



The Effect of Acid Type and pH on The Moisture, Ash, and Protein Content of Protein Isolate from Kecipir Seeds (*Psophocarpus Tetragonolobus L.*)

Ni Putu Vida Indriani Putria^{1*}, I Gusti Ayu Krisma Widya Saraswati², Zaskia Jihan³

Universitas Bali Internasional Muhammadiyah, Indonesia^{1,3}

Universitas Udayana, Indonesia²

Email: vidaindriani@gmail.com^{1*}, krisma.widya@unud.ac.id²

Keywords	Abstract
Protein Isolate; Winged Bean; Acid Type; pH; Plant Protein.	This research aimed to determine the effects of acid type and pH value on the chemical characteristics of winged bean (<i>Psophocarpus tetragonolobus L.</i>) protein isolate, particularly moisture content, ash content, and protein content, as well as to identify the best treatment in the protein precipitation process. The study employed a completely randomized design (CRD) with a factorial pattern consisting of two factors: acid type, namely acetic acid and hydrochloric acid, and precipitation pH values of 4.0, 4.5, and 5.0. Each treatment was replicated three times, resulting in 18 experimental units. The observed parameters included the moisture content, ash content, and protein content of the winged bean protein isolate. Data were analyzed using analysis of variance (ANOVA), followed by a significance test when the treatments showed significant effects. The results indicated that acid type and pH value significantly affected the characteristics of winged bean protein isolate. The best treatment was obtained using hydrochloric acid at pH 4.0, producing a protein isolate with 7.58% moisture content, 3.60% ash content, and 90.04% protein content. The findings suggest that the appropriate selection of acid type and pH adjustment during precipitation can improve the quality of winged bean protein isolate as a potential plant-based protein source for food product development.

INTRODUCTION

Protein isolate is a product obtained through the separation of protein from food materials to achieve a high protein concentration, generally reaching more than 90% protein on a dry basis (De Angelis et al., 2024; Wang et al., 2024). Protein isolates are widely used in the food industry because they possess good functional properties, such as the ability to emulsify, form gels, bind water, and improve the texture of food products (Boye et al., 2010). So far, commercial protein isolates have mostly been derived from soybeans. However, Indonesia's dependence on imported soybeans remains relatively high; therefore, it is necessary to develop alternative local plant-based protein sources that have the potential to be processed into protein isolates.

One potential local plant is winged bean (*Psophocarpus tetragonolobus L.*), a tropical leguminous plant that can grow well under various environmental conditions and has a high protein content. Winged bean seeds are known to contain 30–37% protein and have a relatively

complete composition of essential amino acids, making them a potential alternative protein source to replace soybeans (Singh et al., 2014; Mohanty et al., 2018). In addition, winged bean seeds contain lysine, leucine, isoleucine, phenylalanine, and threonine in sufficiently high amounts, giving them the potential to produce protein isolates with good functional properties for food applications (Nwosu, Ojmelukwe, & Okpara, 2021).

In the food industry, protein isolate is used as an emulsifier, texture-forming agent, water-binding agent, and structure-forming ingredient in various processed products, such as sausages, meatballs, protein drinks, and meat analogues (Day, 2013). The functional properties of proteins are strongly influenced by their molecular structure and the processing conditions during isolation, especially the pH value and type of acid used in the protein precipitation process (Aryee, Agyei, & Udenigwe, 2018). Protein precipitation is generally carried out at the isoelectric point of the protein, which is a condition in which the positive and negative charges of the protein are balanced, resulting in minimal solubility and easier protein precipitation (Damodaran, Parkin, & Fennema, 2017).

Several studies have examined the effects of pH and acid type on the characteristics of protein isolates from various plant sources. Alrosan et al. (2022) reported that protein extraction and precipitation conditions have a significant effect on protein content and the functional properties of legume protein isolates. Karaca, Low, and Nickerson (2011) found that precipitation pH affects the emulsion stability of protein isolates from chickpeas, faba beans, lentils, and peas. Stone et al. (2015) also reported that different extraction methods, including pH variations, affected the functional attributes of pea protein isolates. Triyono (2010) reported that the use of hydrochloric acid resulted in higher protein isolate content than acetic acid in mung bean protein isolate.

However, studies that specifically examine the effects of acid type, namely acetic acid and hydrochloric acid, and pH values of 4.0, 4.5, and 5.0 on the chemical characteristics of protein isolate from winged bean seeds, especially moisture content, ash content, and protein content, are still very limited. Research on winged bean has so far focused more on the overall nutritional content of the seeds or the functional properties of winged bean flour, rather than on optimizing the protein isolation process. In addition, information on optimal precipitation conditions, including acid type and pH, to produce winged bean protein isolates with protein levels above 90% is still not widely available. This research gap forms the basis for the importance of conducting a study to determine the best process conditions in the production of winged bean protein isolate.

This research offers several novelties. First, this study simultaneously examines the interaction between two types of acids, namely acetic acid as a weak acid and hydrochloric acid as a strong acid, and three precipitation pH levels, namely 4.0, 4.5, and 5.0, on the chemical characteristics of winged bean seed protein isolate. Second, this study identifies the optimal precipitation conditions for producing winged bean protein isolate with the highest protein content of 90.04%, which meets the protein isolate standard of at least 90% on a dry basis. Third, this study provides empirical data on the chemical characteristics of winged bean protein isolate, including moisture content, ash content, and protein content, which are not yet widely available in the scientific literature. Fourth, this study uses a factorial completely randomized design (CRD), which allows for a more accurate analysis of the interaction between the two treatment factors.

Although winged bean has great potential as a local source of plant-based protein, research on the influence of acid type and pH value on the characteristics of winged bean protein isolate remains limited. Therefore, this study was conducted to examine the effects of acid type and pH value on the characteristics of winged bean seed protein isolate, especially moisture content, ash content, and protein content, so that optimal process conditions can be obtained for producing high-quality protein isolate. This research has both theoretical and practical benefits. Theoretically, this study enriches knowledge in the field of food science, especially regarding protein isolation techniques from local plant-based sources, namely winged bean, and the influence of processing conditions, including acid type and pH, on the chemical characteristics of protein isolates. The results of this study may also serve as a reference for the development of theories related to the isoelectric points of tropical plant proteins. Practically, this study provides recommendations for the best process conditions, namely the use of HCl at pH 4.0, in the production of winged bean protein isolate that can be adopted by the food industry to produce high-quality protein isolates. The resulting winged bean protein isolate has the potential to be used as a nutrient fortificant, emulsifier, or texture-forming ingredient in various food products, such as sausages, meatballs, protein drinks, and meat analogues, thereby reducing dependence on imported soybeans. This research also contributes to the utilization of Indonesia's local resources, which have not yet been optimally developed.

METHOD

Ingredients

The main material used in this study is 3-4 months old winged seeds with brown criteria obtained from Bongan village, Tabanan, Bali. The chemicals used in this study include acetic acid (glacial), hydrochloric acid (HCl), NaOH, sulfuric acid, boric acid, benzene, hexane (*Merck, Germany*) and aquades (Rofa, Indonesia).

Method

This study uses a Complete Random Design (RAL) factorial pattern consisting of two factors, namely the type of acid and the pH value. The first factor consists of two levels, namely acetic acid (A1) and hydrochloric acid (A2), while the second factor consists of three levels, namely pH 4.0 (P1), pH 4.5 (P2), and pH 5.0 (P3). From these two factors, 6 combinations of treatments were obtained which included A1P1, A1P2, A1P3, A2P1, A2P2, and A2P3, where each treatment was repeated 3 times so that a total of 18 experimental units were obtained.

Manufacture of Fat-Free Winged Seed Flour

The process of making fat-free winged flour begins with soaking the winged seeds in water for 24 hours, followed by boiling for 30 minutes. After that, the skin of the winged seeds is peeled and dried in a drying oven at 50°C for 9-11 hours. The dried winged seeds are then mashed using a *blender* and sifted using a 60-mesh sieve. The next stage of powdered fat is extracted using the organic solvent n-hexane for 2 hours with a ratio of winged flour and organic solvent of 1 : 5. As the final stage of this process, the extracted flour is dried again in the oven at 50°C for 2 hours until fat-free winged bean flour is obtained (Budijanto *et al.*, 2011).

Manufacture of Winged Seed Protein Isolate

The process of isolating winged seed protein is carried out by dissolving lean free flour in aqueducts with a ratio of 1:10 (b/v), then the pH value of the solution is set to 10 using NaOH

1 N. The solution is incubated at a temperature of 50°C for 60 minutes, followed by centrifugation at 3000 rpm for 45 minutes. The filtrate is separated, while the sediment is redissolved in aqueducts to be re-extracted by the procedure of pH 10 setting, heating 50°C for 60 minutes, and re-centrifugation at 3000 rpm for 45 minutes. All the filtrate obtained is filtered using a filter cloth, then the pH is set to 4.0; 4.5; and 5.0 through the addition of HCl or glacial acetic acid according to the treatment, then let it sit for 12 hours until a deposit is formed. The final stage includes centrifugation for 45 minutes at 3000 rpm, sediment washing using aqueducts with pH values according to the treatment, and drying in the oven at 55–60°C for 8 hours (Astawan, 2004; Budijanto *et al.*, 2011).

Observation variables

The observation variables in this study include the analysis of the physicochemical properties of winged seed protein isolate consisting of moisture content, ash content, and protein content.

Moisture content analysis was carried out using the oven method (Sudarmadji *et al.*, 1997). By heating an empty cup at 105°C for 1 hour until a constant weight, then ± 2 grams of sample is dried in the oven at 100°C–105°C for 3–5 hours. The sample was cooled in a desiccant for ± 15 minutes and weighed repeatedly until it reached a constant weight difference of ± 0.0002 g. The percentage of moisture content is calculated based on the ratio of the difference between the initial weight and final weight to the initial weight of the sample.

Ash content analysis using the spraying method (AOAC, 2005). It starts by drying the cup at 100°C–105°C, then 5 grams of samples are burned on *bunsen* until they are smokeless before being put in the oven. The evaporation process is carried out at a temperature of 400°C until the ash is gray, followed by increasing the temperature to 550°C for 12–24 hours until a fixed weight is obtained. The ash content is calculated by dividing the difference between the weight of the cup containing the sample after kilning by the weight of the sample before kiln.

Protein levels are determined through Kjeldahl's macro method (Sudarmadji *et al.*, 1997). Destroyed 0.1 grams of sample using 5 ml of concentrated H₂SO₄ and 0.5-1 gram of Kjeldahl tablets for 90 minutes until clear. The solution that has been diluted with 100 ml of aquades is then distilled with the addition of 25 ml of 50% NaOH solution and the result is contained in 3% boric acid. The final stage is titration using HCl 0.1 N until the color changes from blue to yellowish. Protein content was calculated by multiplying the difference in sample titration volume (ts) and blank (tb) by HCl normality, conversion factor 6.25, and nitrogen molecular weight, which was then divided by the sample weight.

Data analysis

The data from the research were analyzed using Variety Analysis (ANOVA) to determine the diversity of data obtained. If there is a significant difference between treatments, the analysis is followed by the *Duncan's Multiple Range Test* (DMRT) at a significance level of 5% to determine the difference between each treatment. The data analysis in this study was carried out statistically using the SPSS program version 25.0 and presented in the form of a table.

RESULTS AND DISCUSSION

Water Content of Seed Protein Isolate

Data from observations on the moisture content of winged seed protein isolate in various acid types and pH values are presented in Table 1.

Table 1. Average water content of winged seed protein isolate (%)

Types of Acids	pH value			\bar{X} Types of Acids
	4,0	4,5	5,0	
Acetic Acid	8.26 ± 0.34	7.89 ± 0.84	7.59 ± 0.21	7,91
Hydrochloric Acid	8.02 ± 0.31	7.89 ± 0.72	7.58 ± 0.21	7,83
\bar{X} pH value	8,14	7,89	7,59	

Source: Primary data from the research, 2026

The results of the fingerprint showed that the interaction between treatments, acid type, and pH values had an unreal effect ($P > 0.05$) on the moisture content of the lipstick protein isolate. The average value of the water content obtained ranged from 7.58% to 8.26%, with the lowest value in the hydrochloric acid treatment pH 5.0 and the highest value in the acetic acid treatment pH 4.0 (Table 1). This finding is in line with Triyono (2010) that the variation in acid type and pH does not have a real effect on the moisture content of protein isolates. This is suspected because the difference in treatment does not change the capacity of polar amino acids in binding water (Sze-Thao and Sathe in Pratiwi, 2018).

The moisture content of the isolate product is more predominantly influenced by the physical factors of drying. In theory, the increase in temperature and drying duration will increase heat energy to evaporate the liquid mass of the material (Rachmawan, 2001; Taib *et al.*, 1997 in Fitriani, 2008). In this study, all samples were dried at uniform conditions (60°C for 8 hours), so that the amount of water evaporated from each test unit tended to be the same. Controlling water content is crucial because it determines the stability, texture, and shelf life of protein isolates through the prevention of microbial activity and chemical reactions (Amanto *et al.*, 2015; Prasetyo *et al.*, 2019).

Ash Levels of Protein Isolate Seeds

The average value of ash content of winged seed protein isolate resulting from various combinations of acid type treatment and pH values is presented in Table 2.

Table 2. Average value of ash content of winged seed protein isolate (%)

Types of Acids	pH value			\bar{X} Types of Acids
	4,0	4,5	5,0	
Acetic Acid	3.43 ± 0.13	3.07 ± 0.22	2.88 ± 0.16	3,13
Hydrochloric Acid	3.60 ± 0.05	3.19 ± 0.20	2.88 ± 0.14	3,22
\bar{X} pH value	3,52 a	3,14 b	2,88 c	

Source: Primary data from the research, 2026

The results of the variety-based fingerprinting showed that the interaction between treatments and different types of acids had an unreal effect ($P > 0.05$), but the pH value had a real effect ($P < 0.05$) on the ash content of the product. The average value of ash content ranges from 2.88% to 3.60%, with the lowest level at pH 5.0 and the highest level at pH 4.0. The increase in ash content along with the decrease in pH value is caused by an increase in the ability of acids to dissolve natural minerals in materials at higher concentrations (Yusuf *et al.*, 2020). These findings are supported by Wahidin and Ryvandu (2019) and Kesuma *et al.* (2020) which states that acidic conditions increase the dissolution of extracted minerals. In addition, a decrease in pH causes dissociation of protein carboxyl groups so as to increase the absorption of mineral ions such as K, Na, Ca, and Fe in the isolate (Nurdjannah and Usmiati, 2006).

Ash content is an inorganic mineral residue that remains after the combustion process of carbon-free food (Dyahwarni, 2006). The value of the ash content produced reflects the total mineral content, purity level, and cleanliness of the resulting isolate product (Andarwulan *et al.*, 2011).

Protein Isolate Levels of Winged Seed Protein

The average value of the protein content of winged seed protein isolate resulting from various acid type treatments and the pH value are presented in Table 3.

Table 3. Average value of winged seed protein isolate protein content (%)

Types of Acids	pH value		
	4,0	4,5	5,0
Acetic Acid	67.53 b a	60.61 b b	48.17 b c
Hydrochloric Acid	90.04 A a	86.18 A b	79.05 to c

Source: Primary data from the research, 2026

The results of the fingerprint showed that the interaction between the type of acid and the pH value had a real effect ($P < 0.05$) on the protein content of the product. The average protein content ranged from 67.53% to 90.04%, with the highest value obtained in hydrochloric acid (HCl) treatment with a pH value of 4.0 of 90.04%. Protein levels tend to decrease as the pH value of each type of acid increases.

The use of hydrochloric acid results in higher protein levels than acetic acid. This is because HCl is a strong acid that produces excess H^+ ions to neutralize the protein charge so that the isoelectric point is reached faster. In this condition, the proteins form inward folds and settle in larger quantities. On the other hand, acetic acid is a weak acid with lower coagulation and electronegativity, so ionization does not occur perfectly and produces fewer deposits (Triyono, 2010). The use of both types of acids gives optimal results at pH 4.0 because it is the isoelectric point of winged seed protein (Haryasyah, 2009).

Increased protein levels as pH decreases are related to the process of denaturation and coagulation. The addition of acid at high temperatures triggers the breaking of hydrogen bonds and hydrophobic interactions, which then opens up reactive groups in the polypeptide chain to re-bind to form coagulants (Winarno, 1992; Triyono, 2010). At the isoelectric point (pH 4.0), the protein is neutrally charged so that its solubility reaches its lowest point and deposition occurs optimally (Haryasyah, 2009). On the other hand, staying away from isoelectric points

will increase the solubility of proteins due to the repulsion force between similar charges that cause molecules to decompose easily (Damodaran, 1996; Hapsari and Rosida, 2014).

CONCLUSION

This study concluded that the interaction between acid type and pH value had a significant effect on protein content but did not significantly affect the moisture content or ash content of winged bean seed protein isolate. Partially, pH value had a significant effect on ash content, with more acidic conditions increasing the mineral content of the product. The best results were obtained from the combination of hydrochloric acid (HCl) at pH 4.0, which produced the highest protein content of 90.03%, with a moisture content of 7.58% and an ash content of 3.60%. This isolate has high potential for application in the food industry as a nutritional fortification ingredient. Further research on functional properties, such as emulsion capacity, is strongly recommended to expand the utilization of this isolate.

REFERENCE

- Alrosan, M., Tan, T. C., Mat Easa, A., Gammoh, S., & Alu'datt, M. H. (2022). Functional properties of protein isolates recovered from legume seeds: Processing and potential food applications. *Food Reviews International*, 38(6), 1183–1205. <https://doi.org/10.1080/87559129.2020.1733594>
- Amanto, B. S., Atmaka, W., & Siswanti. (2015). Physicochemical characteristics of local legume protein isolate as a functional food. *Journal of Agricultural Product Technology*, 8(2), 85–92.
- Andarwulan, N., Kusnandar, F., & Herawati, D. (2011). *Food Analysis*. Dian Rakyat.
- AOAC (Association of Official Analytical Chemists). (2005). *Official methods of analysis of AOAC International* (18th ed.). AOAC International.
- Aryee, A. N. A., Agyei, D., & Udenigwe, C. C. (2018). Impact of processing on the chemistry and functionality of food proteins. In *Proteins in Food Processing* (2nd ed., pp. 27–45). Woodhead Publishing.
- Boye, J., Wijesinha-Bettoni, R., & Burlingame, B. (2010). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition*, 108(S2), S183–S211. <https://doi.org/10.1017/S0007114512002309>
- Damodaran, S. (1996). Amino acids, peptides, and proteins. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed., pp. 321–429). Marcel Dekker.
- Damodaran, S., Parkin, K. L., & Fennema, O. R. (2017). *Fennema's Food Chemistry* (5th ed.). CRC Press.
- Day, L. (2013). Proteins from land plants – Potential resources for human nutrition and food security. *Trends in Food Science & Technology*, 32(1), 25–42. <https://doi.org/10.1016/j.tifs.2013.05.005>
- De Angelis, D., Latrofa, V., Caponio, F., Pasqualone, A., & Summo, C. (2024). Techno-functional properties of dry-fractionated plant-based proteins and application in food product development: A review. *Journal of the Science of Food and Agriculture*, 104(4), 1884–1896.
- Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1), 1–42. <https://doi.org/10.2307/3001478>
- Dyahwarni, A. (2006). *Determination of ash levels in foodstuffs*. Faculty of Agricultural Technology, Gadjah Mada University.

- Fitriani, S. (2008). Effect of temperature and drying time on some qualities of candied star fruit (*Averrhoa bilimbi* L.). *Journal of Sage*, 7(1), 32–37.
- Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research* (2nd ed.). John Wiley & Sons.
- Hapsari, A. M., & Rosida, D. F. (2014). The effect of pH of precipitation on the characteristics of jackfruit seed protein isolate. *Journal of Food and Agroindustry*, 2(4), 30–36.
- Haryasyah. (2009). *Isolation and protein characterization of winged seed (Psophocarpus tetragonolobus L.)*. Thesis. Bogor Agricultural University.
- Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*, 44(9), 2742–2750. <https://doi.org/10.1016/j.foodres.2011.06.012>
- Kesuma, D. P., Widowati, S., & Syarief, R. (2020). Effect of extraction conditions on the characteristics of local legume protein isolates. *Journal of Food*, 29(2), 101–110. <https://doi.org/10.33964/jp.v29i2.463>
- Mohanty, C. S., Pradhan, R. C., Singh, V., Rout, P. K., & Behera, M. (2018). Physicochemical analysis of winged bean (*Psophocarpus tetragonolobus* L.) seeds from India. *Legume Research*, 41(2), 307–312. <https://doi.org/10.18805/LR-3792>
- Montgomery, D. C. (2017). *Design and analysis of experiments* (9th ed.). John Wiley & Sons.
- Nurdjannah, N., & Usmiati, S. (2006). Functional properties of vegetable protein isolate and its application to food products. *Journal of Agricultural Research and Development*, 25(2), 45–51.
- Nwosu, J. N., Ojmelukwe, P. C., & Okpara, C. C. (2021). Nutritional and functional properties of winged bean (*Psophocarpus tetragonolobus*) flour and protein products: A review. *Journal of Food Biochemistry*, 45(7), e13739. <https://doi.org/10.1111/jfbc.13739>
- Prasetyo, D. I., Utami, R., & Anandito, B. K. (2019). The effect of moisture content on the storage stability of high-protein food products. *Journal of Agricultural Product Technology*, 12(1), 11–18. <https://doi.org/10.20961/jthp.v12i1.29004>
- Pratiwi, N. K. A. (2018). *The characteristics of kidney bean protein isolate with the deposition pH treatment are different*. Thesis. Udayana University.
- Rachmawan, O. (2001). *Drying, cooling and packaging of agricultural commodities*. Department of National Education.
- Singh, A., Sharma, S., & Singh, B. (2014). Comparative studies on proximate composition, amino acid profile and anti-nutritional factors in selected legumes. *Journal of Food Science and Technology*, 51(9), 1973–1979. <https://doi.org/10.1007/s13197-012-0728-1>
- Steel, R. G. D., & Torrie, J. H. (1980). *Principles and procedures of statistics: A biometrical approach* (2nd ed.). McGraw-Hill.
- Stone, A. K., Karalash, A., Tyler, R. T., Warkentin, T. D., & Nickerson, M. T. (2015). Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food Research International*, 76, 31–38. <https://doi.org/10.1016/j.foodres.2014.11.017>
- Sudarmadji, S., Haryono, B., & Suhardi. (1997). *Analytical procedures for foodstuffs and agriculture* (4th ed.). Liberty.
- Sulaiman, A., & Sari, D. K. (2018). Analysis of food protein content using the Kjeldahl method. *Journal of Food Technology*, 9(2), 71–77.
- Triyono, A. (2010). To study the effect of the addition of several acids on the protein isolation process on mung bean protein isolate flour (*Phaseolus radiatus* L.). *Chemical and Process Engineering Seminar 2010*. Diponegoro University.

- Wahidin, M., & Ryvandu, A. (2019). Effect of acidic conditions on the mineral characteristics of vegetable protein isolates. *Journal of Halal Agroindustry*, 5(1), 55–62. <https://doi.org/10.30997/jah.v5i1.1187>
- Wang, Z. L., Tang, X., Wang, M., She, Y. X., Yang, B. R., Sheng, Q. H., & Abd El-Aty, A. M. (2024). β -Lactoglobulin separation from whey protein: A comprehensive review of isolation and purification techniques and future perspectives. *Journal of Dairy Science*, 107(12), 11785–11795.
- Winarno, F. G. (1992). *Food chemistry and nutrition*. Gramedia Pustaka Utama.
- Winarno, F. G. (2008). *Food Chemistry and Nutrition* (revised edition). Gramedia Pustaka Utama.
- Yusuf, M., Rahman, A., & Nurhayati. (2020). Effect of pH extraction on mineral content and protein isolate characteristics of legumes. *Journal of Food Technology and Industry*, 31(1), 45–52. <https://doi.org/10.6066/jtip.2020.31.1.45>