ELSEVIER

Contents lists available at ScienceDirect

### LWT



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

# Salt-soluble protein extracts from *Hermetia illucens* and *Bombyx mori* for high protein pasta production

Alyssa Hidalgo<sup>a</sup>, Marco Cullere<sup>b</sup>, Antonella Dalle Zotte<sup>b</sup>, Gabriella Pasini<sup>c,\*</sup>

<sup>a</sup> Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy

<sup>b</sup> Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Viale dell Università 16, Padova, 35020 Legnaro, Italy

<sup>c</sup> Department of Animal Medicine, Production and Health, University of Padova, Agripolis, Viale dell'Università 16, Padova, 35020 Legnaro, Italy

#### ARTICLE INFO

Keywords: Black soldier Insect powder Novel food Pasta fortification Silkworm

#### ABSTRACT

Black soldier fly (*Hermetia illucens, HI*) and silkworm (*Bombyx mori, BM*) are two promising insect species for possible novel food applications, currently undergoing safety evaluation by European Food Safety Authority (EFSA). Aim of this research was to extract a protein fraction from defatted insect powders using phosphatebuffered saline and to manufacture pasta by replacing 15% semolina with salt-soluble insect protein extract, in order to achieve the claim "high protein". Pasta samples were then analysed to evaluate their technological quality. The high-protein pastas showed darker colour ( $L^*$ : 52.5 < 58.4 < 72.3, for HI, BM, and CP, respectively; p < 0.05), higher fracturability (5.68 and 5.49 > 7.06 N; p < 0.05) and water absorption index (171 and 172 > 149%; p < 0.05) than control pasta. However, pasta firmness and adhesiveness remained unchanged. Black soldier fly and silkworm provide protein extracts suitable for the production of high protein pasta with good technological quality; furthermore, they might have interesting potential for other food applications.

#### CRediT authorship contribution statement

Hidalgo Alyssa: Supervision, Data curation, Writing – review & editing; Cullere Marco: Formal analysis, Visualization; Dalle Zotte Antonella: Formal analysis, Visualization; Pasini Gabriella: Conceptualization, Supervision, Data curation, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition

#### 1. Introduction

World population is projected to reach 9.7 billion people in 2050 (UN - United Nations, 2019). The augmenting demand for food, especially animal-based proteins, is unlikely to be met unless research, innovation and technological development sustain expanding agricultural and zootechnical productions. Plant-based sources are lacking some essential amino acids and B vitamins, and are less digestible than animal proteins (Sarwar, 1997). On the other hand, increasing animal protein production of mainstream livestock species is energy-inefficient and requires vast amounts of inputs (El Hajj et al., 2022). There are clear environmental and economic benefits if traditional sources of animal proteins are replaced by others that require less feed, produce less waste

and result in fewer greenhouse gas emissions (El Hajj et al., 2022). Recently, insects have been considered a viable alternative to meet future food needs and satisfy nutritional requirements. Over 2000 insect species are edible (Van Itterbeeck & Pelozuelo, 2022) and many are consumed in different areas of the world (Baiano, 2020). Insects have a high protein content (on average, around 40–50% based on dry matter), which meets the WHO essential amino acid content requirements (Gravel & Doyen, 2020), and are a good source of fatty acids, microelements, fibre and bioactive compounds such as tocopherols and polyphenols (Nino, Reddivari, Osorio, Kaplan, & Liceaga, 2021; Son, Choi, Hwang, Nho, & Kim, 2020). Furthermore, insects can be fed with agro-industry by-products and farmed in small surfaces (Mancini, Moruzzo, Riccioli, & Paci, 2019), making them ideal candidates for the circular economy concept and competitive to traditional animal-derived foods.

Recognising the importance of increasing and diversifying food sources and also with the aim of counteracting global warming, the European community has recently approved the commercialisation as novel foods of three insects species: *Tenebrio molitor* (yellow mealworm; Regulation EU 2021/882; EU, 2021a), *Locusta migratoria* (grasshopper; Regulation EU 2021/1975; EU, 2021b) and *Acheta domesticus* (house

https://doi.org/10.1016/j.lwt.2023.115507

Received 24 July 2023; Received in revised form 3 November 2023; Accepted 5 November 2023 Available online 11 November 2023 0023-6438/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).



<sup>\*</sup> Corresponding author. Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Viale dell'Università 16, Padova, 35020 Legnaro, Italy.

E-mail address: gabriella.pasini@unipd.it (G. Pasini).

cricket; Regulation EU 2022/188; EU, 2022). Currently, other insects are undergoing safety evaluation by EFSA, including black soldier fly (*Hermetia illucens*) and silkworm (*Bombyx mori*), two promising species for possible novel food applications (Regulation EU 2015/2283; EU, 2015).

Nevertheless, the consumption of whole insects at present is hardly foreseeable for European consumers, due to low consumer acceptance, with food neophobia and disgust being the main constraints (Onwezen, Bouwman, Reinders, & Dagevos, 2021). A smoother approach would be through the utilisation of insect powders in foods. Recently, there has been growing research into the possible application of flour, paste, and protein extracts obtained from different insect species into food preparation, with the focus on technological properties and composition of the final products. For example, A. domesticus or T. molitor flours were added to bread wheat flour to produce breads with acceptable technological characteristics and improved nutritional profile (Cappelli, Oliva, Bonaccorsi, Lorini, & Cini, 2020; González, Garzón, & Rosell, 2019; Kowalski, Mikulec, Mickowska, Skotnicka, & Mazurek, 2022; Osimani et al., 2018). As for the sensory traits of fortified breads, literature results show variation depending on insect species and inclusion level (Osimani et al., 2018; Roncolini et al., 2019). Similarly, black soldier fly prepupae were used to fortified wheat flour to make bread, achieving satisfactory results in terms of rheological and physicochemical characteristics (Montevecchi, Licciardello, Masino, Miron, & Antonelli, 2021), and protein-enriched brown rice cake was obtained by adding Bombay locust powder (Indriani et al., 2020).

Pasta is a popular staple food, particularly suitable for the incorporation of novel ingredients. Recent studies have successfully fortified durum wheat pasta with up to 15% house cricket powder (Duda, Adamczak, Chełminska, Juszkiewicz, & Kowalczewski, 2019). Incorporating 15% grasshopper or yellow mealworm into egg pasta revealed an increase in the nutritional profile (protein, ash and crude fibre content), but harmed cooking performance and overall sensory acceptability (Çabuk & Yilmaz, 2020). More recently, pasta fortification with silkworm pupae flour was proposed to increase the protein daily intake of the consumers (Piazza, Ratti, Girotto, & Cappellozza 2023) while buckwheat pasta enriched with silkworm powder resulted in a suitable, even if less liked, end product (Biró, Fodor, Szedljak, Pásztor-Huszár, & Gerea, 2019). Likewise, protein extract from house cricket (Acheta domesticus) and yellow mealworm (Tenebrio molitor) offer interesting opportunity for pasta formulations, leading to an increased protein content and protein quality (Pasini, Cullere, Vegro, Simonato, & Dalle Zotte, 2022).

Despite the potential commercial relevance, published research about pasta fortification with insects or insect protein extract is still limited, in particular when considering that the use of insect protein extracts may need to minimize the presence of chitin, an exoskeleton indigestible component, and fat, sensitive to rancidity phenomena (Ahmad, Ur-Rehman, Shabbir, Shehzad, & Roberts, 2018; Piazza et al., 2023).

Therefore, this research aimed to i) obtain the powder protein fraction from *Hermetia illucens* and *Bombyx mori*, ii) prepare "high protein" pasta samples with protein powder; iii) evaluate the impact of replacing 15% semolina with salt-soluble insect protein powders on selected technological quality traits of dry pasta.

#### 2. Material and methods

#### 2.1. Raw materials

Wheat semolina (De Cecco, Italia) for pasta production was purchased in a local supermarket. Its composition, as reported on the label, was: total carbohydrates, 680 g/kg; proteins, 140 g/kg; total fat, 15 g/ kg; total dietary fibre, 29 g/kg; 14,520 kJ/kg energy.

Partially defatted black soldier fly larvae (*Hermetia illucens*) powder was purchased from a leading European company specialised in insects as nutritional source (Protix, Dongen, The Netherlands). Further specification about it can be found in the work by Cullere et al. (2016). The defatted silkworm (*Bombyx mori*) powder was provided by the Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

#### 2.2. Protein extraction

The protein from the insect powders was extracted in a 1:8 g/mL phosphate-buffered saline solution (PBS; 0.01 mol/L Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub>, 0.015 mol/L NaCl, pH 8.0) by continuous stirring at 30 °C for 48 h. The samples were then centrifuged at 20,000 g for 30 min, the supernatants were collected by vacuum filtration (GF/A – 90 mm, Whatman; Marlborough, MA, USA), and freeze-dried (Freeze Dryer Modulyo®, Edwards, Milan, Italy) to powder.

#### 2.3. Preparation of pasta samples

The pasta samples were made using a professional pasta-making machine (Lillodue, Bottene, Schio, Italy). The enriched pastas were prepared by replacing semolina with 15% salt-soluble protein extract, necessary to reach the claim "high protein" pasta because Regulation EU 1924/2006 (EU, 2006) states that at least 20% of the energy value of the food must be provided by protein. The control sample was prepared using only wheat semolina. The flour/enriched flour samples were added with water (350 g/kg) at 40 °C, kneaded for 10 min and extruded into a "*tagliatelle*" shape, 5.0 cm long and 3 mm thick. Finally, the freshly extruded pasta samples were air-dried at 50 °C in a Jouan® dryer (Thermo Fisher Scientific, Waltham, USA) for about 12 h to reach a final moisture content of 125 g/kg, as required by Italian law (n.187; (D.P.R. – Presidenza della Repubblica, 2001).

For each sample, three pasta batches were obtained: 1) CP: wheat control pasta, 2) HI: pasta formulated with 15% *Hermetia illucens* salt-soluble protein extract, and 3) BM: pasta formulated with 15% *Bombyx mori* salt-soluble protein extract. Exclusively for sensory tests (visual and smell perception), two additional enriched pasta samples were manufactured 4) pHI: pasta formulated with 15% *Hermetia illucens* flour, and 5) pBM: pasta formulated with 15% *Bombyx mori* flour.

#### 2.4. Proximate composition of insect powders and pasta samples

Analyses of the insect powders were conducted in triplicate following the AOAC (AOAC - Association of Official Analytical Chemists, 2000) procedures to determine the dry matter (method no. 934.01), crude protein (method no. 2001.11) using 6.25 as nitrogen-to-protein conversion factor, crude fibre (method no. 978.10), and ash (method no. 967.05) contents. The ether extract was determined after acid hydrolysis (European Community, 1998).

The chemical composition of pasta samples was performed according to official methods of the American Association of Cereal Chemists (AACC - American Association of Cereal Chemists, 2000) for moisture, protein, total fiber, fat and ash. Total carbohydrates were computed by difference. All determinations were carried out in triplicate.

The energy value in the pasta samples was calculated with the following formula:

Energy value (kJ/g) = 16.74×g protein +16.74×g carbohydrates + 37.66×g fat + 8.37×g fibre.

#### 2.5. Protein content and extraction efficiency of the protein extracts

Total protein was determined as indicated in 2.4. The extraction efficiency was calculated on starting protein basis by the following equation:

Extraction efficiency % = [total extracted protein (g)/total protein content (g)] \* 100.

# 2.6. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The molecular weight (kDa) distribution of the insect protein extract was outlined by SDS-PAGE using 8–16% Mini-PROTEAN® TGX Stain-Free Gel (Bio Rad, Hercules, CA, USA). The samples were dissolved in 0.5 mol/L Tris–HCl Laemmli buffer (Sigma Aldrich, St. Louis, MO, USA) pH 6.8, containing 150 g/L glycerol, 15 g/L SDS and 80 mL/L 2-mercaptoethanol, in ratio 3:1 (v:v), heated at 100 °C in a water bath for 5 min and centrifuged for 3 min at 10.000 g. 30  $\mu$ L of the sample was applied to the gel and the electrophoresis was conducted at a constant current of 48 mA. The molecular weight standard proteins were the Pre-Stained Broad Range Molecular Weight Markers (Bio-Rad). Finally, gels were stained with 0.5 g/L Coomassie Brilliant Blue R-250 (Bio Rad), 50 g/L TCA, 170 mL/L methanol, and 60 mL/L acetic acid, and de-stained in 70 mL/L acetic acid.

#### 2.7. Standard pasta quality parameters

*Colour*. The colour of the raw and cooked pasta was analysed according to the CIELAB system (CIE - Commission Internationale de l'Éclairage, 1976) using a CR-300 colorimeter (Konica Minolta, Tokyo, Japan), taking  $L^*$  value (lightness),  $a^*$  value (redness), and  $b^*$  value (yellowness) at three different points on the sample surface. Each colour value represents the mean of five different measurements. Moreover, to assess colour changes in HI and BM samples compared to the control sample (CP), the following equation was applied:

 $\Delta E \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}$ 

where:  $\Delta E = Total \ colour \ difference$ 

- $\Delta L^* = L^* \text{ control} L^* \text{ treatment}$
- $\Delta a^* = a^* \text{ control} a^* \text{ treatment}$
- $\Delta b^* = b^* \text{ control} b^* \text{ treatment}$

*Pasta cooking quality*. Ten grams of pasta were cooked in 100 mL boiling water. The pasta optimum cooking time (OCT) and cooking loss (CL) were determined accordingly the AACC (AACC - American Association of Cereal Chemists, 2000) methods 44-15 A and 66–50, respectively. Water absorption index was expressed as % increase of pasta weight after cooking.

*Texture Analyses*. Firmness and adhesiveness in cooked pasta were analysed using a TA.XTplus Texture Analyser (Stable Micro System, Ltd., Godalming, UK) equipped with a 490 N load cell. Five cooked and drained pasta stripes of 5 cm length were positioned next to each other on the texture analyser platform. A rectangular probe (35 mm × 50 mm) was used. The test parameters were 1 mm/s test speed and 90% deformation. Firmness was calculated as the maximum force peak required to compress the pasta sample. Adhesiveness was calculated as the maximum peak force curve to separate the probe from the sample's surface. The fracturability of uncooked samples was determined as the maximum force required to break of the samples when was compressed at 1 mm/s test speed with a rectangular probe (35 mm × 50 mm) set at 12 mm width. All the results are reported as the mean value of 8 replicates.

#### 2.8. Visual and smell perception

To determine the consumer's perception, visual and smell evaluations were conducted. Cooked control pasta (CP), pasta with *Hermetia illucens* protein extract (HI) and pasta with *Bombyx mori* protein extract (BM) were tested, along with two additional pasta samples formulated with insect powder (pHI and pBM), added to evaluate any difference with those prepared from insect protein extract. Panelists (n = 20) were given approximately 10 g each of the five cooked samples. The samples were randomly numbered. The panelists were asked to indicate the

liking degree of each sample using the symbols:



#### 2.9. Statistical analysis

One-way analysis of variance (ANOVA) was performed independently for each dependent variable. A post-hoc Tukey's HSD multiple comparison test was used to identify statistically homogeneous subsets at  $\alpha = 0.05$ . The statistical analysis was performed with CoStat Software 6.45 (Minitab Inc., State College, Pennsylvania, USA).

#### 3. Results and discussion

#### 3.1. Chemical composition of insect powder

The partially defatted insect powders showed a high amount of protein (464 and 617 g/kg, for HI and BM, respectively) with a residual amount of lipids (139 and 50 g/kg, respectively; Table 1). Although the chemical composition of insect powder varies depending on insect species, stage of development, environmental conditions and feed (Kouřimská & Adámková, 2016), the protein values were higher and the lipid contents were lower than those reported by other authors in whole insects (Brogan, Park, Matak, & Jaczynski, 2021; González et al., 2019; Montevecchi et al., 2021; Pan et al., 2022), because defatting increases protein content by eliminating one of the constituent (Choi, Wong, & Auh, 2017). This phenomenon was already reported for insects (Bubler, Rumpold, Jander, Rawel, & Schluter, 2016; Yi et al., 2013) as well as for seeds and legumes (Choi et al., 2017; Lam et al., 2018).

#### 3.2. Salt-soluble protein extracts and electrophoretic profile

The extraction efficiency of PBS soluble proteins revealed equivalent results for HI and BM (67.6% and 68.2% respectively) (Table 1), higher than that obtained by Chatsuwan, Nalinanon, Puechkamut, Lamsal, and Pinsirodom (2018) in protein extracts of two types of grasshoppers.

The protein content in freeze-dried extracts was 314 g/kg and 421 g/kg for HI and BM, respectively (Table 1), similar to that obtained by Piazza et al. (2023) in a silkworm flour aqueous extract.

Commonly used protein extraction methods (salt solution, solvent, detergent, alkali) may result in different extraction efficiency due to protein degradation, which is influenced by extraction time, solvents, pH and temperature (Pan et al., 2022). The protein content in the powders, lower than in the defatted insect flours, can be mostly attributable to salts derived from the saline PBS buffer used for extraction; to obtain higher protein concentration, dialysis or protein precipitation should be applied. It is often difficult to compare samples from different studies, however our results show that salt-water solubilisation can be used for proteins separation.

#### Table 1

Proximate composition (g/kg; mean  $\pm$  standard deviation; n = 3) of partially defatted *Hermetia illucens* (HI) and *Bombyx mori* (BM) powders; protein concentration (g/kg) and extraction efficiency (%) of phosphate-buffered saline (PBS)-soluble extract from powder of HI or BM.

Insect species	HI	BM
Dry matter	$946 \pm 0.2$	$947\pm0.2$
Lipid	$139\pm3.0$	$50\pm4.0$
Ash	$69\pm0.3$	$63\pm0.1$
Protein	$464\pm5.0$	$617\pm4.0$
Protein in PBS powder extract	$314 \pm 27.0$	$421 \pm 29.0$
Extraction efficiency	$67.6\pm4.2$	$68.2 \pm 4.9$

The electrophoretic profile and molecular weight distribution of the insect protein fractions are shown in Fig. 1. The gels reveal different intensity proteins bands, more evident in BM, confirming the higher protein content in BM salt-soluble protein powder than in that of HI (Table 1). In any case, the protein mobility was less than 75 kDa for all samples. The stong bands between 10 and 15 kDa were clearly visible both for HI and BM samples, as reported by other authors (Bubler et al., 2016; Janssen, Vincken, Arts, Fogliano, & Lakemond, 2019). Yi et al. (2013) and Nebbia et al. (2019) found proteins bands at ~ 12 and ~15 k Da in *Tenebrio molitor*, which should correspond to haemolymph proteins and cockroach allergen-like protein, respectively.

The bands ranging from 25 to 30 kDa visible in BM, already reported by Brogan et al. (2021), were hypothesized to be cuticle proteins that make up the exoskeleton of insects (Andersen, Hojrup, & Roepstorff, 1995), e.g. chymotrypsin-like protein (24 kDa) (Elpidina et al., 2005). Another band observed at around 70 kDa could belong to hemocyanin



Fig. 1. Electrophoretic profiles and molecular weight (kDa) distribution of insect protein fractions from *Bombyx mori* (BM) and *Hermetia illucens* (HI).

(75 kDa), as reported by Brogan et al. (2021). This band, found in BM, was also prevalent in the HI sample.

#### 3.3. Chemical composition of pasta

The chemical composition and energy values of pasta samples are reported in Table 2. The addition of 15% of insect extracts increased the protein content of pasta from 137 g/kg (CP) to 165 g/kg (HI) or 182 g/kg (BM) (Table 3) allowing the "high protein" claim. The enriched pastas showed also higher ash contents than the control (88 g/kg and 69 g/kg, respectively vs 8 g/kg), arising from the residual PBS buffer.

#### 3.4. Colorimetric profile, cooking quality and texture analysis of pasta

Pasta colour represents a reference quality attribute for the consumers (Costell, Tárrega, & Bayarri, 2010). The addition of insect proteins extracts from HI and BM changed the colour of pasta samples (Fig. 2; Table 3). Specifically, the protein extracts used to fortify pasta led to a significant decrease in brightness (L\* value) and yellowness (b\* value), and a significant redness increase in both raw and cooked pasta  $(a^* \text{ value})$  when compared to CP, as also reported by others author (Duda et al., 2019; Piazza et al., 2023). The darkening of pasta samples can be attributed to the pigments naturally present in the insect powder (Piazza et al., 2023) or to enzymatic browning reactions (Yi et al., 2013). The low temperature applied in this research during pasta drying should have prevented the formation of Maillard reaction products (Anese, Nicoli, Massini, & Lerici, 1999; Brandolini, Lucisano, Mariotti, & Hidalgo, 2018). The total colour difference ( $\Delta E$  values) was high and clearly perceivable to the naked eye ( $\Delta E \ge 3.0$ ; Bellary, Indiramma, Prakash, Baskaran, & Rastogi, 2016).

Pasta texture, in particular fracturability of raw product and firmness and adhesiveness of cooked product, is a quality parameter highly appreciated by the consumers. Raw pasta fracturability is useful to predict pasta behaviour during transport and storage: in fact, a high mechanical strength can reduce breakage during handling. Addition of insect protein extract to pasta samples determined some changes in this parameter (Table 3). The lowest fracturability values were registered for the BM and HI pasta (5.49  $\pm$  0.98 and 5.68  $\pm$  0.98 N respectively), while the CP pasta presented the highest value (7.06 N), significantly different from the others. Enriching wheat pasta with 50, 100, and 200 g/kg spirulina biomass powder reduced fracturability values (Rodríguez De Marco, Steffolani, Martínez, & León, 2014), while a marked matrix weakening was detected with silkworm protein extract addition (Piazza et al., 2023). A fracturability (6.96 N) similar to that of the CP pasta was observed in raw gluten-free pasta (Betrouche et al., 2022); the addition of 150 g/kg tomato pomace produced a fracturability decrease (6.28 N), while the addition of 100 g/kg linseed meal increased it (7.45 N).

The results in Table 3 show that at optimum cooking time the water absorbed by HI and BM pastas was significantly higher than that absorbed by the CP. This result is probably attributable to the water holding capacity of insect proteins which form a structured gel, as

#### Table 2

Chemical composition (g/kg; mean  $\pm$  standard deviation; n = 3) of control pasta (CP), pasta enriched with 15% salt-soluble protein extracts of *Hermetia illucens* (HI) or *Bombyx mori* (BM).

	CP	HI	BM
Moisture	$125\pm1.0$ $^{a}$	$124\pm1.0$ $^{a}$	$124 \pm 1.0$ $^a$
Protein	$137 \pm 1.0^{\rm c}$	$165\pm6.0^{\rm b}$	$182\pm9.0^{a}$
Lipid	$14\pm2.0~^{a}$	$11\pm 6.0$ <sup>b</sup>	$12\pm2.0$ $^{ m b}$
Carbohydrates	690	590	590
Total fiber	$26\pm3.0~^a$	$24\pm9.0~^a$	$25\pm 6.0\ ^{a}$
Ash	$8\pm1.0^{c}$	$88\pm1.0^{\rm a}$	$69\pm1.0^{\mathrm{b}}$
Energy value (kJ/kg)	14560.3	13221.4	13556.2

Values in a row with different superscript letter are significantly different ( $p \le 0.05$ ).

#### Table 3

Technological characteristics (mean  $\pm$  standard deviation; n=3) of raw and cooked control pasta (CP), pasta enriched with 15% salt-soluble protein powders of *Hermetia illucens* (HI) or *Bombyx mori* (BM).

	СР	ні	BM
Raw pasta:			
L*	$76.7 \pm 0.6^{a}$	$63.9\pm0.8^{\rm b}$	$64.5\pm0.2^{b}$
<b>a</b> *	$1.51\pm0.1^{\rm c}$	$9.3\pm0.4^{a}$	$8.1\pm0.5^{\rm b}$
<b>b</b> *	$29.4 \pm 0.4^a$	$21.6\pm0.9^{\rm c}$	$24.2\pm0.6^{\rm b}$
ΔΕ	0	16.9	14.6
Fracturability (N)	$\textbf{7.06} \pm \textbf{0.98}^{a}$	$5.68 \pm 0.98^{\rm b}$	$5.49 \pm 0.98^{\mathrm{b}}$
Cooked pasta:			
L*	$72.3\pm0.2^{\rm a}$	$52.5 \pm 1.1^{\rm c}$	$58.4 \pm 1.5^{\rm b}$
<b>a</b> *	$3.1\pm0.1^{ m b}$	$10.5\pm0.6^{a}$	$11.7\pm0.3^{\rm a}$
<b>b</b> *	$22.2 \pm 0.2^a$	$6.3\pm0.5^{\rm c}$	$8.1\pm0.5^{\rm b}$
ΔΕ	0	26.4	22.4
Optimal cooking time	$11.8\pm0.5^{a}$	$12.7\pm0.5^{a}$	$12.5\pm0.5^{a}$
(min) Water absorption index (%)	$148.8\pm2.3^{b}$	$171.0\pm3.7^{a}$	$172\pm2.8^{a}$
Cooking loss (%)	$4.1\pm0.6^{\rm b}$	$7.2\pm0.2^{\rm a}$	$6.7\pm0.3^{\rm a}$
Firmness (N)	$131.4\pm3.9^{a}$	$138.3\pm7.8^{\text{a}}$	$132.4\pm5.9^{a}$
Adhesiveness (N/s)	$-0.098~\pm$	$-0.098~\pm$	$-0.196~\pm$
	$0.020^{a}$	0.029 <sup>a</sup>	$0.010^{a}$

Values in a row with different superscript letter are significantly different ( $p \le 0.05$ ).

reported by Kumar et al. (2022) for *Hermetia illucens* protein extract, attributable to the hydrophilic amino acid residues. Similarly, Piazza et al. (2023) reported an imbibition value in pasta with silkworm protein extract on average 25% higher than the control one. The well-defined gel, simulating a protein network able to retain water during heating, is also evident in pasta enriched with protein extract from *Acheta domesticus* (Pasini et al., 2022). Moreover, heat is important in gel formation since it promotes protein denaturation (Gravel & Doyen, 2020), which guarantees firmness and adhesiveness, a phenomenon observed by other authors when evaluating the gelation conditions in protein fraction from edible insects (Yi et al., 2013). The texture characteristics of the cooked pastas were similar in all samples and no significant differences were found for firmness and adhesiveness. Nevertheless, the

gluten network dilution led to a significant cooking loss increase in HI and BM enriched-pastas compared to that observed for CP (Table 3), in agreement with the results obtained using insect protein extracts (Pasini et al., 2022; Piazza et al., 2023), spirulina biomass (Rodríguez De Marco et al., 2014) or protein additives of plant origin (Kaur, Sharma, Nagi, & Ranote, 2013).

#### 3.5. Visual and smell perception

*Hermetia illucens* and *Bombyx mori* are undergoing safety evaluation by EFSA; as such, a sensory analysis that includes tasting is still not possible. Nevertheless, it was interesting to visually compare pasta enriched with insect flour and pasta enriched with insect protein extract, considering that both these products could interest the food industry. The panelists, through the visual colour analysis, declared the same appreciation for all the samples, despite a significant difference from CP, and indicated that their colour was similar to that of whole wheat pasta. The panelists reported a better smell perception for pasta enriched with protein extract rather than with insect powder; however, the control pasta was always the most appreciated (Fig. 3). It has to be remembered that previous researches reported the odour as critical point when raw insect flour was used in pasta product (Biró et al., 2019; Çabuk & Yilmaz, 2020).

#### 4. Conclusions

Salt soluble protein extracts from defatted HI and BM insect powders could be used to partially replace wheat flour in pasta making, improving protein content and allowing the "high protein" claim. Hence, the enriched pasta had a higher nutritional value than the traditional product because of the higher protein and lower carbohydrate contents. The texture of insect protein-made pasta was satisfactory in terms of firmness and adhesiveness, despite greater cooking loss than the control pasta. The sensory test revealed a similar visual acceptability for all pasta samples and an acceptable smell of the protein extract enriched pastas, which was significantly better than that of the insect powders enriched pastas. However, further sensory evaluations



Fig. 2. Colour of raw and cooked control pasta (CP) as well as of pastas with 15% *Hermetia illucens* (HI) or *Bombyx mori* (BM) salt-soluble protein extracts. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### Visual appearance



## Smell perception

Fig. 3. Visual appearance and smell perception of (from left to right) control pasta (CP), pasta with 15% Hermetia illucens (pHI) or Bombyx (not Bombix) mori (pBM) powders, Hermetia illucens (HI) or Bombyx (not Bombix) mori (BM) salt-soluble protein extracts.

including a taste test performed by a larger panel of consumers should be performed.

#### 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

Data will be made available on request.

#### Acknowledgments

This research was financially supported by Project BIRD203914 of the University of Padova. The authors thank Mara Vegro to technical assistance and Attawit Kovitvadhi (Kasetsart University, Bangkok, Thailand) for providing the silkworm meal.

#### References

- AACC American Association of Cereal Chemists. (2000). Approved methods of the AACC (10th ed.). St. Paul, MN, USA: American Association of Cereal Chemists.
- Ahmad, N., Ur-Rehman, S., Shabbir, M. A., Shehzad, M. A., & Roberts, T. H. (2018). Fortification of durum wheat semolina with detoxified matri (*Lathyrus sativus*) flour to improve the nutritional properties of pasta. *Journal of Food Science and Technology*, 55(6), 2114–2121.
- Andersen, S., Hojrup, P., & Roepstorff, P. (1995). Insect cuticular proteins. Insect Biochemistry and Molecular Biology, 25, 153–176. https://doi.org/10.1016/0965-1748(94)00052-J
- Anese, M., Nicoli, M. C., Massini, R., & Lerici, C. R. (1999). Effects of drying processing on the Maillard reaction in pasta. *Food Research International*, 32(3), 193–199.
  AOAC - Association of Official Analytical Chemists. (2000). Official methods of analysis
- (17th ed.). Gaithersburg, MD, USA: The Association of Official Analytical Chemists. Baiano, A. (2020). Edible insects: An overview on nutritional characteristics, safety,
- farming, production technologies, regulatory framework, and socio-economic, and ethical implications. *Trends in Food Science & Technology*, 100, 35–50.
- Bellary, A. N., Indiramma, A. R., Prakash, M., Baskaran, R., & Rastogi, N. K. (2016). Anthocyanin infused watermelon rind and its stability during storage. *Innovative*

Food Science & Emerging Technologies, 33, 554–562. https://doi.org/10.1016/j. ifset.2015.10.010

- Betrouche, A., Estivi, L., Colombo, D., Pasini, G., Benatallah, L., Brandolini, A., et al. (2022). Antioxidant properties of gluten-free pasta enriched with vegetable by-products. *Molecules*, *27*, 8993. https://doi.org/10.3390/molecules27248993
  Biró, B., Fodor, R., Szedljak, I., Pásztor-Huszár, K., & Gerea, A. (2019). Buckwheat-pasta
- Biró, B., Fodor, R., Szedljak, I., Pásztor-Huszár, K., & Gerea, A. (2019). Buckwheat-pasta enriched with silkworm powder: Technological analysis and sensory evaluation. *LWT - Food Science and Technology*, 116, Article 108542. https://doi.org/10.1016/j. Iwt.2019.108542
- Brandolini, A., Lucisano, M., Mariotti, M., & Hidalgo, A. (2018). A study on the quality of einkorn (*Triticum monococcum* L. ssp. *monococcum*) pasta. *Journal of Cereal Science*, 82, 57–64. https://doi.org/10.1016/j.jcs.2018.05.010
- Brogan, E. N., Park, Y.-L., Matak, K. E., & Jaczynski, J. (2021). Characterization of protein in cricket (Acheta domesticus), locust (Locusta migratoria), and silk worm pupae (Bombyx mori) insect powders. LWT - Food Science and Technology, 152, Article 112314. https://doi.org/10.1016/j.lwt.2021.112314
- Bubler, S., Rumpold, B., Jander, E., Rawel, H., & Schluter, O. (2016). Recovery and techno functionality of flours and proteins from two edible insect species: Meal worm (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) larvae. *Heliyon*, 2, Article e00218. https://doi.org/10.1016/j.heliyon.2016.e00218
- Çabuk, B., & Yilmaz, B. (2020). Fortification of traditional egg pasta (erişte) with edible insects: Nutritional quality, cooking properties and sensory characteristics evaluation. *Journal of Food Science and Technology*, 57, 2750–2757. https://doi.org/ 10.1007/s13197-020-04315-7
- Cappelli, A., Oliva, N., Bonaccorsi, G., Lorini, C., & Cini, E. (2020). Assessment of the rheological properties and bread characteristics obtained by innovative protein sources (*Cicer arietinum, Acheta domesticus, Tenebrio molitor*): Novel food or potential improvers for wheat flour? *LWT - Food Science and Technology, 118*, Article 108867. https://doi.org/10.1016/j.lwt.2019.108867
- Chatsuwan, N., Nalinanon, S., Puechkamut, Y., Lamsal, B. P., & Pinsirodom, P. (2018). Characteristics, functional properties, and antioxidant activities of water-soluble proteins extracted from grasshoppers, *Patanga succincta* and *Chondracris roseapbrunner. Journal of Chemistry*. , Article 6528312. https://doi.org/10.1155/ 2018/6528312
- Choi, B. D., Wong, N. A. K., & Auh, J. H. (2017). Defatting and sonication enhances protein extraction from edible insects. *Korean Journal for Food Science of Animal Resources*, 37, 955–961. https://doi.org/10.5851/kosfa.2017.37.6.955
- CIE Commission Internationale de l'Éclairage. (1976). Official recommendations on uniform colour space, colour difference equations and metric colour terms, 15. Paris, France: CIE Publication No. https://doi.org/10.1002/j.1520-6378.1977.tb00102.x. E-1.3.1), Suppl. 2; Bureau Central de la CIE.
- Costell, E., Tárrega, A., & Bayarri, S. (2010). Food acceptance: The role of consumer perception and attitudes. *Chemosensory Perception*, 3, 42–50. https://doi.org/ 10.1007/s12078-009-9057-1
- Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S., et al. (2016). Black soldier fly as dietary protein source for broiler quails: Apparent

#### A. Hidalgo et al.

digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *Animal*, *10*, 1923–1930. https://doi.org/10.1017/S1751731116001270

- D.P.R. Presidenza della Repubblica. (2001). Decreto del Presidente della Repubblica 9 febbraio 2001, n. 187. Regolamento per la revisione della normativa sulla produzione e commercializzazione di sfarinati e paste alimentari, a norma dell'articolo 50 della legge 22 febbraio 1994, n. 146, 117. Gazzetta Ufficiale. http://www.gazzettaufficiale.biz /atti/2001/20010117/00160242.htm.
- Duda, A., Adamczak, J., Chełminska, P., Juszkiewicz, J., & Kowalczewski, P. (2019). Quality and nutritional/textural properties of durum wheat pasta enriched with cricket powder. *Foods, 8*, 46. https://doi.org/10.3390/foods8020046
- El Hajj, R., Mhemdi, H., Besombes, C., Allaf, K., Lefrançois, V., & Vorobiev, E. (2022). Edible insects' transformation for feed and food uses: An overview of current insights and future developments in the field. *Processes*, 10, 970. https://doi.org/10.3390/ pr10050970
- Elpidina, E. N., Tsybina, T. A., Dunaevsky, Y. E., Belozersky, M. A., Zhuzhikov, D. P., & Oppert, B. A. (2005). Chymotrypsin-like proteinase from the midgut of *Tenebrio molitor* larvae. *Biochimie*, *87*, 771–779. https://doi.org/10.1016/j. biochi.2005.02.013
- EU. (2006). Regulation (EU) 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union, L404, 9–25. https://faolex.fao.org/docs/pdf/eur68321.pdf.
- EU. (2015). Regulation (EU) 2015/2283 of the European parliament and of the council of 25 november 2015 on novel foods, amending regulation (EU) No 1169/2011 of the European parliament and of the council and repealing regulation (EC) No 258/97 of the European parliament and of the council and commission regulation (EC) No 1852/2001. Official Journal of the European Union, L327, 1–21. https://eur-lex.eur opa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R2283#:~:text=1.,human %20health%20and%20consumers'%20interests.
- EU. (2021a). Commission implementing Regulation (EU) 2021/882 of 1 June 2021 authorising the placing on the market of dried Tenebrio molitor larva as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470, L194 pp. 16–21). Official Journal of the European Union. https://eur-lex.europa.eu/legal-content /EN/TXT/PDF/?uri=CELEX:32021R0882&from=IT.
- EU. (2021b). Commission implementing Regulation (EU) 2021/1975 of 12 November 2021 authorising the placing on the market of frozen, dried and powder forms of *Locusta migratoria* as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470. Official Journal of the European Union, L402, 10–16. https://eur-lex. europa.eu/legal-content/EN/TXT/PDF/2uri=CELEX:32021R1975.
- EU. (2022). Commission implementing Regulation (EU) 2022/188 of 10 February 2022 authorising the placing on the market of frozen, dried and powder forms of Acheta domesticus as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470. Official Journal of the European Union, L30, 108–114. https://eu r-lex.europa.eu/legal-content/EN/TXT/PDF/2uri=CELEX:32022R0188.
- EC European Community. (1998). Commission Directive 98/64/EC of 3 September 1998 establishing Community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feeding stuffs and amending Directive 71/393/EEC. Official Journal of the European Communities, L257, 14–28. https://op. europa.eu/en/publication-detail/-/publication/856db9b7-6f1d-4d6e-a778-c766 c9fa1776.
- González, C. M., Garzón, R., & Rosell, C. M. (2019). Insects as ingredients for bakery goods. A comparison study of H. illucens, A. domestica and T. molitor flours. Innovative Food Science & Emerging Technologies, 51, 205–210. https://doi.org/10.1016/j. ifset.2018.03.021
- Gravel, A., & Doyen, A. (2020). The use of edible insect proteins in food: Challenges and issues related to their functional properties. *Innovative Food Science & Emerging Technologies*, 59, Article 102272. https://doi.org/10.1016/j.ifset.2019.102272
- Indriani, S., Ab Karim, M. S. B., Nalinanon, S., & Karnjanapratum, S. (2020). Quality characteristics of protein-enriched brown rice flour and cake affected by Bombay locust (*Patanga succincta* L.) powder fortification. *LWT - Food Science and Technology*, 119, Article 108876. https://doi.org/10.1016/j.lwt.2019.108876
- Janssen, R. H., Vincken, J. P., Arts, N. J., Fogliano, V., & Lakemond, C. M. (2019). Effect of endogenous phenoloxidase on protein solubility and digestibility after processing of *Tenebrio molitor*, Alphitobius diaperinus and Hermetia illucens. Food Research International, 121, 684–690. https://doi.org/10.1016/j.foodres.2018.12.038
- Kaur, G., Sharma, S., Nagi, H. P. S., & Ranote, P. S. (2013). Enrichment of pasta with different plant proteins. *Journal of Food Science and Technology*, 50, 1000–1005. https://doi.org/10.1007/s13197-011-0404-2

- Kouřimská, L., & Adámková, A. (2016). Nutritional and sensory quality of edible insects. NFS Journal, 4, 22–26. https://doi.org/10.1016/j.nfs.2016.07.001
- Kowalski, S., Mikulec, A., Mickowska, B., Skotnicka, M., & Mazurek, A. (2022). Wheat bread supplementation with various edible insect flours. Influence of chemical composition on nutritional and technological aspects. *LWT - Food Science and Technology*, 159, Article 113220. https://doi.org/10.1016/j.lwt.2022.113220
- Kumar, S., Queiroz, L. S., Marie, R., Nascimento, L. G. L., Mohammadifar, M. A., de Carvalho, A. F., et al. (2022). Gelling properties of black soldier fly (Hermetia illucens) larvae protein after ultrasound treatment. *Food Chemistry*, 386, Article 132826. https://doi.org/10.1016/j.foodchem.2022.132826
- Lam, A. C. Y., Can Karaca, A., Tyler, R. T., & Nickerson, M. T. (2018). Pea protein isolates: Structure, extraction, and functionality. *Food Reviews International*, 34, 126–147. https://doi.org/10.1080/87559129.2016.1242135
- Mancini, S., Moruzzo, R., Riccioli, F., & Paci, G. (2019). European consumers' readiness to adopt insects as food. A review. Food Research International, 122, 661–678. https://doi.org/10.1016/j.foodres.2019.01.041
- Montevecchi, G., Licciardello, F., Masino, F., Miron, L. T., & Antonelli, A. (2021). Fortification of wheat flour with black soldier fly prepupae. Evaluation of technological and nutritional parameters of the intermediate doughs and final baked products. Innovative Food Science & Emerging Technologies, 69, Article 102666. https://doi.org/10.1016/j.ifset.2021.102666
- Nebbia, S., Lamberti, C., Giorgis, V., Giuffrida, M. G., Manfredi, M., Marengo, E., et al. (2019). The cockroach allergen-like protein is involved in primary respiratory and food allergy to yellow mealworm (*Tenebrio molitor*). *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology*, 49, 1379–1382. https://doi.org/10.1111/cea.13461
- Nino, M. C., Reddivari, L., Osorio, C., Kaplan, I., & Liceaga, A. M. (2021). Insects as a source of phenolic compounds and potential health benefits. *Journal of Insects as Food and Feed*, 7, 1077–1087. https://doi.org/10.3920/JIFF2020.0113
- Onwezen, M. C., Bouwman, E. P., Reinders, M. J., & Dagevos, H. (2021). A systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plantbased meat alternatives, and cultured meat. *Appetite*, 159, Article 105058. https:// doi.org/10.1016/j.appet.2020.105058
- Osimani, A., Milanović, V., Cardinali, F., Roncolini, A., Garofalo, C., Clementi, F., et al. (2018). Bread enriched with cricket powder (*Acheta domesticus*): A technological, microbiological and nutritional evaluation. *Innovative Food Science & Emerging Technologies*, 48, 150–163. https://doi.org/10.1016/j.ifset.2018.06.007
- Pan, J., Xu, H., Cheng, Y., Mintah, B. K., Dabbour, M., Yang, F., et al. (2022). Recent insight on edible insect protein: Extraction, functional properties, allergenicity, bioactivity, and applications. *Foods*, 11, 2931. https://doi.org/10.3390/ foods11192931
- Pasini, G., Cullere, M., Vegro, M., Simonato, B., & Dalle Zotte, A. (2022). Potentiality of protein fractions from the house cricket (*Acheta domesticus*) and yellow mealworm (*Tenebrio molitor*) for pasta formulation. *LWT - Food Science and Technology*, 164, Article 113638. https://doi.org/10.1016/j.lwt.2022.113638
- Piazza, L., Ratti, S., Girotto, F., & Cappellozza, S. (2023). Silkworm pupae derivatives as source of high value protein intended for pasta fortification. *Journal of Food Science*, 88(1), 341–355. https://doi.org/10.1111/1750-3841.16420
- Rodríguez De Marco, E., Steffolani, M. E., Martínez, C. S. L., & León, A. E. (2014). Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta. LWT - Food Science and Technology, 58, 102–108. https://doi.org/10.1016/j. lwt.2014.02.054
- Roncolini, A., Milanović, V., Cardinali, F., Osimani, A., Garofalo, C., Sabbatini, R., et al. (2019). Protein fortification with mealworm (*Tenebrio molitor L.*) powder: Effect on textural, microbiological, nutritional and sensory features of bread. *PLoS One, 14*, Article e0211747. https://doi.org/10.1371/journal.pone.0211747
- Sarwar, G. (1997). The protein digestibility-corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. *The Journal of Nutrition*, 127, 758–764.
- Son, Y. J., Choi, S. Y., Hwang, I. K., Nho, C. W., & Kim, S. H. (2020). Could defatted mealworm (*Tenebrio molitor*) and mealworm oil be used as food ingredients? *Foods*, 9, 40. https://doi.org/10.3390/foods9010040
- UN United Nations. (2019). World Population Prospects. Department of Economic and Social Affairs. https://population.un.org/wpp2019.
- Van Itterbeeck, J., & Pelozuelo, L. (2022). How many edible insect species are there? A not so simple question. *Diversity*, 14(2), 143. https://doi.org/10.3390/d14020143
- Yi, L., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Schadler, V., van Huis, A., & van Boekel, M. A. J. S. (2013). Extraction and characterisation of protein fractions from five insect species. *Food Chemistry*, 141, 3341–3348. https://doi.org/10.1016/j. foodchem.2013.05.115