ALGAE CAROTENOID PIGMENTS AS NEW SOURCES OF HALAL BIOACTIVE INGREDIENTS

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ABSTRACT

Halal Active Pharmaceutical Ingredient (API) is a substance used in a finished pharmaceutical product, intended to furnish pharmacological activity or contribute direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease. It also provides a direct effect in restoring, correcting or modifying physiological functions in human beings. Microalgae, which are single-celled microorganisms, are considered to be a rich source of diverse bioactive molecules. They play a vital role in the aquatic food chain as primary producers and can store complex organic compounds in their bodies, which can be released with the help of sunlight. Microalgae have evolved distinctive metabolic pathways, resulting in the production of remarkable secondary metabolites and unique structures that differ from those found in superior plants. Due to the high structural diversity of pigments, microalgae have the potential to produce pharmacologically valuable compounds, making them a promising source of bioactive molecules. This study aimed to examine the carotenoids pigmentation profiling from selected microalgae species subjected to different culture conditions. The methods involve microalgae cell culture mass production, carotenoid sample extraction and HPLC analysis. The findings revealed that the composition and amount of carotenoids differ according to the microalgae species. Genetic factors appear to be a crucial factor in categorizing these species based on their individual carotenoids accumulation. In short, selecting the appropriate species with the ability to accumulate carotenoids is important in determining the sources of pigments for halal bioactive ingredients or for commercial purposes, particularly in the halal market.

Keywords: *Halal Bioactive Ingredient, Halal Market, Halal Pigment, Halal Product, Microalgae*

1. Introduction

Microalgae, which are single-celled microorganisms, are considered to be a rich source of diverse bioactive molecules. They play a vital role in the aquatic food chain as primary producers and can store complex organic compounds in their bodies, which can be released with the help of sunlight (Chacon-Lee & Gonzalez-Marino, 2010; Batista et al., 2013). Microalgae have evolved distinctive metabolic pathways, resulting in the production of remarkable secondary metabolites and unique structures that differ from those found in superior plants. Due to the high structural diversity of pigments, microalgae have the potential to produce pharmacologically valuable compounds, making them a promising source of bioactive molecules (Rodrigues et al., 2015).

Some of these microorganisms produce two families of pigments, namely tetrapyrroles and carotenoids. Cyanobacteria, for example, contain closed tetrapyrroles such as chlorophyll a, and β-carotene, as well as two types of accessory pigments: open tetrapyrroles (phycobilin) and glycosylated xanthophylls (Chen & Blankenship, 2011). Because the compounds derived from microalgae are structurally and bioactively

DOI: https://doi.org/10.59202/jhst/v2i1.669

intriguing, research on microalgae bioproducts has been gaining attention from chemists as a challenging research topic (Heydarizadeh et al., 2013; Zhou & Guo, 2012). Researchers are investigating the potential of different species, which may differ in carotenoid composition, to discover new bioactive compounds (Aluc, 2022).

2. Literature Review

2.1 Natural Pigments

Many studies have been done and identified that fruits and vegetables are the primary sources of natural pigments that can be found in various parts of the plant, such as leaves, stalk, seeds, roots, and flowers. These pigments include carotenoids, myoglobin, chlorophyll, and anthocyanins (Shrikant et al., 2020). Recently scientists discovered microalgae as a source of many different bioactive compounds, including carotenoids, lipids, fatty acids, hydrocarbons, proteins, carbohydrates, and amino acids; meanwhile, carotenoids from algae have achieved commercial recognition in the global market for food and cosmeceutical uses (Ambati et al., 2019).

The two primary categories of pigments responsible for the hues in plants are carotenoids and anthocyanins. Anthocyanins are responsible for the water-soluble, vacuolar pink, red, purple, and blue pigments found in coloured plant pigments, while carotenoids are accountable for the orange and yellow lipid-soluble pigments found in plastids (Van den Berg et al., 2000). Besides, pigments are responsible for the inherent colour of an object or material from natural or synthetic. According to Amchova et al. (2015), natural colourants are typically extracted and concentrated using organic solvents for pigments that are lipophilic and water or lower alcohols for pigments that are water-soluble. However, the law imposes restrictions on permissible colourants, the sources from which they can be derived, the solvents that can be used to extract them, and the quality of the pigment. Despite this, the usage of natural colouring is relatively low due to several constraints, including low heat, light, and pH stability, as well as low tolerance for acidity and high temperatures. Natural food colouring can also quickly fade in the presence of light (Sezgin & Ayyilidz, 2017). Perez-Galvez et al. (2020) suggested that naturally derived colourants are typically less stable to heat, light, and oxygen and may react with other ingredients, causing undesirable colours and flavours to emerge despite their higher manufacturing cost compared to artificial colouring. However, Fernandez-Lopez et al. (2020) noted that genetic modification has been used to increase the concentration of natural colourants in plants, and there is a growing interest in using these procedures to increase the plants' colourant production yield and to find more suitable applications for use in food technology treatments aimed at stabilizing these colourants.

The market now offers natural food colourants that can be practically used in food and other product such as cosmetics and pharmaceuticals. Successful tests have been conducted using natural colourants such as carotenoids and anthocyanins in single-phase colouring systems, such as solid baked goods and liquid beverages (Lin et al., 2016). Natural colours have always played a vital role in our diet, with compounds like chlorophyll, carotenoids, and anthocyanins being consumed daily through the food we eat (Griffiths, 2005). Regulations permit the use of natural colourants such as betaines, quinones, flavonoids, isoprenoids, annatto, red pepper extract, lutein, canthaxanthin, porphyrins, chlorophyllin, copper complexes, caramels, and curcumin (Janiszevska-Turak et al., 2016). Among natural pigments, anthocyanins, carotenoids, phycobiliproteins, betalains, and chlorophylls remain the most widely used in the food industry, even though many other natural pigments have been tested (Luzardo-Ocampo et al., 2021). Pigments

can be classified into four families based on their chemical structure: tetrapyrroles, carotenoids, polyphenolic compounds, and alkaloids, with chlorophyll, beta-carotene, anthocyanins, and betalains being examples of each family, respectively (Schoefs, 2004). Colour additives can be utilized for various purposes such as standardizing raw ingredient colours, giving colour identities to colourless foods, and substituting colour loss during processing or storage (Ronald and Catherine, 2012). Therefore, they can be used for specific purposes based on their colour.

Flavonoids known as anthocyanins are soluble in water and display pH-dependent colours that range from red to blue, and they possess bioactive properties such as antioxidant, anti-inflammatory, hypoglycaemic, and chemo-preventive effects (Chung et al., 2016). These anthocyanins can be found in various foods, including beverages, desserts, ice cream, and dairy products, and are susceptible to a range of pH-dependent colour gradients (Cortez et al., 2017; Meija et al., 2020). Fruits and vegetables are the primary sources of carotenoids, which are known for their red, orange, and yellow colours and contribute to the flavour of food and beverages (Rodriguez, 2019). The yellow, orange, and red shades of carotenoids provide additional health benefits, such as lycopene, a bioactive, red-coloured pigment found in red fruits that possesses antioxidant properties and has been linked to a lower risk of cancer, cardiovascular disease, and diabetes (Eletr et al., 2017).

2.2 Carotenoid and microalgae

Carotenoids are a group of pigments that are lipid-soluble and can be found in various organisms such as plants, algae, bacteria, fungi, and animals. They have different colours ranging from yellow to red and are essential for the health and growth of organisms (Tanaka et al., 2008; Ronald and Catherine, 2012). Unlike animals, plants and other organisms can synthesize carotenoids, and animals obtain them from their diet. Common sources of carotenoids include annatto, paprika, saffron, caramel, chlorophyll, and turmeric. Carotenoids are classified into two groups based on the presence or absence of an oxygen group in their carbon chain: xanthophylls (with oxygen group) such as lutein and zeaxanthin, and carotenes (without oxygen group) such as α-carotene, β-carotene, and lycopene. Carotenoids are composed of eight units of isoprenoids linked together in a symmetrical and linear molecule. The extraction of carotenoids from natural sources involves several pre-treatment steps, and organic solvents are used due to their hydrophobicity. However, the basic cyclic structure of carotenoids is susceptible to changes due to various factors such as hydrogenation, dehydrogenation, cyclization, and oxidation. Their conjugated double-bond structure makes them reactive, and they can be easily isomerized and oxidized (Meija et al., 2020; Oliver and Palou, 2000).

Carotenoids can serve as vitamin supplements since carotene is a precursor of vitamin A, which helps prevent night blindness caused by a vitamin A deficiency (Okafor et al., 2016). In addition to its role in growth and development, immunity, and vision, vitamin A is also a carotenoid with antioxidant and anti-tumour properties that protect against free radicals and singlet oxygen (Malendez-Martinez, 2019; Zeb and Mehmood, 2004). Certain carotenoids like lutein and zeaxanthin are stored in the retina and human lens, which can improve cognitive function in elderly individuals (Hammond et al., 2017; Thane and Reddy, 1997). Under stress conditions, microalgae can produce carotene, while *Haematococcus pluvialis* can produce astaxanthin (Jacob-Lopez et al., 2020). Fucoxanthin, one of the most abundant carotenoids, is primarily extracted from brown microalgae such as *Undaria, Sargassum, Laminaria, Eisenia, Alaria, Cystoseira,* and *Hijikia*, and possesses

photoprotective, anti-obesity, anti-inflammatory, neuroprotective, anti-diabetic, and antioxidant properties when added to food (Lourenco-Lopez et al., 2020).

Carotenoids have been deemed safe by regulatory agencies, including the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), and have been designated as Generally Recognized as Safe (GRAS). However, certain carotenoids have been recommended for acceptable daily intakes, such as lutein (1 mg/kg BW/day), lycopene (0.5 mg/kg BW/day), zeaxanthin (0.75 mg/kg BW/day), α-carotene and β-carotene (15 mg/kg BW/day), bixin (6 mg/kg BW/day), and norbixin (0.4 mg/kg BW/day) (Malendez-Martinez, 2019). Carotenoids are commonly used in meat products, vegetable oils, butter, and dairy products as colourants. However, their utilization as additives and functional ingredients is difficult due to their water insolubility, instability, and low bioavailability. Nevertheless, alternatives such as carotenoid delivery in water-dispersible products, colloidal suspensions, emulsions, and colloidal dispersions have been developed to overcome these limitations (Rodriguez Amaya, 2016).

2.3 Prospects of Carotenoid Derivatives as New and Halal Colorants Agent

Scientists, engineers, and investors are exploring microalgae biomass, insects, and mycoprotein as future food sources to tackle malnutrition. Habib et al. (2008) suggested that future foods should have high protein content, low reliance on chemicals, such as fertilizers and hormones, and a low carbon footprint. It is expected that future food will be in the form of powders, tablets, and capsules made from microalgae biomass. In recent years, there has been a doubling in the number of snacks and drinks containing microalgae, particularly in Western countries. The most popular new products introduced include bakery items, meals, and chocolate confectionery (Kratzer and Murkovic, 2021).

Experts anticipate that the cost of producing microalgae will decrease, and microalgae will be cultivated for protein production, with a range of newly developed microalgae products expected to enter the market soon (Kratzer and Murkovic, 2021). Biotechnological production of carotenoids has expanded due to the ability to use low-cost substrates, the designation of natural substances, and the minimal space required for bio-production (Silva, 2004). Additionally, microalgae are unaffected by environmental factors such as climate, season, or soil composition, and controlled culture conditions further enhance the potential for carotenoid bio-production. Bioprospecting is used to identify the best-starting strain for improving the genotype of the microorganism in a bio-production system. Traditional breeding, mutagenesis, and selection can be used to enhance the strain (Torres-Tiji et al., 2019).

Carotenoids and anthocyanins are the most common colourants used in dairy products. However, the use of phycocyanobilins for blue pigmentation and betacyanins for pH-stable shades has expanded the range of chemical groups that can be incorporated (Luzardo-Ocampo et al., 2021). According to Torres-Tiji et al. (2019), there are numerous molecular tools accessible for modifying the genomes of microalgae. By strategically adjusting genetic information, these tools can be employed to enhance the production of a preferred product. Genetic tools are made up of various technologies needed for successful recombinant gene expression and endogenous gene expression regulation, such as promoters, untranslated regions (UTRs), enhancers, selection markers, and knock-out and knock-down technology. Kratzer and Murkovic (2021) affirm that the availability of suitable genetic tools is crucial for increasing the value of algae as a food source, as the degree and accuracy of genetic modification depend on their availability. Currently, genomic and gene expression data availability for specific algae species, physiological

barriers like cell size or rigid cell walls, and site-directed mutagenesis tools for the desired algae species are limiting these techniques.

Two different violaxanthin or chlorophyll a-binding proteins, VCP1 and VCP2, were also effective in *Nannochloropsis* sp. for stable recombinant expression, according to Barrera et al., (2013) and Qin et al. (2012). Carotenoids are another example of natural products in algae that can be enhanced through metabolic engineering, as demonstrated by overexpression of the *Chlorella zofingiensis* phytoene synthase gene in transgenic *Chlamydomonas reinhardtii* (Cordero et al., 2011). There is tremendous potential to boost the nutritional quality of algae by increasing the accumulation of already present nutrients while also incorporating new ones into an edible and mass-producible system (Torres-Tiji et al., 2019). Because of its promising potential, research in this area has surged recently and is still ongoing.

3. Methodology

All the selected microalgae species were purchased from the Microalgae Bank of University Science Malaysia for further evaluation of the pigmentation profile. Several methods involved in this design experiment as follow:

3.1 Microalgae Selection

Table 1 presents a selection of four species of freshwater blue-green microalgae (*cyanobacteria*) and nine species of green microalgae (*chlorophyta*) that were chosen to analyse their carotenoid content.

Table 1 List of selected green and blue-green microalgae species

Species	Type of Microalgae
Chlorella fusca	Green
Chlorella vulgaris	Green
Selenastrum capricornutum	Green
Pandorina morum	Green
Botryococcus sudeticus	Green
Botryococcus braunii	Green
Chlorococcum sp.	Green
Ankistodesmus sp.	Green
Scenedesmus sp.	Green
Pseudanabaena sp.	Blue green
Synechococcus sp	Blue green
Alkalinema sp.	Blue green
Phormidium sp.	Blue green

3.1 Microalgae Cell Culture Mass Production

Blue-green microalgae were cultured in the standard growth medium, namely Bold Basal Medium (BBM) and BBM Modified, which contains two and three times the nitrate concentration.

Microalgae Inoculation
A volume of 1 ml of algae stock solution was transferred using a pipette into sterile jam jars filled with BBM medium at varying pH levels and mixed thoroughly by swirling.

3.2 Carotenoid Sample Preparation

Microalgae Biomass Freeze Dry
On day 14, the cell culture of microalgae and the growth medium were collected and centrifuged. The resulting microalgae biomass was then collected, weighed, and freeze-dried until it was completely dry and turned into a powder.

Measurement of Carotenoids Pigment Colour Intensity
Green and blue-green microalgae pigments colour intensity was measured by CIELAB.

3.3 Carotenoid Extraction

High–Performance Liquid Chromatography (HPLC) Analysis
The carotenoids were analysed using HPLC, following the procedure outlined in previous studies (Othman, 2009; Radzali et al., 2016). An Agilent model 1200 series was used, which included a quaternary pump with an autosampler injector, micro-digesters, a column compartment equipped with a thermostat, and a diode array detector.

Carotenoid Sample Preparation
The carotenoid extract obtained from algae samples was re-suspended in 200 µl of ethyl acetate and inserted into HPLC for analysis.

4. Results and Discussion

Thirteen microalgae species were evaluated for their carotenoid content in terms of quantity and quality and nine were green algae and four were cyanobacteria. The carotenoid composition of these microalgae species can be classified into four groups, which are detailed in Table 2. Among the microalgae species tested, *Synechococcus* sp., a type of cyanobacteria, had the highest total carotenoid content (7751.87 ± 195.35 µg/g DW), which was significantly higher than all of the other species. In contrast, the lowest total carotenoid concentration was found in *Selenastrum capricornutum*, a type of green algae (40.39 ± 3.89 µg/g DW). Using HPLC analysis, at least six major carotenoid peaks were detected as shown in Figure 1 to Figure 13, including β-cryptoxanthin, β-carotene, zeaxanthin, neoxanthin, lutein, and violaxanthin. Table 2 shows that β-cryptoxanthin was most abundant in *Phormidium* sp. (30.58 µg/g DW), β-carotene was highest in C. vulgaris (356.15 ug/g DW), zeaxanthin was highest in *Synechococcus* sp. (7731.30 µg/g DW), neoxanthin was highest in *Chlorococcum* sp. (129.27 µg/g DW), and lutein and violaxanthin were highest in *C. fusc*a at 220.14 µg/g DW and 307.94 µg/g DW, respectively.

All 13 species can be categorized into several groups based on the accumulation of specific carotenoid pigments as explained in Table 2. *Pandorina morum* and *Chlorococcum* sp. green algae contained five and four individual carotenoid pigments, respectively, with relatively high concentrations of violaxanthin and lutein, compared to neoxanthin, β-carotene, and β-cryptoxanthin. However, six species, namely *C. fusca*, *S. capricornutum*, *Phormidium* sp., *Scenedesmus* sp., *B. sudeticus*, and *C. vulgaris*, were found to have three out of six carotenoid pigments, with relatively high concentrations of

either β-carotene or violaxanthin. The remaining five species, namely *B. braunii*, *Ankistodesmus* sp., *Pseudanabaena* sp., *Synechococcus* sp., and *Alkalinema* sp., accumulated two carotenoid pigments, either β-carotene and zeaxanthin or lutein and violaxanthin. Generally, the highest carotenoid concentrations, either in total or individual carotenoid pigments, were detected in the four-carotenoid pigment group. It can be concluded that the total carotenoid concentration is highly associated with that of individual pigments, especially zeaxanthin. However, the relative distributions of individual carotenoids within each grouping did not necessarily correlate with the levels of total carotenoids.

Table 2 Relative distributions of individual carotenoid from 13 microalgae species

Species	Total Carotenoid (µg/g DW)	β-cryptoxanthin (µg/gDW)	β-carotene (µg/ gDW)	Zeaxanthin (µg/g DW)	Neoxanthin (µg/ gDW)	Lutein (µg/ gDW)	Violaxanthin (µg/g DW)
Species with 5 carotenoid pigments							
Pandorina morum	544.47 ± 5.65	0.48 ± 0.01	53.59 ± 0.64	nd	115.89 ± 1.14	167.13 ± 1.15	207.38 ± 2.71
Species with 4 carotenoid pigments							
Chlorococcum sp.	731.09 ± 66.36	nd	168.78 ± 1.67	nd	129.27 ± 4.35	172.59 ± 38.74	260.45 ± 21.60
Species with 3 carotenoid pigments							
Chlorella fusca	840.82 ± 119.05	nd	312.74 ± 48.76	nd	nd	220.14 ± 47.68	307.94 ± 22.61
Selenastrum capricornutum	40.39 ± 3.89	nd	10.34 ± 1.94	nd	nd	3.42 ± 0.04	26.63 ± 1.91
Phormidium sp	178.09 ± 1.36	30.58 ± 0.47	13.41 ± 0.04	nd	nd	134.10 ± 0.85	nd
Scenedesmus sp.	513.33 ± 9.21	nd	nd	312.89 ± 8.79	nd	130.24 ± 0.20	70.20 ± 0.22
Botryococcus sudeticus	288.4 ± 20.87	0.46 ± 0.01	nd	nd	nd	158.68 ± 8.18	129.26 ± 12.68
Chlorella vulgaris	764.53 ± 50.4	nd	356.15 ± 2.39	nd	nd	186.39 ± 14.30	221.99 ± 33.71
Species with 2 carotenoid pigments							
Botryococcus braunii	273.07 ± 3.93	nd	nd	nd	nd	164.66 ± 1.49	108.41 ± 2.44
Ankistodesmus sp.	262.07 ± 6.97	nd	nd	nd	nd	164.39 ± 1.04	97.68 ± 5.93
Pseudanabaena sp.	1349.69 ± 2.78	nd	2.21 ± 0.32	1347.48 ± 2.46	nd	nd	nd
Synechococcus sp.	7751.87 ± 195.35	nd	20.57 ± 0.32	7731.30 ± 195.03	nd	nd	nd
Alkalinema sp.	1760.77 ± 81.63	nd	7.25 ± 0.91	1753.52 ± 80.72	nd	nd	nd

nd – non-detectable.

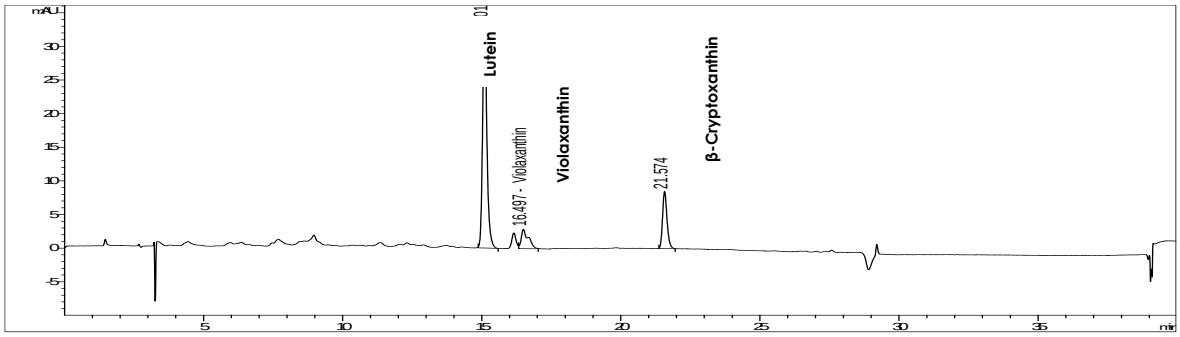

Figure 1 *Botryococcus sudeticus* HPLC chromatogram of lutein, violaxanthin and β-cryptoxanthin

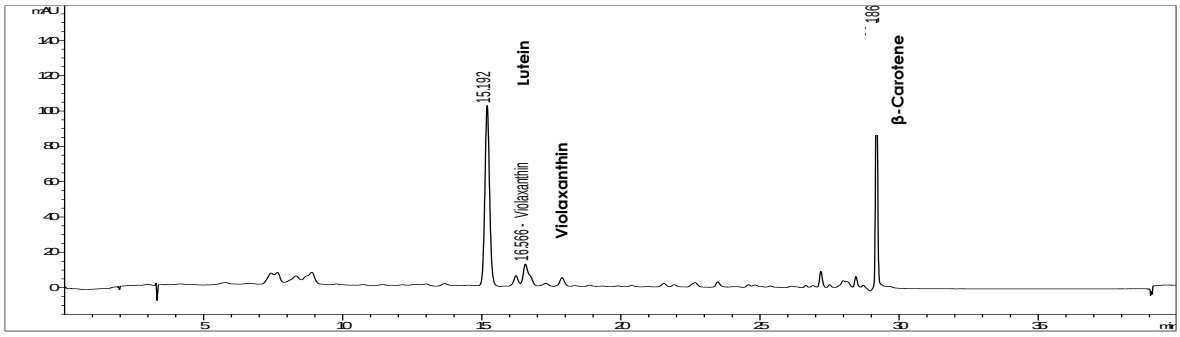

Figure 2 *Chlorella vulgaris* HPLC chromatogram of lutein, violaxanthin and β-carotene

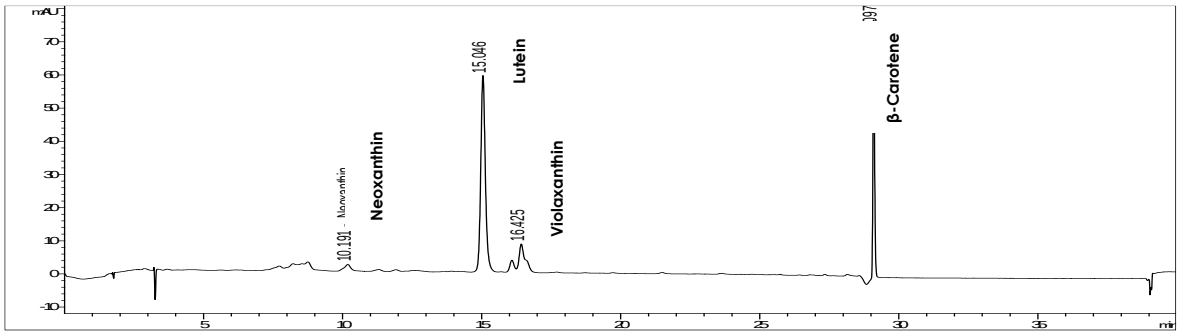

Figure 3 *Chlorococcum sp.* HPLC chromatogram of neoxanthin, lutein, violaxanthin and β-carotene

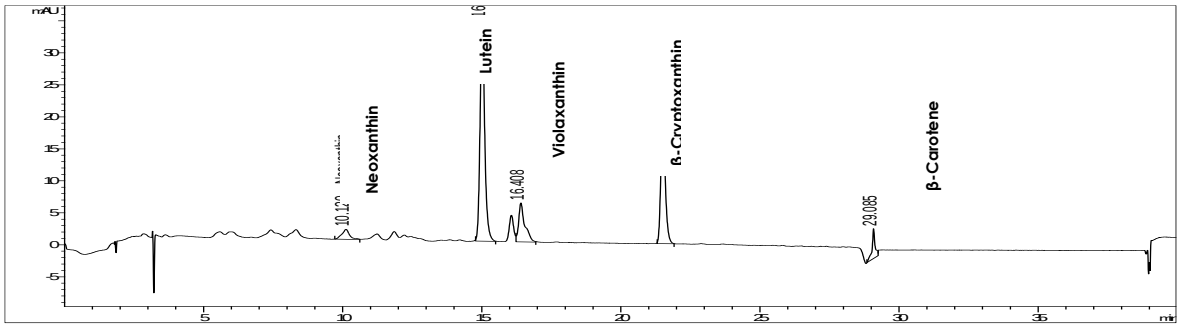

Figure 4 *Pandorina morum* HPLC chromatogram of neoxanthin, lutein, violaxanthin, β-cryptoxanthin and β-carotene

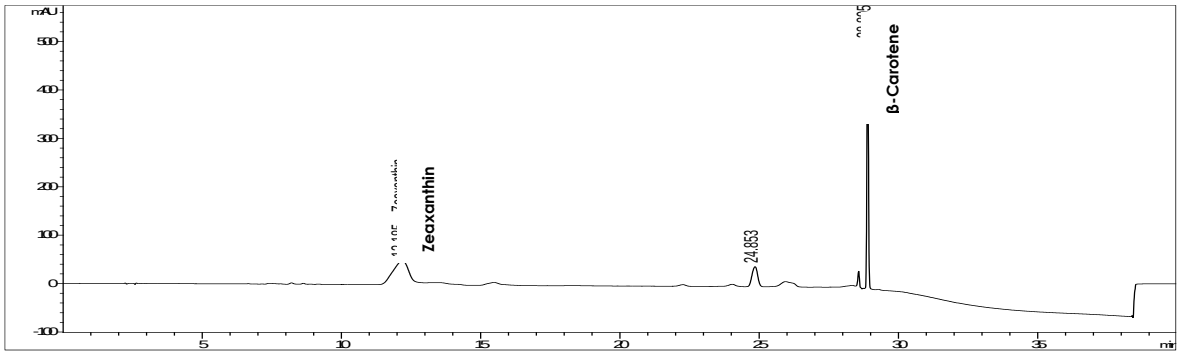

Figure 5 *Synechococcus* sp. HPLC chromatogram of zeaxanthin and β-carotene

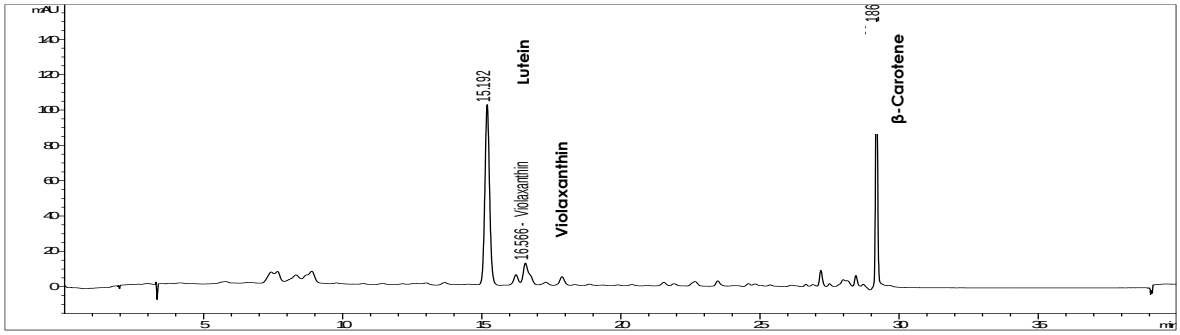

Figure 6 *Chlorella fusca* HPLC chromatogram of lutein, violaxanthin and β-carotene

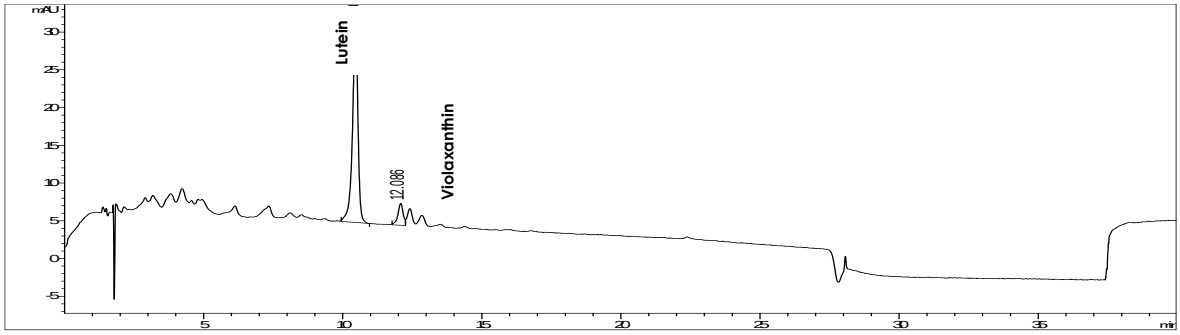

Figure 7 *Botryococcus braunii* HPLC chromatogram of lutein and violaxanthin

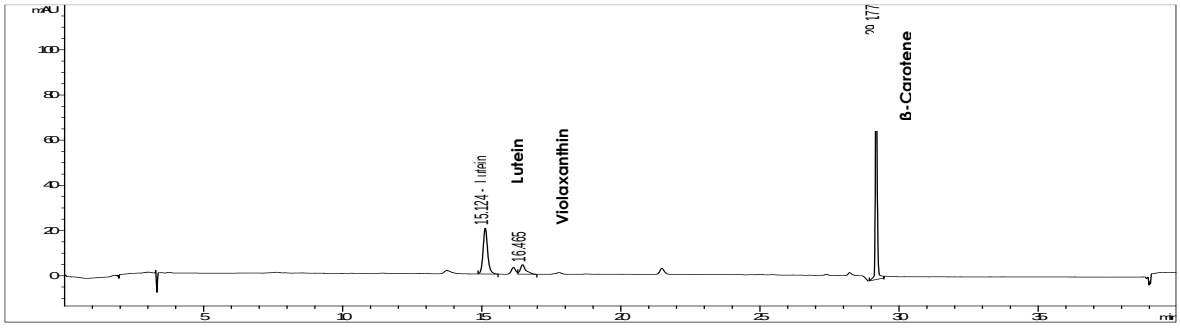

Figure 8 *Selenastrum capricornutum* HPLC chromatogram of lutein, violaxanthin and β-carotene

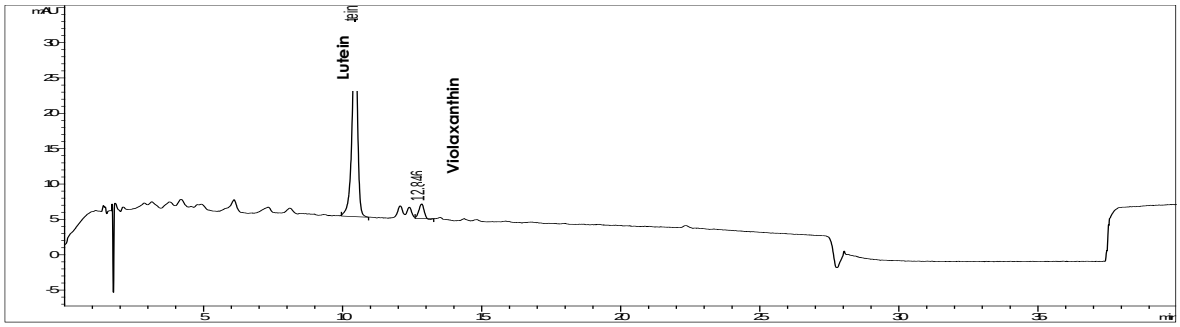

Figure 9 *Ankistodesmus sp.* HPLC chromatogram of lutein and violaxanthin

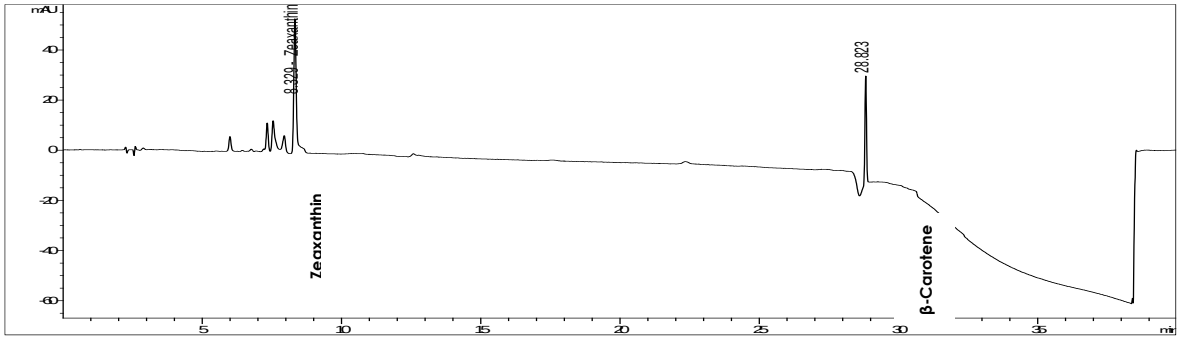

Figure 10 *Pseudanabaena sp.* HPLC chromatogram of zeaxanthin and β-carotene

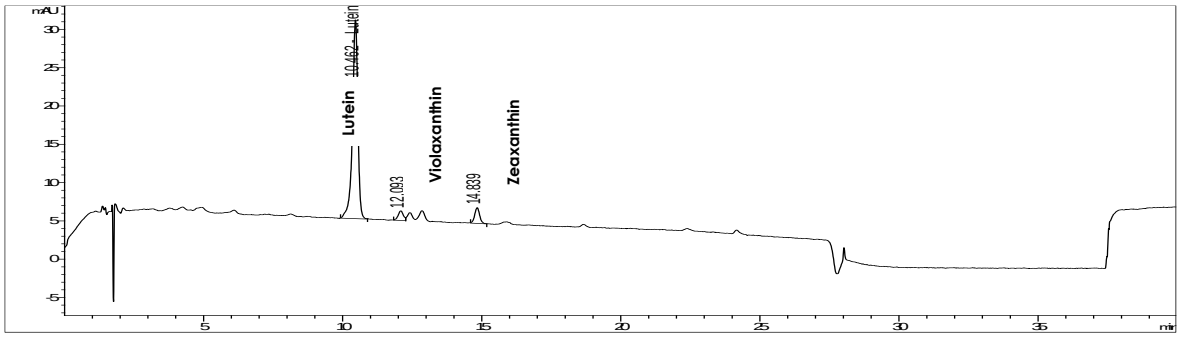

Figure 11 *Scenedesmus* sp. HPLC chromatogram of lutein, violaxanthin and zeaxanthin

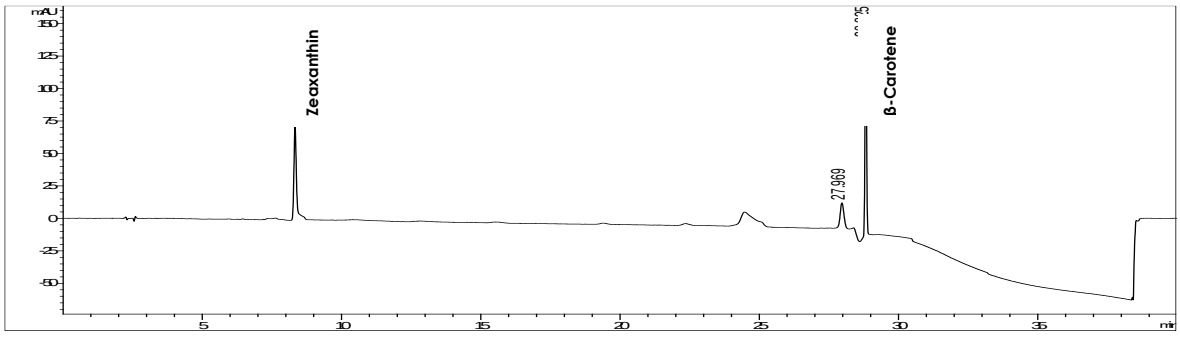

Figure 12 *Alkalinema* sp. HPLC chromatogram of zeaxanthin and β-carotene

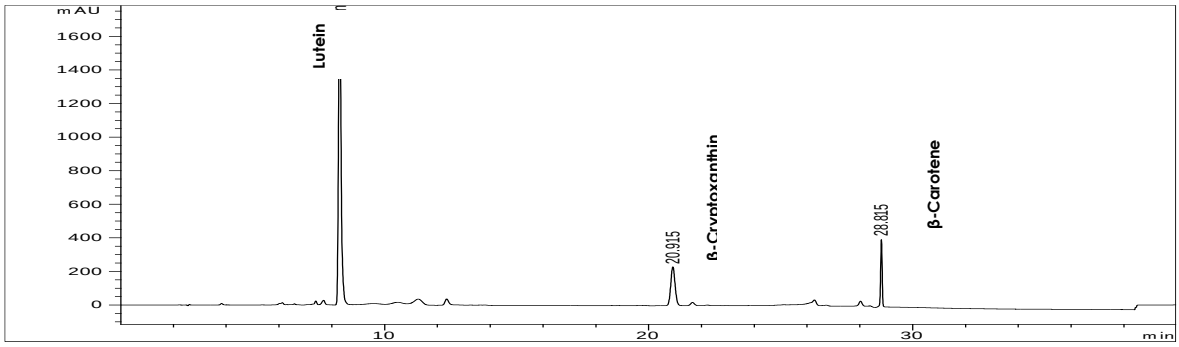

Figure 13 *Phormidium* sp. HPLC chromatogram of lutein, β-cryptoxanthin and β-carotene

According to Borowitzka (2013), out of 750 known carotenoids, 200 are derived from algae species. Algae carotenoids, like those in plants, act as light-harvesting pigments and antioxidants. Carotenoids of the xanthophylls class are abundant in algae and are useful in photosynthesis. For example, fucoxanthin, peridinin and vaucherioxanthin harvest light in the green region of the spectrum (500–550 nm) in algae. Other xanthophylls, such as zeaxanthin (found in green algae) and diatoxanthin + diadinoxanthin (found in chromophytic algae), are involved in non-photochemical quenching of the xanthophyll cycle. In addition to species, autotrophic growth factors such as temperature, light, nitrate, salinity, and iron play a crucial role in regulating the biosynthesis of carotenoids in algae (Minhas et al., 2016).

García-González et al. (2005) and Sanchez et al. (2008) conducted a study on *Dunaliella salina*, which resulted in a higher accumulation of lutein as the temperature rose up to 30ºC. However, Minhas et al. (2016) observed that the autotrophic cultivation of microalgae under temperature stress, for astaxanthin or beta-carotene, is dependent on the species. The availability of light is also considered a crucial limiting factor in several carotenoid biomass production and fatty acids (FAs) (Del Campo et al., 2000). The growth, biomass, and other metabolites of interest in various microalgae species are governed by the light intensity and photoperiod (Abe et al., 2007; Lamers et al., 2012). Lamers et al. (2012) conducted a study on *H. pluvialis*, which indicated a threefold increase in astaxanthin under increasing light intensity.

According to Gomez et al. (2003), increasing light intensity from 100 to 1000 μmol photons/m²/s in *D. salina* led to an increase in beta-carotene content up to 3.1% of dry cell weight. Hadi et al. (2008) found that high light intensity (460 μmol photons/m²/s) caused *Muriellopsis* sp. lutein content to peak. Minhas et al. (2016) noted that astaxanthin, beta-carotene, and lutein production in algae was increased by a combination of high light stress and nitrate. Nitrate was found to be a stress factor affecting not only lipid but also carotenoid accumulation in green algae (Minhas et al., 2016). According to Del Campo et al. (2000), lutein accumulation in microalgae is strongly influenced by nitrogen concentration in the medium. Under limited nitrogen conditions, the cells of aerial microalgae changed from green to red, and carotenoids such as β-carotene, astaxanthin, and canthaxanthin began to accumulate (Abe et al., 2007). Nitrogen-depleted conditions resulted in beta-carotene content rising up to 2.7% of dry cell weight in *D. salina* (Lamers et al., 2012). Lamers et al. (2012) also observed that under nitrogen-depleted conditions in *H. pluvialis* (mainly astaxanthin), carotenoid production was greater, whereas nitrate-deficient conditions led to greater competition for carbon, which is a favourable condition for the synthesis of both carotenoids and proteins.

5. Conclusion

In essence, this investigation completed examines the production of carotenoids from selected microalgae subjected to different culture conditions. The findings reveal that the composition and amount of carotenoids differ according to the microalgae species. Genetic factors appear to be crucial in categorizing these species into four groups based on individual carotenoid accumulation. In addition, the genetic variations in carotenoid content among these microalgae species selection may be attributed to various regulatory factors in the carotenoid biosynthesis pathway. Selecting the appropriate species with the ability to accumulate carotenoids is important in determining the sources of pigments for halal food colourants or for commercial purposes, particularly in the halal market, health benefits, food products, and dye technology for huge production and commercialization.

6. Acknowledgment

The research has been financially reinforced by the International Islamic University of Malaysia (IIUM), under the Research Grant RMCG20-034-0034.

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