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The Effect of Wall Materials and Drying Methods on The Encapsulation *Sardinella lemuru* Smart Flavor

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Abstract— **Antioxidant compounds in food are generally less stable when applied to food, so technology is needed to help antioxidant compounds become more stable during storage, one of which is microencapsulation. The research aims to determine the effect of the wall material ratio and drying methods on the characteristics of *Sardinella lemuru* smart flavor microcapsules. The experimental design used was a two-factor Complete Random Design (CRD): ratio of wall materials (maltodextrin: Arabic gum) and drying methods (spray drying and freeze drying). The research showed that enzyme activity ranged from 14.81-52.64 U/mL; lightness 95.2-100; yield 4.00-17.19%; water content 2.26-9.15%; antioxidant activity 15.75-31.23%; encapsulation efficiency 69.06-78.47%. Microcapsules with the highest water content, lightness, antioxidant activity, and encapsulation efficiency were at the ratio wall materials (maltodextrin: Arabic gum) of 7:3 by spray drying, 9.15%, 100, 31.23%, and 78.47%. On the other hand, the highest yield (17.19%) was at the ratio wall materials (maltodextrin: Arabic gum) 8:2 by freeze-drying. The morphology of the microcapsules by spray drying is spherical, and freeze drying makes it flaky and sharp.**

Keywords— **Microencapsulation, wall materials, drying methods, *Sardinella lemuru*, smart flavor**

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I. INTRODUCTION

Smart Flavor is a technology that produces flavor enhancers that benefit consumer health because they contain essential substances and antioxidants. Smart flavor technology is developed by utilizing local fishery commodities to enrich science and technology treasures, especially in natural flavors [1]. *Sardinella lemuru* is a fishery commodity that has the potential to be developed through smart flavor technology because of its high protein content, which is 20 g/100 g [2]. The high protein content in *Sardinella lemuru* has the potential to produce a hydrolyzed protein with a high yield, which will be the main ingredient in making smart flavors. Hydrolyzed fish protein results from hydrolysis in the form of simple peptides and amino acids which have several functional properties for health [3] such as antihypertensive and antioxidant [4].

Antioxidant compounds in food are generally less stable when applied to food, so technology is needed to help antioxidant compounds become more stable during storage, one of which is microencapsulation [5]. Microencapsulation is a technique of coating liquids, solids, or gases with protective material in the form of a thin layer. Microencapsulation is applied for various purposes, including protecting the core material from degradation, modifying the properties of original material to make it easier to use, releasing the core material slowly at a constant rate, masking unwanted flavors from the material [6], increasing the stability and solubility of ingredients, controlling attack by active compounds, and minimizing nutrient loss [7]. *Sardinella lemuru* contains *trimethyl amine oxide* (TMAO), which causes the fishy aroma to be reduced by the application of microencapsulation [6].

Several methods can do microencapsulation, two of which are spray drying and freeze drying. Spray drying is the most commonly used microencapsulation technique because it can be carried out quickly and cheaply and produces a uniform product but a relatively low yield. While freeze-drying is suitable for heat-sensitive materials, keeping oxidative reactions to a minimum and creating products with a soft texture is expensive [8]. *Sardinella lemuru* smart flavor microcapsules have the potential to be applied in food as an umami taste that can control the gradual release of flavor.

The microencapsulation process requires a non-toxic coating material that does not react easily with the core material. According to [9], the coating material used can consist of one type or a combination of two different types of coating. The combination of two types of coating affects the encapsulation efficiency and is reported to improve the microcapsule properties as expected [10]. The combination of coatings commonly used is maltodextrin and Arabic gum. Previous studies have shown that the combination of these two coatings can produce microcapsules with higher yields [11], better potential for antioxidant activity during storage [12], and better hygroscopicity and antioxidant activity [13] when compared to using only one of the two.

The ratio of coating materials and microencapsulation methods can affect the characteristics of the resulting product. Therefore, it is necessary to conduct further research to determine the effect of ratio wall material and microencapsulation method on the characteristics of microcapsules *Sardinella lemuru* smart flavor.

II. Material and Methods

A. Material

The materials used include *Sardinella lemuru* obtained at Puger Market, calotropin obtained from Watu Ulo Jember Regency, papaya sap obtained from Papaya Gardens in Jember Regency, sugar, salt, garlic, STPP (*Sodium Tripolyphosphate*), CMC (*Carboxy Methyl Cellulose*), maltodextrin, and Arabic gum. Other materials used include 0.05 M phosphate buffer pH 7 (Na_2HPO_4 and NaH_2PO_4), distilled water, Na_2CO_3 0.4 M, NaOH, Folin Ciocalteau, HCL, aquades, DPPH (*1,1-diphenyl-2-picrylhydrazil*), sodium potassium tatrate, BSA (*Bovine serum albumine*), 0.1 mM tyrosine, 0.1 M TCA (*Trichloroacetic acid*), 1% casein, $CuSO_4$, 70% ethanol, ethanol pa, and filter paper.

B. Sample preparation

Microencapsulation of Sardinella lemuru smart flavor

The smart flavor microencapsulation of *Sardinella lemuru* includes several stages. It started with the preparation of protease enzymes from calotropin and papain [14] and production of *Sardinella lemuru* smart flavor using a combination of calotropin and papain [15] with modifications. The wet fish hydrolyzate was added with other ingredients, including 25% sugar, 25% salt, 5% garlic, 5% STPP, and 40% CMC. The resulting dough was dried using an oven at 60 for 24 hours and continued with size reduction 100 mesh sieve. The resulting smart flavor was micro-encapsulated by the spray-drying method [16] and the freeze-drying method [17] with modifications.

First, the wall material was dissolved in distilled water with the formulation of maltodextrin: Arabic gum (7:3, 7.5:2.5, 8:2) (10% total solids in solution) using a homogenizer at 5600 rpm for 5 minutes. The wall solution was added with 10% *Sardinella lemuru* smart flavor from the total wall material solution (w/v) and homogenized at 5600 rpm for 10 minutes. The resulting suspension was dried using a spray dryer and freeze dryer. Drying using a spray dryer was carried out at an inlet temperature of 121ºC, an outlet temperature of 51ºC, an air flow rate of 54 m^3/hour, and a material flow rate of 600 mL/hour. Meanwhile, a freeze dryer was drying at -45ºC for 24 hours.

C. Parameters

Protease enzyme activity [18]

Several treatments for enzyme activity include blanks, tyrosine standards, and samples. The enzyme was replaced with distilled water and 0.1 mM tyrosine for blank and standard treatment. 0.1 mL of the enzyme solution was put into a test tube containing 0.5 mL of casein 1% w/v and 0.5 ml of phosphate buffer pH 7, then incubated at 37ºC for 10 minutes. Furthermore, 1 ml of 0.1 M TCA and 0.1 mL of distilled water were added and then incubated for 10 minutes at 37ºC. After incubation, the solution was centrifuged for 10 minutes at 1000 rpm. The supernatant was taken at 0.75 mL to be added to a test tube, which already contained 2.5 mL of 0.4 M Na_2CO_3, 0.5 mL of Folin Ciocalteau reagent (1:2) was also added, and incubated at 37ºC for 20 minutes. Incubation results were measured using a spectrometer with $\lambda = 670$ nm.

$$UA = (Asp-Abl)/(Ast-Abl) \times P \times 1/T$$

Description:

UA = Enzyme activity unit (Unit/mL)
Asp = Sample absorbance
Abl = Blank absorbance
Ast = Tyrosine standard absorbance
P = Dilution Factor
T = Incubation Time

Color [19]

Color analysis was carried out using a colorimeter. Before measuring the sample, standardization is carried out first by attaching the tool sensor to white paper (L color standard). Next, sample measurements are carried out at three different points by attaching the tool sensor perpendicular to the sample. The L value ranges from 0 to 100, which indicates the color black to white.

Yield [20]
Calculating the yield of microcapsules *Sardinella lemuru* smart flavor refers to [20]. The yield is calculated using the following formula:

Yield % = Microcapsules (g)/Raw material (g) x 100%

Water content [21]
Analysis of water content microcapsules *Sardinella lemuru* smart flavor uses a moisture analyzer. Microcapsules as much as 0.5 grams are placed on an aluminum container and then heated to 130ºC. The water content of microcapsules is indicated by the value displayed on the screen.

Antioxidant activity[22]
One gram of microcapsule was dissolved in 10 mL distilled water, and 0.00197 grams of DPPH was dissolved in 50 mL ethanol pa. Next, 0.1 ml of sample solution was added with 0.9 mL of ethanol pa and homogenized using a vortex. Then, 2 ml of 0.1 mM DPPH solution was added to a test tube and incubated in a dark place for 30 minutes. Furthermore, a blank was made by homogenizing 1 ml of ethanol pa. with 2 ml of 0.1 mM DPPH solution and incubated in a dark place for 30 minutes. All solutions were absorbed at 517 nm.

$$\text{Antioxidant (\%)} = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \times 100$$

Encapsulation efficiency [23]
Analysis of encapsulation efficiency (EE) is based on the difference between total soluble protein content of *Sardinella lemuru* smart flavor (assumed to be A) and soluble protein in the supernatant microcapsules of *Sardinella lemuru* smart flavor (assumed to be B). The A was analyzed by diluting 0.1 g of *Sardinella lemuru* smart flavor in 10 ml of distilled water and then analyzing soluble protein content using method [24]. Meanwhile, B was analyzed by diluting 0.1 g of microcapsules *Sardinella lemuru* smart flavor in 10 ml of distilled water and then separated by centrifugation at 4000 rpm for 10 minutes to form supernatant and residue. The centrifuged supernatant was analyzed for soluble protein using [24].
Measurement of soluble protein content by Lowry carried out by taking 0.125 ml of sample to be reacted with 2.5 ml of Mix-Lowry reagent (50 ml of 2% Na_2CO_3+ 1% $CuSO_4$ + 1% Sodium potassium tartrate), then homogenized and allowed to stand for 10 minutes. Furthermore, 0.25 ml of Folin Ciocalteau was added and allowed to stand for 30 minutes. Then absorbance with a wavelength of 750 nm. The absorbance data obtained were included in the BSA standard curve to calculate the soluble protein content.

$$\% \text{ Encapsulation efficiency} = (A-B)/A \times 100$$

Microcapsule morphology [9]
Microcapsule morphology analysis using a Scanning electron microscope (SEM) which is coated with a thin layer of platinum. The sample is placed on a cylindrical copper plate and inserted into the SEM tool, then the microcapsule morphology is read with a magnification of 2000.

D. Data analysis
The data obtained were processed using Microsoft Excel 2016 and analyzed using the Analysis of Variance Test (ANOVA) at a significance level of 5%. If the results differ significantly, continue with the DMRT (Duncan Multiple Range Test). The data obtained are presented in tables and bar charts to make it easier to analyze. Meanwhile, the business feasibility analysis was processed using Microsoft Excel 2016 and explained descriptively.

III. RESULT AND DISCUSSION

A. Enzyme activites

Protease enzyme activity is a quantitative measure that describes the ability of protease enzymes to hydrolyze proteins into simpler compounds (peptides and amino acids). In **Fig. 1**, the results of the measurement of protease enzyme activity from calotropin and papain were 14.81 U/mL and 52.64 U/mL.

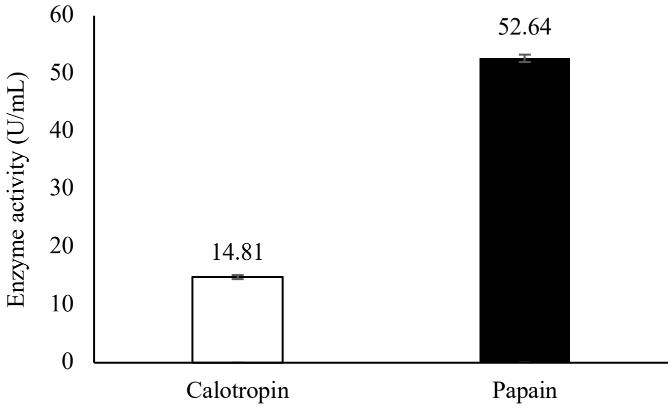


Fig 1. Enzyme activity on protein hydrolysis of *Sardinella lemuru*

The data shows that 1 mL of calotropin can catalyze the hydrolysis reaction to convert 14.81 µmol of protein substrate per minute into protein hydrolyzate. Meanwhile, 1 mL of papain can catalyze the hydrolysis reaction to convert 52.64 µmol of protein substrate per minute into hydrolyzate.
The calotropin strongly indicates the type of exopeptidase enzyme, which cuts the protein structure from the end of the polypeptide chain (both amino and carboxy) substrates to produce amino acids and peptide residues [25]. At the same time, the papain enzyme is a type of endopeptidase enzyme which cuts protein structure randomly on the inside (middle) to produce peptides and polypeptides. [25], combining calotropin and papain for protein hydrolysis can accelerate the breaking of peptide bonds. Using two enzymes together can form simpler proteins than using only one of the enzymes.

B. Color (Lightness)

Lightness (L*) color measurement shows that the product tends to be dark or vice versa. The results of ANOVA analysis at a significant α≤0.05 showed that the ratio of wall materials and microencapsulation methods significantly affected the color of microcapsules. The results of the color analysis of microcapsules *Sardinella lemuru* smart flavor are in **Fig. 2.**

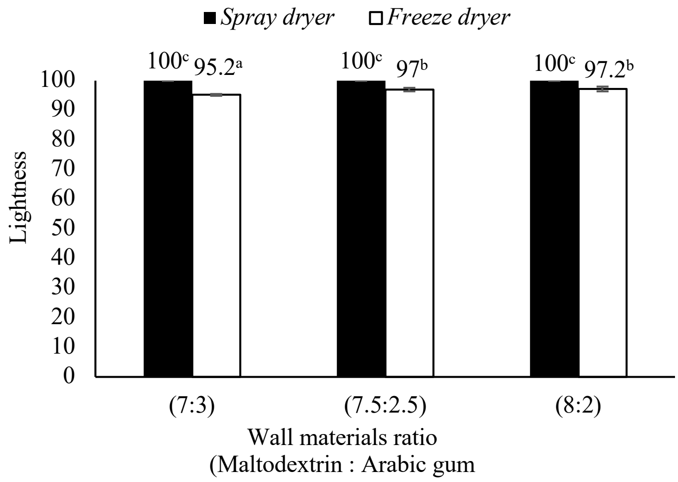


Fig. 2. Lightness of microcapsules *Sardinella lemuru*

The lowest lightness of microcapsules in the freeze dryer with a wall formulation (maltodextrin: Arabic gum) of 7:3 was 95.2 and the highest lightness value in the spray dryer (all formulations). The more maltodextrin added, the higher lightness of microcapsules. This increase in brightness is caused by the color of maltodextrin, which tends to be white so that when added as a wall material, it can increase the brightness of microcapsules [26]. [27] state that lightning increases with increasing maltodextrin and decreasing Arabic gum.

In addition, the lightness of microcapsules is influenced by the drying method. The drying method with a freeze dryer produces a lower lightness than a spray dryer. [28], drying using a spray dryer produces a brighter product than a freeze dryer. According to [29], the high temperature in spray dryer results in the loss of color pigments, so the microcapsules have a high lightness value. At the same time, freeze-dryer maintains the material's color because it uses very low temperatures [30]. According to [31], the high lightness of microcapsules dried using a spray dryer is also caused by the atomization pressure during drying.

C. Yield

A high yield value indicates a more efficient process (Hasrini et al., 2017) [20]. The results of ANOVA analysis at a significant α≤0.05 showed that the ratio of wall materials and microencapsulation methods significantly affected the yield of microcapsules—the yield analysis results of microcapsules *Sardinella lemuru* smart flavor can be seen in **Fig. 3.**

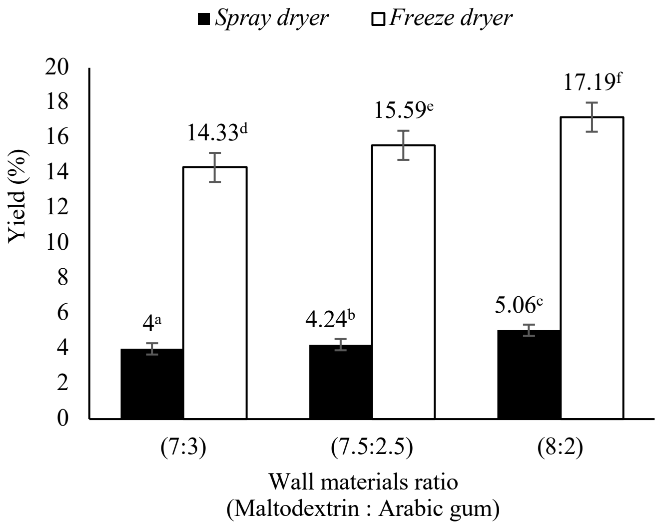


Fig. 3. Yield of microcapsules *Sardinella lemuru*

The lowest yield of microcapsule in spray dryer with wall material (maltodextrin: Arabic gum) 7:3 is 4% and the highest in freeze dryer with wall material (maltodextrin: Arabic gum) 8:2 is 17%. The microcapsule yield increases with increased maltodextrin ratio and the decrease in the Arabic gum ratio as a wall material. Maltodextrin as wall material produces a high yield because it can increase volume and total solids [32]. [13], the use of wall material with a ratio of 8:2 (maltodextrin: Arabic gum) produces a microcapsule yield of 42.6 - 64.9%, while the ratio of 6:4 (maltodextrin: Arabic gum) produces a yield of 38.9 - 58.5%. The results showed lower results due to using only 10g *Sardinella lemuru* concentration with 10g encapsulant material, while other research used 90% main ingredient (90g) and 10% encapsulant (10g).

The microcapsule yield value is influenced by the drying method used. Microencapsulation with a spray dryer produces a lower yield value than a freeze dryer. The low yield in spray dryer is due to the difficulty of taking samples from dryer. More microcapsules fall into the spray dryer chamber or wall sticking than the cyclone [33]. [29] the spray-drying method produced a lower microcapsule yield (58.33 - 70.72%) than the freeze-drying method (86.41 - 93.73%). The study results were lower because the concentration of encapsulant material was only 10g, while other research used 40g with 60g main ingredients.

D. Water content

Water content is an important parameter affecting quality; high water content can disrupt product stability (powder clumping occurs) and cause damage. The results of ANOVA analysis at a significant α≤0.05 showed that the ratio of wall materials and microencapsulation methods significantly affected water content. The results of the water content analysis of microcapsules *Sardinella lemuru* smart flavor can be seen in **Fig. 4.**

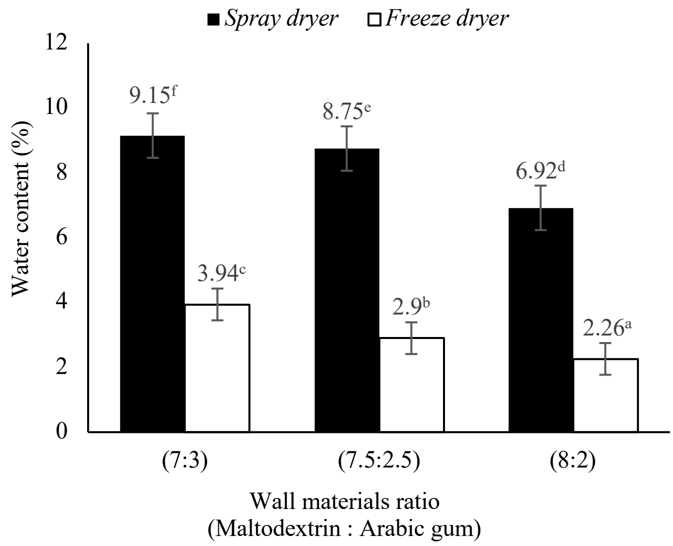


Fig. 4. Water content of microcapsules *Sardinella lemuru*

The lowest water content of microcapsules in freeze dryer with wall material (maltodextrin: Arabic gum) 8:2 is 2.26% and the highest water content in spray dryer with wall material (maltodextrin: Arabic gum) 7:3 is 9.15%. Adding the maltodextrin ratio and reducing Arabic gum as a wall material causes the water content of microcapsules to decrease. This phenomenon is related to the material's molecular structure as a wall. The ability of Arabic gum to bind water is due to its large number of hydroxyl groups, so it can bind large amounts of water strongly [34]. [27], the use of a wall material ratio of 7:3 (maltodextrin: Arabic gum) produces microcapsules with a lower water content of 1.21±0.10% when compared to a ratio of 5:5 (maltodextrin: Arabic gum) of 2.34±0.18%.

A freeze dryer produces lower water content than a spray dryer. Freeze drying occurs through a sublimation process at low temperatures, so water vapor diffuses completely and produces microcapsules with low water content [35]. A spray dryer produces microcapsules with high water content because the inlet temperature influences it during drying. [36] state that an inlet temperature of less than 175ºC produces high water content, while an inlet temperature of more than 175ºC has a low water content.

E. Antioxidant activity

Antioxidants in fish are closely related to protein peptide bonds and amino acids. The results of ANOVA analysis at a significant α≤0.05 showed that the ratio of wall materials and microencapsulation methods significantly affected antioxidant activity. The results of the antioxidant activity of microcapsules *Sardinella lemuru* smart flavor are in **Fig. 5.**

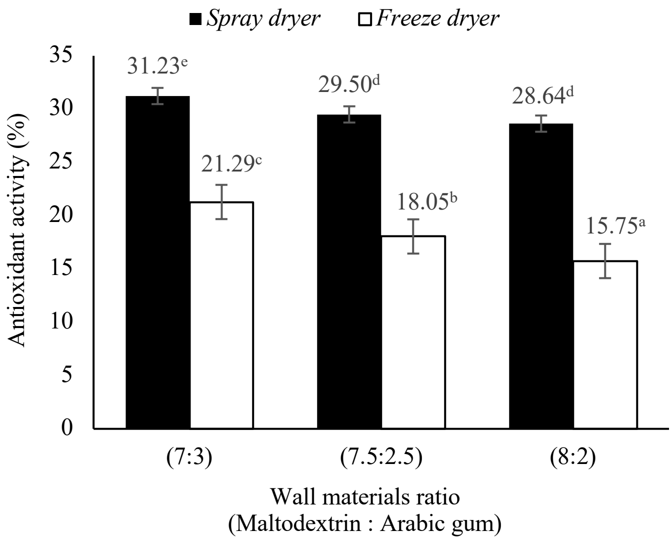


Fig. 5. Antioxidant activity of microcapsules *Sardinella lemuru*

The lowest antioxidant activity of microcapsules in freeze dryer with wall material (maltodextrin: Arabic gum) 8:2 is 15.75% and the highest in spray dryer with wall material (maltodextrin: Arabic gum) 7:3 is 31.2%. Antioxidant activity decreases with a decreasing ratio of Arabic gum as wall material. [37], Arabic gum as a wall material can form good film and structure to maintain core material from the destruction change process and is not easily evaporated/damaged during the drying process.

A spray dryer produces microcapsules with higher antioxidant activity than a freeze dryer. [38] state that high temperatures in spray drying can trigger the Maillard reaction, which causes higher radical scavenging activity. Maillard reactions can produce reductone compounds as antioxidants that can donate hydrogen when reacting with free radicals to become stable [39]. However, using too high a temperature in a spray dryer can damage the core material, so the selection of inlet and outlet temperatures during drying must be adjusted to the characteristics of the material to minimize thermal degradation [29].

F. Encapsulation efficiency

Encapsulation efficiency indicates the ability of the wall material to protect the core material from damage during the drying process [40]. The results of the ANOVA analysis α≤0.05 showed that the ratio of wall materials and microencapsulation methods significantly affected encapsulation efficiency. The results of the encapsulation efficiency of microcapsules *Sardinella lemuru* smart flavor in **Fig. 6.**

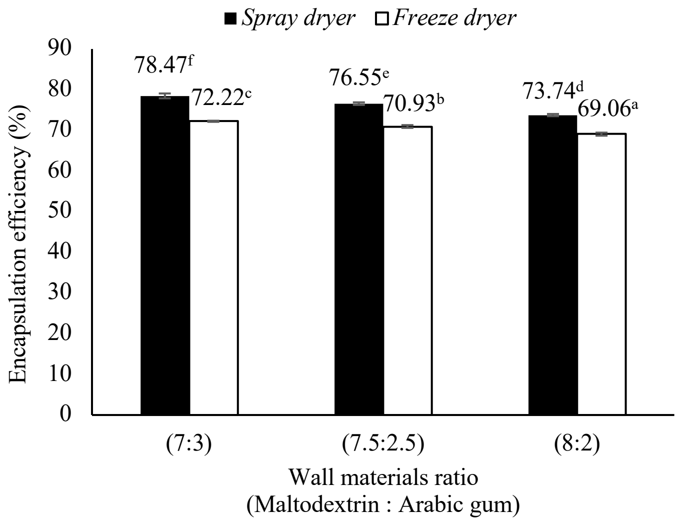


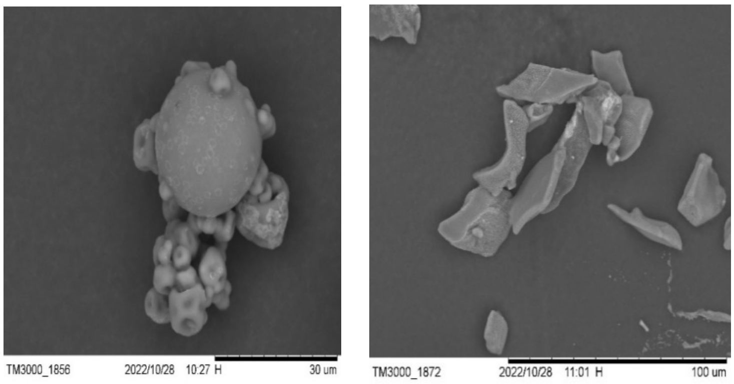
Fig. 6. Encapsulation efficiency of microcapsules *Sardinella lemuru*

The lowest encapsulation efficiency in a freeze dryer with wall material (maltodextrin: Arabic gum) 8:2 is 69.06%, and the highest in a spray dryer with wall material (maltodextrin: Arabic gum) 7:3 is 78.47%. Encapsulation efficiency decreases with the decreasing ratio of Arabic gum as wall material. Arabic gum stabilizes and emulsifies and can prevent core material from reacting with oxygen [41].

A spray dryer produces higher encapsulation efficiency than a freeze dryer. [42] spray dryer provides a higher encapsulation efficiency value than freeze dryer. A spray dryer can form a film layer on the microcapsules in the final drying stage. The trapping of the core material causes low soluble protein on the microcapsule's surface, indicating that the core material is well encapsulated. A freeze dryer takes longer than a spray dryer cause of inconsistent trapping of the core material so that encapsulated low, resulting in high soluble protein on the surface of microcapsules. The high soluble protein on the surface of microcapsules indicates that the wall material is not good at encapsulating the core material [43].

G. Microcapsules morphology

SEM (*Scanning Electron Microscope*) was conducted on microcapsules with each microencapsulation method's (highest antioxidant activity and encapsulation efficiency). The results of the morphology of microcapsules *Sardinella lemuru* smart flavor can be seen in **Fig. 7.**



(a) (b)

Fig. 7. Microcapsul morphology *Sardinella lemuru* smart flavor (a) spray dryer, (b) freeze dryer

The microcapsules of the spray dryer and freeze dryer methods have different particle sizes, namely 30 µm and 100 µm. The microcapsules have met the required size, ranging from 0.2 to 5000 µm [44]. The spray-dryer produces round microcapsules, while the freeze-dryer is shaped like flakes without curves and is porous, sharp, and broken [42]. Figure 7 (a) shows a smooth round shape, but there are wrinkled microcapsules. The smooth round shape is produced due to the atomization process [29]. The thermal dehydration process can cause wrinkles in microcapsules due to rapid moisture loss after cooling [42].

Freeze drying method effectively dries the material matrix by sublimation. After drying, the material can form aggregates but is easily crushed into powder. The microcapsules in Figure 7(b) have a smooth, sharp surface and a rather fragile and porous structure due to the formation of ice crystals during the freezing process and the sublimation process during drying [41].

IV. CONCLUSION

The research showed that enzyme activity ranged from 14.81-52.64 U/mL; lightness 95.2-100; yield 4.00-17.19%; water content 2.26-9.15%; antioxidant activity 15.75-31.23%; encapsulation efficiency 69.06-78.47% and the morphology of the microcapsules from the spray dryer drying was spherical, while the microcapsules from the freeze dryer were shaped like flakes and sharp. The highest antioxidant activity and encapsulation efficiency is at spray dryer with wall material (maltodextrin: Arabic gum, 7:3).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest or personal relationships with other people or organizations that can inappropriately influence this work.

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