

Encapsulation of Gac oil in alginate bead by dripping method

Ta Thi Minh Ngoc^{1,2,*}, Tran Hai Dang³, Huynh Thi Khanh³



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ABSTRACT

Gac oil was encapsulated in the hydrogel bead using alginate as carrying material by dripping method. Different parameters of all three stages of the encapsulation process were investigated: alginate concentration (emulsification stage), nozzle height, extruded pressure (bead formation stage) and calcium flow rate (bead stabilisation stage). Gac oil emulsion was characterised through the droplet's size and emulsion stability. Bead's morphology including size and shape was evaluated by image analysis using ImageJ software. Encapsulation efficiency was evaluated through encapsulated Gac oil and beta-carotene stability during storage at room temperature during 8 weeks. The beta-carotene content was determined through spectrometric absorbance in n-hexane at 452 nm. Results show that alginate is suitable to use as carrying material for Gac oil encapsulation with a moderate encapsulation efficiency of $62.5 \pm 0.8\%$. Gac oil emulsion had a droplet's size in range 1-2 μm and showed a good stability at low alginate concentration. The bead's morphology was highly affected by the height of nozzle and the extruded pressure while the flow rate of gelling solution expressed no influence of the bead's morphology. The most spherical bead and the biggest bead's diameter were obtained with the lowest distance at 150 mm and the lowest pressure at 2 MPa. A smaller size but more irregular shape can be obtained with the higher distances and higher pressures. A progressive decrease in beta-carotene content during storage was observed for both bead's surficial and total level. The degradation of beta-carotene in Gac oil bead fitted the first-order reaction with degradation rate constant k of 0.0020 day^{-1} and a half-life $t_{1/2}$ equal to 347 days which show a good stability of Gac oil in alginate bead.

Key words: Alginate bead, Beta-carotene stability, Dripping method, Gac oil encapsulation

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INTRODUCTION

Gac (*Momordica cochinchinensis* Spreng.) is an indigenous fruit of Vietnam and Gac aril oil contents a rich source of high bioactive compounds such as beta-carotene¹. However, this carotenoid are susceptible to be degraded under light and atmospheric conditions². Encapsulation is an effective technique that can help to protect and stabilise the bioactive compounds. The technique can also transform oil to powder that facilitate its uses in food formulation. Oil encapsulation is normally based on the drop formation technique that can be liquid-liquid or liquid-air dispersion. Among the last group, extrusion dripping method is the most simple to manipulate and easy to scale-up³.

Alginate is a natural polysaccharide extracted from brown algae and is the most popular encapsulant materials used in drop formation technique. The polymer composes of linear unbranched beta-(1,4)-linked D-mannuronic acid (M) and alpha-(1,4)-linked L-guluronic acid (G) residues in different proportion. This structure confers to alginate an excellent ability to instantly cross-link in presence of divalent ions such

as calcium cation to form a biocompatible and thermal stable hydrogel⁴.

Many studies have reported successful oil encapsulation in alginate bead. The encapsulated oils are diverse in origine and chemical composition like fish oil⁵, vegetable oil⁶ or essential oil⁷.

This study investigated the encapsulation of Gac oil, a red vegetable oil rich in carotenoids, in alginate bead by dripping method using scaled-up system that can help to produce a large amount of beads in laboratory. Protective effect of alginate on Gac oil was also evaluated through the stability of its beta-carotene.

MATERIALS AND METHODS

Materials

Gac oil was purchased from Gac Viet Co., Ltd., HoChiMinh city, Vietnam. Sodium alginate was product of Kermel Chemical reagents Co., Ltd., China. Lecithin was purchased from Tran Tien chemical Co., Ltd., HoChiMinh city, Vietnam. Solvents were from Prolabo, France and Labscan, Thailand. Other analysis reagents were products of Xilong Scientific Co., China.

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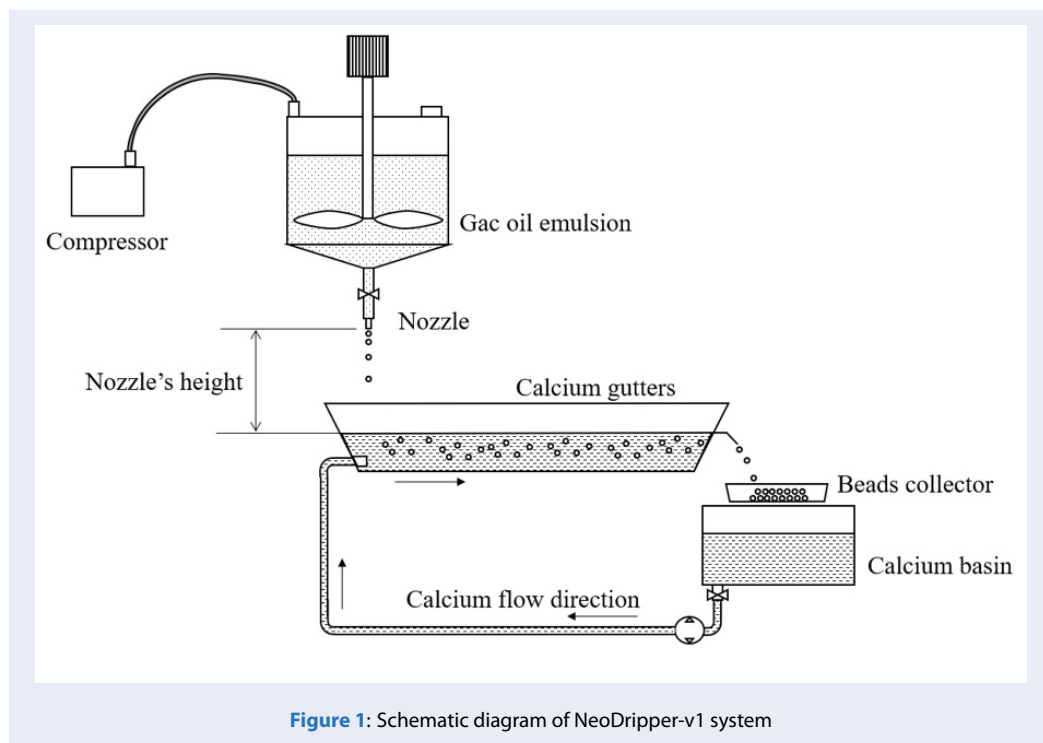


Figure 1: Schematic diagram of NeoDripper-v1 system

Preparation of Gac oil - alginate emulsion and Gac oil bead

Gac oil emulsion was prepared following the method of Tran *et al.*,⁸ with modifications: 25 ml of Gac oil was mixed with 2.5 g lecithin on magnetic agitator plate. The mixture was then added into 500 ml of alginate solution (0.5-4.0 %) and homogenised at 19,000 rpm for 10 minutes using the homogeniser IKA T18 Basic ULTRA_TURRAX. The emulsion was then extruded through a single nozzle using NeoDripper-v1 system and fall down into gelling bath containing 2 % calcium chloride solution which was circulated at different flow rate by a pump (Figure 1). The beads were collected at the end of gutters. The impact of height of nozzle (150-550 mm), extruded pressure (2, 3 and 4 MPa), and calcium flow rate (2-10 l/min) on bead's morphology were explored respectively. The beads were then let to dry at room temperature for 1 day and conserved in PE zipped bag for stability analysis.

Gac oil – alginate emulsion characterisation

The emulsion characteristics were analysed including droplet's size and viscosity as described by Tran *et al.*,⁸. The emulsion droplet size was determined under microscope, using the software Motic Image version 2.0. Viscosity of emulsions was measured using the viscometer DV-I Prime Brookfield.

The emulsion stability was determined by observing the appearance of the boundary line as described by Chan *et al.*,⁹ as followed: the emulsion was filled in Nessler tube and left at room temperature to observe phase separation through appearance of phase's visible boundary for 1 h (as the bead preparation is accomplished in this time). The emulsion stability (ES) was calculated based on the volume of remaining emulsion (V_1) and on the initial emulsion volume (V_0) as following equation:

$$ES (\%) = V_1/V_0 * 100$$

Gac oil – alginate bead characterisation

Morphology of the encapsulated beads were analysed by image analysis¹⁰. A digital camera is used to take photo of fresh bead under a black box. Size and shape are determined using ImageJ software version 14.6. Size of bead was determined by circle equivalent (CE) diameter which is the diameter of a circle with the same area as the particle. Circularity of beads was determined as circumference of equivalent area circle divided by the actual perimeter of the particle and was calculated as following equation:

$$Circularity = \frac{2(\pi * area)^{1/2}}{Perimeter}$$

A circularity > 0.96 is considered as primary sphere; in range of 0.89-0.96 is considered as primary misshapen and <0.89 is considered as agglomerates¹¹.

Determination of Gac oil bead's beta-carotene content and encapsulation efficiency

The beta-carotene content in beads was extracted with n-hexane and determined through absorbance at 452 nm with absorption coefficient ($A^{1\%}_{1\text{cm}}$) in hexane equal to 2592¹². About 0.5 g of bead was used for total beta-carotene determination. Extraction was with n-hexane (25 ml) until the solution became completely colorless. The surficial beta-carotene content was determined by gently shaking the beads (0.5 g) with n-hexane (10 ml) for 10 times without bead destruction. The bead's moisture content was determined by drying to constant mass. Beta-carotene content was calculated as mg/ g of dried weight of bead.

Encapsulation efficiencies (EEs) were determined based on the retention of unextractable beta-carotene in the bead as following equation:

$$EE (\%) = (BC_{total} - BC_{surficial})/BC_{total} * 100$$

where BC_{total} is the total beta-carotene and $BC_{surficial}$ is the surficial beta-carotene content of Gac oil - alginate beads.

Calculation of degradation rate constant and half-life of beta-carotene in Gac oil bead

The Gac oil bead was stored in PE zipped bag at room temperature ($27 \pm 2^\circ\text{C}$) and out of light cover. The degradation of beta-carotene in beads was calculated by using standard equation for first-order kinetic model as following equation:

$$\ln C = \ln C_0 - kt$$

where C is the beta-carotene content at time t ; C_0 is beta-carotene content at time zero; t is the storage time (days) and k is the degradation rate constant (day^{-1}). The last one was obtained from the slope of a plot of $\ln C/C_0$ versus time.

Statistical analysis

The results are reported as averages and standard deviations, and the differences among treatments were calculated based on an analysis of variance (ANOVA) and a post-hoc Duncan test with a confidence level of 95 %. These analyses were carried out using statistical analysis software SPSS, IBM Corporation, Armonk, NY, USA.

RESULTS AND DISCUSSION

Formulation of Gac oil – alginate emulsion

Alginate is a popular polymer used in encapsulation by dripping method as its solution is viscous at low

concentration and its ability to form instantly intermolecule crosslinkage in calcium bath. To encapsulate Gac oil in alginate bead, the very first step is dispersing the oil into alginate solution to form a stable emulsion. A range of 0.5; 1.0; 2.0 and 4.0 % of alginate solution was studied. The viscosity of emulsion made from 0.5 % alginate solution was 92.2 ± 6.9 cP and increased rapidly with higher concentrations. The emulsion with 1.0 % alginate solution had a viscosity of 156.7 ± 5.8 cP while with 2.0 % alginate solution, it was up to 1025 ± 7 cP (Figure 2A). Regarding the emulsion stability (ES), at alginate concentration of 0.5 %, the ES was 53 ± 3 % that showed an unstable emulsion. For concentration from 1.0 %, the ES was 100 % and emulsions were stable up to 24 h. The relation between high viscosity of emulsion and its stability was also reported for the Gac oil emulsion formed with chitosan solution and gelatin solution¹³.

Droplet size is one of the most important characteristic relating to emulsion stability. In theory, the smaller is the droplet size, the higher stable is the emulsion. The droplet size obtained with alginate solution in this study was 10 folds smaller than the one obtained with chitosan or gelatin solution at the same viscosity as reported by Tran *et al.*,⁸ and was decreased with alginate concentration. As shown in the Figure 2B, at time zero, the droplet size of 0.5 % alginate solution was $2.83 \pm 0.33 \mu\text{m}$ while droplet size of 1.0 % alginate solution was $1.64 \pm 0.28 \mu\text{m}$. This parameter continued to decrease to 1.20 ± 0.03 and $1.00 \pm 0.02 \mu\text{m}$ for alginate solution of 2.0 and 4.0 % respectively. For 0.5 % alginate solution, after 1 h, the emulsion had undergone the creaming effect and the bigger droplets were floated and coalesced on the upper phase as the droplet size of the remaining emulsion was decreased to $1.40 \pm 0.05 \mu\text{m}$. Otherwise, results show that there was no significant change in droplet size of emulsion formed with higher alginate concentrations of 1.0-4.0 %.

Formation of Gac oil - alginate bead

Obtaining a small and spherical bead is not only a trend when producing mono-dispersed beads due to anesthetic quality but also required for development of reproducible reaction, free flow particles or to maintain a dust-free environment in production line. The morphology of hydrogel bead can be affected by the parameters of the dripping system⁴ such as the nozzle's height and the extruded pressure during the formation stages or the flow rate of gelling solution during the stabilisation stage.

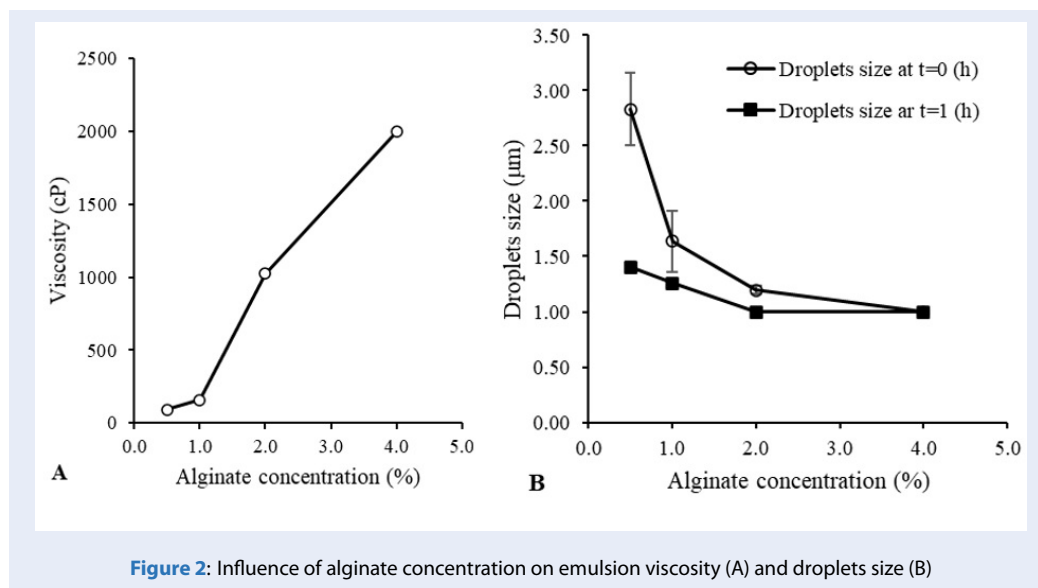


Figure 2: Influence of alginate concentration on emulsion viscosity (A) and droplets size (B)

Results show that the flow rate in range of 2-10 l/min did not affect the size and shape of the beads (Figure 3C and Figure 3F). This may be explained by an instant crosslinkage between alginate and calcium⁴. Otherwise, this is not the case of nozzle's height and extruded pressure. In increasing the height of nozzle as 150, 160, and 180 mm, the bead's diameter decreased as 2.01 ± 0.06 , 1.64 ± 0.15 and 1.47 ± 0.06 mm, respectively. At 200 mm, the bead's diameter fall to 0.70 ± 0.03 mm and was stable with continuous increase in the nozzle height up to 550 mm (Figure 3A). Figure 3B shows the effect of three extruded pressures on the bead size. At 2 MPa, the bead's diameter was 0.85 ± 0.04 mm. As the pressure increased to 3-4 MPa, the bead's diameter decreased to 0.68 ± 0.04 and 0.63 ± 0.06 mm, respectively. A similar decrease in bead's circularity with increase in nozzle's height and extruded pressure was observed as shown in the Figure 3D and Figure 3E. A shorter distance and lower pressure resulted in a more spherical bead. In overall, the obtained bead has a circularity in range of 0.89-0.94 which show a primary misshapen of the bead's shape.

Encapsulation efficiency and stability of beta-carotene in Gac oil bead

The Gac oil bead was produced with 2 % alginate solution using NeoDripping-v1 system with nozzle's height of 200 mm, 3 MPa extruded pressure and 6 l/min flow rate. These were dripping conditions that gave the smallest bead with diameter of 0.70 ± 0.03 mm and a sufficient circularity of 0.90 ± 0.01 . With

this condition, the obtained bead had 0.84 ± 0.01 mg/g in total beta-carotene content and encapsulation efficiency of beta-carotene was 62.5 ± 0.8 %. In comparison with our previous work on encapsulation of Gac oil in different natural polymers, this is not a good beta-carotene retention. A higher encapsulation efficiency around 80.8-81.4 % was obtained with chitosan bead prepared by dripping method¹² or gelatin microcapsules formed by double emulsion method¹⁴. This lower encapsulation efficiency may be due to the porous character of alginate hydrogel during dehydration.

The Gac oil bead's moisture was 9.4 ± 0.6 % at the first week and were relatively stable through storage under room temperature condition as it was 8.1 ± 0.4 % after 8 weeks. The total beta-carotene in beads was determined during storage. Results in the Figure 4 show that the degradation of beta-carotene in the alginate beads during storage fitted a first-order reaction. This finding is consistent with the other studies on beta-carotene stability^{15,16}.

The degradation rate constant k and half-life $t_{1/2}$ of beta-carotene in alginate bead were 0.0020 day^{-1} and 347 days, respectively. Nguyen and Tran (2010) has reported a decrease of 63.7 % in beta-carotene content in Gac oil stored at room temperature for only 3 months¹⁷. These data show a protectant effect of Gac oil encapsulation using alginate as carrying material. Anyway, this protective property of alginate is less effective than other polysaccharide like chitosan or gum Arabic in combination with whey protein concentrate (Table 1). This fact may be due to the porous matrix formed during the dehydration of alginate hydrogel.

As shown in the Figure 4, the plots of $\ln C/C_0$ versus time with total beta-carotene content and with surficial beta-carotene content in bead were superposed which corresponds to a similar degradation rate constant of beta-carotene on the bead's surface and inside the bead. Several authors have proposed to use a complementary polymer as a "filler" to overcome this porous character. Adding compounds that can retain water like glycerol or starch was reported as positive in solving the problem¹⁸.

CONCLUSION

Gac oil was successfully encapsulated in alginate bead. Otherwise, further works are needed to ameliorate the encapsulation efficiency and the stability of encapsulated Gac oil.

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CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

AUTHORS' CONTRIBUTIONS

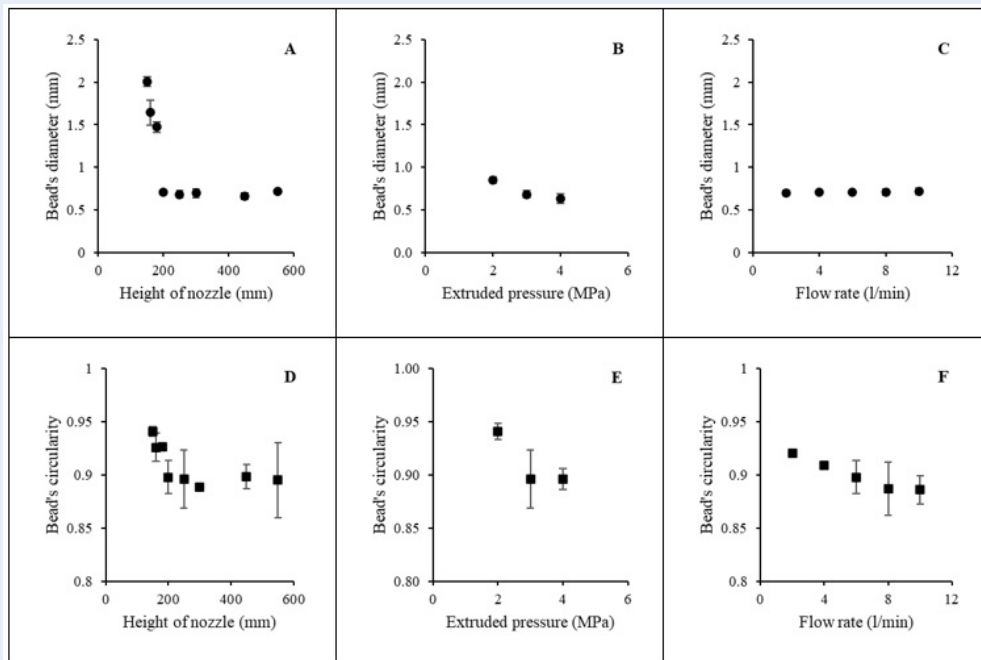
Ta Thi Minh Ngoc participated in project conception, designing the experiments and interpreting the data as well as preparing the manuscript.

Tran Hai Dang was responsible of NeoDripper-v1 development and assisted in designing experiments.

Huynh Thi Khanh participated in doing and collecting experiments data.

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Alginate concentration was 2 %.

Dripping conditions were: (A, D) extruded pressured 3 MPa, flow rate 6 l/min; (B, E) nozzle's height 250 mm; flow rate 6 l/min; (C, F) nozzle's height 200 mm; extruded pressured 3 MPa.

Figure 3: Influence of system parameters on Gac oil - alginate beads morphology: (A, D) Size and shape of bead in function of nozzle's height; (B, E) Size and shape of bead in function of extruded pressure; (C, F) Size and shape of bead in function of flow rate.

Table 1: Degradation kinetic of beta-carotene in encapsulated Gac oil

	k (day ⁻¹)	t _{1/2} (days)	Correlation coefficient (R ²)	References
Gac oil encapsulated in alginate bead	0.0020	347	0.991	This study
Gac oil encapsulated in whey protein concentrate/ gum Arabic	0.0013	533	0.969	Kha <i>et al.</i> , ¹⁹
Gac oil encapsulated in chitosan bead	0.001*	769	-	Ta <i>et al.</i> , ¹²

*k calculated at 25°C

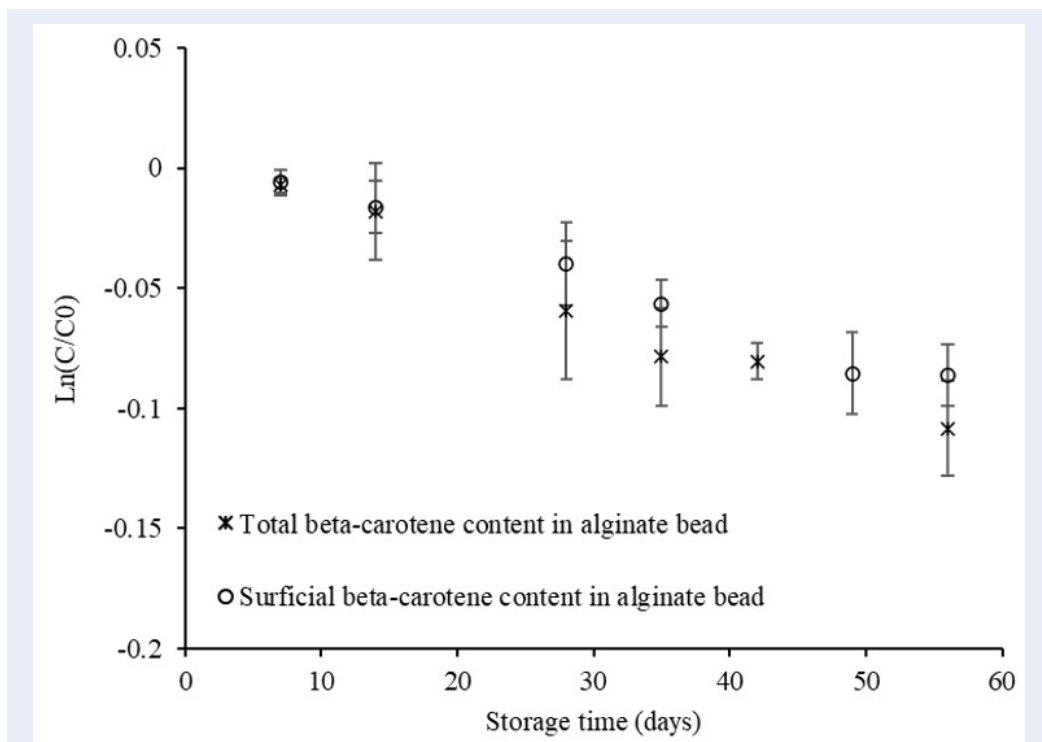


Figure 4: First-order degradation plot for beta-carotene in alginate beads under room temperature storage condition.

Nghiên cứu tạo hạt dầu gấc - alginate bằng phương pháp nhỏ giọt

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TÓM TẮT

Bài báo này nghiên cứu khả năng bao gói dầu gấc trong hạt gel alginate theo phương pháp nhỏ giọt. Nghiên cứu xác định ảnh hưởng của các thông số trong 3 giai đoạn của quá trình tạo hạt bao gồm nồng độ alginate (giai đoạn nhũ hóa), chiều cao đầu phun, áp suất phun (giai đoạn tạo hạt) và tốc độ dòng chảy của canxi (giai đoạn ổn định hạt). Nhũ tương dầu gấc được đánh giá thông qua kích thước hạt nhũ tương và độ bền nhũ tương. Kích thước và hình dạng hạt dầu gấc được đánh giá theo phương pháp phân tích hình ảnh sử dụng phần mềm ImageJ. Hiệu quả quá trình bao gói được đánh giá qua hàm lượng dầu gấc trong hạt và độ ổn định của beta-carotene trong hạt bảo quản ở nhiệt độ phòng trong 8 tuần. Beta-carotene trong hạt được trích ly bằng n-hexane và xác định hàm lượng bằng phương pháp đo độ hấp thụ quang tại bước sóng 452 nm. Kết quả cho thấy có thể sử dụng alginate làm vật liệu bao gói dầu gấc với hiệu quả bao gói đạt mức trung bình $62.5 \pm 0.8\%$. Nhũ tương dầu gấc có kích thước giọt nhũ tương từ 1-2 μm và độ bền cao ngay ở nồng độ alginate thấp. Hình dạng và kích thước hạt chịu ảnh hưởng của chiều cao đầu phun và áp suất phun trong khi tốc độ dòng chảy của canxi không có ảnh hưởng. Chiều cao đầu phun 150 mm và áp suất 2 MPa cho hạt có kích thước và độ tròn lớn nhất. Kích thước hạt sẽ giảm xuống và hạt méo hơn khi tăng chiều cao đầu phun và áp suất phun. Hàm lượng beta-carotene tổng số cũng như bề mặt của hạt giảm dần theo thời gian bảo quản. Tốc độ phân hủy beta-carotene trong hạt tuân theo hàm bậc nhất, với hằng số tốc độ phản ứng phân hủy ở nhiệt độ phòng 0.0020 (1/ngày) với thời gian bán hủy tương ứng 347 ngày.

Từ khóa: Hạt alginate, Độ ổn định beta-carotene, phương pháp nhỏ giọt, bao gói dầu gấc

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