	150	4.50 ± 0.50^{A}	4.00 ± 0.71^{A}	4.25 ± 0.43^{A}	4.25 ± 0.43^{A}	3.75 ± 0.43^{A}
	160	4.00 ± 0.00^{A}	3.75 ± 0.43^{A}	4.50 ± 0.50^{A}	4.50 ± 0.50^{A}	3.75 ± 0.43^{A}
	170	4.25 ± 0.43^{A}	4.50 ± 0.50^{A}	4.50 ± 0.50^{A}	4.50 ± 0.50^{A}	4.25 ± 0.43^{A}
	180	3.75 ± 0.43^{A}	3.50 ± 1.18^{A}	4.50 ± 0.50^{A}	4.50 ± 0.50^{A}	3.50 ± 0.87^{A}
	190	4.50 ± 0.50^{A}	4.50 ± 0.50^{A}	4.00 ± 0.71^{A}	4.00 ± 0.71^{A}	4.50 ± 0.50^{A}
	200	4.50 ± 0.50^{A}	4.75 ± 0.43^{A}	4.00 ± 0.71^{A}	4.00 ± 0.71^{A}	4.25 ± 0.83^{A}

Different superscript lowercase and uppercase letters show the significant differences between the samples colored with spray-dried *N. oculata* and *I. galbana*, respectively, ($p < 0.05$). Data are the means ± SD of three replicates

Cohesiveness is the extent to which a material can be deformed before breaking (Szczesniak 2002). This textural parameter, indicative of the power of internal bonds that make up the structure of a food matrix, was determined to be below 0.86 in previous studies for chewing gum (Santos et al. 2014). In this study, lower values (0.153–0.262) were detected. Low cohesiveness values may be advantageous for releasing compounds that are present in the chewing gum matrix and released during the oral cavity process.

As a result, the changes in the determined textural parameters and the obtained results did not constitute an obstacle to the use of *N. oculata* and *I. galbana* as colorants for chewing gums.

The colors of the gum samples can be named khaki or light khaki based on the US Federal Standard Color Guides (2008) definitions. In both microalgae, degradation of chlorophyll-*a* and carotenoids in the biomass composition was thought to be effective in coloring. Based on a^*, b^*, and C^* values in chewing gum samples, 0.5 g $(100\ g)^{-1}$ concentration and 180 to 190 °C spray dryer inlet temperature were the best conditions for achieving green color using *N. oculata*. For *I. galbana*, it is advantageous to use the same concentration and 170 to 190 °C inlet spray dryer temperatures. These results showed compatibility with pigment concentrations of dried microalgae samples. Importantly, this showed that algal biomass pigments were not affected by the chewing gum production process indicating the suitability of using microalgae as a colorant agent in chewing gums.

As mentioned in texture analysis results, there were significant differences between the instrumentally measured parameters of chewiness and adhesiveness. However, no difference was found in the results of sensory analysis. This could be due to the fact that the mastication is a dynamic process and in this process, chewing gums are affected by saliva components as well as possible chemical and structural changes due to oral cavity temperature. However, further studies could be useful for researchers in this area to determine the optimum TPA methods enabling correlation between TPA parameters and sensory analysis.

In conclusion, microalgae are a potential bioactive ingredient source for the food industry due to their biochemical

compositions and consumer expectations. One of the uses of this source is the natural colorant in different food matrices. The obtained results confirmed that the biomass of *N. oculata* and *I. galbana* species can be used as a colorant in chewing gum matrix and that the production process did not cause color pigment degradation. However, in the production of colorants from these and other microalga species, the use of the spray dryer technique requires species-based optimization. It is also useful to investigate color stability of chewing gums during storage process with further studies.

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