7.2. CHARACTERISTICS OF FUNCTIONAL PROPERTIES OF BEANS PROTEIN RESULTED EXTRACTION OF HYDROCHLORIC ACID

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Submission date: 05-Jan-2021 11:23AM (UTC+0700)
Submission ID: 1483165158
File name: 7.2.CHARACTERISTICS_OF_FUNCTIONAL_PROPERTIES_OF_BEANS.pdf (462.59K)
Word count: 5485
Character count: 28590

CHARACTERISTICS OF FUNCTIONAL PROPERTIES OF BEANS PROTEIN RESULTED EXTRACTION OF HYDROCHLORIC ACID

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abstract

Kind of beans locally as cowpea (Vigna unguiculata), green beans (Phaseolus radiates L.) and red beans (Phaseolus vulgaris L.) has a protein content ranging from 20% -30% so it can be used as a substitute for soy protein concentrates. This study aims to determine the functional properties of protein concentrates local nuts using hydrochloric acid extraction. Using hydrochloric acid concentration of 3%, 5% and 7%. The results showed that the optimal protein concentrates obtained from the green beans in the extraction process using hydrochloric acid 3%. The results of proximate analysis of protein concentrates ie protein content (21.67%), fat content (1.69%), water content (10.36%), ash content (1.14%) with the functional properties of the protein concentrates Bulk density (0.79 g / mL), oil absorption capacity (2.09 mL / g), water absorption (2.73 mL/g), power foam (4.20%) and emulsion capacity (23.62%). Results optimal protein concentrates from cowpea obtained in the extraction process using hydrochloric acid 7%. The results of proximate analysis of protein concentrates ie protein content (21.21%), fat content (1.61%), water content (12.98%), ash content (1.10%) with the functional properties of the protein concentrates bulk density (0.77 g/mL), oil absorption capacity (1.82 mL/g), water absorption (2.73 mL/g), power foam (0.97%) and emulsion capacity (5.38%). Results optimal protein concentrates obtained from red beans in the extraction process using hydrochloric acid 3%. The results of proximate analysis of protein concentrates ie protein content (21.18 %), fat content (2.07%), water content (10.23%), ash content (1.06%) with the functional properties of the protein concentrates bulk density (0.75 g/mL), oil absorption capacity (1.91 mL/g), water absorption (3.67 mL/g), power foam (1.00%) and emulsion capacity (5.61%)

Keywords: green beans, cowpea, red bean and hydrochloric acid

Introduction

Types of beans such as cowpea (Vigna unguiculata), green beans (Phaseolus radiates L.) and red bean (Phaseolus vulgaris L.) can be used as a substitute for soybean. The content of protein and amino acids that resemble soy beans are potential local nuts as an alternative source of vegetable protein concentrates to replace soy products. Beans can be used as a product of protein concentrates to be applied to various types of food products. Bridson (1995) state That the hydrolysis of proteins, which breaks them down to their constituent amino acids and peptides, can be achieved by the use of strong acids, strong bases or proteolytic enzymes, there are three main methods of hydrolysis of proteins[

Separation of proteins from fat, water and reducing sugar produces a product that is resistant to storage. Separate proteins (concentrates) forms paste or powder, and has a higher protein content than the original material. The principle of protein isolation comprises the steps of protein extraction in the extraction medium, removal of insoluble material by centrifuge, filtration, combination, recipitation, washing and drying concentrates (Natarjan, 1980 in Kartika, 2009). Principles used to isolate total protein is whole bean protein precipitation at isoelectric point is pH where the entire protein clumping. Selection of acidic as pH during extraction where the majority of negatively charged amino acids at pH above the isoelectric point, like charges tend to repel, causing minimumya interactions between amino acid residues which means to increase protein solubility (Cheftel et al , 1985).

Protein extraction capability is influenced by several factors, including particle size, flour age, previous heat treatment, dilution ratio, pH and ionic strength of the medium extraction (Kinsella, 1979). Hydrochloric acid is a strong acid because it fully dissociates in water and harmless compared to other strong acids, non-reactive and non-toxic. Hydrochloric acid in the medium concentration is

stable enough to be stored and continue to maintain concentration. Therefore, hydrochloric acid is an acid reagent which is very good and is often used in chemical analysis to hydrolyze samples analysis The influence of the isoelectric point is the value of a protein that gives an important influence on the biochemical properties of proteins that can be used in the process of purification and electrophoresis (Poejiadi, 1994). The charge of a protein depends on the pH value of the medium where he is. At the isoelectric point, a protein showing repulsion least because the protein will have the lowest solubility and easy to settle. These characteristics are very useful in the process of protein crystallization. When the pH of the solution reaches a certain isoelectric point, the protein will precipitate and separate from other proteins that have different isoelectric points.

Methodology

Beans defatted flour

Beans sorted and weighed. Beans soaked in water for 4 hours at a ratio of 1: 3 was then performed by boiling for 30 minutes. Beans shelled and dried in a dryer 50 °C for 3 h. Beans mashed with a disk mill and fat extracted with an organic solvent hexane 1: 4 for 4 h gradually. Defatted beans flour dried at 50 °C for 6 h.

Beans protein concentrates

Defatted flour was suspended with distilled water 10 g / 100 mL and extracted using hydrochloric acid concentration of 3%, 5% and 7% with a ratio of 1: 5 with pH adjustment 4 - 5. The suspension was heated with a water bath at 50 ° C for 1 hour. After cold it neutralized with 1 N NaOH solution Suspension neutral conditions was centrifuged at 3000 rpm for 25 minutes. The filtrate was centrifuged again at 3000 rpm for 25 minutes. The first and second precipitate precipitate washing three times with water 80 °C and dried on temperature 50 ° C for 5 h.

Analysis of Water Absorption (Lin et al, 1974)

A total of 1 g of sample and 10 mL of distilled water for 2 minutes divortex, diiamkan for 1 hour at room temperature and then centrifuged at 3000 rpm for 25 minutes. Filtrate volume was measured. Water absorption was calculated by the following equation:

$$\text{Water absorption (mL water / g sample)} = \frac{\text{10 mL - vol filtrate (mL)}}{\text{W sample (g)}}$$

Analysis of Oil Absorption (Soluski and Fleming, 1977)

A total of 0.5 g of sample plus 3 ml of soybean oil divortex for 2 minutes and then allowed to stand at room temperature. The mixture was centrifuged at 3000 rpm for 25 minutes. Supernatant was poured into 10 ml measuring cup and observed free oil volume oil absorption measurements performed two repetitions with the following equation:

$$\text{Oil absorption capacity (mL / g sample)} = \frac{\text{3 mL of oil - filtrate}}{\text{W sample (g)}}$$

Capacity Analysis and Emulsion Stability (Franzen and Kinsella, 1979)

A total of 0.5 g of sample and 25 ml of water is set pH to 8 while stirring with a magnetic stirrer 5 min. A total of 25 ml of the sample solution plus 25 ml of soybean oil was dispersed in a blender for 1 min, then centrifuged at 3000 rpm for 10 min. The volume of the emulsion can be measured by the equation:

$$\text{Emulsion capacity ct} = \frac{\text{V x 100\%}}{\text{V tot.t}}$$

Specification:

VCT = volume of the mixture emulsifies
Vtot.t = total volume in the tube

The emulsion is formed saved some at room temperature. The volume of the emulsion was observed on the clock to 0.5, 1, 2, 4, 6 and then recorded and emulsion stability curve

Capacity and Stability Foam (Widowati et al, 1998)

A total of 2 g of sample was dissolved in 100 mL of distilled water and homogenized with a magnetic stirrer for ± 1 min . The solution was then adjusted to pH 8 and shaken with waring belnder for 2 min. Foam volume was calculated by the following equation:

$$\text{Foaming capacity (\%)} = \frac{Vbsd \times 100\%}{Valp}$$

Specification:

Vbsd = volume of foam before whipped
Valp = initial volume of protein solution

Preparation and HPLC Analysis of Amino Acid in beans Protein Concentrate

0.1 g sample is taken and added 2 mL of Performat vortex. the mixture was hydrolyzed for 16 h at a temperature of 0 °C. Then added 0.3 mL of 48% HBr and incubated at a temperature of 0 °C for 15 minutes. The solution was dried in a rotary evaporator at 60 °C. The filtrate was pipetted 500 µL and added 40 µm AABA and aquabidest 460 µl. Furthermore, a solution of 10 µl pipette and added 70 µl AccQ-Fluor Borate and vortex. Fluor AReagent 20 µl vortex taken and then allowed to stand 1 '. Reagents were incubated at 55 °C for 10 '. Samples prepared injected into the HPLC. HPLC conditions : colom AccQtag column (3.9 x 150 mm), temperature 37 °C, mobile phase acetonitril 60% - Accq Tag Eluent A, Gradient system, flow rate 1.0 mL/min, detector Fluorescense, excitation 250 nm, emission395 nm and 5 µl injection volume.

Result and Discussion

Protein content

The highest protein content of green beans at a concentration of 3% hydrochloric acid in the amount of 21.67% db was at pH 5.3. The highest protein content of cowpea at 7% hydrochloric acid concentration of 21.21% db was at pH 4.2, while the highest protein content of beans at a concentration of 3% hydrochloric acid 21.18% db was at pH 4.9. Proteins can be denatured by the addition of an acid solution and heating to food that has a high protein content. Protein levels at each concentration of hydrochloric acid and nuts can be seen in Figure 1

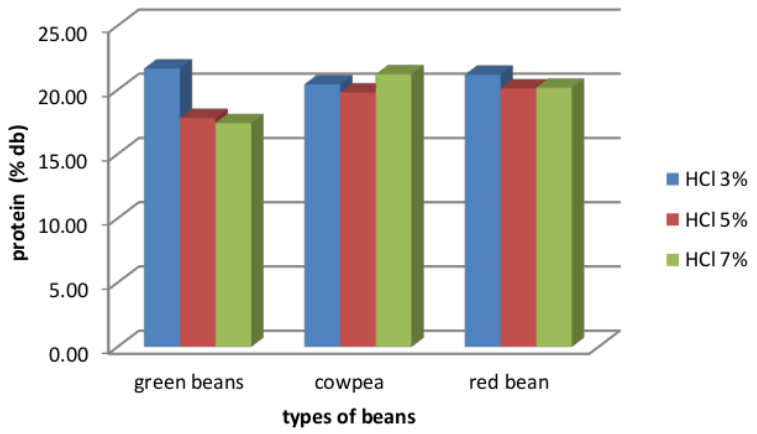

Figure 1. Protein content of beans protein concentrate

Lehninger (1982) states that the effect of pH based on the difference in affinity of the most powerful among the same protein occurred at the isoelectric pH while the pH above and below the isoelectric point, the protein will change the charge that causes decreased affinity between protein molecules, so readily biodegradable molecules. The further distinction pH of the isoelectric point of the protein solubility increases. Kurniati (2009) which states that the protein extraction using a strong acid (HCl) resulted in the addition of excess H^+ ions that neutralize the protein and the achievement of the isoelectric pH. Ionic properties of the protein if the protein contains a lot of the acid has a low isoelectric point. Suhardi (1992) states the isoelectric point is the pH in the form of amphoteric (cations and anions) and at the isoelectric point of the protein solubility decreases and reaches the lowest number, the protein will precipitate and agglomerate.

Table 1. Amino acid composition of cowpea beans protein

No	Amino acid	Result (%)
1	Aspartate	1.34
	Glutamate	2.37
	Serin	1.06
	Glysine	0.79
	Histidine	0.63
	Arginine	1.23
	Threonine	0.86
	Alanine	0.76
	Proline	0.71
	Falin	1.06
	Tyrosine	0.67
	Isoleucine	0.93
	Leucine	1.72
	Phenilalanin	1.42
	Lisin (Lysine HCl)	1.10
	Cystine	0.02
	Methionine	0.29
	Total	16.98

The cowpea (*Vigna unguiculata* L.) contained 41.99% globulin, 10.11% albumin, and 7.81% glutelin (Shoshima et al ., 2005). On the other hand, in the species *Vigna aconitifolia* L., the second principal group is the glutelin fraction (27.83 ± 0.27%), followed by albumins (5.06 ± 0.27%) (Sathe; Venkatachalan, 2007).

In the protein concentrate of baru nuts, the globulin fraction increased and the albumin decreased, while the glutelins remained practically constant. The prolamin fraction was not detected in the concentrate. The dialyzable fraction, 9.27% in the flour, increased to 22.81% in the concentrate, representing the efficient soluble protein extraction of low molecular weight in salt. The final residue decreased to 10.94% in the defatted flour and 2.12% in the concentrate (Guimarães et. al, 2012)

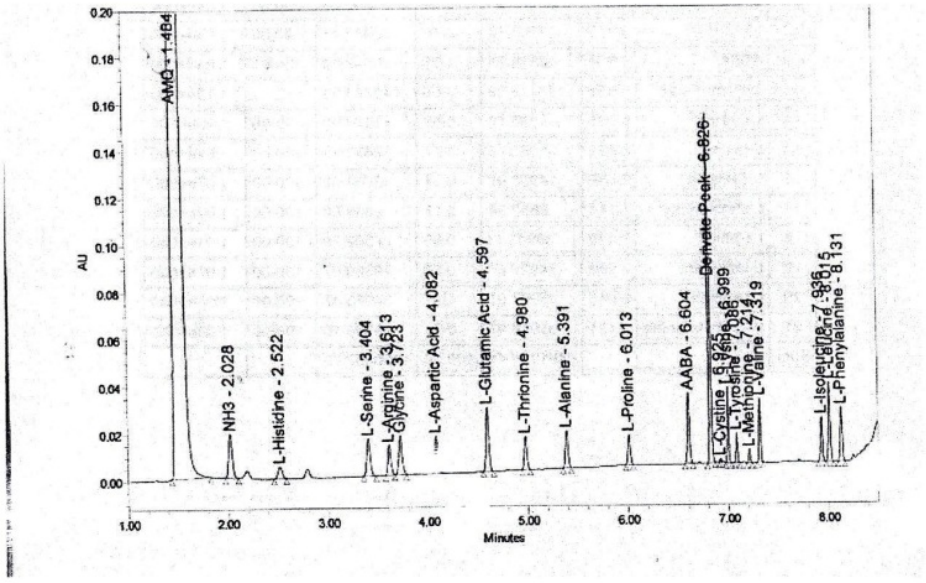

Figure 2. Chromatogram of cowpea beans amino acid

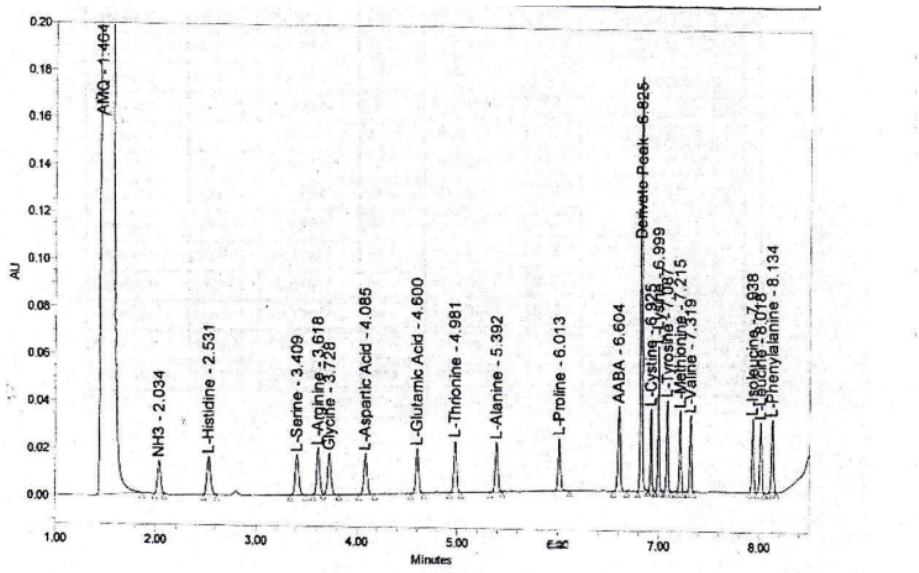

Figure 3. Chromatogram of amino acid standart

Acid or base treatment of plant (soybean, corn) or animal (casein) proteins brings about desirable changes in flavor, texture, and solubility. Such treatments also destroy toxins and trypsin inhibitors and are used to prepare protein isolatesn (Friedman and Liardon, 1985)

One of the principals advantages of acid as compared with base hydrolysis is that the optical activity of the amino acids is not destroyed in the process (Haurowitz, 1955), on the other hand, acid hydrolysis destroys tryptophan and partially destroys cystine, serine, and threonine. Asparagine and glutamine are converted to their acidic form(Bridson, 1995; Haurowitz, 1955)

The ash, soluble solids, and polyphenol contents have no influence on their protein digestibility ((Herna´ndez *et al*., 1997). The comparison between the dietary fiber content of the concentrates and their digestibility values reveals an inversely proportional relationship. Correlation analyses were run on the insoluble, soluble, and total dietary fiber contents (Herna´ndez *et al*., 1995). Subba Rau *et al.* (1972) concluded that there was an inverse relationship between the ash content, polyphenol content, and soluble solids content and the nutritive value.

Water Absorption

Water absorption is the ability of the protein to bind water during application of forces, pressure, centrifugation or heating. Factors that affect water absorption wss the protein concentration, pH and ionic strength and thermal effects. Water absorption in each concentration of hydrochloric acid and nuts can be seen in Figure 4.

In Figure 4 shows that the highest rate of water absorption in the green beans at 7% hydrochloric acid concentration was 2.73 mL / g, cowpea at a concentration of 5% hydrochloric acid was 3.13 mL / g, red beans on the concentration of hydrochloric acid 5 % was 4.11 mL / g. The absorption of red bean protein concentrate has the highest water absorption followed by cowpea and mung bean. Although the value of the functional properties may be quite variable, depending on the technique and conditions of the assays, it can be inferred that the performance of the proteins in the defatted flour was similar to that of soybean (223%, Sosulski (1976) and 175% Sosulski; McCurdy (1987))

The pH 5 showed the lowest protein solubility, slightly more than 20%, that this pH value is near the isoelectric point of protein isolates from amaranth (4.6) resulting in aggregation and precipitation of the great part of the proteins (Mizubuti, 2000).

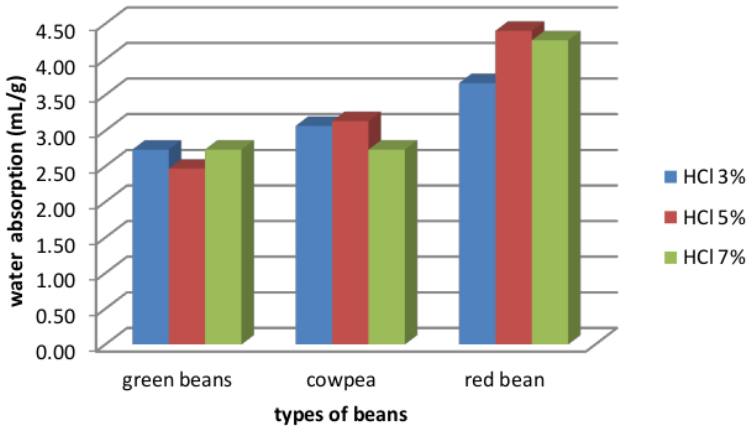

Figure 4. Water absorption of beans protein concentrate

Protein solubility is an important factor for the optimization of functional properties. A more soluble product is also more easily formulated for some foods. Hence, almost all concentrates and isolates are neutralized and sold as proteinates. The major form is sodium, potassium, and calcium proteinate, but other proteinates are available (Wolf; Cowan, 1971).

Water absorption ability of a protein concentrate, caused by a polar amino acid is more dominant. Suwarno (2003) stated that the binding of water depends on the composition and conformation of protein molecules. The interaction between water and polar groups of the side chains of proteins can occur through hydrogen bonds. The amount of water that can be retained by the protein depends on the amino acid composition, surface hydrophobicity, and processing. The amount of water that is bound to be increased if the polarity of the protein increases. water absorption related to the amount of polar amino acid groups present in the protein molecule. Polar amino acid groups, such as hydroxyl, amino, carboxyl, and sulfihidril provide hydrophilic properties of the protein molecule that can absorb and bind water.

Budijanto, et al (2011) stated that the amino acid composition of proteins affect water absorption properties of protein concentrates. Protein concentrates contain many polar amino acids (glutamic acid, aspartic acid and lysine) so as to improve the ability of water absorption. The amount of water that is bound by a protein depends on the composition of the polar amino acids. Sorgentini *et al.* (1991) has studied hydration properties of soy protein isolates are particularly good in products with a high degree of protein denaturation. Surowka K., Żmudzinski D. (2004) The extruded protein concentrates belong hydration properties are also affected by non protein constituents, chiefly pectic substances and hemicelluloses. Surowka (1997) states the hydrolysis leads to a deformation of the protein matrix and, partly, to the transfer of some amount of protein substances into the solution, it should theoretically, decrease hydration capabilities of the extrudates protein.

Protein solubility is influenced by the hydrophilicity/hydrophobicity balance, which depends on the amino acid composition, particularly at the protein surface (Moure et al.,2006). The presence of a low number of hydrophobic residues; the elevated charge and the electrostatic repulsion and ionic hydration occurring at pH above and below the isoelectric pH favour higher solubility. Protein solubility is also influenced by production method and in particular by denaturation due to alterations in the hydrophobicity/hydrophilicity ratio of the surface. A highly soluble protein is required in order to obtain optimum functionality required in gelation, solubility, emulsifying acitivity, foaming and lipoxygenase activity (Riaz, 2006). Soluble protein preparations are easier to incorporate in food systems, unlike those with low solubility indices which have limited functional properties and more limited food uses.

Oil Absorption

Oil absorption capacity is physically oil binding by proteins. Oil absorption capacity at each concentration of hydrochloric acid and nuts can be seen in Figure 5. In Figure 5 shows that the highest oil absorption value of green beans at a concentration of 3% hydrochloric acid in the amount of 2.09 mL / g, cowpea at a concentration of 5% hydrochloric acid for 2,01ml / g and red beans with hydrochloric acid concentration of 3% by 1,91ml / g. In the green bean protein concentrate highest ability to absorb oil. It is alleged in the green bean non-polar amino acids such as phenylalanine dominant so that the absorption ability of oil / fat increased. Lin et al (1974) state oil absorption capacity of a protein depends on its structure. Structure which is the type of lipoprotein lipolytic suspected to contain a non-polar amino acids (glycine, alanine, phenylalanine, tryptophan, valine, leucine and proline) with a protein content of nonpolar branch dominant, contributing to the increase in oil absorption capacity.

Oil absorption in the defatted flour was in the literature for soybean 130% and 56%. The water absorption capacity for the defatted flour of peas and fava beans was 78 and 72%, respectively, and the values found for oil were 41 and 47% (SOSULSKI; McCURDY, 1987).

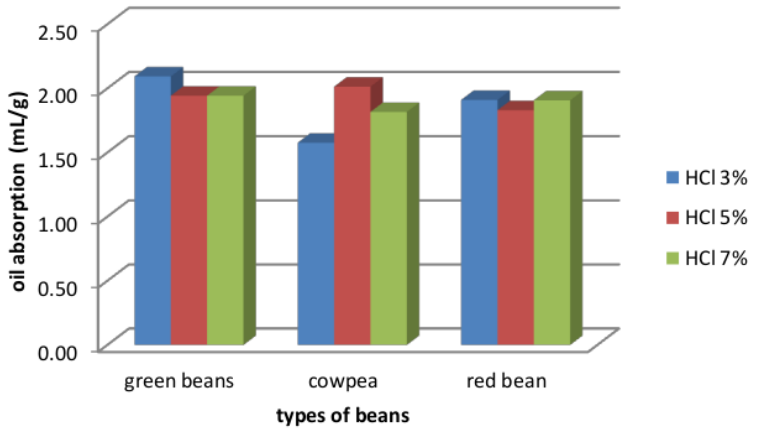

Figure 5. Oil absorption of beans protein concentrate

Oil absorption ability of a protein can be caused by the denaturation process in the protein so that the opening of the conformational structure of the protein. This is in accordance with the opinion Suwarno (2003) which states that the denaturation of the protein can increase the protein's ability to bind fat due to the opening of the protein structure that describes the amino acids that are non-polar. According Zayas (1997) stated that the oil absorption capacity of a protein is affected by sources of protein, the protein particle size, shape processing, other additives, temperature and degree of denaturation.

Kartika (2009) which states that the structure of the proteins that are lipolytic are the type of lipoprotein which is thought to contain a non-polar amino acids (glycine, alanine, phenylalanine, tryptophan, valine, leucine and proline) with a protein content of nonpolar branch dominant, contributing to an increase in absorption of oil.

Foam Power

Foam is a two-phase system of air cells are separated by a thin continuous layer of liquid. Foam power demonstrates the ability of proteins to produce a foam surface area / unit weight of protein to stabilize the film or coating the surface of the internal and external forces. Power foam at each concentration of hydrochloric acid on the type of bean can be seen in Figure 6.

In Figure 6 shows that the value of the highest scum on green beans at a concentration of 3% hydrochloric acid is equal to 4.20%, followed by red beans on the use of HCl 7%. This was due to the concentration of hydrochloric acid 3% opening of bonds in the protein molecule, resulting in the intake air that expands protein. Foaming ability increases when the protein concentration increased, along

with the results of this study indicate the concentration of hydrochloric acid 3% have a high protein value.

Foam formation mechanism begins with the opening of the bond in the protein molecule so that the protein chain becomes longer, later air enters open between protein molecules and proteins to survive so that the volume expands. Zayas (1997) states that the foaming ability increases when the protein concentration also increased because it will increase the thickness of the interfacial layer of film on Cherry and Mc Watters, (1981).

The increase in the proportion of protein in the concentrate appears to influence positively the formation capacity and stability of baru foam due to the presence of soluble sugars, which increase viscosity (Guimaraes et al 2012).

In contrast, the foam formation capacity of the winged bean (*Psophocarpus tetragonolobus* (L.) DC) showed a 76% volume increase at pH 4.6 and 150% at pH 9.8 for the concentrate, while for the defatted flour, foam formation reached 52% at pH 6.0 (Narayana; Narasinga, 1982). Soybean (*Glicina max* (L.) Merril) flour and protein concentrate had an increase of 70 and 170%, respectively, in the volume of foam (LIN et al., 1974). Protein concentrates of the "carioca" variety of the common bean showed a low foam stability at 30, 60, 90, and 12 minutes with values of 1.28, 3.18. 4.43, and 6.63%, respectively (Donadel; Ferreira, 1999).

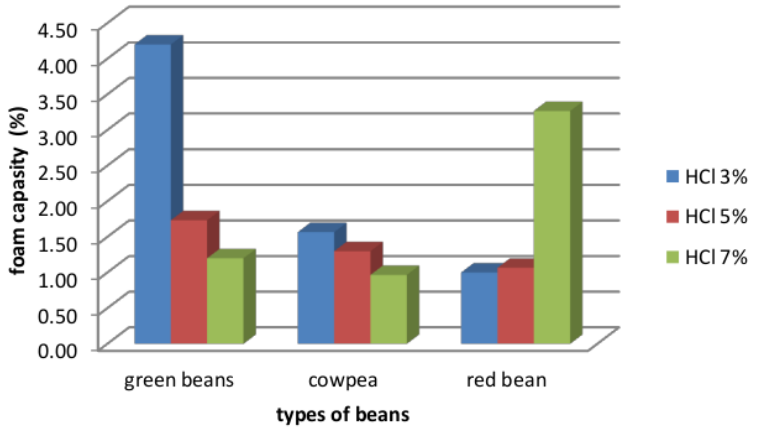

Figure 6. foam capasity of beans protein concentrate

Declining foam (0.97%) in cowpea with the use of hydrochloric acid 7% and red beans (1.0%) on the use of HCl 3% due to have a high fat content so weaken the interaction of proteins in the foaming and instability film is formed. foam instability influenced by the presence of residual fat on protein concentrate which causes the lining of the film is too thin to stabilize bubbles (Zayas, 1997). Dedin et al (2013) has studied that the highest foam capacity of leucaena concentrate at leather pineapple waste enzyme treatment of concentration of 100 mg / 100g with a long incubation of 48 hours of 8%. Dedin et al (2014, unpublised) has studied the value of the highest foam concentrates protein membrane filtration results in pressure 3.66 atm with a separation of 45 minutes is 9.8%. The results of this study, the value is smaller than previous studies. The small number of foam power these results allegedly by hydrochloric acid extraction are not coagulated at the protein isoelectric point so much that shipped with the filtrate.

Soy proteins were suggested to have poor foaming properties due to their large, compact structure (GERMAN *et al.*1985). Enzyme treatment increased the height of foam (HF),but foam stability was markedly decreased in each instance. The foem stability of the unhydrolysed protein of DSF was 148 min/100 mm while that of the hydrolysates in the presence of Alcalase, Flavourzyme and Noozym after 8 hrs was 17.2,32.4 and 2.8 min/100 mm, respectively (*Hrcková 2002)*. The paper by TURNER (1969) indicated that to make a stable foam partially hydrolysed protein is needed to increase the foam expansion and some larger protein components are needed to stabilize the foam. The foaming ability of protein improved in hydrolysis in the presence of Alcalase but foam stability decreased. When the smaller peptides produced after hydrolysis were separated by ultrafiltration, the foam stability of permeate was improved (PANYAM & KILARA 1996). In the present study, there are no

larger protein components present in the hydrolysates so that they could not stabilize the foam. Enzymatic hydrolysis of soy proteins can be used to produce a product with good whipping properties (TURNER 1969; GUNTHER 1972).

Capacity emulsion

Emulsions are, from the physicochemical point of view, thermodynamically unstable systems rapidly or slowly separating into two immiscible phases accordng to The kinetic stability. Mechanisms of physical destabilisation of emulsions include oil droplets size variation processes such as flocculation, and Coalescence and particle migration phenomena like sedimentation and creaming (Comas et al. 2006). Emulsion is a dispersion or suspension of a liquid in another liquid, the molecules do not dissolve each other. Emulsion capacity on each bean protein concentrate can be seen in Figure 7.

In Figure 8 shows the value of the highest emulsion capacity of green beans group on the use of hydrochloric acid 3% at 23.62%. This is because at a concentration of 3% hydrochloric acid emulsion activity increases with increasing levels of protein and fat content, resulting in fat-water emulsion is formed. Lowest emulsion capacity cowpea at 7% hydrochloric acid concentration of 5.38% and red beans (5.61%) on the use of 3% hydrochloric acid. This is due to an imbalance of protein amino acid number hydrophilic and lipophilic emulsifier resulting labor power can not be bound either in oil or water, forming a matrix that is less resulting in a less stable emulsion.

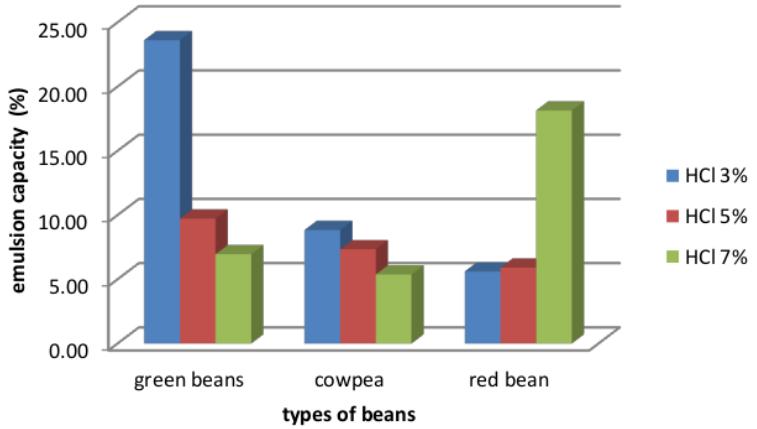

Figure 7. Emulsion capasity of beans protein concentrate

Zayas (1997) which states the balance of hydrophilic-lipophilic amino acid is closely connected with the ability to lower the surface tension as a function of emulsion formation. Components of hydrophilic-lipophilic amino acid protein capable of binding to the oil and water as well with water mechanism would bind the hydrophilic chain and oil on the lipophilic chain. Wolf and Cowman, (1971) which states that the protein has a role in the activity of the emulsion is formed emulsion of fat and water, protein will gather in fat-water interface and the surface low pressure. the solubility and emulsifying properties of Soy Protein Isolate were closely correl ated in the acid condition (Lixia Mu et al.2011).

Defatted protein concentrate of baru nuts (*Dipteryx alata* Vog) flour, the emulsifying activity was 51.00 ± 0.76%, and the emulsion stability was 50.0 ± 0.47% (Guimaraes et al. 2012). The emulsifying activity of the protein concentrate of the "carioca" variety of the bean (*Phaseolus vulgaris*) was very close to that of baru, 50.16% (Donadel; Ferreira, 1999), while the emulsion stability of this bean was lower than that of them, reaching 23.6%.

Conclusion

Breaking down some of the amino acids, completely and breaking down some other amino-acids partially. Functional properties of green beans in oil absorption, foam power and emulsion capacity superior than cowpea and red beans.

Acknowledgments

This research was supported entirely by the Directorate General of Higher Education, Ministry of Education and Culture

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7.2. CHARACTERISTICS OF FUNCTIONAL PROPERTIES OF BEANS PROTEIN RESULTED EXTRACTION OF HYDROCHLORIC ACID

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