MICROENCAPSULATION OF GAC OIL IN CHITOSAN BEAD

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ABSTRACT

Gac oil is a Vietnamese traditional product that contains a high concentration of interesting carotenoids (beta-carotene and lycopene), which are very useful in cancer treatment and anti-aged protection due to their strong antioxidant activity. However, its hydrophobicity restrains its uses in food applications which are normally aqueous systems. This problem is possibly overcome with the help of microencapsulation technology. In this paper, Gac oil was encapsulated in chitosan beads using dripping method. Gac oil emulsified in chitosan 2 % was dropped into alkaline solution. The bead’s formation was evaluated through the decrease of bead weight and pH of alkaline solution. Chitosan beads expressed a good protection to Gac oil with a calculated half-life of beta-carotene up to 2 years. Encapsulation yield and encapsulation efficiency were also determined.

Keywords: Gac oil, beta-carotene, encapsulation, chitosan bead, dripping method.

1. INTRODUCTION

Microencapsulation is a well-known technique, which not only provides protection against environment stress to bioactive ingredients, but also be capable in transforming them into a new form for wider application. In fact, liquid (hydrophilic or hydrophobe) can be transformed in to free-flow powder with designed particle size with a reasonable method. Dripping is a classical method which bases on formation of droplets formed when coming out of a nozzle and harden in a suitable solution. Chitosan is now emerging as a new promising material for encapsulation and food applications [1, 2]. Several methods using chitosan for encapsulation were reported such as covalent crosslink, emulsion crosslink, solvent evaporation, coacervation/precipitation, dripping …. Hardening agent could be alkaline solution, polyanion agent or aldehyde agents.

Gac fruit (Momordica cochinchinensis Spreng) is a popular fruit in Vietnam which possesses numerous bioactivities such as antioxidant, anticancer and source of carotenoids
including beta-carotene and lycopene [3]. Gac oil extracted from Gac aril is longtime used in Vietnamese cuisine but only to fortify infant and children food by preventing vitamin A deficiency [4]. In the last decades, due to several studies on carotenoids content in Gac oil [5 – 8], Gac became a “fruit from heaven” and several products from Gac were available in the market such as frozen Gac fruit, oil fortified with Gac oil for kid and Gac oil capsule to use as compliment in cancer treatment. However, application of Gac oil in food industries is still limited as its oily existence which is difficult to disperse into food systems that usually compose high water content.

Encapsulation of Gac oil was reported with success using spray-drying or ionic gelation. For spray-drying method, encapsulation efficiency of beta-carotene was about 70.4 % when using maltodextrine [9] or 82.76 % when using mixture of gum acacia and whey isolated protein [10]. For ionic gelation method, Gac oil encapsulated in gelatin/ carrageenan bead with beta-carotene content up to 0.06 (mg/g) [11].

In this study, the use of chitosan to encapsulate Gac oil by dripping method was investigated. Schematic of the process was described in Fig. 1. Formed beads were collected, dried and analyzed for encapsulation efficiency and effectiveness of protection of chitosan on Gac oil during storage.

![Figure 1. Shematic of dripping method in case of Gac oil – chitosan bead formation.](image)

2. MATERIALS AND METHODS

2.1. Materials

Gac oil was kindly given by Food Industry Research Institute (FIRI). Chitosan was produced from shrimp shell in NhaTrang University. Standard beta-carotene was purchased from Sigma. Other solvents and chemicals were purchased from Sharlau and Prolabo with technical grade.

2.2. Preparation of Gac oil – chitosan bead

Gac oil emulsion was prepared in polymer’s solution (2 % chitosan dissolved in acetic acid 1 %) with the ratio of 3:100 (v/v). Tween80 was used as surfactant at 5 % in ratio with Gac oil. The mixture was homogenized at 4 bars for 10 minutes using the homogenizer IKA T18 Basic, ULTRA_TURRAX[12]. Gac oil – chitosan bead (microcapsule) was then formed by drop the emulsion into NaOH solution (0.1 – 2.0 N), stirring at room temperature. Beads were then harvested, dried overnight and analyzed for encapsulation yield and encapsulation efficiency.
2.3. Determination of beta-carotene content of microcapsules

Total beta-carotene contains in microcapsules was extracted from beads using n-hexan [13] with modified: m (g) of grounded beads (about 0.2 g) was extracted with V (ml) n-hexan until colorless. Surface beta-carotene was extracted by adding intact bead into n-hexan and gently shaken for 5 min. Extract’s absorbance at 452 nm was registered using Carry UV – VIS spectrometer and the content of beta-carotene was calculated using following equation with absorption coefficient (A% cm) of beta-carotene equal to 2592 (cm⁻¹):

\[
\text{Total beta-carotene content (µg/g)} = \frac{\text{Absorbance}}{2592 \times \frac{m}{V} \times 10^4}
\]

Encapsulation yield (EY) and encapsulation efficiency (EE) was calculated as followed:

\[
\text{EY} (%) = \frac{\text{Total beta-carotene in bead}}{\text{Total beta-carotene in emulsion}} \times 100
\]

\[
\text{EE} (%) = \frac{\text{Total beta-carotene content} - \text{Surface beta-carotene content}}{\text{Total beta-carotene content}} \times 100
\]

2.4. Determination of effectiveness of protection by chitosan beads on Gac oil

Beads after dried was bottled and placed in oven at 45 °C. The beta-carotene retention was determined in times as described in 2.3. Reaction coefficient rate was calculated follow Vant’Hoff equation.

3. RESULTS AND DISCUSSION

3.1. Influence of NaOH concentration on Gac oil – chitosan beads formation

Chitosan beads were formed in different NaOH concentrations from 0.1 N to 2 N. At low concentrations (< 0.5 N), the drops were broken immediately when touching solution even without stirring. At high concentrations (2N), capsules were hardened rapidly within seconds so that we could not measure its mass reduction. At moderate concentration of NaOH (0.5 – 1 N), bead’s weight loss was observed (Fig. 2). This loss was rapid in the first 5 minutes and attainted to balance after 30 minutes. It seems have no significant difference between two tested NaOH concentrations. The weight loss was about 30 % after 60 minutes.

Hardening of the bead was obtained by de-protonated of amino groups in chitosan molecule structure in basic solution [14]. The loss in weight of microcapsule corresponds to water go-out into solution which reduces its pH. A same trend in reduction of pH in compare with the reduction of capsule’s weight confirmed this argument (Fig. 3).
Encapsulation yield and encapsulation efficiency of the process

Encapsulation yield was determined as percent of beta-carotene encapsulated while encapsulation efficiency was determined as ratio of beta-carotene inside capsule to total carotene encapsulated (Tab. 1). In this study, the EY obtained was high, about 94 – 96 %. Normally, EY obtained using dripping method was reported in range 20 – 50 % [15]. Gac oil is hydrophobe while alkaline solution is hydrophile so that there is no affinity for oil transfer from Gac oil – chitosan emulsion into alkaline phase which is the only reason to loss oil during encapsulation. Encapsulation efficiency was about 80.5 % which means there was non-encapsulated carotenoids on the surface of microcapsules. The same result was observed by Tran et al., when using direct gelation method to encapsulation Gac oil in gelatin – carrageenan matrice with EE about 80 – 81 % [11]. SEM images showed that it seemed to have oil precipitated on the surface of capsules (Fig. 4).

Recently, Kha et al., has studied encapsulation of Gac oil in whey protein concentrate – gum acacia by spray-drying method [16]. The EE obtained was varied from 73.59 to 96.67 % while the EY obtained was varied from 30.49 to 48.74 %. Otherwise, concentration of encapsulating material used in this studied was up to 30 % (w/w) and oil load ratio was up to 0.36 (w/w). In our study, the concentration of chitosan was 2 % and oil load was 2.5 (w/w).

Table 1. Encapsulation yield and encapsulation efficiency.

<table>
<thead>
<tr>
<th>Concentration of NaOH (N)</th>
<th>0.5</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total beta-carotene added (µg/g)</td>
<td>122.8 ± 0.4</td>
<td>120.9 ± 1.2</td>
</tr>
<tr>
<td>Total beta-carotene content (µg/g)</td>
<td>115.4 ± 3.5</td>
<td>116.1 ± 3.5</td>
</tr>
<tr>
<td>Surface beta-carotene content (µg/g)</td>
<td>21.8 ± 4.7</td>
<td>22.3 ± 3.2</td>
</tr>
<tr>
<td>EY (%)</td>
<td>94.0 ± 3.2</td>
<td>96.0 ± 1.9</td>
</tr>
<tr>
<td>EE (%)</td>
<td>81.1 ± 4.7</td>
<td>80.8 ± 3.3</td>
</tr>
</tbody>
</table>
3.3. Effectiveness of protection by chitosan microcapsules on beta-carotene of Gac oil

The effectiveness of protection by chitosan beads on carotenoids in Gac oil was tested under accelerated condition. A slight decrease in beta-carotene concentration was observed (Tab. 2). Water content of beads was also slightly increased but there was no effect of bead's agglomeration. After 45 days stored at 45 °C, loss of content of beta-carotene was about 5 – 6 %. We have also calculated the reaction coefficient rate of the Vant'Hoff equation to estimate the half-life of beta-carotene in beads at 25 °C (Tab. 3). The result showed that the beads could retain up to 92 % of beta-carotene after 3 months of storage at room temperature. It needs about 2 years to loss 50 % of beta-carotene in these beads. Normally, Gac oil stored at room temperature loses rapidly its carotenoids content. After Nguyễn et al., Gac oil stored without nitrogen treatment lost up to 63.7 % of its content in beta-carotene and 47 % if treated with nitrogen after 3 months at room temperature [17].

Table 2. Carotenoids retention in chitosan bead conserved at 45°C.

<table>
<thead>
<tr>
<th></th>
<th>Concentration of beta-carotene (μg/g)</th>
<th>Beta-carotene retention (%)</th>
<th>Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initialization</td>
<td>118.06</td>
<td>100</td>
<td>4.63</td>
</tr>
<tr>
<td>After 20 days</td>
<td>114.73</td>
<td>97.2</td>
<td>5.79</td>
</tr>
<tr>
<td>After 45 days</td>
<td>111.49</td>
<td>94.4</td>
<td>6.11</td>
</tr>
</tbody>
</table>

Table 3. Calculated half-time of beta-carotene in chitosan beads.

<table>
<thead>
<tr>
<th>Reaction coefficient rate at 25°C (1/ day)</th>
<th>Calculated concentration of beta-carotene after 3 months (μg/g)</th>
<th>Beta-carotene retention (%)</th>
<th>Calculated half-time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>108.86</td>
<td>92.2</td>
<td>769</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

We have successfully encapsulated Gac oil in chitosan beads. Despite the surface of beads was still rough, a high loading rate was obtained. A good protection was also observed with half-time up to 2 years.
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Từ khóa: dầu gấc, beta-carotene, vi nang hòa, hạt chitosan, phương pháp nhỏ giọt.