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Optimization of Encapsulating Lemuru Fish Protein Hydrolysate Process by Spray-Drying Using Response Surface Method

Running title: Optimization of Lemuru Fish Protein Hydolysate Encapsulation

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SUMMARY

Research background. Encapsulating Lemuru fish protein hydrolysate is important to maintaining its stability. However, optimal conditions for the encapsulation process of Lemuru fish protein hydrolysate using statistical methods remain unexplored. This study aims to address this problem by optimizing the encapsulation conditions.

Experimental approach. Maltodextrin and gum Arabic were used as carrier agents, with mass per volume ratio ranging from 10 to 30 %, and spray dryer inlet temperatures between 90 and 100

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°C. By employing the Response Surface Methodology (RSM), this research analyzes the main interactions of these variables.

Results and conclusions. Our findings indicate that mass per volume ratio of maltodextrin of 25 % and inlet temperature of 100 °C are the optimal conditions for fish protein hydrolysate encapsulation. The optimal conditions achieved a high desirability index of 0.864, indicating an effective balance between yield, solubility and hygroscopicity. The actual measurements also fall well within the confidence interval of the predicted values, confirming the robustness of the model and the reliability of the predicted optimal encapsulation conditions. Characterizations were conducted using FTIR, SEM, and PSA to validate these results, comparing encapsulated fish protein hydrolysate with its non-encapsulated counterpart. The encapsulated fish protein hydrolysate exhibited distinct features, such as the presence of functional groups from maltodextrin, interconnected particle, and more homogenous and narrower particle size distribution.

Novelty and scientific contribution: Lemuru fish protein hydrolysate encapsulation process using maltodextrin with mass per volume ratio of 25 % and inlet temperature 80 °C was successful in improving the properties of the protein hydrolysate. Further research should explore the functional properties of fish protein hydrolysate.

Keywords: encapsulation; protein hydrolysate; Lemuru fish; spray drying; RSM

INTRODUCTION

The significant health benefits drive the increasing global interest in functional food and nutraceuticals. These foods, enriched with bioactive compounds, play a crucial role in enhancing nutrition, preventing diseases, and promoting overall health. A key contributor to the development of functional food is the fishery industry, particularly in countries like Indonesia where fishery productivity significantly increased, from less than 7 million tonnes in earlier years to over 7,2 million tonnes in 2021 (*1*). Among the diverse marine offerings, the Lemuru fish, a small pelagic fish species, stands out due to its significant contribution to Indonesia's marine market. The nutritional profile of Lemuru fish, a species of small pelagic fish, is prominent; it is rich in high-quality protein, essential amino acids, vitamins, and nutrients, making it an excellent candidate for the formulation of fish protein hydrolysate (*2,3*).

Fish protein hydrolysate is a mixture of low molecular mass peptides containing 2–20 amino acids that are created through acid, alkaline, or enzymatic hydrolysis. Among these methods, enzymatic hydrolysis is the most promising one for producing fish protein hydrolysate that is highly

functional and nutritious. Fish protein hydrolysate has the potential to be used in the development of nutraceuticals and pharmaceuticals, as it is effective in treating cardiovascular disease, cancer, and inflammation (*4*). Furthermore, fish protein hydrolysate exhibits various biological activities such as antioxidant, antihypertensive, and anti-obesity, making it a good alternative for functional food production (*5*). These benefit makes a growth in food-protease industry as a nutraceutical market (*6*). Despite these benefits, fish protein hydrolysates are unstable due to their high protein content. This instability leads to several challenges, such as susceptibility to oxidative degradation, limited shelf life, and the retention of undesirable flavour, which can significantly impact their use in food products. These issues highlight the need for advanced processing techniques like encapsulation to preserve fish protein hydrolysate's integrity and functional properties (*7*).

In recent years, the encapsulation of protein hydrolysates has been a subject of extensive research, employing diverse methodologies to optimize this process. Spray drying has emerged as the most common technique in encapsulating protein hydrolysates due to its ability to rapidly and efficiently convert liquid hydrolysate mixtures into stable powdered forms (*8*). Recent studies on protein hydrolysate encapsulation mainly focus on the effects of carrier type, mass per volume ratio, and temperature using descriptive methods (*7,9–11*). However, there is limited research on optimizing encapsulation conditions at lower temperatures to enhance product quality and preserve protein hydrolysate. These approaches are crucial because of a more precise and scientifically grounded understanding of the encapsulation parameters, potentially enhancing the quality of the encapsulated product while mitigating the risks of thermal degradation and ensuring the preservation of sensitive protein hydrolysate.

This study aims to optimize the Lemuru fish protein hydrolysate encapsulation process using a spray dryer set to a maximum temperature of 100 °C. Engaging in low-temperature spray drying at 90–100 °C presents a milder procedure conducive to safeguarding fragile entities like enzymes, probiotics, and proteins. The 90–100 °C temperature range was selected to maintain protein stability. We hypothesize that the reduced temperatures could amplify the transformation rate of volatile fluids into powders by curtailing evaporation. The increased temperature will break down the protein and reduce its functional properties (*12,13*). Furthermore, this technique provides numerous operational advantages, encompassing enhanced thermal efficacy, noteworthy energy conservation, diminished risks of corrosion, and expedited processing durations, all of which enhance the effectiveness and sustainability of the drying operation.

By employing Response Surface Methodology (RSM) and Box Behnken Design (BBD), we evaluated the impact of carrier agent type, mass per volume ratio, and inlet temperature on the

encapsulation efficiency of gum Arabic and maltodextrin were selected as the liquid feeds due to their low viscosity and high solubility in water (*14*). The study aims to identify the optimal encapsulation conditions that maintain the functional integrity of fish protein hydrolysate, contributing to the functional food development field and introducing a new strategy for fish protein hydrolysate encapsulation.

MATERIALS AND METHODS

Materials

Fresh Lemuru fish were purchased from a local market in Bandung City, Indonesia. Chemicals used in the encapsulation process included Alcalase enzyme (Xian Arisun ChemParm Co Ltd, Shaanxi, China), maltodextrin from Tokopedia Indonesia, with a dextrose equivalent (DE) of 10–12, and gum Arabic) (PT Brataco, Bekasi, Indonesia).

Preparation of Lemuru fish protein hydrolysate

Lemuru fish protein hydrolysate were produced using Priatni *et al.* (*10*) method. Frozen Lemuru are thawed and mixed with distilled water in a ratio of 1:4, and homogenized by a blender. The pH of the mixture was adjusted to 6.0 using 0.1 M HCl solution. The hydrolysis process performed using a 15 L capacity of bioreactor unit (manufactured by CV Bangun Rahmat Teknik, Bandung Indonesia). The hydrolysis was carried out by adding 0.75 % (*m/V*) of Alcalase enzyme to the fish slurry and stirred in a reactor at 50 °C for 6 h; then the hydrolysis process was stopped by heating at 85 °C for 15 min. Finally, the fish protein hydrolysate Lemuru were filtered using a vacuum filter and stored at 20 °C.

Encapsulation of Lemuru fish protein hydrolysate using spray drying method

Encapsulation process of Lemuru fish protein hydrolysate were produced according to Kurozawa *et al.* (*15*). Carrier agents (maltodextrin, gum Arabic, and maltodextrin and gum Arabic) with the mass per volume ratio of 10, 20 and 30 % (*m*/*V*) were added directly to the Lemuru fish protein hydrolysate, and mixed under stirring until dissolve completely. The encapsulation process performed using a spray dryer unit (manufactured by CV. Mitra Sentosa, Malang Indonesia), specifically designed for fish protein hydrolysate processing. The mixture (1 L) was fed into the drying chamber for 2–3 h and the dried product was finally collected for analysis. Inlet air temperature of

spray dryer are varied at 90, 95 or 100 °C and outlet temperature is 80 °C with a feed flow rate of about 200 mL/h. The capacity of spray dryer was 1 L/h.

Optimization of Lemuru fish protein hydrolysate using Response Surface-Box Behnken Design method

Response Surface Method and Box Behnken Design (RSM-BBD) were used to identify the optimum encapsulation conditions for Lemuru fish protein hydrolysate using the spray drying method. This statistical approach facilitated the modeling and analysis of three critical process variables: the carrier agent type, the carrier mass per volume ratio, and the spray drying inlet temperature. RSM-BBD was designed for several experiments for the optimization process. The experiment was done in 15 repetitions. The optimal process is determined by evaluating some desirable responses, including high solubility, high yield, and low hygroscopicity. The experimental yield, hygroscopicity, and solubility data were subjected to a comprehensive multivariate regression analysis using Minitab software v. 19.0 (*10*). This analysis describes the quantitative relationship between the process variables and each response. The multivariant regression model is:

$$y=\beta_0+\sum_{i=1}^{q}\beta_i x_i+\sum_{i=1}^{q}\beta_{ii}x_i^2+\ldots+\sum_{i<j}\sum\beta_{ij}x_i x_j+e \quad /1/$$

where y is the response, β_0 is offset term; β_i, β_{ii}, β_{ij}, are the regression coefficients; and x_i, x_j are the levels of the independent variables.

We conducted a validation experiment to empirically verify the predicted optimal parameters upon establishing the optimal conditions. This involved comparing the experimental results under these conditions against the predictions generated by Minitab software v. 19.0 (*10*), ensuring that the identified parameters lead to the desired encapsulation performance.

Yield analysis

According to Agatha *et al.* (*16*), the spray-dried encapsulated of Lemuru fish protein hydrolysate was collected and weighed with an analytical scale. Yield (*Y*) percentage (*m/m*) of encapsulated Lemuru fish protein hydrolysate was calculated with following equation:

$$Y=\frac{m_2}{m_1}\ \text{x}\ 100 \quad /2/$$

m_1 is the mass of the Lemuru fish protein hydrolysate in liquid form and carrier agent as fed in spray dryer; and m_2 is the mass of the dried encapsulated Lemuru fish protein hydrolysate from spray dryer.

Hygroscopicity analysis

According to the method of Sarabandi *et al.* (*17*) with modification, hygroscopicity of the encapsulated sample was determined by weighing 2 g of the samples placed in a Petri dish and stored in a desiccator containing saturated NaCl solution under a relative humidity of 76 % at room temperature (25 °C). After seven days, the samples were weighed on an analytical balance. The hygroscopicity of the Lemuru fish protein hydrolysate was calculated with following equation:

$$\text{Hygroscopicity} = \left(\frac{(m_2 - m_1)}{m_1}\right) \cdot 100 \quad /3/$$

m_1 is the mass of Lemuru fish protein hydrolysate at the first day (2 g) and m_2 is the final product mass after being stored for seven days.

Water solubility

With modification, water solubility was determined according to Sarabandi *et al.* (*17*). The sample powder (1 g) was dissolved with 100 mL of distilled water using a magnetic stirrer at 1450 rpm for 4 min. The mixture was centrifuge using centrifuge (TOMY, MX-301, Tokyo, Japan) for four minutes at 3000×*g*. The supernatant (25 mL) was oven-dried at 105 °C for 3 to 5 h, and weighed on an analytical balance to determine the dry mass of the insoluble protein. The solubility percentage (*m/m*) was calculated with following equation:

$$\text{Solubility} = 100 - \left(\frac{m_1 - m_2}{m_1} \cdot 100\right) \quad /4/$$

m_1 is the initial mass of Lemuru fish protein hydrolysate before drying process (1 g); m_2 is the final product mass after drying process.

Functional group analysis by Fourier Transform Infra-Red (FTIR)

Functional groups present in both encapsulated and non-encapsulated Lemuru fish protein

hydrolysate were characterized using Fourier Transform Infrared Spectroscopy (Thermo Scientific, Nicolet, iS5 iD5 ATR). This method was adapted and modified from the procedure outlined by Priatni *et al.* (*18)*. The sample powders were homogeneously dispersed in potassium bromide (KBr) to form discs. The spectral data were collected over a comprehensive wavelength range from 4000 to 400 cm^{-1} at the room temperature. The functional group of encapsulated fish protein hydrolysate was compared to non-encapsulated fish protein hydrolysate.

Morphological surface of the fish protein hydrolysate particle by Scanning Electron Microscope (SEM)

Particle morphology of the encapsulated and non-encapsulated fish protein hydrolysate was evaluated by SEM (JSM-IT30, Jeol Ltd., Akhishima, Tokyo, Japan). The sample were put on the sample holder using double conductive tape. The powders were coated with gold under vacuum conditions, and then examined by SEM.

Particle size distribution

According to Priatni *et al.* (*18*) with modification, particle size of both encapsulated and non-encapsulated fish protein hydrolysate was evaluated by *Particle Size Analyzer* (PSA) (Zetasizer Nano ZS Malvern, PANalytical, Worcestershire, UK). An amount of sample (1 g) was mixed with 10 mL distilled water, centrifuged at 3075×*g* for 30 min, and examined with PSA. Particle size distribution analyzed the percentage (%) of intensity.

Statistical analysis

The data obtained were analyzed using analysis of variance (ANOVA) and by the 5 % level. All data analysis was done using the Minitab 19.0 program (*10*).

RESULTS AND DISCUSSION

Response data analysis (yield, hygroscopicity, solubility)

In our study, the spray-dried Lemuru fish protein hydrolysate yield encapsulated within a temperature range of 90 to 100 °C exhibited a broad variation from 0.49 to 3.26 % (*m*/*m*). At an inlet temperature of 90 °C, combining gum arabic and maltodextrin as carrier agents resulted in yields lower than 1.6 %. This observation is in contrast to the findings of Hau *et al.* (*19*) who reported yields of 0.6 to 1.6 % (*m/V*) for protein hydrolysate derived from Yellowstripe scad fish at an inlet temperature

of 80 °C, using an ultrasonic spray dryer. The lower yields at 90 °C indicate insufficient energy for optimal water evaporation, which is a significant factor in higher yields in spray drying. At such low temperatures, the evaporation rate slows, increasing the finished product's moisture content and the potential of water molecules attaching to the drying chamber's interior, resulting in increased product loss (*20*). Conversely, higher inlet temperatures generate more thermal energy, and destabilizing water molecules. This increased energy facilitates the breaking of hydrogen bonds between water and active groups in the hydrolysate, allowing for more efficient and complete water evaporation (*20*).

Response Surface Methodology (RSM) further elucidated the relationship between yield and the pivotal variables of carrier agent type, mass per volume ratio, and inlet temperature (Fig. 1). The yield surface plot in Fig. 1a, indicates a negative correlation between gum Arabic mass per volume ratio and yield. This negative correlation is caused by gum Arabic high sugar content that induces stickiness and product adherence to the chamber walls at higher temperature, thus diminishing yield. The combination of maltodextrin and gum Arabic will increase yield content compared to the only use of gum Arabic, as mentioned by Yarlina *et al.* (*22*). An increase in the maltodextrin mass per volume ratio shows an increase of the yield content. This result is similar to observations from a study on spray-dried red dragon fruit kombucha powder, which noted a similar trend (*16*). Fig. 1b illustrates yield enhancement to above 2.4 % (*m*/*m*) When the inlet temperature is adjusted from 90 to 100 °C, attributable to improved moisture evaporation and a resultant drier product *(23*). However, Fig. 1c presents an opposing scenario where an increase in inlet temperature coupled with higher carrier mass per volume ratio precipitates a notable yield decline from 2.2 to 1 % (*m/m*), underscoring the complex mechanism of the encapsulation process and the critical balance required between temperature, carrier mass per volume ratio, and encapsulation efficacy.

Fig. 1

The hygroscopic nature of food products is an intrinsic property that significantly influences their quality, shelf-life, and storage conditions. Hygroscopicity determines how much a product can absorb moisture from the surrounding environment, which is a critical factor in product stability and longevity (*24*). The hygroscopicity of the encapsulated Lemuru fish protein hydrolysate ranged from 4.53 to 20.16 % (*m/m*). This variation can be attributed to the inherent hydrophilic properties of the encapsulating agents used, on both carrier agents, maltodextrin and gum Arabic.

The response surface plots in Fig. 2a show that the increased level of gum Arabic mass per volume ratio will decrease the hygroscopicity of the encapsulated Lemuru fish protein hydrolysate. Higher amounts of gum Arabic effectively function as a barrier to moisture absorption. It creates a

protective layer around the particles, preventing their exposure to environmental moisture (*25*). The combination of maltodextrin and gum Arabic decreases the hygroscopicity of the encapsulated Lemuru fish protein hydrolysate. These results could be explained by the ability of maltodextrin to reduce hygroscopicity (*26*). This is in line with the findings of Sukri *et al*. (*27*), which suggest that the higher quantity of hydrophilic groups in gum Arabic compared to maltodextrin allows for enhanced interaction with water, leading to increased moisture absorption.

In Fig. 2b, a direct correlation is demonstrated between inlet temperature and hygroscopicity, with an increase from 4.5 to 16 % (*m/m*) observed as temperatures rise from 90 to 96 °C. This relationship is presumably due to a decreased water vapor pressure gradient between the powder and the ambient atmosphere, leading to heightened equilibrium moisture content (*28*). However, a decline in hygroscopicity is noted at temperatures exceeding 96 °C, a finding that echoes the results of Reshan Jayawardena *et al.* (*29*), who reported reduced hygroscopicity in spray-dried beef lung powder with elevated drying temperatures. This reduction is potentially due to protein denaturation and increased surface hydrophobicity, which can repel water molecules (*29*).

Fig. 2c indicates that as carrier mass per volume ratio increases, the hygroscopicity of the encapsulated Lemuru fish protein hydrolysate tends to decrease. Reduced water uptake results from the ability of the carrier to interact with the protein hydrolysate hydrophilic groups. Furthermore, at an inlet temperature of 95 °C, protein denaturation increases in frequency, which probably contributes to this decreased hygroscopicity. These findings support the theory that protein structure undergoes substantial changes at higher temperatures (*30*).

Fig. 2

The solubility of fish protein hydrolysate in water is a critical determinant of its functional performance in food applications. High solubility is essential for effectively incorporating fish protein hydrolysate into diverse food matrices, where it serves as an integral component in forming emulsions, foams, and gels, contributing to the texture and stability of the final product (*31*). The solubility of encapsulated Lemuru fish protein hydrolysate lies within the range of 69.40 to 79.69 % (*m/m*), highlighting its suitability for such applications. It has been observed that the encapsulation process utilizing maltodextrin as the carrier agent significantly enhances solubility compared to gum Arabic. This could be attributed to the lower molecular mass and higher dextrose equivalent of maltodextrin, which confers water compatibility and reduces intermolecular bonding, promoting a more dispersed and soluble end product.

Fig. 3a illustrates a decline in the solubility of encapsulated Lemuru fish protein hydrolysate with increasing maltodextrin concentration. This trend suggests that maltodextrin may interact with the hydrophilic sites of the protein, potentially forming a semi-impermeable matrix that inhibits the interaction of protein with water molecules. As the concentration of maltodextrin rises, the barrier effect intensifies, leading to reduced solubility in aqueous environments, which aligns with the observations made by Ningsih *et al.* (*32*). Conversely, encapsulation with gum Arabic, which is rich in dietary fibers, appears to enhance the dissolution of Lemuru fish protein hydrolysate, possibly due to the ability of hydrocolloids to improve water retention and solubility of the protein.

It is important to recognize that both maltodextrin and gum Arabic play essential roles in preserving the structural integrity of the protein during the drying process. Their protective effects are crucial for maintaining the functional solubility characteristics of the protein, as mentioned by Siregar *et al.* (*33*). According to Fig. 3b, solubility is seen to exceed 79 % (*m/m*) as the drying inlet temperature increases. This increase in solubility can be attributed to the diminished hygroscopic nature of the powder at elevated temperatures, which positively influences solubility, in line with findings from Araújo *et al.* (*21*). However, gum Arabic-encapsulated Lemuru fish protein hydrolysate shows a significant decrease in solubility when processed at temperatures ranging from 90 to 96 °C. This decrease in solubility could be caused by particle agglomeration, which complicates particle reconstitution in water. Fig. 3c reinforces this point by demonstrating how agglomeration at lower temperatures reduces solubility. Beyond 96 °C, hydrogen bonds between hydrophilic groups and water molecules are likely to be disrupted, resulting in increased water evaporation and a decrease in the dissolving ability of protein.

Fig. 3

RSM-Box Behnken Design result

The fish protein hydrolysate encapsulation process can be effectively modeled using the Response Surface Methodology (RSM) Box-Behnken design, as demonstrated by the regression equation expressed as a second-order polynomial equation. Table S1 is the inputted experimental data from responses for RSM optimization.

As shown in Table S2, this equation incorporates constant terms, linear and quadratic coefficients, and cross-product interaction terms to account for the interdependent effects of carrier agent type (x1), carrier concentration (x2), and inlet temperature (x3). Table 1 shows the non-significant lack of fit values for the yield and solubility responses, with the regression model close to

1 and p-values greater than 0.05, indicating that the model's predictions are consistent with the experimental data and supporting the model's statistical robustness. The model clarifies a significant interaction ($p \leq 0.05$) between the type of carrier agent and its concentration, especially for the yield response. This indicates that these factors work together to influence the yield of encapsulated fish protein hydrolysate and that maximizing the encapsulation process depends critically on how these variables interact.

Based on the RSM-Box Behnken analysis, the optimized conditions for encapsulating fish protein hydrolysate are identified when using maltodextrin as the carrier agent at a 25 % (*m/v*) concentration and an inlet temperature setting of 100 °C. These parameters achieved a high desirability index of 0.864, suggesting an effective balance between yield, solubility, and hygroscopicity (Fig S1). Further, all the responses of the products were validated through laboratory experiments. The actual and predicted values are present in Table S3. Data showed that the actual and predicted values were in the range (95 % prediction interval); thus, the reliability of the optimized condition was verified. The predicted response values were obtained by calculating the experiment data in Table S1 using the equations in Table S2. The validation was done by comparing actual and prediction values of each response.

Due to their favorable physicochemical properties, Maltodextrin is frequently selected as encapsulating matrices in spray drying applications. They show high solubility and low hygroscopicity, which are critical for the stability of encapsulated products. Additionally, maltodextrins maintain low viscosity at elevated concentrations, facilitating efficient spray drying processes (*34*). Kurozawa *et al.* (*15*) highlighted the superiority of maltodextrin as an encapsulating agent for chicken meat protein hydrolysate in spray drying, noting its ability to yield products with lower hygroscopicity than gum Arabic. This is particularly beneficial for the storage and handling of dried products. Furthermore, the consensus from various studies suggests that the optimal mass per volume ratio range for maltodextrin as an encapsulant lies between 25 and 30 %. Within this mass per volume ratio threshold, maltodextrin provides optimal powder recovery and encapsulation efficiency, as evidenced by the high-quality characteristics of the encapsulated material (*35*). The ability of maltodextrin to form an amorphous and porous matrix contributes to its effectiveness in encapsulation, supporting the entrapment of volatile compounds and the protection of sensitive ingredients from oxidative damage.

Utilizing the robust Response Surface Methodology (RSM)-Box Behnken Design optimization framework, an encapsulated Lemuru fish protein hydrolysate sample was selected and produced under the most favorable conditions. These conditions were determined to be a 25 % maltodextrin mass per volume ratio and a spray drying inlet temperature of 100 °C, predicted to have the highest

encapsulation efficiency and product characteristics. We assume that utilizing a lower temperature of 100 °C for spray drying fish protein hydrolysate is advantageous for several reasons. In our study, a spray dryer was designed for inlet temperatures between 90 and 100 °C. It ensures the preservation of sensitive bioactive compounds, which might otherwise degrade at higher temperatures, thus maintaining the fish protein hydrolysate's nutritional and functional integrity. This temperature optimizes moisture removal efficiency without the excessive energy costs associated with higher temperatures, making the process more economical and environmentally sustainable.

Table 1

Functional groups

Fig. 4 presents the Fourier Transform Infrared (FTIR) spectra of Lemuru fish protein hydrolysate in both encapsulated and non-encapsulated forms. The spectra reveal characteristic absorption bands about various functional groups. For both sample types, NH3 deformation in free amino acids like lysine is indicated within the 1050-1000 cm^{-1} spectral region. The encapsulated fish protein hydrolysate displays a C-N stretch at 1078 cm^{-1}, slightly shifted from the 1076.75 cm^{-1} peak of the non-encapsulated fish protein hydrolysate, suggesting a potential interaction of the carrier matrix with the amino groups. Amide I bands, indicative of protein secondary structure, appear at 1631 cm^{-1} for the encapsulated fish protein hydrolysate and 1594 cm^{-1} for the non-encapsulated fish protein hydrolysate, which could imply some conformational changes due to the encapsulation process. The peaks at 1335 cm^{-1} and 1366 cm^{-1} are attributed to O-H bending vibrations in alcohol functional groups, a sign of polysaccharides like maltodextrin in the encapsulated fish protein hydrolysate. Furthermore, the broader O-H stretching vibration observed at 3324 cm^{-1} for encapsulated fish protein hydrolysate, compared to 3253 cm^{-1} for non-encapsulated fish protein hydrolysate, along with a shift in the C-H stretching vibrations from 2898 cm^{-1} to 2923 cm^{-1}, indicates an increased number of hydroxyl groups due to encapsulation. These O-H and N-H functional groups are integral to the water-binding capacity of fish protein hydrolysate, with the observed shifts suggesting that encapsulation may influence the moisture interaction and solubility of the hydrolysate.

The FTIR spectral analysis between 3750–1000 cm^{-1} wavelengths shows a decrease in transmittance for the encapsulated Lemuru fish protein hydrolysate compared to its non-encapsulated counterpart. This reduction in transmittance indicates a denser molecular packing within the encapsulated product, likely due to the formation of a molecular complex between the maltodextrin polysaccharide carrier and the core protein molecules (*36*). Distinct differences in functional groups

are evident when comparing encapsulated and non-encapsulated Lemuru fish protein hydrolysate. The encapsulated form shows a C-O stretching band at 1205 cm^{-1}, characteristic of the glycosidic linkages in glucose unit of maltodextrin, which consistent with Maqsoudlo *et al.* (*37*). According to Castro-Cabado (*38*), the peaks at 998 and 1103 cm^{-1} in the encapsulated fish protein hydrolysate spectra can be interpreted as the stretching vibrations of anhydroglucose rings, suggesting the formation of these structures as a result of the encapsulation process. This formation occurs through the glycosidic bonding of glucose units from maltodextrin with protein molecules, coupled with the release of water molecules. The O-H stretching vibration in the encapsulated fish protein hydrolysate shifts to 3324 cm^{-1}, indicating potential hydrogen bonding interactions between the fish protein hydrolysate and maltodextrin. The sharper and more defined peak at this wavenumber for the encapsulated fish protein hydrolysate, compared to the broader peak at 3253 cm^{-1} for the non-encapsulated version, implies a modified hydrogen bonding environment. This sharpening of the O-H peak is indicative of a structured environment around hydroxyl groups, which may correlate with an enhanced water solubility of the encapsulated product.

Fig. 4

Particle morphology

Fig. 5 presents SEM images that contrast the microstructure of non-encapsulated and optimally encapsulated Lemuru fish protein hydrolysate. Quantitative image analysis indicates that the average particle size for the non-encapsulated fish protein hydrolysate is approximately 13.53 µm, whereas the encapsulated fish protein hydrolysate exhibits a slightly reduced average size of 12.2 µm. This size reduction in the encapsulated particles can be attributed to the densification effect during the encapsulation process. Moreover, the encapsulated particles display a characteristically wrinkled surface topology, a consequence of the rapid moisture evaporation facilitated by high-temperature spray drying and the formation of a carbohydrate matrix from maltodextrin. This surface morphology is indicative of the film-forming and drying kinetics that are unique to the encapsulation process.

In the SEM image, the non-encapsulated fish protein hydrolysate particles appear to have a smoother surface texture than encapsulated fish protein hydrolysate. This effect occurs as the thermal expansion of trapped gum arabics within the droplets exerts pressure on the forming particle walls, leading to a smooth and taut surface. Fig. 5a shows the presence of link bridges between particles of non-encapsulated fish protein hydrolysate, likely due to the higher hygroscopicity of the material. These link bridges, as observed in the findings of Kurozawa *et al.* (*34*), are attributed to the tendency of non-encapsulated protein hydrolysates to absorb moisture, leading to particle agglomeration (*34*).

The encapsulation process, particularly with maltodextrin, is inferred to reduce these hygroscopic interactions, thereby diminishing the tendency for link bridge formation and potentially enhancing the powder's flowability and stability.

Fig. 5

Particle size distribution

Particle size analysis, as depicted in Fig. 6, reveals a narrower particle size distribution for Lemuru fish protein hydrolysate encapsulated with a 25 % maltodextrin mass per volume ratio, with sizes ranging from 396.1 to 1281 nm and a mean diameter of 520.2 nm, indicating a Z-average value. In contrast, the non-encapsulated fish protein hydrolysate samples demonstrated a broader particle size distribution, from 255.0 to 1718 nm, and a larger mean particle diameter of 428.8 nm, as evidenced by their respective Z-average. The encapsulated samples show a higher uniformity in particle size distribution than their non-encapsulated counterparts. This uniformity can be attributed to the viscosity of the fish protein hydrolysate-maltodextrin mixture, which forms a more cohesive matrix during spray drying. The higher viscosity aids in stabilizing the droplets as they pass through the nozzle, resulting in less expansion and more uniform particle sizes despite the forces of thermal expansion (*37*). The reduction size of encapsulated particles and narrow distribution contribute to an increased specific surface area, which is hypothesized to enhance the solubility of the encapsulated Lemuru fish protein hydrolysate. A larger surface area facilitates a greater interaction with the solvent, thereby potentially increasing the rate and extent of dissolution.

Fig. 6

CONCLUSIONS

In conclusion, this study has effectively optimized Lemuru fish protein hydrolysate's encapsulation process using spray drying techniques. Using the RSM-Box Behnken Design, it was found that the mass per volume ratio of maltodextrin of 25 % and an inlet temperature of 100 °C are the ideal conditions for achieving the best quality of the encapsulated product. Under these conditions, a significant interaction ($p \leq 0.05$) was observed between the carrier agent type and mass per volume ratio influencing the yield of encapsulated fish protein hydrolysate. Moreover, the model predictions for yield and solubility were validated, as indicated by a non-significant lack of fit values ($p > 0.05$). It was shown that encapsulated Lemuru fish protein hydrolysate exhibits lower hygroscopicity compared to its non-encapsulated counterpart, which is evidenced by the reduced formation of link bridges and highlighted in SEM imaging. Further analytical evaluations using FTIR and PSA suggested that

encapsulation enhances the solubility of Lemuru fish protein hydrolysate, potentially due to the increased presence of O-H functional groups and a greater specific surface area provided by the maltodextrin carrier. These findings reinforce the value of encapsulation in improving the functional properties of fish protein hydrolysate and provide an understanding of the physical and chemical characteristics of food application.

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CONFLICT OF INTEREST

We declare all authors have no conflict interest.

SUPPLEMENTARY MATERIALS

Supplementary materials are available at: www.ftb.com.hr.

AUTHORS' CONTRIBUTION

All of the authors made significant contributions to the study. Ayu Hanifah did the experiments, analysis data, and paper. Sri Priatni prompted the idea and the co-author helped with the experiments, analysis data and paper. Wawan Kosasih and Diah Ratnaningrum assisted co-author with the experiments and prepared the sample for the research. The spray dryer was designed and created by Herlian Eriska. Dian Andriani, and Sri Priatni revised the experiment method and paper. Yelliantty was prompted the research idea and experiment method.

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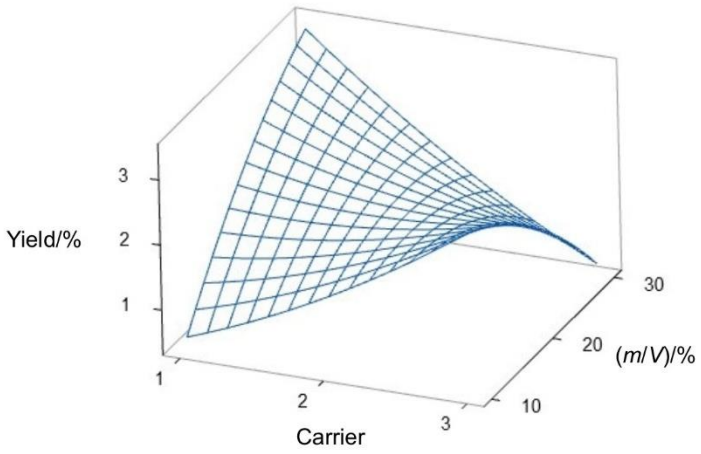
Table 1. The p-values of each response

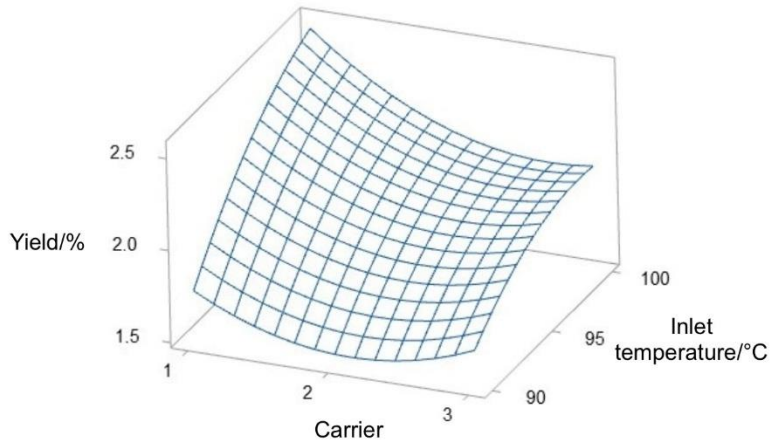
Term	Yield/%	Hygroscopicity/%	Solubility/%
	p value		
Model	0.182	0.791	0.206
Carrier agent (x_1)	0.557	0.590	0.204
Carrier mass per volume ratio (x_2)	0.609	0.254	0.567
Inlet temperature (x_3)	0.281	0.663	0.220
x_1 x_1	0.617	0.731	0.684
x_2 x_2	0.402	0.445	0.811
x_3 x_3	0.745	0.300	0.075
x_1 x_2	**0.011**	0.723	0.060
x_1 x_3	0.762	0.603	0.209

x_2 x_3	0.227	0.844	0.226
Lack of fit	**0.093**	0.050	**0.781**
Regression model	0.80	0.50	0.79

a)



b)



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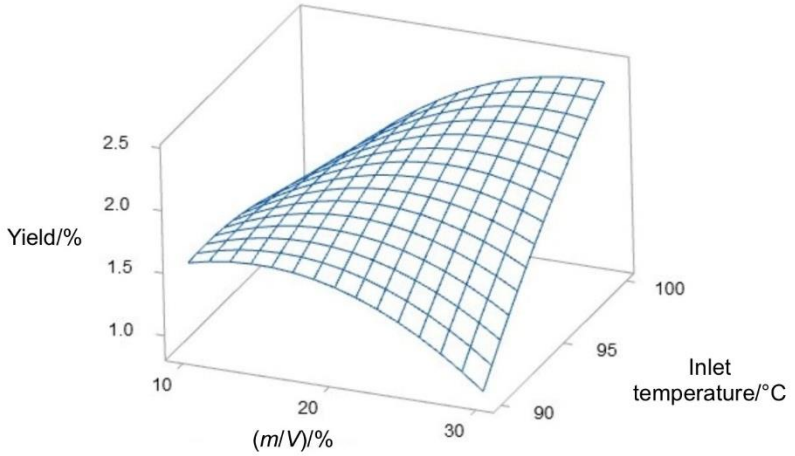


Fig. 1. Surface plots of yield responses as a function of carrier: a) maltodextrin, b) maltodextrin and gum Arabic, and c) gum Arabic

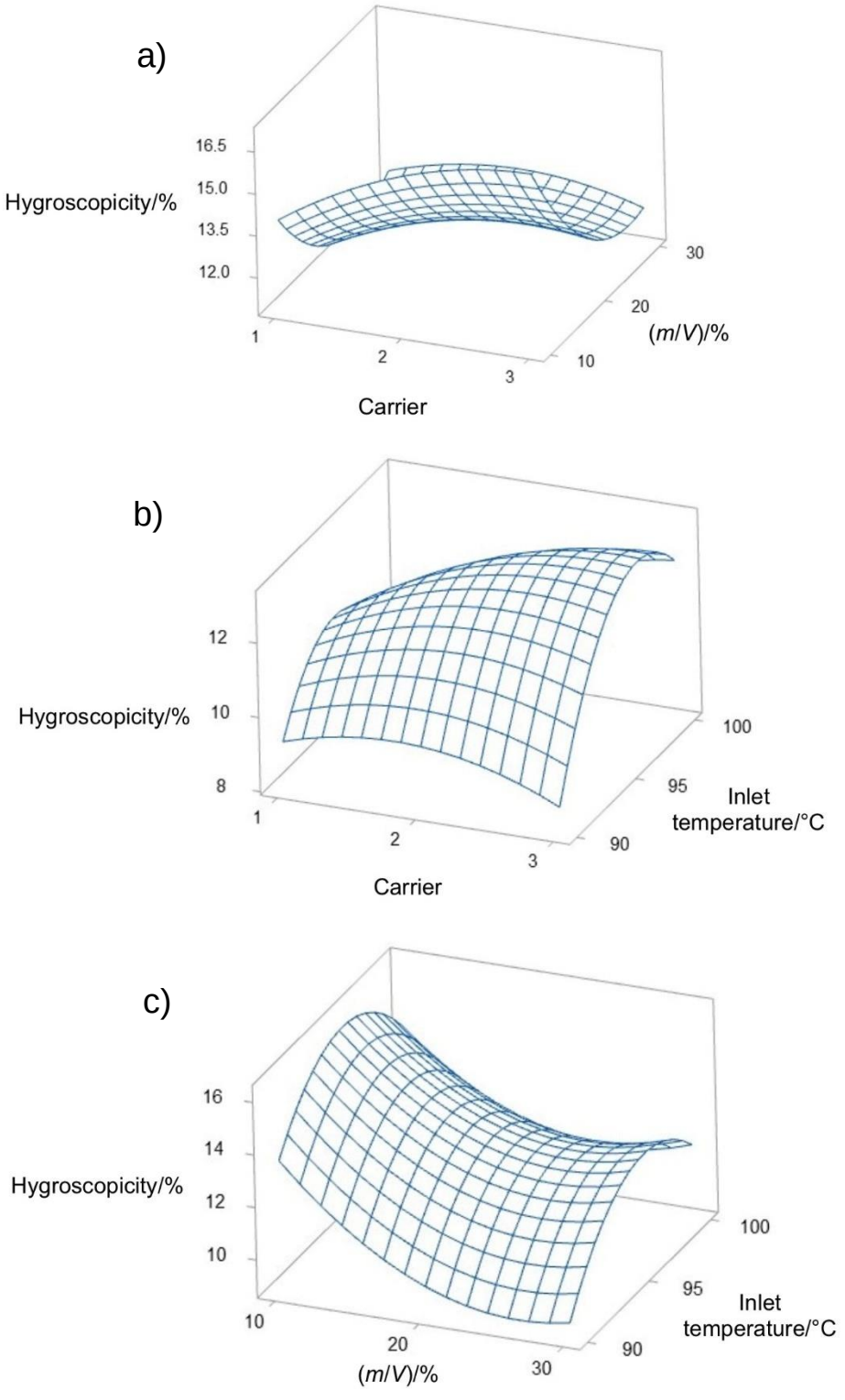


Fig. 2. Surface plots of hygroscopicity responses as a function of carrier: a) maltodextrin, b) maltodextrin and gum Arabic, and c) gum Arabic

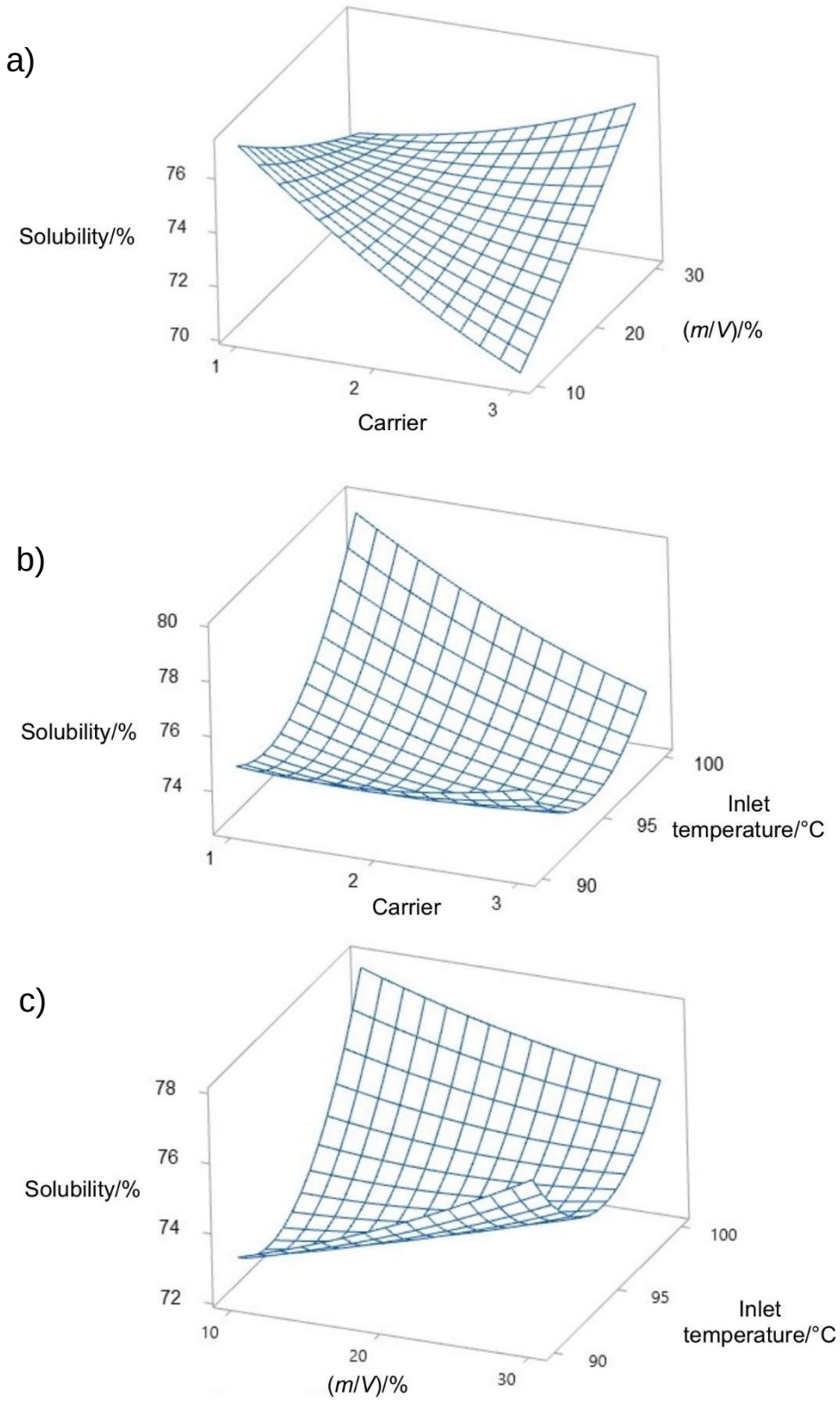
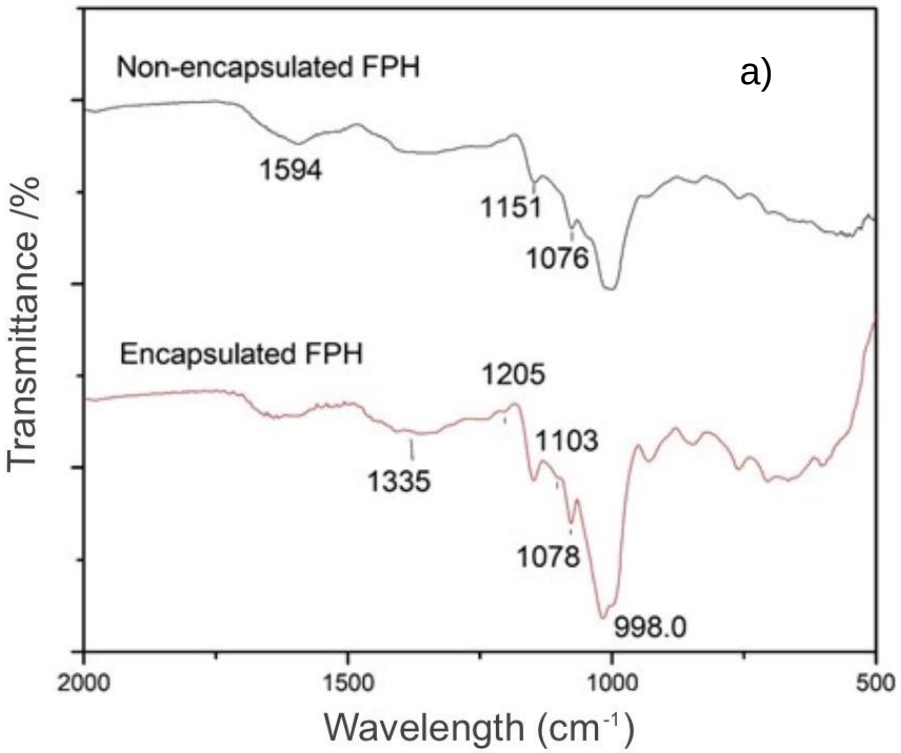


Fig. 3. Surface plots of solubility responses as a function of carrier: a) maltodextrin, b) maltodextrin and gum Arabic, and c) gum Arabic



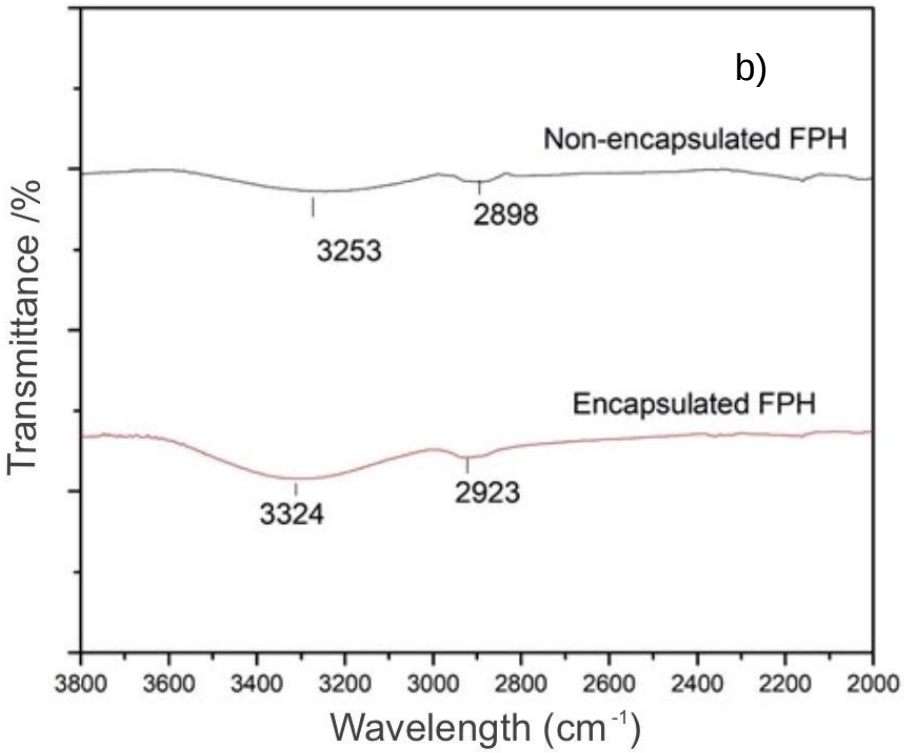


Fig. 4. FTIR spectra of non-encapsulated and encapsulated Lemuru fish protein hydrolysate in the range of: a) 500 to 2000 cm^{-1} and b) 2000 to 3000 cm^{-1}

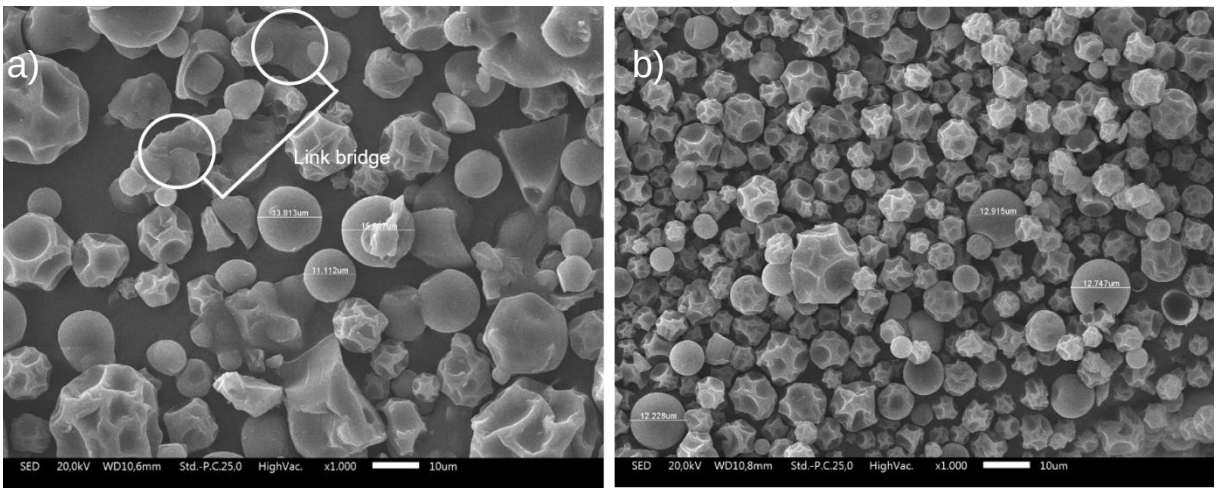


Fig. 5. SEM photographs of: a) non-encapsulated and b) encapsulated Lemuru fish protein hydrolysate with 1000×magnification

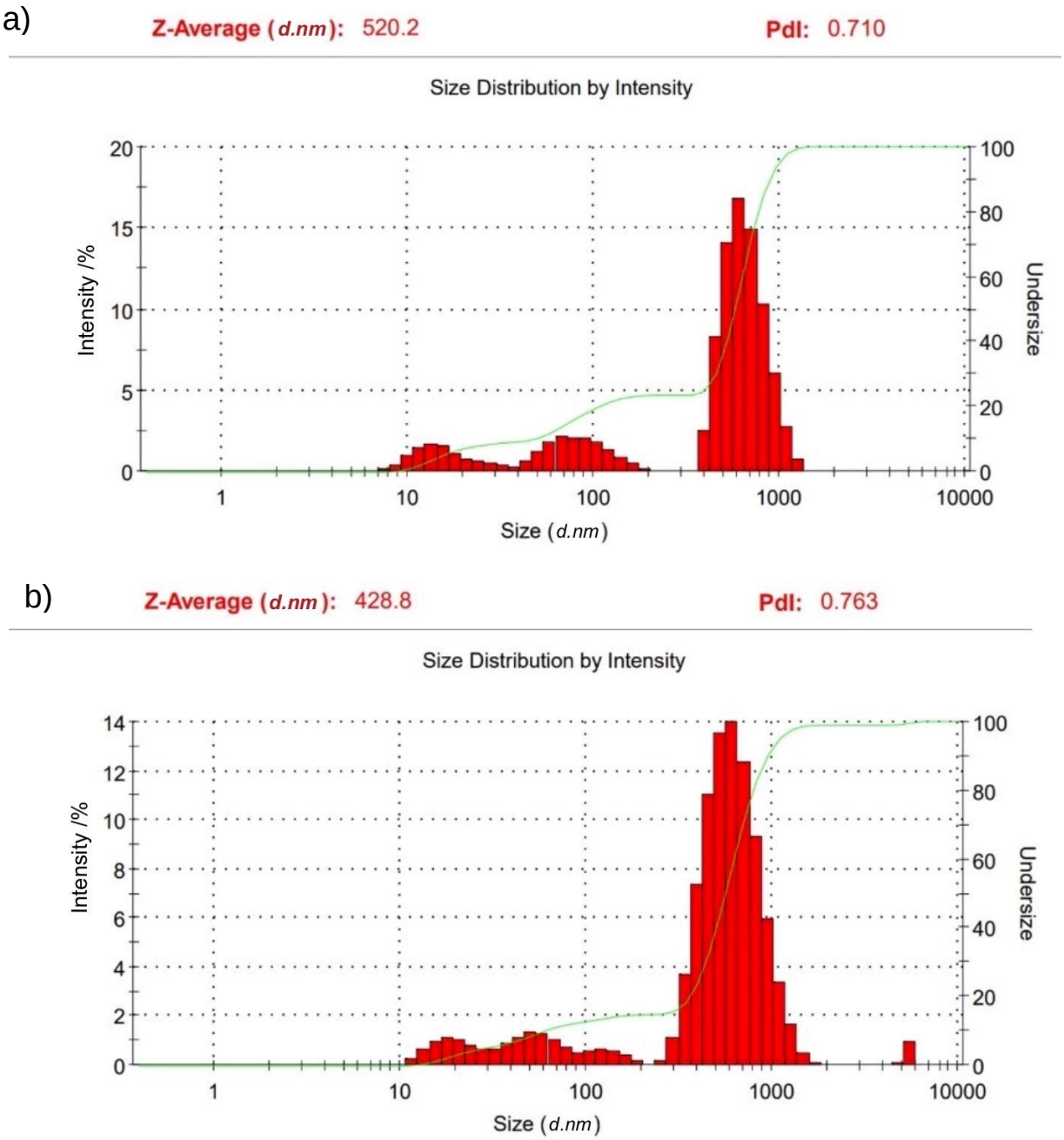


Fig. 6. PSA result of: a) encapsulated and b) non-encapsulated Lemuru fish protein hydrolysate

SUPPLEMENTARY MATERIAL

Tabel S1. Experimental data for the optimization using RSM

Run	Carrier	(*m*/*V*)/%	Inlet temperature/°C	Yield/%	Hygroscopicity/%	Solubility/%
1	Carrier 2	10	100	1.394	12.185	78.684
2	Carrier 1	10	95	0.505	11.710	76.084
3	Carrier 2	30	90	0.694	11.080	75.920
4	Carrier 1	20	100	2.353	12.450	79.692
5	Carrier 3	20	100	1.403	10.900	74.713
6	Carrier 3	20	90	1.858	4.525	75.403
7	Carrier 2	20	95	2.117	12.810	75.043
8	Carrier 2	20	95	1.633	11.525	74.609
9	Carrier 1	20	90	2.384	10.465	74.713
10	Carrier 3	10	95	3.264	20.155	69.403
11	Carrier 3	30	95	0.489	14.155	76.880
12	Carrier 1	30	95	2.951	8.680	74.027
13	Carrier 2	30	100	2.981	10.305	74.963
14	Carrier 2	10	90	0.931	14.600	74.217
15	Carrier 2	20	95	2.052	13.815	70.547

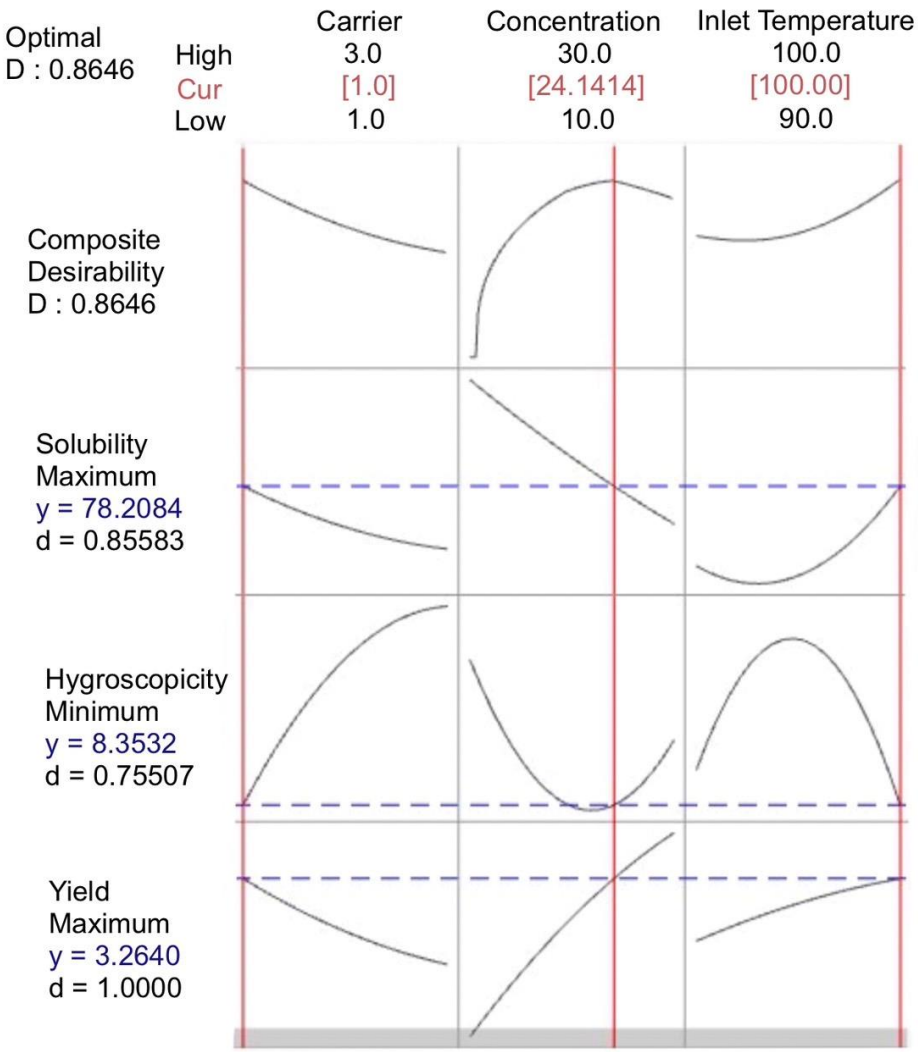
Carrier 1=maltodextrin, carrier 2=maltodextrin and gum Arabic, carrier 3=gum Arabic

Table S2. Regression equation of each response

Response	Regression equation
Yield/%	$y = 1.93 - 0.147x_1 + 0.126x_2 + 0.284x_3 + 0.18(x_1)^2 - 0.317(x_2)^2 - 0.119(x_3)^2 - 1.311x_1x_2 - 0.107x_1x_3 - 0.457x_2x_3$
Hygroscopicity/%	$y = 12.72 + 0.80x_1 - 1.80x_2 + 0.65x_3 - 0.75(x_1)^2 + 1.71(x_2)^2 - 2.38(x_3)^2 - 0.74x_1x_2 + 1.10x_1x_3 + 0.41x_2x_3$
Solubility/%	$0 - 1.014x_1 + 0.425x_2 + 0.975x_3 + 0.44(x_1)^2 + 0.26(x_2)^2 + 2.29(x_3)^2 + 2.383x_1x_2 - 1.418x_1x_3 - 1.356x_2x_3$

Table S3. Prediction and actual value of each response

Response	Prediction	Actual	95 % PI Low	95 % PI High
Hygroscopicity/%	8.3532	7.66	5.37	22.08
Solubility/%	78.2084	76.20	71.39	85.03
Yield/%	3.2766	3.07	0.9628	5.585



Carrier [1]: maltodextrin

Fig. S1. The desirability of the responses