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Effects of organic solvent on functional properties of defatted proteins extracted from *Protaetia brevitarsis* larvae

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ABSTRACT

This study investigated the effect of aqueous fat separation and defatting using organic solvents (99% methanol, ethanol, and *n*-hexane) on the characteristics and functionality of proteins extracted from *Protaetia brevitarsis*. The defatting efficiency, amino acid composition, protein solubility, and technical properties were the highest when proteins were defatted using *n*-hexane. Proteins defatted using ethanol were similar in foam capacity and emulsifying capacity. Surface hydrophobicity decreased when using organic solvents, and excessive fat content disrupted the functional properties of the extracted proteins. Proteins extracted using the different solvents displayed different pH values. The pH of the aqueous extract was the lowest. CIE L* a* b* color values also differed using the different extraction methods. Although *n*-hexane might be the most efficient solvent for defatting the proteins extracted from edible insects, ethanol could also be used to obtain similar foam and emulsifying capacities.

1. Introduction

Interest in edible insects and their use in food preparations have increased owing to food security. Edible insects are popular as major protein sources in various cultures in Asia and Africa (Yoon et al., 2020). Edible insects have been cooked whole with different methods include frying, roasting, and grilling (Feng et al., 2018). However, this consumption is not readily accepted by western civilization or people influenced by western culture (van Huis, 2013).

In western culture, insects can be considered unclean with an unpalatable appearance and processed insect-based foods could be a way to increase consumer acceptance of edible insects (Kim, Yong, Kim, Kim, & Choi, 2019; van Huis, 2013). Development of protein processing technologies that might overcome consumer prejudice concerning edible insects has been intensively studied (Mishyna, Chen, & Benjamin, 2020).

Protaetia brevitarsis (PB) has a excellent antithrombotic activity, and so has been used as a traditional medicine in East Asia (Lee, Lee, Kim, Hwang, Na & Bae, 2017). Furthermore, the technical properties of protein extracted from PB are higher than the properties of protein extracted from *Tenebrio molitor* (Kim et al., 2019). PB could be a potential food ingredient and protein source.

Previous studies of edible insects used hexane as the defatting solvent to enhance the functional properties of the extracted proteins (Mishyna, Martinez, Chen & Benjamin, 2019; Yi, Lakemond, Sagis, Eisner-Schadler, van Huis & van Boekel, 2013). Although hexane is the most frequently used solvent in other food industries, there are some critical disadvantages for the use of hexane as a defatting solvent (Russin, Boye, Arcand & Rajamohamed, 2011). Residual hexane could be detrimental to human health and regulation of the residue might be an obstacle to the use of edible insects as foodstuffs (Russin et al., 2011). Furthermore, because hexane is derived from fossil fuel, air pollution and its flammability could be problematic (Gandhi et al., 2003; Rosenthal et al., 1996). These drawbacks have prompted searches for alternative solvents and their effects on protein functionality have been investigated in various ways (L'Hocine, Boye & Arcand, 2006).

Among the alternatives, ethanol and methanol have been used for the processing of vegetable protein. Aqueous extraction can also be used to separate oil and protein from vegetable seeds based on the insolubility of oil in water (Russin et al., 2011). The effects of organic solvents on the protein characteristics of PB are unknown, as is the solvent efficiency for defatting. The present study investigated the effects of organic defatting solvents focused on proximate composition, amino acid composition, and technical properties of extracted PB

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proteins.

2. Materials and methods

2.1. Materials

Ten kilograms of frozen PB larvae were obtained in triplicate from a local market (Farm bang, Korea). The larvae were grown in cement boxes with fermented sawdust on the floor for three months. Larvae at the last instar stage were washed, degutted, and frozen. Methanol, ethanol, *n*-hexane, and other experimental reagents were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Reagents used for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) were obtained from Bio-Rad Lab, Inc. (Hercules, CA, USA).

2.2. Defatting, processing, and protein extraction

All extraction processes were conducted within 24 h after purchased of the frozen larvae. Twenty stainless steel trays, each containing 500 g of frozen larvae, were placed in a model LP100 freeze-dryer (Ilshinbiobase Co., Dongducheon, Korea) and freeze-dried (−30 °C for 60 min, −20 °C for 600 min, −10 °C for 600 min, and 0 °C for 600 min). The freeze-dried larvae were stored at − 20 °C and defatted within a week. The proximate composition values of the freeze-dried larvae were: moisture, 4.0 ± 0.1%; protein, 61.1 ± 0.4%; fat, 21.2 ± 0.2%; and ash, 5.2 ± 0.2%.

The defatting and extraction processes were performed with minor modifications of the methods of Yi, Lakemond, Sagis, Eisner-Schadler, van Huis, and van Boekel (2013) and Mishyna et al. (2019). For defatting of edible insects, 200 g of ground larvae and 1000 mL of 99% organic solvent (methanol, ethanol, or *n*-hexane) were stirred for 1 h at ambient temperature (20 °C). The fat-containing organic solvent was drained from a glass beaker. This procedure was repeated five times and was stopped when a clear solvent was obtained. Residual solvent was volatilized in a fume hood for 12 h at ambient temperature (20 °C).

To extract protein, 100 g of defatted samples and 200 mL of distilled water (pH 6.86) were homogenized at 10,000 rpm for 2 min using a model AM-11 device (Nihonseiki Kaisha Ltd., Tokyo, Japan) and centrifuged at 15,000 × *g* for 30 min at 2 °C using a Sorvall RC5C Plus apparatus (Thermo Fisher Scientific, Waltham, MA, USA). The supernatant was filtered using a 500 μm-pore size steel sieve, frozen at − 20 °C, and then freeze-dried as described above. The powder was considered extracted protein (EP).

To investigate the effects of aqueous extraction, intact insect powder (not defatted) was homogenized with distilled water for 2 min at 10,000 rpm and centrifuged at 15,000×*g* for 30 min. After filtering through a 500 μm-pore size steel sieve, the supernatant was poured into a separatory funnel and held for 12 h at 2 °C. The aqueous phase was used as the EP solution. This solution was also freeze-dried and stored before analysis within 24 h. The aqueous EP and EP from PB defatted by methanol, ethanol, and *n*-hexane were designated Aq, Meth, Eth, and Hex, respectively.

2.3. Proximate and amino acid compositions and their protein quality

2.3.1. Proximate composition

The standard chemical composition analysis method of AOAC (2000) was used to investigate the proximate composition of EP. A model SW-90D drying oven (Sang Woo Scientific Co., Bucheon, Korea), solvent extraction system (Soxtec® Avanti 2050 Auto System; Foss Tecator AB, Höganäs, Sweden), auto protein analyzer (Kjeltec® 2300 Analyzer Unit; Foss Tecator AB), and incinerator were used to determine crude moisture, fat, protein, and ash contents of EP.

2.3.2. Amino acid and protein quality

Ten milliliters of 6 M HCl containing EP in sealed ampoules were hydrolyzed under nitrogen at 105 °C for 24 h. Hydrolysates were evaporated under vacuum at 40 °C and dissolved in 3–5 mL of 0.02 M HCl. A model L-8800 amino acid analyzer (Hitachi, Tokyo, Japan) was used to determine the amino acid composition of EP and filtration using a 0.20 μm membrane filter (Thomas Scientific, Waltham, MA, USA) was performed using an ion-exchange resin column (4.6 mm i.d. × 60 mm) as previously described (Fountoulakis & Lahm, 1998). The final concentration was calculated as mg/g. Protein quality in terms of the essential amino acid index was calculated according to FAO/WHO/UNU (1985).

2.4. Protein characteristics and functionality

2.4.1. Surface hydrophobicity

Surface hydrophobicity of edible insects was measured a described by Chelh, Gatellier and Santé-Lhoutellier (2006). Briefly, 0.5 g of EP powder was suspended in 100 mL of 0.025 M phosphate buffer (pH 7.4). A 200 μL volume of 1 mg/mL bromophenol blue (BPB; Bio-Rad Inc.) and 1 mL of the prepared sample was agitated at ambient temperature (20 °C) for 10 min and centrifuged at 2,000 × *g* for 15 min. As a control, 1 mL of 0.025 M phosphate buffer (pH 7.4) and 200 μL of BPB was thoroughly mixed. The absorbance was measured using an Optizen 2120 UV plus ultraviolet/visible (UV/VIS) spectrophotometer (Mecasys Co. Ltd., Daejeon, Korea) at 595 nm after 10-fold dilution. BPB bound protein (μg) was calculated as:

$$\text{BPB bound}(\mu g) = \frac{\text{absorbance of control at 595nm - absorbance of sample at 595nm}}{\text{absorbance of control at 595 nm}} \times 200$$

2.4.2. Protein solubility

EP was diluted with distilled water (pH 6.86) and the concentration was normalized to 1% (w/v). Protein concentration was measured using the Bradford method (Kruger, 2009) with bovine serum albumin as the standard solution.

2.4.3. SDS-PAGE

SDS-PAGE was performed according to the method of Laemmli (1970). Briefly, after heating the mixture of 20 μg of EP and 20 μL of sample buffer at 100 °C for 5 min, the protein bands of the cooled sample were separated on 10% Mini-PROTEIN® TGX™ Gels (Bio-Rad, Inc.). After staining protein bands with Coomassie Brilliant Blue R 250, the bands were marked with Precision Plus Protein™ dual-color standards (Bio-Rad, Inc.).

2.4.4. pH measurements

A model 340 pH meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) was used to determine the pH value of a 1% (w/v) EP solution in distilled water.

2.4.5. Color measurements

A model CR-410 colorimeter attached to a model CR-A50 granular attachment (Minolta, Tokyo, Japan) was used to measure CIE (International Commission on Illumination) L*a*b*color values. The standard conditions used were D50 illuminant and 2° observer. CIE L*a*b* values of the calibrated white plate were 97.83, −0.43, and 1.98, respectively. A total of 50 mL of the sample was poured into the CR-A50 attachment and color values were detected as previously described (Kalušević et al., 2017). The ΔE of the detected color values were calculated to evaluate the color difference between Aq and other treatments according to the CIE76 color difference formula established by the International Commission on Illumination in 1976.

2.4.6. Foam capacity and stability

Foam capacity and stability were determined as described by Mishyna, Martinez, et al. (2019). After dilution with distilled water, 1% (w/v) EP was added to a 50 mL conical tube and homogenized at 12,000 rpm for 2 min. The foaming formation ratio, termed the foam capacity, was calculated as a percentage. Each foamed solution was held for 2, 5, 10, 20, 30, and 60 min to determine foaming stability.

2.4.7. Emulsion capacity and emulsion stability

Emulsion capacity and emulsion stability were determined as previously described by Pearce and Kinsella (1978) with minor modifications. In detail, these modifications were the change of peanut oil to olive oil and the change of 0.1% (w/v) SDS to 0.3% (w/v) (Mishyna, Martinez, et al., 2019). A total of 10 mL of 1% (w/v) EP solution and 1 mL of pure olive oil was homogenized at 18,000 rpm for 2 min then incubated for 10 min at ambient temperature. The volume of the emulsified layer and the initial volume of an unhomogenized solution was used to determine the emulsion capacity (%). To determine the emulsion stability of the EP solution, 50 μL of emulsion was added to 10 mL of the 0.3% (w/v) SDS solution and inverted several times. Changes in the absorption of each inverted solution were detected at 500 nm and the absorption of inverted solutions at each incubation time was detected and calculated as a percentage.

2.5. Statistical analysis

SPSS statistics 20 software (SPSS Inc., Chicago, IL, USA) was used to statistically analyze the data. One-way analysis of variance with Tukey's range test was performed ($P < 0.05$). To compare effect of organic solvent, defatting methods were considered as fixed effects. All experiments were tested in triplicate and EPs were also extracted at each replicate. Data are expressed as the mean ± standard deviation ($n = 3$) and replicates were considered as random effects.

3. Results and discussion

3.1. Proximate and amino acid composition and their protein quality

3.1.1. Proximate composition

The significant differences among treatments were observed in proximate composition (Table 1). Although all samples were dried and stored under identical conditions, the moisture content of the samples varied significantly. The Eth extract displayed the highest moisture content and the Aq extract the lowest ($P < 0.05$). These findings indicated that moisture might be absorbed from the atmosphere by the protein powder, which might cause powder sticking and caking problems (Foster, Bronlund & Paterson, 2005). Crude protein composition was the highest in the Hex extract and the lowest in the Aq and Eth extracts ($P < 0.05$). This was because the Aq extract had the highest crude fat content and the Eth extract had the highest moisture content ($P < 0.05$). Since a high-level crude protein might enhance the functionality of the extracted powder, the Hex extract might have the highest functionality. The fat content of Aq was the highest ($P < 0.05$), indicating that the defatting efficiency of aqueous extracts was not greater than the efficiency of organic solvent defatting. Fat globules decrease the technical properties of protein because the structure and large molecular size of the globules can disrupt the interaction between protein molecules (Rousseau, 2000). Ash contents of Meth and Eth extracts were the highest, and the ash content of the Aq extract was the lowest ($P < 0.05$). Increased ash contents might detrimentally affect the solubility of the EP (Pietrysiak, Smith, Smith & Ganjyal, 2018).

3.1.2. Amino acid profile and protein quality

The amino acid composition could help determine the protein quality, protein conversion factor, and indirect protein functional properties of edible insects (Janssen, Vincken, van den Broek, Fogliano & Lakemond, 2017; Yi et al., 2013). The amino acid composition of EP was significantly different based on the organic solvent used for extraction (Table 1). Essential amino acids (EAA) cannot be synthesized by organisms. Thus, the intake of EAA is important for human nutrition and has been recommended (FAO/WHO/UNU, 1985). In this study, when the composition of EAA was compared, the sum of the EAA was greatest in the Hex extract and smallest in the Meth extract ($P < 0.05$). The findings were also observed for the total amino acids and EP quality. However, the sum of EAA of all extracts was lower than the required EAA (271 mg/g). Ghosh, Lee, Jung and Meyer-Rochow (2017) described that the EAA of PB (167.8 mg/g) were lower than the EAA from larvae of *Allomyrina dichotoma* (AD) and *Tenebrio molitor* (TM). The authors also determined that the EAA from AD and TM were also lower than the recommended level of EAA. However, EAA from extracted PB protein was higher than the AD and TM EAA, because protein extraction is dependent on protein solubility (Kim et al., 2019). The observations that the sum of the total amino acids was < 1000 mg/g, with the highest value being 463.8 mg/g, might indicate that EP powder contained a significant non-protein nitrogen fraction, such as chitin, which can be dissolved in distilled water and detected by the Kjeldahl method. According to Janssen et al. (2017), protein in insects can be detected along with the chitin glycoprotein using the Kjeldahl method. The actual protein contents in insects might be lower than the estimated value.

Hydrophobic amino acids are essential enhancers of protein functionality (Li, Wang, Kong, Shi & Xia, 2019). Therefore, the sum of hydrophobic amino acids can be useful for predicting protein functional properties. The bold text in Table 1 presents the data of hydrophobic amino acids. The Hex extract contained the highest level of hydrophobic amino acids compared with other treatments, followed by Aq. However, a more significant amount of fat, which is globular in shape, might interrupt protein functionality in the Aq extract (Rousseau, 2000).

3.2. Protein characteristics and functionality

3.2.1. Surface hydrophobicity and protein solubility

Surface hydrophobicity and protein solubility data of protein extracted from PB are presented in Table 2. Aq extracts displayed the highest surface hydrophobicity, followed by Meth and Hex extracts ($P < 0.05$). When proteins denature, the surface hydrophobicity might increase because of the exposure of previously hidden hydrophobic sites (Chelh et al., 2006). In general, aqueous defatting only minimally alters protein structure (Russin et al., 2011). However, Aq extracts displayed the highest surface hydrophobicity values ($P < 0.05$). The result might be due to the high-fat contents of Aq extracts. Fatty amines can be dyed by BPB in aqueous solution (Larrick, 1963). This might explain the high surface hydrophobicity value of the Aq extracts. Eth extracts displayed the lowest surface hydrophobicity, even though the hydrophobic amino acid composition was higher than the Meth extract. Furthermore, the protein solubility of Eth extracts was higher than the solubility of the Meth extracts. These findings might be due to structural modification by organic solvents (L'Hocine et al., 2006). Organic solvents affect the surface hydrophobicity of proteins because internal disulfide bonds are affected by organic acids. Interfacial tension is altered, and this influence differs by the type of organic solvent and protein sources (Martínez-Aragón et al., 2009). Surface hydrophobicity of a protein is a major characteristic that can determine functional properties (Chelh et al., 2006).

Protein solubility has been investigated in distilled water. Protein solubility influences protein functionality (Choi et al., 2011). According to Zhao, Vázquez-Gutiérrez, Johansson, Landberg, and Langton (2016), the higher salt concentration of the buffer can lessen the protein solubility of mealworm protein extracts. The Meth and Eth extracts had the highest ash contents ($P < 0.05$; Table 1). When dissolved in distilled water, the ash component might affect the ionic strength of protein

Table 1
Effect of different defatting solvent on proximate composition, amino acid profile, and protein quality of extracted protein from edible insect.

	Aq[1]	Meth	Eth	Hex	Reference value[2]
Proximate composition (%)					
Moisture	6.41 ± 0.28^{c}	9.43 ± 0.81^{b}	12.85 ± 0.61^{a}	9.16 ± 0.01^{b}	
Protein	68.77 ± 0.37^{c}	70.41 ± 0.38^{b}	68.39 ± 0.31^{c}	77.62 ± 0.36^{a}	
Fat	14.82 ± 0.16^{a}	1.17 ± 0.54^{b}	0.13 ± 0.06^{c}	0.32 ± 0.24^{c}	
Ash	10.00 ± 0.10^{c}	19.00 ± 0.17^{a}	18.63 ± 0.25^{a}	12.90 ± 0.11^{b}	
Amino acid profile (mg/g)					
Essential amino acid (EAA)					
His	15.51 ± 0.01^{c}	12.30 ± 0.04^{d}	17.89 ± 0.02^{b}	18.77 ± 0.11^{a}	15
Ile	11.17 ± 0.17^{b}	6.85 ± 0.09^{d}	9.30 ± 0.06^{c}	16.39 ± 0.23^{a}	30
Leu	23.52 ± 0.01^{b}	9.69 ± 0.18^{d}	10.78 ± 0.02^{c}	25.61 ± 0.40^{a}	59
Lys	29.38 ± 0.16^{b}	17.08 ± 0.01^{c}	16.81 ± 0.05^{c}	33.48 ± 0.19^{a}	45
Met + Cys	4.52 ± 0.02^{b}	2.54 ± 0.10^{c}	2.81 ± 0.25^{c}	8.51 ± 0.23^{a}	22
Phe + Tyr	30.64 ± 0.37^{a}	14.19 ± 0.53^{b}	13.84 ± 0.37^{b}	30.98 ± 0.54^{a}	38
Thr	17.75 ± 0.32^{b}	10.22 ± 0.09^{c}	9.78 ± 0.03^{c}	21.25 ± 0.03^{a}	23
Val	14.42 ± 0.29^{b}	8.81 ± 0.03^{c}	14.35 ± 0.07^{b}	21.22 ± 0.01^{a}	39
Sum of EAA	146.91 ± 0.56^{b}	81.67 ± 1.05^{d}	95.56 ± 0.61^{c}	176.22 ± 1.71^{a}	271
Non-essential amino acid					
Ala	28.61 ± 0.02^{b}	15.79 ± 0.06^{d}	27.81 ± 0.04^{c}	32.71 ± 0.12^{a}	
Arg	24.56 ± 0.26^{b}	23.55 ± 0.20^{c}	21.29 ± 0.08^{d}	28.12 ± 0.28^{a}	
Asp	36.99 ± 0.24^{b}	22.36 ± 0.01^{c}	18.90 ± 0.14^{d}	39.55 ± 0.76^{a}	
Glu	62.80 ± 1.24^{b}	46.40 ± 0.05^{d}	55.46 ± 0.25^{c}	72.61 ± 0.37^{a}	
Pro	39.81 ± 0.44^{c}	26.34 ± 0.11^{d}	69.93 ± 0.88^{a}	50.86 ± 2.28^{b}	
Gly	28.50 ± 0.11^{c}	26.75 ± 0.17^{d}	35.46 ± 0.01^{a}	34.49 ± 0.33^{b}	
Ser	25.68 ± 0.17^{b}	21.18 ± 0.01^{d}	22.37 ± 0.12^{c}	29.22 ± 0.12^{a}	
Sum of total AA	393.85 ± 1.39^{b}	264.04 ± 1.02^{d}	346.77 ± 0.93^{c}	463.79 ± 5.73^{a}	
Protein quality	23.87 ± 0.10^{b}	15.15 ± 0.19^{d}	17.24 ± 0.19^{c}	29.15 ± 0.25^{a}	39.81

All values are mean ± standard deviation of three replicates (n = 3).
[a-d]Means within a row with different letters are significantly different ($P < 0.05$).
Hydrophobic amino acid is presented in bold text.
[1] Aq: aqueous extraction, Meth: methanol, Eth: ethanol, Hex: hexane.
[2] Reference values of essential amino acid requirement for human were from FAO/WHO/UNU (1985).

solutions and ultimately protein solubility.

3.2.2. SDS-PAGE

SDS-PAGE was performed to investigate the effects of defatting methods on molecular weight and patterns of EP (Fig. 1). Aq extracts displayed widely dispersed protein bands from 10 to 250 kDa. Hex extracts did as well, but the thickness of the protein bands was lower than those of the Aq extract, except for bands of approximately 75 kDa. Hydrophobicity of protein surfaces might have affected the SDS-PAGE results due to the binding of BPB to hydrophobic sites on the protein surface (Chelh et al., 2006). Although the surface hydrophobicity of the Hex EP was not significantly different from those of Meth EP ($P > 0.05$), the protein bands in Hex extracts were more varied and thicker than those from Meth extracts. This finding might reflect increased protein solubility and content of hydrophobic amino acids in Hex extracts compared to Meth extracts. Major protein bands of the Aq and Hex extracts were commonly observed at approximately 25, 37, 75, and 250 kDa. Protein bands exceeding 100 kDa were not observed in the Meth and Eth extracts, and Meth extracts did not contain protein bands exceeding 50 kDa. The surface hydrophobicity, protein solubility, and SDS-PAGE results indicated that pronounced protein solubility affected the wide distribution of protein bands, and that surface hydrophobicity might not affect protein distribution. However, the higher surface hydrophobicity of proteins extracted from PB produced thicker protein bands of approximately 10–15 kDa. Therefore, low molecular weight proteins might more closely correlate with protein surface hydrophobicity than higher molecular weight proteins. Hemolymph, which has a positive effect on protein functionality, was observed at < 30 kDa (Kim, Lee & Kim, 2004; Pagare & Martin, 2013).

3.2.3. pH and color

The pH of EP powder is an essential property for determining protein functionality because the ionic strength of proteins can be changed by altering the pH (Lee et al., 2017). In general, there is a close

Table 2
Effect of different defatting solvent on surface hydrophobicity, protein solubility, and color characteristics of extracted protein solution from edible insect.

	Aq[1]	Meth	Eth	Hex
Surface hydrophobicity (BPB bound, μg)	39.69 ± 2.71^{a}	14.87 ± 1.27^{b}	10.80 ± 0.28^{c}	15.58 ± 0.65^{b}
Protein solubility (mg/ml)	66.80 ± 1.42^{b}	14.90 ± 6.96^{d}	32.14 ± 4.20^{c}	82.43 ± 0.04^{a}
pH	6.67 ± 0.01^{d}	7.02 ± 0.01^{a}	6.86 ± 0.01^{c}	6.94 ± 0.01^{b}
CIE L*	18.89 ± 0.11^{b}	15.39 ± 0.03^{c}	19.70 ± 0.42^{a}	14.71 ± 0.11^{d}
CIE a*	1.31 ± 0.11^{c}	2.67 ± 0.08^{a}	1.41 ± 0.04^{c}	1.75 ± 0.12^{b}
CIE b*	0.99 ± 0.09^{b}	1.55 ± 0.03^{a}	0.16 ± 0.06^{c}	−0.04 ± 0.08^{d}
Color difference (ΔE)[2]	–	3.90 ± 0.04^{b}	1.23 ± 0.30^{c}	4.52 ± 0.10^{a}

All values are mean ± standard deviation of three replicates (n = 3).
[a-d]Means within a row with different letters are significantly different ($P < 0.05$).
[1] Aq: aqueous extraction, Meth: methanol, Eth: ethanol, Hex: hexane.
[2] The color value of Aq was used as standard to compare color difference between other treatment and color difference was calculated according to CIE76 ΔE formula ($(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$).

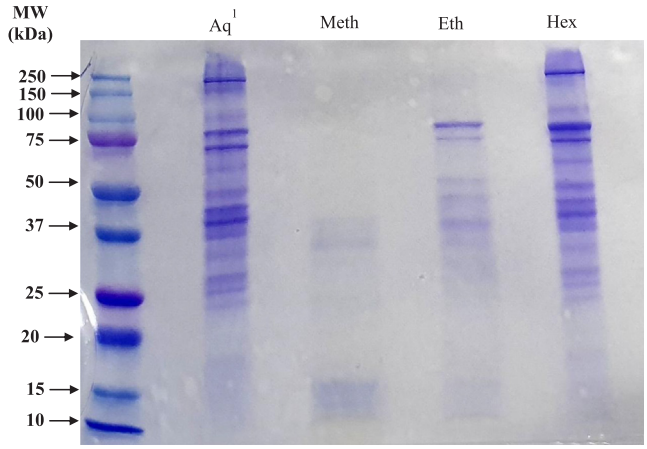

Fig. 1. Effect of different defatting solvent on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-page) of extracted protein from edible insect. [1] Aq: aqueous extraction, Meth: methanol, Eth: ethanol, Hex: hexane.

relationship between protein solubility and buffer solution pH, and these characteristics affect protein functionality (Mishyna et al., 2019; Yi et al., 2013). The pH value was the highest for Meth extracts and the lowest for Aq extracts ($P < 0.05$; Table 2). Alkaline conditions of samples can enhance the functionality of proteins from edible insects (Mishynaet al., 2019; Yi et al., 2013). Therefore, Aq extracts, which presently displayed the lowest pH, might have the lowest protein functionality value.

The color of insects or extracts from insects generally results from different types of melanin pigments and protein aggregates (Atkinson, Brown & Gilby, 1973; Wittkopp & Beldade, 2009). The CIE L*a*b* and color difference (ΔE) values of the extracts differed by treatment (Table 2). The color difference of the treatments was compared with Aq because the Aq extraction results in minimal protein denaturation (Russin et al., 2011). The lightness (CIE L*) value was the highest for Eth extracts and lowest for Hex extracts ($P < 0.05$). The redness (CIE a*) value was the highest for Meth extracts and the lowest for Aq extracts. The yellowness (CIE b*) value was the highest for Meth extracts and the lowest for Hex extracts ($P < 0.05$). These differences were due to protein aggregation, rather than differences in the types of melanin pigments extracted, because PB larvae of the same age and physical condition were used for all experiments. Hex extracts displayed the highest color difference, followed by Meth and Eth extracts ($P < 0.05$). A similar tendency was found for surface hydrophobicity (Table 2). The findings indicate that the structure of insect proteins could be most affected by Hex extraction, followed by Meth and Eth extractions.

3.2.4. Foam capacity and foam stability

Foam capacity and foam stability of EP powder defatted with the various solvents are shown in Fig. 2. Aq extracts had the lowest foam capacity (20.0 ± 7.1%, $P < 0.05$). Meth, Eth, and Hex extracts of defatted insects were not significantly different (72.2 ± 7.9,

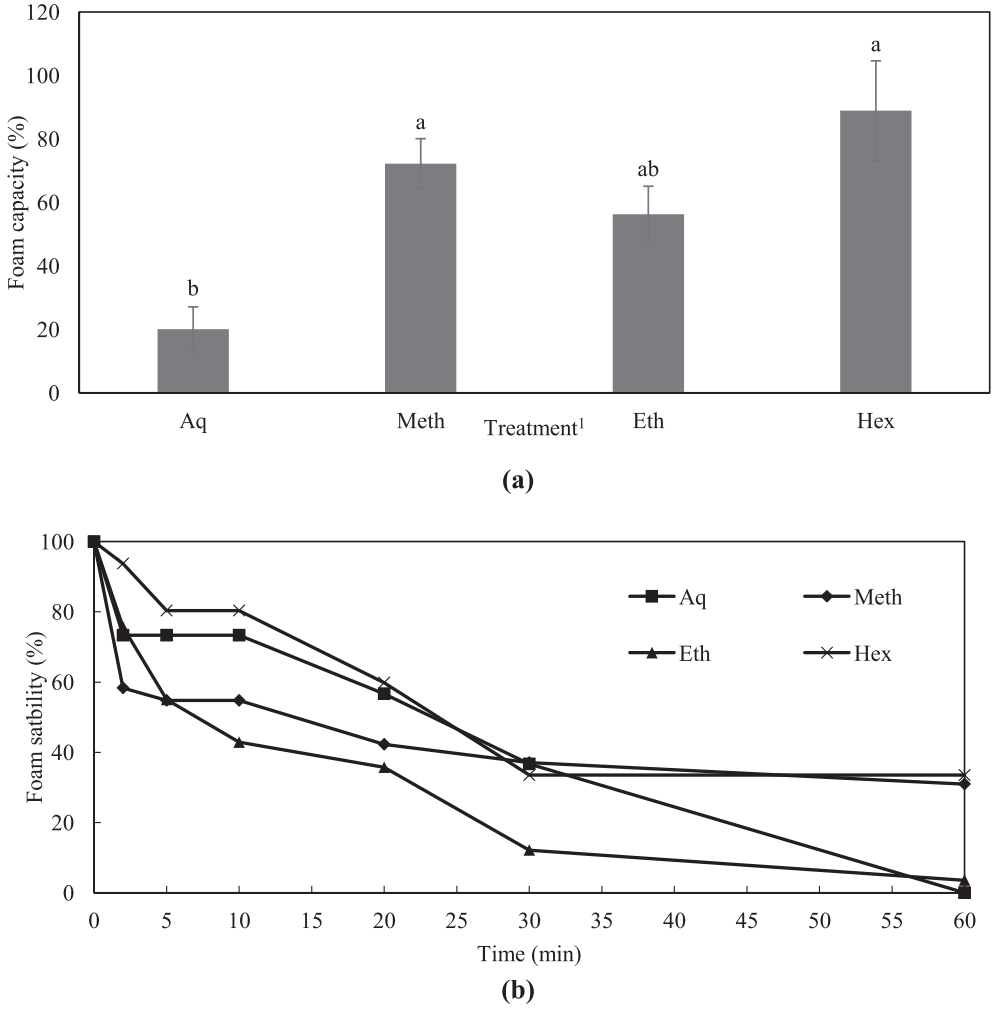

Fig. 2. Effect of different defatting solvent on foam capacity (a) and stability (b) of extracted protein solution from edible insect. [a,b] Different letters on top of columns meant significant difference ($P < 0.05$). [1]Aq: aqueous extraction, Meth: methanol, Eth: ethanol, Hex: hexane.

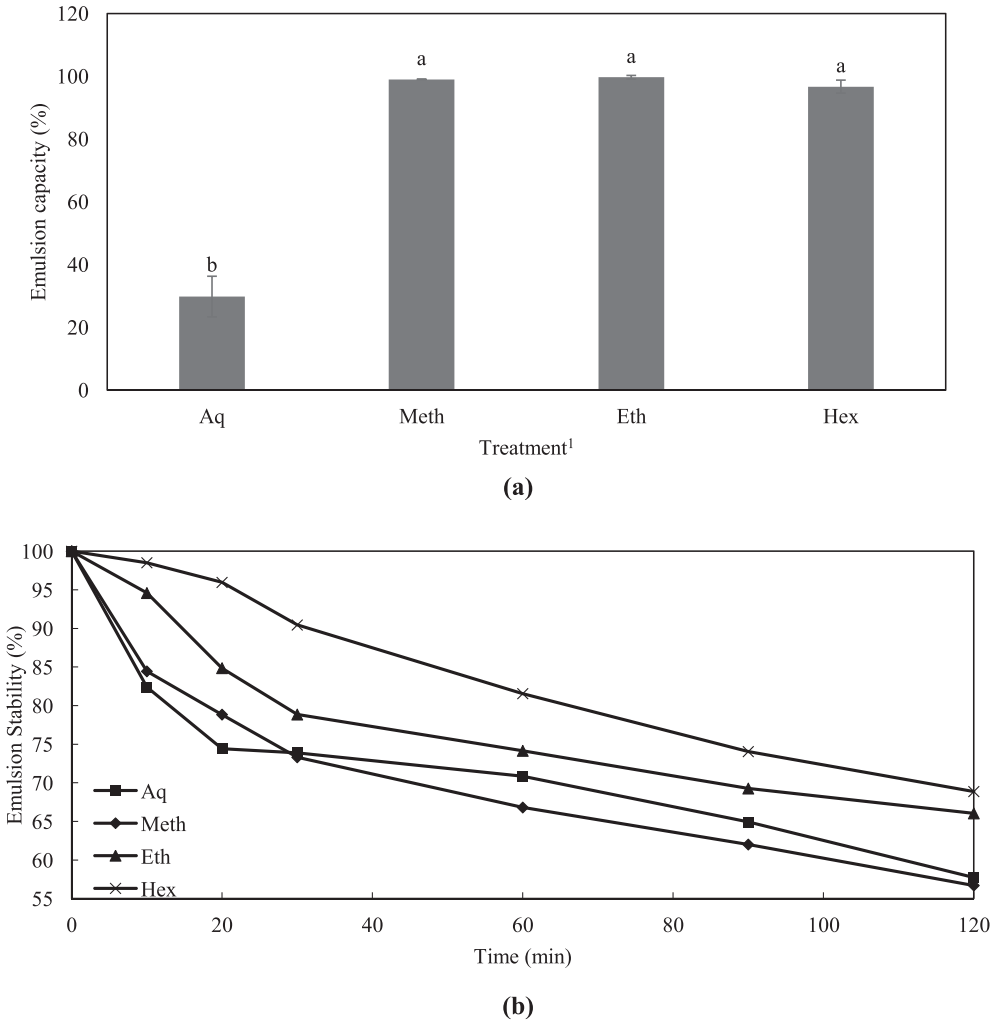

Fig. 3. Effect of different defatting solvent on emulsion capacity (a) and emulsion stability (b) of extracted protein solution from edible insect. [a, b] Different letters on top of columns meant significant difference ($P < 0.05$). [1]Aq: aqueous extraction, Meth: methanol, Eth: ethanol, Hex: hexane.

56.3 ± 8.8, and 88.9 ± 15.7, respectively; $P > 0.05$). Surface hydrophobicity and protein solubility significantly affected foaming ability. The high-fat content in the Aq extract might interrupt foam formation (Russin et al., 2011). Mishyna et al. (2019) reported that defatted insect powder had a higher foam capacity, which was dependent on higher protein contents and alteration of the protein characteristics during the extraction process. Although Eth extracts displayed lower surface hydrophobicity values, the foam capacity of Eth was similar to Meth due to higher protein solubility of Eth compared with Meth. The collective findings indicated that extracts could have a high foam capacity with defatting, even when surface hydrophobicity and protein solubility decreased. Foam stability displayed a similar tendency to foam capacity. Foam stability of Eth extracts was higher than Aq extracts before 10 min incubation, but lower at 10 min. This finding might be due to lower surface hydrophobicity and protein solubility. Foam could readily form because of the absence of fat globules, but the sustainability of protein molecules in the Eth extract was not favorable for foam formation (See Fig. 2).

3.2.5. Emulsion capacity and emulsion stability

Emulsion capacity and stability were not significantly different among the Meth, Eth, and Hex extracts ($P < 0.05$) (Fig. 3). Aq extracts had the lowest values in both emulsion characteristics ($P < 0.05$). When determining the emulsion capacity, the absorption ability of protein surfaces in the emulsified interface is important. Fat or oil contents are important for emulsification because proteins have limited oil and water binding capacities (Rousseau, 2000). Aq extracts displayed the highest fat content, and the emulsion capacity might be reduced. Mishyna et al. (2019) reported that excessively high hydrophobicity was not proper in emulsions compared with lower hydrophobicity. Excessive hydrophobic protein produces a strong binding force between proteins, rather than decreasing interfacial tension. Although there were no significant differences in the emulsion capabilities of the Meth, Eth, and Hex extracts, the emulsion stability differed according to the defatting method used. The emulsion stability of the Hex extract was always the greatest. This was also the case for foam stability. Several factors can disrupt emulsion stability. The emulsion stability of Aq extracts, which had the highest fat content, was distinct from foam stability. Aq extracts had lower emulsion stability than Meth extracts. Molecular weight and protein distribution might be causes of the decreased emulsion stability. Proteins with a low molecular weight of < 37 kDa might display enhanced protein emulsion stability (Damodaran, 2005). As shown in Fig. 1, protein bands < 37 kDa in Eth extracts were thicker than those in Meth extracts and fainter than those in the Hex and AQ extracts. Meth extracts displayed a reduced emulsion stability compared with the Eth and Hex extracts.

4. Conclusion

The effects of various defatting methods (aqueous, methanol,

ethanol, and *n*-hexane) on characteristics and functional properties of extracted *Protaetia brevitarsis* larval proteins were investigated. The collective results implicate hexane extract as the optimal solvent for protein processing in terms of protein functional properties, such as foam and emulsion characteristics and essential amino acid index. However, residual hexane has to be controlled in the human diet because of its health and environmental hazards. Although the protein contents, quality, and stability and of ethanol extract was lower than hexane extract, foam and emulsion capacity were similar to hexane extract when defatting using ethanol. Therefore, using ethanol as a defatting solvent might be the appropriate choice, even though the properties of ethanol extract were not as optimal as those of hexane extract.

CRediT authorship contribution statement

Tae-Kyung Kim: Investigation, Data curation, Methodology, Writing - original draft. **Hae In Yong:** Conceptualization, Data curation, Methodology. **Young-Boong Kim:** Data curation, Methodology, Formal analysis. **Samooel Jung:** Data curation, Methodology, Writing - review & editing. **Hyun-Wook Kim:** Methodology, Data curation. **Yun-Sang Choi:** Conceptualization, Supervision, Validation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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