Research Article

DOI:10.22146/ifnp.70884 https://journal.ugm.ac.id/ifnp

ISSN 2597-9388

Indonesian Food and Nutrition Progress

Optimization of Anti-Nutritional Removal, Protein Extraction, and Modification of Functional Properties of Cowpea (*Vigna unguiculata* L.) Protein With Sonication

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ABSTRACT: Cowpea has high protein but contains some anti-nutritional compounds that lower the nutritional quality It is necessary.to reduce the anti-nutritional in cowpea. The functional properties of protein are essential, affecting its role in the food matrix system. Modifying functional properties by sonication can improve its role as a food ingredient. This study aims to investigate the optimum anti-nutritional removal and protein extraction treatments and modify protein's functional properties with sonication. Removal of anti-nutritional was conducted by a combination of soaking for 24, 48, and 72 hours with 5% NaHCO3 solution and boiling for 0, 2.5, and 5 minutes. The protein extraction was conducted by adjusting the pH to various pH at 2, 4, 6, 8, 10, 11, and 12. Soaking for 48 hours with 5% $NaHCO_3$ significantly reduced HCN level to 0.72 ppm, phytic acid of 9.62 mg/g, and trypsin inhibitor of 7.02 mg/g. The protein extraction was done using optimum pH of 12, while precipitation was at pH 4 with a protein concentration of 70.9%. Sonication with a power of 80% (168 W) for 30 minutes suggested an increase in the water-holding capacity, oil-holding capacity, emulsion stability, and foaming capacity-stability rather than untreated.

Keywords: *Anti-nutrition, removal, cowpea protein, protein extraction. functional properties*

INTRODUCTION

Indonesian cowpea (*Vigna unguiculata)* is a legume species with a brown colour with one of its varieties, KT-6 (Hetharie et al., 2015). Cowpea is rich in carbohydrates and protein (Gondwe et al., 2019). In addition, this legume has low low-fat content, suggesting that it can be used as a protein source in the diet for people with dyslipidemia (Seidu et al., 2015). The protein from cowpea can be utilized as food derivatives that are important to provide an alternative protein source rather than soybean protein imported to Indonesia.

Despite some merits, unfortunately, cowpea contains some anti-nutritional compounds and natural toxins present in the seed, such as cyanide acid (HCN), trypsin inhibitor, and phytic acid (Khattab & Arntfield, 2009). These compounds' presence may diminish the nutritional quality and sensory acceptance as those compounds exhibit a bitter taste (Ngatchic et al., 2013). Hence, it needs some pretreatment before processing this seed for ingredients in food products. The treatments include soaking in water, boiling, or fermenting can be applied to diminish the anti-nutrition content (Wijatniko & Agnes Murdiati, 2017). The presence of alkali compound in the soaking water might affect the reduction of the anti-nutritional substances in seeds since these substances might interfere and reacts with alkali compound to produce the salts that dissolved during soaking (Avilés-Gaxiola et al., 2018; Ertaş & Türker, 2012; Nur Irzam, 2014). In addition, the research about eliminating natural toxins and anti-nutritional substances in cowpeas is still limited. Therefore, it is important to emphasize the elimination of those substances before bringing cowpea for use. This step is required considering that cowpea has good potential as a food ingredient.

One of the prospective derivative products from cowpea is protein isolate. Protein isolates in the food industry can be applied as complementary ingredients in processed meat products to provide good quality products, such as sausage and chicken nuggets (Canti & Murdiati, 2015). The high protein content and its functionality make it a proper binding agent to stabilize emulsions, increase the water binding capacity of meat products, reduce shrinkage during cooking, increase flavour and enhance the characteristics of sliced products (Y. Zhang et al., 2014). Protein isolates widely used in the food industry are soybean protein isolates, but unfortunately, they have low productivity. Therefore, it is necessary to investigate the alternatives to substitute soy protein isolates. The main feature of protein isolates that can be applied to various food production with desired functional properties, such as emulsion stability, emulsion, and water holding capacity (Nwachukwu & Aluko, 2021).

The functional properties of a protein can be modified through physical hydrolysis, one of which is sonication treatment (K. Zhang et al., 2022). Sonication treatment affected the functional and physicochemical properties of protein isolates due to disruption of the conformation of the protein structure(Zhu et al., 2018). Ultrasonic treatment altered the physicochemical and functional properties of sunflower seed protein isolates (Malik et al., 2017). Ultrasound waves from sonication cause the formation of cavitation bubbles which produce high shear energy and turbulence in the protein structure, which will change its functional properties (Byanju et al., 2020). The solubility of protein isolates increased due to the particle size reduction, creating an increase in protein-water interactions. Emulsion capacity and stability have increased after sonication, which is correlated to the increased surface hydrophobicity, so the protein interactions became stronger with the oil-water interface. It will significantly impact the strength of capacity and stability of the foam,

which increased due to increased surface hydrophobicity of proteins (Resendiz-Vazquez et al., 2017). This research was conducted to optimize the elimination of anti-nutritional substances in cowpeas and protein extraction, followed by optimization of sonication treatment to modify the functional properties of cowpea protein isolate. Subsequently, the functional properties of protein isolate were evaluated, including water-holding capacity (WHC), oil-holding capacity (OHC), emulsion capacity and stability, and foaming capacity and stability.

MATERIALS AND METHODS

Materials

Cowpea was obtained from a traditional market in Yogyakarta. The chemicals used are pro analysis grade which included; n-hexane (Merck Millipore), concentrated H_2SO_4 (Sigma-Aldrich), HCl 37% (Merck Millipore), trichloroacetic acid (Sigma-Aldrich), boric acid crystal (Sigma-Aldrich), picric acid (Sigma-Aldrich), KCN (Merck Millipore), bromocresol green-methyl red (BCG-MR) (Sigma-Aldrich), petroleum ether (JT Baker), catalyst (HgO and K_2SO_4) (Sigma-Aldrich), NaOH-$Na_2S_2O_3$ (Sigma-Aldrich), and NaOH crystals (Merck Millipore).

Preparation of Cowpea Flours and Elimination of Antinutritional Substance

Cowpea flour was prepared according to the preparation of jack bean flour (Wijatniko & Murdiati, 2019) with modification. Cowpea seeds were soaked in 3 steps. Step one was soaking the seeds in water for 24, 48 h, and 72. The soaking time of seeds that showed the lowest anti-nutritional content was subsequently treated to step 2. Step 2 was soaking the seeds using 0, 2,5%, and 5% $NaHCO_3$ solution. The concentration of $NaHCO_3$ solution showed the lowest anti-nutritional content at step 2 and will be used for soaking treatment in step 3. Step 3 consisted of boiling the seeds from step 2 with the 3-time course (0, 2.5, and 5 minutes). The seeds were then dried in a cabinet drier (50 ^{0}C) for 24 h, followed by grinding and sieving in a 60 mesh sieve. Cowpea flour was kept in a closed container to prevent moisture absorption from the environment into flour.

Isolation of Cowpea Protein

Protein isolation of cowpea was conducted by adopting the jack bean protein isolate procedure from the previous study (Canti & Murdiati, 2015) with some modifications. Before protein extraction, fat in cowpea flour was diminished by defatting with n-hexane with powder to hexane ratio of 1: 3 (w/v). Defatting was done twice, followed by drying the defatted white cowpea powder at a cabinet dryer at a temperature of 50^{0}C for 2 hours. Extraction of cowpea protein was initiated by making a mixture of defatted cowpea powder and distilled water with the ratio of 1:4 (w/v), followed by adjusting the pH of the mixture to various levels of pH (2, 4, 6, 8, 10 and 12) with 1 N NaOH. Subsequently, the mixture was stirred for 1 hour to allow the protein from cowpea powder to dissolve in water. After the stirring, the protein fraction was separated by centrifugation at 4000 rpm for 30 minutes to obtain supernatant (protein solution). The pH of the supernatant was then adjusted to 4.5 to precipitate the protein, followed by centrifugation at 4000 rpm for 30 minutes to obtain protein precipitate (natant). The protein precipitate was then stored at -50^{0}C prior to analysis.

Physical hydrolysis of cowpea protein isolate

Physical hydrolysis of cowpea protein isolate was conducted with sonication according to the previous study (Zhu et al., 2018) with modification. Sonication was carried out with a 40 kHz bath sonicator (Elma Transsonic Digital/T 760 DH), with power levels of 40% (84 W), 60% (126 W), and 80% (168 W) and time levels of 10, 20, and 30 minutes. The Sonicator bath was filled with distilled water, and the temperature was conditioned to room temperature (± 27ºC).

Chemical dan anti-nutritional analysis

Standard AOAC methods (AOAC, 1995) were used to determine cowpea seeds' moisture, protein, fat, and ash. Protein content was calculated as nitrogen (factor 6.25), and carbohydrate content was estimated by difference (100-crude protein-crude lipid-crude protein-ash-moisture).

Anti-nutritional content from the cowpea flour was evaluated by determining the level of HCN content (Okoliet & Ugochukwu, 1989), phytic acid (Davies and Reid, 1979), and trypsin inhibitor (Benjakul et al., 2000).

Physico-chemical and functional analysis

Protein hydrolysis of the samples was evaluated by determining the degree of hydrolysis (DH) (Silvestre et al., 2013). A total of 500 µL sample was added with 500 µL 20% (w/v) TCA (trichloroacetic acid). The mixture was then allowed to stand for 30 minutes for precipitation. After that, the mixture was centrifuged at 7800 g for 15 minutes. The soluble protein content in the supernatant was determined with bovine serum albumin as standard (Lowry et al., 1951). The DH was calculated by equation 1:

$$DH(\%) = \frac{\text{soluble protein in the supernatant (after TCA addition) } (mg)}{\text{total protein content } (mg)} \times 100\%$$

Sample with the optimum protein digestibility will be further analyzed with functional properties, which comprise water holding capacity (WHC), oil holding capacity (OHC), emulsion capacity and stability, and foam capacity and stability.

Data Analysis

All experiments were conducted in three replicates with triplicate analysis in each measured. The statistical analysis was performed using ANOVA and Duncan test at the 5% probability to determine the significant differences ($p<0.05$) for the concentration of anti-nutrition compound (HCN, phytic acid, and trypsin inhibitor), protein solubility, and degree of hydrolysis. Student's t-test was used to determine the significant differences ($p<0.05$) in the functional properties of the protein.

RESULT AND DISCUSSION

Proximate Analysis of Cowpea

Proximate analysis of cowpea seed is presented in Table 1. The protein content of cowpea seed was in the range of another type of underutilized legumes, such as jack bean (Farinde et al., 2018; Purwandari et al., 2021). However, cowpea seed has a relatively low-fat content and is far below the soybean. Instead, the fat content of cowpea was similar to jack bean (Wijatniko & Agnes Murdiati, 2017). The presence of fat in abundance might interfere with the extraction of the protein, so that it can impair protein isolation. Cowpea bean has a high

amount of carbohydrates which can be utilized as a starch-based food ingredient based its starch. Protein in cowpea might also contribute to determining food quality properties as it is characterized by its protein component (Kumar et al., 2022).

Table 1. Proximate Composition of Cowpea Seeds

Component	Percentage
Water (%wb)	10,67 ± 0,01
Ash (%db)	3,62 ± 0,13
Protein (%db)	25,62 ± 2,12
Fat (%db)	1,24 ± 0,02
Carbohydrate (*by difference*) (%db)	58,84 ± 2,02

Anti-nutritional Content

HCN Content

Tables 2, 3, and 4 showed the HCN content in cowpea flour from steps 1, 2, and 3 soaking. The HCN content decreased significantly after soaking for 24, 48, and 72 hr. The lowest HCN content was shown at the soaking for 48 hr. In general, the HCN content gradually decreased as the soaking time increased. HCN is a natural toxin commonly presented in legumes and plays a role in internal defense from pests. However, soaking of cowpea seed with 5% $NaHCO_3$ solution for 48 hours (without boiling) resulted in the most potent elimination of HCN content with 0,72 ppm. Prior to processing, HCN content in legumes should be reduced by several treatments, such as soaking, fermentation, blanching, and drying. Previous studies suggested that soaking jack bean seed effectively diminished HCN content as it dissolved in soaking water (Canti & Murdiati, 2015; Ngatchic et al., 2013; Wijatniko & Agnes Murdiati, 2017). Soaking in water enabled the HCN reduction since HCN dissolved in the water during soaking, resulting in the diminishing of HCN content (Safdar et al., 2020). Soaking in NaHCO3 enhanced the HCN removal caused by the acid-base reaction between $NaHCO_3$ and HCN to form sodium cyanide salt (NaCN) (Kalpanadevi & Mohan, 2013). While the NaCN immersed in the soaking water, the higher the concentration of $NaHCO_3$ in the soaking water, the greater the HCN compounds reacted with NaHCO3. However, the combination of soaking and boiling in this study suggested no effect on the HCN reduction, which might be explained by the low concentration of HCN obtained after step 2 soaking. Hence, the changes in HCN might not be distinctly observed. In addition, fermentation might also contribute to the elimination of HCN due to enzyme activity that allows the degradation of HCN. During soaking, spontaneous fermentation might appear, which alters the structure of HCN. Hence the HCN concentration might be significantly reduced. (Sridhar & Seena, 2006).

Table 2. Anti-nutritional Content of Cowpea Seeds with Soaking Treatment Step 1

Soaking Treatment (Step 1)	HCN Content **(ppm)**	Phytic Acid Content **(mg/g)**	Trypsin Inhibitor **(mg/g)**
Without soaking	6,62 ± 0,22 ^{d}	13,7835 ± 0,12163 ^{d}	14,6177 ± 0,06599 ^{d}
24 hours soaking in water	5,79 ± 0,17 ^{c}	12,4188 ± 0,1213 ^{c}	12,6559 ± 0,0549 ^{c}
48 hours soaking in water	4,35 ± 0,49 ^{a}	10,5597 ± 0,07653 ^{a}	11,3204 ± 0,0624 ^{a}
72 hours soaking in water	5,07 ± 0,04 ^{b}	11,4787 ± 0,1010 ^{b}	13,8718 ± 0,1333 ^{b}

Means ± standard deviation with different superscript letters in the column are significantly different (one way ANOVA and Duncan test, $p< 0.05$)

Table 3. Anti-nutritional Content of Cowpea Seeds with Soaking Treatment Step 2

Soaking Treatment (Step 2)	HCN Content **(ppm)**	Phytic Acid Content **(mg/g)**	Trypsin Inhibitor **(mg/g)**
48 hours soaking in water	4,35 ± 0,49 ^{c}	10,5597 ± 0,07653 ^{c}	11,3204 ± 0,0624 ^{c}
48 hours soaking in 2,5% $NaHCO_3$ solution	1,60 ± 0,22 ^{b}	10,2176 ± 0,100973 ^{b}	9,2549 ± 0,07699 ^{b}
48 hours soaking in 5% $NaHCO_3$ solution	0,72 ± 0,01 ^{a}	9,6208 ± 0,09928 ^{a}	7,0201 ± 0,0947 ^{a}

Means ± standard deviation with different superscript letters in the column are significantly different (one way ANOVA and Duncan test, $p< 0.05$)

Table 4. Anti-nutritional Content of Cowpea Seeds with Soaking Treatment Step 3

Soaking Treatment (Step 3)	HCN Content **(ppm)**	Phytic Acid Content **(mg/g)**	Trypsin Inhibitor **(mg/g)**
48 hours soaking in 5% $NaHCO_3$ solution without boiling treatment	0,72 ± 0,01 ^{a}	9,6208 ± 0,0928 ^{a}	7,0201 ± 0,0947 ^{a}
48 hours soaking in 5% $NaHCO_3$ solution with 2.5 minutes boiling	1,14 ± 0,02 ^{b}	11,2987 ± 0,51064 ^{b}	8,0185 ± 0,03256 ^{b}
48 hours soaking in 5% $NaHCO_3$ solution with 5 minutes boiling	1,28 ± 0,02 ^{c}	12,7773 ± 0,3629 ^{c}	8,4437 ± 0,0231 ^{c}

Means ± standard deviation with different superscript letters in the column are significantly different (one way ANOVA and Duncan test, $p< 0.05$)

Phytic Acid Content

Phytic acid in cowpea flour from steps 1, 2, and 3 soaking are presented in Tables 2, 3, and 4. The result showed that soaking cowpea seed with time variations significantly decreased the phytic acid content. However, soaking cowpea seed with 5% $NaHCO_3$ solution for 48 hours (without boiling) indicated the most potent treatment to reduce phytic acid content to 9,6208 mg/g. The reduction of phytic acid might be explained due to the activity of endogenous phytase in cowpea seeds. The endogenous phytase in seeds will be activated as the main factor responsible for the hydrolysis of phytic acid during soaking, which caused a significant reduction of phytic acid content in lima beans (Ogungbemi et al., 2022). The longer the soaking time, the greater enzyme activity to hydrolyze the phytic acid in food grains (Gupta et al., 2015). In addition, phytic acid presents in seeds were primarily water-soluble. The presence of Na+ ion from 5% $NaHCO_3$ soaking water induced disruption to the cell wall components from seeds such as pectin, fat, and protein that increased the permeability of the cell wall (Nur Irzam, 2014). The greater the damage to the cell wall by Na ions, the maximum imbibition will occur, enabling the phytic acid to dissolve in the soaking water. Surprisingly, the boiling treatment showed no effect on phytic acid reduction, which might be explained by the short-term boiling time in this study (Ertaş & Türker, 2012).

Trypsin Inhibitor Content

The trypsin inhibitor content in cowpea that was treated with different soaking times (Tables 2, 3, and 4) showed a significant reduction with the soaking duration of 24 hours and 48 hours. However, soaking cowpea seed with 5% $NaHCO_3$ solution for 48 hours (without boiling) resulted in the most potent elimination of trypsin inhibitor content with 7,0201 mg/g. Imbibition of water to the seeds during soaking allowed the trypsin inhibitor to dissolve in water since this compound was soluble in water (Shi et al., 2017). The trypsin inhibitor content significantly decreased with the increasing concentration of $NaHCO_3$. This might be explained by the addition of $NaHCO_3$, which creates an alkali environment that caused loss of enzyme activity due to electrostatic interactions in the active side of the antitrypsin compound (Avilés-Gaxiola et al., 2018). However, boiling treatment did not show any reduction in trypsin inhibitor, which might be due to the insufficiency of the boiling process in this study (Ertaş & Türker, 2012).

Protein Solubility

Extraction of cowpea bean protein was conducted based on its solubility in water. Adjusting the pH of the aqueous fraction might impact the protein's solubility as it increases its solubility in an alkaline environment. The solubility of cowpea protein can be seen in Fig. 1. Protein solubility increased concomitantly with the rise of pH. However, the protein solubility reached the highest at pH 12, suggesting that the optimum condition for extracting the protein from cowpea was pH 12. Instead, the solubility of white lima bean protein was the lowest in aqueous at pH 4. It might be explained that the solubility of protein tended to decline when it reached the isoelectric point of the protein. At this phase, the protein-protein interaction was higher than the protein affinity to the aqueous phase. Hence the pattern that showed in Fig. 1 indicating indicated a common sense on the behaviour of the protein. This result was in accordance with the study conducted on jack bean (Canti & Murdiati, 2015).

An increase in the protein solubility as the pH increase might be attributed to the ionic charge between the water phase and net charge at protein which resulting strong interaction between protein and water. This condition made the protein from the matrix of seeds dissolve in the water phase (Ferreira Machado et al., 2007).

Instead, a dramatic decline of protein solubility at pH 4 indicated weak interaction between water and protein. The protein tended to make aggregate by the formation of linkage between chains and thus eventually affecting the solubility of the protein. As a consequence of the strong force among protein, protein underwent a series of aggregations, so it elucidating elucidated the diminished protein solubility (Ebert et al., 2020)

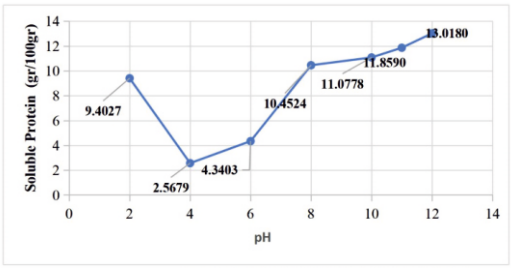

Figure 1. Protein Solubility of Cowpea

Means ± standard deviation with different superscript letters in the column are significantly different (one way ANOVA and Duncan test, $p< 0.05$)

Degree of hydrolysis of cowpea protein hydrolysates

The degree of hydrolysis (DH) analysis was carried out on cowpea protein concentrate after sonication treatment. DH determination was based on the ratio of soluble protein content generated as the consequence of cowpea protein hydrolysis by sonication compared to its original protein content. This study showed that sonication power and time did not significantly affect the DH based on the two-way ANOVA test. There was no interaction between power and sonication time to the degree of hydrolysis of cowpea protein hydrolysate ($p <0.05$).

Sonication treatment was responsible for a significant increase in the hydrolysis of protein concentrate compared to controls. The degree of hydrolysis increased along with the sonication power and time elevation. However, sonication treatment with a power level of 80% (168 W) for 30 minutes showed the highest DH. It also stipulated that the total amount of soluble protein after sonication (80% -168 W for 30 minutes) was the highest compared to other treatments. This result was in line with the previous study on the sonication of soy protein and whey protein which suggests that the physical and functionality of protein structure is altered by sonication (Arzeni et al., 2012)

An increase in the degree of hydrolysis can be attributed to the disruption of several insoluble protein aggregates with high

molecular weight (O'Sullivan et al., 2016). Those proteins were diminished in molecular weight due to cavitation, turbulence, and friction generated during sonication (Hu et al., 2015).

Functional Properties of Cowpea Protein

Water Holding Capacity (WHC)

Water holding capacity (WHC) of protein demonstrates the ability of a protein to be incorporated into food formulas that contain water. The WHC of cowpea protein concentrate increased significantly after hydrolysis treatment with sonication at 80% (168 W) for 30 minutes. WHC is affected by the composition and conformation of protein molecules through hydrogen bonds. In addition, the WHC content of cowpea protein concentrate can be attributed to the differences in protein fractions, which allow the variation in the number of water-binding sites on the protein molecule. Sonication might alter the conformation of the protein, which is associated with the functional properties of the protein that enhance the ability for water-binding sites at the cowpea protein (Hu et al., 2013). In addition, external factors such as stirring speed, pH, and protein concentration can enhance the WHC (Chel-Guerrero et al., 2002).

Oil Holding Capacity (WHC)

Oil holding capacity is the ability of a protein to absorb the oil. Various food matrices contain oil (fat) that can interact with the protein. This experiment showed that the OHC of cowpea protein concentrate increased significantly after sonication for 80% (168 W) for 30 minutes compared to the untreated. The oil absorption ability of cowpea protein depends on the protein structure. The lipophilic structure plays an important role in oil absorption, which is affected by the number of non-polar protein branches. When its number is more dominant, it contributes to the increase in oil absorption. Sonication caused partial globular protein opening, which is also associated with the exposure of some non-polar groups to the surrounding water phase (Hao et al., 2013). This non-polar group had a feature of absorbing oil so that the absorption power of protein extracts increased after sonication treatment increases. In addition, water-insoluble hydrophobic proteins can absorb large amounts of oil (Stone et al., 2015). The high OHC value in cowpea protein concentrate makes it a potential source as an ingredient in the food system, especially in flavour retention, palatability improvement, and prolonging the shelf life of meat products through reduced water and oil loss (Chel-Guerrero et al., 2002). A high OHC value is preferable in the protein isolate as it is important in food formulation, so it can be used as a binder in sausages, increasing protein binding capacity to protein and fat. The interaction of water and oil with protein is necessary for the food system because it influences the flavour and texture of a food product (Foh et al., 2012).

Foaming Capacity and Stability

Foaming capacity (FC) and foam stability (FS) are essential parameters in the characterization of the functional properties of the protein that determine the quality of food products (Cano-Medina et al., 2011). The foam capacity and stability of the sample with sonication were significantly higher than the untreated. The ability of cowpea protein to form foam is related to lowered surface tension in the air/water interface due to the absorption of protein molecules (Wang et al., 2019). This condition caused an intense interaction with the water-air interface that caused foam formation. Proteins with foaming properties can reduce the tension at the water-oil interface and thus prevent coalescence (Lazidis et al., 2016). There is a correlation between foam formation and flexible protein molecules, which reduce surface tension. In addition, low foam formation could be attributed to the high globular protein, which resists surface denaturation (Mune & Sogi, 2016). The basic requirements of a preferred protein foaming agent are the ability to absorb rapidly at the water-air interface during boiling, (rapid conformational change and rearrangement at the interface, and a cohesive viscoelastic film through intermolecular interactions (Cano-Medina et al., 2011).

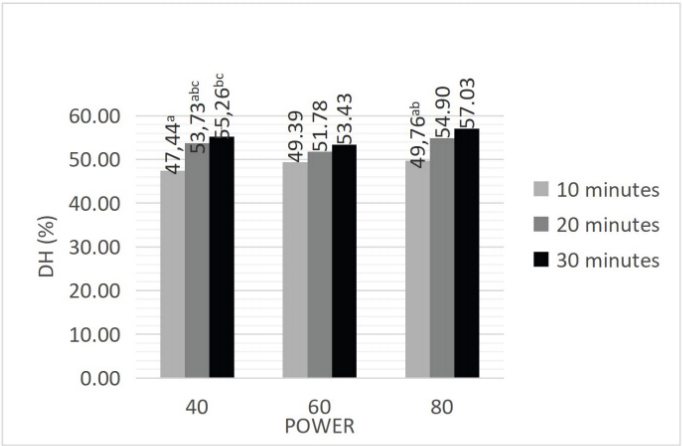

Fig. 2. Degree of hydrolysis of cowpea protein hydrolysate.
Different superscripts indicated significantly different (one way ANOVA and Duncan test, $p < 0.05$)

CONCLUSION

Soaking cowpea seeds in 5% $NaHCO_3$ solution for 48 without boiling treatment showed the most potent reduction of anti-nutritional content in cowpea, with HCN of 0.72 ± 0.01 ppm, phytic acid 9.6208 ± 0.0928 mg/g and trypsin inhibitor 7.0201 ± 0.0947 mg/g. The optimum pH for protein extraction of cowpea was achieved at pH 12, and the lowest protein solubility was observed at pH 4. Hydrolysis of cowpea protein concentrate with sonication for 80% (168 W) for 30 minutes indicated a promising protein functionality with significantly higher of water holding capacity, oil-holding capacity, emulsion stability, and foam stability than the untreated sample, which contributed to the prospective utilization of cowpea protein as a binder in the food system.

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