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REVIEW ARTICLE

The Stability of Phycocyanin, Phycoerythrin, and Astaxanthin from Algae Towards Temperature, pH, Light, and Oxygen as a Commercial Natural Food Colorant

Bryan Ashley Goyudianto[1], Catarina Meliana[1], Debby Muliani[1], Jeslin[1], Yohana Elma Sadeli[1], Nanda Rizqia Pradana Ratnasari[2*]

[1]Departement of Food Science and Nutrition, Indonesia International Institute for Life Sciences, Jakarta, Indonesia

[2]Departement of Bioinformatics, Indonesia International Institute for Life Sciences, Jakarta, Indonesia

**Corresponding author. Email: nanda.ratnasari@i3l.ac.id*

ABSTRACT

Nowadays, food industries are exploring more about naturally-derived colorants. Algae is proposed to be an excellent alternative source for natural colorants as it needs lesser biomass. Phycocyanin, phycoerythrin, and astaxanthin are commercially used blue-green, red, and red-orange algae-sourced pigments due to their high protein yield, health benefits, and ease of extractions methods. A literature survey conducted using Google Scholar and ScienceDirect database with inclusion and exclusion criteria gained 44 papers used as primary references to assess those algae pigments' stability towards temperature, pH, light, and oxygen for food applications. Low pH levels and addition of preservatives (sugar, citric acid) or polyhydric alcohols enhance phycocyanin range of stability (pH of 5–6 and >40°C with pH >5 or <3). Phycoerythrin's stability at -20 to 4°C and neutral pH is improved by adding additives (citric acid, benzoic acid) or nanofibers, cross-linking method, complex formation, and microencapsulation. Phycocyanin and phycoerythrin's light stability depend on the light's composition, quality, and quantity; hence, utilization of dark-colored packaging to prevent light exposure is done. Astaxanthin's instability towards light exposure (causing photoexcitation), temperature of >30°C, and pH of >4 can be solved through chitosan solution coating and microencapsulation using various wall materials and complex formation. Phycocyanin is unaffected against oxygen (unlike phycoerythrin and astaxanthin), yet all of them exert antioxidant properties. Therefore, the inconsistency of these colorants' stability depending on food processing conditions demand further development through research to widen their commercial food applications.

Keywords: phycocyanin; phycoerythrin; astaxanthin; stability; natural food colorant

INTRODUCTION

Algae serves as the most abundant, accessible, and diverse source of biologically natural active metabolites due to its complex composition and valuable chemical fractions that can be utilized as the primary biomass feedstock and terrestrial biomass from forest or agricultural origin (Dominguez, 2013). Algae

refer to the aquatic, oxygen-evolving photosynthetic autotrophs, and morphologically simple organisms, ranging from microscopic and unicellular (microalgae) to macroscopic and multicellular (macroalgae) (Guiry, 2012). Based on its color, algae can be classified into several groups, such as (1) Rhodophyta (red algae) that often have brilliant color due to the domination of phycoerythrin and phycocyanin pigments; (2) Chlorophyta (green algae), which contains chlorophyll *a* and *b*; (3) Phaeophyta (brown algae) that have fucoxanthin pigment; (4) cyanobacteria (blue-green algae) which provide chlorophyll *a*, carotenoids, phycobilin, and phycoerythrin; and (5) cryptophyta or cryptomonads (algae that have plastids) that have phycobilin pigments (Matos, 2017; Domingues, 2013).

The purpose of having colorants in food and beverage products is for appearance enhancement. Kovač *et al.* (2013) mentioned that nowadays, the food and beverage industries are exploring more about naturally derived colorants due to consumer's negative perception towards artificial colorants in terms of health issues and industries' preference towards natural colorants for a few applications. Therefore, algae are proposed to be an excellent alternative source for natural colorants as plant-derived natural colorants require larger biomass. Not only widely known for its chlorophylls, but algae also provide other types of pigments, mainly carotenoids, and phycobiliproteins.

Phycocyanin and phycoerythrin are blue-green and red photosynthetic pigments belonging to the phycobiliproteins (PBP) family and found in microalgae, cyanobacteria, rhodophytes, and cryptomonads (Hsieh-Lo *et al.*, 2019). It was also stated that high protein yield, various health benefits, and their ease of extractions methods are the primary reasons for their commercial usage. Astaxanthin, a red-orange keto carotenoid pigment, can also be commercialized as food colorants due to its higher stability than other carotenoids, high tinctorial properties, safety, and health benefits (Desai *et al.*, 2016; Ambati *et al.*, 2014; Rodriguez-Amaya, 2016). Therefore, this review aims to discuss the stability of phycocyanin, phycoerythrin, and astaxanthin towards common food processing conditions (e.g., temperature, pH, light, and oxygen) as commercial natural food colorants.

METHODOLOGY

A complete search of the Google Scholar and ScienceDirect database was carried out using [TITLE-ABS-KEY "pigment name" "food colorant" "stability" "temperature" "pH" "light" "oxygen"] as the search query, which resulted in 599 documents generated after limiting the search timescale from 2010 to 2020 in Google Scholar and applying filters in ScienceDirect specifically for review articles and research articles and limiting the subject areas to agricultural, biological sciences, and chemistry. It should be noted that the result would vary if different search parameters were used. From those 599 documents, only 44 papers were chosen as the inclusion criteria are only English research articles and literature reviews that explore the stability of phycocyanin, phycoerythrin, and astaxanthin towards temperature, pH, light, and oxygen for food and beverage industry applications, including methods to improve its stability in the previously mentioned factors. Therefore, the exclusion criteria are all the literature that does not fulfill the inclusion criteria (Figure 1).

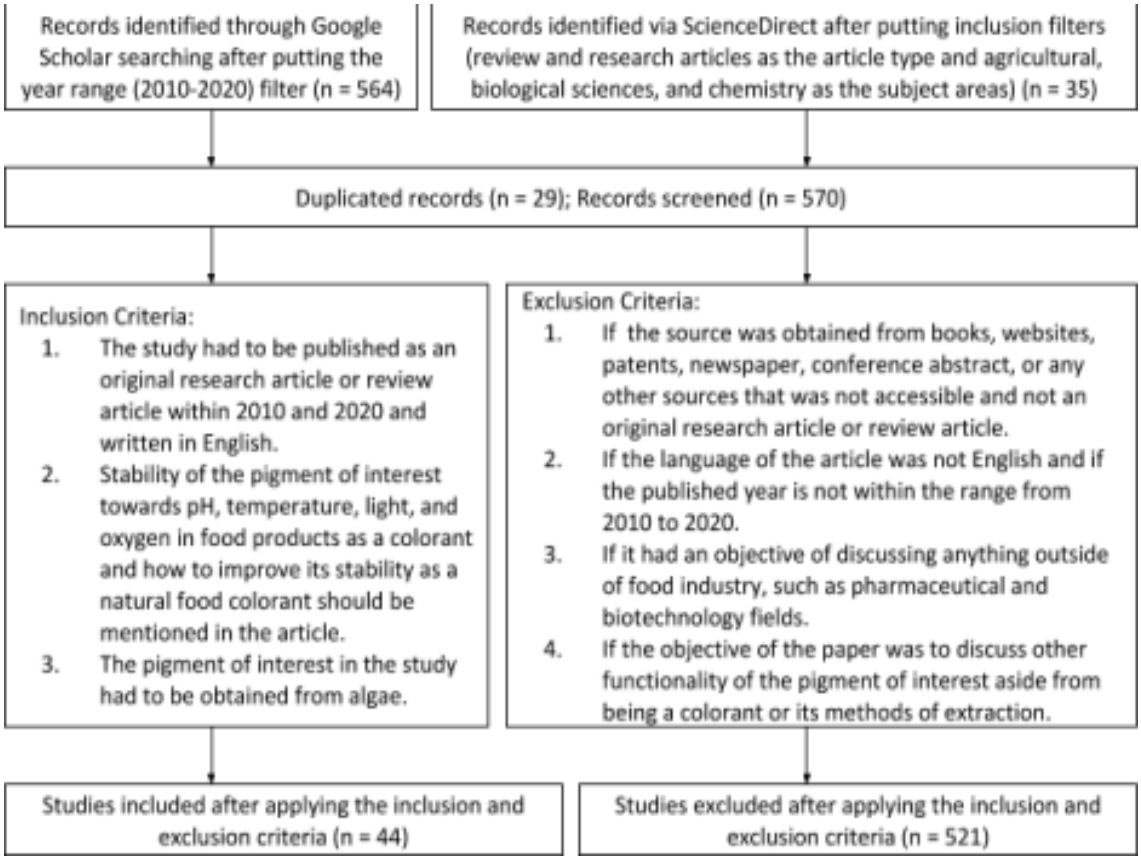

Figure 1. A flowchart of the resource selection process

PHYCOCYANIN

Phycocyanin, as the natural blue colorant sourced from cyanobacteria, has been used mainly as a colorant in health drinks, beverages, confectionery, and fermented milk products. Other than its therapeutic properties (e.g., antioxidant, anti-inflammatory, anti-viral, anti-cancer, and cholesterol-lowering effects), food-grade phycocyanin's market value is around US$ 367 per kilogram, which is relatively accessible (Kannaujiya *et al.*, 2017; Chaiklahan *et al.*, 2012). Phycocyanin is also known as a more promising natural colorant that provides a bright blue color compared to gardenia and indigo. Several challenges have been discovered as the stability of phycocyanin depends mostly on the aggregation state of the protein, which is influenced by parameters such as temperature, pH, light, and oxygen concentration (Pandey *et al.*, 2013; Kannaujiya *et al.*, 2017). Phycocyanin that will be explained is sourced from *Arthrospira platensis* (Spirulina), which is one of the major sources of phycocyanin with up to 20% protein fraction (Pan-utai *et al.*, 2017; Jurić *et al.*, 2020; Chaiklahan *et al.*, 2012).

Stability in food processing conditions

Temperature and pH – Despite it being utilized as a blue colorant in some food and beverages, phycocyanin's bright blue color is unstable to heat, minimizing its usage in food products that demand high thermal processing (e.g., cooking or sterilization). Martelli *et al.* (2014) and Sigurdson *et al.* (2017) stated that the denaturation temperature of phycocyanin at pH 7 was 57.5°C, 61.8°C at pH 5, and 49.9°C at pH 9, meaning that phycocyanin is slightly more stable in acidic than in neutral or alkaline pH.

Chaiklahan *et al.* (2012) and Lang *et al.* (2020) also reported that the highest stability of phycocyanin solution was in the pH range of 5.5 to 6.0 at 45°C for 30 mins and at low temperature (4°C). Hence, the higher the working temperature and the lower the pH, the more stable the aqueous extract of phycocyanin will be, meaning that the degradation temperature and pH were inversely proportional to the degradation of phycocyanin. Phycocyanin's discoloration from bright blue to faint blue after heating at 80°C can be minimized by forming of covalent crosslinks with bifunctional reagents, such as Dithiobis-succinimidyl-propionate (DSP). Due to its high toxicity, DSP addition can be substituted with methylglyoxal or MGO (usually in Manuka honey) to enhance the thermal stability of phycocyanin at neutral pH from 57.5°C to 62.8°C as it can stabilize the tertiary or quaternary structure of phycocyanin by forming intramolecular cross-links between two groups on a single protein (Martelli *et al.*, 2014). It was also stated that Manuka honey or other honey could further enhance the thermal stability to 90°C. Thus, choosing honey rather than MGO alone to improve phycocyanin's thermal stability is more preferred. Another discovery regarding phycocyanin's thermal stability is that its stabilization is more linked to the concentration of sugar in solution than the sugar's type as the same type of sugar exerts the same effect, yet more concentration of sugar exerts more stabilization. Hence, a large concentration of sugars dramatically raises the thermal stability and shelf life of phycocyanin, making it suitable for sweet food applications (e.g., confectionery and pastry). Hadiyanto *et al.* (2018) also proved that adding glucose or fructose could enhance activation energy up to four-fold due to polymerization of protein phycocyanin by sugar, preventing the damage to phycocyanin structure. Microencapsulation of phycocyanin can improve thermal stability (until 80°C) while increasing its shelf life as it protects the protein nativity and structure at high temperature (Pradeep & Nayak, 2019). Encapsulation of phycocyanin stabilizes the pigments in the matrix without any chemical changes due to their electrostatic interactions. Those encapsulations' best stability is at pH 6.5 (both pH 7 and 4.5 as the lowest stability), indicating that encapsulated phycocyanin is unstable at too high or low pH value. A study done by Zhang *et al.* (2020) proved that the addition of a minimum 10% of whey protein is more effective than pea and egg white proteins to solve the aggregation problem of phycocyanin at low pH (pH 3.0). It was also said that undenatured whey protein is more effective than its denatured and hydrolyzed forms, and complete whey protein is better than BSA or whey protein without glycomacropeptides in giving phycocyanin stability at low pH. This study also proves that phycocyanin encapsulation with BSA, immunoglobulins, α-lactalbumin, β-lactoglobulin, and glycomacropeptides may help the stability in low pH. Therefore, this study proves that phycocyanin can be used as a blue colorant in acidified beverages. Pan-utai *et al.* (2017) mentioned that preserving phycocyanin color after its extraction and improving its shelf life can be done by adding stabilizing agents or preservatives (e.g., glucose, sucrose, citric acid, sorbic acid, sodium chloride, ascorbic acid, and sodium azide) to protect the structure of the protein chains. This study also proves that citric acid (4 mg/mL) is more effective in protecting phycocyanin structure than calcium chloride and sucrose at 35°C because citric acid serves as a chelator that lowers the pH. Therefore, adding citric acid as edible preservatives to phycocyanin enhances its stability in high

thermal processing and extraction for food-grade applications. Based on those studies, phycocyanin structure is greatly affected by both temperature and pH, with both factors having an inverted effect. Phycocyanin can maintain its stability in the pH range of 5–6 as its protein structure is unfolded at lower pH. In terms of temperature, high thermal treatment (higher than 40^{o}C) at pH >5 or <3 is detrimental to phycocyanin's color. Hence, its stability will increase at higher temperatures when the pH levels are low (de Morais *et al.*, 2018). Adding preservatives (sugar or citric acid) or polyhydric alcohols, which act as protein-stabilizing agents in the food industry, can moderately increase this pigment's thermal stability and shelf life. In terms of production, temperature as one of the most critical factors in production of Cyanobacteria will determine the diversity and metabolic products; thus, the optimal temperature for the growth or compounds production of different organisms is needed. Temperature can also affect the respiration rate, membrane stability, and nutrient uptake. The study about the effect of temperature on the production of phycocyanin itself has not been deeply discovered. However, most phycocyanin production can be enhanced by equal or higher than 30^{o}C. Pagels *et al.* (2019) stated that *Spirulina platensis* could grow in an extensive range of temperature, yet not below 20^{o}C or above 40^{o}C as those temperatures are not suitable for growing conditions. On the other hand, the effect of pH on the Cyanobacteria production is due to its role in all metabolic activities such as the growth, biomass productivity, chemical dissociation and physiologic effects of the organism. Mainly, the optimal condition for Cyanobacteria to produce phycocyanin is under an alkaline or neutral environment. Even though pH may be fundamental to Cyanobacteria production, the studies of pH on phycobiliprotein production have received little attention.

Light – As one of the photosynthetic pigments in algae, C-phycocyanin has a function to trap light energy and transfer it to the other cell pigment during photosynthesis. The light itself also plays a role in regulating enzymatic activities and influences the microalgae cell growth and C-PC production (de Morais *et al.*, 2018). This is because the phycocyanin can absorb parts of the wavelength that are inadequately utilized by chlorophyll (Bachchhav *et al.*, 2016). Most microalgae species that grow strictly autotrophic will use sunlight as its energy source and carbon dioxide to form new organic matter, leading to a limited amount of biomass yields as the light's penetration is inversely proportional to the cell concentration. On the other hand, heterotrophic and mixotrophic microalgae may result in higher growth rates and biomass yield by using glucose, fructose, or glycerol as the energy source instead of light (Rahman *et al.*, 2019). Cyanobacteria, as the photosynthetic organisms, use light as the fundamental factor that determines their growth and survival in terms of production. Therefore, phycocyanin production is directly related and greatly influenced by the composition of the light provided to the culture of Cyanobacteria. As phycocyanin is a pigment that absorbs the yellow-red spectrum (with red as the most optimum), Cyanobacteria also require a specific range of light to guarantee their photosynthetic efficiency (Pagels *et al.*, 2019). It was also supported by Bachchhav *et al.* (2016) that LEDs that offer different wavelength distribution can be used to enhance the phycocyanin accumulation in the cells. Yellow-light emitting diodes (LEDs) have the highest C-PC production, followed by red, white, blue, and green LEDs. The study of Pagels *et al.* (2019) also stated that

the optimization of light to enhance phycocyanin production by Cyanobacteria is mainly correlated to light quality (spectra composition), intensity, or the period of exposition. In terms of light quality, it is strongly related to the wavelength and has been shown to increase the production of specific compounds, such as the supplementation of UV-B radiation in *Spirulina platensis*. Moreover, the light intensity is also one of the most important factors to achieve a sustainable cyanobacteria culture as inadequate amounts of light caused the cyanobacterium to get light and energy to grow using alternative ways. On the other hand, an adequate amount of light can lead to photoinhibition as the flux energy of light is higher than the organisms can handle, overwhelming the charge inside the cell, resulting in the increased level of ROS and creating a toxic environment that leads to cell death. De Morais *et al.* (2018) also stated that high light intensity might lead to a decreased number of chromophore proteins, resulting in fewer phycobilisomes per cell. Furthermore, photoperiod and the total light the organism receives per day is essential to determine the balance between the photoautotrophic and heterotrophic metabolisms. It has been observed that the most optimal condition for the production of phycocyanin is a photoperiod of 8 hours of dark and 16 hours of light (Pagels *et al.*, 2019).

Oxygen – Phycocyanin is not affected by oxygen as it is a protein; instead, it is more stable to oxidative stress due to its free radical scavenger properties that can neutralise the reactive molecules and decreasing the level of oxidation (Prado *et al.*, 2018; Pagels *et al.*, 2019). Hardiyanto *et al.* (2018) also reported that phycocyanin have properties to capture oxygen radicals as it contains an open tetrapyrrole chain that can bind a peroxy radical by donating its hydrogen atoms that are bonded in the 10th carbon atom of a tetrapyrrole molecule.

PHYCOERYTHRIN

Phycoerythrins sourced from microalgae, Rhodophyta, and cyanobacteria are one of the most well-known PBPs produced commercially as a natural red colorant commonly applied in confections, gelatin desserts, and dairy products (Christaki, Bonos, & Florou-Paneri, 2015). The red color of phycoerythrin is given by the chromophore (phycoerythrobilin) of the PBP, which is linked to the apoprotein by a thioether bond to cysteine residues (Hsieh-Lo *et al.*, 2019). In addition to its health benefits (antioxidant, anti-inflammatory, anti-cancer, hepatoprotective), the commercial use of phycoerythrin as a natural food colorant is of great interest due to the abundant availability of microalgae in the aquatic ecosystem, its simple growth requirements, rapid growth rate, and compliancy to controlled laboratory environment (Pandey *et al.*, 2013). However, the stability of phycoerythrin against temperature, pH, light, and oxygen has limited their applications in foods, which were discussed further in the following paragraphs, along with ways to improve their stability.

Stability in food processing conditions

Temperature and pH – The research from Pereira *et al.* (2020), which extracted phycoerythrin from red algae (R-PE) *Gracilaria gracilis* showed that more accentuated degradation of phycoerythrin occurs at room temperature, 40°C, pH of 4 and 8. In contrast, the phycoerythrin sample is more stable at a pH of 6.9 and -2°C (Pereira *et al.*, 2020). This result aligned with a study from Hsieh-Lo *et al.* (2019)

and Kannaujiya & Sinha (2016), where R-PE from *Grateloupia turuturu*, B-PE from Bangiales, and C-PE from *Nostoc sp.* were found the most stable at 4°C. Specifically, Hsieh-Lo *et al.* (2019) showed that both R-PE and B-PE could stabilize at 4°C for 48 hours and -20°C for two weeks. A slight difference was found in R-PE from *Portieria hornemannii*, where it can only tolerate temperature up to 30°C (Senthilkumar *et al.*, 2013). It also can remain stable at temperature up to 40°C, but slow degradation still occurs, leading to the loss of stability. In comparison, other research from Pumas *et al.* (2012) showed that C-PE from *Leptolyngbya sp.* can retain up to 80% stability at 60°C for 30 minutes along with its antioxidant activity. In addition to optimum pH, Hsieh-Lo *et al.* (2019) also showed that phycoerythrin from *Grateloupia turuturu* (R-PE) and cyanobacteria (C-PE) were most stable at a pH of 7. Findings from Nowruzi *et al.* (2020) showed that phycoerythrins in low pH conditions could denature them into individual subunits, and fluctuations in the pH value can change the phycoerythrin chromophore structure, decreasing its stability. Surprisingly, the formation of hexameric structure in phycoerythrin obtained from Bangiales (B-PE) is stable in pH 4.0 - 10.0, making it more applicable in the food industry (Hsieh-Lo *et al.*, 2019). As stated by Hsieh-Lo *et al.* (2019), Nowruzi *et al.* (2020), and Senthilkumar *et al.* (2013), preservatives like citric acid and glucose in phycoerythrin will improve its thermal stability. This was strengthened by research from Mishra *et al.* (2010), where C-PE in both with and without preservatives were being compared at temperatures of 0 ± 5°C and 35 ± 5°C. Different types of preservatives were used, such as citric acid, sodium chloride, calcium chloride, and sucrose. The result showed that after 45 days of incubation at 4°C, citric acid has the highest stability at 0 ± 5°C and declined stability at 35 ± 5°C in comparison with other preservatives. The same topic is also done in Kannaujiya & Sinha (2016), where C-PEs from *Nostoc sp.* were compared with and without preservatives. Treated PEs will be added with 0.5 mM, 2.5 mM, and 5 mM of citric acid, sucrose, ascorbic acid, and benzoic acid. All C-PEs samples will be incubated for 30 days at 4, 25, and 40°C. The result indicates that the rate of degradation in treated C-PEs was lower compared to control C-PEs (without preservative), where the rate declined as the concentration of preservative increased at all temperatures. Even though all preservatives used possess a decline in the degradation rate, C-PE with 5 mM benzoic acid had the lowest degradation rate at all temperatures. Furthermore, Kannaujiya & Sinha (2016) also showed that ascorbic acid and calcium chloride have the lowest stability than other preservatives. Hsieh-Lo *et al.* (2019) also stated that using glucose, sucrose, and sodium chloride significantly increases the phycoerythrin thermal stability than sorbic acid and sodium azide. The use of preservatives in PE for food application is considered essential as phycoerythrin is very sensitive to temperature, and the processing itself needs to be commercially viable (Nowruzi *et al.*, 2020). Besides, Hsieh-Lo *et al.* (2019) also stated that nanofibers are also used to improve the phycoerythrin stability by decreasing the system enthalpy and forming multiple unions polymer. Other non-additives methods such as cross-linking method, complex formation, and microencapsulation are also done to increase phycoerythrin's thermal stability. The mechanism of the cross-linking method to improve stability is by reinforcing the folded structure of the protein. Similar to cross-linking, complex formation enhances phycoerythrin's color and stability by covalent bonds between

proteins and polysaccharides. Lastly, microencapsulation works by controlling the release of bioactive compounds. However, this method is hard to achieve, as it requires high encapsulation efficiency (Hsieh-Lo *et al.*, 2019).

Light – In an experiment conducted by Munier *et al.* (2014), phycoerythrins obtained from Bangiales (B-PE) and red algae Rhodophyta (R-PE) were exposed to light and discovered that long periods (> 48 hours) exposure to light caused a decrease in saturation and stability as the pigments are more likely to lose their chromophores. They also mentioned that the pigments were stable for up to 8 h of exposure to light as more than half of the absorbance intensity was reduced after 6 hours of light exposure. The photochemical stability of R-PE can be improved by its incorporation in gelatin-based films, which induces protein-protein interactions that provide a suitable environment for R-PE as it mimics the natural environment in algae, resulting in up to 8 months of R-PE remaining photochemically stable under ambient conditions (Bharmoria *et al.*, 2020). Alternatively, the utilization of amber/dark-colored bottles during packing to protect against light may improve the stability of C-PE and R-PE (Mishra *et al.*, 2010). Upon exposure to ultraviolet (UV) radiation, phycoerythrin exhibited bright-yellow fluorescence, which enabled exploitations of this property through their applications in lollipops, soft drinks, hard sugar-drop candies, and alcoholic beverages (Mandal, Chanu, & Chaurasia, 2020). However, Rastogi, Sonani, & Madamwar (2015) reported that UV radiances significantly affect the integrity of phycoerythrin and phycocyanin, specifically, the bilin chromophores that are covalently attached. It was also mentioned that UV-B (280–315 nm) severely and rapidly inhibited the fluorescence activity of both phycoerythrin and phycocyanin compared to UV-A (315–400 nm) and photosynthetically active radiation (PAR). Additionally, UV-B exposure almost eliminated the chromophore bands associated with α and β-subunit of PE, leading to a 96.8% decrease in the content within 5 hours. This phenomenon can occur due to UV-B having absorption maxima in the range of short wavelength UV radiation, which directly affects the vital cellular components. On the contrary, UV-A and PAR indirectly induce DNA damage by generating singlet oxygen (O_2) or reactive oxygen species (ROS) through indirect photosensitizing reactions.

Oxygen – C-PE was also observed to be extremely sensitive towards oxygen, leading up to 90-95% loss (Mishra *et al.*, 2010). However, Sonani *et al.* (2014) reported that C-PE demonstrated an antioxidant activity due to their native folding, enabling it to scavenge reactive oxygen species (ROS) through redox reaction.

ASTAXANTHIN

Astaxanthin or 3,3'-dihydroxy-β-β'-carotene-4-4'-dione is a xanthophyll carotenoid found in a wide variety of microorganisms (green and red algae, fungi, bacteria) and marine animals. Due to its antioxidant activity, astaxanthin consumption has been linked to prevent and reduce the risk of various diseases in humans. Thus, the use of astaxanthin as a nutritional supplement is rapidly growing in foods, feeds, nutraceuticals, and pharmaceuticals. Not limited to its health benefits, astaxanthin has also been used in industries as pigmentation and additives. When using astaxanthin, emulsions are needed for dissolving it in food matrices. However, emulsions and their unsaturated

properties will decompose astaxanthin easily when exposed to heat, light, and oxygen (Ambati *et al.*, 2014; Mezquita *et al.*, 2013; Mezquita *et al.*, 2020).

Stability in food processing conditions

Temperature – According to Ambati *et al.* (2014), astaxanthin derived from *Haematococcus* was stable at temperatures between 70-90^{o}C when stored in rice bran, gingelly, and palm oil carriers with a retention rate of astaxanthin between 84 - 90%. When the temperature is further elevated to 120 and 150^{o}C, astaxanthin content is decreased. These stability conditions contrast with astaxanthin extracted from *Phaffia rhodozyma*, which had higher stabilities at lower temperatures in pH 4. The stability of astaxanthin from this source was shown to be amplified when stored at 4^{o}C and 25^{o}C when present in a complex mixture form with hydroxypropyl-β-cyclodextrin and water (Ambati *et al.*, 2014). Another paper by Soukoulis & Bohn (2017) which reviewed the utilization of micro- and nano- encapsulation of carotenoids, mentioned similar information where encapsulated astaxanthin complexed with β-cyclodextrin showed a 7-9 fold increase in stability towards heat, UV light, and oxygen. The fold increase in astaxanthin's stability depends on the structure of the cyclodextrin structure, specifically depending on the ring size or hydroxypropylation/methylation. The study also stated that increasing the storage temperature of astaxanthin nanodispersions by 10^{o}C increased its rate of oxidation, depending on the identity of stabilizers used in the nanodispersion. For example, nanodispersions stabilized with tween 20 and sodium caseinates experienced a 7- and 24- fold increase in astaxanthin oxidation, respectively. Hence, the addition of free radicals scavenging agents, such as antioxidants, effectively prolongs the shelf life of astaxanthin nanodispersions (Soukoulis & Bohn, 2017). Mezquita *et al.* (2013) conducted a study to comprehend the effects of cold storage on astaxanthin oleoresin's stability when applied to different types of milk. The study found that storing milk added with astaxanthin at a temperature of 5 ± 2^{o}C for 7 days showed no significant color changes in any of the 3 color matrices. It was also found that there was an inverse correlation between the pigment retention rate with the fat level of the milk (Mezquita *et al.*, 2013). Another similar study done by the same authors (2014) assessed the viability of astaxanthin oleoresin and its stability when applied to yogurt. It was found that astaxanthin remained stable throughout the 4 weeks test period with no significant color changes in any of the 3 color coordinates (Mezquita *et al.*, 2014). Özkan & Bilek (2014) discussed the potential application of microencapsulation procedures for astaxanthin. This approach is discussed due to the proneness of *trans* carotenoids, such as astaxanthin, to undergo isomerization to their *cis*- state in response to high temperature conditions (Özkan & Bilek, 2014). This information is strengthened by another research conducted by Saini *et al.* (2018), who found that astaxanthin extraction at temperatures above 30^{o}C resulted in a higher proportion of the *cis*-isomer produced (Saini *et al.*, 2018). The utilization of microencapsulation will protect the biological activity of astaxanthin from environmental factors, hence enhancing the physicochemical stability of the pigment (Özkan & Bilek, 2014). This information is supported by the study done by Bustos-Garza *et al.* (2013), which assessed the stability of astaxanthin in different materials. It was found that the depletion in the concentration of astaxanthin increases as temperature rises. The study results showed that astaxanthin

encapsulated with 100% whey protein retained the most concentration of astaxanthin when exposed to temperatures of 30, 40, and 50°C. The mechanism of protection by whey protein is due to the formation of complexes between astaxanthin and protein through hydrophobic interactions, which may shield the astaxanthin from degradation (Bustos-Garza *et al.*, 2013). Another research utilizing gelatin-cashew gum complex to encapsulate astaxanthin indicated a significantly improved stability compared to a non-encapsulated sample. The study showed that encapsulated astaxanthin contained 47% of the initial level by the end of the storage period, while non-encapsulated astaxanthin contained 0% of the initial level. In addition to the prolongation of astaxanthin's shelf-life, the encapsulation also improved the coloring capacity when applied to yogurt. Hence, the encapsulation of astaxanthin extended the shelf-life of astaxanthin and refined its coloring capacity (Gomez-Estaca *et al.*, 2016).

pH – In addition to temperature stability, Bustos-Garza *et al.* (2013) also assessed the pH stability of astaxanthin after microencapsulation using whey protein microcapsules. The study showed that microencapsulated astaxanthin is the most stable at pH 6 with a half-life value of 170 hours, and least stable at pH 3 with a half-life value of 61 hours at 25°C. The authors speculated that strong hydrophobic interaction between the pigment, the whey protein matrix, and the emulsifier is closer to the isoelectric point of whey protein, which affected the pigment coating. Hence, extreme pH values degrade the pigment and alter astaxanthin's stability (Bustos-Garza *et al.*, 2013). Another study provided by Zhou *et al.* (2018) stated that astaxanthin was more stable at low pH and became unstable in alkaline media (Zhou *et al.*, 2018). This information can be strengthened by two different studies, which highlighted that astaxanthin is highly stable at pH 4 and suitable for application in orange juice and skimmed milk due to its ascorbic acid content (Ambati *et al.*, 2014; Anarjan & Tan, 2013). Other than lowering the pH of the orange juice, ascorbic acid also possesses radical scavenging activity, retarding free radical-induced astaxanthin autoxidation (Anarjan & Tan, 2013).

Light – Mezquita *et al.* (2020) conducted research highlighting the effect of light and dark storage conditions on astaxanthin degradation. The study showed that samples exposed to light stress had greater astaxanthin degradation rates than samples stored in dark conditions; this is due to the promotion of cis-trans isomers formation through photoexcitation, leading to rupturing of double bonds, and then leading to photodestruction of the molecule. The molecule's photodestruction will ultimately signify that the pigment lost its color (Mezquita *et al.*, 2020). Another review by Soukolis & Bohn (2017) discussed the potential of astaxanthin coating using chitosan solution in glacial acetic acid. The coating was shown to be able to improve the chemical stability of astaxanthin under different storage conditions, showing high astaxanthin retention after exposure to light and oxygen (Soukoulis & Bohn, 2017).

Oxygen – A study conducted by Bassijeh *et al.* (2020) assessed the viability of using complex coacervates of whey protein isolate and Persian gum as emulsion stabilizers of encapsulated astaxanthin. The study revealed that encapsulated astaxanthin emulsions stabilized with a 1:4 ratio of whey protein isolate to Persian gum led to emulsions more stable to oxidative degradation (Bassijeh *et al.*, 2020). The mechanism of action of the multilayer emulsions

was believed to be caused by the formation of an impervious layer of Persian gum around the particles, protecting astaxanthin from oxidative damage. In addition to the refined oxidative stability, the ratio of 1:4 whey protein isolates to Persian gum can also produce emulsions where it enables their color retention characteristics better than the non-encapsulated astaxanthin (Bassijeh *et al.*, 2020). In comparison to the other types of carotenoids, astaxanthin has a stronger antioxidant activity. This is due to its chemical structure that contains 13 unsaturated conjugated double bonds made from non-paired electrons that have active electronic effects. Besides providing electrons for free radicals, they can also combine with free radicals forming harmless adducts, thus eliminating free radicals and/or terminating the chain reaction of free radicals. Besides, AST's superior position in the cell membrane also contributes to the stronger antioxidant activity as long chains of alkaline can capture free radicals between the phospholipid bilayers on the cell membrane. It also coincides with the hydroxyl and ketone groups on the ion one rings at both ends of the AST molecule endow it with high antioxidant activity leading to scavenging both free radicals inside and outside the cell membrane (Zhao *et al.*, 2019).

CONCLUSION

Pigments derived from algae as alternatives of commercial natural food colorants display an excellent potential due to their therapeutic effects and tinctorial properties. Phycocyanin, providing a bright blue color, is greatly affected by high temperature and high pH. It remains stable at a pH range of 5-6, yet not at higher than 40^{o}C with pH >5 or <3. Hence, low pH levels and the addition of preservatives (sugar or citric acid) or polyhydric alcohols can improve thermal stability. Phycocyanin's light stability mainly depends on the composition, quality, and quantity of light. It is a protein (i.e., unaffected by oxygen), more stable to oxidative stress due to its free radical scavenger properties.

Phycoerythrin, a widely used natural red colorant, is most stable at low temperatures (-20 to 4°C) and neutral pH, which can be improved by adding additives (citric acid and benzoic acid) or nanofibers, cross-linking method, complex formation, and microencapsulation. Utilization of dark-colored packaging is done to prevent light exposure as phycoerythrin only has 8 hours of light stability. Its fluorescence activity can be further inhibited by UV-B exposure rather than UV-A and PAR.

Astaxanthin's industry application is still limited, as its stability is heavily impacted by external conditions (temperature, light, and oxygen). It is the most stable at 30°C and pH of 4, gradually deteriorating with rising temperature and pH. Color loss due to photoexcitation occurs when light is present during storage. Using chitosan solution coating, microencapsulation with various wall materials and complex formation can improve its stability, enhancing its retention rate. As phycoerythrin, astaxanthin is also very sensitive to oxygen despite its relatively stronger antioxidant activity than other carotenoids.

There is still a lack of research on these pigment's stability in terms of food application usage. Future research should focus on new sustainable ways to stabilize these algae-derived pigments to widen their commercial use as a natural food colorant.

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