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RESEARCH ARTICLE

Comparison Between B-Cyclodextrin and Maltodextrin as Encapsulant Agents of Vacuum Dried Nutmeg Seed Oleoresin

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ABSTRACT

Nutmeg seed oleoresin is widely applied in the food and beverage industry because of its unique flavor and aroma. However, oleoresin has less stable properties to heat, oxygen, and light. Therefore, an encapsulation process is needed to protect oleoresin. The extraction of nutmeg seed oleoresin is carried out by ultrasonication method (Ultrasound Assisted Extraction) because of its lower energy consumption, shorter operating time, and can be carried out at low temperatures and pressures, making it suitable for the extraction of compounds that are sensitive to high temperatures. Nutmeg seed oleoresin encapsulation was carried out by the vacuum drying method using two types of encapsulants (β-cyclodextrins and maltodextrin) at two treatment levels (7 grams and 10 grams) with two levels of stirring time (5 minutes and 15 minutes). This study aims to study the comparison between β-cyclodextrin and maltodextrin as encapsulant agents of vacuum dried nutmeg seed oleoresin. The moisture content and antioxidant activity of β-cyclodextrin microencapsulates was higher than maltodextrin. While, trapped oil was higher on maltodextrin encapsulation than β-cyclodextrin. The antioxidant activity of microencapsulates was higher in β-cyclodextrin encapsulant than maltodextrin. The microencapsulate with the best trapped oil value was obtained in the treatment of 7 grams maltodextrin 5 minutes stirring time which produced 15.7% trapped oil. This treatment resulted in antioxidant activity of 47.94%.

KEYWORDS: *Nutmeg seed, Microencapsulation, Maltodextrin, β-cyclodextrin, Vacuum drying*

Introduction

Nutmeg is a spice plant native from Moluccas, Indonesia. Indonesia is known as the center of nutmeg plant species diversity and the biggest producer of nutmeg mace and nutmeg oil. Of the six types of nutmeg in Maluku, *Myristica fragrans* is the one that has the greatest economic value and is known to excel in the world market because of its distinctive aroma and high in oil yield. Nutmeg processing into dried simplicia (spices or herbs that presented in the dry from) and powder for industrial raw materials already produced. Another alternative that can be done is to process nutmeg into the oleoresin.

Oleoresin is pale yellow thick liquid which has distinctive aroma. Oleoresin also has the similar taste and aroma as the original material which consists of a mixture of essential oils that contribute to volatile aroma and resins and other non-volatile compounds. Oleoresin is obtained by extraction from nutmeg seeds or mace with organic solvents such as ethanol (Santoso, 2020) and hexane (Ananingsih, 2020).

Nutmeg oleoresin can be applied in the food and beverage industry as flavor enhancer and aromatic agent, in the drug and pharmaceutical industry as ingredients, and in the

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soap and cosmetic industries (Baihaqi et al., 2018). The use of oleoresin is considered superior compared to the whole form of nutmeg since oleoresin has a standardized flavor and aroma, is more hygienic, is sterile from bacteria and fungi, has small volume, is free from enzymes, and contains natural antioxidants (Nurdjannah, 2007). Antioxidant properties come from eugenol, isoeugenol, and phenolic compounds in nutmeg oil (Gupta and Rajpurohit, 2011).

However, oleoresin is easily damaged when exposed to heat, oxygen, and sunlight, so a method to protect oleoresin is needed. One such method is encapsulation. Encapsulation is a coating method for sensitive and easily degraded materials. The core materials are coated with coating material or encapsulant. Two types of widely used encapsulant are maltodextrin and β-cyclodextrin. Maltodextrin is widely used as a wall material because it has good stability in oil and water emulsions, good solubility in water, posseses the ability to inhibit oxidation reactions, and ease of handling during the process (Ezhilarasi et al., 2013). β-cyclodextrin is used because its ability to form a coating cavity which result in good encapsulation ability (Hadian et al., 2018). β-cyclodextrin cavity also has hydrophilic and hydrophobic properties on both sides which posseses the ability to encapsulate lipophilic core compounds in water emulsions (Crini et al., 2018). β-cyclodecxtrin forms an inclusion complex in encapsulation process. It contains seven D-glucose units that connected by α-1,4 linkages (Chew et al., 2018).

Vacuum drying is used because it can produce better product quality since the taste and nutritional content in the core material are not damaged by high temperatures during the drying process (Rezvankhah et al., 2019). Microencapsulated nutmeg seed butter was successfully processed by vacuum drying at 50°C for 48 hours (Santoso et.al., 2020). Vacuum drying removes water by lowering the partial pressure of water vapor from the air in the drying chamber. Vacuum drying reduced the exposure of oxygen and it is used to retain vitamins in the fruit during dehydration (Demarchi et al., 2018). Therefore, it is necessary to conduct a research to determine the nutmeg oleoresin encapsulation with maltodextrin and β-cyclodextrin as encapsulants with encapsulant amount and stirring time variations using vacuum drying.

Materials and Method

Materials

Materials used in this experiment were nutmeg seed (from Selayar Island), ethanol solvent 96%, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution, methanol 99.98%, maltodextrin (DE 15-20), β-cyclodextrin, and whey protein isolate.

Extraction of Nutmeg Oleoresin

A total of 1 kg of dried nutmeg seeds were ground with herbs grinder (GEA spice herb grinder IC-10B) and sieved through 80 mesh siever (MBT type AG 515). The large (residual part) of nutmeg seeds were mashed with blender and sifted again. A total of 20 g of nutmeg powder was dissolved in 200 mL of 96% ethanol. Extraction was carried out with an Ultrasonic Cleaner UC-10SD at 50 °C for 37.5 min with frequency of 45 kHz, ultrasonic power 100 W. The extract was filtered using Whatman filter paper no. 1. Ethanol in the filtrate was separated by rotary vacuum evaporator (40°C, speed 52 rpm, and pressure 0.09MPa) to obtain oleoresin.

Moisture Content Analysis

Moisture content analysis was carried out with moisture analyzer. The moisture analyzer was turned on and an empty aluminum pan was placed inside it. A total of 1 gram of encapsulant was placed on the aluminum pan of moisture analyzer.

Analysis of Antioxidant Activity using Free Radical DPPH (2,2-diphenyl-1-pycrylhidrazyl)

For oven drying treatment, as much as 0.025 grams of encapsulate was dissolved in 10 mL of 99.98% methanol and allowed to stand for 2 hours. For vacuum drying treatment, 0.5 grams of encapsulate was added to 5 mL of methanol and allowed to stand for 1 hour. A total of 0.1 mL of the solution was added with 3.9 mL of DPPH solution and homogenized with vortex for 30 seconds and then incubated in a dark room at 25 ˚C for 30 minutes. The absorbance of the sample was measured with a spectrophotometer at a wavelength of 517 nm. Blanks were made from 0.1 mL of methanol to which 3.9 mL of DPPH solution was added in the same method. The absorbance of the blank was recorded as the control absorbance. Antioxidant activity was calculated as % inhibition according to the formula as mentioned in Eq. (1) (Ginting et al., 2018 with modifications).

$$Antioxidant\ Activity\ (\%inhibition) = \frac{control\ absorbance - sampel\ absorbance}{control\ absorbance} \text{ x } 100\% \quad (1)$$

Trapped Oil Analysis

Firstly, surface oil was measured. One gram of encapsulate was put into a centrifuge tube and 5 mL of ethanol was added and centrifuged at 1700 rpm for 15 minutes. The sample was filtered with filter paper and washed with 15 ml of ethanol. The filtrate was transferred to a porcelain cup that had been dried in oven for 24 hours and the initial weight had been known. The sample in the cup was dried in the oven for 24 hours and was then put in a desiccator for 15 minutes and weighed as the final weight. The amount of surface oil was calculated by a subtraction of final weight and initial weight of cup as mentioned in Eq. (2). The percentage of surface oil was calculated using the equation as shown in Eq. (3).

$$Surface\ oil\ =\ final\ weight\ of\ cup - initial\ weight\ of\ cup \quad (2)$$

$$\%\ Surface\ oil = \frac{surface\ oil\ weight\ (g)}{sample\ weight(g)}\ x\ 100\% \quad (3)$$

Then, total oil analysis was conducted. One gram of encapsulate was put into an erlenmeyer and added with 20 mL of ethanol and then put into a water bath of Ultrasonic Cleaner UC-10SD at 50 ˚C for 45 minutes at a frequency of 45 kHz. The sample was filtered with filter paper and the filtrate was transferred to a porcelain cup that had been oven-baked for 24 hours which its initial weight was known. The sample in the cup was then dried in the oven for 24 hours and placed on a desiccator for 15 minutes. The cup containing the sample was weighed and recorded as the final weight. The amount of total oil was calculated by a subtraction of final weight and initial weight of cup as mentioned in Eq. (4). The percentage of total oil was calculated using the equation as shown in Eq. (5). Trapped oil was calculated by a subtraction of total oil and surface oil as mentioned in Eq. (6). The percentage of trapped oil was calculated using the equation as shown in Eq. (7) (Azari, 2020; Jayanudin et al., 2017).

$$Total\ oil\ =\ final\ weight\ of\ cup - \ initial\ weight\ of\ cup \quad (4)$$

$$\%\ Total\ oil = \frac{total\ oil\ weight\ (g)}{sample\ weight\ (g)} \text{ x } 100\% \quad (5)$$

$$Trapped\ Oil\ =\ Total\ oil - Surface\ oil \quad (6)$$

$$\% \, Trapped \, oil = \frac{trapped \, oil \, weight \, (g)}{sample \, weight \, (g)} \text{x } 100\% \quad (7)$$

Encapsulant Morphological Analysis using Scanning Electron Microscopy (SEM)

Morphological analysis of nutmeg oleoresin microcapsules was carried out using Scanning Electron Microscopy (SEM). SEM was coated with a thin layer of platinum with a high vacuum mode of 3.0 nm (30 kV) and a low vacuum mode of 4.0 nm (30 kV), and voltage acceleration of 0.5 to 30 kV. SEM was turned on and the microencapsulate was inserted into the specimen chamber. The image display would appear on the monitor screen in the most appropriate magnification (Jayanudin et al., 2017 with modification).

Data Analysis

Data analysis was performed using parametric statistics with SPSS (Statistical Product and Service Solutions) program version 13.0. There were three independent variables consist of encapsulants, amount of encapsulant, and stirring time. The significance difference between treatments was carried out by independent t-test.

Results and Discussion

Moisture Content

Moisture content is essential in determining the quality and stability of food products during handling, processing, and storage (Vikas et al., 2018). Based on Indonesian National Standard (SNI 01-3709-1995), the maximum moisture content allowed for spice powder is 12%. In this study, the nutmeg oleoresin microencapsulates met the moisture content requirements based on SNI.

Table 1. Moisture content (%) of microencapsulated nutmeg seed oleoresin.

Treatments		Encapsulant agents	
Encapsulant Amount (g)	Stirring Time (minutes)	β-Cyclodextrin (%)	Maltodextrin (%)
7	5	8.27±0.83[a,1]	5.40±0.56[a,2]
10	5	9.31±1.29[a,1]	5.94±0.08[a,2]
7	15	8.04±0.64[a,1]	5.60±0.84[a,2]
10	15	8.32±0.14[a,1]	6.10±0.42[a,2]

According to the independent sample t-test, there was a significant difference between treatments of maltodextrin and β-cyclodextrin encapsulant on the moisture content of microencapsulates (Table 1). Maltodextrin encapsulant results in lower water content. The moisture content of maltodextrin microencapsulation was also lower (5.4% to 6.1%) than β-cyclodextrin (8.04% to 9.31%).

According to the independent sample t-test, there was a significant difference between treatments of encapsulant types (maltodextrin and β-cyclodextrin) on the moisture content of vacuum-dried microencapsulates (see Table 1). The maltodextrin encapsulation resulted in lower water content. In this research, maltodextrin encapsulants showed lower moisture content (between 5.4% to 6.1%) than β-cyclodextrin (between 8.04% to 9.31%). The higher moisture content of β-cyclodextrin microencapsulates is caused by the cyclodextrin structure. Cyclodextrin can form a hydrophobic cavity on the inner side and hydrophilic on the outer side (Crini et al., 2018). The hydrophobic part of a β-cyclodextrin cavity will be occupied by less polar guest molecules so that the water inside the cavity becomes more

challenging to evaporate (Saffarionpour, 2019; Niu et al., 2018). Moreover, β-cyclodextrin also absorbs water leading to higher moisture content (Yu et al., 2021). It revealed that the utilization of β-cyclodextrin in polyacrylonitrile (PAN) nanofibrous membranes in textile showed a higher water vapor transport rate compared to PAN membrane alone due to the improved membrane's pore size and because of the hydrophilicity properties of β-cyclodextrin (Yu et al., 2021).

In contrast, maltodextrin has a lower hygroscopicity (Azari, 2020). Therefore, the moisture content of maltodextrin as microencapsulate agent could be maintained during the drying process and storage, which are susceptible to air exposure. Furthermore, maltodextrin also has a higher water evaporation rate because the water inside maltodextrin is easier to evaporate (Santoso et al., 2020). This property contributes to the lower moisture content of maltodextrin microencapsulates.

The results show that encapsulant amount and stirring time variations did not significantly differ in moisture content. This result is supported by Muchtadi et al. (2015) research, which mentioned that the moisture content of palm oil microencapsulates did not show any significant difference between the homogenization treatments. Homogenization and stirring treatment aimed to perform reduction and uniformity of oil droplet size to be encapsulated. However, the moisture content of microencapsulates was more affected by the encapsulants treatment than stirring time variation (Muchtadi et al., 2015). Another factor that affects the moisture content of microencapsulates is the drying process and the storage conditions (Alvarez-Henao et al., 2018).

Trapped Oil

Trapped oil denote the amount of oil covered in the encapsulates. Trapped oil is an important factor of microencapsulation efficiency. The higher the amount of trapped oil, the better the microencapsulation efficiency. Trapped oil is obtained from the subtraction of total oil and surface oil. Total oil shows the total amount of oil in the microencapsulates whilst surface oil represents the amount of oil on the surface of microencapsulates. Surface oil affects the stability of microencapsulates during storage. Oil on the surface is not covered by encapsulant thus it is more susceptible to oxidation which cause the oil to be more easily damaged (Pourashouri et al., 2014 in Jayanudin et al., 2017). Thus, the higher the percentage of surface oil, the lower the encapsulation efficiency.

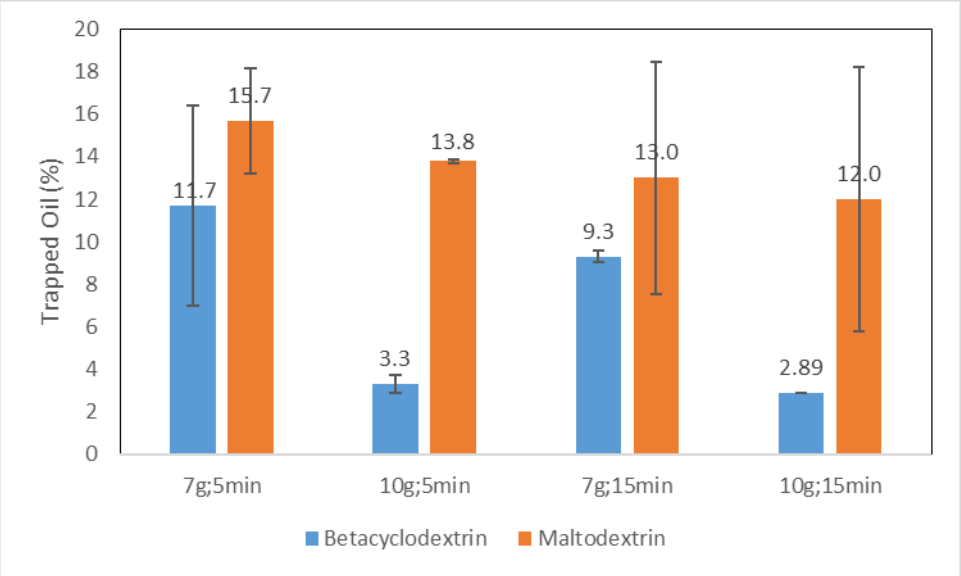

Figure 1. Trapped oil (%) of microencapsulated nutmeg seed oleoresin.

Based on Figure 1, the results of trapped oil microencapsulate with maltodextrin encapsulant type were higher than β-Cyclodextrin. Trapped oil microencapsulate with maltodextrin encapsulant type was in the range of 12.00% to 15.70% while microencapsulate β-Cyclodextrin produces trapped oil which was in the range of 3.30% to 11.70%.

The microencapsulates with maltodextrin tended to have a higher trapped oil than β-cyclodextrin (Figure 1). This happened because the large viscosity of emulsions with maltodextrin encapsulant causes the formation of thick microencapsulate walls. The thick walls of microencapsulates can prevent the migration or transfer of oleoresins to the outside of the microencapsulated walls, resulting in lower surface oil values. The lower the surface oil value causes the greater the value of trapped oil or the amount of oleoresin oil trapped in the microencapsulates.

It can be seen in Figure 1 that microencapsulates both with maltodextrin and β-cyclodextrin showed smaller trapped oil results at a stirring time of 15 minutes than 5 minutes. It happened because the longer stirring time can cause heat due to mechanical forces during stirring. The heat that arises can lower the viscosity of the emulsion so that a thin microencapsulate wall is produced. Oleoresins more easily penetrate thin microencapsulate walls. Therefore, the value of surface oil can be greater and decrease the value of trapped oil.

A treatment of with 10 g encapsulant tended to have lower trapped oil than 7 g encapsulant. This could be possible due to the use of 4 g WPI as an emulsifier with a larger amount of coating material (10 g) was insufficient to form a fairly stable emulsion. Therefore, the oleoresin could not be coated completely and thus the value of trapped oil became low. Research conducted by Assagaf (2013) reported that too little WPC amount will cause an imperfect wall layer formation because WPC is not able to coat oleoresin droplets on the lipophilic side. The stability of the emulsion before the drying process is strongly related to the film formation process during the drying process. Stable emulsion before drying process can increase the value of trapped oil in the resulting microencapsulated.

Antioxidant Activity

Antioxidants are the chemical substances which donate one or more electrons to stabilize or prevent the formation of free radicals (Ginting et al., 2018). Analysis of antioxidant activity in nutmeg oleoresin microencapsulates is very important since nutmeg possesses some natural antioxidant compounds. Eugenol and isoeugenol are antioxidant compounds found in nutmeg seed essential oil (Warsito, 2021). Eugenol and β-caryophillene have hydrogen atoms in benzylic and allylic functional groups that can neutralize peroxyl radicals (Warsito, 2021).

Phenolic compounds also contribute to antioxidant activity of nutmeg (Malik et al., 2021). Antioxidant activity is reported to have a positive correlation with the amount of tannin, total flavonoid and total phenolic compounds of nutmeg flesh extract (Antasionasti et al., 2021). Lignans and essential oils such as eugenol in aromatic plant are components that contribute to phenolic compounds (Chiorcea-Paquim et al., 2020). Myrisfragransin is nutmeg lignan which consists of meso-dihydroguaiaretic acid, erythro-austrobailignan-6, and argenteane as the main components (Gupta and Rajpurohit, 2011). Lignans as secondary plant metabolites are phenolic compounds that act as antioxidants due to the structure of two phenylpropane units in their skeleton and various molecular backbone structures depending on each plant families (Chiorcea-Paquim et al., 2020). Lignan also mentioned as one of the antioxidant potential in nutmeg seeds (Verma et al., 2021). The antioxidant potential of lignans especially comes from the two cinnamic acids structure (Tan et al., 2013). Caffeic acid and catechins are other two phenolic components in nutmeg fruit extract of seed, fleshy pericarp (husk) and mace (Pandey et al., 2015). Caffeic acid as

a phenolic constituent was found as much as 68.17 mg/g in nutmeg seed extract (Odubanjo et al., 2017). The catechol structure of caffeic acid compounds donates electrons to free radicals of reactive oxygen species or lipid peroxyl groups which resulting in nutmeg antioxidant activity (Verma et al., 2021).

Table 2. Antioxidant activity (%) of microencapsulated nutmeg oleoresin.

Treatments		Encapsulant Agents	
Encapsulant Amount (g)	Stirring Time (minute)	β- Cyclodextrin (%)	Maltodextrin (%)
7	5	88.33 ±0.14	47.94 ±5.54
10	5	88.42 ±0.04	37.48 ±0.38
7	15	88.01 ±0.96	42.22 ±4.75
10	15	87.06 ±1.82	35.47 ±0.06

There was a significant difference between the treatment of encapsulant types (maltodextrin and β-cyclodextrin) on the antioxidant activity of microencapsulates. The value of antioxidant activity of β-cyclodextrin was higher (87.06%-88.42%) than maltodextrin (35.47%-47.94%). This happens because β-cyclodextrin forms cavities in microencapsulates (Crini et al., 2018). β-cyclodextrin protects oleoresins by forming covalently stable complex bonds (Yonata, 2020). A better property of thermal stability of β-cyclodextrin also supports the protection of antioxidant compounds, where the encapsulation process involves drying at 50˚C (Vikas et al., 2018). Anggrahini et al. (2007) in Sembiring et al. (2020) explained that the quality of the chemical content in nutmeg will decrease when the drying temperature exceeds 45˚C. The lower antioxidant activity of maltodextrin encapsulation may occur due to the thickness of microencapsulates wall which could affect the oil extraction from the microencapsulates. Assagaf (2013) stated that WPI (Whey Protein Isolates) forms wall that coats the oleoresin droplet on the lipophilic side, while the hydrophilic side will bind to maltodextrin.

Microencapsulates Morphological Analysis Using SEM

Using SEM (Scanning Electron Microscope), the morphology of nutmeg oleoresin microencapsulate was observed in the size between 10 µm to 50 µm, thus it can be classified as microencapsulates since the particle size was less than 100 µm (Huang et al., 2020).

In vacuum-dried with maltodextrin (Figure 2A), cracks were observed on the surface of the microencapsulates. Cracks on the microencapsulates wall may be formed due to the low foam stability during drying. Low foam stability can not maintain its structure during drying and results in broken morphological structure as also reported in freeze-drying foam mats encapsulation of blueberry juice (Darniadi et al., 2020). These cracks may be formed as the result of size reduction of dried encapsulates at the late stage of encapsulation. During the process of encapsulation, the dried encapsulates were mashed with blender to reduce and uniform the size of encapsulates. The blender may cause cracks on some microencapsulates walls. The presence of cracks may increase the oxygen permeability of the encapsulates wall which can oxidize and degrade the core material of microencapsulates during storage (Dadi et al., 2020).

In β-cyclodextrin encapsulation (Figure 2B), smoother surface without shrinkage of microencapsulates wall was observed. This may happen due to the rigid structure of β-cyclodextrin as the coating material. β-cyclodextrin consists of seven hydrogen bonds which can produce complete hydrogen bond on the secondary side or the outer side and results in forming the rigid structure (Crini et al., 2018; Duchene and Bochot, 2016). The smooth surface supports better protection againts oxidation of oleoresin as the core

material since smooth surface has smaller surface area than irregular and non-uniform shape surface (Dadi et al., 2020).

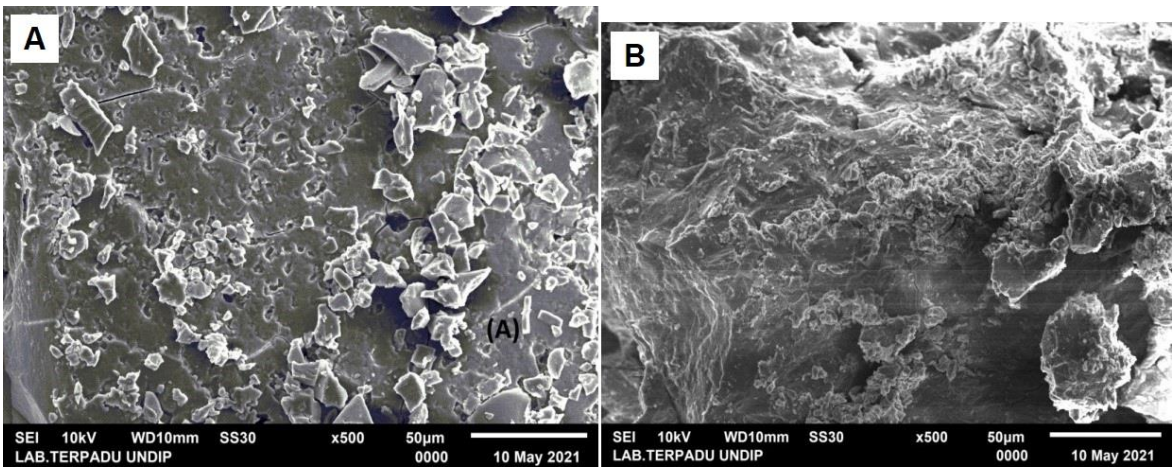

Figure 2. SEM micrograph of microencapsulated nutmeg oleoresin in 500 times magnification: Vacuum-dried microencapsulate with maltodextrin (A) and β-cyclodextrin (B). Samples of 7 grams maltodextrin with 5 minutes stirring time and 7 grams of β-cyclodextrin with 15 minutes stirring time were used for this morphological analysis.

Cracks were observed on the walls of maltodextrin encapsulant in vacuum-dried microencapsulates (Figure 2A). Shrinkage occurred in vacuum-dried microencapsulates using maltodextrin and β-cyclodextrin (Figure 2A and 2B). In β-cyclodextrin encapsulant with vacuum drying, smoother wall surface were observed (Figure 2B).

Conclusion

Encapsulant type showed significant differences in moisture content, trapped oil and antioxidant activity of microencapsulates. The moisture content of maltodextrin microencapsulates was lower (5.94%-6.10%) than β-cyclodextrin (8.04%-9.31%). Trapped oil was higher on maltodextrin encapsulation (12.0%-15.7%) than β-cyclodextrin (3.3%-11.7%). The longer stirring time reduced trapped oil. The antioxidant activity of microencapsulates was higher in β-cyclodextrin encapsulant (86.28%-88.42%) than maltodextrin (35.47%-47.94%). The microencapsulate with the best trapped oil results were obtained in the treatment of 7 grams maltodextrin with 5 minutes stirring time which produced 15.7% trapped oil and 47.94% antioxidant activity.

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Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contributions

Bong Y. A. Santoso and Bernardine A. A. Konstantia conducted the experiment and calculations. Victoria Kristina Ananingsih, Bernadeta Soedarini, and Sumardi, developed the research method and conducted the statistical analysis, wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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