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Protein quality and physical characteristic of wood grasshopper (*Melanoplus cinereus*) hydrolysate flour

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Abstract

Indonesia is a tropical country with high biodiversity including insects. Insects are referred to as a good source of protein to overcome various nutritional problems. Wood grasshoppers (*Melanoplus cinereus*) have been consumed for a long time as a source of protein with limited digestibility due to its high chitin content. In this research, wood grasshoppers flour was added with bromelain enzyme with various concentrations 0%, 4%, 5%, 6% (w/v) to produce hydrolyzed flour in order to improve its protein quality. The manufacture of wood grasshoppers hydrolyzate flour was carried out by first dissolving the flour with water, then adjusting it at pH 7. Then, various concentrations of the bromelain enzyme and inucbated 7 hrs at 55°C. The final product obtained was the hydrolyzed flour in freeze-dried form. These flour were analyzed for their proximate analysis, protein quality (soluble protein, protein digestibility, amino acids total), antioxidant activity and physical properties (pH, color quantification). All these results were compared to obtain the best flour quality. The protein digestibility and color quality improved by the increasing bromelain concentration. However, soluble protein, amino acid total, and pH of wood grasshopper's hydrolysate flour decreased. Variation of bromelain concentration gave a significant difference on water content, carbohydrates, fats, protein, protein soluble, protein digestibility, antioxidant activity, redness and yellowness. Nonetheless ash content, pH and lightness of these flour were comparable. In conclusion, the best protein digestibility of wood grasshopper's hydrolysate flour was 6% of bromelain concentration, which was 51.33%.

1. Introduction

Wood grasshopper (*Melanoplus cinereus*) is one of the local high-protein animal foods that are commonly found in the Gunung Kidul area, Yogyakarta-Indonesia. The utilization of wood grasshopper in the area is still limited as snacks, side dishes, and souvenir foods for adults (Kuntadi *et al*., 2018).Wood grasshopper in Gunung Kidul, which have been processed into flour, contain protein of 76.69%; carbohydrates of 9.62%; the fat of 6.9%; ash content of 2.8%; and energy of 407.3 kcal per 100 g (Blásquez *et al*., 2012; Ruiz *et al*., 2015). The amount of protein indicated that the wood grasshopper protein was higher than beef protein (20 - 55%), poultry (18.7 - 20.8%), fish (12.9 - 18.4%), tempeh (13.84%) and tofu (10,1%). One study reported that wood grasshopper contain the highest protein and the lowest fat compared to crickets, silkworms, and Hongkong caterpillars (Kuntadi *et al*., 2018). Wood grasshopper also have nine essential amino acids that the body cannot synthesize. It includes phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine and histidine (Paul *et al*., 2016).

Besides being high in protein, grasshopper have antioxidant activity in their bioactive peptides (de Castro *et al*., 2018). The study reported that the grasshopper (*Schistocerca gregaria*) hydrolyzed bioactive peptide showed an antioxidant activity value of 27.5 ppm IC50 after being tested by the ABTS method (Zienlinska and Baraniak, 2017). Several amino acids that have antioxidant activity include histidine, cysteine, lysine, methionine, tyrosine and tryptophan (de Castro *et al*., 2018).

One of the criteria to determine the nutritional value

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of a food is protein quality (Adhikari *et al*., 2022). Protein quality can be determined by the amount of soluble protein, protein digestibility, and essential amino acid content (Martono *et al*., 2012; Adhikari *et al*., 2022). The study conducted by Kinyuru *et al*. (2010) proved that the protein digestibility of the green grasshopper *Ruspolia differences* was 82.34%, and the protein digestibility of the brown grasshopper was 85.67%. Paul *et al*. (2016) conducted a study on the protein digestibility of grasshopper flour species *Chorthippus paralleleus*, which has a protein digestibility of 97%. Although the protein digestibility of wood grasshopper is lower than other grasshopper species, its abundant availability is one of the potential food ingredients to be used as protein sources.

One methods that can be applied to improve protein quality is enzymatic protein hydrolysis (Liceaga *et al*., 2018). The process of hydrolysis allows proteins break down by proteolytic enzymes with the end products of amino acids and peptides (Tapal and Tiku, 2019). The proteolytic enzyme was used in this study is the bromelain enzyme because it does not cause a bitter taste like the papain enzyme (Arshad *et al*., 2014). The hydrolisis process of protein might increase the amount of soluble protein therefore it would easily absorbed by the digestive system due to enzyme protein cleavage, thereby reducing molecular protein weight, resulting in increased soluble protein levels (Tavano, 2013). Research on protein hydrolysis of Tempe gembus showed that there was an increase in soluble protein levels after the hydrolysis process from 0.6% to 0.78 (Agustina *et al*., 2018). Protein hydrolysis also affects protein digestibility because it can decompose complex-shaped proteins into simpler amino acids and peptides so that they are more easily digested by the body (Haslina *et al*., 2006). A study by Koopman and Crombach (2009) proved that enzymatically hydrolyzed casein can increase the rate of digestibility and absorption of protein in the intestine and the availability of postprandial amino acids, as indicated by an increase in the rate of amino acids to skeletal muscle.

Research on the wood grasshopper hydrolyzed flour needs to be carried out because the use of the bromelain enzyme to produce bioactive peptides from wood grasshopper has never been reported. This research was conducted to examine the effect of variations in enzyme concentration on nutrient content, protein quality (soluble protein, protein digestibility, amino acids total), antioxidant activity, physical properties (pH, and color quantification) of wood grasshopper flour hydrolyzate in freeze-dried form.

2. Materials and methods

2.1 Materials

The materials used in this study were wood grasshopper (*Melanoplus cinereus*), distilled water, bromelain enzyme "Pinecaps", 12.5 N NaOH, CH_3COOH, ABTS, potassium persulfate, ethanol, *Bradford* reagent, Bovine Serum Albumin (BSA), HCl, Indicator PP, H_2SO_4, K_2SO_4, K_2SO_4, $CUSO_4$, CBB, 85% ortho-phosphoric acid, enzyme pepsin, TCA, whaffhole buffer pH 2, Orthophthalaldehyde, Tetrahydrofuran (THF), amino acid standard solution 0.5 mol/mL, boric acid, 30% Brij-30 solution, 2-mercaptoethanol, Na-EDTA, and 0.15 M NaCl. The equipment used during the research were digital scales, blender, spoon, stirring rod, pH meter, incubator, freeze dryer, freezer, centrifugation, magnetic stirrer, vortex mixer, test tube, micropipette, Erlenmeyer, beaker, measuring cup, measuring flask, cuvette, Erlenmeyer, pipette, mortar, centrifugation, Kjeldahl flask, funnel, distillation set, volume pipette, Pasteur pipette, soxhlet, HPLC, screw tube and UV-Vis spectrophotometer

2.2 Methods

This was an experimental study with a one-factor, completely randomized design (CRD) with four variations of bromelain enzyme concentrations of 0%, 4%, 5% and 6%. The proximate analysis includes protein content using the Kjeldahl method, fat content using the Soxhlet method, carbohydrate using the by difference method, and ash and water content using the gravimetric method. The amount of soluble protein was tested using the Bradford method, the digestibility of the protein was tested using the in vitro method enzymatically with the enzyme pepsin, the amino acid profile was tested using High-Performance Liquid Chromatography (HPLC), and the pH value was measured using a pH meter, the color was measured using a Colorimeter and activity analysis. Antioxidant to obtain the IC_{50} value using the 2,2-Azinobis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) method.

2.3 Process of making wood grasshopper hydrolyzed flour

The production of wood grasshopper hydrolyzed flour were made by mixing 90 g of wood grasshopper with 180 mL of distilled water (1:2) and then mashed using a blender. The mashed sample was added with 12.5 N NaOH until the pH of the solution became 7 so that the bromelain enzyme could work optimally. The sample was added with different concentrations of bromelain enzyme (0% ascontrol, 4%, 5% and 6% (w/v), respectively). After adding the bromelain enzyme, the sample was incubated in an incubator for 7 hrs at 55^{o}C.

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After incubation, the sample was heated at 90°C for 20 mins to inactivate the bromelain enzyme. The wood grasshopper's protein hydrolyzate slurry sample was dried by freeze drying at -73°C. The dry hydrolyzate sample was mashed with a mortar and then sieved using an 80 mesh sieve to become flour. Each sample was analyzed with three repetitions in duplicate.

2.4 Water content

A total of 2 g of the wood grasshopper hydrolyzed flour was weighed using an aluminum crucible that had been dried and its weight known. The crucible was then dried in an oven at a temperature of 105 - 110°C for three hrs. The crucible was removed and cooled in a desiccator and then weighed. Drying was continued again, and every 1/2 hr was cooled and weighed until a constant weight was obtained. The water content was calculated using the formula:

$$\text{Water contents (\%)} = \frac{\text{Initial weight} - \text{Constant weight}}{\text{Initial weight}} \times 100\%$$

2.5 Ash content

A total of 2 g of the sample was weighed in a porcelain crucible that had been dried. The crucible was then ignited and ashed in an ashing furnace at 600°C for four hrs. The crucible was then removed and cooled in a desiccator, then weighed. The ashing was continued again, and every 1/2 hr was cooled and weighed until a constant weight was obtained. The ash content was calculated using the formula:

$$\text{Ash contents (\%)} = \frac{(\text{Crucible weight} + \text{ash}) - \text{Empty weight}}{\text{Sample weight}} \times 100\%$$

2.6 Protein content

Approximately 3 g of sample was weighed in a beaker. Next, 20 mL of concentrated H_2SO_4, 5 g of K_2SO_4, 0.5 g of $CuSO_4$ and a few boiling stones into a Kjeldahl flask. The Kjeldahl flask was placed on the stand with an inclination of 45° and the funnel cup was attached to the mouth of the flask. The mixture was heated until turned transparent green (~ 75 mins). The solution was then cooled and transferred into a 500 mL round bottom flask. Distilled water was added until the volume is about half of the flask. Then, 100 mL of 40% NaOH solution and a few boiling stones were added and the distillation process was performed. The distillate was collected in an Erlenmeyer containing 50 mL of 0.1000 N HCl solution and added with three drops of phenolphthalein indicator solution. The solution was titrated with with 0.1000 N NaOH solution with phenolphthalein indicator until the end point. The protein content was calculated using the formula:

$$\text{Protein contents} = \frac{(V \times N)\text{HCl} - (V \times N)\text{NaOH} \times 14 \times 6.25}{\text{Sample weight}} \times 100\%$$

Where V = Volume (mL), N = Normality (N) and 6.25 = Protein Equivalence.

2.7 Fat content

Fat content analysis was carried out using the Soxhlet method. The flask was dried in the oven at 105˚C for 30 mins. The flask was then cooled in the desiccator and weighed with a leaded filter paper tied with fat-free cotton wool. The solvent was poured into the flask and the Soxhlet extraction apparatus was set up. The flask was heated for extraction for 3-4 hrs (5-6 cycles). The collected solvent was distilled and dried in the oven at 105˚C to constant weight. The weights were measured after cooling in the dessicator for 30 mins. The fat content was calculated using the formula:

$$\text{Fat contents (\%)} = \frac{\text{fat weight (g)} \times 100\%}{\text{sample weight (g)}} = \frac{(b - a) \times 100\%}{\text{sample weight (g)}}$$

2.8 Carbohydrate content

The following calculation determined the carbohydrate content: 100 – (water content + ash content + fat content + protein content).

2.9 Soluble protein analysis procedure

Bradford's reagent was made by weighing 10 mg of CBB, adding 95% ethanol and 10 mL of 85% ortho-phosphoric acid, and then homogenized. After that, it was dissolved with up to 100 mL and homogenized. The solution was filtered and stored in a dark bottle at 4°C.

A total of 50 mg of wood grasshopper hydrolyzed flour was diluted using NaCl until the volume reached 50 mL (1000 ppm concentration). After that, the sample was centrifuged at 4500 rpm for 20 mins. The filtrate was separated from the precipitated and a total of 100 µl was taken. Bradford's reagent was add and incubated for 5 mins at room temperature. After that, the absorbance was taken using a spectrophotometer at a wavelength (λ) = 595 nm.

2.10 Amino acid analysis

Amino acid analysis can be done by utilizing the pre-column reaction of amino groups with specific reagents to form a fluorescence compound. Sample preparation was carried out by determining the protein content using the Kjeldahl method. The sample containing 6 mg of protein was put into a screw tube, and 2 mL of 6 N HCl was added. The screw tube containing the sample solution flowed with nitrogen gas for 0.5 - 1 min and then closed immediately. The closed tube was put in an oven at 110°C for 24 hrs to carry out the hydrolysis stage. The hydrolyzed sample was cooled at room temperature and transferred quantitatively to a rotary evaporator flask. The threaded tube was rinsed with

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distilled water 2 - 3 times. The rinse solution was then combined into a rotary evaporator flask and dried. The dried sample was added with 0.01 N HCl to 10 mL.

The hydrolyzed amino acid sample was soluble in 10 mL of 0.01 N HCL and filtered using millipore paper. After that, add Buffer Potassium Borate pH 10.4 with a ratio of 1:1. Then, add 5 µL of sample and 25 µL of OPA reagent, and leave for 1 min for complete derivatization. A total of 5 µL was injected into the HPLC and waited until the amino acid separation was complete.

$$\text{Amino acid (\%)} = \frac{\text{µmol AA x Mr AA}}{\text{µG sample}} \times 100$$

2.11 Protein digestibility analysis

A total of 5 g of the sample was weighed and put into an Erlenmeyer. After that, 20 mL of whaffhole buffer pH 2 was added. Then, 2 mL of 1% pepsin enzyme was added to the solution and incubated at 40°C for 1 hr. Next, the solution was centrifuged and added with 5 mL of 50% trichloroacetic acid (TCA) to precipitate undigested protein. The solution was allowed to stand for 1 hr to settle the undigested protein completely. A total of 5 mL of the filtrate was taken to analyze the total protein content.

$$\text{Protein Digestivity} = \frac{N\ Digestible\ protein}{N\ total\ protein} \times 100\%$$

2.12 pH analysis

Wood grasshopper hydrolyzed flour was dissolved in water in a ratio of 1:1. After that, it was homogenized using a vortex to form a solution. The pH meter electrode was inserted into the solution to obtain the pH

2.13 Color analysis

Samples were taken sufficiently and placed under the light into a test box. The sample was placed according to the coordinates on the cellphone connected to the colorimeter application to obtain the values.

2.14 Statistical analysis

Data on protein content, protein digestibility, and yellowish color, which were normally distributed, were analyzed by the one-way ANOVA test followed by the Tukey post hoc test. The data of water content, fat content, soluble protein, and antioxidant activity were analyzed by a one-way ANOVA test and followed by the Duncan test. Carbohydrate content and reddish color data were analyzed using the one-way ANOVA test, followed by the LSD test. A one-way ANOVA test was used to analyze data on ash content, color brightness, and pH.

3. Results and discussion

3.1 Proximate analysis

Table 1 shows the significant differences in water, carbohydrate, protein, and fat content in each treatment group ($p<0.005$). In the wood grasshopper hydrolyzed flour, there was an increase in water content which was inversely proportional to the levels of protein, carbohydrates, fat, and ash. The higher the water content, the lower the protein, carbohydrate, fat, and ash content (Buckle *et al*., 1987).This is because if the water content is high, the dry weight is low, so the levels of protein, carbohydrates, fat, and ash in the dry weight are low. So the drier the material, the higher the protein, carbohydrate, fat, and ash content (Laksono *et al*., 2012).

The water content contained in the wood grasshopper hydrolyzed flour product ranged from 9.03-17.03% per 90 g of wood grasshopper hydrolyzed flour. The control treatment had the lowest water content value, which was 9.03%, while the highest water content was in the 4% concentration of wood grasshopper hydrolyzate treatment, which was 17.03%. The water content increases as the enzyme concentration increases because adding bromelain enzyme can affect the resulting liquid. It is known that in the hydrolysis reaction, protein compounds can be broken down into simpler and more soluble compounds, increasing the volume of fluid which increases the product's water content (Wijayanti *et al*., 2016).

The ash content of a food ingredient shows the number of minerals contained in a material (Winarno,

Table 1. Nutritional content of wood grasshopper hydrolyzed flour

Nutritional Content	Treatment				
	Bromelain 0%	Bromelain 4%	Bromelain 5%	Bromelain 5%	*p*
Water (%)	9.03±0.91^{a}	17.03±0.83^{b}	16.51±1.83^{b}	16.93±2.44^{b}	0.001*
Ash (%)	4.03±0.04	3.74±1.45	2.72±0.05	2.706±0.03	0.120*
Carbohydrate (%)	19.25±1.64^{x}	28.4 ±1.57^{y}	31.85±0.47^{z}	34.63±2.21^{z}	0.000*
Protein (%)	60.98±1.76^{d}	46.92±1.08^{e}	44.79±2.35^{e}	42.27±2.03^{e}	0.000*
Fat (%)	6.71±1.15^{a}	3.88±0.53^{b}	4.13±0.85^{b}	3.45±0.91^{b}	0.008*

Values are presented as mean±SD. Values with different superscript letters (a, b) are statistically significantly different analyzed with the Duncan's test ($p<0.05$). Values with different superscript letters (x, y, z) are statistically significantly different analyzed with the LSD test ($p<0.05$). Values with different superscript letters (d, e) are statistically significantly different analyzed with the Tukey test ($p<0.05$). *Statistically significantly different.

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1995). The ash content of the grasshopper protein hydrolyzate flour product ranged from 2.7-4.03% per 90 g of grasshopper hydrolyzate. Based on the results of statistical analysis showed that there was no significant difference between the four treatment groups (p = 0.120). The ash content of the wood grasshopper hydrolyzed flour decreased compared to the control. The ash content decreased due to the use of 50% NaOH solution and a temperature of more than 80°C during the hydrolysis process, thereby eliminating acetyl groups and inorganic minerals through acid and alkaline treatment (Dompeipen *et al*., 2016).

The fat content of the wood grasshopper hydrolyzed flour ranged from 3.45 to 6.71% per 90 g of wood grasshopper hydrolyzed flour. In addition, there was a decrease in fat content from 6.71 g (control) to 3.88 g (bromelain 4%), 4.13 g (bromelain 5%), and 3.45 g (bromelain 6%) due to changes in the structure during the enzymatic hydrolysis process. Myofibril proteins are degraded so much and reduced during the hydrolysis process. During the hydrolysis process, these membranes gather and form insoluble bubbles, resulting in the loss of the fat membrane, which results in a decrease in fat content (Sahidi and Han, 1995). Fat content in protein hydrolyzate is an important component. Protein hydrolyzate products with low-fat content are more stable and durable when compared to hydrolyzate products with high-fat content. In addition, the low-fat content of the hydrolyzate product can be used as a low-fat diet with a fat content of less than 5% (Pigott and Tucker, 1990).

The protein content of wood grasshopper hydrolyzed flour ranged from 42.27 to 60.98%. The control treatment had the highest value, 60.98%, while the lowest protein content was in the wood grasshopper hydrolyzed flour with 6% bromelain, 42.27%. A decrease in protein content is influenced by increasing water content because there is an inverse relationship between water and protein content (Wijayanti *et al*., 2016).

The carbohydrate content of wood grasshopper hydrolyzed flour ranged from 19.25-34.63% per 90 g of wood grasshopper hydrolyzed flour. The highest value was owned by the 6% bromelain treatment, which was 34.63%, while the lowest carbohydrate content was in the control treatment, which was 19.24%. The increase in carbohydrate content in wood grasshopper hydrolyzed flour was due to the high chitin content of 87.3 g (Wang and Zhai, 2007; Kaya *et al*., 2015). Bromelain enzymes have the ability as chitinolytic enzymes and proteolytic enzymes. This enzyme can hydrolyze chitin and protein-bound in food (Tolaimate *et al*., 2000). Research by Wang *et al*. (2008) showed that the optimum reaction time for hydrolysis of chitin with bromelain enzyme was 24 hrs, while for amino acids, it was 48 hrs. This is what causes the carbohydrate content of wood grasshopper hydrolyzed flour to increase. Increasing the concentration of the bromelain enzyme also increases the carbohydrate content because more chitin is hydrolyzed.

3.2 Soluble protein

The amount of soluble protein in the wood grasshopper hydrolyzed flour ranged from 0.0090 to 0.0140%. It tended to decrease as the concentration of the bromelain enzyme added in the hydrolysis process increased. The amount of soluble protein refers to the total amount of protein in a material that enters the solution (Zayas, 1997). The difference in the decrease in protein content of the wood grasshopper hydrolyzed flour in Table 2 shows a significant difference (p = 0.034). This study's reduction of soluble protein was possible because the total protein also decreased due to the deproteination reaction.

Table 2. Soluble protein of the wood grasshopper hydrolyzed flour.

Treatment	Soluble Protein (%)
Bromelain 0%	0.0140 ± 0.0014^{a}
Bromelain 4%	0.0096 ± 0.0008^{b}
Bromelain 5%	0.0090 ± 0.0025^{b}
Bromelain 6%	0.0103 ± 0.0018^{b}
P	0.034*

Values are presented as mean±SD. Values with different superscript letters are statistically significantly different analyzed with the Tukey test ($p<0.05$). *Statistically significantly different.

The deproteination process occurs when the bromelain enzyme hydrolyzes the grasshopper protein that covers chitin. Proteins that have been hydrolyzed and have a negative charge can bind to Na^{+} derived from 12.5 N NaOH, which was added during sample preparation. The bond between Na^{+} and protein forms sodium proteinate (Dompeipen *et al*., 2016). The principle of the deproteination process is the process of releasing protein and chitin bonds. This process is generally carried out by treatment using a NaOH solution and a relatively long time (Dompeipen *et al*., 2016). The higher the bromelain enzyme concentration, the higher the protein and sodium proteinate hydrolyzed by the bromelain enzyme. Enzyme activity is influenced by several factors, one of which is enzyme concentration. The concentration of the enzyme is directly proportional to the rate of the reaction. If the concentration of the enzyme is increased, the reaction rate can increase. High enzyme concentration causes the enzyme's ability to degrade substrates to be more optimal (Robinson, 2015).

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In addition, the decrease in soluble protein occurred due to the higher bromelain enzyme concentration resulting in more soluble protein. However, the greater the interaction between soluble proteins can cause a decrease in solvent activity, so the solubility of proteins in the solvent decreases. In the end, the protein becomes precipitated directly (Semba *et al*., 2016).

3.3 Amino acid profile

The number of amino acids wood grasshopper hydrolyzed flour in Table 3 ranged from 43.04–61.87%. The control wood grasshopper flour had the highest total amino acid, 61.87%, while the wood grasshopper flour with 5% bromelain enzyme had the lowest total amino acid, which was 43.04%. The total amino acid in the wood grasshopper hydrolyzed flour decreased along with increased bromelain enzyme concentration. However, there was an inconsistency: an increase in total amino acids in hydrolyzed flour with 6% bromelain.

Wood grasshopper hydrolyzed flour with 5% bromelain had the lowest total amino acids due to the less perfect process of refining grasshopper with water than control flour, wood grasshopper hydrolyzed flour with 4% bromelain, and wood grasshopper hydrolyzed flour with 6% bromelain, thereby reducing the protein surface area to be broken down by the enzyme. The decrease in total amino acids was caused by reduced total protein and increased bromelain enzyme. This is related to amino acids being the building blocks of protein so that the number of amino acids is proportional to the total amount of protein. The amino acid reduction mechanism is the same as the total protein reduction mechanism; the protein that the bromelain enzyme has broken down turns into sodium proteinate because it binds to Na^+ ions from 12.5 N NaOH used during sample preparation.

Wood grasshopper hydrolyzed flour has an increase in the amino acid arginine. The increase in amino acid arginine was due to the bromelain enzyme's preference for cutting the peptide bond at the carbonyl end of arginine so that the higher the concentration of the enzyme used, the more small peptides that were liberated and containing the amino acid arginine at the carbonyl end. This is in agreement with research on the hydrolysis of brown rice using the bromelain enzyme, which showed that there is an increase in lysine-containing peptides that have antioxidant activity after hydrolysis (Selamassakul *et al*., 2016). In addition to the increase in arginine, hydrolyzed flour also experienced an increase in the amino acid methionine.

A protein can differ from other proteins because each has a different amino acid sequence (Albert *et al*., 2002). The increase in methionine in wood grasshopper hydrolyzed flour can be influenced by the amino acid composition of the wood grasshopper protein and the bromelain enzyme used. The increase in methionine in this study may occur due to the effect of cutting the bromelain enzyme at the carbonyl end of arginine on the amino acid sequence of wood grasshopper protein. This allows an increase in the amino acid arginine and amino acid methionine after the hydrolysis process.

One indicator of protein quality is the presence of complete and balanced essential amino acids. The value

Table 3. Amino acid profile wood grasshopper hydrolyzed flour.

Amino Acids	Treatment				Standard WHO/ FAO/UNU 2007
	Bromelain 0%	Bromelain 4%	Bromelain 5%	Bromelain 6%	
Essential Amino Acids					
Histidine	1.79	1.45	1.20	1.19	1.90
Threonine	1.85	1.48	1.41	1.41	2.90
Methionine	0.19	0.33	0.18	0.38	1.60
Valine	3.64	2.97	2.62	2.70	4.80
Phenylalanine	1.96	1.32	1.39	1.38	
Phenylalanine+ Tyrosine (Aromatic amino acids)	5.02	3.91	3.35	3.49	4.70
Isoleucine	5.37	4.49	3.82	3.95	3.80
Lysine	3.03	2.12	2.17	2.08	5.60
Leucine	11.36	9.41	7.85	8.32	7.30
Non Essential Amino Acids					
Aspartic acid	3.84	3.12	2.94	2.93	
Glutamic acid	7.16	4.94	5.03	4.99	
Serine	1.74	1.58	1.45	1.45	
Glycine	4.75	3.54	3.18	3.34	
Alanine	10.71	8.10	6.40	7.41	
Arginine	1.42	2.11	1.45	1.43	
Tyrosine	3.06	2.59	1.96	2.11	
Amino Acids Total (%)	61.87	49.55	43.04	45.08	

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(score) of the nutritional quality of the protein was declared if the essential amino acids were the most deficient compared to the FAO/WHO standard amino acids. The chemical score of wood grasshopper hydrolyzed flour increased along with the increase in the bromelain enzyme. Wood grasshopper hydrolyzed flour increased its chemical score from 11.9% to 23.8% with the limiting amino acid methionine. The chemical score of wood grasshopper hydrolyzed flour can be seen in Table 4.

The amino acid methionine increased along with the increase in the bromelain enzyme, although the hydrolyzed flour with 5% bromelain enzyme decreased. The increase in the amino acid methionine increased the chemical score, which indicated an increase in the protein quality of the wood grasshopper hydrolyzed flour. The highest amino acid found in wood grasshopper hydrolysate flour with variations in bromelain enzyme concentrations was leucine. High leucine content can make wood grasshopper hydrolyzed flour a source of leucine which is beneficial for the growth process of children to prevent malnutrition problems.

3.4 Protein digestibility

Protein digestibility is the ability of a protein to be hydrolyzed into amino acids by digestive enzymes (Muchtadi, 1989). Protein digestibility of wood grasshopper hydrolyzed flour ranged from 44.65–51.33%. Table 5 shows a significant difference ($p = 0.014$) in each group of wood grasshopper hydrolyzate treatment using different concentrations of the bromelain enzyme. The highest protein digestibility was found in wood grasshopper hydrolyzed flour with 6% bromelain, which was 51.33%, while the lowest protein digestibility was found in wood grasshopper hydrolyzed flour with 5% bromelain, which was 44.65%. Tukey's further test showed a significant difference between wood grasshopper hydrolyzed flour with 5% bromelain and wood grasshopper hydrolyzed flour with 6% bromelain.

Protein digestibility of wood grasshopper hydrolyzed flour increased by the concentration of the bromelain enzymes. However, there was a decrease in wood grasshopper hydrolyzed flour with 5% bromelain, which was caused by an incomplete destruction process, so the protein structure did not decompose completely. This can inhibit protease enzymes in hydrolyzing peptides, resulting in decreased protein digestibility in wood grasshopper hydrolyzed flour with 5% bromelain. Size reduction (smoothing) can make proteins in the form of long complex polypeptide strands into simple long strands (Anam *et al*., 2010). Grasshopper structure that is not completely decomposed can inhibit protease enzymes in hydrolyzing peptides and decrease protein digestibility in hydrolyzed flour with 5% bromelain.

Although there was a decrease in protein digestibility in wood grasshopper hydrolyzed flour with 5% bromelain, there was an increase in protein digestibility in wood grasshopper hydrolyzed flour with 4% bromelain and wood grasshopper hydrolyzed flour with 6% bromelain. The increase in protein digestibility of wood grasshopper hydrolyzed flour was caused by using a temperature of 55°C, which can open the random structure of the protein so that the bromelain enzyme can hydrolyze it. The increase in the bromelain enzyme resulted in more broken peptide bonds and simpler amino acid sequences. Simpler amino acids can facilitate the pepsin enzyme to hydrolyze peptide bonds, thereby increasing the digestibility of protein digestibility of wood grasshopper hydrolyzed flour (Nadzirah *et al*.,

Table 5. Protein digestibility of wood grasshopper hydrolyzed flour.

Treatment	Protein Digestibility (%)
Bromelain 0%	46.41±2.23[a]
Bromelain 4%	48.25±0.61[a]
Bromelain 5%	44.65±2.09[a]
Bromelain 6%	51.33±2.14[b]

Values are presented as mean±SD. Values with different superscript letters are statistically significantly different analyzed with the Tukey test ($p<0.05$).

Table 4. Wood grasshopper hydrolyzed flour chemical score.

Essential Amino Acids	Amino Acids Score			
	Bromelain 0%	Bromelain 4%	Bromelain 5%	Bromelain 6%
Histidine	94.2	76.3	63.2	62.6
Threonine	63.8	51	48.6	48.6
Methionine	11.9	20.6	11.3	23.8
Valine	75.8	61.9	54.6	56.3
Phenylalanine+ Tyrosine (Aromatic amino acids)	100	83.2	71.3	74.3
Isoleucine	100	100	100	100
Lysine	54.1	37.9	38.8	37.1
Leucine	100	100	100	100
Flour chemistry score (limiting amino acid: methionine)	**11.9**	**20.6**	**11.3**	**23.8**

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2016). The higher the digestibility of protein, the higher the level of bioavailability of amino acids (Muchtadi, 2008).

3.5 pH value

The pH value of wood grasshopper hydrolyzed flour ranged from 5.54–6.94. Based on Table 6, the highest pH value was found in control (6.90), while the lowest was found in hydrolyzed flour with 6% bromelain, which was 5.41. The bromelain enzyme concentration did not give a significant difference (p = 0.09) to the pH value of wood grasshopper hydrolyzed flour. The higher the enzyme concentration, the lower the pH value of the wood grasshopper hydrolyzed flour. This happens because the higher the enzyme concentration can increase the enzyme's work, and the more hydrogen ions are released in the hydrolysis process so that the pH decreases. When the protease enzyme breaks the peptide bond, the carboxylate group is released, which can release some hydrogen ions, which results in a decrease in pH (Belinda and Yunianta, 2016).

Table 6. pH value of wood grasshopper hydrolyzed flour.

Treatment	pH
Bromelain 0%	6.94±0.20
Bromelain 4%	5.96±0.65
Bromelain 5%	5.91±0.46
Bromelain 6%	5.54±0.71
p	0.070*

Values are presented as mean±SD. *Statistically significantly different.

3.6 Color

The color aspect of wood grasshopper hydrolyzed flour consists of brightness, redness and yellowness values. Color values are measured using a digital colormeter. The value of brightness, redness and yellowness of wood grasshopper hydrolyzed flour increased along with the increase in the bromelain enzyme (Table 7).

Table 7. Color of wood grasshopper hydrolyzed flour.

Treatment	Brightness	Readness	Yellowness
Bromelain 0%	21.66±1.52	10.33±0.57^{a}	20.33±1.52^{a}
Bromelain 4%	23.33±7.09	14±1.73^{b}	22.66±6.50^{a}
Bromelain 5%	29±2	13±1^{b}	27.66±1.15^{a}
Bromelain 6%	31±2	13±1^{b}	29.66±1.52^{b}
P	0.054*	0.023*	0.037*

Values are presented as mean±SD. Values with different superscript letters are statistically significantly different (p<0.05). *Statistically significantly different.

The brightness value of protein hydrolyzate flour ranged from 21.66–31. The increase in the bromelain enzyme concentration increased the brightness of the wood grasshopper hydrolyzed flour product, so it can be concluded that the increase in the bromelain enzyme could make the wood grasshopper hydrolyzed flour brighter. The statistical analysis results showed no significant difference in the four wood grasshopper hydrolyzed flour (p = 0.054).

The reddish value of wood grasshopper hydrolyzed flour ranged from 20.33 to 29.66. The bromelain enzyme concentration increased the redness value of wood grasshopper hydrolyzed flour. The higher the bromelain enzyme concentration, the wood grasshopper hydrolyzed flour changed color from dark brown to reddish-orange. Statistical analysis showed that the variation of bromelain enzyme concentration gave a significant difference (p = 0.023) to the reddish value of wood grasshopper hydrolyzed flour.

The yellowness value of wood grasshopper hydrolyzed flour ranged from 10.33-14. The bromelain enzyme concentration increased the yellowish value of the wood grasshopper hydrolyzed flour. The higher the bromelain enzyme, the more yellow the wheat flour is in the wood grasshopper hydrolyzed flour. Statistical analysis showed that the bromelain enzyme concentration variation significantly (p = 0.037) to the yellowness value of wood grasshopper hydrolyzed flour.

The higher the bromelain enzyme concentration, the color of the wood grasshopper hydrolyzed flour gradually changed from dark brown to bright orange. A similar increase in color also occurred in the study of (Nadzirah *et al*., 2016) regarding beef that was added with 0.17% bromelain enzyme and heated at 60^{o}C for 10 mins had a lighter color than control beef or that was not treated with bromelain enzyme. This is because beef is oxidized to form metmyoglobin (metMb) which has a pale red color, and there is an increase in light reflection from light scattering by denatured proteins. In addition, the color change from dark brown to bright orange is thought to occur due to the influence of the bromelain enzyme, which has an original bright orange color. In terms of color, the bromelain enzyme can be an alternative to improve the color quality of wood grasshopper hydrolyzed flour because this enzyme can change the color of flour from dark brown to bright orange so that it will make food products more attractive.

4. Conclusion

Variations in bromelain enzyme concentration gave significant differences in antioxidant activity, protein, fat, carbohydrate, water content, amount of soluble protein, protein digestibility, amino acid profile and color of wood grasshopper hydrolyzate flour. The increase in bromelain enzyme concentration increased protein digestibility, protein chemistry score, and color

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value but decreased soluble protein, total amino acids, and pH of wood grasshopper hydrolyzed flour. Bromelain enzyme concentration that has the best digestibility was 6%.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this paper.

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