STABILITY OF ANTHOCYANIN IN MULBERRY FRUITS EXTRACT ADSORBED ON CALCIUM ALGINATE BEADS

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Abstract

Anthocyanins from water extracts of the mulberry fruits (*Morus alba L.*/Jul cultivars) were adsorbed on calcium alginate beads. The beads were produced by external gelation using different concentrations of alginate solution. The calcium alginate beads were studied on capability of water, mulberry extract adsorption and anthocyanin stability at high temperature. The adsorption ability of the bead was found to be depended on the pH of the aqueous media. Mulberry extracts (pH4.3) had the highest adsorption at 1684.30±154.80 %(wt/wt). Beads (1.5%Alginate, ALG) average size were at 415.63±13.09 μm. Water (pH6.8) adsorption of the same type of beads was at 242.68±2.59 %(wt/wt) (325.00±17.09 μm). Anthocyanin encapsulation efficiency was at 17.08±0.25 mg/g Dry weight. Stability of adsorbed anthocyanin on calcium alginate bead was studied. After heat exposure at 40 ^{o}C and 100^{o}C for 10 hrs, anthocyanin was at 92%wt and 24%wt, respectively. Calcium alginate beads enhanced anthocyanin stability in the beads at these conditions by 10-40%. These results provided the useful data for process and storage condition of mulberry products.

Keywords: calcium alginate, anthocyanins, mulberry extracts

Introduction

Hydrogel is a commonly used for bioactive agents and cell encapsulation due to its capability to absorb large amount of water or biological fluids. Among many materials, calcium alginate hydrogel is the most widely used due to several advantages such as non-toxicity, biocompatible, easily produced; thermally and chemically stable (Chan et al. 2010). Alginate is a natural biopolymer extracted from brown algae. It is composed of linear chains of the α-l-guluronic acid (G) and the β-d-mannuronic acid (M). Alginates form ionic reversible hydrogels in the presence of divalent cations like Ca^{2+}. The interaction with blocks of G monomers in alginate resulting in ionic bridges between different polymer chains so called "egg-box model" (Chan et al. 2009) (Figure 1).

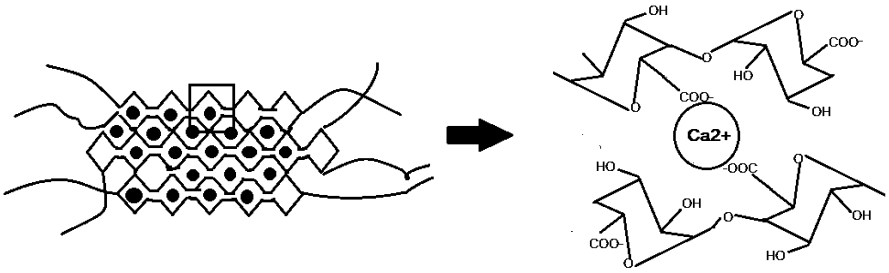

Figure 1 Structure of "egg-box model" of ionic interactions between Ca^{2+} and alginic acid (adapted from Dupuis et al. 2006)

Anthocyanin is one of important naturally derived antioxidants. It is water soluble pigment found in variety of vegetables and fruits. Mulberry fruit is rich in anthocyanins. There are various types of anthocyanins found in mulberry fruits extracts, of these 98% were cyanidin-3-glucoside and cyanidin-3-rutinoside (Qin et al. 2010). Anthocyanin can be considered for uses in cosmetics, nutraceutical and food industry. It has been reported for its anti-inflammatory, anti-cancer and anti-tyrosinase activities (Aramwit et al. 2010). However, anthocyanins are very unstable for storage and processing. They are sensitive to pH, oxygen, light, temperature and the present of chemicals (Tanon et al. 2008).

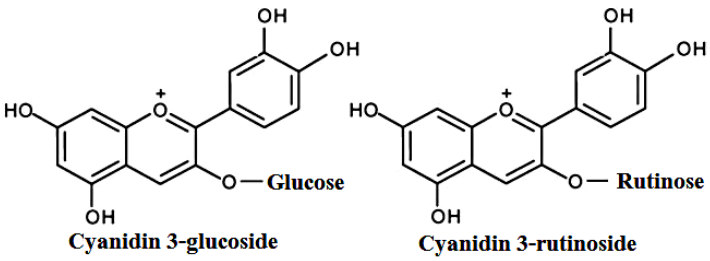

Figure 2 Structure of major anthocyanin found in mulberry fruits (Butt et al. 2008)

The aim of this study was to enhance stability of anthocyanin using adsorption on calcium alginate beads. The effect of alginate concentration and nitrogen gas flow rate on calcium alginate beads formation, adsorption of mulberry extracts and water on beads were evaluated. The beads were exposed to heat for extended time and their anthocyanins contents were evaluated in comparison to the free mulberry fruits extracts solution.

Methodology

Preparation of mulberry fruits extracts

Mulberry fruits powder (freeze dried, received from Chul Thai Agro-Industry Co, Ltd., Thailand) was dissolved in deionized (DI) water at 10%wt. The extracts were protected from lights at time of experiment. The extracts were centrifuged at 4500g for 20 min at 4°C and the supernatants were filtered through a filter paper.

Preparation of calcium alginate beads

Alginate solution (1.0, 1.5, 2.0 and 2.5 %w/v) was prepared from medium viscosity alginic acid (sodium salt, Sigma-Aldrich, USA). The solution was sprayed through two nozzles into a $CaCl_2$ (0.1M) bath (Figure 3). Flow rate of nitrogen gas (10, and 15 L/min) were used to minimize oxidation. The calcium alginate beads were hardened in $CaCl_2$ solution for 30 minutes. The beads were oven dried at 60°C for 4 hours and were kept dried for the experiments.

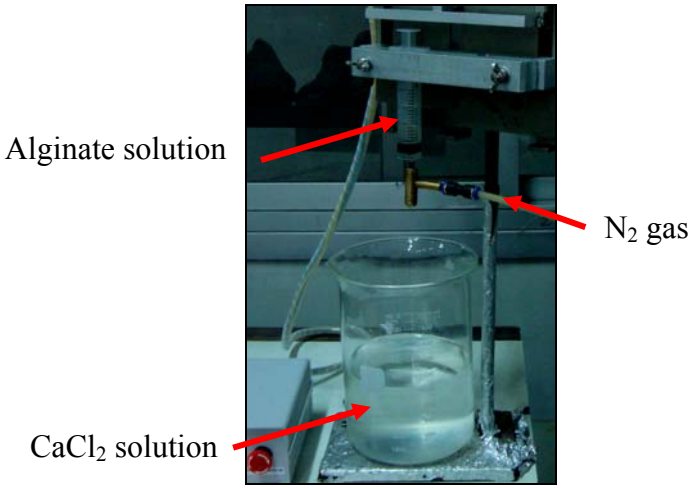

Figure 3 Preparation calcium alginate beads using external gelation method

Adsorption of water/mulberry extracts on calcium alginate beads
Dried calcium alginate beads were immersed in DI water or mulberry fruit extracts solution (10%w/v) for 2 hours at 4°C. The adsorbed beads were freeze-dried for 48 hours.

Particle size analysis
Sizes of the obtained beads were estimated determined using Mastersizer 2000 (Malvern Instruments Ltd., UK). The beads were suspended in deionized water and were stirred at 1,750 rpm during the analysis at room temperature.

Morphological analysis
Oven dried calcium alginate beads were observed under on scanning electron microscope (SEM, JSM-5410LV, Japan) to examine their shape and morphology. Mulberry fruits extract adsorbed on calcium alginate beads were observed under an optical microscopy (Nikon elipse, TS100, Japan).

Anthocyanin contents
Total anthocyanins content was evaluated using the pH-differential method (Durst and Wrolstad 2005). The mulberry fruits extracts were dissolved in 0.025M potassium chloride buffer (pH 1.0) and 0.4M sodium acetate buffer (pH 4.5). These two buffers represent the conditions when anthocyanin would be the most and the least stable. The absorbance was measured using spectrophotometry at 520 and 700 nm. The absorbance (A) of the diluted sample was then calculated as follows:

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5} \quad \text{.....(1)}$$

Total anthocyanins were calculated as cyanidin-3-glucoside according to the following equation:

$$\text{Total anthocyanins (mg/L)} = A \times MW \times DF \times 1000/(\varepsilon \times 1) \quad \text{.....(2)}$$

Where MW = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; 1 = path length in cm; ε = 26,900 molar extinction coefficient in L/mol/cm; 1000 = conversion from gram to milligram.

Total anthocyanin content on calcium alginate beads

Total anthocyanin content was determined by the spectrophotometric method at 530 nm. About 10 mg of mulberry fruit powder was extracted two times with 10 ml of a HCl/water/ethanol solution (1/29/70) (Tanon et al. 2010). The extracts were centrifuged for 10 min at 10,000g and the light absorbents were recorded in a Thermo spectrophotometer (Thermo Spectronic, Genesys 10UV scanning, USA). Total anthocyanin content was expressed as cyanidin-3-glucoside as previously described by Tanon et al (2010). The results were expressed in mg/g of dried calcium alginate beads.

Stability of anthocyanins

Stability of anthocyanins from mulberry fruit extract solution and the adsorbed extracts on calcium alginate beads were evaluated at 40 and 100^{o}C. The samples, protected from lights, were placed in an oven and were rapidly cooled in an ice bath. Anthocyanin content was evaluated using pH-differential method as previously.

Statistical analysis

All statistical calculations were performed using MINITAB (Statistical software, version 15.1.1, PA, USA). The differences of data analyzed using ANOVA were considered at $p < 0.05$ ($n = 3$).

ResultsMorphology of calcium alginate beads.

Table 1 shows shapes and average size of calcium alginate beads prepared at difference alginate concentration and flow rate of N_2 gas. An alginate concentration of 1.0%w/v and 1.5% w/v were found to give spherical bead shape at 10 L/min of N_2 gas. However, the beads became non spherical when the alginate concentration increased at the same flow rate of nitrogen gas. Increasing the flow rate of N_2 gas (10 and 15 L/min) resulted in production of small size beads and aggregated beads. Using spraying technique, the liquid droplets were formed by their surface tension force. The increases in the viscosity (increases of the concentration of alginate) were found to be important to rheological property of the bead formation (Lee et al. 1996). It was found that the N_2 gas flow rate required to form spherical beads were at 10 L/min for 1.0 and at 1.5%w/v of alginate and 15 L/min for 2.0 and 2.5%w/v of alginate. The average sizes of the bead were produced at 325.00±17.09, 415.63±13.09, 302.30±12.90 and 291.78±05.56 μm, respectively.

Table 1 Morphology of calcium alginate beads prepared by external gelation at difference alginate concentrations and N_2 flow rate

Samples	Alginate concentration (%w/v)	N_2 gas flow rate (L/min)	Bead shape	Average size (μm)
1.0%ALG	1.0	10	round	325.00±17.09^{a}
1.5%ALG	1.5	10	round	415.63±13.09^{b}
2.0%ALG	2.0	15	pear-like	302.30±12.90^{a}
2.5%ALG	2.5	15	round mixed pear-like	291.78±05.56^{a}

ALG= alginate, a and b represents the significant difference at p<0.05

Calcium alginate beads after oven dried at 60°C about 4 hours followed by the fully adsorption of DI water (pH6.8) are shown in Figure 4. After preparation calcium alginate bead had spherical shape (results are not shown here), however the oven bead were shrunk and decreased in size. More irregular surfaces were observed on the beads prepared using high alginate concentrations.

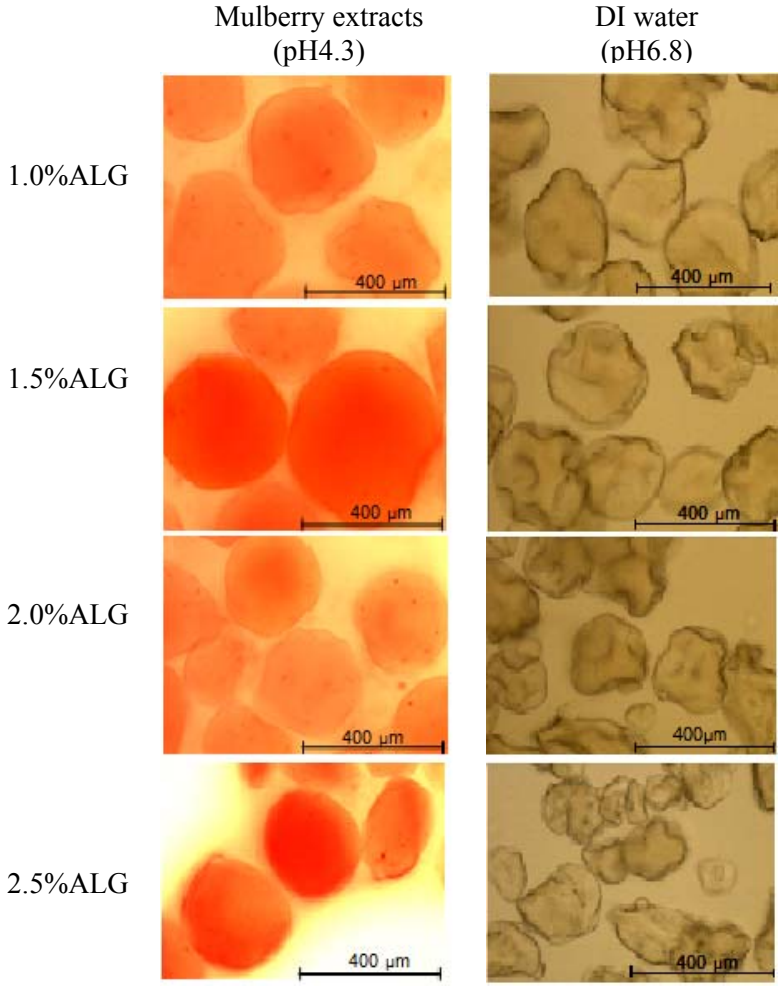

Figure 4 Mulberry extracts and DI water adsorption of calcium alginate beads (1.0, 1.5, 2.0 and 2.5%ALG) for 4 hours (37°C)

Mulberry extracts and water adsorption.

The calcium alginate beads had higher ability in adsorption of mulberry extract (pH 4.3) than the adsorption of DI water (pH 6.8) (Table 3). Characteristics of mulberry fruits extracts were analysed and shown in Table 2 (Yamdech 2012). Water adsorption of the samples was not significantly different while the adsorption of mulberry extracts was. The mulberry extracts adsorption was at 1269-1632%wt. The beads swelled more in mulberry extracts than in DI water (Figure 4). This indicated the effect of pH on swelling of the beads

Table 2 Properties of mulberry fruits extract from mulberry fruits powder.

Density (g/ml)	0.988±0.016
Brix (%)	7.68±0.17
pH	4.04±0.01
Titration acidity (%w/v)	0.80±0.01
Reducing sugars (mg Glucose/g DW)*	127.26±9.29
Anthocyanin (mg/DW)*	12.14±0.77
IC_{50} (mg/ml)	0.28±0.02

** DW = dried weight*

Table 3 Adsorption of mulberry extracts (pH4.3) and DI water (pH6.8) on dried calcium alginate beads

Samples	Adsorption	
	DI water (%wt)	Mulberry extracts (%wt)
1.0%ALG	242.13±2.59[a]	1419.86±225.10[b,c]
1.5%ALG	210.68±19.61[a]	1632.07±122.52[c]
2.0%ALG	236.14±39.53[a]	1373.74±55.70[b,c]
2.5%ALG	234.04±27.08[a]	1269.11.88±48.74[d]

a-d represents the significant difference at p<0.05

Anthocyanin content in calcium alginate beads.

Beads produced with 1.5%ALG could encapsulate the highest anthocyanin content (17.08±0.25 mg/g dry weight**),** while the beads produced by 1.0, 2.0 and 2.5%ALG had lower anthocyanin content (Figure 5). It was noted that 1.5%ALG given the biggest bead size (Table 1). This might effects encapsulation efficiency and stability of anthocyanin.

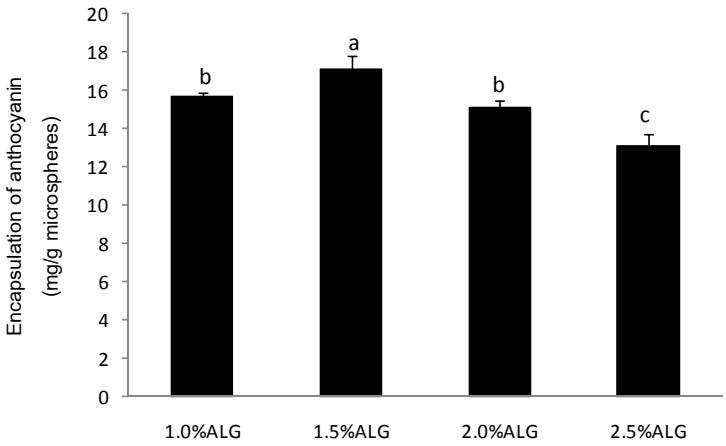

Figure 5 Encapsulation of anthocyanin on calcium alginate beads dried (a-c represents the significant difference at p<0.05)

Stability of anthocyanin on calcium alginate dried bead.

Anthocyanin stability was shown to be influenced by temperature. In fruit extract solution at 40 °C and 100°C (temperature for storage and processing), anthocyanin were at 84.7%wt and 3.69%wt, respectively (Figure 6. (a) and (b)). The faster anthocyanin degradation at higher temperature may relate to presence of sugar which can result in the Maillard reaction. This generally occurs during food processing at high temperature for extended period of time (Tanon et al. 2010). Increasing time of heat exposure increased degradation of anthocyanins. The original anthocyanin loading on calcium alginate beads were at 17.08±0.25 (mg/g Dry weight). For the mulberry fruit extracts adsorbed on calcium alginate beads, the anthocyanin content was significantly different from the original contents. Degradation of anthocyanin adsorbed on calcium alginate beads were lower compared to those of the anthocyanin solutions. This effects was clearly observable of the long time of heat exposure, such as at 10 hrs. Anthocyanin were at 98.8 and 24.1%wt for storage at 40°C and 100°C, respectively. These results showed high temperature and time of heat exposure affected greatly on stability of anthocyanin in the fruit extracts. Adsorption on calcium alginate beads could enhance stability of the anthocyanin content. Calcium alginate beads could reduce the reaction between anthocyanin and oxygen (Tanon et al. 2010).

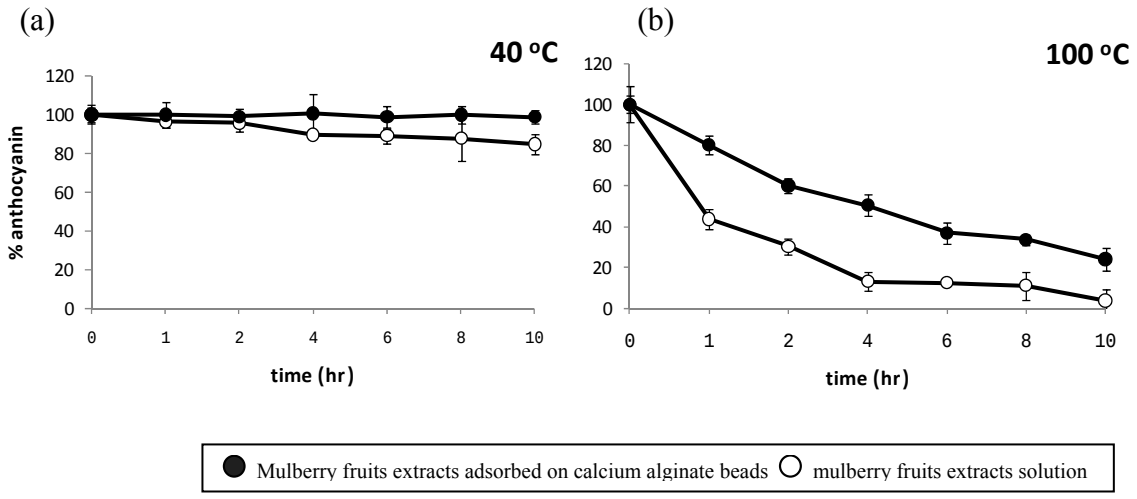

Figure 6 Stability of anthocyanin in mulberry fruit extracts in different time at 40°C (a) and 100°C (b)

Conclusion

Calcium alginate beads were prepared by external gelation method. We proposed to use them to enhance stability of anthocyanins in mulberry fruit extracts. The morphology and anthocyanin adsorption of calcium alginate beads depended on alginate concentration and nitrogen gas flow rate. Adsorption of mulberry fruits extracts on calcium alginate beads could enhance stability of anthocyanin at high temperature. The technique described in this study could benefit food industry.

Acknowledgements

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