Special Issue: Novel Advances in Agricultural Science and Technology for Sustainable Farming in Tropical Region

Functional Properties of Chemically Modified Cowpea Protein Isolates Effects of Reagent Type and Concentration

Choirul Anam*[1], Yuli Witono[2], Mariyatul Qibtiyah[1], Dian Eka Kusumawati[1], Istiqomah Istiqomah[1], Emmy Hamidah[1], W.ahyu Mushollaeni[3], Miftha Churochman[4], Asma Nisar[5], Sayekti Nugrahaning Widi[1] and Trias Agung Pakarti[6,7]

[1]Universitas Islam Darul 'Ulum, Jl. Airlangga No.03, Lamongan, 62253, East Java, Indonesia; [2]University of Jember, Jl. Kalimantan No.37, Jember, 68121, East Java, Indonesia; [3]University of Tribhuwana Tunggadewi Malang 65144, East Java, Indonesia; [4]University of Muhammadiyah Malang Campus 2, Jl Bendungan Sutami No.188, Malang 65145, East Java, Indonesia; [5]University of Lahore, 1-Km Defence Road, 54590 Lahore, Punjab, Pakistan; [6]University of Brawijaya, Jl. Veteran No.10 11, Malang 65145, East Java, Indonesia; [7]Mayantara School, Jl. Puncak Mandala No.40A, Malang 65146, East Java, Indonesia.

Abstract |Cowpea, a potential protein source, is produced in small scale in Indonesia. Most cowpea protein isolate used in food production has been imported. Opting on 0.35 % $CaSO_4$, 0.35 % $MgSO_4$, and 7 % CH_3COOH modifiers, this research focused on discovering the most suitable chemical combination to draw the best features in cowpea protein isolate. Locally grown cowpea was selected as the sample and set into Treatment A (0.35 % $CaSO_4$ and 7 % CH_3COOH), Treatment B (0.35 % $MgSO_4$ and 7 % CH_3COOH), and Treatment C (0.35 % $MgSO_4$ and 0.35 % $CaSO_4$). The materials used included cowpea, $CaSO_4$, $MgSO_4$, CH_3COOH, 70 % acetone, NaOH 0.1 N, HCl 1 N, H_2SO_4, phosphate buffer 0.1 M pH 7, and phosphate buffer 0.05 M pH 7. The methods comprised three main stages: cowpea protein isolation, chemical modification, and functional property assessment. Each treatment was tested in three repetitions for foaming capacity, foaming stability, oil holding capacity, water holding capacity, emulsion capacity, and emulsion stability. Data obtained were analyzed through ANOVA using DSAASTAT version 1.101, followed by Duncan's test at 5 % level before table and histogram presentation and descriptive discussion. It is reported that $MgSO_4$ and $CaSO_4$ combination referred as Treatment C is the optimal modification for cowpea protein isolate with foaming capacity rate at 227 mL g^{-1} (wb), foaming stability rate at 30 % (wb), oil holding capacity rate at 92.46 % (wb), water holding capacity at 60.67 % (wb), emulsion capacity rate at 2.87 m^2 g^{-1} (wb), and emulsion stability rate at 67.20 h (wb). The modified isolate has the potential for application in food products. Further studies are needed to evaluate digestibility, sensory properties, and industrial feasibility to support healthy local food innovation, reduce dependence on imported protein, and achieve Sustainable Development Goals (SDGs).

Received | April 02 2025; **Accepted** | Jun 30, 2025; Published | September 29, 2025
***Correspondence** | Choirul Anam, Faculty of Agriculture and Animal Science, Universitas Islam Darul 'Ulum, Jl. Airlangga No.03, Lamongan, 62253, East Java, Indonesia. **Email** choirulanam@unisda.ac.id
Citation | Anam C., Y. Witono, M. Qibtiyah, D.E. Kusumawati, I. Istiqomah, E. Hamidah, W. Mushollaeni, M. Churochman, A. Nisar, S.N. Widi and T.A. Pakarti. 2025. Functional properties of chemically modified cowpea protein isolates effects of reagent type and concentration. Sarhad Jurnal of Agriculture, 39 (Special issue 1): 208-219.
DOI | https://dx.doi.org/10.17582/journal.sja/2023/39/s1.208.219
Keywords |Black-eye pea, Food ingredient, Healthy flour, Potential protein flour, Sustainable development goals, *Vigna unguiculata*

Introduction

Cowpea [(*Vigna unguiculata*) (L.) Walp.] with common name: black-eye pea, southern pea, niebe, and crowder pea (in Indonesian: kacang tunggak, kacang tolo, and kacang merah kecil) is a *leguminoceae* crop that is strongly potential to be a protein source (Mfeka *et al*., 2019). It has yet not been commercially developed by farmers despite being cultivated in Indonesia for quite some time. With protein content varied between 22.37 % and 24.54 % (Gerrano *et al*., 2022), cowpea planted in Brazil holds 20.82 % to 26.92 % (Freitas *et al*., 2022) while in Indonesia is of 22.90 % in addition to 11 % water, 1.4 % fat, and 61.60 % carbohydrate (Kanetro, 2017). Its lower fat content compared to other legumes should reduce the risk commonly occur after consuming fatty foods. Moreover, cowpea contains more vitamin B1 than mung bean [*Vigna radiata* (L.) R. Wilczek] while its protein is rich in essential amino acids , such as lysin, aspartat acid, and glutamat (Rosida *et al*., 2013; Setyobudi *et al*. 2023, 2024a).

Food processing is no longer limited to conventional methods technology currently allows legumes to be stored in the form of food ingredients, *e.g*. flour, concentrate, or protein isolate (Burger *et al*., 2022; Damat *et al*., 2023; Strauch and Lila, 2021; Witono *et al*., 2014). Protein isolate is an isolation product of legumes generally employing extraction (alkaline-based) and coagulation (acid-based) methods with a standard protein content of 90 %. Most protein isolate in Indonesian market is imported due to inadequate local supply (Bahar and Witono, 2015). After undergoing an acetone wash and 8 h drying at 50 °C, cowpea contains 16.638 % protein isolate yield as well as 7.932 % water, 90.305 % protein, 1.050 % fat, 8.784 % carbohydrate, and 3.852 % ash (Anam *et al*., 2013). To compare, a green pea (*Lathyrus oleraceus* Lam.) protein isolate product in Chicago, USA is of 81.3 % protein, 7 % fat, and 9 % fiber (Masia *et al*., 2022).

Further on cowpea protein isolate, it is also noted to contain more 7S globulin fraction than 11S one. However, its gelatinization ability is as low as 4 gf. Also recorded technical functional characteristics are pH saturation (pH 8), foam capacity (68 mL g^{-1}), foam stability (8 %), oil holding capacity (84.89 %), water holding capacity (136.61 %), emulsion capacity (2.41 m^2 g^{-1}) and emulsion stability (78.15 h) (Witono *et al*., 2014).

Chemical modification on functional features (Wu *et al*., 2023) with CH_3COOH, $CaSO_4$ (Chang *et al*., 2011; Hou *et al*., 1997), and $MgSO_4$ is a way to boost gelatinization ability of protein (Arabi *et al*., 2020; Bangar *et al*., 2022; Hsieh *et al*., 2022). Acetic acid (CH_3COOH) reduces the negative charge to enhance gelatinization, calcium sulfate ($CaSO_4$) cross-links Ca^+ ion and carboxyl group to aggregate and form gel. Adding magnesium sulfate ($MgSO_4$) has the same effect as the latter. Concentrations of 3 % to 7 % for acetic acid and 0.25 % to 0.35 % for both calcium sulfate and magnesium sulfate allow a wide range of modification results to explore leading to an optimal protein modification process.

The best modification on cowpea is of 0.35 % $CaSO_4$ and 0.35 % $MgSO_4$ combination, resulting in an isolate of 13.9 gf mm^{-1} gel strength containing 81.04 % water, 6.77 % ash (db), 92.53 % protein (db), and soluble protein in pH 8 at 22.47 mg g^{-1} (db) (Anam *et al*., 2024). Since the functional features of the aforementioned treatment are unknown, this study aims to reveal them in modified cowpea protein isolate in accordance with certain chemical types and concentrations. This research is urgently needed to support the development of healthy local plant based protein ingredients, enhance the competitiveness of indigenous legumes, and contribute to reducing dependency on imported protein isolates in the Indonesian food industry. The broader goal is to achieve Sustainable Development Goals, especially the 2nd Goal: Zero hunger; the Goal 3: Good health and well being; the Goal 9: industry, innovation, and infrastructure; and the Goal 12: Responsible consumption and production (United Nations, 2025).

Materials and Methods

The exploration on varied chemical types and concentrations in modifying cowpea protein isolate had been performed between May and August 2022 in the Food Processing and Agricultural Product Engineering Laboratory, the Food Chemistry and Biochemistry Laboratory, and the Integrated Analysis Laboratory of the Faculty of Agriculture of Universitas Jember, focusing on foam capacity, foam stability, oil holding capacity, water holding capacity, emulsion capacity and emulsion stability.

Materials

Cowpea sample was locally obtained from Malang, East Java, Indonesia. Chemicals involved were aquadest, aceton 70 %, NaOH 0.1 N, HCL 1 N, H_2SO_4, phosphate buffer 0.1 M pH 7, phosphate buffer 0.05 M pH 7, CH_3COOH, $CaSO_4$, and $MgSO_4$ all of pro analiysis quality purchased from PT. Pash Mitra Mandiri, Bandung, West Java. Equipments used were blender (GMC, China), filtering cloth, pH meter (Jen Way 3320, Germany), centrifuge (Medifringer Gyrozen 2236 HR, South Korea), centrifuge tube (China), spatula, water bath (Memmert, Jerman), stirrer (SM 24, Germany), stirrer shaft (SM 24, Germany), analytic scale (Ohaus Ap-310-O, AS), vortex (Eppendorf, Germany), spectrophotometer (Genesis 10 UV, USA), and glassware.

Methods

This study employed a Completely Randomized Design (CRD) with a single factor: the type and combination of chemical modifiers. The experiment consisted of three treatments, each replicated three times, resulting in a total of nine experimental units. The treatments were as follows: Treatment A = 0.35 % calcium sulfate ($CaSO_4$) + 7 % acetic acid (CH_3COOH); Treatment B = 0.35 % magnesium sulfate ($MgSO_4$) + 7 % acetic acid (CH_3COOH); and Treatment C = 0.35 % calcium sulfate ($CaSO_4$) + 0.35 % magnesium sulfate ($MgSO_4$). Each experimental unit consisted of 100 g of dried cowpea seeds, which were processed into protein isolate and then chemically modified according to the designated treatment. The variables in this study were: i) Independent variable: The type and concentration of chemical reagents ($CaSO_4$, $MgSO_4$, CH_3COOH); and ii) Dependent variables: Foaming capacity, foaming stability, oil holding capacity (OHC), water holding capacity (WHC), emulsion capacity, and emulsion stability The experimental procedure was carried out in three main stages: i) Protein Isolation: Cowpea protein isolate was extracted using an alkaline extraction and acid precipitation method, following Witono *et al*. (2014) and Shanthakumar *et al*. (2022); ii) Chemical Modification: The extracted protein isolate was modified using specific combinations and concentrations of $CaSO_4$, $MgSO_4$, and CH_3COOH according to the treatment groups; and iii) Functional Property Analysis: The modified protein isolate was evaluated for its functional properties, including foaming capacity and stability, OHC, WHC, emulsion capacity, and emulsion stability. Measurements and calculations were performed using standardized analytical methods based on relevant scientific literature.

Sample preparation (Witono et al., 2014)

An sample of 100 g cowpea was soaked in water for ± 5 h. Once drained, the rind was extracted at the ratio of 3:1 (w w^{-1}) and filtered. The supernatan was mixed with NaOH 0.1 N at the ratio of 0.5:1 in a glass beaker to optimally solve the protein in pH 8, then heated at 55 °C for 30 min. Centrifugation followed at 2 000 rpm (1 Hz = 60 rpm) for 10 min. HCl 1 N was added to bring protein precipitation to the isoelectric point of pH 5 before the second centrifugation at 2 000 rpm for 10 min. After purified using acetone 70 % at the ratio of 3:1, homogenously stirred for 20 min , and centrifugated at 2 000 rpm for another 10 min, cowpea protein isolate was ready (Shanthakumar *et al*., 2022).

Step 1: Sample grouping modification (Anam et al., 2024)

In treatment A, $CaSO_4$ 0.35 % and CH_3COOH 7 % were combined. Treatment B received $MgSO_4$ 0.35 % and CH_3COOH 7 %, while $MgSO_4$ 0.35 % and $CaSO_4$ 0.35 % were administered in Treatment C. After homogenously mixed using a spatula, each treatment was heated at 70 °C for 15 min to denaturate protein, allowing molecular interaction to form modified cowpea protein isolate gel.

Step 2: Functional feature analysis

Foam capacity and foam stability (Damat et al., 2000; Zayas, 1997)

The amount of 0.5 g sample and 100 mL phosphate buffer 0.1 M pH 7 were mixed in a 250 mL measuring cup (scaled prior to the process) and stirred for 10 min. Aerator worked in the mixture for 60 s to form foam, of which height was recorded. Both stirrer and aerator were then stopped for 2 min and the foam height reduction was also recorded. The figures were then calculated based on Equation (1) and Equation (2)

$$Foaming\ capacity\ (m\ g{-}1) = \frac{(after - aeration\ volume\ -\ basic\ volume)}{sample\ weight} \quad ...(1)$$

$$Foaming\ stability(\%) = \frac{(foam\ height\ reduction\ volume - basic\ volume)}{sample\ weight} \quad ....(2)$$

Emulsion capacity and emulsion stability (Anam et al., 2023a; Parkington et al., 2000)

An amount of 0.2 g sample dan 100 mL phosphate buffer 0.05 M pH 7 were mixed for 15 min, and then blended with 25 mL cooking oil for 3 min. Next, 1 mL of it was procured for emulsion capacity test, while another 1 mL was taken after resting for 10 min for emulsion stability test. 5 mL Sodium Dodecyl Sulfate SDS 0.1 % was added in each solution prior to vortex round, then absorbance test was performed at 500 nm. The results were processed as per Equation (3) and Equation (4):

$$EAI = \frac{2 \ x \ 2{,}303}{c \ x \ (1 - \varphi) \ x \ 10^{4}} \ x \ abs \ x \ dilution \ \ldots\ldots\ldots\ldots (3)$$

$$ESI = \frac{abs. emulsion \ capacity \ x \ 10}{abs. emulsion \ stability - abs. emulsion \ capacity} \ \ldots\ldots\ldots (4)$$

Notes:

- c = protein concentration (g mL^{-1})
- φ = oil volume fraction (mL/mL) of emulsion
- abs = absorbance
- dilution = solution fraction (SDS + emulsion)
- EAI = Emulsifying Activity Index
- ESI= Emulsifying Stabilitation Index

Oil holding capacity (Anam et al., 2025; Zayas, 1997)

In a prescaled clean centrifugal tube (a g), 2 g sample (b g) and 14 g cooking oil (7× of sample volume) were vortex-mixed and centrifuged for 5 min s at 2 000 rpm. The precipitation was then measured after separated from its supernatant (c g) and run in Equation (5) (Anam *et al*., 2023b):

$$\% \ OHC = \frac{(c - a) - b}{b} x \ 100 \ \% \ \ldots\ldots\ldots (5)$$

Water holding capacity (Ma et al., 2022)

As must as 2 g sample (bg) and 14 g aquadest (7× of sample volume) were vortex-mixed in a prescaled clean centrifugal tube (a g), and centrifuged for 5 min at 2 000 rpm. The precipitation was then measured after separated from its supernatant (c g) and evaluated using Equation (6):

$$\% \ OHC = \frac{(c - a) - b}{b} x \ 100 \ \% \ \ldots\ldots\ldots (6)$$

Data analysis

Laboratory experimental research design was utilized, and the data were computed on Microsoft Excel to be presented in the form of tables and graphs. ANOVA test One Way (DSAASTAT 1.101 version, Italy) and Duncan test (p ≤ 0.05) ensured any significance should different results appeared (Damat *et al*., 2021).

Results and Discussion

Preliminary test (stage 1 and stage 2)

In the first stage, the optimal treatment for cowpea protein isolate was determined based on the firmness and gel strength resulting from the application of different coagulating agents. The objective was to identify which concentration and type of modifier could produce the most compact, rigid, and stable gel structure, as these characteristics are essential indicators of protein denaturation effectiveness and network formation, which directly affect functional properties. Among the tested acid concentrations (3 %, 5 %, and 7 % CH_3COOH), the 7 % CH_3COOH treatment was found to be the most effective. It produced gels with the highest viscosity and firmness, indicating optimal protein precipitation at pH levels conducive to isoelectric point aggregation. This level of acidity effectively disrupted protein solubility and promoted dense gel network formation. For the salt-based coagulants, both $CaSO_4$ and $MgSO_4$ were tested at 0.25 %, 0.30 %, and 0.35 %. The 0.35 % concentration of both salts yielded the strongest and most cohesive gel structures, suggesting that this concentration is most effective in inducing protein cross-linking through ionic interaction, which enhances the protein matrix's integrity. At this concentration, the divalent cations (Ca^{2+} and Mg^{2+}) facilitated stronger electrostatic interactions between protein molecules, leading to better gel compactness. Thus, CH_3COOH at 7 % and $CaSO_4$ and $MgSO_4$ at 0.35 % were considered effective in enhancing the gelation capacity, structural integrity, and compactness of cowpea protein isolate. These properties are critical for ensuring good functional behavior in food.

The second stage revealed treatment C of $CaSO_4$ 0.35 % and $MgSO_4$ 0.35 % combination to be the best modification. The gel structure of 13.9 gf. $(0.1 \ mm)^{-1}$ is the most compact and, therefore, the most beneficial when applied in the food industry. Having the lowest water content at 81.04 %, this isolate should be the most stable with the longest shelf-life. The highest ash content at 6.77 % (db) points to a significant amount of essential minerals. Its highest protein content at 92.53 % (db) is undoubtedly a feasible protein concentration, with soluble protein content in pH 8 at 22.47 m g^{-1} (dB), showing its solubility in specific pH environments critical to several food production applications (Anam *et al.*, 2024).

Functional property analysis

Foaming capacity and foaming stability

Two key functional properties of protein in aerated food systems are foaming capacity, which reflects the ability of a protein to entrap air during whipping, and foaming stability, which refers to the protein's ability to maintain the foam structure over time (Stone *et al.*, 2015). In this study, the foaming capacity of modified cowpea protein isolate ranged from 187 mg g^{-1} to 227 mg g^{-1}, with a statistically significant difference among treatments ($P < 0.05$), as shown in Figure 1.

Note:

- A = CH_3COOH - $CaSO_4$ combination; B = CH_3COOH - $MgSO_4$ combination; C = $CaSO_4$ -$MgSO_4$ combination
- Numbers followed by the same letter indicate no significant difference with the Duncan 5 % test.

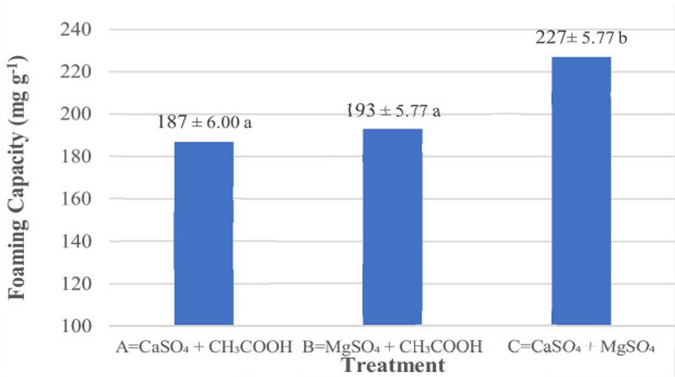

Figure 1: *Foaming capacity of modified cowpea protein isolate.*

Treatment C (0.35 % $CaSO_4$ + 0.35 % $MgSO_4$) demonstrated the highest foaming capacity at 227 mg g^{-1}, followed by Treatment B (0.35 % $MgSO_4$ + 7 % CH_3COOH) and Treatment A (0.35 % $CaSO_4$ + 7 % CH_3COOH). The enhanced foaming performance in Treatment C may be attributed to the synergistic cross-linking effect of Ca^{2+} and Mg^{2+}ions, which bind to negatively charged carboxyl groups in proteins, leading to more compact protein networks capable of stabilizing air bubbles (Arabi *et al.*, 2020). These findings are consistent with previous research: The foaming capacity of unmodified cowpea isolate was reported at only 68 mL g^{-1} (Witono *et al.*, 2014), While Soy (*Glycine max Merr.*) protein isolate reached 136 mL g^{-1}, and Lima bean (*Phaseolus lunatus* L.) protein isolate was recorded at 425 mg g^{-1} (Ajayi *et al.*, 2025), further supporting the functional enhancement through chemical modification.

Regarding foaming stability, the modified cowpea protein isolates showed a range from 20 % to 30 % (Figure 2), although no statistically significant differences were observed among treatments ($P > 0.05$).

Note:

- A = CH_3COOH - $CaSO_4$ combination; B = CH_3COOH - $MgSO_4$ combination; C = $CaSO_4$ -$MgSO_4$ combination

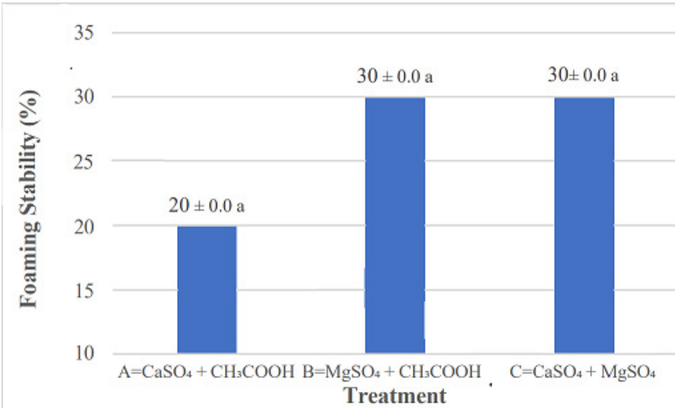

Figure 2: *Foaming stability of modified cowpea protein isolate.*

Treatments B and C exhibited the highest foam stability (30 %), while Treatment A displayed the lowest (20 %). The differences in stability may be due to variations in protein solubility and globulin content, which affect the interfacial film strength (Chao *et al.*, 2018). These results align with Nazar and Usha (2018), who reported that legume protein isolates enriched with 7S and 11S globulins exhibit better foam stability due to their surface-active properties.

In terms of application: Treatments B and C with higher foam stability are suitable for aerated food products requiring stable foam structures, such as mousses, whipped toppings, ice cream, and sausage emulsions, Whereas Treatment A, with quicker foam collapse, is more appropriate for bakery applications such as cakes, where controlled defoaming during baking is desirable.

The improved foaming capacity and stability of cowpea protein isolate particularly in Treatment C suggest its promising potential as a local, plant-based alternative to imported protein isolates in functional food development. However, this study is limited by the absence of evaluation on: Digestibility, Sensory acceptability, and Long-term shelf-life of the modified isolates. Future studies should investigate these parameters to fully assess the commercial applicability of modified cowpea protein isolates in food systems.

Emulsion capacity and emulsion stability

Chemically modified cowpea protein isolates exhibited significantly different emulsion capacities ($P < 0.05$), ranging from 2870 m^2 kg^{-1} to 5400 m^2 kg^{-1} (Figure 3). Treatment A ($CH_3COOH + CaSO_4$) showed the highest emulsion capacity (5400 m^2 kg^{-1}), significantly outperforming Treatments B and C. This result suggests that acetic acid and calcium sulfate effectively improve interfacial properties of proteins for emulsion formation.

Note:

- A = CH_3COOH - $CaSO_4$ combination; B = CH_3COOH - $MgSO_4$ combination; C = $CaSO_4$ -$MgSO_4$ combination
- Numbers followed by the same letter indicate no significant difference with the Duncan 5 % test.

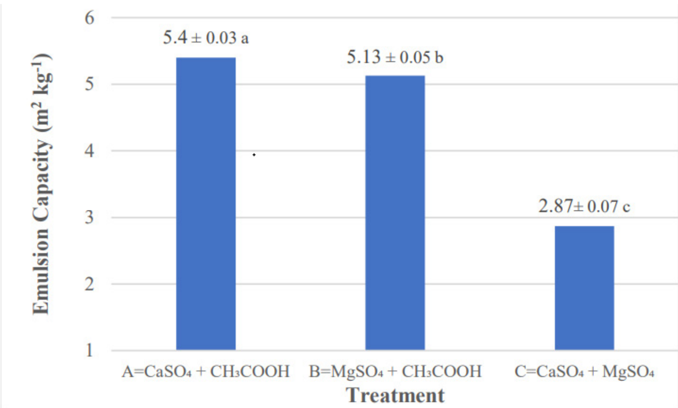

Figure 3: *Emulsion capacity of modified cowpea protein isolate.*

The superior performance of Treatment A supports Witono *et al.* (2014), who found that protein complexity enhances emulsification, and aligns with D'Alessio *et al.* (2022), who demonstrated that proteins with greater solubility and surface activity form finer emulsions. This finding is also consistent with the emulsion capacity of soybean protein isolates (4 500 m^2 kg^{-1} to 5 600 m^2 kg^{-1}) reported by Fu *et al.* (2020), indicating that modified cowpea protein can match commercial plant protein performance.

Emulsion stability results ranged from 67.20 h to 77.41 h (Figure 4), with Treatment A again producing the most stable emulsion. The presence of globulins 7S and 11S, known to improve interfacial film formation and retard coalescence, likely contributed to this stability (Chao *et al*., 2018; Witono *et al*., 2014). Park *et al.* (2025) also emphasized that legume proteins with higher globulin content enhance emulsion durability.

Note:

- A = CH_3COOH - $CaSO_4$ combination; B = CH_3COOH - $MgSO_4$ combination; C = $CaSO_4$ -$MgSO_4$ combination.
- Numbers followed by the same letter indicate no significant difference with the Duncan 5 % test.

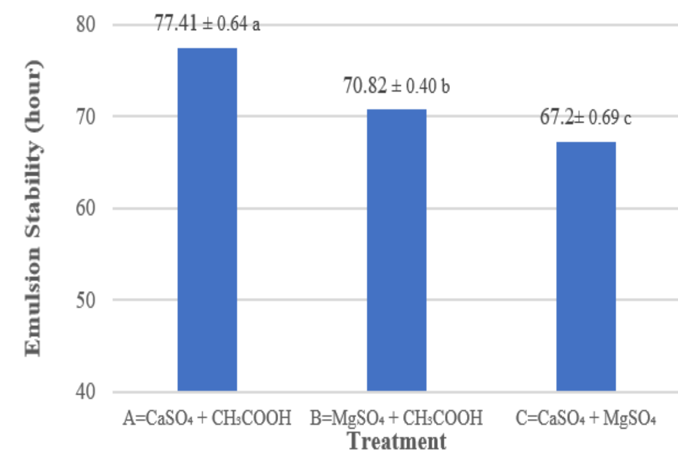

Figure 4: *Emulsion stability of modified cowpea protein isolate*

Despite promising results, the study has limitations. Measurements were taken under controlled conditions, which may not reflect real food systems with variable pH, salt, or fat contents. Microstructure and droplet size distribution were not assessed, which are key parameters in determining long-term emulsion behavior. Future studies should examine stability in actual food matrices, explore sensory implications, and evaluate encapsulation potential for functional food development. Scaling up and shelf-life assessments in commercial formulations are also warranted.

Oil holding capacity (OHC)

Oil holding capacity (OHC) indicates a protein's ability to retain oil within a food matrix, which is vital for texture and flavor retention in high-fat food systems. In this study, OHC values of chemically modified cowpea protein isolates ranged from 82.16 % to 92.46 %, with significant differences among treatments ($P < 0.05$), as shown in Figure 5.

Note:
- A = CH_3COOH - $CaSO_4$ combination; B = CH_3COOH - $MgSO_4$ combination; C = $CaSO_4$ -$MgSO_4$ combination.
- Numbers followed by the same letter indicate no significant difference with the Duncan 5 % test.

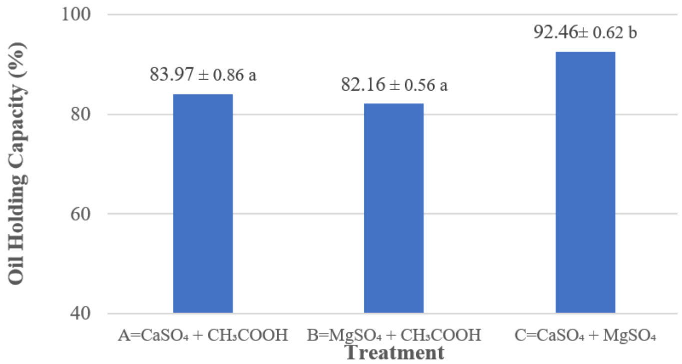

Figure 5: *Oil holding capacity of modified cowpea protein isolate.*

Treatment C (combination of 0.35 % $CaSO_4$ and 0.35 % $MgSO_4$) showed the highest OHC value of 92.46 %, significantly greater than Treatment A (CH_3COOH + $CaSO_4$) and Treatment B (CH_3COOH + $MgSO_4$). This superior performance may be attributed to the synergistic effect of divalent salts, which promotes the formation of a denser and more uniform protein gel network, increasing surface area for oil entrapment. According to Arabi *et al.* (2020), smaller protein particle size enhances the interaction with oil due to increased surface reactivity. These findings align with Wisaniyasa and Suter (2016), who reported that common bean sprout powder had an OHC of 89.84 %, and regular bean powder had 86.03 %. Furthermore, the OHC of modified cowpea protein in this study surpassed that of soy protein isolate (84.89 %) as previously reported by Witono *et al.* (2014), indicating its potential use in high-fat plant-based products such as vegan sausages, spreads, and bakery fillings. This highlights the role of salt-induced protein gelation in enhancing fat-binding capacity, a crucial feature for texture control in formulated foods (D'Alessio *et al.*, 2022).

Future studies should investigate: i) The correlation between protein microstructure (*e.g.*, via SEM) and OHC, ii) Performance in real food systems under industrial conditions, iii) Digestibility and sensory acceptance of oil rich formulations using this modified isolate. Limitation: This study did not include microstructural characterization of the modified proteins, which would have helped explain the observed oil retention mechanism at the molecular and physical levels.

Water holding capacity (WHC)

Water holding capacity reflects a protein's ability to retain moisture within a food matrix, a critical attribute in formulating plant based foods. In this study, WHC values of the chemically modified cowpea protein isolate ranged from 5.82 % to 60.67 %, showing significant differences among treatments ($P < 0.05$) as shown in Figure 6.

Note:
- A = CH_3COOH - $CaSO_4$ combination; B = CH_3COOH - $MgSO_4$ combination; C = $CaSO_4$ -$MgSO_4$ combination
- Numbers followed by the same letter indicate no significant difference with the Duncan 5 % test.

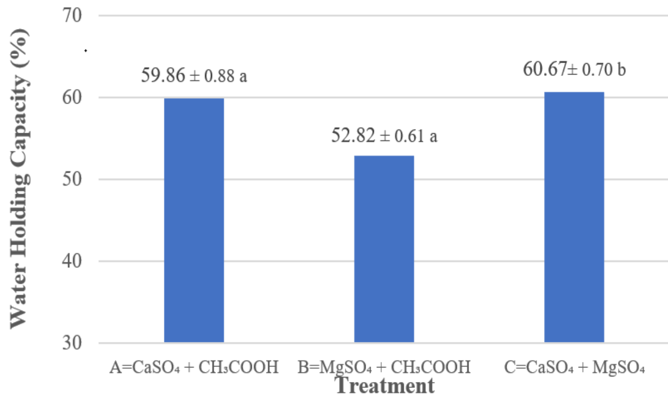

Figure 6: *Water holding capacity of modified cowpea protein isolate.*

Treatment C (0.35 % $CaSO_4$ + 0.35 % $MgSO_4$) yielded the highest WHC at 60.67 %, significantly higher than Treatment B (CH_3COOH + $MgSO_4$), though not significantly different from Treatment A (CH_3COOH + $CaSO_4$). This indicates that salt-based coagulants ($CaSO_4$ and $MgSO_4$) are more effective in enhancing water retention than acid-based coagulants. Both calcium and magnesium ions promote protein cross linking and structure stabilization, allowing proteins to trap more water molecules during gel formation. According to Witono *et al.* (2014), higher protein content and more compact gel networks result in better water retention. The trend is consistent with Gao *et al.* (2021), who reported that increasing salt concentration improved WHC in silver carp (*Hypophthalmichthys molitrix* Valenciennes,

1844) surimi from 85.02 % to 93.63 %. Likewise, Sosa *et al.* (2020) demonstrated that modified legume proteins could retain water efficiently, supporting their application in moisture-sensitive products like nuggets and meat substitutes.

However, this study did not include microstructural analysis (*e.g.*, via SEM) or test WHC in real food systems. Thus, further research is recommended to: i) Correlate protein gel microstructure with WHC behavior, ii) Evaluate WHC in formulated foods like sauces or protein drinks, and iii) Assess WHC under processing conditions such as heating and storage.

To achieve the goal of producing healthy flour related to SDGs, a Standard Operating Procedure (SOP) must be established, especially to minimize new ubiquitoes pollutants (Chen, *et al.*, 2022; Setyobudi, *et al.*, 2024b, 2024c), *i.e.*, microplastics (MPs). This pollutant has been reported to contaminate soybeans and their derivatives (Setyobudi *et al.*, 2024d). Several researchers, including Hermayanti *et al.* (2024) and Garfansa *et al.* (2024), have reported deaths in experimental animals due to MPs contamination.

Conclusions and Recommendations

The optimal chemical modification for cowpea protein isolate was found in Treatment C, engaging 0.35 % $CaSO_4$ and 0.35 % $MgSO_4$ combination. The tested functional properties are of foaming capacity rate at 227 mL g^{-1} (wb), foaming stability rate at 30 % (wb), oil holding capacity rate at 92.46 % (wb), water holding capacity at 60.67 % (wb), emulsion capacity rate at 2.87 m^2 g^{-1} (wb), and emulsion stability rate at 67.20 h (wb). It is recommended that modified cowpea protein isolate (especially Treatment C) be utilized by the food industry as an alternative to imported protein. Further research is needed on digestibility, sensory characteristics, and industrial feasibility. Government policy support is essential to promote innovation, production, and the use of local legumes as part of a national strategy for protein self-sufficiency and sustainable food industry development.

Acknowledgments

This research was supported by funding from the Universitas Islam Darul 'Ulum, Lamongan, Indonesia (0234/U/K.1/XII/2021/SK). Thank you to RP Editage for preparing and guiding the scientific writing of this manuscript.

Novelty Statement

This research develops cowpea protein isolate as a functional food ingredient through chemical modification with an optimal combination of $CaSO_4$ and $MgSO_4$ (0.35 %), which successfully increases the foam capacity, emulsion, and oil and water absorption. This finding opens up opportunities for using cowpeas in high value added food products while supporting the sustainability of agriculture in dry areas and increasing the economic value of cowpeas.

Authors' Contribution

Choirul Anam: Designed the study, performed a literature search, wrote the manuscript draft and manuscript revised.
Yuli Witono: Supervised the experiment, analyzed and interpreted the data, and provided comments and suggestions for the whole work.
W.ahyu Mushollaeni, Miftha Churochman, and Asma Nisar: Manuscript review.
Emmy Hamidah, Mariyatul Qibtiyah, and Dian Eka Kusumawati: Conducted the experiments and collected the data.
Istiqomah Istiqomah and Sayekti Nugrahaning Widi: Resources, Grammarly, and Turnitin.
Trias Agung Pakarti: Formatted manuscript and edited in English.
All authors have read and approved the final manuscript

Generative AI and AI-assisted technology statement
The authors stated that they didn't use generative AI and AI-assisted technology in preparing this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

References

Anam, C., Y. Witono and K.W. Bachtiar. 2013. Functional protein isolation technology from cowpea (*Vigna unguiculata L.*) as food ingredient. Proceedings of the national seminar on the role of food technology and nutrition quality, safety and halalness of local food products, 39–45. Faculty of Agricultural Technology, Andalas University, Padang, Indonesia.

Anam, C., N. Harini, D. Damat, R.H. Setyobudi, I. Ekawati, T. Liblik, E.D. Purbajanti, H. Bernedektus, L.M. Souripet, A. Fauzi, A.R. Farzana, R. Tonda, I. Iswahyudi, A. Amiroh, M. Qibtiyah, D.E. Kusumawati, I. Istiqomah and E. Hamidah. 2023a. Functional characteristics of trash fish in Lamongan Regency, East Java, Indonesia. E3S Web Conf., 432(00007): 1–12. https://doi.org/10.1051/e3sconf/202343200007

Anam, C., M.F.M. Atoum, N. Harini, D. Damat, R.H. Setyobudi, A. Wahyudi, A.D. Pamujiati, N. Kuswardhani, Y. Witono, R. Tonda, H. Prasetyo, I. Ekawati, E.D. Purbajanti, Z. Vincevica Gaile, T. Liblik, A. Fauzi, H. Hadinoto, N.S. Sebayang, E. Suhesti, A. Putri, and F. Munsif. 2023b. Chemical and functional properties of myofibrillar protein from selected species of trash fish. Jord. J. Biol. Sci., 16(2): 267 – 277. https://doi.org/10.54319/jjbs/160211

Anam, C., E. Hamidah, D.E. Kusumawati, I. Istiqomah, M. Qibtiyah and A. Amiroh. 2024. Modified cowpea protein isolate through types and concentrations of chemicals. Agroin. 18(2): 419–428. https://doi.org/10.21107/agrointek.v18i2.16837

Ajayi, F.F., P. Mudgil and S. Maqsood. 2025. Delving into the green extraction of proteins from underutilized lima beans using deep eutectic solvent versus conventional methods: A comprehensive investigation of physicochemical, structural, and functional properties. Food Chem., 484: 144262. https://doi.org/10.1016/j.foodchem.2025.144262

Arabi, S.H., D. Haselberger and D. Hinderberger. 2020. The effect of ethanol on gelation, nanoscopic, and macroscopic properties of serum albumin hydrogels. Molecul., 25(1927): 1–14. https://doi.org/10.3390/molecules25081927

Arteaga, V.G., S. Kraus, M. Schott, I Murany, U. Schweiggert-Weisz and P. Eisner. 2021. Screening of twelve pea *(Pisum sativum L.)* cultivars and their isolates focusing on the protein characterization, functionality, and sensory profiles. Foods., 10(758): 1–17.

Bahar, A. and Y. Witono. 2015. Process optimization of tempeh protein isolate from soybean (*Glycine max Merr*) and cowpea (*Vigna unguiculata*) mixture. Int. J. Adv. Sci. Eng. Inf. Technol., 5(2): 139–143. https://doi.org/10.18517/ijaseit.5.2.501

Bangar, S.P., O.J. Esua, N. Sharma and R. Thirumdas. 2022. Ultrasound-assisted modification of gelation properties of proteins: A review. J. Textu. Stud., 53(6): 763–774. https://doi.org/10.1111/jtxs.12674

Guzman, M.D.B., S. Luna Suarez, A.S.H. Cazares, J.A.H. Corredor, J.V.H. Contreras and M.A.R. Corripio. 2022. Comparison of the physicochemical and functional properties of flour and protein isolate from moringa (*Moringa oleifera Lam.*) leaves. Int. J. Food Prop., 25(1): 733–747. https://doi.org/10.1080/10942912.2022.2058533

Burger, T.G., I. Singh, C. Mayfield, J.L. Baumert and Y. Zhang. 2022. The impact of spray drying conditions on the physicochemical and emulsification properties of pea protein isolate. L.W.T., 153(20):112495. https://doi.org/10.1016/j.lwt.2021.112495

Chang, Y.H., S.Y. Shiau, F.B. Chen and Lin. 2011. Effect of microbial transglutaminase on the rheological and textural characteristics of black soybean packed tofu coagulating with agar. L.W.T., 44(4): 1107–1112. https://doi.org/10.1016/j.lwt.2010.10.020

Chao, D., S. Jung and R.E. Aluko. 2018. Physicochemical and functional properties of high pressure treated isolated pea protein. Innov. Food Sci. Emerg. Technol., 45: 179–185. https://doi.org/10.1016/j.ifset.2017.10.014

Chen, K., R. Tang, Y. Luo, Y. Chen, A. EI-Naggar, J. Du, A. Bu, Y. Yan, X. Lu, Y. Cai, S.X. Chang. 2022. Transcriptomic and metabolic responses of earthworms to contaminated soil with polypropylene and polyethylene microplastics at environmentally relevant concentrations. J. Hazard. Mater. 427: 128176. https://doi.org/10.1016/j.jhazmat.2021.128176

Damat, D., R.H. Setyobudi, P. Soni, A. Tain, H. Handjani and U. Chasanah. 2020. Modified arrowroot starch and glucomannan for preserving physicochemical properties of sweet bread. Cienc. Agrotec., 44: 1–8. https://doi.org/10.1590/1413-7054202044014820

Damat, D., R.H. Setyobudi, J. Burlakovs, Z.V. Gaile, D.D. Siskawardani, R. Anggriani and A. Tain. 2021. Characterization properties of extruded analog rice developed from arrowroot starch with addition of seaweed and spices. S. J.Agric., 37(Special issue 1): 159–170. https://dx.doi.org/10.17582/journal.sja/2021.37.s1.159.170

Damat, D., R.H. Setyobudi, N. Harini, A. Asmawati, S. Anwar, C.Z. Mahesah, M. Wachid, E. Andoko and A.T. Salsabila. 2023. Characteristics of gluten free biscuit from purple sweet flour, rice brands and coffee cherry flour. E3S Web Conf., 432(0008): 1–9. https://doi.org/10.1051/e3sconf/202343200008

Alessio, G.D., F. Flamminii, M. Faieta, P. Pittia and C.D. Di Mattia. 2022. Pea protein isolates: emulsification properties as affected by preliminary pretreatments. Ital. J. Food Sci. 34(4): 25–32. https://doi.org/10.15586/ijfs.v34i4.2259

Anam, C., D. Damat, R.H. Setyobudi, I. Ekawati, P.G. Adinurani, S. Anwar, R. Tonda, W. Mushollaeni, and M.T. Shidq. 2025. Optimized processing techniques for enhancing fillet yield from low-value fish in Lamongan, East Java, Indonesia. Proc. Pak. Acad. Sci.: B 62(2): 101–114. http://doi.org/10.53560/PPASB(62-2)957

Damat, D., R.H. Setyobudi, P. Soni, A. Tain, H. Handjani and U. Chasanah. 2020. Modified arrowroot starch and glucomannan for preserving physicochemical properties of sweet bread. Cienc. Agrotech., 44: 1–8. https://doi.org/10.1590/1413-7054202044014820

Freitas, T.K.T., F. de Oliveira Gomes, M. dos Santos Araújo, I.C.V. Silva, D.J.S. Silva, K.J. Damasceno-Silva and M. de Moura Rocha. 2022. Potential of cowpea genotypes for nutrient biofortifi cation and cooking quality. Rev. Cienc. Agron. 53: 1–11. https://doi.org/10.5935/1806-6690.20220040

Fu, L., H. Zhiyong, Z. Maomao, Q. Fang and C. Chen. 2020. Effects of soy protein composition in recombined soy-based cream on the stability and physical properties of whipping cream. J. Sci. Food Agric. 100(6): 2315–2811. https://doi.org/10.1002/jsfa.10305

Gao, X., Y. Xie, T. Yin, Y. Hu, J. You, S. Xiong and R. Liu. 2021. Effect of high intensity ultrasound on gelation properties of silver carp surimi with different salt contents. Ultrason. Sonochem. 70(105326). https://doi.org/10.1016/j.ultsonch.2020.105326

Garfansa, M.P., R.H. Setyobudi, I. Iswahyudi, S. Anwar, D. Damat, T. Liwang, M.S. Susanti,, D. Hermayanti, M. Setiawani, T.D.N. Subchi, D. Mariyam, Y.M. Ananda, S.K. Wahono, A. Basir, M. Musrif, and A.S. Khan. 2024. Potential impact microplastic polyethylene terephthalate on mice. Sarhad J. Agri. 39(1): 47–60. https://dx.doi.org/10.17582/journal.sja/2023/39/s1.47.60

Gerrano, A.S., Z.G., Thungo, H. Shimelis, J. Mashilo, and I. Mathew. 2022. Genotype-by-environment interaction for the contents of micro-nutrients and protein in the green pods of cowpea (*Vigna unguiculata* L. Walp.). Agric. (Switz.), 12(4): 531. https://doi.org/10.3390/agriculture12040531

Hansen, L., F. Bu and B.P. Ismail. 2022. Structure-function guided extraction and scale-up of pea protein isolate production. Foods., 11(23): https://doi.org/10.3390/foods11233773

Hermayanti, D., R.H. Setyobudi, S. Anwar, M.P. Garfansa, I. Iswahyudi, M. Setiawan, T. Liwang, T.D.N. Subchi, L. Zalizar and P.G. Adinurani. 2024. The effect of polyethylene terephthalate microplastics on the growth of mice. Bio. Web Conf., 104(00005): 1–17. https://doi.org/10.1051/bioconf/202410400005

Hou, H.J., K.C. Chang and M.C. Shih. 1997. Yield and textural properties of soft tofu as affected by coagulation method. J. Food Sci., 62(4): 824–827. https://doi.org/10.1111/j.1365-2621.1997.tb15464.x

Hsieh, K.C., T.C. Lin and M.I. Kuo. 2022. Effect of whole chia seed flour on gelling properties, microstructure and texture modification of tofu. L.W.T., 154(12676): https://doi.org/10.1016/j.lwt.2021.112676

Iqbal, M.A., M.H. Siddiqui, S. Afzal, Z. Ahmad, Q. Maqsood and R.Di. Khan. 2018. Forage productivity of cowpea [*Vigna unguiculata (L.) Walp*] cultivars improves by optimization of spatial arrangements. Rev. Mex. Cienc. Pecu., 9(2): 203–219. https://doi.org/10.22319/rmcp.v9i2.4335

Iseki, K., O. Olaleye and H. Ishikawa. 2020. Intra-plant variation in seed weight and seed protein content of cowpea. Plant Prod. Sci., 23(1): 103–113. https://doi.org/10.1080/1343943X.2019.1677161

Kanetro, B. 2017. Processing technology and functional foods of nuts.p. 71–76. Plantaxia.

Yogyakarta.
Lam, A.C.Y., A.C. Karaca, R.T. Tyler and M.T. Nickerson. 2018. Pea protein isolates: Structure, extraction, and functionality. Food Rev. Int., 34(2): 126–147. https://doi.org/10.1080/87559129.2016.1242135
Ma, K.K., L. Grossmann, A.A. Nolden, D.J. McClements and A.J. Kinchla. 2022. Functional and physical properties of commercial pulse proteins compared to soy derived protein. J. Fut. Food., 6 (100155). https://doi.org/10.1016/j.fufo.2022.100155
Masia, C., P.E. Jensen, I.L. Petersen and P. Buldo. 2022. Design of a functional pea protein matrix for fermented plant-based cheese. Foods., 11(2): 1–13. https://doi.org/10.3390/foods11020178
Matemu, A., S. Nakamura and S. Katayama. 2021. Health benefits of antioxidative peptides derived from legume proteins with a high amino acid score. Antioxid., 10(2): 1–17. https://doi.org/10.3390/antiox10020316
Mfeka, N., R.A. Mulidzi, and F.B. Lewu. 2019. Growth and yield parameters of three cowpea (*Vigna unguiculata* L. Walp) lines as affected by planting date and zinc application rate. South African J. Sci., 115(1): 1–8. https://doi.org/10.17159/sajs.2019/4474
Mohammed, M., S.K. Jaiswal and F.D. Dakora. 2018. Distribution and correlation between phylogeny and functional traits of cowpea (*Vigna unguiculata L. Walp*.) nodulating microsymbionts from Ghana and South Africa. Sci. Rep., 8(1): 1–19. https://doi.org/10.1038/s41598-018-36324-0
Nazar, H.A. and A. Usha. 2018. Biochemical and functional properties of proteins from common Legumes (*Vigna mungo, Vigna radiata, Cajanus cajan* and *Cicer arietinum*). Res. J. Biotechnol., 13(6): 20-29.
Onofri, A. and E. Pannacci. 2014. Spreadsheet tools for biometry classes in crop science programmes. Commun. Biometr. Crop Sci., 9(2): 3–13.
Park, J., T. Umeda, T. Araki and I. Kobayashi. 2025. Formulation characteristics and physical stability of legume protein-stabilized monodisperse oil in water emulsions prepared using the microchannel emulsification method. Innovative Food Sci. Emer. Technol., 104062. https://doi.org/10.1016/j.ifset.2025.104062
Parkington, J.K., Y.L. Xiong, S.P. Blanchard, S. Xiong, B. Wang, S. Srinivan and G.W. Froning. 2000. Food chemistry and technology: Chemical and functional properties of oxidatively modified beef heard surimi stored at 2 °C. Food Sci., 65 (3): 428–433.
Rosida, D.F., Q. Hardiyanti, and Murtiningsih. 2013. Study of the impact of cowpea substitution on the physical quality and chemical properties of tofu. J. Food Technol., 5(2): 138–149. https://doi.org/10.33005/jtp.v5i2.413
Saad, A.M., R.A. Elmassry, K.M. Wahdan and A.O. Osman. 2017. Chemical characterization, antibacterial and antioxidant activity of 7S and 11S globulins isolated from pea (*Pisum sativum*). Zagazig. J. Agric. Res., 44(6): 2221–2230. https://doi.org/10.21608/zjar.2017.51290
Sandjian, M.E. and K.D. Martinez. 2025. Emulsions, foams, and gels structure design formulated with soy protein isolate and calcium for vegan consumers. Food Human., 4: 100490. https://doi.org/10.1016/j.foohum.2024.100490
Setyobudi, R.H., D. Damat, S. Anwar, A. Fauzi, T. Liwang, L. Zalizar, Y.A. Nugroho, M.A. Wedyan, M. Setiawan, S. Husen, D. Hermayanti, T.D.N. Nur Subchi, P.G. Adinurani, E.D. Septia, D. Mariyam, I.R. Utarid, I. Ekawati, R. Tonda, E.D. Purbajanti, S. Suherman, M.S. Susanti, T.A. Pakarti, I. Iswahyudi, B.A. Prahardika and A.R Farzana. 2023. Amino acid profiles of coffee cherry flour from different origins: A comparative approach. E3S Web Conf., 432 (00032): 1–11. https://doi.org/10.1051/e3sconf/202343200032
Setyobudi, R.H., S. Anwar, M. Ali Wedyan, D. Damat, Y. A. Nugroho, T. Liwang, P.G. Adinurani, S.K. Wahono, E.S. Savitri, B.A. Prahardika, I.R. Utarid, I. Iswahyudi and H.A. Rachmawati. 2024a. Characterization of amino acids in coffee cherry flour from different coffee cultivation areas: As potential functional food. S. J. Agric., 39 (Special issue 1): 36–46. https://dx.doi.org/10.17582/journal.sja/2023/39/s1.36.46
Setyobudi, R.H., I. Iswahyudi, D. Damat, M.P. Garfansa, D. Hermayanti, S.D. Ramadani, Z. Vincevica Gaile, L.R. Nurfadilah, E.S. Savitri and Z. Meylanzharie. 2024b. Presence of microplastics in water, soil, organic fertilizer,

and potato plants on potato plantations. Environ. Qual. Manage., 34(2): e70001. https://doi.org/10.1002/tqem.70001

Setyobudi, R.H., S. Anwar, I. Iswahyudi, S. Husen, D. Damat, M.P. Garfansa, P.G. Adinurani, M. Mel, T. Liwang, R. Aprilianti, T.D.N. Subchi, M. Setiawan, D. Hermayanti, D. Mariyam, B.A. Prahardika, Z. Vincevica Gaile, S.K. Wahono, T.N. Punjungsari, A. Fauzi, I. Andini, N.R. Malihah, I. Ekawati, D.D. Sulistyoningrum and Y.A.C. Ekalaturrahmah 2024c. Identification and quantification of microplastics contamination in potato from Malang Raya, Indonesia. Bio. Web Conf., 104(00036): 1–15. https://doi.org/10.1051/bioconf/202410400036

Setyobudi, R.H., S. Anwar, M.P. Garfansa, T. Liwang, I. Iswahyudi, D. Damat, E.S. Savitri, S.K. Wahono, L. Latipun, P.G. Adinurani, T.D.N. Subchi, M. Setiawan, D. Hermayanti, D. Mariyam, A. Fauzi, Z Vincevica-Gaile, M. Churochman, D. Sulistyoningrum, A.R. Farzana and I.O. Dewi. 2024d. Microplastic debris in palm cooking oil: A call for research. Bio. Web Conf., 104(00037): 1–17. https://doi.org/10.1051/bioconf/202410400037

Shanthakumar, P., J. Klepacka, A. Bains, P. Chawla, S.B. Dhull and A. Najda. 2022. The current situation of pea protein and its application in the food industry. Molecul., 27(554): 777–782. https://doi.org/10.1016/b978-0-08-099925-8.00065-x

Sosa, E.F., C. Thompson, M.G. Chaves, B.A. Acevedo and M.V. Avanza. 2020. Legume seeds treated by high hydrostatic pressure: Effect on functional properties of flours. Food Bioproc. Tech., 13: 323–340. https://doi.org/10.1007/s11947-019-02386-9

Stone, A.K., A. Karalash, R.T. Tyler, T.D. Warkentin and M.T. Nickerson. 2015. Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. Food Res. Int., 76(P1): 31–38. https://doi.org/10.1016/j.foodres.2014.11.017

Strauch, R.C. and M.A. Lila. 2021. Pea protein isolate characteristics modulate functional properties of pea protein cranberry polyphenol particles. Food Sci. Nutr., 9(7): 3740–3751. https://doi.org/10.1002/fsn3.2335

United Nations. 2025. The 17th goal. UN Department of Economic and Social Affairs Sustainable Development.

Wisaniyasa, N.W. and I.K. Suter. 2016. Study of functional and chemical properties of red bean (*Phaseolus vulgaris L.*) sprouts flour. Sci. Med. Food Technol., 3(1): 26–34.

Witono, Y., C. Anam, H. Herlina and A.D. Pamujiati. 2014. Chemical and functional properties of protein isolate from cowpea (*Vigna unguiculata*). Int J. Adv. Sci. Eng. Inf. Technol., 4(2): 94–98. https://doi.org/10.18517/ijaseit.4.2.377

Wu, D.T., W.X. Li, J.J. Wan, Y.C. Hu, R.Y. Gan and L. Zou. 2023. A Comprehensive review of pea (*Pisum sativum L.*): chemical composition, processing, health benefits, and food applications. Foods., 12(13): 2527. https://doi.org/10.3390/foods12132527

Zayas, J.F. 1997. Functionality of protein in food. Springer Science and Business Media, Berlin, Germany.