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# Antioxidant Activity and Total Phenolic of Encapsulated Stingless Bee Propolis by Spray Drying Method

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## ABSTRACT

The development of propolis in food products is still very limited due to its strong taste and aroma. The encapsulation of propolis using the spray drying technique can be an alternative to avoid undesirable sensory characteristics. The aim of this study is to obtain encapsulated *Trigona itama* stingless bee propolis powder through the spray drying method. Propolis was extracted with a water solvent by the ultrasound method, then dried through the spray drying technique using a mixture of maltodextrin and Arabic gum as a coating agent. The propolis encapsulation consisted of three formulas with the ratio of propolis and coating agents as follows: F1 (1:1), F2 (1:2), and F3 (1:3). The morphology profile was analyzed by the Scanning Electron Microscope (SEM) images, while analysis of total flavonoids and total phenols were conducted by using the AlCl3 and Follin-Ciocalteu methods. Antioxidant activity was analyzed using the DPPH method. The results demonstrated that all formulas were well encapsulated, which was indicated by a uniform spherical shape in SEM images analysis. F3 had the highest yield (65.22%) and the lowest moisture content (3.89%), while F1 had the highest solubility (98.96%) compared to other formulas. The F1 also had the highest antioxidant activity (1692.131 mg/l), total flavonoid (0.80 mg/g QE), total phenol (3.81 mg/g GAE), and encapsulation efficiency (81.69%). Analysis of variance showed that the type of formula significantly affected all physical and chemical characteristics (p=0.000), except moisture content (p=0.165) and solubility (p=0.127). Therefore, F1 was the best formula for obtaining encapsulated propolis due to its high antioxidant activity, total flavonoid and phenolic content.

**Keywords**: encapsulation, propolis, spray drying, stingless bee, *Trigona itama*

## INTRODUCTION

Propolis or bee glue is a complex resinous substance produced by bees and used by humans for its medicinal function (Bachesvki *et al.* 2020). Propolis is also known as a natural product that has potential as a drug in humans and animals. Since ancient times, Egyptians have used propolis as a preservative balm. Moreover, Greeks and Romans physicians used propolis as an antiseptic and cicatrizant agent (Sforcin & Bankova 2011). Propolis has various benefits, namely as an antitumor, anti-inflammatory, antioxidant, antibacterial, antiviral, antiemesis, antiparasitic, immunomodulatory, and antifungal (Sforcin & Bankova 2011; Fikri *et al.* 2018). These benefits are obtained because of the presence of the chemical compounds contained in propolis, such as flavonoids, phenyl and esters, terpenes, lignans, stilbene, sugars, hydrocarbons, and minerals (Huang *et al.* 2014). Halim *et al.* (2012) also found that Indonesian and Brazilian propolis contain phenolic compounds, α-amyrin, cylolanost, and pyrimidines. These chemical compounds have the potential to turn propolis into a functional food. However, the use of propolis as a food ingredient is still very limited due to its strong taste and aroma (Nori *et al.* 2011).

Propolis encapsulation through the spray drying technique can be used as an alternative to avoid unwanted taste and aroma. This technique is able to protect the active compounds in propolis, increase solubility, avoid unwanted flavours, as well as being easy and inexpensive in its application (Poshadri & Kunia 2010; Busch

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*et al.* 2017). The coating agents used in the spray drying technique are very important; maltodextrin and arabic gums are commonly used as coating agents. Maltodextrin has high solubility and good retention of bioactive compounds (Busch *et al.* 2017).

Propolis also has the benefit as a natural antioxidant activity agent (Mat Nafi *et al.* 2019; Abdullah *et al.* 2019). According to Mat Nafi *et al.* (2019), propolis from *Trigona itama* has the potential to act as an antioxidant agent compared to other types of bees. Propolis extract from this bee could produce 30 µg/ml $IC_{50}$ and 85.69 percent inhibition. This antioxidant activity in propolis is closely related to its polyphenol content such as flavonoids and phenols (Da Silva *et al.* 2013). Another research from Da Silva *et al.* (2013) showed that encapsulated propolis using the spray drying method produced 1,500 µg/ml to 5,000 µg/ml. Therefore, the aim of this study was to obtain an encapsulated *Trigona itama* stingless bee propolis powder through the spray drying method. Maltodextrin and Arabic gum were used as coating agents. The antioxidant capacity and physicochemical characteristics of encapsulated propolis were also determined.

## METHODS

### Design, location, and time

The design of this study was experimental design. The research was carried out at the Nutrition Analysis Laboratory, Department of Community Nutrition, Faculty of Human Ecology, Advanced Research Laboratory IPB University, Bogor Postharvest Center, and SEAFAST Center for Research and Community Service Program at IPB University. The study was conducted from April 2019 to January 2020.

### Materials and tools

The material used in this study was raw propolis from *Trigona itama* stingless bee. The propolis was collected by bee-keepers in Bintan Island, Riau Archipelago Province, Indonesia. Distilled water was used in the propolis extraction procedure. Maltodextrin with Dextrose Equivalent 10–12 (Qinhuangdao LihuaStrach Co., LTD, China) and arabic gum (Sigma-Aldrich) were used as the coating agent for propolis encapsulation. The materials used for the total phenol and flavonoid assays were Folin-Ciocalteu 7.5%, distilled water, NaOH 1%, $C_7H_6O_5$, metanolp.a, larutan HMT, HCl 25%, $CH_3COOH$ 5%, $AlCl_3$ 2%, and acetone. The antioxidant activity assay used DPPH, ethanol p.a, ascorbic acid, and distilled water.

### Procedures

### Propolis extraction

Propolis was extracted with distilled water using an ultrasonic bath (OVAN ATM 10l, Badalona, Barcelona). This extraction method referred to Fikri *et al.* (2018) with a slight modification. Raw propolis was crushed into small pieces and homogenized. The propolis was dissolved in distilled water (1:10) in an Erlenmeyer flask. It was sonicated using an ultrasonic bath for 3 h, then filtered with Whatman No. 41. Afterwards, the filtrate was evaporated using a vacuum evaporator at 60°C for 1 h.

### Propolis encapsulation

Propolis encapsulation was carried out by using the spray drying method as referred by Busch *et al.* (2017) with a slight modification. Modifications were made in the form of the amount of propolis, the amount of coating agent, and a shorter stirring time with greater speed. Propolis encapsulation consisted of three formulas namely F1, F2, and F3. Each formula was distinguished by the amount of coating material, where the ratio of the propolis extract and coating agent were as follows: F1 (1:1), F2 (1:2), and F3 (1:3). The encapsulation was prepared by mixing the coating agent of maltodextrin (F1: 10; F2: 20; F3: 30) and Arabic gum (F1: 0.1; F2: 0.2; F3: 0.3) in 100 ml of bi-distilled water. It was stirred at 10,000 rpm for 15 min and 15,000 for 2 min by a homogenizer (Wiggen Houser D500; Germany). Then, 10 ml of propolis extract was added and stirred again at 15,000 rpm for 5 min. Subsequently, it was filtered twice with Whatman No. 41 to get rid of any remaining solids. The operational conditions of the mini spray dryer (Büchi B190, Flawil, Switzerland) were as follows: nozzle diameter of 0.7 mm; flow rate of 15 ml/min; inlet temperature of 125°C–135°C; and outlet temperature of 78°C–82°C. Propolis encapsulation was performed with three replications.

### Yield

The yield of propolis extract and encapsulated propolis was determined by

weighing the dry extract or encapsulated propolis and then calculated with the following formula:

$$Extract\ yield(\%) = \frac{Weight\ of\ propolis\ extract(g)}{Weight\ of\ raw\ propolis(g)} \times 100$$

$$Encapsulated\ propolis\ yield(\%) = \frac{Weight\ of\ encapsulated\ propolis(g)}{Weight\ of\ propolis\ extract\ and\ coating\ agent(g)} \times 100$$

**Moisture content**

The moisture content of the sample was tested using the gravimetric method (AOAC 2005). An aluminium dish was heated in an oven at 130°C for 15 min. A cup was then cooled in a desiccator and weighed. A sample of 2 g was weighed with aluminium plates. The sample was dried in an oven at 130°C for 3 h, then cooled in a desiccator. The sample was weighed and process repeated until a stable weight was obtained. Three replications were applied for this analysis.

**Solubility**

The solubility of propolis referred to AOAC 2005. First, encapsulated propolis was weighed to 0.75 g, then dissolved in 100 ml of distilled water. The solution was filtered using a vacuum pump and Whatman filter paper No. 42. The filter paper was first dried in an oven at 105°C for 30 min then weighed. After the filtering process, the filter paper and residue were dried again in an oven at 105°C for about 3h. The filter paper was cooled in a desiccator for 15 min, then weighed until a stable weight was obtained. The analysis was carried out in three replications.

**Total flavonoid assay**

Total flavonoids were referred from the National Agency for Drug and Food Control (NADFC 2004) using the $AlCl_3$ method with three replications. Samples were hydrolyzed with HMT, HCl, and aceton for 30 min. The mixtures were filtered, then diluted with acetone to produce ethyl acetate fraction. $AlCl_3$ was added to the fraction and incubated at room temperature for 30 min. After incubation, absorbance was measured at 425 nm with a spectrophotometer. Quercetin was used as the standard solution.

**Total phenolic assay**

Total phenolic was determined by using the Follin-Ciocalteu method (Marinova *et al.* 2005). Ten milligrams of propolis was placed into a 25 ml volumetric flask and re-dissolved in methanol. One ml of mixture was mixed with 5 ml of Folin-Ciocalteu 7.5%, then homogenized and incubated at room temperature and kept in the dark for 8 min. After incubation, 4 ml of NaOH 1% was added to the mixture, homogenized, then incubated again for 1 h. Absorbance was then measured at 730 nm with a spectrophotometer. Gallic acid was used as the standard solution. Three replications were performed for total the phenolic assay.

**Antioxidant activity assay**

The antioxidant activity assay utilized the DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich, USA) spectrophotometric method which referred to Salazar-Aranda *et al.* (2011) with three replications. Propolis was re-dissolved in ethanol (1 mg $ml^{-1}$) at different concentrations (100–3,000 mg $l^{-1}$). Then, samples were added with 125 µM DPPH in the microplate, homogenized and kept at dark room temperature for 30 min. The absorbance was measured at 517 nm in the microplate reader (Biotech Instruments, Winooski, USA). The positive control in this analysis was ascorbic acid.

**Encapsulation efficiency**

Encapsulation efficiency (% encapsulation) was calculated following Busch *et al.* (2017) based on the total amount of phenolin encapsulated propolis per total amount of phenol in propolis extract. Encapsulation efficiency can be calculated by the following formula:

$$\%Encapsulation = \frac{X}{Y} \times 100\%$$

X: The total amount of phenol in encapsulated propolis (%).
Y: The total amount of phenol in propolis extract (%).

**Morphological profile with scanning electron microscope (SEM)**

The surface of the encapsulated propolis was observed by SEM (ZEISS EVO® MA 10). Samples were prepared in the form of dry powder, then sprinkled as thin as possible on the specimen holder that had been coated with carbon tabs. The sample was then coated with gold using a sputter coater for 60 sec. Next, the sample was installed in the stage for SEM analysis. The stage was then put into a chamber and the image taken with the

SEM tool. Samples were seen at a magnification of 5,000 times with 16 kV voltage. The analysis results were obtained in the form of three-dimensional propolis particle images. A round and relatively uniform shape indicates that the sample had been well encapsulated.

**Data analysis**

All of the data were expressed as the mean±SEM. All data were analyzed using one-way analysis of variance (ANOVA) followed by the Duncan's test using SPSS 25 for mac. Probability values less than 0.05 ($p<0.05$) were set as the significance limit.

## RESULTS AND DISCUSSION

**Propolis yield**

Yield is a comparison of the amount of extract or encapsulated propolis from the extraction and encapsulation process of propolis. A higher yield indicates a larger amount of encapsulated propolis. *Trigona itama* propolis extract produced a yield of 13.73±0.33 percent. F3 had the highest yield value of 65.22%, as shown in Table 1, Meanwhile, F2 and F1 had a yield of 36.82% and 32.27%, respectively. Based on the analysis of variance, the formulation of encapsulated propolis had a significant effect on the yield value. The yield of F3 was almost similar to the study of Pratami *et al.* (2019) i.e. 65.63%.

**Moisture content**

The encapsulated propolis moisture content was important to know as a parameter of sample stability during storage. High moisture content can shorten the shelf life of samples. Conversely, the lower the moisture content, the longer the shelf life. Table 1 shows that F3 had the lowest moisture content at 3.89%. Meanwhile, the moisture content of F1 and F2 were 5.52% and 4.39%, respectively. The analysis of variance showed that the formulation of encapsulated propolis did not have a significant effect on moisture content ($p=0.165$). Other research conducted by Pratami *et al.* (2019) related to encapsulated propolis through the spray drying technique resulted in moisture content ranging from 2.04% to 6.69%. Busch *et al.* (2017) argued that encapsulated propolis with maltodextrin and Arabic gum coatings had a moisture content of 2.21%. There is still no moisture content standard to be followed for encapsulated propolis.

**Solubility**

In this study, all formulas were well dissolved with the water solvent. The higher the solubility value, the better the ability to release active compounds. Results in Table 1 showed that there was high solubility for all formulas, ranging between 98.12–98.96%. This shows that the three formulas could be well dissolved in water. Furthermore, all the active ingredients present in the encapsulated propolis were well released. The analysis of variance showed that the encapsulated propolis formulation did not have a significant effect on its solubility level ($p=0.127$). As a comparison, other research conducted by Pratami *et al.* (2019) showed that the solubility of encapsulated propolis ranged from 74.01–88.96%.

**Antioxidant activity**

Antioxidant activity is indicated by the value of $IC_{50}$, where the greater the value, the

Table 1. Physical characteristics of encapsulated propolis

| Treatment (ratio of propolis:coating agent) | Yield (%) | Moisture content (%) | Solubility (%) |
|---|---|---|---|
| F1 (1:1) | 32.27±0.27[a] | 5.52±0.62[a] | 98.96±0.08[a] |
| F2 (1:2) | 36.82±0.19[b] | 4.39±0.29[a] | 98.19±0.24[a] |
| F3 (1:3) | 65.22±0.25c | 3.89±0.61[a] | 98.12±0.39[a] |

*All of the data were expressed as means±SEM from n=3; Mean values with different letters (a–c) within a row were significantly different at a level of $p<0.05$ by ANOVA followed by Duncan's multiple range test

Table 2. Chemical characteristics of extracted and encapsulated propolis

| Sample | $IC_{50}$ (mg/l) | Total flavonoids (mg/g QE) | Total phenolics (mg/g GAE) | Encapsulation efficiency |
|---|---|---|---|---|
| PE | 965.889±8.136[a] | 1.60±0.0004[d] | 4.67±0.001[d] | - |
| F1 (1:1) | 1692.131±24.035[b] | 0.80±0.0002[c] | 3.81±0.005[c] | 81.693[c] |
| F2 (1:2) | 2933.121±21.430[c] | 0.22±0.0003[b] | 2.35±0.002[b] | 50.249[b] |
| F3 (1:3) | >4000 | 0.18±0.000[1a] | 1.74±0.001[a] | 37.375[a] |

*All of the data were expressed as means±SEM from n=3; Mean values with different letters (a–d) within a row were significantly different at a level of $p<0.05$ by ANOVA followed by Duncan's multiple range test; PE: Propolis Extract

weaker the antioxidant activity. Conversely, a small $IC_{50}$ value indicates strong antioxidant activity. The antioxidant activity of the propolis extract in Table 2 was 965.889 mg/l. The results of the analysis of variance showed that there was a significant effect of the type of sample on the antioxidant activity of propolis (p=0.000). This was due to the different amounts of propolis extract in each formula. In propolis encapsulation, the amount of propolis extract in F1 was the same as the amount of coating agent, while F2 and F3 had less amount of propolis extract. Thus, F1 had a higher level of antioxidant activity.

The antioxidant activity in this study was almost the same as the study conducted by Fikri *et al.* (2019), where propolis extracted with water solvents had $IC_{50}$ values ranging from 503.93–1027.29 mg/l. In addition, Abdullah *et al.* (2019) conducted an antioxidant analysis of *Trigona itama* propolis in Brunei Darussalam which was extracted with different amounts of ethanol solvents with $IC_{50}$ values in the study ranging from 76.5–1905 mg/l.

In this study, three encapsulated propolis formulas were made from propolis extract coated with maltodextrin and arabic gum in different amounts. Table 2 illustrates the data on the $IC_{50}$ values of encapsulated propolis F1, F2, and F3. F1 had the highest $IC_{50}$ values compared to other formulas, measured at 1692.131 mg/l. While the $IC_{50}$ of the F2 and F3 formulas were 2933.121 mg/l and>4,000 mg/l. This value indicates that the antioxidant capacity of F2 and F3 were relatively weak. The analysis of variance showed that the type of sample significantly affected antioxidant activity (p=0.000). Research conducted by Da Silva *et al.* (2013) relating to encapsulated propolis by spray drying showed antioxidant values ranging from 1,500 mg/l to 5,000 mg/l. In the study, propolis was coated with starch and Arabic gum, where propolis extract coated with starch (1:4) had the highest antioxidant activity.

Antioxidant activity in propolis is beneficial to human health. Research from Zhao *et al.* (2016) showed that Brazilian green propolis given to 32 patients with Type 2 Diabetes Mellitus was effective in improving the antioxidant function. This was indicated by an increase in serum glutathione, polyphenols, and anti-inflammatory cytokines. Mujica *et al.* (2017) evaluated the effect of propolis solution by oral administration on the oxidative status and lipid profile in 35 Chilean people. The propolis was given twice a day for 90 days at 15 drops each time. Results showed that the propolis could increase HDL-c by 22%, increase glutathione levels by 175%, and decrease Thiobarbituric Acid Reactive Substances (TBARS) amounts by 67%. It was concluded that propolis had positive effects on oxidative status and improved lipid profile, which may potentially reduce the risk of cardiovascular disease.

**Total flavonoids and phenolics**

The total flavonoids and total phenols of the *Trigona itama* propolis extract from Riau

Archipelago Province were 1.60 mg/g QE and 4.67 mg/g GAE, respectively. Fikri *et al.* (2019) found that *Trigona itama* propolis from South Kalimantan Province extracted with water solvent had a flavonoid level of 1.42 mg/g QE. After the extraction process, the propolis was encapsulated and made into three formulas (F1, F2, and F3) with different amounts of coating agents. Table 2 shows a significant difference in total flavonoids and total phenols (p=0.000). F1 had higher total flavonoids and phenols compared to the other formulas. This was because the propolis extract and coating agent had the same ratio, while F2 and F3 had more coating agent than propolis extract in the encapsulation process. Another study by Busch *et al.* (2017) related to encapsulated propolis resulted in a total phenol level of 0.26%.

**Encapsulation efficiency**

Encapsulation efficiency is a comparison between the amount of active compound contained in encapsulated propolis with the propolis extract. This shows the percentage of the amount of active compound (total phenol) that was successfully protected by the coating agent during the drying process. The percentage of encapsulation efficiency is important to know because it shows the success level of the encapsulation process. A high percentage value indicates that the propolis is well coated. The results in Table 2 showed that F1 had the highest efficiency of 81.69%. While the efficiency of F2 and F3 were only 50.25% and 37.38%, respectively.

The percentage of encapsulation efficiency is related to the proportion of coating agent amount and core material. The F1 had a balanced proportion of material that was 1:1. This allows more core material to be encapsulated with the coating agents. A similar study was conducted by Pratami *et al.* (2019), where propolis with maltodextrin and Arabic gum as coating agents in different amounts resulted in an encapsulation efficiency of 33.81%–81.48%.

**Morphological profile**

Morphology of encapsulated propolis was analyzed using a Scanning Electron Microscope (SEM). A morphological profile was performed because it can influence the characteristics of encapsulated propolis, such as the retention and rate of active compound release (Ali *et al.* 2014). The SEM results of all formulas can be seen in Figure 1. F1 had diameters of 1.6–8.6 µm, F2 1.9–8.5 µm, and F3 1.2–5.3 µm. These three formulas tend to be uniformly spherical in shape; some surfaces were joined together and non-porous with a dented surface. A dented surface is caused by the quick evaporation of water during the spray drying process (Ali *et al.* 2014). Evaporation of water occurs due to the use of high temperatures during the drying process (125$^{o}$C–135$^{o}$C). The morphological profile of all formulas indicated that the core material was well encapsulated. This was also due to the absence of a continuous gap on the surface wall of encapsulated propolis. Almost the same morphology was shown by Pratami *et al.* (2019), where encapsulated propolis from Tetragonula bees with a coating material of maltodextrin and Arabic gum was shown to have a spherical shape. Another study by Busch *et al.* (2017) also showed a similar morphology.

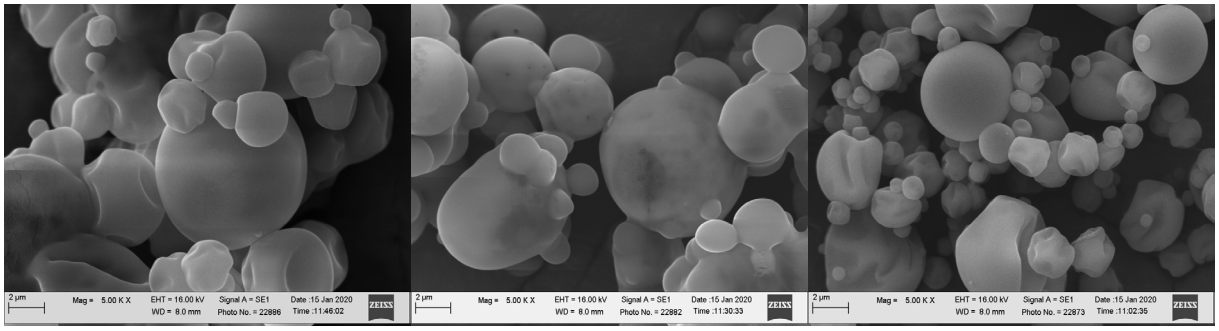


Figure 1. Morphology of encapsulated propolis with a magnification of 5000x

## CONCLUSION

The formula with a 1:1 ratio of propolis extract and coating agents (F1) was the best formula to obtain encapsulated propolis. Analysis of variance of the three encapsulated propolis formulas showed that the formula did not have a significant effect on moisture content and solubility, except for yield value. Meanwhile, there was a relationship between the encapsulated propolis formula and the chemical characteristics of the extract and the encapsulated propolis. The F1 formula had higher solubility, higher antioxidant activity, and higher total flavonoid and phenol compared to the other formulas. This was influenced by the balanced proportion between the amount of propolis extract and the coating agent, thus allowing more propolis extract to be encapsulated.

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## AUTHOR DISCLOSURES

The authors have no conflict of interest.

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