

Microencapsulation of rambutan seed oil by spray-drying using different protein preparations

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<u>Abstract</u>

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Microencapsulation Protein Rambutan seed oil Spray-drying Rambutan is a popular fruit widely cultivated in South East Asia. Rambutan seed is a by-product in canned fruit industry. The seed is rich in oil content. Rambutan seed oil contained high level of unsaturated fatty acids which are highly susceptible to microbiological, chemical and biochemical deteriorations. Oil microencapsulation is a popular method to prevent the change of oil quality during preservation. In this study, different protein preparations were alternatively used in microencapsulation of rambutan seed oil by spray-drying. Sodium caseinate, gelatin and soy protein isolate resulted in similar microencapsulation efficiency (67.3-69.9%) while the highest microencapsulation efficiency (73.6%) was obtained for whey protein isolate. The microencapsulation yield of sodium caseinate and gelatin was very low (25.6-28.5%) due to their high viscosity emulsions. Increased microencapsulation yield was observed for soy protein isolate (59.3%) while whey protein isolate showed the best microencapsulation yield (72.5%). Whey protein isolate also demonstrated the highest protective ability against oil oxidation during the accelerated storage of the spray-dried powder. Decrease in the ratio of rambutan seed oil/whey protein isolate from 1/1 to 1/4 enhanced the microencapsulation efficiency by 18% but did not change the microencapsulation yield.

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Introduction

Rambutan (Nephelium lappaceum L.) is a tropical fruit widely cultivated in South East Asia. In Vietnam, the plantation area is 91,200 hectares and the annual productivity of rambutan fruit is approximately 649,300 tons (Ton et al., 2013). Rambutan seed is a by-product in canned fruit industry. The percentage of seed in rambutan fruit varied from 7 to 10% (Arenas et al., 2010). The seed is rich in oil content which is approximately 33.4% dry weight (Solís-Fuentes et al., 2010). Previous studies revealed that physicochemical and thermal characteristics of rambutan seed oil may become interesting for specific applications in several segments of the food industry (Solís-Fuentes et al., 2010; Sirisompong et al., 2011). However, rambutan seed oil contains high level of unsaturated fatty acids including oleic acid (36.8%), linolenic acid (6.5%), linoleic acid (1.4%), erucic acid (0.7%) and palmitoleic acid (0.5%) (Sirisompong et al., 2011) which are highly susceptible to microbiological, chemical and biochemical deteriorations (Gharsallaoui et al., 2007). Protection of rambutan seed oil against deterioration has not been reported.

Many attempts have been performed to prevent

the change of food lipid during preservation. Lipid microencapsulation by spray-drying has become a potential method. In this method, lipid (core material) is packaged within a wall material; the product is in powder form and the particle size varies from 1 to 5000 μ m. The method consists of three steps: mixing lipid and wall solution for preparation of an oil-inwater emulsion, homogenization and spray-drying of the emulsion with hot air for formation of lipid powder (Jafari *et al.*, 2008).

Wall materials used in lipid microencapsulation by spray-drying are divided into two main groups: carbohydrates and proteins. Carbohydrate material offers the advantage of being relatively inexpensive, bland in flavor, low viscosity at high solids for spray-drying. However, carbohydrates lack of any emulsifying properties and typically result in poor retention of lipid during spray-drying (Gharsallaoui et al., 2007). On the contrary, functional properties of proteins including film formation, emulsification and stabilization of emulsion droplets, exhibit many desirable characteristics of a wall material for lipid microencapsulation (Nerestenko et al., 2013a). Different protein preparations such as casein, whey protein, gelatin, soy protein, pea protein and cereal protein were tested for microencapsulation of different fats and oils (Jafari *et al.*, 2008; Nerestenko *et al.*, 2013a). Nevertheless, the ability of protein preparations for rambutan seed oil microencapsulation has never been compared.

In this work, for the first time, rambutan seed oil was microencapsulated by spray-drying. The objective of this study was to compare the ability of different protein preparations for rambutan seed oil microencapsulation.

Materials and Methods

Materials

Seeds of rambutan (Nephelium lappaceum L.) fruits were originated from a canned fruit processing plant in Dong Nai province. The seeds were firstly washed with potable water and the kernels were manually separated from the seeds. The kernels were ground and subsequently dried at 40°C to a moisture content of 10%. Lipid extraction from the kernels was performed with hexan under the following conditions: material and solvent ratio of 1:10 (w/w), temperature of 40°C and time of 36 h. After extraction, the liquid phase was separated by filtration. Hexan was removed from the rambutan seed oil by distillation at 60°C. The physico-chemical characteristics of rambutan seed oil were as follows: moisture content: 0.2%; acidic value: 0.35 mg KOH/ kg oil; peroxide value: 4.25 meq/kg oil; iodine value: 41.6 g/100g oil.

Sodium caseinate (Protein content: 92% on dry basis) was supplied by Avani Food Products company (India). Whey protein isolate (Protein content: 88.8% on dry basis) was purchased from Boomer company (Australia). Gelatin (Bloom index: 250, viscosity of 6.67% gelatin solution at 60°C: 5.0 mPa.s) was originated from Cartino gelatin company Limited (Thailand). Soy protein isolate (Protein content: 90% on dry basis) was supplied by Foodchem International Corporation (China). All solvents and chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich (The United States).

Procedure of rambutan seed oil microencapsulation

Microencapsulation of rambutan seed oil was performed as follows: 10% (w/w) protein solution (De Barros Fernandes *et al.*, 2014) was prepared by dissolving protein preparation in distilled water, stirring at 50°C and 750 rpm for 6 h. Rambutan oil was then added to protein solutions and the total solid of the mixtures obtained was 20% (w/w). The weight ratio of lipid to protein was therefore 1:1 (De Barros Fernandes *et al.*, 2014). All samples were treated with Heidolph Diax 900 mechanical homogenizer (Labexchange, Germany) at 8000 rpm for 5 min and with high-pressure homogenizer Model 1000 (APV, Denmark) at 200 bar for 3 recirculation (Ahn et al., 2008). Finally, emulsion samples were spraydried by a Mobile Minor-Model E spray-drier (Niro A/S, Denmark). The emulsions were fed into the chamber at the rate of 1.6 L/h by a 505S peristaltic pump (Matson-Marlow, England). The atomization was performed by TS Minor MO2/B rotary atomizer. The drying took place with an air inlet temperature of 180°C, air outlet temperature of 60°C (Wang *et al.*, 2011) and air pressure of 3 bar at the atomizer. The powder of each run was collected for further analysis.

Evaluation of microencapsulation of rambutan seed oil by various protein preparations

In this section, different protein preparations including whey protein concentrate, sodium caseinate, gelatin and soy protein concentrate were used as wall materials. Before the spray-drying, viscosity of the emulsion samples was determined. At the end of the spray-drying, powder samples were analyzed to measure total and surface oil level, peroxide value, size range and volume mean diameter of the particles.

For understanding further of the microencapsulating ability of different protein preparations, oil powder samples were stored at 60°C under vapor-saturated conditions for 30 days. In order to achieve vapor-saturated condition, a waterfilled metal container was put inside an incubator UM 500 (Memmert, Germany); the incubator was set at 60°C and no air circulation working mode. The water container was never empty during the experimentation. The air in incubator was always kept at relative humidity close to 1. Powder samples, sealed in High Density PolyEthylen HDPE package (with vapor-permeability of 15 g/m^2 .day), were stored in this incubator in order to accelerate oil oxidation. During the accelerated storage, samples were taken every five days to determine the peroxide value.

Effects of core/wall ratio on microencapsulation of rambutan seed oil

Based on the results of the previous section, one protein preparation was selected for rambutan seed oil microencapsulation in this experiment. Four emulsion samples were prepared. The weight ratio of lipid to protein in the emulsion samples was 1/1, 1/2, 1/3 and 1/4, respectively. The total solid of all emulsion samples was fixed at 20% (w/w). Therefore, the protein concentration of the wall solutions was 10, 13.33, 15 and 16% (w/w), respectively. Other operating conditions were similar to those in the previous section. Powder samples were subjected to

similar analyses as mentioned above.

Analytical methods

Total solid content of oil-in-water emulsion (g/L) was determined by drying at 110±3°C until constant weight (Lakshanasomya et al., 2011). Total oil content of oil-in-water emulsion (g/L) was determined by using a method proposed by Lakshanasomya et al. (2011) with slight modification. Ten milliliters of emulsion was taken into the oil extraction flask for analysis. Firstly, 1.5 mL of ammonium hydroxide was added and mixed followed by 10 mL of alcohol (9%) and the contents were again well mixed. Secondly, 25 mL diethyl ether was added to the flask; it was then shook vigorously for 1 min. Finally, 25 mL of light petroleum ether (b.p. 40-60°C) was added and the flask was shook vigorously for 1 min. After separation was complete, the oil solution was transferred into a Petri dish and the Petri dish was dried at 102±2°C for 1 h and weighed. The total oil content was calculated as the difference between weight of Petri dish with oil and weight of initial Petri dish.

Viscosity of oil-in-water emulsion (cP) was measured at 30±2°C using Brookfield viscometer DV1 with spindle no. 1 and rotation rate of 100 rpm. Moisture content of the oil powder (%) was evaluated by drying at 110±2°C until constant weight (Lakshanasomya *et al.*, 2011).

The oil on the surface of the powder particles (g/g) was determined by a method suggested by Young *et al.* (1993). One gram of the powder was accurately weighed into the extraction flask. Subsequently, 25 mL of petroleum ether (b.p. 40–60°C) was added and the mixture was shook vigorously for 10 min. The mixture was then filtered through a cloth. The filtrate was transferred into the Petri dish, dried at $102\pm2^{\circ}$ C for 1 h and weighed. The surface oil content was calculated as the difference between weight of Petri dish with oil and weight of initial Petri dish.

The total oil content of the spray-dried powder (g/g) was determined by using a method described by Young *et al.* (1993). One gram of the powder was accurately weighed into the oil extraction flask. Water was added to complete the volume to 10 mL and mixed. The total oil content in the emulsion was determined by using a method proposed by Lakshanasomya *et al.* (2011).

The encapsulated oil content (g/g) was calculated as a difference of the total oil content and the surface oil content of the powder obtained. Particle size distributions of the spray-dried powder samples were analyzed by using a Model LA 920 laser diffraction particle analyzer (Horiba, Japan). A small sample was suspended in 99.5% ethanol using magnetic agitation and the distribution of particle size was monitored during three successive measurements. The volume mean diameter of spray-dried powder particles (μ m) was expressed as D_{4.3} (De Brouckere mean diameter).

Microencapsulation efficiency and microencapsulation yield of rambutan seed oil

Microencapsulation efficiency and yield were calculated by formulas reported by Shu *et al.* (2006). Microencapsulation efficiency was defined as a ratio between the mass of the encapsulated oil and the mass of the total oil in the spray-dried powder. Microencapsulation yield was defined as a ratio between the mass of the total oil of the spray-dried powder and the mass of the total oil of the emulsion before spray drying.

Statistical analysis

All experiments were performed in triplicate. Mean values were considered significantly different when P<0.05. One-way analysis of variance was performed using the software Statgraphics Centurion XV.

Results and Discussion

Evaluation of microencapsulation of rambutan seed oil by various protein preparations

Table 1 shows that sodium caseinate, gelatin soy protein isolate resulted in similar and microencapsulation efficiency for rambutan seed oil (p>0.05). Whey protein isolate demonstrated higher oil microencapsulation efficiency (p<0.05). According to Jafari et al. (2008), functional properties of proteins including solubility, film formation, emulsification and stabilization of emulsion droplets, exhibited many desirable characteristics for a wall material; these proteins changed their structure during emulsification through unfolding and adsorption at the oil-water interface and subsequently formed resistant multilayer around oil droplets. That would stabilize oil-in-water emulsion and improve lipid microencapsulation. The microencapsulation efficiency of whey protein for rambutan seed oil in this study was higher than that for flaxseed oil (Tonon et al., 2012) but lower than that for linoleic acid (Jimenez et al., 2004). It can be explained by difference in protein level and chemical composition of the whey protein preparations, emulsification and spray-drying conditions.

Peroxide value is a standard index to monitor lipid deterioration (Wang *et al.*, 2011). The peroxide value of the spray-dried powder with whey protein isolate was the lowest (Table 1). Use of other protein

	Whey protein isolate	Sodium caseinate	Gelatin	Soy protein isolate
Microencapsulation efficiency (%)	73.6 ± 1.4^{a}	69.9 ± 0.9^{b}	67.7 ± 1.1 ^b	67.3 ± 2.2 ^b
Microencapsulation yield (%)	$72.5\pm3.5^{\mathtt{a}}$	$28.5 \pm 1.2^{\circ}$	$25.6 \pm 3.7^{\circ}$	$59.3 \pm 7.1^{ m b}$
Moisture content of the oil powder (%)	4.0 ± 0.2^{a}	4.9 ± 0.1^{b}	$5.5 \pm 0.2^{\circ}$	$4.2\pm0.1^{\text{a}}$
Peroxide value of the spray-dried powder (meq/kg oil)	$5.26\pm0.16^{\text{a}}$	7.58 ± 0.11^{b}	7.92 ± 0.08^{b}	7.54 ± 0.11^{b}
Size range of the powder particle (um)	8 - 344	10 - 1460	9 - 1532	7 - 394
Volume mean diameter of the powder particle d _{4,3} (μm)	30ª	453 ^b	456 ^b	59 ^c

Table 1. Microencapsulation of rambutan oil with different protein preparations

Values with different small letters in the same row are significantly different (P < 0.05).

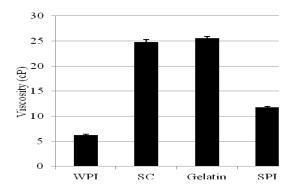


Figure 1. Viscosity of oil-in-water emulsion before the spray-drying (WPI: whey protein isolate, SC: sodium caseinate, SPI: soy protein isolate)

preparations led to statistically higher peroxide value (p<0.05). This result was in accordance with lipid microencapsulation efficiency of the protein preparations. High microencapsulation efficiency implies low surface oil content in the spray-dried powder. As a result, prevention of lipid oxidation during the spray-drying would be enhanced. Similar behavior was recently reported in microencapsulation of tilapia oil (Huang *et al.*, 2014).

Table 1 also demonstrates that the powder samples microencapsulated with gelatin and sodium caseinate showed higher moisture content than those microencapsulated with whey protein and soy protein; however the difference was low. In addition, gelatin and sodium caseinate resulted in very low microencapsulation yield (25.6% and 28.5%), large size range (9-1532 μ m and 10-1460 μ m) and high mean size of the powder particles (456 µm and 453 μm) (Table 1). On the other hand, Figure 1 shows that both gelatin and casein produced oil-in-water emulsions with very high viscosity. Previously, Bhandari et al. (1992) reported the formation of large spray-dried particles from highly viscous feeds in microencapsulation of citral and linalyl acetate. In our study, stringy particles were obviously observed and many particles were stuck on the drying chamber wall during the spray-drying. This phenomenon reduced the microencapsulation yield. Gharsallaoui et al. (2007) explained that high viscosity emulsion interfered with the atomization process and led to formation of elongated droplets which adversely affected the drying rate. On the contrary, whey and soy protein isolate produced the spray-dried powders with much smaller size, more narrow size range (Table 1) and the initial feeds with whey or soy protein isolate demonstrated much lower viscosity (Figure 1). Whey protein isolate resulted in the highest lipid microencapsulation yield (72.5%). Nevertheless, this value was lower than that in the study of Young et al. (1993) who used whey protein isolate for microencapsulation of anhydrous milk fat. It was due to difference in microencapsulation condition; the protein concentration in the initial wall solution in the study of Young et al. (1993) was double than that in our study. The higher the lipid microencapsulation yield, the lower the lipid loss during the spray-drying and the better the economic efficiency of the process.

Figure 2 presents the change in peroxide value of the rambutan seed oil powder during the accelerated storage. For all samples, the peroxide value gradually increased during the time due to oil oxidation. Whey protein isolate showed the best protective ability against oil oxidation. It was due to low surface oil content as well as low peroxide value of the powder at the beginning of the storage. In addition, whey protein showed antioxidant activity (Gad et al., 2011) that would contribute to prevention of oil oxidation in the spray-dried powder. Although gelatin, sodium caseinate and soy protein isolate resulted in similar peroxide value of the powder at the beginning of the storage, their protective abilities against oil oxidation were very different. Gelatin demonstrated the highest oil oxidation followed by casein and soy protein isolate.

Effects of core/wall ratio on microencapsulation of rambutan seed oil

was used as wall material)							
Core/wall ratio	1/1	1/2	1/3	1/4			
Microencapsulation efficiency (%)	72.5 ± 1.9^{a}	76.2 ± 2.6^{b}	$81.5 \pm 2.5^{\circ}$	85.6±1.4 ^d			
Microencapsulation yield (%)	71.6 ± 2.2^{a}	73.4 ± 3.4^{a}	72.8 ± 0.6^{a}	$74.5\pm3.5^{\text{a}}$			
Moisture content of the oil powder (%)	$3.9\pm0.2^{\text{a}}$	$4.1\pm0.1^{\texttt{a}}$	4.2 ± 0.2^{a}	4.3 ± 0.3^{a}			
Peroxide value of the spray-dried powder (meq/kg oil)	5.51 ± 0.25^{a}	$5.56\pm0.28^{\text{a}}$	5.35 ± 0.28^{a}	5.26 ± 0.16^{a}			
Size range of the powder particle (µm)	8 - 350	9 - 334	9 - 328	8 - 322			
Volume mean diameter of the powder particle (µm)	31ª	28ª	27ª	25ª			

Table 2. Effects of core/wall ratio on the microencapsulation of rambutan oil (Whey protein isolate was used as wall material)

Values with different small letters in the same row are significantly different (P < 0.05).

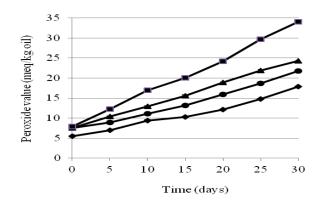


Figure 2. Change in peroxide value of the spray-dried powder lipid during the accelerated storage at 60°C; the wall materials for rambutan oil microencapsulation were as follows: (\bullet) Whey protein isolate; (\bullet) Soy protein isolate, (\bullet) Sodium caseinate, (\blacksquare) Gelatin

When the weight ratio of core to wall material decreased from 1/1 to 1/4, the microencapsulation efficiency for rambutan oil increased by 18% (Table 2). Similar observation was reported by various researchers for microencapsulation of flaxseed oil (Tonon *et al.*, 2012), fish oil (Tan *et al.*, 2005) and linoleic acid (Minemoto *et al.*, 2002). According to Minemoto *et al.* (2002), at low core/wall ratio, the amount of wall material is not enough to fully cover the oil droplets and this insufficiency may result in a decrease in microencapsulation efficiency.

As mentioned above, the higher the microencapsulation efficiency, the lower the surface oil content of the spray-dried powder. However, Table 2 shows that the peroxide value of the four powder samples obtained was statistically similar (p>0.05). It was probably due to short microencapsulation time. Similar result was reported in microencapsulation of menhaden oil when mixture of sodium caseinate and carbohydrate was used as wall material (Hogan *et al.*, 2003).

Decrease in core/wall ratio from 1/1 to 1/4 did not change the microencapsulation yield, the

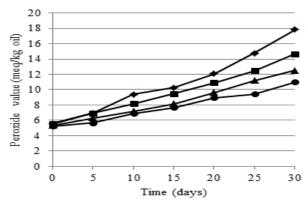


Figure 3. Change in peroxide value of the spray-dried powder lipid during the accelerated storage at 60oC; whey protein isolate was used as wall material; the core/wall ratios were as follows: (\bullet) 1/1, (\blacksquare) 1/2, (\bullet) 1/3, (\bullet) 1/4.

moisture content of powder samples as well as the size range and the mean size of the spray-dried particles (Table 2). Our experimental results showed that the viscosity of the four emulsion samples was similar (p>0.05). Nesterenko *et al.* (2013b) also reported unchanged particle mean size and retention efficiency in α -tocopherol microencapsulation when the core/wall ratio varied from 2/1 to 1/1 and 1/2.

Change in peroxide value of oil powder samples microencapsulated with different core/wall ratios during the accelerated storage is presented in Figure 3. The lower the core/wall ratio, the better the protective ability against oil oxidation. It was due to lower surface oil content in the spray-dried particles with high wall material level. Nevertheless, low core/wall ratio reduced the oil level in the powder obtained and increased the wall material level used for microencapsulation.

Conclusion

In microencapsulation of rambutan seed oil by spray-drying, whey protein isolate resulted in

the best microencapsulation efficiency as well as microencapsulation yield in comparison with sodium caseinate, gelatin and soy protein isolate. Whey protein isolate also showed the highest protective ability against oil oxidation during the accelerated storage of the rambutan seed oil powder. Change in the ratio of rambutan seed oil and whey protein isolate from 1/1 to 1/4 significantly improved the microencapsulation efficiency but did not affect the microencapsulation yield. Whey protein was a potential wall material for microencapsulation of rambutan seed oil.

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