TẠP CHÍ KHOA HỌC
TRƯỜNG ĐẠI HỌC SƯ PHẠM TP HỒ CHÍ MINH
Tập 23, Số 9 (2026): 1984-1995
Website: https://journal.hcmue.edu.vn
ISSN: 2734-9918

HO CHI MINH CITY UNIVERSITY OF EDUCATION
JOURNAL OF SCIENCE
Vol. 23, No. 9 (2026): 1984-1995
https://doi.org/10.54607/hcmue.js.23.9.5328(2026)

Research Article

ULTRASOUND-ASSISTED PROTEIN EXTRACTION FROM DEFATTED PEANUT FLOUR (*ARACHIS HYPOGAEA*) FOR LIQUID AMINOS PRODUCTION

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Received: January 29, 2026; Revised: June 26, 2026; Accepted: June 30, 2026

ABSTRACT

Defatted peanut flour, the primary byproduct of peanut oil processing, is a rich precursor for umami peptides due to its high content of acidic amino acids, which play key roles in umami taste formation. In this study, protein was extracted from defatted peanut flour (DPF) using ultrasound-assisted extraction (UAE) and used to produce liquid aminos. The effects of key technological parameters were evaluated, establishing the optimal extraction conditions as follows: a solid-to-solvent ratio of 1:15 (w/v), pH 12, an extraction temperature of 80 °C, and an ultrasonication duration of 20 min. Under these optimized conditions, the protein recovery yield reached 87.00 ± 7.21%. The resulting protein extract was hydrolyzed with Protease A (1% w/w) at 60 °C for 4 h and subsequently used to produce liquid aminos. The resulting liquid aminos demonstrated high nutritional quality, with a total nitrogen content of 4.69 ± 0.02 g/100 mL and an amino acid nitrogen content of 1.134 ± 0.02 g/L. These findings suggest that DPF is an effective substrate for generating a nitrogen-rich condiment through enzymatic hydrolysis.

Keywords: amino acid nitrogen; defatted peanut meal; enzymatic hydrolysis; ultrasound-assisted extraction; Liquid Aminos

1. Introduction

Vietnam has strong advantages in agriculture, with a wide range of agricultural products produced in large quantities, including oilseed crops. During oil extraction, a significant amount of by-products is generated, many of which may cause environmental pollution if not properly managed. Currently, only a small portion of these by-products is

Cite this article as: Nguyen, V. A., Nguyen, K. P., Nguyen, C. B., & Vu, T. N. A. (2026). Ultrasound-assisted protein extraction from defatted peanut flour (*Arachis hypogaea*) for liquid aminos production. *Ho Chi Minh City University of Education Journal of Science, 23*(9), 1984-1995. https://doi.org/10.54607/hcmue.js.23.9.5328(2026)

used as animal feed or fertilizer, while most oilseed cakes remaining after pressing are treated as agricultural waste. These by-products generally contain high protein levels, and recovering and reusing their protein could provide considerable economic and social benefits (Aït-Kaddour et al., 2024; Sá et al., 2021).

Peanut (*Arachis hypogaea L.*) is a widely cultivated, short-cycle industrial crop in Vietnam. Defatted peanut flour contains approximately 45–60% protein, 22–30% carbohydrates, 3.8–7.5% crude fiber, and 4–6% minerals (Galanakis, 2019). This protein fraction is rich in arginine and histidine, as well as substantial amounts of other amino acids. Structurally, most peanut proteins are glycoproteins containing small amounts of neutral and amino sugars (Kholief, 1987). With its favorable functional characteristics, including solubility, emulsifying performance, foaming ability, and water-holding capacity, peanut protein is increasingly recognized as a promising plant-based protein source for various food applications (H. Zhang et al., 2024).

Various conventional methods for peanut protein extraction have been studied, predominantly focusing on alkaline extraction followed by acid precipitation (S. Cui et al., 2023). To overcome the limitations of conventional methods—such as high chemical consumption, long processing times, and potential protein denaturation—several techniques have been developed to enhance extraction, including microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), and ultrasound-assisted extraction (UAE). While MAE can lead to localized overheating and EAE often incurs high operational costs, UAE is attractive because of its cost-effectiveness, environmental friendliness, and ability to preserve protein integrity (Chemat et al., 2017). The acoustic cavitation generated by ultrasound effectively disrupts plant cell walls and facilitates rapid mass transfer at lower temperatures (Suchintita Das et al., 2022). In recent years, UAE has been applied to peanut protein extraction, demonstrating significant improvements in both recovery yield and the functional properties of the resulting proteins (Sun et al., 2021). However, a major limitation in the current literature is that most of these studies rely on probe-type (horn) sonication. While effective at the laboratory scale, probe ultrasound poses substantial challenges for industrial scaling due to low processing capacity and intense localized heating. Furthermore, previous research has largely focused solely on the extraction process or the structural characterization of the protein, with very little attention given to its direct application in specific food products. In Vietnamese cuisine, dipping sauces are commonly consumed; traditionally, soy sauce—produced through soybean fermentation—is a dark brown liquid. Liquid aminos are considered a healthy, gluten-free alternative to soy sauce and other seasonings. While they may offer certain health benefits, such as reducing appetite, they can also pose risks for some individuals. Data on using peanut protein as a primary ingredient for liquid aminos production remain scarce (L. Zhang et al., 2022).

In one of the few studies we consulted on peanut-based liquid aminos, Yao et al. (2024) reported that peanut flour was blended with soybean meal and roasted wheat, followed by a 90-day koji fermentation during the primary fermentation stage. This fermentation method requires stringent control over raw material blending ratios, microbial activity, and incubation conditions to mitigate matrix aggregation, which can hinder microbial respiration. In contrast, extracting protein from peanut meal prior to hydrolysis facilitates better control over the purity of the nitrogenous solution, allowing the final product to achieve high nitrogen levels depending on the initial concentration of the extracted protein.

Therefore, this study aims to determine the optimal protein extraction conditions and to evaluate the feasibility of using the resulting protein solution for liquid aminos production.

2. Materials and methods

2.1. Materials

Defatted peanut flour was obtained from a peanut oil production facility in Dak Lak Province, Vietnam. The material was milled to a particle size of 0.1 mm, vacuum-packaged, and stored at room temperature for 3 months prior to the extraction experiments.

Protease A "Amano" 2SD and Alcalase 2.4L were sourced from Amano Enzyme Inc., Nagoya, Japan. All analytical-grade chemicals used for extraction and analysis were purchased from Xilong Scientific, Guangdong, China. Food-grade additives used for product formulation, including refined cane sugar, edible salt (NaCl), caramel color class III, and synthetic soy sauce flavor, were obtained from local suppliers in Ho Chi Minh City, Vietnam, and complied with Vietnam’s food safety standards.

2.2. Methods

In this study, an ultrasonic cleaning bath (GTsonic T20, China; dimensions: 505 × 300 × 200 mm) served as the ultrasound source. The device was operated in continuous sweep mode at a constant ultrasonic output of 300 W and a fixed frequency of 37 kHz during all experiments. The ultrasonic bath was heated to the target temperatures according to the investigated conditions. A single-factor experimental design was used, in which the optimal level determined from each stage was used for subsequent experiments.

The ground material was mixed with distilled water at solid-to-solvent ratios of 1:6, 1:8, 1:10, 1:12, 1:15, and 1:20 (w/v). The pH was adjusted to 7, 8, 9, 10, 11, and 12 using NaOH. UAE was performed at bath temperatures of 40, 50, 60, 70, and 80 °C for 0, 10, 20, 30, 40, and 50 min. After sonication, samples were centrifuged at 4,000 rpm for 20 min to remove solid residues. The resulting supernatant was diluted with distilled water to a final volume of 100 mL, and protein content was determined using the Kjeldahl method following AOAC standards (2001.11, 2005).

The resulting protein-containing extract was adjusted to pH 7.0 and used to evaluate the degree of hydrolysis (DH) using different methods. For chemical hydrolysis, 7 N HCl was added, and the reaction was carried out for 1 h at ambient temperature. The solution was

then neutralized with Na_2CO_3 and filtered to remove solids; the filtrate was analyzed for formol nitrogen content to determine the DH. For enzymatic hydrolysis, two enzymes—Alcalase 2.4L and Protease A "Amano" 2SD—were applied at a concentration of 1%. Hydrolysis was performed at 60 °C for 1 h, followed by filtration. The formol nitrogen content of the filtrate was then measured to evaluate the DH. Following the selection of Protease A 2SD, the hydrolysis conditions were further optimized using a sequential single-factor experimental design. First, to determine the optimal enzyme concentration, Protease A 2SD was added to the protein extract at varying concentrations of 0.75, 1.0, 1.25, 1.50, and 1.75% (w/w). For this step, the reaction was maintained at a constant temperature of 60 °C and a fixed duration of 3 h. Subsequently, using the newly determined optimal enzyme concentration, the hydrolysis time was varied over 1, 2, 3, 4, and 5 h while keeping the temperature constant at 60 °C. Following each hydrolysis experiment, the enzymes were inactivated by heating the mixture at 90 °C for 15 min. The mixtures were then filtered, and the degree of hydrolysis was evaluated by measuring the amino acid nitrogen (AAN) content via the formol titration method.

The optimal protein hydrolysate was blended with specific food-grade additives to achieve the sensory and preservation qualities of standard liquid aminos. The formulation consisted of the base protein hydrolysate supplemented with 13% (w/v) NaCl, 5% (w/v) refined sugar, 0.5% (v/v) caramel color class III, and 0.01% (v/v) synthetic liquid aminos flavor. The mixture was continuously stirred until all solid additives were fully dissolved and uniformly dispersed. After homogenization, the solution was filled into sterilized glass bottles and pasteurized at 85 °C for 15 min to obtain the final product. The final product was subjected to physicochemical analysis according to the TCVN 1763:2008 standard for soy sauce. Product stability was assessed by diluting the solution (0, 2, 5, and 10 times), followed by the addition of 13% salt, stirring, and visual observation.

The chemical composition of the raw material was determined using AOAC protocols. Moisture was measured by oven drying to a constant weight (AOAC 925.10, 2005); lipids were quantified by Soxhlet extraction (AOAC 945.16, 2005); total carbohydrates were analyzed using the Lane–Eynon method (AOAC 968.28–1969, 2000); ash content was measured by high-temperature incineration (AOAC 923.03, 2005); and total protein was measured by the Kjeldahl method (AOAC 2001.11, 2005). Formol nitrogen was quantified using the Sorensen method (AOAC, 1995). Protein content in DPF was estimated using a nitrogen-to-protein conversion factor of 6.25.

Protein recovery yield (H) was defined as the ratio of the amount of protein recovered from 1 g of sample after extraction (h) to the total protein content in the initial DPF (H_0), as shown in Equation 1.

$$\mathrm{H}(\%) = \frac{\mathrm{h}}{\mathrm{h}_0} \times 100 \quad (1)$$

The formol nitrogen content (g/mL) in the solution was calculated using Equation 2:

$$gN = \frac{(V - V_0) \times 0.0028}{V_m} \times 1000 \quad (2)$$

All experiments were performed in triplicate, and the results are reported as the mean ± standard deviation (*SD*). Statistical analyses were performed using Microsoft Excel 2019 and Minitab 19.

3. Results and discussion

3.1. Chemical Composition of the Raw Material

The chemical composition of raw materials can vary depending on several factors. Table 1 summarizes the chemical composition of the DPF examined in this study.

Table 1. *Chemical Composition of the Raw Material (% Dry Weight Basis)*

Component (%)	Defatted peanut meal
Moisture content	8.8 ± 0.07
Protein	41.93 ± 0.30
Fat	6.86 ± 0.15
Ash	4.45 ± 0.03
Carbohydrate	37.96 ± 0.14

The analysis of chemical composition (Table 1) indicates that the crude protein content of DPF was largely preserved after processing. These peanut proteins are classified by solubility into albumins (water-soluble, ~10%) and globulins (salt-soluble, ~90%) (Ahmed & Young, 1982). The high protein level on a dry-weight basis indicates that DPF is a suitable substrate for plant protein extraction.

3.2. Effects of Process Parameters on Ultrasound-Assisted Protein Recovery Yield

3.2.1. Effect of Solid-to-Solvent Ratio *on Protein Recovery Yield*

The effect of different solid-to-solvent ratios on protein recovery yield is presented in Figure 1A.

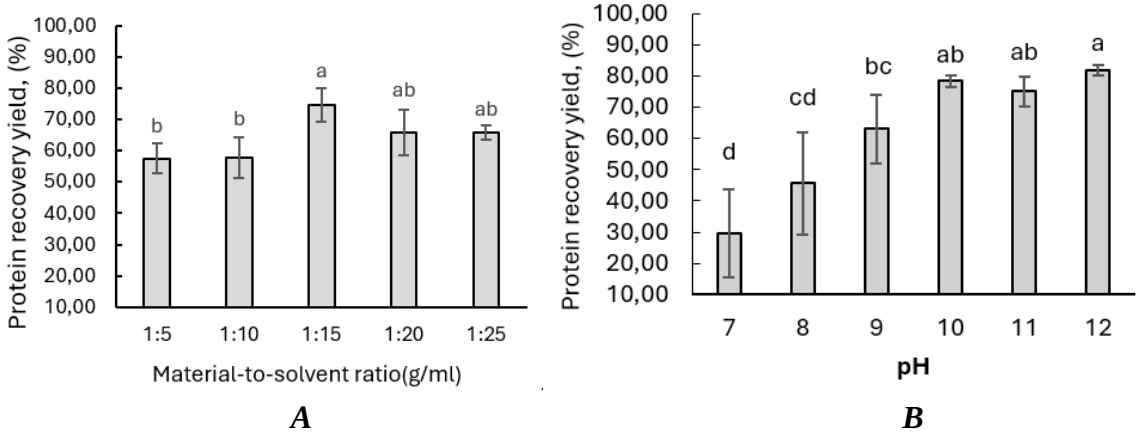


Figure 1. *Effects of Solid-to-Solvent Ratio (A) and pH (B) on Protein Recovery Yield From DPF*
Note. Constant extraction parameters were (A) pH 11, 60 °C, and 40 min and (B) a solid-to-solvent ratio of 1:15, 60 °C, and 40 min. Values with different lowercase letters differ significantly ($p < .05$)

The results indicated that the solid-to-solvent ratio significantly influenced the protein recovery yield ($p < .05$). When the solid-to-solvent ratio changed from 1:10 to 1:15, the protein recovery yield rose significantly and reached its peak at a ratio of 1:15, with a yield of 74.67 ± 5.45%. Further increases in the solvent volume did not result in a statistically significant difference in protein recovery yield.

During the extraction process, soluble compounds diffuse from the solid matrix into the liquid phase. Increasing the solvent volume relative to the solid mass typically improves extraction efficiency, as a steeper concentration gradient between the solid matrix and the solvent enhances mass transfer (Suchintita Das et al., 2022). However, excessive solvent volumes dilute the extract; once equilibrium is reached, mass transfer is reduced and extraction efficiency plateaus. Therefore, a solid-to-solvent ratio of 1:15 was identified as optimal for protein recovery from DPF.

3.2.2. Effect of Extraction pH on Protein Recovery Yield

The effect of extraction pH on protein recovery yield from DPF is presented in Figure 1B. The results indicated that extraction pH significantly influenced the protein recovery yield ($p < .05$). Previous studies have reported that an aqueous alkaline solution is suitable for protein extraction, as it preserves protein functional properties and nutritional value. When the pH approaches the isoelectric point, proteins lose their net charge, minimizing electrostatic repulsion between molecules and triggering protein precipitation, which subsequently decreases the recovery yield (Alibhai et al., 2006; Flaibam & Goldbeck, 2024).

Therefore, an alkaline environment is advantageous for enhancing protein extraction efficiency. As the pH increases, the protein recovery yield exhibits an upward trend because higher alkalinity promotes matrix structural disruption, facilitating protein release into the liquid phase. Furthermore, recent studies have confirmed that protein solubility is significantly enhanced at higher pH values (De Schouwer et al., 2019). Consequently, pH 12 was selected as the optimal condition for subsequent experiments on DPF. Strongly alkaline conditions (such as pH 12) could induce significant protein denaturation, leading to the loss of functional properties such as foaming, gelation, and emulsification, and posing a risk to nutritional value via amino acid racemization. However, in the context of liquid aminos production, preserving intact functional properties is unnecessary. Instead, the severe structural unfolding and denaturation caused by the pH 12 environment, combined with the acoustic cavitation of ultrasound, are advantageous for the subsequent processing step.

3.2.3. Effect of Ultrasonic Extraction Temperature on Protein Recovery Yield From DPF

When an ultrasonic bath is used, the extraction temperature is governed by two simultaneous processes: heat generation resulting from ultrasonic cavitation and temperature stabilization via the electric heating element. The intense heat generated by the implosion of cavitation bubbles within the solution promotes cell membrane disruption, thereby facilitating the mass transfer of target compounds into the liquid phase (Chemat et al., 2017;

Shen et al., 2023). Concurrently, the elevated bath temperature enhances the solubility and diffusion of these compounds. The influence of ultrasonic temperature on the protein recovery yield from DPF is illustrated in Figure 2A. The results indicated that the ultrasonic extraction temperature significantly influenced the protein recovery yield ($p < .05$). When the temperature increased to 80 °C, the protein recovery yield peaked at 82.35 ± 1.48%. At lower tested temperatures, the recovery yields showed no statistically significant differences. Elevating the ultrasonic temperature enhanced extraction efficiency, primarily by reducing solvent viscosity and increasing molecular mobility.

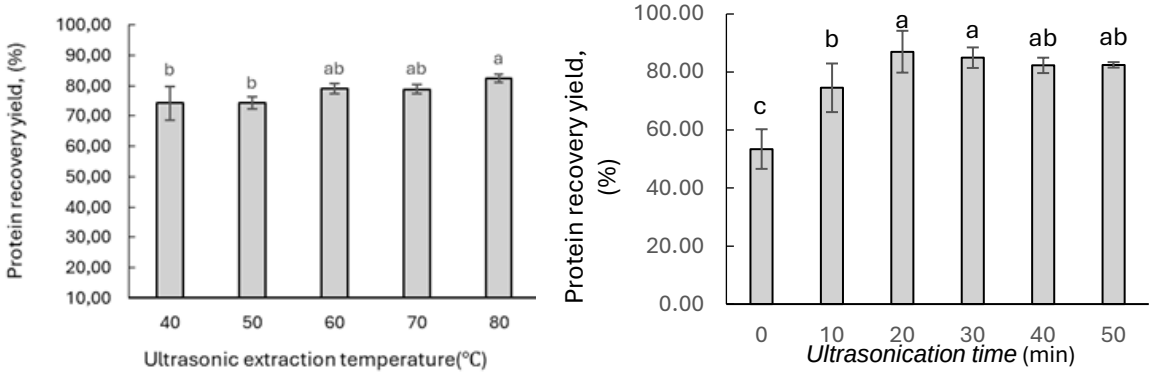


Figure 2. *Effects of Ultrasonic Extraction Temperature (A) and Ultrasonication Time (B) on Protein Recovery Yield From DPF*

Note. Constant extraction parameters were (A) a solid-to-solvent ratio of 1:15, pH 12, and 40 min and (B) a solid-to-solvent ratio of 1:15, pH 12, and 80 °C. Values with different lowercase letters differ significantly ($p < .05$)

Ultrasonic temperature plays a critical role in protein recovery, as the exposure of the matrix to high-frequency ultrasonic waves generates cavitation bubbles, turbulence, and shear forces that effectively disrupt the matrix structure (Chemat et al., 2017). In particular, ultrasonic treatment induces acoustic cavitation through the formation and implosion of microbubbles, which facilitates protein release (Shen et al., 2023). Therefore, increasing the extraction temperature is an effective way to enhance protein recovery during UAE. Accordingly, an ultrasonic temperature of 80 °C was chosen as the optimal condition for extracting proteins from DPF.

3.2.4. Ultrasonication Time

The effect of ultrasonication time on protein recovery yield from DPF is shown in Figure 2B. The findings demonstrated that ultrasonication significantly affected the protein recovery yield ($p < .05$). The protein recovery yield increased gradually, reaching a maximum of 87.00 ± 8.21% at 20 min. Prolonging the ultrasonication time beyond this point did not result in a statistically significant difference in the protein recovery yield.

Increasing the ultrasonication time enhances the disruption of the matrix structure, facilitating solvent diffusion into the particles and thereby improving protein recovery.

However, the observed plateau or slight decrease in protein recovery yield during extended ultrasonication times may be attributed to sonochemical effects, specifically the increased concentration of hydroxyl free radicals in the extract. These radicals can react with the functional groups of protein molecules, triggering protein aggregation or coagulation (Jambrak et al., 2009). Suchintita Das et al. (2022) also reported that, for certain materials, excessive extraction durations lead to foaming and protein aggregation, reducing mass transfer and subsequently lowering the protein recovery yield. Therefore, an ultrasonication time of 20 min was selected as optimal for protein extraction from DPF.

3.3. Application of the Extracted Protein Solution in Liquid Aminos Production

3.3.1. Protein Hydrolysis Method

The effect of the hydrolysis method on the amino acid content obtained from DPF was studied. Hydrolysis was performed under comparable experimental conditions; specifically, chemical hydrolysis was performed using 7 N HCl for 2 h, while enzymatic hydrolysis involved the addition of 1% (w/w) enzyme at 50 °C for 2 h. The results indicated that protein hydrolysis using Protease A 2SD yielded total protein and amino acid nitrogen (AAN) contents of 20.65 ± 0.04% and 0.826 ± 0.02%, respectively. These values were higher than those obtained from chemical hydrolysis and Alcalase-mediated hydrolysis, with an AAN content that was 0.168% higher. This difference may be attributed to enzyme specificity and the accumulation of peptides in the hydrolysate, which could induce feedback inhibition of enzyme activity (Tran et al., 2024). Protease A 2SD, acting as a neutral protease with both exopeptidase and endopeptidase activities, efficiently catalyzes the cleavage of specific peptide bonds (Q. Cui et al., 2022). Based on these findings, Protease A 2SD was selected for further studies.

3.3.2. Effect of Enzyme Concentration on Hydrolysis Efficiency

The effect of different enzyme concentrations on the amino acid content of peanut meal is shown in Figure 3A.

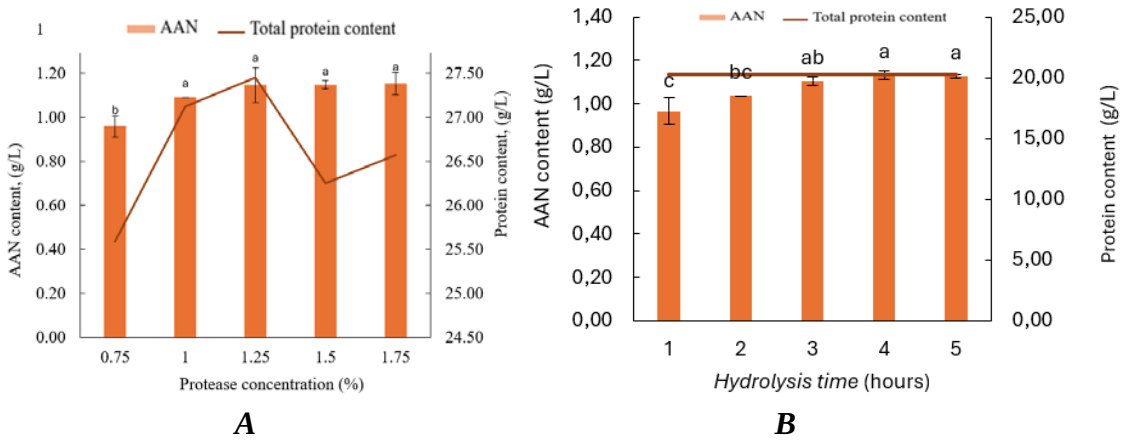


Figure 3. *Effects of Enzyme Concentration (A) and Hydrolysis Time (B) on the Hydrolysis of Peanut Meal*

Note. Values with different letters differ significantly ($p < .05$)

As the enzyme concentration increased, the AAN content exhibited an upward trend. Specifically, when the enzyme concentration was raised from 0.75% to 1% (w/w), the AAN content increased by 0.133 g/L, reaching a peak of 1.092 ± 0.003 g/L at the 1% concentration. Further increases in enzyme concentration did not result in statistically significant differences in AAN levels. Enzyme concentration plays a critical role in determining DH. An increase in DH implies the cleavage of peptide bonds, leading to shorter peptide chains and, consequently, a higher concentration of free amino acids (Ningrum et al., 2023). This finding is consistent with the results reported by Tadesse and Emire (2020).

3.3.3. Hydrolysis Time

The results concerning the effect of hydrolysis time on the amino acid content of peanut meal are presented in Figure 3B.

The AAN content tended to increase with extended hydrolysis duration. Specifically, at a hydrolysis time of 4 h, the AAN content derived from peanut meal reached a peak of 1.134 ± 0.02 g/L. Beyond this point, further increases in hydrolysis time resulted in no statistically significant differences.

3.3.4. Quality Assessment of the Liquid Aminos Product

The protein hydrolysate was blended with salt, sugar, class III caramel color, and synthetic liquid aminos flavor; subsequently, the mixture was bottled and labeled to obtain the final liquid aminos product. The liquid aminos produced by enzymatic hydrolysis of the peanut protein solution were analyzed according to the TCVN 1763:2008 standard. All quality parameters met the required specifications (Table 2).

Table 2. *Quality Assessment of the Experimental Liquid Aminos*

Parameter	TCVN 1763:2008	Experimental liquid aminos
Total nitrogen content (g/100 mL)	0.4	4.69
Soluble solids content excluding salt (g/100 mL)	8	10.36
Salt content (NaCl) (g/100 mL)	13 – 22	13
Acidity (calculated as acetic acid) (g/100 mL)	0.8 – 1.6	0.87

Total nitrogen (TN) content is a critical indicator for evaluating the quality of liquid aminos. The measured TN levels were higher than those of several commercial products, such as Chin-su (1.68 g/100 mL), Maggi (3 g/100 mL), and Nam Duong soybean soy sauce (2.4 g/100 mL). However, they were lower than those of Nam Duong concentrated soy sauce (4 g/100 mL) and Nam Duong premium soy sauce (5.8 g/100 mL).

Furthermore, the evaluation of the degree of hydrolysis (DH) showed that the protein hydrolysis efficiency of the peanut extract reached 36.09%. This indicates that the protein was partially hydrolyzed into amino acids. Typically, DH for such processes ranges from 1% to 38%. A study by Sun (2011) reported that pretreatment with low-concentration acid (0.2 N HCl) can enhance DH values. Specifically, DH increased from 9.6% (control) to 14.3%.

4. Conclusion

Ultrasound-assisted extraction of protein from peanut meal demonstrated high efficiency, simplicity, and resource-saving potential, with a high protein recovery yield. The findings indicate that the solid-to-solvent ratio, pH, temperature, and ultrasonication duration significantly influence the extraction efficiency. Specifically, the optimal parameters were established as a solid-to-solvent ratio of 1:15, pH 12, an extraction temperature of 80 °C, and a duration of 20 min, resulting in a protein recovery yield of 87.00 ± 7.21%.

Subsequently, the resulting protein solution was hydrolyzed using Protease A "Amano" 2SD (1% w/w) for 4 h at 60 °C. The final total nitrogen and amino acid nitrogen contents reached 4.69 ± 0.02 g/100 mL and 1.134 ± 0.02 g/L, respectively. These findings indicate that peanut meal, owing to its high protein content (~50%), can be effectively used for liquid aminos production. This study provides a theoretical and practical basis for optimizing enzymatic hydrolysis conditions to facilitate the industrial production of high-protein liquid aminos from sustainable plant-based resources.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

❖ ***Acknowledgments:*** *This research is funded by Ton Duc Thang University under grant number FOSTECT.2025.08.*

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TRÍCH LI PROTEIN CÓ HỖ TRỢ SIÊU ÂM TỪ BÃ ĐẬU PHỘNG (*ARACHIS HYPOGAEA*) TÁCH BÉO ỨNG DỤNG TRONG SẢN XUẤT NƯỚC CHẤM

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Ngày nhận bài: 29-01-2026; Ngày nhận bài sửa: 26-6-2026; Ngày duyệt đăng: 30-6-2026

TÓM TẮT

Bã đậu phộng đã tách béo – phụ phẩm chính trong quá trình sản xuất dầu đậu phộng – là nguồn nguyên liệu dồi dào để tạo ra các peptide mang vị umami nhờ hàm lượng cao các axit amin có tính axit, vốn đóng vai trò then chốt trong việc hình thành vị umami. Trong nghiên cứu này, protein từ bã đậu phộng đã tách béo (DPF) được chiết xuất bằng phương pháp hỗ trợ siêu âm (UAE) và được sử dụng để sản xuất nước chấm (liquid aminos). Các thông số kĩ thuật chính đã được đánh giá để xác định điều kiện chiết xuất tốt nhất, bao gồm: tỉ lệ nguyên liệu/dung môi 1:15 (w/v), pH 12, nhiệt độ chiết xuất 80 °C và thời gian siêu âm 20 phút. Với các điều kiện tối ưu này, hiệu suất thu hồi protein đạt 87.00 ± 7.21%. Dịch chiết protein thu được sau đó được thủy phân bằng enzyme Protease A (1% w/w) ở 60 °C trong 4 giờ để sản xuất nước chấm. Sản phẩm thu được có chất lượng dinh dưỡng cao, với hàm lượng nitơ tổng số là 4.69 ± 0.02 g/100 mL và hàm lượng nitơ dạng axit amin là 1.134 ± 0.02 g/L. Những kết quả này cho thấy DPF là nguồn nguyên liệu hiệu quả để sản xuất loại gia vị giàu nitơ thông qua quá trình thủy phân bằng enzyme.

Từ khóa: amino acid; bã đậu phộng đã tách béo; thủy phân bằng enzyme; chiết xuất hỗ trợ siêu âm; nước chấm