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A comparative study on the functional properties of mealworm (*Tenebrio molitor*) larvae and soybean protein isolates and hydrolysates

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Abstract

The present work aimed to compare the functional and antioxidant properties of mealworm larvae and soybean proteins at different processing steps. The mealworm larvae protein isolate (MPI) was hydrolysed with 2% alcalase at pH 8 and 60°C for 3 h to produce mealworm protein hydrolysate (MPH). The content of amino acids were higher in MPI than in soybean protein isolates (SPI), except for those of threonine, arginine, glutamic acid, and serine. MPI contained a higher amount of hydrophobic amino acids (941.4 µmol/L) than hydrophilic amino acids (697.1 µmol/L). The emulsifying activity, stability, and fat absorption capacity of MPI were higher than those of SPI, whereas their water absorption and holding capacities were similar. Alcalase hydrolysis increased MPI solubility. MPI showed lower solubility at pH 3 - 9 than that of SPI, whereas MPH had higher solubility than that of soy protein hydrolysate (SPH). The foam expansion capacity and foam stability of MPI were lower than those of SPI, but hydrolysis improved those of MPI. MPI formed a gel at pH 5, 7, and 9 at 15% concentration or at pH 7 and 9 at 10% concentration. However, MPH showed no gel formation under any conditions. The total phenolic content and antioxidant capacity of MPI were higher than those of SPI. The DPPH activity of MPH (70%) was higher than that of MPI (18%), SPI (12%), or SPH (34%). MPI can be used as an alternative to SI. Alcalase hydrolysis can increase the antioxidant effect, digestibility, and functionality of MPI as a sustainable ingredient in high value-added products.

Introduction

Edible insects have recently received attention as novel sustainable ingredients owing to their high nutritional value, particularly as an alternative protein sources for animals (da Silva Lucasa *et al.,* 2020). Moreover, insects are considered economical and environmental-friendly materials because they require minimal feeding and maintenance as well as emit less greenhouse gases when compared with conventional livestock. Only a small amount of insect proteins can provide the recommended daily protein amount, and they are more digestible with better amino acid compositions than plant-based proteins (Yi *et al.,* 2016). Many studies have focused on edible insects for preparing conventional foods such as meat products and analogues, snacks, pastas, and breads to improve their nutritional values, particularly based on their functional properties (Gravel and Doyen, 2020). In South Korea, locusts, crickets, silkworm (Bombyx sp.) pupae, white-backed silkworm (*Bombyx* sp.), mealworm (*Tenebrio molitor*) larvae, beetle (*Protaetia brevitarsis*) larvae, and rhinoceros beetle (*Allomyrina dichotoma*) larvae are approved as edible insects. Among them, mealworm larvae have attracted attention because of their wide distribution and strong reproductive ability.

Protein is one of the most important components that can improve the physicochemical and sensory properties of food while providing nutrients (Foegeding, 2015). Several studies have aimed to maximise the efficiency of proteins when they are used as ingredients in food formulations. Enzymatic hydrolysis is an effective way to improve and modify the functional properties of proteins such as casein (Kumar *et al.,* 2016), whey protein (Sinha *et al.,* 2007), and soy protein (Coscueta *et al.,* 2019). Moreover, insect-derived ingredients such as protein isolates and hydrolysates could be one of the best ways to increase the acceptance of insect foods by consumers as compared to the use of whole insects as food materials. Further studies on mealworm protein isolate (MPI) and mealworm protein hydrolysate (MPH) as food ingredients are thus needed for the optimisation of edible insects to achieve desirable functional and nutritional properties, and also to formulate new products.

Therefore, the present work aimed to assess

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the potential of mealworm larvae protein as a functional ingredient in food formulations, particularly as a substitute for soybean protein, which lacks certain essential amino acids, and is less digestible than animal protein. The physicochemical properties of mealworm larvae and soy proteins were compared at different processing steps. Enzymatic hydrolysis was performed to improve the functionality of mealworm proteins. The functional properties and antioxidant capacity of MPH were also evaluated.

Materials and methods

Preparation of soybean and mealworm larvae isolates

Soybean flour (Ssalnongbu, Geochang-gun, Korea) (protein, 38%; fat, 18.2%; and carbohydrate 34%) was defatted at 25°C using 99% ethyl ether at a ratio of 1:3 for 30 min in a shaking water bath. The extraction process was repeated three times to yield defatted soybean (protein, 45%; fat, 1.5%; and carbohydrate, 38%). To obtain a soy protein isolate (SPI; protein, 91%; fat, 0.0%; and carbohydrate, 5.4%), defatted soybean was dispersed in water at a ratio of 1:10. After adjusting the pH to 9, the mixture was shaken for 50 min at 45°C, and then centrifuged twice at 4,000 *g* for 10 min at 4°C. The supernatant was collected, adjusted to pH 4.5, and centrifuged again at 4,000 *g* for 10 min at 4°C. The precipitate obtained was dialysed using a dialysis bag (12 kDa MWCO; Sigma-Aldrich Chemical Co., St. Louis, MO, USA) against distilled water for 12 h, freeze-dried, and stored at -20°C until further analyses.

Mealworm larvae powder (Edible-Bug Co., Seoul, Korea) (protein, 48%; fat, 28.8%; and carbohydrate, 11%) was defatted at 40°C using 99.5% ethanol at a ratio of 1:5 for 60 min in a shaking water bath for three times to yield defatted mealworm (protein, 70%; fat, 1.3%; and carbohydrate, 16%). To obtain mealworm larvae protein isolate (MPI; protein, 91%; fat, 0.1%), defatted mealworm larvae powder was dispersed in 1 N NaOH at a ratio of 1:12 and heated at 70°C in a shaking water bath for 1.5 h. Following centrifugation at 3,000 *g* for 10 min, the supernatant was adjusted to pH 4.4, and centrifuged again at 3,500 *g* for 10 min at 4°C. The precipitate obtained was dialysed using a dialysis bag (12 kDa MWCO) against distilled water for 24 h, freeze-dried, and stored at -20°C until further analyses.

Enzymatic hydrolysis of SPI and MPI

Soy protein hydrolysate (SPH; protein, 83%; fat, 0.0%; and carbohydrate, 7.7%) and mealworm larvae hydrolysate (MPH; protein, 80%; fat, 0.0%; and carbohydrate, 10%) were obtained following the method described by de Oliveira *et al.* (2015) with slight modifications. Briefly, 5 g isolated protein was dissolved in 100 mL distilled water, pre-treated at 85°C for 20 min, cooled to 60°C, and adjusted to pH 8.0 with 1 N NaOH. Next, 2% alcalase (2.4 L food-grade; Novozymes, Bagsvaerd, Denmark) was added and mixed for 3 h in a water bath at 60°C, and centrifuged at 4,000 *g* for 10 min to obtain a hydrolysate that was freeze-dried and stored at -20°C. SDS-PAGE was performed using a 12% running gel to confirm the hydrolysis of SPH and MPH. SPH and MPH had smaller molecular weight bands (6 and 17 kDa) than SPI and MPI.

Amino acid composition analysis

The amino acid composition of protein isolates and hydrolysates was analysed by high performance liquid chromatography (YL9100 Plus HPLC, YL Instruments, Anyang, Korea) using the AccQ•Tag Column (3.9 × 150 mm; Waters, Milford, MA, USA) following pre-injection derivatisation. Each sample was hydrolysed with 6 N HCl at 105°C for 24 h in a vacuum hydrolysis tube (ThermoFisher Scientific, Waltham, MA, USA). The determination was monitored by fluorescence detection (P4025, JASCO International Co., Easton, MD, USA) at 37°C, with a 250 nm excitation wavelength and 395 nm emission wavelength. Gradients of mobile phase A (10% A buffer) (Waters, Milford, MA, USA) and mobile phase B (60% acetonitrile) were employed at a flow rate of 1.5 mL/min with a 10 µL of injection volume. The concentration of each amino acid in the samples was determined by calibrating with standard amino acids (Waters, Milford, MA, USA) using a software (YL Clarity, YL instruments).

Protein solubility

The protein solubility was determined using the procedure described by Zhao *et al.* (2016). Samples (1 g) were dispersed in 40 mL distilled water, and the pH was adjusted to 3, 5, 7, and 9, followed by mixing at room temperature for 30 min. Following centrifugation at 2,100 *g* for 30 min, the supernatant was weighed to calculate the soluble protein content, and the total protein content was measured using the Kjeldahl method with a nitrogen conversion factor of 6.25. The protein solubility index was calculated by dividing the soluble protein content with the total protein content, and multiplying by 100.

Emulsifying activity and emulsion stability

Samples (1 g) were dissolved in 20 mL

distilled water, and mixed with 20 mL soybean oil, followed by homogenisation at 11,000 *g* for 2 min using a homogeniser (T25 digital Ultra-Turrax; IKA Ltd., Staufen, Germany), and centrifugation at 1,500 *g* for 5 min. The emulsifying activity (EA, %) was calculated as the height of the emulsion layer in the centrifuge tube divided by the height of the total liquid in the centrifuge tube. To determine the emulsion stability (ES), the centrifuge tube containing the sample and soybean oil was placed in a water bath (80°C, 30 min), and then cooled to room temperature. The height of the emulsion layer was measured following centrifugation at 1,500 *g* for 5 min. The ES (%) was calculated as the height of the emulsion layer after 30 min divided by the height of the initial emulsion layer.

Fat absorption capacity (FAC), water absorption capacity (WAC), and water holding capacity (WHC)

To determine the FAC, a mixture of 0.5 mL sample and 5 mL soybean oil was centrifuged at 2,100 *g* for 5 min, and the pellet was weighed after discarding the supernatant. The FAC (g/g) was calculated as the weight of the oil absorbed divided by the weight of the sample. To determine the WAC, 1 g sample was mixed with 10 mL distilled water, stirred for 5 min, and centrifuged at 2,100 *g* for 10 min. The discharged water was transferred to a cylinder, and the volume was measured. The WAC (mL/g) was determined as the volume of water absorbed divided by the weight of the sample. To determine the WHC, 1 g sample was mixed with 30 mL distilled water, heated in a water bath at 60°C for 30 min, cooled for 30 min, and centrifuged at 3,000 *g* for 5 min. The supernatant was removed, and its weight was measured. The WHC (g/g) was expressed as the weight of the water absorbed divided by the weight of the sample.

Foam expansion capacity (FEC) and foam stability (FS)

The FEC and FS were measured following the procedure of Cano-Medina *et al.* (2011) with slight modifications. Briefly, 3% (w/v) solutions were agitated in a cylinder at 10,000 rpm for 5 min. The FEC (%) and FS (%) were calculated using Eq. 1 and Eq. 2, respectively:

$$FEC\ (\%) = \frac{Volume\ after\ agitation - Volume\ before\ agitation}{Volume\ before\ agitation} \times 100 \quad \text{(Eq. 1)}$$

$$FS\ (\%) = \frac{Residual\ foam\ volume\ after\ 2\ hours}{Volume\ after\ agitation} \times 100 \quad \text{(Eq. 2)}$$

Gel formation

Visual observation of gelation based on pH and substrate concentration was assessed following the method described by Yi *et al.* (2013) with slight modifications. Briefly, 5, 10, and 15% (w/v) protein isolates were maintained at pH 3, 5, 7, and 9 by adding 1 M HCl/NaOH. Few drops of paraffin oil were added to the solutions to prevent evaporation, and the solutions were heated at 86 ± 1°C for 10 min. After heating, the samples were cooled overnight at 4°C.

Total phenolic content, total flavonoid content, and antioxidant capacities

Total phenolic content and flavonoid content were measured following the method reported by Kim *et al.* (2019b) with slight modifications. The extract was prepared by heating 1 g sample with 25 mL of 80% ethanol at 75°C for 2 h. To determine the total phenolic content, 300 µL sample extract was reacted with 1 mL Folin-Ciocalteu reagent, and then mixed with 3 mL of 10% Na_2CO_3 and 10 mL distilled water. Following incubation at room temperature for 2 h, the absorbance was measured at 765 nm using a UV/visible spectrophotometer (Ultrospec 2100 Pro; Biochrom Co. Ltd., Cambridge, England). To determine the flavonoid content, 0.5 mL extract was incubated with 5 mL of 90% diethylene glycol and 500 µL of 1 N NaOH at 37°C for 1 h. The absorbance was then measured at 510 nm using a UV/visible spectrophotometer (Ultrospec 2100 Pro). A calibration curve of gallic acid and catechin was used as the standard for total phenolic content and flavonoid content, respectively. The antioxidant capacities of the protein isolates and hydrolysates were assessed based on the DPPH radical scavenging activity using a UV/visible spectrophotometer (Ultrospec 2100 Pro) (Kim *et al.,* 2019b).

In vitro ileal digestibility (IVID)

The IVID was evaluated following the method described by Kong *et al.* (2015) with slight modifications. Sample dry matter (DRM) was added to sodium phosphate buffer (0.1 M, pH 6) and 0.2 M HCl solution. After adjusting the pH to 2, pepsin (Sigma-Aldrich Chemical Co.) and chloramphenicol (Sigma-Aldrich Chemical Co.) solutions were added and incubated at 39°C for 6 h. Then, sodium phosphate buffer (0.2 M, pH 6.8) and 0.6 M NaOH solution were added, and the pH was adjusted to 6.8. Pancreatin solution (pancreatin from porcine pancreas; Sigma-Aldrich Chemical Co.) was added to the samples. Following incubation at 39°C for 18 h, 20% sulfosalicylic acid solution was added and incubated for 30 min at room temperature. The

samples were filtered using a glass filter crucible (FN1200-2G; Corning Life Science Co., Oneonta, New York, USA) containing 500 mg Celite. The test flask sample was washed with 1% sulfosalicylic acid solution, 95% ethanol, and 99.5% acetone, and added to the crucibles. The DRM of the residuals was recorded after drying overnight at 105°C. The IVID of DRM (%) was calculated using Eq. 3:

$$\frac{IVID\ of\ sample}{dry\ matter\ (\%)} = \frac{DRM - (Residue\ DRM - Blank\ DRM)}{DRM} \times 100 \quad \text{(Eq. 3)}$$

Statistical analysis

All statistical analyses were performed using SPSS 24.0 for Windows (SPSS Inc., Chicago, IL, USA). Soybean and mealworm larvae samples were compared using one-way analysis of variance (ANOVA) and Duncan's multiple range test, with the level of significance set at $p < 0.05$. Different samples with the same treatment were compared using an independent sample *t*-test, with the level of significance set at $p < 0.05$.

Results and discussion

Amino acid composition and protein pattern

The amino acid compositions of soybean and mealworm larvae are presented in Table 1. MPI had an amino acid profile similar to that of SPI. MPI contained 17 amino acids, which included eight essential and nine non-essential amino acids, similar to the findings reported by Wu *et al.* (2020). Tryptophan was not detected in SPI and MPI. The

Table 1. Amino acid composition of soybean and mealworm larvae protein isolates and hydrolysates.

Amino acid	SPI	SPH	MPI	MPH
Histidine	38.5	53.7	51.8	58.8
Isoleucine	51.9	48.8	53.6	65.5
Leucine	129.9	174.8	176.2	206.7
Lysine	119.4	209.1	129.3	158.4
Methionine	15.7	28.6	34.5	49.5
Phenylalanine	64.3	88.8	93	99.1
Tryptophan	ND	ND	ND	ND
Threonine	46.3	65.5	27.3	27.7
Valine	99.4	107.7	126.8	162.3
Sum of EAA	565.4	777	692.5	828
Alanine	109.9	155.5	178.9	208.2
Arginine	41.8	59.2	24.6	25
Aspartic acid	214.9	325.2	222.8	268.8
Cysteine	41.1	67.5	43.1	39
Glutamic acid	284.3	377.2	213.2	250.8
Glycine	127.8	197.7	172.2	197.1
Proline	97.2	137.2	106.2	129.1
Serine	130.6	196.6	54.6	64
Tyrosine	48.8	66	136.1	160.6
Total AA	1661.8	2359.1	1844.2	2170.6
Hydrophobic amino acid	696.1	939.1	941.4	1117.5
Hydrophilic amino acid	766	1098	697.1	810.9
BCAA	281.2	331.3	356.6	434.5

SPI = soybean protein isolate; SPH = soybean protein hydrolysate; MPI = mealworm larvae protein isolate; MPH = mealworm larvae protein hydrolysate, and ND = not detectable.

predominant essential amino acids in SPI and MPI were leucine (129.9 and 176.2 µmol/L, respectively), lysine (119.4 and 129.3 µmol/L, respectively), and valine (99.4 and 126.8 µmol/L, respectively). The content of most amino acids were higher in MPI than in SPI, except for those of threonine, arginine, glutamic acid, and serine. The content and total number of essential amino acids in MPI were comparable to the recommendations for humans, similar to SPI (FAO, 2013; Zhao *et al.,* 2016; Wu *et al.,* 2020). SPI contained a higher amount of hydrophilic amino acids (766 µmol/L) than hydrophobic amino acids (696.1 µmol/L), whereas MPI contained a higher amount of hydrophobic amino acids (941.4 µmol/L) than hydrophilic amino acids (697.1 µmol/L). The amino acid contents of protein hydrolysates in SPI and MPI were higher than those of protein isolates for all amino acids except isoleucine and cysteine. Isoleucine content decreased in SPH to 48.8 µmol/L, but it increased to 65.5 µmol/L in MPH. Cysteine showed the opposite tendency; its levels increased in SPH but decreased in MPH. In both isolates and hydrolysates, mealworm had higher branched chain amino acid (BCAA) content than soybean (281.2 and 331.3 µmol/L in soybean and 356.6 and 434.4 µmol/L in mealworm, respectively). BCAA, an essential amino acid present in muscle protein, is oxidised in the skeletal muscle, and involved in muscle energy production, thus preventing muscle loss (Hormoznejad *et al.,* 2019). In general, protein functionality and bioactivity, including antioxidant activities are governed by amino acid composition, molecular weight distribution, and amino acid sequence (Chatsuwan *et al.,* 2018).

Protein solubility

The variation in soybean and mealworm larvae protein solubility at different pH values are shown in Figure 1. The solubilities of SPI and MPI at pH 3 were 57.0% and 23.4%, respectively. The solubilities of SPI and MPI were the lowest and highest at pH 5 (SPI, 2.83%; MPI, 8.04%) and pH 9 (SPI, 92.6%; MPI, 70.8%), respectively. These results indicated that the pI values of SPI and MPI were near pH 5, which is consistent with previous results showing that the pI values of SPI and MPI were 4.5 and 4.8, respectively. The solubilities of protein hydrolysates were significantly ($p < 0.05$) higher than those of protein isolates at all pH values. The highest increase in solubility was observed at pH 5 (SPI, 25-fold increase; MPI, 4-fold increase). This could be explained by the expansion of the spatial protein structure following enzymatic hydrolysis, thus improving their molecular flexibilities. Relaxed protein structures are known to improve solubility (Jiang *et al.,* 2018). The solubility of MPH was lower at pH 3 (63.9%) and pH 5 (32.9%) but higher at pH 7 (99%) and pH 9 (99.6%) than that of SPH. The improved solubility of mealworm protein by enzymatic hydrolysis at pH 3 - 9, except at pH 5, makes mealworm protein a suitable candidate for many food applications.

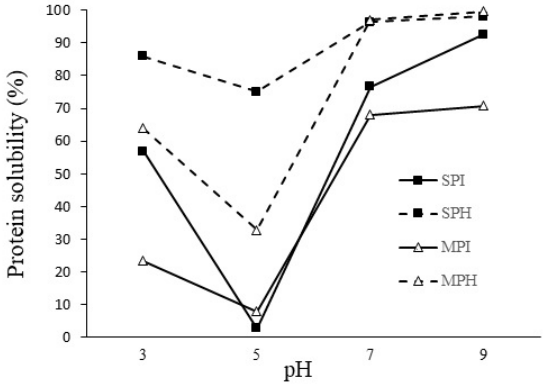

Figure 1. Solubilities of soybean and mealworm larvae protein isolates and hydrolysates. SPI = soybean protein isolate; SPH = soybean protein hydrolysate; MPI = mealworm larvae protein isolate; and MPH = mealworm larvae protein hydrolysate.

Foam expansion capacity (FEC) and foam stability (FS)

The FEC and FS of soybean and mealworm larvae protein are shown in Figure 2. MPI (18.67%) showed a lower FEC than that of SPI (58.33%). FEC significantly ($p < 0.05$) increased after enzymatic hydrolysis; however, the FEC of MPH (104.44%) was higher than that of SPH (94.44%). This can result in an exposed functional group and decreased molecular weight with hydrolysis, which facilitates the hydrophobic-hydrophilic balance and diffusion and adsorption at the interface (Wouters *et al.,* 2016; Phongthai *et al.,* 2020). In contrast, the FS of mealworm was different from that of soybean (Figure 2B). Hydrolysis decreased the FS of SPH (89.86%) when compared with that of SPI (97.35%), but the FS of MPI (89.86%) and MPH (89.21%) were similar. A reduced FS may be related to the decreased molecular weight, whereas increased FS can be affected by the hydrophobic interactions between hydrolysates, which lead to stable air bubbles (Wouters *et al.,* 2018). The increased hydrophobic amino acid content in MPH (Table 1) could affect foam stability.

Emulsifying activity (EA) and emulsion stability (ES)

Mealworm larvae proteins had significantly ($p < 0.05$) lower EA (90.6% for full-fat soybean vs. 54.1% for full-fat mealworm; 93.2% for defatted

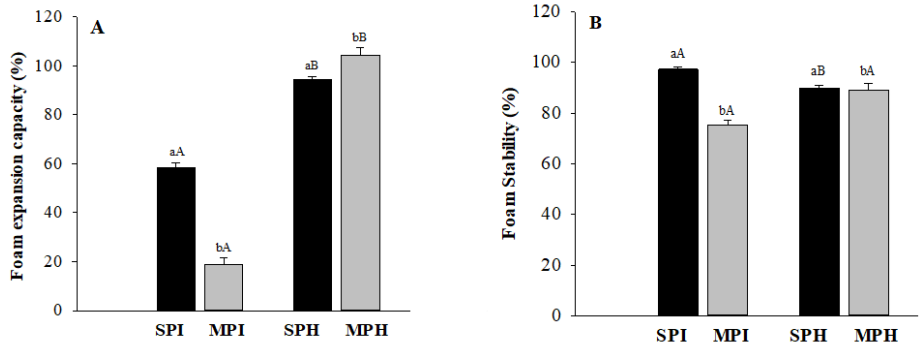

Figure 2. Foam expansion capacity (A) and foam stability (B) of soybean and mealworm larvae protein isolates and hydrolysates.

SPI = soybean protein isolate; SPH = soybean protein hydrolysate; MPI = mealworm larvae protein isolate; MPH = mealworm larvae protein hydrolysate. [a-b] Different superscript letters for different samples with the same treatment are significantly different by *t*-test ($p < 0.05$). [A-B] Different superscript letters for the same sample with different treatments are significantly different by *t*-test ($p < 0.05$).

soybean vs. 56.3% for defatted mealworm) than that of soybean proteins (Table 1). Defatting had little effect on emulsifying activities. However, the EA of MPI increased to 92.3%, whereas that of SPI decreased to 74.2%. This might be because EA is affected by molecular structure and protein content. Globulin, the main component of SPI, is not well emulsified, leading to the low EA of SPI (Xie *et al.,* 2000). In contrast, MPI has more hydrophobic amino acids at the protein surface (da Silva Lucasa *et al.,* 2020). Following hydrolysis, the EA of MPH decreased to 89.1%, whereas that of SPH increased to 83.5%. Small protein size by hydrolysis leads to easier diffusion, thus affecting emulsion formation (Gravel and Doyen, 2020). ES showed a tendency different from that of the emulsifying properties (Table 1). Mealworm larvae proteins had significantly ($p < 0.05$) higher ES values (84.4% for full-fat soybean vs. 92.4% for full-fat mealworm; 82.2% for defatted soybean vs. 89.9% for defatted mealworm) than those of soybean proteins. Defatting resulted in a slight decrease in ES. The ES of SPI (89.0%) increased, whereas that of MPI (89.3%) did not change. There were no significant differences in ES between SPI and MPI, which is consistent with the results of previous studies (Zielińska *et al.,* 2018). Following hydrolysis, the ES of SPH decreased ($p < 0.05$) to 60.3%, whereas that of MPH increased to 90.3%. The increased ES of MPH might be attributed to the alcalase cleavage site, which has a hydrophobic amino acid peptide bond, resulting in more hydrophobic amino acids in the hydrolysed product (Table 1) that can maintain the emulsion layer stability at the oil-water interface. In contrast, the reduced EA of SPH might be because of excessive protein hydrolysis and degradation of subunits into small peptides that are not conducive for adsorbing or diffusing proteins at the oil-water interface, thus reducing the ES (Jiang *et al.,* 2018). Since mealworm larvae proteins have higher ES than that of soybean proteins, they could be used as natural ingredients to maintain product stability.

Fat absorption capacity (FAC), water absorption capacity (WAC), and water holding capacity (WHC)

FACs of soybean and mealworm larvae proteins showed a similar increasing tendency following different treatments (Table 2). For full-fat samples, the FAC of FM (1.76 g/g) was lower than that of FS (2.01 g/g). After defatting, the FACs of mealworm and soybean increased to 2.25 and 2.35 g/g ($p > 0.05$), respectively. The oil absorption capacity is negatively influenced by fat content (Wani *et al.,* 2015). The FAC of MPI (4.39 g/g) was 1.3 times higher than that of SPI (3.29 g/g), whereas the FACs of the protein hydrolysates significantly ($p < 0.05$) increased. The FAC of MPH increased to 5.66 g/g, which was higher than that of SPH (4.59 g/g). Small and hydrophobic proteins hold more fat than their hydrophilic counterparts (Gravel and Doyen, 2020). Hydrolysis can lead to protein unfolding, thus exposing hydrophobic groups. This can result in an increased oil retention ability, as shown by the high oil absorption capacity of hydrolysates (Wani *et al.,* 2015). FAC affects taste retention and soft texture in food formulations, resulting in palatable food (Aremu *et al.,* 2007). Therefore, MPI and MPH could be used to promote the acceptability of insect proteins owing to their FAC in certain types of foods.

The interaction between protein and water are indicated by WAC and WHC. The former is related to chemical bonding, whereas the latter is associated with physical retention (Hua and Gu, 1999). The WACs of soybean and mealworm larvae proteins showed similar increasing tendencies after different treatments (Table 2). The WAC of full-fat mealworm (1.27 mL/g) was twice that of full-fat soybean

Table 2. Functional properties of soybean and mealworm larvae proteins.

Sample		EA (%)	ES (%)	FAC (g/g)	WAC (mL/g)	WHC (g/g)
Full-fat	soybean	90.60 ± 1.22^{aB}	84.44 ± 0.97^{bB}	2.01 ± 0.10^{aD}	2.57 ± 0.06^{aC}	2.40 ± 0.08^{aC}
	mealworm	54.11 ± 1.02^{bD}	92.40 ± 0.16^{aA}	1.76 ± 0.02^{bD}	1.27 ± 0.06^{bC}	1.91 ± 0.01^{bC}
Defatted	soybean	93.15 ± 1.14^{aA}	82.22 ± 1.19^{bC}	2.35 ± 0.09^{aC}	4.03 ± 0.06^{aA}	3.34 ± 0.07^{aB}
	mealworm	56.33 ± 0.48^{bC}	89.89 ± 0.20^{aC}	2.25 ± 0.05^{aC}	2.13 ± 0.23^{dB}	2.55 ± 0.13^{bB}
Protein isolate	soybean	74.20 ± 1.00^{bD}	88.95 ± 0.61^{aA}	3.29 ± 0.18^{bB}	3.47 ± 0.12^{aB}	3.56 ± 0.06^{aA}
	mealworm	92.33 ± 1.13^{aA}	89.33 ± 0.22^{ac}	4.39 ± 0.09^{aB}	3.67 ± 0.06^{aA}	3.12 ± 0.04^{aA}
Protein hydrolysate	soybean	83.45 ± 0.95^{bC}	60.33 ± 0.57^{bD}	4.59 ± 0.04^{bA}	ND	ND
	mealworm	89.05 ± 1.12^{aB}	90.32 ± 0.14^{aB}	5.66 ± 0.02^{aA}	ND	ND

EA = emulsifying activity; ES = emulsion stability; FAC = fat absorption capacity; WAC = water absorption capacity; WHC = water holding capacity; and ND = not detectable. $^{a-b}$ Different superscript letters for different samples with the same treatment are significantly different by *t*-test ($p < 0.05$). $^{A-D}$ Different superscript letters for the same sample with different treatments are significantly different by Duncan's multiple range test ($p < 0.05$).

(2.57 mL/g). After the defatting process, WACs of soybean and mealworm increased approximately 1.6 times as compared to those of full-fat samples ($p < 0.05$). Such increases in water absorption in defatted samples might be due to the exposure of water-binding sites of protein side chains previously blocked in a lipophilic environment (Du *et al.,* 2012). The WAC of MPI significantly ($p < 0.05$) increased to 3.67 mL/g. Water absorption is also related to the amino acid composition of the proteins. The amino acids of soybean proteins have high hydrophilic or polar amino acid content, such as aspartic acid and glutamic acid, thus leading to the high WAC of soybean (Hua and Gu, 1999). The data obtained in the present work revealed that SPI had higher hydrophilic amino acid content than MPI, particularly that of glutamic acid (SPI, 284.3 μmol/L; MPI, 213.2 μmol/L) (Table 1). The WHCs of soybean and mealworm larvae proteins showed similar tendencies to those of WACs (Table 2). The WHC of full-fat mealworm (1.91 g/g) was lower than that of full-fat soybean (2.4 g/g). After defatting, the WHCs of soybean and mealworm increased approximately 1.3 times as compared to those of full-fat samples ($p < 0.05$). The WHC of MPI significantly ($p < 0.05$) increased to 3.12 g/g, which was similar to that of SPI. Thus, MPI, similar to SPI, can be used in meat analogues to reduce moisture loss and improve product quality (Smetana *et al.,* 2018).

Gel formation

The visual appearance of the protein isolates after heating is described in Table 3. In general, the factors affecting gel formation include protein concentration, pH, and thermal treatment (Yi *et al.,* 2013). Gel formation increases with increasing

Table 3. Gel formation from soybean and mealworm larvae protein isolate solutions.

Concentration (%)	Sample	pH 3	pH 5	pH 7	pH 9
5	SPI	A	A	O	X
	MPI	X	X	X	X
10	SPI	A	O	O	O
	MPI	A	A	O	O
15	SPI	A	O	O	O
	MPI	A	O	O	O

X = no gel formation; A = aggregation; O = gel formation; SPI = soybean protein isolate; and MPI = mealworm larvae protein isolate.

dispersion concentration. At pH 3, no gel formation was observed owing to the increased charge, which prevented the proteins from gelling. At pH 5, which is the pI of soy and mealworm larvae protein, all samples except 5% MPI formed an aggregate or gel. Close to the pI, the net charge is zero, which leads to the development of a weak repulsive force that results in a dense aggregate formation (Yi *et al.,* 2013). Except at a concentration of 5%, all dispersions formed aggregates or gels at pH 7 and 9. However, MPH did not form gels by decreasing the average molecular mass, thereby limiting the ability to form strong networks (Wouters *et al.,* 2016).

Total phenolic and flavonoid contents and antioxidant activities

The total phenolic content of MPI (4.85 mg GAE/g) was four times higher than that of SPI (1.07 mg GAE/g) (Figure 3A). Phenolic compounds are secondary metabolites preserved in plants that have antioxidant effects (Lee *et al.,* 2015). Kim *et al.*

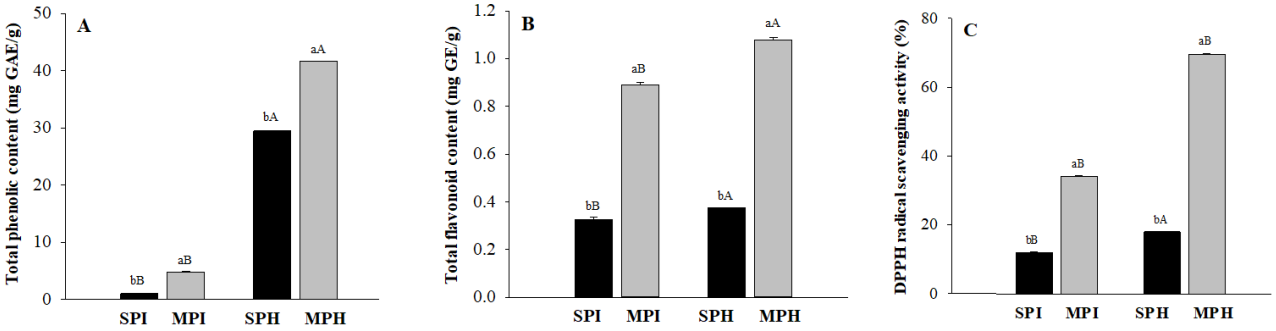

Figure 3. Total phenolic content (A), flavonoid content (B), and DPPH radical activity (C) of soybean and mealworm larvae protein isolates and hydrolysates.
SPI = soybean protein isolate; SPH = soybean protein hydrolysate; MPI = mealworm larvae protein isolate; MPH = mealworm larvae protein hydrolysate. [a-b] Different superscript letters for different samples with the same treatment are significantly different by *t*-test ($p < 0.05$). [A-B] Different superscript letters for the same sample with different treatments are significantly different by *t*-test ($p < 0.05$).

(2019a) also reported similar total phenolic content in full-fat mealworm (3.1 mg GAE/g), which was higher than that in whey protein concentrate (1.16 mg/g). In particular, there was no difference in terms of phenolic content between full-fat mealworm and protein isolate (3.11 mg GAE/g), thus indicating that most phenolic compounds in mealworms exist as proteins, rather than in other insect parts. After hydrolysis, the total phenolic content significantly ($p < 0.05$) increased to 29.5 and 41.7 mg GAE/g for SPH and MPH, respectively. During hydrolysis, the bound polyphenols are released in a free state (Yan *et al.,* 2015). The total flavonoid and phenolic contents of the protein isolates and hydrolysates showed similar tendencies (Figure 3B). The total flavonoid content of MPI (0.89 mg GE/g) was 2.5 times higher than that of SPI (0.35 mg GE/g). After hydrolysis, the total flavonoid content significantly ($p < 0.05$) increased. This might be because hydrolysis can increase aglycone production from protein flavonoids. The total flavonoid content of MPH was 2.9 times higher than that of SPH.

DPPH radical scavenging activity showed tendencies similar to those of total phenolic content and flavonoid content (Figure 3C). The DPPH radical scavenging activity of MPI (34.36%) was higher ($p < 0.05$) than that of SPI (12.21%). Wu *et al.* (2020) reported that that the relative phenol and butylated hydroxytoluene (which is used as an antioxidant food additive) content in mealworm larvae was found to be the highest (Figure 3A). The DPPH radical scavenging activity and polyphenol content of mealworms were highly correlated ($r = 0.96$). Mealworm proteins are known to have high contents of sulphur-containing amino acids that act as potential antioxidants by removing reactive oxygen species (Wu *et al.,* 2020). The methionine and cysteine contents were 15.7 and 41.1 µmol/L in SPI, and 34.5 and 43.1 µmol/L in MPI, respectively (Table 1). After hydrolysis, the DPPH radical scavenging activities significantly ($p < 0.05$) increased. In particular, the DPPH radical scavenging activity of MPH increased to 69.9%, which was 3.8 times higher than that of SPH (18.2%). Similar results have been reported previously, showing that the DPPH radical scavenging activity of a protein hydrolysate produced by alcalase significantly improved as compared to that of casein (Kumar *et al.,* 2016). Protein structures might change after enzymatic hydrolysis, exposing more amino acid residues by electron-radical reactions. More stable products may be generated, thus preventing free radical chain reactions (Hall *et al.,* 2017). In addition, the hydrophobic and aromatic amino acids in mealworm proteins can increase their antioxidant properties through their electron-donating ability or direct lipid radical scavenging ability (da Silva Lucasa *et al.,* 2020). The high antioxidant capacities of MPI and MPH indicate that they could be used as functional ingredients with bioactive properties, such as anti-inflammatory and anti-cancer effects (da Silva Lucas *et al.,* 2020).

In vitro ileal digestibility (IVID)

The IVID of soybean and mealworm larvae is shown in Figure 4. In both samples, IVID significantly ($p < 0.05$) increased with protein extraction and hydrolysis. The IVID of mealworm larvae (73.05%) was higher than that of soybean (48.20%). This may be related to the anti-nutritional factors present in soybean, including phytate, trypsin inhibitors, and tannins (Adeyemo and Onilude, 2013). After protein extraction, the IVID of SPI (79.14%) and MPI (75.07%) were similar. However, enzymatic hydrolysis improved the IVID of both SPH (96.37%) and MPH (96.33%). The increased IVID of hydrolysates was owing to low-molecular-weight

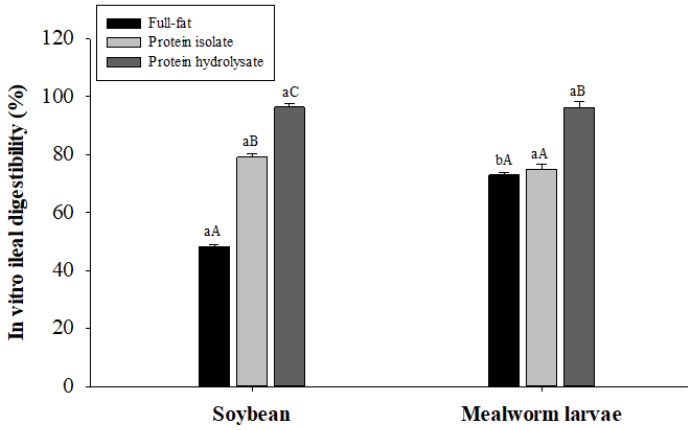

Figure 4. The *in vitro* ileal digestibility of soybean and mealworm larvae protein isolates and hydrolysates. SPI = soybean protein isolate; SPH = soybean protein hydrolysate; MPI = mealworm larvae protein isolate; MPH = mealworm larvae protein hydrolysate. [a-b] Different superscript letters for different samples with the same treatment are significantly different by *t*-test ($p < 0.05$). [A-B] Different superscript letters for the same sample with different treatments are significantly different by Duncan's multiple range ($p < 0.05$).

peptides produced during hydrolysis, which are easily digested as compared to protein or raw materials (Aryee and Boye, 2016).

Conclusion

Mealworm larvae proteins were investigated as alternatives to soybean proteins based on their functional antioxidant properties and digestibility. The results showed that MPI had higher emulsion capacity, stability, and fat absorption capacity than those of SPI, whereas its water absorption and holding capacity, foam expansion capacity, and stability were similar to or lower than those of SPI. Further hydrolysis by alcalase improved the solubility and foam expansion capacity of MPI, as well as its antioxidant effects. These results demonstrated that mealworm larvae proteins have good functional and antioxidant properties based on compatible amino acid profiles and high digestibility, and could be used as protein ingredients in high value-added food products. Moreover, because the consumer acceptability of insect-based foods is low, the use of hydrolysates might be one of the best ways to approach consumers and gain acceptance.

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