

Impact of conventional processing on allergenic proteins in grasshopper-based meat analog

Anny Nila Syauqiyyah¹, Eny Palupi^{1*}, Chomdao Sinthuvanich², Rimbawan Rimbawan¹, and Ade Chandra Iwansyah³

¹Department of Nutrition Science, IPB University, 16680 Bogor, Indonesia

²Department of Biochemistry, Kasetsart University, 10900 Bangkok, Thailand

³Research Center for Food Technology and Processing, National Research and Innovation Agency Indonesia, 55861 Yogyakarta, Indonesia

Abstract. Edible insects are increasingly recognised as sustainable protein sources due to their high nutritional value and low environmental impact. However, the allergenic potential of insect-derived ingredients, particularly after processing, remains poorly understood. This study assessed the effect of conventional processing on protein distribution and allergenicity in grasshopper (*Valanga nigricornis*) based meat analog compared with unprocessed grasshopper powder. Protein extraction using PBS (pH 7.4) separated samples into supernatant and pellet fractions, followed by protein quantification, SDS-PAGE profiling, and immunodetection with anti-tropomyosin and anti-arginine kinase antibodies. Pellet fractions showed higher protein concentrations in both grasshopper powder (48.56 mg/mL) and meat analog (69.04 mg/mL) than in supernatants (4.03 mg/mL and 2.39 mg/mL), indicating the predominance of insoluble proteins. SDS-PAGE revealed reduced solubility and increased aggregation in the processed analogs. Western blotting detected tropomyosin (~36–38 kDa) mainly in the supernatant, with decreased intensity after processing. Arginine kinase (~40 kDa) appeared in both fractions, suggesting its partial entrapment in the insoluble matrix. Overall, processing reduces but does not eliminate allergenic proteins, highlighting the need for thorough allergen risk assessment in the development of insect-based meat analogs.

1 Introduction

The continuous rise in global population, projected to reach 9.7 billion by 2050, has intensified the demand for accessible, safe, and sustainable protein sources. Indonesia, as the fourth most populous nation, faces persistent nutritional challenges, with more than 53% of its population consuming protein below recommended levels and high rates of stunting (30.8%) and underweight (17.7%) still reported [1]. These nutritional deficits are further compounded by inequalities in protein accessibility across socioeconomic groups. At the

* Corresponding author: enypalupi@apps.ipb.ac.id

same time, conventional livestock production imposes substantial environmental burdens, contributing up to 20% of total greenhouse gas emissions while requiring vast land and water resources. These factors collectively underscore the urgent need for alternative protein sources that are both nutritionally adequate and environmentally sustainable.

Edible insects have gained increasing attention as a promising protein alternative due to their high protein content, balanced amino acid profile, and lower ecological footprint. Insect farming requires 75% less water, 59% less land, and generates 65% fewer greenhouse gas emissions compared with beef production [1]. Among the notable species, the grasshopper *Valanga nigricornis* demonstrates high protein content comparable to poultry and ruminant meats and provides essential polyunsaturated fatty acids such as α -linolenic acid (ALA) [2]. These characteristics make insects valuable candidates for the development of processed food products such as meat analogs, which aim to deliver desirable texture and sensory properties while offering sustainable nutrition.

However, a major limitation in the utilization of insect-derived ingredients is their allergenic potential. Several insect proteins, including tropomyosin and arginine kinase, exhibit strong homology to allergens found in crustaceans and other arthropods, which may cause IgE-mediated cross-reactivity [3]. This concern remains one of the key barriers to broader social acceptance of insect-based foods. As insects are increasingly incorporated into structured products such as meat analogs, understanding how processing affects the solubility, stability, and immunoreactivity of these allergenic proteins becomes crucial for ensuring consumer safety [4].

Processing is known to induce substantial transformations in protein structure through denaturation, aggregation, hydrolysis, and epitope masking. Such modifications can either reduce or preserve allergenic epitopes depending on processing conditions. While fermentation with lactic acid bacteria, such as *Lactobacillus plantarum*, has been shown to enhance protein digestibility and modify protein structure due to its proteolytic activity [5], evidence regarding how conventional processing, such as heating, mixing, or structuring, affects allergenic proteins in insect-based foods remains limited. Moreover, interactions between insect proteins and other components in complex matrices like meat analogs may further influence protein extractability and detection.

Given the rapid growth of insect-based food innovation and the necessity of ensuring consumer safety, it is critical to evaluate how processing alters the distribution and immunoreactivity of key insect allergens. Therefore, this study aims to determine the impact of conventional processing on protein solubility, structural integrity, and detectability of tropomyosin and arginine kinase in *Valanga nigricornis*-based meat analog compared with unprocessed grasshopper powder. Through protein quantification, SDS-PAGE profiling, and immunoblot analysis, this research provides essential insights for allergen risk assessment and supports the safe development of sustainable insect-based protein products.

2 Methods

2.1 Sample preparation

Grasshopper (*Valanga nigricornis*) powder was used as the unprocessed baseline sample, while a processed grasshopper-based meat analog was prepared as the treatment sample. Fresh Javanese grasshoppers were thoroughly washed, soaked in a 0.3% sodium bicarbonate solution for 10 minutes, and blanched at 70–80 °C for 3 minutes. The blanched grasshoppers were dried in a tray dryer at 50 °C for approximately 24 hours, then ground with a laboratory blender and sieved using a 100-mesh sieve to obtain fine homogenized grasshopper powder. This powder served as the unprocessed reference sample [6].

Supporting ingredients for the meat analog included kidney-bean tempeh flour and glucomannan. Kidney beans were washed, boiled for 10 minutes, soaked in a 0.5% vinegar solution (pH 4–5) for 7 hours, dehulled, and steamed at 100 °C for 15 minutes. After cooling, a 0.2% (w/w) *Rhizopus* starter was added, followed by fermentation at 25–27 °C for 48 hours. The resulting tempeh was dried at 50 °C for 24 hours, ground, and sieved with a 100-mesh sieve. Glucomannan powder (Ikarie brand, 100% purity) was used as a gel-forming agent. To prepare the meat analog, glucomannan was mixed with ice water until a gel formed. Grasshopper powder, kidney-bean tempeh flour, gluten, spices (onion, garlic, tomato sauce, soy sauce, pepper, salt, beef extract powder), cooking oil, and 1% (w/w) transglutaminase were added and homogenized using a food processor. The dough was kneaded until smooth and shaped into 80 g patties. Enzymatic cross-linking was optimized by steaming the patties at 45 °C for 30 minutes, followed by a final steaming at 100 °C for 30 minutes to complete cooking and protein structuring. The cooked patties were dried and milled to achieve a uniform powder comparable in particle size to the unprocessed grasshopper sample. All samples were stored at 4 °C in airtight polyethylene containers prior to analysis to prevent microbial growth and protein degradation [7].

2.2 Protein extraction

Protein extraction was conducted using phosphate-buffered saline (PBS, pH 7.4) to separate proteins based on their solubility. Prior to extraction, all dried samples were finely milled using a laboratory grinder and passed through a 100-mesh sieve to ensure uniform particle size. Each sample (10 g) was first defatted using food-grade ethanol at a ratio of 1:5 (w/v). The mixture was stirred for 10 minutes at room temperature, after which the solvent was decanted. The defatting procedure was repeated twice to ensure maximal lipid removal. The resulting pellet was then air-dried in a fume hood for 3–4 hours to eliminate residual solvent. For protein extraction, 10 g of the defatted powder was suspended in 100 mL of PBS buffer (pH 7.4). The suspension was homogenized using a tissue blender for 5 minutes to ensure complete dispersion of the sample in the extraction buffer. The mixture was incubated on an orbital shaker for 30 minutes at 4 °C to facilitate protein solubilization, followed by centrifugation at 12,000 × g for 15 minutes at 4 °C. After centrifugation, the supernatant was carefully collected as the soluble protein fraction. The pellet, representing the insoluble protein fraction, was washed once with PBS (1:3 w/v), vortexed briefly, and centrifuged again under the same conditions to remove residual soluble proteins. Both fractions were transferred into labeled tubes. The soluble fraction was stored at –20 °C for subsequent quantification and characterization, while the insoluble pellet was lyophilized and kept at –20 °C until analysis. Protein concentration in the soluble fraction was determined using the Bicinchoninic Acid (BCA) assay with bovine serum albumin (BSA) as the standard.

2.3 Protein quantification

Protein concentrations in both supernatant and pellet fractions were quantified using the Bradford assay, with bovine serum albumin (BSA) serving as the standard calibration protein. Each sample was incubated with Bradford reagent, and absorbance was measured at 595 nm using a spectrophotometer. The absorbance values were compared to the BSA standard curve to determine final protein concentrations. This analysis allowed the assessment of protein distribution differences between unprocessed grasshopper powder and the processed meat analog.

2.4 SDS-PAGE analysis

Protein profiling was conducted using SDS-PAGE following the Laemmli electrophoresis protocol. All reagents and materials for SDS-PAGE were sourced from Abcam plc, Cambridge, United Kingdom. Samples from both fractions were mixed with sample buffer containing SDS and β -mercaptoethanol and subsequently heated to ensure complete denaturation. Each sample was loaded into gel wells alongside molecular weight markers, and electrophoresis was run at a constant voltage until optimal band separation was achieved. Gels were stained with Coomassie Brilliant Blue and destained to visualize protein bands clearly. Band patterns were examined to evaluate changes in solubility, degradation, and aggregation induced by conventional processing.

2.5 Immunoblotting (Western Blot)

The identification of allergenic proteins, particularly tropomyosin and arginine kinase, was performed via Western blotting. After SDS-PAGE separation, proteins were transferred onto PVDF membranes using a semi-dry transfer system. All Western blot reagents and apparatus were obtained from Bio-Rad Laboratories Ltd., Hercules, California, USA. Membranes were blocked to prevent nonspecific binding and incubated with primary antibodies specific to tropomyosin and arginine kinase. Following washing, membranes were incubated with enzyme-conjugated secondary antibodies to amplify detection signals. Chemiluminescent substrate was applied for visualization, and band intensities were analyzed to compare allergen detectability between unprocessed and processed samples. This technique provided insights into structural integrity and immunoreactivity of target allergens after processing.

2.6 Data interpretation

All data generated from protein quantification, SDS-PAGE profiles, and Western blot signals were analyzed descriptively to determine the effects of processing on protein distribution and allergenicity. Comparisons between unprocessed grasshopper powder and the processed meat analog were evaluated based on protein concentration differences, solubility patterns, aggregation presence, and allergen band intensity. These interpretations were used to assess whether conventional processing reduced, maintained, or altered the presence of tropomyosin and arginine kinase, two major insect allergens, in *Valanga nigricornis*.

3 Results and discussion

3.1 Protein quality and solubility distribution

Protein quantification revealed that the majority of proteins in both grasshopper powder and the grasshopper-based meat analog were localized in the pellet fraction, whereas the supernatant contained considerably lower protein concentrations (4.03 vs. 2.39 mg/mL). In contrast, pellet fractions showed markedly higher levels (48.56 vs. 69.04 mg/mL), indicating that most proteins were in an insoluble state following processing. This distribution pattern demonstrates that extensive denaturation and aggregation occurred during the processing of the meat analog, resulting in reduced solubility and greater sedimentation during centrifugation. Such behavior is consistent with previous findings on plant-based meat analogs, in which thermal and mechanical processing commonly reduces protein solubility and promotes insoluble matrix formation [8].

The reduction of soluble proteins in the meat analog aligns with literature on high-moisture extrusion (HME), where heat and intense shear trigger protein unfolding and aggregation, producing structures that fail to dissolve in mild buffers such as PBS or even SDS [9]. This effect is reflected in the weak protein bands observed in the supernatant of the meat analog (Lane 2), suggesting that most proteins underwent structural transformation that shifted them from the soluble phase into the insoluble protein network. Prior studies demonstrate that during HME, unfolding caused by thermal–mechanical input promotes the formation of disulfide bonds, hydrophobic interactions, and hydrogen bonding, yielding dense, aggregated protein [10]. This mechanism explains the strong protein signals seen in the pellet fraction (Lane 4), which contains accumulated insoluble proteins that were integrated into the structured network of the meat analog. Furthermore, extrusion-driven restructuring—producing anisotropic, fibrous textures resembling meat—has been well-documented to reduce solubility by stabilizing protein–protein cross-linking [11].

Similarly, thermomechanical processing increases hydrophobic interactions and disulfide cross-linking, reducing extractability and promoting accumulation in the solid fraction [12]. Overall, these findings show that conventional processing substantially reduces protein solubility, leading to a predominance of aggregated, insoluble proteins in the pellet fraction. The degree of solubility loss and protein redistribution observed in this study mirrors the biochemical mechanisms described in extrusion-based analog meat systems and confirms that the processing of *Valanga nigricornis*–based meat analog induces extensive structural modification.

3.2 SDS-PAGE protein profiling

The SDS-PAGE results further support the observed protein redistribution. The supernatant of grasshopper powder displayed distinct bands ranging from >100 kDa to approximately 20–30 kDa, reflecting the diversity of soluble proteins typically found in unprocessed insect tissue. In contrast, the supernatant of the meat analog exhibited noticeably weaker and fewer bands, indicating that processing substantially reduced solubility or caused extensive aggregation beyond the resolving capacity of SDS-PAGE.

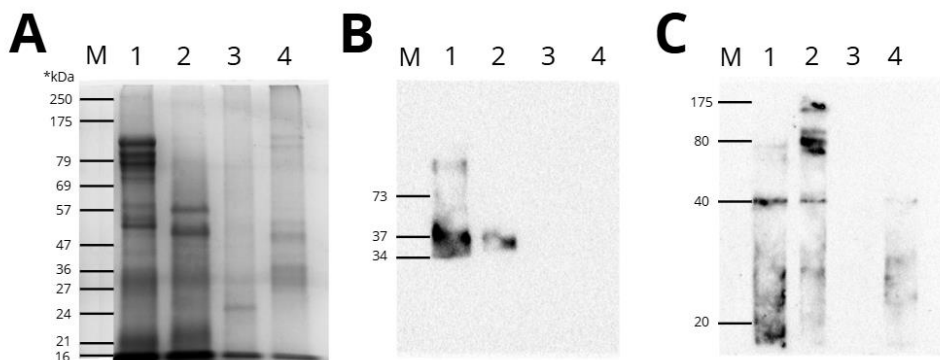


Fig. 1. Analysis of crude protein extracts from grasshopper (*Valanga nigricornis*). (A) SDS-PAGE; (B) Immunoblotting probed with an anti-shrimp tropomyosin monoclonal antibody; (C) Immunoblotting probed with an anti-prawn arginine kinase polyclonal antibody. Lane M: protein marker; lane 1: supernatant of grasshopper powder; lane 2: supernatant of meat analog; lane 3: pellet of grasshopper powder; lane 4: pellet of meat analog. Protein loading for SDS-PAGE was 40 µg per lane, for immunoblotting analysis was 40 µg per lane.

The pellet of grasshopper powder showed minimal band intensity, consistent with the dominance of non-protein components such as chitin in the insoluble portion. Conversely,

the pellet of the meat analog exhibited prominent and multiple protein bands, consistent with the aggregation and cross-linking expected from thermomechanical processing. These results follow the pattern described in literature where extrusion restructures proteins into dense networks, yielding insoluble aggregates that remain detectable on SDS-PAGE despite being absent from the soluble fraction [10, 13].

The markedly reduced solubility of meat analog proteins, combined with increased pellet-associated bands, reflects the extrusion-induced formation of anisotropic structures, which arise from heat-induced unfolding and subsequent intermolecular cross-linking [11]. Thus, the SDS-PAGE data corroborate the shift of proteins from soluble to insoluble fractions due to processing-induced structural modification.

3.3 Immunoblotting of tropomyosin and arginine kinase

Immunoblotting for tropomyosin revealed strong immunoreactive bands at ~36–38 kDa in the supernatant of grasshopper powder, consistent with its role as a major pan-allergen in arthropods. In the meat analog supernatant, band intensity noticeably decreased, suggesting that processing led to partial denaturation or epitope masking that reduced antibody recognition. Tropomyosin was absent from both pellet fractions, confirming that this protein is predominantly soluble and does not stably incorporate into insoluble matrices after processing. These observations align with evidence that tropomyosin is a heat-stable allergen whose immunoreactivity can persist despite moderate degradation, as shown in *Hermetia illucens* [12].

For arginine kinase (AK), immunoblots detected strong reactive bands at ~40 kDa in the supernatant of grasshopper powder and weaker, but still detectable, bands in the supernatant of the meat analog. Interestingly, AK also appeared in the pellet fraction of the meat analog but not in the pellet of grasshopper powder. This suggests partial entrapment of AK within insoluble aggregates formed during processing, reflecting its known structural stability and potential for cross-linking under thermomechanical conditions. The persistence of AK immunoreactivity agrees with reports demonstrating its resilience to heat and digestion, as well as its well-established cross-reactivity with allergens from crustaceans and mites [12, 14]. Cross-reactive IgE binding at ~40 kDa has been repeatedly documented in various insect species, reinforcing AK's classification as a dominant allergen among edible insects. The present study also confirms that both tropomyosin and AK remain detectable after processing, although their distribution and solubility may shift [15].

4 Conclusion

This study demonstrated that conventional processing substantially alters protein distribution and solubility in *Valanga nigricornis*-based meat analogs, promoting protein aggregation into insoluble fractions while reducing soluble protein content. Although processing modified the solubility and immunoreactivity of key insect allergens, tropomyosin and arginine kinase remained detectable, with tropomyosin showing reduced immunoreactivity and arginine kinase present in both soluble and insoluble fractions. Overall, these findings indicate that processing can modify but not eliminate allergenic proteins, underscoring the need for comprehensive allergen risk assessment and the exploration of advanced strategies, such as controlled hydrolysis or fermentation, to further reduce allergenicity while maintaining nutritional quality.

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