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Determination of natural carotenoid pigments from freshwater green algae as potential halal food colorants

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Abstract

It is noted that nowadays, halal products are gaining wider recognition as a new benchmark for safety and quality assurance. As a consequence of these additional pigment needs, the demand in isolated natural colorants has increased as compared with synthetic dyes. The aim of the research is to explore new sources of pigments to be used as halal food colorants. This quest is not only directed in finding natural alternatives for synthetic dyes, but also with the aim to discover new taxons for the pigment production, for instance from microalgae. Therefore, a total of six freshwater algae species were evaluated quantitative and qualitatively using HPLC for carotenoids pigment. Three main carotenoids were identified in *Chlorella fusca, Chlorella vulgaris, Selenastrum capricornutum, Pandorina morum, Botryococcus sudeticus* and *Chlorococcum* sp. which are lutein, β-cryptoxanthin and β-carotene. The ratio of these carotenoids varies between species where lutein was detected substantially higher in *Chlorella fusca* (69.54±11.29µg/g DW); β-cryptoxanthin in *Pandorina morum* (1.24±0.33 µg/g DW) whereas β-carotene in *Chlorella vulgaris* (18.42±9.2 ug/g DW). The significant outcome of the research will be new findings of new natural carotenoid pigment sources as potential food colorants and bioactive compounds which can be beneficial to halal health promoting products industry, food products and dye technology which covers not only the Shariah requirement, but also the hygiene, purification and safety aspects.

Introduction

The market for certified halal food and products is growing robustly, both domestically and internationally (Zakaria and Buang, 2004). The uses of synthetic food colorant such as sudan I, II and III as food colorant had raise health issue and in fact the uses of these types of food colorant had been banned in certain country. International Agency for research on cancer had declared that these type of colorant as category 3 carcinogen which means that it can make some alteration to human gene and hence stimulate the growth of cancerous cell. Regardless to that their consumption is not shariah compliant for Muslim consumer.

Plants produce biochemical that are of importance in the healthcare, food, flavour and cosmetics industries. The extraction of photosynthetic pigments from plants can be used as natural food colorant, an alternative for the harmful synthetic food colorant. Currently, this type of biochemical and many other natural products are produced solely from massive quantities of whole parts of plant. Recent advances in molecular biology, enzymology, physiology and fermentation technology of plant cell cultures suggest that this systems will become a viable source of important natural products (Dicosmo *et al.*, 1995). Plant pigments are labile: they can be easily altered and even destroyed on the basis of their chemical structures. Pigments can be classed into four families, i.e. tetrapyrroles (e.g. chlorophyll), carotenoids (e.g. beta-carotene), polyphenolic compounds (e.g. anthocyanins), and alkaloids (e.g. betalains) (Schoefs, 2004). Colorant from plants commonly is made of carotenoids and anthocyanin. Carotenoids are responsible for the orange and yellow lipid soluble pigments in plastids.

Green algae (chlorophyta) is known to benefit in human dietary as they have high nutritional values that related to its composition of biochemical which contain source of proteins, carbohydrates, lipids and vitamins. Recently, studies found that microalgae pigments can be used as an alternative ingredients in food industries (Christaki *et al.*, 2015) such as *Chlorella vurgaris* has been found that having collagen properties (Koller *et al.,* 2014). In 1997, 2400 t microalgal biomass were produced and commercialized as “health food” in Japan and

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indicates that is trending as a fashionable "health food" (Plaza *et al.*, 2008).

Antioxidant that is very crucial in fighting against free radical in our body also can be extracted from algae. That antioxidant is came from the microalgae photosynthetic pigments which is name as carotenoid (Zaho *et al.*, 2004; Plaza *et al.*, 2009; Goh *et al.*, 2010; Hajimahmoodi *et al.*, 2009; Goiris *et al.*, 2012). It is one of the biological constituent that can be extracted from green microalgae due to its characteristic which resemble higher level plants. Membrane bound chloroplast and nuclei presence in this type of green microalgae and carotenoid can be found in that particular organelle. In Israel and Australia, *Dunaliella Salina* was cultured to obtained their Beta carotene (Borowitzka *et al.,* 1990). Studies also had extracted carotenoid from many types of microalgae. Some of the studies uses industrial waste water to culture microalgae and extracted their carotenoid (Rodrigues *et al.*, 2014) and they found that *P. autumnale* having the potential to the production of microalgal carotenoid in agroindustrial wastewater. Therefore, the aim of this study is to explore new sources of pigments to be used as halal food colorants. Six species of freshwater green microalgae (chlorophyta) had been profiled their carotenoid contents in this study which from the *Chlorella fusca, Chlorella vulgaris, Selenastrum, Pandorinamorum* and *Chlorococcum* species. The microalgae were cultured in a sterile optimise condition and the carotenoid content was analyse via high performance liquid chromatography (HPLC).

Materials and methods

Mass production of microalgae cell culture

Microalgae cell culture was incubated in Bold's Basal Medium in a growth room at 24ºC day and night temperature, with a 16-h photoperiod at 80-85 µmol m-2 s-1 under cool white fluorescent light.

Extraction of carotenoid pigments

The extraction procedure followed the methods described by Othman (2009). For each sample, 1.0 g of powdered freeze-dried material was rehydrated by adding 1 ml of distilled water, followed by 5 ml of an acetone. The solution was stand overnight in darkness at room temperature. The following day the samples was vortexed and centrifuged for 2 minutes at 13 500 x g and the supernatant was collected and extracted with hexane. The supernatant transferred into a 50ml centrifuged tube and was dried by Nitrogen Oxide (NOx) gas.

Saponification

20 ul ethyl acetate, 380 ul (Acetone(9) : H_2O(1)) and 400 ul 10% Potassium hydroxide:Methanol was added to the tube. Then, the mixture was allowed to stand overnight before it was extracted with 10% sodium chloride, hexane and 4 ml 10% butylatedhydroxytoluene (Butnariu, 2016). The mixture was centrifuged and the supernatant was collected and dried using NOx. The carotenoid content in the sample then was analysed by the aid of High Performance Liquid Chromatography (HPLC).

Result and discussion

Total carotenoid analysis recorded that *Chlorella vurgaris* contained highest total carotenoid (81.81± 32.60µg/g DW) which is consist of lutein (63.39 ±5.99 µg/g DW) and beta carotene (18.42 ± 5.31 µg/g DW). The least carotenoid content were detected in *Butryococcusseduticus* species (53.96±29.44 µg/g) as listed in Table 1.

The peak of HPLC analysis recorded that three types of carotenoid can be detected and showed the peak of lutein, beta carotene and beta cryptoxanthin. Among all of those carotenoid, lutein was detected in all species of microalgae that been analyse. Figure 1 shows the *Chlorella fusca* recorded containing highest lutein (69.53 ±9.56µg/g) content amongs all species of microalgae. Only two species that been analysed contain beta cryptoxanthin which is *Butryococcusseduticus* (1.99±0.21) and *Pandorinamorum* (2.38 ± 0.32µg/g) shows in Figure 2. Beta carotene in figure 3 was presence in four species of microalgae that been cultured which is *Chlorella fusca* (10.01 ± 1.27µg/g), *Chlorella vurgaris* (18.42 ± 5.31µg/g), *Selenestrum* (13.54 ± 5.36µg/g) and *Pandorinamorum* (2.82 ±1.43µg/g). *Pandorinamorum* showed having the peaks of lutein, beta carotene and beta cryptoxanthin.

In terms of health benefits, lutein is beneficial to delay the onset of the cataract hence reducing the risk of cataract occurance (Nwachukwu *et al.*, 2015). According to WHO, they estimated that 285 millions of people in this world suffered from visually-impaired problems hence this finding may overcome this problems (Nwachukwu *et al*., 2015). Studies reported that the consumption of beta carotene may increase the bone mineral density (Chen *et al*., 2015) besides providing cardioprotective effect to reduces the risk of heart disease (Csepanyi *et al*., 2015). In other hands, the anti-proliferative activity of beta cryptoxanthin was discovered via the mitochondrial pathway of apoptosis against the *Adenocarcinoma* Caco-2 cell (Cilla *et al.,* 2015).

Table 1. Distribution of total and individual carotenoid content (µg/g DW) in six species of freshwater green microalgae

Species	Total Carotenoid (µg/g DW)	lutein (µg/g DW)	β-cryptoxanthin (µg/g DW)	β-carotene (µg/g DW)
Chorella fusca	79.55 ±37.58	69.53 ±9.56	Nd	10.01 ± 1.27
Chlorella vurgaris	81.81± 32.60	63.39 ±5.99	Nd	18.42 ± 5.31
Selenestrum	76.22 ±32.97	62.67 ±18.05	Nd	13.54 ± 5.36
Butryococcussudeticus	53.96 ±29.44	51.96 ±3.90	1.99 ±0.21	nd
Pandorinamorum	56.82 ±28.29	51.61 ±5.68	2.38 ± 0.32	2.82 ±1.43
Chlorococcus	62.66 ±36.18	62.66 ±5.83	Nd	nd

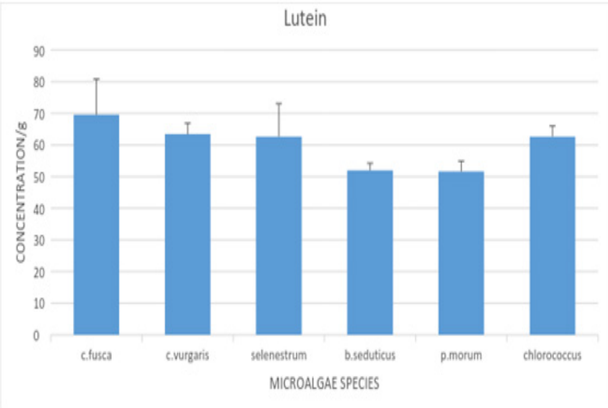

Figure 1. Lutein content among six species of freshwater green microalgae

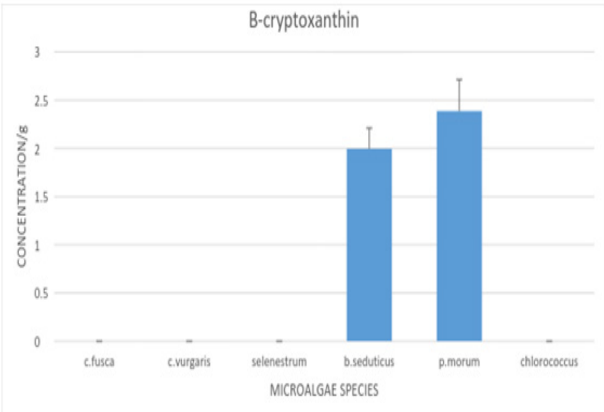

Figure 2. B-cryptoxanthin content among six species of freshwater green microalgae

According to Quran chapter 16: verse 114, "therefore eat of what Allah has given you, lawful and good (things), and give thanks for Allah's favor if him do you serve". Hence, this give the perception that consuming synthetic food colorant is against this Quran verse as give diverse bad side effect to human health. The findings of the natural food colorants from this studies is crucial to Muslim consumer as they seek lawful things to be consume. The natural food colorants from this microalgae not only lawful and halal for them nevertheless maintaining their good health by the benefits of the carotenoid that

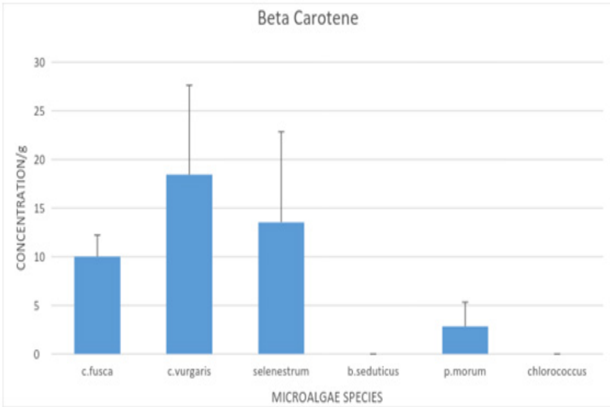

Figure 3. Beta carotene content among six species of freshwater green microalgae

been stated above. Food colorant that been obtained from microalgae in this study halal in the perspective of its sources as well as the method of extraction.

Conclusion

Carotenoid can be extracted from the microalgae and from this study *Chlorella vurgaris* recorded the highest carotenoid content. Moreover, lutein can be obtained from all six species of microalgae and furthermore *Pandorinamorum* contain all 3 types of carotenoid observed which are lutein, beta carotene and also beta cryptoxanthin. The carotenoid that been extracted from the culture of microalgae not only halal but give diverse health benefits to human. Hence, in a nutshell microalgae have the potential as of the sources of the halal food colorant.

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References

Butnariu, M. 2016. Methods of Analysis (Extraction, Separation, Identification and Quantification) of Carotenoids from Natural Products. Journal of Ecosystem and Ecography 6(2).

Borowitzka, M.A., Borowitzka, L.J. and Kessly, D. 1990. Effects of salinity increase on carotenoid accumulation in the green alga *Dunaliella salina*, Journal of Applied Phycology 2: 111–119.

Chen, G., Zhu, Y.-Y., Cao, Y., Liu, J., Shi, W., Liu, Z. and Chen, Y. 2015. Association of dietary consumption and serum levels of vitamin A and β-carotene with bone mineral density in Chinese adults. Bone 79: 110–115. *http://doi.org/10.1016/j.bone.2015.05.028*

Christaki, E., Bonos, E. and Florou-Paneri, P. 2015. Handbook of Marine Microalgae. Handbook of Marine Microalgae. US: Elsevier. *http://doi.org/10.1016/*B978-0-12-800776-1.00014-5

Cilla, A., Attanzio, A., Barberá, R., Tesoriere, L. and Livrea, M. A. 2015. Anti-proliferative effect of main dietary phytosterols and β-cryptoxanthin alone or combined in human colon cancer Caco-2 cells through cytosolic Ca^{+2} – and oxidative stress-induced apoptosis. Journal of Functional Foods 12: 282–293. *http://doi.org/10.1016/j.jff.*2014.12.001

Csepanyi, E., Czompa, A., Haines, D., Lekli, I., Bakondi, E., Balla, G., Tosaki, A. and Bak, I. 2015. Cardiovascular effects of low versus high-dose beta-carotene in a rat model. Pharmacological Research 100: 148–156. *http://doi.org/10.1016/j.phrs*.2015.07.021

DiCosmo, F. and Misawa, M. 1995. Plant cell and tissue culture: alternatives for metabolite production. Biotechnology Advances 13 (3): 425-53.

Fatimah, A.M.Z., Norazianand, M.H. and Rashidi, O. 2012. Identification of carotenoid composition in selected "ulam" or traditional vegetables in Malaysia. International Food Research Journal 19(2): 527–530.

Goh, S.H., Yusoff, F.M. and Loh, S.P.A. 2010. A comparison of the antioxidant properties and total phenolic content in a diatom *Chaetoceros* sp. and a green microalga, *Nannochloropsis* sp. Journal of Agricultural Science 2: 123–130.

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., De Brabanter, J. and De Cooman, L. 2012. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. Journal of Applied Phycology 24 (6): 1477–1486.

Hajimahmoodi, M., Faramarzi, M.A., Mohammadi, N. Soltani, N., Oveisi, M.R. and Nafissi-Varcheh, N. 2009. Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. Journal of Applied Phycology 22: 43–50

Koller, M., Muhr, A. and Braunegg, G. 2014. Microalgae as versatile cellular factories for valued products. Algal Research 6: 52–63. *http://doi.org/10.1016/j.*algal.2014.09.002

Nwachukwu, I.D., Udenigwe, C.C. and Aluko, R.E. 2015. Lutein and zeaxanthin: production technology, bioavailability, mechanisms of action, visual function, and health claim status. Trends in Food Science and Technology. *http://doi.org/10.1016/j.tifs.*2015.12.005

Othman, R. 2009. Biochemistry and genetics of carotenoid composition in potato tubers. New Zealand: Lincoln University, PhD. Dissertation.

Plaza, M., Cifuentes, A. and Ibáñez, E. 2008. In the search of new functional food ingredients from algae. Trends in Food Science and Technology 19 (1): 31–39.

Plaza, M., Herrero, M., Cifuentes, A. and Ibáñez, E. 2009. Innovative natural functional ingredients from microalgae. Journal of Agricultural and Food Chemistry 5: 7159–7170

Rodrigues, D.B., Flores, É.M.M., Barin, J.S., Mercadante, A.Z., Jacob-Lopes, E. and Zepka, L.Q. 2014. Production of carotenoids from microalgae cultivated using agroindustrial wastes. Food Research International 65: 144–148. *http://doi.org/10.1016/j.*foodres.2014.06.037

Schoefs, B. 2004. Determination of pigments in vegetables. Journal of Chromatography A 1054(1-2): 217–226.

Zaho, X., Xue, C.H., Li, Z.J., Cai, Y.P., Liu, H.Y. and Qi, H.T. 2004. Antioxidant and hepatoprotective activities of low molecular weight sulfated polysaccharide from *Laminaria japonica*. Journal of Applied Phycology 16: 111–115

Zakaria Z. and Buang A.H. 2004. Some Assessments on the Adequacy of Regulatory and Supervisory Framework. International Seminar on Halal Food and Products: Challenges and Prospects in the Global Market, 28-29 Sept 2004. Malaysia: Marriott Putrajaya.