

## ENCAPSULATION OF RED GINGER OLEORESIN (*ZINGIBER OFFICINALE VAR RUBRUM*) WITH CHITOSAN AS WALL MATERIAL

JAYANUDIN<sup>1</sup>, ROCHMADI<sup>2</sup>

<sup>1</sup>Chemical Engineering Department, University of Sultan Ageng Tirtayasa. <sup>2</sup>Chemical Engineering Department, Gadjah Mada University  
Email: jayanudin@untirta.ac.id

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### ABSTRACT

**Objective:** This research aims to determine the effect of the spray drying condition against encapsulation efficiency and characterization microcapsules of red ginger oleoresin.

**Methods:** Preparation of encapsulation begun with the formation of emulsions by mixing red ginger oleoresin with chitosan solution which was dissolved with acetic acid 2% (v/v). The weight ratio of chitosan with red ginger oleoresin was 1: 1, 2: 1 and 3: 1 and then stirred using a homogenizer while added 2 ml tween 80 for 10 min. The size of emulsion droplet was measured using nanoparticle analyzer (NPA). The emulsion is formed and then inserted into the feed tank of a spray dryer. Inlet temperature of the spray dryer used in the 180 °C, 190 °C and 200 °C; and the spray dryer outlet temperature was 85 °C, feed rate at 2 L/h. The microcapsules formed were then analyzed encapsulation efficiency and characterization using scanning electron microscopy (SEM) and fourier transform infrared spectroscopy (FTIR).

**Results:** Based on the research that has been done, the smallest effective diameter of the emulsion droplets was 216.4±1.5 nm and the largest was 2109.2±46.1 nm. The value of encapsulation efficiency ranged between 83.33±0.42%-99.15±0.02%. Increasing the weight ratio of chitosan with red ginger oleoresin and increase the spray drying inlet temperature, the encapsulation efficiency is also increased. The highest encapsulation efficiency was 99.15±0.02% occurred at 200 °C of spray drying inlet temperature and the weight ratio of chitosan with red ginger oleoresin of 3:1. Morphology analysis of the surface of microcapsules using scanning electron microscope (SEM) showed that the inlet temperature of 200 °C was obtained microcapsules with smooth surfaces. The Fourier transforms infrared spectroscopy (FTIR) analysis results indicating the absence of new compounds is formed.

**Conclusion:** This research indicates that the spray drying conditions affecting the encapsulation efficiency and morphological characteristics of the red ginger oleoresin microcapsules.

**Keywords:** Chitosan, Encapsulation efficiency, Red ginger oleoresin, Spray drying, Surface oil, Total oil

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### INTRODUCTION

Red ginger (*Zingiber officinale var. rubrum*) was one of the families of *Zingiberaceae*. Red ginger can be used to flavourings in food and beverages also as a medicine. Ginger can be consumed as a paste, dried powder, candy (crystallized ginger) or as a flavoring of tea. Ginger also used as a seasoning for industries of food, beverage, and fragrance, in China for its pungent constituents and aromatic volatile constituents [1]. Red ginger (*Zingiber officinale var. rubrum*) is part of the ginger varieties that have a spicier flavour, the smaller size of the rhizome with red leather. Phenol and flavonoid compound red ginger is larger than the white ginger (*Zingiber officinale roscoe*) [2].

Ginger contains a volatile oil about 1% to 3% and also contains a non-volatile oil called oleoresin. The main components were identified from the ginger are sesquiterpene hydrocarbon and phenolic compounds which are *gingerol* and *shogaol* [3-5]. Ginger oleoresin is obtained from dried ginger by extraction using an organic solvent. Oleoresin is a viscous liquid, sticky and reddish brown [6]. The oleoresin contains the organoleptically volatile oil and pungent principles, together with fatty oil, resin, palmitic acid, carbohydrates and free fatty acids [7].

Bioactive components in ginger oleoresin can be used as an antioxidant [8-9], even antioxidant activity of ginger oleoresin was more effective than the others oleoresin and almost the same with the synthesis of antioxidants [10]. The component of *6-shogaol* in red ginger oleoresin has effectively inhibited the growth of prostate cancer cells [11]. The compounds of *6-shogaol* can be used to inhibit breast cancer cells and colon [12], and also effective in treating colon cancer [13].

Bioactive contained in ginger oleoresin such as *gingerol*, *shogaol* and *zingerone* that produce a hot sensation in the mouth. The *gingerol*, a series of chemical homologues differentiated by the length of their unbranched alkyl chains, were identified as the major active components in the fresh rhizome. *Shogaol* was formed from dehydration of *gingerol* during the heating process and storage [14].

The weakness of oleoresin are sensitive to light, heat and oxygen that can cause degradation of the bioactive component. Oleoresin degradation can be caused by changes in polymer involving fatty oil component of monoterpinic hydrocarbons (e. g., *α-pinene*, *β-pinene*, *limonene*, *sabinene*) [15]. The *shogaol* also susceptible to acidic pH and heat treatment, they probably transform to non-pungent polymers. The pungency of oleoresins decreases steadily on storage as the *gingerol* are first transformed to the *shogaol*, which are in turn degraded [7, 16]. Encapsulation was a method that can be used to protect the active components oleoresin [17].

Encapsulation is a technique of coating the active ingredient of solids, liquids and gases using a thin layer as a wall material [18-19]. Encapsulation is used to protect the active ingredients such as polyphenols, enzyme, and antioxidant. Encapsulation is also used for controlled release [20]. The core can be composed of one or several materials and coating can be single or double layered [21]. This technique has been widely used for coating the organoleptic properties such as flavor and aroma [22]. Microcapsules or microspheres size are ideally less than 200 μm [23].

The selection of wall material for encapsulation is one of the most important factors, as it relates to the encapsulation efficiency, protection and controlled release. Wall material serves to protect the core material from environmental influences such as light, heat,

air oxidation, and the effects of acid and alkaline chemicals. Chitosan is one of the wall materials that can be used for the encapsulation process because chitosan are biocompatible, biodegradable and low toxic. Chitosan in recent years has been used for the development of oral drug delivery systems [24]. Chitosan can be used for the preparation of the mucoadhesive microspheres. Therefore, it is necessary to develop an oral drug delivery system that is comfortable for the patient [25].

Generally, methods of encapsulation are distinguished after the emulsion formation processes are chemical and physical methods. Spray drying is one of the physical method most widely used and the oldest method of encapsulation process which was discovered in 1930 [26]. The basic principle of spray drying is the process of spraying the material through the atomizer and then contacted with hot air to produce a fine powder. The resulting particle sizes of the spray drying are between 10-50 µm and a large size of 2-3 mm [20, 27-28]. Although many techniques have been developed for microencapsulation, spray drying is the technology most commonly used in the food and pharmaceutical industries because it's low cost and the available equipment [22].

Effect of spray drying condition such as inlet temperature will have a significant impact, such as drying rate, product quality based on the encapsulation efficiency and morphology of microcapsules. This study aims to determine the effect of the spray drying condition and the weight ratio of wall material and ginger oleoresin as a core material to produce microcapsules with a higher encapsulation efficiency and analysis characterization of red ginger oleoresin microcapsules using scanning electron microscope (SEM) and Fourier transforms infrared spectroscopy (FTIR).

## MATERIALS AND METHODS

### Materials

Red ginger oleoresin was obtained from CV. M and H Farm, food grade chitosan was obtained from CV. Bio Chitosan Indonesia, n-hexane from Merck, acetic acid 100% from Merck and tween 80 from Sigma-Aldrich.

### Preparation of emulsion

The process of emulsion formation refers to previously reported methods [29]. Chitosan solution with a concentration of 2% (g/v) was prepared by dissolving 20 g of chitosan with 2% (v/v) acetic acid, than 1000 ml chitosan solution mixed with red ginger oleoresin with a weight of 20 g, 10 g and 6.7 g to make the ratio of chitosan: oleoresin were 1: 1, 2: 1 and 3: 1, and also added 2 ml of tween 80. The mixture was stirred using an Ika Werk Ultra Turrax homogenizer at 20,000 rpm for 10 min to form an emulsion. Droplet size of the emulsion was then analyzed using the Nano Particle Analyzer (NPA).

### Encapsulation of red ginger oleoresin

The red ginger oleoresin encapsulation process using spray drying method was a modification of previous research [30-31]. The emulsion of red ginger oleoresin entered in the feed tank of a spray dryer and then flowed into the atomizer to contact with hot air. Inlet air temperature variations used in the spray dryer was 180 °C, 190 °C and 200 °C; outlet air temperature was maintained at 85 °C, feed rate at 2 L/h. Microcapsules powder that is formed then analyzed for encapsulation efficiency and characterization using scanning electron microscope (SEM and fourier transform infrared spectroscopy (FTIR).

### Emulsion droplet size analysis

Emulsion droplet size analysis used the nanoparticle analyzer (NPA) Brookhaven 90 plus, with the following specifications: size range: 2 nm to 3 µm, diffusion coefficient range: 10<sup>-6</sup> to 10<sup>-9</sup> cm<sup>2</sup>/sec, accuracy: ±1 to 2% with monodisperse samples, repeatability: ±1 to 2% with dust free samples, temperature control (optional): 5 to 75 °C on steps of 0.1 °C, sample volume: 3 ml, results: mean and standard deviation calculated for size distribution by weight assuming a lognormal distribution.

### Scanning electron microscopy (SEM)

Powder morphology of the red ginger oleoresin microcapsules was analysed using scanning electron microscopy (SEM) JEOL-type JSM-

6510LV in coated with a thin layer of platinum, resolution: high vacuum mode: 3.0 nm (30 kV) and low vacuum mode: 4.0 nm (30 kV), accelerating voltage: 0.5 to 30 kV.

### Fourier transforms infrared spectroscopy (FTIR)

Analysis of the interaction between the components in the microcapsules was performed using fourier transform infrared spectroscopy (FTIR) using KBr pellets, in a Shimadzu IR spectrophotometer, operating between 500 and 4000 cm<sup>-1</sup>.

### Surface oil and total oil analyses

The methods used for the analysis of surface oil and total oil modification from the previous methods [32]. Surface oil analysis method was a powder with weight 3±0.1 g be entered in a conical flask containing 30 ml of hexane was then stirred for 10 min. The suspension was then filtered and the residue rinsed thrice by passing 20 ml of hexane through each time. Powder residue was then dried for 30 min and weighed. The amount of surface oil was calculated by the difference in weight of the microsphere, before and after washing.

The total oil was determined using soxhlet extraction unit. Accurately weighed microspheres (3 g) were extracted using 100 ml hexane for 4 h to ensure complete oil extraction. After extraction, the oil exhausted powder was air-dried to constant weight.

### Encapsulation efficiency

Encapsulation efficiency was calculated using equation from previous methods [30]:

$$EE = \frac{\text{Total oil-Surface oil}}{\text{Total oil}} \times 100\% \dots\dots 1)$$

Surface oil = Original weight-Final weight of microspheres . . . . . 2)

Total oil = Original weight-Weight of soxhlet extracted micro-spheres 3)

## RESULTS AND DISCUSSION

### Droplet size of emulsion

The initial preparation of the encapsulation process was the formation of an emulsion from the mixture solution of chitosan and red ginger oleoresin. In this research also used tween 80 that serves to stabilize the emulsion. Tween 80 is a non-ionic surfactant to emulsify faster with small droplet size [34]. Fig. 1 shows that the emulsion droplet size tends to increase with increasing the ratio between chitosan and red ginger oleoresin. Increases in the ratio indicate increased concentrations of the chitosan solution. The decrease of the wall material concentration can decrease the viscosity, this causes the emulsion droplet size becomes smaller [35]. The increase in viscosity would produce emulsions with larger droplet size and would be produced large microspheres [36].

Besides the influence of the wall material viscosity against of the emulsion droplet size, another factor is the effect of stirring speed. Increase stirring speed will decrease emulsion droplet size and create a more stable emulsion. If the agitation time is too long, emulsifier effectiveness will be decreased because the intense stirring will cause the emulsifier to drop out from the oil-water interface [37]. The size of emulsion droplets in various ratios of oleoresin and chitosan can be summarized in table 1.

### Surface oil

The success of the encapsulation process is determined by the surface oil from the microcapsules. Surface oil is the amount of oil on the surface of the microcapsules are not coated, this would be detrimental because it will be damaged due to environmental influences. The relatively low encapsulation efficiency (EE) values obtained indicate that high surface oil values would be detrimental to the oxidative stability of the microcapsules [38]. Surface oil was the amount of oil on the surface of the microcapsules that was not coated. The oil on the surface will be damaged due to environmental influences. Encapsulation efficiency will be low, if the amount of surface oil to be great. The high surface oil would be detrimental to the oxidative stability of the microcapsules [38].

Based on research that has been conducted, the largest surface oil was obtained from the ratio of chitosan: red ginger oleoresin is 1: 1 with the

inlet temperature of the spray dryer at 180 °C and in the ratio 2: 1 with the inlet temperature at 190 °C were 0.05±0.004 grams and 0.05±0.006 grams.

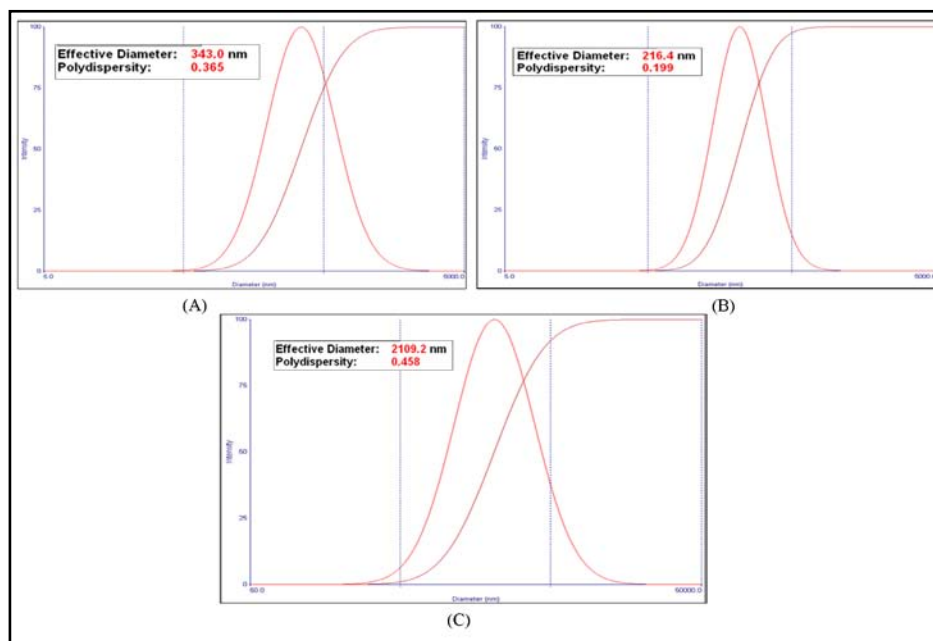


Fig. 1: The emulsion droplet size with different ratio of chitosan: red ginger oleoresin, (A) 1: 1; (B) 2: 1; (C) 3: 1

Table 1: Droplet size of emulsion

Ratio (Chitosan: Oleoresin)	Droplet size (nm) <sup>a</sup>
1:1	343±1.5
2:1	216.4±0.9
3:1	2109.2±46.1

<sup>a</sup>mean±SEM, n = 5

The lowest surface oil obtained at a ratio of chitosan with red ginger oleoresin was 3: 1 at the inlet temperature spray drying of 200 °C that was equal to 0.010 ±0.005 grams. The same result has been reported [31], shows that the declining value of the oil surface due to an increase the ratio of sodium caseinate (wall material): red ginger oleoresin. Increase in the ratio of chitosan with red ginger oleoresin would increase the volume and viscosity of chitosan for coating oleoresin. Increase in the amount of chitosan would increase the wall thickness of microcapsule and reduce the migration of oleoresin to the particle surface. Conversely, a decrease in a number of chitosan microcapsules would make the walls become thinner, so would make oleoresins migrated out during the drying process. Another reason was decreased in the amount of chitosan can cause increased amount of oleoresin was not coated, so it would be stick on the surface of the particles.

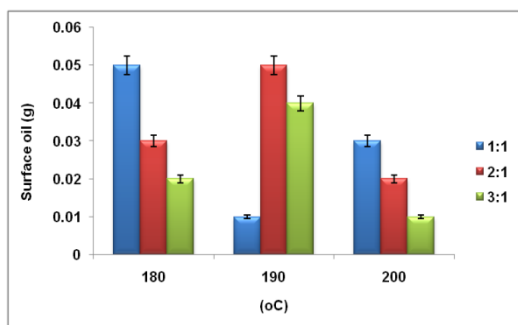


Fig. 2: The effect of spray dryer temperature on the surface oil of microcapsules, \*Error Bars represent standard deviation

The value of surface oil was also influenced by the inlet temperature of the spray dryer. Fig. 2 shows that increasing the inlet temperature of spray dryer causes decreased the value of the surface oil at any ratio chitosan with red ginger oleoresin. The temperature in the spray dryer will affect the rate of evaporation of the solvent in emulsion droplets [30], [33]. Increasing the inlet temperature of the spray dryer it would increase the rate of evaporation. This makes the wall material would be faster to harden. It would be reduce the migration rate of oleoresin to the microcapsules surface.

**Total oil**

The effect of chitosan: red ginger oleoresin ratio and the inlet temperature of the spray dryer to the total oil can be seen in fig. 3. The highest value of total oil obtained from the ratio of chitosan: red ginger oleoresin was 3: 1 with the inlet temperature of spray dryer at 200 °C in the amount of 1.18±0.02 g.

The lowest value of total oil obtained from 0.3 g derived from the ratio of chitosan: oleoresin of 1: 1 with a temperature at 180 °C.

Fig. 3 shows that in general, increasing the ratio of chitosan: oleoresin could increase total oil. Ratio of chitosan: red ginger oleoresin decline may reduce the ability of chitosan to coat oleoresin, so a lot of oleoresin was not coated and would evaporate during the drying process. Another factor that is caused by a decrease in the ratio of chitosan: oleoresin was produced microcapsule walls become thin and may cause the walls of the microcapsules become cracked when the drying temperature increases.

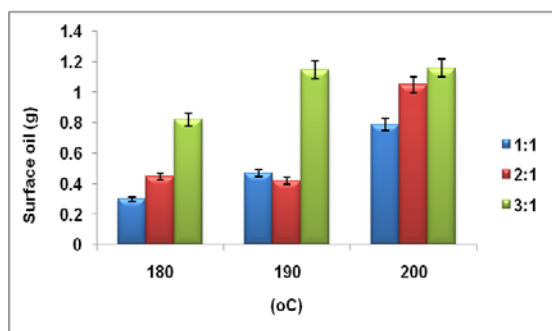


Fig. 4: Total oil microcapsules at various inlet temperature of the spray dryer, \*Error Bars represent standard deviation

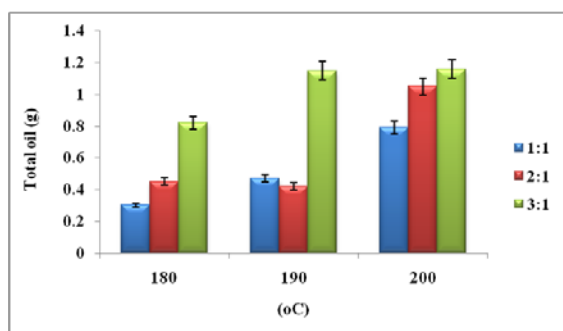


Fig. 4: Total oil microcapsules at various inlet temperature of the spray dryer, \*Error Bars represent standard deviation

Effect of the chitosan: red ginger oleoresin ratio and the inlet temperature spray drying to the total oil can be seen in fig. 3. The highest value of total oil obtained from the ratio of chitosan: red ginger oleoresin at 3: 1 with 200 °C of the inlet temperature spray

drying in the amount of  $1.18 \pm 0.02$  g. While the lowest value of total oil obtained of  $0.3 \pm 0.06$  g derived from the ratio of chitosan: oleoresin at 1: 1 and the temperature of 180 °C.

Fig. 3 shows that in general, increasing the ratio of chitosan: oleoresin could increase the total oil. Ratio of chitosan: red ginger oleoresin decline may reduce the ability of chitosan to coat oleoresin, so that a lot of oleoresin was not coated and would be evaporate during the drying process. Another influence, due to the decrease in the ratio of chitosan: Oleoresin is a microcapsule wall becomes thin and could cause the walls of the microcapsules become cracked during the drying process.

When the walls of the microcapsules already cracked, then the value of total oil become low. Reduction in the amount of coating material makes total oil becomes low [39], said that when the amount of coating material was reduced, then the total oil value will be lower. Consequently the amount of oleoresin that coated would be reduced and oleoresin which was not coated will evaporate during the spray drying process.

The rapid drying rate would also possible to make the crust more firms and no further leaching of volatile would happen to the surface [40]. The high inlet temperatures lead to the formation of the semipermeable membrane on the surface of the droplet getting faster. This caused the breakage of the product during the spray drying process [41]. Fig. 3 shows that at 200 °C, total oil produced was higher than the temperature of 180 °C and 190 °C. This means that chitosan was able to withstand at 200 °C without cracking.

#### Encapsulation efficiency

The high encapsulation efficiency of the microcapsule can be affected by different parameters, such as low solubility of the polymer in organic solvent, high solubility of organic solvent in water, a high concentration of polymer, the rate of solvent removal and rate solidification of microcapsule. The values of encapsulation efficiency in table 2 were calculated based on the total oil and surface oil using equation 1. The encapsulation efficiency values obtained from this research were ranging from  $83.33 \pm 0.42\%$  -  $99.15 \pm 0.02\%$ .

Table 2: Encapsulation efficiency

Ratio (chitosan: oleoresin)	Temperature ( °C)	Surface oil (g) <sup>a</sup>	Total oil (g) <sup>a</sup>	Encapsulation efficiency (%) <sup>a</sup>
1:1	180	$0.05 \pm 0.004$	$0.3 \pm 0.06$	$83.33 \pm 0.42$
	190	$0.01 \pm 0.012$	$0.47 \pm 0.003$	$97.87 \pm 0.21$
	200	$0.03 \pm 0.004$	$0.79 \pm 0.05$	$96.20 \pm 0.85$
2:1	180	$0.03 \pm 0.002$	$0.45 \pm 0.04$	$93.33 \pm 0.32$
	190	$0.05 \pm 0.006$	$0.42 \pm 0.01$	$88.10 \pm 0.31$
	200	$0.02 \pm 0.003$	$1.05 \pm 0.2$	$98.10 \pm 0.54$
3:1	180	$0.02 \pm 0.005$	$0.82 \pm 0.02$	$97.56 \pm 0.46$
	190	$0.04 \pm 0.003$	$1.15 \pm 0.05$	$96.52 \pm 0.35$
	200	$0.01 \pm 0.005$	$1.18 \pm 0.02$	$99.15 \pm 0.02$

<sup>a</sup>Mean±SD, n = 3

The lowest of encapsulation efficiency values obtained from the ratio of chitosan: red ginger oleoresin at 1: 1 with temperature of 180 °C, while the highest value obtained from the ratio of chitosan: oleoresin at 3: 1 and temperature of 200 °C. Increase the ratio of chitosan: red ginger oleoresin would increase the viscosity. When the emulsion droplets contact with the hot air, the diffusion rate of red ginger oleoresin would decrease to the droplet surface, it was indicated by an increase in total oil. Increasing the viscosity of the polymer solution, the diffusion of the drug will be delayed through the polymer membrane [42]. After the polymer solution is compacted, packaged drugs are not easily escaped from the polymer and thus the encapsulation efficiency remains quite high.

The same result has been reported previously [43]. The result of encapsulation efficiency of linoleic acid decreased when the weight of core was increased. The amount of gum arabic as wall materials was not enough for coating all of oil, so the value of encapsulation efficiency was decrease.

#### Scanning electron microscope (SEM) analysis

Scanning electron microscopy (SEM) analysis was used to analyze the morphology of the microcapsules. This characteristic analysis was conducted to determine the inlet temperature of a spray dryer and the ratio of chitosan: red ginger oleoresin to the condition of the microcapsules.

Fig. 4 shows that the morphology of the microcapsules was affected by changes of the inlet temperature of a spray dryer and the ratio of chitosan: red ginger oleoresin. At the inlet temperatures were 180 °C and 190 °C have produced microcapsules rough surface and wrinkled. This happens because, at low temperatures, the drying rate would decrease so that the solidification process of microcapsules would take longer [44, 45]. Wrinkles microcapsules could be caused by the diffusion of oleoresin from the microcapsules because of slow drying rate. Another cause was the low temperature makes the microcapsule wall would not withstand for a vacuum

pressure conditions during the analysis process SEM. Surface morphology of microcapsules that was produced from inlet temperature at 200 °C had a smooth surface, spherical shape and there were no wrinkles.

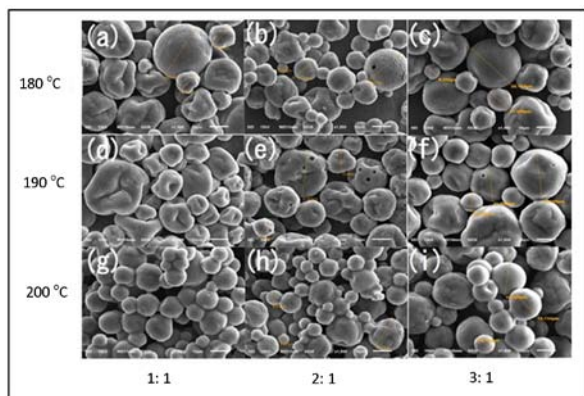


Fig. 5: Morphology of microcapsules

#### Fourier transform infrared spectroscopy (FTIR) analysis

Analysis of the interaction of the components in the microcapsules could see in fig. 6 was the result of Fourier transform infrared spectroscopy (FTIR) analysis.

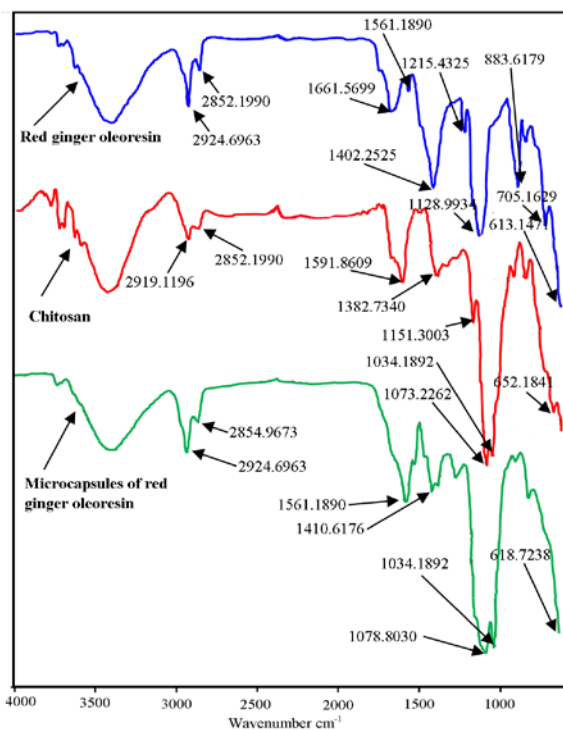


Fig. 6: Fourier transform infrared spectroscopy (FTIR) analysis

The spectrum of chitosan in fig. 6 shows, the O-H group on the wavenumber of 3433.8 cm<sup>-1</sup>. C-H emerged in wavenumber of 2919.1196 cm<sup>-1</sup>. N-H groups in wavenumber of 1591.189 cm<sup>-1</sup> which was the bending vibration absorption peaks of the amine group and the low intensity of the CH group of CH<sub>3</sub> groups in the area the wavenumber from 652.1841 to 1034.1892 cm<sup>-1</sup>. The spectrum of red ginger oleoresin showed an OH-H group at wavenumber 2924.7 cm<sup>-1</sup>. The bond of C=C at a wavenumber of 1661.57 cm<sup>-1</sup>. The bond C=C aromatic in the band 1561.19 cm<sup>-1</sup>. The bond of C-O-C at

wavenumber 1215.43 cm<sup>-1</sup>, and the O-H group at wavenumber 613.14 to 883.61 cm<sup>-1</sup>.

FTIR analysis shows that the microcapsules did not appear new peaks for their new functional groups, only a change in the peak width and slightly shifting the frequency. These results indicated the absence of any significant interaction between the microcapsules of red ginger oleoresin with chitosan and red ginger oleoresin.

#### CONCLUSION

Based on research that has been done, increasing the inlet temperature of the spray dryer then the encapsulation efficiency was increased, the highest encapsulation efficiency values obtained in spray dryer inlet temperature of 200 °C with a ratio at 3:1. The results of the microcapsules characterization using SEM showed that the surface morphology of the microcapsules was smooth and without wrinkles obtained at 200 °C and the results of FTIR analysis showed that there were no new functional groups.

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#### CONFLICT OF INTERESTS

Declare no conflict of interest was reported by the authors.

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