Encapsulation of *Piper sarmentosum* within ca-alginate beads

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Introduction

Piper sarmentosum is one of the most abundant and widely used traditional herbal medicines in Malaysia and regional countries. 'From head to toe', the plant has multiple functional and remedial properties, for instance, healing fever, toothache, asthma, anti-malarial activity, neuromuscular blocking activity, hypoglycemic effect and so forth (Rahman et al., 1999). Extracts of the plant are rich in certain important bioactive compound such as alkaloids, amides, prophenylphenols, steroids, miscellaneous compounds including hydrocinnamic acid and oxalic acids (Masuda et al, 1991).

Encapsulation technology has gained significant interest from pharmaceutical and food industries. Herbal extracts or antioxidants are encapsulated for the major purposes of controlled release/delivery, extending shelf-life, providing protection to the essence and improving particle qualities (Shu et al., 2006; Kosaraju et al., 2006). Hydrogel is commonly used as encapsulation material due to its capability of absorbing large amounts of water or biological fluids (Peppas et al., 2000). Among all hydrogel, alginate is the most widely used due to several advantageous features such as non-toxicity, biocompatible, abundant, cheap and its ability to react with divalent cations, mainly calcium, to form stable gels (Chan et al., 2006).

Since most of the liquid herbal concentrates are prepared through aqueous extraction, encapsulation of these soluble essences could be easily achieved by absorption with alginate hydrogel beads. The aim of this study is to investigate the effect of process variables (i.e. alginate type or M/G ratio, alginate concentration, essence concentration, bead size and bead water content) on encapsulation efficiency of *Piper sarmentosum* liquid extract. Since the herbal extract contains many different bioactive compounds, the interactions between the chemical groups and the ca-alginate gel matrix was also studied.

Material and methods

Materials: Sodium alginates of high guluronic acid content (Manugel GHB), denoted as high-G and high mannuronic content (Manugel DH), denoted as high-M were obtain from ISP Technologies Inc, UK. *Piper sarmentosum* liquid extracts were prepared and donated by Furley, Malaysia.

Experimental setup & design: Blank ca-alginate beads were first prepared by using the extrusion method. The beads were then dried with tissue paper to remove the free water. One gram of the beads were loaded into a cylinder and followed by injection of 2 g of *Piper sarmentosum* liquid extract. The cylinder was gently tapped until uniform packing was achieved. The beads immersed in the liquid extracts were left in a dark cabinet. After 1 hour, the excess extract was withdrawn with the help an air-compressor (Figure 1). The mass of encapsulated *Piper sarmentosum* was then determined. Encapsulation efficiency of *Piper sarmentosum* within the ca-alginate beads was determined by using the following equation: mass of extract absorbed (g) / mass of beads (g). All experiments were carried out at room temperature, 25 ± 1 ºC. The experimental design of the study is shown in Table 1. The chemical interactions between the extract and ca-alginate matrix were analyzed by using Fourier transform infrared spectroscopy (FTIR).

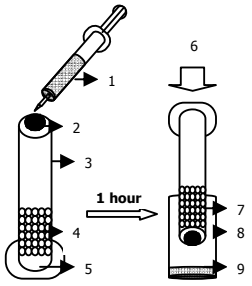

Figure 1: Experimental setup of this study: (1) syringe containing *Piper sarmentosum* liquid extract; (2) sieve; (3) cylinder; (4) blank ca-alginate beads; (5) cap; (6) connected to low air flow compressor; (7) encapsulated *Piper sarmentosum*; (8) universal bottle; (9) left-over *Piper sarmentosum* extract

Table 1: Experiemental design and manipulated variables of this study

No	Immersion time	Alg. type	Bead diameter, mm	Alg. conc., %	Beads water content (g)/ wet beads (g)	Extract conc., %	Injected liquid extract, g
1			1.9				
		High-G	2.2	2	1	1x	
		High-M	3.3				
2				2			
		High-G	2.2	3	1	1x	
		High-M		4			
	1 hour			5			2
3					0.25	1x	
		High-G	2.2	2	0.50		
		High-M			0.75		
					1		
4		High-G				1x	
		High-M	2.2	2	1	0.75x	
						0.50x	
						0.25x	

Note: x designates original concentration of *Piper sarmentosum* liquid extract

Results and Discussion

In general, high-M alginate showed higher encapsulation efficiency of *Piper sarmentosum* than that of high-G alginate (Figure 2a-d). The encapsulation efficiencies of high-M and high-G alginate beads were about 80% and 20% respectively. The encapsulation efficiency was found to be affected by the concentration of high-M alginate where it gradually increased when the concentration was higher (Figure 2a). Meanwhile, the encapsulation efficiency was less affected by the concentration of high-G alginate. The effect of extract concentration on the encapsulation efficiency is shown in Figure 2b. The extract concentration has no significant effect on the encapsulation efficiency of high-G alginate beads. On the other hand, the encapsulation efficiencies of high-M alginate beads were unchanged when the extract concentration was above 50% of its original strength. However, the encapsulation efficiency was lower when the extract concentration was diluted to 25% of the original extract concentration. It was also found that the encapsulation efficiency was the lowest when diameter of the bead was the largest although this effect was less clear for high-G alginate beads (Figure 2c). Furthermore, the bead water content was found to have significant effect on both on the encapsulation efficiency of both alginate types. It was observed that the drier beads showed

higher encapsulation efficiency. On the other hand, the FTIR spectra shows that the characteristic peaks at 1610, 1500, 1380, 1240, 1065, 760, 625 cm^{-1} of *Piper sarmentosum* liquid extract and those encapsulated within ca-alginate matrix remained unaltered (Figure 3).

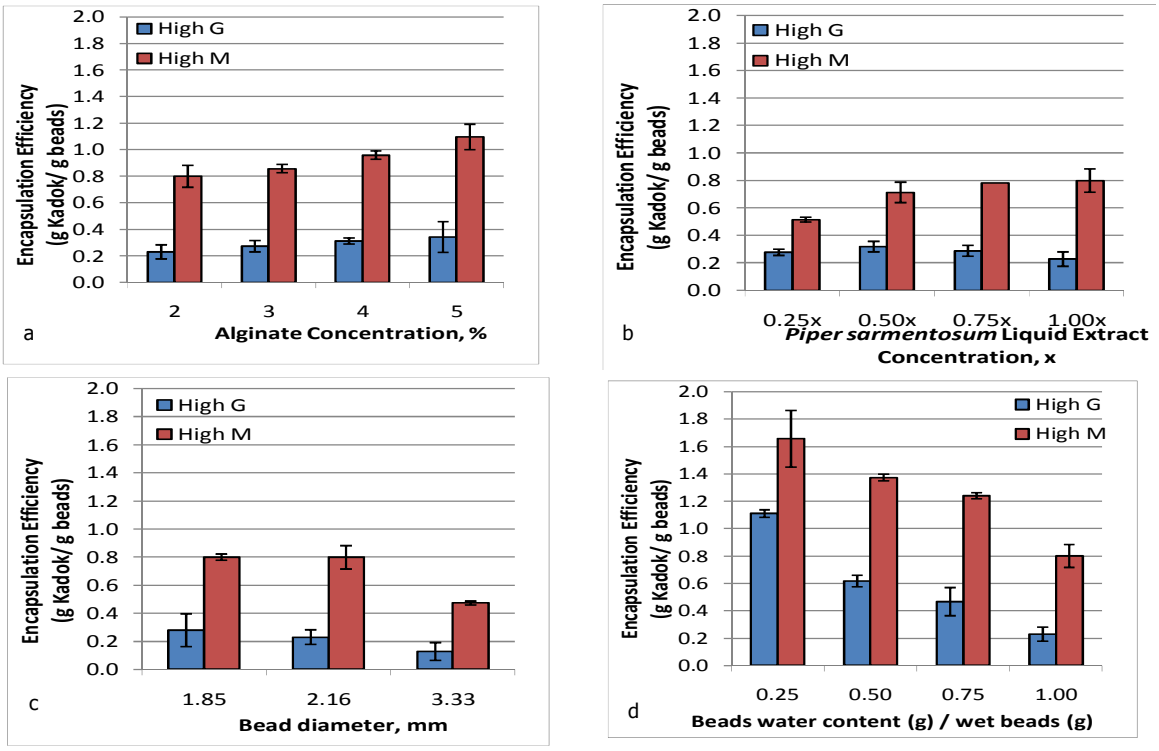

Figure 2: Effect of (a) alginate concentration, (b) extract concentration, (c) bead diameter and (d) beads water content (g)/ wet beads (g) on encapsulation efficiency

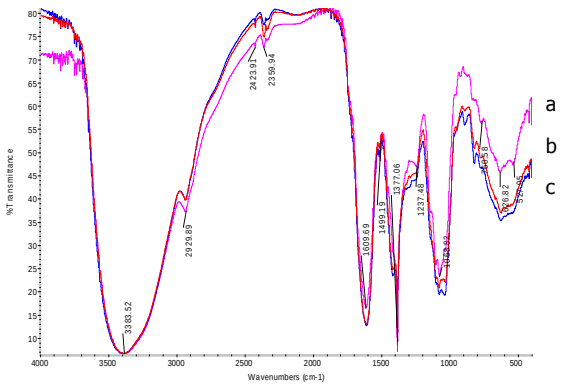

Figure 3: FTIR spectra of *Piper sarmentosum* in (a) liquid extract, encapsulated within (b) high-G alginate and (c) high-M alginate

The encapsulation efficiency was found to be mainly affected by the alginate M/G content and the water content of the alginate beads. High-M alginate beads are generally known to have lower porosity and higher swelling capability (i.e. more final volume) if compared to the high-G alginate beads (Storr et al., 2001; I. Donati et al., 2005). The lower porosity could be due to mannuronic acid contents that do not take part in the cross-linking process. On the other hand, the beads made of high-M alginate were generally found to swell to a greater extent than that of the high-G alginate

(data not shown) and thus increased the volume of the beads. These attributes could contribute to the higher absorption capability and thus better encapsulation efficiency. Furthermore, it is known that hydrogel contains mostly water. Therefore, the removal of the free water from the alginate beads could enhance their absorption capability since the original space occupied by the water could be replaced with the liquid herbal extract. On the other hand, it was found that the bead size has affected the encapsulation efficiency. This observation was unexpected since the volume of total beads per mass of beads should remain constant regardless of the bead size. The effect could be due to the higher interstitial spaces between the smaller beads if compared to larger beads. Therefore, more liquid could fill the space between the ca-alginate beads. In addition, the FTIR spectra show no chemical interactions between the bioactive compounds of *Piper sarmentosum* and the ca-alginate hydrogel matrix regardless of the M/G ratio. Similar finding has also been reported where no chemical interaction was found between a plant-derived essence (i.e. *Azadirachta Indica* A. Juss.) and the alginate based hydrogel matrix (Anandrao *et al.*, 2000). Therefore, this suggests that ca-alginate hydrogel matrix is a suitable material for encapsulating bioactive compounds extracted from plant.

Conclusions

In general, the encapsulation efficiency of high-M alginate beads was higher than that of the high-G alginate beads. The encapsulation efficiency of high-M alginate beads was dependant on alginate concentration, extract concentration and bead diameter. However, these variables had minimal effect on the encapsulation efficiency of the high-G alginate beads. Nevertheless, both type of alginate beads showed higher encapsulation efficiency at drier state. FTIR spectra showed no chemical interactions between the bioactive compounds of *Piper sarmentosum* and ca-alginate hydrogel matrix.

Acknowledgement

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