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EFFECT OF HYDROLYSIS TIME ON ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF JACK BEAN (*Canavalia ensiformis*) PROTEIN HYDROLYSATE

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Abstract

Jack Bean or Kacang Koro (*Canavalia ensiformis*) is one of the under-exploited tropical dry beans. This study was aimed to determine the effect of hydrolysis time on physicochemical properties, antioxidative and antimicrobial activity of Jack Bean protein hydrolysate (JBPH). The physicochemical properties of JBPH were evaluated based on protein content, WHC, OHC, degree of hydrolysis, foam stability and foaming capacity. The antioxidative activity of JBPH was measured using DPPH, hydroxyl radical scavenging, superoxide radical scavenging and FRAP. Well diffusion method was used to study antimicrobial activity of JBPH. The highest protein content (33.16±0.03%) obtained in JBPH that hydrolysed for 150 min. The degree of hydrolysis was showed for JBPH highest at 120 min (51.79±0.28%). The size of the microstructure of JBPH analysed using SEM were decrease with hydrolysis time. FTIR analysis confirmed that JBPH comprised of three major components (Region I, II and III). Water holding capacity of JBPH was the highest for the sample hydrolysed for 60 min (63.87±0.72%) while oil holding capacity depicts the highest by it at 180 min (57.17±1.19%). Foaming capacity and foam stability decreased with hydrolysis time. JBPH produced at 120 min hydrolysis time showed the highest inhibition toward DPPH (42.44%) and hydroxyl radicals (20.01%). FRAP and superoxide radical scavenging, JBPH at 90 min showed the highest inhibition (91.15±0.05 µM and 64.33%). JBPH also showed antimicrobial properties by inhibits the growth of *P. aeruginosa*. The best hydrolysis time to produce JBPH with the highest physicochemical properties was found at 120 min.

INTRODUCTION

A growing global population, combined with factors such as changing socio-demographics, has increased pressure on the world's resources not only to provide more, but also different types of food. Increased demand for protein-based especially on animals is expected to have a negative impact on the environment [1]. Animal proteins were expensive and relatively hard to obtain, leading to increased research around the world on vegetable protein sources, especially legumes [2]. However, conventional legumes such as soybeans could not meet dietary protein requirements in many countries [3]. A more sustainable production of existing protein sources and much-needed alternative sources for direct human consumption.

Kacang Koro or Jack Bean is one of the legumes that may be underused to reduce protein deficiency in human nutrition, particularly in developing countries [3]. Jack Bean (*Canavalia ensiformis*) is one of Africa, Asia, West Indies, Latin America and India's most widely distributed under-exploited tropical dry beans [4]. [5] reported that Jack Bean contains 25% of crude protein. Protein is hydrolyzed chemically or enzymatically to improve the functional properties of the protein. Compared to other common legumes, Jack bean seeds have a high protein nutritional profile. The Jack Bean protein hydrolysate could be a substitute for soy protein and animal protein in the food supply. Raw Jack Bean's crude protein content is 29.8g / kg and the moderate intake of Jack Bean significantly increased the total dietary protein consumption [6]. In the food processing industries, however, the high protein content of Jack Bean has so far been less used. This research was conducted to determine the effects of different enzymatic hydrolysis time on JBPH's physicochemical, functional properties and antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Materials

Jack Bean was collected from Kuala Berang, Terengganu. Before enzymatic hydrolysis, the Jack Bean was ground into flour. The JBPH was extracted using Alcalase enzyme (Merck, USA).

Methods

Protein extraction and hydrolysis
Protein extraction was carried out using a modified [2] method. A flour: water dispersion (1:6 w / v) was prepared and its pH with NaOH 1 N was adjusted to 11. After 1 h of soaking, the solid fraction was separate from the protein and starch fraction of the liquid. It was then centrifuged at 1317 g for 12 min. The protein-rich sample was mixed with 100 ml sodium phosphate buffer (1:2 w / v) at 25ºC for 5 min. The solution was set to pH 8 (optimal pH for Alcalase enzyme). The hydrolysis process was performed at different times (60 min, 90 min, 120 min, 150 min and 180 min). Using a water bath at 90 ° C, heat treatment was applied for 10 min to denature the enzyme. The solution was then centrifuged at 10,000 rpm for 20 min. The sample was filtered and gathered. To obtain JBPH in the form of powder, the sample was then freeze-dried at -54 ° C and vacuum setting at 0.25 mbar.

Yield
The yield analysis was conducted on raw Jack Bean to obtain the percentage of flour. In this analysis, 10 kg of raw Jack Bean were used. The exact weight was recorded as raw Jack Bean pre-milled and milled flour Jack Bean weight as recorded.

Degree of hydrolysis (DH)
Approximately 0.5 g of sample dissolved with 10 ml of sodium phosphate buffer solution [7]. Then 5 ml of 10% Trichloroacetic acid (TCA) was added to the sample solution. The solution was kept at room temperature for 30 min. The sample was then centrifuged at 4000 rpm for 5 min. Determination of protein content of the samples was carried out in triplicates using Kjedahl method (AOAC, 2000).

Microstructure determination
Electron Microscope [8] was used to determine the microstructure properties. The sample was applied to the sticker surface of a specimen holder. Before analyzing using SEM (Jeol-6360, USA), using Auto Fine Coater, and the sample was coated with 99% pure gold.

Determination of functional groups
The structural properties of JBPH was studied using Fourier Transform Infrared Spectroscopy (FTIR) (Nicolet, Thermo Electron, USA). The lyophilized JBPH was measured by the FTIR spectrophotometer for its infrared spectra in the absorption of 4000-500 cm^{-1}. The scanning was done with a 4 cm^{-1} resolution and the FTIR spectrum was an average of 32 scans [9]. The functional group and vibration mode were identified based on the peak of interest at specific wavelengths and absorption [10].

Water Holding Capacity (WHC)
In 5 ml of distilled water, a suspension of 0.5 g JBPH (dry weight) was added. At 30 min the mixture was left standing. It was centrifuged at 5000 g for 30 min (Hettich Universal 32, Germany). The tube was drained for 10 min and the free water was discarded. The pallet left was weighed and calculated in the centrifuge tube [11].

Oil Holding Capacity (OHC)
Oil holding capacity was performed using a [12], whereby 0.5 g of the sample was weighed and then stirred for 30 min in 5 ml of maize oil. Then these protein suspensions were centrifuged at 5000 g for 30 min (Hettich Universal 32, Germany), and measured the volume of the supernatant.

Foaming Capacity and Foam stability
In addition to the sodium phosphate buffer, a 20 ml sample of 5 percent (w/v) suspension was prepared. The suspension was placed in a beaker of 100 ml and was homogenized for 2 min at 17 500 rpm using homogenizer (IKA, Germany), and the volume of foam was recorded after 30 s. Foam stability was determined at 3 min after homogenization based on residual foam volume [12]. The volume of foam remaining after 3 min was determined as a percentage of the initial volume of foam using the equation below.
Foaming capacity = (volume of foam after 30s) / (original volume of protein solution) x 100
Foam stability = (volume of foam after 3 min) / (volume of foam after 30 s) x 100

Protein content
The content of proteins was measured using the Kjedahl method (AOAC, 2000).

Scavenging effect on DPPH free radical
DPPH (2, 2-dipheny l-2- picrylhydrazyl) radical scavenging assay was carried using a reported method with some modifications [13] and [14]. At the same quantity of 2 ml of 0.1 mM DPPH solution in methanol, 1 ml of sample solution was added. The reduction of the DPPH free radical was measured at 517 nm after 30 min of incubation at 25ºC.

Superoxide radical scavenging activity (SRSA)
The SRSA was determined by measuring pyrogallol autoxidation inhibition using Marklund and Marklund's slightly modified method [15]. In a freshly prepared 90 µl of 3 mM pyrogallol (in 10 mM HCl), a sample solution with a volume of 0.3 ml (5 mg / ml) and 2.61 ml of 50 mM phosphate buffer (pH 8.2) was added. After incubation for 4 min, 100 µl of 0.2 M ascorbic acid was added to the mixture and measured at 325 nm.

Hydroxyl radical scavenging activity
The hydrogen peroxide scavenging test was performed after the [16] procedure. A solution of H_2O_2 (43 mM) in phosphate buffer (0.1 M, pH 7.4) was prepared for this purpose. Samples were added to 2 mL of H_2O_2 solution at the 4mg / mL concentration in the 2.4 mL phosphate buffer. It was kept at room temperature for 10 min. The reaction mixture's absorbance value was recorded at 230 nm.

Ferric reducing antioxidant power (FRAP)
The ferric reduction of antioxidant power (FRAP) assay as described by [17] was used to measure the antioxidant activity of the extracts with some modifications. The FRAP reagent consisted of 10 ml TPTZ (10 mM, 40 mM hydrochloric acid), 10 ml ferric chloride (20 mM) and 100 ml acetate buffer (0.3 M, 3.6 pH). Extracting 1 ml solutions at various concentrations was added to

the 5 ml FRAP reagent and the mixture was incubated for 20 min at 37°C and measured at 593 nm.

Antimicrobial activity assay: Agar Well diffusion method
Agar well diffusion method [18] has been used to determine JBPH's antimicrobial activity. On Muller-Hinton agar (MHA) plates containing 6 mm wells, 100 ml of bacterial suspension were spread. Twenty microliters of each extract were poured into each well and plates were aerobically incubated for 24 h at 37 °C. Wells containing JBPH without zones of inhibition were considered as negative results.

Data analysis
The statistical analysis for JBPH was completed using MINITAB software at the confidence level at $\alpha \leq 0.05$. In these samples, the hydrolysis time was used as a parameter. The data obtained were analyzed using ANOVA one-way. Mean comparisons were made using Fisher's LSD and the data was displayed as mean ± standard deviation.

RESULTS AND DISCUSSION

Yield of powder hydrolysate

The efficiency of hydrolysis time on obtaining JBPH was determined using yield of powdered JBPH. The highest yield was achieved by JBPH that hydrolysed for 150 min. The yield for JBPH was obtained at varying hydrolysis time from 4 to 5 %. A steady increase was observed in the yield of powdered JBPH with hydrolysis time until 150 min. The yields of powder JBPH might increase because of the increase in peptides and amino acid present. The increased hydrolysis time causes the protein to split into smaller peptides and amino acids [19].

Protein content of JBPH

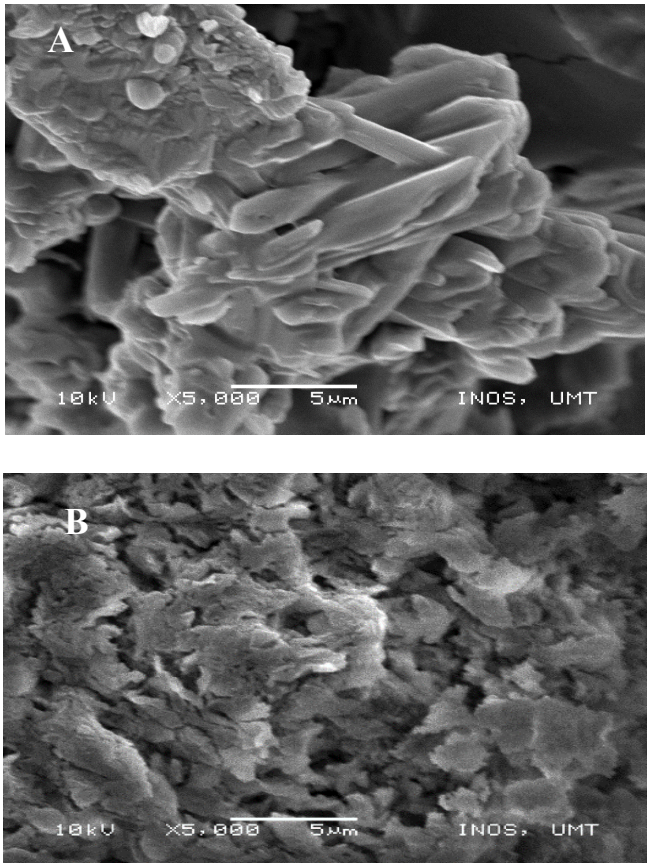
There was a significant difference in the hydrolysis time of JBPH at 90 min, 120 min, and 150 min. In contrast, the protein content of the sample at 90 min and 120 min was not significantly different. The increase in protein content of JBPH was observed up to 150 min. This may be because more peptide bond has been cleaved and peptide and amino acids have increased nitrogen concentration. Previous studies have shown that more protein molecules were released longer than the hydrolysis period [20]. JBPH's protein content increases up to 150 min with hydrolysis time. The more peptide bonds cleaved in whey protein hydrolysate; the more formal titration can determine amino nitrogen [21].

Degree of hydrolysis of JBPH

The degree of hydrolysis plays an important role in determining JBPH's functional properties [21]. Data in **Table 1** shows that 60 min, 90 min and 120 min hydrolysis time increase in the degree of hydrolysis for the sample. This could be due to the concentration of enzymes and the content of proteins. However, the degree of hydrolysis began to decline at 150 min and 180 min for the hydrolysed sample. The decline in the degree of hydrolysis may be due to protein molecules denaturation. At 60 min hydrolysis time, the minimum degree of hydrolysis occurs. Several protein substrates such as peanut [22], rice protein [23] and corn protein [24] have been reported to have similar results.

Microstructure of JBPH

JBPH structure is one of the key roles in determining its functional properties. Scanning electron microscope (SEM) has thus been used to show changes in JBPH microstructure at different times of hydrolysis. **Figure 1** illustrates the image with 5000× magnification of JBPH, which showed irregular shape at different time of hydrolysis. This may be because of freezing drying processes causing samples to change into powder form. The "collapse-building" shape of the freeze-dried protein hydrolysate [25]. Changes in morphology may affect protein hydrolysates' functional properties [26]. Irregular shapes may help to trap water and oil molecules in more exposed amino acids outwards. **Figure 1** also shows that with hydrolysis time, the sample size decreases. The JBPH microstructure in 60 min is larger, while the JBPH is dispersed at 180 min and smaller in size. The size and structure had a major impact on JBPH's functional properties, such as solubility. A previous study reported that larger plant proteins generally have lower solubility than smaller ones because smaller weight protein entropy decreases [27].



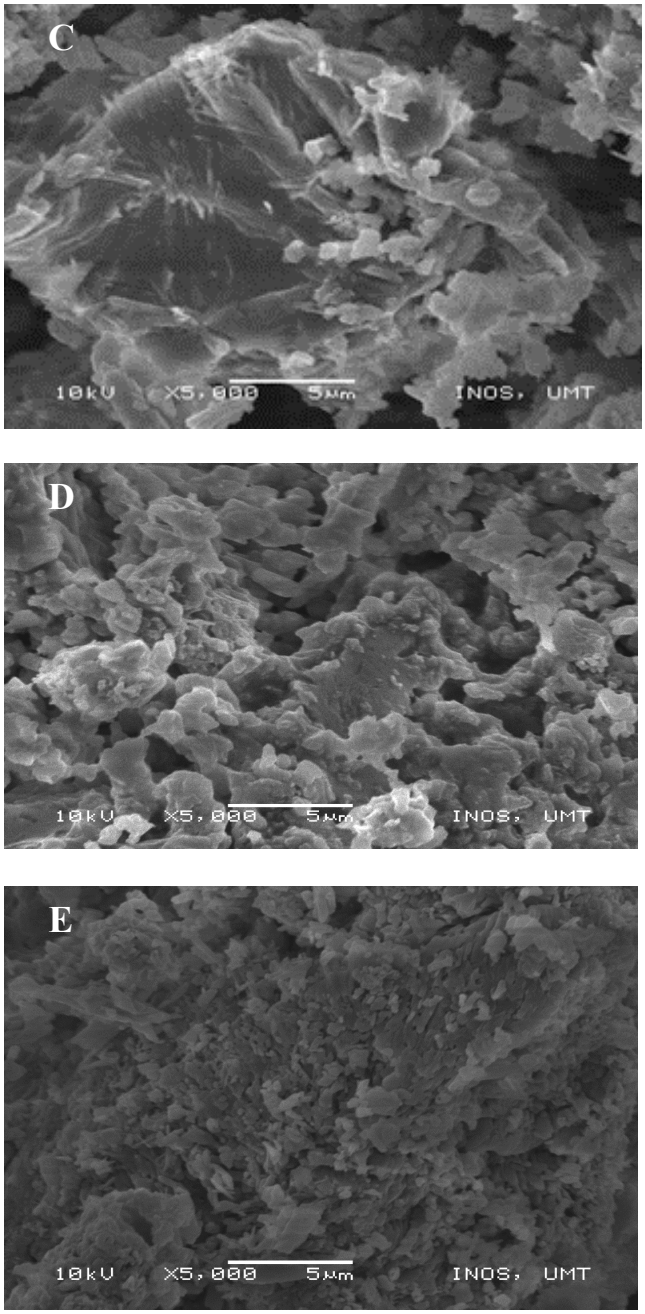


Figure 1. SEM images of powdered JBPH. Note: A=60 min, B=90 min, C=120 min, D=150 min, E=180 min [Hydrolysis time]

Functional groups of JBPH

The properties of JBPH in terms of purity and functional group were analyzed using FTIR. FTIR was used to determine physico-chemical, morphological, chemical, structural and intermolecular cross-linking of foods and biomaterials [28]. There were three major peaks present in the protein hydrolysate. These regions represent the bonds Amide A and B, Amide I, II and III; and Amide IV and V [29]. **Table 2** shows that Amide I, II and III absorption by JBPH samples were observed (1613.20 to 1638.81 cm^{-1}). The Amide I, II and III (1700–1600 cm^{-1}) of proteins are found to be most sensitive for determination of secondary structure (β-sheet, α-helix, coils and turn) [30]. The FTIR absorption by Cucurbitaceae seeds protein hydrolysate showed similar observation at 1650.77, 1650.77 and 1649.80 cm^{-1} (Region II) same as JBPH [31]. Amide IV and V represent choline asymmetric stretch, O=C-N deformation and CH_2 rocking [29]. The data also shows that the peak at 1633 cm-1 indicating the presence of aromatic rings of phenolic compounds [32].

Table 1. Yield, protein concentration of JBPH

Sample	Yield (%)	Protein content (%)	Degree of hydrolysis (%DH)
Raw seed		35.84±0.04	
A	4.85±0.01^{e}	28.47±0.13^{c}	40.03±2.34^{b}
B	4.94±0.01^{d}	29.06±0.27^{bc}	41.91±2.55^{b}
C	5.15±0.02^{c}	29.75±0.27^{b}	52.32±3.51^{a}
D	5.33±0.01^{a}	33.16±0.13^{a}	51.86±2.37^{a}
E	5.20±0.01^{b}	32.66±0.13^{a}	51.90±3.11^{a}

Note: A=60 min, B=90 min, C=120 min, D=150 min, E=180 min [Hydrolysis time] All values given are means of triplicate results. Standard deviation (mean ± SD) is included for each average. Means with different letter are significantly different ($p < 0.05$).

Table 2. FTIR results for powdered JBPH

Sample	Region I Amide A and B	Region II Amide I, II and III	Region III Amide IV and V
A	3454.31	1613.20	864.60
B	3450.97	1628.48	863.74
C	3435.34	1633.50	862.16
D	3258.36	1633.11	862.74
E	3432.49	1638.81	815.06

Note: A=60 min, B=90 min, C=120 min, D=150 min, E=180 min [Hydrolysis time]

Water and Oil Holding Capacity of powdered JBPH

Water holding capacity (WHC) and oil holding capacity (OHC) are important for determining the texture properties of food products such as juiciness, mouthfeel and tenderness which can be used as a meat protein alternative [27]. Data in **Table 3** shows that JBPH hydrolyzed at 60 min exhibited the highest water holding capacity (63.9%) compared to other samples. This due to the presence of a high concentration of polar groups, which cause the protein-water interaction of polar groups and hydrophobicity in the sample [27]. However, the water holding capacity was decreased for JBPH at 90 min, 120 min and 150 min. This may occur because the hydrophobic group was higher in the sample. The molecules size decrease with hydrolysis time as shown in SEM might also influence the ability to trap water. **Table 3** also depicts that the JBPH at 180 min had higher WHC (61.6%) compared to another protein hydrolysis at 90 min, 120 min and 150 min. The sudden increase of WHC at 180 min hydrolysis time might happen because of the balancing of hydrophilic amino acid with hydrophobic amino acids.

Table 3. Water holding capacity (WHC), oil holding capacity (OHC), foaming capacity (FC) and foaming ability (FA) of JBPH

Sample	WHC (%)	OHC (%)	FC (%)	FA
A	63.87±0.72^{a}	40.00±1.00^{c}	90.00±1.00^{a}	43.00±1.00^{a}
B	54.23±0.60^{c}	31.80±0.35^{d}	38.00±1.00^{b}	37.00±1.00^{a}
C	43.57±0.93^{e}	38.97±0.40^{c}	30.00±1.00^{c}	36.00±1.00^{c}
D	51.80±1.32^{d}	46.57±1.10^{b}	22.00±1.00^{d}	29.00±1.00^{d}
E	61.60±0.53^{b}	57.17±1.19^{a}	15.00±1.00^{e}	22.00±1.00^{e}

Note: A=60 min, B=90 min, C=120 min, D=150 min, E=180 min [Hydrolysis time] WHC = Water holding capacity, OHC = Oil holding capacity, FC= Foaming capacity, FA= Foaming ability. All values given are means of triplicate results. Standard deviation (mean ± SD) is included for each average. Means with different letter are significantly different ($p < 0.05$).

Oil holding capacity is defined as the amount of oil that is retained by protein, which bound by hydrophobic interaction between oil and protein [33]. Data showed that there was a significant difference between JBPH hydrolysed at different times. JBPH at 180 min hydrolysate shows the highest oil holding capacity compared to other samples. Hydrolysis of different plants such as chickpea [34] and barley [35] increases the OHC. **Table 3** shows that JBPH at 90 min has the lowest OHC (31%). It reflects that OHC depends heavily on the releases of hydrophobic groups from the hydrolysis process. The mechanism of OHC is attributed to the combination of physical entrapment of oil and the hydrophobicity of the material [36].

Comparing the WHC and OHC of JBPH, it appears that the sample at different hydrolysis time had good WHC than OHC. However, JBPH still possessed remarkable OHC properties. The decrease and increase in the WHC and OHC depends on the release of ionizable polar group and balance of hydrophilic and hydrophobic group [36].

Foaming capacity and foam stability of powdered JBPH

The relation between the degree of hydrolysis and the foaming capacity and foam stability were inversely proportional (**Table 3**). There is a relation between the degree of hydrolysis and foaming properties [37]. **Table 3** also showed that the JBPH had the lowest foaming ability and stability at 90 min, 120 min, 150 min, and 180 min. JBPH prepared at 180 min hydrolysis time does not form any foam. This may occur because the peptides and amino acid present in it after further hydrolysis had lost the foaming properties. The decrease in foaming of the more extensively hydrolysed sample suggests that lower surfactant activity of smaller polypeptide chains [38].

Antioxidative properties of powdered JBPH

DPPH (2, 2-dipheny l-2- picrylhydrazyl) radical scavenging activity of powdered JBPH

JBPH produced at 120 min shows strong inhibition of DPPH radicals, which was significantly higher than that of other samples. **Table 4** shows the increase in the degree of hydrolysis causing increased DPPH radical scavenging activities. A similar result has also been reported for porcine plasma protein [39]. Changes in size, level and composition of free amino acids and small peptides affect the antioxidative activity [40]. Peptides that are composed of hydrophobic and aromatic amino acids in their chain can easily scavenge the DPPH radical [41]. JBPH at 120 min might have higher hydrophobic amino acids as it is also had the lowest water holding capacity at 120 min.

Table 4. Antioxidant properties of JBPH

Hydrolysis time	FRAP value (μM)	Inhibition of DPPH (%)	Superoxide radical scavenging (%)	Hydrogen peroxide (%)
A	89.28±0.05^{c}	26.48±0.35^{f}	44.24±0.55^{d}	8.32±0.15^{g}
B	91.15±0.05^{b}	34.86±0.45^{d}	65.56±0.66^{c}	10.52±0.23^{f}
C	88.15±0.18^{d}	43.51±0.77^{b}	41.32±0.35^{e}	21.34±0.32^{c}
D	80.58±0.13^{e}	38.56±0.46^{c}	29.76±0.55^{f}	15.36±0.66^{d}
E	77.18±0.10^{f}	19.57±0.24^{g}	5.15±0.15^{g}	12.18±0.18^{e}
BHT	105.67±3.36^{a}	45.03±0.18^{a}	95.12±0.96^{a}	53.12±0.26^{b}
α-Tocopherol	112.67±2.15^{a}	31.68±0.45^{e}	92.67±0.85^{b}	58.87±0.47^{a}

Note: A=60 min, B=90 min, C=120 min, D=150 min, E=180 min [Hydrolysis time] All values given are means of triplicate results. Standard deviation (mean ± SD) is included for each average. Means with different letter are significantly different ($p < 0.05$).

Superoxide radical scavenging activity of powdered JBPH

Table 4 also shows that JBPH at 90 min hydrolysis time exhibited the strongest inhibition (64%) compared to other samples. The percentage of inhibition decreases with further hydrolysis time at 120 min, 150 min and 180 min. Mungbean meal protein hydrolysate also had high superoxide radical scavenging activity with 65.10% [42]. The JBPH at 180 min showed a significant decrease in the percentage of inhibition. This might due to the fact that, more amino acids and smaller peptide releases at 180 min hydrolysis time. These released amino acids might do not possess superoxide scavenging properties. The antioxidant activity could be contributed by the presence of free amino acids and small peptides, which can effectively scavenge radicals [43].

Free radical scavenging activity of hydrogen peroxide

Table 4 shows the largest proportion of JBPH inhibition display at 120 min as it also had the lowest degree of hydrolysis. The scavenging activity decreases after reaching a peak of about 20% inhibition. The antioxidant properties of peptides may dependent on their amino acid composition, structure, hydrophobicity, and other factors [44]. Although radical JBPH scavenge hydroxyl was inhibited, only 20% of radical hydroxyl was inhibited. Mungbean meal protein hydrolysate also had a low capacity to respond with hydroxyl radicals by donating an electron or double bond to become stable molecules of hydroxycyclohexadienyl radicals [42].

Ferric Reducing Antioxidant Power (FRAP) of JBPH

Table 4 also shows that JBPH prepared at 90 min exhibited the strongest antioxidant power on reducing ferric ions compared to other samples. The highest FRAP value obtained by all JBPH reflects that it can be a good antioxidant agent for reducing ferric ion. The data also shows that lower peptides (at 180 min) exhibited better energy reduction than large fractions of molecular weight (at 60 min). Similar results were obtained in African yam bean seed protein hydrolysate with a decrease in the molecular size of protein [45]. Small size protein was easy to reduce iron as more amino acid exposed to the environment. [46] reported that the rapeseed fractions showing the strongest reduction power also contained a higher amount of hydrophobic amino acids, which were suggested to be responsible for the strong reduction power of peptides.

Antimicrobial properties of Jack Bean JBPH

JBPH inhibition capacity was assessed against three pathogenic food bacteria namely *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using a well diffusion technique. **Table 5** shows that the only prone microorganism inhibited by JBPH was *P. aeruginosa*, while there was no inhibition for *E. coli* and *S. aureus*. Similarly, protein hydrolysates from germinated protein lablab bean pancreatin hydrolysis, pigeon pea, and kidney bean also demonstrate no antibacterial activity towards *E. coli* and *S. aureus* [47]. It appears that protein hydrolysate antimicrobial characteristics affect gram-negative bacteria (*P. aeruginosa*) more than gram-negative bacteria (*S. aureus*). This could be due to the Gram-positive bacteria's cell wall which is high in peptidoglycan. A particular action mechanism for *Staphylococcus* spp. or *E. coli* could be clarified by variations in Gram-negative and Gram-positive cell wall constitutions [48].

Table 5. Antimicrobial activity of powdered JBPH

Hydrolysis time	Inhibition zone (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
A	NI	NI	10.96 ± 0.36^{c}
B	NI	NI	11.74 ± 0.39^{bc}
C	NI	NI	13.07 ± 0.42^{a}
D	NI	NI	12.34 ± 0.35^{ab}
E	NI	NI	12.97 ± 0.06^{a}
Penicillin	52±0.05	-	50±0.10
Chloramphenicol	-	43±0.04	-

Note: A=60 min, B=90 min, C=120 min, D=150 min, E=180 min [Hydrolysis time]
NI= No Inhibition
All values are means of triplicate analysis. For each average, the standard deviation (mean ± SD) is included. Means with distinct letters are considerably distinct ($p < 0.05$)

CONCLUSION

Protein hydrolysate was effectively obtained from Jack bean using various hydrolysis time. It was found that the best hydrolysis time to produce JBPH with the finest physical features was 120 min, while the size of the structure decreased with hydrolysis time and showed irregular shape like “collapse building”. JBPH's foaming ability and stabilization very small and reduce with hydrolysis time. JBPH prepared at 120 min hydrolysis time showed the highest inhibition toward DPPH and hydroxyl radicals. Overall, JBPH good antioxidant agent for ferric reducing and superoxide radical scavenging activity. Only *P. aeruginosa* can be inhibited by JBPH. However, there was inhibition showed by all samples only towards *P. aeruginosa.* This shows that a hydrolysis time of 120 min is seen promising to generate protein hydrolysate from Jack Bean as a prospective source of functional food ingredients.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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