Volume IV, No. 1: 31–38 (2020)
ISSN 2507-9638

THE CLSU INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLOGY
www.clsu-ijst.org

Microalgal Pigments Carotenoids and Chlorophyll as Potential Natural Colorants to Cakes

Wan Jing Soh[1], Sze Hui Jong[*1], Soo Yee Peng[1], and Hazel Monica Matias-Peralta[2]

[1]Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, UTHM Pagoh Campus, Pagoh Higher Education Hub, KM 1, Jalan Panchor, 84600 Muar, Johor, Malaysia.
[2]College of Fisheries, Freshwater Aquaculture Center, Central Luzon State University, Science City of Munoz, 3120 Nueva Ecija, Philippines.

Email for correspondence: jongszehui@gmail.com

Submitted November 25, 2019, Accepted June 2, 2020, Published online July 31, 2020.

Abstract

The study was conducted to determine the suitability of carotenoid and chlorophyll pigments of the freshwater microalgae, *Scenedesmus* sp. as natural cake colorants to replace synthetic ones. First, the pigments were extracted from microalgal powder to be used in evaluating its concentration and also its antioxidant activities by DPPH assay. The microalgal powder and synthetic colorant were incorporated into two different cakes. Both cakes were used to perform color analysis and sensory test to assess the consumer acceptance towards the enhanced microalgal butter cake. The results showed microalgal powder contained 4.16 ± 0.012 mg/g of chlorophyll a, 3.30 ± 0.005 mg/g of chlorophyll b, and 0.40 ± 0.003 mg/g of carotenoids. Screening of antioxidant activities through DPPH assay showed lower antioxidant activities (10.93 ± 0.59 %) of the microalgal extract than commercial antioxidant (51.79 ±14.2%). However, the color analysis proved that microalgal powder provided a more intense (p<0.05) green coloration (>L*: -a*: >b*) to the cake as compared to synthetic colorant. Sensory test also depicted no significant difference (p>0.05) on the preference by the panelists between a normal cake with synthetic color or cake with microalgal powder as a colorant. The result of this study revealed a good potential of using microalgal powder as colorant for cake and pastry preparation.

Key Words: carotenoids, chlorophyll, colorant, microalgae, *Scenedesmus* sp.

Introduction

Food colorant is a food additive which gives the respective color to foods and beverages. It is found in the form of dye, powder, or liquid and is necessary to be included as one of the additives in commercial food products to impart color to the food, to compensate the color loss during the process, to enhance the naturally occurring color of food products, and to ensure the color uniformity within different batches of same products (Amchova et al., 2015). Food colorants can be derived from natural sources or synthetically made (Zahra et al., 2015). In fact, most of food industry tend to choose synthetic colorants instead of natural since they are cost-effective with higher stability and tinctorial power as compared to the latter when they are incorporated

DOI: https://doi.org/10.22137/ijst.2020.v4n1.03

in foods (Oplatowska-Stachowiak & Elliott, 2015). However, the use of synthetic colorant in commercial food is controversial due to the possible hazardous effect that cannot be underestimated. Previous studies have shown that toxic substances might be generated from the reaction between the food and synthetic colorant which triggered allergic reactions, inflammation, or even cancer in certain people (Bachalla, 2016). It was also reported that synthetic colorant may cause toxicological effect in humans after long-term use (Ghany, 2015).

Considering the negative implication promoted by uncontrolled consumption of synthetic colorant, the use of natural colorant as a substitute is encouraged since it has less negative effect on human health and the environment (Ghany, 2015). Natural colorant can be derived from plants, microorganisms, and also algae. In the past decade, different species of microalgae became the alternative sources of natural colorants due to the pigments present such as chlorophyll, carotenoids, and phycobiliproteins (Gouveia et al., 2008). The microalgae-based food has been used to enhance palatability, nutritional properties, as well as storage stability (Gutierrez, 2014). Aside from being a colorant to food, these pigments possess anti-carcinogenic, anti-inflammatory, anti-fungal, antioxidant, and anticancer properties (Kuczynska et al., 2015). The antioxidant activities of pigments might also help in reducing the lipid oxidation in food products (Akbarirad et al., 2016). A previous study showed that microalgae are more appropriately used in pigment extraction since it is easier for largescale production of microalgal biomass and the pigments are derived from a renewable and sustainable source (Ornelas-Soto et al., 2020). Therefore, this study was conducted to investigate the suitability of using carotenoids and chlorophyll pigments derived from *Scenedesmus* sp. powder as a natural colorant to replace the use of synthetic ones.

Materials and Methods

Pigment Extraction and Quantification

The extraction method was adapted from Ishaq et al. (2015) with some modification. In this method, 0.75 g microalgal powder was put in a test tube and added with 2 ml to 3 ml of 100% aqueous acetone solution. Next, the solution was macerated using Teflon grinder for five minutes. The sample was then transferred to a screw-cap centrifuge tube while the remaining solution was rinsed with a few millilitres of 100% aqueous acetone and added with the rest of the extraction slurry. Next, 100% aqueous acetone was added until the total volume of the solution reached 10 ml. The samples were then steeped for 2 hours at 4 degree Celsius inside the dark room. After that, centrifugation was done in closed tubes for 20 minutes at 3000 rpm to clear the solution. The clarified extract was put into a clean, calibrated, 15-ml screw-cap centrifuge tube before quantification. Dilution was made on the extract with a dilution factor of 10-1. The diluted extract was measure using UV-Visible spectrophotometer (T60U, PG Instruments, UK) at the appropriate wavelength and pigment concentration was quantified accordingly (Table 1).

Table 1

Empirical Formulae for the Quantification of Microalgal Pigments (Dere et al., 1998)

Type of pigments	Formulae (mg/L)	
$C_{chlorophyll\text{-}a}$ (C_a)	$11.75 \cdot A_{662} - 2.35 \cdot A_{645}$	(1)
$C_{chlorophyll\text{-}b}$ (C_b)	$18.61 \cdot A_{645} - 3.96 \cdot A_{662}$	(2)
$C_{carotenoids}$ (C_{x+c})	$(1000 \cdot A_{470} - 2.270 \cdot C_a - 81.4 \cdot C_b)/227$	(3)

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The DPPH assay was performed based on a procedure as described previously (Katalinic et al., 2006) with some modification. The sample extract was prepared at a concentration of 1 mg/ml through dilution of extract using acetone. Next, 77 µl of diluted sample extract was mixed with 3 ml of 6 x 10-5 M methanolic DPPH solution in the test tube and allowed to stand for 15 minutes at room temperature inside the dark room. The mixture was then transferred to a cuvette and the absorbance value was taken at 515 nm using UV-visible spectrophotometer (T60U, PG Instruments, UK). The absorbance value of methanolic DPPH solution without sample extract served as control. All the tests were performed in triplicate. The result was expressed in percentage inhibition (I%) which was calculated using the following equation (Uma et al., 2011):

$$I\% = \frac{\text{Abs (control)-Abs (sample)}}{\text{Abs (control)}} \times 100 \quad (4)$$

Where:
Abs (control) = absorbance value of the control (methanolic DPPH solution)
Abs (sample) = absorbance value of sample.

Preparation of Butter Cake

The method of making butter cake was adapted from Marina et al. (2016) with slight modification. The ingredients required were cake flour (110 g), self-rising flour (110 g), baking powder (3 g), butter (150 g), whole eggs (150 g), sugar (100 g), and milk (125 ml). The mixture was prepared by creaming butter with sugar with an electric mixer (MK-GB1WTZ, Panasonic, Japan) for 8 minutes. Then, the rest of the wet ingredients were added slowly under constant mixing. The other dry ingredients were also added while creaming on medium. Next, 3.0 g of synthetic food coloring powder was added to the batter and mixed well. The batter was poured into cake pans and baked in the electric oven (EEO-A2815(SV), Elba, Malaysia) for approximately 45 minutes at 180 degree Celsius. The procedure was repeated this time adding the microalgal powder to replace the synthetic food coloring powder as food colorant during mixing.

Color Analysis of the Butter Cake

The color of cake from each sample were evaluated using a color spectrophotometer (EZ 4500L, HunterLab, USA) (Hafez, 2012). Standardization and calibration were done before using the instrument. The cake sample were taken from the center of each cake and put inside the petri dish. The readings were taken and tabulated. The parameters used to evaluate color was L (lightness), a (greenness and redness), and b (yellowness and blueness).

Sensory Test

The affective test was carried out to indicate consumer acceptance towards the enhanced microalgal butter cake (Singh-Ackbarali & Maharaj, 2014). In this test, the panelists involved were 75 students from Universiti Tun Hussein Onn Malaysia. Enhanced microalgal butter cake and ordinary butter cake were used as samples. The samples were labelled with three-digit code before presenting to the panelists. Acceptance test was carried out using a hedonic scale from 1–9 to evaluate the degree of liking towards the color, aroma, taste, texture, and overall acceptability of both cakes. The 9-point hedonic scale represents: 9 – like very much; 8 – like much; 7 – like moderately; 6 – like slightly; 5 – neither like nor dislike; 4 – dislike slightly; 3 – dislike moderately; 2 – dislike much; 1 – dislike very much. The data collected were analyzed using t-tests on means for two different samples (Lawless & Heymann, 2010). T-tests were done to compare the results of acceptance test of both cakes, with significant difference at $p<0.05$.

Statistical Analysis

Three replicates were required for all measurements to be used in statistical analysis (except for sensory test with n=75), and the values were reported as mean ± the standard deviation (SD) (n=3). The obtained data were analyzed using t-tests. Statistical analysis was performed using Microsoft Office Excel 2013.

Results and Discussion

Solvent extraction with mechanical disruption were used to isolate the carotenoids and chlorophyll pigments from the *Scenedesmus* sp. powder as this species may contain rigid cell walls which are highly resistant to disruption (Kendir & Ugurlu, 2018). The use of mechanical grinding (using tissue grinder) enables the cell wall to be fully disrupted in order to release the pigments from microalgal powder as well as improve the extraction efficiency and the amount of extracted pigments (Baldev et al., 2014). Freeze-dried form of microalgal powder was used in the extraction since it may improve the recovery of polar and non-polar pigments as well as prevent the degradation of chlorophyll to form chlorophyll derivatives like pheopigment due to the presence of chlorophyllase and the presence of water (Safafar et al., 2015). Absolute (100%) acetone was used in extraction to prevent product degradation (Hosikian et al., 2010). *Scenedesmus* sp. possessed a higher amount of chlorophyll pigments than carotenoids, which gave the green appearance to the microalgae itself (Table 2). Moreover, these highly valuable metabolites help to prevent oxidation, microbial growth, and also act as coloring agent in certain food (Ishaq et al., 2016).

Table 2

Concentration of Chlorophylls and Carotenoids in Scenedesmus *sp.*

Type of pigment	Concentration (mg/g)
Chlorophyll a	4.16 ± 0.012
Chlorophyll b	3.30 ± 0.005
Carotenoids	0.40 ± 0.003

Values are presented as mean ± standard deviation.

DPPH assay is used to evaluate the sample capacity to scavenge free radicals since the presence of antioxidant compound in the sample may reduce the DPPH radical in methanolic solution, causing color change from violet to yellow (Abdelazim et al., 2013). This assay can determine the antioxidant capacity which involves single electron transfer (SET) mechanism and hydrogen atom transfer (HAT) mechanism (Liang & Kitts, 2014).

The microalgal extract (1 mg/ml) exerted a percentage inhibition of 10.93 ± 0.59 %, which was considered low in terms of antioxidant capacity. Although the microalgal pigments might contribute to the antioxidant activity, these pigments were added in a very low amount (Table 2). Thus, they may not have served efficiently in promoting antioxidant activity. Likewise, the green color of the extract may have possibly cause interference to DPPH absorption which affected the accuracy of measured antioxidant activity (Liang & Kitts, 2014). In fact, previous study also showed that natural chlorophyll a and chlorophyll b depicted lower antioxidant activity (Mishra et al., 2011).

The result of the sensory test showed that majority of consumers prefer natural coloring agents due to the potential toxic, carcinogenic, and mutagenic effect of using synthetic colorant to humans (Amchova et al., 2015). There was enough evidences proving that certain valuable bioactive compounds in *Scenedesmus*

sp. can be utilized as food colorants in food products, for instance, beta carotene and chlorophylls (Ishaq et al., 2016). In a color analysis, the L* indicates lightness, a* represents redness (positive a* value) and greenness (negative a* value), while b* indicates yellowness (positive b* value) and blueness (negative b* value). The color of cakes was affected by the types of colorant used. The microalgal cake was significantly (p<0.05) different from the synthetic cake coloring for its lightness, greenness, and yellowness. The butter cake with microalgal powder as colorant provided a darker and greener color than the butter cake with synthetic food color since microalgal cake had lower L* and -a* value (Table 3). The microalgal powder was naturally dark green in color, contributing to the greener coloration of the cake. Besides, oxidation of microalgal pigments, browning reaction due to caramelization of sucrose, as well as Maillard reaction that happened during baking may have increased the intensity of color in the microalgal cake preparation (Ghanbari & Habibi, 2015; Mohamad et al., 2015). In short, it was inferred that the microalgal powder could really be used as a natural colorant to food products and it gave a greener appearance to the cake in a natural way as compared to the synthetic food color.

Table 3

L, a* and b* Value of the Butter Cakes*

Color	Butter cake with microalgae powder	Butter cake with synthetic food color
L*	52.36 ± 0.44	62.03 ± 0.68
a*	-1.53 ± 0.06	0.03 ± 0.06
b*	30.63 ± 0.41	34.44 ± 0.08

Values are presented as mean ± standard deviation.

An acceptance test was performed on 75 panelists among the students of Universiti Tun Hussein Onn Malaysia to determine the degree of liking over the enhanced butter cake added with microalgal powder and control butter cake added with synthetic colorant for its color, aroma, flavor, texture, and overall acceptability. According to the panelists, the aroma and overall acceptability of the butter cake with microalgal powder were comparable to the butter cake with synthetic colorant (Table 4). The addition of microalgal powder enhanced the appearance by giving the butter cake a dark green color, as well as the flavor of the cake since it gave a matcha-like taste to the cake. Furthermore, the butter cake with microalgal powder was moister than the butter cake with synthetic colorant. This also gave the microalgal cake a softer texture as compared to normal butter cake. Overall, there was no significant difference (p<0.05) between the color, aroma, taste, texture, and overall acceptability of butter cake with microalgal powder and normal butter cake (Table 4). Thus, we could infer that both cakes were well accepted by the consumers.

Identification of synthetic food colors adulteration by paper chromatography and spectrophotometric methods. Sensory score of butter cake with microalgal powder and butter cake with synthetic colorant

Table 4

Sensory Score of Butter Cake with Microalgal Powder and Butter Cake with Synthetic Colorant

Attributes	Butter Cake with Microalgal Powder	Butter Cake with Synthetic colorant
Color	6.72 ± 1.48	6.32 ± 1.70
Aroma	6.41 ± 1.63	6.83 ± 1.42
Flavor	6.91 ± 1.50	6.79 ± 1.53
Texture	6.99 ± 1.61	6.95 ± 1.58
Overall acceptability	7.20 ± 1.51	7.29 ± 1.39

Values are presented as mean ± standard deviation.

Conclusion

The results indicated that *Scenedesmus* sp. could be used as natural colorants to food products since it possessed chlorophyll and carotenoids pigments. Besides, the microalgal powder provided more intense coloration and a moister and softer texture to the cake. In addition, the microalgal powder showed antioxidant activity in DPPH assay although the percentage inhibition was considered lower compared to the synthetic antioxidant. Furthermore, the sensory test revealed that the butter cake with microalgal powder was accepted by the panelists as much as the ordinary butter cake. Further work is required to determine the market price to be appropriated when using microalgal powder as a natural colorant to cakes and pastries since it is expected to be more expensive in terms of production than synthetic food color.

Acknowledgements

The authors would like to acknowledge FRGS grant (Vot 4176) and the Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM) for the use of laboratory facilities and technical assistance.

References

Abdelazim, A. A., Mahmoud, A., & Ramadan-Hassanien, M. F. (2013). Oxidative stability of vegetable oils as affected by sesame extracts during accelerated oxidative storage. *Journal of Food Science and Technology*, *50*(5), 868–878. https://doi.org/10.1007/s13197-011-0419-8

Akbarirad, H., Gohari Ardabili, A., Kazemeini, S. M., & Mousavi Khaneghah, A. (2016). An overview on some of important sources of natural antioxidants. *International Food Research Journal*, *23*(3), 928–933.

Amchova, P., Kotolova, H., & Ruda-Kucerova, J. (2015). Health safety issues of synthetic food colorants. *Regulatory Toxicology and Pharmacology*, *73*(3), 914–922. https://doi.org/10.1016/j.yrtph.2015.09.026

Bachalla, N. (2016). Identification of synthetic food colors adulteration by paper chromatography and spectrophotometric methods. *International Archives of Integrated Medicine*, *3*(6), 182–191. http://iaimjournal.com/

Baldev, E., Mubarakali, D., Dhivya, M., Kanimozhi, M., Shakena-Fathima, T., Alharbi, N. S., Arunachalam, C., Alharbi, S. A., & Thajuddin, N. (2014). Facile and novel strategy for methods of extraction of biofuel grade lipids from microalgae—An experimental report. *International Journal of Biotechnology for Wellness Industries*, *3*, 121–127. https://doi.org/10.6000/1927-3037.2014.03.04.2

Dere, Ş., Güneş, T., & Sivaci, R. (1998). Spectrophotometric determination of Chlorophyll-A, B, and total carotenoid contents of some algae species using different solvents. *Turkish Journal of Botany*, *22*, 13–17.

Ghanbari, S., & Habibi, N. (2015). Study of texture and color of the sponge cake produced using the different levels of soy flour. *Indian Journal of Fundamental and Applied Life Sciences*, *5*(4), 1084–1089. www.cibtech.org/sp.ed/jls/2015/04/jls.htm

Ghany, T. M. A. (2015). Safe food additives: A review by biological and chemical research. *Journal of Biological and Chemical Research*, *32*(1), 402–437.

Gouveia, L., Batista, A. P., Sousa, I., Raymundo, A., & Bandarra, N. M. (2008). Microalgae in novel food product. In K.N. Papadopoulos. *Food chemistry resarch developments* (75–112). Nova Science Publishers, Inc.

Gutierrez, A. M. (2014). *Effects of lipid oxidation initiators and antioxidants on the total antioxidant capacity of milk and oxidation products during storage*. [Master's thesis, Iowa State University, Ames, United States]. Retrieved from https://lib.dr.iastate.edu/

Hosikian, A., Lim, S., Halim, R., & Danquah, M. K. (2010). Chlorophyll extraction from microalgae: A review on the process engineering aspects. *International Journal of Chemical Engineering*, 1–11. https://doi.org/10.1155/2010/391632

Ishaq, A. G., Matias-Peralta, H. M., & Basri, H. (2016). Bioactive compounds from green microalga – Scenedesmus and its potential applications: A brief review. *Pertanika Journal of Tropical Agricultural Science*, *39*(1), 1-16.

Ishaq, A. G., Matias-Peralta, H. M., Basri, H., & Muhammad, M. N. (2015). Antibacterial activity of freshwater microalga Scenedesmus sp. on foodborne pathogens Staphylococcus aureus and Salmonella sp. *Journal of Science and Technology*, *7*(2), 858–871.

Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*, *94*(4), 550–557. https://doi.org/10.1016/j.foodchem.2004.12.004

Kendir, E., & Ugurlu, A. (2018). A comprehensive review on pretreatment of microalgae for biogas production. *International Journal of Energy Research*, *42*(12), 3711–3731. https://doi.org/10.1002/er.4100

Kuczynska, P., Jemiola-Rzeminska, M., & Strzalka, K. (2015). Photosynthetic pigments in diatoms. *Marine Drugs*, *13*(9), 5847–5881. https://doi.org/10.3390/md13095847

Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food.* (2nd ed.). Springer Science & Business Media, LLC. https://doi.org/10.1007/978-1-4419-6488-5_1

Liang, N., & Kitts, D. D. (2014). Antioxidant property of coffee components: Assessment of methods that define mechanism of action. *Molecules*, *19*(11), 19180–19208. https://doi.org/10.3390/molecules191119180

Marina, A. M., Nurhanan, A. R., Rosli, W., & Nurul Ain, O. (2016). Physical properties and microstructure of butter cake added with Persea americana puree (Sifat Fizikal dan Mikrostruktur Kek Mentega yang Ditambah dengan Puri Persea americana). *Sains Malaysiana, 45*(7), 1105-1111.

Mishra, V. K., Bachheti, R. K., & Husen, A. (2011). Medicinal uses of chlorophyll: A critical overview. In H. Le & E. Salcedo (Eds.), *Chlorophyll: Structure, function and medicinal uses.* (pp. 177–196). Nova Science Publishers, Inc.

Mohamad, R. A., Taip, F. S., Mazlina, S., Kamal, M., & Bejo, S. K. (2015). Color and volume development of cake baking and its influence on cake qualities. *Journal of Applied Science and Agriculture*, *10*(5), 63–68. www.aensiweb.com/JASA

Oplatowska-Stachowiak, M., & Elliott, C. (2015). Food colours: Existing and emerging food safety concerns. *Critical Reviews in Food Science and Nutrition*, *57*(3), 524–548. https://doi.org/10.1080/10408398.2014.889652

Ornelas-Soto, N., Orona Navar, A., López Luke, T., Pacheco, A., & Aguilar-Hernández, I. (2020). Dye Sensitized Solar Cell (DSSC) by using a natural pigment from microalgae. *International Journal of Chemical Engineering and Applications*, *11*(1), 14–17. https://doi.org/10.18178/ijcea.2020.11.1.772

Safafar, H., Wagenen, J. Van, Møller, P., & Jacobsen, C. (2015). Carotenoids, phenolic compounds, and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Marine Drugs*, *13*(12), 7339–7356. https://doi.org/10.3390/md13127069

Singh-Ackbarali, D., & Maharaj, R. (2014). Sensory evaluation as a tool in determining acceptability of innovative products developed by undergraduate students in Food Science and Technology at the University of Trinidad and Tobago. *Journal of Curriculum and Teaching*, *3*(1), 10–27. https://doi.org/10.5430/jct.v3n1p10

Uma, R., Sivasubramanian, V., & Devaraj, S. N. (2011). Evaluation of in vitro antioxidant activities and antiproliferative activity of green microalgae, *Desmococcus olivaceous* and *Chlorococcum humicola*. *Journal of Algal Biomass Utilization*, *2*(3), 82–93.

Zahra, N., Fatima, Z., Kalim, I., & Saeed, K. (2015). Identification of synthetic food dyes in beverages by thin layer chromatography. *Pakistan Journal of Food Sciences*, *25*(4), 178-181.