B-05

RECENT DEVELOPMENT OF CAROTENOIDS ENCAPSULATION TECHNOLOGY

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Abstract

Carotenoids are natural pigments to be responsible for yellow-orange-red color in many organisms. They are being widely used not only for natural colorant in foods and beverages, but also for health. By scavenging singlet oxygen and peroxyl radicals, carotenoids play important biologic activities associated with antioxidant properties. A diet rich in fruits and vegetables full of carotenoids content will strengthening immune system, decreasing risk of degenerative illnesses such as cancer, preventing risk of cardiovascular disease, preventing macular degeneration, and reducing risk of cataracts. However, carotenoids are unstable molecules sensitive to light, temperature and or extreme pH in the presence of oxygen. Encapsulation has become an important technology to overcome the instability problem, helping to protect those carotenoids pigment under the desired conditions, especially when they are ingested in the body. For the encapsulation purposes, an optimized design of capsules, choosing the best encapsulation process and conditions are important factors to be considered. This paper discusses application of carotenoids encapsulation technique in this field.

Keywords: Encapsulation, carotenoid, technique, stability

INTRODUCTION

Carotenoids are organic pigment distributed widely in nature. The present of this pigment is characterized by yellow, orange to red color in many organisms [1]. Carotenoids can be found in plant chromoplasts (higher plant) and other photosynthetic organisms, such as mosses, fern, algae, and phototropic bacteria. The main function of carotenoids is light harvesting and photo-protection in photosynthesis. In light harvesting function, carotenoids harvest solar energy and converted to singlet excited state to drive photosynthesis machine. While in photo-protection function, carotenoids dissipates excess energy through singlet triplet conversion as heat [2].

Carotenoids cannot be synthesized by non-photosynthetic organisms. The presence of carotenoids in animals and human tissues is due to dietary intake from substance that contains this pigment [1]. More than 600 carotenoids have been characterized and they can be classified into two classes: carotenes that are hydrocarbons such as α-carotene, β-carotene, lycopene; and xanthophylls that are oxygenated derivatives such as lutein, β-cryptoxanthin, and zeaxanthin [3]. Xanthophylls are more polar than carotenes because the hydroxyl groups in their structure. Many sources of carotenoids that familiar for us are presented in Table 1. Bonnie (2007) [5] reported that the richest source of carotenoids is palm oil (*Elaeis guineensis*) containing 500-700 ppm of carotenoids.

Based on manufacturing process, carotenoids belong to nature-identical color. This pigment is found in nature and can be synthesized [6]. Naturally, β-carotene and β-

cryptoxanthin are responsible for orange color; α-carotene and α-cryptoxanthin are responsible for yellow color; lutein, zeaxanthin, and cryptoxanthins are respon sible for yellow-orange color; while lycopene and astaxanthin are responsible for red color [7,8]. Currently, the use of natural colorant in various food, drink, cosmetic, animal feed, and pharmaceutical industry has increased. This is because the safety of natural colorant is more credible than synthetic colorant.

A diet rich in fruits and vegetables full of carotenoids content is very important for our health. β-carotene is major source of precursor vitamin A. In the body, β-carotene will be converted to retinal by enzyme 15,15'-dioxygenase. By consuming β- carotene, the risk of vitamin A toxicity can be avoided. It will inhibit the enzyme when we have vitamin A adequately [9]. On the other hand, lutein and zeaxanthin are two major components of macular pigment that protect against development of aged-related macular degeneration (AMD) by filtering blue light which can damage cells [10]. In addition, carotenoids also play as antioxidant through ability to quench singlet oxygen [11] and scavenge free radicals [12]. Such characteristics make carotenoids able to maintain our health by strengthening immune system [13], decreasing risk of degenerative illness [14], preventing risk of cardiovascular disease [15], preventing macular degeneration [16], and reducing risk of cataracts [17].

Table 1. Carotenoids in Vegetables

Item	Carotenoid *(μg (100 g fresh product)$^{-1}$)*					
	α-Carotene	**β-Carotene**	**γ-Carotene**	**Cryptoxanthin**	**Lutein + Zeaxanthin**	**Lycopene**
Potato						
New (Aug)	-	3.2	-	-	13	-
Old (March)	tr	7.7	-	nd	60	-
Carrot	530	7600	nd	-	300	-
Rutabaga	-	tr	nd	nd	-	-
Turnip	tr	72	-	-	tr	-
Cabbage						
White	tr	66	nd	-	150	-
Red	tr	15	-	nd	26	-
Chinese	tr	15	-	tr	40	-
Cauliflower	-	11	-	-	33	-
Broccoli	tr	1000	nd	24	1800	-
Brussels sprouts	-	430	-	nd	920	-
Spinach	-	3300	-	-	4400	-
Lettuce						
Leaf	tr	980	-	-	1800	-
American	-	73	-	nd	250	-
Celery	-	2900	nd	nd	7200	-
Celeriac	-	-	nd	nd	tr	-
Yellow onion	-	6.9	-	-	16	-
Leek	-	1000	nd	-	1900	-
Radish	-	9.4	-	-	12	-
Bean	39	180	nd	-	440	-
Dill	-	4500	-	-	6700	-
Parsley	-	5600	-	-	10200	-
Cucumber	tr	130	-	-	470	-
Tomato	-	660	170	nd	100	3100
Pepper						
Red	nd	2900	nd	nd	nd	nd
Yellow	92	150	-	-	770	-
Green	tr	240	nd	-	700	-

* From Gross (1991) [4].

Notes: - = not detected at a detection limit of 0.5 μg (100 g)$^{-1}$; tr = trace; nd = not determinated

Red pepper contains capsanthin 3200 μg $(100\ g)^{-1}$

DISCUSSION

Carotenoid structure

General formula of carotenoids is $C_{40}H_{56}$, they exist in *trans*- or *cis*- isomeric forms. In nature, *trans*- isomer is more often found. It is more stable compared to *cis*-isomer [1]. However, the formation of *cis*- isomer as isolation artifact can be triggered during manufacturing processes promoted by light, heat, and various catalysts [11].

Carotenoids pigment is a diverse group of lipophilic compounds. They are polyenes consisting of 3 to 13 conjugated double bounds with tail-to-tail linkage but sometimes there are 6 carbon ring structures at one or both ends of molecule. The visible colors found in plants are due to conjugated double bonds of carotenoids that absorb light [3]. For example, the orange color of carrot (*Daucus carota* L.) is caused by eleven conjugated double bounds in β-carotene structure, as shown in Figure 1. Consequently, such structure makes carotenoids insoluble in aqueous systems thus they have poor intake in the body [18]. On the other hand, the highly conjugated structure of carotenoids makes them very unstable and can be easily degraded when exposed by light, temperature and or extreme pH in the presence of oxygen [19].

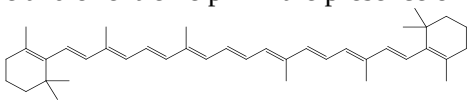

Figure 1. Chemical stucture of β-carotene [3]

General principal of encapsulation

Industrial use of carotenoids involves their application in nutrient supplementation, pharmaceutical purposes, food colorants and in animal feeds [20]. Commercial demand of food supplement rich of carotenoids is continuously increasing. However, it has been mainly met by chemical synthesis and to a minor extent by extraction from natural sources. Due to instability problem of carotenoids, the commercial carotenoids have reached limitation.

During ingestion of carotenoids, efficiency of absorption is strongly dependent on type of food, way of food processed and amount of accompanying dietary lipids [20]. Unlu *et al*., (2005) [21] reported that consumption of avocado as a lipid source with carotenoids-rich foods enhances carotenoids absorption in humans. When carotenoids are added as a food supplement, problem of intestinal absorption can be overcome by an appropriate formulation. Therefore, in order to deal with instability problem of carotenoids, encapsulation has become an important tool.

Encapsulation is defined as a technology to entrap a component (core material) in particle within barrier (coating material). This system may protect its contain from environment which is susceptible to oxygen, water, and light, etc. Besides that, this system can be used as a mean to improve self-life and control release rates under specific condition [22,23]. The initial idea of encapsulation concept came from cell model which has semi-permeable membrane that protect nucleus from external medium and controls the entrance and exit of substances [24].

The encapsulation processes of carotenoids need to be done in order to keep solubility and stability. A research that conducted by de Paz *et al*., (2013) [25] gives an overview about the influence of encapsulated β-carotene efficiency with organic-water ratio, result shows that micellar particle size of the encapsulated β-carotene was increased as well as the organic-water ratio. It implied that β-carotene solubility was increased by encapsulation process. On the other hand, Barbosa *et al*., (2005) [26] reported that decay rate of bixin molecules (derived carotenoids) in encapsulated system is lower than non-encapsulated system under illumination. It indicated that bixin molecules in the encapsulated system are well protected from oxidative

and photochemical degradation.

The developments of nano or microcapsulation are based on their size. Microcapsules usually having particle size between 0.2 and 5,000 μm, and macrocapsules are bigger than 5,000 μm. Capsules smaller than 0,2 μm are referred to as nanocapsules [27].

Selection of appropriate barrier is important for efficiency of encapsulation process. Particle in the encapsulated system must have a high encapsulation rate, good polydispersity index (PDI), and high self-life [22,23]. Although composition and structure of barrier are strongly influence the encapsulation efficiency, operating condition during the process such as temperature, pH, pressure, and humidity may also take some effects [28]. Therefore to obtain an optimized design capsules, selection of appropriate technique and condition in this process are critical stages.

Encapsulation techniques

There are some different techniques encapsulation that continuously been developed over time. The first used encapsulation technique was coacervation that evolving until the use of supercritical fluids in modern encapsulation process. According to nature of combination between coating material and core material, encapsulation technique has been classified into three categories. They are physical process, chemical process, and physical-chemical process [29] which is presented in Table 2.

Table 2. Clasification of encapsulation technique according to the nature of combination between coating material and core material

Category	Technique
Physical process	Spray-coating (spray-drying, spray-cooling/chilling) Extrusion coating Centrifugal and rotational suspension separation Fluidized bed coating Liophilization Cocrystallization
Chemical process	Inclusion complexation Emulsion polymerization
Physical-chemical process	Coacervation Emulsion phase separation Liposome entrapment

*From Jackson and Lee (1991) [29]

In this study, we will discuss several techniques that commonly used. At the end of this study, we will also discuss supercritical fluid encapsulation technique as the latest technique in this field.

Spray-coating

Spray-coating is one several technique in the encapsulation which involves dispersion of core material in a coating material followed by atomization process using air desiccant that is sprayed into mixture solution to produce powder particles. There are two different methods depending on the air desiccant; spray-drying if the air desiccant is hot, and spray-cooling/ chilling if the air desiccant is cold [30].

Spray-drying is a conventional encapsulation method that commonly used in large-scale production of encapsulated compounds. The determination of appropriate coating material influences stability and solubility of the core material. The most frequently used are gums, maltrodextrin (MD) with different dextrose equivalent (DE), some proteins and their blends [26].

As compared to the other encapsulation methods, spray-drying is relatively simple. It has high availability equipment, wide choice of coating materials with various range cost, and high throughput of finished product with good stability [31]. Moreover, the final product that produced through this method has low water activity and reduced the weight which makes them easier in storage and transportation [32]. However, controlling particle size in this process is

difficult, and yields of small batches are moderate [33].

According to the high temperature of this process, spray-drying also described as a harsh-drying method. This can damage or inactivate the highly temperature- sensitive compounds such as vitamin, enzyme, flavors, pigments, and essential oils during the process. In order to encapsulate such compounds, spray-cooling/chilling may be appropriate method [29]. Roustapour*et al*., (2009) [34] reported that as the rapid evaporation keeps the droplets temperature relatively low, the product quality is not significantly or negatively affected. Several encapsulation studies using spray-drying at lower temperature have been successfully done to antioxidant substances such as lycopene, anthocyanins, and β-carotene [35-37].

Inclusion complexation

Inclusion complexation (molecular inclusion) is the encapsulation technique using β-cyclodextrin as coating material. This technique involves hydrophilic/ hydrophobic interaction of coating material to core material [35]. Particular polar molecules are entrapped through a hydropobic interaction inside β-cyclodextrin cavity replacing water molecule. The core material is diluted to the coating material (β-cyclodextrin) that have been diluted before in aqueous system, and then dried by conventional method to obtain powder form [38].

Nowadays, the encapsulation of many bioactive compounds using this technique has been developed. Technically, this method is more appropriate for unstable substances since there is no heat stage compared with spray-drying [38]. The influence between spray-drying and molecular inclusion followed by freeze- drying of lycopene encapsulation has been studied by Nunes *et al*., (2007) [35]. Result shows that molecular inclusion is not necessary better than spray-drying. Through molecular inclusion, purity of lycopene decreased from 97.7% to 91.3%. The low temperature used in this technique causes the mixture solution takes a long time, allowing lycopene to reach the isomer with a consequent of increasing *cis*-lycopene [35]. However this result is inconsistent with the previously reported result.

Supercritical fluid encapsulation technique

Supercritical fluid is any substance at a temperature and pressure above its thermodynamic critical point. The form of supercritical is between gas and liquid phase, it can transform into a gas or a liquid when temperature and pressure changed. This substance can pass through solids like a gas, and dissolve objects like a liquid. Such capability makes it much developed as separating agent replacing the organic solvents in several techniques.

Supercritical fluid is also known as "greener solvent". It can minimize the amount of potentially harmful residues in capsules [39]. Moreover, it can also avoid several technical problems such as poor control of particle size and morphology, degradation of thermolabile compounds, low encapsulation efficiency, and low yield [40]. According to Cocero *et al*., (2009) [41], the use of carbon dioxide (CO_2) as supercritical fluid in the encapsulation process can avoid the degradation of thermolabile substance. This is because the supercritical region of CO_2 is at moderate pressure and temperature which safe for such substance. Therefore, supercritical fluid is more appropriate method to carry out the optimum encapsulation of carotenoids.

Table 3. Classification of Encapsulation Using Supercritical Method

Role of The Supercritical Fluid	Method
Solvent	Rapid Expansion Supercritical Solutions (RESS) Supercritical Solvent Impregnation (SSI)
Solute	Particles from Gas Saturated Solutions (PGSS)
Antisolvent	Supercritical Anti Solvent (SAS) Supercritical Fluid Extraction of Emulsions (SFEE)

*From Martin and Cocero (2008) [42]

Table 3 shows several methods using supercritical fluids based on their each role. In recent years, the development of encapsulation technology has been generally using Rapid Expansion Supercritical Solutions (RESS) and Supercritical Anti Solvent (SAS) methods [42]. In RESS method, supercritical fluid acts as a secondary solvent that dissolve mixture solution of core and coating material. The co-precipitation process of both substances occurs by reduction of solvent power of the supercritical fluid [43]. In other hand, supercritical fluid acts as an antisolvent in SAS method. The addition of supercritical fluid as second solvent caused the co-precipitation of core and coating material in mixture solution [44].

CONCLUSION AND SUGGESTION

Carotenoids are natural pigment important for human body. This pigment can function to maintain our health by several roles. However, poor solubility in aqueous system and instability to light, temperature, and extreme pH make them sensitive to damage and poor intake in the body. In order to overcome these problems, keeping certain amount of carotenoids in long-term storage, encapsulation technology is necessary to apply in several industries. There are several encapsulation techniques that continuously been developed over time. Spray-drying and molecular inclusion techniques are commonly used. Supercritical fluid encapsulation technique is a new technique in this field that is used to replacing organic solvent in several techniques. This method is environmental friendly and can avoid several technical problems. Therefore, in order to protect carotenoids from adverse condition, supercritical fluid encapsulation technique hopefully can be adopted by industry in the coming years.

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