Bioencapsulation of Beta-Carotene in Three Different Methods

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INTRODUCTION

Carotenoids comprise a widespread class of natural pigments, primarily used by industry as colorants in various food and drinks. In literature carotenoids have been reported to act as chain breaking antioxidants under specific conditions. However due to their highly conjugated structure, carotenoids are very unstable and can be easily degraded when exposed to oxygen or light during storage or manufacture of foods. This can cause the loss of their nutritive and biological desirable properties as well as the production of undesirable flavor or aroma compounds. For these reasons, these compounds are not usually handled in their crystalline form but rather as encapsulated forms.

Encapsulation entraps the sensitive bioactive ingredient in a coating material in order to protect its biological activity from environmental factors and enhance its physicochemical stability. Numerous wall materials or encapsulating agents are available for food application. Maltodextrin, alginate and gums are most commonly used materials. In this study encapsulation studies were performed by three methods: Spray drying, freeze drying, alginate entrapment.

MATERIAL AND METHODS

Encapsulation of β-carotene

For spray drying experiments, β-carotene, rosemary extract and emulsifier were dissolved in sunflower seed oil. Acacia gum and maltodextrin were added to this mixture. Then, the mixture was dissolved in water at room temperature (Amount of water added: 1:1).The solution was vigorously homogenized at 2500 rpm for 10 min. Homogenized mixture was maintained under slow agitation during spray-drying. The homogenized mixture was microencapsulated in a pilot spray drier. Processing conditions for spray drier was determined as 25000 rpm for spray drier disk rotation, 210-220°C and 100-110°C for entrance and exit air temperature, respectively. Feed flow was applied as 10-20%.The dried microcapsules were mixed with anticaking agent silicon dioxide (E551), packed and stored at room temperature.

Another encapsulation method that was applied is freze drying microspheres. For this reason, 0.2 g of β-carotene was added to 350 ml of an aqueous solution of 20% maltodextrin Tween 80 was added as emulsifier at ratio of 0.06%., agitated with mixer (2500 rpm for 10 min), and frozen at -20 °C for 24 hours . After freze drying, the pellet was broken into powder, washed with hexane and dehydrated under vacuum over $MgClO_4$

Another encapsulation method for β-carotene is calcium alginate entrapment. Sodium alginate was solubilized with deionised water (20g/l) and combined with β-carotene at ratio of 0,1% in mixture and 1,5% Tween 80. The mixture were extruded through a syringe into a 0,05M CaCl2 solution Calcium alginate beads were hardened for 30 min and rinsed with buffer solution (acetic-acetate,

pH 5,5) harvested from solution and freeze dried (Deladino et al., 2008; Higuera-Ciapara et al., 2004; Han et al., 2008).

Carotenoid concentration

Total initial carotenoids concentration was determined accordingly. 50 mg powder was dispersed in 2,5 ml of water and 25 ml of hexane was added to test tube. The tube then was sealed and agitated at 500 rpm for 30 min. The hexane fraction was measures at 455 nm (Desobry et al., 1997)

Surface carotenoids was determined in the finished powders by weighing 50 mg powder into test tubes and extracted with 25 ml of hexane. After 15 sec shaking at 100 rpm and the carotene concentration in the supernatant was measured at 455 nm. The percentage of surface carotene was determined by dividing the surface concentration by the total β-carotene concentration in the powder (Desobry et al., 1997)

Encapsulation efficiency(EE) was calculated as follows (Rodriguez-Huezo, 2004)

EE=[(total carotenoids-surface carotenoids)/total carotenoids]x100

Scanning electron microscopy

Scanning electron microscope (SEM) was used to study the morphological properties of dried encapsulated materials. Powder particles were attached to SEM strubs of 1" diameter using a two sided adhesive tape. The samples were then sputter coated with gold and examined at 200x and 350x magnifications. An acceleration potential of 20 kV was used during micrograph.

Water solubility

One gram of powder was mixed with 100 ml of water using a magnetic stirrer at room temperature for 30 min. A 30 ml of aliquot of solution was transferred to a 50 ml centrifuge tube and centrifuged at speed 430 g for 15 min. A 10 ml of aliquot of the supernatant was evaporated on a steam bath and dried in an oven at 110C for overnight (Loksuwan, 2007). The cold water solubility was calculated as CWS%= (grams of solid in supernatant x10)/ grams of sample x100 (Singh and Singh, 2003).

Active agent release

10 mg of alginate beads were placed in test tubes containing 5 ml of phosphate buffer (pH=7,4 were assayed at 37C for 24 hours. Sodium citrate (%10 w/v) used as calcium chelator and total dissolution was obtained after 20 min immersion. The absorbance of supernatant at 455 nm was recorded (Giunchedi et al., 2000; Deladino et al., 2008).

RESULTS AND DISCUSSION

Moisture content of spray dried powders was found as 6,07±0,07 %. Total carotene value was found as 80,61 ± 0,077 % and surface carotene was determined as 30,32 ± 0,007 % by using standard B-carotene curve (y=0,038abs-0,022; R^2= 0,966). Encapsulation efficiency was found as %64,953. These values are higher than those found in Loksuwan, 2007 where total carotene and surface carotene was found as 46,74% and 73,02% respectively. The use of Tween 80 and acacia gum may increase the amount of encapsulated material in this study. It was reported that maltodextrin with emulsifier Tween 80 had the ability to encapsulate higher amount of carotenoid than maltodextrin alone (Barbosa et al., 2005). Acacia gum is a well known effective wall material and it has been used with maltodextrin to increase emulsification stability. Cold water solubility of spray dried microencapsulated powders was determined as 87,38±0,17 %. Cold water solubility depends on

granular structure and polymer type used in the wall material. The homogeneity of granule size will also affect the solubility. SEM views of spray dried encapsulated carotenes are shown in Figure 1a and Figure 1 b.

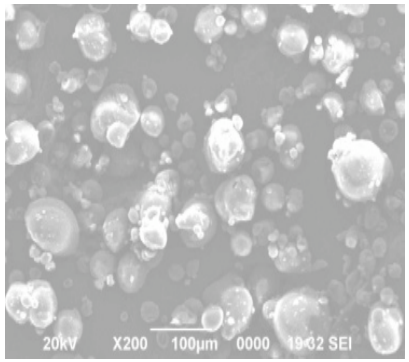

Figure 1.a. 200xmagnification

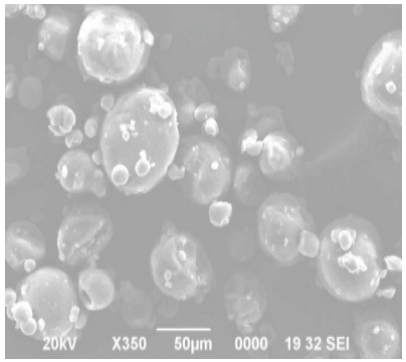

Figure 1.b. 350xmagnification

Microcapsules showed more heterogeneous size, rounded shape with smooth surface with no obvious dents. Formation of dented surface of spray dried particles was attributed to the shrinkage during the drying process. The lower amount of DE in maltodextrin means the greater amount of low molecular weight sugar. These low molecular weight sugars may act as a plasticizer during the shrinkage of the surface during drying. However, higher DE maltodextrins lower the glass transition temperature since the molecular weight decreases, the material would have higher hygroscopicity and it can cake.

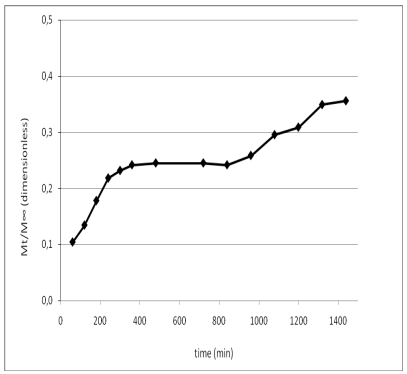

Figure 2 shows Mt/M∞ as a function of time where Mt is the mass of B-carotene released in buffer solution (pH=7,4) at time t and M∞ is the maximum mass of B-carotene released after capsule disintegration with sodium citrate. The release increased with time, and after 24 hours 35% of active compound released compared to M∞.

Figure 2. Release profile (pH=7.4) of alginate matrices: Mt/M∞ vs. time

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CONCLUSION

Carotenoid encapsulation protects its activity from environmental factors and enhances its physicochemical stability. Numerous wall materials or encapsulating agents are available for food application. Maltodextrin, alginate and gums are most commonly used materials. Increased level of total carotene of spray dried powders might be attributed to the use of Tween 80 and acacia gum in the composition of wall material in addition to maltodextrin. Cold water solubility was found as 87,38±0,17 % which depends on granular structure and polymer type used in the wall material. Alginate is water soluble naturally occurring anionic polysaccharide and has the ability of forming gels in the presence of divalent cations by ionotropic gelation. Alginate is insoluble at low pH conditions whereas its dissolution occurs at higher pH ranges. Alginate has been used for prolonged drug release where its dissolution occurs in gastrointestinal tract rather than gastric conditions where pH is 1,2. In this study it is found that after 24 hours agitation in phosphate buffer (pH=7,4) 35% of active compound released compared to maximum release obtained by sodium citrate dissolution.

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