

The Effect of Spray Drying Temperature on the Emulsifying Properties of Jack Bean Protein Concentrate

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Abstract—Jack bean (*Canavalia ensiformis* L.) is one of the local legumes that has the potential to be developed as a plant-based emulsifier. Currently, the spray drying method is widely used in the food industry. The setting conditions, in this case the inlet temperature, affect the characteristics of the protein and the emulsifying properties of jack bean protein. This study evaluated the effect of the spray drying inlet temperature (temperatures 160 °C and 210 °C) on protein content, color (L^* , a^* , b^* values), Amadori and Melanoidin content, as well as jack bean protein solubility. This study also evaluated an Emulsion Activity Index (EAI), creaming index, adsorbed protein, and optical microscopy of jack bean protein emulsions. The results showed that the protein content of jack bean protein dried at different temperatures was also significantly different ($p < 0.05$). Similarly with color, solubility, and Amadori and Melanoidin compounds. Jack bean protein extract dried at 160 °C had higher protein content and solubility. Upon application in emulsion system, both protein samples displayed high Emulsifying Activity Index (EAI) of 191.34 m²/g for jack bean protein dried at 160 °C and 159 m²/g of which dried at 210 °C. Nevertheless, the stability of the protein-stabilized emulsions were still low as indicated by creaming index values reaching 72% and 71% after one hour of observation due to the large particle size. This result suggested that spray drying at 160 °C provided better techno-functionality as compared to at 210 °C.

Keywords—Jack bean, spray dryer, inlet temperature, protein, emulsifying properties

I. INTRODUCTION

Recently, the shift from animal-based protein consumption to plant-based protein sources has been increasingly promoted. The shift in consumption patterns is due to the high price of animal protein sources and their impact on health, the environment, and climate [1]. Jack bean (*Canavalia ensiformis* L.) is a potential plant-based protein source due to its high protein content [2]. Jack bean consists of protein (28.8–39.3%), carbohydrates (46–65%), fiber (4.7–11.4%), and fat (1–4%) [3]. The relatively high protein content in jack beans makes them a promising candidate as a plant-based emulsifier in food products. According to the research of [4], Jack bean protein extracted using various methods has an emulsifying activity above 100 m²/g.

An emulsion is a multiphase dispersion consisting of two immiscible liquids, in which one phase is dispersed as fine droplets within the other phase. Based on the dispersed phase, emulsions are classified as Oil-in-Water (O/W) or Water-in-Oil (W/O) systems, where oil or water serves as the continuous phase [5]. The formation and stability of emulsions are significantly influenced by the characteristics of the interface layer that separates the dispersed phase and

the continuous phase. This influence is mainly determined by the interaction between the emulsifier and the two phases [6]. Protein as an emulsifier has amphiphilic properties, meaning it has hydrophilic and hydrophobic groups that are distributed and adsorbed on the surface droplets [7]. The emulsifying properties of a protein are influenced by its characteristics and techno-functional properties, such as the solubility of the protein.

Spray drying is one of the most popular drying methods in the food industry because the process is efficient and can affect the properties of the resulting product [8]. In addition, spray drying conditions affect the techno-functional properties of protein through mechanisms of hydrophobic interaction, denaturation, and aggregation [9]. One of the conditions that needs to be controlled in the use of spray drying is the inlet temperature. The use of high temperatures can induce denaturation, aggregation or the Maillard reaction during the drying process. According to research [9], an increase in the inlet temperature of spray drying causes an increase in surface hydrophobicity, protein particle size, and emulsion droplet size of PPI. However, studies investigating the effect of spray drying temperature on the emulsifying properties of jack bean protein concentrates are still limited.

Therefore, the purpose of this research was to evaluate the effect of spray drying inlet temperature on the characteristic protein and the emulsifying properties of jack bean protein concentrates. Jack bean extract was dried using spray drying at inlet temperatures of 160 °C and 210 °C. The information obtained in this study can be used to optimize the spray drying process conditions for jack bean protein concentrates and as material for future research.

II. MATERIALS AND METHODS

A. Materials

Jack bean (*Canavalia ensiformis* L.) from Gunung Kidul, Indonesia. Sunflower oil from a local market. Sodium Dodecyl Sulfate (SDS), di-Sodium hydrogen phosphate anhydrous, Sodium dihydrogen phosphate monohydrate, and NaOH were obtained from Merck KgaA (Darmstadt, Germany). HCl was obtained from Mallinckrodt (Staines-upon-Thames, UK).

B. Jack Bean Protein Concentrate (JPC) Extraction

The production of JPC followed the method [4], with slight modifications. Jack beans were peeled and dried, then ground using a grinder and sieved using a 60-mesh sieve. The jack bean flour is suspended using distilled water at a ratio of 1:10 and adjusted to a pH 9 using 1M NaOH, then stirred for 1h at

RT. The suspension was extracted using ultrasonic-assisted extraction with a probe-type sonicator (UP200St Hielscher, Germany) at 100% amplitude and duty cycle, 30 ± 5 °C for 10 min. After ultrasonic treatment, the solution was separated by centrifugation at 8000 rpm for 10 min to collect the supernatant. The supernatant was adjusted to pH 4.2 using 1M HCl and left to stand for 1h, then centrifuged again. The resulting precipitate was rinsed and its pH adjusted to pH 7. The sample was dried using spray drying (Buchi mini spray dryer B-290, Switzerland) at inlet temperatures of 160 °C and 210 °C.

C. Determination of Protein Content and Recovery Rate

Crude protein content was analyzed using the Kjeldahl method in accordance with AOAC (2005) procedures. Protein recovery rate was calculated based on the following equation.

$$\text{Protein recovery rate (\%)} = \frac{E_w \times E_p \times 100}{R_w \times R_p} \quad (1)$$

E_w means weight (g) of concentrate protein on a dry basis; R_w means initial weight (g) of raw material; E_p means protein content (%) of concentrate protein; R_p means protein content (%) in initial raw material.

D. Color

Color measurement of the powder prefers the method described by [10]. Samples were placed in closed plastic petri dishes. Color measurement is performed from the bottom of the petri dish using a colorimeter (Minolta Model CM-2500D Spectrophotometer, Konica Minolta Sensing Inc., Osaka, Japan) with SCI (Specular Component Included). Color development is expressed as ΔE (Eq. (2)), where L^* represents lightness, a^* represents redness, and b^* represents yellowness.

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (2)$$

E. Solubility

Solubility refers to a modified method [11] with slight modifications. A protein solution (0.1%, phosphate buffer pH 7) was prepared. The solution was stirred for 2 h at room temperature, then centrifuged at 11,000 g for 10 min. The protein content of the supernatant was measured using a simplified Lowry method.

F. Measurement of Amadori Compounds and Melanoidins

The Maillard reaction produces Amadori and Melanoidin that can be measured at wavelengths of 304 and 420 nm [12]. JPC (1 mg/mL) is dissolved in distilled water, then its absorbance is measured using a UV-Vis spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, Thermo Scientific, USA).

G. Emulsion Preparation

Emulsion preparation refers to the method [4] with slight modifications. The emulsion was prepared by mixing 80 mL of protein suspension (0.1%, phosphate buffer pH 7) with 20 mL of sunflower oil using an Ultra-Turrax T50 digital homogenizer at 10,000 rpm for 4 min.

H. Emulsion Activity Index (EAI)

EAI of JPC was evaluated according to the method [4]. 50 μ L of emulsion was taken from the lower third of the tube and added to 4.950 μ L of SDS solution (1.0%). The absorbance of the emulsion was measured at 500 nm. EAI was calculated using the equation as follows:

$$\text{EAI (m}^2/\text{g)} = (2 \times 2.303) / (C \times (1 - \phi) \times 1000) \times A_0 \times D \quad (3)$$

A_0 is the absorbance at 0 min, C is the amount of protein per unit volume (0.1%), ϕ is the oil fraction (0.2), and D is the dilution factor ($d = 100$).

I. Creaming Stability

Creaming stability was evaluated according to the method [13] with slight modifications. Fresh emulsion is placed in a 15 mL Falcon tube, and cream layer separation is observed for 7 h at room temperature. Every hour, the height of the serum layer (H_c) and the total emulsion height (H_e) are measured using a ruler (mm) to determine the creaming index (H).

$$H (\%) = \frac{H_c \times 100}{H_e} \quad (4)$$

J. Optical Microscopy

The optical image of the emulsion droplets was measured qualitatively using a digital microscope (Nikon 80i, Japan) with 40 $\times$ magnification.

K. Adsorbed Protein

Adsorbed Protein (AP) refers to the method [14] with slight modifications. 1 mL of each sample was placed in a microtube and centrifuged at 7500 g for 30 min at 25 °C. The supernatant was filtered using a 0.22 μ m filter syringe and then tested for protein content using a simplified Lowry method. AP (%) was calculated based on the following equation.

$$\text{AP (\%)} = \frac{(C_o - C_f) \times 100}{C_o} \quad (5)$$

C_o was the initial protein concentration (mg/mL) of JPC solutions, and C_f was the protein concentration of filtrate protein (mg/mL).

L. Statistical Analysis

Experiments were conducted in triplicate unless otherwise stated. Results are expressed as mean $\pm$ standard deviation, and data analysis was performed using SPSS statistical software (SPSS 27.0, IBM). Comparison of mean values was performed to determine significant differences using an independent t -test at a 95% confidence level ($p < 0.05$).

III. RESULTS AND DISCUSSION

A. Protein Content and Recovery Rate

The protein content and protein recovery rate of jack bean protein concentrate are presented in Table 1. The protein content of jack bean protein concentrates dried using the spray drying inlet temperature at 210 °C (E2) was significantly different ($p < 0.05$) compared to samples dried

at 160 °C (E1). The protein content in sample E2 was higher, reaching 81.95%, while in sample E1 it only reached 71.84%. This is thought to be due to the use of very high temperatures, which increase drying efficiency and thus reduce protein loss during the drying process. The use of high temperatures results in more efficient mass and heat transfer and reduces the likelihood of particles sticking to the walls of the drying chamber [15]. Meanwhile, the recovery rate of sample E1 was not statistically significantly different ($p > 0.05$) from that of sample E2.

Table 1. Protein content and recovery rate of JPC with different inlet temperatures

Treatments	Protein Recovery Rate (%)	Protein Content (%)
E1	36.86 ± 0.94 ^a	71.84 ± 0.65 ^b
E2	37.99 ± 1.31 ^a	81.95 ± 2.72 ^a

Different letters indicate a significant difference ($p < 0.05$).

B. Color

The color of the samples compared to the biomass is shown in the color development (ΔE). Table 2 shows that sample E2 experienced a decrease in L^* value and an increase in b^* value. The color of the sample appeared darker and yellowish. The decrease in L^* value is related to lysine availability [16]. An increase in inlet temperature will cause greater heat exposure to the particles, so that the Maillard reaction occurs more intensively [17]. The use of a very high inlet temperature induces the Maillard reaction not only in the early stages but also continues to the final stage. This is in line with the results of the Amadori and melanoidin tests on both samples.

Table 2. Color development of JPC with different inlet temperatures

Treatments	ΔL^*	Δa^*	Δb^*	ΔE
E1	0.98 ± 0.02 ^a	0.07 ± 0.02 ^a	-1.20 ± 0.13 ^a	1.55 ± 0.09 ^a
E2	-0.38 ± 0.10 ^b	0.30 ± 0.07 ^b	0.98 ± 0.06 ^b	1.10 ± 0.05 ^b

Different letters indicate a significant difference ($p < 0.05$).

C. Amadori and Melanoidin

Fig. 1 shows that the spray drying inlet temperature has a significant effect ($p < 0.05$) on the Maillard reaction that occurs in jack bean protein concentrate. Sample E2 had higher Amadori and Melanoidin values than sample E1. This indicates that at a spray drying inlet temperature of 210 °C, tends to induce Maillard reaction more intensively than the 160 °C. These results are consistent with the color test on each sample. Increasing temperature can accelerate reaction rates by increasing the movement of reactant molecules and promoting the formation of reactive intermediates. This results in increased concentrations of the Amadori complex and Melanoidins [18].

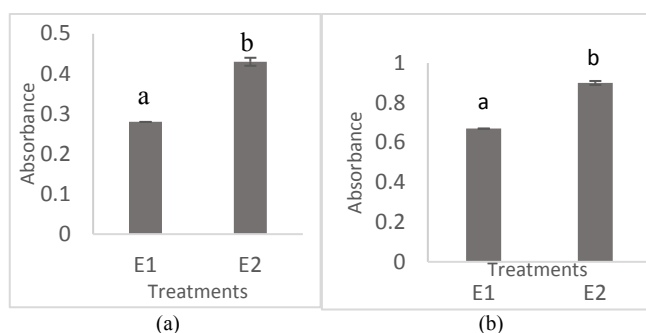


Fig. 1. Formation of compounds. (a) Amadori (A_{304}), (b) Melanoidin (A_{420}).

D. Solubility

The solubility of protein will affect its techno-functional properties, especially its emulsification ability. The solubility values of jack bean protein concentrate dried using the spray drying method at temperatures of 160 °C and 210 °C can be seen in Fig. 2. The figure shows that the solubility (%) of jack bean protein concentrate dried at 160 °C is higher than that of jack bean protein concentrate dried at 210 °C. The solubility value of the protein concentrates dried at 160 °C reached 45.02%, while that dried at 210 °C was only 18.03%. This may be due to the use of excessively high temperatures, which caused excessive denaturation of the protein, thereby reducing its solubility [19]. This denaturation causes the opening of hydrophobic groups that were previously protected within the protein structure, thereby increasing protein-protein interactions and aggregate formation. This aggregation inhibits the diffusion of protein molecules into the aqueous phase, which ultimately reduces protein solubility [20].

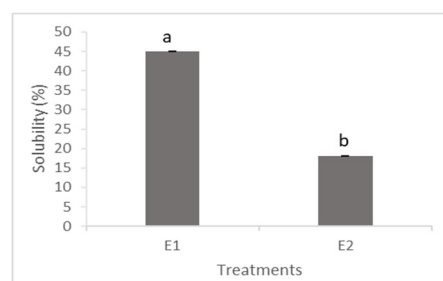


Fig. 2. Differences in protein solubility of JPC at different inlet temperature.

E. EAI

The effect of spray drying temperature on the emulsification activity of jack bean protein concentrate has been explored (Fig. 3). The emulsification activity of protein dried by spray drying at 160 °C was higher than that of jack bean protein dried at 210 °C ($p < 0.05$). These results are consistent with the solubility values of the two samples. Higher solubility values allow for rapid protein migration and adsorption at the water/oil interface to form a film layer around oil droplets [21]. The EAI value of sample E1 reached 191.34 m^2/g , while sample E2 was 159 m^2/g . Based on previous research by Ajayi [4], Jack bean protein emulsion extracted using a similar method but with a different drying technique showed an EAI value of 125.28 m^2/g .

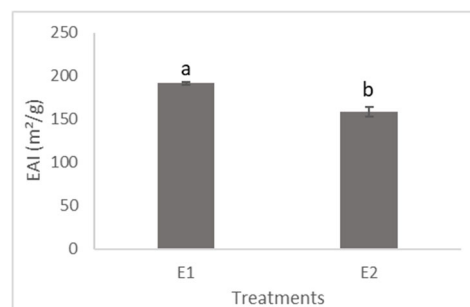


Fig. 3. Emulsion Activity Index (EAI) value in m^2/g .

F. Creaming Stability

Creaming is a common form of instability that occurs in

oil-in-water emulsions [7]. Creaming occurs due to the difference in density between oil and water, which is influenced by gravitational forces. The creaming value of jack bean protein concentrate emulsions was measured during 7 h of storage. Based on Fig. 4, the creaming index values of emulsion samples E1 and E2 did not differ significantly ($p < 0.05$). Although the EAI test results for sample E1 showed a higher EAI value, both samples showed instability even in the first hour of observation. This condition is thought to be caused by the relatively large droplet size in both samples. Based on Stokes' Law, the rate of creaming is influenced by droplet size and viscosity. Larger droplets move faster than smaller droplets [13]. Hence, a future work to optimize the homogenization process of JBC stabilized emulsion is required, in order to produce smaller particle size with better emulsion stability.

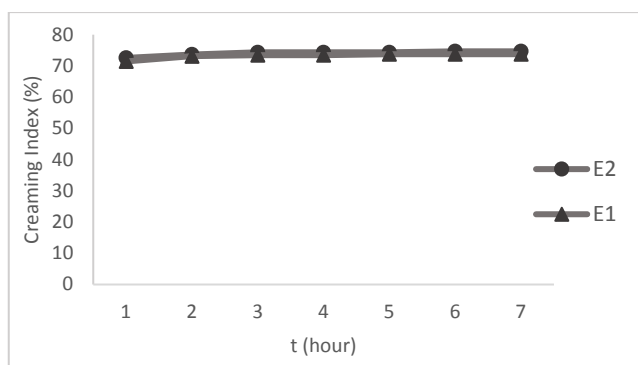
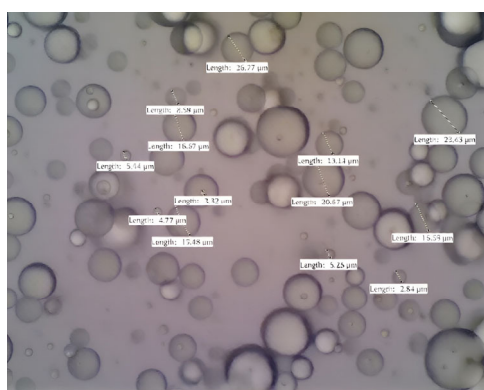


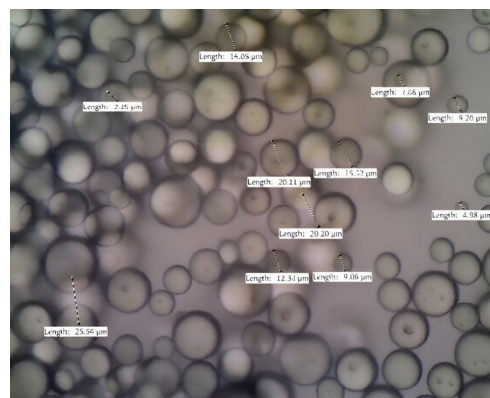
Fig. 4. Changes creaming index of JPC with different inlet temperature during storage.

G. Optical Microscopy

Optical microscope images of jack bean protein concentrate emulsion droplets can be seen in Fig. 5. Both images show that jack bean protein concentrate emulsions dried using the spray drying method at temperatures of 160 °C and 210 °C have fairly large droplet sizes. This is thought to be caused by the emulsion production process that only uses Ultra-Turrax, which is not enough to produce smaller droplets. The use of Ultra-Turrax produces an emulsion with a larger droplet size, thus triggering coalescence [22]. In addition, increasing the inlet temperature of the spray dryer also affects the droplet size. According to research [9]. Increasing the inlet temperature of the spray dryer increases the droplet size of the pea protein isolate emulsion.



(a) 160 °C



(b) 210 °C

Fig. 5. Optical microscopy images of oils in water emulsions by JPC with different spray drying temperatures.

H. H.Adsorbed Protein

The protein adsorption (AP%) percentage is shown in Fig. 6. AP% increased significantly ($p < 0.05$) in dried jack bean protein concentrate emulsions at an inlet temperature of 210 °C. This is thought to be because the use of high temperatures causes the globular folds to unfold, exposing these groups to the surface of the protein molecule [23]. This change can increase protein flexibility and facilitate the formation of an oil/water interface in the protein membrane [24].

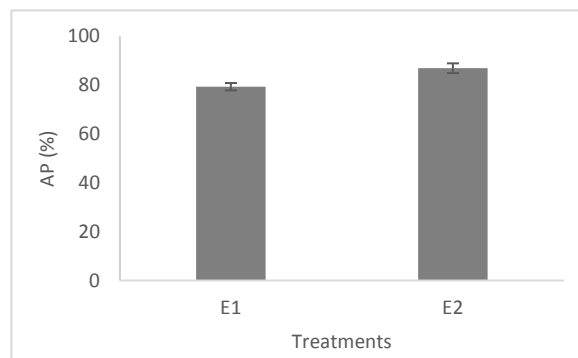


Fig. 6. Adsorbed protein of emulsion by JPC with different inlet temperature.

IV. CONCLUSION

The results of color and Maillard reaction product testing indicate that spray-dried jack bean protein exhibits the Maillard reaction. Furthermore, temperature differences affect protein content and the protein's ability to act as an emulsifier. Based on the EAI value, sample E1 showed higher emulsifying activity compared to sample E2. However, both samples exhibited low stability, indicated by instability after one hour of observation and a relatively large second droplet size. This study can be used as a reference for further research in a wider temperature range and more in-depth testing.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Fiki Ni'matul J: conducted the experiments, analyzed the data, and wrote the journal manuscript. Qurrotul A: provided suggestions, helped develop the idea and reviewing. Arima Diah S: provided feedback on the research results. All authors

had approved the final version.

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