STABILIZATION OF BETACYANINS FROM RED BEET ENCAPSULATED IN CHITOSAN-ALGINATE GEL BEADS

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ABSTRACT

Betacyanins are organic compounds that contribute to the redness of red beetroot; these compounds are also used as a natural colorant. Studies on enhancing the stability of natural colorants are pivotal for obtaining and utilizing the natural food colorant from stable local resources and catching up with the recent trends. In this study, the betacyanins extract was obtained by cold maceration method with the obtained betacyanins content was 24.93 ± 0.20 mg/L; later, betacyanins was encapsulated in alginate-chitosan gel beads with the encapsulation efficiency was 90.85 ± 0.96%. Betacyanins encapsulation by medium molecular weight chitosan-coated alginate gel bead showed the ability to enhance betacyanins stability after extraction. The suitable chitosan concentration was 1.5%, and the first-order kinetic model was used to describe the thermal degradation of betacyanins; the rate constant and half-time period were calculated according to the first-order model.

Keywords: red beet root, natural colorant, alginate-chitosan gel bead.

1. INTRODUCTION

Natural colorants are drawing attention because these compounds do not have adverse health effects; some natural colorants possess potent antioxidant activities that could be used in food processing and functional food development [1]. Natural pigments have been applied in the food industry as colorants, including anthocyanin, betalain, chlorophyll, carotenoid, carminic acid, and curcuminoid [2]. Betalain is a group of natural pigments consisting of two main compounds: betaxanthins and betacyanins. These compounds mainly exist in red beetroot, dragon fruit, prickly pear, and flowers from the Caryophyllaceae family. Red beetroot is accessible and contains numerous bioactive compounds among those betalain sources. Betalain is a potential compound used as a food colorant or ingredient for functional food due to its antioxidant, anti-inflammatory, and anti-tumor activities [3]. However, after being extracted, betalain is highly susceptible to the external environment and has low stability compared to artificial synthetic colorants; these are also the main downsides of natural colorants.

Encapsulation methods were used to protect the bioactive compounds, which are sensitive to light, moisture, and temperature; the improving stability and control release of encapsulated compounds were proved. Selecting appropriate encapsulants and encapsulating methods depends on the product end use; several factors such as the concentration, release mechanism, physical and chemical stability, and operation cost should be carefully examined [4]. Gel alginate is one of the ionic gels commonly applied in encapsulating bioactive compounds because of its eco-friendly, low-cost, unsophisticated encapsulation procedure. The ionic gelation of alginate was formed by the interaction of divalent cations such as Ca^{2+} with α-L-guluronic acid chains [5].

Chitosan is a natural polysaccharide consisting of N-acetyl-D-glucosamine and D-glucosamine; these two monosaccharides are linked by β-1,4-glycoside bonding. Chitosan can create an ionic complex with anionic surfactants (sodium dodecyl stearate, sodium stearoyl lactylate, lecithin) or anionic polymer like alginate. Complex formation ability depends on the molecular weight and cationic density of chitosan; the protonation of amine groups at low pH creates the polycation characteristic. Therefore, chitosan can form a polyelectrolyte complex with natural polyanion compounds [6, 7].

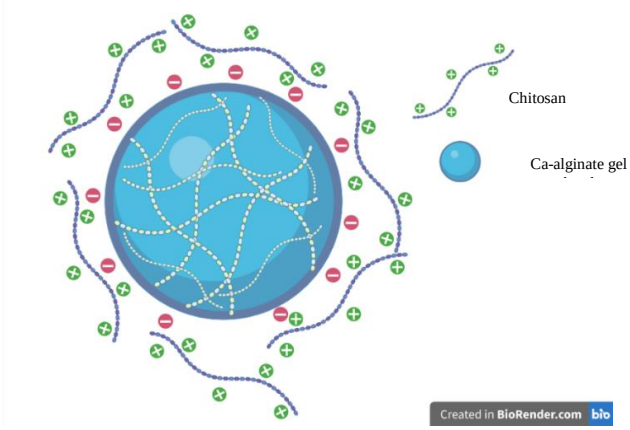


Figure 1. Polyelectrolyte complex of chitosan and ca-alginate bead.

Recently studies on the colorant encapsulation by ca-alginate gel beads and chitosan-coated ca-alginate beads showed the possibility of enhancing the stability and reducing the degradation of the encapsulated compounds. The encapsulation of sensitive colorants like betalain (amaranth leaves, prickly pear), and anthocyanin (hibiscus, mulberry) in gel beads could enhance the stability during storage and color food products like beverages and gummy candy [8-11]. Furthermore, chitosan-alginate beads could aid the release of the encapsulated bioactive compound and probiotic [12, 13].

This study aims to encapsulate betacyanins from red beetroot into chitosan-coated ca-alginate beads, examine the factors that affect the encapsulation efficiency, and investigate the ability to enhance the thermal stability of encapsulated betacyanins.

2. MATERIALS AND METHODS

2.1. Material and chemicals

2.1.1. Preparation of material

Red beetroot originated from Da Lat (Lam Dong province) and was purchased from a local market and supermarket in District 8th Ho Chi Minh City; the average quantity per purchase was 2-3 kilograms; red beetroot was later transported to the food processing laboratory - Saigon Technology University. The raw material was washed, peeled, and diced with dimensions 2×2×2 cm. Pretreated material was packed in zip-lock pouches and stored at -18^{o}C. The frozen raw material's moisture is 91.79 ± 0.79%.

2.1.2. Chemicals

Sodium alginate (Shanghai Lanji - China), Methanol (CH3OH), Trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H2O$), Citric acid monohydrate (C6H8O7.H2O), Sodium acetate trihydrate (CH3COONa).3H2O, Acetic acid (CH3COOH) were purchased from Xilong

Scientific Co., Ltd. (China), Calcium lactate ($C_6H_{10}CaO_6$) was supplied by PATH Co., Ltd (Vietnam), distilled water (Food microbiology laboratory – Saigon Technology University). Low molecular weight (LW; Deacetylation degree: 91.05%), medium molecular weight (MW; Deacetylation degree: 90.49%), and high molecular weight chitosan (HW; Deacetylation degree: 84.43%) were supplied by Vietnam Food Joint Stock Company – VNF (Vietnam).

2.2. Extraction of betacyanins

Betacyanins extract from red beetroot was obtained by maceration method without heating. The ratio of solvent volume and material weight (v/w) was fixed at 30:1; this mixture was dispersed by high-shear disperser IKA T25D (Germany) at 3000 rpm in 3 minutes. The dispersed mixture was placed at room temperature for 20 minutes, then filtrated by quantitative filter paper (20-25 µm) and stored in a refrigerator (7-10^{o}C).

2.3. Betacyanins encapsulation in chitosan-alginate gel beads

Sodium alginate was hydrated with citrate buffer (pH 5.5) 24 hours before forming gel beads. The sodium alginate solution was pumped dropwise into a calcium lactate 4% solution bath by a peristaltic pump with the fixed flowrate (5.06g/min); the ionic gelation was done in an hour, then the blank gel beads were drained and washed with distilled water. Encapsulation was conducted by submerging the blank ca-alginate beads in betacyanins extract in a cool and dark place with the ratio of extract volume and gel beads weight was 2:1; the temperature for encapsulation was 12^{o}C. The initial betacyanins content in the extract was 24.93 ± 0.20 mg/L. Before coating ca-alginate beads with different molecular weight chitosan, chitosan was hydrated with 0.1M acetic acid solution (pH 2.92 ± 0.04); the ratio of chitosan solution volume and gel beads weight was 3:1, and the complex formation was conducted in 2 minutes.

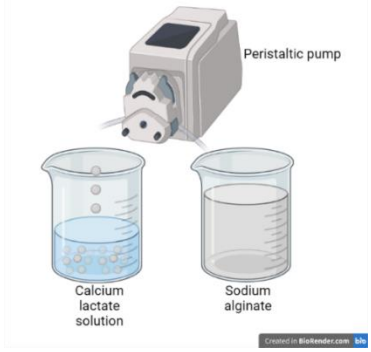


Figure 2. Ca-alginate gel beads formation.

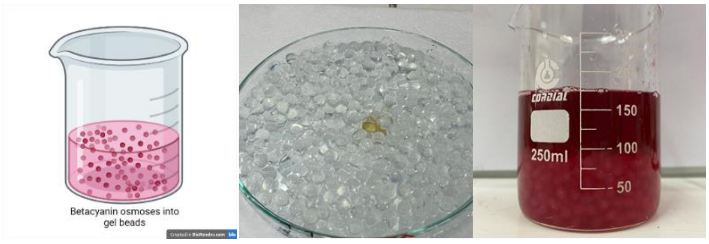


Figure 3. Blank ca-alginate gel beads were submerged in betacyanins extract.

2.4. Determination of moisture

The sample was dehydrated at 105^{o}C, and the sample's moisture was calculated according to the following equation [14]:

$$W = \frac{m_1 - m_2}{m_1} \times 100\%$$

Where: m_1 is sample mass before dehydration (g);

m_2 is sample mass after dehydration (g).

2.5. Determination of betacyanins

Betacyanins in the sample was conducted by spectrophotometry method [9, 15, 16]. Betacyanins extract was diluted with distilled water; then, the diluted extract was transferred into a macro cuvette and measured at wavelength 538 nm. The betacyanins content in the extract was calculated according to equation (1); equation (2) and (3) were used to determine the betacyanins content in the solid sample:

$$\text{Betacyanins (mg/L)} = (A_{538} \times MW \times 1000 \times DF)/(\varepsilon \times L) \quad (1)$$

$$\text{Betacyanins (mg/100gFW)} = (A_{538} \times MW \times V_a \times 100 \times DF)/(\varepsilon \times L \times W_{FW}) \quad (2);$$

$$\text{Betacyanins (mg/100gDW)} = (A_{538} \times MW \times V_a \times 100 \times DF)/(\varepsilon \times L \times W_{DW}) \quad (3)$$

Where: FW is fresh weight; DW is dried weight.

A_{538} is optical absorption of extract at 538 nm;

MV is molecular weight of betacyanins (M = 550 g/mol);

DF is a dilute factor;

ε is a molar absorption coefficient in water (ε = 60000 L/mol.cm);

L is the thickness of the cuvette (d = 1 cm);

V_a is volume of extractant (mL);

W_{FW} is sample mass (g);

W_{DW} is dried sample mass (g).

2.6. Determination of encapsulation efficiency

Encapsulation efficiency (EE%) was determined by the percentage of encapsulated betacyanins and initial betacyanins content in the extract. The determination of encapsulation efficiency followed the method of Vinh Truong et al. [17] with slight modification. 2g of beads were dispersed with citrate buffer (pH 5.5), then filtrated by quantitative filter paper (20-25 µm). The clear filtrate was measured by spectrophotometry at the wavelength of 538nm. The following equation calculated encapsulation efficiency:

$$\text{EE \%} = \frac{Betacyanin\ content\ in\ beads}{Total\ betacyanin} \times 100$$

2.7. Degradation kinetic of betacyanins

The first-order degradation kinetic model was used to describe the degradation of betacyanins, and the rate constant and half-time period were calculated. The method to determine the kinetic of betacyanins degradation was accorded to the studies of Li et al. [18], and Yang et al. [19] 2g of chitosan-alginate beads were placed in a static temperature chamber at 45°C; the total investigation period was 180 minutes. The control sample was conducted with the same procedure; the control in this study was gel beads without chitosan coating. The betacyanins content of samples was measured every 30 minutes; the gel beads were completely dispersed with citrate buffer by IKA T25D high-shear disperser.

First order kinetic model equation: $ln\frac{C_t}{C_o} = -kt$

Half-time period $t_{1/2} = ln\frac{k}{2}$ (h)

Where: C_0 is the initial betacyanins content in gel beads (mg/100g);

C_t is betacyanins content at the time t (mg/100g);

k is degradation rate constant (h^{-1})

t is the observed time (h).

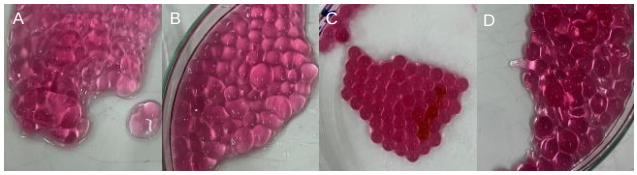
2.8. Statistical analysis

The presented results of this study were the average value of three replications and the standard deviation. One-way analysis (ANOVA) followed by a Turkey test to determine the significant difference between the mean values with the p-value smaller than 0.05. The statistical results were done by JMP software version 13.

3. RESULT AND DISCUSSION

3.1. Effect of alginate concentration on betacyanins encapsulation efficiency

The results showed that the shape of gel beads was significantly affected by alginate concentration; the gel beads' shape and size were more homogenously by increasing alginate concentration. However, high alginate concentration adversely influenced the gelation due to the high viscosity of the alginate solution. The homogenous size and shape of the gel beads were given by 2% of alginate; the recovery of the gel beads was highest without bursting during the washing stage. The beads with lower appropriate concentrations show a lack of rigidity; the loose structure of the beads can be explained by the lack of interaction between carboxyl groups and divalent cations [20]. With 3% alginate concentration, the gel beads were formed into a tadpole shape, and the gel beads were the firmest compared to the other samples. The tadpole shape of the high alginate concentration was reported in previous studies; this can be explained by the high viscosity of alginate solution leading to unevenly interact of cation $Ca2^+$ and alginate when dropping the alginate solution into calcium lactate [21, 22]. The hardness of the gel beads was closely related to the alginate concentration; a previous study by Maleki et al. [23] reported that higher alginate concentration would increase the hardness of gel beads, so the bursting of the hydrogel during forming and storage could be prevented. This study's appropriate alginate concentration for gelation agreed with previous studies that applied ca-alginate in compound encapsulation [11, 20]. The shapes of betacyanins encapsulated gel beads were shown in Figure 4.



(A) 0.75%; (B) 1%; (C) 2%; (D) 3% of alginate concentration

Figure 4. Ca-alginate gel beads with different concentrations of alginate

The blank gel beads were immersed in betacyanins extract for 4 hours. The effect of alginate concentration on the betacyanins encapsulation efficiency was presented in Figure 5. Alginate concentration significantly affected the encapsulation effectiveness; the efficiency tends to increase when alginate concentration increases. This study's results showed that the lowest encapsulation efficiency and encapsulated betacyanins content were achieved from

0.75% alginate beads. The highest encapsulation efficiency was observed in the 2% alginate beads; nevertheless, the efficiency decreased significantly when the 3% alginate beads were used to encapsulate betacyanins.

The diffusion of betacyanins into gel bead and leaching out of the gel bead co-occur while soaking the blank beads in the betacyanins extract; therefore, the loose structure of the low alginate concentration beads with large surface porosity leads to the low ability to encapsulate betacyanins. On the contrary, the firm structure of the high alginate concentration gel beads with dense polymer chains and small surface pores size improves the betacyanins encapsulation. The previous studies also showed that increasing alginate concentration with a fixed concentration of cation Ca^{2+} improved the encapsulation efficiency [20, 24, 25]. However, the gel beads formed by the excess alginate concentration may hinder the diffusion of betacyanins into the gel beads due to the high density of polymer chains; this study's results agreed with the previous research of Nguyen et al [11]. Based on the observed results, the appropriate alginate concentration for betacyanins encapsulating and further experiments was 2 %; this alginate concentration was similar to the study on watermelon's lycopene encapsulation by Sampaio et al. [26] and the encapsulation of betacyanins from red dragon fruit of Farahnaz et al. [27].

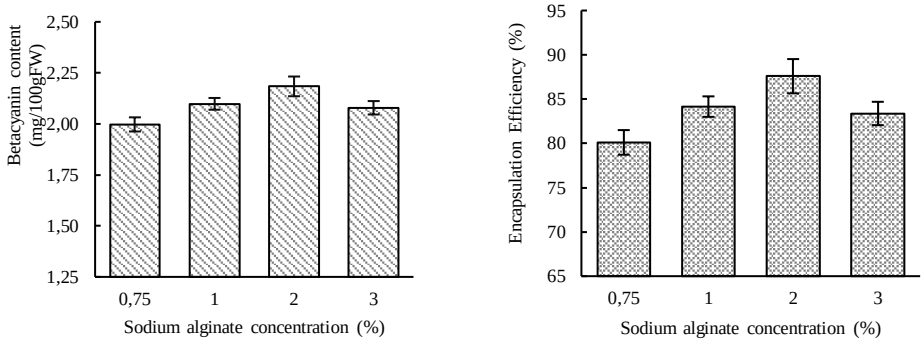


Figure 5. Effect of alginate concentration on encapsulation efficiency and betacyanins content in bead

3.2. Effect of soaking time on betacyanins encapsulation efficiency

The results showed that betacyanins content in beads and encapsulation efficiency tends to increase while extending the soaking time and reach the highest values at 5 hours of soaking; the betacyanins content in beads and encapsulation efficiency were 2.27 ± 0.02 mg/100g and 90.85 ± 0.96%, respectively. With continuous soaking time, the betacyanins encapsulated in beads and encapsulation efficiency decrease significantly. Data were presented in the diagrams in Figure 6.

The betacyanins was diffused into the gel matrix while soaking the blank gel beads in betacyanins extract. The gel beads with porosity structure aid the diffusion rate into alginate beads [28]. The gradient concentration is the main driver for the diffusion of betacyanins into gel beads. The encapsulation efficiency increases upwardly in the first stage because betacyanins penetration into the gel beads was favorable due to high osmosis pressure [11]. Nonetheless, the encapsulation efficiency tends to decrease when extending the soaking time due to the swelling of gel beads during the soaking and the equilibrium of solute concentration between the beads and extract.

The decrease in encapsulation efficiency and betacyanins content in beads can be explained by the betacyanins leaching out of the gel beads due to the degradation of betacyanins in extract when increasing the soaking time. At this stage, the betacyanins content in the extract was lower than the betacyanins content in the gel beads. This result trend agreed

with the research of Nguyen et al. [11]; the anthocyanin was rapidly diffused into the blank gel beads and then slowed down at the end. However, the soaking time in this study was shorter, and encapsulation efficiency was higher than the reported encapsulation efficiency in the research of Nguyen et al. [11].

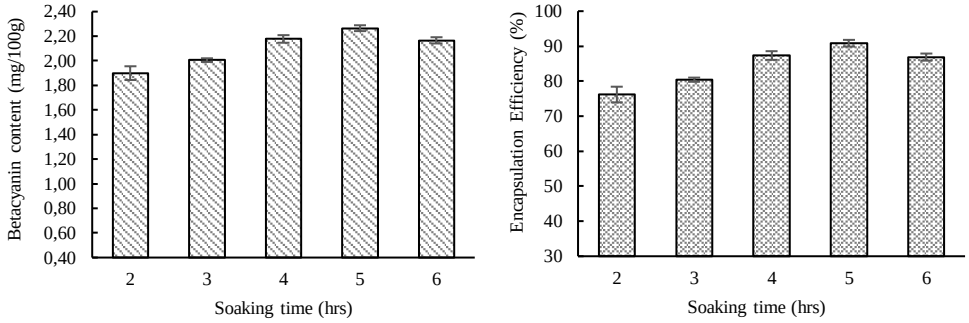


Figure 6. Effect of soaking time on the betacyanins content in bead and encapsulation efficiency

3.3. Degradation kinetic of betacyanins in gel beads

The parameters of degradation kinetic of betacyanins in chitosan-alginate beads, control (beads without chitosan coating), and the extract were presented in Table 1. The degradation kinetic was described according to the first-order model, and the degradation model equations are illustrated in Figure 7. The results showed that the first-order model fitted to describe the degradation of betacyanins with a high coefficient of determination (0.974-0.999). The enhanced thermal stability of encapsulated betacyanins indicates by a lower degradation rate constant (k) and a more significant half-time period ($t_{1/2}$).

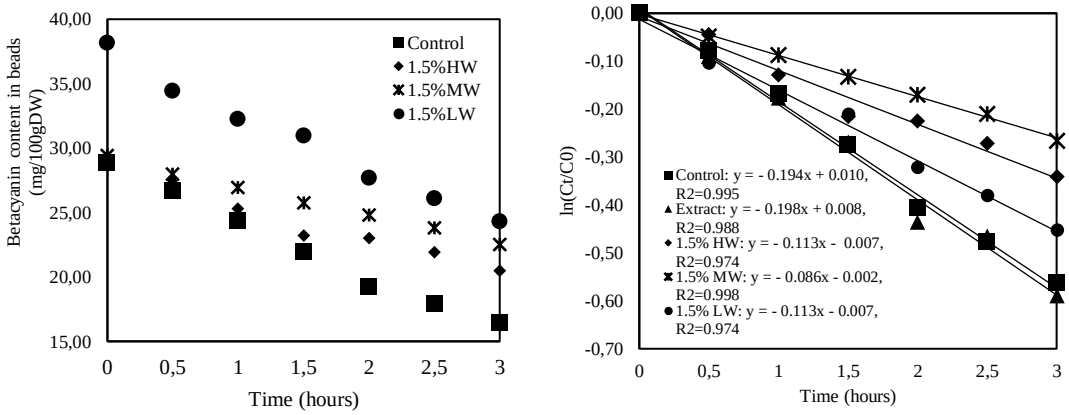


Figure 7. Diagram of the degradation kinetics of betacyanins in extract and encapsulated beads; (■)alginate; (▲)betacyanins extract; (◆)1.5%LW chitosan-alginate; (✱)1.5%MW chitosan-alginate; (●)1.5% HW chitosan-alginate.

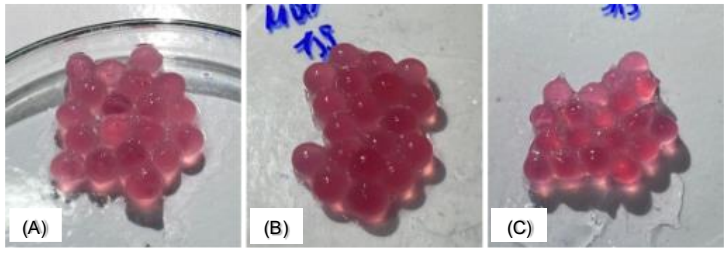


Figure 8. Betacyanins encapsulated in chitosan-alginate beads.
(A) LW chitosan-alginate; (B) MW chitosan-alginate; (C) HW chitosan-alginate.

The degradation rate constant *k* of chitosan-alginate beads tends to decrease and concurrently increases the half-time period with the increasing chitosan concentration. The chitosan-coated beads generally have a slower degradation rate than the control sample and extract. The chitosan polyelectrolyte complex with surface alginate beads showed the ability to hinder the effect of the environment on the encapsulated betacyanins. Betacyanins content in the extract was half degraded in 3.5 hours due to its environmental susceptibility, and the stability of betacyanins in the control beads was not significantly higher than extract. The concentration of the coating chitosan significantly influences the stability of betacyanins. Low chitosan concentration insufficiency covers alginate beads and forms a thinner coating layer; therefore, the stability of low coating chitosan concentration was lower than the high coating chitosan concentration. The stability will improve when the chitosan concentration increases; the results showed that the stability of betacyanins in gel beads coated with 1.5% chitosan was higher than in gel beads coated with 0.75% chitosan. The degradation rate of 1.5% chitosan-coated beads was slower 2 to 3 times than the 0.75%-coated beads. The sufficient chitosan concentration to form the polyelectrolyte complex is crucial for enhancing the stability of encapsulated compounds. Insufficient chitosan concentration may cause the leaching of the encapsulated compounds during the coating stage. This study trend is coherent with previous studies; the chitosan film covering the gel beads can absorb heat and protect the gel from the air, reducing the oxidation of sensitive compounds [29, 30].

Table 1. Betacyanins degradation kinetic parameters

Sample	Equations	Degradation rate constant – k (h^{-1})	Half-time period – $t_{1/2}$ (h)	R^2
Extract	y = - 0.199x + 0.008	0.199	3.488	0.988
Control	y = - 0.194x + 0.010	0.194	3.566	0.995
0.75 LW	y = - 0.234x + 0.009	0.234	2.957	0.996
0.75 MW	y = - 0.170x + 0.010	0.170	4.084	0.996
0.75 HW	y = - 0.169x - 0.005	0.169	4.103	0.993
1 LW	y = - 0.184x - 0.022	0.184	3.759	0.986
1 MW	y = - 0.122x + 0.004	0.122	5.670	0.999
1 HW	y = - 0.151x - 0.000	0.151	4.605	0.987
1.25 LW	y = - 0.163x + 0.011	0.163	4.265	0.986
1.25 MW	y = - 0.088x + 0.001	0.088	7.885	0.996
1.25 HW	y = - 0.122x - 0.007	0.122	5.683	0.981
1.5 LW	y = - 0.148x - 0.013	0.148	4.699	0.992
1.5 MW	y = - 0.086x - 0.002	0.086	8.055	0.998
1.5 HW	y = - 0.113x - 0.007	0.113	6.162	0.974

The different molecular weight of chitosan also affects the stability of betacyanins. The stability of betacyanins encapsulated in gel beads coated with MW chitosan was highest, followed by the HW and LW chitosan. Previous studies also reported that the molecular weight and deacetylation degree of chitosan significantly influence the characteristics of polyelectrolyte complex film; therefore, the protection ability depends on the chitosan properties. The high deacetylation degree increases the electrostatic force between chitosan and alginate, and the low molecular weight of chitosan decreases the layer's thickness [31].

The observed results are consistent with previous studies; the beads coated with MW and HW chitosan can better protect against the degradation of betacyanins than LW chitosan-coated beads due to the thickness of the coated chitosan. The betacyanins protection in the MW chitosan beads was more effective than in HW chitosan beads can be explained by two main reasons. Firstly, the degree of deacylation of MW chitosan was higher than HW chitosan; secondly, the HW chitosan chains need more time to arrange and interact with the alginate gel beads during the coating stage [32, 33].

4. CONCLUSION

This study proved the ability to stabilize the betacyanins extracted from red beetroot by encapsulating it in the MW chitosan-alginate gel beads. The encapsulation efficiency in this study was $90.85 \pm 0.96\%$, and the degradation rate of encapsulated betacyanins in MW chitosan-alginate beads ($k = 0.086$) was two-fold slower than in uncoated samples ($k = 0.194$) and extract ($k = 0.199$). However, several evaluations on the stability of the encapsulated betacyanins in different food matrixes are necessary for betacyanins application. Furthermore, other factors that affect the stability of betacyanins, such as pH, oxygen, and light, need to assess in future studies.

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TÓM TẮT

NGHIÊN CỨU ỔN ĐỊNH BETACYANIN TỪ CỦ DỀN BẰNG PHƯƠNG PHÁP BAO GÓI TRONG HẠT GEL ALGINATE-CHITOSAN

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Betacyanin là hợp chất hữu cơ đóng vai trò tạo màu đỏ trong củ dền; đây cũng là nhóm chất màu tự nhiên có thể dùng làm chất tạo màu thực phẩm. Để việc khai thác và tận dụng chất màu thực phẩm tự nhiên từ nguồn nguyên liệu ổn định tại địa phương được khả thi và bắt kịp xu hướng trong những năm gần đây, các nghiên cứu giúp tăng độ ổn định của chất màu tự nhiên là công việc cần được thực hiện. Trong nghiên cứu này, dịch chiết giàu betacyanin được thu nhận bằng phương pháp ngâm chiết không gia nhiệt với hàm lượng betacyanin thu được là 24,93 ± 0,20 mg/L; sau đó, betacyanin được bao gói trong hạt gel alginate-chitosan hiệu suất bao gói đạt được là 90,85 ± 0,96%. Phương pháp bao gói betacyanin trong hạt gel alginate phủ chitosan phân tử lượng trung bình cho thấy khả năng tăng độ ổn định của betacyanin sau khi trích ly. Nồng độ chitosan phù hợp để phản ứng với alginate được xác định là 1,5% và mô hình động học bậc nhất được sử dụng để mô tả quá trình thoái hóa nhiệt của betacyanin với hằng số tốc độ thoái hóa và chu kỳ bán rã được xác định thông qua phương trình động học.

Từ khóa: Củ dền, chất màu tự nhiên, hạt gel alginate-chitosan.