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# Combination of whey protein and carbohydrate for microencapsulation of pumpkin (*Cucurbita* spp.) seed oil by spray-drying

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**Abstract**

In this study, pumpkin seed oil was microencapsulated by spray-drying for stabilization of oil quality during the storage. Different carbohydrates including gum arabic, modified corn starch, modified manioc starch, maltodextrin, sucrose, maltose, lactose and glucose were alternatively combined with whey protein isolate for microencapsulation of pumpkin seed oil. Mixture of whey protein and maltodextrin generated the spray-dried powder with high microencapsulation efficiency as well as high microencapsulation yield. This wall system also produced the powder with the lowest peroxide value at the end of the acceleration storage. The appropriate whey protein/maltodextrin ratio was 1/3 under which the microencapsulation efficiency and yield for pumpkin seed oil were 94.5% and 65.6%, respectively. Microencapsulation was a potential method for protection of pumpkin seed oil against deterioration.



## Introduction

Pumpkin (*Cucurbita* spp.) is a vegetable widely cultivated in the world. Pumpkin seed is a by-product in the production of frozen pumpkin dice, dried pumpkin dice and pumpkin puree (Tsao and Lo, 2004). The main component in pumpkin seed is lipid, the level of which is approximately 51%. Pumpkin seed oil is rich in unsaturated fatty acids including linoleic acid (55.6% of total lipid) and oleic acid (20.4% of total lipid). Pumpkin seed oil has been produced worldwide due to high nutritional effects for human health (El-Adawy and Taha, 2001). However, oil with high unsaturated fatty acid level is susceptible to microbiological, chemical and biochemical deterioration (Ton *et al*., 2016). Protection of pumpkin seed oil against deterioration has not been reported.

Lipid microencapsulation by spray-drying has been a potential method to prevent lipid change during food preservation. In this method, lipid (core material) is packaged within a wall material and solid powder of microparticles is obtained. This method involves three basic steps: mixing lipid and wall solution for preparation of an oil-in-water emulsion, homogenization and spray-drying of the emulsion with hot air for formation of lipid powder (Jafari *et al*., 2008).

Criteria for wall materials used in oil microencapsulation consisted of high emulsifying properties, high water solubility, low viscosity and high film-forming properties (Sheu and Rosenberg, 1995). Proteins including sodium caseinate, whey protein, soy protein and gelatin exhibited desirable characteristics of wall materials (Jafari *et al*., 2008; Ton *et al.*, 2016). Among protein preparations, whey proteins have been shown to be an excellent wall material for oil microencapsulation (Bae and Lee, 2008). Carbohydrates are generally added as a secondary wall material (a filler) to improve drying properties of sprayed droplets by enhancing the formation of dry crust around drying droplets and increase the oxidative stability of microencapsulated oils by reducing oxygen permeability of wall matrix (Kagami *et al.*, 2003; Bae and Lee, 2008). Carbohydrates used in lipid microencapsulation can be divided into two major groups: low molecular weight carbohydrates (glucose, maltose, lactose, maltodextrin) and high molecular weight carbohydrates (modified starch, gum arabic) (Sheu and Rosenberg, 1995; Bae and Lee, 2008). Nevertheless, comparison on carbohydrate use in combination of whey protein for lipid microencapsulation has not been mentioned.

In this work, for the first time, pumpkin seed oil was microencapsulated by spray-drying. Whey protein was combined with different carbohydrates as wall systems. The objective of this study was to

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select the appropriate carbohydrate for combination with whey protein in the microencapsulation of pumpkin seed oil.

## Materials and Methods

*Materials*

Seeds of pumpkin *(Cucurbita pepo)* were originated from a pumpkin processing plant in Ho Chi Minh City. 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%. Oil extraction from the kernels was performed with hexane 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. Hexane was removed from the pumpkin seed oil by vacuum distillation at 60°C and 10 mbar. The physico-chemical characteristics of pumpkin seed oil were as follows: moisture content: 0.2%; acidic value: 2.3 mg KOH/kg oil; peroxide value: 20.3 meq/kg oil; iodine value: 50.8 g/100g oil.

Whey protein concentrate (Protein: 80%, ash: 5%) was purchased from Davisco Foods International, Inc. (The United States). Gum arabic (Purity degree: 99.5%, heavy metals: less than 20ppm, pH: 4.2-4.8) was supplied by Jumbo Acacia Co., Ltd (Thailand). Maltodextrin (Dextrose equivalent: 12, heavy metals: less than 5ppm, pH: 4.5-5.5) was originated from Roquette Frères (France). Modified corn starch - acetylated distarch adipate E1422 (Purity degree: 99.5%, heavy metals: less than 5 ppm, pH: 7.5-8.0) was purchased from Roquette Frères (France). Modified manioc starch - hydroxypropyl distarch phosphate E1442 (Purity degree: 99.5%, heavy metals: less than 5 ppm, pH: 5.1) was supplied by Ajinomoto (Vietnam). Glucose (Purity degree: 99.5%, heavy metals: less than 5 ppm) was originated from P.S.C. Starch Product PCL (Thailand). Sucrose (Purity degree: 99.9%, ash: 0.05%) was purchased from Bien hoa Sugar Jointstock Company (Vietnam). Lactose (Purity degree: 99%, heavy metals: less than 5ppm) was supplied from Roquette Frères (France). Maltose (Dextrose equivalent: 42, total solid: 80oBx) was originated from Bien hoa Sugar Jointstock Company (Vietnam). All solvents and chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich (The United States).

*Procedure of pumpkin seed oil microencapsulation*

Firstly, 5% (w/w) protein solution was prepared by dissolving protein preparation in distilled water, stirring at 50°C and 750 rpm for 6 h on C-MAG HS4 heating magnetic stirrer (IKA, Malaysia). Carbohydrate was then dissolved in the protein solution; the weight ratio of protein/carbohydrate in the wall solution was 1/1. Pumpkin seed oil was added to the wall solution and the total solid of the mixture obtained was 15 % (w/w). All samples were subsequently treated with Heidolph Diax 900 mechanical homogenizer (Labexchange, Germany) at 3000 rpm for 5 min and with high-pressure homogenizer Model 1000 (APV, Denmark) at 300 bar for three recirculations. Finally, emulsion samples were spray-dried by a Mobile Minor-Model E spray-drier (Niro A/S, Denmark). The emulsions were fed into the chamber at the rate of 0.8 L/h by a 505S peristaltic pump (Matson-Marlow, England). The drying took place with an air inlet temperature of 160°C, air outlet temperature of 60°C and air pressure of 3 bar at the atomizer. The powder of each run was collected, sealed in glass jar and stored at 4°C until further analysis.

*Selection of carbohydrate for combination with whey protein in microencapsulation of pumpkin seed oil*

In this section, different carbohydrates including gum arabic, modified corn starch, modified manioc starch, maltodextrin, glucose, sucrose, lactose, maltose were alternatively combined with whey protein isolate for microencapsulation of pumpkin seed oil. At the end of the spray-drying, powder samples were analyzed to measure total and surface oil level, moisture content, acidic and peroxide value. Some samples were taken for morphological analysis under scanning electron microscope.

For further understanding of the microencapsulating ability of different wall systems, oil powder samples were stored at 60°C under vapor-saturated conditions for 30 days. In order to achieve vapor-saturated condition, a water-filled 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 80%. 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. Control sample with liquid pumpkin seed oil was also performed under the same conditions. During the accelerated storage, samples were taken every five days to determine the peroxide value.

*Effects of whey protein/carbohydrate ratio on microencapsulation of pumpkin seed oil*

Based on the results of the previous section, one carbohydrate was selected for combination with whey protein for pumpkin seed oil microencapsulation in this experiment. Four emulsion samples were prepared. The total solid of all emulsion samples was fixed at 15% (w/w). The weight ratio of lipid to protein in the emulsion samples was fixed at 1/2. The weight ratio of protein to carbohydrate was 1/1, 1/2, 1/3 and 1/4. Therefore, the protein concentration of the wall solutions was 5, 3.33, 2.5 and 2%, 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 was determined by drying at 102±2°C until constant weight (Lakshanasomya *et al*., 2011). Total oil content of oil-in-water emulsion was determined by 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 (95 % v/v) and the contents were again well mixed. Secondly, 25 mL diethyl ether was added to the flask; it was then shaken 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.

The oil on the surface of the powder particles 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±2°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 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 then determined by a method proposed by Lakshanasomya *et al*. (2011).

The encapsulated oil content was calculated as a difference of the total oil content and the surface oil content of the powder obtained. Scanning electron microscopy of pumpkin seed oil powder was performed by the following procedure. The powder sample was placed on one surface of a double faced adhesive tape and coated with gold by using an ion coater E-102 (Hitachi- Japan). The S-4800 model scanning electron microscope (Hitachi, Japan) was used to observe the outer surface of the pumpkin seed oil powder particles. The examination was operated at an accelerating voltage of 2 kV. The S-4800 software (Hitachi, Japan) was used to present the micrographs of the powder microstructure.

*Microencapsulation efficiency and microencapsulation yield of pumpkin 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

*Selection of carbohydrate for combination with whey protein in microencapsulation of pumpkin seed oil*

Figure 1 shows that sample with lactose demonstrated the lowest microencapsulation yield 45.7%. It was due to low solubility of lactose in the wall solution (Vega and Roos, 2006). Better microencapsulation yield was observed for samples with modified corn starch, modified manioc starch, maltodextrin, glucose, sucrose and maltose. The highest microencapsulation yield was achieved for sample with gum arabic (65.7%). The higher the lipid microencapsulation yield, the lower the oil loss during the spray-drying and the better the recovery yield of the process.

The combination of whey protein and monosaccharide (glucose), disaccharide (maltose, lactose, sucrose) or maltodextrin resulted in very high

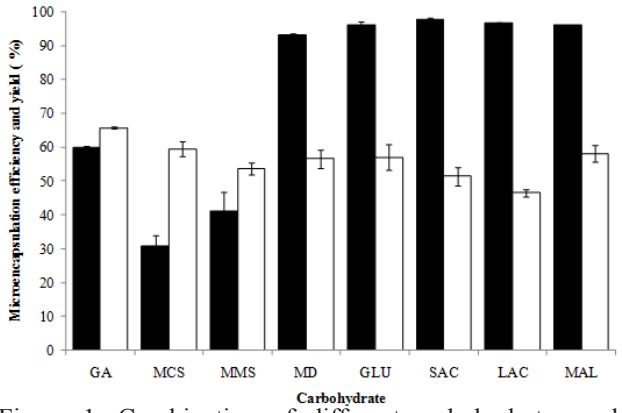
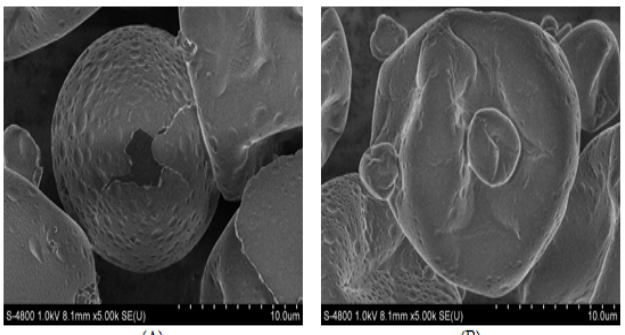



Figure 1. Combination of different carbohydrates and whey protein for microencapsulation of pumpkin seed oil (Black bars: microencapsulation efficiency, white bars: microencapsulation yield; GA: gum arabic, MCS: modified corn starch, MMS: modified manioc starch, MD: maltodextrin, GLU: glucose, SAC: sucrose, LAC: lactose, MAL: maltose)

microencapsulation efficiency which varied from 93.0 to 97.8% (Figure 1). High microencapsulation efficiency of whey protein-lactose and whey protein-maltodextrin mixtures was previously reported in the spray-drying of red-fleshed pitaya (Hylocereus polyrhizus) seed oil (Lim *et al*., 2012). According to Jafari *et al*. (2008), whey protein changed structure during emulsification through unfolding and adsorption at the oil-water interface and subsequently formed resistant multilayer around oil droplets. In addition, carbohydrate in the continuous phase of the spray-dried powder particles reduced oil globule coalescence in the emulsion formation and spray-drying steps (Keogh, 2005). As a result, protein-carbohydrate mixture was a good wall for oil microencapsulation.

Although whey protein and gum arabic mixture showed the best microencapsulation yield, their microencapsulation efficiency was low (Figure 1). According to Klein *et al.* (2010), the interaction of whey protein and gum arabic was rather weak and it can be deducted that the two polymers could not generate resistant multilayer around oil droplets. Low microencapsulation efficiency was also noted for mixture of whey protein and other high molecular weight carbohydrates including modified corn and manioc starch. In order to clarify the packaging of pumpkin seed oil within the wall materials, the morphology of spray-dried powder samples was investigated with scanning electron microscope. Crack was clearly observed on the surface of the particles spray-dried with whey protein and modified corn starch (Figure 2). This result was appropriate for low microencapsulation efficiency of this combination (30.7%). On the




(A) (B)

Figure 2. Morphology of pumpkin seed oil powder spray-dried with (A) whey protein and modified corn starch, (B) whey protein and maltodextrin

contrary, particle surface of the sample using whey protein and maltodextrin was intact and undamaged which corresponded with high microencapsulation efficiency of whey protein and maltodextrin. Sheu and Rosenberg (1998) also mentioned that using maltodextrin limited the formation of surface cracks in the microencapsulation of ethyl caprylate ester.

The analytical results showed that moisture content of all spray-dried powder samples was varied from 2.8 to 3.4%. Similar moisture content was also noted when whey protein and various carbohydrates were used in microencapsulation of avocado oil (Bae and Lee, 2008) and red-fleshed pitaya (Hylocereus polyrhizus) seed oil (Lim *et al*., 2012). Low moisture content would prevent chemical changes in pumpkin seed oil powder during the preservation. In addition, all powder samples showed nearly similar acidic and peroxide values. Comparison with the acidic value (2.3 mg KOH/kg oil) and peroxide value (20.3 meq/kg oil) of the initial pumpkin seed oil, it can be noted that the microencapsulation did not change the pumpkin seed oil quality. It can be explained that spray-drying time was very short (Jafari *et al*., 2008) and that could prevent the hydrolytic and oxidative reactions of pumpkin seed oil during the microencapsulation.

Oil deterioration may be evaluated by the change in peroxide value (Bae and Lee, 2008). The evolution of peroxide value of pumpkin seed oil powder during the accelerated storage is visualized in Figure 3. The peroxide value of the control with non-microencapsulated pumpkin seed oil quickly augmented and achieved approximately 125 meq/kg oil at the end of the accelerated storage. This result was in accordance with the findings of Hogan *et al.* (2003) who investigated the microencapsulation of fish oil. On the contrary, slight increase in peroxide value was observed for all microencapsulated powder samples. Among 8 combinations tested, whey protein and maltodextrin showed the lowest peroxide value at the end of the accelerated storage. This combination

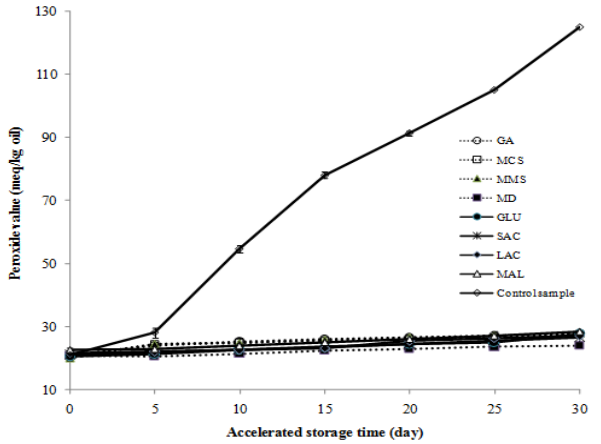



Figure 3. Change in peroxide value of pumpkin seed oil powder during the accelerated storage (GA: gum arabic, MCS: modified corn starch, MMS: modified manioc starch, MD: maltodextrin, GLU: glucose, SAC: sucrose, LAC: lactose, MAL: maltose; Control sample: pumpkin seed oil was not microencapsulated)

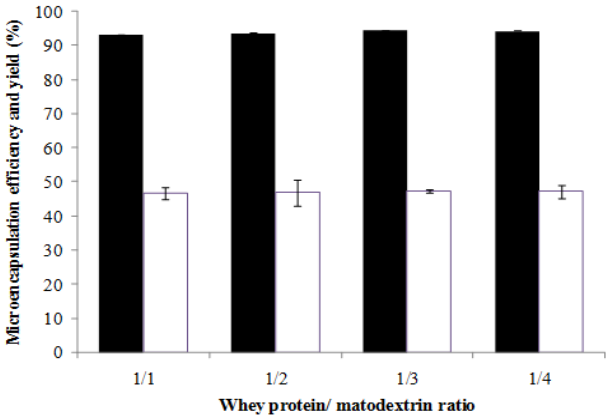



Figure 4. Effect of whey protein/maltodextrin ratio on the microencapsulation efficiency and yield for pumpkin seed oil (Black bars: microencapsulation efficiency, white bas: microencapsulation yield)

was therefore used in the next experiment.

*Effects of whey protein/maltodextrin ratio on microencapsulation of pumpkin seed oil*

Figure 4 presents that decrease in whey protein/ maltodextrin ratio from 1/1 to 1/4 did not change the microencapsulation yield. To our knowledge, there have been so few studies which reported the effect of protein/carbohydrate ratio on oil microencapsulation yield (Vega and Roos, 2006). High maltodextrin ratio in the wall system could reduce the production cost since maltodextrin is much cheaper than whey protein preparation.

When the whey protein/maltodextrin ratio reduced from 1/1 to 1/3, the analysis of variance showed that the microencapsulation efficiency slightly augmented from 93.4 to 94.5%. Further decrease in whey protein/maltodextrin ratio from

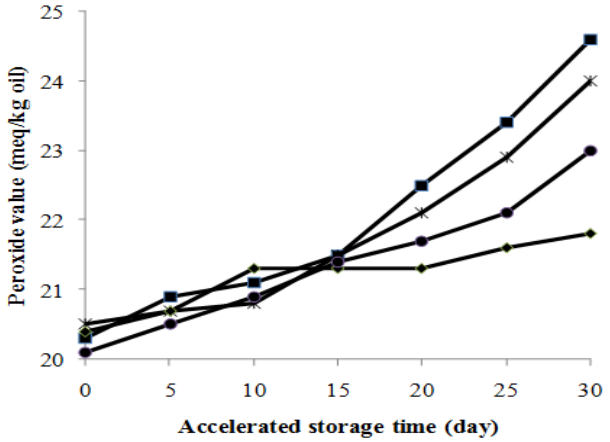



Figure 5. Effect of whey protein/maltodextrin ratio on the evolution of peroxide value of pumpkin seed oil powder during the accelerated storage; Whey protein/ maltodextrin ratio (■): 1/1, (*): 1/2, (◆):1/3; (●): 1/4

1/3 to 1/4 did not change the microencapsulation efficiency. Previously, Sheu and Rosenberg (1995) reported an increased microencapsulated ethyl caprylate level in the spray-dried powder when the whey protein/maltodextrin (DE=10) decreased from 1/1 to 1/9.

When the whey protein/maltodextrin ratio varied from 1/1 to 1/4, the resultant powder samples showed similar acidic value (0.2 mg KOH/kg oil) and peroxide value (20.3 meq/kg oil). Similar results also reported in fish oil microencapsulation when the sodium caseinate and maltodextrin varied from 1/4 to 1/49 (Hogan *et al.*, 2003).

Figure 5 demonstrates that the peroxide value of all powder samples was slightly increased during the accelerated storage.

Sample with the whey protein/maltodextrin ratio of 1/3 showed the lowest peroxide value. Although the microencapsulation efficiency was statistically similar when the whey protein/maltodextrin ratio decreased from 1/3 to 1/4, low protein ratio in the wall system resulted in enhanced peroxide value at the end the accelerated storage. It can be suggested that the protein level in wall system must be higher than the perception threshold; lower protein level in the wall system would not prevent oil change in the spray-dried powder although the microencapsulation efficiency could achieve high level at the end of the spray-drying. The appropriate whey protein/maltodextrin ratio for pumpkin seed oil microencapsulation was therefore 1/3; the microencapsulation efficiency and yield for pumpkin seed oil were 94.5% and 65.6%, respectively.

## Conclusion

Combination of whey protein with high molecular weight carbohydrates such as modified corn starch, modified manioc starch or gum arabic resulted in low microencapsulation efficiency (30.7- 59.8%) for pumpkin seed oil. However, the microencapsulation efficiency was improved to 93.0-97.8% when mixture of whey protein with lower molecular weight carbohydrates including maltodextrin, sucrose, lactose, maltose or glucose was used. Change in whey protein/maltodextrin ratio from 1/1 to 1/4 did not modify the microencapsulation yield but affected the microencapsulation efficiency. The appropriate whey protein/maltodextrin ratio was 1/3 under which the microencapsulation efficiency and yield for pumpkin seed oil were 94.5% and 65.6%, respectively. Microencapsulation was potential method for protection of pumpkin seed oil against deterioration.

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