Use of edible insects in thermal-induced protein gels containing porcine myofibrillar protein

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Abstract

Edible insects are considered an environmentally friendly source of protein; however, products containing only edible insects are associated with poor quality and processing properties. Therefore, we examined and compared the thermal stability and rheological properties among thermal-induced myofibrillar protein gels prepared using porcine myofibril protein and five different edible insect species: larvae: *Tenebrio molitor* L., *Protaetia brevitarsis*, and *Allomyrina dichotoma* and adult insects: *Gryllus bimaculatus* and *Oxyachinensis sinuosa*. The myofibrillar protein gels containing edible insects had a higher pH than that of the control. The water-holding capacity of the myofibrillar gel containing *G. bimaculatus* (T4) was higher than that of the other samples. The hardness, cohesiveness, gumminess, and chewiness of the thermal-induced myofibrillar gel containing T4 were the highest. The myofibrillar gel containing edible insects had a higher peak temperature than that of the control, except for T4, as well as a similar or lower enthalpy, which is required for denaturation. All myofibrillar gels with/without edible insects exhibited Hershel-Bulkley fluid properties, and rigid structures were developed during heating. The myofibrillar protein gels containing T4 revealed excellent processing properties. Therefore, myofibrillar protein gels containing T4 may be used for producing protein-rich foods.

Keywords: thermal-induced gel, myofibrillar protein, edible insect, thermal stability, rheological property

1. Introduction

Myofibrillar protein is considered an important protein owing to its gel-forming ability in meat protein (Kim *et al.*, 2021a), and it affects the processing of emulsion meat products (McCord *et al.*, 1998). Myofibrillar proteins comprise approximately 60% of total meat protein (Choi *et al.*, 2011) and play an important role in improving functional properties of meat foods such as gel-forming ability, water-binding ability, and emulsifying properties (Li *et al.*, 2020). It is well known that myofibrillar protein, a salt-soluble protein, forms a gel via thermal denaturation, and this protein gel has a stable three-dimensional network structure (Choi *et al.*, 2011). The myofibrillar protein gel network structure depends on the protein concentration, pH, and actomyosin solubility (Yasui *et al.*, 1982). Xu and Xu (2021) reported that myofibrillar proteins are primarily

responsible for processing yield, texture properties, and organoleptic characteristics of emulsion meat products. Few studies have reported a new approach to improve the functional properties of myofibrillar proteins with addition of polyphenols or polysaccharides (Cheng *et al.*, 2020; Zhuang *et al.*, 2020). However, the addition of non-protein fraction such as polyphenols or polysaccharides could increase the processing cost or decrease protein nutritional value in essential amino acid. Therefore, other approach should be conducted to improve functional properties of myofibrillar protein.

Edible insects are considered as a food source as they provide food security (Kim *et al.*, 2021a); studies have additionally evaluated insect proteins as an alternative to animal protein (Kim *et al.*, 2019a). Edible insect protein is an environmentally friendly protein since its production

can reduce environmental pollution compared to that created by animal protein production (Köhler *et al.*, 2019). Additionally, production of edible insect protein is effective in maintaining carbon neutrality, as it can reduce carbon dioxide emissions (Ham *et al.*, 2021). However, edible insect proteins are associated with technical limitations and cannot completely replace animal protein at present (Choi *et al.*, 2017). Kim *et al.* (2019b) reported that a product containing only edible insects results in poor quality and processing properties. Edible insect processing technologies are currently under research to overcome technical limitations (Kim *et al.*, 2021a; Park *et al.*, 2017). Thus, this study investigated and compared the properties between porcine myofibrillar proteins and edible insect proteins. The aim of this study was to evaluate and compare the physicochemical properties of thermal-induced protein gels between porcine myofibril and edible insect proteins obtained from different insect species to enhance their rheological properties.

2. Materials and methods

Porcine myofibrillar protein extraction

Porcine myofibrillar protein extraction was performed as described by Choi *et al.* (2011). The freeze-dried porcine myofibrillar protein was prepared as below method. Firstly, fresh pork ham (*M. biceps femoris*, *M. semitendinosus*, and *M. semimembranosus*) was obtained from local market A and ham was ground through 8 mm grinder. To dissolve sarcoplasmic protein, one parts of ground meat was dissolved in 4 parts of 0.1 M KCl in 0.01 M phosphate buffer at pH 7.4. After centrifuging at 15,000×g using continuous centrifuge (HC-140HO, Hanil SME, Anyang, Korea) collected residue was re-dissolved in 4 times of 0.1 M KCl in 0.01 M phosphate buffer. Same procedure was repeated 2 times and lastly, remained salt was removed by distilled water using continuous centrifuge (HC-140HO). Residue was considered as myofibrillar protein (MP) and freeze-dried MP was dissolved in 0.58 M saline solution at 30 mg/ml.

Preparation of edible insects

The larvae of three insects (*Tenebrio molitor* L., *Protaetia brevitarsis*, and *Allomyrina dichotoma*) and two adult insects (*Gryllus bimaculatus* and *Oxyachinensis sinuosa*) commonly used in industrial applications were selected. An important factor in selecting edible insects is whether they can be used easily as well as for industrial use. Edible

insects were purchased from Farm Bang (Suncheon, South Korea). The fasting insects were obtained and stored at -15 °C. The insects were freeze-dried and pulverised to obtain an insect powder. Pulverised insects were filtered through a sieve (20 mesh) and used for preparation of myofibrillar protein gels.

Gel preparation

Thermal-induced myofibrillar gel was prepared as described by Choi *et al.* (2011). To prepared mixed gel, 1% of insect powder was added into 30 mg/ml of myofibrillar protein gel. Control was prepared without any edible insect. The insect species which added in myofibril gel were *T. molitor* larvae, *P. brevitarsis* larvae, *A. dichotoma* larvae, *G. bimaculatus* adult, *O. sinuosa* adult and treatments named T1, T2, T3, T4, and T5 in order. The mixed porcine myofibril protein and edible insects were transferred into 20 mm diameter glass gelling tubes. The gel solution containing porcine myofibrillar protein and edible insects was centrifuged at 800×g for 10 min to remove air bubbles (4 °C). The sealed tubes containing the gel solution were heated at 75 °C for 30 min.

pH

The pH values of the non-thermal and thermal-induced myofibrillar gel containing edible insect samples were measured in a mixture comprising 5 g of sample and 50 ml distilled water 20 ml using a pH meter.

Colour

The lightness (L*), redness (a*), and yellowness (b*) values of the non-thermal and thermal-induced myofibrillar gel containing edible insect samples were measured (Illuminant C) using a colourimeter (CR-410; Minolta Co., Osaka, Japan).

Water-holding capacity

The water-holding capacity (WHC) of the thermal-induced myofibrillar gel containing edible insect samples was determined gravimetrically as described by Kocher and Foegeding (1993). After cover 1 g of heat-induced gel using filter paper (No. 4, Whatman Ltd. Kent, UK), samples were centrifuged at 6,710×g for 10 min. The difference weight of filter paper before and after centrifugation was considered as moisture loss and it was compared with protein gel weight (1). The WHC was calculated as percentage (%).

$$\text{WHC (\%)} = \frac{\text{Weight of sample before centrifugation} - \text{moisture loss content after centrifugation}}{\text{Weight of sample}} \times 100$$

Texture profile analysis

The textural properties of the thermal-induced myofibrillar gel containing edible insect samples were measured using a texture analyser (TA-XT2i; Stable Micro Systems, Surrey, England) with 25.4 mm diameter cylinder probe. The texture profile analysis conditions were as follows: height of myofibrillar gel, 15 mm; maximum load, 2 kg; force, 10 g; pre-test speed, 3.0 mm/s; post-test speed, 5.0 mm/s; distance, 10.0 mm; and head speed, 3.0 mm/s (Bourne *et al.*, 1978).

Differential scanning calorimetry

The thermal properties of the myofibril gels were estimated via differential scanning calorimetry (DSC; DSC 400 furnace; PerkinElmer, Waltham, MA, USA) which calibrated by indium standard. The non-heated samples (30 mg) were placed in an aluminium pan and sealed tightly, and the thermal properties at 20–100 °C were evaluated based on heating at 10 °C/min. The onset, peak, and end temperatures were detected using Pyris data analysis software (PerkinElmer), and enthalpy (ΔH) was calculated using the same software (Kim *et al.*, 2021a).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

The protein molecular weight distribution was estimated via sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). All reagents and materials were obtained from Bio-Rad (Hercules, CA, USA). Protein concentration of the mixtures of MP and insect powder was regulated to 1 mg/ml solutions using bicinchoninic acid protein assay. Sample buffer and 20 μ g of sample was mixed and β -mercaptoethanol was used to reduce protein; Precision Plus Protein™ Dual Color Standards (Bio-Rad Laboratories, Hercules, CA, USA) were loaded onto Mini-PROTEIN® TGXTM gels (Bio-Rad Laboratories) after heating at 100 °C for 5 min. After electrophoresis at 100 mA, the gels were stained with 0.25% Coomassie Brilliant Blue R-250 (Bio-Rad Laboratories) and destained.

Evaluation of rheological properties

The flow curves of the non-heated gel samples were characterised using a modular compact rheometer (MCR 102; Anton Paar GmbH, Graz, Austria). The measurements were performed using a parallel plate with a diameter of 25 mm and a gap of 1 mm at different shear rates ranging from 0.01–100 s⁻¹ at 25 °C. The Herschel-Bulkley model was applied to analyse the obtained results (Gomathy *et al.*, 2015; Štreimikytė *et al.*, 2020), by using the following equation:

$$\sigma = \sigma_0 + K(\dot{\gamma})^n$$

where σ and σ_0 are the shear stress (Pa) and the yield stress (Pa), respectively; K is the consistency coefficient (Pa·sⁿ); $\dot{\gamma}$ is the shear rate (s⁻¹); and n is the flow behaviour index.

A temperature sweep test was performed using the same equipment at a strain of 0.5% in the temperature range of 10–90 °C. The storage modulus (G'), loss modulus (G''), and loss factor ($\tan \delta = G''/G'$) were recorded using Rheocompass 1.19 (Anton Paar GmbH, Graz, Austria) during the measurements at a constant heating rate of 2 °C/min.

Statistical analysis

Data were obtained from triplicate experiments. All data were analysed using the general linear model procedure in SPSS (ver. 20.0; SPSS Inc., Chicago, IL, USA). The five edible insect species were considered as the main effects, and the replicates and other uncontrollable external factors were considered as randomised effects. Duncan's multiple range test was performed to investigate the significant differences between samples ($P < 0.05$).

3. Results and discussion

pH, colour, and water-holding capacity

The pH, colour, and WHC of the thermal-induced myofibrillar gel containing proteins of five different edible insect species are shown in Table 1. The pH of the non-thermal and thermal-induced myofibrillar protein gels ranged from 6.13–6.51 and from 6.20–6.59, respectively. The myofibrillar protein gels containing edible insects had a higher pH than that of the control gels ($P < 0.05$). Kim *et al.* (2020) reported that the pH of emulsion systems replacing myofibrillar protein with *T. molitor* is significantly higher because of the high pH of *T. molitor* components. Similarly, the results in our study were found to be affected by the myofibrillar protein extraction buffer solution, and it is thought to be affected by the pH of the edible insects. These results are in agreement with those of Choi *et al.* (2011), who indicated that the pH of heated myofibrillar gel systems is higher than that of unheated myofibrillar gel systems.

The L^* of the control myofibrillar gel and gel containing *G. bimaculatus* (T4) was higher than that of the other samples. The L^* of the control was the highest among those of the heated samples ($P < 0.05$). When heat was applied, the pH tended to decrease and the L^* tended to increase. According to Choi *et al.* (2011), L^* increases due to the scattering of water molecules when heated. The a^* and b^* of the gels containing *T. molitor* larvae (T2) and *O. sinuosa* (T5) were the highest ($P < 0.05$). This result may be due to the different colours of edible insect species (Kim *et al.*,

Table 1. pH, colour, and water holding capacity (WHC) of thermal-induced myofibrillar protein gels containing edible insects.¹

Treatments ²	Control	T1	T2	T3	T4	T5
Raw						
pH	6.13±0.02 ^e	6.24±0.01 ^c	6.24±0.01 ^c	6.35±0.01 ^b	6.51±0.02 ^a	6.20±0.02 ^d
CIE L*	59.75±0.73 ^a	52.30±2.19 ^{bc}	53.38±2.88 ^b	48.78±0.79 ^c	58.32±1.27 ^a	43.15±4.07 ^d
CIE a*	-1.50±0.12 ^f	0.15±0.17 ^d	3.05±0.12 ^a	1.20±0.06 ^c	-0.42±0.08 ^e	2.71±0.19 ^b
CIE b*	1.93±0.28 ^e	8.38±0.61 ^c	17.53±1.48 ^a	10.92±0.56 ^b	5.51±0.45 ^d	12.01±5.15 ^b
Heated						
pH	6.20±0.01 ^f	6.29±0.01 ^e	6.30±0.01 ^d	6.56±0.01 ^b	6.59±0.01 ^a	6.33±0.01 ^c
CIE L*	80.56±1.09 ^a	69.98±1.65 ^c	63.11±0.52 ^d	58.46±0.31 ^e	75.28±0.50 ^b	59.18±2.13 ^e
CIE a*	2.92±0.06 ^e	0.36±0.95 ^c	4.47±0.47 ^a	1.71±0.04 ^b	0.85±0.29 ^d	4.93±1.09 ^a
CIE b*	3.94±0.36 ^d	12.79±2.75 ^b	20.04±0.93 ^a	13.28±0.24 ^b	7.50±0.45 ^c	17.19±2.73 ^a
WHC(%)	56.50±2.07 ^b	58.38±1.76 ^b	52.64±2.64 ^c	56.91±1.68 ^b	62.59±1.01 ^a	55.72±1.64 ^{bc}

¹ All values are presented as the mean ± standard deviation of three replicates (n=3). Means within a row with different letters are significantly different ($P<0.05$).

² Control = gel without edible insect; T1 = 1% *Tenebrio molitor* larvae; T2 = 1% *Protaetia brevitarsis* larvae; T3 = 1% *Allomyrina dichotoma* larvae; T4 = 1% *Gryllus bimaculatus* adult; T5 = 1% *Oxyachinensis sinuosa* adult.

2021a). Melanin is a major insect pigment produced by biosynthesis pathways (Wittkopp and Beldade, 2009).

The WHC of the thermal-induced myofibrillar gels ranged from 52.64–62.59%. The WHC of the myofibrillar gel containing T4 samples was higher than that of the other samples ($P<0.05$). *G. bimaculatus* contains a large amount of chitin (Chae *et al.*, 2018), which is known to have a high WHC (Chang *et al.*, 1994). According to Choi *et al.* (2011), gel formation occurs during protein denaturation followed by irreversible aggregation, and the thermal-induced myofibrillar protein gel forms a three-dimensional network in which water is trapped. Although WHC of treatments was higher ($P<0.05$) or similar ($P>0.05$) with control, WHC of T2 was lower than control ($P<0.05$). It might be due to poor insect-protein interaction with other protein and water than porcine myofibrillar protein (Scholliers *et al.*, 2020). Thus, the high WHC of myofibrillar gels containing edible insects may be advantageous in the application of

various products by improving the processing potential of insect products.

Texture profile analysis

The texture profiles of the thermal-induced myofibrillar gels containing five different edible insect species exhibited considerable differences among treatments (Table 2). The hardness, cohesiveness, gumminess, and chewiness of the control thermal-induced myofibrillar gel (without edible insects) were higher ($P<0.05$) than those of the thermal-induced myofibrillar gels containing edible insects, except for myofibrillar gel containing T4. The hardness, cohesiveness, gumminess, and chewiness of the thermal-induced myofibrillar gel containing T4 were the highest ($P<0.05$). The springiness of the thermal-induced myofibrillar gels ranged from 0.84–0.96, and that of the thermal-induced myofibrillar gel containing *P. brevitarsis* (T2) was the lowest ($P<0.05$). Kim *et al.*

Table 2. Texture profile analysis of thermal-induced myofibrillar protein gels containing edible insects.¹

Treatments ²	Control	T1	T2	T3	T4	T5
Hardness (g)	40.59±1.24 ^b	28.64±1.31 ^d	17.31±1.26 ^e	32.65±1.78 ^c	48.68±1.15 ^a	17.05±1.32 ^e
Springiness	0.96±0.03 ^a	0.92±0.05 ^b	0.84±0.04 ^c	0.92±0.05 ^b	0.95±0.05 ^a	0.93±0.04 ^{ab}
Cohesiveness	0.43±0.01 ^b	0.39±0.01 ^c	0.36±0.03 ^d	0.37±0.01 ^d	0.47±0.01 ^a	0.39±0.02 ^c
Gumminess (g)	17.27±0.38 ^b	11.19±0.61 ^c	6.27±0.67 ^d	12.17±0.83 ^c	22.70±0.64 ^a	6.68±0.54 ^d
Chewiness (g)	16.55±0.56 ^b	10.32±0.25 ^c	5.23±0.62 ^e	11.18±1.22 ^c	21.58±0.96 ^a	6.19±0.56 ^d

¹ All values are presented as the mean ± standard deviation of three replicates (n=3). Values within a row with different letters are significantly different ($P<0.05$).

² Control = gel without edible insect; T1 = 1% *Tenebrio molitor* larvae; T2 = 1% *Protaetia brevitarsis* larvae; T3 = 1% *Allomyrina dichotoma* larvae; T4 = 1% *Gryllus bimaculatus* adult; T5 = 1% *Oxyachinensis sinuosa* adult.

(2021b) reported that the hardness of a gel containing *A. dichotoma* was higher than that of gels containing *T. molitor* and *P. brevitarsis*, and significant differences in texture profiles were confirmed in gels prepared only using edible insect proteins. Thus, it was confirmed that the structure and physical properties of the gel are affected by the insect protein, which is similar to the results of this study. According to Choi *et al.* (2011), the texture profile of thermal-induced gels is affected by the protein concentration and protein interaction. Westphalen *et al.* (2006) reported that the texture profiles of thermal-induced gel containing porcine myofibrillar protein is affected by muscle type, and it contributes to the texture profile which is also affected by the gelling capacity of protein interaction. Nuckles *et al.* (1991) noted that the gelation of thermal-induced myofibrillar gel is one of the most substantial functional properties of meat emulsion products due to its desirable textural properties. Thus, it is possible that texture profiles of thermal-induced gels may be attributed to the interaction between porcine myofibrillar protein and edible insect protein, and it may expand its utilisation as an edible insect protein.

Differential scanning calorimetry

Differences and changes in the structural characteristics of proteins can be induced by heating; denaturation temperature and enthalpy can be detected via DSC (Kazemi *et al.*, 2011; Quinn *et al.*, 1980). Generally, myosin and actin are denatured at approximately 55 °C and 78–80 °C, respectively (Kazemi *et al.*, 2011). Denaturation of the control gel started at approximately 52.43 °C and ended at approximately 76.11 °C (Table 3). Therefore, the control gel followed the general thermal characteristics of myofibrillar protein. Thermal properties of proteins are affected by the type of protein, protein hydration, hydrophobic residue, hydrophilic residue, and added ingredients (Parniakov *et al.*, 2018; Quinn *et al.*, 1980). Therefore, protein-protein interactions, protein-water interactions, and protein residues may change with the addition of edible insects. All treatments except for T4 had higher peak temperatures than

those of the control ($P<0.05$) and a similar ($P>0.05$) or lower ($P<0.05$) enthalpy (ΔH), which is the energy required for denaturation. When proteins are heated, highly denatured proteins require a lower enthalpy than that required by proteins denatured to a lower extent (Kazemi *et al.*, 2011). These results showed that the thermal properties of myofibrillar protein can be affected by the addition of edible insects and by the interaction with myofibrillar protein and processed insects. However, a similar peak temperature between the control and T4 was observed ($P>0.05$), and T4 had a higher enthalpy than that of the control ($P<0.05$). This higher value of enthalpy indicates a more complex protein structure. Therefore, T4 may form a more compact gel than that formed by other treatments.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

The molecular weight distribution of edible insects and myofibrillar proteins of various edible insects is shown in Figure 1. When focused on protein distribution of edible insects, their patterns were different by species. The major bands of *T. molitor* were observed below 15 kDa before and after reducing. High intense protein bands were observed between 75 and 100 kDa in *P. brevitarsis* and the difference between reducing and non-reducing was slight; protein bands under 20 kDa was observed after reducing. In *A. dichotoma*, protein band above 250 kDa was observed before reducing, but this band was disappeared after reducing. The clear changes after reducing in protein bands were observed in *G. bimaculatus* compared with other species. The protein bands between 75 and 150 kDa was reduced to 37–50 kDa by mercaptoethanol and this protein molecular weight was similar with actin component in meat (Choi *et al.*, 2011). *O. sinuosa* had the highest intensity above 250 kDa and disulphide bonds induced by heating between proteins might be a reason of increased molecular weight because novel bands were observed between 100–250 kDa. Although the exact protein by molecular weight was not identified in this study, this difference might affect gel properties of myofibrillar protein due to changes in various

Table 3. Differential scanning calorimetry of thermal-induced myofibrillar protein gels containing edible insects.¹

Treatments ²	Control	T1	T2	T3	T4	T5
Onset temperature (°C)	52.43±0.78 ^{ab}	54.40±3.35 ^{ab}	54.88±1.73 ^a	53.69±0.83 ^{ab}	50.50±1.05 ^b	52.74±0.35 ^{ab}
Peak temperature (°C)	66.52±0.52 ^b	71.40±0.53 ^a	71.21±0.56 ^a	70.27±0.71 ^a	66.66±1.29 ^b	69.90±0.58 ^a
End temperature (°C)	76.11±0.65 ^b	79.40±1.21 ^a	78.71±1.48 ^a	77.89±0.52 ^{ab}	78.54±0.81 ^a	78.08±0.53 ^{ab}
Delta H (J/g)	0.60±0.01 ^b	0.52±0.09 ^{bc}	0.47±0.03 ^c	0.48±0.03 ^{bc}	0.97±0.04 ^a	0.52±0.05 ^{bc}

¹ All values are presented as the mean ± standard deviation of three replicates (n=3). Values within a row with different letters are significantly different ($P<0.05$).

² Control = gel without edible insect; T1 = 1% *Tenebrio molitor* larvae; T2 = 1% *Protaetia brevitarsis* larvae; T3 = 1% *Allomyrina dichotoma* larvae; T4 = 1% *Gryllus bimaculatus* adult; T5 = 1% *Oxychinensis sinuosa* adult.

Figure 1. Protein bands obtained using non-reduced and reduced SDS-PAGE for edible insects (A and B) and thermal-induced myofibrillar protein gels containing edible insects (C and D). Con, gel without edible insect; T1, 1% *Tenebrio molitor* larvae; T2, 1% *Protetia brevitarsis* larvae; T3, 1% *Allomyrina dichotoma* larvae; T4, 1% *Gryllus bimaculatus* adult.; T5, 1% *Oxyachinensis sinuosa* adult.; SDS-PAGE = sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

interaction (ionic bonds, hydrophilic bonds, hydrophobic interaction, and disulphide bonds) between proteins (Zayas, 2012).

Myosin heavy chain (205 kDa), actin (45 kDa), tropomyosin (34 kDa), troponin-1 (21 kDa), and myosin light chain (22, 18, and 16 kDa) are composed of general myofibrillar proteins (Choi *et al.*, 2011). The interaction and ratio between myosin (205 kDa) and actin (45 kDa) is critical when forming compact gel structure (Samejima *et al.*, 1981). When the molecular distribution was compared using non-reduced SDS-PAGE, the treatment groups showed different distributions from that of the control. In addition, high intensity around molecular weight of abovementioned proteins in treatments also might be due to myofibrillar protein. Large bands between 75 and 100 kDa were observed for T1, T2, and T3, and large protein bands of approximately 10 kDa were also observed for T1 and T2. After the reduction of disulphide bonds, protein bands were separated more clearly because almost heat-induced protein interactions linked by disulphide bonds (Yasui *et al.*, 1982). Protein bands of control and treatments that were above 250

kDa and between 37 and 50 kDa appeared faint. Thus, these faint bands could explain that myosin and actin proteins were interacted with disulphide bonds. When compared to the patterns of the control and treatments, new protein patterns except for T4 samples were observed, and certain protein bands appeared faint or were not observed. Protein bands observed between 15 and 20 kDa for the control were not observed for T1 and T2, and protein bands at 20 and 25 kDa appeared faint. In contrast, protein bands at 75 kDa were thicker than those of the control. Although protein bands of *P. brevitarsis* between 10–15 kDa was not observed before reducing, novel protein bands were observed between 10–15 kDa after reducing. In addition, protein bands observed for the control were not observed for the treatments, especially myosin light chain. In general, insects are processed without any separation of components such as meat, fat, organs, and blood, even, digestive enzymes because of their small body size. Therefore, various components obtained from insects could affect myofibril protein interaction such as binding inhibiting or degradation by enzymes during manufacturing. These changes can affect molecular weights of proteins and the

difference in SDS-PAGE could be occurred (Vasilescu *et al.*, 2004). Therefore, various uncharacterised components in edible insects can bind or lose protein interactions and affect molecular weights. The protein molecular weight distribution of T4 was similar to that of the control, and the band intensity was strong. When the hardness of the protein gel was observed, T4 had the highest hardness value compared to that of other treatments ($P < 0.05$). Therefore, the addition of *G. bimaculatus* may enhance myofibrillar protein interaction, and changes in protein patterns based on insect composition may negatively affect the formation of a compact gel.

Rheological properties

The rheological properties of myofibrillar proteins containing five different edible insect species are shown in Figure 2. The changes in viscosity of all samples when shear stress was applied at 25 °C are shown in Figure 2A. The viscosity of all samples, including the control, decreased as the shear rate increased, indicating a shear-thinning behaviour. A minor difference between the viscosities of the samples was observed. This indicates that the addition of edible insect proteins to myofibrillar protein derived from lean meat did not affect the apparent viscosity based on the shear rate. In general, most food materials and polymer suspensions are shear-thinning fluids. Likewise, the flow

behaviour index (n) of the control, T1, T2, T3, T4, and T5 samples was lesser than 1 (0.150 ± 0.015 , 0.166 ± 0.010 , 0.104 ± 0.039 , 0.144 ± 0.032 , 0.143 ± 0.013 , and 0.106 ± 0.002 , respectively), indicating non-Newtonian and pseudoplastic behaviour (Gomathy *et al.*, 2015). However, none of the samples flowed until the shear stress reached specific values (yield stress), and the samples exhibited solid-like behaviour below the values (Figure 2B). When flow curves do not pass through the origin, this implies the presence of yield stress (Štreimikytė *et al.*, 2020). The yield stress values of the control, T1, T2, T3, T4, and T5 samples were 30.1 ± 1.5 , 25.4 ± 0.2 , 24.7 ± 0.1 , 24.9 ± 0.9 , 29.0 ± 1.7 , and 26.6 ± 0.3 Pa, respectively. The control and T4 samples began flowing (fluid behaviour) at a higher shear stress than that of other samples ($P < 0.05$). This was in agreement with the hardness results of the samples. These rheological properties indicate that myofibrillar protein gels containing five different insect species represent Hershel-Bulkley fluids; they sustained their initial structure below the yield stress, and their viscosities decreased as the shear rate increased. However, there were differences between the control and treatment samples. The shear stress of the control sample steadily increased as the shear rate increased; however, the slope of the curve with the shear rate increased. In contrast, the treatment samples exhibited complex patterns. At low shear rates, the shear stresses of the treated samples increased more sharply than those of the control sample

Figure 2. Rheological properties of the myofibrillar protein gels containing edible insects. Variations in (A) viscosity and (B) shear stress are presented as a function of shear rate. Variations in (C) storage (G') and loss (G'') modulus, and variations in (D) loss factor ($\tan \delta$) are presented as a function of temperature. Control, gel without edible insect; T1, 1% *Tenebrio molitor* larvae; T2, 1% *Protaetia brevitarsis* larvae; T3, 1% *Allomyrina dichotoma* larvae; T4, 1% *Gryllus bimaculatus* adult; T5, 1% *Oxyachinensis sinuosa* adult.

and were maintained or slightly decreased at high shear rates. In addition, consistency coefficient (K) of myofibrillar proteins with edible insect species (70.03 ± 1.43 , 74.78 ± 1.89 , 75.16 ± 1.56 , 70.42 ± 6.38 , and 73.05 ± 4.43 Pa $\cdot$ s n , respectively) were higher than K of the control sample (61.96 ± 3.78 Pa $\cdot$ s n). These differences ($P < 0.05$) may be due to changes in protein-protein interactions due to the increase in the myosin light chain content of edible insect proteins (section 3.4). The changes in G' , G'' , and loss factor ($\tan \delta = G''/G'$) as a function of temperature are presented in Figure 2C and 2D). All samples showed similar trends with increasing temperature from 10 to 90 °C. The apparent onset of gelation occurred at approximately 40 °C. During the heating process, myosin loses its α -helix structure, followed by intermolecular association and aggregation, stabilisation of covalent (disulphides) and noncovalent interactions, and development of a rigid structure (Zayas, 2012). The G' and G'' values decreased in the temperature range of 40–60 °C and increased above 60 °C due to the formation of a rigid structure. As shown in Figure 2D, the loss factor of control sample was slightly lower than the values of other samples containing edible insect proteins in temperature range of 10 to 40 °C ($P < 0.05$). The loss factors of each sample at 40 °C were 0.509 ± 0.021 , 0.604 ± 0.006 , 0.618 ± 0.005 , 0.607 ± 0.004 , 0.592 ± 0.003 , and 0.604 ± 0.002 , respectively. Gel formation in meat products is generally related to the gel-forming properties of myosin and actomyosin (Zayas, 2012). Samejima *et al.* (1981) reported that myosin heavy chains are mainly responsible for the thermal gelation of myosin, and the rigidity of the gel during heating is affected by the myosin/actin ratio. Yasui *et al.* (1982) reported that the maximum increase in gel rigidity is obtained at a myosin-to-actin ratio of 15. In addition, the loss factor of all samples reached the highest value at approximately 47 °C and then decreased sharply until temperature increased to 60 °C. The loss factors of each sample at the end of the temperature (90 °C) sweeps were 0.056 ± 0.003 , 0.063 ± 0.002 , 0.069 ± 0.001 , 0.060 ± 0.001 , 0.055 ± 0.004 , and 0.060 ± 0.001 , respectively. The value of $\tan \delta$, which is smaller than 1, indicates a solid-like behaviour (Lee *et al.*, 2018). Thus, the very low values of $\tan \delta$ at high temperatures indicate that the network reached a high degree of development.

4. Conclusions

The study on thermal-induced protein gels showed that the interaction between porcine myofibril protein and edible insect species considerably influences thermal stability and rheological properties. The thermal-induced myofibrillar protein gel was prepared from five different edible insect species (larvae: *T. molitor*, *P. brevitarsis*, and *A. dichotoma*; adults: *G. bimaculatus*, and *O. sinuosa*). The gels showed different thermal stabilities and rheological properties depending on the edible insect species used. These results suggest that the thermal-induced myofibrillar protein gel prepared using *G. bimaculatus* protein resources may have

excellent processing functionality. Furthermore, edible insect proteins can replace animal proteins and various protein resource foods.

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Conflicts of interest

The authors declare no conflicts of interest.

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