Food Chemistry 134 (2012) 1453–1460

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Influence of polysaccharides and storage during processing on the properties of mango seed kernel extract (microencapsulation)

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ARTICLE INFO

*Article history:*
Received 14 June 2011
Received in revised form 19 February 2012
Accepted 12 March 2012
Available online 30 March 2012

*Keywords:*
Gum arabic
Maltodextrin
Alginate
Emulsion
Encapsulation
Response surface methodology

ABSTRACT

Extracts from mango (*Mangifera indica* Linn.) cultivar Chok–Anan seed kernels were studied as active substances, since they are known as a good source of phenolic antioxidants with metal chelating and tyrosinase inhibitory activities. Response surface methodology (RSM) was used to investigate the effect of a combination of polysaccharides selected from gum arabic, maltodextrin and alginate on droplet size distribution, encapsulation efficiency (EE), stability and viscosity of W/O/W emulsions. In addition, the effects of stored emulsion on the properties of the encapsulated powder were studied. The results showed that there were interactions between polysaccharides which affected droplet size distribution, stability, viscosity and EE of multiple emulsions. The RSM showed a good fit to the proposed model with $R^2 > 0.83$, 0.79 and 0.69 for viscosity, stability and EE, respectively, with significant correlations ($p < 0.05$). The formulation which gave an optimal coating material was also a suitable coating mixture for preparation of encapsulated mango seed kernel extract powder. Moreover, if the polysaccharide combination is not appropriate for coating, the storage after emulsion preparation will have a greater effect on the properties of the encapsulated emulsion and powder.



## 1. Introduction

The extract from mango seed kernel was shown to be good source of phenolic antioxidants with metal chelating and tyrosinase inhibitory activities (Maisuthisakul & Gordon, 2009). The components present in mango seed kernel extract (MSKE) included gallotannins and condensed tannin-related polyphenols (Arogba, 1997). The extract from the mango seed kernel cultivar, Chok–Anan, contained total phenolics at a concentration of 194 mg GAE/g dry weight of mango seed kernel (MSK), of which 85.7% was identified as methyl gallate (Maisuthisakul & Gordon, 2011). In addition, 1,2,3,4,6-penta-*O*-galloyl-beta-D-glucopyranose, methyl gallate and gallic acid have been identified as components of an ethanolic extract of the Thai mango seed kernel cultivar, Fahlun (Nithitanakool, Pithayanukul, & Bavovada, 2009).

The effectiveness of products depends on preserving the stability, bioactivity and bioavailability of the active ingredients. One method to improve the stability of phenolic compounds is microencapsulation, which entraps a sensitive ingredient inside a coating. These coatings can be prepared from gums, proteins, polysaccharides, lipids and synthetic polymers (Mozafari et al., 2008). Various techniques are employed to microencapsulate food ingredients. Spray-drying is the most common technique in the food industry. In this process, the sensitive ingredient is mixed or homogenised in a solution containing macromolecules and emulsifiers to form a stable emulsion. The emulsion is then fed into a spray-dryer where it is converted to a dried particle.

Double emulsions offer the opportunity to improve the stability of sensitive phenolic ingredients before spray-drying. They are complex systems in which the droplets of the dispersed phase contain even smaller dispersed droplets themselves. The most common double emulsions are of water-in-oil in water (W/O/W) but, in some specific applications, O/W/O emulsions can also be prepared. During years of investigations to improve the stability and to control sustained and prolonged release of active components from double emulsions, the effects of encapsulation materials, including polysaccharides and proteins, emulsifier types, and homogenisation techniques, have been extensively reported. In addition, the stability of phenolic products (which have been encapsulated by spray-drying) has been widely reported (Fang & Bhandari, 2010). Processing steps, including emulsion preparation, storage time before spray-drying, and spray-drying conditions, are relevant to the stability of the encapsulated product.

Numerous coating materials or encapsulating agents are available for food applications. Gum arabic, maltodextrin, xanthan gum, and emulsifying starches are most commonly used as coating materials. In the last 10 years there has been a progressive increase in the number of publications related to the combination of polysaccharides as wall materials. Gum arabic (GA) has been recognised as

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http://dx.doi.org/10.1016/j.foodchem.2012.03.054

an effective encapsulating agent for many years and is still a good choice as an encapsulating agent, due to its ability to stabilise emulsions and facilitate good volatile retention. GA can used as coating materials combined with xanthan gum (Mirhosseini, Tan, Hamid, & Yusof, 2008), maltodextrin (Gomes et al., 2010), or GA with modified starch and maltodextrin (Krishnan, Bhosale, & Singhal, 2005). Problems associated with the use of GA in microencapsulation are high cost and limited supply. Maltodextrins (MD) are produced by partial hydrolysis of starch with acid or enzymes. Hydrolyzed starches are reported to improve the shelf life of orange oil (Anandaraman & Reineccius, 1986) and carrot carotenoids (Wagner & Warthesen, 1995). Problems associated with the use of hydrolyzed starches in microencapsulation are lack of emulsifying properties and poor flavor retention (Reineccius, 1988). Alginates (ALGs) are hydrophilic colloidal carbohydrates. ALG is reported as wall material in combination with chitosan (Belščak-Cvitanović et al., 2011), cyclodextrin (Pluemsab, Sakairi, & Furuike, 2005). ALG is of interest as a potential biopolymer film or coating component because of its unique colloidal properties, which include thickening, stabilizing, suspending, film-forming, gel-producing, and emulsion-stabilizing (Hambleton, Debeaufort, Bonnotte, & Voilley, 2009).

Despite a large number of studies performed on polysaccharides and their combinations (as wall materials of encapsulation products), there is a lack of sufficient knowledge on the formulation of a desirable microcapsulation of mango seed kernel extract and the storage effect. The aim of this study was to develop a system for emulsion stabilisation based on formulations containing GA, MD and xanthan gum as coating materials in W/O/W emulsions containing phenolic mango seed kernel extract, which was optimised with respect to the stability of the emulsion and the encapsulation efficiency by using response surface methodology (RSM). In addition, the effect of storage time after emulsion preparation but before spray-drying was studied.

## 2. Materials and methods

### 2.1. Materials

Sun-dried seeds, obtained as by-products from ripened mango (*M. indica* cultivar Chok–Anan), in March–June 2011, were donated by a mango processing manufacturer in Thailand. The seeds were washed and sun-dried in the greenhouse for 3 days and the kernels were removed manually from the seeds for further extraction. The moisture content of dried mango seed kernel, determined according to AOAC (1990), was 9.81 ± 0.34% on a dry weight basis. The dried material was kept in a freezer at −20 °C for up to 2 months before use.

Folin Ciocalteu reagent, sodium carbonate and low viscosity sodium alginate were purchased from Sigma Chemical Co., Ltd. (St. Louis, USA). Maltodextrin (DE = 16–20, 5% moisture) was purchased from Natural Starch and Chemical Company (Sydney, Australia). Gum arabic (food grade) was provided by Colloides Naturels International Co. (Rouen, France). Gelatin (Type A: Bloom 100) was obtained from Venford Holdings Ltd. (Hong Kong, China). Sodium chloride was purchased from Merck Co., Ltd. (Schuchardt, Germany). Polyglycerol polyricinoleate (PGPR, 4150, HLB ≈ 3) was obtained from Palsgaard (New Jersey, USA). The emulsifier used was Tween 80 (Fisher Scientific, New Jersey, USA). The other chemicals and solvents used in this experiment were of analytical grade, purchased from Sigma–Aldrich Co., Ltd. (Steinheim, Germany). Deionised water (electrical conductivity < 2 μS $cm^{-1}$) was used throughout all the experiments.

### 2.2. Extraction of antioxidant from mango seed kernel

The frozen kernel (80 g) was blended for 1 min with 95% ethanol and refluxed with 1.2 M hydrochloric acid in ethanol for 3 h. The supernatant, after filtration through cheesecloth and Whatman No 4 filter paper, was evaporated under vacuum. The residue was dried in a freeze-dryer and stored in aluminum foil after flushing with nitrogen at −20 °C until analysis (Maisuthisakul & Gordon, 2009).

### 2.3. Preparation of $W_1/O/W_2$ emulsions

$W_1/O/W_2$ emulsions were prepared using the modified two-step emulsification method described by Fechner, Knoth, Scherze, and Muschiolik (2007). The primary water-in-oil ($W_1/O$) emulsion (300 g) was prepared by initially mixing the inner aqueous phase with the oil phase. The mixtures were homogenised with a high-pressure homogenizer (Armfield model FT9, UK), twice at 3000 psi. The inner phase ($W_1/O$) was prepared from 1% (w/w) hydrating gelatin (0.6%, w/w); NaCl, (1.68%, w/w); refined soybean oil, (2.33%, w/w); PGPR; MSKE, (0.16%, w/w) and deionised water. The inner aqueous phase ($W_1$) was prepared by mixing gelatin in water at 60 °C for 15 min, using moderate magnetic stirring. After that, the extract and NaCl solution were added and the solution was kept warm for 5 min. The oil phase was refined soybean oil containing the hydrophobic emulsifier PGPR and was heated to 60 °C for 10 min.

The composition of the outer phase ($W_2$) was as shown in Table 1, 20 Samples containing GA (0–20%, w/w), MD (0–30%, w/w) and ALG (0–0.5%, w/w) were prepared for the optimisation procedure, based on a three factor central composite design (CCD). The studied range was chosen from the results of a preliminary study. One constraint for the emulsion formulation was to ensure that viscosity was not too high for spray-drying. Subsequently, the $W_1/O$ emulsion was gradually added to the outer phase ($W_2$), and mixed with a hand-held laboratory homogenizer at 10,000 rpm for 2 min (PRO200 Homogenizer, Pro Scientific, CT, USA) to produce the final double emulsion. The droplet size distribution of the W/O/W emulsions and the encapsulation efficiency were measured. The prepared emulsions were stored at 30 °C and 4 °C for 0 and 24 h before being analyzed for stability, apparent viscosity and by microscopic observation. The encapsulation efficiency of the 24 h-stored emulsion was also measured.

### 2.4. Preparation of spray-dried encapsulated powders

The selected emulsions were spray-dried, using a Nitro Minor Dryer (Gea Nitro A/S, Denmark) pilot scale spray-dryer. The run numbers 7, 9, 11, 13 were selected for spray-drying. The dispersion was fed by a peristaltic pump at a fixed rate of 30–35 ml/min. Drying was carried out in the concurrent mode. Inlet and outlet air temperatures were 180 °C and 90 °C, respectively. The microencapsulated powder was collected at the dryer's cyclone. The microcapsules were analyzed for water activity and by scanning electron microscopy.

### 2.5. Determination of droplet size distribution

The droplet size distribution of the emulsion was determined by a static light-scattering technique, with a Malvern Instruments particle and droplet sizer (S version 2.19, He–Ne laser source; wavelength 633 nm, beam length 2.40 mm). The mean droplet diameter (μm) of the emulsion was characterised by $d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$. The parameter $n_i$ is the number of droplets with diameter $d_i$.

### 2.6. Determination of encapsulation efficiency of the $W_1/O/W_2$ emulsions during emulsification and storage

The multiple emulsions were characterised in term of encapsulation efficiency (EE) by measuring the concentration of phenolics

**Table 1**
Experimental values of response variables obtained from the central composite experiment design at 4 °C.

| Run number | Independent variables (%, w/w) | | | Response variables | | | | |
|---|---|---|---|---|---|---|---|---|
| | GA ($X_1$) | MD ($X_2$) | ALG ($X_3$) | Creaming index (%) | Viscosity (η, cPs) | | Encapsulation efficiency (*EE*, %) | |
| | | | | 24 h | 0 h | 24 h | 0 h | 24 h |
| 1 | 5.95 | 8.91 | 0.15 | 61.1 ± 0.5 | 48.0 ± 1.1 | 63.3 ± 1.4 | 90.1 ± 0.8 | 80.1 ± 0.5 |
| 2 | 15.95 | 8.91 | 0.15 | 23.8 ± 0.7 | 173.8 ± 1.2 | 323.8 ± 1.2 | 94.5 ± 0.9 | 92.3 ± 0.6 |
| 3 | 5.95 | 23.90 | 0.15 | 39.0 ± 0.9 | 85.3 ± 1.5 | 169.3 ± 1.7 | 95.7 ± 1.1 | 92.9 ± 0.8 |
| 4 | 15.95 | 23.90 | 0.15 | 25.0 ± 0.3 | 407.8 ± 1.5 | 758.0 ± 1.0 | 95.6 ± 1.4 | 94.1 ± 0.3 |
| 5 | 5.95 | 8.91 | 0.40 | 43.8 ± 0.4 | 62.8 ± 1.6 | 103.8 ± 0.8 | 94.2 ± 1.1 | 91.7 ± 0.7 |
| 6 | 15.95 | 8.91 | 0.40 | 24.4 ± 0.3 | 166.0 ± 1.4 | 361.5 ± 0.4 | 95.6 ± 1.2 | 93.1 ± 0.2 |
| 7 | 5.95 | 23.90 | 0.40 | 17.8 ± 0.7 | 155.0 ± 1.2 | 325.5 ± 1.5 | 95.1 ± 1.3 | 94.2 ± 0.5 |
| 8 | 15.95 | 23.90 | 0.40 | 7.0 ± 0.1 | 390.5 ± 1.7 | 1626.7 ± 1.2 | 96.3 ± 1.2 | 95.2 ± 0.3 |
| 9 | 0.00 | 15.00 | 0.25 | 50.0 ± 0.3 | 50.7 ± 1.0 | 451.8 ± 1.2 | 90.8 ± 1.0 | 82.1 ± 0.4 |
| 10 | 20.00 | 15.00 | 0.25 | 14.3 ± 0.5 | 515.0 ± 0.8 | 1075.8 ± 1.0 | 95.7 ± 0.7 | 94.6 ± 0.7 |
| 11 | 10.00 | 0.00 | 0.25 | 55.0 ± 0.4 | 47.0 ± 0.4 | 82.3 ± 1.1 | 90.8 ± 0.9 | 85.7 ± 0.8 |
| 12 | 10.00 | 30.00 | 0.25 | 16.2 ± 0.7 | 256.5 ± 1.5 | 997.0 ± 1.3 | 95.2 ± 1.3 | 94.4 ± 0.9 |
| 13 | 10.00 | 15.00 | 0.00 | 65.8 ± 0.3 | 72.6 ± 1.2 | 131.8 ± 1.4 | 85.6 ± 1.1 | 72.3 ± 0.2 |
| 14 | 10.00 | 15.00 | 0.50 | 22.5 ± 0.4 | 197.5 ± 1.2 | 318.3 ± 1.2 | 95.8 ± 1.4 | 94.2 ± 0.8 |
| 15 | 10.00 | 15.00 | 0.25 | 50.0 ± 0.8 | 105.5 ± 1.0 | 162.8 ± 1.3 | 90.1 ± 0.5 | 83.7 ± 0.1 |
| 16 | 10.00 | 15.00 | 0.25 | 48.6 ± 0.9 | 101.2 ± 1.1 | 190.0 ± 1.6 | 92.3 ± 1.4 | 85.7 ± 0.4 |
| 17 | 10.00 | 15.00 | 0.25 | 51.4 ± 0.1 | 105.9 ± 1.3 | 169.8 ± 1.2 | 93.2 ± 0.6 | 86.7 ± 0.6 |
| 18 | 10.00 | 15.00 | 0.25 | 47.0 ± 0.2 | 104.0 ± 1.4 | 200.0 ± 1.1 | 91.5 ± 0.8 | 84.2 ± 0.7 |
| 19 | 10.00 | 15.00 | 0.25 | 45.0 ± 0.4 | 103.3 ± 1.2 | 167.5 ± 1.3 | 90.1 ± 0.9 | 83.4 ± 0.3 |
| 20 | 10.00 | 15.00 | 0.25 | 49.0 ± 0.3 | 102.0 ± 1.1 | 195.1 ± 1.1 | 92.3 ± 1.1 | 86.0 ± 0.4 |

leaked immediately after preparation of the double emulsion and after a storage period of 24 h. Briefly, an aliquot (5 g) of $W_1/O/W_2$ emulsion was diluted with 5 g of deionised water. The mixture was centrifuged at 30,000*g* at 4 °C for 30 min, after which the lower layer was carefully removed and filtered using a 0.22 μm Millipore syringe filter (Milex-GV, Millipore, MA, USA). The phenolic content was determined with Folin Ciocalteu's reagent. The total phenolic concentration in all samples was expressed as mg of methyl gallate equivalent per g dry weight of MSKE, using a linear equation. The total phenolic content of each sample recovered (TPs) by centrifugation was calculated using the equation $TPs = [TP_e - TP_o]$, where $TP_e$ was the total phenolic content of the broken emulsion sample after syringe filtration and $TP_o$ was the total phenolics of the broken emulsion sample not containing phenolics extract after syringe filtration. The EE (%) was then calculated, using the equation $EE\ (\%) = [1 - (TP_s/TP_p)] \times 100$, where $TP_p$ was the total phenolic content of the inner aqueous phase of the emulsion system after syringe filtration.

### 2.7. Measurement of stability

Ten grammes of $W_1/O/W_2$ emulsion samples were transferred into a test tube (internal diameter 13 mm, height 150 mm), tightly sealed with a plastic cap. The creaming stability was measured by visual observation of the emulsions for 10 h. After storage at 4 °C and 30 °C, emulsions were separated into an optically opaque 'cream' layer at the top and a transparent (or turbid) 'serum' layer at the bottom. The serum layer was defined as the sum of the turbid and transparent layers. The total height of the emulsion ($H_E$) and the height of the serum layer ($H_S$) were measured. The extent of creaming was characterised by the creaming index (%) = $100 \times (H_S/H_E)$. The creaming index provided indirect information about the extent of droplet aggregation in an emulsion: the faster the creaming, the higher the creaming index, and the larger the particle size (Surh, Ward, & McClements, 2006).

### 2.8. Determination of apparent viscosity

The viscosities of the freshly prepared emulsion samples were determined at 25 °C with the aid of a Brookfield DV-II, LV viscometer (Brookfield Engineering Laboratories, USA), equipped with concentric cylinder geometry. The viscosity (η) was obtained in terms of centipoise (cPs).

### 2.9. Emulsion photomicrographs

The diluted double emulsions (one part emulsion and nine parts deionised water) were inserted between two glass microscope slides and observed with an Olympus BX61 light microscope (Germany), equipped with Pro-Microscan Microscope Digital Camera model DCM35 (Oplenic, Japan), to record the photomicrograph. The samples were analyzed at room temperature.

### 2.10. Determination of UV spectrum

The freshly prepared aqueous 1% gum arabic, 10% MD, 0.1% ALG and their mixtures including aqueous 24 h aqueous storage of the same combination were used to observe the UV spectrum between 210 and 400 nm.

### 2.11. Determination of water activity

Water activity was measured using an aqualab analyzer (Decagon Devices, USA), at 25 °C, after stabilisation of the samples at this temperature for 1 h.

### 2.12. Scanning electron microscopy (SEM) of encapsulated materials

The outer structures of the microcapsules were studied by SEM. Freeze-dried particles were mounted on metal stubs and coated with gold (~20 nm thickness) using an ion coater (IB-2, Eiko Engineering Co., Ltd., Japan). Samples were then observed using a JEOL JSM5600LV scanning electron microscope (JEOL, Japan) at an acceleration potential of 10 kV. Pictures were captured by automatic image-capturing software (JEOL, Japan).

### 2.13. Experimental design and statistical analysis

The effects of three independent variables, namely GA content, MD content and ALG content, on droplet size distribution, stability, apparent viscosity and encapsulation efficiency of the double emulsion were studied using a three factor CCD. Twenty emulsion

samples were established, based on the CCD with three independent variables at five levels on each variable. The center point was repeated six times to calculate the reproducibility of the method. Multiple regression analysis was applied for prediction of the linear, quadratic and interaction terms of the independent variables in the RSM. Regression analysis was performed to estimate the response function as polynomial model:

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j \quad (1)$$

where $Y$ is response calculated by the model, $\beta_0$ is a constant, and $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ are linear, squared and interaction coefficients, respectively. Data were modeled by multiple regression analysis, adopting stepwise analysis. The variables significant at $p < 0.05$ levels were only selected for the model construction. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of the model was checked, accounting for lack of fit, adequate precision and pure error. Experimental data were

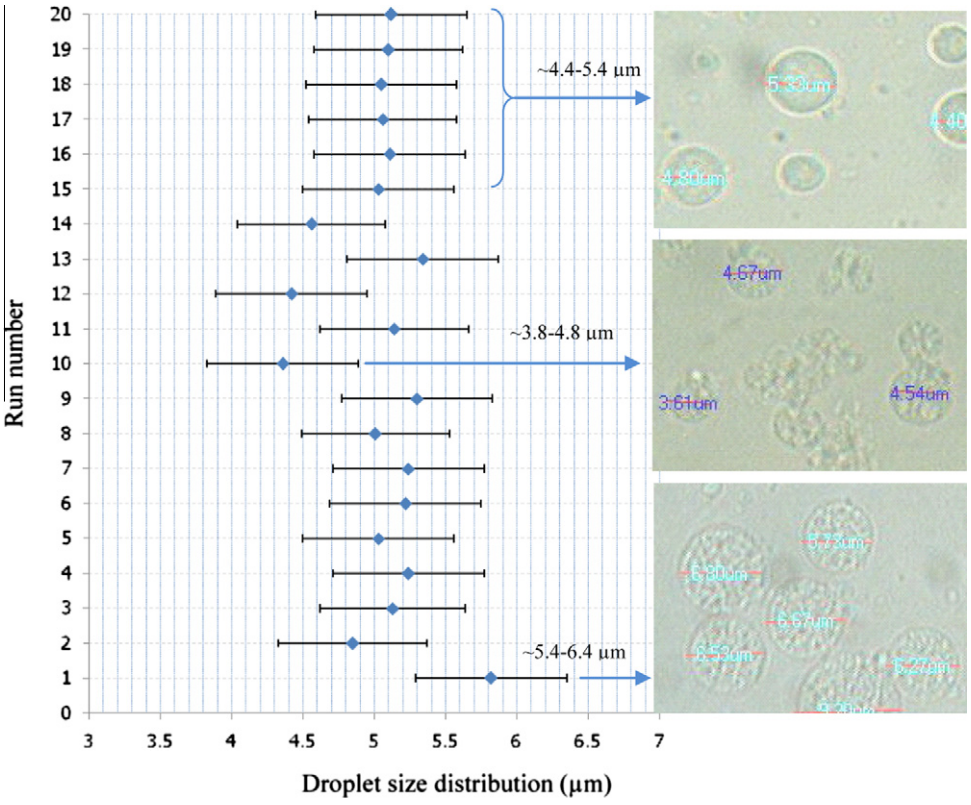


**Fig. 1.** Effect of polysaccharide combinations on the droplet size distribution of W/O/W emulsions. The polysaccharide composition for each run number is given in Table 1.

**Table 2**
Experimental values of response variables obtained from the central composite experiment design at 30 °C for 24 h.

| Run number | Independent variables (%, w/w) | | | Response variables | | |
|---|---|---|---|---|---|---|
| | GA ($X_1$) | MD ($X_2$) | ALG ($X_3$) | Creaming index (%) 24 h | Viscosity (η, cPs) 24 h | Encapsulation efficiency (*EE*, %) 24 h |
| 1 | 5.95 | 8.91 | 0.15 | 66.4 ± 0.2 | 63.5 ± 1.4 | 75.3 ± 0.4 |
| 2 | 15.95 | 8.91 | 0.15 | 39.5 ± 0.4 | 186.4 ± 0.3 | 89.1 ± 0.5 |
| 3 | 5.95 | 23.90 | 0.15 | 51.3 ± 0.6 | 120.5 ± 0.6 | 89.3 ± 0.5 |
| 4 | 15.95 | 23.90 | 0.15 | 52.5 ± 0.3 | 629.5 ± 1.0 | 91.2 ± 0.4 |
| 5 | 5.95 | 8.91 | 0.40 | 48.8 ± 0.1 | 78.8 ± 0.8 | 89.5 ± 0.2 |
| 6 | 15.95 | 8.91 | 0.40 | 25.9 ± 0.3 | 200.8 ± 0.9 | 90.3 ± 0.4 |
| 7 | 5.95 | 23.90 | 0.40 | 19.8 ± 0.2 | 219.4 ± 0.6 | 91.7 ± 0.3 |
| 8 | 15.95 | 23.90 | 0.40 | 9.5 ± 0.2 | 729.3 ± 1.0 | 92.1 ± 0.4 |
| 9 | 0.00 | 15.00 | 0.25 | 66.9 ± 0.2 | 63.9 ± 1.2 | 78.1 ± 0.4 |
| 10 | 20.00 | 15.00 | 0.25 | 17.4 ± 0.3 | 641.2 ± 1.0 | 92.2 ± 0.5 |
| 11 | 10.00 | 0.00 | 0.25 | 64.8 ± 0.2 | 89.9 ± 1.1 | 80.1 ± 0.2 |
| 12 | 10.00 | 30.00 | 0.25 | 22.9 ± 0.4 | 572.2 ± 1.3 | 91.9 ± 0.3 |
| 13 | 10.00 | 15.00 | 0.00 | 79.5 ± 0.3 | 94.4 ± 1.4 | 67.3 ± 0.8 |
| 14 | 10.00 | 15.00 | 0.50 | 31.1 ± 0.1 | 275.8 ± 1.2 | 90.9 ± 0.2 |
| 15 | 10.00 | 15.00 | 0.25 | 66.9 ± 0.4 | 140.0 ± 1.3 | 78.7 ± 0.4 |
| 16 | 10.00 | 15.00 | 0.25 | 54.3 ± 0.3 | 147.1 ± 1.4 | 80.3 ± 0.3 |
| 17 | 10.00 | 15.00 | 0.25 | 63.2 ± 0.1 | 159.7 ± 1.4 | 82.1 ± 0.8 |
| 18 | 10.00 | 15.00 | 0.25 | 52.1 ± 0.2 | 145.3 ± 1.1 | 80.7 ± 0.4 |
| 19 | 10.00 | 15.00 | 0.25 | 55.5 ± 0.4 | 159.7 ± 1.3 | 80.2 ± 0.1 |
| 20 | 10.00 | 15.00 | 0.25 | 58.2 ± 0.2 | 146.3 ± 1.1 | 82.3 ± 0.4 |

compared with the fitted values predicted by the models in order to verify the adequacy of the regression models.

Each experiment was repeated in triplicate and mean values, including the pooled standard error of the mean (SEM), were then determined.

## 3. Results and discussion

### 3.1. Role of combination of coating materials in $W_1/O/W_2$ emulsion properties

The initial droplet size distribution is an important characteristic of stabilised water-in-oil-in-water emulsions. The average droplet size was used to compare the changes in particle size for all emulsions which contained different concentrations of each polysaccharide mixture. The results showed that the combination of polysaccharides affected the size distribution of the emulsions. The particle size distribution for the emulsions showed a similar principal peak between 4.36 and 5.82 μm. The particle sizes can be classified into three groups. They are small (~3.6–4.6 μm), medium (~4.4–5.4 μm) and large (~5.4–6.4 μm) size. Microscopic investigation of the structure of the W/O/W emulsion showed that the microencapsulated emulsion had been formed from several W/O droplets in water (Fig. 1). The finest droplet size distribution was found in run number 10, in which the coating contained GA 20% (w/w), MD 15% (w/w) and ALG 0.25% (w/w). There was no trend in the effect of each polysaccharide on emulsion size. This result can be attributed to interactions between polysaccharides. GA and ALG are anionic polymers which are good candidates for coacervation reactions. They form gels under appropriate preparation conditions, which also qualifies them as encapsulation or entrapment materials (Risch & Reineccius, 1995). MD is considered as a good encapsulating agent because it has good solubility, and solutions exhibit low viscosity at high solids content, but it lacks the interfacial properties required for high microencapsulation efficiency, which is generally associated with other encapsulating agents, such as GA (Yoshii et al., 2001). MD characterisation study published by Raja, Sankarikutty, Sreekuma, Jayalekshmy, and Narayanan (1989) showed that MD with dextrose equivalence between 10 and 20 are suitable for use in coatings. MD samples have the highest capacity of encapsulating agents because MD can be dispersed in water at concentrations up to 35.5% of the solution without haze formation. In addition, it is known that ALG has gelling properties that could stabilise emulsions towards flocculation and coalescence (Dalgleish, 2006).

The most obvious manifestation of emulsion instability is droplet size distribution and creaming index. Samples with a smaller particle size were more stable, as reflected in the lower creaming index. Typically, the stability of all samples was influenced by the coating materials used. This study also showed that storage temperature (4 °C and 30 °C) affected emulsion stability (Tables 1 and 2). At the same concentration of each polysaccharide, most of samples at 4 °C showed lower values of creaming index than that at 30 °C. These represent the higher stability at lower temperature storage. However, with storage at 30 °C, the stability of emulsion containing GA, MD and ALG as wall material exhibited different effects from that at 4 °C. These results suggested that the storage temperature is an important factor affecting to stability.

Considering to interaction between polysaccharides, GA and MD exhibited greater effects than those of the others (Table 3). The coefficient of interaction between GA and MD exhibited higher than the others. MD is used as a bulking agent to assist the gelling action of GA (Weiss, Scherze, & Muschiolik, 2005).

**Table 3**
Regression coefficients, $R^2$, adjusted $R^2$ and lack of fit for the final reduced models.

| Regression coefficient | Creaming index at 4 °C for 24 h ($Y_1$, %) | Viscosity at 4 °C for 0 h ($Y_2$, cPs) | Encapsulation efficiency at 4 °C for 24 h ($Y_3$, %) |
|---|---|---|---|
| $b_0$ | 48.46 | 105.88 | 84.88 |
| $b_1$ | −5.07 | 72.96 | 1.08 |
| $b_2$ | −9.49 | 68.85 | 2.48 |
| $b_3$ | −9.43 | 19.73 | 3.78 |
| $b_1^2$ | −13.12 | 108.86 | 3.95 |
| $b_2^2$ | −4.30 | 2.44 | 2.23 |
| $b_3^2$ | −1.28 | −3.47 | −0.17 |
| $b_{12}$ | 3.99 | 41.13 | −1.43 |
| $b_{13}$ | 2.64 | −13.70 | −1.37 |
| $b_{23}$ | −2.81 | 5.68 | −1.25 |
| $R^2$ | 0.8872 | 0.9133 | 0.8335 |
| $R^2$ (adj) | 0.7858 | 0.8352 | 0.6837 |
| Regression ($p$-value) | 0.0011 | 0.0003 | 0.0045 |
| Lack of fit ($F$-value) | 27.27 | 1807.87 | 12.39 |
| Lack of fit ($p$-value) | 0.0012 | <0.0001 | 0.0046 |

$b_i$, The estimated regression coefficient for the main linear effects.
$b_i^i$, The estimated regression coefficient for the quadratic effects.
$b_{ij}$, The estimated regression coefficient for the interaction effects.
1, Gum arabic; 2, maltodextrin; 3, alginate.

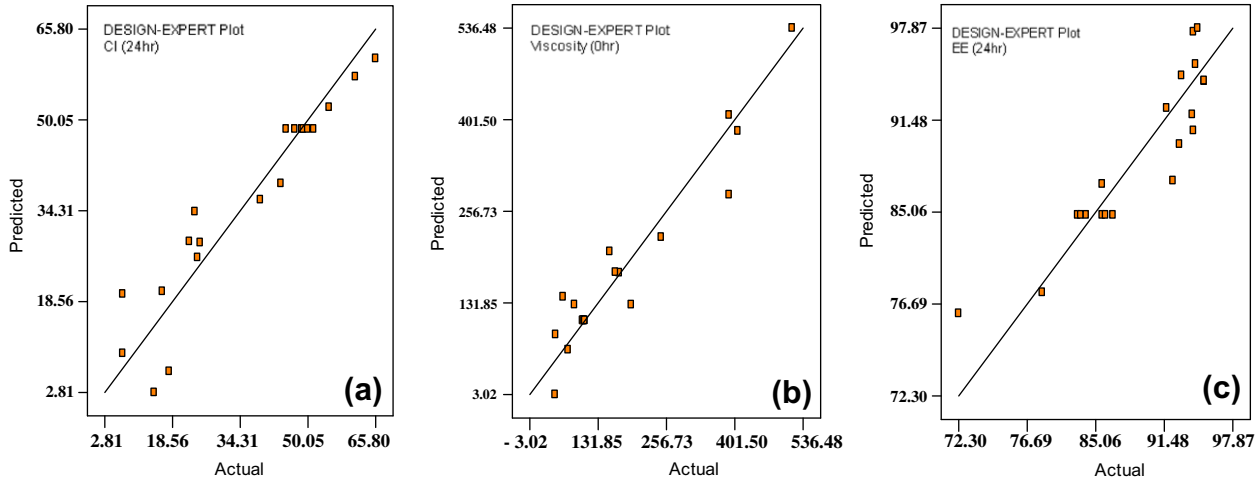


**Fig. 2.** Predicted and experimental values of (a) emulsion stability, (b) viscosity and (c) encapsulation efficiency of W/O/W emulsions containing mango seed kernel obtained from the central composite experimental design with storage at 30 °C.

As shown in Table 1, the encapsulation efficiency varied from 72.3% to 95.8% and was influenced by the gum formulation. The EE of multiple emulsions at the initial stage was different from that after a 24 h storage. The emulsion formulation which produced good stability also gave high encapsulation efficiency (Tables 1 and 2). Encapsulating agents of good quality increase the effectiveness of the coating, resulting in the reduction of phenolic loss during storage. These results are in agreement with a previous report that the effect of the gelling capacity on encapsulation becomes considerably more important during longer storage periods in the preparation of multiple emulsion systems (Weiss et al., 2005).

### 3.2. Optimisation using response surface analysis

The values of creaming index, viscosity and encapsulation efficiency, for each trial of the experimental design, are shown in Tables 1 and 2. The linear, quadratic and interaction effects of GA ($X_1$), MD ($X_2$), and ALG ($X_3$) on each of the response variables are given in Table 3. The $R^2$ and adjusted $R^2$ values for the significant ($p < 0.05$) response surface models varied from 0.834–0.913 and 0.684–0.835, respectively. These values were obtained for creaming stability with storage at 4 °C for 24 h, viscosity at 4 °C for 0 h and encapsulation efficiency at 4 °C for 24 h. These selected variables gave the highest values of $R^2$ and adjusted $R^2$ for each response variable studied. All models were significant for all variables (creaming stability ($p = 0.0011$), viscosity ($p = 0.0003$) and encapsulation efficiency ($p = 0.0045$)) (Table 3). Therefore, the RSM was able to predict most of the variation (68–84%) of the properties of the multiple emulsions as a function of the polysaccharide variables. All interaction effects of the polysaccharide matrix were insignificant ($p > 0.05$) in their effects on creaming stability, viscosity and encapsulation efficiency of the multiple emulsions. The effect of ALG was highly significant ($p < 0.05$) for encapsulation efficiency (Table 3), considering the highest coefficient of main linear effects.

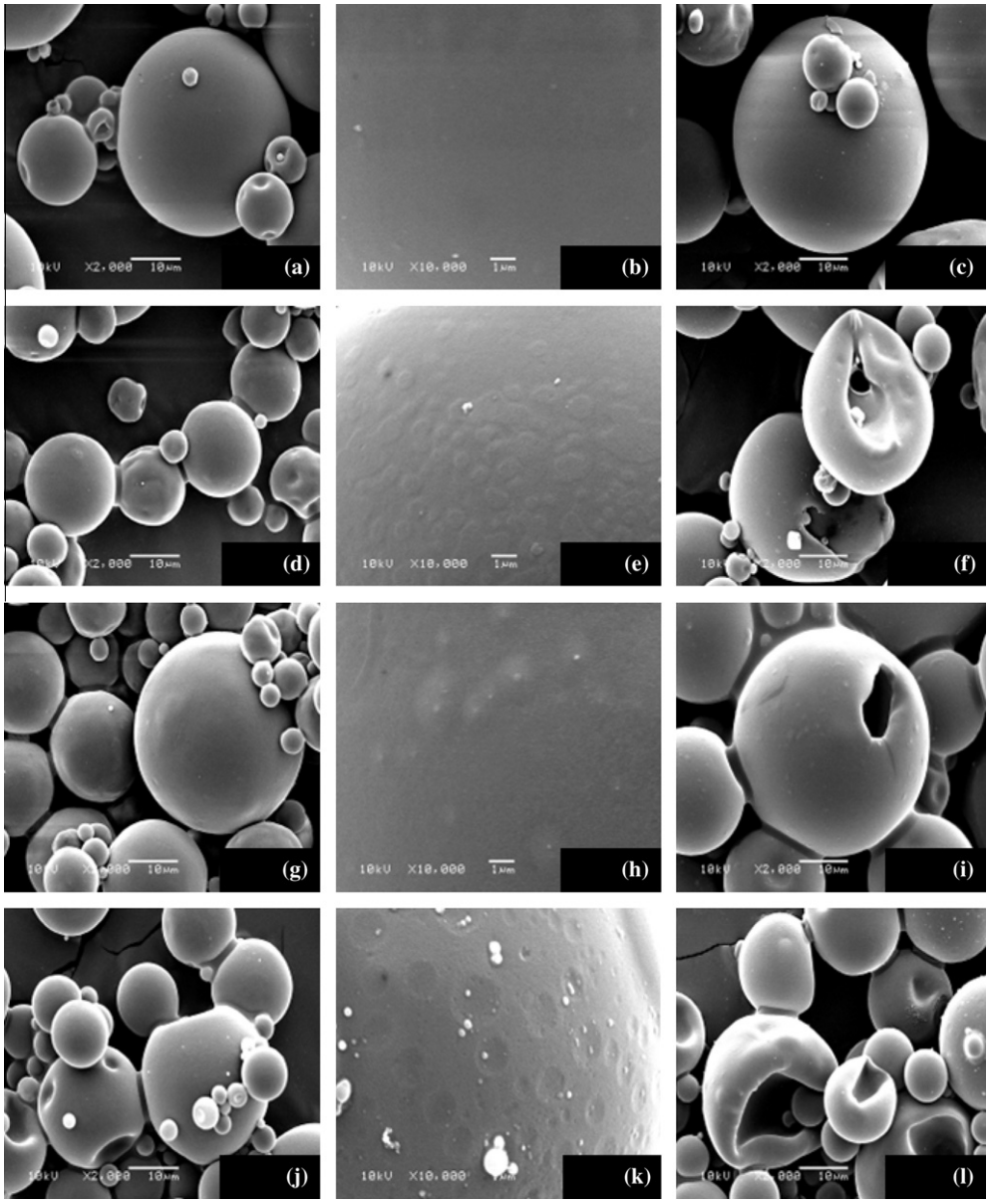


**Fig. 3.** Scanning electron micrographs of dry microcapsules prepared with (a) 5.95% GA + 23.9% MD + 0.4% ALG from fresh emulsion preparation (AMG); (b) surface of AMG dried microcapsules; and (c) microcapsules from AMG emulsion after storage for 24 h; (d) with 15.0% MD + 0.25% alginate from fresh emulsion preparation (MA); (e) surface of MA dried microcapsules and (f) microcapsules from MA emulsion after storage for 24 h; (g) microcapsules with 10.0% GA + 0.25% alginate from fresh emulsion preparation (AA); (h) surface of AA dried microcapsules; and (i) microcapsules from AA emulsion after storage for 24 h; (j) microcapsules with 10.0% GA + 15.00% MD from fresh emulsion preparation (AM), (k) surface of AM dried microcapsules and (l) microcapsules from AM emulsion after storage for 24 h.

Similar results have also been reported by others (Hambleton et al., 2009; Weiss et al., 2005). These results can be explained by the fact that ALG solutions can form gels, and hence ALG can reduce the release of phenolic components from the inner phase to the outer phase of the emulsion. ALG is of interest as a potential biopolymer film or coating component because of its unique colloidal properties, which include thickening, stabilizing, suspending, film-forming, gel-producing, and emulsion-stabilizing (Fabra, Talens, & Chiralt, 2008). The quadratic model of GA showed a significant ($p < 0.05$) but variable effect on the response variables. The fitted models were suitable, showing significant regression, no lack of fit and satisfactory coefficients (Table 3).

The predicted values were obtained by a model-fitting technique, using Design Expert Software. Fitting of the data to various models (linear, two factorial, quadratic and cubic) and their subsequent ANOVA showed that the quadratic polynomial model was most suitable. There was a good relationship between predicted values and experimental values (Fig. 2). The model was used to find an optimal region for the response variable studied and to define the relationship between three independent variables and the response variables. The optimum mixture was found to be 5.95% GA, 23.90% MD and 0.11% ALG. This mixture gave the best stability, and encapsulation efficiency in the required region of viscosity (<300 cPs).

For validation the model, the adequacy of the response surface equations was checked by the comparison of experimental and fitted values predicted by the response regression models. No significant difference ($p > 0.05$) was found between the experimental and predicted values (data not shown).

### 3.3. Microcapsule production by spray drying

Run number 7, which was composed of 5.95% GA, 23.90% MD and 0.40% ALG, showed the optimal formulation, which embraced high stability and encapsulation efficiency of the W/O/W emulsion obtained, including appropriate viscosity for injection into the spray-dryer. Normally, the solution for spray-drying should not be more viscous than 300 cPs. Other runs such as runs 8, 10 (Table 1) showed higher stability and encapsulation efficiency but the viscosity was too high for pumping by the spray-dryer. Runs 9, 11 and 13 (Table 1) showed the effects of binary mixtures of polysaccharides as coating materials. The creaming index of emulsions containing GA and MD exhibited a high value or less stability than those of emulsions containing other binary mixtures of polysaccharides (Table 1). These results confirmed that ALG affected to stability of emulsions more than did GA and MD.

Microcapsules containing mango seed kernel extract (MSKE) showed similar values of water activity (in the range 0.19–0.21) for all polysaccharide mixtures. Fig. 3 shows the scanning electron micrographs of the dry emulsion powders obtained by spray-drying. Fig. 3a, d, g and j were obtained from the freshly prepared dried emulsion run numbers 7, 9, 11 and 13 (Table 1). They consisted of

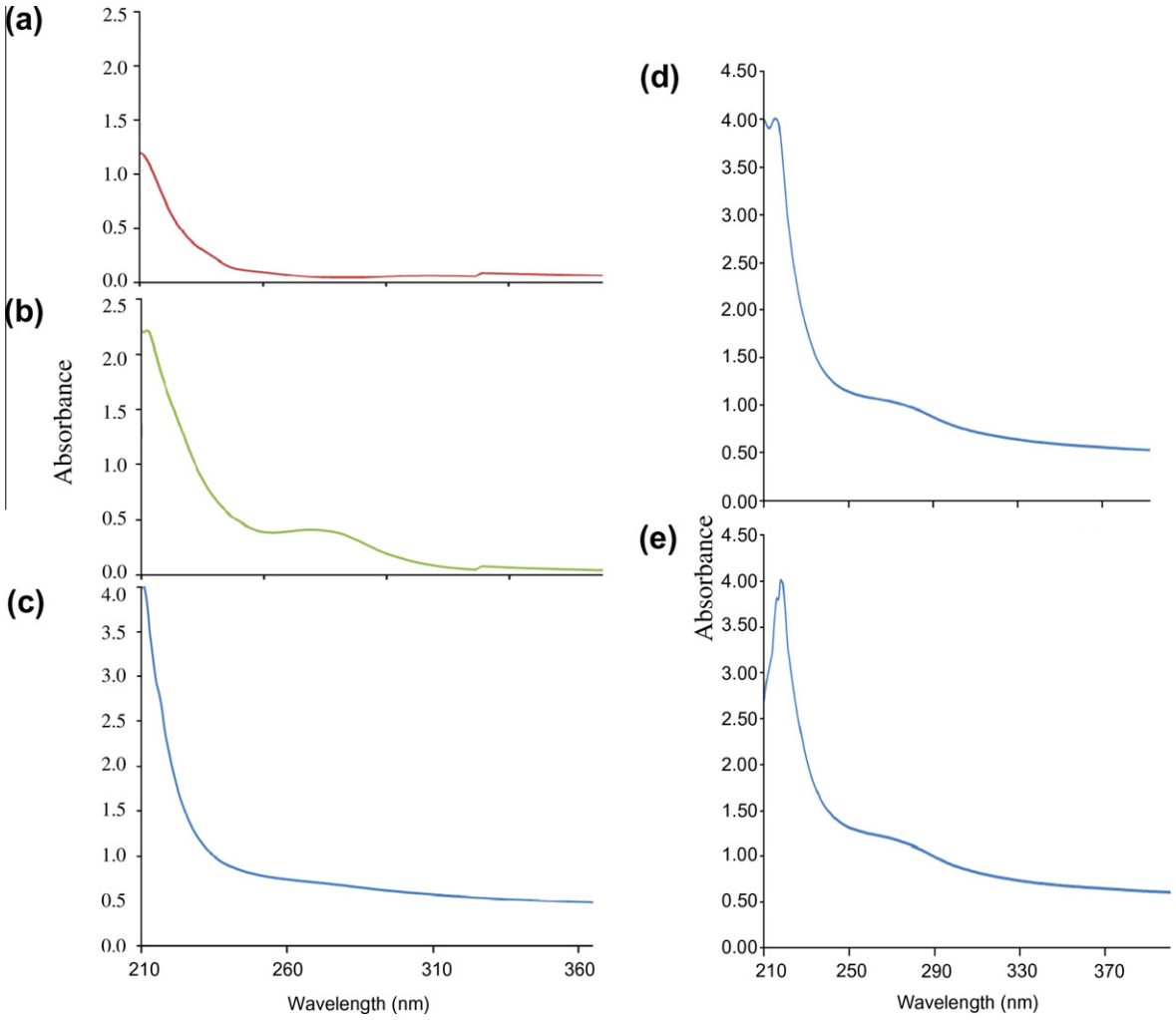


**Fig. 4.** UV spectrum of freshly prepared solution of (a) 1%GA, (b) 10%MD, (c) 0.1%ALG, (d) a freshly prepared mixture of 1%GA + 10%MD + 0.1%ALG and (e) a storage mixture of the same combination for 24 h.

well-separated spherical particles, having similar diameters (4.4–5.8 μm), rounded shape, and a smooth surface, with no obvious indentations. However, the surface of the powder was smoother in the microcapsules prepared from 5.95% GA, 23.9% MD and 0.4% ALG (Fig. 3b) than with the others (Fig. 3e, h and k). The amorphous glasses produced in the dehydration process protect the entrapped molecules from the effects of heat and oxygen (Roos, 1997). The resulting powders had particles of various sizes, which agreed with the results obtained for the particle size distribution.

It should be noted that, following spray-drying, the surface morphology and inner structure of spray-dried powders depend mainly on the properties of the coatings (Hecht & King, 2000). The coating formulation of run number 13 (Fig. 3k, Table 1) which was not composed of ALG, produced a porous surface. This is consistent with the fact that ALG in emulsified alginate edible films produces packaging with good mechanical and water barrier properties. These observations could be used to explain why ALG showed a higher efficiency for entrapment.

Fig. 3c, f, i and l show the powder obtained from emulsions stored for 24 h before drying. Fig. 3c shows the powders composed of 5.95% gum arabic, 23.9% MD and 0.4% ALG which were the most suitable coating mixture for encapsulated mango seed kernel extract after 24 h of storage. The other coating formulations showed wrinkle and broken microcapsules (Fig. 3f, i and l). These results confirmed that the storage time during emulsion processing before drying affected the properties of microcapsules. Moreover, if the coating mixtures are not stable or an optimal composition, the storage time will have a greater effect on the encapsulation property than that for optimal formulations.

The UV spectrum of each freshly prepared polysaccharide and a mixture, including its storage mixture, were used to measure UV spectrum to show the interaction of these polysaccharides (Fig. 4). The UV spectrum of a freshly prepared mixture of GA, MD and ALG was different from the storage one (Fig. 4d and e). These results confirmed that there were interaction between GA, MD and ALG and the interactions were changed during storage. Hence, the storage time after emulsion preparation before spray-drying is also an important factor.

## 4. Conclusions

Combinations of polysaccharides containing gum arabic, maltodextrin and alginate, and storage temperature and time during processing affected the properties and stability of multiple emulsions and the encapsulated powder. The emulsion formulations which produced good stability also gave high encapsulation efficiency in the emulsion and strong coating protection in the powder. The optimal mixture for multiple emulsions was 5.95% gum arabic, 23.9% maltodextrin and 0.11% alginate. This mixture gave the best stability, and encapsulation efficiency in the required region of viscosity (<300 cPs) of the multiple emulsion. After emulsion preparation, the drying process should be completed rapidly to obtain the best encapsulated powder.

## Acknowledgements

This research was supported by the research fund of the University of the Thai Chamber of Commerce.

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