Effect of conjugated protein composed of porcine myofibril and *Protaetia brevitarsis* protein on protein functionality

T.-K. Kim¹, H.-I. Yong¹, J.-M. Sung¹, H.W. Jang² and Y.-S. Choi^{1*}

¹Research Group of Food Processing, Korea Food Research Institute, Wanju 55365, Republic of Korea; ²Department of Food Science and Biotechnology, Sungshin Women's University, Seoul 01133, Republic of Korea; kcys0517@kfri.re.kr

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Abstract

The replacement of conventional protein supply has been studied in the context of continuous population growth and the consequent environmental problems. Among protein substitutes, edible insects have gained relevance due to their high breeding efficiency. This study was conducted to estimate the effect of the partial replacement of porcine myofibril protein (pork protein; PP) with protein extracted from *Protaetia brevitarsis* (insect protein; IP) in protein-containing solutions and gels. The protein concentration was regulated at 30 and 1 mg/ml for gels and solutions, respectively, and the replacement percentages were increased by 20%. Gels containing IP showed a lower viscosity and thermal stability; the texture and surface of the gels were negatively impacted as the replacement percentage increased. Therefore, heating is not an appropriate method to induce the gelation of IP containing solutions. However, the replacement of PP with IP increased the foam capacity and emulsion capacity in solutions. In conclusion, we show that the partial replacement of PP with IP positively impacts on protein solutions but impairs their gelation.

Keywords: edible insect, protein characteristics, functional properties, defatting

1. Introduction

Various alternative diet protein sources such as plant protein, cultured meat, and edible insects (non-livestock-based) have been studied to cope with the various environmental problems associated with livestock farming (Kumar *et al.*, 2017; Post, 2012). Among these sources, edible insects have a lower feed conversion ratio than conventional livestock such as beef, pork, poultry, and lamb (Janssen *et al.*, 2017). With this particular advantage, many studies have suggested that edible insects, particularly their protein extracts, can be used as food enhancers such as emulsifiers, foaming agents, and binders (Kim *et al.*, 2019b; Mishyna *et al.*, 2019; Yi *et al.*, 2013). Among edible insects, *Protaetia brevitarsis*, *Tenebrio molitor*, and *Allomyrina dichotoma* are well-known; the first at the larva stage has the highest essential amino acid index, foaming properties, and emulsifying properties (Kim *et al.*, 2020). In addition, it has been used as a therapeutic agent in East Asia because of its high antithrombotic and antioxidant properties (Ghosh

et al., 2017; Lee *et al.*, 2017). Therefore, *P. brevitarsis*, called white-spotted flower chafer beetle, can be used as meat alternatives to prepare processed meat products.

In the meat gelation process, myofibrillar protein in skeletal muscle is important, as it has good gelling properties with the heating condition and can be extracted easily in salting conditions (Macfarlane *et al.*, 1977). When separated protein, that proteins called muscular protein are also composed in insect; of note, the saline solution can also extract insect proteins (IPs) (Kim *et al.*, 2019a; Yi *et al.*, 2016). Edible insects can either be used as additives or as direct meat substitution agents. On the one hand, some studies reported that the addition of edible insects to sausages or meat emulsions increased the quality of processed meat products (Kim *et al.*, 2016; Park *et al.*, 2017). On the other hand, the substitution of pork meat by mealworms did not affect the quality of a meat emulsion; in fact, an excessive substitute content decreased the quality of the meat emulsion (Choi *et al.*, 2017). Furthermore,

sausages consisting of only edible insects were of poor quality (Bessa *et al.*, 2019). Therefore, optimisation of the processing conditions is required to potentiate the use of edible insects in meat products. Therefore, this study aimed to evaluate the effects of replacing the porcine myofibrillar protein (pork protein; PP) with proteins obtained from *P. brevitarsis* (IP).

2. Materials and methods

Materials

Freeze-dried *P. brevitarsis* on the third instar stage and pork meat were obtained from a conventional market. The chemical agents used in this study, except for those used in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA); the chemicals (tris-glycine electrophoresis, Coomassie Brilliant Blue R-250, and Precision Plus Protein™ Dual Color Standard) and gels (12% Mini-PROTEIN® TGXTM gels) for SDS-PAGE were obtained from Bio-rad Lab., Inc. (Hercules, CA, USA).

Protein extraction

The excessive fat components from larvae were removed using n-hexane. Briefly, insect powder was mixed with n-hexane (5-fold) and stirred at ambient temperature for 1 h under a fume hood and repeated 5 times, and the defatted powder was left under the fume hood for another 12 h to remove the residual hexane. Subsequently, the defatted larvae and pork meat were dissolved in 0.58 M saline solution (0.49 M NaCl, 17.8 mM $\text{Na}_5\text{P}_3\text{O}_{10}$, and 1 mM NaN_3 ; pH 8.3; 2 °C) at 1:2 (w/v), and kept at 2 °C for 12 h. The solutions were then centrifuged at 15,000×g for 30 min at 2 °C, and the pH of the supernatants was adjusted to 5.4. The obtained residue was washed using distilled water, and the same processing was repeated three times to remove salts in extracts (Jiang *et al.*, 2009). Finally, the salt-free proteins were freeze-dried, stored at -20 °C and used within a week.

Protein gel and solution preparation

The IP and PP powder were dissolved in the saline solution used in protein extraction at 30 and 1 mg/ml to evaluate the gelation and solution properties, respectively. The solution was then homogenised at 4,000 rpm for 1 min. Subsequently, the prepared PP and IP were mixed in a sequential replacing order, where the quantity of PP was replaced with IP following a 20% increment (100:0, 80:20, 60:40, 40:60, 20:80, 0:100), and the end-products were labelled as IP0, IP20, IP40, IP60, IP80, and IP100, respectively. The contents were transferred to polyethylene bags and heated at 75 °C for 30 min, followed by cooling

in an ice bath inducing thermal aggregation to obtain the heat-induced gels. Unheated samples were used as controls for differential scanning calorimetry (DSC) and apparent viscosity assays. The heated samples were also analysed to estimate gel solubility, texture profile analysis, and scanning electron microscopy (SEM). The foam and emulsion properties were estimated using the protein solutions. Furthermore, SDS-PAGE was performed to determine the molecular weight distribution of proteins in the preparations.

Gel solubility

Gel solubility was evaluated using 5 different solutions (0.05 M NaCl; SA, 0.6 M NaCl; SB, 0.6 M NaCl+1.5 M urea; SC, 0.6 M NaCl+8 M urea; SD, and 0.6 M NaCl+8 M urea+0.5 M 2-β-mercaptoethanol; SE) (Lin *et al.*, 2019). Two grams of each sample and 10 ml of each solution were mixed at 300 rpm at 2 °C for 3 h using a shaker (MaXshake™, Daihan Scientific, Seoul, Korea). The solutions were then centrifuged at 20,000×g for 15 min, and the protein concentration of the supernatants was measured using the Bradford method (Kruger, 2009).

Apparent viscosity

The apparent viscosity of the samples was evaluated following the method reported by Alavi *et al.* (2020). Briefly, the samples were homogenised at a pre-shear rate of 50 s⁻¹, and the viscosity of the samples was measured at a shear rate of 1–70 s⁻¹ at 15 °C using an HB-DV3T system with an SC4-29 spindle (Brookfield Engineering Inc., Stoughton, MA, USA). Data were converted to Pa*s using an analysis software (Brookfield Engineering Inc.).

Scanning electron microscopy

First, the samples were fixed using Karnovsky fixative at 4 °C for 12 h and washed 3 times using 0.05 M sodium cacodylate buffer at room temperature for 10 min, followed by a second fixation using osmium tetroxide in 0.1 M sodium cacodylate buffer at 4 °C for 2 h. Subsequently, the samples were washed with distilled water and then dehydrated with 30, 50, 70, 80, 90, and 99.8% ethanol (Uengkimbuan *et al.*, 2006). Micrographs of samples were taken using an S-2380N apparatus (Hitachi, Tokyo, Japan) after coating with a layer of gold (E-1010, Hitachi).

Differential scanning calorimetry

The thermal properties of the samples were estimated using a differential scanning calorimeter (PerkinElmer, Waltham, MA, USA). The empty aluminium pan as a reference and the sample pans containing 20 mg of samples were used. The temperature range was 20–100 °C at a heating rate of 10 °C/min. The obtained data were analysed using Pyris

(PerkinElmer) and the thermal properties of samples were estimated according to the method of Kazemi *et al.* (2011).

Texture profile analysis

The heat-induced gel was cut into 25-mm diameter cylinders with a height of 2 cm. A texture analyser (TA-XT2i, Stable Micro Systems, Surrey, England) was used to estimate the texture. The detailed conditions were as follows: pretest speed 2 mm/s; test speed 2 mm/s; post-test speed 5 mm/s; 50% strain; force 10 g (Bourne *et al.*, 1978).

SDS-PAGE

Twenty micrograms of samples and 4× sample buffer under non-reducing conditions were mixed and heated at 100 °C for 5 min. Mini-PROTEIN® TGX™ gels (12%) and tris-glycine electrophoresis buffer were used to run the samples at 80 mA. Protein patterns were stained using Coomassie Brilliant Blue R-250, and the Precision Plus Protein™ Dual Color Standard was used as the molecular weight marker (Laemmli, 1970).

Foaming capacity and foam stability

Ten millilitres of each sample were loaded onto a conical tube and homogenised at 12,000 rpm for 2 min. The foam volume was recorded and used to calculate foaming capacity (percentage; versus the initial volume); the decreased volumes at 2, 5, 10, 20, 30, and 60 min after homogenisation were used to calculate foam stability (percentage; versus the initial foam volume) (Mishyna *et al.*, 2019).

Emulsifying capacity and emulsion stability

To 10 ml of samples, 1 ml of pure olive oil was added and homogenised at 18,000 rpm for 2 min. The difference between the emulsion layer volume and the initial volume was calculated as the percentage. After adding 50 µl sample to 10 ml of SDS solution, the absorbance at 500 nm was recorded at 5, 10, 20, 30, 60, 90, and 120 min and compared with the initial absorbance of the mixed samples (Pearce and Kinsella, 1978).

Statistical analysis

The one-way analysis of variance (ANOVA) with Duncan's multiple range test was conducted to evaluate significant differences among samples. A *P*-value of less than 0.05 was defined as statistically significant. The IBM SPSS statistics 20 software (IBM Corp., Armonk, NY, USA) was used for all the statistical analyses. The content of protein sources from pork and insects and the buffer type in the context of gel solubility were considered as fixed effects, while the other variants were considered as random effects.

3. Results and discussion

Gel solubility

The gelling properties can be determined via the analysis of ionic bonds, hydrogen bonds, hydrophobic bonds, and disulphide bonds (Zayas, 2012). In this study, five different solutions – SA, SB, SC, SD, and SE – were used to break ionic, hydrogen, hydrophobic, and disulphide bonds of gel (Lin *et al.*, 2019). Solubility in SA represents the non-specific association, whereas the differential solubility in SA and SB, SB and SC, SC and SD, SD and SE were related with ionic bonds, hydrogen bonds, hydrophobic bonds, and disulphide bonds, respectively (Gomez-Guillen *et al.*, 1997). All protein combinations in this study showed the highest value for hydrophobic bonds (Table 1), suggesting that hydrophobic bonds had a close relationship with the gelation of PP and IP (Lin *et al.*, 2019). In addition, the replacement of IP with PP has increased the disulphide bonds in all combinations, which could be attributed to the changes in protein structure during the heat processing of the gels (Lin *et al.*, 2019). These findings are consistent with Wang *et al.* (2017), who reported that heating reduced the ionic bonds and hydrogen bonds while increasing the disulphide and hydrophobic bonds in wheat gluten gelation (Wang *et al.*, 2017).

Moreover, the non-specific association, ionic bonds, hydrogen bonds, hydrophobic bonds, and disulphide bonds were slightly increased with increasing IP content (*P*<0.05; Table 1). These results could be due to the interference of other components that could have reacted with the Bradford reagent, besides protein (Janssen *et al.*, 2017). In insects, there are abundant amounts of acetyl glucosamine components such as chitin, and these components also have ionic bonds, hydrogen bonds, hydrophobic bonds, and disulphide bonds (Pillai *et al.*, 2009). The chitin content in extracted *P. brevitarsis* protein powder has been reported to be >3% (Kim *et al.*, 2020). Although chitin is insoluble in distilled water, low molecular size chitin components of 25–75 kDa might be present within the supernatants since whole insects were used for protein extraction. Of note, these non-protein fractions could inhibit the enhancement of protein function (Kim *et al.*, 2020). However, further investigations could help understand the exact effects of chitin on protein function.

Apparent viscosity

Generally, flow behaviour and gelling properties are closely related since the cross-linking strength between proteins affects them both (Zayas, 2012). Therefore, the apparent viscosity was measured to estimate the gelling properties. The apparent viscosity of the gels prepared from all combinations showed a decreasing tendency with increasing rotation time. It was noteworthy that the apparent viscosity of the gels containing IP0 and IP20 had a

Table 1. Effect of partial replacement of porcine myofibril protein by *Protaetia brevitarsis* protein on gel solubility of heat-induced gel.^{1,2,3,4}

	IP0	IP20	IP40	IP60	IP80	IP100
Nonspecific association (mg/ml)	1.60±0.16 ^{Ec}	1.96±0.14 ^{Cc}	1.75±0.14 ^{Ec}	2.19±0.37 ^{Dc}	4.86±0.21 ^{Bd}	5.24±0.16 ^{Abc}
Ionic bonds (mg/ml)	1.40±0.27 ^{Ec}	2.76±0.70 ^{Db}	2.14±0.13 ^{Dc}	3.67±0.70 ^{Cb}	5.41±0.20 ^{Ac}	5.06±0.23 ^{Bc}
Hydrogen bonds (mg/ml)	1.59±0.13 ^{Ec}	2.01±0.34 ^{Dc}	2.13±0.03 ^{Dc}	4.27±0.81 ^{Cb}	6.04±0.34 ^{Aab}	5.28±0.18 ^{Bbc}
Hydrophobic bonds (mg/ml)	2.70±0.13 ^{Da}	3.80±0.11 ^{Ca}	5.88±0.39 ^{Ba}	6.19±0.17 ^{Aa}	6.36±0.34 ^{Aa}	6.32±0.18 ^{Aa}
Disulphide bonds (mg/ml)	1.99±0.25 ^{Cb}	3.34±0.38 ^{Ba}	3.99±0.78 ^{Bb}	3.91±1.65 ^{Bb}	5.96±0.25 ^{Ab}	5.52±0.32 ^{Ab}

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

² IP and number meant insect protein and percentage of insect extracted powder in gel.

³ Means within a row with different capital letters are significantly different ($P<0.05$).

⁴ Means within a column with different lower case letters are significantly different ($P<0.05$).

similar decreasing tendency with increasing rotation time; however, it was decreased with increasing contents of IP in gels containing a higher proportion of IP powder (40% and over) (Figure 1). These results indicated that the apparent viscosities of the IP gels are lower than that of PP gel. Similar to our findings, a previous study has shown a lower apparent viscosity of a mealworm emulsion than that of an emulsion composed of pork meat (Choi *et al.*, 2017), which could be explained by the lower water-binding capacity of insect versus pork meat (Kim *et al.*, 2016). Furthermore, it has been shown that the higher hydrophobicity of proteins leads to a higher apparent viscosity; however, our results did not show this tendency, which could also be due to the non-protein fractions mentioned above, inhibiting the cross-linking between proteins.

Scanning electron microscopy

The cross-linking between the proteins was detected by SEM. IP0 had a compact and smooth surface appearance. However, the addition of IP turned the surface rough; moreover, the increase in IP content increased the porosity of the protein gel (Figure 2). Therefore, the high solubility of hydrophobic and disulphide bonds of IP may not be appropriate for gelation. Here we observed some linear and globular-shaped structures in the micrographs of gels, representing the long-chain Poly-N-acetyl glucosamine molecules and proteins (Pillai *et al.*, 2009; Uengkimbuan *et al.*, 2006). Interestingly, the collapse of round structures was also observed with an increasing IP content indicating the inhibition of the thermal gelling reaction, and the decreased

Figure 1. Effect of partial replacement of porcine myofibril protein by *Protaetia brevitarsis* protein on apparent viscosity of gel. IP and number meant insect protein and percentage of insect extracted powder in gel.

gelling reaction could lead to poor gelling properties (Zayas, 2012). Taken together, these findings demonstrate that IPs might not be appropriate to form compact gel products.

Differential scanning calorimetry

DSC was used to estimate the thermal stability of proteins (Heussen *et al.*, 2011) and deduce the effects of the heating process on protein denaturation and aggregation. The DSC analysis revealed a reducing effect of heating on all the parameters used to measure the thermal stability, including the onset temperature, peak temperature, end temperature, and change in enthalpy (ΔH) (Table 2). It has been shown that partial protein unfolding, the formation of new bonds between proteins, and the protein hydration proceed during thermal processing; protein thermal stability depends on protein hydration and the hydrophilic and hydrophobic reaction residue (Parniakov *et al.*, 2018). Moreover, the variation in the above-mentioned thermal properties may depend on the type of protein (Quinn *et al.*, 1980). In the present study, despite the high value of hydrophobic bonds, the IPs had a poor flow behaviour (Figure 1) and showed collapsed micrographs (Figure 2), which could explain the low thermal stability of IP-based gels. In addition, the reduced thermal stability of the IP-based gels could also be attributed to the low cross-linking power of IP. Furthermore, the peak temperature (denaturation temperature) was the highest in IP0, IP20, IP40, and IP 60 ($P < 0.05$), and the onset

temperatures of IP0, IP20, and IP40 were not significantly different ($P > 0.05$). These results suggest that PP can increase the denaturation temperature and thermal stability in the context of gelation. However, the ΔH of IP40 was lower than that of IP0 and IP20 ($P < 0.05$). This decreased ΔH value could be related to protein unfolding and the interaction between the proteins (Parniakov *et al.*, 2018). In addition, the increase in gel solubility and decrease in thermal properties could be attributed to the chitin content in edible insects (Kim *et al.*, 2020). However, the thermal stability of chitin is very high, and it does not degrade at over 300 °C (Guinesi and Cavaleiro, 2006); therefore, the poor gel properties of the IP-based gels in this study could probably be due to some other components in IP whose structure was degraded at 75 °C.

Texture profile analysis

During thermal gelation, the denaturation of the components determines gel formation and the textural properties of the gel (Zayas, 2012). The hardness of the gel decreased rapidly with the replacement by IP (Table 3), consequently decreasing the gumminess and chewiness. Although the springiness was not different among treatments except for IP100, the other parameters were lower in gels containing IP than in gels containing 100% PP. Importantly, these results align with those obtained in other studies, in the context of the substitution of meat by proteins extracted from the

Figure 2. Effect of partial replacement of porcine myofibril protein by *Protactia brevitarsis* protein on scanning electrical microscope of gel. IP and number meant insect protein and percentage of insect extracted powder in gel.

Table 2. Effect of partial replacement of porcine myofibril protein by *Protaetia brevitarsis* protein on thermal properties of gel.^{1,2,3}

	IP0	IP20	IP40	IP60	IP80	IP100
Onset temperature (°C)	57.87±1.32 ^a	56.98±1.55 ^a	57.59±1.50 ^a	51.13±0.55 ^b	46.46±0.80 ^c	44.97±0.68 ^c
Peak temperature (°C)	68.52±0.27 ^a	68.54±0.14 ^a	70.18±0.77 ^a	68.14±0.48 ^a	56.90±1.85 ^b	54.78±2.42 ^b
End temperature (°C)	79.76±2.19 ^a	77.29±1.72 ^{ab}	77.32±1.28 ^{ab}	76.00±0.80 ^{ab}	73.61±2.68 ^b	62.82±2.14 ^c
Delta H (J/g)	1.64±0.05 ^a	1.35±0.29 ^a	0.77±0.39 ^b	0.75±0.06 ^b	0.43±0.08 ^{bc}	0.17±0.06 ^c

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

² IP and number meant insect protein and percentage of insect extracted powder in gel.

³ Means within a column with different lower case letters are significantly different ($P<0.05$).

larvae of other insect species, such as mealworms and black soldier flies (Bessa *et al.*, 2019; Choi *et al.*, 2017). These studies showed that the poor cross-linking properties of edible IP might rapidly decrease the quality of meat proteins in the context of meat products.

As Line *et al.* (2019) reported, the higher the hydrophobicity of the gels, the higher is their strength (Lin *et al.*, 2019). However, despite the increased hydrophobic and disulphide bonds imparted by the substitution by IP, the gel strength was decreased in this study. As observed by DSC, IP reduced the ΔH value, which indicated lower unfolding of IP via heat-treatment than PP. Therefore, it was speculated that the lower unfolding rate, together with the non-protein fraction with high thermal stability, probably could have led to increased gel solubility. Furthermore, these non-protein fractions might inhibit protein-protein interactions or protein-water interactions, impair gel formation, and negatively impact the textural properties (Zayas, 2012).

SDS-PAGE

The molecular weight of proteins determines their functional properties; higher molecular weight proteins promote a stronger gel network (Wang and Damodaran,

1990). As shown in Figure 3, the bands between 25 and 50 kDa and above 100 kDa were weaker with an increase in the percentage of IP in the protein gels, while the bands between 50 and 75 kDa were strong. Furthermore, widely spread but faint bands (<15 kDa) were more intense, which was further increased with an increase in IP content. These results suggest that the molecular weight of IP is widely distributed. Pork meat consists of typical proteins such as myosin, actin, and sarcoplasmic components, whereas insect extracts may contain various components such as haemolymph proteins, enzymes, chitin, and various salt-soluble molecules (Yi *et al.*, 2013, 2016). The observed protein between 14 and 32 kDa could be an insect cuticle protein containing chitin; the increase in band intensity could, therefore, be explained by an increase in chitin content with an increasing IP content (Yi *et al.*, 2013). Although proteins with low molecular weights may be irrelevant in the context of gel formation, they may contribute to the enhancement of protein functionality in solution at low protein concentration (Zayas, 2012). In fact, the hydrophobicity and protein concentration may have a critical impact on the foam and emulsion properties of protein solutions because excessive cross-linking was shown to interrupt the formation of stable foam and emulsion states (Baeza *et al.*, 2004; Zayas, 2012).

Table 3. Effect of partial replacement of porcine myofibril protein by *Protaetia brevitarsis* protein on textural properties of gel.^{1,2,3}

	IP0	IP20	IP40	IP60	IP80	IP100
Hardness (kg)	5.63±0.21 ^a	0.39±0.12 ^b	0.33±0.01 ^{bc}	0.08±0.02 ^c	0.08±0.01 ^c	0.07±0.03 ^c
Springiness	0.99±0.02 ^a	0.94±0.04 ^a	0.94±0.04 ^a	0.89±0.04 ^a	0.93±0.01 ^a	0.63±0.18 ^b
Cohesiveness	0.85±0.07 ^a	0.59±0.01 ^b	0.50±0.01 ^{bc}	0.48±0.04 ^{bcd}	0.43±0.08 ^{cd}	0.37±0.01 ^d
Gumminess (kg)	4.82±0.58 ^a	0.19±0.05 ^b	0.12±0.01 ^b	0.04±0.01 ^b	0.04±0.01 ^b	0.03±0.02 ^b
Chewiness (kg)	4.76±0.65 ^a	0.18±0.05 ^b	0.12±0.01 ^b	0.04±0.01 ^b	0.04±0.01 ^b	0.02±0.02 ^b

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

² IP and number meant insect protein and percentage of insect extracted powder in gel.

³ Means within a column with different lower case letters are significantly different ($P<0.05$).

Figure 3. Effect of partial replacement of porcine myofibril protein by *Protactia brevitarsis* protein on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of solution. IP and number meant insect protein and percentage of insect extracted powder in solution.

Foaming capacity and foam stability

The formation of foam is an important factor in the determination of the physicochemical properties of proteins. Foam formation may be affected by pH, ionic strength, protein structure, and protein hydrophobicity (Zayas, 2012). IP0 had the lowest, and IP60, IP80, and IP100 the highest foaming capacity ($P<0.05$; Figure 4A). These results are probably due to the different structural characteristics of IP and PP. Because high surface viscosity decreases the molecular flexibility of interfacial film during

foam formation, low viscous proteins have a higher capacity to foam with larger bubbles (Poole *et al.*, 1984; Zayas, 2012). Furthermore, as shown in Figure 2, the linear structure was observed in gels containing IP. These unfolded structures have a higher hydrophobicity than folded proteins, affecting an interfacial film strength (Zayas, 2012). Therefore, the foaming capacity of the solution containing IP was increased with the increase in the replacement content. However, large-sized bubbles may have poor foam stability; large bubbles and high value in initial foam volume usually show a decreased thickness of the protein film at the interface (Zayas, 2012). It was observed that the foam of IP0 remained over 60% until 60 min, but more than half of the foam collapsed after 60 min in other treatments (Figure 4B). Since the size of the proteins also affects the thickness of the foam film, the high content of PP in the solution might have a positive effect on foam stability instead of IP.

Emulsifying capacity and emulsion stability

When PP was substituted by IP, the emulsifying capacity of the mixed protein was increased (Figure 5A) with increasing IP content. IP80 and IP100 showed the highest ($P<0.05$), whereas IP0, IP20, and IP40 showed the lowest emulsifying capacity ($P<0.05$). Among the various factors that could affect emulsifying capacity, protein hydrophobicity is one of the most important factors – proteins with high hydrophobicity usually correlate with an increased emulsifying capacity (Kim *et al.*, 2019a). As shown in Table 1, the replacement of PP with IP increased the hydrophobic bonds. Furthermore, the flexibility of proteins is important, particularly while considering the dispersion of oil droplets in water (Zayas, 2012). Concerning emulsion stability, IP had a negative effect,

Figure 4. Effect of partial replacement of porcine myofibril protein by *Protactia brevitarsis* protein on (A) foaming capacity and (B) foam stability of solution. Different letters on top of column means significant difference ($P<0.05$). IP and number meant insect protein and percentage of insect extracted powder in solution.

and the emulsion stability sharply decreased when IP and PP were mixed after 30 min (Figure 5B). Furthermore, when emulsions were generated using only one type of protein, the IP emulsion was more stable than the PP emulsion for 120 min, indicating that the interfacial film was easily disrupted only when IP and PP were mixed. These results might be due to protein denaturation, as observed by DSC (Table 2). With the addition of IP, ΔH was rapidly decreased, and this result indicated that myofibrillar protein could denature easily when mixed with IP. High denatured myosin in myofibrillar protein could induce the large size of cross-linked protein, and too large size of protein could induce the loss of molecular flexibility (Xia *et al.*, 2010). Therefore, the low emulsion stability of the mixed protein solutions with highly denatured protein compared with pure protein solutions (IP0 and IP100) could be due to the loss of molecular flexibility that could have induced unstable emulsion formation (Zayas, 2012).

4. Conclusion

This study was conducted to estimate the effect of the partial replacement of PP with IP on the gelation and solution properties. Despite the higher amount of hydrophobic and disulphide bonds in the gels containing IP, they showed low viscosity, thermal stability, and a poor texture. However, the solution properties such as foaming and emulsion capacities increased when PP was replaced with IP. Overall, these findings demonstrate that the proteins extracted from *P. brevitarsis* were not sufficient to form a hard heat-induced gel; however, they are more suitable for use in a solution state with high physicochemical functionality.

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Conflict of interest

The authors declare no conflict of interest.

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Figure 5. Effect of partial replacement of porcine myofibril protein by *Protactia brevitarsis* protein on (A) emulsifying capacity and (B) emulsion stability of solution. Different letters on top of column means significant difference ($P < 0.05$). IP and number meant insect protein and percentage of insect extracted powder in solution.

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