Innovative Food Science and Emerging Technologies 87 (2023) 103407

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Functional properties and essential amino acid composition of proteins extracted from black soldier fly larvae reared on canteen leftovers

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ARTICLE INFO

Keywords:
Insect proteins
Amino acid composition
Protein functionalities
Foaming capacity
Foaming stability

ABSTRACT

This study investigated the functional properties and essential amino acid composition of proteins extracted from black soldier fly larvae which represent a good source of proteins (30.12% dry matter). The proteins extracted in alkaline conditions (pH 11) were then isolated using two different recovery methods, (i) ultrafiltration, and (ii) isoelectric precipitation. Ultrafiltration provided higher purity of proteins (96.42%) but a lower extraction yield (24.30%) compared to isoelectric precipitation which provided a protein purity of 76.02% and higher extraction yield (37.22%). All essential amino acids were present in adequate quantities for human requirements. The fraction of proteins obtained by ultrafiltration had significantly higher oil holding capacity and foaming capacity than isoelectrically precipitated proteins. The protein fractions obtained by ultrafiltration and isoelectric precipitation had oil holding capacity of 125.8% and 81.6%, while the foaming capacity was 141.9% and 114.3%, respectively. These technological functionalities can be used to improve human food characteristics, thus resulting in enhanced consumer acceptance.

Industrial relevance: The food industry seeks alternative and sustainable sources of proteins, such as insect proteins, to reduce the environmental impact (i.e., greenhouse gas emissions). Consumers' acceptance is the main barrier to adopting edible insects in commercial applications. The acceptance increases when the insect proteins are incorporated in food products as ingredients rather than consuming the whole insect. Hence, this study focuses not only on extraction of proteins but also on functional properties of those proteins making it easier to target specific food formulations (i.e., whipped toppings), which require specific functionalities (i.e., foaming capacity and stability). The protein purity was increased by including an ultrafiltration step. The proteins obtained through the ultrafiltration method showed better oil holding capacity and foaming stability compared to proteins obtained by isoelectric precipitation. The strategies assessed in the present study help enhance the purity of larval proteins and improve their functional properties, thereby opening up new opportunities to incorporate this ingredient into targeted food formulations and improve consumer acceptance.

1. Introduction

The world population is projected to reach 9.7 billion by 2050 and 11.2 billion by 2100, thus increasing concerns over food availability. This will reflect in high demands for sustainable sources of proteins since the growing demand for proteins cannot be met by current agricultural practice. Moreover, agriculture already has a major global environmental impact leading to greenhouse gas (GHG) emissions and use of agrochemicals that pollute fresh, marine, and terrestrial ecosystems (Tilman & Clark, 2014). Aside from plant-based proteins, in-vitro cultured meat, seaweed, and macroalgae (Aimutis, 2022; Chriki & Hocquette, 2020; Montevecchi et al., 2022), novel and environmentally friendly protein sources such as insects (Miron et al., 2023; van Huis, 2020), and single cell protein (Thiviya et al., 2022) have been suggested as promising alternative sources.

Insects have been claimed as a sustainable protein alternative to

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https://doi.org/10.1016/j.ifset.2023.103407
Received 24 September 2022; Received in revised form 18 May 2023; Accepted 1 June 2023
Available online 7 June 2023

enhance food and feed security due to their nutritional composition and ease of rearing (van Huis et al., 2013). Black soldier fly larvae (BSFL), *Hermetia illucens* (Diptera: Stratiomyidae), are capable of efficiently converting a wide variety of organic materials like seasonal agri-food leftovers (Barbi et al., 2020), animal manure (Bortolini et al., 2020), and municipal organic waste (Shumo et al., 2019) into protein-based and fat-rich biomass. The rearing substrates influence the protein fraction of BSFL, and its protein content ranges from 37.8 to 46.0% on dry matter basis (Singh & Kumari, 2019). Moreover, BSFL proteins contain all essential amino acids required by humans (Montevecchi et al., 2021). Due to their high protein content, the BSFL was used as a feed source for pigs, poultry, fish, and crustaceans (Makkar et al., 2014).

The use of BSFL for human consumption is challenging because of regulations and consumer acceptance. Currently, BSFL is not considered on the list of accepted edible insects in the novel foods' regulation of European Union, and it is subject to novel foods' authorization process (Bessa et al., 2020). Moreover, consumer acceptance is another issue that should be addressed. Most people in Western countries are reluctant to try or consume insects, which are often met with disgust or uneasiness among consumers. Thus, consumer acceptance remains one of the largest barriers to commercialization of insects (Hartmann & Siegrist, 2017). However, several studies have shown a more positive attitude toward eating insects and a lower incidence of disgust when insects are incorporated into food as processed ingredients rather than consuming the whole insect (La Barbera et al., 2018).

To effectively use insect proteins as food ingredients, they must be extracted, and their functional properties need to be fully understood. Therefore, extraction and recovery of BSFL proteins is necessary and there are several approaches of protein extraction from BSFL reported in the literature (Bußler et al., 2016; Caligiani et al., 2018; Janssen et al., 2019; Leni et al., 2020; Mintah et al., 2020; Miron et al., 2019; Smets et al., 2020). The quality of extracts is influenced by the extraction procedure and a dark color is formed during extraction because of the presence of endogenous phenol oxidases in BSFL (Janssen et al., 2019). The use of phenol oxidases' inhibitors like sodium ascorbate and ethylenediaminetetraacetic acid (EDTA) significantly reduces the amount of browning (Caligiani et al., 2018). The authors reported that in the defatting procedure of BSFL before extraction, the extraction yield is affected by both the ratio of alkali solution to sample and the extraction time. Alternatively, it is likely that a blanching treatment carried out on the larvae, such as that adopted by Montevecchi et al. (2020) can partially or totally deactivate the biochemical processes triggered by the larval phenol oxidases.

Most of the studies concerning BSFL focused on rearing, composition, nutritional aspects, safety, and protein extraction. However, the functionality of BSFL-extracted proteins also needs to be addressed to broaden the technological potential of this food ingredient. (Bessa et al., 2020). To our knowledge, there is very little information concerning the functionality of BSFL proteins. Recently, there have been authors showing that functionality of insect proteins is affected by insect processing and protein extraction condition (Kumar et al., 2022).

This study was aimed to investigate the functionality of proteins extracted from BSFL reared on canteen leftovers. The proteins were sequentially extracted in alkaline conditions (pH 11) followed by two different recovery methods, namely (i) ultrafiltration and (ii) isoelectric precipitation. The functional properties of proteins in terms of protein solubility, foaming properties, water and oil binding capacity were determined. It was hypothesized that an ultrafiltration step would improve protein purity and functional properties of the proteins compared to an isoelectric precipitation step.

2. Materials and methods

2.1. Materials

Soy protein isolate (SPI) and pea protein isolate (PPI) used as reference samples were purchased from Myprotein (Northwich, Cheshire, UK). Bovine serum albumin (BSA), hexane, potassium metabisulfite ($K_2S_2O_5$), sodium dodecyl sulfate (SDS), sodium hydroxide (NaOH), and tris(hydroxymethyl)aminomethane (TRIS) were purchased from Fischer Scientific (Landsmeer, The Netherlands). The reagents A and B for protein assay were purchased from Bio-Rad Laboratories (Lunteren, The Netherlands).

The BSFL were provided by University of Modena and Reggio Emilia, Italy. BSFL were reared on a random mixture of food collected in a kitchen canteen (CIRFOOD s.c., Reggio Emilia, Italy), including animal and vegetal products. More details on the nature and composition of these leftovers have been reported in Montevecchi et al. (2023).

The larval rearing was performed in a climate chamber at 27.0 ± 0.5 °C and 70 ± 10% RH. At the end of the experiment, when the insects reached the desired developmental stage, larvae were cleaned and stabilized through heat as described by Hadj Saadoun et al. (2020). Therefore, the BSFL were frozen (−20 °C) and sent by courier to Zetadec (Wageningen, The Netherlands).

Milli-Q water-quality was obtained through a Barnstead Smart2Pure water purification system (Thermo Fischer Scientific, Landsmeer, The Netherlands).

2.2. Proximate analysis

Crude protein, crude fat, crude ash, crude fiber content, and residual moisture of freeze dried BSFL were analyzed according to EC (2009). In short, the moisture content was determined by drying the BSFL in an oven at 103 °C up to constant weight. The crude protein content was determined according to the Kjeldahl method on the basis of nitrogen content, and the conversion factor of 4.67 was used to calculate the crude protein content, as suggested by Jonas-Levi and Martinez (2017). The crude fat was determined using a Soxhlet extractor apparatus with petroleum ether as solvent and quantified gravimetrically. The crude ash content was analyzed by incineration in a muffle furnace at 550 °C for 3.5 h. Crude fiber was determined by sequential digestion of sample with H_2SO_4 and KOH using a fiber glass container drying in an oven to constant weight at 130 °C and then placed in a muffle furnace and incinerated up to constant weight at 500 °C. The crude fiber was quantified gravimetrically. Other components were calculated by difference.

2.3. Extraction of black soldier fly protein

Fig. 1 shows an overview of the sequential extraction of lipid and protein from BSFL. The protocol developed by Smets et al. (2020), with minor adjustments, was employed to dehydrate and defat the gross sample of BSFL, until obtaining the protein extract. Briefly, the freeze-dried larvae were ground using a Retsch mill (Verder Scientific, Haan, Germany) over a sieve size of 4 mm. The ground sample was used for lipid extraction by Soxhlet with hexane for 18 h at 80 °C and then, the lipid fraction was kept in an oven for 6 h at 50 °C to evaporate the sample to dryness.

Defatted larvae (DBSF) were ground using a Retsch mill over a sieve size of 0.2 mm. Fifty (50) g of defatted and ground larvae were dispersed in 1000 mL milli-Q water (material to solvent ratio = 1:20) and the pH was adjusted to 11 using 1 M NaOH. The dispersion was stirred on a magnetic stirrer for 2 h at 25 °C to dissolve the protein fraction. This step was followed by centrifugation at 5000 rpm for 30 min to separate the chitinous pellet from the protein aqueous extract. All the protein extraction processes were conducted using 10 mM potassium metabisulfite ($K_2S_2O_5$) to prevent protein browning.

Afterward, the aqueous protein extract was separated into two aliquots in order to be subjected to two different methods of protein recovery, ultrafiltration and isoelectric precipitation. The extraction yield of proteins was calculated according to Eq. (1):

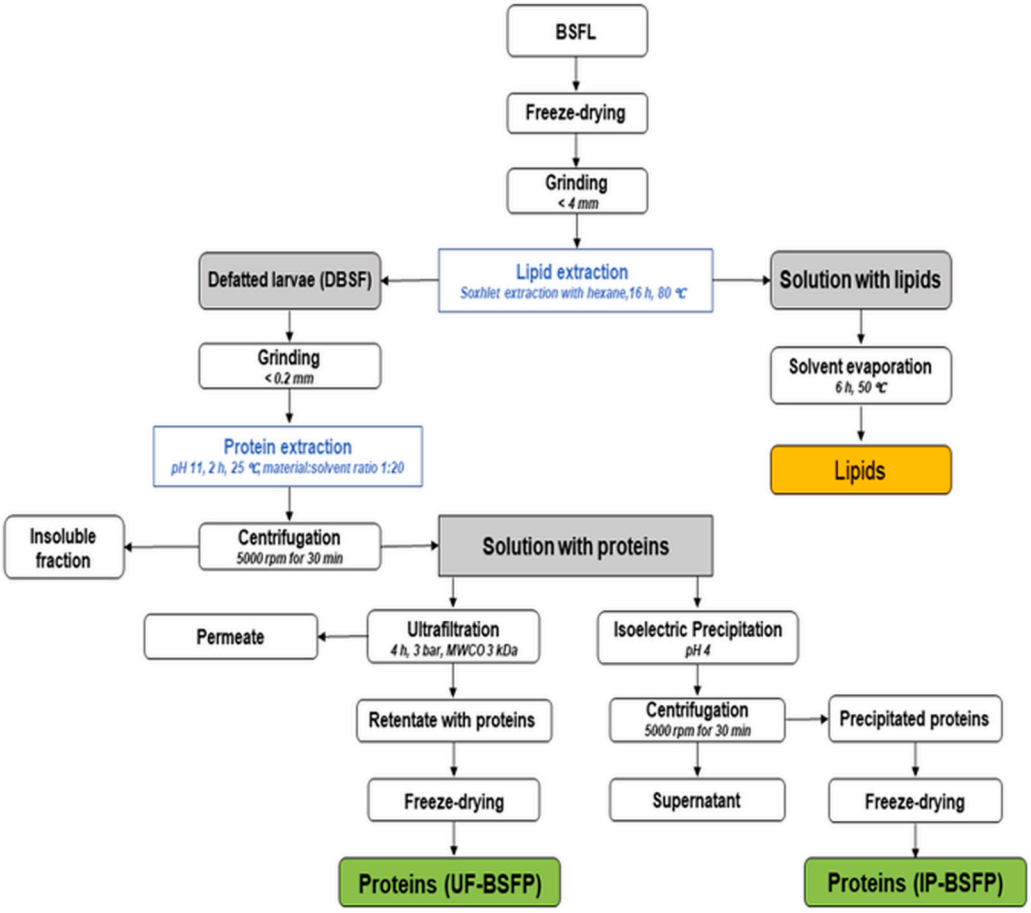

Fig. 1. Schematic representation of proteins extraction from black soldier fly larvae (*Hermetia illucens*).

$$Extraction\,yield\,(\%) = \frac{mass\,protein\,sample}{starting\,total\,protein\,content\,in\,freeze-dried\,BSFL} \times 100 \quad (1)$$

The extraction procedure was performed in duplicate, and the results of the extraction yields are presented as mean values ± standard deviations.

2.3.1. Black soldier fly protein isolation through ultrafiltration (UF-BSFP)

The first one consisted in the ultrafiltration of a part of aqueous protein extract at 3 bars for 4 h using an Amicon® stirred cell (Merck Millipore, Burlington, Massachusetts, USA) and an Ultracel® ultrafiltration membrane (Merck Millipore, Burlington, Massachusetts, USA) with molecular weight cut-off (MWCO) of 3 kDa. Then, the retentate containing UF-BSFP was freeze-dried and, finally, stored at 4 °C for further analysis.

2.3.2. Black soldier fly protein isolation through isoelectric precipitation (IP-BSFP)

The second recovery method consisted in the isoelectric precipitation. Part of the aqueous protein extract was adjusted to pH 4 with 1 M HCl to precipitate the protein fraction. Subsequently, the suspension was centrifuged at 5000 rpm for 30 min and the pellet of IP-BSFP was freeze-dried thereafter. This sample was also stored in the fridge at 4 °C for further analysis.

2.4. Protein purity

The Lowry BioRad DC™ protein assay (Bio-Rad Laboratories, Philadelphia, USA) was used to determine the purity of protein samples obtained by both ultrafiltration and isoelectric precipitation. Thus, 6 mg of each freeze-dried protein sample (UF-BSFP and IP-BSFP) were added to 1 mL of lysis buffer I (60 mM Tris pH 9, 2% SDS) in Eppendorf tubes. The Eppendorf tubes were mixed by a Retsch vortex mixer (Verder Scientific, Haan, Germany) for 1 min. The samples were incubated at 100 °C for 30 min, followed by centrifugation for 10 min at 3500 rpm. Then, the supernatant was diluted 10 times with milli-Q water, hence 100 µL of diluted supernatant were added to a test tube to which 0.5 mL reagent A and 4 mL reagent B were added. The protein quantification was performed using bovine serum albumin as a protein standard. Absorbance was measured after 15 min at 750 nm using a spectrophotometer (Buck Scientific Cecil, Norwalk, USA).

The protein purity was calculated according to the Eq. (2):

$$Protein\ purity\ (\%) = \frac{mass\ protein\ sample}{mass\ total\ dry\ weight\ sample} \times 100 \quad (2)$$

The assay was performed in triplicate and the results are presented as mean values ± standard deviations.

2.5. Total amino acids profile

Amino acid composition of protein extract was analyzed according to EC (2009). Because of the low extraction yield, the quantity needed to determine the amino acid profile of UF-BSFP did not suffice. Thus, only the amino profile of the proteins obtained by isoelectric precipitation (IP-BSFP) was evaluated.

Protein quality was evaluated through the essential amino acid index (EAAI). EAAI compares the content of all essential amino acids with a reference protein or, as in the present study, with the values for human requirements (Smith, 2017). EAAI estimates the potential of using IP-

BSFP as a protein source for human consumption, without correcting for protein digestibility. EAAI was calculated with the Eq. (3):

$$EAAI = \sqrt[9]{\left(\frac{g\ of\ one\ out\ of\ nine\ essential\ amino\ acids\ in\ 1\ g\ BSFP}{g\ of\ one\ out\ of\ nine\ essential\ amino\ acids\ needed\ in\ 1\ g\ reference\ protein}\right)} \times (etc.for\ each\ of\ the\ other\ eight\ essential\ AA) \quad (3)$$

2.6. Protein solubility

Protein solubility indices were determined at various pH values ranging from 2 to 11. In short, 100 mg of protein sample was dispersed in 10 mL milli-Q water and the pH was adjusted to the desired value using 1 M NaOH or 1 M HCl. Then, the samples were centrifuged at 5000 rpm for 30 min. The amount of protein in the supernatant was determined by Lowry BioRad DC™ protein assay. Protein solubility is expressed as percentage of soluble to total protein. The experiments were carried out in duplicate and the results are presented as mean values.

2.7. Water holding capacity

Water holding capacity (WHC) was determined in duplicate according to AACC method 56-30.01 (AACC International, 2000). Enough water was added to over saturate the sample (0.5 g), but not to cause a liquid dispersion. The hydrated samples were centrifuged at 4300 rpm for 5 min and the supernatant was removed. The experiment was performed in triplicate and the results are presented as mean values ± standard deviations.

The WHC (%) was calculated as follows:

$$WHC\ (\%) = \frac{final\ weight - initial\ weight}{initial\ weight} \times 100 \quad (4)$$

2.8. Oil holding capacity

Oil holding capacity (OHC) was determined according to the method of Haque and Mozaffar (1992) with a slight modification. Each sample (0.25 g) was added to 1.5 mL of sunflower oil and mixed for 1 min in a vortex mixer at room temperature. After having centrifugated at 6000 rpm for 15 min the precipitate was weighed. The experiment was performed in triplicate and the results are presented as mean values ± standard deviations.

The oil holding capacity was calculated through the following equation:

$$OHC\ (\%) = \frac{final\ weight - initial\ weight}{initial\ weight} \times 100 \quad (5)$$

2.9. Foaming properties

Foaming capacity (FC) and foam stability (FS) were studied in duplicate using the method of Zielińska et al. (2018) with slight modifications. Fifty milliliters of a 1% (*w/v*) sample were homogenized in a measuring cylinder with Ultra-turrax homogenizer (Silent Crusher M, Heidolph, Germany) at 20,000 rpm for 2 min. The experiment was performed in triplicate and the results are presented as mean values ± standard deviations.

The total volume of whipped sample was read immediately (V_0) and 30 min after whipping (V_{30}). The foaming capacity (FC) was calculated using the following equation:

$$FC\ (\%) = \frac{V_0 - V}{V} \times 100 \quad (6)$$

The foam stability (FS) was calculated through the following equation:

$$FS\ (\%) = \frac{V_{30}}{V_0} \times 100 \quad (7)$$

where:

V – volume before whipping (mL);
V_0 – volume immediately after whipping (mL);
V_{30} – volume after standing 30 min (mL).

2.10. Statistical analysis

Data were statistically evaluated by one-way analysis of variance (ANOVA) using R statistical language and environment (R Development Core Team, 2009). Before carrying out the ANOVA, normal distribution and homoscedasticity of the data were favorably verified. The statistically significant differences among samples were determined using the Tukey's test, *p*-values ($p \leq 0.05$) were considered significant.

3. Results and discussion

3.1. Proximate composition

The proximate composition of freeze-dried BSFL was important to set up the fractionation procedure. The results in terms of crude protein, crude fat, crude ash, crude fiber, and other components (calculated by difference) are given in Table 1. BSFL contains high levels of fat (40.63 g/100 $g_{freeze\text{-}dried\ BSFL\ sample}$) and protein (30.12 g/100 $g_{freeze\text{-}dried\ BSFL\ sample}$). BSFL also contains 4.77 g/100 $g_{freeze\text{-}dried\ BSFL\ sample}$ ash and 7.02 g/100 $g_{freeze\text{-}dried\ BSFL\ sample}$ crude fiber. The fat content is similar to that reported by Smets et al. (2020) and higher compared with Mintah et al. (2020) who found a fat content of 32.09% in BSFL.

The crude protein content is reasonably in line with that found by (Mintah et al., 2020), who used 6.25 as a conversion factor, while it is lower than what was found by Smets et al. (2020), who used 4.67. However, the crude protein values in the present study are in line with what has been reported by Smets et al. (2020) on BSF prepupae and pupae. Therefore, there might be an effect due to the progress of the specific preimaginal stage. The studies cited used organic material/food waste from restaurants or supermarkets for rearing BSFL.

Table 1
Proximate composition of freeze-dried BSFL. The standard deviation was within 10% for all parameters.

Component	Composition (g/100 $g_{freeze\text{-}dried\ samples}$)
Crude protein	30.12
Crude fat	40.63
Crude ash	4.77
Crude fiber	7.02
Residual moisture of the freeze-dried samples	2.78
Other components (e.g., carbohydrates, minerals, and vitamins) (calculated by difference)	14.68

Table 2
Extraction yield and purity of protein samples obtained by isoelectric precipitation (IP-BSFP) and ultrafiltration (UF-BSFP).

Sample	Extraction yield (% - see Eq. (1))	Protein purity (% - see Eq. (2))
IP-BSFP	37.22 ± 1.50	76.02 ± 0.72
UF-BSFP	24.30 ± 1.53	96.42 ± 0.87

3.2. Extraction yield and protein purity

Considering the high-fat content of BSFL, a defatting step was necessary since the fat can interfere with protein extraction. Defatted BSFL was then subjected to protein extraction at pH 11 and then two different recovery methods were applied. The first one involved isoelectric precipitation at pH 4, while the second procedure replaced the isoelectric precipitation with the ultrafiltration process. Ultrafiltration was introduced in this study with the main goal of obtaining proteins with high purity in order to improve the functional properties of proteins.

The effect of the specific method on extraction yields and protein purity was examined, and results are presented in Table 2. An extraction yield of 37.22% and a purity of 76.02% were attained by isoelectric precipitation, while a lower extraction yield (24.30%) but a higher purity of proteins (96.42%) was achieved through ultrafiltration. Thus, ultrafiltration removed the impurities and significantly increased the protein purity ($p \leq 0.01$) by 20.40%. However, the extraction yield was significantly lower ($p \leq 0.001$) and could be explained by the removal of impurities by ultrafiltration. Ultrafiltration has not been used before for extraction of proteins from BSFL, while this method has already been used for extraction of legume proteins, such as pea, chickpea, and lentil (Boye et al., 2010). In these cases, the ultrafiltration process has been shown to improve the protein concentration with values between 2.2 and 9.5% compared with isoelectric precipitation.

The extraction yields obtained in this study are the same range as Smets et al. (2020), who obtained an extraction yield of 27.58%. However, the extraction yields are lower compared to extraction yields (41–61%) found by Mintah et al. (2020), who extracted the protein fraction in alkaline conditions and temperatures between 30 and 70 °C. Caligiani et al. (2018) obtained even higher extraction yields using trichloroacetic acid as a protein-precipitating agent (73% yield) and the stepwise protein extraction, also known as Osborne fractionation (yield > 85%).

In the present study, the protein purity obtained by isoelectric precipitation was in accordance with Mintah et al. (2020) who obtained protein contents of 80.42% and 76.91% in alkaline conditions at 40 °C. The protein contents obtained in the present study were higher than other values reported in the literature. To make a comparison, Baigts-Allende et al. (2021), Janssen et al. (2017), Kumar et al. (2022), Mshayisa et al. (2022), and Queiroz et al. (2021) have reported protein contents of BSFL ranging between 55.18 and 73.35%.

3.3. Amino acid composition

The BSFL protein quality was evaluated through amino acid composition. Table 3 displays the amino acid profile of the IP-BSFP, which was found to be a good source of essential amino acids. In addition, all essential amino acids of IP-BSFP were in quantities exceeding those needed to meet human requirements. In particular, the sum of essential amino acids (53.76 g/100 g) was almost double that recommended (minimum 27.7 g/100 g) to meet human needs (FAO/WHO/UNU, 1985). Furthermore, the sum of essential amino acids was higher than the figures reported by Baigts-Allende et al. (2021) and Queiroz et al. (2021), which found values of 32.93 and 42.42 g/100 g, respectively.

Noteworthy, the content of lysine (8.19 g/100 g IP-BSFP) was much higher than the recommended value of 4.5 g/100 g. Lysine is considered a limiting amino acid in staple-food cereals, such as maize, rice, and wheat (Mshayisa et al., 2022) and is of particular interest in developing countries because of low content in people's diet (Bessa et al., 2020).

Table 3
Amino acid (AA) profile expressed as a percentage (g of amino acid/100 g protein dry weight - DW) in samples of proteins obtained by isoelectric precipitation (IP-BSFP) compared to human daily requirements. The standard deviation was within 10%. EAAI: essential amino acid index. Sum of essential AA, sum of non-essential AA, and EAAI are reported in bold.

Amino acid	Composition ($g_{amino\ acid}$/100 $g_{protein\ DW}$)	
	IP-BSFP	Human daily requirements FAO/WHO/UNU (1985)
Essential AA		
Histidine	3.02	1.5
Leucine	7.87	5.9
Isoleucine	5.21	3.0
Lysine	8.19	4.5
Methionine + cystine	3.41	2.2
Phenylalanine + tyrosine	14.28	3.8
Threonine	4.46	2.3
Valine	5.74	3.9
Tryptophan	1.57	0.6
Sum essential AA	**53.76**	**27.7**
Non-essential AA		
Alanine	4.26	
Arginine	5.74	
Aspartic acid	12.98	
Glutamic acid	11.30	
Glycine	4.36	
Proline	3.93	
Serine	3.68	
Sum non-essential AA	**46.24**	
EAAI	**1.94**	**1.0**

The calculated essential amino acid index (EAAI) was 1.94. This value is higher than those reported for proteins extracted from other insects such as lesser mealworm (*Alphitobius diaperinus*) (1.65), yellow mealworm (*Tenebrio molitor*) (1.60), superworm (*Zophobas morio*) (1.66), edible cricket (*Acheta domesticus*) (1.39) and soybean (1.56) and is very similar to casein (1.93) (Yi et al., 2013). Moreover, lower EAAI values (1.50 and 1.44) for proteins extracted from BSFL were reported by Huang et al. (2019).

3.4. Protein solubility

In addition to providing energy and nutrition, food ingredients must also provide functional properties. The development of new protein ingredients hinges on protein solubility, as it impacts the entirety of the functional properties, e.g., foaming, gelling, emulsifying, water, and fat holding capacity, making it the utmost functional property (Voudouris et al., 2017). The solubility of defatted larvae (DBSF), isoelectric precipitated proteins (IP-BSFP), and proteins obtained by ultrafiltration (UF-BSFP) was evaluated over a pH range of 2–11.

Fig. 2 shows the results of protein solubility. The sample DBSF showed the lowest solubility (18.7%) at pH 4, while IP-BSFP and UF-BSFP showed the lowest solubility (6.2 and 5.8%) at pH 5. These solubility values at isoelectric point (pI) resembled the pI of major protein sources such as soybean (4.5), casein (4.6), and meat proteins (5.0) (Mishyna et al., 2019). As for DBSF, the highest solubility was found at pH values of 9–11.

As for IP-BSFP and UF-BSFP, a high solubility (89.0 and 92.6%) was observed at pH 2, thus suggesting that extraction of proteins from BSFL at this acidic pH might be a good option for further research since most of the extraction protocols found in the literature have been done in alkaline conditions. The solubility of IP-BSFP and UF-BSFP decreases at pH values between 3 and 7 and increases again in alkaline conditions (pH 8–11).

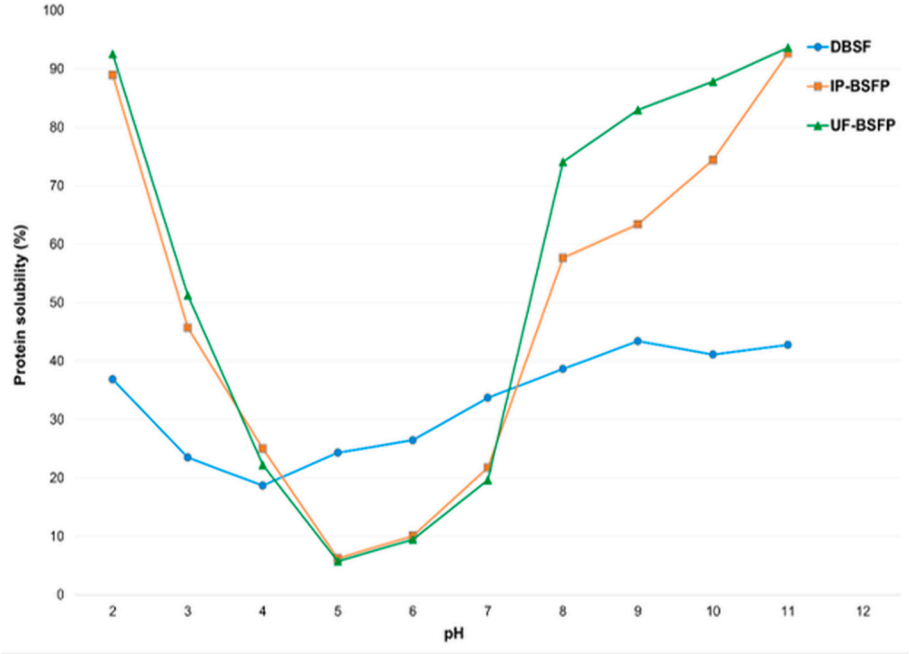

Fig. 2. Protein solubility (expressed as percentage of soluble to total protein) of defatted larvae (DBSF), proteins obtained by isoelectric precipitation (IP-BSFP), and proteins obtained by ultrafiltration (UF-BSFP) as a function of the aqueous solution pH. The standard deviation (not shown) was within 10%.

pH is a parameter that affects another crucial phenomenon, namely enzymatic browning. Monophenols, which include the essential amino acid L-tyrosine, can be subjected to enzymatic oxidation. In addition, browning reaction can have direct and possible important consequences on insect protein quality. Modifications induced by phenol oxidase (but also by other enzymes) might eventually affect protein quality, extractability, and digestibility (Leni et al., 2019).

The data present in the literature indicate that phenol oxidases have an optimal pH value around 6.5, while they are not active at values lower than 2.5 or higher than 8.5 (Mizobutsi et al., 2010; Yang et al., 2000). However, the protein solubilization step is placed at the end of the flow chart, while a suitable killing system of the larvae manages to avoid enzymatic oxidation.

Leni et al. (2019) showed a considerable involvement of phenol oxidase during killing of the larvae through freezing and grinding. In addition, they concluded that blanching represents a suitable way to inactivate phenol oxidase, thus fully inhibiting browning reaction and other metabolic pathways activated by the stress induced by the slow killing mode using freezing. Furthermore, Montevecchi et al. (2020) showed that the killing of the larvae through blanching also resulted in considerable benefits from the point of view of deactivation of lipases, thus preventing the hydrolysis of the fatty acids included in the

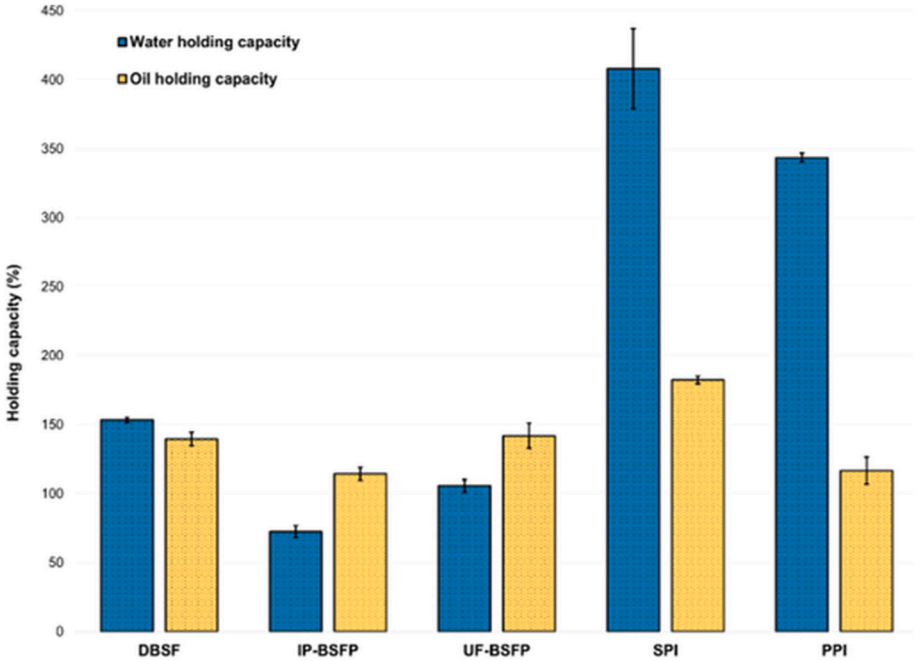

Fig. 3. Water holding capacity (WHC % – see Eq. (4)) and oil holding capacity (OHC % – see Eq. (5)) of defatted larvae (DBSF), proteins obtained by isoelectric precipitation (IP-BSFP), proteins obtained by ultrafiltration (UF-BSFP) and two reference samples: soy protein isolate (SPI) and pea protein isolate (PPI).

triglycerides.

The highest solubility (92.7 and 93.6%, respectively) of IP-BSFP and UF-BSFP was observed at pH 11, the pH that was also selected for extraction of proteins in the present study. A similar protein solubility pattern has been noticed in other BSFL studies (Bußler et al., 2016; Mshayisa et al., 2022; Smets et al., 2020) and other insects such as yellow mealworm (*Tenebrio molitor*) (Zhao et al., 2016; Zielińska et al., 2018) and yellow grasshopper (*Schistocerca gregaria*) (Mishyna et al., 2019; Zielińska et al., 2018). These studies showed that solubility is pH dependent and that protein-protein interactions occurs near pI where protein's net charge is neutral, thus resulting in protein precipitation, while extreme pH values yield charged protein surfaces that repel each other and increase solubility (Gravel & Doyen, 2020).

3.5. Water and oil holding capacity

Water holding capacity (WHC) was determined in this study because it is associated with gelation properties, improved texture and moisture which is of great importance in food formulation (Gravel & Doyen, 2020). WHC is influenced by many characteristics such as amino acid profile, protein concentration, charge characteristics, conformation, hydrophobicity, pH, temperature, and ionic strength (Naik et al., 2012). On the other hand, the oil holding capacity was also measured in this study because it is important in food applications to enhance palatability and mouthfeel, as well as retention of flavor (El Nasri & El Tinay, 2007).

Fig. 3 shows the WHC and OHC of DBSF, IP-BSFP, and UF-BSFP. Other two proteins, namely soy protein isolate (SPI) and pea protein isolate (PPI), were included in this study as reference samples because are extensively used in the food industry. The WHC of DBSF, IP-BSFP, and UF-BSFP were 153.5%, 72.5%, and 105.7%, respectively. Significant differences were found between all samples. Thus, DBSF showed the WHC significantly higher ($p \leq 0.001$), compared with UF-BSFP and IP-BSFP and the sample UF-BSFP had a significantly higher WHC ($p \leq 0.001$) compared with IP-BSFP. However, all samples had significantly lower WHC ($p \leq 0.001$) than both reference samples, namely SPI (408.0%) and PPI (343.5%).

The WHC of IP-BSFP is similar to the values (approx. 70%) reported by Bußler et al. (2016) and UF-BSFP is similar to the values (approx. 100%) obtained by Kumar et al. (2022). The WHC values for IP-BSFP and UF-BSFP obtained are lower compared to Mshayisa et al. (2022) that reported WHC values ranging from approximatively 300 to 550%, and Mintah et al. (2020) that reported WHC values of 132% and 136%.

In the present study, the results related to the WHC were also lower than other insects such as yellow mealworm (187% and 395%) (Zhao et al., 2016; Zielińska et al., 2018), yellow grasshopper (231%) (Zielińska et al., 2018), and edible cricket (273%) (Ndiritu et al., 2017). This could be due to different insect species and/or extraction techniques.

The OHC of DBSF, IP-BSFP, and UF-BSFP were 139.5%, 114.3%, and 141.9% respectively. The OHC of IP-BSFP was significantly lower ($p \leq 0.001$) than DBSF and UF-BSFP. The samples DSFP and UF-BSFP were not significantly different ($p > 0.05$) and this could be due to the fact that the oil holding capacity is mainly attributed to the physical entrapment of oil itself (Kinsella, 1976). The OHC of samples DBSF, IP-BSFP, and UF-BSFP was significantly lower ($p \leq 0.001$) compared with SPI. The OHC of samples DBSF and UF-BSFP was significantly higher ($p \leq 0.01$) than PPI.

In the present study, the OHC values were higher than the OHC values (80–90%) of BSFL obtained by Bußler et al. (2016) and lower than the OHC values (270 and 340%) reported by Mintah et al. (2020). Since DBSF and UF-BSFP showed a good oil binding capacity, these protein substances could be conveniently used in food formulations such as meats, sausages, cakes, and bakeries (Voudouris et al., 2017).

3.6. Foaming properties

The foaming properties of DSFP, IP-BSFP, UF-BSFP, and two reference samples were also investigated. Foam formation relies on a variety of factors such as protein structure since foam formation implies protein unfolding to ensure better absorption at the air-water interface (Gravel & Doyen, 2020).

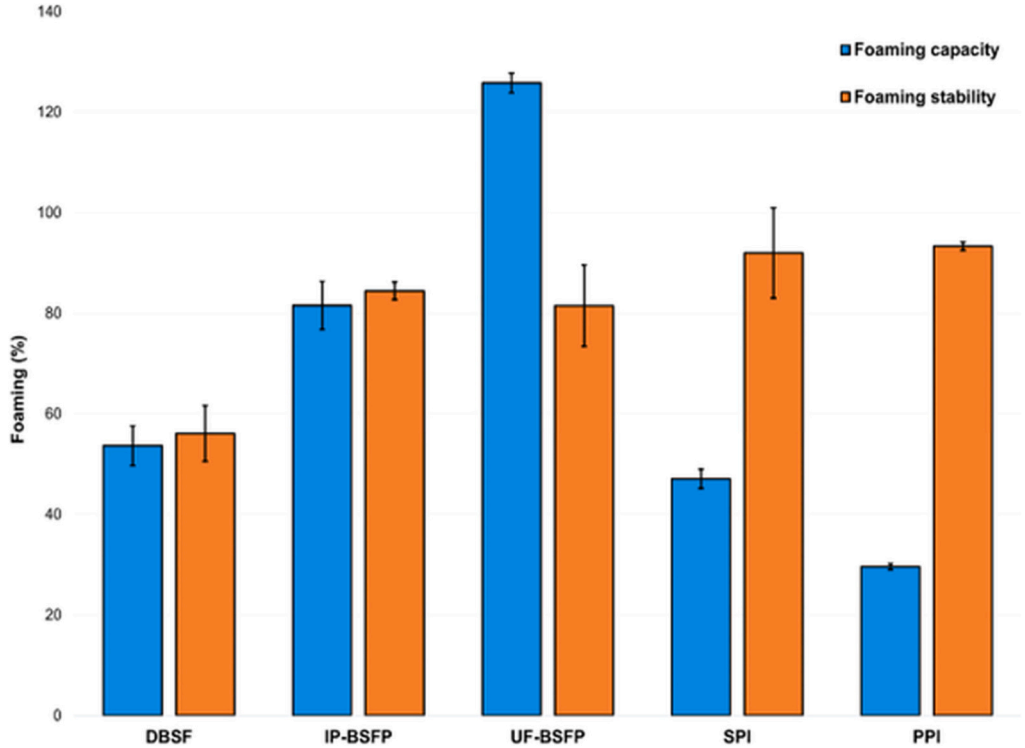

Fig. 4. Foaming capacity (FC % – see Eq. (6)) and foaming stability (FS % – see Eq. (7)) of defatted larvae (DBSF), proteins obtained by precipitation (IP-BSFP), proteins obtained by ultrafiltration (UF-BSFP) and two reference samples: soy protein isolate (SPI) and pea protein isolate (PPI).

Fig. 4 depicts the results of the foaming capacity and stability. The highest foaming capacity was observed in UF-BSFP (125.8%). This was significantly higher ($p \leq 0.001$) than DBSF (53.7%), IP-BSFP (81.6%), SPI (47.1%), and PPI (29.6%). Thus, the ultrafiltration step increased the foaming capacity, and this improvement could be related to protein concentration. Indeed, the protein concentration besides other factors (i.e., intermolecular interactions, charge, molecular size, surface hydrophobicity, ionic strength) influences the stabilization of the network that entraps air bubbles (Wagner & Guéguen, 1999).

In the literature, lower foaming capacity has been observed in studies of BSFL. Queiroz et al. (2021) reported foaming capacity from approximately 60% to 75% and Mshayisa et al. (2022) obtained foaming capacity between approximately 76% and 78%. Mintah et al. (2020) tested foaming capacity of BSFL proteins at different pH values (2, 4, 6, 8, and 10) and showed also low foaming capacity ranging from 5.55 to 31.82%. Lower foaming capacity than other study has been reported for other insects such as yellow grasshopper (approximatively 30–90%) (Mishyna et al., 2019; Zielińska et al., 2018) and edible crickets (11.11%) (Ndiritu et al., 2017).

Although the proteins obtained in this study showed good foaming capacity, the rapid air bubble formation does not always imply foam stability, which is also a parameter of paramount importance in food formulation (Gravel & Doyen, 2020). Therefore, an evaluation of stability was necessary as well. The lowest foaming stability was shown by DBSF that was significantly lower than all other samples ($p \leq 0.001$). The foaming stability of all other samples was not significant different ($p > 0.05$). The samples IP-BSFP and UF-BSFP showed good foaming stability values (84.4% and 81.5%). However, these values were slightly lower compared with values obtained by Mshayisa et al. (2022) (approximatively 90 and 100%) for BSFL; as well as slightly lower than the values found by Shevkani et al. (2015) in kidney bean (92%) and field pea (95%) samples. When testing the foaming stability at different pH values (2, 4, 6, 8 and 10), Mintah et al. (2020) showed higher values (88.88 and 90.91%) at pH 4 and lower foaming stability (between 10 and 65%) for all other pH values.

Current industrial trends are directed toward exploration of alternative proteins to replace foaming agents (i.e., egg proteins) in food products (Hall et al., 2017). Considering that UF-BSFP showed a good foaming capacity (125.8%) and stability (81.5%), this insect protein can represent a suitable alternative to replace conventional proteins in those food formulations that need foaming agents such as whipped toppings, desserts, and cakes (Voudouris et al., 2017).

4. Conclusions

The BSFL reared on canteen waste represent a good source of fat and proteins. A defatting step was necessary to improve protein extraction. Ultrafiltration proved to be a more efficient extraction method of larval protein than isoelectric precipitation. All essential amino acids required in a healthy diet were present in the BSFL proteins, and their sum was higher than the recommendation of FAO/WHO/UNU for human requirements.

The BSFL protein fraction showed good oil holding capacity making them suitable for food applications such meats, meats, sausages, cakes, and bakeries.Ultrafiltration improved not only the protein purity but also the foaming capacity and stability of proteins providing valuable sources of foaming agents for food formulations like whipped toppings, desserts, and cakes. Therefore, the proteins obtained in the present study displayed high protein purity, good nutritional values, oil holding capacity, foaming capacity and stability that can be considered valuable sources of protein ingredients in food systems. These results might help improve consumer acceptance, which is critical for the development of alternative and sustainable ingredients for the food industry.

CRediT authorship contribution statement

Author Contributions: conceptualization, LUM, GM; methodology, LUM, GB; validation, GM; formal analysis, LUM, LIM; investigation, LUM, GM, GB; data curation, LUM, GM; writing—original draft preparation, LUM; writing—review and editing, GM, MT; supervision, LM, AA, MT, project administration, AA; funding acquisition, GB, LM, AA, MT. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Acknowledgments

Results incorporated in this publication originate from the SCALIBUR project, which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 817788.

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