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ENCAPSULATION OF A MIXTURE OF NONI (*Morinda citrifolia* L.) FRUIT EXTRACT AND PATCHOULI (*Pogostemon cablin*) OIL WITH MALTODEXTRIN AND ITS ANTIOXIDANT ACTIVITY

Enkapsulasi Campuran Ekstrak Buah Noni (*Morinda Citrifolia* L.) dan Minyak Nilam (*Pogostemon cablin*) Berasaskan Maltodextrin dan Aktiviti Antioksidannya

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Abstract

Excessive free radical compounds in the human body can cause various dangerous diseases. This can be prevented by consuming antioxidant-rich foods, one of which is noni fruit with its pungent odor and taste that many people dislike. To overcome this issue, noni fruit can be encapsulated. In this study, noni fruit extract was encapsulated with maltodextrin with the addition of patchouli oil. The extraction of noni fruit was done by the maceration method. Research maltodextrin in this study was produced from partial hydrolysis of cassava starch using 0.5% α-amylase enzyme, achieving a Dextrose Equivalent (DE) value below 20. However, as it is insoluble in water, this maltodextrin cannot be used as an encapsulating agent. Therefore, the encapsulation stage was carried out using the freeze-drying method on with commercial maltodextrin as a coating agent, tween 80 as an emulsifier, and noni fruit extract and patchouli oil as the core ingredients in a ratio of 4:1 and 1:1. The highest encapsulation efficiency of 91.35% was achieved in the encapsulation of noni fruit extract without the addition of patchouli oil. Nevertheless, the organoleptic test results showed that patchouli oil positively reduced the unpleasant odor of noni fruit extract. Furthermore, the SEM test results indicated irregular particle shapes and fractures without any pores, while the results of the antioxidant activity test using the DPPH method revealed that the addition of patchouli oil in a ratio of 1:1 made a positive contribution to increasing the antioxidant activity of encapsulated noni extract, with an IC_{50} value of 50.80 µg/mL.

Keywords: antioxidant, encapsulation, maltodextrin, noni, patchouli oil

Abstrak

Sebatian radikal bebas yang berlebihan dalam tubuh manusia boleh menyebabkan berbagai penyakit berbahaya. Ini boleh dicegah dengan pengambilan makanan yang kaya dengan antioksidan, salah satunya ialah buah mengkudu dengan bau dan rasa yang menyengat yang tidak disukai orang ramai. Untuk mengatasi masalah ini, buah mengkudu boleh dikapsulkan. Dalam kajian ini, ekstrak buah mengkudu dikapsulkan dengan maltodekstrin dengan penambahan minyak nilam. Pengekstrakan buah mengkudu dijalankan dengan kaedah maserasi. Maltodekstrin penyelidikan dalam kajian ini dihasilkan daripada hidrolisis separa kanji ubi kayu menggunakan enzim α-amilase 0.5%, mencapai nilai *Dextrose Equivalent* (DE) di bawah 20. Namun, kerana ia tidak larut dalam air, maltodekstrin ini tidak boleh digunakan sebagai agen pengekapsul. Oleh kerana itu, enkapsulasi dijalankan

menggunakan kaedah pengeringan beku dengan maltodekstrin komersial sebagai agen salutan, tween 80 sebagai pengemulsi, dan ekstrak buah mengkudu, dan minyak nilam sebagai bahan teras dalam nisbah 4:1 dan 1:1. Kecekapan enkapsulasi tertinggi iaitu 91.35% dicapai dalam pengkapsulan ekstrak buah mengkudu tanpa penambahan minyak nilam. Namun begitu, keputusan ujian organoleptik menunjukkan bahawa minyak nilam secara positif mengurangkan bau yang tidak menyenangkan daripada ekstrak buah mengkudu. Tambahan pula, keputusan ujian SEM menunjukkan bentuk zarah yang tidak teratur dan patah tanpa sebarang liang, manakala keputusan ujian aktiviti antioksidan menggunakan kaedah DPPH mendedahkan bahawa penambahan minyak nilam dalam nisbah 1:1 memberi sumbangan positif kepada peningkatan aktiviti antioksidan kapsul ekstrak mengkudu, dengan nilai IC_{50} 50.80 µg/mL.

Kata Kunci: antioksidan, enkapsulasi, maltodekstrin, mengkudu, minyak nilam

Introduction

Free radicals are reactive and unstable molecules with one or more unpaired electrons in their outermost orbitals that play a role in oxidative tissue damage and pathological processes in the human body by attacking lipid compounds, proteins, and DNA, thus causing various dangerous diseases such as cancer, cardiovascular diseases, and diabetes mellitus. Therefore, antioxidants are essential for the human body as they can inhibit oxidation through reactions with free radicals to form non-reactive and stable free radicals. One of the antioxidant-rich fruits in Indonesia is noni fruit (*Morinda citrifolia* L.), which is known to contain more than 160 phytoconstituents. One of the antioxidants contained in noni fruit is ascorbic acid, reaching 24-158 mg per 100 grams of noni fruit [1]. This content is higher than that of several other fruits such as tangerine (3.716 mg/100 grams), persimmon (2.878 mg/100 grams), and tamarillo (11.328 mg/100 grams) [2].

However, the caproic acid content in noni fruit produces an unpleasant odor and bitter taste, making people reluctant to consume it. This can be circumvented by encapsulation, a method of coating compounds with certain wall materials to reduce damage to the compounds and improve their characteristics and organoleptic properties [3]. The encapsulating agent used in this study is maltodextrin, which has low viscosity, high solubility, and high binding capacity in forming a microcapsule matrix [3]. Maltodextrin can be produced through enzymatic processes or acid hydrolysis of starch. In Indonesia, one of the extremely abundant sources of starch is cassava. Due to its high starch content, namely around 85%, cassava has the potential to be developed for use in various applications. Further utilization of cassava starch in the industry is expected to increase the economic value of this root vegetable [4]. This study attempts to produce maltodextrin from cassava starch for use in the encapsulation of noni fruit extract.

In this study, encapsulation was performed using the freeze-drying method with the addition of patchouli oil to suppress the unpleasant odor of noni fruit extract and increase the antioxidant activity of the encapsulation results. The use of the freeze-drying method is considered the most suitable for encapsulating compounds that are volatile and sensitive to heat or temperature, such as compounds found in essential oils. When compared with other methods, freeze drying has the advantage of not using high temperatures like other methods so that it can protect sensitive bioactive compounds, the implementation steps are limited compared to other methods and the product can be reshaped quickly and easily. Meanwhile, other methods such as spray drying, extrusion and pan-coating have the disadvantage of removing a lot of material because they use high temperatures during the process and produce a lot of residue [22] This study focuses on changes in the organoleptic properties of noni fruit extract in three different encapsulation variations, especially the decrease in the unpleasant odor and bitter taste of noni fruit extract and the increase in antioxidant activity of the encapsulation results with *freeze drying* method for encapsulation.

Materials and Methods

Materials

Noni (*Morinda cirrifolia* L.) fruit powder, which is the main ingredient in this study, was purchased at Pasar Gede Surakarta, Central Java, Indonesia and macerated

with 96% ethanol solvent (Merck). Commercial patchouli oil was obtained from Happy Green Patchouli Oil e-commerce store. The encapsulation process used Tween 80 and commercial Maltodextrin with DE value of 10-12 purchased from Indoplant e-commerce store. Meanwhile, the alternative maltodextrin in this study was produced from partial hydrolysis of cassava starch and distilled water (Aquadest) with 500 grams of α-amylase enzyme from Micro Bio e-commerce store, D(+)-Glucose anhydrous (Merck), Fehling A or $CuSO_4$ (Merck), Fehling B or KOH and $NaKC_4H_4O_6.4H_2O$ (Merck), n-Hexane (Merck), 200 ppm anhydrous $CaCl_2$ (Merck), 0.1 N Hydrochloric acid or HCl (Merck), 0.1 N Sodium hydroxide or NaOH (Merck), and universal pH paper. Furthermore, the antioxidant testing employed the DPPH method with methanol p.a. (Merck), 2,2-diphenyl-1-picrylhydrazyl or DPPH (Merck), and dimethylsulfoxide ($(CH_3)_2SO$) (Merck).

Instrumentations

This study utilized the following instruments: Scanning Electron Microscope (Jeol JSM-60 SEM), UV-Vis Spectrophotometer (Hitachi UH5300), Particle Size Analyzer (Malvern Zetasizer Nano ZS), GC-MS Spectrophotometer (Shimadzu QP2010S), and Freeze Dryer (Telstar LyoQuest).

Making maltodextrin from cassava starch

Maltodextrin was made based on the literature with several changes to the parameters such as the concentration of the enzyme used. First step is mixing 30% of fine-textured cassava starch in a 200 ppm $CaCl_2$ solution. After that, 0.1 N NaOH was added to the solution to reach pH 7. In the previous method, 0.1% enzyme was used. Meanwhile, in this study, α-amylase enzymes were used at 0.5% of the solution volume. The mixture was heated at 85°C for 120 minutes with a stirring process and cooled rapidly until the temperature dropped to 30 °C. The pH was readjusted to 3-4 by adding 0.1 N HCl. After leaving it at room temperature for 15-30 minutes, 0.1 N NaOH was added to the mixture until the pH became 4.5-6. The maltodextrin obtained was dried in an oven at 50–60 °C for 24 hours [5]. Afterwards, it was crushed and sieved with a 100-mesh sieve to obtain fine maltodextrin. Lastly, an analysis of the Dextrose Equivalent (DE) value and particle size was carried out using Particle Size Analyzer (PSA).

Determination of the physiochemical properties of maltodextrin

The DE value of the maltodextrin was determined by Lane-Eynon Titration based on the SNI 01-2892-1992 standard as a quantitative method of testing reducing sugars. Meanwhile, the solubility of the maltodextrin in water was determined by a solubility test [6], and the results were adjusted to the quality requirements for maltodextrin solubility by the National Standardization Agency of Indonesia (*Badan Standardisasi Nasional*/BSN).

Encapsulation of a mixture of noni fruit extract and patchouli oil

The ratio between encapsulating agent, core ingredients, and Tween 80 in this study is 4:1:1. A total of 30 mL of maltodextrin solution was added to 0.943 mL of Tween 80 then homogenized at a speed of 750 rpm for 15 minutes. Next, comparative modifications were carried out at the stage of adding the core ingredients to determine the effect of the amount of core ingredients on the antioxidant activity and organoleptic properties. Some of these variations are: (1) 100% noni fruit extract without the addition of patchouli oil; (2) 0.8 grams of noni extract and 0.2 grams of patchouli oil in a ratio of 4:1; and (3) 0.5 grams of noni extract and 0.5 grams of patchouli oil in a ratio of 1:1. After that, the mixture was homogenized at a speed of 750-1000 rpm for 30 minutes at room temperature until an emulsion phase was formed. The final stage was drying with a freezer dryer for 3 days. The encapsulation results were then analyzed for yield, organoleptic properties, encapsulation efficiency, microcapsule morphology, and antioxidant activity [7, 8].

Analysis of encapsulation

Changes in the organoleptic properties of noni extract before and after encapsulation with the addition of patchouli oil were determined by carrying out organoleptic tests in the form of observing the taste, color, aroma, texture and overall hedonic test (liking test) which was attended by 15 panelists who had fulfilled the requirements of the Quality Management

Module. Organoleptic Physics (2013). Then, an Encapsulation efficiency analysis was carried out to determine the success of the encapsulation process for noni extract and patchouli oil which was carried out by calculating the percentage of the core ingredients of each noni fruit extract and patchouli oil encapsulated by maltodextrin using the division formula between the difference in total extract used and surface oil content by total extract [10]. The encapsulation results were also analyzed using SEM instruments to compare the morphology of the particles resulting from freeze drying between the encapsulation results without the addition of patchouli oil and with the addition of patchouli oil. Then the particle size was analyzed using PSA to compare the particle size of the maltodextrin before and after encapsulation [14].

Testing of antioxidant activity with the DPPH method

In this study, the positive control was vitamin C, whereas the blank solution was methanol. The concentrations of the test sample solutions were 5, 10, 15, 20, and 25 ppm, prepared by dissolving 100 ppm of test samples in 5 mL of DMSO. Each concentration variation was added to the reagent solution of 0.2 mM DPPH and incubated for 30 minutes in a dark room. The absorbance was then measured using a UV-Vis spectrophotometer at a wavelength of 517 nm [11].

Results and Discussion

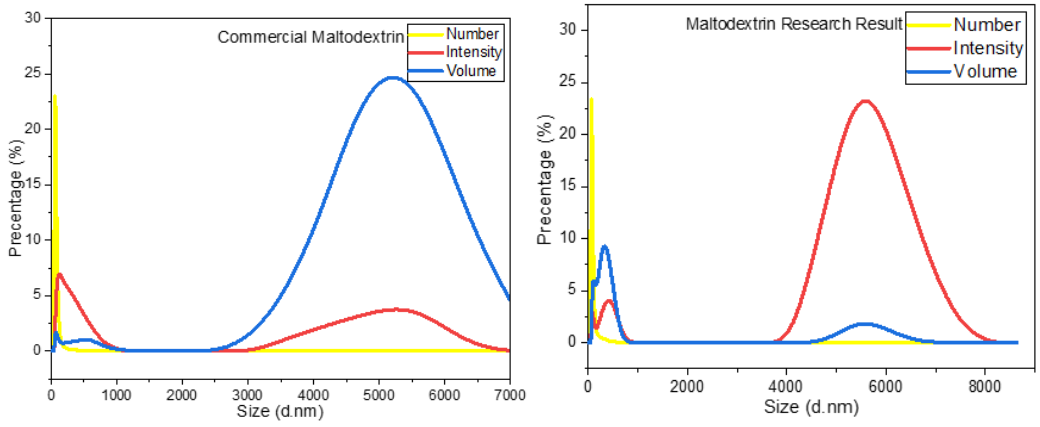
Making maltodextrin

Hydrolysis of cassava starch with α-amylase enzyme at a concentration of 0.5% of the solution volume in this study produced maltodextrin with different physical properties from those of commercial cassava starch-based maltodextrin. The differences in the physical properties of the two maltodextrins can be seen in Table 1.

Table 1**.** Physical properties of maltodextrin

Parameter	**Research Maltodextrin**	**Commercial Maltodextrin**
Color	Cream	White
Flavor	No flavor	No flavor
Solubility in water	63%	98.85%
Texture	Rugged and not sticky	Smooth and slightly sticky

According to the National Standardization Agency of Indonesia (BSN), the standard for maltodextrin solubility is in the range of 97-98%. This means that the maltodextrin produced in this study does not meet the specified quality requirement. Maltodextrin's solubility can be affected by its particle size; the larger the particle size, the smaller the surface area, and the longer it takes for water to absorb the starch particles. In other words, if the particle size is smaller, the hydration rate of the flour will increase [12]. Based on the PSA results, the average particle diameters for commercial maltodextrin and research maltodextrin were 214.3 and 415.9 nm, respectively.



(a) (b)

Figure 1. Graph of particle size distribution in commercial maltodextrin (a) and research maltodextrin (b)

From the PSA results for research maltodextrin (Figure 1b), the highest distribution peak was based on intensity in the particle size range of 5000-6000 nm, signifying that the intensity of photon absorption at these particle sizes is higher than at other particle sizes. Meanwhile, for commercial maltodextrin (Figure 1a), the highest distribution peak was based on volume in the particle size range of 3500-5500 nm, indicating that these particle sizes have a larger total volume than other particle sizes. The presence of larger particles with high intensity can cause the solubility of maltodextrin to decrease. The polydispersity index (Pd index) values of commercial maltodextrin and research maltodextrin were 0.380 and 0.424, respectively. The Pd index value of below 0.5 suggests that the homogeneous dispersion system formed is monodisperse and does not settle. The dispersion results of both maltodextrins are relatively good since these values reflect a fairly narrow particle size distribution and a homogeneous particle size diameter.

Furthermore, based on the analysis results, the DE values of research maltodextrin and commercial maltodextrin were 9.2 and 9.6, respectively. The difference in DE values for the two maltodextrins can be influenced by the concentration of the α-amylase enzyme and the hydrolysis time. With increasing hydrolysis time and α-amylase enzyme concentration, the DE value also increase as more substrates are hydrolyzed over a longer time and at higher enzyme concentrations, thus increasing the numbers of short polymers (monosaccharides, disaccharides, and oligosaccharides) formed in the maltodextrin. Based on the Food and Drug Administration (FDA) standards, maltodextrin has a DE value range of <20 [13]. Hence, the hydrolyzed maltodextrin and commercial maltodextrin in this study have met the FDA standards in terms of their DE values. However, as the maltodextrin produced in this study did not meet the BSN standards for its solubility, it could not be used as encapsulating agent in the encapsulation of noni fruit extract. Therefore, commercial maltodextrin was used as the encapsulating agent in this study.

Compound content of patchouli oil

This study used commercial patchouli oil with the following specifications:

Botanical name : *Pogostemon cablin*
Product Code : 150028
Batch Number : 220408/177366
Appearance : Clear liquid
Color : Yellow-Brown
Smell : Leafy, Earthy, Sweet, and Woody
Production Time : 08 April 2022

Based on its specifications presented in Table 2, the density and color of commercial patchouli oil have met the SNI 06-2385-2006 standards, namely 0.9328-0.9652 g/mL at the same temperature and light yellow to reddish brown.

Table 2**.** Specifications of commercial patchouli oil

Test Item	**Specification**	**Result**
Density (20°C)	0.9328-0.9652	0.9427
Specific Gravity (20°C)	0.9345-0.9669	0.9444
Refractive Index (20°C)	1.4921-1.5225	1.5041
Solubility	Not Soluble in Water	Not Soluble in Water

The results of GC-MS analysis of patchouli oil compounds are displayed in Figure 2 and Table 3.

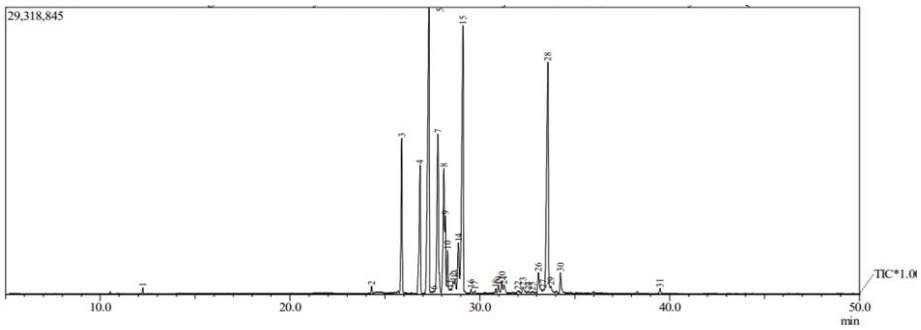
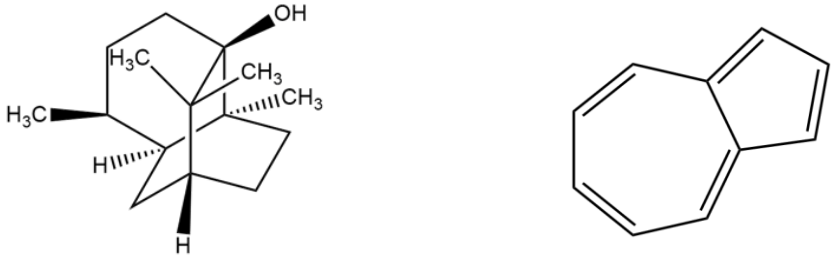


Figure 2. Chromatogram of commercial patchouli oil compounds

Table 3**.** GC-MS analysis of commercial patchouli (*Pogostemon cablin*) oil

RT	Compounds	Area (%)	SI
27.320	Azulene	19.52	92
29.113	delta-Guaiene	16.69	94
33.587	Patchouli Alcohol	16.62	92
27.794	Seychellene	10.03	86
28.104	1H-3a,7-Methanoazulene	7.00	87
25.884	1H-Cyclopropa[a]naphthalene	6.85	91
26.854	trans-Caryophyllene	6.82	93
28.872	Alloaromadendrene	3.11	88
33.090	1H-Cycloprop[e]azulen-4-ol	1.47	84
34.253	2H-Pyran-2,4(3H)-dione	1.08	80
28.690	2,4-Diisopropenyl-1-methyl-1-vinyl-cyclohexane	0.81	90
8.602	1-Iodo-2-methylnonane	0.69	81
31.160	(-)-Caryophyllene oxide	0.63	90
31.273	Pentadecane (CAS) n-Pentadecane	0.59	96
28.439	gamma-Gurjunene	0.30	92
39.497	Octadecanoic Acid	0.27	92

As seen in Table 3, commercial patchouli oil has a patchouli alcohol content of 16.62%, and the highest compound is azulene with a percentage of 19.52%. The chemical structures of patchouli alcohol and azulene compounds are presented in Figure 3.



(a) (b)

Figure 3. Chemical structures of patchouli alcohol (a) and azulene (b)

Encapsulation efficiency of a mixture of noni fruit extract and patchouli oil

Encapsulation efficiency states the percentage of noni fruit extract and patchouli oil that can be encapsulated from the total core ingredients used [14]. The yield and efficiency of the encapsulation samples in this study are shown in Table 4.

The decrease in encapsulation efficiency in the three variations can be caused by the increase in encapsulation surface area in each variation. Overall, all encapsulation samples are considered efficient as they range from 80% to 97% [14].

Table 4. Encapsulation yields and efficiencies

Encapsulation Sample	Yield (%)	Encapsulation Efficiency (%)
Noni Fruit Extract	91.35	91.77
Noni Fruit Extract and Patchouli Oil (4:1)	88.03	Noni Fruit Extract: 83.5% Patchouli Oil: 90.78%
Noni Fruit Extract and Patchouli Oil (1:1)	83.51	Noni Fruit Extract: 90.34% Patchouli Oil: 87.15%

Organoleptic test

Organoleptic tests with 15 panelists were carried out on five samples: (1) noni fruit extract before encapsulation; (2) encapsulation of noni fruit extract; (3) encapsulation of a mixture of noni fruit extract and patchouli oil (4:1); and (4) encapsulation of a mixture of noni fruit extract and patchouli oil (1:1). The results of the organoleptic tests show differences in taste, aroma, color, and texture between noni fruit extract before and after being encapsulated together with patchouli oil. After encapsulation, the bitter taste of noni fruit extract is reduced, but the addition of patchouli oil brings back the bitterness because patchouli oil has a bitter taste (*pungent taste*) [15]. Furthermore, the unpleasant odor of noni fruit extract also decreases after encapsulation with the addition of patchouli oil. Meanwhile, based on the results of the favorability test, 15 panelists preferred the encapsulation of pure noni fruit extract (without the addition of patchouli oil) and the encapsulation of noni fruit extract with the addition of patchouli oil in the smallest ratio of 4:1. The results of the organoleptic tests are presented in graphs as displayed in Figure 4.

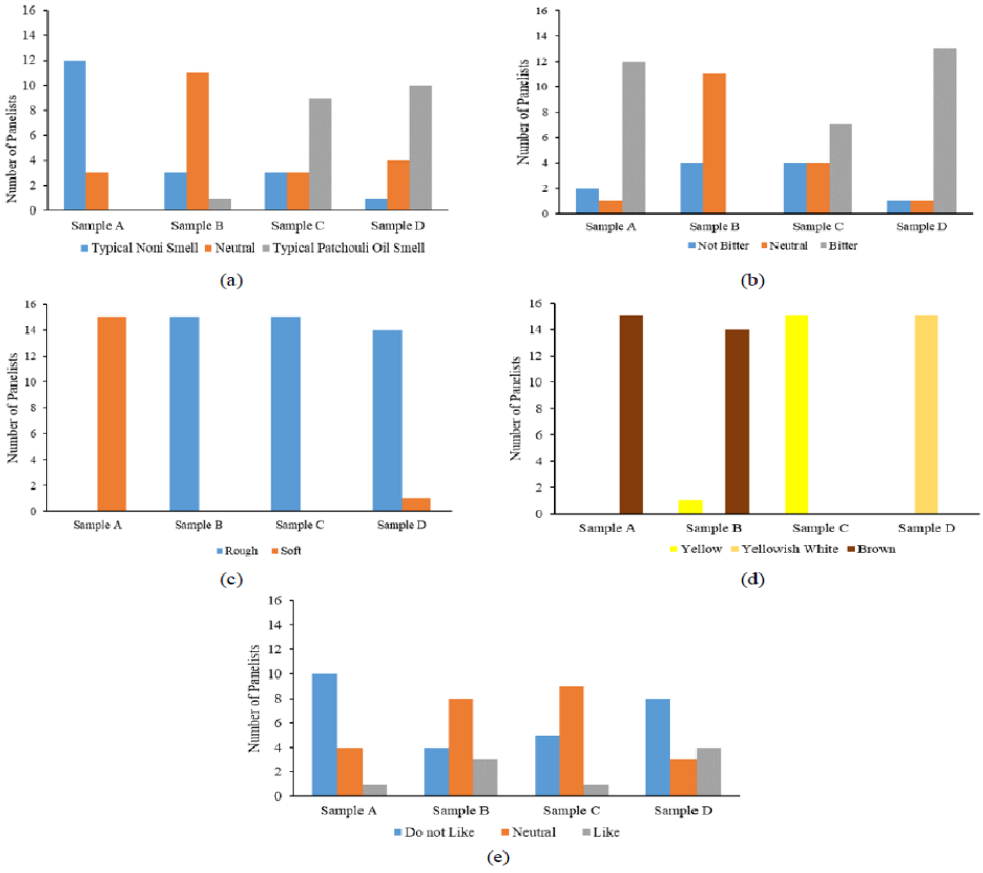


Figure 4. Graphs of organoleptic test results for aroma (a), taste (b), texture (c), color (d), and favorability (e)

Microcapsule morphology using SEM

The morphological analysis results of the microcapsules using SEM reveal an appearance that is not much different from one another at 500x magnification with a scale of 50 μm, as displayed in Figure 5. The resulting microcapsules are irregular, shaped like fractures, and have diverse sizes. This can be caused by incomplete freeze-drying process (the result is not completely dry) and uneven stirring with a magnetic stirrer. Wrinkles formed in the encapsulation of noni fruit extract and patchouli oil may occur due to the inappropriate composition and the lack of emulsifying ability of the maltodextrin used as encapsulating agent [7]. In addition, the particle surface of the three microcapsules did not show detectable pores, signifying that the solvent did not evaporate too quickly during the drying process. The results of the morphological analysis in this study are consistent with those of a previous study that encapsulated cold-pressed seed oils with various unsaturated fatty acids using the same freeze-drying method [16].

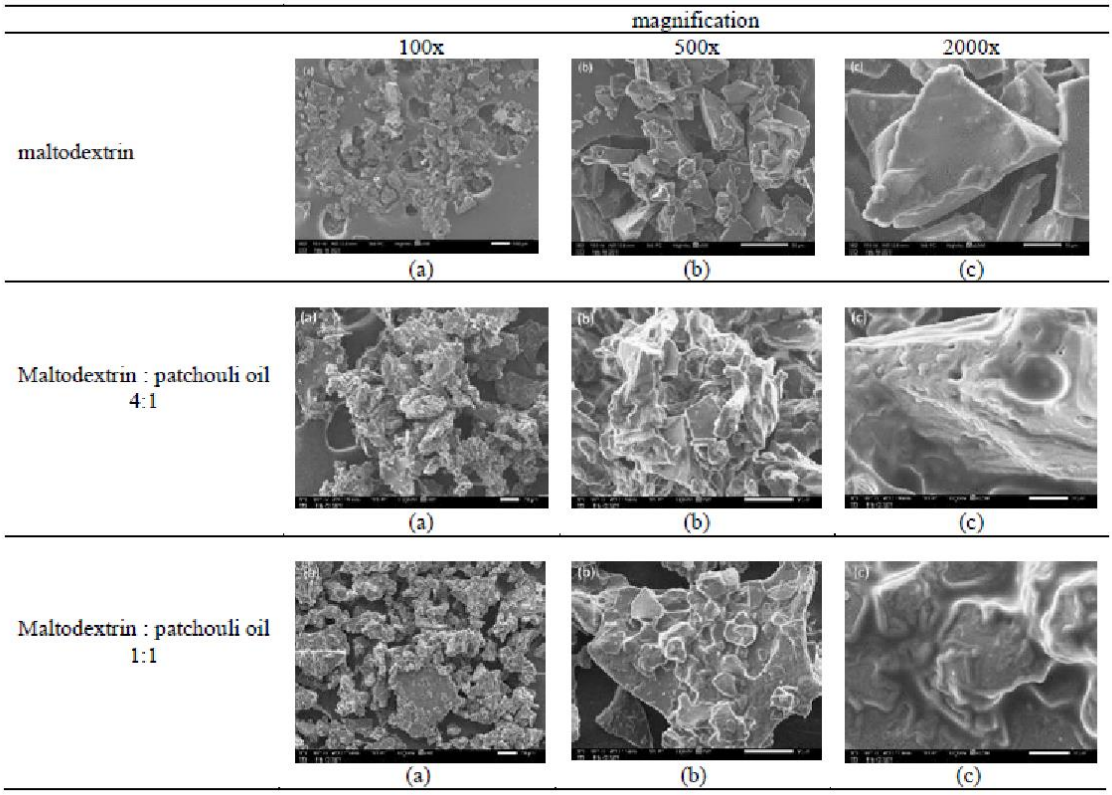


Figure 5. Morphology of noni fruit extract microcapsules with maltodextrin

Antioxidant activity test of the encapsulation

The success of an encapsulation process can be seen from several parameters, i.e., organoleptic properties, encapsulation efficiency, micro-particle characteristics, stability, release after consumption [17], and the antioxidant activity [18]. In this study, the antioxidant activity test was performed using the DPPH method, whose is that antioxidant compounds will donate hydrogen atoms and bond with free electrons in radical compounds, causing the free radical (diphenylpicrylhydrazyl) to change into a more stable non-radical compound (diphenylpicrylhydrazine) [19]. The sample absorbance results are used to determine the IC_{50} values of the encapsulation samples, which are shown in Table 5.

Table 5. IC_{50} values of encapsulation samples

Sample	IC_{50} (µg/mL)	Category
Noni Fruit Extract	61.74	Intermediate
Maltodextrin	1083346.9	Very weak
Encapsulation of Noni Fruit Extract with Maltodextrin	553.24	Very weak
Encapsulation of a Mixture of Noni Fruit Extract and Patchouli Oil (4:1)	88.30	Intermediate
Encapsulation of a Mixture of Noni Fruit Extract and Patchouli Oil (1:1)	50.80	Intermediate
Ascorbic Acid	1.10	Very strong

After encapsulation, the IC_{50} value of noni fruit extract experienced a significant increase. This indicates weak antioxidant activity as maltodextrin encapsulates the noni fruit extract and its active compounds. In this regard, the ratio of the core ingredients greatly influences the antioxidant activity of encapsulation result; the higher the concentration of maltodextrin compared to the core ingredients, the less fruit extract is encapsulated, resulting in the decreased amount of active compounds that act as antioxidants [20]. In this study, the ratio of coating material to core ingredients is 80:20, producing a higher IC_{50} value after encapsulation and resulting in lower antioxidant activity. However, the addition of patchouli oil has been found to contribute positively to increasing the antioxidant activity of encapsulated noni fruit extract. Due to the presence of patchouli alcohol which has hydroxy groups in phenols and terpenes, patchouli oil is known to have high antioxidant properties. As mentioned previously, the patchouli oil used in this study contains 16.62% Patchouli alcohol. Additionally, the patchouli oil also has an IC_{50} value of 22.45 µg/mL, which falls into the 'strong' category [21]. The results of this research indicate that the addition of patchouli oil to the encapsulation of noni extract is able to reduce the unpleasant aroma of noni extract and is able to increase the antioxidant activity of noni extract when combined. In previous research, modifications were also made to the encapsulation of noni extract, namely by adding lemon peel and it gave positive results to eliminate the unpleasant aroma of noni extract. However, this research did not include the antioxidant activity of the encapsulation results so it cannot be said to have potential as an antioxidant [23].

Conclusion

Based on the research that has been carried out, it can be concluded that Maltodextrin with a Dextrose Equivalent (DE) value of less than 20 can be produced through the partial hydrolysis process of cassava starch using the α-amylase enzyme with a concentration of 0.5% of the solution volume. The addition of patchouli oil to the encapsulation process of noni extract with maltodextrin has a positive effect on reducing the unpleasant aroma of noni extract and there is an increase in the antioxidant activity value of noni extract. Therefore, this research can be continued and developed as a solution for consuming noni fruit and preventing diseases caused by free radicals.

Acknowledgement

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