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Original research article

Influence of Whey Protein on Stability of α-Mangostin Entrapped in Alginate/Whey Protein Beads

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ABSTRACT

Although encapsulation efficiency of various biopolymers has been documented, no attempts have been made to investigate stability of α-mangostin entrapped and an encapsulation efficiency of biopolymer beads in different conditions. In this study, the efficiency of whey protein (W) and Alginate (A) to encapsulate α-mangostin was assessed through the investigation of the α-mangostin content stored at different conditions. Particle size and stiffness of biopolymer beads were measured. An increase in whey protein content enhanced gel viscosity, which resulted in particles with significantly higher dimension size and weight ($p<0.05$). A decrease in encapsulation efficiency was found at higher temperatures and lower pH values. However, higher encapsulation efficiency was observed in gel beads which contained whey protein (50A : 50W and 60A : 40W) at all conditions. An increased protein content resulted in a higher gel network density and retention of the α-mangostin in the beads. The released α-mangostin content decreased with increasing protein content at all conditions during storage. The results confirmed that the α-mangostin stability was significantly governed by both conditions and the use of whey protein to protect the target compound.

Keywords: Whey protein; Alginate; α-mangostin; Encapsulation; Biopolymer; Bead

1. Introduction

Bioactive plant phenolic compounds such as xanthones are mostly found in mangosteen pericarp. Mangosteen (*Garcinia mangostana* L.) or “Mang Khut” is a tropical fruit in Southeast Asia [1]. Xanthones are a class of phenolic compounds, which are one of the most

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potent natural antioxidants. In several studies, xanthones have shown remarkable pharmacological effects, i.e., antioxidant, anticancer, anti-inflammatory and antibacterial, which are related to potential health [2-4]. The α-mangostin is one of the six xanthones reported to show the highest antioxidant activity [1,5]. However, xanthones probably undergo degradation during the processes and storage including environmental conditions in the human gastrointestinal tract. Moreover, most of phenolic antioxidants often show low stability after extraction and digestion due to the acidic pH values below 3. Rapid degradation of bioactive ingredients could result from environmental conditions such as pH, temperature, oxygen, light, etc. [6-9]. Therefore, the objective of this study was to determine the stability of α-mangostin entrapped in spherical biopolymer beads for application as food additives and to investigate the encapsulation efficiency for α-mangostin release.

2. Materials and Methods

2.1 Materials

Biopolymer beads were formed using whey protein concentrate (W) containing 80% protein (Vicchi Enterprise Co., Ltd., Bangkok, Thailand). Calcium chloride ($CaCl_2$), sodium alginate (A) was purchased from Fisher Scientific, Pittsburgh, PA, USA. Dried powder of mangosteen pericarp extract used as a model water-soluble core (model release compound) was commercially supplied by Thiptipa Co., Ltd. (Bangkok, Thailand) and purified α-mangostin (99.9%) was also purchased from Sigma-Aldrich, St. Louis, MO. Sodium azide was purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.1.1 Preparation of biopolymer beads

A mixture of whey protein (W) and sodium alginate (A) solution used as a wall material of biopolymer beads was prepared by the method of Wichchukit et al., [10] with some modifications. A solution of 20% (w/w) whey protein was dispersed in distilled water at 40°C and allowed to stand at room temperature for a complete protein hydration. Then, the whey protein solution was heated at 80°C for 30 min for proteinase denaturation described by Egan et al., [11]. The denatured whey protein solution was cooled to 30°C and held at that temperature until use. Sodium alginate solution (1.5% w/w) was prepared using distilled water (40°C) and stirred overnight. Sodium azide (0.02% w/w) was added to the mixture of whey protein and sodium alginate solution as an antimicrobial agent. Three biopolymer solutions were prepared by mixing the A solution with denatured whey protein solution at following ratios of sodium alginate to whey protein as 50A: 50W, 60A: 40W and 100A: 0W. For sustained release experiments, mangosteen pericarp extract (10% w/w) was added to the mixture of sodium alginate and whey protein solution.

To form biopolymer beads according to the method of Cheng et al., [12] with some modifications, each polymer solution was dropped slowly through a 21 gauge needle (0.8 mm diameter) from a 10 cm falling distance into 200 mL of mildly agitated 0.25 M $CaCl_2$ solution using a peristaltic pump (Harvard Apparatus, Mills, MA, USA) with a rate of 0.64 ml/min at room temperature (25°C). The formed beads were mildly stirred at room temperature for 45 min. After 45 min, the beads were filtered and washed with distilled water to eliminate excess free Ca^{2+} retained in the beads.

2.1.2 Particle size measurement

The sizes of gel beads were determined by an optical microscope (E100 LED, Nikon, USA.) according to the method of Nithitanakool et al., [7] with some modifications. Thirty beads randomly selected were captured through a digital camera (Moticam 2300) connected with a microscope, and the diameter of each gel bead was measured by a pre-calibrated image analysis program (Motic Image Plus, Version 2.0). The assay was carried out in triplicate and the results were expressed as mean values and standard deviations (SD).

2.1.3 Determination of bead stiffness

Stiffness of biopolymer beads was measured by a Texture Analyzer TA XT-2 (Stable Micro Systems Ltd., Godalming, Surrey UK) according to the method of Betz and Kulozik [6] with some modifications. The gel strength or stiffness of gel beads was determined by using a 5 mm probe with 0.5 $mm.s^{-1}$ compression speed and 10 mm depth. The maximum of the force-time was referred to as stiffness (N). The measurement was carried out in triplicate and the results were expressed as mean values and standard deviations (SD).

2.1.4 Determination of the encapsulation-related stability of biopolymer beads

The effect of biopolymer gel entrapment on stability of α-mangostin was determined according to the method of Betz and Kulozik [6] with some modifications. In the present study, the formed biopolymer beads with different ratios of sodium alginate and whey protein (50A : 50W, 60A : 40W and 100A : 0W) were stored at two different temperatures (4 and 30°C) and two different pH values (1.2 and 7.4) for 14 days. In the first set of experiments, each sample of biopolymer beads (10 beads) was transferred into a glass vial with a stopper containing 10 mL of aqueous hydrochloric acid solution (pH 1.2) and stored at 4 and 30°C in the dark. Similarly, each sample of beads was placed in a glass vial containing 10 mL of phosphate buffer saline (PBS) solution (pH 7.4) and kept at the temperatures of 4 and 30°C in the dark for the second set of experiments. All sample beads were withdrawn at specific time intervals (0, 2, 4, 10, 12 and 14 days). Each sample of the beads was decomposed with an Ultra Turrax (IKA-Werke GmbH & Co., KG, Staufen, Germany)and then centrifuged (ALC International, PK-131R, MI, Italy) at 19,000 rpm for 10 min. The α-mangostin of the supernatant was analyzed by using HPLC. All measurements were carried out on triplicate samples. The decrease of α-mangostin content during storage C (t = n) was related to the α-mangostin content before storage C (t = 0) as in Eq. (2.1):

$$Relative\ \alpha - \text{mangostin content} = \frac{c\,(t=n)}{c\,(t=0)} \quad (2.1)$$

2.1.5 Analysis of α-mangostin content

The α-mangostin content of a sample was determined by HPLC according to the method of Silalai et al., [9] with some modifications. Each sample (approximately 200 mg) was dissolved in 10 mL of 95% (v/v) ethanol and extracted in an ultrasonic bath (Lennox, 2645 Ultrawave, Cardiff, UK) at 30 kHz for 15 min at room temperature. The mixture was then centrifuged (ALC International, PK-131R, MI, Italy) at 10,000 rpm for 5 min. The collected supernatant was transferred to a 10-mL volumetric flask; the extract was filled up to the final volume of 10 mL with 95% (v/v) ethanol. Prior to injection (10 mL) the extract was filtered through a 0.45-mm nylon membrane filter (Millipore Corp., Bedford, MA). The α-mangostin content was analyzed via an HPLC system consisting of a pump and controller (Agilent 1200 series, Agilent Technologis, Palo Alto, CA) equipped with a diode array detector (detection at 254 nm). Symmetry® C18 5 µm (4.6 x 150 mm) (Agilent Zorbax, Agilent Technologis, Palo Alto, CA) was used for the analysis of α-mangostin. The mobile phase comprised A: 0.1% formic acid in HPLC water and B: methanol. The mobile phase was applied by gradient elution at following proportions: 35% A/65% B (v/v) to 10% A/90% B (v/v) in 1800 s at a flow rate 1 mL/min (0.0167 mL/s). The mobile phase was degased by an ultrasonic bath at 30 kHz for 900 s at room temperature. The injection volume, 10 mL purified α-mangostin assay, was performed as a standard curve with different concentrations (0, 25, 50, 75, 100, 125, 150, 175 and 200 mg/L).

2.1.6 Encapsulation efficiency

Encapsulation efficiency (*EE*) was calculated according to the method of Shah et al., [14] with some modifications. In the present study, the encapsulation efficiency of gel beads with and without protein is defined as the α-mangostin retention in the gel beads. The α-mangostin retention is equal to the ratio of the actual loading of α-mangostin to the theoretical loading of α-mangostin Eq. (2.2).

$$\alpha - \text{mangostin retention } (\%) = \frac{\textit{Actual loading of } \alpha\textit{-mangostin}}{\textit{Theoretical loading of } \alpha\textit{-mangostin}}\ x\ 100 \qquad (2.2)$$

2.1.7 Statistical analysis

All data were analyzed using SPSS 16.0 for Windows® (SPSS Inc., Chicago, IL) and presented as mean values with standard deviations. Differences between mean values were established using Duncan's new multiple range tests at a confidence level of 95%.

3. Results and Discussion

3.1 Particle size of biopolymer beads

As expected, protein content has a significant effect on mean particle size of the biopolymer beads. The mean diameters of the beads were larger with higher protein content (Table 1). The factor contributing to the increase in mean particle size was probably due to the viscosity of the liquid drop phase with increasing protein content, which caused the formation of a larger particle of each gel bead [12,13]. Changes in mean particle size or gel droplet size with increasing protein content affected the mean weight of the droplet particle. The bigger the mean particle size, the higher its mean weight (Table 1). The mean diameters of bead size for 50A : 50W, 60A : 40W and 100A : 0W were 3.05, 2.59 and 2.2 mm, and the mean weights of the beads were 19.82, 16.11 and 12.14 mg, respectively. This result was consistent with the study of Wichukit et al., [10] indicating that an increase in whey protein content enhanced gel viscosity, which resulted in significantly higher dimension size and weight particles ($p<0.05$).

Table 1. Physical properties of gel beads with different protein contents.

Treatment	Mean diameter (mm)	Mean weight (mg)	Encapsulation Efficiency (%)
50A : 50W	3.05 ± 0.15^a	19.82 ± 2.27^a	94.3 ± 3.7^a
60A : 40W	2.59 ± 0.17^b	16.11 ± 1.36^b	76.9 ± 1.9^b
100A : 0W	2.28 ± 0.12^c	12.14 ± 1.29^c	63.8 ± 2.8^c

Note: Each value represents the mean ± SD (n = 3).

3.2 Encapsulation efficiency of gel beads

The encapsulation efficiency (% *EE*) of gel beads can be calculated from Eq. (2.2) as the ratio of actual to theoretical of α-mangostin content in the gel beads. The actual loading of α-mangostin was higher in the beads with whey protein than in those without whey protein. The actual loading of α-mangostin in the beads with 50% whey protein (50A : 50W) was 77 - 82%, with 40% whey protein (60A : 40W) it was 63 – 66% and without whey protein (100A : 0W) it was 51 – 56%. In addition, the actual loading of α-mangostin in the beads (53 - 78%) was lower than the theoretical loading (83%), corresponding to the encapsulation efficiency (% *EE*) of the beads. The encapsulation efficiency is given in Table 1. The *α*-mangostin entrapped in protein gel was lower in the system without whey protein (100A : 0W) indicating the encapsulation efficiency. The % *EE* of the beads increased from 63.8 to 94.3%, when protein content was added from 0 to 50% whey protein. The *α*-mangostin in the beads (50A : 50W) was the highest, while the % *EE* of the beads without protein (100A : 0W) was the lowest. It showed the effect of protein on the encapsulation efficiency that molecules of protein may undergo self-assembling process during hydration and heating [6]. This can result in formation of strong structure entrapping *α*-mangostin in the beads.

Table 2. Stiffness and *α*-mangostin stability of gel beads with and without whey protein kept at different conditions at 14-day storage. (n = 3)

	Treatment	*α*-mangostin content (%)	Stiffness (N)
At 4ºC in pH 7.4	50A : 50W	97.0 ± 1.5	14.8 ± 0.8
	60A : 40W	78.8 ± 0.7	9.7 ± 0.5
	100A : 0W	32.7 ± 1.1	4.1 ± 0.4
At 30ºC in pH 7.4	50A : 50W	90.4 ± 1.1	13.4 ± 0.5
	60A : 40W	75.9 ± 1.1	9.0 ± 0.6
	100A : 0W	28.5 ± 1.3	3.8 ± 0.4
At 4ºC in pH 1.2	50A : 50W	85.6 ± 1.2	12.3 ± 0.4
	60A : 40W	70.2 ± 1.7	11.2 ± 0.4
	100A : 0W	25.6 ± 1.3	2.5 ± 0.3
At 30ºC in pH 1.2	50A : 50W	80.0 ± 0.8	11.9 ± 0.4
	60A : 40W	65.2 ± 1.7	9.5 ± 0.5
	100A : 0W	24.7 ± 1.2	2.0 ± 0.5

Note: Each value represents the mean ± SD (n = 3).

3.3 Effect of whey protein on the α-mangostin stability during storage

Temperature and pH affected *α*-mangostin entrapped in the systems associated with encapsulation efficiency of protein, which could be observed from the stability of *α*-mangostin content in the beads (Table 2). A decrease in *α*-mangostin content was found at higher temperatures and lower pH values. The lowest content of *α*-mangostin was found at 30ºC and pH 1.2, while the highest content was found at 4ºC and pH 7.4 as a result of accelerating degradation by temperature and acid. However, higher *α*-mangostin content was observed in gel beads that contained whey protein (50A : 50W and 60A : 40W) at all conditions. This was consistent with the study of Betz and Kulozik [6] indicating gel strength of whey protein. Associated with the rise in gel strength, an increased protein content is expected to result in a higher gel network density measured by stiffness and, thus, in a favored core material retention of the gel beads.

In addition, the *α*-mangostin stability in gel beads with 0, 40 and 50% (w/w) protein during storage was shown in Figure 1. Temperature and pH-dependent degradation of *α*-mangostin followed the first-order kinetics according to the earlier study [9]. Therefore, the first-order fitting of data was carried out in the present study (Table 3). In regards to the storage conditions, gel-entrapped *α*-mangostin content decreased during storage. The released *α*-mangostin content decreased dramatically with increasing protein content at all conditions during storage.

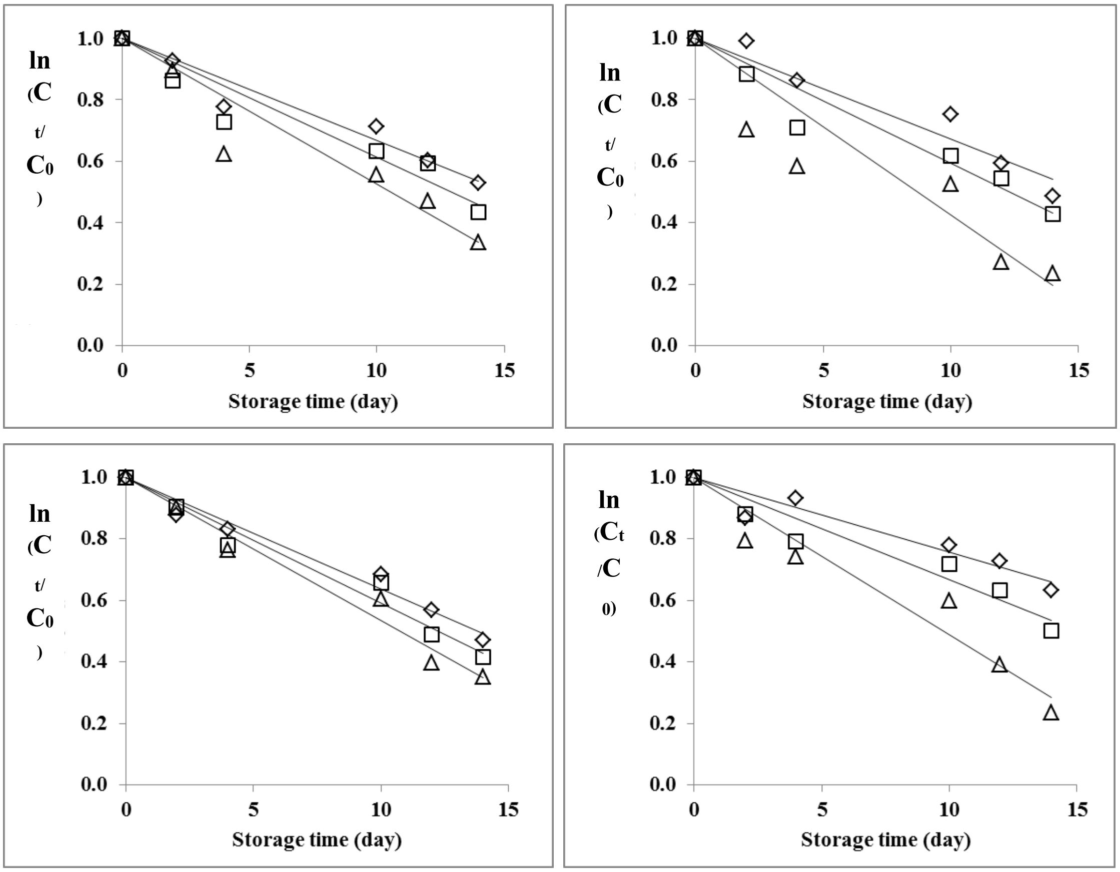

Fig. 1. Relative α-mangostin content of alginate (A) and whey protein (W) gels in aqueous solution at pH 1.2 and pH 7.0 with 5% (w/w) mangosteen pericarb extract content during storage at different temperatures (4°C and 30°C): ◇ 50A:50W; □ 60A:40W; △ 100A:0W.

Kinetic rates of α-mangostin in all conditions are given in Table 3. They indicate that the degradation rate of α-mangostin was higher in gel beads containing only sodium alginate (100A:0W), whereas a slight decrease in degradation rates of α-mangostin was found in the gel beads containing whey protein (50A:50W and 60A:40W). As already discussed in a previous study [6], protein would enhance gel strength leading to a higher gel network density and causing a decreased α-mangostin degradation. Therefore, the results indicated addition of whey protein could reduce the α-mangostin degradation at all pH and temperatures. The results confirmed that the α-mangostin stability was significantly governed by both conditions and the use of whey protein to protect the target compound.

Table 3. Kinetic rates of α-mangostin content of alginate (A) and whey protein (W) gels with 5%(w/w) mangosteen pericarb extract content during storage at different conditions (4°C and 30°C).

Condition	Treatment	Rate	R^2
At 4°C in pH 7.4	50A:50W	0.0308	0.9462
	60A:40W	0.0336	0.9261
	100A:0W	0.0420	0.9081
At 30°C in pH 7.4	50A:50W	0.0356	0.9516
	60A:40W	0.0360	0.9405
	100A:0W	0.0463	0.8791
At 4°C in pH 1.2	50A:50W	0.0342	0.9757
	60A:40W	0.0394	0.9712
	100A:0W	0.0454	0.9746
At 30°C in pH 1.2	50A:50W	0.0224	0.9054
	60A:40W	0.0297	0.9355
	100A:0W	0.0471	0.9396

4. Conclusion

Encapsulation efficiency decreased with increasing temperature and decreasing pH in all treatments. However, it was found that the addition of whey protein can retain α-mangostin content in the beads during storage at all pH and temperatures. The results indicated that the α-mangostin stability was significantly governed by both conditions and the use of whey protein to protect the sensitive ingredients from severe environment.

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