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RESEARCH PAPER

Extraction of natural pigments from marine algae

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استخلاص ملوّنات طبيعية من طحالب بحرية

تنوير علام ولبنى نجم وأحمد الحراصي

Abstract. The pigment content in microalgae is a specific feature of each species. Pigments from natural sources are gaining more importance mainly due to health and environmental issues. Algae contain a wide range of pigments. Three major classes of pigments are chlorophylls, carotenoids (carotenes and xanthophylls) and phycobilins (Phycocyanin and phycoerythrin). Phycocyanin and phycoerythrin belong to the major class of phycobilins photosynthetic pigment while fucoxanthin and peridinin belong to carotenoid group of photosynthetic pigment. Macro- and microalgae (including cyanobacteria) have been recognized to provide a wide diversity of metabolites including pigments for energy capture and photo-protection.

Keywords: Chlorophyll; phycobillins; microalgae; cyanobacteria; pigments

المستخلص: للصبغيات في الطحالب المجهرية ميزة خاصّة تميّز كل نوع منها. والملوّنات الطبيعية تكتسب أهميّة كبيرة بسبب آثارها الإيجابية على الصحّة والبيئة. تحتوي الطحالب على أنواع كثيرة من الصبغيات. ثلاثة أكبر أقسام الصبغيات هي: كلوروفيلات (اليخضور)، كاروتينويدات (كاورتين، زونتفيل) وَ فيكوبيلينات (فيكوسينين ، فيكوارترين). فيكوسينين وَ فيكوارترين تنتميان إلى القسم الأكبر من صبغيات فيكوبيلينات التمثيل الضوئي. كما أنّ فيكوزونتين وَ بريدينين تنتميان إلى مجموعة صبغة كاروتينويد التمثيل الضوئي. الطحالب الكبرى والمجهرية بما فيها البكتيريا الزرقاء (سيانوبكتيريا) تُعرف بقدرتها على توفير كميّة متنوّعة من المستقلبات بما فيها صبغيات التقاط الطاقة و الحماية بفعل الضوء.

الكلمات المفتاحية: كلوروفيلات (اليخضور)، فيكوبيلينات، الطحالب المجهرية، البكتيريا الزرقاء (سيانوبكتيريا)، الصبغيات.

Introduction

There are two types of pigments: natural and synthetic. Synthetic pigments are mainly coal tar derivatives made from chemicals which are by products of coal distillation. Many synthetic dyes are controversial and banned in many countries for use in food products because of safety concerns. Use of these synthetic pigments in personal care products also adds health risks. Some of these dyes contain impurities like lead acetate which are toxic to nervous system. Some commonly used synthetic pigments are allergens, irritants and some others are known carcinogens. Thus, there is an increasing demand for natural pigments percieved as less toxic for use in food products, pharmaceuticals and cosmetics. Many plants contain dyes and pigments (other than chlorophyll) which may serve as colorants and may have other roles e.g.; in photosynthesis, insect attractants etc. Natural pigments represent an apparently more sustainable sources of colorants than synthetic counterparts.

Other than higher plants (Angiosperm and gymnosperm), microalgae are good alternatives of carotenoids and phycobiliproteins for natural colors. Microalgae belong to an heterogenous group of microorganisms. Microalgae are small, unicellular monocellular or multicellular, autotrophic, colorful and grow generally in water and they may be either eukaryotic or prokaryotic. Production of pigments from microalgae has a number of advantages such as cheaper and easy production, easier extraction, higher yields, no lack of raw materials and no seasonal variations. The status of microalgal applications in aquaculture, food, speciality chemicals and environmental applications has been reviewed (Apt and Behrens, 1991; Muller-Feuga, 2000; Pulz et al., 2001; Benemann et al., 2002). In this review our focus is on extraction of microalgal pigments as natural colors, factors affecting their yield, extraction methods and their applications. Major pigments of the microalgae which are used as pigments are carotenoids and phycobiliproteins.

The pigments are characteristic of certain algal groups as indicated in Table 1 (Dring 1982). Chlorophylls and carotenes are generally fat soluble molecules that can be extracted from thylakoid membranes with organic solvents such as acetone, methanol or dimethyl sulfoxide The phycobilins and peridinin, in contrast, are water soluble and can be extracted from algal tissues after the organic solvent extraction of chlorophyll in those tissues.

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Objectives

Main objective of this work is to summarize the Natural Pigments from Marine species of microalgae.

Common Algal Pigments

The following pigments are industrially important products.

Chlorophylls

This photosynthetic green pigment is mainly derived from *Chlorella* spp. Chlorophyll as a food colorant is found to exhibit anti-mutagenic property (Fig.1,2). This is accomplished by inducing production of Carcinogen Detoxifying Enzymes, and thereby reducing the risk of cancer.

β-Carotene

Dunaliella salina a halophilic green algae is used for β -carotene production. This pigment is used mainly as food colorant that imparts a Yellow-Orange color. Apart from its use as a colorant, *D. Solina* is used popularly as a nutraceutical additive because it is rich in *Vitamin A*.

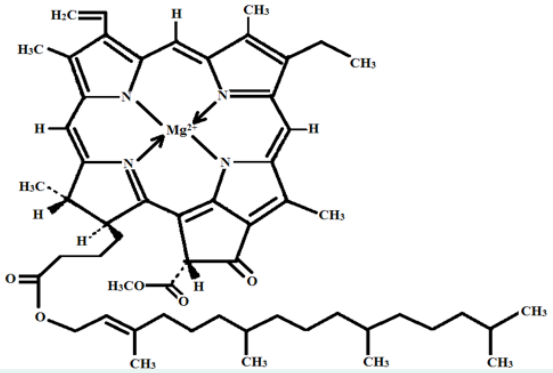

Figure 1. Chemical structure of Chlorophyll *a*.

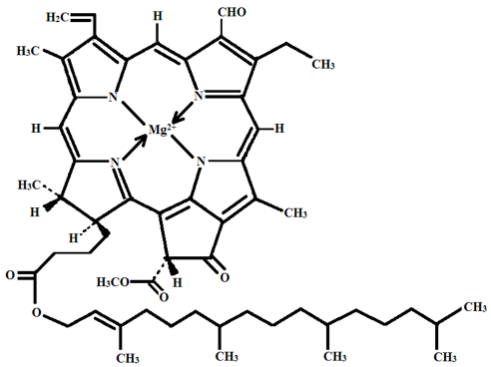

Figure 2. Chemical structure of Chlorophyll *b*.

Fucoxanthin

This pigment, derived from Phaeophytes, is used for coloring food products brown. This fat reducing properties are well documented.

Peridinin

Peridinin is a light-harvesting Apocarotenoid, a pigment associated with chlorophyll. The most popular algal source of this pigment is the dinoflagellate, *Amphidini-*

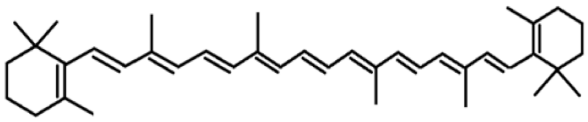

Figure 3. Chemical structure of β-Carotene.

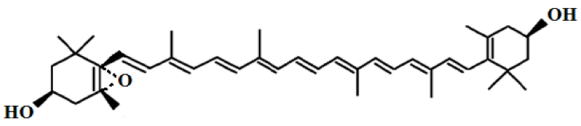

Figure 4. Chemical structure of Antheraxanthin.

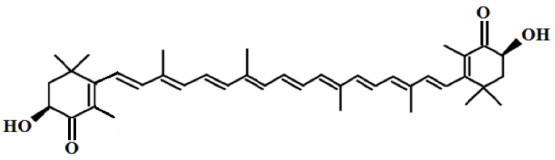

Figure 5. Chemical structure of Astaxanthin.

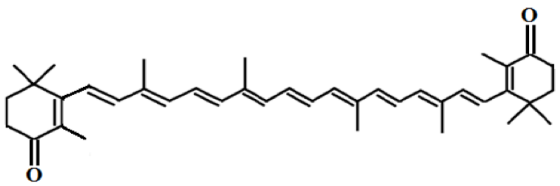

Figure 6. Chemical structure of Cantaxanthin.

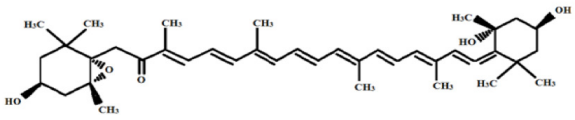

Figure 7. Chemical structure of Fucoxanthinol.

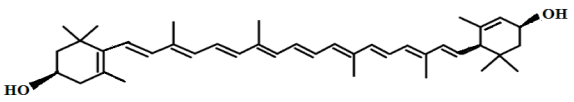

Figure 8. Chemical structure of Lutein.

um carterae (Hofmann et.al, 1996) but is found in many other species.

Phycoerythrin

Red pigment, phycoerythrin is extracted from red algae (Rhodophyta). The species most commonly used for phycoerythrin production is *Porphyridium cruentum*. It is cultured in artificial seawater with added Potassium Nitrate and optimum temperature of growth for Porphyridium is 21°C.

Phycocyanin

Blue pigment, Phycocyanins are derived from blue green algae (Cyanophyta). The most popular algal source of this pigment is *Spirulina platensis*. It requires an alkaline pH range of 7.2 to 9.0 and a salinity of 30 g/L. In the wild, *Spirulina* grows at 27°C.

Extraction of Algal Pigment

Chlorophylls and carotenoids are generally fat soluble molecules and can be extracted from thylakoid membranes with organic solvents such as acetone, methanol or dimethyl sulfoxide.

The phycobilins (Phycoerythrin & Phycocyanin) and peridinin, in contrast, are water soluble and can be extracted from algal tissues after the organic solvent extraction of chlorophyll in those tissues.

Chlorophylls

Industrial extraction of these pigments involves homogenization (disintegration) of algal biomass, followed by solvent treatment using an organic solvent mixture (Chloroform-Hexane-Ether-Methanol) (Jaffrey and Humphrey, 1975; Strickland and Parsons, 1968; UNESCO, 1966; Mackinney, 1941; Porra et. al. 1989; Lichtenthaler and Wellburn, 1983; Kaczmar, 2004). Pigments can be extracted from seaweeds by a variety of techniques. It is important to note that light, heat, extremes of pH, and oxygen cause the destruction of pigment extracts. The extracts should be kept cold and worked with in the lowest light possible throughout the procedure. The rationale behind the extraction techniques is to disrupt cell integrity as much as possible, thereby removing

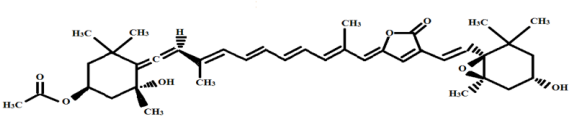

Figure 9. Chemical structure of Peridinin

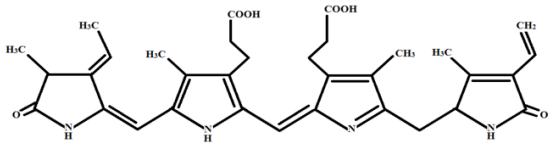

Figure 10. Chemical structure of Phycoerythrin

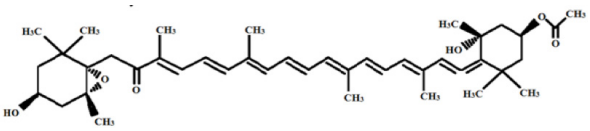

Figure 11. Chemical structure of Chemical structure of Fucoxanthin.

pigment molecules from intrinsic membrane proteins. Freezing the tissue with liquid nitrogen, and grinding the still frozen tissue in with a mortar and pestle or blender, overcomes some of the problems of working with material that produces large amounts of viscous polysaccharides. "Freeze-thawing" tissue also breaks down cellular membranes, but may liberate more polysaccharides. Finely ground tissue can be then homogenized in organic solvent to further disrupt cellular membranes, and to liberate pigment molecules from the light harvesting pigment protein complexes.

Once the pigments are extracted into appropriate

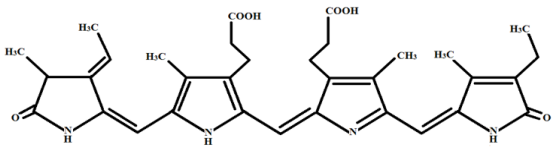

Figure 12. Chemical structure of Phycocyanin.

solvents they can be separated chromatographically by TLC or HPLC for spectral analysis and identification. Pigment concentrations in hydrocarbon solvents can be estimated with various emparical formulae linking absorbances at different wave lenghts to concentrations. However, these formulas are predictive and may overestimate some pigment concentrations (Seely et al. 1972). Uncoupling pigments from the pigment binding proteins can change the absorption patterns of the pigments, resulting in shifts in maxima from 10 to 50 nm, when compared with spectra measured for intact tissues.

Carotenoids

Carotenoids are lipophilic colored compounds that are found in higher plants (gymnosperms & angiosperms) and algae as well as in non-photosynthetic organisms like fungi and bacteria. Carotenoids are found in the form of isomers, viz. all trans, 9-cis, 13-cis, 5-cis forms (Wang et al., 1994) More than 600 carotenoids are known (some important ones are, β-carotene, astaxanthin, cantaxanthin, lutein etc.) and their chemical structure is based on a 40-carbon polyene which is the backbone of the molecule (Fig. 1). The polyene system imparts carotenoids their distinctive molecular structure, their chemical properties and their light absorbing characteristics. The hydrocarbon carotenoids are named carotenes, whereas oxygenated derivatives are known as xanthophylls. In xanthophylls, oxygen can be present as OH groups (as in canthaxanthin), or as combination of both as in astaxanthin (Huguera-Ciapara et al., 2006).

At present carotenoid production from microalgae refers only to astaxanthin and β-carotene from *Haematococcus pluvialis* and *Dunaliella salina*, respectively. In astaxanthin producing organisms like *Phaffia rhodozyma* (yeast) or *H. pluvialis* (algae), carotenoid are located in cytoplasmic lipid globules (Lang, 1968; Johnson and An, 1991). Such extra-plastidic carotenoids are also referred to as secondary carotenoids (Grung et al., 1992). *H. pluvialis* represents the richest biological source of this pigment and is being cultivated at large scale by several companies, using different approaches. Commercially grown *H. pluvialis* can accumulate > 30 g of astaxanthin kg^{-1} dry biomass (Olaizola and Huntley, 2003). Another important source for the production of β-carotene is the green, unicellular alga *Dunaliella salina*. β-carotene obtained from *Dunaliella* has many advantages like increased absorption by human body, high efficiency, isomeric composition and it can be produced up to 14% of dry wt. of the biomass in a very short time (Metting, 1996).

Table 1. Pigment composition of several algal groups (During 1982)

Division	Common Name	Botanical Name	Major Pigment
Chlorophyta	Green algae	Chlorella sp.	Chlorophyll b
Charophyta	Charophytes	Spirogyra	Chlorophyll b
Euglenophyta	Euglenoids	Euglena gracilis	Chlorophyll b
Phaeophyta	Brown algae	Fucus vesiculosus	Chlorophyll c1 + c2, Fucoxanthin
Chrysophyta	Yellow-brown or golden brown algae	Dunaliella salina	Chlorophyll c1 + c2, Fucoxanthin
Pyrrhophyta	Dinoflagellates	Amphidinium carterae	Chlorophyll c2, Peridinin
Cryptophyta	Cryptomonads	Cryptomonas sp.	Chlorophyll c2, Phycobilins
Rhodophyta	Red algae	Porphyridium cruentum	Phycoerythrin, Phycocyanin
Cyanophyta	Blue-green algae	Spirulina platensis	Phycoerythrin, Phycocyanin

The carotenoid pigment astaxanthin has important applications in the cosmetics, nutraceuticals, food and feed industries. Astaxanthin is a strong colouring agent and a potent antioxidant (Guireen et al., 2003). Contrary to advantages using microalgae as source of natural colourants, some disadvantages have also been reported. Production of microalgae at large scale is associated with disadvantages like little process control (Borowitzka, 1992), high CO_2 consumption with low efficiency (Chaumont, 1993), contamination problems and optimal requirements of high amounts of salt, water and solar radiation (Ogbonna and Tanaka, 2000).

For these reasons, alternative strategies/improvement of operating systems such as extensive open ponds (Pulz, 2001, Gomez and Gonzalez, 2004), natural ponds (Gomez and Gonzalez, 2004), paddle wheel driven raceway/ ponds (Pulz, 2001), tubular photo bioreactors (Garcia-Gonzalez et al., 2005), large bags (Pulz, 2001) were suggested and tried to increase the β-carotene production. Extraction efficiency and productivity of β-carotene from Dunaliella can be enhanced many folds by using a biphasic bioreactor consisting of an aqueous and a biocompatible organic phase (Hejazi et al., 2002, 2003, 2004). Nowadays industries use closed tubular bioreactors for the production of carotenoids (Gonzalez et al., 2005). This bioreactor has been found preferable for biomass and astaxanthin production from *H. pluvialis* (Lopez et al., 2006).

Extraction of Carotenoid Pigments

Extraction and purification are two steps in carotenoid production from microalgae (Lee et al., 1999).

First Step

Biomass is separated from liquid media by centrifugation. Some alternative methods like flocculation, filtration etc. can also be used (Molina Grima et al., 2004).

Second Step

Separated biomass needs to be quickly processed to avoid spoiling. The most acceptable methods are spray-drying, drum drying, freeze drying (lyophilization) and sun drying. β-carotene is extracted from wet *Dunaliella* paste by different processes, using vegetable oils with or without chemicals, liquid or supercritical CO_2 extraction, crystallization and others. The supercritical fluid extraction of carotenoids from the microalgae *D. salina*, *C. vulgaris, Spirulina pacifica,* and *Nannochloropsis gaditana* has been reported by many workers with promising results (Lorenzo et al., 1991; Mendes et al., 1995, 2003; Careri et al., 2001; Macias-Sanchez et al., 2005). Dynamic extraction of carotenoids with supercritical CO_2 from a marine strain of *Synechococcus* sp. was investigated with regard to operation pressure and temperature effects on extraction efficiency (Montero et al., 2005). A biphasic aqueous/organic system to force the extraction of β-carotene into the medium is applied to *Dunaliella* cultures. In this system, a biocompatible organic solvent is in contact with aqueous phase where the cells develop accumulation of pigments, β-carotene is continuously extracted into the organic phase overcoming low water solubility of the product and facilitating product recovery and continuous operation (Salter and Kell, 1995; Hejazi et al., 2002, 2003).

Extraction Methods

The extraction techniques of cell components usually make use of chemical, mechanical and/or enzymatic proceses. In this work only the chemical and mechanical procedures were used, alone or simultaneously, with the aim of maximizing the extraction process efficiency. Figure 13 shows a diagram where the different elementthat were used to extract then identify and quantify pigments in the microalgae, are presented under a structured sequence. The following methods were used to analyze the algal pigments in different extracts. (Table 2, 3 & 4)

Thin Layer Chromatography Concentration of Carotenoid

The carotenoid content of seaweeds was determined by the method of Kirk and Allen, 1965. The extract that was used for the chlorophyll estimation was used for carotenoid estimation also. The same chlorophyll extract was measured at 480nm in UV-spectrophotometer to estimate the carotenoid containing the following formula (Eq. 1).

$$\text{Carotenoid } (\mu g/g) = A480 + (0.114 \times A663) - (0.638 \times A645) \quad \text{Eq. (1)}$$

A = Absorbance at respective wavelengths (nm).

Applications of Carotenoids

Nutritional Value: Most of the natural pigments have high nutritional value unlike their synthetic counterparts (Jin et al., 2003) because synthetic pigments con-

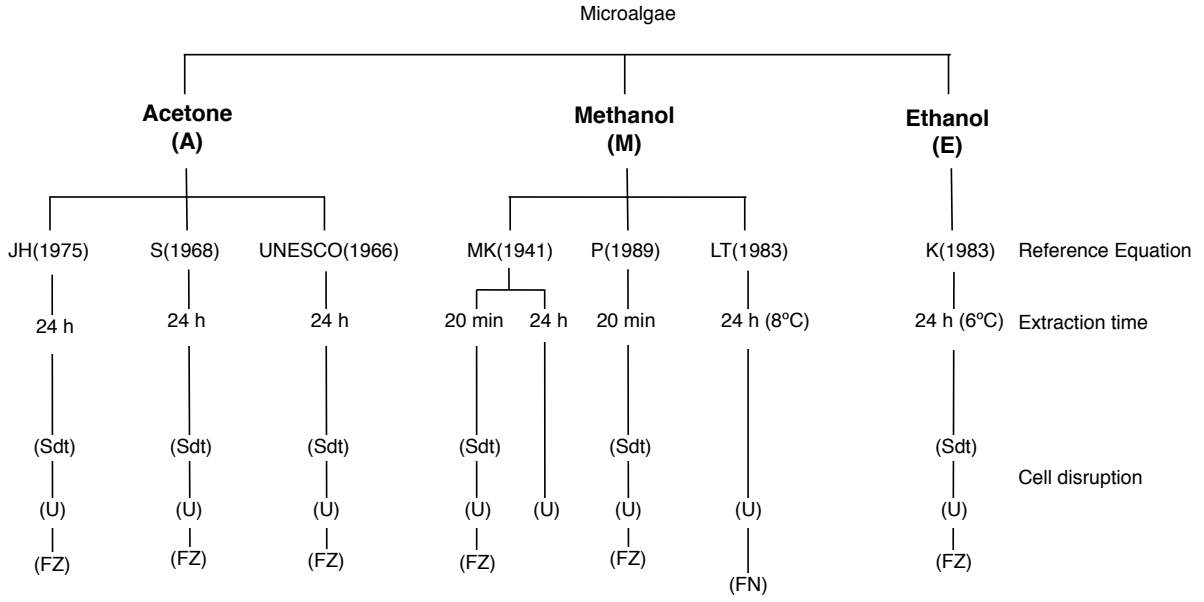

Figure 13. Items tested to compare different methods of pigments evaluation. Abbreviations: St : Standard, U: Ultrasound, F: Freezing/Unfreezing, FN: Freezing/Unfreezing with liquid N_2

Table 2. Empirical equations used to evaluate the concentration of pigments using methanol, acetone and ethanol as extraction solvent.

Acetone	
Jeffrey and Humphrey (1975)	*µg Chlorophyll/mL medium = (11.85 A664 – 1.54 A647 – 0.08 A630) v/(lV)*
Strickland and Parsons (1968)	*µg Chlorophyll/mL medium = (11.66 A665 – 1.31 A645 – 0.14 A630) v/(lV)*
UNESCO (1966)	*µg Chlorophyll/mL medium = (11.64 A663 – 2.16 A645 – 0.10 A630) v/(lV)*
Methanol	
Mackinney (1941)	*µg Chlorophyll/mL medium = 13.43 A665 v/(lV)*
Porra et.al. (1989)	*µg Chlorophyll/ mLmedium = (16.29 A665 – 8.54 A652) v/(lV)*
Lichtenthaler (1983)	*µg Chlorophyll/mL medium = 15.65 A666* *µg total carotenoids/mL medium = [(1000 A470 – 44.76 A666)/221]*
Ethanol	
Kaczmar (2004)	*µg Chlorophyll/mL medium = (11.64 A663 – 2.16 A645 – 0.10 A630) v/(lV)*

A is the absorbance at respective wave lengths (nm) , v means the volume of solvent used (mL), l is the spectrophotometric cell length (cm) and V is the sample volume (mL).

tain mainly trans-forms and natural pigments cis-form (Von Laar et al., 1996).

Antioxidant and anticancer properties: β-carotene has been shown to have antioxidant and anticancer properties (Becker, 2004).

Pigmentation in fish: Major application of astaxanthin carotenoid is as pigmentation source in aquaculture, primary salmon trout and red sea bream (Guerin et al., 2003; Cysewski and Lorenz, 2004).

Eco-friendliness: The process of manufacturing of natural pigments from algae does not involve the application of hazardous chemicals. The majority of the biomass are biodegradable and can also be reused as fodder, bio-fertilizers, etc.

Non-Toxicity and non-carcinogenicity: Natural pigments derived from algae have been certified as safe for application as food colorants.

Table 3. R_f Values of different pigments

S. No.	Pigment	R_f Value	Solvent System
1	Chlorophyll a	0.68	7:3 (Petroleum ether : Acetone)
2	Chlorophyll b	0.54	7:3 (Petroleum ether : Acetone)
3	Chlorophyll c	0.03	7:3 (Petroleum ether : Acetone)
4	β-carotene	0.94	7:3 (Petroleum ether : Acetone)
5	Fucoxanthin	0.51	7:3 (Petroleum ether : Acetone)
6	Lutein	0.43	7:3 (Petroleum ether : Acetone)
7	Violaxanthin	0.22	7:3 (Petroleum ether : Acetone)
8	Neoxanthin	0.08	7:3 (Petroleum ether : Acetone)

Dyes: Chlorophyll Derivatives (Chlorophyllin) are used for dyeing of fabrics such as wool, acetate derivatives and cotton.

Pharmaceuticals: β-carotene has market applications like food coloring agent, as provitamin A (retinol) in food and animal feed, as an additive to cosmetics and multivitamin preparations and as a health food product under the antioxidant claim (Johnson and Schroeder, 1996; Edge et al., 1997).

Cosmetics: Algal pigments are used for adding exotic pigments to soaps, shampoo, hand wash. Macroalgae are a source of good pigments for various hair coloring

Table 4. Wavelength maxima for pigments in various solvents.

S. No.	Pigment	Wavelength maxima	Solvent
1	Chlorophyll *a*	428.5, 660.5	diethyl ether
2	Chlorophyll *c1*	629.1	100 % acetone
3	Chlorophyll *b*	452.5, 642	diethyl ether
4	Chlorophyll *c2*	630.6	90% acetone
5	Chlorophyll *c2*	629.6	100% acetone
6	Chlorophyll *c2*	630.9	90% acetone
7	Chlorophyll *c*	447, 533 or 449, 635	90% acetone
8	β carotene	452, 470	Ethanol
9	Lutein	446, 474	Ethanol
10	Violaxanthin	442, 470	Ethanol
11	Neoxanthin	437, 466	Ethanol
12	Myxoxanthophyll	445, 471, 503	Ethanol
13	Siphonoxanthin	455	Ethanol
14	Peridinin	455	Ethanol

products due to their long lasting properties.Xanthophylls, astaxanthin has many applications in cosmetics products.

Paint Additives: Beer yeast diatoms are also used in paint additives, other than algal pigments, due to the iridescent nature of their silica shells.

Feed industries: Xanthophylls, astaxanthin has many applications in feed industries like as poultry. Major application of this carotenoid is the pigmentation in egg yolk.

Phycobiliproteins

Structure of Phycobiliproteins

The phycobiliproteins are antennae protein pigments found in cyanobacteria, rhodophytes, cryptomonads and cyanelles (Glazer, 1994). The phycobiliproteins are present as phycobilisomes anchored on the thylakoid membranes and lie adjacent to the photosynthetic reaction centre of the PS II in cyanobacteria and red algae. These chromoproteins are classified into 3 groups based on the presence of different chromophores among them (Gantt, 1980, 1994; Glazer, 1985; Zilinskas, 1986; Rowan, 1989; Sidler 1994; Mac Coll, 1998; Ducret et al., 1998). These groups are (1) Phycoerythrin (PE) λmax 480 nm-570 nm; (2) Phycocyanin (PC) λmax 590-630 nm and phycoerythrocyanin (PEC) λmax 630-665 nm (3) Allophycocyanin (APC) λmax 620-665 nm. Core of phycobiliproteins is composed of allophycocyanin from which arise six rods of varying length consisting of phycocyanins to the proximal side of the core and phycoerythrins to the distal side of the core (Fig. 14).

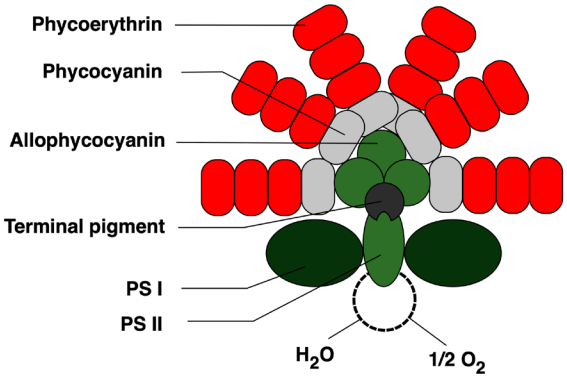

Figure 14. Schematic structure of the pigments in a Phycobilisome.

Extraction and purification methods

Phycocyanin

Phycocyanin is water-soluble and can be easily extracted as a protein-pigment complex (Chaiklahan et al., 2012). Phycocyanin was extracted from the wet biomass of Spirulina using the following methods:

Extraction was done using 100mM phosphate buffer (pH 7.0) at a ratio of 1:100 (w/v) with continuous stirring at 300 rpm at room temperature for 4 hrs. the sample was centrifuges at 4800 × g for 15 minutes to remove cell debris. The crude extract was first filtered through a 5 µm membrane at flow rate of 150 mL min^{-1}.and then through 0.8/0.2 µm membrane at flow rate of 100 mL min^{-1}. The phycocyanin was then filtered again through a membrane with a molecular cut-off of 50 kDa at 69 kPa and 75 mL min^{-1}. Finally the filtrate was lyophilized to get the phycocyanin powder.

Mechanical cell disintegration methods are currently preferred for large-scale operations (Gacesa and Hubble, 1990; Kula and Schutte, 1987) since a complete disintegration of the biomass is desired, with high product and activity yields.

Allophycocyanin, a bluish green protein and CPC, a blue protein have the major absorption (λmax) in the visible region of 650-655 nm and 610-620 nm, respectively, with emission light at 660 nm and 637 nm respectively (Bryant et al., 1979; Sekar and Chandramohan, 2007). Determinations of these phycobiliproteins by spectrophotometry have been assessed by different authors (Furuki et al., 2003; Chaiklahan et al., 2012). The purity ratio of the phycocyanin extract is determined by the A620/A280 ratio. High purity in the extract refers to high purity ratios (Chaiklahan et al., 2012). Absorbance ratio ≥ 0.7 refers to food grade pigment, while reagent and analytical grade correspond to 3.9 and ≥ 4.0 respectively (Borowitzka, 2013).

C-phycocyanin concentration

The C-phycocyanin concentration (CPC) in $mg.mL^{-1}$ was calculated from the optical densities at 652 and 620 nm, using Eq. 2 (Bennett and Bogorad, 1973):

$$CPC = (OD620 - 0.474OD652) / 5.34 \quad \text{Eq.(2)}$$

Extraction yield: the extraction yield was calculated using Eq. 3 (Silveira et al., 2007).

$$Yield = (CPC)V/DB \quad \text{Eq.(3)}$$

where Yield is the extraction yield of phycocyanin in mg of C-phycocyanin /dry biomass (g), V is the solvent volume (mL) and DB is the dry biomass (g).

Calculation of Phycocyanin content (C-PC): (Kursar and Alberte 1983)

The C-PC and APC concentration (µg/ml) were de-

termined spectrophotometrically from Equations 1 and 2.

$$C\text{-}PC = 166(A618) - 108(A650) \quad \text{Eq.(4)}$$

$$A\text{-}PC = 200(A650) - 52.3(A618) \quad \text{Eq.(5)}$$

Where A refers to absorption at the indicated wave lengths

Phycoerythrin

Phycoerythrin is a red coloured phycobiliprotein with absorption maxima range at 565 nm. Purity is usually determined as the absorbance ratio of A565/A280 which defines the relationship between the presence of phycoerythrin and other contaminating proteins. A purity ratio A565/A280 >4 corresponds to diagnostics and pharmaceutical grade phycoerythrin (Benavides and Rito-Palomares, 2004).

Phycoerythrin is an intracellular protein, the general purification process relies in three stages: 1. protein extraction by cell disruption, 2. primary recovery and 3. purification. Disruption methods like sonication, mechanical maceration and lysozyme treatment have been successfully used to extract phycoerythrin from microalgae. Choosing the right cell disruption method has a significant impact in the recovery of the overall process.

Benavides and Rito-Palomares (2006) used aqueous two-phase system (ATPS) to concentrate and purify phycoerythrin. Aqueous two-phase system is an advantageous technique due to its biocompatibility and can easily be scaled. The authors found that it is possible to concentrate phycoerythrin in the PEG-rich top phase using a PEG 1450-phosphate system. The system constructed with a volume ratio (Vr) of 1, PEG 1450 of 24.9% (w/w), phosphate concentration of 12.6% (w/w) and pH value of 8 allowed the recovery of phycoerythrin with a 2.9 purity ratio.

Purification is achieved by chromatographic methods like ion exchange chromatography, hydroxyapatite chromatography, gel filtration and expanded bed absorption chromatography.

Calculation of Phycoerythrin content (PE): (Beer and Eshel, 1985)

The following equations are proposed for correct calculations of pigment concentrations (E, phycoerythrin; C, phycocyanin, mg ml-1 in red algal crude extracts:

$$PE\ (mg/ml) = [(A564 - A592) - (A455 - A592) \times 0.2] \times 0.12 \quad \text{Eq.(6)}$$

$$C\ (mg/ml) = [(A618 - A645) - (A592 - A645) \times 0.15] \times 0.15 \quad \text{Eq.(7)}$$

Where A refers to absorption at the indicated wave lengths.

Applications

In Food Coloring: One of the most important application of phycocyanin is its use in food items. It is used as a colourant in chewing gums, popscicles, candies, soft drinks, dairy products and cosmetic also in the industry for lipsticks and eye liners. The major organisms exploited are Spirulina for phycocyanin and the red alga Porphyridium for phycoerythrin (Roman et al., 2002).

Dyes: Phycobiliproteins are used for dyeing of fabrics such as wool and cotton in Japan, Thailand and China. Phycocyanin derived from *S. platensis* is used as a natural pigment in food items such as chewing gums, dairy products and jellies (Santago-Santos et al., 2004), as a dye in pharmaceutical and cosmetic industry (Batista et al., 2006). Pure phycobiliproteins are also widely used as fluorescent labeling agents (Glazer, 1994; Telford et al., 2001). Due to their antioxidant and anti-inflammatory properties, both C-PC and APC are also potential therapeutic agents. (Zhang et al., 2000, Romay et al., 2003).

Phycocyanin colorants in general are non-toxic and non-carcinogenic. Uses of phycocyanin in foods include the coloring of fermented milk products, ice creams, chewing gum, soft drinks, alcoholic drinks, desserts, sweet cake decoration, and milk shakes.

Conclusion

Important pigments (chlorophyll *a, b* and *c*, β-carotene, astaxanthin, xanthophylls, and phycobiliproteins) are produced by many microalgae. Synthetic pigments are used in food, cosmetics, beverages , nutraceutical and pharmaceutical industries. Synthetic pigments are having harmful effects, natural pigments become an attractive option from microalgal pigments. Though algal pigments have the drawback of unstable at high temperature, an effective solution involves using thermophilic algal pigments. Due to increased interest in bio-fuels and food supplements of algal origin, in the recent times, there is widening scope for industries to exploit the availability of other algal products, mainly dyes, fodder and bio-plastics. Investing in the fields of algal pigments production would both increase profitability and reduce wastage of resources (in the form of expelled biomass used as bio fertilizers).

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