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Effect of Blanching on the Protein Profile of Bitter Leaf (*Vernonia amygdalina)* Analyzed by SDS-PAGE

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Abstract
Blanching is widely employed as a pre-treatment for leafy vegetables to inactivate enzymes and preserve quality. However, its effect on protein integrity in polyphenol rich species remains inadequately characterized. This study evaluated the effect of blanching on the protein profile and protein content of bitter leaf (*Vernonia amygdalina*). Blanched and non-blanched leaves were extracted using a TCA-based protocol, and protein profiles were analyzed by SDS-PAGE, while protein content was determined by the Kjeldahl method in triplicate. SDS-PAGE revealed distinct protein bands only in blanched samples, whereas non-blanched samples showed no visible bands. Kjeldahl analysis indicated that non-blanched samples had slightly higher protein content (19.20±0.13%) than blanched samples (18.24±0.21%, $p < 0.05$). These findings suggest that blanching improves protein extractability and electrophoretic detectability, although it slightly reduces total Kjeldahl protein content. Combined qualitative and quantitative approaches are essential for accurate protein assessment in polyphenol-rich plant materials.

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INTRODUCTION

Bitter leaf (*Vernonia amygdalina*) is a widely used in Africa as both food and medicine and is recognized for its considerable crude protein content, highlighting its potential as a plant-based protein source (Ugbogu et al., 2021). In recent years, this species has also been introduced and utilized in several regions of Indonesia, where it is commonly known as "African leaf" and is available in many areas as a local herbal resource (Cornelia et al., 2025). At the same time, *V. amygdalina* is rich in phytochemicals such as flavonoids, tannins, phenolic acids and other polyphenols, which contribute to its pharmacological activities but can complicate analytical characterization of its proteins (Ugbogu et al., 2021).

Protein analysis in polyphenol-rich plant matrices is challenging because secondary metabolites can bind to proteins and modify their structure, solubility and detectability. Studies on protein-polyphenol systems show that these interactions, driven by both non-covalent forces such as hydrogen bonding, hydrophobic and electrostatic interactions. Covalent linkages via oxidized phenolic intermediates, can alter protein conformation, thermal stability, solubility and electrophoretic behavior, thereby affecting functional properties and in vitro digestibility (Oseghale et al., 2024). Such complexation may interfere with methods such as SDS-PAGE and Kjeldahl-based protein determination by promoting aggregation, precipitation or masking of reactive groups (Peter Achunike Akah, 2024).

Thermal and aqueous treatments like blanching are commonly used in leafy vegetables to inactivate enzymes, reduce antinutritional factors and limit oxidative reactions that can drive protein-polyphenol crosslinking (Obi, P. U. et al., 2024). In *V.amygdalina*, processing methods including blanching have been reported to modify both nutritional composition and

phytochemical levels, indicating that heat and water treatments can significantly impact protein content and associated metabolites (Edo et al., 2023). Blanching can inactivate endogenous enzymes and modify protein-polyphenol interactions, with reported effects on protein solubility, stability and electrophoretic patterns in seaweeds (Trigo et al., 2023).

Protein extraction and clean-up strategies, particularly TCA precipitation is widely used to remove interfering compounds (e.g., salts, lipids, polyphenols) and obtain high-quality protein extracts for gel-based proteomics (Niu et al., 2018). Yet, the interplay between blanching, protein content (e.g., Kjeldahl) and electrophoretic detectability (SDS-PAGE) in polyphenol-rich matrices such as *V.amygdalina* remains poorly defined. Based on this gap, the present study aims to evaluate the effect of blanching on protein electrophoretic detectability using SDS-PAGE and on total protein content measured by the Kjeldahl method in bitter leaf grown and utilized in Papua, Indonesia.

METHOD

This study aimed to evaluate the effect of blanching on protein content and characteristics of bitter leaf (*Vernonia amygdalina*). Protein was extracted using the TCA method, quantified by the Kjeldahl method, profiled by SDS-PAGE, and analyzed statistically to assess treatment effects.

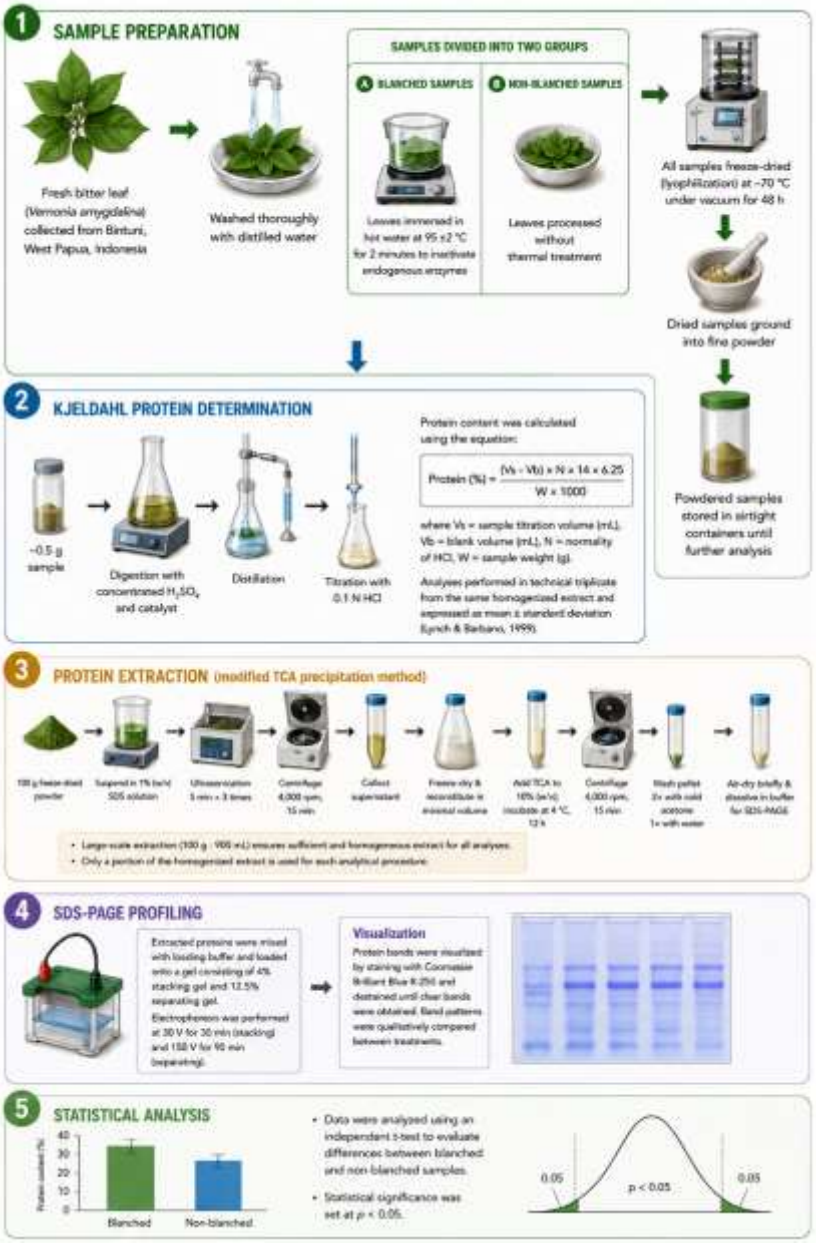


Figure 1. Research workflow for protein analysis of bitter leaf (*Vernonia amygdalina*) under blanched and non-blanched treatments.

Sample Preparation

Fresh bitter leaf (*Vernonia amygdalina*) samples were collected from Bintuni, West Papua, Indonesia. The sample were washed thoroughly with distilled after to remove surface contaminants. The samples were divided into two groups,

Blanched samples

Leaves were immersed in hot water at 95±2°C for 2 minutes to inactivate endogenous enzymes such as proteases and polyphenol oxidase.

Non-blanched samples

Leaves were processed without thermal treatment.

After treatment, all samples were immediately subjected to freeze-drying (lyophilization) at -70°C and vacuum pressure for 48 hours to preserve protein structure and prevent biochemical degradation. The deried samples were then ground into fine powder using laboratory grinder. The powdered samples were stored in airtight containers until futher analysis.

Kjeldahl Protein Determination

Protein content was determined using the Kjeldahl method based on total nitrogen analysis, a long-established reference procedure for protein determination in foods and other biological materials. Approximately 0.5 g of sample was digested with concentrated sulfuric acid in the presence of a catalyst, followed by distillation and titration using 0.1 N HCl.

Protein content was calculated using the equation:

$$\text{Protein (\%)} = \frac{(Vs - Vb)\ x\ N\ x\ 14\ x\ 6.25}{W\ x\ 1000}$$

where Vs is the sample titration volume (mL), Vb is the blank volume (mL), N is the normality of HCl, and W is the sample weight (g). Kjeldahl analyses were performed in technical triplicate from the same homogenized extract and expressed as mean ± standard deviation.(Lynch & Barbano, 1999).

Protein Extraction

Protein extraction was performed using a modified trichloroacetic acid (TCA) precipitation method. A batch extraction approach was used to obtain homogeneous protein extracts for all downstream analyses. Approximately 100 g of freeze-dried bitter leaf powder was used to prepare a homogenized extraction stock by suspension in 900 mL of 1% (w/v) SDS solution. Only a portion of the homogenized extract was used for each analytical procedure by ultrasonication for 5 minutes, repeated three times. The homogenate was centrifuged at 4,000 rpm for 15 minutes, and the supernatant was collected. The extract was freeze-dried and reconstituted in a minimal volume, followed by the addition of TCA to a final concentration of 10% (w/v). The mixture was incubated at 4 °C for 12 hours and centrifuged at 4,000 rpm for 15 minutes to obtain the protein pellet. The pellet was washed twice with cold acetone and once with distilled water, then briefly air-dried and dissolved in buffer for SDS-PAGE analysis.

SDS-PAGE Profiling

Protein profiling was carried out using SDS-PAGE following a modified Laemmli method. Extracted proteins were dissolved in 1% (w/v) SDS solution with concentrations of 100 mg/mL (non-blanched sample) and 40 mg/mL (blanched sample). Non-blanched extracts exhibited poor solubility and weak electrophoretic migration; therefore, higher stock concentrations were required to obtain detectable protein bands. In contrast, blanched extracts produced clearer and readily soluble protein solutions, allowing distinct bands to be observed at lower concentrations. Final loading volume and protein amount were adjusted prior to electrophoresis.

Samples were mixed with loading buffer and loaded onto a gel consisting of a 4% stacking gel and a 12.5% separating gel (Achilli et al., 2018). Electrophoresis was performed at 30 V for 30 min (stacking) and 150 V for 90 min (separating). Protein bands were visualized using a molecular weight marker, stained with Coomassie Brilliant Blue R-250, and destained until clear bands were obtained. Band patterns were qualitatively compared between treatments.

Statistical Analysis

Data were analyzed using an independent t-test to evaluate differences between blanched and non-blanched samples. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Blanching on Protein Extraction Yield and Solubility

The TCA extraction yield of proteins from bitter leaf (*Vernonia amygdalina*) under blanching and non-blanching treatments is presented in (Figure 2). Blanching resulted in a higher

extraction yield (5.3±0.5%) compared to non-blanching (2.1±1.0%). The trend consistently indicated improved protein recovery following blanching. The modified TCA-SDS protocol successfully extracted protein fractions from both treatments. However, differences were observed in protein resolubilzation and extract appearance. Blanched sample produces pellets that were easily resolubilized in SDS-based buffers, resulting in clear and slightly viscous extracts. In contrast, non-blanched sample formed pellets that were difficult to dissolve and produced turbid, brownish suspensions. Similar findings have been reported in recalcitrant plant tissues, where protein solubility depends on the composition of the sample matrix (Niu et al., 2018). The turbidity observed in non-blanched extracts suggest the presence of phenolic compounds another interfering substances (Lee et al., 2025).

These observations show that protein extractability is not determined solely by extraction chemistry but also by the biochemical state of the tissue prior to extraction. In polyphenol-rich plant materials, proteins readily interact with secondary metabolites. Oxidation of polyphenols to reactive quinones leads to covalent protein-polyhenols crosslinked aggregates, which lower protein solubility and impair redissolution after precipitation (K. Li et al., 2024). This process has been widely described in both model and food systems. (Y. Li et al., 2016). Although, TCA-based precipitation helps remove or exposed for extended periods (Y. Li et al., 2016).

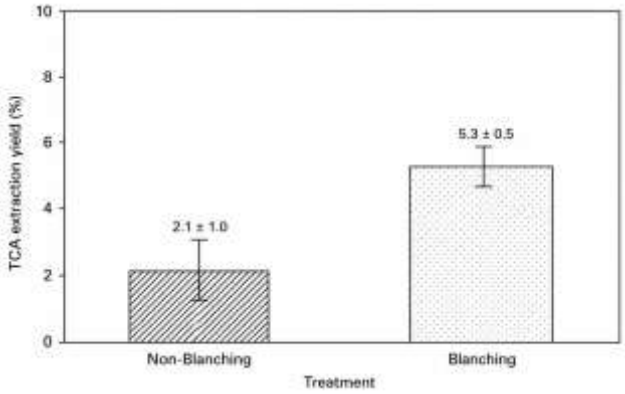


Figure 2. TCA extraction yield of proteins from bitter leaf (*Vernonia amygdalina*) under blanching and non blanching treatments. Values are expressed as mean ± SD (*n*=2)

Blanching likely improved protein extraction by inactivating oxidative enzymes such as polyphenol oxidase (PPO), thereby reducing quinone formation and protein–polyphenol interactions (Lee et al., 2025). In addition, thermal treatment may distrupt cell structures making proteins more accessible for extraction (Lee et al., 2025; Wohlt et al., 2021). Therefore, the higher yield and better solubility observed in blanched samples can be attributed to reduce oxidative interaction and improved protein accessibility.

The improved solubility and clarity of protein extracts obtained from blanched samples are expected to directly influence downstream electrophoretic profiling. Proteins that are fully solubilized and free from phenolic interference generally produce sharper, more distinct bands during SDS-PAGE analysis, whereas poorly solubilized or aggregated proteins may result in smeared or faint band patterns. Therefore, differences in extraction quality between treatments are likely to be reflected in the SDS-PAGE profiles, providing further insight into protein integrity and extract suitability for analytical applications.

Effect of Blanching on SDS-PAGE Protein Profile

Electrophoretic analysis revealed striking differences in protein profiles between treatments (Figure 3). Blanched samples displayed well-resolved protein bands over a broad molecular weight range (10-100 kDa) with prominent bands around 55–60 kDa and additional bands in the 15-25 kDa region, These bands are consistent with major leaf proteins, particularly the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and smaller polypeptides. (Di Stefano et al., 2018). Similar banding patterns in this range have been reported for leaf protein concentrates and RuBisCO-rich extracts from diverse green biomasses (Ducrocq et al., 2022). Blanched samples exhibited sharp, well-resolved bands spanning approximately 10–100 kDa, indicating efficient extraction and electrophoretic separation. A dominant band at ~55–60 kDa was consistently observed, corresponding to RuBisCO, the most abundant soluble protein in green leaf tissue. Additional discrete bands in the 15–25 kDa region likely represent the RuBisCO small subunit and other soluble leaf polypeptides. The preservation of distinct banding patterns suggests minimal aggregation, effective denaturation, and uniform SDS binding, reflecting structurally intact and electrophoretically competent proteins.

In contrast, non-blanched samples displayed severe disruption of electrophoretic resolution. Proteins were largely retained within the stacking gel or appeared as diffuse high-molecular-weight smears with an absence of clearly defined RuBisCO bands. In several replicates, negligible band migration into the resolving gel was observed despite equivalent protein loading, indicating profound impairment of protein solubility and/or electrophoretic mobility. The loss of discrete banding and accumulation of material at the gel interface are characteristic of covalent aggregation and incomplete SDS-mediated dissociation. High molecular weight smears and loss of discrete RuBisCO bands have likewise been observed in polyphenol-rich leaf exctracts subject to oxidative crosslinking (Menzel et al., 2016; Zheng et al., 2025).

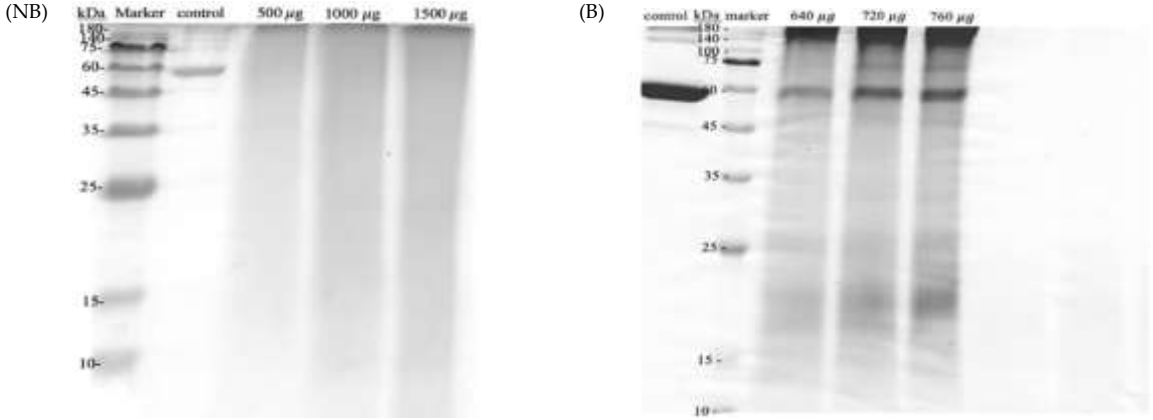


Figure 3. SDS-PAGE profiles of protein extracts from bitter leaf (*Vernonia amygdalina*) showing qualitative differences in protein detectability and electrophoretic behavior between treatments. NB: non-blanched; B: blanched. Blanched samples show sharp, well-resolved bands (10–100 kDa), including a dominant ~55–60 kDa band (RuBisCO), whereas non-blanched samples exhibit smearing and poor migration, indicating reduced solubility and protein aggregation.

Mechanistically, these observations are consistent with extensive oxidative protein modification occurring in non-blanched tissue. In intact leaf matrices, polyphenol oxidase (PPO) catalyzes the oxidation of endogenous phenolic substrates to highly reactive o-quinones (Zheng et al., 2025). Theses electrophilic quinones readily undergo nucleophilic addition with amino acid side chains such as lysine, cysteine, and tyrosine, forming covalent pretein-phenolic adducts and promoting intermolecular crosslinking (Zheng et al., 2025).

Such reactions generate high-molecular-weight aggregates and insoluble complexes and are well documented to involve abundant stromal proteins such as RuBisCO/RBCL in plant systems, which shift from ~55 kDa to high-molecular-weight complexes on gels when PPO is active (Tran et al., 2025). Covalent crosslinking and phenolic adduct formation alter protein conformation, reduce solubility, and interfere with SDS binding, leading to streaking, smearing, and loss of discrete bands in SDS-PAGE of phenolic-rich plant extracts (Zheng et al., 2025).

The absensce or weakening of resolvable RuBisCO bands in non-blanched samples is therefore consistent with oxidative polymerization and aggregation, in line with reports where PPO and related oxidative enzymes, thereby limiting quinone formation at early processing stages (Tilley et al., 2023). Blancing and related pre-treatments are widely recognized to suppress PPO/POD activity, reduce enzymatic browning and improve product quality in fruits and vegetables. By reducing enzymatic oxidation, blanching decreases covalent protein-polyphenol complex formation and preserves proteins in a more soluble, extractable state, which in turn restores clearer, more reproducible protein patterns in SDS-PAGE and improves detectability of RuBisCO and other major leaf proteins (Tilley et al., 2023; Tran et al., 2025).

Discrepancy between Kjeldahl Protein Content and SDS-PAGE Detectability

Total protein content determined by the Kjeldahl method showed a statistically significant but modest difference between treatments (Figure 4). Non-blanched samples exhibited slightly higher protein content (19.20±0.13) than blanched samples (18.24±0.21) (Table 1), corresponding to a

small absolute decrease (0.96%). This limited reduction indicates that blanching had minimal impact on total nitrogen content. Similar behavior has been reported where blanching or mild thermal treatment alters protein structure and distribution but does not markedly reduce total protein or amino acid levels in plant and marine biomasses (Trigo et al., 2023).

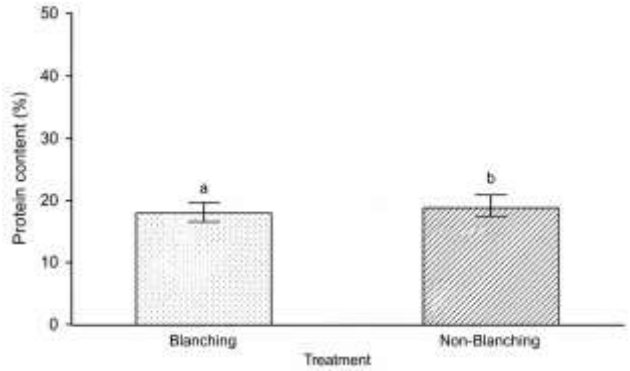
The slight decrease after blanching can be explained by processing-induces losses of soluble nitrogenous constituents. Thermal treatment in aqueous systems can promote leaching of low molecular weight nitrogen compounds such as free amino acids, small peptides, and other non-protein nitrogen (NPN) fractions into the surrounding medium (Barea et al., 2023). Heat-induced denaturation can also increase protein susceptibility to diffusion and leaching, as shown for blanched navy beans where processing waters contained highly modified protein fragment and amino acid-derived products. Nevertheless, the small magnitude of reduction observed here suggests high nitrogen retention under the applied blanching conditions, consistent with studies in seaweed and other matrices where appropriately controlled blanching retained most of the total protein/nitrogen (Trigo et al., 2023).

These quantitative Kjeldahl findings contrast with SDS-PAGE result. Kjeldahl quantifies total nitrogen and converts it to crude protein, without distinguishing intact proteins from peptides, free amino acids, or other non-protein nitrogen (NPN), therefore overestimates true protein when NPN is present (Simonne et al., 1997). It also cannot differentiate native proteins from proteolyzed or structurally modified form (Di Marzo et al., 2021). In milk, most casein proteolysis products (89% of CNPP) remain in the Kjeldahl non-casein precipitate, so casein as a percentage of true protein measured by Kjeldahl underestimates proteolytic damage relative to SDS-PAGE (Di Marzo et al., 2021). Similar overestimation occurs in plant matrices, where NPN and other nitrogen-containing compounds substantially bias nitrogen-based protein values (Magomya et al., 2014).

Table 1. Protein Content using Kjeldahl

Treatment	Protein Content			
	Rep 1	Rep 2	Rep 3	Mean*
Blanching	18.36	18.00	18.36	18.24±0.21
Non-Blanching	19.27	19.05	19.27	19.20±0.13

**Values are expressed as mean ± SD (technical triplicate, n = 3)*



**Values are expressed as mean ± SD from technical triplicate analyses of the same homogenized extract. Bars with different letters are significantly different (p < 0.05)*

Figure 4. Protein content of blanched and non-blanched bitter leaf samples.

In non-blanched bitter leaf, the slightly higher Kjeldahl protein content likely reflects contributions form NPN and oxidatively modified protein-polyphenol complexes rather than fully recoverable protein, consistent with reports that NPN (free amino acids, urea, other small nitrogenous molecules) can form a considerable fraction of total nitrogen in foods. Oxidation of polyphenols to quinones promotes covalent reactions with nucleophilic amino acid side chains, generating high-molecular-weight aggregates and conjugates, as shown for pea, soy, and other plant proteins (Zhao et al., 2020). These nitrogen-rich aggregates are fully counted by Kjeldahl but often become poorly soluble and show reduced migration or smeared high molecular weight material on SDS-PAGE (Qiu et al., 2025).

Conversely, SDS-PAGE visualizes only proteins that are soluble under denaturing, SDS-

containing conditions, and is widely used to characterize protein integrity, composition, and aggregation in dairy, legumens, pulses, and insects (Di Marzo et al., 2021; Zhao et al., 2020). Insoluble or heavily crosslinked proteins are underrepresented, while intact or extractable proteins appear as discrete bands. Discrepancies between relatively stable Kjeldahl nitrogen and altered SDS-PAGE profiles have been observed in dairy and multiple processed protein systems, where heating, high pressure, or other treatments change solubility and aggregation without large changes in total nitrogen (Kiełczewska et al., 2021). Overall, Kjeldahl reflects total nitrogen (including NPN and modified forms), whereas SDS-PAGE better represents protein quality, integrity, and extractability; combining both methods is therefore recommended when evaluating protein in polyphenol-rich plant systems. The concentrations were optimized primarily to obtain detectable electrophoretic bands under different extract solubility conditions, the SDS-PAGE results should be interpreted qualitatively rather than as a direct quantitative comparison of band intensity between treatments.

CONCLUSION

Blanching slightly reduces Kjeldahl protein content in bitter leaf (*Vernonia amygdalina*) but markedly improved protein extraction yield, solubility, and SDS-PAGE detectability. The higher protein value in non-blanched samples likely reflects non-protein nitrogen (NPN) and oxidatively modified protein-polyphenol complexes rather than functional protein. This explains the discrepancy between Kjeldahl and SDS-PAGE results, where only soluble, structurally intact proteins are effectively resolved. Overall, Kjeldahl reflects total nitrogen, whereas SDS-PAGE better represents protein extractability and electrophoretic detectability. Therefore, combining both method is essential for accurate protein evaluation in polyphenol-rich plant matrices.

RECOMMENDATION

Future studies should integrate Kjeldahl with complementary structural and functional techniques (e.g., SDS-PAGE, amino acid analysis, and chromatography) to more accurately distinguish true protein from non-protein nitrogen (NPN) and modified aggregates in polyphenol-rich matrices. Optimization of blanching conditions is recommended to balance nitrogen retention with improved protein solubility, while additional strategies such as the use of phenolic-binding agents or modified extraction protocols may further enhance protein recovery. Validation across different plant sources and processing conditions is also necessary to confirm broader applicability. Importantly, future work should link these structural improvements to functional outcomes, including digestibility, bioavailability, and techno-functional properties, to strengthen the relevance of protein quality assessment for food and bioprocessing applications.

BIBLIOGRAPHY

Achilli, C., Ciana, A., & Minetti, G. (2018). Oxidation of cysteine-rich proteins during gel electrophoresis. *Journal of Biological Methods*, *5*(4), 1. https://doi.org/10.14440/jbm.2018.275

Barea, P., Melgosa, R., Illera, A. E., Alonso-Riaño, P., Díaz de Cerio, E., Benito-Román, O., Beltrán, S., & Teresa Sanz, M. (2023). Production of small peptides and low molecular weight amino acids by subcritical water from fish meal: Effect of pressurization agent. *Food Chemistry*, *418*. https://doi.org/10.1016/j.foodchem.2023.135925

Cornelia, M., Agustini, I., & Sugata, M. (2025). Characterization of bitter leaf (Vernonia amygdalina Delile) extract, lemon (Citrus limon Osbeck), and honey in functional beverages making. *BIO Web of Conferences*, *169*. https://doi.org/10.1051/bioconf/202516904005

Di Marzo, L., Pranata, J., & Barbano, D. M. (2021). Measurement of casein in milk by Kjeldahl and sodium dodecyl sulfate–polyacrylamide gel electrophoresis. *Journal of Dairy Science*, *104*(7), 7448–7456. https://doi.org/10.3168/jds.2020-18794

Di Stefano, E., Agyei, D., Njoku, E. N., & Udenigwe, C. C. (2018). Plant RuBisCo: An Underutilized Protein for Food Applications. In *JAOCS, Journal of the American Oil Chemists' Society* (Vol. 95, Number 8, pp. 1063–1074). Wiley-Blackwell. https://doi.org/10.1002/aocs.12104

Ducrocq, M., Morel, M. H., Anton, M., Micard, V., Guyot, S., Beaumal, V., Solé-Jamault, V., & Boire, A. (2022). Biochemical and physical–chemical characterisation of leaf proteins extracted from Cichorium endivia leaves. *Food Chemistry*, *381*. https://doi.org/10.1016/j.foodchem.2022.132254

Edo, G. I., Samuel, P. O., Jikah, A. N., Onoharigho, F. O., Idu, L. I., Obasohan, P., Opiti, A. R., Electric, J., Ikpekoro, V. O., Otunuya, C. F., Ugbuwe, E., Ongulu, J., Ijide, M., Nwaose, I. D., Ajakaye, S. R., & Owigho, J. E. (2023). Biological and bioactive

components of bitter leaf (Vernonia amygdalina leaf): Insight on health and nutritional benefits. A review. In *Food Chemistry Advances* (Vol. 3). Elsevier Ltd. https://doi.org/10.1016/j.focha.2023.100488

Kiełczewska, K., Kowalik, J., Dabrowska¸, A., Jankowska, A., & Wachowska, M. (2021). The effect of high-pressure treatment and skimming on caprine milk proteins. *Applied Sciences (Switzerland)*, *11*(13). https://doi.org/10.3390/app11135982

Lee, B. J., Siow, L. F., Cheong, K. W., & Thoo, Y. Y. (2025). Effect of Indirect-Contact blanching treatment on phytochemicals, antioxidant activities, and enzyme inactivation of calamansi waste. *Journal of Food Measurement and Characterization*, *19*(6), 4244–4256. https://doi.org/10.1007/s11694-025-03250-5

Li, K., Yuan, X., Zhao, J., Ren, J., Ma, L., Liao, X., Hu, X., Chen, F., & Ji, J. (2024). Covalent conjugate of pea protein induced by cyanidin-3-O-glucoside quinone: The structural formation and functional properties. *Food Hydrocolloids*, *153*. https://doi.org/10.1016/j.foodhyd.2024.110047

Li, Y., Jongberg, S., Andersen, M. L., Davies, M. J., & Lund, M. N. (2016). Quinone-induced protein modifications: Kinetic preference for reaction of 1,2-benzoquinones with thiol groups in proteins. *Free Radical Biology and Medicine*, *97*, 148–157. https://doi.org/10.1016/j.freeradbiomed.2016.05.019

Lynch, J. M., & Barbano, D. M. (1999). Kjeldahl Nitrogen Analysis as a Reference Method for Protein Determination in Dairy Products. In *Journal of AOAC International* (Vol. 82, Number 6). https://academic.oup.com/jaoac/article-abstract/82/6/1389/5683920

Magomya, A. M., Kubmarawa, D., Ndahi, J. A., & Yebpella, G. G. (2014). Determination Of Plant Proteins Via The Kjeldahl Method And Amino Acid Analysis: A Comparative Study. *International Journal of Scientific & Technology Research*, *3*. www.ijstr.org

Menzel, S., Holland, T., Boes, A., Spiegel, H., Bolzenius, J., Fischer, R., & Buyel, J. F. (2016). Optimized blanching reduces the host cell protein content and substantially enhances the recovery and stability of two plant-derived malaria vaccine candidates. *Frontiers in Plant Science*, *7*(FEB2016). https://doi.org/10.3389/fpls.2016.00159

Niu, L., Zhang, H., Wu, Z., Wang, Y., Liu, H., Wu, X., & Wang, W. (2018). Modified TCA/acetone precipitation of plant proteins for proteomic analysis. *PLoS ONE*, *13*(12). https://doi.org/10.1371/journal.pone.0202238

Obi, P. U., Babagana, M., Idris, I., Hadiza, M., Nma, E. M., & Nadhiekhan, A. (2024). Analysis of Proximate, Mineral and Phytochemical Composition of Fresh and Dry Vernonia amygdalina (Bitter Leaf) in Bida Metropolis, Niger State. *UMYU Scientifica*, *3*(1), 88–94. https://doi.org/10.56919/usci.2431.010

Oseghale, I. D., Lawal, O. P., Ubebe, D. O., Orjiewulu, V. C., Igunma, A. A., Odey, O. P., Ajibola, D. T., Chima, D. I., Tuador, N. K., & Ani, C. P. (2024). Ethnomedicinal and Phytopharmacological Aspects of Vernonia amygdalina (Bitter Leaf) Utilized as a Traditional Medicinal Herb. *Asian Journal of Research in Biochemistry*, *14*(6), 41–57. https://doi.org/10.9734/ajrb/2024/v14i6326

Peter Achunike Akah. (2024). Vernonia amygdalina Del (Bitter leaf) a traditional anti-diabetic gold mine - Mini Review. *Journal of Ayurveda and Integrated Medical Sciences*, *9*(1), 170–175. https://doi.org/10.21760/jaims.9.1.24

Qiu, C., Meng, Y., Zhang, Z., Li, X., McClements, D. J., Li, G., Jiang, L., Wen, J., Jin, Z., & Ji, H. (2025). Enhancement of soy protein functionality by conjugation or complexation with polysaccharides or polyphenols: A review. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 24, Number 1). John Wiley and Sons Inc. https://doi.org/10.1111/1541-4337.70095

Simonne, A. H., Simonne, E. H., Eitenmiller, R. R., Mills, H. A., & Cresman, C. P. (1997). Could the Dumas Method Replace the Kjeldahl Digestion for Nitrogen and Crude Protein Determinations in Foods? *Journal of the Science of Food and Agriculture*, *73*(1), 39–45. https://doi.org/10.1002/(sici)1097-0010(199701)73:1<39::aid-jsfa717>3.0.co;2-4

Tilley, A., McHenry, M. P., McHenry, J. A., Solah, V., & Bayliss, K. (2023). Enzymatic browning: The role of substrates in polyphenol oxidase mediated browning: Mechanisms of enzymatic browning. In *Current Research in Food Science* (Vol. 7). Elsevier B.V. https://doi.org/10.1016/j.crfs.2023.100623

Tran, T., Xu, Z., Coupland, J., & Zhang, Y. (2025). Acid-Adapted Polyphenol Oxidases from Agricultural Wastes: Extraction, Characterization, and Application in Plant Protein Crosslinking. *Foods*, *14*(19). https://doi.org/10.3390/foods14193312

Trigo, J. P., Stedt, K., Schmidt, A. E. M., Kollander, B., Edlund, U., Nylund, G., Pavia, H., Abdollahi, M., & Undeland, I. (2023). Mild blanching prior to pH-shift processing of Saccharina latissima retains protein extraction yields and amino acid levels of extracts while minimizing iodine content. *Food Chemistry*, *404*. https://doi.org/10.1016/j.foodchem.2022.134576

Ugbogu, E. A., Emmanuel, O., Dike, E. D., Agi, G. O., Ugbogu, O. C., Ibe, C., & Iweala, E. J. (2021). The Phytochemistry, Ethnobotanical, and Pharmacological Potentials of the Medicinal Plant-Vernonia amygdalina L. (bitter Leaf). *Clinical Complementary Medicine and Pharmacology*, *1*(1), 100006. https://doi.org/10.1016/j.ccmp.2021.100006

Wohlt, D., Schwarz, E., Schieber, A., & Bader-Mittermaier, S. (2021). Effects of extraction

conditions on banana peel polyphenol oxidase activity and insights into inactivation kinetics using thermal and cold plasma treatment. *Foods*, *10*(5). https://doi.org/10.3390/foods10051022

Zhao, H., Shen, C., Wu, Z., Zhang, Z., & Xu, C. (2020). Comparison of wheat, soybean, rice, and pea protein properties for effective applications in food products. *Journal of Food Biochemistry*, *44*(4). https://doi.org/10.1111/jfbc.13157

Zheng, K., Kaschani, F., Watts, E. C., Kaiser, M., & van der Hoorn, R. A. L. (2025). *Polyphenol oxidase depletion in* Nicotiana benthamiana *enhances recombinant protein purification and preserves native protein integrity*. https://doi.org/10.1101/2025.09.28.679031