Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.1590/fst.41817
(cc) BY

Porphyridum Cruentum as a natural colorant in chewing gum

Omer Said TOKER[1]*

Abstract

In this study, usage possibility of the dried *Porphyridium cruentum* type microalga biomass in the chewing gum formulation as a natural colorant was investigated. For this aim, the effect of different spray dryer inlet temperatures (150-200 °C) on pigment (total carotenoid and chlorophyll-a) quantities and color properties (L^*, a^*, b^* and C^*) of *Porphyridium cruentum* type microalga biomass were examined. *P. cruentum* were cultured using the tubular photobioreactor and harvested by centrifugation. The dried microalga was then added to chewing gum at different concentrations (0.5% and 1.0% w:w) as a natural red colorant. The amount of the total carotenoids in dried biomass ranged from 112.6 to 419.9 μg g^{-1} and chlorophyll-a from 511 to 1513 μg g^{-1}. Considering sensorial analysis, algal taste increased with increasing microalga concentration ($p < 0.05$). The studied factors had no significant effect on cohesiveness, (0.186-0.254), springiness (0.713-0.806) and resilience (0.014-0.015) parameters in chewing gums ($p < 0.05$). However, a^* value in chewing gum samples increased with drying temperature and usage amount (from 10.5 to 18.7) which increased red color intensity. According to the findings of the present study, it can be concluded that *P. cruentum* biomass had a potential use in chewing gum matrix.

Keywords: microalgae; coloring agent; natural; spray dryer; confectionery.

Practical Application: Microalgae can be used in food products for improving both functional and color characteristics.

1 Introduction

Microalgae can naturally accumulate considerable amounts of protein, pigment, fatty acids, vitamins, antibiotics, hydrocarbons, polysaccharides and many other metabolites in the cell. For this reason, they have been investigated due to their functional properties for more than 100 years. Commercial interest in algal biomass and their metabolites such as proteins, lipids, starch, glycerol, natural pigments and biopolymers is continuously increasing. Most of the algae are capable of producing commercially valuable compounds such as vitamins or pigments at high amounts. For this reason, microalgae have become one of the most studied biological materials. Carotenoids are oil-soluble molecules that can be produced only by phytoplankton, algae, plants and a limited number of fungi and bacteria (Baysal & Ersus, 1999; Horrobin, 1999). In plants and algae, carotenoids together with chlorophyll and other pigments have a vital importance in photosynthetic processes.

Color is one of the most important characteristics of foods, being considered as a quality indicator that determines their acceptance (Azeredo, 2009; Chranioti et al., 2015). The synthetic colorants have been widely applied for coloring purposes of food products, however, their use is a controversial issue in the food industry due to their toxicological potential on human health (Mizutani, 2009). Microalgae are recognized as an excellent source of natural colorings and it is expected that they will surpass synthetics as well as other natural sources due to their sustainability of production and renewable nature (Dufossé et al., 2005). There are some attempts to incorporate the different food products such as pasta (Fradique et al., 2010), biscuits (Gouveia et al., 2007), puddings/gelled desserts (Batista et al., 2008), mayonnaises/salad dressings (Gouveia et al., 2006), chewing gum (Palabiyik et al., 2018) and bread (Graça et al., 2018) with micoralgae for different purposes. All of the studies indicated that usage of microlagae in food producs has been promising issue in the industry.

P. cruentum (Figure 1) is a natural red microalgae species and has protein content of 28-39%, carbohydrate content of 40-57%, and total lipids between 9-14% of dry weight. Containing tocopherol, vitamin K and large amounts of carotenoids (Becker, 1994), *P. cruentum* could be a healthy candidate for natural red colorant in foods, and also the algae contains significant amounts of tocopherol and both eicopentanoic acid (EPA) and arachidonic acid (AA), which can be used for human and animal nutrition (Durmaz et al., 2007).

Spray drying, freeze drying and drum drying can be mentioned as the main techniques that can be used for microalgae biomass drying. Spray dried powder has got a standardized particle size making a treatment of grinding unnecessary. Each droplet is in contact with drying steam for such a short time making it suitable for drying compounds such as pigments which are sensitive to heat (Olafsson, 2013). However, the process stability of the pigment level is also important.

In the present study, usage possibilities of spray dried microalgae biomass was used in the formulation of a chewing

Received 20 Dec., 2017
Accepted 19 Mar., 2018
[1] *Food Engineering Department, Chemical and Metallurgical Engineering Faculty, Yildiz Technical University, Istanbul, Turkey*
*Corresponding author: os.toker85@gmail.com; stoker@yildiz.edu.tr

Figure 1. Microscopic images of *Porphyridum cruentum*.

gum as a natural colorant agent. Chewing gum typically includes sugar, polyols, gum base, aroma, acidulants, colors and sweeteners (Valduga et al., 2012). General production process of the chewing gum is composed of heating of the gum base to 70-120 °C depending on the chewing gum type, mixing of plasticizer and emulsifier with heated gum base, kneading after addition of sugar and colorants, addition of antioxidants, humectants and fillers and then mixing, which is followed by extruding and cutting process (Konar et al., 2016). In previous study, *Isochrysis galbana* and *Nannochloropsis oculata* microalgae biomass were used in the chewing gum formulation to improve green color of the products (Palabiyik et al., 2018). In the previous study, the mentioned microalgae biomass was added for providing green color; however, in this research, *P. cruentum* was used for red color.

The present study is composed of two parts. Firstly, the effects of spray drying inlet temperature on the pigment (total carotenoid and chlorophyll-a) amounts and color properties of *P. cruentum* microalga biomass were investigated. Then, these biomasses were added to chewing gum formulation at different concentrations to study the effects on the color, texture and sensory properties of the chewing gum samples.

2 Materials and methods

2.1 Microalgae cultures and culture conditions

Porphyridium cruentum was obtained from the Microalgal Biotechnology Laboratory at Ben-Gurion University, Israel. Culture was kept illuminated with halogen lamp (Philips Halogen lamp 500W E40 38x215mm) in this study. Illumination was measured as 200 μmol $m^{-2}s^{-1}$ light intensity. These lamps were placed into glass tubes system to avoid heating in the vicinity of the reactor. Culture medium (F/2 medium (Guillard & Ryther, 1962) was added 1 mL per liter daily. All cultures were maintained at 3.5% salinity and temperature of 20 ± 1 °C under 24 h light regime. The tubular photobioreactor was inoculated and operated in batch mode for 11 days.

2.2 Growing and harvesting of microalga

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 Plexiglas 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 was used for mixing, degassing and heat exchange of culture medium. The pH control unit was set at 7.5 and automatically injection of pure industrial-grade CO_2 gas at 5 L min^{-1}.

It is not possible to completely sterilize the tubular bioreactor, but in this study, the tubular photobioreactor was disinfected by using sodium hypochlorite overnight and neutralized for 2 h with sodium thiosulfate. While preparing the tubular photobioreactor for *microalga* culture, marine water was sterilized by passing through 0.02 μm filtration system and also sterilized marine water was used for addition 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).

2.3 Spray drying

Spray drying process was performed in a laboratory scale spray dryer (Buchi B290, Huddersfield, England), with a nozzle atomization system with 1.0 μm diameter nozzle. The separated algae biomasses were fed into the main chamber through a peristaltic pump and the feed flow rate was controlled by the pump speed. Compressor air pressure was set to 0.04 MPa. Inlet air temperature were adjusted to 150 °C, 160 °C, 170 °C, 180 °C, 190 °C and 200 °C. Feed flow rate was 12 ± 2 mL/min. Each drying was performed in triplicate.

2.4 Determination of pigment contents of dried microalgae biomass

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. 10 mg sample mixed with 5 ml methanol and the mixture was centrifuged for 10 min at 3500 rpm. After that these samples were read in 475 nm 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 has 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 was calculated using a specific absorption coefficient of 13.9 (Qiang & Richmond, 1994).

2.5 Preparation of chewing gum samples

Chewing gum base (Maykim, Turkey) was heated to 70 °C in an oven and taken out from the oven for the addition of ingredients. Firstly, *microalga* was mixed with glucose syrup. Then, *microalga* (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 minutes again, taken out and mixed 10 minutes to obtain homogeneous mixture. 1 gr of samples was formed from the mixture, molded and stored at room temperature in cap tight containers prior to analysis.

2.6 Determination of color properties of dried microalga biomasses and chewing gum samples

Color parameters of spray dried *microalga* biomasses and produced chewing gum samples were determined using colorimeter (Chroma Meter CR-400, Konica Minolta, Japan). Chroma (C^*) values were calculated using the following Equation 1 (Periche et al., 2015).

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

2.7 Texture analysis of chewing gum samples

Textural properties of the samples were determined using texture analyzer (TA.HD Plus, Stable Micro systems, Surrey, 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 1mm/s, 5mm/s and 5mm/s, respectively. The samples were compressed twice 1 cm inside the samples to calculate textural parameters.

2.8 Sensory properties of chewing gum samples

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

2.9 Statistical analyses

ANOVA was conducted using MINITAB-16 to determine statistically significant effects of the spray drying inlet temperatures and using different concentrations of spray dried biomass on sensory, texture and color properties of chewing gum samples ($p < 0.05$). Significant differences were determined by using Tukey test.

3 Results

3.1 Effect of spray dried inlet temperature on pigment content and color of microalga

General techniques for drying are spray dryer and freeze dryer. Freeze drying is a more common method for *microalga* biomass. However, special preservation conditions and high costs for industrial production are disadvantageous for this technique. In this study, total carotenoid, chlorophyll-a and color characteristics (L^*, a^*, b^* and C^*) of dried biomass obtained from different inlet temperature (150-200 °C) applications were investigated (Table 1).

The amount of carotenoid in dried biomass of *P. cruentum* was determined as 112.6-415.9 μg g^{-1} in dry biomass. The total amounts of chlorophyll-a varied from 511 to 1513 μg g^{-1} dry biomass. The effect of spray dryer inlet temperature on the chlorophyll-a, as well as on the total carotenoid amount was significant ($p < 0.05$). The temperature application above 180 °C showed a dramatic decrease in the total amount of carotenoids, a similar change was observed for chlorophyll-a over 190 °C. Based on both pigment types, it can be stated that application of the inlet temperature of the spray dryer between 170 °C and 180 °C was more advantageous for *P. cruentum* dried biomass.

The color parameters L^* (brightness), a^* (red-green), b^* (yellow-blue) and C^* (chroma) of microalga biomass dried at different temperatures were determined (Table 1). Spray dryer inlet temperature differences did not cause significant difference on a^* and C^* parameters, but the effects on L^* and b^* values were significant ($p < 0.05$). L^* values increased with inlet temperature increase, therefore, brighter biomass was obtained. The change in b^* values was not correlated with temperature differences, but was generally determined to be in the blue zone (-b^*) with near neutrality. a^* values varied in a narrow range in the red region (29.2-33.4).

3.2 Texture of chewing gum samples

Hardness, adhesiveness, cohesiveness, springiness, chewiness and resilience properties of the chewing gum samples containing *P. cruentum* microalga dried at different spray dryer inlet temperatures were investigated using texture profile analysis (TPA) (Table 2). The main quality parameters of gum include textural properties. Hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience value of the chewing gum colored with 0.5% biomass were found to between 710-909 g, 14.6-41.7 g.s,

Table 1. Pigment content, drying efficiency and color properties of spray dried *Porphyridium cruentum*.

Drying Temp. (°C)	Carotenoid (μg g^{-1}*)	Chlorophyll-a (μg g^{-1}*)	L^*	a^*	b^*	C^*
150	396.2 ± 54.9^{a}	1029 ± 255^{ab}	47.6 ± 0.2^{c}	32.0 ± 1.0^{a}	-4.6 ± 0.5^{bc}	32.4 ± 0.9^{a}
160	375.9 ± 14.8^{a}	1307 ± 374^{a}	47.9 ± 0.4^{bc}	30.2 ± 0.8^{a}	-2.8 ± 0.1^{a}	30.3 ± 0.8^{a}
170	399.1 ± 11.5^{a}	1500 ± 434^{a}	47.2 ± 0.6^{c}	30.7 ± 2.8^{a}	-5.1 ± 0.5^{c}	31.1 ± 2.7^{a}
180	415.9 ± 17.9^{a}	1513 ± 62^{a}	49.2 ± 1.9^{bc}	29.2 ± 3.1^{a}	-3.1 ± 0.2^{a}	29.4 ± 3.0^{a}
190	196.6 ± 62.6^{b}	1257 ± 44^{a}	50.5 ± 0.2^{ab}	31.0 ± 0.4^{a}	-3.4 ± 0.8^{ab}	31.2 ± 0.3^{a}
200	112.6 ± 43.5^{b}	511 ± 63^{b}	52.8 ± 1.4^{a}	33.4 ± 3.9^{a}	-4.4 ± 0.3^{bc}	33.7 ± 3.8^{a}

dry bases; Different superscript lowercase letters show the significant differences between the samples ($P < 0.05$); L^*: brightness; a^*: ± red-green; b^*: ± yellow-blue; C: chroma.

Table 2. Textural properties of chewing gum containing *Porphyridium cruentum* biomass dried under various spray dryer inlet temperatures.

Con. (%)	Drying T. (°C)	Hardness (g)	Adhesiveness (g.s)	Springiness	Cohesiveness	Chewiness (g)	Resilience
0.5 g/100 g	150	873 ± 103^{ab}	-21.4 ± 2.0^{ab}	0.713 ± 0.059^{a}	0.194 ± 0.022^{a}	120 ± 9^{abc}	0.014 ± 0.000^{a}
	160	710 ± 37^{ab}	-41.5 ± 6.8^{c}	0.735 ± 0.021^{a}	0.254 ± 0.016^{a}	133 ± 5^{ab}	0.015 ± 0.000^{a}
	170	909 ± 111^{a}	-14.6 ± 4.9^{ab}	0.779 ± 0.014^{a}	0.186 ± 0.006^{a}	131 ± 14^{ab}	0.014 ± 0.000^{a}
	180	758 ± 155^{ab}	-30.4 ± 3.2^{bc}	0.806 ± 0.01^{a}	0.237 ± 0.054^{a}	142 ± 5^{a}	0.015 ± 0.000^{a}
	190	794 ± 158^{ab}	-15.0 ± 2.9^{ab}	0.782 ± 0.017^{a}	0.191 ± 0.036^{a}	121 ± 19^{abc}	0.014 ± 0.001^{a}
	200	710 ± 15^{ab}	-41.7 ± 16.4^{c}	0.748 ± 0.038^{a}	0.231 ± 0.021^{a}	123 ± 20^{abc}	0.015 ± 0.000^{a}
1.0 g/100 g	150	764 ± 134^{ab}	-28.3 ± 5.4^{abc}	0.784 ± 0.05^{a}	0.224 ± 0.046^{a}	132 ± 13^{ab}	0.015 ± 0.001^{a}
	160	667 ± 138^{ab}	-26.8 ± 4.0^{abc}	0.716 ± 0.04^{a}	0.197 ± 0.011^{a}	94 ± 18^{bc}	0.015 ± 0.000^{a}
	170	584 ± 61^{b}	-10.3 ± 0.3^{a}	0.733 ± 0.066^{a}	0.197 ± 0.005^{a}	84 ± 14^{c}	0.015 ± 0.001^{a}
	180	752 ± 30^{ab}	-16.1 ± 8.3^{ab}	0.779 ± 0.014^{a}	0.206 ± 0.015^{a}	121 ± 6^{abc}	0.015 ± 0.001^{a}
	190	715 ± 135^{ab}	-18.0 ± 6.0^{ab}	0.740 ± 0.028^{a}	0.186 ± 0.014^{a}	97 ± 8^{bc}	0.014 ± 0.001^{a}
	200	763 ± 99^{ab}	-32.4 ± 1.3^{bc}	0.743 ± 0.031^{a}	0.216 ± 0.005^{a}	123 ± 18^{abc}	0.014 ± 0.001^{a}

Different superscript lowercase letters show the significant differences between the samples including biomass at same concentration ($P < 0.05$).

0.713-0.806, 0.186-0.254, 120-142 g and 0.014-0.015, respective and those were 584-764 g, 10.3-32.4 g.s., 0.716-0.779, 0.186-0.224, 84-132 g and 0.014-0.015 for the samples including biomass at 1.0%. The low values (84.3-141.5 g) were found for chewiness in all chewing gum samples containing microalga. This could be regarded as a positive result, due to the lower energy requirement during chewing, which can be also proved by low hardness values.

Cohesiveness values were determined at low levels in the all samples (0.186-0.254). This could be regarded as an indication that the internal bonds that made up the samples had low forces. This could cause fast release of bioactive compounds present in chewing gums which might positively increase bioavailability, improving functional properties during the oral process. However, the possibility of fast/short release of aroma compounds is a disadvantage. Because the consumer expectancy in gum is an aroma release spread over the chewing process.

3.3 Color properties of chewing gum samples

The color parameters of L^*, a^*, b^* and C^* were determined in the chewing gum samples including *P. cruentum* dried biomass (Figure 2 and Table 3). L^*, a^*, b^* and C^* values changed between 56.55-61.35, 10.51-14.99, 3.54-7.50 and 12.52-15.41, respectively for the chewing gum samples prepared with 0.5 of biomass. Regarding the samples including microalga at 1.0%, those values were found to between 49.52-54.52, 15.79-18.77, 0.79-3.09 and 16.09-18.81, respectively. Concerning L^* values, biomass concentration and spray dryer inlet temperature significantly affected brightness, and especially the brightness decreased with increasing microalga concentration for all temperature values ($p < 0.05$). a^* value increased with temperature and concentration in general ($p < 0.05$). It can be stated that both factors provided more intense red color formation in chewing gum samples. b^* values significantly decreased with temperature and concentration increase (from 7.5 to 0.8, $p < 0.05$). This positively resulted in obtaining a purer red color.

3.4 Sensory properties of chewing gum samples

Sensorial properties of appearance, chewiness, adhesiveness, algal taste and overall acceptability of chewing gum samples containing 0.5 g/100 g and 1.0 g/100 g *P. cruentum* biomass dried at different spray dryer inlet temperatures were shown in Table 4.

There was no significant difference between the sensorial properties except algal taste in the all samples prepared using *P. cruentum* ($p > 0.05$). Along with the increase in the usage amount of *P. cruentum*, the algal taste of chewing gum samples was more felt. These differences in instrumental color measurements of chewing gum samples, sensorial appearance differences were not significant ($p > 0.05$). Also the difference between adhesiveness and chewiness values determined by TPA was not determined by sensorial properties. This might be due

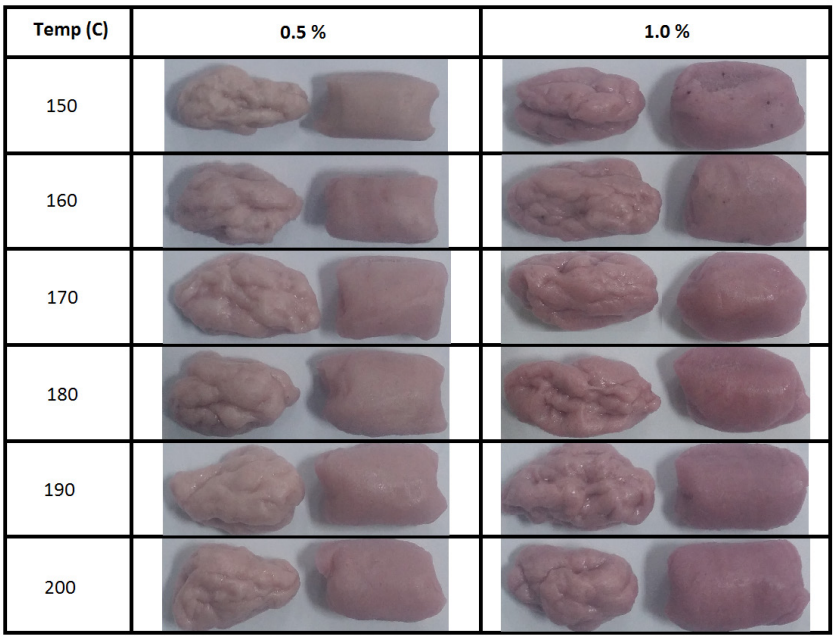

Figure 2. Photograps of chewed/unchewed chewing gums coloured with *Porphyridium cruentum* spry dried at different temperatures.

Table 3. Color properties of chewing gum containing *Porphyridium cruentum* biomass dried under various spray dryer inlet temperatures.

Con. (%)	Drying T. (°C)	L^*	a^*	b^*	C^*
0.5 g/100 g	150	61.4 ± 0.9^{a}	10.5 ± 0.4^{i}	7.5 ± 0.2^{a}	12.9 ± 0.4^{g}
	160	57.7 ± 0.7^{bc}	11.9 ± 0.3^{h}	4.6 ± 0.3^{b}	12.8 ± 0.3^{g}
	170	58.4 ± 0.9^{ab}	12.7 ± 0.3^{gh}	4.0 ± 0.2^{bc}	13.3 ± 0.3^{g}
	180	59.6 ± 0.9^{ab}	11.7 ± 0.6^{hi}	4.4 ± 0.2^{b}	12.5 ± 0.6^{g}
	190	57.2 ± 0.9^{bc}	13.7 ± 0.4^{fg}	4.4 ± 0.4^{b}	14.4 ± 0.4^{ef}
	200	56.6 ± 0.8^{bc}	14.9 ± 0.2^{ef}	3.5 ± 0.2^{cd}	15.4 ± 0.2^{de}
1.0 g/100 g	150	50.7 ± 0.9^{e}	16.6 ± 0.5^{cd}	1.4 ± 0.1^{f}	16.7 ± 0.5^{bcd}
	160	54.5 ± 1.4^{cd}	15.8 ± 0.4^{de}	3.1 ± 0.3^{d}	16.1 ± 0.4^{cd}
	170	51.3 ± 2.1^{e}	17.8 ± 0.9^{abc}	1.0 ± 0.2^{f}	17.8 ± 0.9^{ab}
	180	49.5 ± 0.5^{e}	18.8 ± 0.5^{a}	1.2 ± 0.2^{f}	18.8 ± 0.5^{a}
	190	51.9 ± 1.3^{de}	17.3 ± 0.6^{bc}	2.2 ± 0.3^{e}	17.4 ± 0.7^{abc}
	200	51.5 ± 1.1^{de}	18.1 ± 0.5^{ab}	0.8 ± 0.2^{f}	18.1 ± 0.5^{ab}

Different superscript lowercase letters show the significant differences between the samples ($P < 0.05$); L^*: brightness; a^*: ± red-green; b^*: ±yellow-blue: C^*: chroma.

Table 4. Sensory properties of chewing gum containing *Porphyridium cruentum* biomass dried under various spray dryer inlet temperatures.

Con. (%)	Drying T. (°C)	Apperance	Chewiness	Adhesiveness	Bitterness (algal taste)	Overall Acceptability
0.5 g/100 g	150	4.3 ± 0.4^{a}	4.0 ± 0.7^{a}	4.5 ± 0.5^{a}	5.0 ± 0.0^{a}	4.5 ± 0.5^{a}
	160	4.5 ± 0.9^{a}	4.5 ± 0.5^{a}	4.8 ± 0.4^{a}	5.0 ± 0.0^{a}	4.5 ± 0.5^{a}
	170	4.8 ± 0.4^{a}	4.3 ± 0.4^{a}	4.5 ± 0.5^{a}	5.0 ± 0.0^{a}	4.8 ± 0.4^{a}
	180	4.5 ± 0.9^{a}	4.0 ± 0.7^{a}	4.3 ± 0.4^{a}	4.5 ± 0.4^{ab}	4.5 ± 0.9^{a}
	190	5.0 ± 0.0^{a}	4.5 ± 0.5^{a}	4.8 ± 0.4^{a}	5.0 ± 0.0^{a}	4.5 ± 0.9^{a}
	200	4.8 ± 0.4^{a}	4.0 ± 0.7^{a}	4.5 ± 0.5^{a}	5.0 ± 0.0^{a}	4.0 ± 0.7^{a}
1.0 g/100 g	150	4.8 ± 0.4^{a}	4.8 ± 0.4^{a}	4.0 ± 0.0^{a}	4.0 ± 0.0^{b}	4.8 ± 0.4^{a}
	160	4.8 ± 0.4^{a}	4.3 ± 0.8^{a}	4.0 ± 0.0^{a}	4.0 ± 0.0^{b}	4.3 ± 0.8^{a}
	170	4.8 ± 0.4^{a}	4.8 ± 0.4^{a}	4.5 ± 0.5^{a}	4.5 ± 0.5^{ab}	4.5 ± 0.5^{a}
	180	4.8 ± 0.4^{a}	4.3 ± 0.4^{a}	4.5 ± 0.5^{a}	4.5 ± 0.5^{ab}	4.0 ± 0.7^{a}
	190	4.8 ± 0.4^{a}	3.8 ± 0.4^{a}	4.5 ± 0.5^{a}	4.5 ± 0.5^{ab}	4.0 ± 0.7^{a}
	200	4.5 ± 0.5^{a}	4.8 ± 0.4^{a}	4.5 ± 0.5^{a}	4.5 ± 0.5^{ab}	4.8 ± 0.4^{a}

Different superscript lowercase and uppercase letters show the significant differences between the samples ($P < 0.05$).

to possible sensitivity differences between sensory analysis and the use of TPA technique.

4 Discussion

Spray drying is the most commonly applied method for drying of many different compounds used in the food industry. However, any study where this method used for drying of microalgae was not encountered. Although it is economic and very fast method, thermal stress occurred during drying process caused reduction in carotenoid content in the microalgal biomass (Ryckebosch et al., 2011). The findings found in the literature were consistent with this study as chlorophyll-a content of *P. purpurerum* was found between 506.7 and 1913.3 μg g^{-1} and those were between 136.0 and 396.7 μg g^{-1} for beta carotene content (Coward et al., 2016). In the present study, the highest chlorophyll-a content was found to be 1513 μg g^{-1} and for total carotene was 415.9 μg g^{-1}. Carotenoid and chlorophyll-a content of the biomass increased with increasing temperature up to 180 °C and higher temperature levels caused to decrease in pigment content, indicating that degradation of the pigments might have due to sensitivity of those compounds. The studies related with usage of the microalgae in the different formulations indicated that microalgae can be used in the formulations for improving nutritional, functional and sensory properties of the products. For this aim, drying procedure should be considered since it directly affects the composition of the biomass.

It was observed that concentration of *P. cruentum* biomass and drying conditions did not have significant effects on cohesiveness, (0.186-0.254), springiness (0.713-0.806) and resilience (0.014-0.015) parameters in the all chewing gum samples ($p > 0.05$). Santos et al. (2014) studied the chewing gum samples including microcapsules of xylitol and menthol to prolong the duration of the cooling sensation. In their obtained samples, the hardness (11373-16253 g), chewiness (4972-9354 g), cohesiveness (0.80-0.86) and springiness (0.53-0.67) were measured. Springiness values of the samples containing *P. cruentum* biomass were found to be consistent with those of that study. However, hardness, chewiness and cohesiveness were found at lower levels. Concerning hardness values, it was determined that only chewing gums containing *P. cruentum* biomass dried at 170 °C differed from the other chewing gums ($p < 0.05$). In general, chewiness values decreased with the increase in concentration of biomass dried at the same temperature. This suggested that addition of algal biomass had an effect on chewiness and structural properties of chewing gum. However, significant differences in adhesiveness values ($p < 0.05$) were not associated with the algal biomass concentration and the spray dryer inlet temperature.

As a result of the texture data, it could be stated that *P. cruentum* biomass could be used as a colorant in the chewing gum. Nevertheless, it was also possible to modify the textural properties of chewing gums containing the microalga biomass by composition optimization including the components such as elastomer (Potineni & Peterson, 2008), aroma carrier solvents (Baek et al., 1999), texturizers or fillers (Raithore, 2012) or emulsifiers (Estruch, 2008). Studies have shown that there is an effect of texture on the release and perception of aroma in foods and model systems (Raithore, 2012). Partitioning of flavor compounds is affected by the composition of food, and the resistance to mass transfer by its texture. However, although these important relationships between textures and various quality parameters exist, the number of studies examining chewing gum samples using the TPA technique is limited. In this work, it was aimed to obtain chewing gum with red color which can allow the use of flavors associated with fruits and blends in chewing gums (Hearty et al., 2014). The number of studies in which the color qualities of gum samples were determined by using instrumental techniques was very limited. Color parameters of the chewing gum were also generally affected by microalgae concentration and drying temperature of the microalgae. Such result was expected when considering the influence of drying process on the pigment composition and color properties of the dried biomass. Lightness of the chewing gum decreased with increase in concentration of biomass in the formulation. One of the main advantage of the microalgae usage in the chewing gum is that as can be seen from Figure 2, the color of the chewed and unchewed samples was very similar to each other, indicating stability of the coloring pigments present in dried microalgae biomass in chewing gum matrix during chewing process. However, the color of the commercial chewing gums disappear in short times as a result of chewing.

Along with the increase in the usage amount of *P. cruentum*, the algal taste of chewing gum samples were more felt. This showed the need to determine the optimum level of usage to improve the color effect as well as the taste and functional properties of the products prepared with the bioactive components. Because consumers generally prefer food properties that are closest to conventional forms in functional foods (Konar et al., 2016).

It was demonstrated that *P. cruentum* algal biomass can be successfully used to give red color to chewing gums. Obtaining of stable coloring is an important innovation for these type of products. Also sensorial properties could not be affected significantly when concentration of microalga was optimized. In addition, it will be useful to study color stability under different storage conditions with further future work.

5 Conclusions

In this study, color properties of spray dried *Porphyridium cruentum* microalga species and the possibility of its use in chewing gum sample to give red color were investigated. With the increase in spray dryer inlet temperature total carotenoid and chlorophyll-a amount decreased significantly ($p < 0.05$). However, this caused increase in L^* value, and other color parameters (a^*, b^* and C^*) were not affected in microalga biomass. Textural parameters were not affected by the usage level of microalga biomass except chewiness. Increase in concentration of microalga biomass in chewing gum decreased chewiness values ($p < 0.05$). Color of the chewing gum samples were significantly changed by the drying conditions and concentration of *P. cruentum* which showed that desired color in chewing gum can be achieved by optimization of the studied factors. The addition of *P. cruentum* biomass to chewing gum is an interesting tool for providing nutritional supplementation with biological active compounds as antioxidant and fatty acids. However, %1 *P. cruentum* usage caused strange unwanted taste in chewing gums.

References

Azeredo, H. M. C. (2009). Betalains: properties, sources, applications and stability—a review. *International Journal of Food Science & Technology*, 44(12), 2365-2376. http://dx.doi.org/10.1111/j.1365-2621.2007.01668.x.

Baek, I., Linforth, R. S. T., Blake, A., & Taylor, A. J. (1999). Sensory perception is related to the rate of volatile concentration in-nose during eating of model gels. *Chemical Senses*, 24(2), 155-160. PMID: 10321816.

Batista, A. P., Gouveia, L., Nunes, M. C., Franco, J. M., & Raymundo, A. (2008). Microalgae biomass as a novel functional ingredient in mixed gel systems. In P. A. Williams, & G. O. Phillips (Eds.), *Gums and stabilizers for the food industry* (Vol. 14, pp. 487-494). Cambridge, UK: RSC Publishing.

Baysal, T., & Ersus, S. (1999). Karotenoidler ve insan sağlığı. *Gıda Dergisi*, 24(3), 177-185.

Becker, E. W. (1994). *Microalgae biotechnology and microbiology*. Cambridge: Cambridge University Press.

Chranioti, C., Nikoloudaki, A., & Tzia, C. (2015). Saffron and beetroot extracts encapsulated in maltodextrin, gum arabic, modified starch and chitosan: Incorporation in chewing gum system. *Carbohydrate Polymers*, 127, 252-263. http://dx.doi.org/10.1016/j.carbpol.2015.03.049. PMid:25965482.

Coward, T., Fuentes-Grünewald, C., Silkina, A., Oatley-Radcliffe, D. L., Llewellyn, G., & Lovitt, R. W. (2016). Utilising light-emitting diodes of specific narrow wavelengths for the optimization and co-production of multiple high-value compounds in Porphyridium purpureum. *Bioresource Technology*, 221, 607-615. http://dx.doi.org/10.1016/j.biortech.2016.09.093. PMid:27693726.

Dufossé, L., Galaup, P., Yaron, A., Arad, S. M., Blanc, P., Chidambara Murthy, K. N., & Ravishankar, G. A. (2005). Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? *Trends in Food Science & Technology*, 16(9), 389-406. http://dx.doi.org/10.1016/j.tifs.2005.02.006.

Durmaz, Y., Monteiro, M., Bandarra, N., Gokpinar, S., & Isik, O. (2007). The effect of low temperature on fatty acid composition and tocopherols of the red microalga, *Porphyridium cruentum. Journal of Applied Phycology*, 19(3), 223-227. http://dx.doi.org/10.1007/s10811-006-9127-6.

Estruch, R. A. (2008). Gum base. In D. Fritz (Ed.), *The formulation and production of chewing gum and bubble gum* (pp. 93-118). Loughton, England: Kennedy's Books, Ltd.

Fradique, M., Batista, A. P., Nunes, M. C., Gouveia, L., Bandarra, N. M., & Raymundo, A. (2010). Incorporation of Chlorella vulgaris and Spirulina maxima biomass in pasta products. Part 1: Preparation and evaluation. *Journal of the Science of Food and Agriculture*, 90(10), 1656-1664. http://dx.doi.org/10.1002/jsfa.3999. PMid:20564448.

Gouveia, L., Batista, A. P., Miranda, A., Empis, J., & Raymundo, A. (2007). Chlorella vulgaris biomass used as colouring source in traditional butter cookies. *Innovative Food Science & Emerging Technologies*, 8(3), 433-436. http://dx.doi.org/10.1016/j.ifset.2007.03.026.

Gouveia, L., Gomes, E., & Empis, J. (1997). Use of Chlorella vulgaris in rainbow trout, Oncorhynchus mykiss, diets to enhance muscle pigmentation. *Journal of Applied Aquaculture*, 7(2), 61-70. http://dx.doi.org/10.1300/J028v07n02_07.

Gouveia, L., Raymundo, A., Batista, A. P., Sousa, I., & Empis, J. (2006). Chlorella vulgaris and Haematococcus pluvialis biomass as colouring and antioxidant in food emulsions. *European Food Research and Technology*, 222(3-4), 362-367. http://dx.doi.org/10.1007/s00217-005-0105-z.

Graça, C., Fradinho, P., Sousa, I., & Raymundo, A. (2018). Impact of Chlorella vulgaris on the rheology of wheat flour dough and bread texture. *Lebensmittel-Wissenschaft + Technologie*, 89, 466-474. http://dx.doi.org/10.1016/j.lwt.2017.11.024.

Guillard, R. R., & Ryther, J. H. (1962). Studies of marine plankotic diatoms: I. Cyclotella Nana Hustedt, and Detonula Confervacea (CLEVE) Gran. *Canadian Journal of Microbiology*, 8(2), 229-239. http://dx.doi.org/10.1139/m62-029. PMid:13902807.

Hearty, A., Lau, A., & Roberts, A. (2014). Chewing gum intake in Europe: a survey of intakes in France, Germany, Italy, Spain and the UK. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 31(7), 1147-1157. PMid:24720761.

Horrobin, D. F. (1999). Lipid metabolism, human evolution and schizophrenia. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, 60(5-6), 431-437. http://dx.doi.org/10.1016/S0952-3278(99)80024-6. PMid:10471133.

Konar, N., Palabiyik, I., Toker, O. S., & Sagdic, O. (2016). Chewing gum: production, quality parameters and pppurtinities for delivering bioactive compounds. *Trends in Food Science & Technology*, 55, 29-38. http://dx.doi.org/10.1016/j.tifs.2016.07.003.

Mizutani, T. (2009). Toxicity of xanthene food dyes by inhibition of human drug-metabolizing enzymes in a noncompetitive manner. *Journal of Environmental and Public Health*, 2009, 953952. http://dx.doi.org/10.1155/2009/953952. PMid:20041016.

Olafsson, S. F. (2013). *Downstream process design for microalgae* (Master's thesis). University of Iceland, Reykjavic, Iceland.

Palabiyik, I., Durmaz, Y., Öner, B., Toker, O. S., Coksari, G., Konar, N., & Tamtürk, F. (2018). Using spray-dried microalgae as a natural coloring agent in chewing gum: effects on color, sensory, and textural properties. *Journal of Applied Phycology*, 30(2), 1031-1039. http://dx.doi.org/10.1007/s10811-017-1324-y.

Periche, A., Heredia, A., Escriche, I., Andrés, A., & Castelló, M. L. (2015). Potential use of isomaltulose to produce healtier marshmallows. *Lebensmittel-Wissenschaft + Technologie*, 62(1), 605-612. http://dx.doi.org/10.1016/j.lwt.2014.12.024.

Potineni, R. V., & Peterson, D. G. (2008). Mechanisms of flavor release in chewing gum: cinnamaldehyde. *Journal of Agricultural and Food Chemistry*, 56(9), 3260-3267. http://dx.doi.org/10.1021/jf0727847. PMid:18426214.

Qiang, H., & Richmond, A. (1994). Optimizing the population density in Isochrysis galbana grown outdoors in a glass column photobioreactor. *Journal of Applied Phycology*, 6(4), 391-396. http://dx.doi.org/10.1007/BF02182155.

Raithore, S. (2012). *Effect of polyols on flavor release during mastication of sugar-free confections* (Ph.D. thesis). University of Minnesota, Minneapolis.

Ryckebosch, E., Muylaert, K., Eeckhout, M., Ruyssen, T., & Foubert, I. (2011). Influence of drying and storage on lipid and carotenoid stability of the microalga Phaeodactylum tricornutum. *Journal of Agricultural and Food Chemistry*, 59(20), 11063-11069. http://dx.doi.org/10.1021/jf2025456. PMid:21866882.

Santos, M. G., Carpinteiro, D. A., Thomazini, M., Rocha-Selmi, G. A., da Cruz, A. G., Rodrigues, C. E. C., & Favaro-Trindade, C. S. (2014). Coencapsulation of xylitol and menthol by double emulsion followed by complex coacervation and microcapsule application in chewing gum. *Food Research International*, 66, 454-462. http://dx.doi.org/10.1016/j.foodres.2014.10.010.

Valduga, E., Lazzari, M. R., Vardanega, R., & Di Luccio, M. (2012). Evaluation of sugar inversion in chewing gum added of sodium lactate. *Journal of Food Process Engineering*, 35(1), 37-53. http://dx.doi.org/10.1111/j.1745-4530.2009.00570.x.