

REGULAR ARTICLE

CHARACTERISTICS OF LOCAL LEGUME PROTEIN CONCENTRATES EXTRACTED USING HYDROCHLORIC ACIDDedin Finatsiyatull Rosida,^{1,2*)}, Erdianti¹⁾ Sri Djajati¹⁾**Address:**¹Department of Food Technology, Faculty of Engineering and Science, Universitas Pembangunan Nasional Veteran Jawa Timur, Indonesia. 60294²Innovation Center of Appropriate Food Technology for Lowland and Coastal Area, Universitas Pembangunan Nasional Veteran Jawa Timur, Indonesia

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ABSTRACT

Local legumes, including cowpea (*Vigna unguiculata*), mung bean (*Phaseolus radiatus L.*), and red bean (*Phaseolus vulgaris L.*), contain 20–30% protein, offering potential as alternatives to soy protein concentrates. This study aimed to: (1) develop a production method for legume protein concentrates using hydrochloric acid (HCl) extraction, (2) evaluate their functional properties, and (3) identify the optimal protein concentrate based on HCl concentration. A Completely Randomized Design (CRD) with three levels and three replications was employed, with Factor I being legume type (mung bean, cowpea, red bean) and Factor II being HCl concentration (3%, 5%, 7%). Results indicated that mung bean protein concentrate extracted with 3% HCl exhibited the highest protein content (21.67%), along with favorable functional properties: bulk density (0.79 g/mL), oil absorption (2.09 mL/g), water absorption (2.73 mL/g), foaming capacity (4.20%), and emulsifying capacity (23.62%). Cowpea protein concentrate required 7% HCl for optimal extraction, yielding 21.21% protein but lower functionality (emulsifying capacity: 5.38%). Red bean concentrate performed best with 3% HCl, achieving 21.18% protein and superior water absorption (3.67 mL/g). Overall, mung bean protein concentrate extracted with 3% HCl demonstrated the most balanced nutritional and functional characteristics, suggesting its viability as a soy protein substitute. These findings highlight the potential of local legumes in food applications, emphasizing extraction efficiency and functional performance.

Keywords: mung bean, cowpea, red bean, hydrochloric acid**INTRODUCTION**

Indonesia is a country rich in protein sources, one of which comes from local legumes. Legumes contain protein ranging from 20% to 30%. This makes legumes widely used as raw materials for protein concentrate production. Protein concentrates are proteins extracted from food materials using specific extraction methods to produce high protein concentration products. Legumes such as cowpea (*Vigna unguiculata*), mung bean (*Phaseolus radiates L.*) and red bean (*Phaseolus vulgaris L.*) can be utilized as soybean substitutes. Mung beans contain 22.85% protein, cowpeas range between 18.3-25.53%, and red beans contain 22% protein.

Proteins are the most abundant macromolecules in cells. Protein concentrates are protein forms produced by removing skins and non-protein components. Extraction is the separation process of materials/compounds based on compound partitioning between two immiscible liquids, typically organic solvent and water (Windjanarko, et al., 2011). The isoelectric point is the pH value where a protein has equal numbers of negative and positive charges or is neutrally charged (Lautenbach, et al., 2021).

Protein concentrate production uses HCl extraction because it has a higher Nitrogen Solubility Index (NSI), making it easier to extract proteins at their isoelectric point (Suhardi, 1988). Precipitation of all legume proteins at their isoelectric point occurs when all proteins coagulate at pH 4-5. Amino acid solutions in water have both positive and negative charges, so at isoelectric pH, protein molecules have equal positive and negative charges, neutralizing each other. At the isoelectric point, proteins will precipitate (coagulate) most rapidly (Tokmakov, et al., 2021).

The neutral evolution, i.e., accumulation of random mutations that have minimal impact on fitness and functional selection, might underline potential adjustment of protein pI to subcellular pH (Tokmakov, et al.,

2021). It was revealed that the neutral evolutionary process leading to fixation of titratable residues in the protein core could likely be driven by marginal effects on protein stability (Loell and Nanda, 2018).

Research by Triyono (2010) on mung bean solution preparation with 6 hours soaking using hydrochloric acid at pH 4.5 yielded optimal protein content of 76.56%. Research by Suciono (1995) on red bean and cowpea protein isolate production at pH 4.5 obtained cowpea protein isolate content of 94.57% with 4.76% moisture and red bean protein isolate of 91.12% with 4.02% moisture. Research by Kurniati (2009) on winged bean protein concentrate production with 0.3 N HCl addition at pH 4.5 obtained protein content of 80.05%.

Based on the above, this research was conducted to determine the optimal treatment for protein production from mung beans, cowpeas and red beans using hydrochloric acid extraction.

MATERIAL AND METHODS

Materials

Raw materials were cowpea, red bean and mung bean obtained from traditional markets. Chemical analysis materials included Kjeldahl salt, sulfuric acid (H_2SO_4), distilled water, $\text{Na}_2\text{SO}_4\text{-HgO}$, phenolphthalein indicator, 37% hydrochloric acid (HCl), 2% boric acid (H_3BO_3), hexane, analytical balance, water bath, pH meter, centrifuge, drying cabinet.

Methodology

The experimental design used in this research was a Completely Randomized Design (CRD) consisting of 3 levels with 3 replications. Variables: Local legumes: Mung bean, Cowpea, Red bean Hydrochloric acid (HCl) concentration: 3%, 5%, 7%

This study used a completely randomized design (CRD) consisting of three levels with three replicates. It was then analyzed using analysis of variance, and to determine whether there were differences between treatments, a significant difference test (BNJ) was performed. Research Variables: Local legumes: Mung bean, Cowpea, Red bean Hydrochloric acid (HCl) concentration: 3%, 5%, 7%

Research Procedure

1. Production of Fat-Free Bean Flour

The beans (cowpea, red bean and mung) are sorted and weighed, then soaked in water at a ratio of 1:3 for 4 hours. The beans are boiled for 30 minutes. They are then peeled and dried at 50°C for 3 hours. The dried nuts are ground in a blender. The fat is extracted from the nut flour using organic hexane solvent at a ratio of 1:4 for 4 hours in stages. The nut flour is dried at 50°C for 6 hours. The fat-free flour with a mesh size of 100 is stored in a sealed container.

2. Production of Legume Seed Protein Concentrate

Legume flour was added to distilled water so that the concentration becomes 10 g/100 ml. The suspension was extracted using hydrochloric acid according to the treatment (concentrations of 3%, 5%, and 7%) at a ratio of 1:5 (hydrochloric acid : bean flour) with pH adjustment to 4–5. The suspension was heated in a water bath at 50°C for 1 hour. The solution was cooled to room temperature and neutralized with NaOH 1 N solution, then centrifuged at 3000 rpm for 25 minutes. The first filtrate was centrifuged at 3000 rpm for 25 minutes to obtain the filtrate and precipitate. The first and second precipitates were washed with 80°C water three times. The precipitate was dried at 50°C for 5 hours. The precipitate was analyzed for protein content, fat content, moisture content, ash content, bulk density, oil absorption capacity, water absorption capacity, foaming capacity, emulsion capacity and stability, and organoleptic properties (color and odor).

RESULTS AND DISCUSSION

A. Raw Material Analysis Results

The protein and amino acid content, which is similar to that of soybeans, shows the potential of local nuts as an alternative source of vegetable protein to replace soybean concentrate products. Nuts can be used to make protein concentrate products for use in various types of food products. Beans such as cowpeas (*Vigna unguiculata*), mung beans (*Phaseolus radiates L.*) and red beans (*Phaseolus vulgaris L.*) can be used as substitutes for soybeans.

Protein concentrate is a protein extracted from food ingredients using a specific extraction method to produce a product with a high protein concentration. These proteins are widely used as raw materials in the food industry because they have certain functional properties that can improve the quality of food products.

Before making protein concentrate from three types of local beans, the beans were first ground into flour with the fat removed. The defatted flour from the beans is tested for moisture, ash, fat, and protein content (Table 1.)

Table 1. Raw Material Analysis Results

Legume type	Result of analysis			
	Water (%)	Ash (%)	fat (%)	Protein (% db)
Mug bean	8.02 ± 0.04	1.11 ± 0.06	1.22 ± 0.15	25.33 ± 0.17
Cowpea	10.73 ± 0.08	2.36 ± 0.02	1.40 ± 0.57	22.98 ± 0.49
Red Bean	12.71 ± 0.04	1.69 ± 0.08	1.46 ± 0.01	21.30 ± 0.33

The obtained legume flour satisfies the requirements for defatted flour, exhibiting fat content below the 1.5% threshold established by USDA (2002), as referenced by Kartika (2009).

B. Protein Concentrate Analysis Results

Structural and colloidal stability of proteins at different surfaces and interfaces is of great importance in many fields including medical, pharmaceutical, or material science. Due to their flexibility, proteins tend to respond to their environmental conditions and can undergo structural and conformational changes. For instance, alterations in physiological factors such as temperature, ions concentration, or pH as well as the adsorption to an interface can initiate protein aggregation. Therefore, at different surfaces and interfaces the characterization of the structural and colloidal stability of proteins, which is mainly influenced by their electrostatic and hydrophobic interactions, is of fundamental importance (Lautenbach, et. al., 2021).

Protein Content

The initial protein content of Mung beans, Cowpea, and red beans decreased after undergoing extraction with hydrochloric acid. This is thought to be because during extraction or concentrate production, a lot of protein is dissolved and carried over into each stage of the process, resulting in a significant loss of protein.

Anglemier (1976) states that most legume proteins are globulins that precipitate at pH 4.1, while other proteins such as protease, prolamin, and albumin are water-soluble. Therefore, the decrease in protein content during processing is likely due to the breakdown of protein structural bonds and the solubility of protein components in water.

On Table 2 demonstrates that the optimal protein extraction from mung beans occurred at 3% HCl concentration, yielding 21.67% (db) protein content. This correlates with the observed isoelectric point pH of 5.3 at this acid concentration, which facilitated protein coagulation.

Table 2. Chemical characteristics in legumes Protein Isolates as Affected by Hydrochloric Acid Concentration

Treatment research					
Legume type	HCL Concentration	Protein(%)	Lipd (%)	Moisture (%)	Bulk Density (g/mL)
Mung bean	3%	21.67 ± 0.02 ^b	16.90 ± 0.13 ^b	10.36 ± 0.27 ^a	0.79 ± 0.03 ^a
Mung bean	5%	17.82 ± 0.66 ^a	20.12 ± 0.08 ^a	10.59 ± 1.58 ^a	0.85 ± 0.02 ^a
Mung bean	7%	17.42 ± 0.09 ^a	20.17 ± 0.09 ^a	12.71 ± 0.79 ^a	0.81 ± 0.02 ^a
Cowpea	3%	20.44 ± 0.06 ^b	1.23 ± 0.21 ^a	12.35 ± 0.23 ^a	0.70 ± 0.01 ^b
Cowpea	5%	19.80 ± 0.01 ^a	1.58 ± 0.16 ^a	13.26 ± 1.77 ^a	0.65 ± 0.01 ^a
Cowpea	7%	21.21 ± 0.08 ^c	1.61 ± 0.14 ^a	12.98 ± 0.12 ^a	0.77 ± 0.01 ^c
Red Bean	3%	21.18 ± 0.13 ^b	2.07 ± 0.02 ^c	10.23 ± 0.89 ^a	0.75 ± 0.01 ^a
Red Bean	5%	20.12 ± 0.08 ^a	1.80 ± 0.08 ^b	9.87 ± 0.06 ^a	0.77 ± 0.01 ^a
Red Bean	7%	20.17 ± 0.09 ^a	1.48 ± 0.03 ^a	12.93 ± 0.73 ^a	0.76 ± 0.01 ^a

Note. Means with different letter superscripts differ significantly at $p < .05$ according to Fisher's LSD test.

Lehninger (1990) state the effect of pH is based on the strongest attractive forces between identical proteins occurring at the isoelectric pH. Table 2 showed that the highest protein content in cowpeas was obtained at 7% hydrochloric acid concentration, reaching 21.21% (db). This is presumably because in this study, the isoelectric point pH of cowpeas at 7% hydrochloric acid concentration was 4.23, resulting in coagulation. The use of strong acid (HCl) for protein extraction results in excess H⁺ ions that neutralize the proteins and achieve the isoelectric pH. As presented in Table 2, the optimal protein extraction yield (21.18%

db) from red beans occurred at 3% HCl, corresponding to their isoelectric pH of 4.93 where maximum protein precipitation occurs (Kurniati, 2009). The isoelectric point is the state where pH reaches an amphoteric form (with equal numbers of cations and anions), and at this isoelectric point, protein solubility decreases to its lowest level, causing proteins to precipitate and coagulate (Suhardi, 1988).

Interaction with a surface, proteins usually adopt a different conformation than those in the bulk solution, to minimize their free energy. For instance, Roach et al. (2005) showed that increased surface hydrophobicity increases the adsorption affinity of amphiphilic proteins and triggers structural changes. Meanwhile, electrostatic attraction or repulsion not only affects the adsorption of proteins on surfaces but also alters proteins' structural and conformational stability. The magnitude of electrostatic interactions between proteins and surfaces and between protein molecules can be altered either by the ionic content or the pH value of the surrounding medium. At pH values close to the isoelectric point (IEP), proteins possess a net neutral charge whereas at pH values above and below IEP they exhibit net negative and net positive charge, respectively. The IEP of proteins is strongly influenced by the composition of amino acids, their local distribution in protein structure, as well as the structural conformation of proteins (Lautenbach et al., 2021).

Fat Content

Table 2 showed that the highest fat content in the mung bean group was obtained at a 3% hydrochloric acid concentration, reaching 1.69%. This is presumably because the addition of 3% hydrochloric acid caused protein denaturation and coagulation, which exposed hydrophobic groups, thereby increasing their capacity to bind fat.

Protein denaturation can enhance the protein's fat-binding capacity due to the exposure of hydrophobic groups in the protein material (Suwarno, 2003). Table 2 showed that the highest fat content in cowpeas was obtained at 7% hydrochloric acid concentration, reaching 1.61%. This is presumably because higher concentrations of hydrochloric acid can increase protein content proportionally with the increase in fat content.

Fat content increases with rising pH up to a certain threshold corresponding to the protein content of the protein concentrate. The fat present in this protein concentrate consists of fatty acids bound to proteins through acid extraction processes. Table 2 showed that the highest fat content in red beans was achieved at 3% hydrochloric acid concentration, measuring 2.07%. This is presumably because the addition of 3% hydrochloric acid induced protein denaturation and coagulation, exposing hydrophobic groups that consequently enhanced fat-binding capacity. As reported by Suwarno (2003), the unfolding of protein structures during denaturation reveals hydrophobic regions that improve fat absorption properties. Whey protein concentrates with protein content of 70–80 % may be used to improve fat and water absorption in different food products, the formulation of which includes dry milk whey or dry fat-free milk. It is evident that further use of dry concentrates in the production of other food products, for instance, meat products or bakery goods, are directly related to the properties, conditioned by the physical and chemical composition of concentrates (Minorova, et al. 2017).

Moisture Content

Table 2 showed that the highest moisture content in mung beans was achieved at 7% hydrochloric acid concentration, measuring 12.71%. This is presumably because mung beans contain abundant polar amino acids, and the addition of hydrochloric acid enhances their water-binding capacity. The hydrophilic properties of proteins arise from polar side chains along the peptide backbone, specifically carboxyl and amino groups (Damodaran and Paraf, 1997).

Table 2 showed that the highest moisture content in cowpeas was obtained at 5% hydrochloric acid concentration, reaching 13.26%. This is presumably because at 5% HCl concentration, the intermolecular attractive forces decrease as the pH moves further from the isoelectric point, thereby enabling greater water retention capacity.

Suhardi (1988) posited that the attractive forces between proteins intensify at the isoelectric point, resulting in gel contraction. Conversely, reducing these attractive forces by adjusting the pH away from the isoelectric point enables the gel to encapsulate a substantial volume of water.

As indicated in Table 2, the highest moisture content observed in the red bean group was at a hydrochloric acid concentration of 7%, with a value of 12.93%. It is hypothesized that this phenomenon is attributable to the prevalence of polar amino acids in red beans, which enhances their water-binding capacity upon the addition of hydrochloric acid. Water binding capacity is influenced by the composition of polar amino acids (Suwarno, 2003).

Bulk Density

Table 2 showed that the highest bulk density value (0.85 g/mL) was observed in the mung bean group treated with 5% hydrochloric acid. This difference in the bulk density of the protein concentrate is presumed to be influenced by two factors: (1) increased intermolecular attractive forces between particles, and (2) moisture content. Specifically, lower moisture content results in higher bulk density.

Wirakartakusumah et al. (1992) proposed that water absorption correlates with enhanced cohesivity, mainly attributed to the formation of liquid bridges among particles.

As presented in Table 2, the maximum bulk density (0.77 g/mL) was achieved in cowpea samples treated with 7% hydrochloric acid. This can be explained by the decreased porosity and lower moisture content at this acid concentration, which substantially influences the particle mass per unit volume. Wirakartakusumah et al. (1992) defined porosity as the portion not occupied by particles or solid materials.

On Table 2 demonstrates that the highest bulk density (0.77 g/mL) was achieved in the red bean group with 5% hydrochloric acid concentration. This difference is presumably due to variations in intermolecular attractive forces and higher moisture content at 5% hydrochloric acid concentration, resulting in lower particle mass per unit volume. Peleg and Bagley (1983) as cited in Kartika (2009) explained that the bulk density of food materials depends on both the intensity of intermolecular attractive forces and particle size distribution.

Oil Absorption Capacity

Table 3. shows that the highest oil absorption capacity (2.09 mL/g) was observed in the mung bean group treated with 3% hydrochloric acid. This phenomenon is presumably due to the dominance of non-polar amino acids (particularly phenylalanine) in mung beans, which enhances their oil/fat absorption capability. Oil Absorption Capacity (OAC) of Red Bean Protein Concentrate at Various Hydrochloric Acid Concentrations

Table 3. functional characteristics of legume protein concentrates

Treatment research					
Legume type	HCL Concentration	Oil Absorption Capacity (mL/g)	Water Absorption Capacity(mL/g)	Foaming Capacity (%)	Emulsifying Capacity (%)
Mung bean	3%	2.09 ± 0.09 ^a	2.73 ± 0.12 ^a	4.20 ± 1.08 ^b	23.62 ± 5.94 ^b
Mung bean	5%	1.94 ± 0.14 ^a	2.47 ± 0.23 ^a	1.73 ± 0.64 ^a	9.72 ± 3.68 ^a
Mung bean	7%	1.93 ± 0.07 ^a	2.73 ± 0.23 ^a	1.20 ± 0.87 ^a	6.95 ± 4.81 ^a
Cowpea	3%	1.58 ± 0.04 ^a	3.07 ± 0.12 ^b	1.57 ± 0.55 ^a	8.83 ± 2.92 ^a
Cowpea	5%	2.01 ± 0.06 ^c	3.13 ± 0.12 ^b	1.30 ± 0.10 ^a	7.34 ± 0.49 ^a
Cowpea	7%	1.82 ± 0.87 ^b	2.73 ± 0.12 ^a	0.97 ± 0.21 ^a	5.38 ± 1.18 ^a
Red Bean	3%	1.91 ± 0.10 ^a	3.67 ± 0.12 ^a	1.00 ± 0.20 ^a	5.61 ± 0.97 ^a
Red Bean	5%	1.83 ± 0.03 ^a	4.40 ± 0.87 ^a	1.07 ± 0.29 ^a	5.91 ± 1.46 ^a
Red Bean	7%	1.81 ± 0.03 ^a	4.27 ± 1.10 ^a	3.27 ± 1.20 ^a	18.13 ± 6.69 ^b

Note. Means with different letter superscripts differ significantly at $p < .05$ according to Fisher's LSD test.

The oil absorption capacity of a protein depends on its structure. On Table 3 showed that the highest oil absorption capacity in cowpeas was achieved at 5% hydrochloric acid concentration, measuring 2.01 ml/g. This is presumably because during processing with 5% hydrochloric acid concentration, protein denaturation occurs, thereby enhancing the protein's ability to bind more fat through exposure of non-polar amino acids. According to Suwarno (2003), protein denaturation enhances fat-binding capacity by unfolding protein structures and exposing non-polar amino acids.

Table 3 showed that the highest oil absorption capacity in red beans was achieved at 3% hydrochloric acid concentration, measuring 1.91 ml/g. This is presumably because red beans contain predominantly non-polar amino acids such as leucine, which increases oil/fat absorption. This finding is consistent with the study results showing that 3% hydrochloric acid concentration yields both high protein content and high fat content. Kartika (2009) state lipolytic protein structures, specifically lipoprotein types, are presumed to contain non-polar amino acids.

Water Absorption Capacity

Table 3 showed that the highest water absorption capacity in mung beans was achieved at 7% hydrochloric acid concentration, measuring 2.73 ml/g. This is presumably because mung beans contain predominantly polar amino acids, and the addition of 7% hydrochloric acid enhances their water-binding capacity. This finding is consistent with the study results showing that 7% hydrochloric acid concentration yields higher moisture content. According to Suwarno (2003), water binding capacity depends on the composition and conformation of protein molecules.

Table 3 showed that the highest water absorption capacity in cowpeas was achieved at 5% hydrochloric acid concentration, measuring 3.13 ml/g. This is presumably because the water absorption capacity of protein concentrate at 5% hydrochloric acid concentration is influenced by the predominance of polar amino acids, consistent with this study's findings showing that 3% hydrochloric acid concentration yields higher moisture content. According to Suwarno (2003), water absorption capacity is related to the number of polar amino acid groups present in the protein molecule.

Table 3 showed that the highest water absorption capacity in red beans was achieved at 5% hydrochloric acid concentration, measuring 4.11 ml/g. This is presumably because the 5% hydrochloric acid concentration has the ability to prevent water loss from the material structure due to the predominance of polar amino acid

properties. The amino acid composition of proteins influences the water absorption capacity of protein concentrates.

Foaming Capacity

On Table 3 shows that the highest foaming capacity in mung beans was achieved at 3% hydrochloric acid concentration, measuring 4.20%. This is presumably because at 3% hydrochloric acid concentration, protein molecular bonds unfold, allowing air incorporation and consequent protein expansion. The foam formation mechanism begins with the unfolding of protein molecular bonds, causing protein volume expansion.

Table 3 showed that the lowest foaming capacity in cowpeas was obtained at 7% hydrochloric acid concentration, measuring 0.97%. This is because the reduced foaming capacity at 7% hydrochloric acid concentration results from high fat content, which weakens protein interactions during foam formation. According to Kartika (2009), foaming capacity decreases due to high fat content in protein concentrates, which weakens protein-protein interactions by disrupting hydrophobic surfaces.

Table 3 showed that the lowest foaming capacity in red beans was obtained at 3% hydrochloric acid concentration, measuring 1.0%. This is presumably because the 3% hydrochloric acid concentration results in high fat content, leading to instability of the formed film.

According to Zayas (1997), foam instability is influenced by residual fat in protein concentrates, which creates films that are too thin to stabilize bubbles. The increase in protein content in concentrates up to 70–80 % leads to a significant increase in the foam-forming, water-retaining, fat-retaining and emulsifying capacities of concentrates in comparison with the milk whey (Minorova, et. al 2017).

Emulsifying Capacity

Table 3 showed that the highest emulsifying capacity in mung beans was achieved at 3% hydrochloric acid concentration, measuring 23.62%. This is because at 3% hydrochloric acid concentration, emulsifying activity increases with rising protein and fat content, resulting in the formation of fat-water emulsions. Wolf and Cowman (1971) state proteins play a role in emulsifying activity by forming emulsions between fat and water, where proteins accumulate at the fat-water interface and low-pressure surfaces.

Table 3. showed that the lowest emulsifying capacity in cowpeas was obtained at 7% hydrochloric acid concentration, measuring 5.38%. This is because emulsifying capacity is influenced by the amino acid composition of the protein, with the lowest capacity at 3% hydrochloric acid concentration resulting from an imbalance between hydrophilic and lipophilic amino acids. The hydrophilic-lipophilic amino acid balance is critically related to surface tension reduction capability, which functions in emulsion formation (Zayas, 1997).

Table 3 showed that the lowest emulsifying capacity in red beans was obtained at 3% hydrochloric acid concentration, measuring 5.61%. This is presumably because at 3% hydrochloric acid concentration, there is an imbalance between hydrophilic and lipophilic amino acids, resulting in the emulsifier's inability to properly bind to both oil and water phases. This leads to the formation of fewer matrices and consequently less stable emulsions. The hydrophilic-lipophilic amino acid balance is closely related to the ability to reduce surface tension, which functions in emulsion formation (Zayas, 1997).

The functional and technological properties of concentrates, characterizing their capability to participate in the surface phenomena while obtaining foam-forming and emulsion systems, including fat- and moisture-retaining properties, were estimated by conventional methods. The foam-forming capability of dry samples was determined by the relative increase in their solution volumes after shaking for 5 min at 800 rpm (Tvorogova AA, 2003). The emulsifying and fat-retaining capabilities of the investigated products were estimated using the emulsion solutions with refined oil according to the method. The moisture-retaining capability of dry products was determined by the increase in the mass of wet precipitate after centrifugation at 8,000 rpm for 15 min (Jasumatsu, 1972).

Organoleptic Analysis

As presented in Table 4, colour evaluation revealed distinct pigment modifications in legume samples following 3% hydrochloric acid treatment:

- a. Mung bean (*Vigna radiata*) protein isolates exhibited an off-white to pale yellow coloration
- b. Red bean (*Phaseolus vulgaris*) protein concentrates demonstrated significant browning, transitioning from their native reddish hue to light brown

This chromatic alteration in red beans is attributed to acid-induced structural changes in anthocyanin pigments at pH 3.0-4.0, facilitating Maillard reaction precursors. The observed color shift aligns with previous findings that food appearance critically influences consumer acceptance, wherein even nutritionally superior products experience reduced palatability when exhibiting non-characteristic pigmentation ($\Delta E > 3.5$ CIELAB units).

Table 4. Scoring Values for Color and Odor of Legume Protein Concentrates

Treatment research			
Legume type	HCL Concentration	Color scoring	Odor scoring
Mung bean	3%	147,5	108,5
Mung bean	5%	146,5	71,5
Mung bean	7%	144,5	70
Cowpea	3%	89	125,5
Cowpea	5%	73	118,5
Cowpea	7%	77,5	66
Red Bean	3%	70	111,5
Red Bean	5%	78	131
Red Bean	7%	74	97,5

Note: Maximum scores represent optimal preference according to hedonic testing.

As evidenced in Table 4, odor sensory evaluation revealed that:

- Cowpea (*Vigna unguiculata*) protein concentrates prepared with 3% hydrochloric acid exhibited minimal beany odor
- Those processed with 7% HCl demonstrated intense beany off-flavors

This odor development is likely attributable to lipoxygenase-mediated oxidation of unsaturated fatty acids during extraction and thermal processing. Shurtleff and Aoyagi (1984) established that legume crushing activates lipoxygenase enzymes, which catalyze the oxidation of linoleic and linolenic acids (C18:2 and C18:3), generating volatile carbonyl compounds responsible for characteristic beany flavors.

CONCLUSIONS

Based on this research, the following conclusions can be drawn:

1. The optimal protein concentrate from mung beans was obtained using 3% HCl extraction with protein content (21.67%), fat (1.69%), moisture (10.36%), bulk density (0.79g/ml), oil absorption (2.09ml/g), water absorption (2.73ml/g), foaming capacity (4.20%) and emulsifying capacity (23.62%).
2. The optimal protein concentrate from cowpea was obtained using 7% HCl extraction with protein content (21.21%), fat (1.61%), moisture (12.98%), bulk density (0.77g/ml), oil absorption (1.82ml/g), water absorption (2.73ml/g), foaming capacity (0.97%) and emulsifying capacity (5.38%).
3. The optimal protein concentrate from red beans was obtained using 3% HCl extraction with protein content (21.18%), fat (2.07%), moisture (10.23%), bulk density (0.75g/ml), oil absorption (1.91ml/g), water absorption (3.67ml/g), foaming capacity (1.00%) and emulsifying capacity (5.61%).

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