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Original Article

MICROENCAPSULATION OF ASTAXANTHINUSING IONOTROPIC GELATION METHOD ISOLATED FROM THREE CRAB VARIETIES

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ABSTRACT

Objective: The objective of the present study was to prepare microencapsulated astaxanthin isolated from three crab varieties.

Methods: Astaxanthin was isolated from *Portunus sanguinolentus* (Three Spotted Crab), *Callinectes sapidus* (Blue Crab), and *Paralithodes brevipes* (Spiny King Crab) using hexane: isopropanol and microencapsulated astaxanthin were prepared by 2% sodium alginate and 3% calcium chloride using ionotropic gelation method. The prepared microsphere was evaluated for SEM analysis, drug content, and encapsulation efficiency and swelling studies.

Results: The microspheres obtained were heterogeneous in size, round shaped with smooth surface and with no obvious dents. The practical drug content varied between 35.25 mg to 42.21 mg. The encapsulation efficiency was found to be higher in blue crab (90.42 %) when compared with other crab varieties along with astaxanthin standard. The result showed that the swelling ratio at pH 1.2 was lesser than at pH 7.4.

Conclusion: Thus the result gained revealed that the microencapsulated astaxanthin could support the concept of sustained release in drug therapy.

Keywords: Microsphere, Astaxanthin, Ionotropic gelation, Drug therap**y.**

INTRODUCTION

Astaxanthin is one of the carotenoid pigments found in aquatic animals such as crab, shrimp, salmon, and many other organisms [1, 2]. Astaxanthin is water insoluble, which results in the low absorption and bioavailability [3]. In recent years, this compound has gained popularity as a nutraceuticals and pharmaceutical ingredient. The presence of double bonds in its molecular structure has made astaxanthin susceptible to oxidants, light and heat, resulting in poor quality products with reduced health promoting properties. Therefore, free form of astaxanthin requires some forms of protection from chemical damage or modification, before it can be applied in functional foods [4].

Microencapsulation is one of the most frequently employed techniques used to overcome the stability problems of these components. It has been applied by the food and cosmetics industries to control the delivery of molecules and to protect them from oxidation. Various methods have been used, including liposome entrapment, spray drying, extrusion, or inclusion complexation [5]. For these purposes, different food grade polymers, i.e., β-cyclodextrins, chitosan, or alginate, have been applied [6-8]. Particularly, alginate gels have been used to improve the qualitative properties of numerous functional foods [9, 10].

Sodium alginate is an anionic polymer which can be easily cross-linked with calcium chloride. This is because that the calcium ions are bound to carboxylate residues of both mannuronic acid and glucournic acid which are components of sodium alginate. Here it is the interaction of calcium ions with glucournic acid that contributes to the complexation mechanism. This complexation between calcium ions and sodium alginate leads to controlled release of drugs [8].

Incorporation of astaxanthin into polymer matrix may protect astaxanthin from light, high temperature and oxygen, in addition to improving its solubility and bioavailability. The solubility and stability of astaxanthin when encapsulated by diverse polymers have been well studied [11].

Hence, the main objective of the present investigation is to encapsulate the isolated astaxanthin from three crab varieties into sodium alginate microsphere.

MATERIALS AND METHODS

Materials

Sodium alginate and calcium chloride were purchased from Trident Scientific Company, Park town, Chennai. Standard astaxanthin was collected from Nootrabiolabs, US. All the chemicals and solvents used were of analytical grade.

Preparation of sample source

The *Portunus sanguinolentus* (Three Spotted Crab), *Callinectes sapidus* (Blue Crab), *Paralithodes brevipes* (Spiny King Crab) were collected from Gandhi market, Trichy, Tamilnadu, India. The crabs were transported to the laboratory in a sterile container filled with ice. The shells generated from the crabs such as cephalothorax, abdominal portion and pincers were removed. They were washed under running water, air dried in the shade and powdered.

Extraction of astaxanthin using Hexane and Isopropanol

50 ml of hexane: isopropanol solvent mixture in the ratio of 30: 20 v/v was prepared. 5gram of each samples were weighed and mixed with 10 ml of the solvent mixture. It is placed in magnetic stirrer for proper mixing. Again 10 ml of solvent was added and kept in magnetic stirrer. This process was repeated until the colors disappear. The solvent extract was washed with 50 ml of saline and the hexane layer was separated and evaporated to dryness. The astaxanthin extracted was subjected to quantification and further studies [12].

Quantification of astaxanthin

The extracted astaxanthin is re-dissolved in 3 ml of acetone and read @ 470 nm [13].

$$AST\ (\mu g/g) = \frac{A \times D \times 10^6}{100 \times G \times d \times E\ ^{1\%}{}_1cm}$$

Where,

AST is astaxanthin concentration in µg/g

A is absorbance

D is volume of extract in acetone

10^6 is dilution multiple

G is weight of sample in g

D is the cuvette width (1 cm)

E is extinction coefficient 2100.

OD @470 nm

Encapsulation of astaxanthin using ionotropic gelation techniques

The astaxanthin microspheres were prepared by ionotropic gelation techniques. 2 % sodium alginate was placed in magnetic stirrer. To that, 2 gram of astaxanthin and 50 ml of 1.5 % tween 80 was added slowly and allowed to mix for 45 min in magnetic stirrer. Then, the polymer drug solution was added drop wise by using the syringe of 22G in diameter from a height of about 15 cm into a beaker containing 3% of calcium chloride with continuous stirring by magnetic stirrer. Calcium alginate beads were hardened for 30 min, filtered by using Whatman filter paper no.1 and rinsed with buffer solution (acetic-acetate, pH 5.5) harvested from solution and allowed to dry at about 30 to 40 ℃ and stored in well closed container which is used for further studies [14].

SEM analysis

The particle size, shape and surface morphology of microsphere was examined by scanning electron microscopy. The samples were coated with gold which is attached to SEM strubs and examined at 1500 X. An acceleration potential of 10kV was used during micrograph.

Estimation of drug content in microsphere Microsphere (50 mg) was powdered and transferred into a 50 ml volumetric flask. The volume was made up to the mark with phosphate buffer 7.4 and kept for 12 h with occasional shaking and filtered. Then the drug content was analyzed spectrophotometrically at 478 nm using a single beam U.V./Visible spectrophotometer [15].

The encapsulation efficiency was calculated using the formula:

$$\%E\ E = \frac{\text{Practical drug content}}{\text{Theoretical drug content x 100}}$$

Swelling studies

A known weight (50 mg) of microsphere was placed in a glass vial containing 10 ml hydrochloric acid buffer pH 1.2 and phosphate buffer pH 7.4 at 37+0.5˚C with occasionals hacking. The microcapsules were periodically removed, weighed and again allowed for swelling until equilibrium weight was attained. Finally, the weight of swollen microsphere was recorded after a time period of 4 h and the swelling ratio (SR) was then calculated from the formula:

$$\text{Swelling ratio (SR)} = \frac{W_e - W_o}{W_o}$$

Where,

W_o =initial weight of the dry microsphere.

W_e =weight of the swollen microsphere at equilibrium swelling in the media [15].

Table 1: Amount of astaxanthin obtained from hexane: isopropanol extract

Extraction using hexane: isopropanol	Astaxanthin (µg/g)
Portunus sanguinolentus (Three Spotted Crab)	31.23
Callinectus sapidus (Blue Crab)	48.41
Paralithodus brevipes (Spiny King Crab)	20.15

RESULTS AND DISCUSSION

Quantification of astaxanthin

Table 1 represents the amount of astaxanthin (µg/g) obtained from hexane: isopropanol solvents. The concentration of astaxanthin obtained from *Portunus sanguinolentus* (Three Spotted Crab), *Callinectes sapidus* (Blue Crab), and *Paralithodes brevipes* (Spiny King Crab) using Hexane: Isopropanol solvent was found to be 31.23 (µg/g), 48.41 (µg/g) and 20.15 (µg/g). The highest yield of astaxanthin was observed in blue crab due to the reason that, astaxanthin a red pigment absorb the blue light from the environment, which bind with the protein crustacyanin to form an astaxanthin-crustacyanin complex with an appearance of greenish blue.

The present result was correlated with the findings of Sachindra *et al.,* 2005a [16] who reported that a 50:50 mixture of Isopropanol and Hexane gave the highest yield (43.91µg/g wet waste) of carotenoid compared to acetone (40.60µg/g wet waste). The carotenoid content in *Callinectes sapidus* (Blue Crab), was 4.63 mg/g according to Coral-Hinostroza GN and Bjerkeng B, 2002 [17] which is interrelated with the present results.

SEM analysis of microspheres

Scanning electron microscope depicts the morphological features of microspheres. SEM analysis of microencapsulated astaxanthin standard and astaxanthin isolated from different crab varieties such as *Portunus sanginolentus* (Three Spotted Crab), *Callinectes sapidus* (Blue Crab), *Paralithodes brevipes* (Spiny King Crab) were represented in fig no. 1a to fig no. 1d.

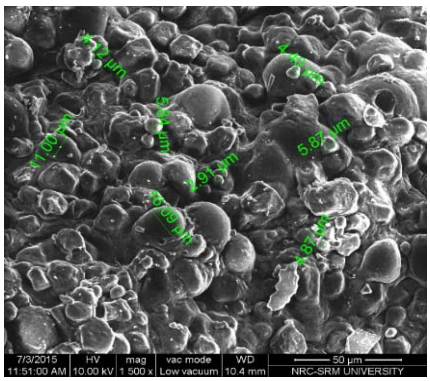

Fig. 1a: SEM view of microencapsulated astaxanthin standard

The size of microencapsulated astaxanthin standard ranges from 2.91 µm to 16.09 µm and the mean of the particle size was about 5.355 µm which is represented in fig no. 1a.

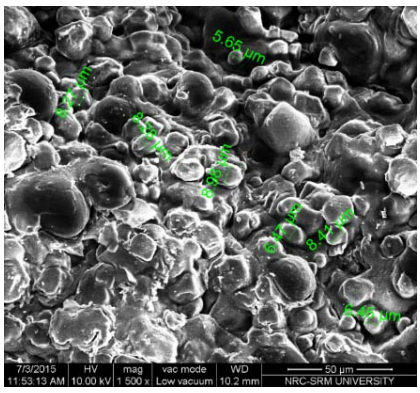

Fig. 1b: SEM view of microencapsulated astaxanthin isolated from *Portunus Sanginolentus* (Three Spotted Crab)

In the SEM analysis of microencapsulated astaxanthin isolated from *Portunus sanginolentus* (Three Spotted Crab) the size of the microparticle ranges from 5.65 µm to 8.98 µm which is indicated in fig no. 1b.

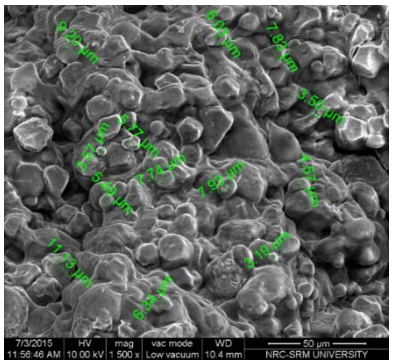

Fig. 1c: SEM view of microencapsulated astaxanthin isolated from *Callinectes Sapidus* (Blue Crab)

The microparticle in the range of 3.19 µm to 11.13 µm were seen in microencapsulated astaxanthin isolated from *Callinectes sapidus* (Blue Crab) which is shown in fig no. 1c with mean size of 6.03 µm.

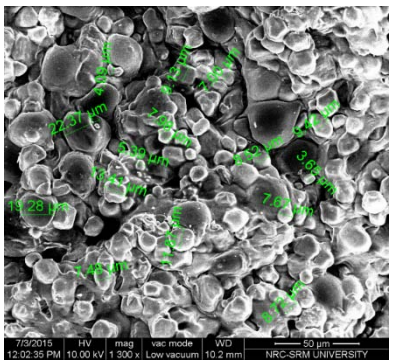

Fig. 1d: SEM view of microencapsulated astaxanthin isolated from *Paralithodes Brevipes* (Spiny King Crab)

The maximum of above 22.37 µm sizes of microparticle were detected in SEM analysis of microencapsulated astaxanthin isolated from *Paralithodes brevipes* (Spiny King Crab) that ranges from 3.41 to 22.37 µm which is depicted in fig no. 1d.

In the present investigation, concentration of sodium alginate and calcium chloride was statistically significant and tween 80 has acted as a best emulsifier. By viewing various literature regarding microencapsulation using sodium alginate and calcium chloride, the usage of 2% sodium alginate and 3% of calcium chloride were fixed.

Microencapsulation prepared by this method showed more heterogeneous size, rounded shape with the smooth surface with no obvious dents. Futher using prepared microspheres the studies such as evaluation of physiochemical properties like entrapment efficiency and swelling ratio was performed.

Estimation of drug content and entrapment efficiency of microsphere

The pigment entrapped in microsphere correlates with the concentration of pigment present in the microsphere. The study performed with 50 mg of microsphere and evaluated data is represented in table no. 2. From the data it is viewed that, the practical drug content varied from 35.25 mg to 45.21 mg. Table 2 shows the percentage of encapsulation was greater in *Callinectes sapidus* (Blue Crab) with 90% when compared with other crabs showing 77% for *Portunus sanguinolentus* (Three Spotted Crab), 71% for *Paralithodes brevipes* (Spiny King Crab) and 79% for astaxanthin standard. The reason for increased entrapment is predicted as sodium alginate and calcium chloride have mediated a good cross-linking reaction by holding the dye within it. The greater the rigidity of microspheres enabled the greater entrapment efficiency.

The present results were similar to the findings of Chan, 2011 [8] who reported that the entrapment efficiency of palm oil in calcium alginate beads was up to 90%. The present study was also correlated with the study of Taksima T *et al.,* 2015 [18] where they recorded the % entrapment efficiency in the range of 85% to 91%. The lower concentration of alginate or higher core materials was reported to cause an insufficient cross linking between the alginate and calcium ions at the droplet surface which resulted in the formation of loose calcium alginate hydrogel wall barrier [8].

Table 2: Estimation of drug content in microsphere

Samples	Practical drug content(mg)	Theoretical drug content (mg)	% OfEntrapmet Efficiency
Astaxanthin standard	39.45	50	78.9%
Portunus sanguinolentus (Three Spotted Crab)	38.48	50	76.96%
Callinectes sapidus (Blue Crab)	45.21	50	90.42%
Paralithodes brevipes (Spiny King Crab)	35.25	50	70.5%

Table 3: Swelling studies of astaxanthin standard in pH 1.2 (HCL) and pH 7.4 (Phospahte buffer)

Minutes	Swelling studies	
	pH 1.2	pH 7.4
30	1.4	2.1
60	1.8	2.6
90	2.2	3.4
120	2.4	4.6
150	2.4	4.6
180	2.4	4.6

Table 4: Swelling studies of astaxanthin isolated from*Portunus sanguinolentus* (Three spotted crab) in pH 1.2 (HCL) and pH 7.4 (Phospahte buffer)

Minutes	Swelling studies	
	pH 1.2	pH 7.4
30	1.2	2.4
60	1.4	3.0
90	1.9	3.8
120	2.3	4.9
150	2.3	4.9
180	2.3	4.9

Swelling studies of microspheres

Swelling studies were carried out, to know the behavior of sodium alginate micro particles during gastro-intestinal passage. The swelling studies were done by incubating micro particles in hydrochloric acid buffer pH 1.2 and phosphate buffer pH 7.4 at 37 °C. Comparison plots of swelling studies of sodium alginate–astaxanthin micro particles isolated from three varieties of crabs along with the standard are shown in Table. 3 to 6 and the values were tabulated in table. 3 to 6.

Swelling studies of astaxanthin standard in pH 1.2 ranges from 1.4 to 2.4 and in pH 7.4 ranges from 2.1 to 4.6 which is tabulated in table 3. Sodium alginate microparticles of astaxanthin standard showed higher swelling ratio value of 4.6 at the end of 3 h in phospahte buffer pH 7.4 and as 2.4 at the end of 3 h in HCl buffer pH 7.4 which is shown in table 3. Swelling studies of astaxanthin isolated from *Portunus sanginolentus* (Three spotted crab) in pH 1.2 ranged from 1.2 to 2.3 and in pH 7.4 ranged from 2.4 to 4.9 which is tabulated in

table 4 and table 4. Swelling studies of astaxanthin isolated from *Callinectes sapidus* (Blue Crab) in pH 1.2 ranged from 0.8 to 2.2 and in pH 7.4 ranges from 2.8 to 4.8 which is tabulated in table.5 and were represented in table 5. Swelling studies of astaxanthin isolated from *Paralithodes brevipes* (Spiny king Crab) in pH 1.2 ranged from 1.3 to 2.8 and in pH 7.4 ranged from 2.4 to 4.2 which is tabulated in table 6 and shown in table 6.

Table 5: Swelling studies of astaxanthin isolated from *Callinectes sapidus* (Blue Crab) in pH 1.2 (HCL) and pH 7.4 (Phospahte buffer)

Minutes	Swelling studies	
	pH 1.2	pH 7.4
30	0.8	2.8
60	1.2	3.2
90	1.7	4.0
120	2.2	4.8
150	2.2	4.8
180	2.2	4.8

Table 6: Swelling studies of astaxanthin isolated from *Paralithodes brevipes* (Spiny King Crab)

Minutes	Swelling studies	
	pH 1.2	pH 7.4
30	1.3	2.4
60	1.6	3.0
90	2.1	3.6
120	2.8	4.2
150	2.8	4.2
180	2.8	4.2

Thus, the results gained revealed that the swelling studies of sodium alginate were found to be higher in pH 7.4 due to erosion when compared to pH 1.2 due to shrinkage of sodium alginate gel network. At gastric pH even at the end of 3 h, the rate of swelling was lesser revealing us that the rate of release by encapsulated dye takes the longer time in the intestine which could support the concept of sustained release in drug therapy.

The reason was predicted as at gastric pH sodium alginate is usually converted to insouble alginic acid, thus reducing the rate of swelling and releasing the dye in a steady manner. But at alkaline pH sodium algiante dissolves easily, thus gets swelled and releases the encapsulated dye in a faster rate which is not applicable for commercialization. Thus, for the further study the gastric pH can be maintained. The present findings were interconnected with the findings of [15].

CONCLUSION

The present investigation has showed a good % of astaxanthin yield from the crab shell waste. Also, the isolated astaxanthin was successfully encapsulated in sodium alginate gel beads with good release rate. Thus the present work has acted as the good substitute for chemical dyes and used in drug therapy.

CONFLICT OF INTERESTS

Declared None

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