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Protein Isolate of Jack Bean Tempeh (*Canavalia ensiformis*) by Spray Drying Method with Variation of Inlet Temperature

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***Abstract*—Protein isolation from beans is commonly carried out to increase protein availability and digestibility. Protein isolates made through the spray drying process have functional properties and characteristics. The variation of spray drying inlet air temperatures affects the properties of jack bean tempeh protein isolate (JTPI). The protein of jack bean tempeh was extracted and isolated using the method of the isoelectric point approach. The isoelectric point of jack bean tempeh was determined at a pH of 4.20 using the turbidimetry method. The research aimed to identify different inlet spray drying air temperatures on the physical characteristics and functional properties of JTPI. The spray drying method of JTPI was carried out using variations in inlet temperatures were 140°C, 150°C, and 160°C, and then moisture content, protein content, water holding capacity (WHC), and microstructure by Scanning Electron Microscopy were determined. The results showed that the 150°C inlet air temperature variation gave the lowest value for JTPI moisture content (3,91±0.04%). In comparison, the 160°C inlet air temperature variation gave the highest value for JTPI protein content (49.6±0, 30%) and JTPI water holding capacity (3.89±0.03 ml/g). The microstructure of JTPI obtained was porous, with a more spherical shape found at lower inlet temperature but wrinkled at the higher inlet temperature. The inlet temperature also affects the particle size JTPI. The inlet temperature of 160°C can be carried out to produce JTPI, which requires both high protein content and water holding capacity.**

***Keywords*— Jack bean tempeh; inlet temperature; isoelectric point; protein isolate; spray drying.**

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I. INTRODUCTION

Protein is one of the macromolecular components found in food. Protein is essential in the body; fulfilling protein nutrition through food consumption needs to be done [1]. Recommended daily protein intake is 0.8 g per kg [2]. The main protein process synthesizes new proteins, such as muscle, bone, skin, and blood. The body uses the protein consumed to repair damaged tissue, store genetic information, and help the regeneration process of hormones, enzymes, and antibodies [3].

Commonly, vegetable protein can be found chiefly in bean products, and the protein content in beans can reach up to 40% [4], [5]. Soybean is the primary type of bean mainly used for food products in Indonesia. However, soybean production in Indonesia is minimal so it can experience scarcity. A total of 2.67 tons of soybean needed in 2019 were imported from subtropical countries that produce soybeans, such as the United States, Brazil, and Argentina [6]. Thus, using local beans as an alternative to soybean is required, which is jack bean.

The jack bean (*Canavalia ensiformis*) has a relatively high protein content of 28,9-35 [7]. The protein content of jack beans is relatively higher than any other local beans, such as mung beans and red beans. Jack bean has been cultivated in at least nine provinces in Indonesia. In Central Java, jack bean is available since it has been cultivated in approximately 12 districts with a yield of at least 216 tons per harvest from a total land area of 24 hectares.

The process of fermenting legume or bean protein in tempeh can increase the digestibility of protein in the body. Protease enzymes break down protein molecules during fermentation into peptides and amino acids [8], [9]. The tempeh fermentation increased the free amino acid content

[10], [11]. The process of tempeh fermentation has also been shown to increase protein digestibility in the body [12].

Tempeh has several advantages compared to other processed legume products such as tofu. All parts of the beans except the husk are used in making tempeh, making minimum to no nutritional components wasted. In contrast to the tofu-making process, where waste is separated, it still contains a high 21.10% protein [13], [14]. The protein content in soybean-based tempeh is 18%, while in tofu, it only reaches around 8-10% [13], [15]. Thus, transforming beans into tempeh is considered better in maintaining protein components.

The isolation of protein from beans is commonly carried out to increase its protein availability. Making protein isolate also aims to increase the application of protein in processed food products. Protein isolate has a perfect role in providing functional characteristics of food products [16]. Functional properties of protein isolate include water-solubility, emulsifying ability, foaming ability, oil holding capacity (OHC), and water holding capacity (WHC) [17].

The spray drying method offers several advantages, including low production costs, a fast-heating process, better ability to maintain the final product's sensory and nutritional quality, and precise control over foods that are generally difficult to dry [18], [19]. The relatively fast spray drying process can also prevent the denaturation of protein isolate products due to the heating process [20].

The spray drying process affects the physical properties of protein isolates [21]. The spray drying method also produces protein isolates with a smaller particle diameter than other drying methods. The use of spray drying in the manufacture of soy protein isolates resulted in low hygroscopicity. Protein isolates made through the spray drying process have better carrier masking properties than freeze-drying, resulting in a non-bitter taste and retaining its properties after the encapsulation process [19], [20].

The parameter critical in the spray drying method is the inlet temperature. The higher inlet temperature will provide a drier product because the force in the process gives heat and mass transfer between air and droplets [22], [23]. However, protein is susceptible to denaturation under heat; thus, the control of inlet air temperature used needs to be done to maintain its quality [24].

The use of different inlet air temperatures in spray drying can affect the physical characteristic and functional properties of protein isolates and other products [25]–[27]. The inlet temperature in spray drying can affect the particle size of the protein produced. High inlet air temperature can also lead to oxidation of fatty compounds in the material, resulting in an off-flavor on the product [28], [29]. Thus, it is essential t to regulate the inlet temperature in the spray drying method to obtain protein isolates with the expected quality.

II. Materials and Methods

The research was conducted from November 2020 to June 2021 at several laboratories in Universitas Padjadjaran. The equipment used in this research are Mini Spray Dryer B-290, UV-VIS spectrophotometer, SEM (Scanning Electron Microscopy) TM 3000, grinder, vortex, centrifuge, centrifugation tube, 80 mesh filter, glass beaker, agitator, analytical balance, oven, blower oven, Erlenmeyer, pH meter, measuring cup, desiccator, spatula, hot plate stirrer, agitator, and magnetic bar. The materials used in this study were jack beans (*Canavalia ensiformis*) obtained from farmers in Salatiga Central Java, distilled water, NaOH, HCl, H_2SO_4, Kjeldahl tablets, and H_3BO_3. The research was carried out using the experimental method with descriptive analysis. The spray drying process of jack beans tempeh protein isolate was carried out at three different levels of inlet air temperature, namely 140°C, 150°C, and 160°C.

A. Jack Bean Tempeh and Flour Preparation

The jack beans are weighed and washed thoroughly. The beans are boiled for 30 minutes, then soaked in water for 24 hours. The water is replaced periodically. The soaking process can be carried out to help hydrate the jack beans and reduce HCN levels. After soaking, the jack beans are peeled, chopped, and washed thoroughly. The jack beans are then steamed for 45 minutes. The streaming process helped soften the beans' tissue. The yeast is added with a 1% concentration, then wrapped in a zip lock bag and punched some holes using a knife or needle. The jack beans are then fermented at 27°C for 36 hours [30].

The fully fermented jack bean tempeh is sliced with approximately 0,5 cm thickness. The jack beans tempeh is then steam-blanched at 80°C for 10 minutes. The jack bean is dried using a blower oven at a temperature of 50°C for 12 hours or until the tempeh is thoroughly dried. The dried jack bean tempeh is then ground with the grinder and sieved using an 80-mesh sieve.

B. Determination of Isoelectric Point

The isoelectric point is determined using the turbidimetry method [31], [32]. The isoelectric point of jack bean tempeh is determined by varying the pH of isolation at 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6 and then the absorbance level for every pH used were read using UV-VIS spectrophotometry at wavelength of 320 nm.

C. Isolation Protein of the Jack Bean Tempeh

The process of making jack bean tempeh protein isolate with slight modifications [33]. Jack bean tempeh flour was dissolved using distilled water with a dissolution ratio of 1:10 (w/v), then stirred by a stirrer. The initial pH of the solution mixture was recorded for later use. 2N NaOH solution was added to pH 10. The solution mixture was then conditioned at 30°C and the extraction process was carried out for 30 minutes. The solution was centrifuged at 4000 rpm for 15 minutes, and the supernatant was taken. The supernatant was then added with 2N HCl until it reached pH 4.2, then stirred for 10 minutes. Centrifugation was carried out again at 4000 rpm for 15 minutes. The filtrate acquired is the protein isolate of jack beans tempeh.

D. Spray Drying Method

The spray drying method of jack bean tempeh protein isolates was modified [25], [29]. The isolate protein of jack bean tempeh was dissolved by distilled water with ratio of 1:3 (w/v). Neutralization of protein isolate that have a low pH with the addition of 1 N NaOH to the initial pH. The solution was homogenized for 10 minutes. Furthermore, the process of drying the sample of protein isolate solution using spray drying was carried out. In this study, three variations of the inlet spray drying temperature were carried out, namely 140°C, 150°C, and 160°C.

E. Observation

The observation parameters of this research includes: moisture content [34], protein content [35], water holding capacity (WHC) [36], [37], and microstructure [34].

III. Results and Discussion

A. Determination of Isoelectric Point

The isoelectric point is determined using the turbidimetry method [31], [32]. In this method, the isoelectric point of jack bean tempeh is determined using the absorbance of a UV-VIS spectrophotometer with a wavelength of 320 nm. Suspension of protein isolate at its isoelectric point will give the highest absorbance value. The high absorbance value indicates the turbidity of the suspension. This is due to the aggregation and coagulation of protein molecules so that the protein is difficult to dissolve in water. The pH of the isolation on the absorbance of the suspension of the protein isolate of jack bean tempeh is shown in Figure 1.

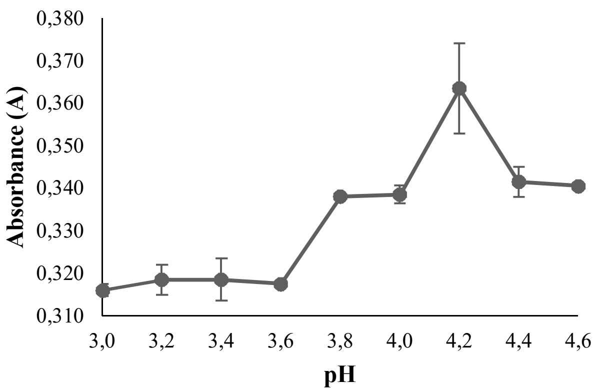

Fig. 1 Effect of isolation pH on absorbance level of jack bean tempeh using the turbidimetry method

The highest jack bean tempeh protein absorbance value was shown at pH 4.2. Thus, it can be concluded that the isoelectric point of jack bean tempeh protein is at a pH value of 4.2. Determination of the isoelectric point of jack bean protein has been previously carried out, namely at pH 4.9 [39]. The isoelectric point obtained in this study has a lower pH value. It can be altered by breaking down protein into amino acids and peptides carried out by protease enzymes produced by microorganisms during the tempeh fermentation process, thus eventually lowering its pH [40], [41].

B. Moisture Content

The moisture content of jack bean tempeh protein isolate is shown in Table I. Based on Table I, the moisture content of jack bean tempeh protein isolates for each inlet air temperature given ranges from 3,91% to 5,27%. The inlet air temperature of 150°C gave the lowest moisture content of jack bean tempeh protein isolate, while the inlet air temperature of 160°C gave the highest result.

TABLE I
Moisture Content

Variation	Moisture Content (%)
140°C	4,90 ± 0,12
150°C	3,91 ± 0,04
160°C	5,27 ± 0,02

Table II shows that the moisture content of jack bean tempeh protein isolates for each inlet air temperature ranges from 3,91% to 5,27%. The lowest moisture content of jack bean protein isolate was at the inlet air temperature of 150 °C, while the intake air temperature of 160 °C had the highest result.

There is a slight difference between the result of this research and previous studies, where if the inlet temperature increase in the spray drying gives the lower moisture content in the dried product [23], [42]. This difference may be related to the lower moisture content of the product, and the increased porosity of the material can increase the hygroscopicity of the resulting product. There is an increase in the hygroscopicity of the final product due to an increase in the inlet temperature [23]. In this condition, the moisture content of the product increases due to the absorption of water from the environment during the storage process due to different moisture concentrations between the product and the environment [43], [44]. Thus, further action is needed regarding storage conditions and the use of appropriate packaging; thus, the increase in water content in the product can be avoided.

C. Protein Content

The protein isolates content of jack bean tempeh was analyzed using the Kjeldahl method. The results of the protein content analysis are shown in Table II.

TABLE II
Protein Content

Variation	Protein Content (%)
140°C	44,12 ± 0,01
150°C	46,62 ± 0,34
160°C	49,60 ± 0,30

The jack bean tempeh isolates' protein content ranges from 44,12 to 49,62%. The results indicate that the increase of inlet air temperature significantly affects the protein isolates content of the jack bean tempeh. This is indicate that higher drying temperature gives a greater protein content in the resulting product [37], [45].

During the drying process, some proteins can be trapped inside the spray dryer, thus decreasing the amount of total protein. The exertion of heat in the jack bean protein isolates can cause changes in their properties. During spray drying, the interaction between heat from drying air and the food products can induce a sticky layer primarily consisting of protein and carbohydrates, thus reducing the total mass of protein in the dried product [46], [47].

The difference in protein content can also be influenced by the amount of non-protein components such as carbohydrates. Carbohydrate components dominate the formation of a sticky layer on the spray dryer, thus carbohydrate components are more likely to be trapped inside the spray dryer [46]. Higher protein content in the final product may indicate lower levels of other non-protein components, such as carbohydrates, which can happen due to the sticky layer formation, as previously mentioned.

D. Microstructural Analysis

Microstructural analysis of jack bean tempeh protein isolate was carried out using Scanning Electron Microscopy (SEM). SEM aimed to identify and observe the morphological structure of the particles. Microstructure morphology can be used to determine the optimum conditions for the spray drying method to produce desired specifications [48].

The particles of jack bean tempeh isolate protein dried at an inlet air temperature of 140°C has a more spherical shape (Fig. 2), in contrast with the variation of air inlet temperature 150°C (Fig. 3) and 160°C (Fig. 4), which are dominated by wrinkled or shrinking particles. The porous particle structure and porous structure increase when using higher intake air temperatures. In addition, the variation in the air inlet temperature also affects the particle size.

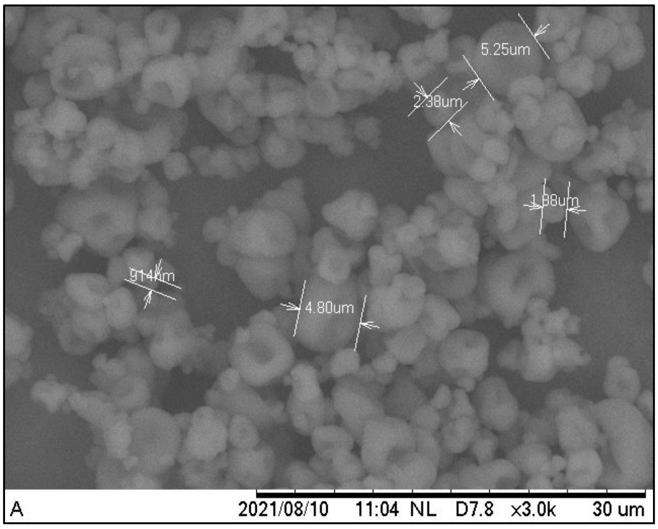

Fig. 2 Microstructure of jack bean tempeh protein isolate with a variation of 140°C inlet air temperature observed using SEM with 3000x magnification

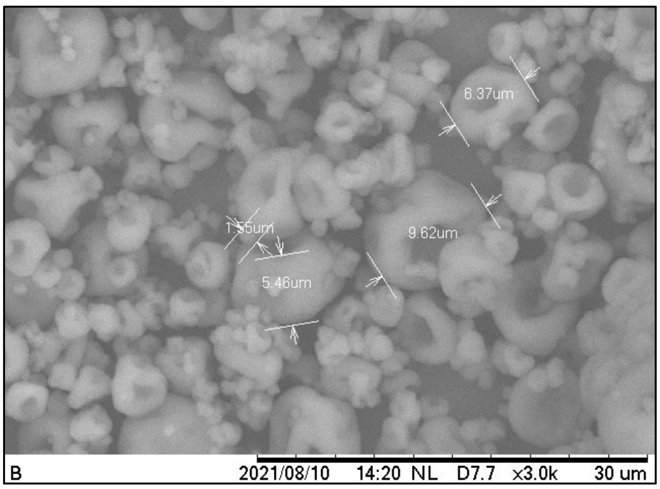

Fig. 3 Microstructure of jack bean tempeh protein isolate with a variation of 150°C inlet air temperature observed using SEM with 3000x magnification

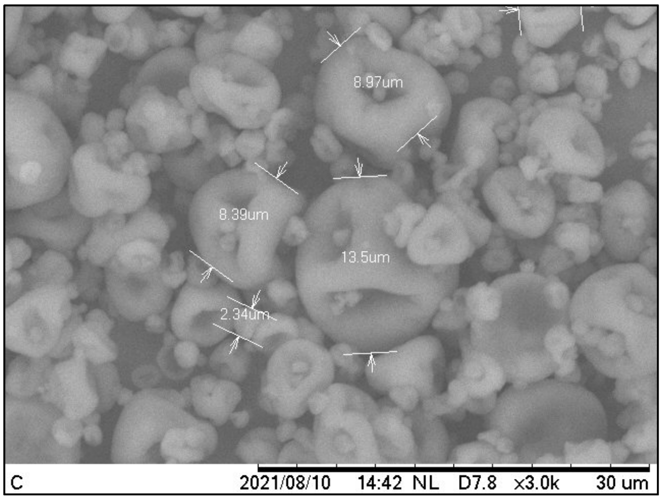

Fig. 4 Microstructure of jack bean tempeh protein isolate with a variation of 160°C inlet air temperature observed using SEM with 3000x magnification

The use of inlet air temperature of 140°C results in relatively small particles, where the largest particle diameter can reach 5.25 μm, and the smallest particle diameter reaches 914 nm. In the 150°C variations, the diameter of the largest particles can reach 9.62 μm, while the small particles can reach 1.55 μm. The 160°C inlet air temperature variation produced the largest particles, ranging from 13.5 μm for the largest size up to 2.34 μm for the smallest size.

Different inlet air temperatures affect the particle morphology of the jack bean tempeh protein isolates produced. Increasing the inlet air temperature will increase the number of porous or hollow particles formed on the spray drying method [31], [49]. High inlet air temperature provides a faster drying rate, thus making the process of moisture evaporation more quickly. This condition then promotes faster particle shell formation, which eventually leads to the formation of hollow space inside particles [50].

The inlet of air temperature affects the particle size products. The higher inlet temperature increased the particle size of the jack bean tempeh protein isolate produced. The increase in the inlet air temperature causes increases in the volume of the formation of hollow space inside particles. The increase of the inlet air temperature can also promote a rapid formation of the dried layers at the droplet surface. The dried layers at the droplet surface cause the moisture to not be out from the droplet, which is that the particle size increases [49].

A lower inlet temperature resulted in more spherical particles, while a high temperature produced shrinking or wrinkled particles. This wrinkled particle structure is related to the formation of hollow space inside the particles due to high temperatures. The formation of hollowness in the particles dried at high temperatures occurs early, causing particles to blast and occasionally contract [51].

E. Water Holding Capacity

Water holding capacity (WHC) is a measurement of the total amount of water absorbed by each gram of the dried powder protein [52]. Food proteins can directly interact with water due to its hydrophilicity [52]. Water holding capacity can affect the flavor and texture of food products due to the interaction of protein and water in these food products [53].

The water holding capacity of the jack bean tempeh protein isolates is shown in Table III.

TABLE III
WATER HOLDING CAPACITY

Variation	Water Holding Capacity (ml/g)
140°C	3,79 ± 0,03
150°C	3,68 ± 0,01
160°C	3,89 ± 0,03

Based on Table III, inlet air temperature affects the water holding capacity of the protein isolate jack bean tempeh. The protein isolate jack bean tempeh's water holding capacity ranges from 3,68 ml/g to 3,89 ml/g. The air inlet temperature of 160°C resulted in jack bean tempeh protein isolate giving the highest water holding capacity, while the lowest result was inlet temperature of 150°C. The jack bean tempeh protein isolates that were dried using an inlet air temperature of 160°C are suitable for products requiring high water holding capacity.

Water holding capacity is influenced by the morphological structure of the particles [54]. Based on this research, the drying process method using an inlet air temperature of 160°C can provide a more porous particle of jack bean tempeh protein isolate (Fig. 4). The porosity of particles can facilitate water to enter more easily and then form a hydrogen bond with protein. Particle size can also play an important role in determining water holding capacity [55], [56]. The use of 140°C inlet air temperature resulted in isolates with smaller particle sizes than other treatments. The smaller particle size causes a greater total surface area of particles [57]. This provides more contact between particles and water, increasing the amount of water absorbed by the material. This condition could be why the jack bean tempeh protein isolate dried using an inlet temperature of 140° C has a relatively higher water holding capacity than using an inlet air temperature of 150° C.

The protein content can influence the difference in the water holding capacity of the protein isolate jack bean tempeh. Based on Table II, the protein isolates of jack bean tempeh at an inlet air temperature of 160°C had the highest protein content, thus providing the highest value for water holding capacity. The protein content is believed to influence the water holding capacity. Protein can form hydrogen bonds with water; higher protein content can increase the water-binding process during hydration [58].

Differences in protein content necessarily indicate the differences in the composition of non-protein components. The presence of non-protein components, such as carbohydrates, is one of the intrinsic factors of water-holding capacity [53]. Carbohydrates are known for their ability to inhibit the interaction of water and protein. Water molecules that proteins should take up are replaced by saccharides, thus maintaining their conformity [37].

IV. CONCLUSION

The variations of the inlet air temperature affect the physical characteristic and properties of the jack bean tempeh protein isolate. The inlet air temperature can be adjusted to obtain the final product with desired characteristics with the spray drying method. The characteristics of the final product of jack bean tempeh protein isolate can be used to determine its application in food products.

The jack bean tempeh protein isolate (JTPI) particles obtained is porous. The particle shape indicated was predominantly spherical at the inlet air temperature of 140°C and more wrinkled at the inlet air temperature treatment of 150°C and 160°C. The variations inlet temperature of 150°C gave the lowest moisture content (3,91±0.04%), while inlet air temperature of 160 °C gave the highest value for protein content (49.6±0.30 %) and water holding capacity (3.89±0.03 ml/g). JTPI dried using an inlet air temperature of 160°C has more hollow or porous particles, thereby increasing the water absorption process.

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