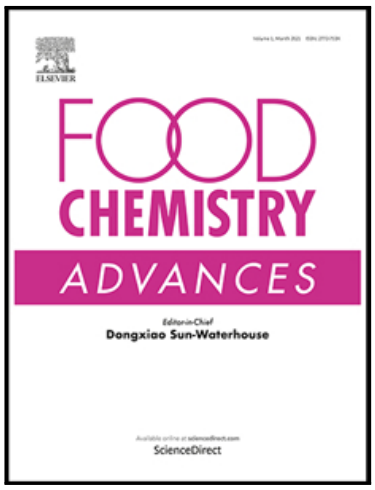

Extraction and Characterization of Gelatin Derived from Acetic Acid-Treated Black Soldier Fly Larvae

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Highlights:

- Investigated the potential of black soldier fly larvae (BSFL) as a source of gelatin.
- Examined the extraction process of BSFL gelatin with acetic acid pretreatment.
- Characterized the physicochemical properties of BSFL gelatin and compared it with commercial porcine gelatin.
- Found that BSFL gelatin is heat-stable and has a high melting temperature, rough microstructure, and low foaming properties.
- Demonstrated that BSFL gelatin can be used as a novel techno-functional ingredient in the food industry due to its high nutritional value and low cost.

Extraction and Characterization of Gelatin Derived from Acetic Acid-Treated Black Soldier Fly Larvae

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Abstract

Gelatin is a low-cost and widely used soluble protein compound derived from collagen. Black soldier fly (*Hermetia illucens*) larvae (BSFL) are a potential alternative source of gelatin due to their high nutritional value. This study investigated the effect of acetic acid pretreatment concentration, time, and centrifugation speed on the yield of BSFL gelatin and characterized its physicochemical properties. BSFL gelatin extracted with 0.05mol/L acetic acid for 3 h and centrifuged at 3000× g (BSFL3) had the highest yield of 1.085%±0.2%. The BSFL3 gelatin had lower protein content and amino acids than commercial porcine gelatin, specifically proline and hydroxyproline. It also had a high melting temperature of 135.12±1.04°C, viscosity of 1.69±0.05 mPa·s, and rough microstructure with low foaming properties. The BSFL gelatin is high in K (680/01 mg/kg) and contains less Na, Mg, Ca, and P. Field emission scanning electron microscopy (FE-SEM) and energy dispersive X-ray (EDX) revealed that BSFL3 gelatin has high potassium content. The results suggest that BSFL gelatin can be used as a novel techno-functional ingredient in the food industry.

Keywords: Black soldier fly larvae; Gelatin; protein; Physicochemical properties

1. Introduction

Gelatin is a gelling, foaming, and emulsifying agent which can be extracted from mammalian collagen-containing tissues, such as bovine hides and porcine skins (I.J.Haug & K.I.Draget, 2011). However, certain religions prohibit using gelatin derived from these sources, which are also subject to infectious diseases such as bovine spongiform encephalopathy, BSE. Substitutes derived from fish and avian sources exist but cannot satisfy market demand due to the low availability of sources for the former and high production costs for the latter. Therefore, alternative sources of gelatin are needed.

Gelatin extracted from edible insects has been identified as a potential alternative due to its high availability and low costs. Globally, insects are consumed as part of regular diets, ranging from locusts and silkworms in Thailand to beetles in South Korea (Köhler, Kariuki, Lambert & Biesalski, 2019; Ghosh, Lee, Jung, & Meyer-Rochow, 2017; van Huis et al., 2013). Furthermore, edible insects are more environmentally friendly than conventional livestock, given that they require minimal space for breeding, less water consumption, and have low greenhouse gas emissions (Rumpold & Schlüter, 2013). Additionally, Mariod et al. (2011b) have reported the possibility of extracting gelatin from two edible insects, the melon bug (*Aspongubus viduatus*) and the sorghum bug (*Agonoscelis pubescens*). The yield of gelatin extracted from the melon bugs and sorghum bugs was 3% and 3.04%, respectively. The gelatins derived from these insects were comprised mainly of 40 kDa molecules. Because of their work, it can be seen that edible insects are a potential alternative source of gelatin.

In this study, black soldier fly (*Hermetia illucens*) larvae (BSFL) have been identified as a potential alternative source of gelatin because of their high protein content, abundance, cost-efficiency, and eco-friendliness. Smets et al. (2020) have reported that the proximate composition of BSF varies at different developmental stages, such as larvae, prepupae, and

pupae. The prepupae showed the highest lipid content (47.65% w/w), whereas the larvae showed the highest protein content (38.86% w/w). This is significant because these insect proteins contain 18 amino acids, half of which are essential because the human body cannot synthesize them. These nine essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Köhler et al., 2019). The gelatin polypeptide chain comprises amino acid sequences such as glycine-hydroxyproline-proline or glycine-proline-proline (Siregar & Suprayitno, 2019). Proline and hydroxyproline are important amino acids that determine the thermoreversible properties of gelatin. The extraction parameters influence the quality and extraction yield of the gelatin (Mariod et al., 2011b).

Pretreatment is vital to facilitate the breakdown of collagen, allowing it to denature into gelatin through the cleaving of the inter- and intra-molecular bonds. This is done by unwinding the triple helix structure of collagen, which causes it to swell. This swelling reduces the denaturation temperature, which allows the gelatin to be extracted under mild conditions. This study used acetic acid as a suitable pretreatment because it restrains protease activity, reduces enzyme degradation in gelatin, and cleaves the crosslinks in collagen with minimal breakdown of the peptide chains (Ghassem, Mamot, & Babji, 2014). Therefore, the effects of different concentrations of acetic acid (0.05, 0.1, 0.2, 0.5, 1, and 2 mol/L), times (0.5 and 3 h), and centrifugation speeds (1000× g, 3000× g, and 10,000× g) on the yield of gelatin from BSFL were investigated. The physicochemical properties of the BSFL gelatin were also compared to a control sample of type A porcine skin gelatin.

2. Materials & Methods

2.1. Materials

Live BSFL were supplied by Green Soldier Biotech PLT-Biovae (Johor Bharu, Malaysia). Coomassie brilliant blue R-250 dye, acetonitrile, bromophenol blue, sodium lauryl sulfate, acrylamide/bis-acrylamide, isopropyl alcohol, Type A gelatin from porcine skin, protein marker, and trypsin from porcine pancreas (T7409) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Glacial acetic acid, glycine, β-mercaptoethanol, tris base, tris-hydrochloride, and ammonium persulfate were purchased from Merck Sdn Bhd (Selangor, Malaysia). All reagents used were of analytical grade.

2.2. Sample Preparation

The live BSFL were fasted overnight to reduce their gut microbiota. They were washed with running tap water to remove impurities and then blanched in boiled distilled water at 98±1°C for 1 min. After blanching, the BSFL were kept in a blast freezer, (ColdRoomMaster, Subang Jaya, Malaysia) then stored at −20°C (Value Lab Upright Freezers, Thermo Scientific, Waltham, MA, USA) for no more than one month. Prior to extraction, the frozen BSFL were thawed at room temperature.

2.3. BSFL Gelatin Extraction

The gelatin extraction was performed according to Mariod et al. (2011b) with slight modifications. The BSFL were treated with acetic acid (1:2 w/v) to study the impact of acid concentration (0.05 to 2.0 mol/L) and the pretreatment time in an acid medium (0.5 and 3 h) on gelatin yield. The pretreated BSFL were rinsed with distilled water three times before they were blended with distilled water (1:2 w/v) using a blender (Sharp, Shah Alam, Malaysia). For the thermal treatment, around 450 g of the pretreated and blended BSFL mixture was

placed into 250 ml of conical flasks and put into a water bath shaker (Memmert, Germany) at 55°C for 16 h to break down the protein. The thermal treated BSFL was filtered and centrifuged (Thermo Scientific Fiberlite F13-14x50cy, Thermo Scientific, Waltham, MA, USA) at 1000× g (BSFL1), 3000× g (BSFL3), and 10,000× g (BSFL10) for 30 min at 15°C. After removing the lipids and pellets that floated to the top, the excess water in the supernatant was removed by a rotary evaporator (Heidolph, Schwabach, Germany). The concentrated sample was kept at −80°C in a freezer (Ultra-Low Temperature Freezer MDF-U74V, Panasonic, Gunma, Japan) for 2 days. Subsequently, it was freeze-dried with a Labogene Scanvac Coolsafe Touch 110-4, DK-3450 (Allerød, Denmark) at −102°C for 3 days to obtain gelatin samples with moisture contents ranging from 4%–10% (w/w) for storage. The freeze-dried BSFL gelatin was ground, weighed, and stored at room temperature for further analysis. Gelatin solutions (6.67% w/v) were prepared by dissolving these powders in distilled water to test their physicochemical properties.

2.4. Yield of Gelatin

The yield of extracted BSFL gelatin was determined using Equation 1:

$$\text{Yield of gelatin (\%)} = \left(\frac{\text{weight of extracted BSFL gelatin powder}}{\text{weight of raw BSFL}}\right) \times 100 \quad (1)$$

Gelatin is a hygroscopic food additive. Therefore, the freeze-dried BSFL gelatin was stored in a desiccator with silica gel at room temperature. This storage method is essential to minimize the exposure of the extracted gelatin powder to the moisture in the air, which will cause it to become sticky.

2.5. Proximate Composition

The moisture, ash, and total fat contents of the gelatin were determined according to the AOAC methods 950.46, 945.46, and 963.15, respectively (AOAC, 2019). The protein

content was determined according to Malaysia Standard MS 807:1983 and calculated with a conversion factor of 6.25. The total carbohydrates was calculated by the difference method (FAO Food and Nutrition Paper 77, 2003). The energy of the gelatin was calculated based on the factors 4, 4, and 9 cal/g for carbohydrates, protein, and fat, respectively.

2.6. Amino Acid Composition

The amino acid composition of extracted BSFL gelatin was determined through high-performance liquid chromatography (HPLC) with a photometric diode array (PDA) detector (LCMS-2020, Shimadzu, Kyoto, Japan), following the protocols established by Nemati, Oveisi, Abdollahi, & Sabzevari (2004). For this analysis, approximately 1 g of BSFL gelatin was hydrolyzed in a screw-cap tube with 15 mL of 6 mol/L HCl in an oven (Generation 2012, Memmert, Buechenbach, Germany) at 110°C for 24 h. After hydrolysis, the HCl was removed by evaporation with a stream of nitrogen. After the HCl was removed, the residue was re-dissolved in 0.1mol/L HCl and filtered through a 0.45 µm cellulose membrane before loading into an HPLC vial for analysis. For the solvent mobile phase, deionized water (mobile phase A) and 60% (v/v) acetonitrile (mobile phase B) were used. A C18 column (150 mm × 3.9 mm) (Shimadzu, Kyoto, Japan) was used. For data acquisition, the Shimadzu software was employed (Raja Mohd Hafidz, Yaakob, Amin, & Noorfaizan, 2011).

2.7. Electrophoretic Profile

The protein molecular weight of BSFL gelatin was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, or SDS-PAGE (Gimenez, Turnay, Lizarbe, Montero, & Gomez-Guillen, 2005). A 4% (w/v) acrylamide stacking gel and a 12% (w/v) separating gel were prepared. The BSFL gelatin (2 mg/mL) was mixed at a ratio of 1:2 with a sample buffer containing deionized water, 0.5 mol/L Tris-HCL (pH 6.8), glycerol, 10% (w/v)

SDS, ß-mercaptoethanol, and 1% (w/v) bromophenol blue (v/v). The mixtures were heated at 95°C for 5 min before 10 µL of each sample was loaded into the sample wells. The samples were run at 100 V and 20 mA in a Mini-PROTEAN Tetra Cell unit (1658004EDU, Bio-Rad, Hercules, CA, USA) for 1.5 h until the blue band reached the bottom of the gel. The gel was stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in a 40% (v/v) methanol and 10% (v/v) acetic acid solution for 1 hour, followed by de-staining in a solution containing 40% (v/v) methanol and 10% (v/v) acetic acid for 12 h. The molecular weight distribution of the BSFL gelatin was identified by referring to the protein marker (94964, Sigma-Aldrich, St. Louis, MO, USA). The protein marker has two colored reference bands, 75 kDa (red) and 25 kDa (green). The molecular weight distribution of porcine type A gelatin (CAS No. 9000-70-8, Sigma-Aldrich, St. Louis, MO, USA) was also observed in the same gel for comparison.

2.8. Viscosity

The viscosities of both BSFL and porcine gelatin solutions (6.67%w/v) were determined using an Anton Paar ViscoQC 300 rotational viscometer with a Peltier device, PTD 80 set at 40±1°C (GMIA, 2019). The gelatin solutions were measured using spindle DG26 (Anton Paar, Graz, Austria) at 100 rpm and recorded in millipascal seconds (mPa·s).

2.9. pH

The pH of the extracted BSFL3 gelatin solution was measured using a calibrated pH meter (pH 700 Meter, Thermo Scientific, Eutech, Waltham, MA, USA) at 55±1°C (GMIA, 2019). The gelatin solution was prepared by dissolving 1.06 g of the gelatin powder into 105 mL of distilled water in a beaker.

2.10. Mineral Composition

The mineral compositions (K, Mg, Na, and P) were determined according to AOAC Method 975.03 (AOAC, 2000) using an inductively coupled plasma optical emission spectrometer, ICP-OES (7300 Dual View, Perkin Elmer, Waltham, MA, USA). The 1-m CzernyTurner monochromator is based on a holographic grating with 1800 grooves mm^{-1}. Tygon-type pump tubing was used to transport the freeze-dried gelatin. Ca was measured using an in-house method, STP/FD/029/V1, based on AOAC 968.08 (AOAC, 2000).

2.11. Differential Scanning Calorimetry (DSC)

The thermal stability of the gelatins was analyzed using a differential scanning calorimetry instrument (DSC, MicroCal PEAQ-DSC Automated system, Mettler Toledo, Columbus, OH, USA) attached to a thermal analyzer. A mechanical cooling regulator was used to cool the samples to −90°C. Approximately 10 mg of freeze-dried BSFL gelatin was loaded into a 30-uL aluminum crucible. The crucible was then sealed with a compatible lid and placed into the DSC for analysis. An empty sealed aluminum crucible was placed next to the sealed crucible containing the sample, serving as a reference. The crucibles were cooled to −90°C at 5°C/min. After equilibration for 10 min, the crucibles were scanned from 0 to 170°C at a heating rate of 10°C/min. The glass transition temperature and melting temperature of the extracted gelatins were evaluated in a nitrogen gas environment at a flow rate of 50 mL/min using the DSC analysis program STARe system (DSC, MicroCal PEAQ-DSC Automated system, Mettler Toledo, Columbus, OH, USA). The peak temperature in the endotherm was recorded as the helix-coil transition temperature, T_m.

2.12. Color and Clarity

The BSFL gelatin solution (6.67% w/v) was placed on a glass Petri dish and measured using a colorimeter (Color Flex EZ, Hunter Lab, Reston, VA, USA). The colorimeter was calibrated using standard black and white tiles. The CIE color values L*(lightness), a* (red/green), and b* (yellow/blue) were recorded. The clarity of the 6.67% BSFL gelatin solutions was determined by measuring the transmittance (T%) at 620 nm with a spectrophotometer (GENESYS 10S UV-Vis, Thermo Scientific, Waltham, MA, USA) (GMIA, 2019). The gelatin solutions were maintained at 45±1°C and placed into a quartz cuvette for analysis, and distilled water was used as the blank. The color and clarity of porcine gelatin were also tested for comparison.

2.13. Foaming Ability (FA)

The foaming abilities (FAs) of both porcine and extracted BSFL gelatins were determined based on the method of Jridi et al. (2014) with slight modifications. The initial volume of the gelatin solution, B (30 mL, 1% w/v), was added into a 100-mL cylinder. Then, the sample was aerated by a homogenizer (T 25 digital Ultra-Turrax, IKA, Staufen, Germany) equipped with spindle S 25 N at 12,000 rpm for 1 min. The total volume after whipping, A, was measured. The FA (% volume) was measured in terms of the volume of the liquid. FA was calculated according to Equation 2:

$$\text{FA}\ (\%)\ = \frac{(\text{A}-\text{B})}{\text{B}} \text{x}\ 100 \qquad (2)$$

where A and B are the volumes after and before whipping (in mL), respectively.

2.14. Foaming Stability (FS)

The foaming stability (FS) (% volume) is expressed as the percentage of liquid drainage relative to the initial liquid volume after standing for 30 min.

The gelatin solutions were placed at room temperature for 30 min, and the volume of the foam after standing, C, was recorded. FS was calculated according to Equation 3:

$$\text{FS}\ (\%)\ = \frac{(\text{C}-\text{B})}{\text{B}} \text{x}\ 100 \qquad (3)$$

where B is the initial volume of the gelatin solution and C is the volume of the foam after standing.

2.15. Field Emission Scanning Electron Microscopy (FE-SEM)

The microstructure of the freeze-dried BSFL gelatin powder was observed using field emission scanning electron microscopy (FE-SEM, Hitachi SU8010, Tokyo, Japan), based on the method of Rhim et al. (2013), with slight modifications. The BSFL gelatin was coated with a thin platinum (Pt) layer by sputtering (Q150R S, Quorum, Darmstadt, Germany) for 38 s at 30 mA. After coating, the morphology was observed at an acceleration voltage of 5.0 kV.

2.16. Energy Dispersive X-ray (EDX) Analysis

An energy dispersive X-ray (EDX) analyzer (Xmax50 EDX, Oxford-Horiba Inca, High Wycombe, UK) mounted on the FE-SEM was used to determine the elemental composition, including Ca, K, Mg, Na, and P, semi-quantitatively (Mozafari, Rabiee, Azami, & Maleknia, 2010).

2.17. Statistical Analysis

Statistical evaluation of the obtained experimental results was carried out using SPSS Statistics 20 (IBM, Armonk, NY, USA). The data for all the analyses are presented as the means of triplicate measurements. For pair-wise comparisons, the t-test was used. The data were also analyzed by analysis of variance (ANOVA). Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Yield of Extracted BSFL Gelatin

The yield of gelatin is undoubtedly important for mass industrial production. The yield of gelatin is influenced by the age and species of raw materials, their proximate content, the collagen content, and the extraction parameters (Songchotikunpan, Tattiyakul, & Supaphol, 2008). The yield of extracted BSFL gelatin was calculated and is shown in Table 1. The concentration of acetic acid used for pretreatment showed no significant difference ($p > 0.05$) in extraction yields. This finding disagrees with the results of a study by Siregar and Suprayitno (2019) that stated the yield of gelatin could be reduced by pretreatment with low or high concentrations (i.e., 0.5 and 1.5%) of acetic acid and showed that pretreatment with 1% acetic acid solution produced the highest yield. Mariod et al. (2011b) have reported that alkaline and acid pretreatment enables the removal of non-collagenous proteins and the loss of collagen during insect gelatin extraction. For weak acid extraction, Mariod et al. (2011b) have shown that the yield of sorghum bug gelatin (2.68%) is higher than that of melon bug gelatin (2.5%). In this study, all the BSFL pretreated with varied acetic acid concentrations had a lower gelatin yield than sorghum and melon bugs. This may be due to the freeze-drying process, which reduces the moisture content of the extracted BSFL gelatin (Table 2).

The yield of gelatin is also affected by and the pretreatment time. Hence, various pretreatment times (0.5 and 3 h) and concentrations of acetic acid in the pretreatment solutions (0.05, 1, and 6 mol/L) were studied. Despite the use of a higher concentration of acetic acid (6 mol/L), extraction times and concentrations showed no significant difference ($p > 0.05$) in the extraction yield (Table 1). It is hypothesized that the collagen structure in BSFL remains unchanged in different concentrations of acetic acid, and the hydrolysis process cannot occur even if treated over a prolonged period. Furthermore, the protein

structure will denature if the pretreatment solution concentration is too high and the pretreatment time is too long. Hence, a pretreatment solution with a lower acetic concentration (0.05 M) and a pretreatment time of 3 h was chosen for subsequent studies.

The impact of centrifugation speed on BSFL gelatin extracted with 0.05 mol/L acetic acid for 3 h was also studied. The yields in percentage were calculated and are shown in Table 1. The supernatant of BSFL1 (0.05 M, 3 h, 1000× g) was cloudy, and no pellets formed. Therefore, the centrifugation speed used for BSFL1 was insufficient to extract high-quality gelatin. The data showed no significant differences ($p > 0.05$) for various extraction times, centrifugation speeds, and concentrations.

3.2. Proximate Composition

The proximate composition of the BSFL gelatins (BSFL 3 and BSFL 10) produced following acetic acid pretreatment was assessed. Table 2 shows that BSFL3 had the highest protein content (55.31% w/w), moisture content (6.62% w/w), and total carbohydrates (19.59% w/w), while BSFL 10 had the highest ash content, 25.19% (w/w). Among the macronutrients, protein is the major constituent of BSFL gelatin. A study of edible insect gelatin has reported that the protein contents of sorghum and melon bugs were 28.2% and 27.0% (w/w), respectively (Mariod et al., 2011b). These findings indicate that gelatin derived from edible insects has a lower protein content than mammalian gelatins, such as porcine gelatin (91.3%±0.14% w/w) (Ninan, Joseph, & Aliyamveettil, 2014). This observation follows the findings of recent studies, where the protein contents of BSFL protein extract were 42% and 61% (w/w) (Huang, et al., 2019; Queiroz, et al., 2021). Although the BSFL protein extract obtained in this study has a lower protein content than mammalian gelatin, its protein content was higher than that of other edible insects, such as sorghum and melon bugs (Mariod et al., 2011b).

BSFL gelatin was also high in total carbohydrate content (Table 2), with at least 15.77% (w/w) carbohydrate content. The total carbohydrates of BSFL gelatins was higher than gelatin derived from other edible insects, such as sorghum and melon bugs, which contained 4.4% and 7.0% (w/w) carbohydrate, respectively (Mariod et al., 2011b).

Overall, the total fat content of BSFL gelatin was lower than that of some edible insects and porcine gelatin (0.78%±0.05%) (Ninan, Joseph, & Aliyamveettil, 2014). The gelatins from other edible insects, such as sorghum and melon bugs, were reported to contain 57.3% and 54.2% (w/w) fat, respectively (Mariod et al., 2011b). In this study, the BSFL gelatins were centrifuged for 30 min at 15°C. The centrifugation parameters, such as time and temperature, effectively isolated the fat, which was removed before the next extraction step. Determining the fat content of gelatin is essential because the storage period of the gelatin is easily influenced by the abundance of fat (Siregar & Suprayitno, 2019). Gelatin with a lower fat content avoids lipid oxidation and can be stored longer without affecting its quality and properties. In addition, BSFL3 and BSFL10 showed 1258.72 and 1175.08 kJ/100g of energy.

Non-proteinaceous substances within gelatin are predominantly moisture content and mineral ash. The ash contents of BSFL3 gelatin (18.31±0.06 %) were higher than that of the porcine gelatin (1.02%±0.04% w/w) (Ninan, Joseph, & Aliyamveettil, 2014). However, BSFL protein extract contains approximately 14.4% (w/w) ash (Queiroz, et al., 2021). These data show that BSFL have high ash content in both protein extracts and gelatin. The ash content likely accounts for the minerals in the gelatin, whereas the higher mineral content is hypothesized in BSFL gelatin due to its high ash content. Hence, the mineral composition of the BSFL gelatin was examined later in this study. Generally, commercial gelatin is subjected to ion exchange to eliminate excessive salt content and reduce the ash content to 2%–3% (I.J.Haug & K.I.Draget, 2011).

The moisture content of BSFL gelatin was determined as the percentage of weight loss of the sample after drying at high temperatures. The moisture content of BSFL10 (4.68% w/w) was the lowest, followed by BSFL3 (6.62% w/w) and porcine gelatin (8.4% w/w) (Ninan et al., 2014). Similarly, the moisture contents of gelatins made from sorghum and melon bugs were 7.6% and 8.3% (w/w), respectively (Mariod et al., 2011b). This is in line with the moisture content of commercial gelatin, which is 4%–10% after drying.

3.3. Amino Acid Composition

The amino acid composition of gelatin is responsible for its chemical and physical properties. The amino acid profiles of BSFL3 and BSFL10 gelatin are given in Table 2. The amino acid analysis is expressed as residues per 1000 amino acid residues. The quantity of amino acids in BSFL gelatins differed from those in the porcine gelatin. The amino acid sequence of gelatin is Gly-X-Y, where X is proline and Y is hydroxyproline (Siregar & Suprayitno, 2019). Glycine, proline, and hydroxyproline are the three predominant amino acids of gelatin. Glycine is located at every third position of the triple helical structure of collagen, while the hydroxyl group of hydroxyproline stabilizes the triple helical structure through hydrogen bonding. The quantity of amino acids mainly determines the thermoreversible property of gelatin. The amounts of amino acids in BSFL3, such as proline and hydroxyproline, were higher than those in BSFL10.

Compared with mammalian gelatin, BSFL3 consists of 46 residues/1000 residues of glycine, 82.49 residues/1000 residues of proline, and 1.77 residues/1000 residues of hydroxyproline. In contrast, porcine gelatin is composed of 330 residues/1000 residues of glycine, 132 residues/1000 residues of proline, and 91 residues/1000 residues of hydroxyproline (Eastoe & Leach, 1977). The amino acids form the gelatin structure for gelling; hence, lower amino acid content affects the chemical and functional properties of the

gelatin (Amiza & Siti Aishah, 2011). In this study, the levels of the three predominant amino acids of BSFL3 were noticeably lower than those of porcine gelatin. This agrees with the low total protein content of the BSFL gelatin in proximate analysis. Compared to BSFL10, BSFL3 gelatin has better quality and was chosen for further analysis of the physicochemical properties.

3.4. Electrophoretic Profile

For the electrophoretic profile, SDS-PAGE was used to estimate the relative molecular weight and identify the relative abundance of major proteins in BSFL gelatin. Gelatin has a basic element, the α-chain, with a molecular weight of 95 kDa. Gelatin consists of α-chains, dimers (β-chains), trimers (γ-chain)s, and some low molecular weight protein fractions. BSFL3 gelatin showed a major protein fraction at 75 kDa (Figure 1). Proteins with a molecular weight of 75 kDa to 17 kDa were found in BSFL3 gelatin. This molecular weight distribution indicates that the triple helical collagen broke down and was converted into gelatin by cleavage of inter- and intrachain cross-linkages.

The porcine gelatin, P, showed a protein fraction with higher molecular weight than BSFL gelatin in the same gel. The protein fractions of BSFL3 gelatin had lower molecular weight than the porcine gelatin. This data indicates that the protein fractions of insect gelatin had lower molecular weight than mammalian gelatin and, specifically, porcine gelatin. This agreed with Mariod et al. (2011b), who have stated that gelatins extracted from melon and sorghum bugs have low molecular weight protein chains. Both showed a significant mass fraction at 40 kDa.

The molecular weight of gelatins might differ slightly due to their sources, pretreatment parameters, and extraction procedures (Muyonga, Cole, & Duodu, 2004b; Bigi, Panzavolta, & Rubini, 2004). Therefore, the length of polypeptide chains and the functional

properties of the extracted gelatins will vary. The presence of small peptides, especially those with a molecular weight lower than 120 kDa, has been attributed to protein denaturation during thermal extraction (Chandra & Shamasundar, 2015; Lin, Chiou, & Sung, 2015). Gelatin is prone to denaturation during elevated thermal treatment (Bosch & Gielens, 2003). The hot air-dried gelatin bands were severely blurred, whereas freeze-dried gelatin from the same source showed clear bands with less blurring (Tkaczewska, Wielgosz, Kulawik, & Zając, 2019). The molecular weight of gelatin affects the gel strength of gelatin extracted from various sources (See, Ghassem, Mamot, & Babji, 2014).

3.5. Viscosity

Viscosity can be considered as the hydrodynamic volume of the molecules in a solution. The viscosities of porcine gelatin and BSFL3 gelatin were 7.48±0.25 and 1.69±0.05 mPa·s, respectively (Table 3). These results are in accordance with GMIA 2019, which shows that the viscosity of type A gelatin ranges from 1.5 to 7.5 mPa·s. A recent study has reported that the viscosity of gelatin is slightly affected by the molecular weight and size distribution of the gelatin (Sperling, 2006). High viscosity was attributed to the high molecular weight fraction (I.J.Haug & K.I.Draget, 2011). In the present study, the low viscosity of BSFL3 gelatin is in accordance with the low molecular weight distribution observed in the electrophoretic profile.

3.6. pH

The pH identifies the charge state of the extracted gelatin in its natural form. The pH of extracted BSFL3 gelatin was 4.38±0.17 while the pH of 1.5% type A porcine gelatin solution was 3.8–5.5 at 25°C, based on the product information from Sigma-Aldrich (Sigma-Aldrich, 2022). The types and concentrations of pretreatment chemicals influence the pH of

the gelatin sample. GMIA (2019) has also reported a pH range of 3.8–5.5 for edible type A gelatin and 5.0–7.5 for edible type B gelatin. In this study, the pH of BSFL3 was within the pH range for type A gelatin. Gelatin is stable over a broad pH range. However, the pH of gelatin significantly increased when it was exposed to an elevated temperature, such as thermal treatment at 55°C (Lim & Mohammad, 2011). The differences in gelatin yield and gel strength can be affected by the pH of the extracted gelatin (See, Ghassem, Mamot, & Babji, 2014). Gelatin at pH close to neutral showed maximum gel strength and viscosity, while gelatins with extreme pH exhibited low gel strength and viscosity because they had degraded into an abundance of low molecular weight peptides.

3.7. Mineral Composition

The source of the raw material, such as insects or mammals, influences the mineral composition of gelatin. The higher the mineral composition, the higher the ash content. Hence, the mineral composition of BSFL-derived gelatin in this study was compared with those of other edible insects. Among the edible insects, the quantities of Na, Mg, Ca, and P in BSFL3 gelatin were lower than in gelatins from sorghum and melon bugs, but K was higher (Table 4). BSFL3 gelatin possessed the highest concentration of K (680.01 mg/kg), followed by sorghum (412.52 mg/kg) and melon bugs (200.08 mg/kg). Potassium is vital for regulating the heartbeat, muscles, and nerves. It also improves protein synthesis and metabolizes carbohydrates in the body. The differences in the mineral composition of gelatins are likely influenced by the diet of the insects used as raw materials. Also, minerals can be lost during the extraction process.

3.8. Differential Scanning Calorimetry (DSC)

Gelatin can form a unique thermoreversible gel associated with a "melt-in-the-mouth" texture (Mariod & Adam, 2013; Stevens, 2009). DSC uses thermal analysis to measure the physical changes of samples with increasing temperature over time. The melting point, T_m, of BSFL3 gelatin was 135.12±1.04°C, the peak temperature at which the helical structures of BSFL3 gelatin melted. These findings agree with the results from Mariod et al. (2011b), who reported T_m of 136.5, 58.2, and 120.5°C in melon bug gelatins and 136.5, 135.2, and 120.5°C in sorghum bug gelatins after different extraction parameters had been used (Mariod et al., 2011b). The insect gelatins exhibited similar T_m. However, the T_m of insect gelatin is higher than that of type A pigskin gelatin (91°C) (Bigi, Bracci, Cojazzi, Panzavolta, & Roveri, 1998). Therefore, the T_m of gelatins is influenced by the raw materials and extraction parameters.

3.9. Color and Clarity

The color and clarity values of BSFL3 gelatin are shown in Table 3. BSFL gelatin has a lower L* value (48.79) but higher a* (8.12) and b* (26.63) values than porcine gelatin (L*, 90.25); (a*, 0); (b*, 10.55). The lower L* value paired with higher b* values imply lower lightness with increased yellowness in BSFL3 gelatin. The drying method of the extraction of gelatin contributes to its color. It has been reported that freeze-dried gelatin has higher L* and b* values than hot air-dried gelatin (Tkaczewska, Wielgosz, Kulawik, & Zając, 2019). Different drying parameters, such as pressure, temperature, and time, can lead to different gelatin colors. The color can also be affected by the source of the gelatin. However, the color of the gelatin does not influence its chemical and functional properties (Rahman & Jamalulail, 2012; Songchotikunpan, Tattiyakul, & Supaphol, 2008).

Furthermore, the clarity is low due to insoluble particles scattering light, leading to cloudiness. The clarity of the gelatin solution is ideally equal to that of distilled water.

However, this is difficult to achieve because of the presence of protein molecules and the mineral content. Therefore, measuring the deviation from the ideal using a spectrophotometer is essential. A significant difference in clarity was observed between the BSFL3 and porcine gelatins. The BSFL gelatin was brown after extraction. As noted above, BSFL3 has a high percentage of potassium, K, which could increase turbidity.

3.10. FA

Foam is formed as air is injected into water by a mixer, for example, a homogenizer. There are three critical steps involved in foam formation. First, the proteins within the sample must be partially unfolded, exposing more hydrophobic groups. Second, the protein molecules move toward the interface. Last, they bond with one another. Foaming properties are determined by the adsorption rate of protein molecules attached to the air–water interface. In this study, at the same gelatin concentration, the FAs of porcine gelatin and BSFL3 were 51.11%±5.09% and 35.56%±1.92%, respectively, as shown in Table 3. This finding indicates that BSFL3 has lower FA ($p<0.05$) than porcine gelatin. The FA of gelatin can be improved by exposing more hydrophobic groups of the protein molecules (Cole, 2000). BSFL3 gelatin has lower FA because of its low protein content in this study. Hence, BSFL3 gelatin might not be a good foaming agent.

3.11. Foaming Stability

The foaming stability (FS) was analyzed to investigate the link between protein concentration and protein unfolding at different interfaces (Wierenga, Egmond, Voragen, & Jongh, 2006; Delahaije, Gruppen, Giuseppin, & Wierenga, 2015). The FS of BSFL3 gelatin (1.11%±1.92%) was significantly lower ($p<0.05$) than that of the porcine gelatin (15.56%±1.92%) at the same concentration of gelatin (Table 3). The lower FS observed in

BSFL3 is attributed to its low protein concentration, which resulted in insufficient protein molecules spreading over the interfacial area to stabilize the foam. Therefore, a higher protein concentration will likely contribute to a higher FS as the adsorption process becomes more rapid than the protein unfolding process.

3.12. FE-SEM analysis

The surface microstructure of BSFL3 gelatin is shown in Figure 2a. BSFL gelatin had a rough surface with many protein fibrils and voids. As mentioned above, the major constituent in BSFL gelatin is protein. Among the gelatins of BSFL, sorghum bug, and melon bug, the melon bug had the lowest protein content. The SEM image of melon bug gelatin presented a smoother morphology with fewer protein fibrils and voids. The moisture content of the melon bug was the highest (8.3%); therefore, it has a smooth and sticky structure due to the behavior of gelatin (Mariod et al., 2011a).

3.13. EDX

The elemental maps obtained by EDX indicate the distribution of elements on the sample surface. The elemental percentages are summarized in Table 4. An example spectrum containing various elements is presented in Figure 2b, and a sample elemental map is shown in Figure 2c. In addition to C and O, K had the highest concentration among the major elements and exhibited the most abundance (many bright spots) in maps. This observation agreed with the result of the mineral composition analysis, which showed that K was most abundant in BSFL3 gelatin. Mariod et al. (2011a) have stated that defatted insect protein contained smaller quantities of various major elements and elements such as Al, Fe, Mg, and Na were not present in the defatted insects.

4. Conclusions

In conclusion, this study found that BSFL was an alternative gelatin source and demonstrated a suitable extraction method for BSFL gelatin. BSFL gelatin extracted by 0.05mol/L acetic acid for 3 h of pretreatment and centrifuged at 3000× g had the highest yield. This extraction method is suitable for mass production in the food industry. BSFL gelatin and gelatin hydrolysate could be used as novel techno-functional ingredients in the food industry after undergoing additional treatments to improve their foaming ability, such as trypsin treatment. However, this study is limited to BSFL gelatin treated with a mild acid; strong acid and alkaline pretreatment solutions could also be used in future studies. BSFL gelatin holds great potential in the food industry and warrants further investigation and development.

Declaration of interest

The authors declare that there is no conflict of interest.

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Tables

Table 1. The yield of BSFL gelatin from different pretreatment acid concentrations, times, and centrifugation speeds.

Factors	Parameters	[1]Yield, % w/w
Concentration	0.05 M	1.077±0.1[a]
	0.10 M	1.149±0.2[a]
	0.20 M	1.167±0.2[a]
	0.50 M	1.085±0.2[a]
	1.00 M	1.157±0.0[a]
	2.00 M	1.271±0.4[a]
Time	0.05 M 0.5 h	1.085±0.2[a]
	0.05 M 3 h	1.221±0.1[a]
	1 M 0.5 h	1.138±0.0[a]
	1 M 3 h	1.076±0.3[a]
	6 M 0.5 h	1.063±0.2[a]
	6 M 3 h	1.328±0.3[a]
Centrifugation Speed	0.05 M 3 h 1000× g	1.605±0.0[a]
	0.05 M 3 h 3000× g	1.085±0.2[a]
	0.05 M 3 h 10,000× g	1.061±0.1[a]

[1]Mean±SD (n = 3). Significant differences ($p<0.05$) are indicated by different superscript letters within the same column of each factor.

Table 2. Proximate analysis and amino acid profiling of BSFL3, BSFL10, and commercial porcine gelatins on a dry basis.

	Gelatins		
Components	**[*]Porcine**	**[1]BSFL3**	**[1]BSFL10**
Total Protein Content,% w/w	91.3±0.14	55.31±0.12[a]	54.24±0.12[b]
Total Fat Content,% w/w	0.78±0.05	0.17±0.02[a]	0.12±0.02[a]
Moisture Content,% w/w	8.4±0.20	6.62±0.07[a]	4.68±0.03[b]
Ash Content,% w/w	1.02±0.04	18.31±0.06[b]	25.19±0.03[a]
Total Carbohydrate,% w/w	-	19.59±0.02[a]	15.77±0.04[b]
Energy, kJ/100g	-	1258.72±0.04[a]	1175.08±0.01[b]
Amino Acid (residues per 1000 total amino acid residues)	**[**]Porcine**	**BSFL3**	**BSFL10**
Alanine	112	192.93	132.65
Valine	26	43.04	49.63
Leucine	24	38.47	34.63
Isoleucine	10	29.17	29.27
Phenylalanine	14	30.44	24.45
Methionine	4	11.51	7.6
Proline	132	82.49	68.99
Glycine	330	46	51.8
Serine	35	26.27	30.72
Threonine	18	35.81	33.57
Tyrosine	3	54.09	52.63
Aspartic Acid	46	102.52	93.25
Glutamic Acid	72	152.45	246.42
Lysine	27	70.84	64.8
Arginine	49	30.52	30.71
Histidine	4	32.36	21.21
Hydroxyproline	91	1.77	1.71
Cysteine	0	19.26	25.96

Significant differences ($p<0.05$) are presented as different superscript letters in the same row.
[*]Adapted from Ninan, Joseph, & Aliyamveettil, 2014.
[**]Adapted from Eastoe & Leach, 1977.

Table 3. Physicochemical properties of BSFL3 gelatin.

Tests	[1]Porcine	[1]BSFL3
Viscosity, mPa·s	7.48±0.25[a]	1.69±0.05[b]
Lightness, L*	90.25±0.39[a]	48.79±0.10[b]
Redness, a*	0.00[b]	8.12±0.01[a]
Yellowness, b*	10.55±0.46[b]	26.63±0.04[a]
Transmittance,%	76.90±0.20[a]	2.07±0.72[b]
Foaming Ability,%	51.11±5.09[a]	35.56±1.92[b]
Foaming Stability,%	15.56±1.92[a]	1.11±1.92[b]

[1]Mean±SD (n = 3). Values followed by different superscript lowercase letters in the same row were significantly different ($p<0.05$).

Table 4. Elemental composition of BSFL3 gelatin tested by ICP-OES and EDX in weight and atomic percentages.

Elements tested by ICP-OES	[*]Sorghum Bug Gelatin, mg/kg	[*]Melon Bug Gelatin, mg/kg	[1]BSFL3 Gelatin, mg/kg	
Na	340.41±0.62[a]	401.10±0.60[b]	79.75±0.01[c]	
Mg	309.22±0.61[a]	301.10±0.61[b]	99.09±0.01[c]	
K	412.52±0.72[a]	200.08±0.63[b]	680.01±0.01[c]	
Ca	759.51±0.64[a]	1021.21±0.52[b]	312.24±0.01[c]	
P	923.11±0.63[a]	1234.33±0.63[b]	56.72±0.01[c]	
Elements tested by EDX			**Weight,%**	**Atomic,%**
C			55.88±3.16	62.98±2.56
O			35.22±2.37	31.12±2.47
Na			0.16±0.01	0.10±0.01
Mg			0.82±0.04	0.48±0.03
P			0.41±0.03	0.19±0.02
K			3.91±0.78	1.42±0.31
Ca			1.71±0.17	0.60±0.07
Pt			1.70±0.03	0.13±0.01

[1]Mean±SD (n = 3). Values followed by different superscript letters in the same row are significantly different ($p<0.05$).

[*]Adapted from Mariod & Adam, 2013.

Figures

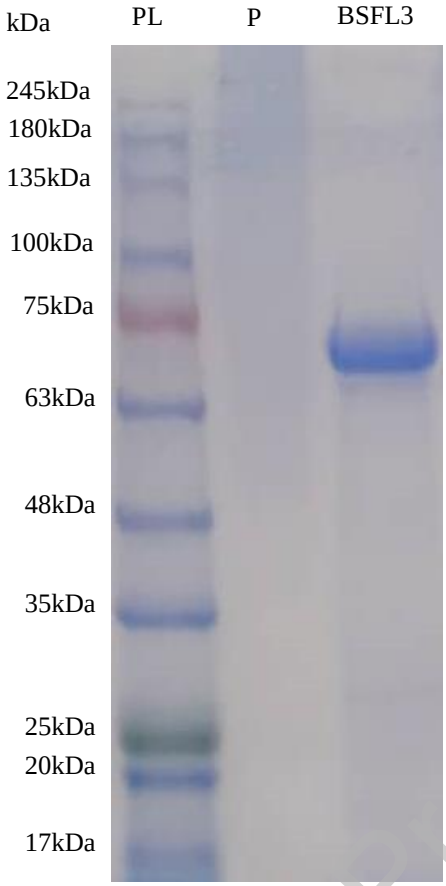

Figure 1. The molecular weight distribution of the standard protein ladder (PL), porcine (P), and BSFL3.

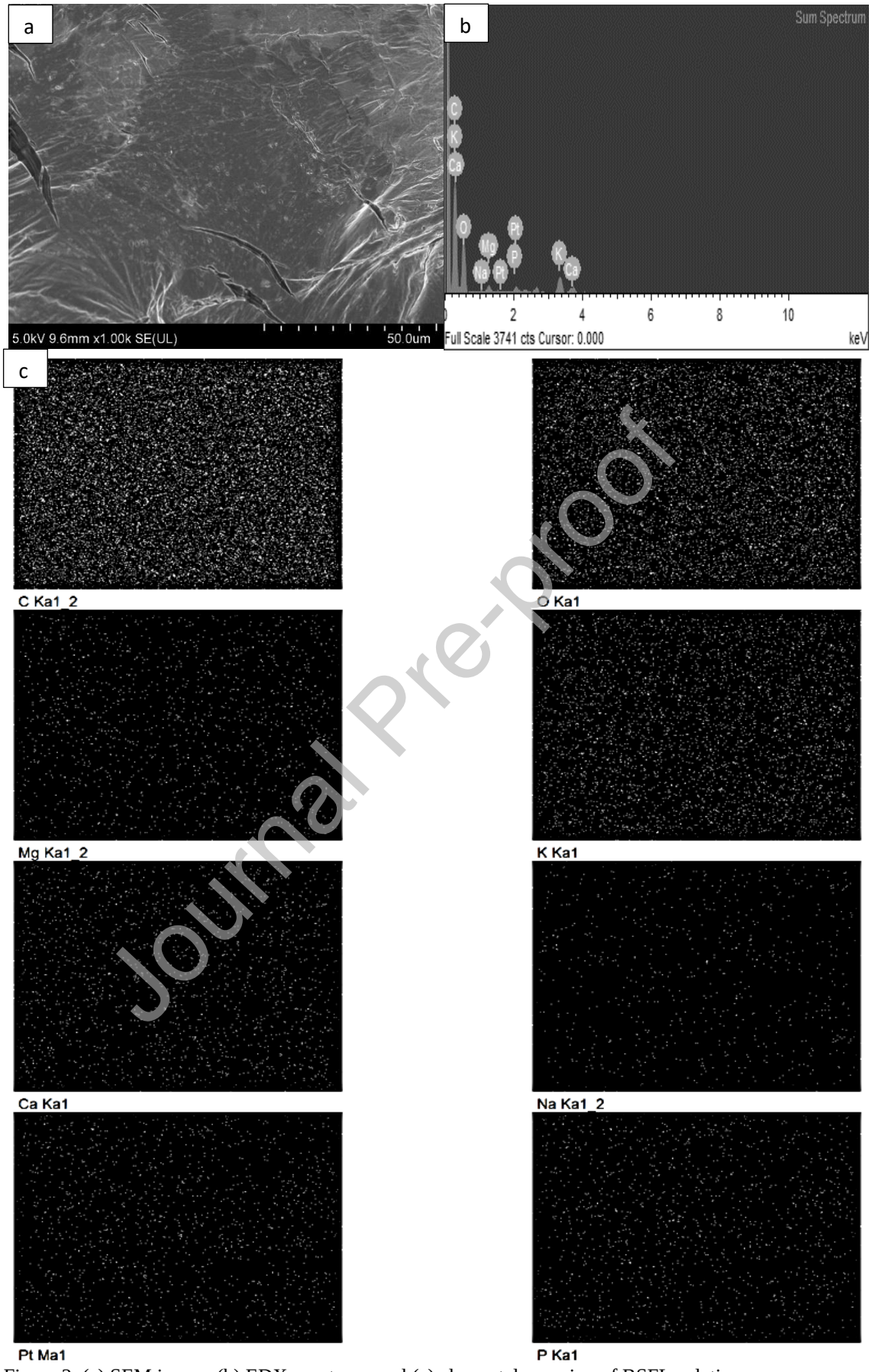

Figure 2. (a) SEM image, (b) EDX spectrum, and (c) elemental mapping of BSFL gelatin.

Authorship Statement

Lee-Kee Chua: Conceptualization, Investigation, Data curation and Writing-Original draft,

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Declaration of interest: None