



Research Article

Optimization of Protein Extraction from Red Lima Bean (*Phaseolus lunatus* L.) and Its Application as a Binder in Chicken Nuggets**Bambang Dwi Wijatniko^{1,2*}, Amelia Susilo¹⁾, Agnes Murdiati¹⁾**¹ Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta² Center of Excellence for Indonesian Gastronomy, Universitas Gadjah Mada, Yogyakarta*Corresponding email: bambangdw92@ugm.ac.id**ABSTRACT**

Red lima bean (*Phaseolus lunatus* L.) is an underutilized legume that is well adapted to marginal environments and possesses high nutritional value. However, its application remains limited due to the presence of antinutritional compounds, particularly hydrogen cyanide (HCN). This study aimed to optimize protein extraction from red lima beans and to evaluate the functional performance of the resulting protein concentrate as a binder in chicken nuggets. Protein extraction was optimized using alkaline solubilization followed by isoelectric precipitation. A soaking pre-treatment for 24 h reduced the HCN content to 6.40 ppm. Optimal extraction was achieved at pH 10, with subsequent precipitation at pH 4, yielding a protein concentrate containing 49.32% protein. The functional properties of the protein concentrate were evaluated through partial to full substitution of soy protein isolate as a binder in chicken nuggets. In addition, physicochemical and sensory properties were analyzed. The results demonstrated that red lima bean protein concentrate exhibited effective binding functionality in chicken nuggets. A substitution level of 50% (w/w) with soy protein isolate resulted in the highest sensory acceptance without compromising product quality. These findings highlight the potential of red lima bean protein as a sustainable and functional ingredient in meat products, supporting the valorization of underutilized legumes and the development of alternative plant-based binders.

Keywords: protein extraction, protein concentrate, red lima bean, binder, chicken nuggets**1. INTRODUCTION**

The growing demand for sustainable and diversified protein sources has intensified interest in underutilized legumes, particularly those adapted to marginal environments. Indonesia is endowed with a wide variety of indigenous legumes, including lima beans (*Phaseolus lunatus* L.), which are well suited to dryland cultivation and has relatively high productivity, with yield reaching up to 1700 kg/ha. Among these, red lima bean represents a promising yet underexploited resource due to its favorable nutritional composition,

consisting of approximately 21.40% protein, 60.55% carbohydrates, and low lipid content (1.07%) [1]. Such a composition highlights its potential as an alternative plant-based protein source for food applications [2].

However, the broader utilization of red lima beans is constrained by the presence of antinutritional compounds, particularly cyanogenic glycosides that release hydrogen cyanide (HCN), with reported levels as high as 26.22 mg/g [3]. This compound brings issue on the safety, so that appropriate processing strategies are required to reduce it while preserving nutritional and functional properties. Conventional treatments, such as soaking, thermal processing, and fermentation, have been shown to reduce HCN levels. After the removal of HCN content, the proteins can be isolated and utilized for various functional applications in food systems. Since protein yield and quality are highly dependent on the extraction method employed, optimizing extraction parameters is essential not only for improving recovery efficiency but also for modulating the techno-functional characteristics of its protein product [4]. Previous studies have suggested that combined extraction approaches, particularly alkaline treatment coupled with ultrasonication, can enhance protein recovery, increase extraction yield, and improve the nutritional properties of sunflower proteins [5].

Among various food ingredients, protein isolates have gained considerable attention because of their desirable techno-functional properties, including water-holding capacity, oil-binding ability, emulsifying performance, and gel-forming characteristics [6]. These properties are highly dependent on protein structure, which can be modulated through physicochemical treatments such as pH adjustment, ionic strength variation, and thermal processing [7]. Among various methods, alkaline extraction followed by isoelectric precipitation remains one of the most commonly employed techniques for plant protein isolation, as it enables selective solubilization and recovery of proteins with relatively high yield [4]. Nevertheless, extraction conditions, particularly pH, play a crucial role in determining protein yield, composition, and functionality. Excessively alkaline conditions may induce protein denaturation, aggregation, or chemical modification, thereby impairing functional performance in food systems. Therefore, optimization of extraction parameters is essential to achieve an optimal balance between protein recovery and functional quality.

Currently, soy protein isolate (SPI) dominates the global market as a functional plant protein ingredient due to its well-established techno-functional properties. However, reliance

on imported soybean in many countries, including Indonesia, raises economic and sustainability concerns. This situation has driven the exploration of locally available legumes as alternative protein sources. In this context, red lima bean protein presents a viable and sustainable substitute, particularly if its functional performance can match or complement that of soy protein.

One of the key applications of protein isolates in the food industry is their use as binders in meat products, such as chicken nuggets. Nuggets are structured as complex oil-in-water emulsions, in which myofibrillar proteins, non-meat proteins, and water form a continuous matrix that stabilizes dispersed fat globules [8]. The presence of non-meat proteins, such as plant protein isolates, can significantly influence emulsion stability, water-holding capacity, cooking yield, texture, and sensory properties. Protein-based binders contribute to reducing cooking losses, improving slice ability, enhancing juiciness, and stabilizing the overall product structure [9]. Given the high protein content of red lima beans, their proteins are expected to exhibit functional properties suitable for such applications.

Despite the promising potential of red lima bean (*Phaseolus lunatus* L.), studies on the optimization of protein extraction and its application as a functional food ingredient remain limited. In particular, there is a lack of comprehensive information regarding the reduction of HCN, optimization of extraction conditions, and the techno-functional properties of protein extracted from red lima bean as a binder in food systems. Furthermore, the substitution of soy protein isolate with red lima bean protein in meat products has not been investigated. Therefore, this study aimed to produce protein isolate from red lima beans through optimized extraction processes and to evaluate the effect of its incorporation as a binder in chicken nugget products.

2. MATERIALS AND METHODS

2.1. Materials

Red lima beans were obtained from a traditional market in Yogyakarta. Other ingredients used in this study included commercial soy protein isolate (Solpro 920), chicken meat, tapioca flour, iced water, seasoning, salt, and baking soda.

All chemicals used were of analytical grade. n-Hexane, concentrated H₂SO₄, HCl, boric acid crystals, and bromocresol green–methyl red (BCG–MR) were purchased from Merck Millipore (Burlington, MA, USA). Petroleum ether; catalysts (HgO and K₂SO₄); NaOH–Na₂S₂O₃;

NaOH crystals; bovine serum albumin (BSA) powder; Na_2CO_3 crystals; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ crystals; $\text{Na}_2\text{Tartrate} \cdot 2\text{H}_2\text{O}$ crystals; Folin–Ciocalteu solution; KCN crystals; and picric acid solution were obtained from Sigma-Aldrich (Missouri, USA)

2.2. Preparation of Red lima bean Flour

Red lima bean flour was prepared following the method described for jack bean flour (Wijatniko et al., 2018) with slight modifications. Red lima bean seeds were soaked in water for a maximum of 72 h, with dehulling performed after the first 24 h of soaking. The dehulled seeds were then dried in a cabinet dryer at 50 °C for 24 h, followed by grinding and sieving through a 60-mesh sieve. The resulting red lima bean flour was stored in an airtight container to prevent moisture absorption.

2.3. Isolation of Red Lima Bean Protein

Protein isolation from red lima bean was carried out by adopting the method for jack bean protein isolate with slight modifications [10]. Prior to extraction, lipids in red lima bean flour were removed by defatting with n-hexane at a flour-to-solvent ratio of 1:3 (w/v). The defatting process was performed twice, followed by drying the defatted flour in a cabinet dryer at 50 °C for 2 h. Protein extraction was conducted by dispersing the defatted flour in distilled water at a ratio of 1:4 (w/v), followed by adjusting the pH of the suspension to different levels (pH 2, 4, 6, 8, 10, and 12) using 1 N NaOH. The mixture was then stirred for 1 h to facilitate protein solubilization. After extraction, the suspension was centrifuged at 4000 rpm for 30 min to separate the supernatant (protein extract) from the residue. The pH of the collected supernatant was subsequently adjusted to 4 to induce isoelectric precipitation of proteins. The mixture was then centrifuged again at 4000 rpm for 30 min to obtain the protein precipitate. The resulting protein concentrate was stored at –50 °C prior to further analysis.

2.4. Production of Chicken Nugget

Chicken nuggets were prepared using a modified formulation incorporating soy protein isolate and red lima bean protein concentrate. The ratios of soy protein isolate to red lima bean protein concentrate were 100:0, 75:25, 50:50, 25:75, and 0:100 (w/w). The formulation (Table 2.1) was adapted from previous study with modifications [11]. Fresh chicken meat was trimmed, washed, and cut into small pieces prior to grinding using a food processor. Soy protein isolate and red lima bean protein concentrate were then added according to the specified ratios. Subsequently, salt, pepper, finely ground shallots and garlic, beaten eggs,

tapioca flour, and ice cubes were incorporated into the mixture. The batter was homogenized for approximately 2–3 min until a uniform consistency was achieved. The batter was transferred into an oil-coated mold and steamed at 90–100 °C for 30 min or until fully cooked. After cooking, the product was removed and cooled to room temperature, then cut into pieces measuring approximately 4 × 3 × 1 cm. Each piece was dipped in beaten egg and coated with breadcrumbs. Finally, the nuggets were deep-fried at 170 °C for 2–3 min until golden brown, then removed and drained prior to further analysis.

Table 2.1. Formulation of chicken nuggets with soy protein and red lima bean protein

Ingredients	Protein Isolate Formulation Ratio (Soy Protein : Red Lima Bean Protein)				
	100:0	75:25	50:50	25:75	0:100
Chicken (g)	260	260	260	260	260
Tapioca flour (g)	78	78	78	78	78
Soy protein isolate (g)	8	6	4	2	0
Red lima bean protein concentrate (g)	0	2	4	6	8
Egg (g)	31.2	31.2	31.2	31.2	31.2
Ice (g)	52	52	52	52	52
Salt (g)	7.8	7.8	7.8	7.8	7.8
Red onion (g)	7.8	7.8	7.8	7.8	7.8
Garlic (g)	7.8	7.8	7.8	7.8	7.8
Pepper (g)	2	2	2	2	2
Total (g)	454.4	454.4	454.4	454.4	454.4

2.5. Analysis of Chicken Nuggets

Cooked chicken nuggets were subjected to sensory evaluation comprising both attribute intensity and hedonic (acceptance) tests. The evaluated attributes included color, texture (hardness, chewiness, and cohesiveness), aroma, taste, aftertaste, and overall acceptability.

2.6. Data Analysis

The statistical analysis was performed using ANOVA at the 5% test level. If there is a significant difference, then the analysis was followed by Duncan's test (Duncan Multiple Range Test/DMRT) at the 5% test level.

3. RESULT AND DISCUSSION

3.1. HCN Content of Red Lima Bean

The HCN content of red lima bean is presented in Table 3.1. The HCN content of untreated beans (13.55 ± 1.51 ppm) was significantly higher than that of the flours subjected to soaking

treatments ($p < 0.05$). A progressive reduction in HCN content was observed with increasing soaking duration, decreasing to 6.40 ± 0.15 ppm (24 h), 1.02 ± 0.28 ppm (48 h), and 0.02 ± 0.02 ppm (72 h). This reduction can be attributed to the high water solubility of hydrogen cyanide, which facilitates its leaching into the soaking medium, particularly when the soaking water is periodically replaced [12]. According to established safety guidelines, HCN levels below 10 ppm are considered safe for consumption [13]. Therefore, the 24 h soaking treatment was selected for further processing, as it meets safety standards while maintaining processing efficiency.

Table 3.1. HCN Content of Red Lima Bean

Sample	HCN concentration (ppm)
Red lima bean seeds	13.55 ± 1.51^c
Red lima bean powder (soaking 24 hours)	6.40 ± 0.15^b
Red lima bean powder (soaking 48 hours)	1.02 ± 0.28^a
Red lima bean powder (soaking 72 hours)	0.02 ± 0.02^a

Values are presented as the mean $\pm$ SD ($n = 6$). Means not marked by a common letter are significantly different as determined using the DMRT post-hoc test ($P < 0.05$).

3.2. Chemical Composition of Red Lima Bean

The proximate composition of whole seeds and red lima bean flour is summarized in Table 3.2. Whole seeds contained 13.39% moisture, 3.65% ash, 1.55% fat, 18.74% protein, and 76.06% carbohydrates (by difference). These values are consistent with those previously reported [14]. Processing into flour resulted in notable compositional changes. The moisture content decreased to 8.50%, reflecting water loss during drying and milling. A lower moisture content is important to minimize quality deterioration associated with microbial growth [15]. The protein content increased to 24.98%, which can be attributed to the relative reduction of other components during soaking and drying. Certain soluble sugars and carbohydrates may have leached into the soaking water, thereby contributing to the relative increase in protein content. Furthermore, the reduction in moisture content may have resulted in a pseudo-increase in the proportion of other components, including protein [16]. Conversely, fat and ash contents showed slight decreases, indicating partial leaching or degradation during soaking. The carbohydrate content (by difference) decreased to 70.88%, reflecting shifts in the proportions of other macronutrients. Overall, these results demonstrate that soaking and milling not only reduce antinutritional compounds but also concentrate protein content, thereby enhancing the nutritional quality of the flour.

Table 3.2. Chemical composition of red lima bean

Parameter	Red lima bean seeds	Red lima bean flour
Water (% wb)	13.39 ± 0.07	8.5 ± 0.03
Ash (% db)	3.65 ± 0.12	3.09 ± 0.06
Fat (% db)	1.55 ± 0.04	1.05 ± 0.05
Protein (% db)	18.74 ± 1.19	24.98 ± 0.88
Carbohydrate <i>by difference</i> (% db)	76.06 ± 1.23	70.88 ± 0.96

Values are presented as the mean ± SD (n = 6). Means not marked by a common letter are significantly different as determined using the DMRT post-hoc test (P<0.05).

3.3. Protein Solubility of Red Lima Bean

The solubility profile of red lima bean protein as a function of pH exhibited a typical U-shaped pattern, as shown in Fig. 1. Protein solubility was lowest at pH 4, corresponding to the isoelectric point (pI), at which the net charge of the protein is zero. Under this condition, electrostatic repulsion is minimized, leading to increased protein aggregation [4]. At both acidic (pH 2) and alkaline (pH 10) conditions, protein solubility increased significantly. Under acidic conditions, the presence of excess protons imparts a net positive charge to the protein, enhancing its interaction with water. However, the highest solubility was observed under alkaline conditions (pH 10), where increased negative charges promote protein dispersion and solubilization.

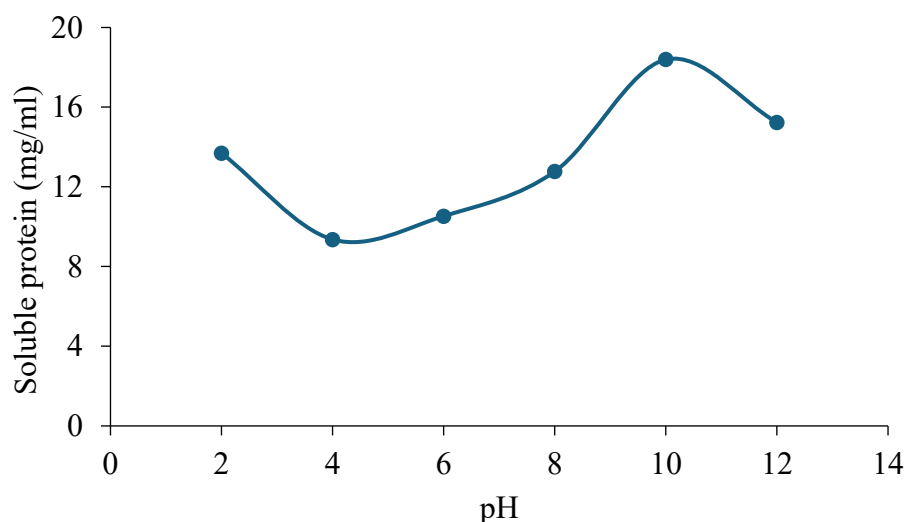


Figure 3.1. Solubility of red lima bean protein at different pH

This finding is consistent with previous studies indicating that alkaline conditions (pH 10–12) are optimal for plant protein extraction due to enhanced solubility [17,18]. Based on these findings, protein extraction was optimized at pH 10, while precipitation was performed at pH 4 to optimized protein recovery.

3.4. Protein Content of Red Lima Bean Protein Concentrate

The protein content of the extracted red lima bean protein was 49.32% (wet basis). This value indicates that the product is a protein concentrate rather than a protein isolate, as isolates typically contain protein levels exceeding 90%. In this study, we obtained the protein concentrate of red lima bean. The reduced protein level identified in the present study, relative to earlier reports, is likely influenced by variations in the source of raw materials, cultivation environments, and differences in processing techniques employed [19]. Additionally, the presence of residual non-protein components in the concentrate may have contributed to the lower protein percentage [20]. Previous study has reported that the combined application of pH-shifting and ultrasonication can improve protein yield and protein concentration in quinoa protein isolates [21]. Since ultrasonication was not applied in the present study, future studies are recommended to investigate the combined use of alkaline extraction and ultrasonication as a strategy to further improve the concentration of red lima bean protein. Despite its relatively lower protein content, the protein concentrate of red lima bean still demonstrates potential as a functional ingredient due to its appreciable protein level and suitability for food applications.

3.5. Sensory Evaluation of Chicken Nuggets

Sensory evaluation is a critical approach for assessing the quality attributes of food products based on human perception, which ultimately determines consumer acceptance or rejection [22]. This evaluation involves multiple sensory attributes, including visual, tactile, olfactory, and gustatory responses. In this study, sensory analysis of chicken nuggets was conducted using both descriptive and hedonic tests performed by 35 untrained panelists using a 5-point scale. All panelists participated voluntarily and were informed about the sensory evaluation procedure before testing. The evaluated attributes included color, texture (hardness, chewiness, and cohesiveness), aroma, taste, aftertaste, and overall acceptability. The nuggets were formulated with varying ratios of soy protein isolate (SPI) and red lima bean protein concentrate (RLBPC), including 100:0, 75:25, 50:50, 25:75, and 0:100 (w/w), to determine the changes of sensory acceptance and properties in the products.

The hedonic test results (Table 3.3) indicated that substitution levels significantly influenced several sensory attributes. Color preference did not differ significantly ($p > 0.05$) among treatments, although the 25:75 formulation showed the highest mean score, while

the 75:25 and 0:100 formulations were less preferred. Texture attributes, including hardness, chewiness, and cohesiveness, were significantly affected by formulation ($p < 0.05$). The 50:50 ratio consistently received the highest preference scores for these parameters, indicating an optimal balance between firmness and chewiness. This finding is consistent with previous studies reporting that partial substitution of soybean protein can modify textural properties in meat products [9]. In contrast, the control sample (100:0) was the least preferred in terms of texture, suggesting that the absence of RLBPC may result in a less desirable structural profile. Variations in textural characteristics are likely associated with the techno-functional behavior of the proteins, including their water-holding, oil-binding, and foaming capacities, which are essential for maintaining the stability and structural integrity of emulsified systems [23]. This result indicated that RLBPC demonstrated techno-functional properties to contribute positively to the textural attributes of chicken nuggets.

Table 3.3. Hedonic test results of chicken nuggets with different ratios of soy protein isolate and red lima bean protein concentrate

Parameter	SPI: RLBPC				
	100:0	75:25	50:50	25:75	0:100
Color	3.60 ± 0.65 ^a	3.49 ± 0.98 ^a	3.74 ± 0.66 ^a	3.86 ± 0.65 ^a	3.49 ± 0.78 ^a
Texture (hardness)	2.11 ± 0.80 ^a	3.40 ± 1.01 ^{bc}	3.80 ± 0.83 ^c	3.63 ± 0.91 ^{bc}	3.29 ± 0.93 ^b
Texture (chewiness)	2.51 ± 0.78 ^a	3.40 ± 0.91 ^{bc}	3.77 ± 0.84 ^c	3.37 ± 0.84 ^{bc}	3.26 ± 0.70 ^b
Texture (cohesiveness)	2.54 ± 0.85 ^a	3.37 ± 0.91 ^b	3.71 ± 0.75 ^b	3.63 ± 0.94 ^b	3.31 ± 0.87 ^b
Aroma	3.40 ± 0.69 ^a	3.49 ± 0.74 ^a	3.94 ± 0.76 ^b	3.77 ± 0.84 ^{ab}	3.46 ± 0.78 ^a
Taste	3.63 ± 0.81 ^{ab}	3.37 ± 0.88 ^a	4.00 ± 0.73 ^b	3.66 ± 0.84 ^{ab}	3.54 ± 0.95 ^a
Aftertaste	3.31 ± 0.72 ^a	3.37 ± 0.73 ^a	3.57 ± 0.92 ^a	3.57 ± 0.92 ^a	3.40 ± 0.85 ^a
Overall acceptability	2.86 ± 0.81 ^a	3.40 ± 0.77 ^b	3.86 ± 0.69 ^c	3.66 ± 0.73 ^{bc}	3.54 ± 0.66 ^{bc}

– Values are presented as the mean ± SD (n = 35). Means not marked by a common letter are significantly different as determined using the DMRT post-hoc test ($P < 0.05$).

– Different superscript letters within the same row indicate significant differences ($p < 0.05$).

– Scale: 1 = dislike very much; 2 = dislike; 3 = neither like nor dislike; 4 = like; 5 = like very much.

Aroma and taste were also influenced by protein substitution. The 50:50 formulation exhibited the highest scores for both attributes, indicating improved flavor acceptability, whereas lower scores were observed in the control and certain other substitution levels. No significant differences in aftertaste were observed among treatments ($p > 0.05$), although the 50:50 and 25:75 formulations showed slightly higher preference scores. Overall acceptability differed significantly among treatments ($p < 0.05$), with the 50:50 formulation achieving the highest score. This finding suggests that partial substitution of soy protein isolate with red lima bean protein concentrate enhances consumer acceptance. In contrast, the 100:0 formulation was the least preferred across most attributes.

Sensory attribute intensity was conducted to evaluate the sensory profile of chicken nuggets among the formula (Table 3.4). Color intensity did not differ significantly among most treatments ($p > 0.05$), although the 25:75 ratio exhibited the highest intensity, while the 75:25 ratio showed the lowest. Texture evaluation revealed that hardness was significantly affected by formulation ($p < 0.05$), with the 100:0 sample exhibiting the highest hardness, whereas the 50:50 formulation showed the lowest, indicating a softer and more desirable texture. Chewiness did not differ significantly among treatments, suggesting that the addition of red lima bean protein concentrate did not adversely affect this attribute. Cohesiveness was highest in the 100:0 formulation and lowest in the 50:50 formulation, indicating that partial substitution reduced structural compactness while improving palatability.

Table 3.4. Sensory evaluation of chicken nuggets with different ratios of soy protein isolate and red lima bean protein concentrate

Parameter	SPI:RLBPC				
	100:0	75:25	50:50	25:75	0:100
Color	4.03 ± 0.86^b	3.57 ± 0.81^a	3.94 ± 0.84^{ab}	4.23 ± 0.73^b	3.60 ± 1.01^a
Texture (hardness)	3.74 ± 0.74^c	2.54 ± 0.95^b	2.14 ± 0.49^a	2.49 ± 0.74^{ab}	2.66 ± 0.84^b
Texture (chewiness)	3.03 ± 1.27^a	3.14 ± 0.94^a	3.14 ± 0.73^a	2.69 ± 0.90^a	3.14 ± 0.85^a
Texture (cohesiveness)	4.37 ± 0.65^b	3.57 ± 0.95^a	3.43 ± 0.65^a	3.54 ± 0.98^a	3.51 ± 0.95^a
Aroma	3.31 ± 0.87^a	3.71 ± 0.86^a	3.71 ± 0.89^a	3.51 ± 0.78^a	3.51 ± 0.89^a
Taste	3.29 ± 0.71^a	3.46 ± 0.85^a	4.06 ± 0.68^b	3.57 ± 0.81^a	3.66 ± 0.91^a
Aftertaste	2.31 ± 0.76^b	2.23 ± 0.88^{ab}	1.86 ± 0.60^a	1.94 ± 0.64^{ab}	2.06 ± 0.73^{ab}
Overall	3.23 ± 0.81^a	3.60 ± 0.91^b	4.03 ± 0.57^c	3.80 ± 0.72^{bc}	3.71 ± 0.71^{bc}

– Values are presented as the mean $\pm$ SD ($n = 35$). Means not marked by a common letter are significantly different as determined using the DMRT post-hoc test ($P < 0.05$).

– Different superscript letters within the same row indicate significant differences ($p < 0.05$).

– Scale interpretation: Color: 1 = very dark, 5 = very bright; Texture (hardness): 1 = very soft, 5 = very hard; Texture (chewiness): 1 = not chewy, 5 = very chewy; Texture (cohesiveness): 1 = not cohesive, 5 = very cohesive; Aroma: 1 = no chicken aroma, 5 = strong chicken aroma; Taste: 1 = no chicken taste, 5 = strong chicken taste; Aftertaste: 1 = not beany, 5 = very beany; Overall quality: 1 = very poor, 5 = very good

Aroma intensity (chicken aroma) did not differ significantly across treatments, indicating that substitution with red lima bean protein concentrate (RLBPC) did not mask the characteristic aroma of chicken. Similarly, taste intensity was significantly enhanced in the 50:50 formulation ($p < 0.05$), suggesting improved flavor perception at this substitution level. Aftertaste, particularly the beany note commonly associated with plant proteins, showed no significant differences among most treatments. However, the control sample (100:0) exhibited the highest aftertaste intensity, while the 50:50 formulation showed the lowest, indicating a more favorable sensory profile. The incorporation of RLBPC into emulsified chicken nuggets did not adversely affect aroma acceptance. Previous studies have reported

that the substitution of legume-based proteins in meat systems generally has minimal impact on aroma attributes [24]. In line with this, the present findings indicate that the addition of RLBPC did not significantly alter flavor perception, particularly with respect to the development of undesirable beany notes. Instead, the observed sensory differences were primarily associated with changes in texture and overall acceptability

These results suggest that RLBPC can be applied at moderate substitution levels without negatively affecting flavor perception, thereby supporting its use as a functional ingredient in emulsified meat products while maintaining acceptable sensory quality. Overall, descriptive evaluation confirmed that the 50:50 formulation achieved the most balanced sensory profile, whereas the control (100:0) exhibited the least desirable characteristics. The improved texture and overall acceptability observed in the 50:50 formulation may be associated with the techno-functional properties of RLBPC, particularly its water-holding and oil-binding capacities, which could enhance moisture retention, fat distribution, and structural stability of the nugget matrix compared to SPI alone. The presence of both hydrophobic and hydrophilic proteins and peptides in the RLBPC may play an essential role in forming the structural matrix responsible for the nugget texture preferred by the panelists. Additionally, balanced combination of RLBPC and SPI may contribute to the stabilization of the emulsion system while preserving desirable aroma and taste attributes, as well as reducing the beany flavor and undesirable aftertaste.

4. CONCLUSION

The reduction of HCN in red lima bean was achieved through soaking for 24 h, resulting in an HCN content of 6.40 ppm. Red lima bean protein concentrate was obtained through alkaline extraction at pH 10, corresponding to the highest protein solubility, followed by isoelectric precipitation at pH 4, where protein solubility was minimal. Based on sensory evaluation, the most preferred formulation was the 50:50 ratio of soy protein isolate to red lima bean protein concentrate. Descriptive analysis indicated that this formulation exhibited a slightly bright color, low hardness, moderately chewy texture, and a compact structure. Furthermore, the characteristic chicken aroma and taste were retained, while no undesirable beany aftertaste was detected. Overall, this formulation demonstrated good sensory quality

and high acceptability, highlighting the potential of red lima bean protein concentrate as a partial substitute for soy protein isolate in emulsified meat products.

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