Techno-functional and rheological properties of *Tenebrio molitor* larvae protein by different extraction methods

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Abstract

Alkaline- or salt-assisted extractions have been widely used to extract edible insect proteins, however, there is a need for extraction techniques that balance cost-efficient production as well as preserving the protein properties. Mealworm proteins (*Tenebrio molitor* larvae) were extracted using three different extraction methods–alkali (AMP), salt (SMP), and water (WMP)–and then physicochemical and techno-functional properties were examined. AMP had high yield, protein, and amino acid contents, whereas WMP had high moisture, ash, and fat contents. SDS-PAGE showed a wide range of molecular weights in WMP whereas mostly low molecular weights were observed in AMP and SMP. AMP had poor protein solubilities compared to SMP and WMP across all pHs. AMP had enhanced water-holding capacity and emulsion stability, whereas WMP had improved oil-holding capacity and foaming properties. WMP formed a gel with and without the transglutaminase. The physicochemical and techno-functional properties demonstrated that water-soluble mealworm protein was superior to alkali- and salt-soluble mealworm proteins. Considering the cost efficiency and minimal impact on the environment as well, a cold press juicer could be utilized for mass production of mealworm protein compared to the conventional methods of protein extraction using alkali and salt.

Introduction

Sustainable food sources, such as insect-based proteins replacing animal-based proteins, have been continuously sought to address the challenges of overpopulation (Van Huis *et al*., 2013). Mealworm (*Tenebrio molitor* larvae), as one of the most common edible insects, is a promising alternative protein containing 14-25 g of protein per 100 g fresh weight, which was comparable to that of raw beef, 19-26 g protein/100 g fresh weight (Van Huis *et al*., 2013). In addition to being rich in fatty acids, minerals, and vitamins, mealworm protein is nutritious by exceeding most of the essential amino acids for humans required by the World Health Organization (Zhao *et al*., 2016). Moreover, farming mealworms is environmentally friendly; the water footprint, global warming potential, and land use per edible mealworms were 3.5, 5.5-12.5, and 7.9-14.1 times lower than those per edible beef, respectively (Miglietta *et al*., 2015; Oonincx and De Boer, 2012). Thus, a number of current researches (177 publications during 2016-2020) have actively investigated the nutritional

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compositions, physicochemical and techno-functional characteristics of mealworm to increase its availability and acceptance as food ingredients (Gkinali *et al*., 2022).

Mealworm protein has the potential as an alternative food constituent with enhanced techno-functionalities compared to animal proteins, although results might be difficult to compare due to variations in methods and calculations. Compared to commercial whey protein isolate, a small quantity of protein extract from mealworms was able to stabilize oil-in-water emulsions with improved interfacial activity and adsorption properties (Gould and Wolf, 2018). The foaming capacity and stability of mealworm proteins were comparable to egg albumin (Borremans *et al*., 2020) and superior to whey protein isolate (Anusha and Negi, 2023). Gelation is a critical attribute in food applications. Recently, mealworm protein has been studied by incorporating it into myofibrillar protein gels of chicken or pork, which had been shown to improve (Wang *et al*., 2023) or impair (Choi *et al*., 2023; Kim *et al*., 2019) the gelling abilities.

A variety of methods have been employed to extract mealworm proteins and investigate the changes in their physicochemical and techno-functional properties. Alkali extraction, followed by isoelectric precipitation, is a widely utilized method to purify and concentrate insect proteins (Pan *et al*., 2022). The addition of salts, mostly NaCl, aids in protein extraction by modifying the ionic strength of media, which promotes protein and water interaction and then improves the characteristics of mealworm proteins (Gkinali *et al*., 2022; Jiang *et al*., 2021; Kim *et al*., 2019). However, the alkali extraction is usually combined with a heating step (Anusha and Negi, 2023; Jiang *et al*., 2021) which possibly alters the mealworm proteins. Additionally, an extra step, such as dialysis during salt extraction, may be necessary to eliminate salt residues that could interfere with protein functionalities, leading to reduced solubility (Zhao *et al*., 2016). Therefore, extraction techniques that balance cost-effective production while preserving the properties of proteins are needed.

This study aimed to compare the physicochemical and techno-functional properties of mealworm proteins extracted by alkali, salt, and water to determine the suitable extraction methods in terms of process efficiency as well as preservation of protein characteristics. Water extraction using a cold press juicer was applied in this study in consideration of its simple and quick processing suitable for large-scale production.

Materials and methods

Preparation of mealworm protein

Dried mealworm (*Tenebrio molitor* larvae) was purchased from Agricultural Co., Ltd. (Soil-Nong, Korea). Ethyl alcohol (99.5%) at a ratio of 1:5 (*w/v*) was used to remove fat from the dried mealworm powder using a shaking incubator at 40℃ for 1 h. The procedure was repeated five times, then the defatted mealworm powder was placed under a fume hood at 20℃ overnight, sieved (0.36 mm), and stored at -20℃ before use.

For an alkali-soluble mealworm protein extraction by the modified method of Zhao *et al*. (2016), the defatted mealworm powder was dispersed in 0.25 M NaOH at a ratio of 1:15 (*w/v*) and shaken at 40℃ for 1 h. Centrifugation was performed twice at 4,360 × *g* at 4℃ for 20 min to collect the supernatant. After adjusting the supernatant pH to 4.4 with 1N HCl and 1N NaOH, the pellet was collected using a centrifuge at 2,136 × *g*, 4℃ for 10 min. Then the pellet was washed with 100 mL of distilled water twice using a centrifuge at 2,407 × *g*, at 4℃ for 10 min to remove salts and freeze-dried for 36 h using a vacuum freeze dryer (EYELA FDU-12AS; Tokyo Riakikai Co., Ltd., Tokyo, Japan). The sample was referred to as alkali-soluble mealworm protein (AMP).

For a salt-soluble mealworm protein extraction by the modified method of Sun and Arntfield (2010), the defatted mealworm powder was mixed with 0.1 M NaCl (pH 8) at a ratio of 3:10 (*w/v*). Extraction was performed in a shaking incubator at 20℃ for 24 h. Then the supernatant was collected after centrifugation (4,500 × *g*, 4℃, 20 min) and freeze-dried. The sample was referred to as salt-soluble mealworm protein (SMP).

For a water-soluble mealworm protein extraction, frozen mealworms (Hek-Nang Co, Korea) were thawed at 20℃ for 6 h, and a liquid was extracted from the frozen mealworms by a juicer (Cold press juicer NJ-120K, NUC Co, Korea). The obtained liquid was sieved through a stainless-steel filter (100 μm), diluted with distilled water containing 0.02% L-ascorbic acid, and centrifuged at 15,000 × *g*, 4℃, 30 min to collect the supernatant. The supernatant was freeze-dried and referred to as water-soluble mealworm protein (WMP).

Proximate composition analysis

Moisture, ash, and crude fat contents were determined according to the method described by Horwitz (2000). Moisture content was examined by heating each sample in an oven at

105°C overnight. Ash content was determined by incinerating each sample in a muffle furnace at 600°C for 6 h. Crude fat content was measured according to the Soxhlet method using an auto extractor (HSOX-6; Hanil Co.; Seoul, Korea). The moisture, ash, and crude fat were measured gravimetrically.

Protein and amino acid composition

Protein content was determined according to the Kjeldahl method using an auto digestor (HDG-P, Hanil Co., Seoul, Korea) and distillation unit (HKD-P, Hanil Co., Seoul, Korea) with conversion factors (5.60 for alkali- and salt-soluble mealworm protein, 4.76 for water-soluble mealworm protein) (Janssen *et al.*, 2017). For an amino acid composition, each sample was hydrolyzed by 6 N HCl at 105°C for 24 h in an acid hydrolysis tube (Thermo Scientific, Rockford, USA), filtered through 0.45 µm filter, and diluted (a dilution factor of 100) with Milli-Q water. A high-performance liquid chromatography (YL 9100, YL instrument, Anyang, Korea) equipped with an AccQ-Tag column (3.9 × 150 mm, Waters, Milford, MA, USA) and a fluorescence detector (FP-4020; Jasco, Tokyo, Japan) were used to determine the amino acid contents. The detection was set at 37°C with 250 nm wavelength of excitation and 395 nm of wavelength of emission according to the manufacturer's protocol (AccQ-Tag Chemistry package, WAT052875, Waters Co). A standard curve was prepared by a standard reagent (Waters Co., Milford, USA) containing 17 amino acids, and the concentration of amino acids in the sample was determined by the standard curve.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

A molecular weight was determined by SDS-PAGE with a modified method of Kao *et al.* (2003). Each sample (0.3 g) was dispersed in 20 mL Tris-glycine buffer (86 mM Tris, 90 mM glycine, and 4 mM Na_2EDTA, pH 8.0) and sonicated at 60°C for 90 min in a sonication water bath (JAC Ultrasonic 2010P; Jinwoo Engineering Co., Ltd.; Gyeonggi, Korea) to fully extract the protein. The dispersion was centrifuged at 12,000 × g, 4°C for 20 min and the protein content was determined by bicinchoninic acid (BCA) assay (Thermo Fisher Scientific), as described by Smith *et al.* (1985). Each dispersion containing 40 µg of protein was mixed with 6x Laemmli loading buffer containing mercaptoethanol, and heated at 95°C for 5 min. After cooling, an aliquot of the sample was loaded on a 4-20% precast gel (Luminano; Seoul, Korea). An aliquot of protein standard

(GenDEPOT; Barker, TX, USA) was loaded into another well of the gel as a molecular-weight marker. Electrophoresis was run at 120 V for 80 min with running buffer (0.025 M Tris-HCl [Sigma-Aldrich Chemical Co.; St. Louis, MO, USA], 0.192 M glycine [Daejung], and 0.1% w/v SDS [Bio-Rad Laboratories Inc.; Hercules, California, USA], pH 8.3). The gel was stained in Coomassie Brilliant Blue R-250 staining solution (Bio-Rad Laboratories Inc.) for 1 h and destained with de-staining solution containing 50% (v/v) methanol and 10% (v/v) acetic acid.

Fourier transform-infrared spectroscopy (FTIR) analysis

FTIR spectra of each sample were scanned using an ATR-FTIR spectrometer (Cary 630; Agilent Inc.; Santa Clara, CA, USA) in the wavelength range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. Data were analyzed using ResolutionsPro Software (version 5.2.0 (CD 846), Agilent Technologies, Inc., Australia) and MicroLab PC software (Agilent Technologies).

Protein solubility

Protein solubility of mealworm protein was measured based on the modified method of Son *et al.* (2020). Each sample (0.5 g) was dispersed in 20 mL of distilled water and the pH value was adjusted to 3, 5, 7, 9, and 12 with 1 N HCl and 1 N NaOH. The dispersion was centrifuged at 2,000 × g, 25°C for 20 min to collect the supernatant. The amount of protein in the supernatant was measured using Bradford assay at 595 nm (Multiskan Sky, Thermo Fisher Scientific Inc.). The protein solubility (%) was calculated as a percentage dividing the soluble protein content at a defined pH by the soluble protein content at pH 12.

Particle size and zeta-potential

Each sample (1%) was dispersed in 0.01 M Tris buffer (pH 8.0) and sonicated in an ultrasonic water bath (JAC Ultrasonic 2010P), followed by agitation and centrifugation at 1,000 × g 25°C for 1 h. After centrifuge, the supernatant was filtered through 0.45 µm filter paper, and the particle size and zeta potential were measured using a Nano-ZS Zetasizer (Nano ZS, Malvern Instruments Ltd., UK).

Sulfhydryl group and disulfide bond contents

Sulfhydryl group (SH) and disulfide bond (SS) contents were determined according to the method described by Deng *et al.* (2019) with some modifications. For a total SH content, each

sample (15 mg) was dispersed in 10 mL Tris-Gly buffer (pH 8.0), added 100 μL of 2-mercaptoethanol, and incubated at 25°C for 1 h. Then, 20 mL of 12% trichloroacetic acid (TCA) was added and centrifuged at 8,000 × g, 25°C for 10 min to separate the precipitate. The precipitate was washed with 10 mL of 12% TCA three times and dissolved in 10 mL Tris-Gly buffer and 50 μL Ellman's reagent (5,5'-dithio-bis-2-nitrobenzoic acid in 4 mg/mL Tris-Gly buffer). The absorbance was measured at 412 nm and the buffer containing Ellman's reagent was used as a blank. For a free SH content, each sample (30 mg) was dissolved in 10 mL of Tris-Gly buffer (pH 8), added 50 μl of Ellman's reagent, incubated at 25°C for 1 h, and centrifuged at 8,000 × g, 25°C for 5 min to collect the supernatant. The absorbance of the supernatant was measured at 412 nm. SH and SS contents were calculated as follows: SH content $(\mu M/g) = (73.53 \times A_{412} \times D) / C)$, where A_{412} is the absorbance at 412 nm, D is the dilution factor, and C is the protein concentration (mg/mL). SS content = (Total SH value – Free SH value) / 2)

Surface hydrophobicity

Surface hydrophobicity was evaluated by a modified method of Wan *et al*. (2021). A set of each mealworm protein dilution (0.02-0.1 mg/mL) were prepared in 0.1 M phosphate buffer (pH 7.4). Then 1 mL of the diluted mealworm protein solution was mixed with 5 μL of 8 mM sodium 8-anilino-1-naphthalenesulfonate (ANS) and incubated in the dark for 10 min. ANS blank was prepared by mixing 1 mL of 0.1 M phosphate buffer and 5 μL of ANS. The fluorescence intensity (FI) of the dispersion was measured by a fluorescence spectrometer (F2500, Perkin Elmer, Watham, MA, USA) with excitation at 390 nm and emission at 470 nm at 25.8°C (Chen *et al*., 2018). A corrected FI was calculated by subtracting FI of ANS blank from FI of protein solution with ANS. A slope of linear regression of the corrected FI versus protein concentration was used as a surface hydrophobicity.

Techno-functional properties

Water and oil holding capacities were determined with a modified method of Stone *et al*. (2015). Each sample (0.5 mg) was suspended in 5 g of water/oil in a 50 mL screw cap centrifuge tube, vortexed for 10 s every 5 min for 30 min, and centrifuged at 2,000 × g for 15 min. The supernatant was carefully removed and the remaining pellet was weighed. Water/ oil holding capacity was calculated as a percentage of the pellet

weight to the original sample weight.

Emulsifying activity (EA) and emulsion stability (ES) were determined by a modified method by Pearce and Kinsella (1978). Each sample (1%, w/v) was prepared in 10 mM sodium phosphate buffer (pH 7.0) and stirred overnight at 4°C. The mealworm protein dispersion (5 g) was homogenized with 5 g of canola oil using a homogenizer (T25 Digital Ultra-Turrax, IKA Ltd., Staufen, Germany) at a speed of 7,200 rpm for 5 min. An aliquot (50 μL) of the emulsion was immediately pipetted from the bottom of the tube, diluted in 7.5 mL of 0.1% sodium dodecyl sulphate (SDS), vortexed for 10 s, and measured at an absorbance of 500 nm (A_0) using a microplate reader (Multiskan; Thermo Fisher Scientific). Emulsifying activity was calculated by multiplying A_0 by 2.303 (Son *et al*., 2020). After the prepared emulsion was allowed to stand for 10 min, the emulsion activity was measured. Emulsion stability was calculated as a percentage of the emulsifying activity at 10 min to the emulsifying activity at 0 min.

Foaming capacity (FC) and foaming stability (FS) were determined by a modified method by Son *et al*. (2020). Each sample (1%) was prepared in 10 mM sodium phosphate buffer (pH 7.0) and stirred overnight at 4°C. An aliquot (15 mL) of the dispersion was transferred into a narrow 500 mL glass beaker and foamed using a homogenizer (T25 Digital Ultra-Turrax, IKA Ltd., Staufen, Germany) at 7,200 rpm for 5 min. After homogenization, the foam was immediately transferred to a 100 mL graduated cylinder and the foam volume was recorded at time zero (V_0) and after 30 min (V_{30}). Foaming capacity was calculated as a percentage of the foam volume (V_0) to the original dispersion volume (V). Foaming stability was calculated as a percentage of the foaming capacity at 30 min (V_{30}) to the foaming capacity at 0 min (V_0).

Rheological properties

The dynamic rheological properties of soluble mealworm protein were determined by a modified method of Zhao *et al*. (2016). Each sample (15%, *w/v*) was dispersed in phosphate buffer (pH 7.0) and incubated at 4°C for 24 h. Then the supernatant was obtained after centrifugation (3,000 × g, 10 min, 4°C) and loaded on a lower plate of parallel-plate geometry (50 mm diameter and a gap of 1mm) which was attached to a rheometer (MCR 302; Anton Paar, GmbH, Graz, Austria). To avoid sample evaporation during measurement, a thin layer of paraffin oil was covered. Samples were equilibrated at 25°C

Table 1. Yield, proximate chemical composition, and amino acid analysis of mealworm proteins extracted by alkali, salt, and water

	AMP	SMP	WMP
Yield (%)	24.12	5.44	3.55
Moisture (%)	5.74±0.18[c]	7.81±0.05[b]	11.94±0.54[a]
Ash (%)	0.66±0.03[c]	9.23±0.03[b]	9.82±0.29[a]
Fat (%)	0.46±0.06[b]	0.21±0.07[c]	15.96±0.18[a]
Protein content (%)	83.06±0.42[a]	56.79±0.01[b]	44.56±0.06[c]
Total amino acids (mg/100g)	106.76±1.09[a]	79.07±1.56[b]	75.70±1.21[c]
Hydrophilic amino acids	41.94±1.02[a]	26.04±0.43[b]	20.60±1.29[c]
Hydrophobic amino acids	41.62±0.78[a]	31.52±0.16[b]	31.39±0.45[b]

Values are mean±standard deviation. [a-c]Different superscript letters in the same row are significantly different (Duncan's test, $p < 0.05$). AMP, alkali soluble mealworm protein; SMP, salt soluble mealworm protein; WMP, water soluble mealworm protein
Hydrophilic amino acids: Aspartic acid, Cysteine, Glutamic acid, Serine, Threonine, Tyrosine
Hydrophobic amino acids: Alanine, Glycine, Isoleucine, Leucine, Methionine, Phenylalanine, Proline, Valine

for 15 min, heated to 95°C at a rate of 5°C/min, kept at 95°C for 5 min and cooled to 25°C at a rate of 5°C/min. During the measurement, storage modulus (G'), loss modulus (G"), loss factor (tan δ), and complex viscosity (η) were determined using a strain of 0.1% which was within the linear viscoelastic range of the samples, and an angular frequency of 20 rad/s. Following the same procedure, the rheological properties of soluble mealworm protein were performed with an addition of transglutaminase (100 U Tgase/g protein; Kinry Food Ingredients Co., Ltd., Shanghai, China) and under incubation at 37°C for 1 h.

Statistical analysis

All experimental values were obtained in triplicate. Statistical analyses were analyzed by one-way analysis of variance with Duncan's multiple-range test which was performed using SPSS Statistical Package (SPSS 27.0; IBM; Armonk, NY, USA). The significance level was set at $p < 0.05$.

Results and Discussion

Yield, proximate composition, and amino acid of mealworm proteins

The yield, proximate chemical composition, and amino acid content of mealworm proteins extracted by alkali, salt, and water are presented in Table 1. Alkaline condition (AMP, 24.12%) the highest amount of protein from mealworms compared to other extraction conditions (SMP: 5.44%, and WMP: 3.55%).

The higher yield of protein from alkali extraction compared to those obtained from salt or aqueous extraction aligned with the results of (Osman and Simon-Sarkadi, 1991), who compared aqueous, salt, and alkaline protein extracts from lupine seeds. The moisture content of AMP, SMP, and WMP were 5.74%, 7.81%, and 11.94%, respectively. The moisture content of AMP and SMP were in accordance with previous studies that reported the moisture contents ranged from 3.08 to 10.33% (Boukil *et al.*, 2022; Gkinali *et al.*, 2022; Kim *et al.*, 2019) whereas that of WMP was relatively high that might be because WMP was extracted frozen mealworms without defatting procedure. The ash content of AMP, SMP, and WMP were 0.66%, 9.23%, and 9.82%, respectively. The fat content of mealworm protein extracted by alkali, salt, and water were 0.46%, 0.21%, and 15.96%, respectively. The highest fat content of water-soluble mealworm protein resulted from its simple extraction process of protein, which did not undergo fat removal. AMP had the highest protein content (83.06%) compared to those of SMP (56.79%) and WMP (44.56%) which were comparable to the results reported by Gkinali *et al.* (2022), who reported protein content of 67-75% for alkali-soluble mealworm protein, 62% for salt-soluble mealworm protein, and 45% for whole mealworm. Ionization of acidic and neutral amino acids at alkaline pH increased the solubility of mealworm proteins which possibly led to the high protein content of AMP (Pan *et al.*, 2022). In addition, alkaline conditions followed by acidic precipitation might remove undesirable components (fat and exoskeleton) and purify the mealworm protein (Brogan *et al.*, 2021). A total of 17

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amino acids, including 8 essential amino acids, were detected in mealworm proteins (data not shown). Total amino acids content of AMP, SMP, and WMP were 106.76 mg/100 g, 79.07 mg/100 g, and 75.70 mg/100 g of mealworm protein, respectively. The amount of hydrophilic amino acids in AMP, SMP, and WMP were 41.94 mg/100 g, 26.04 mg/100 g, and 20.60 mg/100g of mealworm protein, whereas those of hydrophobic amino acids in AMP, SMP, and WMP were 41.62 mg/100 g, 31.52 mg/100 g, and 31.39 mg/100 g of mealworm protein, respectively.

Molecular weight of mealworm proteins

SDS-PAGE of mealworm proteins extracted by alkali, salt, and water are shown in Figure 1 with molecular weight ranged from 6 kDa to 170 kDa. AMP and SMP had multiple bands with a molecular weight less than 56 kDa which suggested that alkali and salt extract several low molecular weight. The major bands of WMP were observed at approximately 70, 43, and below 17 kDa. Also, high molecular weight protein bands ranged from 170 kDa to 95 kDa were observed in WMP whereas AMP and SMP were in absence of high molecular weight protein bands, indicating that starting material (dried mealworm powder) and defatting condition at 40 °C for 1 h might remove high molecular weight of proteins from mealworms. Protein bands observed in *Tenebrio molitor* might originate from; anti-freeze type of proteins (8.5-13 kDa) including hemolymph proteins (12 kDa), cuticle proteins (14-32 kDa) such as chymotrypsin-like proteinase (24 kDa), melanization-inhibiting protein (43 kDa), β-glycosidase (59 kDa), trypsin-like protenase (59 kDa), melanization-engaging types of protein (85 kDa), and vitellogenin-like protein (160 kDa) (Yi *et al.*, 2013).

FTIR

The FTIR spectra of mealworm proteins showed changes in protein structure influenced by the extraction conditions (Figure 2). All spectra of mealworm proteins were dominated by a wide band from 3600 cm^{-1} to 3000 cm^{-1}, which is attributed to amide A (N-H and O-H stretching vibration) (Chen *et al.*, 2017). WMP had unique peaks at 2916 cm^{-1} and 2849 cm^{-1} (peaks 2 and 3, respectively) corresponding to aliphatic bonds (-CH$_2$) of lipids (Gkinali *et al.*, 2022), which was consistent with the high amount of fat in WMP (15.06%) compared to those in AMP (0.46%) and SMP (0.21%). The peaks at 1628 cm^{-1} and 1541 cm^{-1} (peaks 4 and 5, respectively) are linked to proteins, especially amide I (80% C=O stretch, 10% C-N stretch) and amide II (60% N-H bend, 30% C-N stretch, and 10% C-C stretch) (Chen *et al.*,
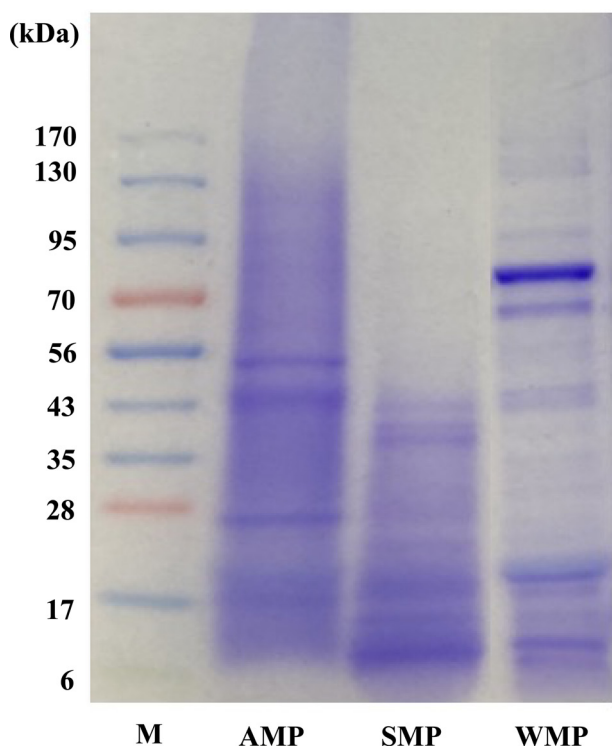
Fig. 1. SDS-PAGE of mealworm proteins extracted by alkali, salt, and water
M, protein marker; alkali soluble mealworm protein (AMP); salt soluble mealworm protein (SMP); water soluble mealworm protein (WMP)
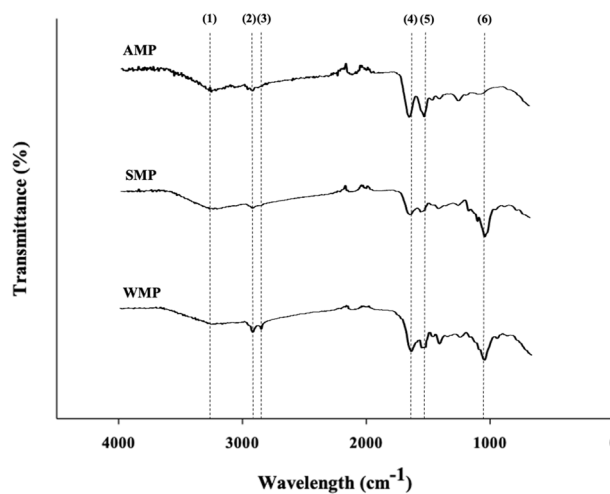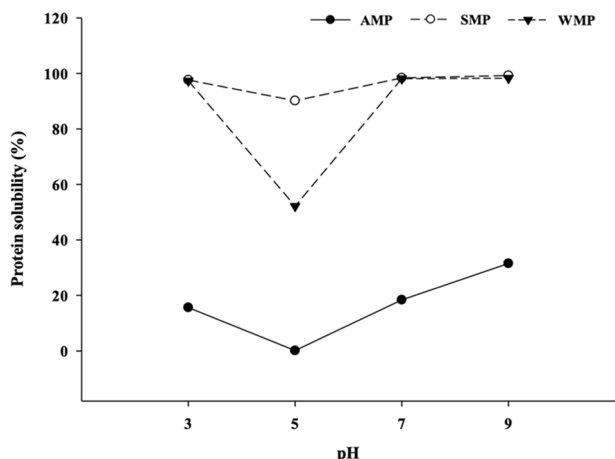

Fig. 2. FTIR spectra of mealworm proteins extracted by alkali, salt, and water
AMP, alkali soluble mealworm protein; SMP, salt soluble mealworm protein; WMP, water soluble mealworm protein. (1) 3274 cm^{-1}; (2) 2916 cm^{-1}; (3) 2849 cm^{-1}; (4) 1628 cm^{-1}; (5) 1541 cm^{-1}; (6) 1039 cm^{-1}

2017). These peaks were stronger in AMP, which was attributed to the higher content of protein (83.06%) compared to those in SMP (56.79%) and WMP (44.56%). The peak 6 (1039 cm^{-1}) was assigned to C-O-C stretching vibration of polysaccharide bonds which might be related to chitin or chitosan from mealworm's cuticles (da Silva Lucas *et al.*, 2021; Gkinali *et al.*, 2022). Alkali-extracted mealworm protein showed no peak at 1039 cm^{-1} whereas salt- and water-extracted mealworm proteins had strong intensities at peak 6, as a result of the harsh protein extraction processes.

Protein solubility of mealworm proteins

The changes in mealworm proteins solubility at different pHs are plotted in Figure 3. All mealworm proteins followed a typical U-shaped solubility with lowest solubility at pH 5 and higher solubilities at pH 3 and 9. The lowest solubility at pH 5 resulted from a decrease in electrostatic repulsion forces among

Fig. 3. Solubility of mealworm proteins extracted by alkali, salt, and water at pH 3, 5, 7, and 9
AMP, alkali soluble mealworm protein; SMP, salt soluble mealworm protein; WMP, water soluble mealworm protein

proteins at isoelectric pH causing protein aggregation (Azagoh *et al.*, 2016). Among samples, AMP had the lower solubility (below 40%) at entire pH ranges compared to WMP and SMP (above 60%). The solubility of AMP at pH 5 was 0.14% whereas those of SMP and PMP were 91.73% and 54.45%. During the extraction process, AMP was treated in alkaline solution at 40°C for 1 h whereas SMP and WMP underwent at room temperature. Heating temperature during extraction might influence the protein solubility which denature and aggregate proteins eventually leading to the low solubility (Stone *et al.*, 2015).

Physicochemical characteristics of mealworm proteins

The physicochemical characteristics of mealworm proteins extracted by alkali, salt, and water are summarized in Table 2. The particle sizes of AMP, SMP, and WMP were 242.75 nm, 128.80 nm, and 260.05 nm, respectively. The size and distribution of particles are important attributes to predict the quality of final products (Son *et al.*, 2019). Zeta-potential is an important indicator of colloidal solubility as high absolute zeta-potential values usually reflect particle repulsion and prevention of protein aggregation (Azagoh *et al.*, 2016; Gkinali *et al.*, 2022). The zeta-potential of mealworm proteins extracted by alkali, salt, and water at pH 8 were -14.90 mV, -15.90 mV, and -10.44 mV, respectively. (Gkinali *et al.*, 2022) reported consistent data of zeta-potential for protein extracted from *Tenebrio molitor* by alkali, isoelectric precipitation, and salt at pH 7 ranged from -11.49 mV to -17.4 mV.

Protein conformation is determined by the sulfhydryl group (SH) and disulfide bond (SS), which dictate the structural stability and rigidity of protein molecules (Hu *et al.*, 2010). The free SH content of AMP, SMP, and WMP were 38.12 μM/g, 40.86 μM/g, and 30.43 μM/g, respectively, whereas the SS

Table 2. Physicochemical characteristics of mealworm proteins extracted by alkali, salt, and water

	AMP	SMP	WMP
Particle size (nm)	242.75±0.49[b]	128.80±0.99[a]	260.05±4.74[c]
Zeta-potential (mV)	-14.90±0.85[b]	-15.90±0.42[b]	-10.44±0.94[a]
Free SH group (μM/g)	38.12±0.01[b]	40.86±0.10[a]	30.43±0.17[c]
SS bond (μM/g)	7.78±0.19[c]	22.22±0.01[b]	30.19±0.08[a]
Surface hydrophobicity (S_0)	$2.8×10^6$±$5.4×10^5$[c]	$7.6×10^6$±$1.2×10^5$[a]	$6.1×10^6$±$4.4×10^5$[b]

Values are mean±standard deviation. [a-c]Different superscript letters in the same row are significantly different (Duncan's test, $p < 0.05$). AMP, alkali soluble mealworm protein; SMP, salt soluble extracted mealworm protein; WMP, water soluble mealworm protein; SH, sulfhydryl group; SS, disulfide bond

Table 3. Techno-functional characteristics of mealworm proteins extracted by alkali, salt, and water

	AMP	SMP	WMP
Water holding capacity (g/g)	3.07±0.00	0.00±0.00	0.00±0.00
Oil holding capacity (g/g)	2.07±0.38[b]	2.29±0.32[b]	3.89±0.34[a]
Emulsifying activity	0.141±0.008[a]	0.144±0.002[a]	0.154±0.009[a]
Emulsion stability (%)	96.41±1.39[a]	92.53±1.84[b]	79.05±1.61[c]
Foaming capacity (%)	31.11±3.85[c]	51.11±7.70[b]	104.44±3.85[a]
Foaming stability (%)	56.67±5.77[b]	30.16±2.75[c]	93.61±0.24[a]

Values are mean±standard deviation. [a-c]Different superscript letters in the same row are significantly different (Duncan's test, $p < 0.05$). AMP, alkali soluble mealworm protein; SMP, salt soluble mealworm protein; WMP, water soluble mealworm protein; ND, not detected

bond content of AMP, SMP, and WMP were 7.78 μM/g, 22.22 μM/g, and 30.19 μM/g, respectively. Proteins with high levels of SH content are more prone to association or aggregation due to the formation of disulfide bonds (Tang *et al*., 2006). Proteins containing more disulfide bonds are likely to be stable in terms of their structure because the peptide chains are tightened by covalent bonds.

Surface hydrophobicity depends on exposed hydrophobic regions at the protein surface (Jiang *et al*., 2021). The surface hydrophobicity of mealworm protein extracted by AMP, SMP, and WMP were $2.8×10^6$, $7.6×10^6$, and $6.1×10^6$, respectively. Relatively high surface hydrophobicity was considered a substantial presence of non-aggregated proteins that exhibit rapid adsorption at the air or water interface. Thus, this parameter can be positively correlated with the techno-functional properties of proteins, such as emulsifying and foaming capacities (Deng *et al*., 2019).

Techno-functional characteristics of mealworm proteins

Techno-functional characteristics of AMP, SMP, and WMP are compared in Table 3. Water holding capacity (WHC) and oil holding capacity (OHC) are capacities to retain water or oil, which may determine the texture, tenderness, and juiciness of foods (Aryee *et al*., 2018). The WHC value of AMP was 3.07, whereas those of SMP and WMP were zero which indicated SMP and WMP were fully soluble in water. The WHC of mealworm proteins was consistent with the protein solubility at pH 7, with low solubility in AMP (below 20%) and high solubilities in SMP and WMP (approximately 100%). While, the OHC value of AMP, SMP, and WMP were 2.07, 2.29, and 3.89, respectively. WMP containing the highest fat content showed the

highest oil-holding capacity value compared to those of AMP and SMP ($p<0.05$). OHC, an ability to capture oil, is usually determined by the number of non-polar side chains present in proteins that bind the fatty acids in the oil (Al-Kahtani and Abou-Arab, 1993).

Emulsifying activity (EA) and emulsion stability (ES) are capacities of protein forming and stabilizing emulsion systems (Gkinali *et al*., 2022). For emulsifying activity, the quantity of oil that can be emulsified per mass of mealworm protein was measured. The EA of AMP, SMP, and WMP were 0.141, 0.144, and 0.154, respectively. Emulsion stability is a measure of strength that a formed emulsion resists to change during time period (Boye *et al*., 2010). The ES of AMP, SMP, and WMP were 96.41%, 92.53%, and 79.05 %, respectively.

Foaming capacity (FC) and foaming stability (FS) measure protein capacity to form and stabilize foams. It is an important factor in improving texture, consistency, and appearance in food applications (Zielińska *et al*., 2018). The FC of AMP, SMP, and WMP were 31.11%, 51.11%, and 104.44%, whereas those of FS were 56.67%, 30.16%, and 93.61%, respectively. WMP had the highest FC and FS compared to those of WMP and SMP ($p<0.05$).

Rheological properties of mealworm proteins

Rheological behaviors of mealworm proteins during oscillatory temperature sweep were presented in Figure 4. Significant differences in heat-induced gel formation were observed among mealworm proteins. AMP did not form a gel possessing no crossover between G' and G'' whereas SMP and WMP had a gelling point during the heating phase (25-95°C) of temperature sweep. This could be attributed to the harsh conditions of the extraction procedure causing a high degree
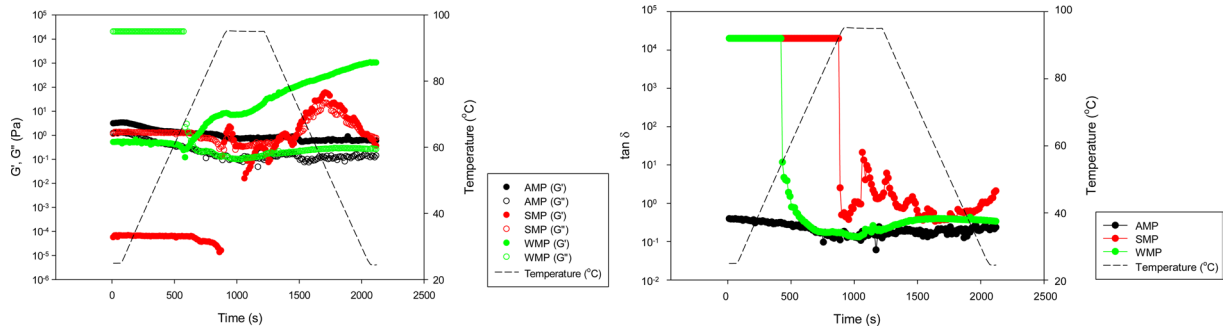
Fig. 4. Rheological behaviors of mealworm proteins extracted by alkali, salt, and water during temperature sweep.
AMP, alkali soluble mealworm protein; SMP, salt soluble mealworm protein; WMP, water soluble mealworm protein.
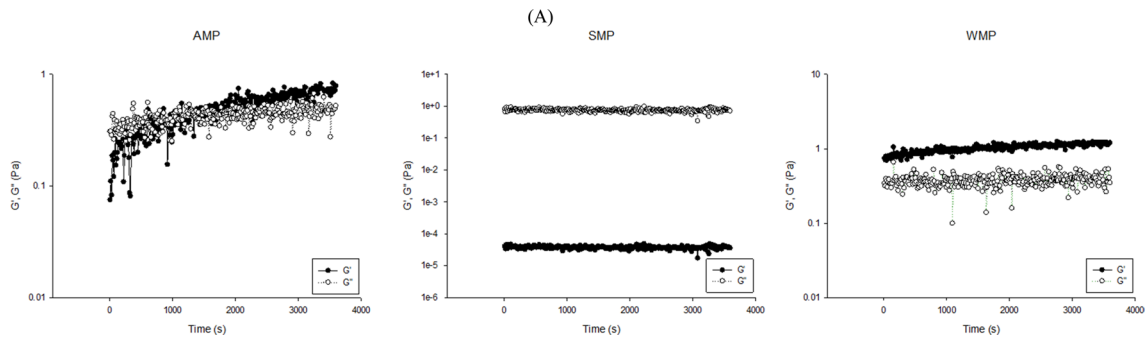
Fig. 5. G' and G" of mealworm proteins extracted by alkali, salt, and water in the presence of transglutaminase.
AMP, alkali soluble mealworm protein; SMP, salt soluble mealworm protein; WMP, water soluble mealworm protein.

of unfolding protein structure, which eventually reduced the gelation ability of protein (Yang *et al.*, 2021). Furthermore, the lowest protein solubility of AMP due to a high degree of protein denaturation might not provide enough protein amount, which is required for gelation. Interestingly, SMP and WMP formed a gel at 94.24°C and 68.21°C, respectively, where the liquid-like system (G'<G") changed to the solid-like system (G'>G") (Al-Kahtani and Abou-Arab, 1993). In the cooling phase (95-25°C) of the temperature sweep, the G′ and G″ values of WMP continued to increase steadily until the end of the cooling phase, whereas those values of SMP were inclined to increase from 95°C to 50-60°C then decrease to 25°C. Continuously increasing G' of WMP indicated that the network structure of WMP slowly formed and rearranged while it was cooled (Sun and Arntfield, 2010). Short-range interactions within the gel primary network, such as van der Waals, hydrogen bonding, and covalent bonds might be attributed (Shevkani *et al.*, 2015).

Rheological behaviors of mealworm proteins in the presence of transglutaminase were plotted in Figure 5. Transglutaminase catalyzes ε-(γ-glutamyl)-lysine crosslinking between glutamine and lysine residues (Schlangen *et al.*, 2023). With an addition of

the enzyme, AMP and WMP formed weak gels with increased G' demonstrating that the development of covalent cross-links converts soluble proteins into insoluble high molecular weight polymers (Sun and Arntfield, 2010). During the incubation, the mealworm proteins and their constituents were preheated which could expose the reactive groups and increase their capacity to react with one another (Gauche *et al.*, 2010). However, SMP did not form a gel with the enzyme because no substantial modulus changes occurred during the measurement. The presence of excessive sodium chloride in SMP might negatively affect the enzyme activity (Pérez-Mateos *et al.*, 2004). Possibly, the lowest amount of glutamic acid (10.33 mg/100 g) and lysine (4.51 mg/100 g) in SMP might not be enough making a gel compared to those of AMP (12.64 mg/100 g and 7.19 mg/100 g) and WMP (12.80 mg/100 g and 6.35 mg/100 g), respectively (data not shown).

Conclusions

The study demonstrated that the extraction processes differed physicochemical and techno-functional properties of mealworm

proteins (*Tenebio molitor* larvae). Among them, water-extracted mealworm protein could be used as an applicable ingredient for protein-fortified foods. Water-soluble mealworm protein showed high moisture, ash, and fat contents and enhanced oil-holding capacity and foaming properties. According to SDS-PAGE and rheology, water-soluble mealworm protein presented a variety of proteins with different molecular weights and formed a gel with and without the transglutaminase. Alkali-soluble mealworm protein was high in protein and amino acid contents but poor in protein solubility and gel-forming. Considering the cost efficiency, minimal impact on the environment, and the enhanced applications of water-soluble mealworm protein, a cold press juicer could be utilized as mass production of mealworm protein compared to the conventional methods of protein extraction using alkali and salt. In addition to the food application aspects, it would be worth to investigate the bioactive properties of mealworm proteins influenced by different extraction processes for future work.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

References

Al-Kahtani HA, Abou-Arab A (1993) Comparison of physical, chemical, and functional properties of Moringa peregrina (Al-Yassar or Al-Ban) and soybean proteins. Cereal Chem 70, 619-626.

Anusha S, Negi PS (2023) Characterization and techno-functional properties of Tenebrio molitor larvae protein concentrate. Food Biosci 54, 102882. https://doi.org/10.1016/j.fbio.2023.102882

Aryee A, Agyei D, Udenigwe C (2018) Impact of processing on the chemistry and functionality of food proteins; in Proteins in food processing. pp. 27-45, Elsevier, Amsterdam.

Azagoh C, Ducept F, Garcia R, Rakotozafy L, Cuvelier ME, Keller S, *et al.* (2016) Extraction and physicochemical characterization

of *Tenebrio molitor* proteins. Food Res Int 88, 24-31. https://doi.org/10.1016/j.foodres.2016.06.010

Borremans A, Bußler S, Sagu ST, Rawel H, Schlüter OK, Leen VC (2020) Effect of blanching plus fermentation on selected functional properties of mealworm (*Tenebrio molitor*) powders. Foods 9, 917. https://doi.org/10.3390/foods9070917

Boukil A, Marciniak A, Mezdour S, Pouliot Y, Doyen A (2022) Effect of high hydrostatic pressure intensity on structural modifications in mealworm (*Tenebrio molitor*) proteins. Foods 11, 956. https://doi.org/10.3390/foods11070956

Boye J, Aksay S, Roufik S, Ribéreau S, Mondor M, Farnworth E, *et al.* (2010) Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. Food Res Int 43, 537-546. https://doi.org/10.1016/j.foodres.2009.07.021

Brogan EN, Park YL, Matak KE, Jaczynski J (2021) Characterization of protein in cricket (*Acheta domesticus*), locust (*Locusta migratoria*), and silk worm pupae (*Bombyx mori*) insect powders. LWT 152, 112314. https://doi.org/10.1016/j.lwt.2021.112314

Chen X, Zhou R, Xu X, Zhou G, Liu D (2017) Structural modification by high-pressure homogenization for improved functional properties of freeze-dried myofibrillar proteins powder. Food Res Int 100, 193-200. https://doi.org/10.1016/j.foodres.2017.07.007

Choi JS, Kim GH, Kim HE, Kim MJ, Chin KB (2023) Evaluation of Gelation Properties of Salt-Soluble Proteins Extracted from *Protaetia brevitarsis* Larvae and *Tenebrio molitor* Larvae and Application to Pork Myofibrillar Protein Gel System. Food Sci Anim Resour 43, 1031-1043. https://doi.org/10.5851/kosfa.2023.e69

da Silva Lucas AJ, Oreste EQ, Costa HLG, López HM, Saad CDM, Prentice C (2021) Extraction, physicochemical characterization, and morphological properties of chitin and chitosan from cuticles of edible insects. Food Chem 343, 128550. https://doi.org/10.1016/j.foodchem.2020.128550

Deng Y, Huang L, Zhang C, Xie P, Cheng J, Wang X, *et al.* (2019) Physicochemical and functional properties of Chinese quince seed protein isolate. Food Chem 283, 539-548. https://doi.org/10.1016/j.foodchem.2019.01.083

Gauche C, Barreto PL, Bordignon-Luiz MT (2010) Effect of thermal treatment on whey protein polymerization by transglutaminase: Implications for functionality in processed dairy foods. LWT 43, 214-219. https://doi.org/10.1016/j.lwt.2009.08.009

Gkinali AA, Matsakidou A, Paraskevopoulou A (2022) Characterization of *Tenebrio molitor* larvae protein preparations obtained by different extraction approaches. Foods 11, 3852. https://doi.org/10.3390/

foods11233852

Gould J, Wolf B (2018) Interfacial and emulsifying properties of mealworm protein at the oil/water interface. Food Hydrocoll 77, 57-65. https://doi.org/10.1016/j.foodhyd.2017.09.018

Horwitz W (2000) Official Methods of Analysis of AOAC International. Journal of AOAC International.

Hu XZ, Cheng YQ, Fan JF, Lu ZH, Yamaki K, Li LT (2010) Effects of drying method on physicochemical and functional properties of soy protein isolates. J Food Process Preserv 34, 520-540. https://doi.org/10.1111/j.1745-4549.2008.00357.x

Janssen RH, Vincken JP, van den Broek LA, Fogliano V, Lakemond CM (2017) Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor, Alphitobius diaperinus*, and *Hermetia illucens*. J Agric Food Chem 65, 2275-2278. https://doi.org/10.1021/acs.jafc.7b00471

Jiang Y, Zhu Y, Zheng Y, Liu Z, Zhong Y, Deng Y, *et al.* (2021) Effects of salting-in/out-assisted extractions on structural, physicochemical and functional properties of *Tenebrio molitor* larvae protein isolates. Food Chem 338, 128158. https://doi.org/10.1016/j.foodchem.2020.128158

Kao FJ, Su NW, Lee MH (2003) Effect of calcium sulfate concentration in soymilk on the microstructure of firm tofu and the protein constitutions in tofu whey. J Agric Food Chem 51, 6211-6216. https://doi.org/10.1021/jf0342021

Kim TK, Yong HI, Jeong CH, Han SG, Kim YB, Paik HD, *et al.* (2019) Technical functional properties of water-and salt-soluble proteins extracted from edible insects. Food Sci Anim Resour 39, 643. https://doi.org/10.5851/kosfa.2019.e56

Miglietta P, De Leo F, Ruberti M, Massari S (2015) Mealworms for food: a water footprint perspective. Water 7, 6190-6203. https://doi.org/doi.org/10.3390/w7116190

Oonincx DG, De Boer IJ (2012) Environmental impact of the production of mealworms as a protein source for humans–a life cycle assessment. PLoS One 7, e51145. https://doi.org/10.1371/journal.pone.0051145

Osman MK, Simon-Sarkadi L (1991) Extraction and isolation of protein from lupine (*Lupinus termis L.*) seeds. Period Polytech Chem Eng 35, 65-70.

Pan J, Xu H, Cheng Y, Mintah BK, Dabbour M, Yang F, *et al.* (2022) Recent Insight on Edible Insect Protein: Extraction, Functional Properties, Allergenicity, Bioactivity, and Applications. Foods 11, 2931. https://doi.org/10.3390/foods11192931

Pearce KN, Kinsella JE (1978) Emulsifying properties of proteins: evaluation of a turbidimetric technique. J Agric Food Chem 26, 716-723. https://doi.org/10.1021/jf60217a041

Pérez-Mateos M, Amato PM, Lanier TC (2004) Gelling properties of Atlantic croaker surimi processed by acid or alkaline solubilization. J Food Sci 69, FCT328-FCT333. https://doi.org/10.1111/j.1365-2621.2004.tb06335.x

Schlangen M, Ribberink MA, Dinani ST, Sagis LM, van der Goot AJ (2023) Mechanical and rheological effects of transglutaminase treatment on dense plant protein blends. Food Hydrocoll 136, 108261. https://doi.org/10.1016/j.foodhyd.2022.108261

Shevkani K, Singh N, Kaur A, Rana JC (2015) Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study. Food Hydrocoll 43, 679-689. https://doi.org/10.1016/j.foodhyd.2014.07.024

Smith Pe, Krohn RI, Hermanson G, Mallia A, Gartner F, Provenzano M, *et al.* (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150, 76-85. https://doi.org/10.1016/0003-2697(85)90442-7

Son YJ, Choi SY, Hwang IK, Nho CW, Kim SH (2020) Could defatted mealworm (*Tenebrio molitor*) and mealworm oil be used as food ingredients? Foods 9, 40. https://doi.org/10.3390/foods9010040

Son YJ, Lee JC, Hwang IK, Nho CW, Kim SH (2019) Physicochemical properties of mealworm (*Tenebrio molitor*) powders manufactured by different industrial processes. LWT 116, 108514. https://doi.org/10.1016/j.lwt.2019.108514

Stone AK, Karalash A, Tyler RT, Warkentin TD, Nickerson MT (2015) Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. Food Res Int 76, 31-38. https://doi.org/10.1016/j.foodres.2014.11.017

Sun XD, Arntfield SD (2010) Gelation properties of salt-extracted pea protein induced by heat treatment. Food Res Int 43, 509-515. https://doi.org/10.1016/j.foodres.2009.09.039

Tang CH, Ten Z, Wang XS, Yang XQ (2006) Physicochemical and functional properties of hemp (*Cannabis sativa* L.) protein isolate. J Agric Food Chem 54, 8945-8950. https://doi.org/10.1021/jf0619176

Van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, *et al.* (2013) Edible insects: future prospects for food and feed security. Food and agriculture organization of the United Nations.

Wan Y, Li Y, Guo S. (2021). Characteristics of soy protein isolate gel induced by glucono-δ-lactone: Effects of the protein concentration during preheating. Food Hydrocoll 113, 106525. https://doi.org/10.1016/j.foodhyd.2020.106525

Wang M, Zhao Y, Wang M, Zhang Z, Huang M, Wang K, *et al.* (2023). Effects of Tenebrio molitor protein emulsion on the properties, and structure of myofibrillar protein gel. LWT 189, 115511. https://doi.org/10.1016/j.lwt.2023.115511

Int. J. Indust. Entomol. Biomater.
Vol. 48, No. (2), pp. 86-97 (2024)

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Yang J, Zamani S, Liang L, Chen L. (2021). Extraction methods significantly impact pea protein composition, structure and gelling properties. Food Hydrocoll 117, 106678. https://doi.org/10.1016/j.foodhyd.2021.106678

Yi L, Lakemond CM, Sagis LM, Eisner-Schadler V, van Huis A, van Boekel MA. (2013). Extraction and characterisation of protein fractions from five insect species. Food Chem 141, 3341-3348. https://doi.org/10.1016/j.foodchem.2013.05.115

Zhao X, Vázquez-Gutiérrez JL, Johansson DP, Landberg R, Langton M. (2016). Yellow mealworm protein for food purposes-extraction and functional properties. PLoS One 11, e0147791. https://doi.org/10.1371/journal.pone.0147791

Zielińska E, Karaś M, Baraniak B. (2018). Comparison of functional properties of edible insects and protein preparations thereof. LWT 91, 168-174. https://doi.org/10.1016/j.lwt.2018.01.058