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Research Article

Acceptability of Different Concentrations of *Chlorella* sp. in Filipino Delicacy Puto as Coloring Agent

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Abstract: Natural colorants play a crucial role in food product development and improvement of health. Microalga *Chlorella* sp. is one of the sources of natural colorant. In this study, different concentrations of microalga *Chlorella* sp. (0.5, 1, and 2%) were added to Puto as coloring agents to evaluate its sensory properties. Pigments such as chlorophyll *a* and total carotenoid quantities of *Chlorella* powder and the experimental group were also investigated. It was found that the natural colorant *Chlorella* sp. at all levels of concentrations did not affect the color properties ($p \geq 0.05$) of the Puto products. However, the smell and texture of Puto differed significantly ($p \leq 0.05$) when 2% *Chlorella* sp. was incorporated. The study also found that the 0.5% and 1% amounts of *Chlorella* sp. component did not significantly affect ($p \geq 0.05$) the Puto's taste and overall acceptability. However, the 2% level of *Chlorella* sp. significantly decreased both overall acceptability and taste attributes. Moreover, *Chlorella* sp. powder constituted 4004.79±119.1 µg g^{-1} chlorophyll *a* and 1442.67±74.41 µg g^{-1} total carotenoids. Chlorophyll *a* amounts in experimental groups varied from 14.34±0.49 µg g^{-1} to 54.06±1.71 µg g^{-1} while total carotenoids amounts were found ranging from 5.59±0.37 µg g^{-1} and 18.06±0.66 µg g^{-1}. Puto used these biomasses at a concentration of 0.5%, 1%, and 2% as natural green colorants. However, chlorophyll *a* and carotenoid pigments level at 2% *Chlorella* sp. were not tolerable for the production of Puto. Hence, the *Chlorella* sp. biomass at 0.5% and 1% would be suitable for use as a natural colorant in the Filipino delicacy Puto.

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1. Introduction

Puto is a Filipino delicacy from the Philippines, also known as a rice cake, that is generally made from flour, usually cooked by steaming, and includes rice-free varieties. It is actually less sweet and fluffy and makes a great base for different toppings and flavors, such as cheese, ube (purple yarn), pandan (screwpine), or salted egg, which gives it different colors. It is believed that the color of food is one of the most crucial factors affecting its safety and quality since the color of food influences one's perception of its taste and safety. Colors used in food can be categorized into natural and synthetic colors (Mortensen, 2006). It is normally possible to use synthetic food colors in foods without further processing since they are usually water-soluble chemicals manufactured in factories (Sadar et al., 2017). Unlike natural colors, which are less stable and more expensive, synthetic colorants can be trusted and are both economical and reliable (Saleem and Umar, 2013). However, human health was adversely affected by synthetic food colorants. There is evidence that synthetic food color additives can sometimes be harmful to the liver, kidneys, and testes (Van Bever et al., 1989; Mahmoud, 2006).

There are a variety of natural food coloring sources, such as fruits, seeds, vegetables, insects, and microbes that do not require chemical treatment (Sadar et al., 2017). Renewable resources are used to produce natural food colorants. Typically, plant material is used as a source of colorants; however, insects, fungi, algae, and cyanobacteria are also employed (Mortensen, 2006). In today's society, the benefits of natural colorants, such as anthocyanins and carotenoids, are further reinforced by scientific results. *Hibiscus* sp. has historically been used in the reduction of liver dysfunction and hypertension due to its presence of 2.5% (dry weight) anthocyanin (Da-Costa-Rocha et al., 2014; Abdallah, 2016; Mushtaq et al., 2016). Natural colors containing carotenoids, such as β-carotene and pro-vitamin A, offer a variety of health benefits (Britton and Khachik, 2009; Bohn, 2012; De Andrade and De Andrade, 2017). For example, lycopene protects against prostate cancer (Aghajanpour et al., 2017; Li et al., 2019), and Lutein reduces cataract incidence (Akhtar and Bryan, 2008; Jia et al., 2017; Zhao et al., 2017). Consequently, Colorants and pigments derived from natural sources can provide important aspects for food product development. According to Durmaz and Bandarra (2017), microalgae are an extremely valuable source of natural pigments.

Due to the fact that microalgae contain a variety of macro- and micronutrients and are increasingly being studied since they can potentially provide humans with health benefits such as antioxidation, anticarcinogenesis, and antihypertensive effects (Pulz and Gross, 2004; Durmaz and Bandarra, 2017; Koyande et al., 2019). Microalgae's natural pigments may have numerous benefits, such as enhancing not only the visual qualities of a product but also its antioxidant capacity and preservation function (Durmaz and Bandarra 2017; Sun et al., 2023).

Biomass from microalgae can be utilized to make a wide range of foods. White chocolate with *Nannochloropsis oculata* (Genc Polat et al., 2020); yogurt with *Spirulina platensis* (Barkallah et al., 2017); pasta with *Isochrysis galbana* and *Diacronema vlkianum* (Fradique et al., 2013); yogurt with *Pavlova lutheri* (Robertson et al., 2016); *Nannochloropsis gaditana, Isochrysis galbana, Tetraselmis suecica, Scenedesmus almeriensis,* and *Isochrysis galbana* in bread (Garcia Segovia et al., 2017); chewing gum with *Isochrysis galbana* and *Nannochloropsis oculata* (Palabiyik et al., 2017); and pasta with *Chlorella vulgaris* and *Spirulina maxima* (Fradique et al., 2010), currently being evaluated.

Chlorella is a eukaryotic green microalga with a spherical shape whose importance has increased commercially and scientifically (Liu and Chen, 2014; De Andrade and De Andrade, 2017). Moreover, *Chlorella* has been used as a source of food and animal feed, as well as a protein source, and can also be used as a natural fuel since it contains a high oil content (Liu and Chen, 2014). Although microalgae have been studied in the literature for their potential use in food products, however, the use of microalgae *Chlorella* sp. as a natural colorant in Filipino delicacies has not been studied. Thus, the main goal of this study was to examine the effects of the natural pigment source *Chlorella* on sensory evaluation and acceptability in the Filipino delicacy Puto as a coloring agent.

2. Material and Methods

2.1. Preparation of chlorella powder

Chlorella sp. biomass was produced at the Faculty of Fisheries, Kastamonu University. The culture procedure was given by Erbil et al. (2021). After harvesting microalgae from the photobioreactor, biomass was separated from water and then dried in an oven at 50 °C for 4 hours. Dried biomass was ground in a coffee grinder.

2.2. Preparation of Puto Filipino delicacy

Puto was made of mixtures of flour, sugar, baking powder, vanilla, egg, milk, and water. The formulation of ingredients is given in Table 1. Three different *Chlorella* sp. concentrations were determined after pre-trials. Each treatment with three replicates was placed into a *Puto* molder and then steamed over medium heat to prevent overcooking or drying out of Puto. The lid of the steamer was covered with a soft cloth to absorb the moisture. After cooking, the Puto was cooled for 5 minutes before removing it from the Puto molder.

Table 1. Composition of product

Ingredients	Group A (0% control)	Group B (0.5% *Chlorella* sp.)	Group C (1% *Chlorella* sp.)	Group D (2% *Chlorella* sp.)
Flour (g)	100	100	100	100
Sugar (g)	50	50	50	50
Baking powder (g)	10	10	10	10
Vanilla (g)	5	5	5	5
Egg (g)	55	55	55	55
Milk (ml)	70	70	70	70
Water (ml)	50	50	50	50
***Chlorella* sp. (g)**	-	1.71	3.43	6.94

2.3. Color measurements

The color values of the Puto products in the control group and incorporated with *Chlorella* sp. were measured with a HunterLab color measurements system (2°-10° observer angle, 10 nm wavelength interval, 400-700 nm wavelength range). A standard white plate (L*=86.6, a*=-0.5, b*=0.5) was used to calibrate the colorimeter. Triplicate measurements for three different parts of the top and bottom of Puto products. L^*, a^*, and b^* were determined, where L^* is the brightness coefficient from dark (0) to bright (100), a^* is the coefficient from red (+) to green (-), and b^* is the coefficient from yellow (+) to blue (-). *Chroma* indicates the intensity of color, whereas *hue* angle is an indication of red versus yellow color. Chroma (C*; Eq. 1), *hue* (h*; Eq. 2), whiteness (W*; Eq. 3), and total color difference (*TCD*; Eq. 4) were obtained by following equations:

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

$$Hue = arctan\frac{b}{a} \quad (2)$$

$$Whiteness = 100 - \sqrt{(100 - L^2) + a^2 + b^2} \quad (3)$$

$$TCD = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (4)$$

2.4. pH measurements

The pH values of the Puto products in the control group and incorporated with *Chlorella* sp. were determined by a pH-meter (IsoLab, METERS" tabletop," Microprocessor pH-meter, Germany). All pH analyses of products were carried out in triplicate.

2.5. Sensory evaluation of Puto

The newly prepared *Puto* delicacies were evaluated by 32 panelists composed of consumers from two nationalities, Filipinos, and Turkish. Following steaming, a 5-point Hedonic scale was used to evaluate the products, varying from extremely like (5) to extremely dislike (1) for appearance/color, flavor/aroma, mouthfeel or texture, taste, and overall acceptability (Blase and Labay, 2017). A random product of three replications was given to each panelist for each Puto treatment. One treatment was served at a time to each panelist in random order. The evaluators were provided with bottled mineral water to rinse their mouths before receiving another set of Puto products every one to two minutes. The researchers were instructed to evaluate each product using a 5-point Hedonic scale in order to eliminate any bias.

2.6. Pigment analysis

Pigment analysis was performed both for *Chlorella* sp. powder and for the Puto experimental groups. A total of 5 ml of methanol was added to the products in glass tubes. Products underwent 10 minutes of ultrasound at 65 °C after 30 seconds of vortexing. Following this, products were vortexed again for 30 seconds, then centrifuged for 10 minutes at 5000 rpm. Supernatants were read in a spectrophotometer, and pigment amounts were calculated by equations 5-6 (Zou and Richmond, 2000; Macıas-Sánchez et al., 2005) given below:

$$\text{Chlorophyll } a \text{ (µg/ml)} = 13.9 \text{ A}_{666} \quad (5)$$

$$\text{Total carotenoids (µg/ml)} = 4.5 \text{ A}_{475} \quad (6)$$

2.7. Statistical analysis

SPSS software version 22.0 was used to analyze the data of experimental results of incorporated *Chlorella* sp. in Puto, including untreated control, by using analysis of variance (ANOVA) at the $p \leq 0.05$ significance level. All experimental values are at least the mean of triplicate determination. Duncan's multiple range test method was applied to rank the mean, and Levene's Test was used to test for homogeneity of variance. The following ranges were used to interpret the mean scores for appearance/color, odor/aroma, mouthfeel/texture, flavor/taste, and general acceptability:

4.50 – 5.00	Extremely like
3.50 – 4.49	Very much like
2.50 – 3.49	Neither like nor dislike
1.50 – 2.49	Very much dislike
0.50 – 1.49	Extremely dislike

3. Results

3.1. Color properties and pH measurement of Puto product

Color properties were measured on the top and bottom parts of the Puto. Table 2 shows the color measurement of the Puto product. On the top part of Puto, the L^* values of group A (66.28±0.36) were significantly ($p \leq 0.05$) different from those of group B, group C, and group D, while on the bottom part of Puto, the L^* values in group A and B were significantly different from those of group C and D ($p \leq 0.05$). In terms of a^* and b^* values, group A was significantly different from groups B, C, and D for both the top and bottom parts of Puto. Moreover, the chroma (C^*), hue (h^*), whiteness (W^*), and total color difference (*TCD*) values were calculated based on the average results of L^*, a^*, and b^* values, which are frequently used to determine how a food product's color tone and saturation can be defined. Table 3 shows the calculated color measurement of the Puto product. According to the results, C^* values ranged from 15.28 – 27.56 on the top part of the Puto, while 14.92 – 27.40 were recorded on the bottom part. The h^* values on the top part of Puto varied from -1.55 to 1.56, while the h^* values on the bottom part of Puto ranged from -1.53 to 1.50. Additionally, W^* values ranged from 38.16 – 62.98 on the top part of the Puto, while W^* values (31.85) of group D is lower than 39.79 in group A at the bottom part of the Puto product. The *TCD* values on the top part of Puto varied from 25.16 to 49.93, while the *TCD* values on the bottom part of Puto ranged from 31.52 to 55.66. Furthermore, Figure 1 shows the pH values of the Puto products incorporated with *Chlorella* sp. Based on the result of the present study, groups A, B, C, and D obtained pHs of 8.97, 9.03, 8.96, and 8.87, respectively.

Table 2. Color measurement of Puto

Color parameters	TOP				BOTTOM			
	Group A (0%)	Group B (0.5%)	Group C (1%)	Group D (2%)	Group A (0%)	Group B (0.5%)	Group C (1%)	Group D (2%)
L*	66.28±0.36^{a}	59.85±0.83^{b}	49.65±0.45^{c}	44.64±1.25^{d}	58.60±1.90^{a}	55.07±1.67^{a}	46.39±1.23^{b}	36.51±1.47^{c}
a*	1.29±0.24^{a}	0.39±0.07^{b}	-0.61±0.09^{c}	-0.47±0.16^{c}	1.08±0.06^{a}	-0.81±0.59^{b}	-1.19±0.34^{b}	-0.98±0.11^{b}
b*	15.23±0.89^{a}	25.66±0.96^{b}	27.29±0.72^{c}	27.55±0.05^{c}	14.88±0.08^{a}	26.93±1.68^{b}	27.38±1.14^{b}	24.75±2.11^{b}

Values followed by the different letters in the same row are significantly differences (P≤0.05). Means (±SE) are based on triplicate analyses. L=lightness (black to white), a=red(+) to green(-) and b=yellow(+) to blue(-). Different letters (a,b,c,d,…) indicate significant differences among the same color parameter in different groups.

Table 3. Calculated color measurement of Puto

Color parameters	TOP				BOTTOM			
	Group A (0%)	Group B (0.5%)	Group C (1%)	Group D (2%)	Group A (0%)	Group B (0.5%)	Group C (1%)	Group D (2%)
Chroma	15.28	25.66	27.3	27.56	14.92	26.94	27.4	24.77
Hue	1.49	1.56	-1.55	-1.55	1.5	-1.54	-1.53	-1.53
Whiteness	62.98	52.35	42.73	38.16	55.99	47.61	39.79	31.85
TCD	25.16	36.74	45.64	49.93	31.52	41.14	48.37	55.66

Chroma (*C**), hue (*h**), whiteness (*W**), and total color difference (*TCD*) values were calculated based on the average results of *L**, *a**, and *b** values.

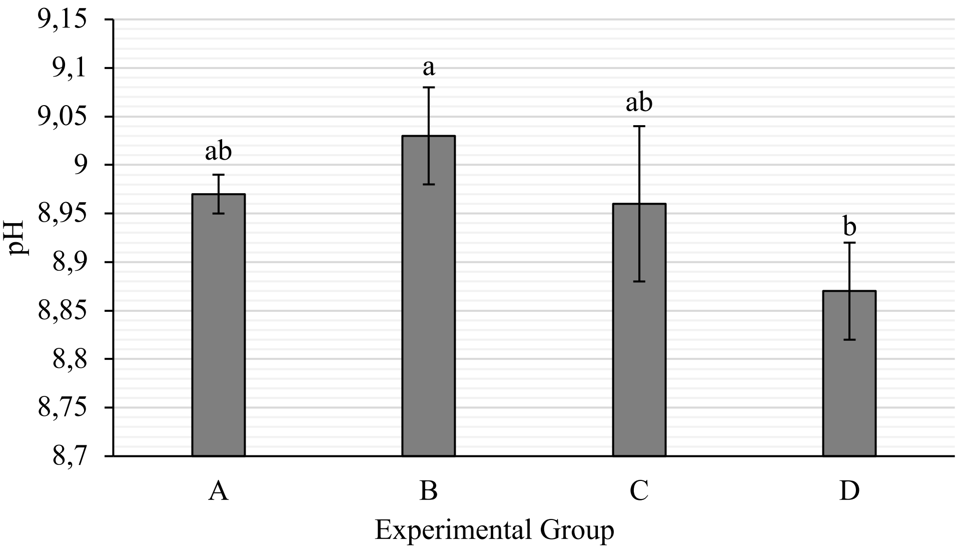

Figure 1. pH measurement of Puto. Group A (0% *Chlorella* sp.), group B (0% *Chlorella* sp.), group C (0% *Chlorella* sp.), and group D (0% *Chlorella* sp.).

3.2. Sensory evaluation of Puto

Sensory evaluation results of the Puto product containing *Chlorella* sp. at different concentrations are presented in Table 4 and Figure 2. The panelists' preferences were not influenced by the color of the product; hence, the appearance/color of the Puto did not differ significantly (p≥0.05). The slightly sweet aroma in group A and group B, with mean scores of 3.66 ± 0.18 and 3.38 ± 0.21, respectively, were significantly higher (p≤0.05) than in group C and group D, with mean scores of 2.97 ± 0.21 and 2.88 ± 0.22, respectively. In terms of mouthfeel or texture of the Puto products, group D and C with a mean score of 3.31 ± 0.17 and 3.53 ± 0.19, respectively, were statistically lower (p≤0.05) than group A, and group B. In addition, the taste or flavor of group A, group B, group C, group D, with mean scores of 3.69 ± 0.18, 3.16 ± 0.22, 3.13 ± 0.24, and 2.84 ± 0.22, respectively, where group A significantly different (p≤0.05) than group D. For overall acceptability scores, group A and group B were significantly different (p≤0.05) from group D, while group C did not significantly different (p≥0.05) from the group D.

Table 4. Sensory mean scores of Puto with different concentrations of *Chlorella* sp.

Treatments	Color / Appearance	Aroma / Odor	Texture / Mouthfeel	Taste / Flavor	Overall Acceptability
Group A	3.94 ± 0.21[a]	3.66 ± 0.18[a]	4.19 ± 0.12[a]	3.69 ± 0.18[a]	3.72 ± 0.17[a]
Group B	3.69 ± 0.17[a]	3.38 ± 0.21[ab]	3.88 ± 0.16[ab]	3.16 ± 0.22[ab]	3.44 ± 0.19[a]
Group C	3.94 ± 0.18[a]	2.97 ± 0.21[b]	3.53 ± 0.19[bc]	3.13 ± 0.24[ab]	3.25 ± 0.21[ab]
Group D	3.69 ± 0.21[a]	2.88 ± 0.22[b]	3.31 ± 0.17[c]	2.84 ± 0.22[b]	2.78 ± 0.216[b]

Columns with the same letters are not significantly different (p≥0.05). Values in SEM (standard error mean), n=32.

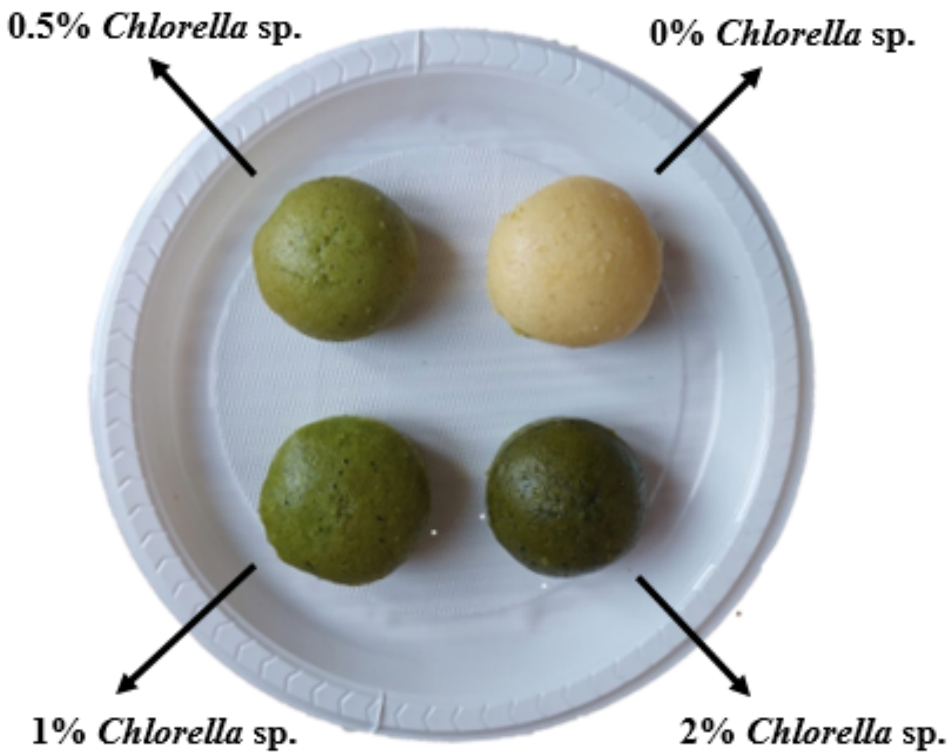

Figure 2. Products of Puto prepared with different concentrations of *Chlorella* sp. (Photograph using Samsung Galaxy A72). Group A (0% *Chlorella* sp.), group B (0% *Chlorella* sp.), group C (0% *Chlorella* sp.), and group D (0% *Chlorella* sp.).

3.3. Pigment analysis

Chlorophyll *a* and total carotenoid amounts of crude *Chlorella* sp. powder and experimental groups were measured. *Chlorella* sp. powder was constituted of 4004.79±119.1 µg g^{-1} chlorophyll *a* and 1442.67±74.41 µg g^{-1} total carotenoids according to pigment analysis. Chlorophyll *a* amounts in experimental groups varied from 14.34±0.49 µg g^{-1} to 54.06±1.71 µg g^{-1}. Also, total carotenoid amounts were found between 5.59±0.37 µg g^{-1} and 18.06±0.66 µg g^{-1} (Table 5).

Table 5. Pigment amounts of crude *Chlorella* sp. and Puto experimental groups

Pigments	Crude *Chlorella* sp.	Puto with 0.5% *Chlorella* sp.	Puto with 1% *Chlorella* sp.	Puto with 2% *Chlorella* sp.
Chlorophyll *a* (µg g^{-1})	4004.79±119.1	14.34±0.49	27.51±1.09	54.06±1.71
Total carotenoids (µg g^{-1})	1442.67±74.41	5.59±0.37	10.28±0.85	18.06±0.66

4. Discussion

Color is a very important aspect of a person's perception of food, serving as a quality indicator that can promote or hinder their acceptance of a food product (Chranioti et al., 2015). Colorants are widely used in food products for coloring, but their toxicological potential on humans is a controversial issue in the food industry (Mizutani, 2009). The color properties of the Puto product incorporated with *Chlorella* sp. were investigated in the present study. The C^*, h^*, and W^* values for each Puto product were calculated based on the average values for the a^*, b^*, and L^* parameters. The values of total color difference (*TCD*) were also calculated based on the a^*, b^*, and L^* values. As a result of the present study, increasing concentrations of *Chlorella* sp. in Puto decreased the color parameters of L^*, a^*, h^*, and W^*. Similar to another study, increasing the concentration of spray-dried *Nannochloropsis oculata* in white chocolate decreased the color parameters of L^*, h^*, and W^* (Genc Polat et al., 2020). In addition, there is considered to be a noticeable color change when the *TCD* value of the product exceeds 3.00 (Witzel et al., 1973; Periche et al., 2015). Thus, products with *TCD* values below 3.00 indicate color stability under the relevant circumstances. Consequently, as *Chlorella* sp. concentration in Puto increases, a total color difference can be easily perceived by the human eye. Moreover, a pH measurement was also conducted on the Puto products. Based on the result of the present study, *Chlorella* sp. incorporated into Puto obtained a pH measurement ranging from 8.87 – 9.03. As a result, it is lower than the study conducted by García-Segovia et al. (2017), in which microalgae such as *Isochrysis galbana*, *Scenedesmus almeriensis*, *Tetraselmis suecica*, *Nannochloropsis gaditana* were incorporated into wheat bread obtained at pH values that ranged from 10.10 to 10.50, which is still considered suitable for human consumption.

As a traditional product of the Philippines, Puto is consumed daily as a snack food, dessert, or even breakfast, served during family gatherings, and can be purchased at almost any grocery store. During Ramadan, Muslims in the southern Philippines also consume Puto with hot coffee or cold beverages as part of their iftar (breaking the fast). Puto is usually flavored or topped with ingredients such as ube, cheese, and salted eggs. However, to ensure that the organoleptic properties of the experimental product were not affected, no other flavors or toppings were used in the present study.

Consumer acceptance of a product is influenced heavily by the color or appearance, and the choice of food can affect consumer perception of subsequent flavors and acceptability of the food (Imram, 1999; Silva et al., 2022). Hence, it is vital to evaluate the sensory properties in developing the new product, optimization process, and re-formulation studies. In this study, a sensory analysis was performed to quantify the appearance, odor, mouthfeel, taste, and overall acceptability properties.

Nowadays, due to food coloring pigments being unstable and deteriorating during processing, colorants are added to food products worldwide to maintain or restore product color uniformity (Silva et al., 2022; Nabi et al., 2023). In order to determine a product's overall appearance, three factors must be considered: its optical properties, its shapes or physical form, and the way it is presented (Hutchings, 1977). Natural colorants are said to be more expensive than synthetic colors; however, microalgae might be a solution to this problem since they can be produced almost everywhere, like non-arable areas. Green food is perceived as healthier, especially among health-conscious consumers (Schuldt, 2013). Trends in the food industry include functional food and green-colored foods, which have more potential health benefits than normal nutrition (Villaró et al., 2021). In microalgae, green pigments provide health benefits for humans, including anticarcinogenesis, antihypersion, and antioxidative effects (Koyande et al., 2019). Typically, a Puto is white in color, though variations may occur due to the flavorings such as pandan (screwpine) and ube (purple yarn). However, *Chlorella* sp. was incorporated at different concentrations (0.5, 1, and 2%) in this experiment, resulting in a change in color. Results found that the color/appearance of Puto products did not affect the preferences of the panelists; hence, Puto's appearance/color did not differ significantly ($p \geq 0.05$) based on the level of the microalgal component. Genc Polat et al. (2020) also stated that using different concentrations (0.125, 0.25, 0.5, and 0.75%) of dried encapsulated microalga *Nannochloropsis oculata* in white chocolate did not affect the appearance of the panelist's references.

The way a person perceives texture is based on their oral responses to touch; auditory stimulation may also determine the deep responses of the masseter muscle (Rustagi, 2020). In addition to the food characteristics, such as its composition and nature, the deformation rate in the mouth can influence texture perception (Mathoniere et al., 2000). Odors can be evaluated based on their hedonic value by our sense of smell (Puleo et al., 2021). Humans share a sense of smell with many animal species that was crucial to their survival and evolution. In reality, it provides information about our surroundings, stimulates our emotional states, allows us to socialize, protects us from risks and stresses (Ludvigson and Rottman, 1989), and helps us avoid food hazards (Stevenson, 2010). It is important to consider the textural and odor attributes when developing new products to ensure their acceptance by consumers (Fradique et al., 2013). Puto is often soft or fluffy in texture with various odors depending on the flavor, such as ube (purple yarn), pandan (screwpine), or salted egg. As the panelists evaluated, adding 0.5% and 1% of *Chlorella* sp. to Puto had no significant effect, as demonstrated in the present study. However, Puto's mouthfeel and odor decreased dramatically with 2% *Chlorella* sp. enriched. It is parallel to the study of Genc Polat et al. (2020), where dried encapsulated *Nannochloropsis oculata* was not found to affect the texture and odor of white chocolate. As far as microalgae biomass for food applications is concerned, they are generally green and have volatile compounds that can cause musty, muddy, and fishy odor (Persson, 1980; Fradique et al., 2013; Andrade et al., 2018; Villaró et al., 2021). In the present study, Puto enriched with 2% *Chlorella* sp. smells slightly seaweedy, which may explain its low acceptance in terms of smell.

Taste is used to identify essential nutrients and toxic compounds. The human tongue can discern five primary flavors: sweet, umami (the taste of amino acids), sour, bitter, and salty (Briand and Salles, 2016). Puto made in this study is actually less sweet and serves as a great base for flavors such as microalgae *Chlorella* sp. The 0.5% and 1% amounts of *Chlorella* sp. component in Puto were not found to affect the significant level of this difference in taste and overall acceptability. However, Barkallah et al. (2017) mentioned that 0.75% and 1% of microalga *Spirulina platensis* concentrations added to yogurt possessed lower sensory properties of flavor and overall acceptability. *Spirulina* supplements produce an off-flavor due to their ability to oxidize lipids and minerals, which act as both pro-oxidants as well as produce metallic compounds (Shimamatsu, 2004). In addition to being strong-colored (generally) and strong smell, microalgae biomass is also disadvantageous for food applications due to its "fishy" taste (Villaró et al., 2021). 2% level of *Chlorella sp.,* in the present study, decreased overall acceptability and taste, according to the panelists. Thus, higher concentrations of *Chlorella* sp. may lead to an undesirable preference among evaluators.

Pigments play a key role in algae photosynthetic activity and have many beneficial biological properties, such as anti-inflammatory, anti-oxidant, anti-angiogenic, anti-obesity, neuroprotective, and anti-cancer properties (Guedes et al., 2011; Ciccone et al., 2013). Carotenoids and chlorophylls found in microalgae are widely used in industries like food, nutraceuticals, pharmaceuticals, cosmetics, and aquaculture (Begum et al., 2016). In photosynthesis, a plant's light processes are supported by chemical energy converted by chlorophyll (Barsanti and Gualtieri, 2005; Oo et al., 2017). The majority of microalgae contain chlorophyll *a*, but Dinophyta have chlorophyll *b* and *c* (Barsanti and Gualtieri, 2005).

In this study, chlorophyll *a* pigment levels were determined in the Puto products. Chlorophyll *a* levels varied from 14.34 to 54.06 µg g^{-1} in the Puto containing 0.5%, 1%, and 2% of *Chlorella*. In white chocolate products containing 0.125, 0.250, 0.500, and 0.750% of spray-dried microalga *Nannochloropsis oculata*, the amount of chlorophyll *a* ranged from 9.60 to 20.5 µg g^{-1}, indicating that the effects of the white chocolate production process on this pigment are tolerable (Genc Polat et al., 2020). In addition, chewing gum was determined to have chlorophyll *a* levels of 7.775-11.377 µg g^{-1} in *Isochrysis galbana* and 1.141-1.836 µg g^{-1} in *Nannochloropsis oculata* biomass (0.5% and 1%) respectively are also acceptable in the chewing gum production (Palabiyik et al., 2018). However, Puto that contains 0.5% and 1% *Chlorella*, with a chlorophyll *a* content ranging from 14.34 to 27.51 µg g^{-1}, is acceptable in the production of Puto. The encapsulation and spray drying of microalgae could be used to increase pigment concentration (Palabiyik et al., 2018; Genc Polat et al., 2020). However, the *Chlorella* sp. used in the current study was powdered using the conventional processing method, which may be the cause of the unsuitable pigment at 2% *Chlorella* sp. concentration.

Pigments called carotenoids, found in microalgae as natural sources of carotenoids, provide powerful antioxidants that prevent many human diseases and maintain good health (Britton and Khachik, 2009; De Andrade and De Andrade, 2017). Generally, they prevent the oxidation of lipids and enhance the stability and functionality of the cells' photosynthetic machinery by shielding them from reactive radicals (Grobbelaar, 2004). Palabiyik et al. (2018) mentioned that chewing gum with 0.5% and 1% biomass of microalgae *Isochrysis galbana* and *Nannochloropsis oculaa* has total carotenoids ranging from 1.984–3.373 µg g^{-1} and 0.378–0.077 µg g^{-1} respectively. Comparatively, it is lower than the present study where Puto enriched with 0.5, 1, 2% of *Chlorella* sp. has total carotenoid pigments varied from 5.59 – 18.06 µg g^{-1}. In general, humans need carotenoids in their diets of between 10 - 20 mg daily, either from b-carotene, lutein, lycopene, a-carotene, or b-cryptoxanthin (O'Neill et al., 2001; Bohn, 2012). Hence, a puto may supply 10-20% of a person's daily carotenoid requirement.

Conclusion

Microalgae offers the opportunity to create bioactive ingredients for the food industry. Natural colorants may be incorporated into a wide variety of foods by using microalgae sources. In this study, 2% of *Chlorella* sp. did not meet the sensory expectations of the panelists due to the slightly seaweedy smell and fishy taste. Thus, higher concentrations of *Chlorella* sp. may lead to an undesirable preference among consumers. However, 0.5% and 1% *Chlorella* sp. biomass had been found to have potential as a colorant for the Filipino delicacy Puto. Hence, incorporating lower concentrations of natural colorant microalga *Chlorella* sp. into Puto is beneficial for human health, as it contains pigments like chlorophyll *a* and carotenoids that facilitate disease protection and immunity enhancement (Liu and Hu, 2013). Consequently, it has been found that *Chlorella* sp. is a potential healthy natural food coloring agent for daily products such as Puto. However, further investigation needs to be done for optimization of the *Chlorella* sp. in the Filipino delicacy Puto.

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