Carotenoids as potential biocolorants: A case study of astaxanthin recovered from shrimp waste

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Introduction

Colorant

Colorant is defined as chemical compounds that absorb light within the range of wavelengths of the visible region (Delgado-vargas & Paredes-Lopez, 2000; Jiménez, 2000). Historically, the art of natural colorant was established alongside the development of *Homo sapiens* civilization (Gulrajani, 1992). This is attributable to the nature of man and their ever-present attraction to colorful and beautiful elements. In the second half of the nineteenth century, the coloring using natural colorant evolved with the invention of synthetic colorant, which provided numerous attractive spectra (Ul-Islam, 2017). Aberoumand (2011) and Mortensen (2006) have stated that colors can be classified into different types of natural, synthetic, nature-identical, and inorganic colorants, respectively. Natural colors are found in renewable resources, oftentimes extracted from plant sources, but also from other sources such as insects, algae, cyanobacteria, and fungi. Meanwhile, synthetic colors are formulated colors that do not originate from nature, such as azo dyes, whereas nature-identical colors are defined as synthesized colors that can also be found in nature, such as carotene, canthaxanthin, and riboflavin. Finally, inorganic colors are derived from metals, such as titanium dioxide, gold, and silver.

Perceptible visual aesthetics in terms of color is one of the most important aspects for product marketability and acceptability, as color itself is ubiquitous and is capable of conveying valuable messages regarding products. A study titled "Impact of Color in Marketing" has revealed the tendency for the average people to make a judgment within 90 seconds of their first impression of a product, where up to 90% of the judgment is influenced solely by the colors (Singh, 2006). The addition of colorants to products is needed because of things such as color replacement for those colors that have vanished during product processing, enhancement of the existing color, and ensuring minimal dissimilarity in the same batch of products (Aberoumand, 2011). Furthermore, the effectiveness and economic factors offered by synthetic colorants have resulted in wide use in various industries, including the food industry. Unfortunately, the application of synthetic colorants or color additives has been reported to negatively contribute toward human development and health, as their toxicity can cause health problems (Bridle & Timberlake, 1997; Samanta & Agarwal, 2009).

Controversial issues regarding the safety of synthetic colorants have drawn great attention among consumers. The European Union (EU) has enforced all food manufacturers to label their products with cautionary clarifications in case of the presence of "Southampton six colorant." These are tartrazine, quinoline yellow, sunset yellow, carmoisine, ponceau red, and allura red (Munawar, Makiah, & Jamil,

2014). Research by Buchweitz, Nagel, Crle, and Kammerer (2012) has shown that children who consume drinks containing azo dyes and benzoic acid may potentially trigger the attention deficit hyperactivity disorder (ADHD). The International Agency for Research on Cancer (IARC, 1975) has previously classified azo dyes of Sudan I, Sudan II, Sudan III, and Sudan IV as human carcinogens. Therefore, the EU has banned the use of azo dye as a food additive or colorant, but the practice of using this dye is still present in some countries, including Malaysia.

Carotenoids as biocolorants

Natural colors produced by living organisms can be categorized into three major groups, which include flavonoids, tetrapyrroles, and tetraterpenoids (Aberoumand, 2011). An example of a flavonoid is anthocyanin, which is responsible for colors, ranging from red to purple, of many fruits such as berries. Meanwhile, chlorophyll is considered as the most important element of the tetrapyrroles that can be found in higher plants. Similar to chlorophyll, tetraterpenoids like carotenoids are widely found as they absorb light as energy during photosynthesis and protect the chlorophyll from photodegradation.

Carotenoids are pigments naturally occurring in several fruits, vegetables, algae, and crustaceans, whereas they are synthesized by all photosynthetic organisms and many nonphotosynthetic bacteria and fungi (Sajilata, Singhal, & Kamat, 2008). Epidemiological studies have shown an inverse relationship between the risk of laryngeal, lung, and colon cancers and the consumption of carotenoid-rich foods (Block, Patterson, & Subar, 1992; Steinmetz & Potter, 1993). Approximately 700 carotenoids have been found, contributing to the different colors of yellow, orange, and red (Fortes, 2006; Mezzomo & Ferreira, 2016; Polyakov et al., 2009; Rodriguez-Amaya, Rodriguez, & Amaya-Farfan, 2006). Carotenoids are liposoluble tetraterpenes originating from the condensation of isoprenyl units that form a series of conjugated double bonds constituting a chromophoric system (Britton, 1995).

Carotenoids are typically categorized according to their functional groups, specifically carotenes and xanthophylls. There are two main classes of naturally occurring carotenoids: β-carotene, α-carotene and lycopene that are hydrocarbons (linear or cyclized at one or both ends of the molecule), and xanthophylls (lutein, zeaxanthin, β-cryptoxanthin and astaxanthin), which are oxygenated derivatives of carotenes (Fortes, 2006; Rivera, Vilaró, & Canela, 2011; Rodriguez-Amaya, 2001). All xanthophylls produced by higher plants, such as violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, and lutein, are also synthesized by green algae (Eonseon, Polle, Lee, Hyun, & Chang, 2003).

β-Carotene is one of the best-known food carotenoids, and it is sometimes found together with α-carotene in certain foods. It can be detected in carrots, mangoes, and apricots, whereas α-carotene is typically found in carrots and pumpkins. Lutein, the dihydroxy derivative of β-carotene is commonly detected in yellow or orange fruits and flowers as well as green vegetables. Lutein is isomeric with zeaxanthin. Specifically, the two carotene alcohols differ from one another just by the shift of a single double bond so that in zeaxanthin, all double bonds are conjugated. Both

zeaxanthin and lutein are important in the prevention of age-related macular degeneration (AMD), the leading cause of blindness (Moeller, Jacques, & Blumberg, 2000; Snodderly, 1995). Zeaxanthin is yellow and is naturally found in corn, egg yolks, as well as some of the orange and yellow vegetables and fruits like alfalfa and marigold flowers (Handelman, Nightingale, Lichtenstein, Schaefer, & Blumberg, 1999; Humphries & Khachik, 2003; Nelis & DeLeenheer, 1991; Sajilata et al., 2008). Zeaxanthin is used as a feed additive and colorant in the food industry for birds, swine, and fish (Hadden et al., 1999). The pigment imparts a yellow coloration to the skin and egg yolks of birds, whereas in pigs and fish it is used for skin pigmentation (Nelis & DeLeenheer, 1991). Meanwhile, lycopene is a typical food carotenoid found in many red fruits and vegetables, such as watermelon, pink guava, grapes, and tomatoes (Fortes, 2006; Rodriguez-Amaya, 2001).

Unique carotenoids like bixin may be sourced in annatto, whereas crocin is present in saffron (Rodriguez-Amaya, 2001). The red pigment from bixin is widely and typically used in the food, pharmaceutical, cosmetic, and textiles industries accordingly (Santos, Albuquerque, & Meireles, 2011). In contrast, astaxanthin is the major form of carotenoid detected in marine animals like salmon, shrimp, lobster, and crab, as well as other microorganisms (Ushakumari & Ramanujan, 2012). Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is classified as one of the lipophilic carotenoids (C_{40}) and is categorized under the xanthophyll group (Rodrigo-Baños, Garbayo, Vílchez, Bonete, & Martínez-Espinosa, 2015). However, the primary carotenoids in a type of food may differ depending on the genetics, locality, seasonality, and handling techniques (Arvayo-Enríquez, Mondaca-Fernández, Gortáres-Moroyoqui, Lopez-Cervantes, & Rodríguez-Ramírez, 2013; Fortes, 2006; Manzi, Flood, Webb, & Mitchell, 2002; Othman, 2009).

Chemical structure of carotenoids

Carotenoids are developed from acyclic C_{40} isoprenoid lycopene, which is also categorized as a tetraterpene (Arvayo-Enríquez et al., 2013). Generally, most of them are lipophilic in nature, which means they are soluble in organic solvents like alcohol, chloroform, ethyl ether, ethyl acetate, and acetone, and they are insoluble in water. Therefore, the best organic solvent to dissolve carotenes is either petroleum ether, hexane, or toluene, whereas methanol and ethanol are the best options for xanthophylls (Rodriguez-Amaya, 2001). Regardless, food carotenoids are mostly found as polytenes in all-*trans* forms made from eight 5-carbon isoprenoid units. However, during processing especially involving thermal conditions, a nonnegligible proportion of *cis*-isomers can be formed (Arvayo-Enríquez et al., 2013; Borel, 2003). Generally, one double bond is added to the molecule during the process of conversion from phytoene to lycopene, which is made up of 3 to 13 conjugated double bonds accordingly. After lycopene, the biosynthetic process will result in enzymatic cyclization of the end groups, which would next create γ-carotene (one beta ring) and β-carotene (two beta rings). The limit of the biosynthetic step is determined by the concentration of carotenoids available in the sources (Fortes,

2006). For example, red tomatoes containing lycopene at a very high concentration are not capable of converting them to β-carotene due to inadequate enzyme activity. According to McClements, Decker, Park, and Weiss (2009), certain cases display the formation of six carbon ring structures at one or both ends of the molecules. Some carotenoid structures are shown in Fig. 1.

Bioactive compound properties in carotenoids

According to Farre et al. (2015), all carotenoids comprise a polyisoprenoid structure containing long, conjugated double bonds at the center of the compound. The acyclic structure is capable of transforming and yielding a large family of more than 800 compounds via cyclization of the end of groups or the introduction of oxygen-rich functional groups (Britton et al., 2004). Therefore, their polyene structure has rendered carotenoids to be recognized as excellent free-radical scavengers with singlet-oxygen quenching properties and the ability to trap peroxyl radicals. In principle, the presence of greater conjugated chains causes greater antioxidant activity in the compound. Besides, the higher polarity of end groups (such as carbonyl and hydroxyl groups) due to β-ionone rings also greatly contribute to such antioxidant function. In fact, extracts from natural sources commonly contain bioactive compounds with antiinflammatory, antitumor, antiobesity, antimicrobial, antibacterial, antiviral, antifungal, and antioxidant properties (da Silva, Rocha-Santos, & Duarte, 2016).

Some carotenoids have been recognized as pro-vitamin A, such as β-carotene, γ-carotene, and β-cryptoxanthin, whereas others such as lycopene and xanthophylls are not recognized (Arvayo-Enríquez et al., 2013; Stahl & Sies, 2005; Borel, 2003). Carotenoids, in particular, have been reported as capable of lessening damage to cell membranes and are linked to receptors triggered by free radicals, controlling cellular immune reactions, and preventing tumor-cell growth (Fortes, 2006). Similarly, the annatto carotenoid has also been reported as having antioxidant activity against free radicals and protecting from sunlight (Santos et al., 2011). Furthermore, numerous epidemiological research studies have shown that dietary carotenoid intake may reduce the risks of some cancer, thus suggesting that antioxidant properties are instrumental for cancer protection. However, the evidence concerning β-carotene, α-carotene, β-cryptoxanthin, lutein, and zeaxanthin in terms of ideal supplementation dosage as cancer protection is still inadequate and requires further research in the context of a well-balanced diet (Fortes, 2006). The sources of some carotenoids and their respective bioactive compounds as reported by researchers are presented in Table 1.

Astaxanthin

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is classified as one of the lipophilic carotenoids (C_{40}) and is categorized under the xanthophyll group (Rodrigo-Baños et al., 2015). Its structure is shown in Fig. 1, whereby it contains a polyene chain that connects two terminal rings and two asymmetric carbons situated

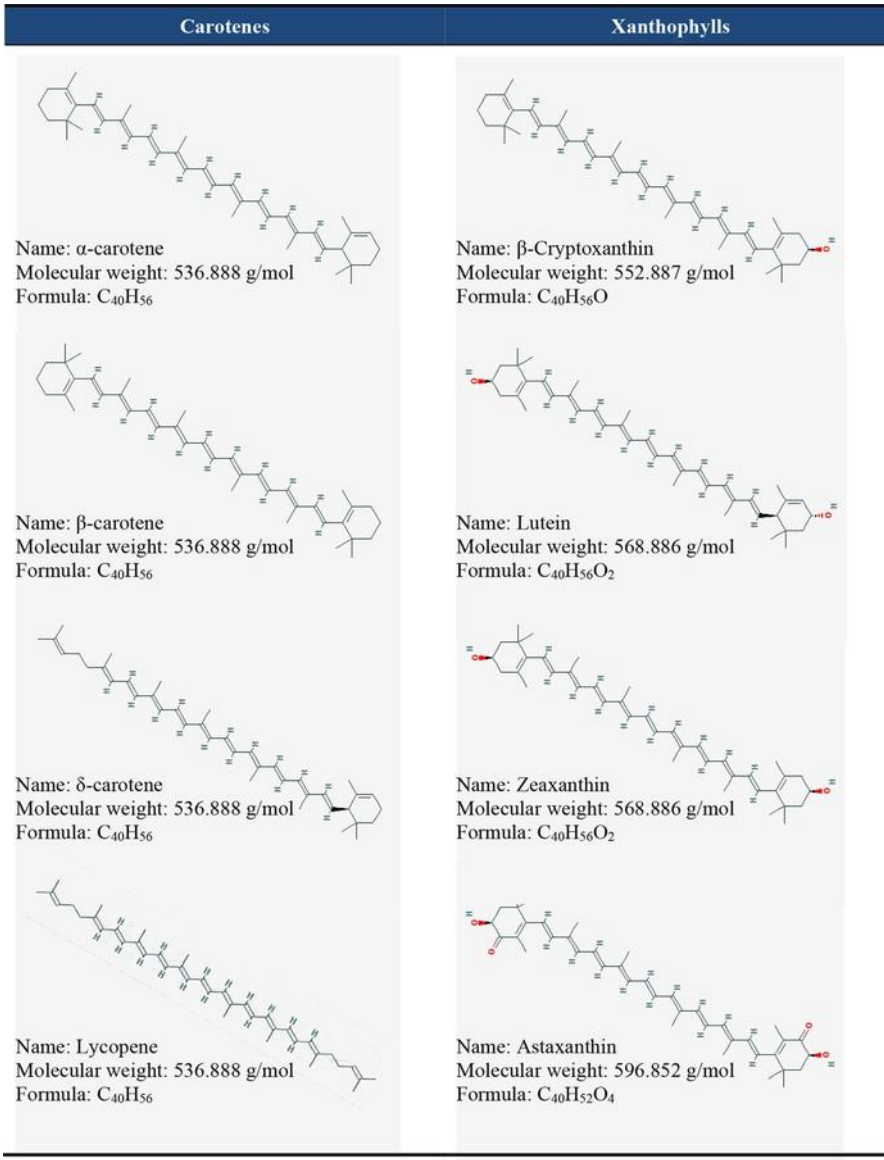

Fig. 1 Examples of carotenoid structure (National Center for Biotechnology Information, 2018).

at 3. The molecular formula for astaxanthin is $C_{40}H_{52}O_4$ and the molar mass is 596.85 g/mol (Ambati, Moi, Ravi, & Aswathanarayana, 2014). This reddish lipophilic carotenoid can be found accumulating predominantly in seafood or crustaceans, such as shrimp, lobster, crab, and salmon (Mezzomo & Ferreira, 2016;

Table 1 Carotenoids and their bioactive compounds

Sources	Carotenoids	Bioactivities	References
Algae (*Haematococcus pluvialis*)	Astaxanthin	Antioxidant	Wang, Yang, Yan, and Yao (2012)
Arabian red shrimp (*Aristeus alcocki*)	Astaxanthin	Antioxidant, antiinflammatory	Sindhu and Sherief (2011)
Aztec marigold (*Tagetes erecta*)	Lutein	Antimutagenicity	González-de-Mejía, Loarca-Piña, and Ramos-Gómez (1997)
Crabs (*Portunus sanguinolentus*, *Callinectes sapidus*, and *Paralithodes brevipes*)	Astaxanthin	Antioxidant, antimicrobial	Suganya and Asheeba (2015)
Green and dark green, leafy vegetables, e.g., spinach, parsley, kale, broccoli, corn, avocado, Brussels sprouts, beans	Lutein	Prevention against age-related Macular Degeneration (AMD), anticancer	Jaswir, Noviendri, Hasrini, and Octavianti (2011)
Green leafy vegetables	α-Carotene	Antioxidants, anticarcinogenic	Jaswir et al. (2011)
Peaches, oranges, tangerines, mangoes, papayas	β-Cryptoxanthin	Antioxidants, anticancer	Jaswir et al. (2011)
Seaweed (*Undaria pinnatifida*)	Fucoxanthin	Anticancer	Liu, Huang, Hosokawa, Miyashita, and Hu (2009)

Table 1 (Continued)

Sources	Carotenoids	Bioactivities	References
Seaweed (*Undaria pinnatifida*)	Fucoxanthin	Antiobesity	Maeda, Hosokawa, Sahima, Funayama, & Miyashita (2005)
Shrimps (*Penaeus brasiliensis* and *Penaeus paulensis*)	Astaxanthin	Antiobesity and hypolipidemic	Mezzomo et al. (2015)
Tomato	Lycopene	Anticancer	Kim, Rao, and Rao (2002)
Tomato	Lycopene	Antioxidant	Erge and Karadeniz, 2011)
Tomato and carrot	Carotenoids	Antioxidant	Patras, Brunton, Da, Butler, and Downey (2009)
Tomatoes, pink-red grapefruit, red-fleshed papayas, watermelon	Lycopene	Antioxidant, anticancer, anticholesterol	Jaswir et al. (2011)
Yellow-orange fruit and vegetables, e.g., carrot, broccoli, spinach, parsley, celery, tomatoes	β-Carotene	Antioxidants	Jaswir et al. (2011)

Radzali, Masturah, Baharin, Rashidi, & Rahman, 2016; Rodriguez-Amaya, 2001; Sui, Yue, Wang, & Han, 2015). Its buildup in the body of some microalgae and phytoplankton is also due to their being the primary source of astaxanthin consumption (Senthamil & Kumaresan, 2015).

Regardless, astaxanthin from shrimp wastes (byproduct) has been reported as containing highly antioxidant activities, which can improve human health through UV-light protection and antiinflammatory activity (Guerin, Huntley, & Olaizola, 2003; Kidd, 2011; Sui et al., 2015; Ushakumari & Ramanujan, 2012; Yamashita, 2013; Yang, Kim, & Lee, 2013). Despite not being a vitamin A precursor, several studies have reported that its biological activity is more potent than other carotenoids (Ambati et al., 2014; Lin, Chen, Chen, Chen, & Ho, 2016; Mezzomo & Ferreira, 2016; Naguib, 2000). According to Miki (1991), astaxanthin's antioxidant activity is

10 times greater than zeaxanthin, lutein, canthaxanthin, β-carotene, and is 100-fold better than α-tocopherol. This is attributable to its unique molecular structure that contains hydroxyl and keto moieties on each ionone ring (Hussein, Sankawa, Goto, Matsumoto, & Watanabe, 2006; Liu & Osawa, 2007), as well as long, conjugated double bonds at the center of the compound (13 conjugated double bonds) (Delgado-vargas & Paredes-Lopez, 2000). Therefore, their nutritional benefits have caused the United States Food and Drug Administration (USFDA) to approve astaxanthin as a color additive (pigment) in animal feeds (Pashkow, Watumull, & Campbell, 2008). Other than that, it is also used widely as a nutritious supplement in food, pharmaceutical products, and nutraceutical products (Ambati et al., 2014).

Astaxanthin production from shrimp waste

Malaysia's fishery sector has contributed extensively to the country's economic growth, with the marine-capture fishery serving as the main supplier for the sector. The aquaculture industry has also expanded significantly to cater to both local and international demands for fish products (*Investment Opportunities in the Aquaculture Industry in Sabah, Malaysia*, 2010). For example, the incentives offered by the National Key Economic Areas (NKEA) have spurred the Northern Corridor Implementation Authority (NCIA) and Hannan Corporation Sdn. Bhd. (HCSB) to invest in developing two phases of shrimp aquaculture complexes located in Perak Darul Ridzuan (Economic Transformation Programme). Both complexes are projected to produce 14,000 tons of white shrimp per year. In 2002, the global aquaculture industry has contributed more than 50 million mt valued at US$60 billion, whereby more than 90% of the amount has been contributed from Asia (Primavera, 2005).

However, the shrimp aquaculture industry specifically has led to serious ecological impacts, one of which is a result of the tremendous amount of shrimp waste produced (Famino, Oduguwa, Onifade, & Olotunde, 2000). Shrimp waste usually consists of the head part from a processed shrimp, called the cephalothorax and the exoskeleton, which generates up to 70% of the raw material (Simpson & Haard, 1985; Quan & Turner, 2009; Mezzomo, Martínez, Maraschin, & Ferreira, 2013). Therefore, researchers have advocated various alternatives to overcome this issue, as well as to attain sustainability in aquacultural practices. For example, shrimp waste has been reported to contain valuable carotenoid compounds that are usable in the food, nutraceuticals, and pharmaceutical industries alike (Ambati et al., 2014; Mezzomo et al., 2013; Sui et al., 2015).

Furthermore, the aquaculture industry typically makes use of synthetic carotenoid as an animal-feed supplement, which constitutes up to 20% of the feed expenses (Quan & Turner, 2009). In particular, astaxanthin is added to the feed to enhance the pigmentation of cultured animals. Thus, this component that is produced from shrimp waste may be used as a colorant in salmonid and crustacean feed (Mezzomo & Ferreira, 2016). These opportunities have increased the demand for colorants or pigments from natural sources, drawing the attention of many researchers to explore the potential natural sources as colorants for various products. In this current work,

the raw material of white shrimp waste, *Litopenaeus vannamei* (Fig. 2), has been specially chosen because the cephalothorax and exoskeleton parts are known to potentially generate other by-products.

Extraction technologies for astaxanthin production

Astaxanthin can be produced through various extraction methods, such as chemical extraction, the alkaline method, soxhlet, ultrasound, oil extraction, or supercritical fluid extraction (Gildberg & Stenberg, 2001; Macías-sanchez et al., 2009; Meyers & Chen, 1985; Mezzomo, Maestri, Dos Santos, Maraschin, & Ferreira, 2011; Sui et al., 2015; Sun, Sangkatumvong, & Shung, 2006). However, the applicability of the extraction methods used is a monumental factor in ensuring product quality (Mezzomo et al., 2013). A large volume of alkaline and acid chemicals are typically utilized in the conventional methods, which not only lead to environmental pollution but may also decrease the quality of the astaxanthin generated (Sui et al., 2015). Furthermore, the toxic organic solvent used during the process may leave traces in the final product that can cause further harm to human health (Mezzomo & Ferreira, 2016). Moreover, the conventional extraction method normally consumes more time and a higher amount of solvent, and it also causes compound degradation (Herrero, Cifuentes, & Iban, 2006). Further investigation on green technologies for carotenoid extraction has steadily amassed scholarly attention with surging demand in the global market trending toward products generated from natural compounds.

Hence, such weaknesses have been addressed and progress toward improvement achieved by the introduction of supercritical fluid extraction (SFE), which allowed a high extraction yield within a shorter time while maintaining the extract compound concomitantly (Santos et al., 2011; Arapitsas & Turner, 2008; Silva, Gamarra,

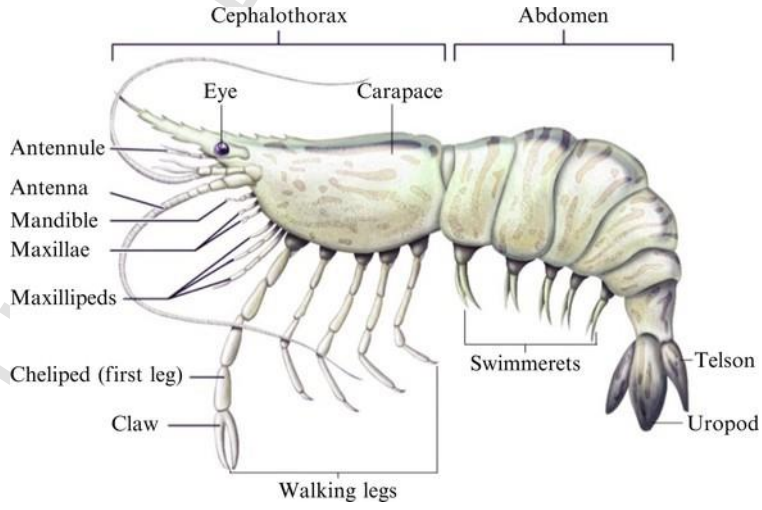

Fig. 2 Anatomy of *L. vannamei*.

Oliveira & Cabral, 2008). Some research has been done on extracting carotenoids from shrimp waste using vegetable oils as cosolvent via supercritical carbon dioxide extraction, such as sunflower oil, soybean oil, palm oil, and flaxseed oil (Sachindra & Mahendrakar, 2005; Handayani, Sutrisno, Indraswati, & Ismadji, 2008; Pu, Bechtel, & Sathivel, 2010). Regardless, SFE technology is well known for its numerous advantages over the conventional extraction methods (Cadoni, De Giorgi, Medda, & Poma, 2000; da Silva et al., 2016; Mezzomo et al., 2013). The use of cheaper, nontoxic, and inflammable gas (i.e., carbon dioxide) has rendered it a paramount element in various industrial applications. Moreover, the high consumption of carbon dioxide on an industrial scale can be controlled by recycling efforts, thus minimizing the operating costs and preventing an environmental carbon footprint. Although the low polarity of the gas complicates the process of extracting compounds with high polarity, cosolvent use coupled with high polarity has made the process more efficient (Herrero et al., 2006).

The low critical temperature and pressure of carbon dioxide at 31.3°C and 72.9 atm, respectively, have allowed the compounds to maintain and preserve their thermally labile samples (Durante et al., 2014). SFE, in particular, can have a shorter extraction time due to high diffusivity and low viscosity of the supercritical fluid, allowing easy diffusion of the fluid through the samples (Herrero et al., 2006). Moreover, manipulation of the pressure and temperature used has allowed the fluid density to be modified and has improved its solubility. Additionally, selective extraction of the compound via SFE is undertaken using a systematic experimental design approach, where response variables of samples can be optimized through modification of parameters such as temperature, pressure, and time. It can also be ensured that the sample output is of high purity. Therefore, the green technology is an efficient alternative for implementation in a wide range of industries, such as dye, food and beverage, pharmaceutical, cosmetic, and others (Cadoni et al., 2000).

Another emerging method of extraction is high-pressure processing (HPP), which is a new and safe technology in research and development that is capable of reducing the loss of bioactive compounds during processing (Tadapaneni, Daryaei, Krishnamurthy, Edirisinghe, & Burton-freeman, 2014). HPP is also widely recognized in the food industry due to its capability to preserve food nutrients, quality, sensory properties, as well as offering a longer shelf life. These properties are attributable to its very minimal effect on the covalent bonds of low molecular-mass compounds (Patras et al., 2009). Furthermore, nonthermal technology has allowed for the quality of thermo-labile compounds to be preserved (Ahmed & Ramaswamy, 2006), whereby the pressure during the process can increase the mass transfer rate, thus enhancing the sample permeability (Xi, 2006). A few studies have reviewed the use of HPP as an extraction method, and one study (Xi, 2013) has used it to extract active compounds from plants. Moreover, Sánchez, Baranda, and Martínez de Marañón (2014) and McInerney, Seccafien, Stewart, and Bird (2007) have also studied the total carotenoid content in several plants extracted using HPP. When HPP has initiated its operation, the different pressures between the internal and external cell membranes have resulted in continuous penetration until the

concentration between them reaches the equilibrium state (Xi, 2006). This process is attainable in a short time. Like SFE, extraction via HPP can also produce high extraction yield despite not being a time-consuming process.

Future prospects of biocolorant for polymer products

When selecting colorant materials used especially for polymer products in contact with food, toys, and graphic-arts tools, the toxicology and environmental aspects should be taken into consideration. Some synthetic colorants contain toxic heavy metals like cadmium and lead, which are illegal for use in these products as per quality-assurance and safety standards outlined by European Union (BS EN 71-3:1995) and International Standards (International Organization for Standardization, ISO 8124-3:1997 Migration of Certain Elements) (Kumar & Pastore, 2006). However, the rising consumer awareness regarding the negative impacts of synthetic-colorant consumption has also increased the demand for natural products, spurring product manufacturers to opt for natural colorants (Samanta & Agarwal, 2009; Santos et al., 2011). This is evident in the various works done on biocolorant application in mitigating environmental pollution caused by industries associated with synthetic colorant (Ali, El-Khatib, El-Mohamedy, & Ramadan, 2014).

Incorporating colorants as an additive in polymers is synonymous with altering the optical properties so as to attract consumers in purchasing the products (Christie, 1994). The technique of colorant incorporation has been selected in accordance with the desired finish and quality in product application. The dispersion process involves the dispersion of colorants into the liquid phase of a polymer, whereby physical retainment is seen in the polymer matrix after solidification concludes. Meanwhile, it may also be retained in the polymer matrix by completely dissolving in a polymeric medium to create an affinity between the colorant and the polymer molecules (Christie, 1994). Incorporating biocolorants from carotenoids with biodegradable polymers has been reported to show a protective element against the oxidation process (Martín, Mattea, Gutiérrez, Miguel, & Cocero, 2007). The application of biocolorant to polymer is a promising invention in various applied sectors, starting from the production of colorant materials in the agrotechnology sector until the final product is manufactured by the polymer industry. Thus, these biocolored polymers may accordingly be applied as safe food-contact items (e.g., packaging, kitchen utensils), baby and kids' items (e.g., toys, polymer clay), medical devices (e.g., implants, gloves, bandages), cosmetics, and pharmaceutical products. Therefore, further research is encouraged to discover the use, challenges, and technologies of carotenoids production and its development as a potential biocolorant for polymer products.

Current research

This chapter aims at exploring the application of carotenoids as biocolorants, focusing on a case study of astaxanthin recovered from shrimp waste. It assesses its chromaticity stability across various environmental factors involving UV irradiation,

heat, salinity, and pH stability tests throughout 4 weeks. The color analysis was performed using the CIELAB system. According to Sant'Anna, Gurak, Ferreira Marczak, and Tessaro (2013) and Torskangerpoll and Andersen (2005), CIELAB is a useful system to describe colors, as it involves the complete visible spectrum of the eye. When a visual assessment is performed on an object, three factors can determine the color output: namely, light source, light-receptor mechanism, and the object itself. Although there are various standards of light source, standard daylight illuminant, D65, is the commonly used option in food color measurement by CIE (Vidal Zaragoza, n.d.).

The CIELAB system can measure the index of lightness (L^*) and two color coordinates (a^* and b^*). The luminosity or lightness is described by the L^* index at the vertical axis, whereby every color is associated with vividness or dullness in the range of black ($L^* = 0$) and white ($L^* = 100$). The positive coordinates of the a* index refer to the reddish colors, whereas its negative coordinates refer to the greenish colors. In contrast, the positive coordinates of the b^*index denote the yellowish colors, whereas its negative coordinates represent the bluish colors. Moreover, important information can be gathered from the values of the chroma (C^*_{ab}) (quantitatively) and the hue (h_{ab}) (qualitatively) (Sant'Anna et al., 2013). Boonsong, Laohakunjit, and Kerdchoechuen (2012) have defined hue angle ($h°$) as an angle ranging from 0° to 360°. Red, orange, and yellow are indicated by angles that range from 0° to 90°, whereas yellow to green is displayed by the angle between 90° and 180°. Meanwhile, green, cyan, and blue ranges between 180° and 270°, whereas blue, purple, magenta, and red are indicated by angles from 270° to 360°.

Experimental design

Raw materials preparation

The raw material of *Litopenaeus vannamei* consists of white shrimp waste made up of the cephalothorax and supplied by the shrimp aquaculture company, Hannan Corporation Sdn. Bhd. The materials are transported to the laboratory in a container filled with ice at a temperature of 4°C. On arrival, the sample is stored in the freezer at a temperature of –20°C prior to further processing, before approximately 30 kg of them are dried using a freeze dryer (FDU-1100, EYELA, Tokyo Rikakikai Co., Japan). This has been done at a temperature of –50°C and vacuum level at 6.5 Pa for three days. At the same time, approximately 30 kg of shrimp waste is dried at 60°C for 1 week in an oven with air circulation (UF55, Memmert) for HPP extraction. Then, the sample is ground into fine powder using a heavy-duty blender (LB20E, Laboratory Blender). The ground sample is conveniently stored under appropriate conditions at a temperature of –20°C to minimize enzymatic reactions (Othman, 2009).

Extraction of astaxanthin by chemical extraction

One g of ground shrimp waste has been added into 50 mL tubes and was rehydrated via the addition of 1 mL distilled water. Then, 5 mL of different solvents [i.e., acetone:methanol mixture (9:1, v/v), acetone, methanol, ethanol] prepared with 0.1% butylated hydroxytoluene (BHT) was added into each tube. BHT is used to avoid any oxidation and isomerization from occurring during the extraction (Arvayo-Enríquez et al., 2013). The samples are then stored overnight in a dark condition at room temperature, and each has been prepared in triplicate. The next day, the samples were vortexed and centrifuged for 2 minutes at 13,500 × *g* (NU-C200R-E, Nuaire, United States), with the resulting supernatant being transferred into 50 mL graduated polypropylene centrifuge tubes. This step is then repeated by adding 5 mL of the same solvent until the supernatant of the sample become colorless (normally 2 or 3 times). Then, an equal volume of hexane and distilled water (5 mL) is added to extract the carotenoid from the combined supernatants. The solution is then allowed to separate, with the upper hexane layer containing the carotenoids being collected. This procedure is repeated with hexane alone until it becomes colorless. After that, the combined upper layer is dried to completion under a gentle stream of oxygen-free nitrogen. Tubes are then capped and sealed with parafilm to exclude oxygen, and they are immediately stored at –20°C before further analysis. Fig. 3 shows the collected astaxanthin and nitrogen-dried astaxanthin.

High-performance liquid chromatography (HPLC) analysis

One mg of dried astaxanthin extract was dissolved in 1 mL ethyl acetate. Then, 450 µL of the sample was added into Whatman Mini-UniPrep Syringeless Filters vials, with the prepared sample being inserted into HPLC for analysis. HPLC employing diode-array detection (DAD) is the most common analytical method in determining the qualitative and quantitative carotenoid profiles. The HPLC analysis was done following the procedure described by previous authors (Othman, 2009; Radzali et al., 2016), with the HPLC Agilent 1200 series (Agilent Technologies, United States) being used to analyze the carotenoid content extracted from *L.*

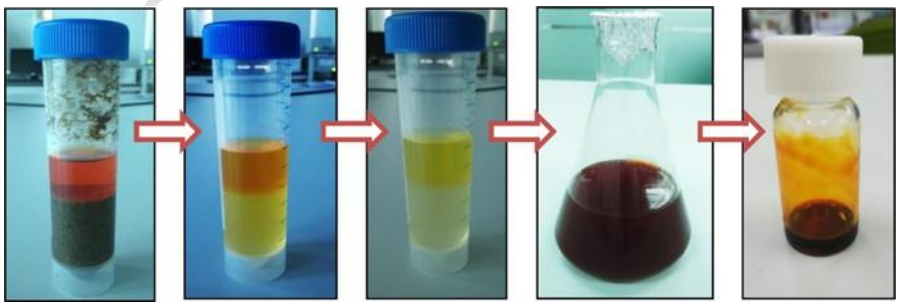

Fig. 3 Astaxanthin extracted using chemical extraction.

vannamei waste. It is equipped with a binary pump with autosampler injector, microvacuum degassers, and a thermostatic column compartment, whereas the reverse-phase column is a ZORBAX Eclipse XDB-C_{18} end capped (5 μm) and sized at 4.6 × 150 mm. An HPLC grade of acetonitrile:water (9:1, v/v) has been prepared as eluent A, whereas the HPLC grade of ethyl acetate is designated as eluent B. Meanwhile, Ultrapure water 18.0 MΩ is prepared using a Milipore S.A.S water purification system.

The solvent gradient used has been established as follows: 0%–40% solvent B (0–20 min), 40%–60% solvent B (20–25 min), 60%–100% solvent B (25–25.1 min), 100% solvent B (25.1–35 min), and 100%–0% solvent B (35–35.1 min). The flow rate is set at 1.0 mL min^{-1} and the injection volume is 10 μL. The column was thermostatic at 25°C, with carotenoid peak identification performed in the range of 350–550 nm. The stop time was set at 40 min, with astaxanthin detection done at the maximum absorption wavelength (480 nm). Triplicate samples were prepared, with three injections being performed for each vial. The concentration of the astaxanthin was determined using the standard curve in regard to its structure and properties. The chromatographic peak is then identified by comparing the retention time and spectra against the astaxanthin standard. The carotenoids concentration has been quantified in terms of microgram per 1.0 g dry weight of sample (μg/g DW).

Astaxanthin pigment preparation for stability test

Astaxanthin extract stored at –20°C have been used for color-stability tests. Three concentrations comprised of 1, 2, and 3 g/L of astaxanthin (Fig. 4) have been prepared by dissolving in ethanol. The chromaticity of the sample without any treatment has been measured as a blank.

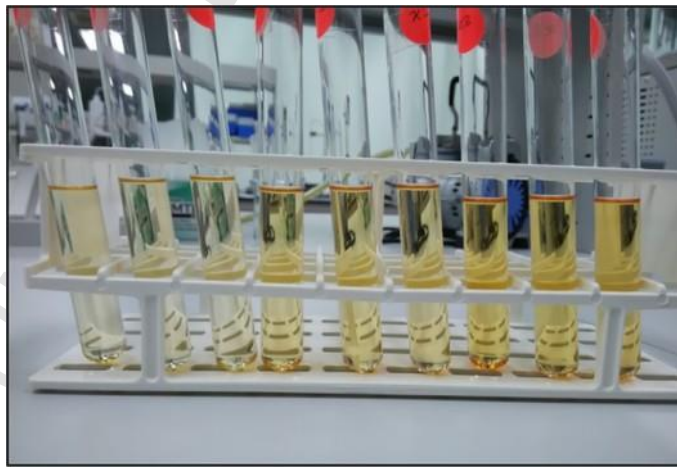

Fig. 4 Prepared 1, 2, and 3 g/L of astaxanthin pigment.

UV irradiation test

All glass tubes containing samples of three different concentrations were placed in a laminar hood and exposed under UVA (long wavelength = 365 nm, intensity 5500 lux) and UVB (middle wavelength = 312 nm, intensity 2900 lux) perpendicularly 10 cm below the light source for 8 hours at room temperature (Fig. 5). The samples were prepared in triplicate and were analyzed hourly to monitor their chromaticity. Any changes in color before and after the experiment have been measured accordingly.

pH test

The pH of samples was adjusted to 3, 5, 7, 9, and 11 using acid hydrochloric (HCl) and sodium hydroxide (NaOH). They were prepared at three concentrations and were exposed under dark and light conditions (cool light of a fluorescent lamp with light intensity, 3100 lux, perpendicularly 30 cm below) at room temperature for 4 weeks. The samples were prepared in triplicate and were analyzed hourly to monitor their chromaticity. The chromaticity of a sample for day 1 was also measured.

Salinity test

Sodium chloride (NaCl) was added into each sample at three concentrations, making the final concentration of the NaCl of 1, 3, and 5 g/L, respectively. Samples were incubated in dark and light conditions at room temperature for 4 weeks. The samples were prepared in triplicate and were analyzed hourly to monitor their chromaticity. The chromaticity of the sample for day 1 was also measured.

Heat test

An astaxanthin sample at concentration 1, 3, and 5 g/L was prepared accordingly. All samples were incubated in the oven at eight points of temperature (i.e., 25°C, 35°C, 45°C, 55°C, 65°C, 75°C, 85°C, and 95°C) for 3 hours. The samples were prepared in

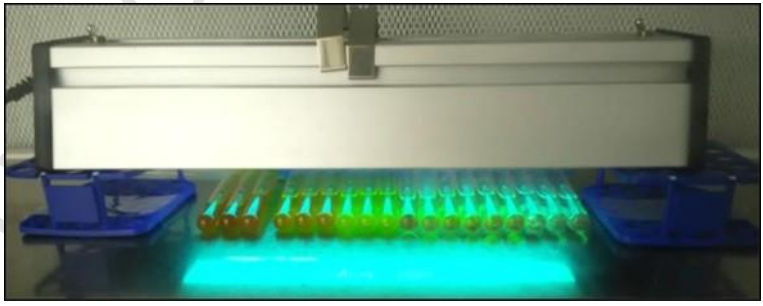

Fig. 5 Samples exposed under UV irradiation.

triplicate and were analyzed hourly to monitor their chromaticity. The changes in color before the experiment were also measured.

Analysis of chromaticity

A total of 1 mL of carotenoid pigment was pipetted into a glass cuvette and was introduced into the CIELab colorimeter (Cary Win UV Color Application, Agilent Technologies). The CIELab system is then used to analyze L^* (lightness), a^* (red-green), and b^* (yellow-blue) values of the samples' colors . Meanwhile, the three measured color parameters are calculated into C^* (Chroma) representing color saturation, h (hue angle) indicating color intensity, and ΔE (total color change) using the following equations (Eq. 1) (Licón, Carmona, Rubio, Molina, & Berruga, 2012; Yawadio & Morita, 2007).

$$\Delta E = \left[\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2 \right]^{1/2} \quad (1)$$

Statistical analysis

One-way analysis of variance (ANOVA) has been conducted using SPSS (version 20) to compare CIELAB parameters in different treatments, and the resulting values have been given as a mean. Significant ($P < .0001$) differences between means of three replicates are subsequently identified using Tukey's test.

Result and discussion

Analysis of total astaxanthin content

HPLC analysis has been performed to characterize the total astaxanthin content extracted from *L. vannamei* waste using a reference calibration curve. Fig. 6 shows the HPLC chromatogram for astaxanthin obtained via chemical extraction. Astaxanthin has been detected at the retention time of 15.674 minutes with a purity greater than 85%, and the amount of total astaxanthin has been calculated on a dry

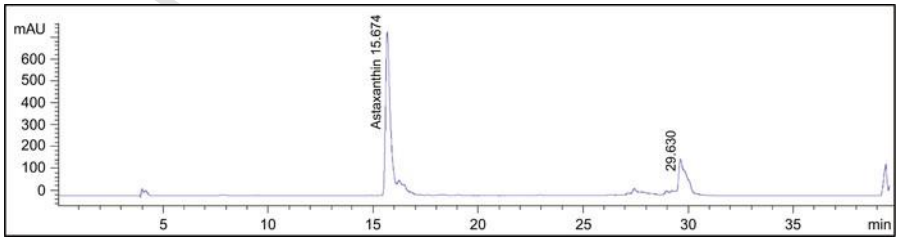

Fig. 6 Chromatogram of total astaxanthin obtained by chemical extraction.

weight (DW) basis. The results obtained have been presented as an average of the three replicates (mean ± standard deviation) and they are demonstrated in Fig. 7. The highest amount of astaxanthin content has been obtained from extraction using 100% acetone, which is 5701.99 ± 261.15 μg/g DW, followed by acetone:methanol (7,3, v/v), ethanol, and methanol (4184.14 ± 312.14, 3100.62 ± 136.66, and 2294.57 ± 165.18 μg/g DW, respectively).

The extraction using solvent typically involves the interaction resulting in an affinity between the sample and the solvent. The low polarity of carotenoid compounds normally makes the extraction more efficient, with solvents, such as petroleum ether and hexane, having low polarities. However, some carotenoids also contain a polar part, thereby rendering a proper selection of solvent to assist in increasing the extraction yield (Arvayo-Enríquez et al., 2013). The result of the chemical extraction has shown that the use of an acetone:methanol mixture (7:3, v/v) and acetone as a solvent has produced a significant amount of total astaxanthin. This result is supported by those obtained by Mezzomo et al. (2011), Sachindra, Bhaskar, and Mahendrakar (2005), Sindhu and Sherief (2011), and Vimala and Paul (2009), whereby astaxanthin is effectively extracted by acetone. The high polarity of acetone renders it the best solvent for the extraction of carotenoids from shrimp waste. This is due to the higher molecular chain in etherified astaxanthin and the presence of a hydrophobic mass surrounding the pigments of xanthophylls, which requires more polar solvents. This will assist the penetration in the sample matrix, which increases the concentration of extraction yield.

However, the result of previous authors has also shown that the use of polar solvent has caused low selectivity, in which other compounds aside from carotenoids are also extracted. This has resulted in an increased yield of extract. In the food and pharmaceutical industries, the use of acetone is intolerable, as its traces found in the final product can consequently cause harm to human health (Arvayo-Enríquez et al., 2013). Hence, there are numerous studies done using environmentally friendly solvents like ethyl acetate and ethanol for carotenoid extraction.

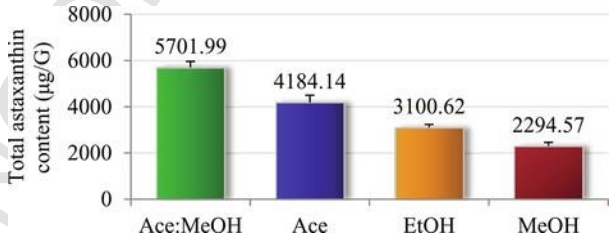

Fig. 7 Total astaxanthin content obtained by the different solvent used.

Color analysis of astaxanthin pigment stability

Salinity test

Color stability is demonstrated by the chromaticity and color saturation graphs of astaxanthin pigment, once added with 1, 3, and 5 g/L NaCl and stored in dark and light conditions. Undertaken across 4 weeks, the results are shown in Figs. 8 and 9, respectively. Color intensity and brightness are responsible for the chromaticity (C^*) values of the pigment, whereas the saturation (s) is defined as the vividness of an area visualized, which is given by the ratio of chromaticity to lightness (Nor, Aziz, Mohd-adnan, Taha, & Arof, 2016). From the results, it can be seen that the values for astaxanthin pigment chromaticity and saturation increase linearly with the concentration of the pigments. Furthermore, the pigments stored in light conditions have shown higher degradation rates than those stored in a dark condition. The graphs have also confirmed that pigment without salt treatment (blank) has shown the least degradation effect, followed by the pigment treated with the sodium chloride (NaCl) concentration of 1, 3, and 5 g/L, respectively, both in dark and light conditions.

By the end of the treatment in a dark condition, the lowest concentration of a pigment treated with 0, 1, 3, and 5 g/L NaCl was obtained as 17.01%, 60.57%, 63.92%, and 70.52% of chromaticity degradation. Meanwhile, the medium concentration of astaxanthin pigments (2 g/L) show 16.80%, 66.87%, 69.71%, and 70.88% degradation, whereas the highest concentration of pigments (3 g/L) show 8.2, 67.49, 67.77, and 72.65% degradation correspondingly. In contrast, the end of the treatment in a light condition resulted in a chromaticity degradation of 32.45, 70.64, 79.14, and 85.90% for the lowest concentration pigments, 57.72, 83.28, 88.33, and 88.07% for medium concentration pigments, and 66.18, 83.94, 87.34, and, 90.76% for the highest concentration pigments, respectively. This corresponded with the concentrations of NaCl across each class. Therefore, the color-difference values (ΔE^*) shown in Fig. 10 support the result of salinity stability tests, where it shows significant changes affected by different pigment concentrations, the presence of NaCl, and the storage condition. Almost consistent increments have also been seen throughout the 4 weeks of experiment duration, despite the smallest differences from 1 week to another.

The statistical analysis is performed via One-Way ANOVA for color changes difference value, indicating any statistically significant differences between pigment concentrations, NaCl concentrations, time, and storage conditions. The output indicated by the P value confirmed a statistically significant difference in the mean of the color difference value between different pigment concentrations, NaCl concentrations, time, and storage conditions, respectively, with a P value less than .0001. Then, a Tukey post hoc test was performed to show how each group differs from one another, revealing statistically significant differences in color values between each group of pigment concentrations of 1, 2, and 3 g/L ($P < .0001$). The test also revealed a statistically significant difference in color value between the 1-

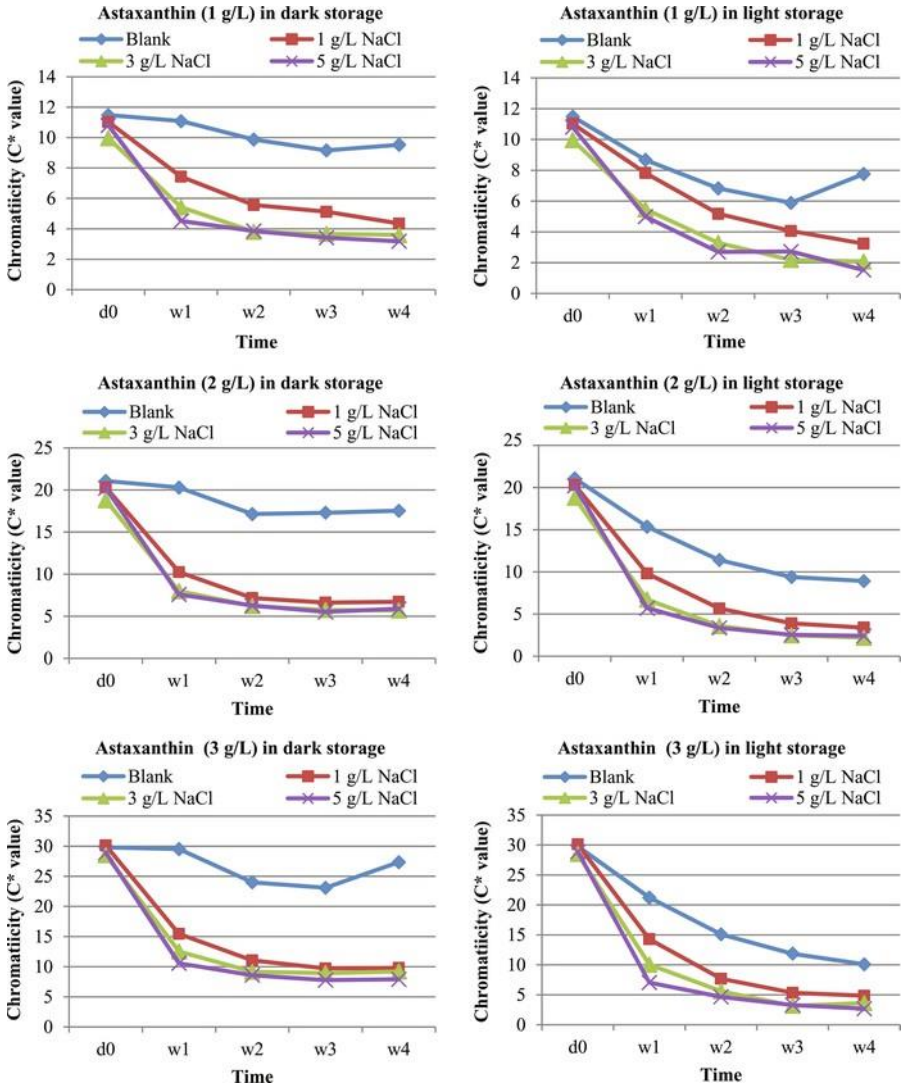

Fig. 8 Chromaticity of astaxanthin pigments at different concentrations 1, 2, 3 g/L and different salinities, stored in dark and light conditions, measured initially and every 1, 2, 3, and 4 weeks.

week group with other groups of 2, 3, and 4 weeks, as well as between the blank with other groups of 1, 3, and 5 g/L NaCl.

From the results, it can be deduced that the pigment degradation is increased in higher concentrations of salt treatment. This is due to salinity being one of the environmental stresses that can destabilize the molecular structure of the pigment (Mohajer, Taha, & Azmi, 2016). Besides, it can be concluded that the presence of

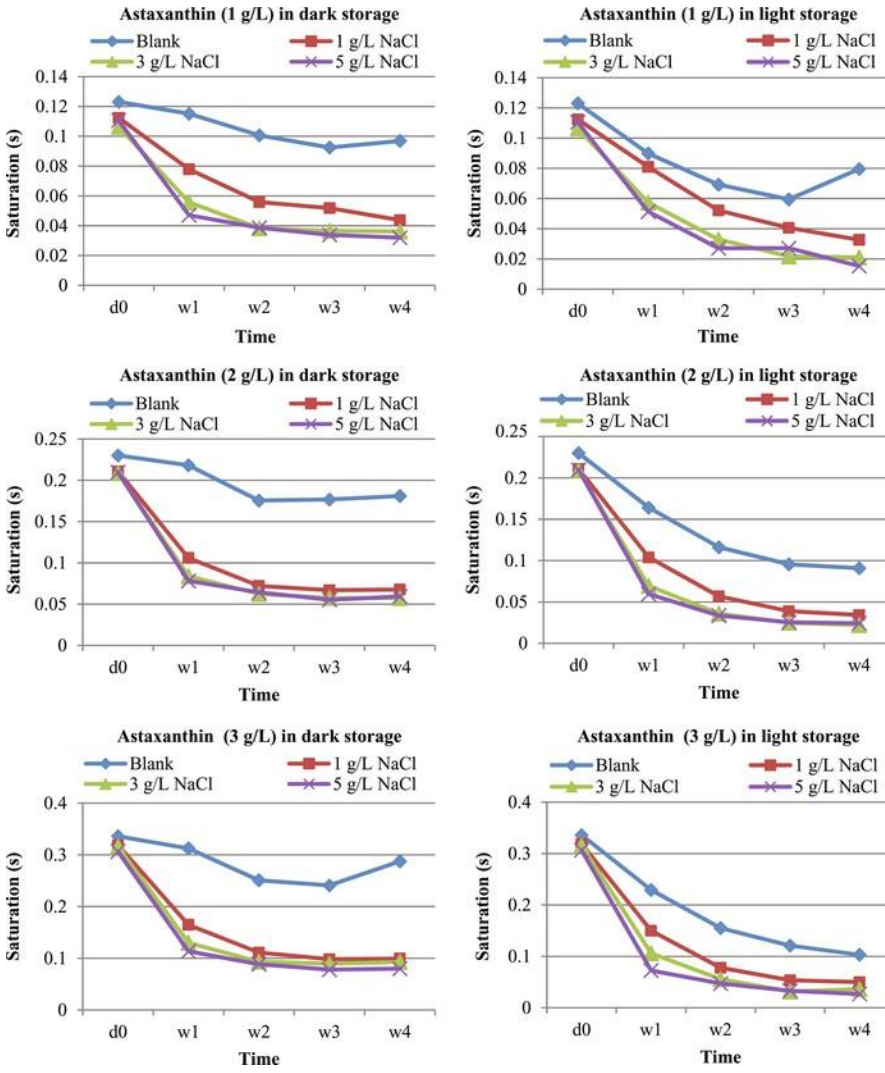

Fig. 9 Color saturation of astaxanthin pigments at different concentrations 1, 2, 3 g/L and different salinities, stored at room temperature, in dark and light conditions, measured initially and every 1, 2, 3, and 4 weeks.

light induces the photodegradation process. Therefore, during processing and storage of the carotenoid products, light exposure should be avoided to minimize quality deterioration.

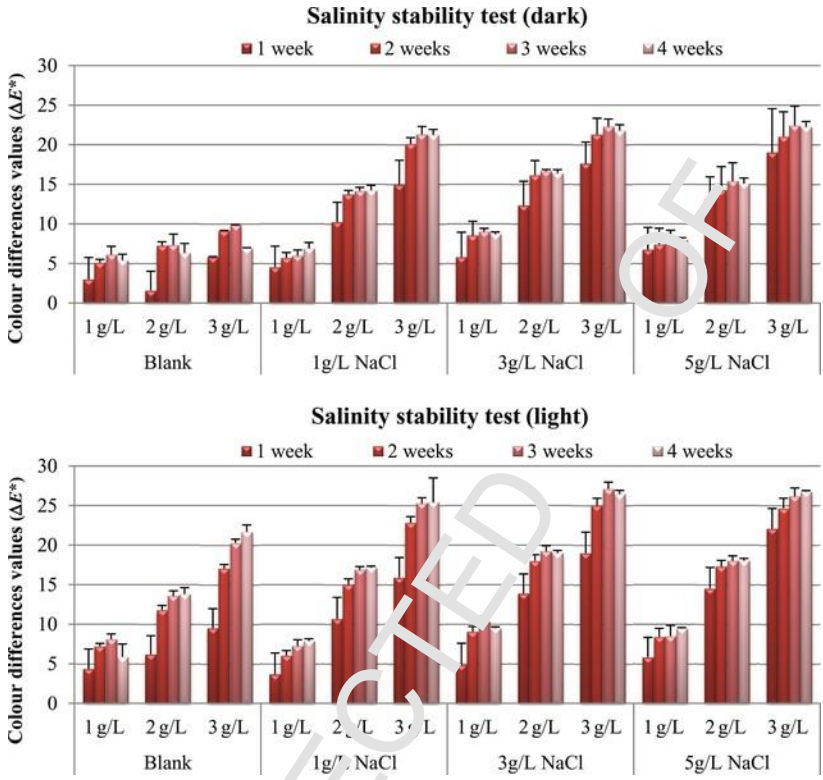

Fig. 10 Color difference values (Δ *E**) of astaxanthin pigments at different concentrations 1, 2, 3 g/L and different salinities, stored at room temperature, in dark and light conditions, measured initially and every 1, 2, 3, and 4 weeks.

pH test

Color stability is demonstrated by the chromaticity and color saturation graphs of the astaxanthin pigment, which is treated with pH 3, 5, 7, 9, and 11 and is stored in dark and light conditions accordingly throughout 4 weeks. The graphs are shown in Figs. 11 and 12, respectively. The values of astaxanthin pigment chromaticity and saturation have increased linearly with the concentration of the pigments, with those stored in light conditions showing a higher degradation rate than the pigments stored in dark conditions. The graphs also demonstrate that the pigment with pH 3 has shown the highest degradation effect compared to those treated with other pHs for both dark and light conditions.

By the end of the treatment in a dark condition, the lowest concentration of pigment treated with pH 3, 5, 7, 9, and 11 has shown 76.19%, 3.45%, 6.69%, 2.87%, and 1.92% of chromaticity changes. Then, the medium concentration of astaxanthin pigments (2 g/L) showed 77.41%, 34.00%, 16.11%, 21.04%, and 18.09% changes,

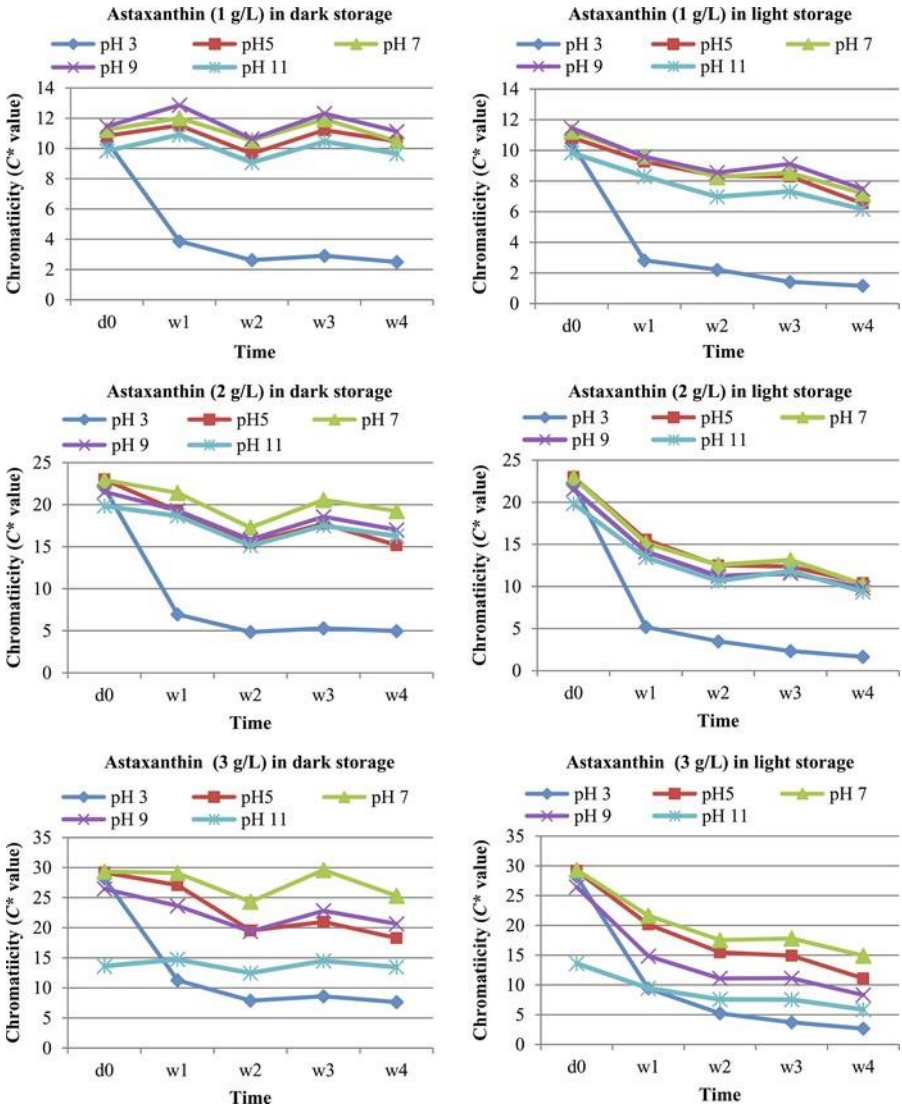

Fig. 11 Chromaticity of astaxanthin pigments at different concentrations 1, 2, 3 g/L and different pH values, stored at room temperature, in dark conditions, measured initially and every 1, 2, 3, and 4 weeks.

whereas the highest concentration of pigments (3 g/L) showed 72.94%, 37.37%, 13.69%, 21.96%, and 1.16% changes correspondingly. In contrast, the end of the treatment in light conditions resulted in chromaticity changes of 88.92%, 39.70%, 36.05%, 34.76%, and 37.45% for the lowest concentration pigments. This is followed by 92.55%, 54.79%, 55.28%, 54.39%, and 52.85% for the medium

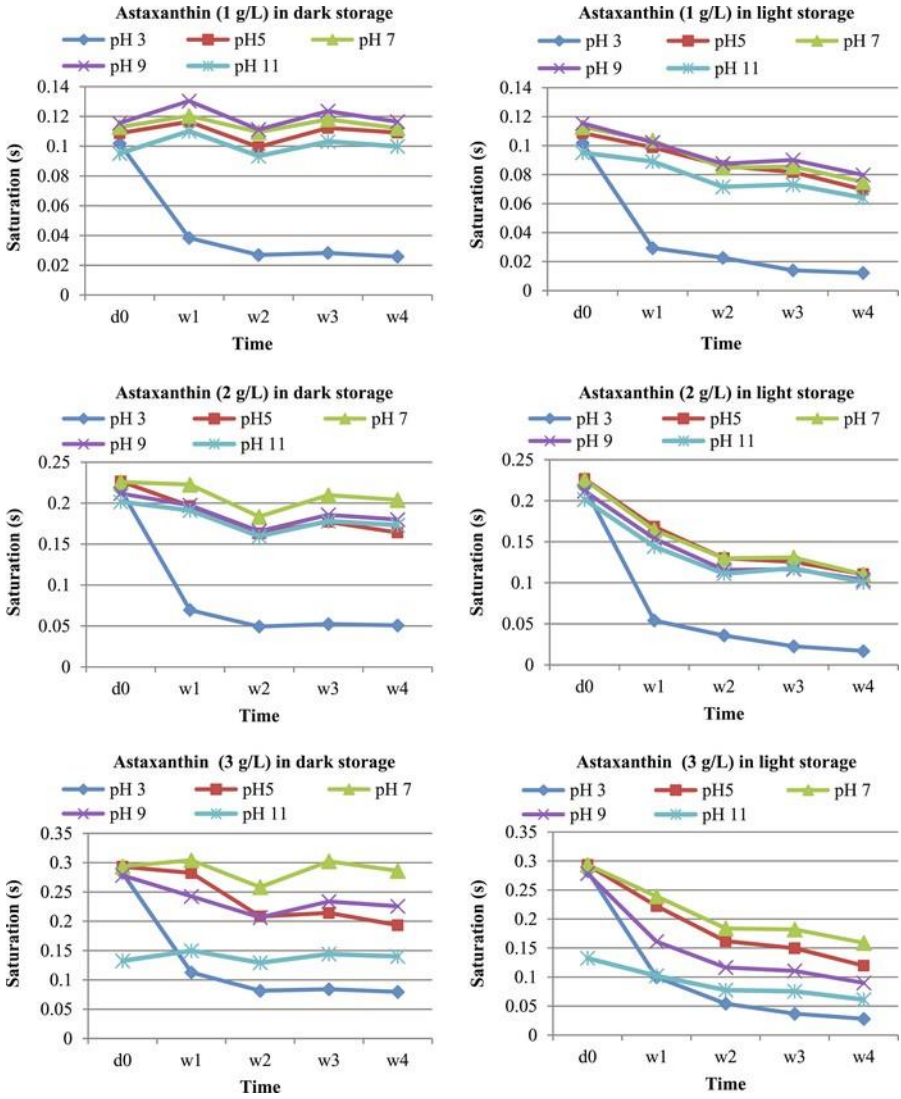

Fig. 12 Color saturation of astaxanthin pigments at different concentrations 1, 2, 3 g/L and different pH values, stored at room temperature, in dark and light conditions, measured initially and every 1, 2, 3, and 4 weeks.

concentration pigments, and 90.51%, 61.94%, 49.16%, 68.56%, and 56.92% for the highest concentration pigments. This corresponded with pH 3, 5, 7, 9, and 11, respectively. The color difference values (ΔE^*) shown in Fig. 13 have supported the result of the pH stability test, whereby the pigment has shown significant changes

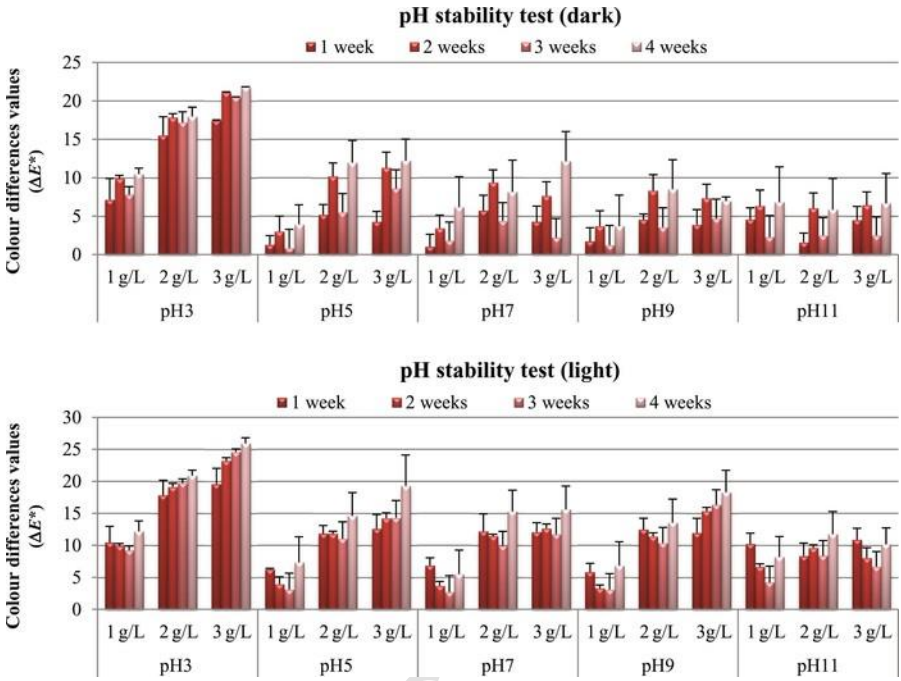

Fig. 13 Color difference values (Δ *E**) of astaxanthin pigment at different concentrations 1, 2, 3 g/L and different pH values, stored at room temperature, in dark and light conditions, measured initially and every 1, 2, 3, and 4 weeks.

when adjusted to very acidic conditions, and it showed fluctuation throughout the treatment period.

The statistical analysis performed via One-Way ANOVA for color-change difference value displayed the presence of any statistically significant difference between astaxanthin pigment concentrations, pHs, time, and storage conditions. The output indicated by the *P* value showed that there is a statistically significant difference ($P < .0001$) in the mean of the color difference value between different pigment concentrations, pHs, time, and storage conditions. The Tukey post hoc test was done showing groups differing for each other, revealing a statistically significant variance ($P < .0001$) in color difference value between the 1 g/L concentration group with 2 and 3 g/L groups. In terms of time, there are statistically significant differences ($P < .0001$) between the 4-week group and the 1-week and 3-week groups. There are also statistically significant differences ($P < .0001$), between pH 3 with each of the other pH groups.

The observed color degradation suggested that the pigment is unstable in highly acidic conditions. This might be due to trans-cis isomerization occurring with the presence of acid (Gliemmo, Latorre, Gerschenson, & Campos, 2009; Rodriguez-Amaya, 2001; Ye & Eitenmiller, 2006; Yip, Joe, Mustapha, Maskat, & Said, 2014). The result of the pH stability test is also in agreement with the studies done by

Rodriguez-Amaya (2001), Ye and Eitenmiller (2006), and Chen, Peng, and Chen (1996), where carotenoids in an alkaline condition are commonly more stable than in acidic conditions. An acidic pH is also believed to create ion pairs that form a carotenoid carbocation [e.g., Car + AH ↔ ($CarH^{+\cdots}A^{-}$) ↔ $CarH^{+}$ + A^{-}] (Boon, Mcclements, Weiss, & Decker, 2010). Besides, it can be concluded that the presence of light has a strong effect on astaxanthin stability, causing photodegradation of the pigment.

Heat test

Color stability is presented by the chromaticity and color saturation graphs of astaxanthin pigment, which are incubated in various temperatures (i.e., 4°C, 25°C, 35°C, 45°C, 65°C, 75°C, 85°C, and 95°C) for 3 hours as shown in Figs. 14 and 15. The values of astaxanthin pigment chromaticity and saturation fluctuated over 3 hours of incubation, but the changes observed are at a very minimal range. The color difference values (ΔE^*) shown in Fig. 16 supported the results of the heat stability test, where the pigment showed insignificant changes across different temperatures and periods of experiments.

The statistical analysis performed by One-Way ANOVA for the color difference value has indicated the presence of a statistically significant difference between astaxanthin pigment concentrations, temperatures, and time. The output indicated by the *P* value showed that there is no statistically significant difference by means of color difference value across different pigment concentrations, temperatures, and time. The Tukey post hoc test is performed afterward, confirming that no groups are statistically different from each other.

The minimal fluctuation trend over 3 hours at all temperatures applied showed the resistance of astaxanthin pigment toward heat. The results of this study are also supported by other research, where the pigment is found stable at 70–90°C in various carriers and is reported to degrade only at 120–150°C (Ambati et al., 2014). This may be due to esterified astaxanthin, which is more stable in nature than free astaxanthin, which has lower stability against light, oxygen, acidity, and heat. This subsequently causes oxidation, degradation, and isomerization processes (Mezzomo et al., 2013; Mezzomo & Ferreira, 2016).

UV irradiation test

The color stability of the astaxanthin pigment that is irradiated under UVA (365 nm) and UVB (312 nm) for 8 hours is exhibited by the chromaticity and color saturation graphs as shown in Figs. 17 and 18. After 8 hours of irradiation under UVA light, the chromaticity of 1, 2, and 3 g/L astaxanthin pigments show 14.54%, 38.71%, and 31.61% of degradation, respectively. Meanwhile, after being irradiated under UVB light, the chromaticity of 1, 2, and 3 g/L astaxanthin pigments shows 39.81%, 30.69%, and 52.67% of degradation, respectively. Therefore, the color difference values (ΔE^*) shown in Fig. 19 have supported the results of the UV stability test,

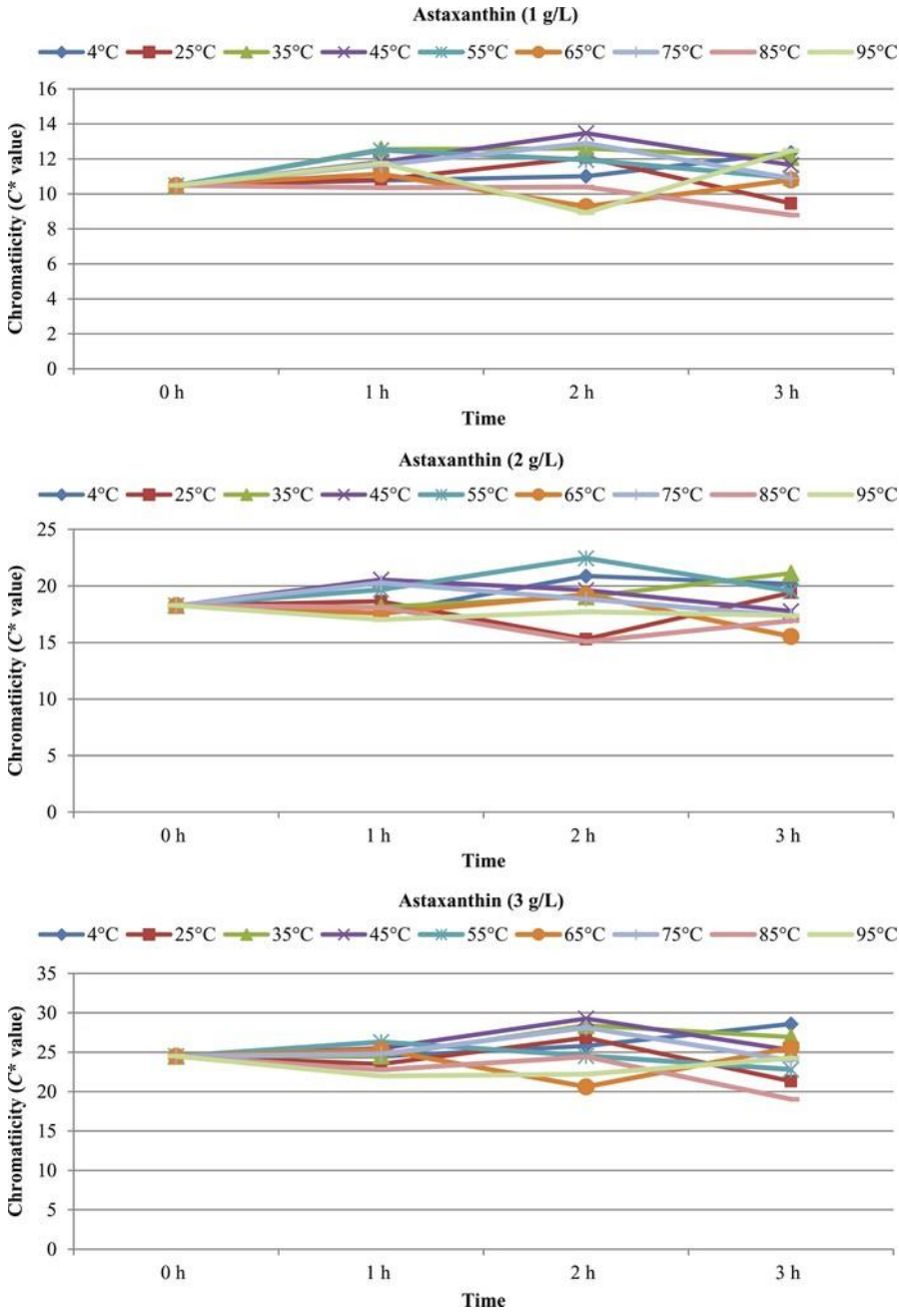

Fig. 14 Chromaticity of astaxanthin pigments at different concentrations 1, 2, 3 g/L incubated in different temperatures measured initially and every 1, 2, and 3 hours.

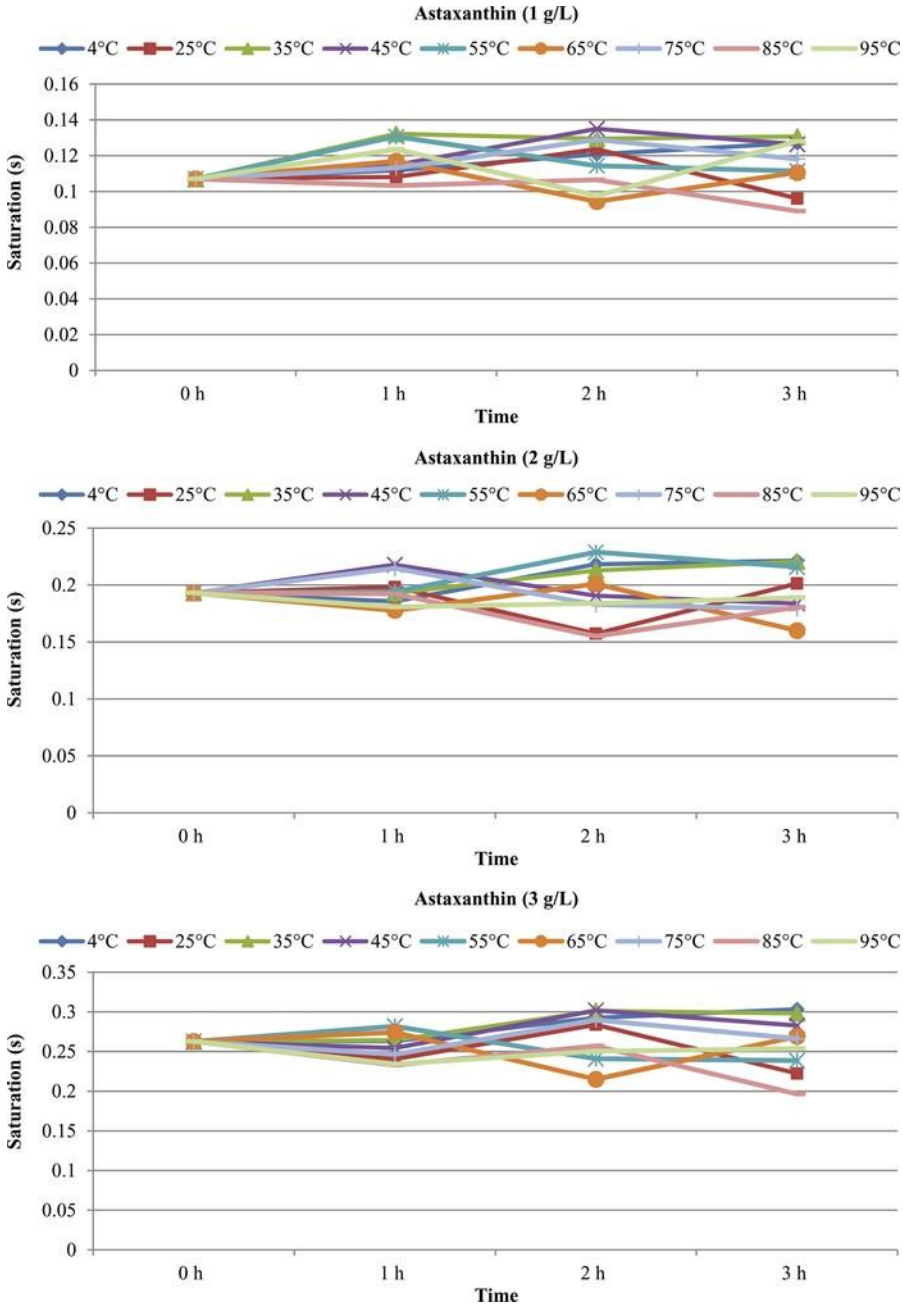

Fig. 15 Color saturation of astaxanthin pigments at different concentrations 1, 2, 3 g/L incubated in different temperatures measured initially and every 1, 2, and 3 hours.

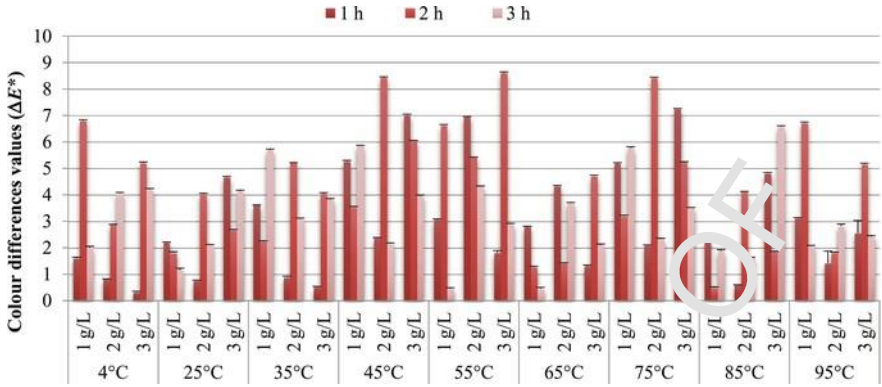

Fig. 16 Color difference values (Δ E*) of astaxanthin pigment at different concentrations 1, 2, 3 g/L temperatures, measured initially and every 1, 2, and 3 hours.

where the highest pigment concentration irradiated under UVB light shows significant changes over 8 hours of experimentation.

The statistical analysis performed using One-Way ANOVA for color-difference value showed whether there is a statistically significant difference between astaxanthin pigment concentrations, UVs, and time. The output indicated by the *P* value shows that there is a statistically significant difference ($P < .0001$), by means of the color-difference value between different pigment concentrations. Then, the Tukey post hoc test is done to show whether different groups differ from each other, revealing a statistically significant difference ($P < .0001$) in color difference value between the 1 g/L concentration group with 2 and 3 g/L groups.

Light exposure can affect the stability of carotenoids (Boon et al., 2010; Rodriguez-Amaya, 2001), as trans-cis photoisomerization may occur (Rodriguez-Amaya, 2001; Yip et al., 2014). The light absorption may also lead to the single oxygen formation resulting in the excited state of the carotenoid molecule. This may subsequently involve a chemical-degradation pathway (Boon et al., 2010; Meléndez-Martínez, Britton, Vicario, & Heredia, 2006).

Conclusion

There are several outcomes of the chromaticity-stability assessment of astaxanthin throughout different periods of exposure to pH, salinity, UV, and heat. Salinity treatment has displayed a significant effect on astaxanthin where the highest degradation rate has been observed at higher concentrations of NaCl given that they are stored in light storage, displaying 91% degradation. NaCl, as one of the known environmental stresses, can destabilize the molecular structure. The highest degradation rate has also been found in pigments stored in light conditions; at an acidic condition, astaxanthin has resulted in chromaticity changes greater than 89%. This is explained by the occurrence of tran-cis isomerization that contributes to the

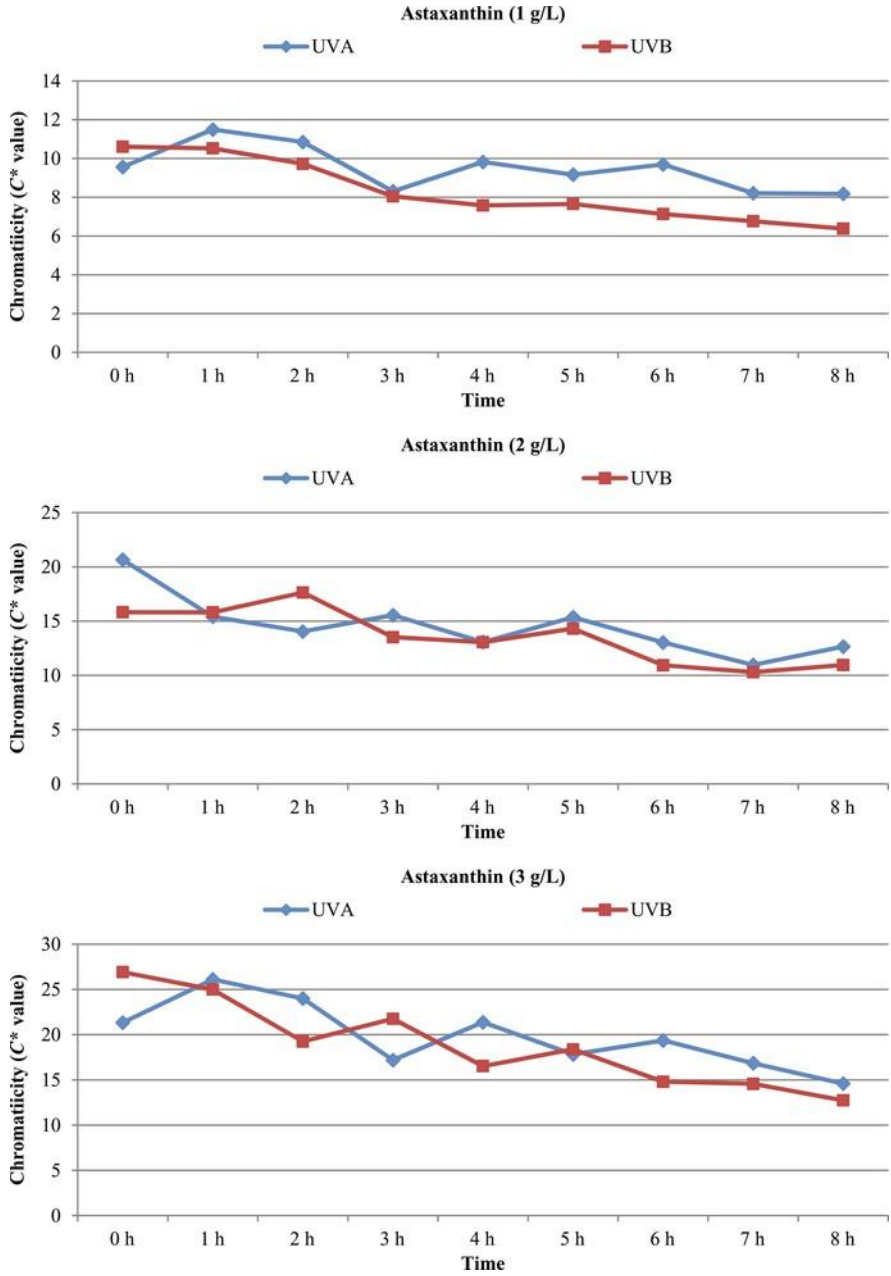

Fig. 17 Chromaticity of astaxanthin pigments at different concentrations 1, 2, 3 g/L exposed under UVA (365 nm) and UVB (312 nm) light, at room temperature, measured every hour for 8 hours.

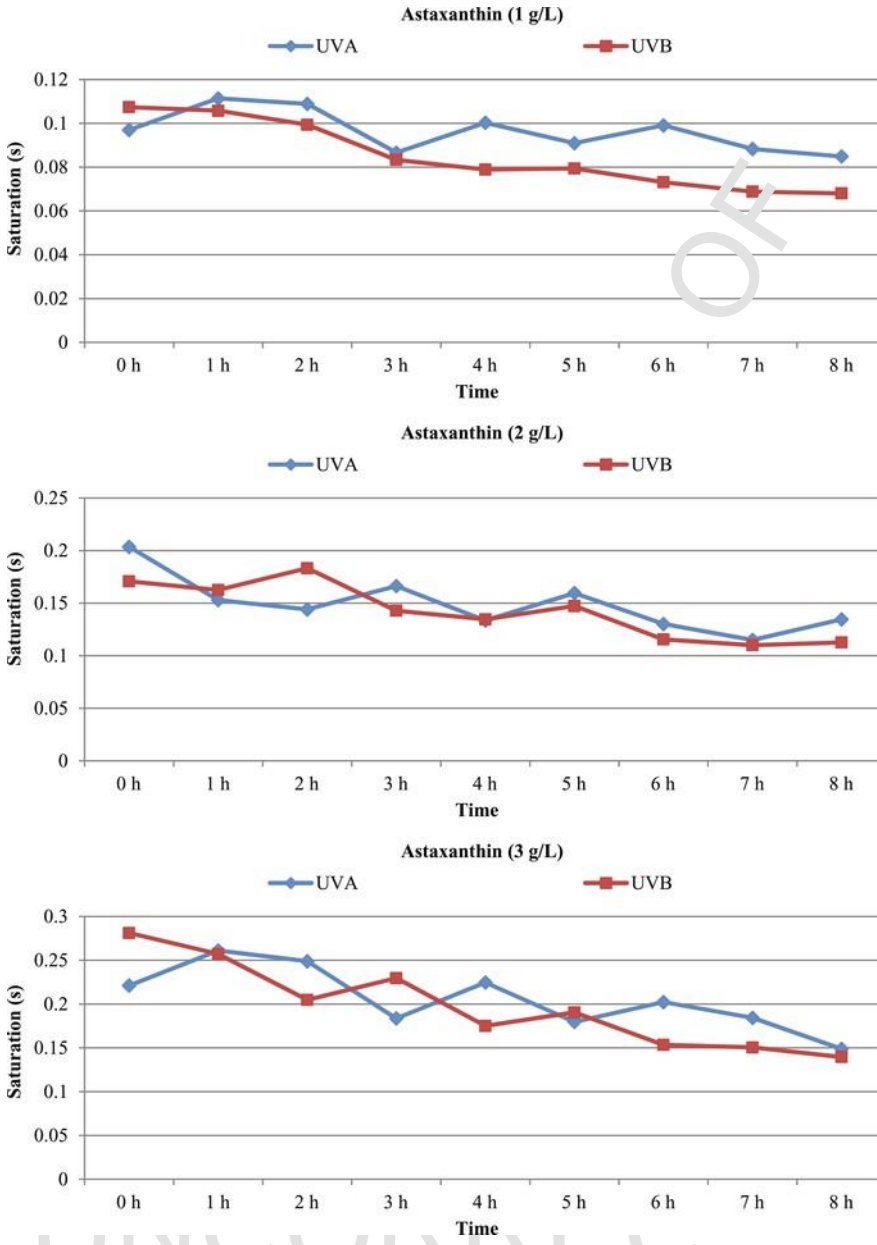

Fig. 18 Color saturation of astaxanthin pigments at different concentrations 1, 2, 3 g/L exposed under UVA (365 nm) and UVB (312 nm) light, at room temperature, measured every hour for 8 hours.

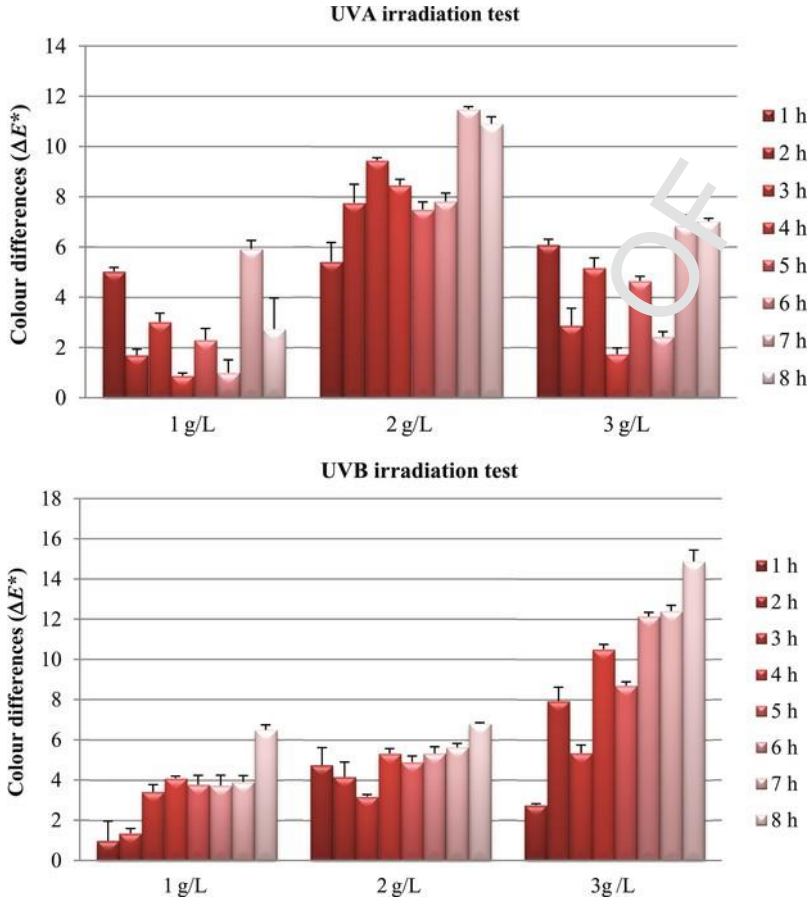

Fig. 19 Color difference values of astaxanthin pigment exposed under UVA (365 nm) and UVB (312 nm).

decoloration of the pigment. The astaxanthin pigment has shown fluctuation over 3 hours of incubation but within a minimal range, indicating its resistance against high temperature. UVA and UVB irradiation can also cause photodegradation of carotenoids. In the UV irradiation stability test, astaxanthin has shown a low degradation rate of only 40%.

Therefore, the properties of carotenoids in terms of color appearance have been correlated with various environmental factors, such as heat, light, oxygen, pH, and others. Color is also attributed to the conjugated double bonds system within their structure, which develops the light-absorbing chromophore that substantiates the carotenoid characterization and quantification. During the production, storage, delivery, and consumption of carotenoid products, various environmental stresses will be encountered and will influence the stability of the pigments. Therefore, this

study has successfully provided a basic understanding regarding the carotenoid pigment stability related to these factors.

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