

## Research Article

### Encapsulation Red Ginger Oleoresin (*Zingiber officinale* var. *Rubrum*) With Chitosan-alginate as Wall Material Using Spray Drying

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**Abstract:** Encapsulation is the process of protecting the active ingredients that is susceptible to environmental influences by using a coating. Red ginger oleoresin contains bioactive components that can be used as natural antioxidants, but sensitive to environmental influences. Chitosan-alginate nanoparticle is used as the coating, because it is safe for consumption and also stable. The purpose of this research was to determine the effect of the Tripolyphosphate (TPP) concentration against the emulsion droplet size and determine the encapsulation efficiency of red ginger oleoresin. Encapsulation method was done by mixing 2% chitosan solution with 1% acetic acid, 1% sodium alginate and 8 g of red ginger oleoresin. The mixture was stirred and added sodium tripolyphosphate (3.5, 4.5 and 5.5%, respectively), emulsion preparation process was done by adding tween 80 (3, 4 and 5%, respectively) and then stirred using homogenizer with a speed of 22,000 rpm, emulsions formed were analyzed using nanoparticle analyzer. Emulsion formed was flowed on to the spray dryer inlet temperature of 180°C to form a powder encapsulation. Powder products were analyzed for determining encapsulation efficiency and morphology of red ginger oleoresin powder using Scanning Electron Microscopy (SEM). Based on the research results, the smallest droplet size of the emulsion was obtained at 481.5 nm and the largest encapsulation efficiency was as high as 70.59%.

**Keywords:** Chitosan, encapsulation, red ginger oleoresin, sodium alginate, tripolyphosphate

## INTRODUCTION

Ginger (*Zingiber officinale* var. *Rubrum*) is a plant that is used as a spice in foods and pharmaceuticals in China, India and Arabic herbal traditions since ancient times as to treat inflammation, arthritis, neurological disease, gingivitis, asthma, stroke and diabetes, or antifatulent carminative, diaphoretic, antispasmodic, expectorant (Ali *et al.*, 2008; Chrubasik *et al.*, 2005; Bellik, 2014; Kizhakkayil and Sasikumar, 2012). Characterization of ginger is apale yellow and flavorful spicy, ginger contains essential oils and oleoresins (Zarate and Yeoman, 1996; Bellik, 2014). The content of essential oil of ginger affects the aroma and produces a yield of 1-3% (Ali *et al.*, 2008; Kiran *et al.*, 2013; Eze and Agbo, 2011). El-Ghorab *et al.* (2010) said that the content of gingers essential oil about 1-4% depending on the type. In addition to essential oils, ginger also contains oleoresin about 4-7.5% (Zarate and Yeoman, 1996; Kiran *et al.*, 2013).

Red ginger (*Zingiber officinale* var. *Rubrum*) is part of the ginger varieties with spicier flavor, rhizome size smaller with red leather. The phenol and flavonoid

content of red ginger is higher than the white ginger (*Zingiber officinale* Roscoe) (Obloh *et al.*, 2012). The antiradical activity of phenol and flavonoid based on the structure relationship between different parts of their chemical structure (Evans *et al.*, 1996). Sivasothy *et al.* (2011) have analyzed the essential oil of red ginger rhizome identified as many as 54 compounds. The oil was very rich in monoterpenoids (81.9%), comprising mainly camphene (14.5%), geranyl acetate (13.7%), geraniol (14.3%), neral (7.7%), geraniol (7.3%) and 1, 8-cineole (5.0%). Red ginger also contains oleoresin which is a viscous liquid that resulting from extraction with organic solvents. Oleoresin contained bioactive components more than the essential oils because they contain volatile and non-volatile components (Onyenekwe, 2000). Volatile component is contained in the essential oils and non-volatile components in oleoresin such as gingerol (Onyenekwe, 2000; Lun *et al.*, 2008; Nwaoha *et al.*, 2013) and shogaol that formed from dehydration of gingerol during heating or storage (Ali *et al.*, 2008; Bhattarai *et al.*, 2007; Wohlmuth *et al.*, 2005). Red ginger oleoresin produce a yield of 13% resulting from

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the extraction with ethanol, red ginger oleoresin analysis using GCMS can be detected as many as 45 compounds with the main compound is shogaol (11:22%) and zingerone (14:13%) (Jayanudin *et al.*, 2013).

Bioactive component contained in the oleoresin can be used as natural antioxidant and antimicrobial (El-Ghorab *et al.*, 2010; Oboh *et al.*, 2012; Sivasothy *et al.*, 2011; Yeh *et al.*, 2014; Dugasani *et al.*, 2010; Pawar *et al.*, 2011). Antioxidant activity of red ginger oleoresin is better than the others oleoresin and has the same activity with synthetic antioxidants (Singh *et al.*, 2008). The weakness of oleoresin is sensitive to light, heat and oxygen, so the bioactive components is easily degraded. It is caused by changes in polymer involving fatty oils and hydrocarbon components of monoterpenes (Balasubramani *et al.*, 2013; Shaikh *et al.*, 2006; Vaidya *et al.*, 2006). One solution of these problems is the encapsulation process (Jayanudin *et al.*, 2013; Vaidya *et al.*, 2006). Encapsulation is a coating technique of the active solid, liquid and gas using thin layer as wall material (Dubey *et al.*, 2009; Venkatesan *et al.*, 2009; Bansode *et al.*, 2010) Encapsulation is used to protect the bioactive compounds (polyphenols, micronutrients, enzyme, antioxidants and nutraceuticals) and also to control releasing at targeted sites (Gouin, 2004). Many research of the encapsulation process using alginate as wall material had been done (Bosio *et al.*, 2014; Santa-Maria *et al.*, 2012; El-Aassara *et al.*, 2014; Sathyabama *et al.*, 2014; Cook *et al.*, 2014; Oliveira *et al.*, 2014). Chitosan also had many uses for encapsulation processes (Wittayasuporn *et al.*, 2010; Trifkovic *et al.*, 2014; Koppolu *et al.*, 2014; Hosseini *et al.*, 2013; Jarudilokkul *et al.*, 2011; Al-Qadi *et al.*, 2012; Klaypradit and Huang, 2008; Choi *et al.*, 2013).

Coating for the encapsulation process used in this research was chitosan-alginate. The combination of the chitosan-alginate as wall material will have ionic bond to each other because of the difference ionic charges. Alginate bears the negative charges while chitosan bears the positive one. Negative charge on the alginate is caused by the presence of carboxyl groups and positive charge of chitosan due to the presence of amino groups (Friedli and Schlager, 2005). The method used for the encapsulation process was spray drying. This method was more efficient to stabilize than a no capsule. The drying process is faster and the solid particles resulted will be more uniform. Encapsulation using spray drying depends also on the previous process as nano emulsion prior to spray drying (Ezhilarasi *et al.*, 2013). Another important factor is the effect of sodium tripolyphosphate concentration on the formation of cross-linked wall material of the encapsulation process. Spray drying is widely used in the encapsulation process (Nadeem *et al.*, 2013; Maciel *et al.*, 2014; Carvalho *et al.*, 2013; Torres *et al.*, 2013; Gallardo *et al.*, 2013; Borrmanna *et al.*, 2013; Anekella and Orsat, 2013; Kha *et al.*, 2014).

The purposes of this research were to determine the effect of sodium tripolyphosphate concentration to the particle size of emulsion, to determine the encapsulation efficiency of the red ginger oleoresin with chitosan-alginate as wall material and to characterize the powder of red ginger oleoresin using Scanning Electron Microscope (SEM).

## 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. MERCK™ n-Hexane p.a., MERCK™ Acetic Acid Glacial 100%, Tween 80 and Sodium tripolyphosphate was analytical grade from Merck.

**Emulsion preparation:** A solution of 2% chitosan was diluted with 1% acetic acid and mixed with 1% sodium alginate solution, then added 8 grams of red ginger oleoresin while stirring. In the Cross-link encapsulation process, a solution of tripolyphosphate with concentration (3.5, 4.5 and 5.5%, respectively) was added to the previous mixture and then stirred using homogenizer with a speed of 22,000 rpm and then tween 80 was added with concentration (3, 4 and 5%, respectively) to form emulsion. Emulsion droplets were analyzed using a nano size analyzer.

**Spray drying operation:** The emulsion was fed to a GEA Niro VERSATILE-SD™ spray dryer. The spray dryer was operated at inlet temperatures 180°C. The feed rate was adjusted to 1 L/h. The product of encapsulation was analyzed with Scanning Electron Microscopy (SEM) to get the morphology of microcapsule.

**Encapsulation efficiency:** Encapsulation Efficiency (NE) was determined according to the method described by Bae and Lee (2008), Tan *et al.* (2005) and Calvo *et al.* (2010). Ten milliliters of hexane was added to 3 g of powder and shaken by hand for 2 min, at room temperature to extract free oil. The solvent mixture was then decanted and filtered through a Whatman No. 1 filter paper and after that, non-capsulated oil (surface oil or free oil) was collected after distillation of hexane at 60°C. The non-capsulated oil or free oil (surface oil) content was then calculated as percentage by the weight difference in the powder before and after extraction and washing with hexane. To measure the encapsulated oil (Total oil), oil was extracted by 4 h Soxhlet of the same powders. After that, hexane was evaporated and internal oil was scaled. Encapsulation Efficiency (EE) was calculated using the following formula:

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

**Droplet size distribution:** The resulting droplet size from the emulsion process was analyzed using Nano Particle Analyzer (NPA) Brookhaven 90 plus, with the

following specifications: Size Range: 2 nm to 3  $\mu\text{m}$ , Diffusion Coefficient Range:  $10^{-6}$  to  $10^{-9}$   $\text{cm}^2/\text{sec}$ , Accuracy:  $\pm 1$  to 2% with monodisperse samples, Repeatability:  $\pm 1$  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 spray dryer products were analyzed 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. The SEM micrographs representing the microstructure of the powders were taken using software installed on a PC connected to the system.

## RESULTS AND DISCUSSION

**Droplet size:** Stages of encapsulation process started with the emulsion formation from a mixture of chitosan-alginate-TPP-Oleoresin of red ginger, emulsifier used is a non-ionic surfactant (tween 80). Emulsions are dispersion system of droplets of a liquid with another immiscible liquid. Emulsion droplet size can be divided into the microemulsion and nano emulsions, the difference lies in the effect of thermodynamic stability. Microemulsion is more stable than the nanoemulsion (McClements, 2011). Emulsifier used in this research is Tween 80 was a non-ionic surfactant because it produces better self-emulsifying formulations with faster emulsification time and smaller oil droplets in water (Eid *et al.*, 2012).

Figure 1 showed the effect of the Tripolyphosphate concentration (TPP) as cross-linked and tween 80 as emulsifier. The increase of the TPP concentration emulsion droplet size getting larger, at the fixed concentration of tween 80. For example Tween 80 at a concentration of 3% with TPP concentration of 3.5% obtained droplet size was 579.5 nm, the TPP concentration of 4.5% was 646.3 nm and TPP concentration of 5.5% was 768.2 nm. The same thing occur in the tween 80 concentration of 4 and 5%, generally droplet size increases with increasing concentration of TPP. The smallest droplet size obtained at tween 80 concentration of 5% and TPP concentration of 4.5% with a droplet size of 481.5 nm. Increasing droplet size of the emulsion of chitosan-alginate-TPP due to the present of cross-linked, so it is difficult to split into small droplets. Increasing the TPP concentration will cause aggregation in the mixture, so that the droplets become larger. Excess TPP may allow an increase in the formation of cross-linking that can lead to the formation of interparticle aggregation. The van der Waals forces are relatively weak attractive forces between atoms or nonpolar molecules, which

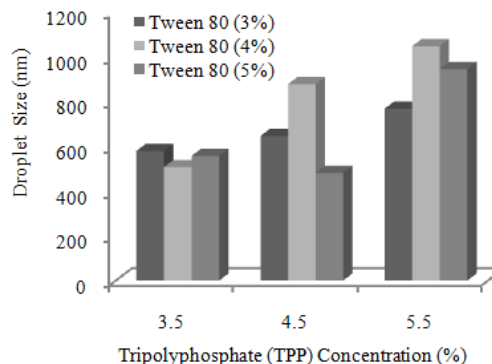


Fig. 1: The influence of TPP concentration on the droplet size of the emulsion at various concentrations of tween 80

increase as the molecular size increases (Jonassen *et al.*, 2012).

Tripolyphosphate (TPP) is a poly anion and can interact with cationic chitosan by electro static force. In addition, reversible physical cross linking by electrostatic interaction, instead of chemical cross linking, is applied to avoid possible toxicity of reagents and other undesirable effects. However, the mechanical strength of these chitosan beads is very poor, so its usage in the pharmaceutical industry is still limited (Shu and Zhu, 2000). TPP is the most extensively used ionic cross-linking agent, because of its non-toxic and multivalent properties (Shu and Zhu, 2002). Aral and Akbuga (1998) have strengthened TPP: chitosan beads by coating sodium alginate on the bead surface to form a polyelectrolyte complex film.

Aggregation is therefore suppressed when the local polymer concentration inside the nano particles is sufficiently high. On the other hand, electrostatic attraction between oppositely charged entities (such as chitosan and TPP) will promote aggregation (Jonassen *et al.*, 2012). Chitosan-TPP nanoparticles are formed by ionic gelation of Chitosan, a mechanism that is driven by the cross-linking of the Chitosan's  $-\text{NH}_3^+$  groups with the  $\text{P}_3\text{O}_5^-$  and  $\text{HP}_3\text{O}_4^-$  ionic species of TPP. The intra- and inter molecular linkages created between the negatively charged groups of TPP. In the presence of alginate, it is expected that the ionic gelation process occurs concomitantly with the complex of the polyelectrolyte with Alginate's  $-\text{COO}^-$  groups (Goycoolea *et al.*, 2009; Schatz *et al.*, 2004).

Illustration of emulsion droplet formation of red ginger oleoresin encapsulation process with chitosan-alginate coating in cross-linked with TPP using tween 80 as emulsifier modified from research of Schutz *et al.* (2011) could be seen in Fig. 2.

Emulsions are mixtures of two immiscible liquids, where one liquid dispersed as a droplet to another liquid due to the presence of a third substances as a stabilizer. Emulsifier concentration will affect the measurement of droplets Fig. 1 shows that in general the greater the concentration of Tween 80 droplet-droplet size becomes larger, it is related to the Critical Micelle Concentration (CMC), which shows the limits of the

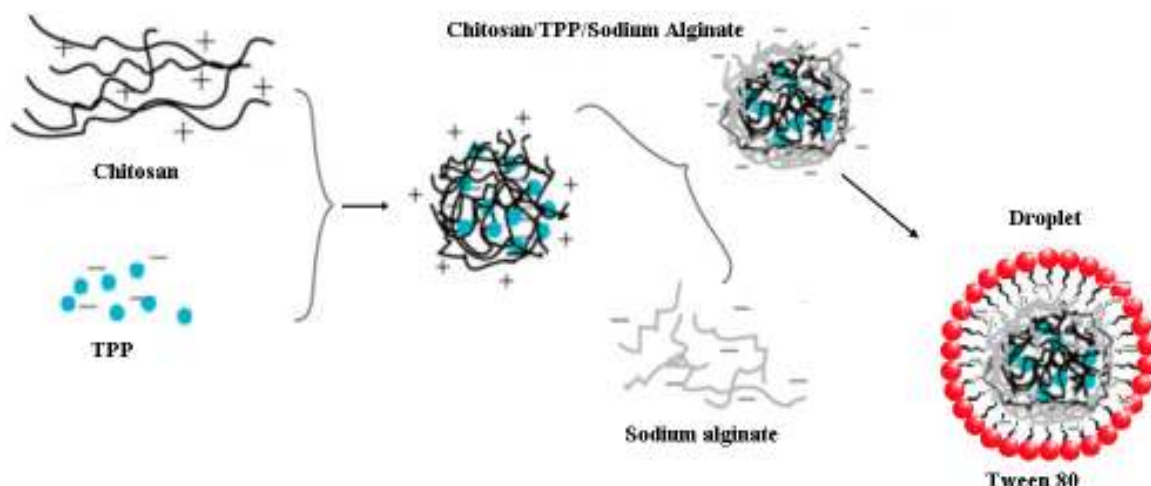


Fig. 2: Illustration of the formation of an emulsion droplet of red ginger oleoresin encapsulation with chitosan-alginate as wall material and cross-linked with TPP using emulsifier of tween 80

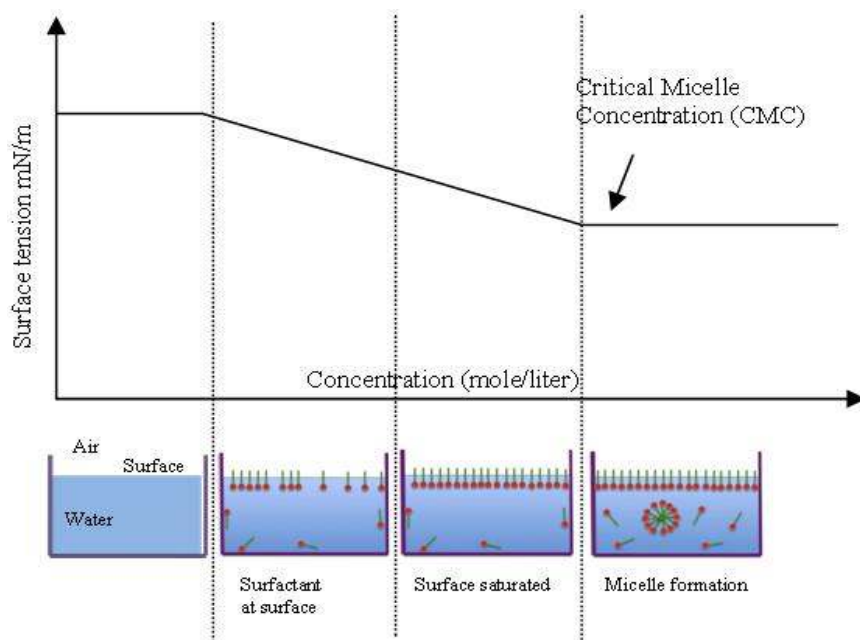


Fig. 3: Graph of critical micelle concentration

critical concentration of emulsifier in the solution, the greater concentration will be occur formation of micelle or aggregate (Picone and Cunha, 2013). As shown in Fig. 3.

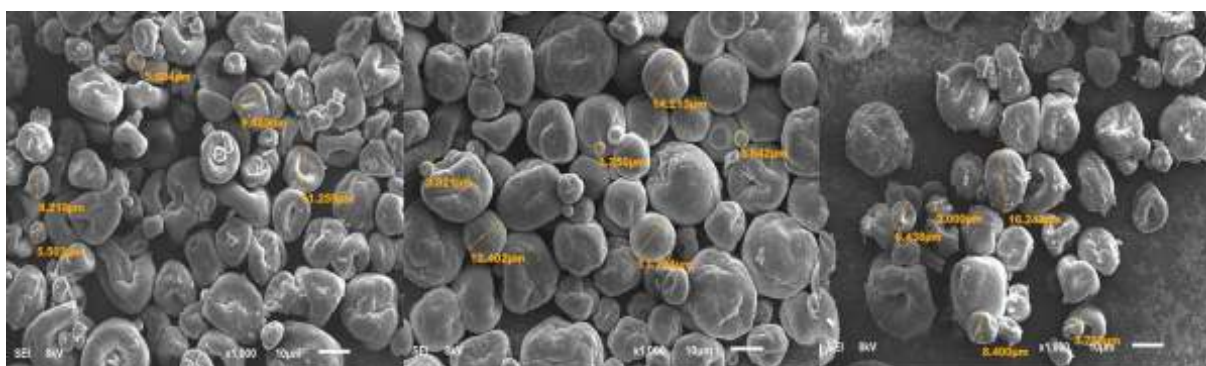
**Encapsulation efficiency:** Encapsulation efficiency is determined by the number on the surface of red ginger oleoresin oil and total oil. Surface oil is the amount of red ginger oleoresin attached to the surface of the microcapsules and the total amount of oil is red ginger oleoresin contained in microcapsule, both contained in or attached to the surface of the microcapsules. Encapsulated efficiency can be seen in Table 1.

Table 1 showed changes in the concentration of TPP effect on the encapsulation efficiency. The greater

concentration of TPP leads to smaller encapsulation efficiency. The use of 5% tween 80 shows different trend, the encapsulation efficiency increases when concentration of TPP gets higher. The more tween 80 in the mixture leads to greater possibility of oleoresin aggregation and makes the oleoresin not coated, so that more oleoresin microcapsules attached to the surface and make the value of surface oil increase, as shown in Table 1. The Encapsulation Efficiency obtained in this experiment, approximately between 59.37-70.59%, is relatively low. It can be caused by the use of chitosan coating in cross-linked with TPP and alginate, so makes it more stable microcapsules. The increase concentration of TPP makes the total oil decreased and increasing surface oil makes encapsulation efficiency

Table 1: Encapsulation efficiency of red ginger oleoresin

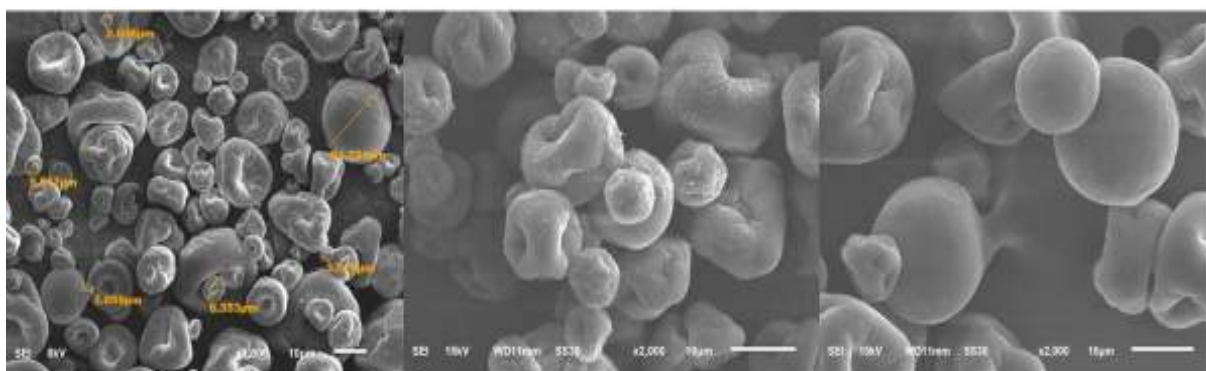
Tween 80 (%)	TPP (%)	Total oil (%)	Surface oil (%)	Encapsulation efficiency (%)
3	3.5	10.30	3.30	67.74
	4.5	10.00	3.33	66.67
	5.5	10.00	4.00	60.00
4	3.5	11.33	3.33	70.59
	4.5	9.67	3.33	65.52
	5.5	10.00	4.00	60.00
5	3.5	10.67	4.33	59.37
	4.5	9.33	3.33	64.29
	5.5	6.67	2.67	60.00



(A)

(B)

(C)



(D)

(E)

(F)



(G)

(H)

(I)

Fig. 4: SEM images of red ginger oleoresin with chitosan-alginate as wall; (A): Tween 80 (3%) TPP (3.5%); (B): Tween 80 (3%), TPP (4.5%); (C): Tween 80 (3%), TPP (5.5%); (D): Tween 80 (4%) TPP (3.5%); (E): Tween 80 (4%), TPP (4.5%); (F): Tween 80 (4%), TPP (5.5%); (G): Tween 80 (5%) TPP (3.5%); (H): Tween 80 (5%), TPP (4.5%); (I): Tween 80 (5%), TPP (5.5%)

value getting smaller. Increasing concentration of TPP will form aggregation, so that the red ginger oleoresin encapsulated decreased and resulted in non-encapsulated oleoresin attached to the surface of the microcapsules. Low Encapsulation Efficiency makes red ginger oleoresin more stick on the surface of microcapsule, so it will be easily oxidized due to direct contact with oxygen from the air (Aghbashlo *et al.*, 2012).

The process of emulsification is an important role in determining the encapsulation efficiency especially for food flavors and oils (Liu *et al.*, 2000, 2001; Saberi *et al.*, 2014). Emulsion droplet size determines the encapsulation efficiency values at various core materials in the drying process (Soottitantawat *et al.*, 2003, 2005). These reports clearly show that reducing emulsion size can result in encapsulated powders with higher retention of volatiles and lower content of non-encapsulated oil at the surface of powder particles. The presence of oil on the surface of the powder particles is the most undesirable property of encapsulated powders (Jafari *et al.*, 2008).

**Powder morphology:** Microcapsules prepared by spray drying of red ginger oleoresin using chitosan-alginate as wall materials were observed for size and shape using the SEM (Fig. 4).

Figure 4 shows the morphology of ginger oleoresin microcapsules, in general, has a spherical shape with a smooth surface, except microcapsules A from Tween 80 (3%) TPP (3.5%), microcapsules produced almost all non-spherical shape. This can be caused by the imperfect process of cross-links between the chitosan-TPP in alginate coated, so that when it is passed to the spray dryer for the drying process, the wall material becomes brittle and microcapsule core here has no red ginger oleoresin. These phenomena can be seen from the analysis of red ginger oleoresin microcapsule morphology on the A. Red ginger oleoresin microcapsule size ranging from 2.0 to 14.21  $\mu\text{m}$ .

## CONCLUSION

Red ginger oleoresin encapsulation process with chitosan-alginate coating in cross-link with the TPP has been successfully done. Changes in the concentration of TPP as across-link and tween 80 as emulsifier effect on emulsion droplet size, the smallest droplet size was obtained at a concentration of 4.5% TPP concentration and 4% tween 80 generated droplet size of 481.5 nm. The biggest of Encapsulation efficiency of red ginger oleoresin was 70.59% and the lowest was 59.7%, this value is still low because the coating of chitosan-alginate and cross-link with the TPP is stable on the mark with the amount of surface oil from the microcapsule obtained relatively small. Morphology of red ginger oleoresin microcapsules generally spherical shape with smooth surface, it indicates that the encapsulation process has been successfully done.

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