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# Effect of extraction condition on technological properties of protein from *Protaetia brevitarsis* larvae

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**RESEARCH ARTICLE**

## Abstract

Alternative food sources are garnering increasing interest owing to overpopulation and environmental stresses. Edible insects are a promising alternative protein source. In this study, we evaluated the effect of pH and NaCl concentration on the quality and technical properties of extracted *Protaetia brevitarsis* protein. Nine different solutions were used to extract edible insect protein (pH 1, 4, 7, 10, and 14 and 0, 0.1, 0.5, and 1 M NaCl). The pH of the extracted protein increased with increasing pH (1.12±0.02 to 12.81±0.03) and decreasing NaCl concentration (6.52±0.02 to 6.89±0.01). Colour difference increased when deviating from neutral pH and 0 M NaCl. Further, surface hydrophobicity (μg) and solubility (mg/ml) of the protein decreased at pH 1 (12.25±1.39 μg and 0.74±0.08 mg/ml) and 14 (20.62±1.48 μg and 0.18±0.02 mg/ml) compared with pH 7 (71.81±1.76 μg and 1.26±0.22 mg/ml). Higher pH and NaCl concentrations yielded higher thermal stability. Foaming capacity was the highest at 0.5 (110.5±0.71%) and 1 M (100.5±10.61%) NaCl, and pH 14 (122.5±3.54%), followed by that at pH 1 (72.50±3.54%), although with low stability. Furthermore, emulsifying capacity and stability of the protein increased when deviating from pH 4. Therefore, the protein of *P. brevitarsis* had high functionality when extracted at highly alkaline conditions using NaCl.

**Keywords:** edible insect, protein extraction, technological properties, pH, NaCl

## 1. Introduction

Alternative methods for stable protein supply must be developed given the environmental issues and increased global population (Choi *et al.*, 2017; Kwak *et al.*, 2020). Among the alternatives, edible insects have arisen as an important protein substitute with a high protein conversion ratio (Post, 2012). Although various researchers have encouraged and emphasised the use of edible insects as food sources due to their high nutritional value (Khampakool *et al.*, 2020), the unappealing appearance and negative image of insects inhibits the promotion of their consumption in the Western society (DeFoliart, 1999; Kim *et al.*, 2019a). Education and exposure might increase the acceptance of edible insects; however, their effectiveness is limited by food neophobia (Kim *et al.*, 2020b; Piha *et al.*, 2018). Although edible insects increase nutritional value when added to conventional food, textural qualities and stability of food enriched with edible insects was lower than the original food (Bessa *et al.*, 2019; Haber *et al.*, 2019). Therefore, technical properties of protein from edible insects must be improved to encourage edible insect consumption as a food source.

Various factors can affect the technical properties of proteins. Control of pH and salt concentration are effective ways to regulate the ionic strength of proteins (Zayas, 2012). Today, optimal conditions for protein extraction have been studied for various protein sources, while also improving the technological properties of the extracted protein (Preece *et al.*, 2017). However, information regarding edible insects is not abundant, and most existing information relates to mealworms (Yi *et al.*, 2013). Among various edible insects, *Protaetia brevitarsis*, which is Coleoptera species and go through complete metamorphosis, is indigenous to East

Asia (Suh and Kang, 2012). This species has been used as traditional medicine and functional food which has abundant antithrombotic activity, and the Ministry of Food and Drug Safety of South Korea listed this insect as a food source (Ghosh *et al.*, 2017; Kim *et al.*, 2015). In addition, *P. brevitarsis* had higher technical properties and nutritional value such as emulsifying capacity, foaming capacity, essential amino acid index than *Tenebrio molitor* when extracted in aqueous conditions (Kim *et al.*, 2019b).

Therefore, the objective of this study was to establish the proper conditions regarding pH and salt concentration when extracting protein from *P. brevitarsis* by evaluating the technical properties and nutritional information.

## 2. Materials and methods

### Materials

Frozen *P. brevitarsis* larvae were obtained from a local market (Farm band, Farmbang, Sunchang, Korea). All chemicals used in this study were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), except for chemicals used for SDS-PAGE (Bio-rad Lab, Inc., Hercules, CA, USA).

### Protein extract preparation

Frozen larvae were freeze-dried (LP100, Ilshinbiobase Co., Dongducheon, Korea) and 500 g of larvae were placed on a tray. Drying conditions were controlled by raising the temperature in steps (-30 °C for 60 min, -20 °C for 600 min, -10 °C for 600 min, and 0 °C for 600 min) at 5 mPa, and dried samples were stored at -20 °C after grinding using a 3 mm plate grinder. With 5 parts of volumes of n-hexane, 1 part of insect powder was stirred at 20 °C for 1 h. After drained n-hexane which contained fat, same procedure repeated five times. And then, residual n-hexane was volatilised in a fume hood at 20 °C for 12 h (Kim *et al.*, 2021). After removing fat composition using n-hexane, defatted insect powder was dissolved in different solutions at 1:2 (w/w) using a high-speed homogeniser (T-25 ULTRA TURRAX Digital homogeniser, IKA, Staufen, Germany), and these mixtures were held at 4 °C for 12 h. The samples were tested separately by solution which had various pH values (pH 1, 4, 7, 10, and 14, regulated by NaOH and HCl) or salt concentrations (0, 0.1, 0.5, and 1 M NaCl). Each dissolved protein was centrifuged at 15,000×*g* for 30 min, and the supernatants were collected. For further analysis, protein concentration was regulated at 1 and 15 mg/ml. Except for differential scanning calorimetry, protein solution at 1 mg/ml prepared to estimate the protein condition and technical functionalities such as pH, colour, surface hydrophobicity, foaming properties, and emulsifying properties. After extraction, samples were hold until their temperature reached at ambient temperature (20 °C) and experiments were performed immediately. Three replicates were performed to procedure extracted protein for each experimental condition.

### pH

pH values of dissolved extracted protein at 1 mg/ml were estimated using a pH meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland).

### Colour

The CIE L*a*b* values of 1 mg/ml protein solutions were measured using CR-410 colorimeter (Minolta, Tokyo, Japan). Illuminant C, 2 degree standard observer, calibration plate, and CR-A50 were used to estimate colour values (Kim, *et al.*, 2020b). Colour difference was calculated according to the CIE 76 ΔE formula, and extracted protein using pH 7 (0 M NaCl) solution was used as the standard.

### Surface hydrophobicity

Surface hydrophobicity of extracted protein samples were evaluated by the method described by Chelh *et al.*, 2006). After protein concentration was regulated at 1 mg/ml, 0.5 g of extracted sample, and 10 ml of buffers which were used to extract protein were vortexed. Then, 1 ml of dispersed sample solution and 200 µl of 1 mg/ml of bromophenol blue (BPB) solution was mixed at atmospheric temperature for 10 min, and each solution was centrifuged at 20 °C for 15 min at 2,000×*g*. Each buffer which was used to extract protein was mixed with BPB solution to be used as a control, and the absorbance of 10-fold diluted samples was measured at 595 nm wavelength. Surface hydrophobicity was calculated according to the equation below.

$$\text{Surface hydrophobicity (BPB, µg)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 200\ \text{µg}$$

### Protein solubility

After supernatant were diluted 80-fold, protein solubility was measured (Kruger, 2009). Five microliters of diluted solution and 200 µl of Bradford solution mixed and their absorbance was measured at 595 nm by UV/VIS spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Daejeon, Korea). Bovine serum albumin was used to generate the standard curve.

### Differential scanning calorimetry

A differential scanning calorimeter (DSC-4000, PerkinElmer, Waltham, MA, USA) was used to estimate the thermal properties of the extracted protein. The temperature range

was 20-100 °C with a heating rate of 5 °C/min. The flow rate of nitrogen gas was set at 20 ml/min and the temperature of the cooler was -80 °C. Thirty milligrams of 15 mg/ml of samples was poured into aluminium pan; an empty pan was used as a reference. Data were collected using Pyros.

### Foaming capacity and foam stability

Ten millilitres of protein solution regulated at 1 mg/ml was homogenised at 12,000 rpm for 2 min using a high-speed homogeniser (T-25 ULTRA TURRAX Digital homogeniser, IKA). The difference of foaming volume and initial volume was calculated as a percentage (foaming capacity) and decreased foaming volume was recorded after 2, 5, 10, 20, 30, and 60 min (foam stability) (Mishyna *et al.*, 2019). All experiments were performed at ambient temperature (20 °C).

### Emulsifying capacity and emulsion stability

One millilitre of olive oil and 10 ml of 1 mg/ml protein solution were homogenised at 18,000 rpm for 2 min using a high-speed homogeniser (T-25 ULTRA TURRAX Digital homogeniser, IKA). After the mixture was held for 10 min, the difference of the separated phase (top phase) and initial volume was calculated as a percentage (emulsifying capacity). Fifty microliter of emulsion and 10 ml of 0.3% sodium dodecyl sulphate were inverted a number of times for mixing, and their absorbance at 500 nm was recorded at 10, 20, 30, 60, 90, and 120 min (Pearce and Kinsella, 1978). The difference of absorbance by time was calculated as a percentage (emulsion stability). All experiments were performed at ambient temperature (20 °C).

### Statistical analysis

The significant effect ($P$<0.05) of pH and NaCl was calculated using one-way analysis of variance (ANOVA) with Duncan's multiple range test for surface hydrophobicity, protein solubility, pH, colour, foaming capacity, and emulsifying capacity. Buffers were fixed terms and other conditions were random terms. Data were calculated as mean ± standard deviation.

## 3. Results and discussion

### pH and colour

pH is a critical factor which can affect the physical characteristics of protein (Zayas, 2012). This value depends on the pH of materials, extracted components, and the external pH of the extractant. pH values of the extracted protein solution are shown in Table 1. When compared to pH values of extracted protein by different pH extractants, pH values of samples significantly increased when the pH buffer was in an alkaline condition ($P$<0.05). However, there were large differences between extracted samples at pH 1 and 4 and extracted samples at pH 10 and 14 over pH 5. Protein has the lowest solubility at the isoelectric point (pI), and a large gap relative to the pI causes higher protein solubility (Zayas, 2012). However, excessive gaps could reduce the protein solubility due to the formation of strong hydrogen bonds between protein and water, and the excessive heating and alkaline conditions of protein can produce de-hydro amino acids which is not digested easily (Gerrard, 2002; Jiang *et al.*, 2009). Differential ionic strength could affect the technical properties of protein. Furthermore, extreme pH condition could induce the structural changes of protein and their technical functionality could be enhanced (Jiang *et al.*, 2009). Therefore, extreme pH condition (pH 1 or pH 14) might enhance the technical properties of insect protein. Low concentration of NaCl could enhance protein technical properties, but excessive NaCl concentration caused an adverse effect on protein technical properties (Zayas, 2012). pH values of the extracted proteins using various salt concentrations were reduced with an increase in salt concentration. This may be due to depolymerised proteins by a change in ionic strength (Puolanne *et al.*, 2001). In general, alkali pH condition enhances the technical properties of protein and the decreased pH condition at 0.5 M and 1 M might negatively affect technical properties of insect protein (Zayas, 2012). However, increased ionic strength by NaCl might reinforce the effect of decreased pH value.

The colour values could be affected by various pigments and protein aggregation (Kim *et al.*, 2021). The colour properties of the extracted proteins are shown in Table 1. In CIE L*, pH had the lowest value of colour among the pH treatments and its value significantly increased with increase in NaCl concentration ($P$<0.05). In CIE a*, pH 7 and 14 had the highest values and pH 1 and 10 had the lowest values ($P$<0.05). The CIE a* value of NaCl treatments had an inverse relationship with those of CIE L*. In CIE b*, pH 14 had the highest value of colour while pH 4 and 7 had the lowest values of the same. ($P$<0.05). NaCl treatments showed a U-shaped trend (0 and 1 M had the highest value, and 0.1 and 0.5 M had the lowest value) ($P$<0.05). Ideal salt concentration might increase protein technical properties, but concentrations over 1 M NaCl could affect it negatively (Zayas, 2012). When comparing colour difference with pH 7, there were no significant differences among values at pH 1, 4, and 10. However, pH 14 had the highest value. This different might be due to CIE b* of extracted protein. The colour of insects is depending on melanin mainly and the stability of melanin which had brown colour is the highest in the alkali condition (Wang *et al.*, 2006; Wittkopp and Beldade, 2009). Therefore, pH 14 had the highest CIE b* value and the highest value in colour difference. Furthermore, metal ions could affect colour of melanin and sodium ion reduced colour pigments (Wang *et al.*, 2006). This change of colour pigments might affect colour

**Table 1. Effect of pH and NaCl concentration on pH and colour properties of extracted protein solutions from edible insects.[1,2]**

| | pH | CIE L* | CIE a* | CIE b* | Colour difference |
|---|---|---|---|---|---|
| Effect of pH | | | | | |
| pH 1 | 1.12±0.02$^{a}$ | 16.65±0.33$^{a}$ | 0.90±0.10$^{c}$ | 0.07±0.05$^{b}$ | 0.61±0.13$^{b}$ |
| pH 4 | 6.73±0.08$^{b}$ | 16.19±0.06$^{b}$ | 1.28±0.06$^{b}$ | 0.00±0.03$^{c}$ | 0.67±0.06$^{b}$ |
| pH 7 | 6.89±0.01$^{c}$ | 16.85±0.16$^{a}$ | 1.38±0.05$^{a}$ | -0.04±0.05$^{c}$ | -[3] |
| pH 10 | 7.01±0.01$^{d}$ | 16.73±0.05$^{a}$ | 0.86±0.09$^{c}$ | 0.12±0.04$^{b}$ | 0.56±0.10$^{b}$ |
| pH 14 | 12.81±0.03$^{e}$ | 16.79±0.02$^{a}$ | 1.35±0.07$^{ab}$ | 0.72±0.06$^{a}$ | 0.76±0.06$^{a}$ |
| Effect of NaCl | | | | | |
| 0 M | 6.89±0.01$^{A}$ | 16.85±0.16$^{C}$ | 1.38±0.05$^{A}$ | -0.04±0.05$^{A}$ | - |
| 0.1 M | 6.87±0.14$^{A}$ | 16.94±0.07$^{C}$ | 1.14±0.06$^{B}$ | -0.12±0.02$^{B}$ | 0.28±0.05$^{B}$ |
| 0.5 M | 6.52±0.02$^{B}$ | 17.29±0.07$^{B}$ | 1.32±0.04$^{AB}$ | -0.15±0.04$^{B}$ | 0.46±0.07$^{B}$ |
| 1 M | 6.52±0.02$^{B}$ | 17.49±0.04$^{A}$ | 0.79±0.34$^{C}$ | -0.01±0.02$^{A}$ | 0.90±0.26$^{A}$ |

[1] All values are mean ± standard deviation of three replicates (n=3).

[2] Means within a column with different letters are significantly different regarding the effect of pH (a-d) or NaCl (A-C) ($P$<0.05).

[3] Colour value of extracted protein solution at pH 7 (0 M NaCl) used as a standard value to calculate colour difference of extracted protein according to the CIE 76 ΔE formula ($(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$).

of extract and colour difference in the NaCl treatments increased with an increase in NaCl concentration. These results showed that the proteins of *P. brevitarsis* changed most at pH 14, and salt concentrations significantly affected its proteins.

### Surface hydrophobicity and protein solubility

Surface hydrophobicity of extracted protein was shown in Table 2. pH 7 resulted in a significantly higher value than pH 1 which had the lowest value ($P$<0.05). Surface hydrophobicity of proteins is changed by protein aggregation, revealed hydrophobicity, and amino acid composition (Zayas, 2012). In general, a high surface hydrophobicity means high denaturation of protein and high protein technical properties (Chelh *et al.*, 2006; Zayas, 2012). Therefore, these results could be apparent that extracted protein in extreme pH condition have poor technical properties. However, since chitin groups have hydrophobic residues and can therefore bind with BPB due to its amino group, surface hydrophobicity of samples may seem abundant (Janssen *et al.*, 2017). The solubility of chitin can be changed with pH 6 as the centre; samples which had an acidic or alkaline pH far from neutrality might have low chitin solubility (Pillai *et al.*, 2009; Roy *et al.*, 2017). Similar results were observed for NaCl treatments. The addition of NaCl could shrink the structure of chitin and chitin content in solution could decrease (Roy *et al.*, 2017). Because chitin can inhibit the improvement of protein technical properties, high hydrophobicity and protein solubility of samples may not mean high functionality of extracted proteins from edible insects (Kim *et al.*, 2020a). Therefore, surface hydrophobicity of extract was not proper to estimate the protein functionality.

**Table 2. Effect of pH and NaCl concentration on surface hydrophobicity and protein solubility of extracted protein solutions from edible insects.[1,2]**

| | Surface hydrophobicity (bromophenol blue bound, µg) | Protein solubility (mg/ml) |
|---|---|---|
| Effect of pH | | |
| pH 1 | 12.25±1.39$^{d}$ | 0.74±0.08$^{b}$ |
| pH 4 | 66.38±2.22$^{b}$ | 1.00±0.02$^{a}$ |
| pH 7 | 71.81±1.76$^{a}$ | 1.26±0.22$^{a}$ |
| pH 10 | 64.34±2.67$^{b}$ | 1.12±0.20$^{a}$ |
| pH 14 | 20.62±1.48$^{c}$ | 0.18±0.02$^{c}$ |
| Effect of NaCl | | |
| 0 M | 71.81±1.76$^{A}$ | 1.26±0.22$^{A}$ |
| 0.1 M | 43.88±6.75$^{B}$ | 1.01±0.11$^{B}$ |
| 0.5 M | 42.77±5.84$^{B}$ | 0.97±0.03$^{B}$ |
| 1 M | 22.03±4.01$^{C}$ | 1.03±0.04$^{AB}$ |

[1] All values are mean ± standard deviation of three replicates (n=3).

[2] Means within a column with different letters are significantly different in regarding the effect of pH (a-d) or NaCl (A-C) ($P$<0.05).

There was a rapid decrease in protein solubility at pH 1 and 14, but the effect of NaCl treatment was minuscule compared to that of pH treatment (Table 2). The protein solubility at pH 14 was the lowest; the highest protein solubility was observed in pH 4, 7, and 10. However, exposure to excessive alkaline or acidic solutions could decrease the protein solubility due to the formation of stronger hydrogen bonds (Jiang *et al.*, 2009). In addition, pH also could affect solubility of chitin contents (Pillai *et*

*al.*, 2009; Roy *et al.*, 2017). Therefore, decreased protein solubility might be due to strong hydrogen bonds and decreased chitin solubility. NaCl treatments, 0 M had the highest protein solubility and was slightly decreased with increased salt concentration ($P<0.05$). In general, increased ionic strength increases protein solubility, but the inverse result was obtained in this study. This result might be due to shrinking of chitin components by NaCl, and decreased solubility of chitin could affect protein solubility. Therefore, the similar value in protein solubility between 0 M and 1 M may mean increased protein solubility with the addition of NaCl ($P>0.05$).

### Thermal properties

The effect of pH and NaCl on the thermal properties of the extracted edible insect protein is shown in Figure 1. Various thermic denatured components were obtained with heating. High differential in the graph and higher denatured temperature (peak point) mean higher thermal stability of the sample (Heussen *et al.*, 2011). As observed in Figure 1A, peak points of pH 1 were the lowest and the overall graph shifted higher temperature with increasing pH value. This result meant the proteins that have low thermal stability could be extracted in acidic conditions, and highly thermal stable proteins could be extracted in alkaline conditions. When comparing the effects of NaCl (Figure 1B), peak points in the graph also shifted with higher temperature, and 1 M NaCl had the highest enthalpy change. Thermal properties might be affected by pH and NaCl concentration due to the difference of dissolved protein (Zayas, 2012). In contrast with the size of mammals, insects are small and have the obstacle of separating proteins by parts of the body. For this reason, in insects that are processed whole, various proteins could be extracted by dissolved solution conditions (Yi *et al.*, 2016).

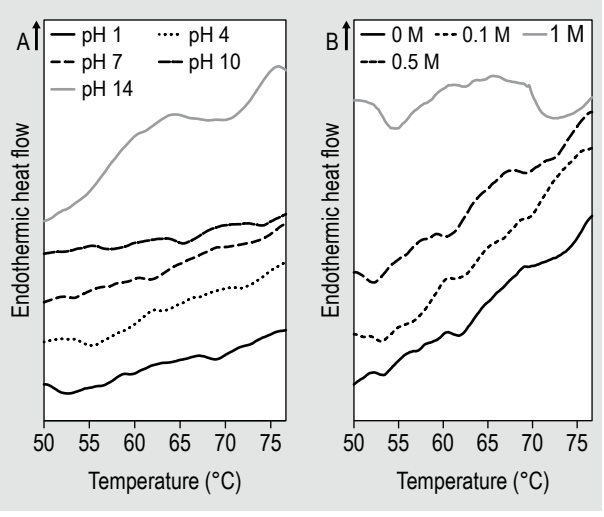


**Figure 1. Effect of (A) pH and (B) NaCl concentration on thermal properties of extracted protein solutions from edible insects.**

When compared thermal properties, alkaline conditions and NaCl addition might improve the thermal properties of edible insects.

### Foaming capacity and foam stability

Foaming properties, including capacity and stability, of the extracted proteins are presented in Figure 2. Because foam could determine the textural properties and appearance of the final food products, foaming capacity and foam stability are an important index regarding the utility of extracted protein. High protein hydrophobicity, flexibility, pH, protein concentration, and non-protein particles may affect these properties (Zayas, 2012). pH and NaCl concentration had significant effects on foaming properties of extracted edible insect proteins (Figure 2). The U-shaped trend was observed for the foaming capacity at different pH values (Figure 2A). Although hydrophobicity and protein solubility were lowest at pH 14 followed by pH 1, pH 14 had the highest value for foaming capacity, and was followed by pH 1 ($P<0.05$). Foaming capacity was also affected by NaCl concentration (Figure 2C). 0.1 M had no significant difference compared to 0 M in foaming capacity ($P>0.05$) and foaming capacity increased with the addition of NaCl over 0.5 M ($P<0.05$). Non-protein components such as chitin or lipid have negative effects on surface tensile strength, and air bubbles may collapse (Kim *et al.*, 2020a). As previously mentioned, surface hydrophobicity of extraction may be affected by chitin content, and their solubility may be changed by pH and NaCl concentration. Therefore, foaming capacity may be increased by varying the pH and NaCl concentration during extraction.

Foam stability might be changed by the thickness of bubble film, protein concentration and solubility, and ionic strength (Zayas, 2012). Foam stability of the treatments is shown in Figure 2B and 2D. Despite the high foaming capacity at pH 14, this pH showed the most unstable foam properties. A similar phenomenon was observed for NaCl treatments. Treatments which had a higher foaming capacity had lower stability. This might be due to the relatively thin protein surface film thickness. Protein concentration of treatments regulated at 1 mg/ml and increased foaming capacity induced the decreased thickness of protein film.

### Emulsifying capacity and emulsion stability

Emulsion properties are the most important characteristics of proteins as food, as balanced, emulsified foods have excellent texture and appearance (Zayas, 2012). Therefore, emulsifying capacity and emulsion stability of extracted proteins were evaluated to estimate the effect of pH and NaCl concentration on the emulsion properties of extracted edible insect proteins (Figure 3). The emulsifying capacities observed at pH 4 and 10 were significantly lower than that

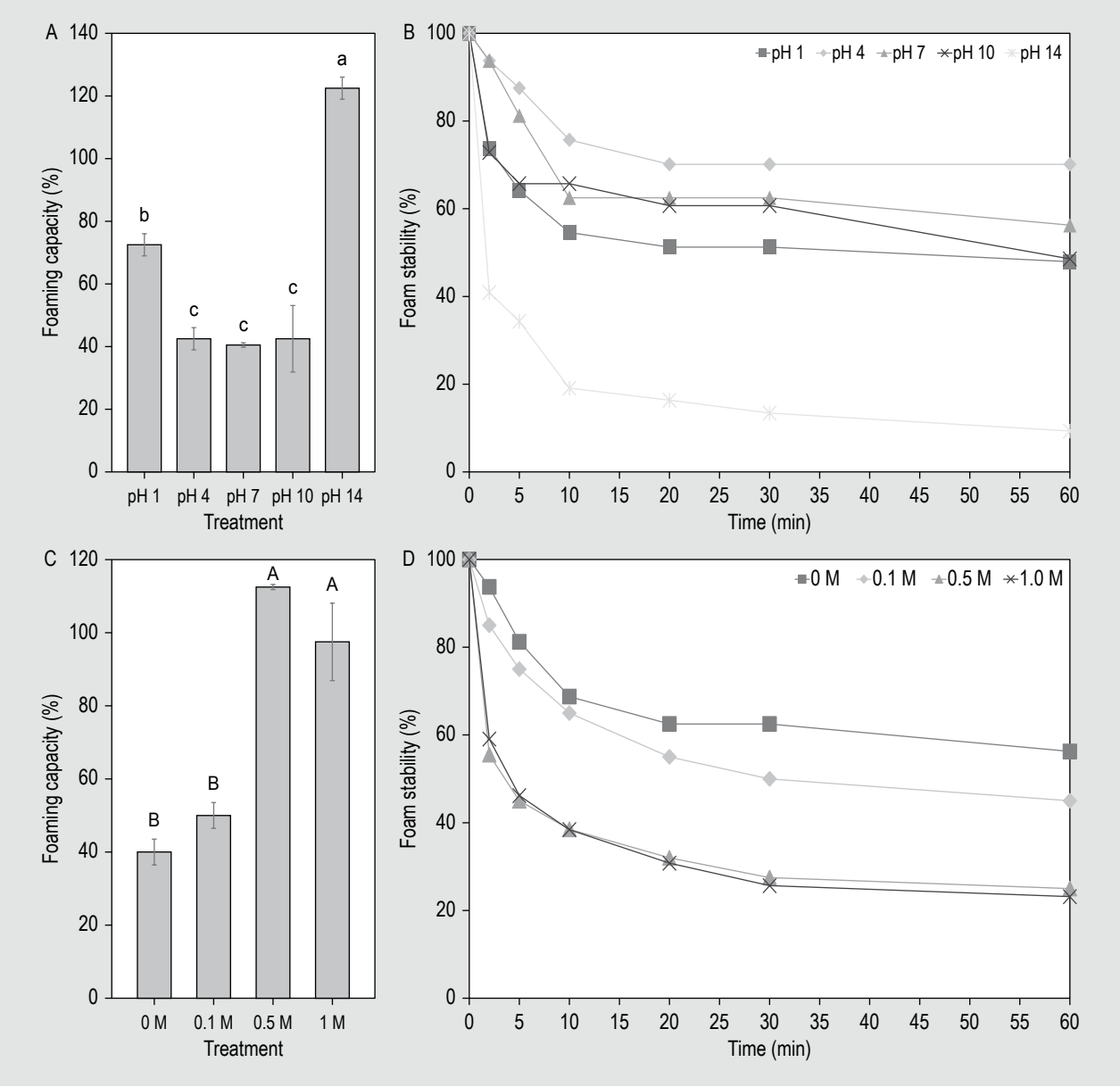


**Figure 2. Effect of pH and NaCl concentration on foaming capacity and foam stability of extracted protein solutions from edible insects. (A) and (B) for pH treatment and (C) and (D) for NaCl treatment. [a-c] Different letters showed significant difference regarding the effect of pH ($P$<0.05). [A,B] Different letters showed significant difference regarding the effect of NaCl ($P$<0.05).**

observed at pH 14 ($P$<0.05; Figure 3A). These results might be due to the pI of the extracted protein. The protein in edible insects had a similar pI at pH 4-5, and emulsifying capacity decreased as pH approached the pI (Yi *et al.*, 2013). Emulsifying capacity in the NaCl treatments decreased with the addition of NaCl ($P$<0.05; Figure 3C). In general, emulsifying capacity of extracted proteins increased with increasing ionic strength (Zayas, 2012). However, excessive addition of NaCl might interrupt the formation of interfacial film due to expanded droplet size in emulsion (Binks *et al.*, 2006). Protein extracted from *P. brevitarsis* had excessive development activity in the diameter of drops in emulsion with NaCl. Therefore, addition of NaCl might not be suitable for the enhancement of the emulsifying capacity of proteins.

Emulsion stability increased with alkaline conditions and the addition of NaCl (Figure 3B and 3D). Emulsions with low stability separated more rapidly than those with high stability and hydrate interfacial could enhance the emulsion stability of edible insect protein emulsion (Zayas, 2012). Increased pH and NaCl concentration have a positive effect on emulsion stability due to decreased coalescence with drops (Fox and Mulvihill, 1982).

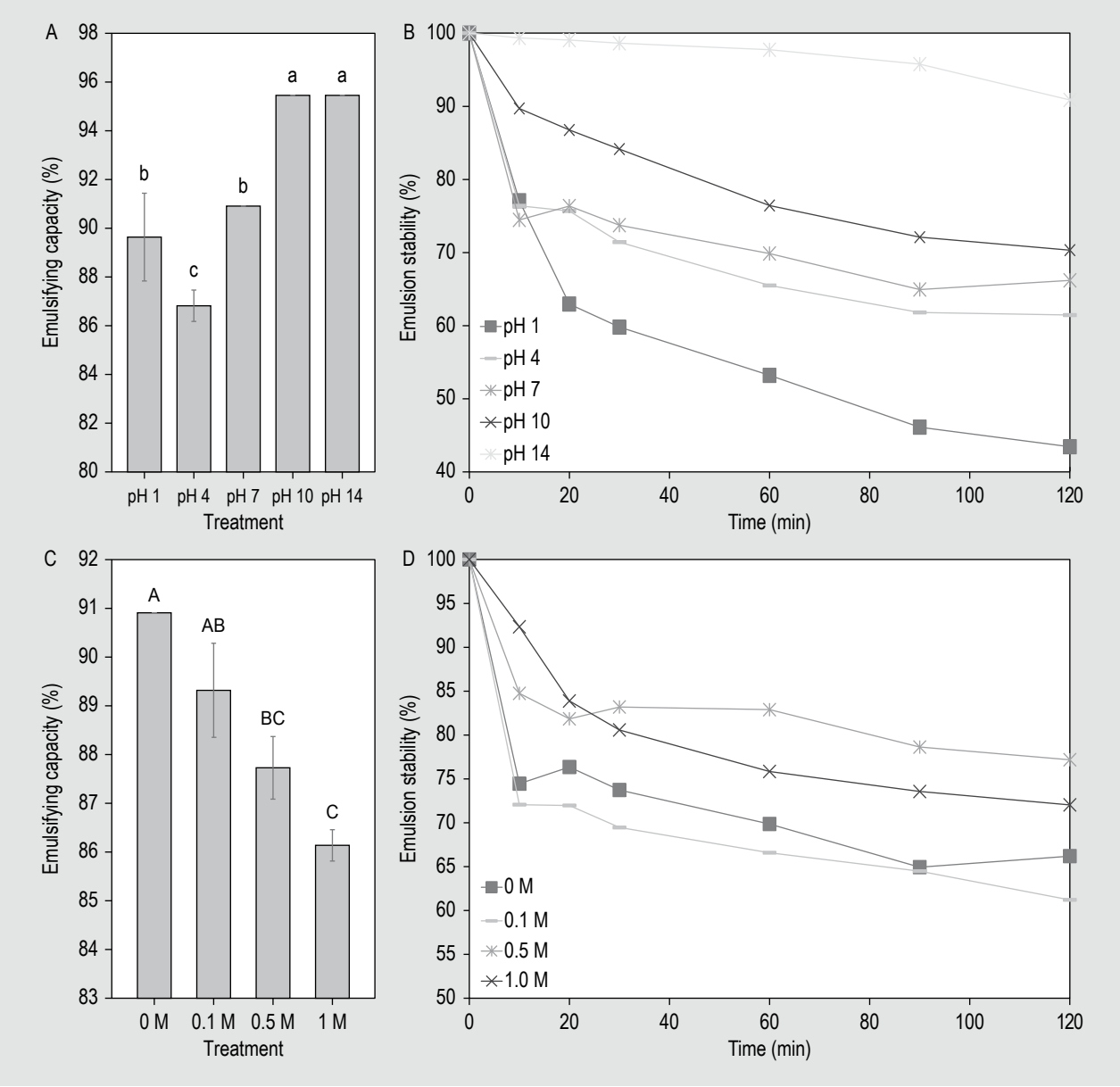


**Figure 3. Effect of pH and NaCl concentration on emulsifying capacity and emulsion stability of extracted protein solutions from edible insects. (A) and (B) for pH treatment and (C) and (D) for NaCl treatment. [a-c] Different letters showed significant difference regarding the effect of pH (*P*<0.05). [A-C] Different letters showed significant difference regarding the effect of NaCl (*P*<0.05).**

## 4. Conclusion

Effects of pH and NaCl on protein functionality of edible insects were studied and pH, colour, protein characteristics, and technical properties were altered by changes in pH and the addition of NaCl. Although surface hydrophobicity and protein solubility decreased in acidic or alkali condition or addition of NaCl, these characteristics might be affected by chitin contents. Decreased chitin solubility might increase technical properties such as foaming capacity and stability. Furthermore, because thermal stability of extracted proteins at alkaline conditions and high NaCl concentration, extreme alkaline condition and 0.5 M NaCl concentration might be more suitable than neutral or acidic conditions and other NaCl concentration.

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