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Antioxidant properties and encapsulation methods of astaxanthin: A review

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Abstract

Astaxanthin is a predominant carotenoid pigment and a highly efficient antioxidant affecting health benefits. It is primarily found in algae or bacterium organisms and is a kind of natural colorants found in marine organisms, including hard-shelled-aquatic animals such as crabs and shrimp, and fish such as salmon and sea bream. Astaxanthin comprises the conjugated double bonds within its structure, which lead to a cause of rising the activation energy from the singlet oxygen; therefore, the singlet oxygen is destroyed by auto-oxidation mechanism. This demonstrates higher antioxidative capacity of astaxanthin compared with β-carotene and vitamin E. However, the presence of light and oxygen causes astaxanthin degradation which affects low absorption rates in the human digestive system. Many encapsulation methods have been applied to enhance astaxanthin physical stability and bioavailability for medical and dietary uses. This review describes and compares various encapsulation methods used to astaxanthin. There are many typical methods for encapsulation that include nanoliposome, drying method, emulsification, coacervation and extrusion coating. In addition, encapsulation methods led to use for in vivo and in vitro studies were also discussed.

Keywords: Bioavailability, antioxidant, encapsulation, astaxanthin

1. Introduction

Astaxanthin is a kind of carotenoids, which distributes widely and naturally in marine organisms including hard-shelled-aquatic animals (crustaceans) such as crabs and shrimp, and fish such as salmon and sea bream. Astaxanthin is a powerful antioxidant, natural antioxidant, and having a superior position within the cell membrane, which is against pro-oxidants and oxidation. Hence, it is benefits to be produced as a supplement for sale in general markets. However, it is destroyed by light, heat and oxidation reactions during its processing or storage since its molecule contains a high unsaturation degree, which can cause higher loss of its biological properties (Martínez-Delgado *et al.,* 2017). Moreover, astaxanthin has low water solubility, which limits its application in aqueous systems (Tropea *et al.,* 2013). There are many encapsulation methods, such as nanoliposome, drying method, emulsification, coacervation and extrusion coating. These methods are interesting for astaxanthin encapsulation to prolong its shelf life.

Thus, this review encompasses the underlying basis for astaxanthin bioactivity and different encapsulation methods for natural astaxanthin obtained from the microalgae, *Haematococcus pluvialis* and marine organisms as well as focusing on astaxanthin sources, structure and chemical properties, antioxidant activities, and encapsulation methods.

2. Synthesis of astaxanthin from *Haematococcus pluvialis*

Astaxanthin is found in a lot of aquatic animals, such as salmonids and crustaceans, as well as *Haematococcus pluvialis* (Tropea *et al*., 2013). *Haematococcus pluvialis* would produce the high contents of astaxanthin (0.5-5.0% (w/w) dry weight) (Table 1), which is green microalgae within phylum Chlorophyta, class Chlorophyceae, order Volvocales, family Haematococcaceae, genus *Haematococcus*, species *pluvialis* (Kamath *et al*., 2008).

These microalgae could move after an ordinary cell and grow in the modified autotrophic bold's basal medium. In the conditions of high intensity of light or oligotrophic conditions, these microalgae would become the cyst cells which have a thickened cell wall (Figure 1). The cyst cells can accumulate carotenoids within the cells such as astaxanthin, violaxanthin, lutein, zeaxanthin, α-carotene and β-carotene (Figure 2) (Kamath *et al*., 2008).

Table 1 Comparison of sources of astaxanthin

Sources	Astaxanthin (%) on the Dry Weight Basis	Reference
Chlorophyceae		
Haematococcus pluvialis	3.8	Ranga *et al.* (2010)
Haematococcus pluvialis (K-0084)	3.8	Aflalo *et al.* (2007)
Haematococcus pluvialis (Local isolation)	3.6	Torzillo *et al.* (2003)
Haematococcus pluvialis (AQSE002)	3.4	Olaizola (2000)
Haematococcus pluvialis (K−0084)	2.7	Wang *et al.* (2013)
Chlorococcum	0.2	Zhang and Lee (1997)
Chlorella zofingiensis	0.001	Zhang *et al.* (1997)
Neochloris wimmeri	0.6	Orosa *et al.* (2000)
Ulvophyceae		
Enteromorpha intestinalis	0.02	Banerjee *et al.* (2009)
Ulva lactuca	0.01	
Florideophyceae		
Catenella repens	0.02	Banerjee *et al.* (2009)
Alphaproteobacteria		
Agrobacterium aurantiacum	0.01	Yokoyama *et al.* (1995)
Paracoccus carotinifaciens (NITE SD 00017)	2.2	
Tremellomycetes		
Xanthophyllomyces dendrorhous (JH)	0.5	EFSA (2007)
Xanthophyllomyces dendrorhous (VKPM Y2476)	0.5	
Labyrinthulomycetes		
Thraustochytrium sp. CHN-3 (FERM P-18556)	0.2	Kim *et al.* (2005)
Malacostraca		
Pandalus borealis	0.12	Kim *et al.* (2005)
Pandalus clarkia	0.015	
Salmonidae		
Oncorhynchus mykiss	0.025	EFSA (2005)
Oncorhynchus nerka	0.039	

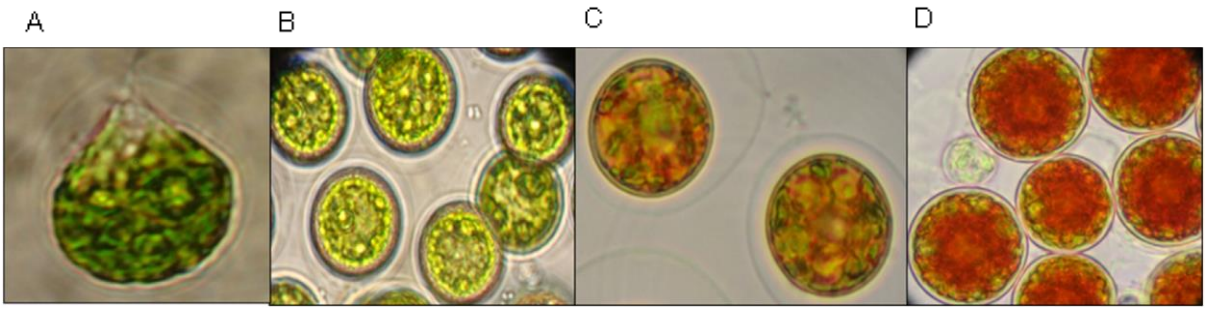

Figure 1 Appearance of *H. pluvialis* obtained from microscope: flagellate cell, (B) green coccoid cell, (C) intermediate cell, (D) cyst cell, (1:10 µm) (Tropea *et al*., 2013)

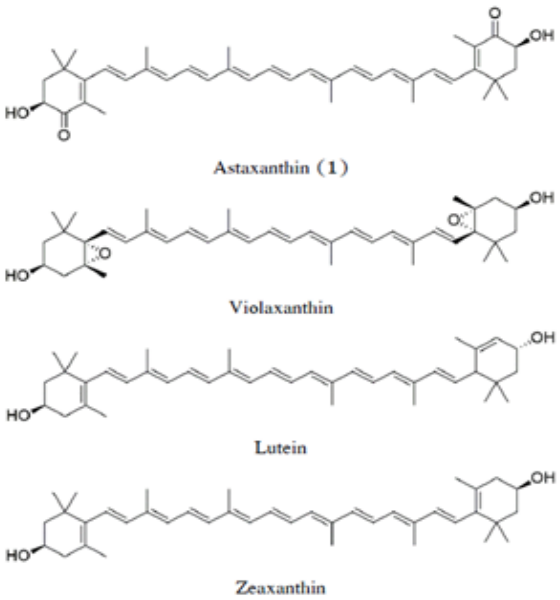

Figure 2 Carotenoids from *H. pluvialis* (Tropea *et al*., 2013)

3. Structure and chemical properties of astaxanthin

Astaxanthin is in a group of xanthophylls since it contains atoms of oxygen. The chemical formula and molecular weight of astaxanthin (3,3′-dihydroxy-β, β'-carotene-4,4′-dione) (Figure 2) are $C_{40}H_{52}O_4$ and 596.84 g.moL^{-1}, respectively. Astaxanthin has a backbone of the aliphatic hydrocarbon which contains conjugated polyene bound with ionone rings, appearing both head and tail of astaxanthin structure, and the hydroxyl group occurred in the alicylic hydrocarbon. Astaxanthin is discovered naturally in a racemic mixture of three main stereoisomers: two of these are enantiomers (3R, 3′R, and 3S, 3′S) and the third is a meso form (3R, 3′S), as a result of the presence of two chiral centers in the C-3 and C-3′ positions (Higuera-Ciapara *et al*., 2006). The solubility of astaxanthin is in descending order: dichloromethane = 30 g/L > chloroform = 10 g/L > dimethyl sulfoxide = 0.5 g/L > acetone = 0.2 g/L (Kamath *et al*., 2008). Astaxanthin is widely and naturally found in 3 kinds as the following: 1) free astaxanthin (5%); 2) monoester (70%); 3) diester (25%) (Miao *et al*., 2006). Nevertheless, the different kinds of astaxanthin are different in each kind of creatures (Figure 3) (Miao *et al*., 2006). The hydroxyl groups on the position of 3 and 3′ carbon atoms indicate the degree of astaxanthin esterification. Astaxanthin

esterification shows absorption and transportation into organism due to change it polarity in descending order: free astaxanthin > monoester > diester (Yuan *et al*., 2011). Compared to astaxanthin sources, free astaxanthin with a higher polarity is the highest occurrence in red yeast; however, monoester and diester astaxanthin in red yeast is found (Higuera-Ciapara *et al*., 2006). Moreover, an 80 percentage of astaxanthin contains the ester of fatty acids, such as C16:0 (7%), C18:0 (6.6%), C19:0 (6.1%), C20:0 (24.7%) and C18:1 (55.7%).

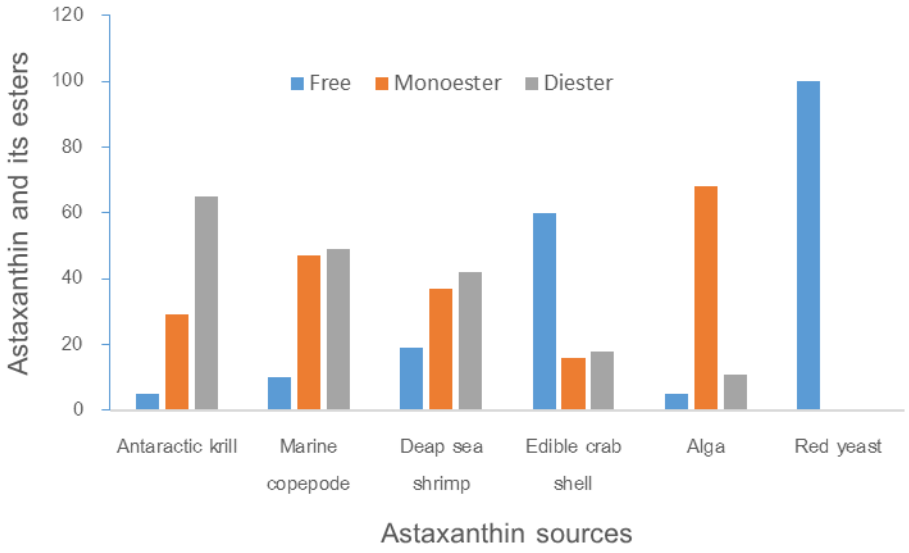

Figure 3 Contents of astaxanthin from various sources in different forms: Free; Monoester; Diester (Durbeej and Eriksson, 2006)

Besides astaxanthin obtained from the carapace of marine crustaceans and microalgae (*Haematococcus pluvialis*), the biosynthesis of astaxanthin would be occurred by some plants (*Adonis aestivalis*) and the marine bacterium (*Agrobacterium aurantiacum*). Among these species, the green microalgae *H. pluvialis* are the richest and traditionally most usable source of natural astaxanthin (80-99%w/w of total carotenoids); they can collect astaxanthin up to 3-5% (w/w) dry cell weight (Ambati *et al*., 2014; Saini and Keum, 2017). In the marine environment, microalgae are the primary producer of astaxanthin, and its ingestion by fish generates astaxanthin in the food chain (Yuan *et al*., 2011). Astaxanthin leads to the natural red, orange, and yellow colors of organisms, because of maximal absorption in the range of 475-500 nm.

In carapace of marine crustaceans, it is found not only astaxanthin but also carotenoproteins. The carotenoproteins are formed as complexes between protein-carotenoid via imine bond. They are responsible for natural colors i.e. green, blue, and purple (Armenta and Guerrero-Legarreta, 2009). In lobster (*Homarus gammarus*) shell, these carotenoproteins have either β-crustacyanin (dimers of apoprotein-astaxanthin) or α-crustacyanin (octamer of β-crustacyanins); therefore, the formation of carotenoproteins indicates the blue coloration of the shell of the lobster which the maximum absorbance of α-crustacyanin is~630 nm and ~580-590 nm of β-crustacyanin (Durbeej and Eriksson, 2006). This protein-induced bathochromic shift in the absorption of α-crustacyanin has been interested by researchers in science from a long

time because it is the most important protein induced spectral shift known (Durbeej and Eriksson, 2006).

4. Extraction of astaxanthin

The different extraction methods such as maceration, percolation, super critical fluid extraction and soxlet indicate rapid extraction; however, these methods usually consume a large number of solvents as well as a risk of thermal denaturation or transformation of chemicals (Holanda and Netto, 2006). Thus, pigment extractability with sensitivity to heat or unsuitable environment such as astaxanthin needs the appropriate solvents. Astaxanthin has been extracted with solvents, acids, edible oils, microwave-assisted and enzyme-assisted approaches (Ambati et al., 2014). Singh and Rather (2018) described that astaxanthin occurred in *H. pluvialis* extracted with different organic solvents combined with HCl was studied. The encysted cells of *H. pluvialis* were done with acetone, DMSO, hexane, methanol combined with pretreating with HCL at 70°C. The extraction with organic solvents without the HCL pretreatment led to extraction efficiency as follows: 55% (acetone), 18.4% (DMSO), 17.5% (hexane) and 10% (methanol). However, HCl pretreatment followed by acetone provided > 90% extraction efficiency of astaxanthin. This is agreement with Dong *et al.* (*2014*), who reported that hydrochloric acid pretreatment followed by acetone extraction (HCl-ACE) could obtain the highest oil yield (33.3% w/v) and astaxanthin content (19.8% w/v) compared with hexane/isopropanol (6:4, v/v) mixture solvents extraction (HEXIPA), methanol extraction followed by acetone extraction (MET-ACE, 2-step extraction). Moreover, astaxanthin extract from HCl-ACE allowed high antioxidant properties. For another solvent, vegetable oils, i.e. soya bean, corn, olive and grape seed were used to be the solvents for astaxanthin extraction from *Haematococcus*. Astaxanthin extracted with olive oil led to the highest recovery of 93% (Kang and Sim, 2008).

5. Antioxidant activity of astaxanthin

Astaxanthin indicates a high efficiency of antioxidant and anti-inflammatory activities. Moreover, it helps not only protection of skin cancer but also diabetic nephropathy, atherosclerosis and hypertension (Yamashita, 2013).

The conjugated double bonds within astaxanthin structure could gain the activation energy from the singlet oxygen so that the singlet oxygen is insensitive to oxidation. Moreover, astaxanthin has peculiar chemical properties based on its molecular structure as showed in Figure 4, which is composed of hydroxyl (OH) and ketone (C=O) moieties on each ionone ring along with an extension of conjugated double bond system. Hence, astaxanthin could indicate the efficiency of astaxanthin with stronger antioxidant activity compared with β-carotene and vitamin E.

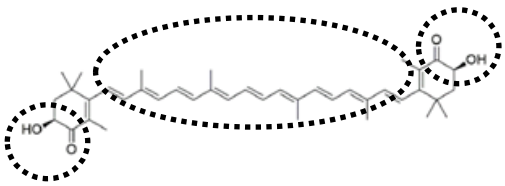

Figure 4 Antioxidant chemical site of astaxanthin (Niizawa *et al.*, 2019)

Determination for antioxidant activities of astaxanthin showing two mechanisms are as follows: 1. quenching of singlet oxygen; 2. inhibition of lipid peroxidation (Durbeej and Eriksson, 2006). Moreover, Yamashita (2013) studied antioxidant capacities of different carotenoids, especially antioxidant capacities of astaxanthin gave 10-fold stronger than those of other carotenoids, i.e. zeaxanthin, lutein, canthaxanthin and β-carotene. Moreover, it was mentioned that astaxanthin had much stronger antioxidant activity than vitamin E and βcarotene, providing different health benefits.

5.1 Singlet oxygen (O_2) quenching ability

Singlet oxygen (O_2) is obtained from photosensitization depending on exposure to light, which could be highly reactive because it would destroy protein structures in the body and oxidize protein residues, i.e. methionine, tryptophan, histidine and cysteine. As well, it would oxidize unsaturated fatty acids of cell membrane becoming lipid peroxides. Determining the principle of singlet oxygen (O_2) quenching ability is using singlet oxygen (O_2) occured by naphthalene-derived endoperoxide which the known amount of carotenoids is included, the quenching rate constant (Kq) is then determined by calculation from the reduction of singlet oxygen-generated infrared chemiluminescence (Higuera-Ciapara *et al*., 2006). Kq is the velocity of quenching reaction between O_2 and and astaxanthin. The reaction efficiency is involved with a lot of conjugated double bonds appeared in the molecule, which leading to their lowest triplet energy level (Durbeej and Eriksson, 2006). Many previous studies, the singlet oxygen quenching abilities of various carotenoids, i.e. astaxanthin, canthaxanthin, zeaxanthin, β-carotene, fucoxanthin and halocynthiaxanthin were compared (Higuera-Ciapara *et al*., 2006). Obviously, the highest Kq value of astaxanthin is 3.3×10^{-9}, while β-carotene has the Kq value of 0.089×10^{-9} as the widely known (Table 2). This high value of Kq indicates increased singlet oxygen quenching ability.

Table 2 Singlet oxygen quenching rate constants (Test Reagent : $CDCl_3/CD_3OD$ (2:1)) (Higuera-Ciapara *et al*., 2006)

Carotenoids	Singlet oxiygen quenching rate10^{-9} (Kq)	
Astaxanthin	3.30	
Cantaxanthin	2.10	Approximantely 40x
Zeaxanthin	0.220	
B-carotene	0.089	
Fucoixanthin	0.009	
Halocynthiaxanthin	0.004	

5.2 Inhibition of Lipid Peroxidation

Lipid peroxidation shows to induce the various diseases, e.g. coronary stenosis, inflammation, cardiovascular disease and cancers. Peroxidation of unsaturated fatty acids leads to destroy the structure of cell membrane producing physical illnesses of receptors and proteins within cell membrane. Xanthophylls, i.e. astaxanthin and zeaxanthin point to the inhibition of lipid peroxidation that against the production of lipid hydroperoxide (LOOH) depended on oxidative damage of fatty acids and other lipids. *In vivo* study, astaxanthin colud inhibit lipid peroxidation in rat hepatic mitochondria induced by ADP/Fe^{2+}. The inhibitory activity (ED_{50}) of

astaxanthin is 1,000 times higher than that of conventional α-tocopherol (Figure 5) (Yamashita, 2013).

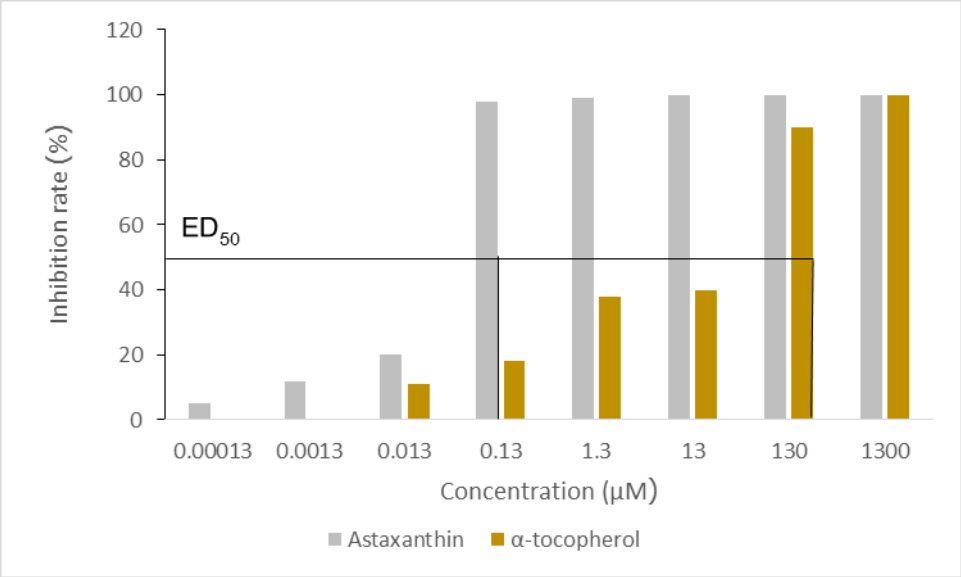

Figure 5 The effect of astaxanthin and α-tocopherol on lipid peroxidation on rat hepatic mitochondria induced by Fe^{2+} (Yamashita, 2013)

6. Encapsulation methods for stabilization of astaxanthin

Encapsulation method indicates very potential for increasing the protection, solubility, stability and available as bioactive component of astaxanthin. Astaxanthin stability and bioavailability are clearly developed by different encapsulation methods and formulations (Anarjan *et al.*, 2011). This part accounts for effect of cost-effective astaxanthin encapsulation formulations including nanoliposome, drying method, emulsification, coacervation and extrusion coating techniques. These techniques have been used in food, drug and functional food products. However, the development of these methods is quite hard to apply in encapsulation of astaxanthin as emulsifying ability, heat treatment and pH affect the production, storage stability and bioavailability of astaxanthin formulations (Mcclements, 2012).

6.1 Coacervation

Among the encapsulation methods, coacervation is a modified emulsification means, which is used for the industrial production of microcapsules (Jyothi *et al.*, 2010). The phenomenon of coacervation is a process of polymer-rich phase (coacervate) balanced with another liquid phase. Coacervation is related to the separation of a liquid phase of a coating material from a complex solution containing various polymers and the enwraping of liquid phase as a uniform layer around suspended core particles (Wilson and Shah, 2007). Commonly, the coacervation means has the driving force which is electrostatic attraction between differently charged molecules. In addition to the electrostatic interactions between biopolymers of opposite charges, the interaction between hydrogen bonding and hydrophobic oppositely impacts on a complex coacervation system. The functional ability of obtained encapsulated substances upon the chemical and surface property of the shell made from biomaterial. The capability of encapsulation enhances with an increase of

surface charges (Fang and Bhandari, 2010). Coacervation can be divided into two types as the following number of the polymers involved, including simple and complex coacervations. Nevertheless, these conditions of the coacervations require the addition of water-soluble substances, non-solvents or inorganic salts. The simple coacervation comprises only one polymer and the polymer phase becomes insoluble or dehydrated as a result of separation of the phase. (Ansel *et al*., 1995). Gomez-Estaca *et al*. (2016) studied encapsulating astaxanthin from shrimp waste by the coacervation method using gelatin. The complex contained the astaxanthin-containing lipid extract from shrimp waste, used as a food ingredient for technological and bioactive applications. The relatively low encapsulation efficiency and an average size of polymorphic microcapsules of astaxanthin were > 59.9% and 32.7 µm, respectively. A study of accelerated stability (43 days / 80% relative humidity/ 36±1°C) indicated that the encapsulation productively decelerated astaxanthin degradation compared with non-encapsulated lipid extract and no lipid oxidation by TBARS during the storage period. The encapsulated astaxanthin showed > 28.6% of the water solubility, indicating a good solubility in yogurt. The color of the encapsulated astaxanthin was improved compared with the non-encapsulated, showing higher chromaticity at increasing concentrations.

The complex coacervation shows the electrostatic attraction between 2 hydrophilic polymers of opposite charges. The polymer is separated and agglomerated in the droplet since one of the charges is neutralized by the other one (Singh *et al*., 2011). This technique needs the interaction of two or more differently charged particles, i.e. alginate (anionic group) and chitosan (cationic group) applied as coating materials (Onder *et al*., 2008). Recently, Taksima *et al*. (2015) reported the natural astaxanthin from shrimp waste encapsulated by cocevation method into alginate-chitosan beads using an ultrasonic atomizer. The encapsulated astaxanthin comprising 1.2% (w/v) alginate and 1.0% w/v chitosan resulted in over 90% of the encapsulation efficiency of astaxanthin and indicated > 50% of DPPH scavenging activity. Moreover, the stability of the encapsulated astaxanthin could be extended with high potential when used as a functional ingredient in yogurt. This indicated the complex coacervation of alginate-chitosan beads leading sufficient protection for astaxanthin as well as a prolonged shelf life.

6.2 Nanoliposome

Encapsulation is a potential method to protect the substances within the core from disappropriate environment. Liposome plays an important role of various advantages, e.g., encapsulating both hydrophilic and hydrophobic substances, target potential, slow-release properties as well as biocompatibility (Tan *et al*., 2016). Liposome directly involves a colloid system obtained from the soluble amphiphilic lipids in organic solvents via self-assembly, which has similar to lipid bilayer structures as cytomembranes. Nevertheless, the application of common liposome is limited by the range of size between 1 µm and 100 µm because of its large particle sizes. Therefore, nanoliposome is developed to be the particle size ≤ 100 nm, which is a novel technique to transport bioactive compounds. According to nanoscopic scale, nanoliposome has notable benefits, showing preferable penetration and special targeting properties (Pan *et al*., 2018). Pan *et al*. (2018) noted that the astaxanthin was encapsulated via nanoliposomes using soybean phosphatidylcholine as a shell material. The astaxanthin-loaded nanoliposomes led to higher encapsulation capacity

and lower particle size with a good dispersion. Based on X-ray diffraction and differential scanning calorimetry (DSC) techniques, the interaction of astaxanthin and the lipid bilayer affected the forming of astaxanthin-loaded nanoliposomes. The particle size of nanoliposomes is a significant cause affecting the absorption and release of core material *in vivo*. Nanoliposomes with less size are able to be the accumulation of core materials at the target site, prolonging its half-life in blood and reducing the absorption of liver and spleen. Hence, it is required to control the particle size of nanoliposomes. The *in vitro*, there are difference of substance release characteristics between astaxanthin encapsulated in nanoliposomes and pure astaxanthin solution are different. The release of astaxanthin from nanoliposomes seems obviously slower than that of free astaxanthin solution (Pan *et al*., 2018). Gong *et al*. (2016) reported that the release rate of astaxanthin within nanoliposomes was 5.32% by weight astaxanthin after 1 h and 19.86% by weight astaxanthin after 8 h; however, the release rate from free astaxanthin solution was 53.39% by weight astaxanthin and 88.50% by weight astaxanthin, respectively. Nanoliposome shows a controlled release property which is similar to previous study (Lu *et al*., 2011). The controlled release behavior of nanoliposomes could be explained as astaxanthin incorporated into lipid bilayer, which inhibits astaxanthin from diffusing too fast into the dialyzate. Thus, the encapsulation of astaxanthin with nanoliposomes is able to be a potential way to release astaxanthin continuously in the body (Saini and Keum, 2017).

6.3 Drying methods

Microencapsulation via spray drying is the most commonly applied method used in the food industry because it is simple and transforms liquids to a solid form. The advantages of this method are easy to apply incorporation and dispersion in food formulations when a few quantities need to be incorporated (Bustos-Garza *et al*., 2013). Microencapsulation of astaxanthin with various particle sizes is obtained from the method of spray drying or freeze drying. Different coatings or wall materials are used to microencapsulate astaxanthin such as soluble corn fiber, whey protein isolates, sodium caseinate, maltodextrin, gum arabica and chitosan (Shen and Quek, 2014; Bustos-Garza *et al*., 2013).

The principle of spray drying method contains 2 steps as follows: 1. the fine emulsion is using 2 steps of homogenization process; 2. the fine emulsion is spray dried using an atomizing nozzle with spray controllers using a rotational speed of~25,000 rpm. Shen and Quek (2014) studied of the blend of milk proteins and soluble corn fiber to encapsulate astaxanthin using spray drying method. The result showed the spray-dried emulsion indicating a high physical stability (≥ 95%). Moreover, Bustos-Garza et al. (2013) studied the astaxanthin encapsulation by spray drying using gum arabic (GA) and/or whey protein (WP) with combination of maltodextrin (MD) or inulin (IN) at 50:50, 25:75 ratios as coating materials. WP alone or blended with GA led to the highest encapsulation yields (61.2-70.1% (w/w) dry weight). However, the microcapsules of astaxanthin with 100% WP exhibited the highest temperature stability, and its pH stability in descending order with half-life values was 6 (169.43 h) > 5(137.38 h) > 4 (93.72 h) > 7 (83.02 h)> 3(61.35 h)

Freeze drying process is commonly used to evaporate solvent, which gives a high payload > 85% (Tachaprutinun *et al*., 2009). In expectation to protect the liposomal stability during freeze-drying, glycerol (42% of dry matter) is needed for

the formulation due to keep completeness of vesicle and prevent sedimentation and leakage of the entrapped bioactive materials (Mozafari, 2005). Glycerol plays a plasticizer role inducing increased hydration of the lipid bilayers (Manca *et al*., 2013). Furthermore, the addition of glycerol inhibits the formation of a dry lyophilized powder, providing samples with a gluey and appearance, as observed by Stark *et al*. (2010). Marin *et al*. (2018) studied astaxanthin extraction from shrimp encapsulated in soy phosphatidylcholine liposomes with the addition of glycerol, which was freeze-dried as well as its application in squid surimi. Freeze-drying encapsulation of astaxanthin increased particle size ranging from 199 nm to 283 nm and slightly decreased zeta potential. As well, the lyophilized liposomes of astaxanthin with 10.5% w/w were incorporated in squid surimi gels. The incorporation of the freeze-dried liposomes caused a slight decrease in gel strength and contributed to maintaining the stability of the gels during long-term frozen storage.

6.4 Emulsification encapsulation

Low energy method for emulsification encapsulation causes to retard the degradation of sensitive bioactive compounds. Low energy emulsification techniques provide long-term storage stability of encapsulated compounds; however, the limitation of emulsification methods depends on various factors, such as temperatures, inappropriate environments, materials and equipments. Among low energy methods, the emulsion technology is one promising technology for the encapsulation of sensitive substances. The advantages of emulsion techniques are nutrients within emulsion droplets indicating quite stable and easy dispersion in the medium. Usually, emulsion systems are composed of two or more immiscible liquids, such as oil and water, where one of liquids is dispersed as small spherical droplets in the other. Emulsion types are obviously classified into 2 main groups as the following: 1. simple or single emulsions; 2. multiple or double emulsions. For simple emulsions, oil-in-water (O/W) emulsion indicates oil droplets dispersed into the continuous aqueous phase; however, water-in-oil (W/O) emulsion is the liquid phase dispersed in the oil (continuous) phase. In multiple emulsions, water-in-oil-in-water (W/O/W) emulsion containing W/O droplets is dispersed in a continuous aqueous phase but oil-in-water-in-oil (O/W/O) emulsion is O/W droplets dispersed in a continuous oil phase. Both W/O emulsion and W/O/W emulsion are normally applied to encapsulate water-soluble substances. The W/O emulsion is appropriately applied for nutrient fortification in oil-based foods, while W/O/W emulsions are properly used in water-based food products. Thus, O/W emulsion and O/W/O emulsion are mostly used for encapsulation of oil-soluble substances. In addition, the O/W emulsion demonstrates to encapsulate the significant nutrients for supplementation in the water-based food products, while O/W/O emulsions are used for fortification in oil-based foods. W/O/W and O/W/O emulsions could be applied as a co-encapsulation system which is encapsulation together of both water-soluble and oil-soluble substances (Vladisavljevic *et al*., 2012). For example, the utilization of straight-through microchannel emulsification (MCE) to encapsulate different extracts of astaxanthin (AXT) in oil-in-water (O/W) emulsion droplets was studied. The different emulsifiers, i.e. 1% w/w of SDS, ML-750, MO-7S, Na-Cs and ML were applied. High droplet productivity was achieved with 1% w/w of ML-750 as the optimized emulsifier in Milli-Q Water. The O/W emulsion droplets was still stable at 25°C with encapsulation efficiency of > 98% at the end of the storage for 15 days

(Khalid *et al*., 2017). The emulsification-evaporation method is an interesting method, which is a novel combination used to prepare astaxanthin nanodispersion applying a three-component emulsifier system comprising Tween 20, sodium caseinate and gum arabic. The response surface methodology (RSM) applied to optimization of the major emulsion ingredients was studied as follows: at 0.02 to 0.38% w/v concentration of astaxanthin; at 0.2 to 3.8% v/v concentration of emulsifier; at 2 to 38% v/v concentration of organic phase (dichloromethane) on nanodispersion properties. The fitted models led to the prediction of the response variables showed high coefficients of determination ($R^2 > 0.930$) for all responses. The overall optimum region demonstrated a desirable astaxanthin nanodispersion obtained from the concentrations at 0.08% (w/v) astaxanthin, 2.5% (v/v) emulsifier and 11.5% (v/v) organic phase (Anarjan *et al*., 2013).

6.5 Extrusion coating

The extrusion encapsulation process has been widely exploited in various industries since 1957 and is presently used today in food industry. The extrusion coating used for encapsulation is different from the extrusion used for cooking and texturizing cereal-based products. Actually, the fun-dement of this method is a relatively low-temperature entrapping manner comprising to force a core material in a molten carbohydrate mass over a series of dies into a bath of dehydrating liquid, showing strength of wall material when approaching in contact with liquids and forming an encapsulated matrix to entrap the inner phase (Desai and Park, 2005). Extrusion encapsulation method is separated into 2 processes as the following: 1. an oil-in-water (O/W) emulsion is obtained, where the oil phase disperses into the continuous phase; 2. the emulsion is delivered to an extrusion process, which consists of small drop size formation using a syringe or an atomizer referring to simple dripping, vibrating jet/nozzle, coaxial airflow, electrostatic extrusion and jet cutting. The drops produced from a gelling solution or they are controlled to a physical process as cooling or heating (Dima *et al*., 2015; Dordevic *et al*., 2014). An important advantage of the extrusion techniques is no addition of severe conditions, either temperature or solvents, and could be adjusted to obtain the desirable temperatures and speeds (Pimentel-Moral *et al*., 2016). For instance, simple dripping carries on low liquid velocity and extrudes liquid sticks for the formation of a drop when the being higher than surface tension, whereas vibrating jet/nozzle and jet cutting are techniques which have a higher production capacity due to a controllable liquid jet break-up (Dordevic *et al*., 2014). Among the extrusion techniques, the vibration technique could be used for large-scale applications when multinozzle modification applied (Prusse *et al*., 2008). However, the processing of extrusion with vibration technique is featured by less severe conditions which insignificantly affects the integrity of most bioactive compounds (Chew and Nyam, 2016).

An extrusion process study has been focused on the development of encapsulated astaxanthin in fish feed. It has been found that astaxanthin was very stable in the extrusion process and the retained value after extrusion was 86% (w/w) astaxanthin. Samples were also collected during feed processing and the vitamin C loss was sitll 93% w/w in the final product (Anderson and Sunderland, 2002). In the food processing, Vakarelova *et al*. (2017) have improved a sustainable method for the extraction and the astaxanthin microencapsulates stability. Almost 2% (w/w) astaxanthin was separated by the high-pressure homogenization from dried

Haematococcus pluvialis cells in soybean oil. Encapsulating the astaxanthin-enriched oil was produced in alginate and low-methoxyl pectin with Ca^{2+}-mediated vibrating-nozzle extrusion technology. The results revealed that the 3% of pectin microbeads was the highest sphericity and oil retention. The microbeads after storage for 52 weeks presented the total-astaxanthin with maximum retention at 94% under the oxygen exposition at 4°C in the absent of light (+O_2/+4°C/-light), wherase the lowest total-astaxanthin retention was 38% at room temperature (+O_2 /room temperature/ -light). The extrusion encapsulations of astaxanthin as previously mentioned are widely used in various processings.

7. Conclusion

Astaxanthin has been used in food and pharmaceutical products because of an excellent antioxidant profile. Nevertheless, its functionality of astaxanthin is highly unstable and is degraded by some food processing procedures or during storage. Various encapsulation methods are requried to be useful techniques to preserve astaxanthin during processing and storage. The suitable condition of these techniques leads to enhance the efficiency and productivity focuses on the biological properties. Each encapsulation technique of astaxanthin has different advantages for applying in different foods or environments (Table 3). Lastly, most bioavailability and digestion studies of encapsulated astaxanthin obtained from activated gastric fluids, or different in vitro assay need to be the useful information for food processing. More accurate bioavailability determination should be required as testing in human cases or at least animal in vivo assay as well as determining the effective bioavailability of specific encapsulation systems.

Table 3 Wall material, encapsulation efficiency, approximate particle size of different encapsulation processes of astaxanthin

Encapsulation process	Wall material	Encapsulation efficiency (%)	Approximate particle size	Reference
Simple coacervation	Gelatin	> 59.9	32.7 µm	Gomez-Estaca *et al.* (2016)
Complex coacervation	Alginate combined with chitosan	> 90	-	Taksima *et al.* (2015)
Nanoliposome	Soybean phosphatidylcholine	> 94	< 100 nm	Pan *et al.* (2018) and Gong *et al.* (2016)
Spray drying method	Milk proteins and soluble corn fiber	> 95	-	Shen and Quek (2014)
Spray drying method	Gum arabic (GA) and/or whey protein (WP) combined with maltodextrin or inulin	100	-	Bustos−Garza *et al.* (2013)
Freeze-drying method	Soy phosphatidylcholine combined with glycerol	> 90	199-283 nm	Marín *et al.* (2018)
Emulsification encapsulation	ML-750 (emulsifier) Tween 20, sodium caseinate and gum arabic	> 98	-	Khalid *et al.* (2017)
Extrusion coating	Alginate and low-methoxyl pectin with Ca^{2+}	94%	-	Vakarelova *et al.* (2017)

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