LWT - Food Science and Technology 164 (2022) 113638

Contents lists available at ScienceDirect

LWT

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

Potentiality of protein fractions from the house cricket (*Acheta domesticus*) and yellow mealworm (*Tenebrio molitor*) for pasta formulation

Gabriella Pasini [a], Marco Cullere [b,*], Mara Vegro [a], Barbara Simonato [c], Antonella Dalle Zotte [b]

[a] Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Viale dell'Università 16, Padova, 35020, Legnaro, Italy
[b] Department of Animal Medicine, Production and Health, University of Padova, Agripolis, Viale dell'Università 16, Padova, 35020, Legnaro, Italy
[c] Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134, Verona, Italy

ARTICLE INFO

Keywords:
Insect powder
Protein extraction
Novel food
Pasta fortification
Technological properties

ABSTRACT

House cricket (*Acheta domesticus*; AD) and yellow mealworm (*Tenebrio molitor*; TM) are two promising insect species for possible novel food applications. In this research the insect protein fractions were extracted, characterised, and used in the manufacturing of pasta by replacing semolina with 14% of powdered proteins. Pasta samples were then analysed to evaluate technological quality aspects. Results showed that insect protein inclusion resulted in a darker (L^* value: 76.7, 53.4, 59.9 for control, AD and TM, respectively) and firmer (12.4, 13.7, 13.8 N: control, AD and TM, respectively) AD and TM pasta, and a higher water absorption index for AD (148, 178, 150%: control, AD and TM, respectively). In conclusion, both extracts offer interesting opportunity for pasta formulations, possibly leading to an improved protein content and quality. From an industrial perspective, the present study demonstrated that the tested edible insects can provide protein extracts for the possible fortification of pasta with high-quality protein and technological traits, thus representing an ingredient with interesting potential for several food applications.

1. Introduction

For centuries, edible insects have been an important source of nutrients for humans worldwide; in fact, a wide variety of species are commonly consumed in many countries such as Asia, Africa, Latin America, and they ensure a valuable supply of nutrients (Belluco et al., 2013). Despite the nutritional composition of insects can display significant variations according to species, development stage, environmental conditions, and substrate on which they feed themselves (Kouřimská & Adámková, 2016), they can generally be considered a significant source of high-quality protein, with an interesting amino acid profile, essential fatty acids, microelements and other bioactive compounds (Elhassan, Wendin, Olsson, & Langton, 2019). The growing scientific and commercial interest regarding the use of insects as food, as well as feed (Dalle Zotte, 2021), is mainly linked to the fact that they can represent a valuable mean to ensure food security in areas subjected to significant demographic growth and resources scarcity, but also to improve the sustainability of feed/food production. In fact, it has been documented that farming insects allows a significant reduction in land and direct water use compared to conventional crops (Van Huis & Oonincx, 2017), and generate fewer emission of greenhouse gases than cattle or pigs (Oonincx et al., 2010). The potential sustainability of insect farming makes them one of the alternative protein sources to face the increase in global protein demand (Berggren, Jansson, & Low, 2019).

The manufacturing and marketing of insect-based food products has become interesting also in Western Countries, where insects can be exploited to enhance the nutritional value of food products. In the EU, the Novel Foods Regulation (Regulation (EU) 2015/2283) has entered into force in 2018 and it explicitly considers whole insects. Furthermore, following the 2021's positive scientific opinions of the European Food Safety Authority about *Locusta migratoria*, *Acheta domesticus* and *Tenebrio molitor*, the European Union (EU) Member States authorities have recently approved the drafts implementing regulation aiming to authorise the commercialisation of *Locusta migratoria* on the EU market (https://ipiff.org/insects-novel-food-eu-legislation), while dried *Tenebrio molitor* and *Acheta domesticus* have already been approved with the Regulation (EU) 2021/882 and Regulation (EU) 2022/188 respectively.

In these Countries, however, insect consumption is still very limited, mainly attributable to cultural habits and bias (Mancini, Moruzzo, Riccioli, & Paci, 2019). From this viewpoint, promoting a positive attitude

* Corresponding author.
E-mail addresses: gabriella.pasini@unipd.it (G. Pasini), marco.cullere@unipd.it (M. Cullere), mara.vegro@unipd.it (M. Vegro), barbara.simonato@univr.it (B. Simonato), antonella.dallezotte@unipd.it (A. Dalle Zotte).

https://doi.org/10.1016/j.lwt.2022.113638
Received 29 April 2022; Received in revised form 30 May 2022; Accepted 4 June 2022
Available online 7 June 2022
0023-6438/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

toward entomophagy could be a goal in stimulating consumers to eat and cook insects (Megido et al., 2016). Up to now, the most promising strategy to increase insect acceptability, and thus the market potential of insects in Western diets, seems to be their processing into powder form to be used as an ingredient for different food products, thus avoiding visual recognition of insects (Balzan, Fasolato, Maniero, & Novelli, 2016).

In the recent past, there has been growing research into the possible application of flour, paste, and protein extracts obtained from different insect species into several food products including bakery goods (da Rosa Machado & Thys, 2019; González, Garzón, & Rosell, 2019), brown rice flour and cake (Indriani, Ab Karim, Nalinanon, & Karnjanapratum, 2020), cereal bars (Ribeiro et al., 2019) and other snacks (Azzollini, Derossi, Fogliano, Lakemond, & Severini, 2018). Among the possible food applications of insects there is pasta, a well-known traditional cereal-based food, characterised by a high palatability, an interesting nutritional profile, and also by a moderate glycaemic index (Bustos, Perez, & Leon, 2015). Being a staple food, pasta could represent an excellent carrier of nutrients and bioactive compounds, and the novel ingredient should be easily incorporated into the pasta microstructure and ensure its technological quality, including colour, cooking time and loss, water absorption, swelling index, and texture profile (De Marco, Steffolani, Martínez, & León, 2014). Despite the popularity and commercial relevance of this food product, published research about its possible fortification with insects is still limited. A recent study has successfully fortified durum wheat pasta with house cricket powder up to an inclusion level of 15% (Duda, Adamczak, Chełminska, Juszkiewicz, & Kowalczewski, 2019), while incorporating grasshopper (*Locusta migratoria*) or yellow mealworm into egg pasta with a 15% inclusion level revealed an improvement in the nutritional profile (protein and fat), but it harmed cooking performance and overall sensory acceptability, the latter likely attributable to the lipid fraction (Çabuk & Yilmaz, 2020). Based on above mentioned, isolating and extracting insect proteins seems theoretically desirable to increase the protein content of pasta. However, the possible impact of extracted insect proteins on the technological traits of pasta is still unknown, yet needs to be studied.

Therefore, the present research aimed to i) evaluate a laboratory-scale manufacturing protocol of a powder enriched with proteins from *Acheta domesticus* and *Tenebrio molitor*; ii) characterise the proteins of insect protein-rich powders; iii) evaluate the impact of the incorporation of 14% of insect protein powders on selected technological quality traits of dry pasta.

2. Material and methods

2.1. Raw materials

Commercial wheat semolina (*Triticum durum*), the raw material for pasta production, was bought in a local supermarket and its composition, as reported on the label, was: 70.8 g/100 g of total carbohydrates, 12.5 g/100 g of proteins, 1.8 g/100 g of total fat and 2.6 g/100 g of total dietary fibre.

Dried larvae of yellow mealworm *(Tenebrio molitor)* and adult house cricket (*Acheta domesticus*) were provided by INEF (Insect Novel Ecologic Food, Piombino Dese, Italy). The dried insect samples were ground with liquid nitrogen to obtain a fine powder.

2.2. Proximate composition of insect powder

Analysis of the insect powder was carried out in triplicate following the AOAC - Association of Official Analytical Chemists (2000) procedures to determine the dry matter (DM; method no. 934.01), crude protein (CP; method no. 2001.11), crude fibre (method no. 978.10), and ash (method no. 967.05) contents. The ether extract was determined after acid hydrolysis (European Community, 1998 19).

2.3. Protein recovery from insect powder and amino acid composition

Insect powder samples from adult house cricket and yellow mealworm larva were defatted using *n*-hexane (ratio 1:10 – sample:solvent). Samples were processed twice in 48 h at room temperature by stirring at 70 RPM/min (M201-OR shaker, MPM Instruments, Milan, Italy), afterward, they were centrifuged (Beckman Coulter, Brea California, USA) at 20,000 g for 30 min. Pellets were recovered and dried under a nitrogen stream at 20 °C to obtain a defatted powder.

The protein from the defatted powder was extracted in PBS buffer (0.01 mol L^{-1} Na_2HPO_4/NaH_2PO_4, 0.015 mol L^{-1} NaCl, pH 7.4), ratio of 1:10 w/v, by continuous stirring at room temperature for 48 h. Samples were then centrifuged at 20,000 g for 30 min, the supernatants were collected by vacuum filtration (GF/A – 90 mm, Whatman; Cytiva, Marlborough, MA), and then freeze-dried (Edwards Italy, Milan, Italy) to recover a fine powder. The protein content of the two extracts was calculated using 6.25 as nitrogen-to-protein conversion factor.

The extraction yield (%) was calculated and expressed on starting weight basis by the following equation: extraction yield % = [weight of extract (g) / weight of sample (g)] * 100

Extraction efficiency was calculated on starting protein basis by the following equation: extraction efficiency % = [total extracted protein (g) / total protein content (g)] * 100

The amino acid composition of the two protein extracts was determined after acid hydrolysis and pre-column derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), separated by RP-HPLC and analysed by UV detection (Agilent 1260 Infinity, Agilent Technologies, Santa Clara, CA, USA) following a method adapted from European Pharmacopoeia (Council of Europe, 2005). Briefly, for Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, and Val determination, protein of the sample was hydrolysed with hydrochloride acid (6 M) at 105 °C for 24h. Cys was determined as the sum of cysteine and cystine, after reaction with dithiodipropionic acid, producing a mixed disulphide, which then underwent acid hydrolysis accordingly. After hydrolysis, the samples were neutralized with sodium hydroxide (8 M), adjusted to volume, and filtered at 0.45 μm. Then, the derivatisation step was conducted according to the manufacturer's instructions (AccQTag Ultra Derivatisation Kit; Waters, Milford, MA).

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

The molecular weight distribution of the protein fraction obtained from insects was visualised with SDS-PAGE using 8–16% TGX Stain-Free Gel (Bio Rad, Hercules, CA). The samples were dissolved in 0.5 M Tris–HCl Laemmli buffer (Sigma Aldrich, St. Louis, MO) pH 6.8, containing 15% (w/v) glycerol, 1.5% (w/v) SDS and 8% 2-mercaptoethanol, in ratio 1:3 (w/v), heated at 100 °C in a water bath for 5 min and centrifuged for 3 min at 10.000 g. Afterward, 30 μL of the solution 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 0.05% (w/v) Coomassie Brilliant Blue R-250 (Bio Rad), 5% (w/v) TCA, 17% (v/v) methanol, and 6% (v/v) acetic acid, and de-stained in 7% (v/v) acetic acid.

2.5. Preparation of pasta samples enriched with insect protein fraction

The pasta was made using a professional Lillodue pasta-making machine (Bottene, Schio, Italy). The samples were prepared with total wheat semolina (Control sample), and by replacing semolina with 14% of insect powdered proteins to reach the claim "high protein" pasta, according to the Regulation (EC) No 1924/2006, at least 20% of the energy value of the food must be provided by protein. Total semolina

and the blend semolina/insect powdered proteins were added with 35% tap water 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, Massachusetts, USA) for about 12 h to reach a final moisture content of 12.5% (w/w), as required by Italian law (DPM February 9, 2001, n.187). For each sample, n = 3 pasta batches were obtained 1) CP: wheat control pasta, 2) AD: pasta formulated with adult house cricket (*Acheta domesticus*) protein extract, and 3) TM: pasta formulated with yellow mealworm (*Tenebrio molitor*) larvae protein extract.

2.6. Standard parameters of pasta quality

Colour. The colour of the raw pasta was analysed according to the CIELAB system (CIE, 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 AD and TM samples compared to the control sample (CP), the following equation was applied:

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

where: ΔE = *Total colour difference*.

$\Delta L^* = L^*$ control$-L^*$ treatment
$\Delta a^* = a^*$ control$-a^*$ treatment
$\Delta b^* = b^*$ control$-b^*$ 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 - American Association of Cereal Chemists (2000) methods 44-15A and 66–50, respectively. Water absorption index was expressed as % increase of pasta weight after cooking.

Texture Analyses. Firmness and adhesiveness in cooked pasta, after removing water excess, were analysed using a TA.XTplus Texture Analyser (Stable Micro System, Ltd., Godalming, UK) equipped with a 50 kg load cell. Five pasta stripes of 5 cm length were positioned parallel next to each other on the texture analyser platform. A rectangular probe (35 mm × 50 mm) was used. The test parameters were: pre-test speed (2 mm/s), test speed (1 mm/s), post-test speed (3 mm/s), percentage deformation (90%). Firmness was calculated as the maximum force peak required to compress the pasta sample. Stickiness was calculated as the maximum peak force to separate the probe from the sample's surface. The results were reported as the mean value of 6 replicates.

2.7. Statistical analysis

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

3. Results and discussion

3.1. Chemical composition of insects

It is well known that the protein-lipid interaction can limit protein solubility (Lam, Can Karaca, Tyler, & Nickerson, 2018; Azagoh et al., 2016) and this phenomenon has already been reported for seeds and legumes (Choi, Wong, & Auh, 2017; Lam 2018), but also for insects (Bubler, Rumpold, Jander, Rawel, & Schluter, 2016; Yi et al., 2013). For this reason, mealworm (*Tenebrio molitor*) larvae and adult house cricket (*Acheta domesticus*) were defatted. After defatting, the insects powder contained high amount of protein (64.9 and 57.9% DM for AD and TM, respectively) with a residual amount of lipids (6.7 and 10.9% DM, respectively; Table 1). The hexane defatting allowed to increase the protein yield of about 8% in both samples compared to the initial values which is a result that could be further improved optimizing the defatting process. In fact, another study considering ethanol-defatted mealworms achieved an efficiency of about 30% (Zhao, Vázquez-Gutiérrez, Johansson, Landberg, & Langton, 2016).

3.2. Protein-enriched fraction, electrophoretic profile and amino acid composition

The yield and extraction efficiency of PBS-soluble proteins from defatted insect powders are shown in Table 2. The extraction yield of AD was 1.5-fold higher than that of TM (64.3% *vs* 41.2%). The proteins were simply extracted in PBS buffer and the difference between samples was probably due to the different protein solubility caused by heat treatment during the insect drying process, and/or interaction between protein and residual fat, as the latter was greater in TM than in AD. Despite the hexane is reported to be the best solvent (L'hocine, Boye, & Arcand, 2006), given the high fat content in edible insects, the defatting step can be difficult and incomplete. Moreover, extraction yields obtained from aqueous, or salt extraction are usually lower than those obtained by alkaline solubilisation (Gravel & Doyen, 2020). In any case, our results revealed an extraction efficiency higher than that obtained by Chatsuwan, Nalinanon, Puechkamut, Lamsal, and Pinsirodom (2018) in protein isolates of two types of grasshoppers. In addition, although the extraction yield was not greater, the extraction efficiency was high (75.2% and 88.7% in AD and TM, respectively) and similar to that obtained by Azagoh et al. (2016). Despite it is difficult to compare samples analysed in different studies, these results show that salt-water solubilisation can be used for proteins separation.

The electrophoretic profiles and molecular weight distribution of insect protein fractions from insect samples are shown in Fig. 1. The gels revealed different proteins mobility, in any case, less than 75 kDa for all samples. Based on the intensity, bands ≤20 kDa were abundant for TM sample. In particular, bands ranging from 12 to 15 kDa are strongly visible and also reported by other authors (Azagoh et al., 2016; Janssen, Vincken, Arts, Fogliano, & Lakemond, 2019; Yi et al., 2013) which could correspond to haemolymph proteins at ~ 12 kDa (Yi et al., 2013), and cockroach allergen-like protein ~15 kDa (Nebbia et al., 2019). Next, bands ranging from 15 to 30 kDa, also reported by Yi et al. (2013), were hypothesized to be larval cuticle proteins that make up the exoskeleton of insects (Andersen, Hojrup, & Roepstorff, 1995), *e.g.* chymotrypsin-like protein (24 kDa) (Elpidina et al., 2005). Other bands observed at around 60 kDa could belong to β-glycosidase (59 kDa), trypsin-like proteinases (59 kDa), and melanisation-engaging types of protein (65 kDa) (Yi et al., 2013). These bands found in TM sample were also prevalent in the AD sample. Similar findings were noted by Bubler et al. (2016) and Brogan (2018).

Table 1
Proximate composition (% dry matter) of defatted adult *Acheta domesticus* (AD) and *Tenebrio molitor* (TM) larvae (mean ± S.D.).

Insect species	AD	TM
Dry matter	93.4 ± 0.02	94.3 ± 0.02
Protein	64.9 ± 0.5	57.9 ± 0.4
Lipids	6.7 ± 0.3	10.9 ± 0.4
Ash	4.4 ± 0.03	4.3 ± 0.01
Fibre	6.2 ± 0.05	11.2 ± 0.07

Table 2
Yield and extraction efficiency of PBS-soluble proteins from defatted adult *Acheta domesticus* (AD) and *Tenebrio molitor* (TM) larvae (mean ± S.D.).

Insect species	AD	TM
Extraction yield, %	64.3 ± 5.4	41.2 ± 4.7
Total protein in PBS powder extract, %	48.8 ± 1.7	51.4 ± 1.9
Extraction efficiency, %	75.2 ± 5.2	88.7 ± 5.9

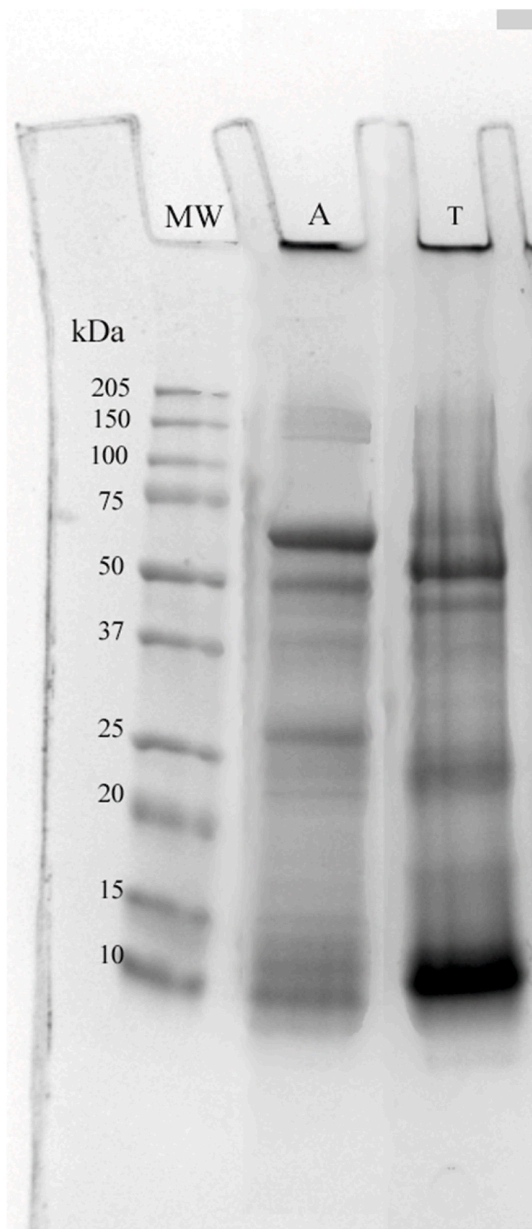

Fig. 1. Electrophoretic profiles and molecular weight distribution of insect protein fractions from insect samples *Acheta domesticus* (AD) and *Tenebrio molitor* (TM).

3.3. *Amino acid composition of the PBS-soluble proteins from defatted insect powder*

PBS-soluble proteins from AD were richer in essential amino acids compared to those from TM (12.4 *vs* 11.2 g/100 g sample for AD and TM, respectively; sum of singles essential amino acids reported in Table 3), with the sole exception of Lysine which was found to be higher in TM compared to AD (6963 vs 4040 mg/100 g sample for TM and AD, respectively). The same trend was observed for the conditionally essential and non-essential amino acids, with the exception of Glutamic acid, higher in TM PBS-soluble proteins compared to AD ones (11908 *vs* 8020 mg/100 g sample, respectively). Overall, the two extracts showed a similar amino acid content, slightly superior in AD compared to TM (35.3 *vs* 33.7 g amino acids/100 g sample, for AD and TM, respectively; sum of amino acids reported in Table 3).

Table 3
Amino acid composition (mg/100 g sample) of PBS-soluble proteins from defatted adult *Acheta domesticus* (AD) and *Tenebrio molitor* (TM) larvae (mean ± S.D.).

Insect species	AD	TM
Essential amino acids:		
Histidine	606 ± 41.1	258 ± 13.9
Isoleucine	2290 ± 155	977 ± 44.8
Leucine	1079 ± 75.4	490 ± 63.3
Lysine	4040 ± 297	6963 ± 221
Methionine	511 ± 38.8	181 ± 19.4
Phenylalanine	1038 ± 65.5	439 ± 34.2
Threonine	924 ± 83.0	683 ± 84.5
Valine	1793 ± 122	1157 ± 84.8
Tryptophan	97.8 ± 8.22	54.5 ± 9.50
Conditionally essential:		
Arginine	1248 ± 98.6	524 ± 34.7
Cysteine	245 ± 33.6	252 ± 15.8
Glycine	2109 ± 123	967 ± 73.5
Proline	2131 ± 120	787 ± 57.1
Tyrosine	987 ± 87.4	849 ± 80.2
Nonessential amino acids:		
Alanine	4035 ± 198	3797 ± 139
Aspartic acid	2867 ± 281	2621 ± 102
Glutamic acid	8020 ± 579	11908 ± 673
Serine	1275 ± 106	746 ± 97.5

In absolute terms, AD and TM generally have a remarkable protein quality, with an essential amino acid composition similar to that recommended by the FAO (Wu et al., 2020). As observed in the present study, also in the above-cited study glutamic acid was the most abundant amino acid, which a distinctive trait of the most studied insect species (Fogang Mba et al., 2017).

3.4. *Colorimetric profile, cooking quality and texture analysis*

Food colour plays an important role in food choice influencing the consumers' attitude towards food, already at purchase (Costell, Tárrega, & Bayarri et al., 2010). The addition of insect protein extract from AD and TM changed the colour of the raw pasta samples which became similar to the colour of the whole wheat pasta already known by consumers (Fig. 2; Table 4).

Specifically, insect protein extracts led to a darker (L^* value: 22% in TM and −30% in AD pasta compared to CP) and redder (a^* value) pasta colour. In reverse, yellowness (b^* value) was less pronounced in TM but similar between CP and AD. Overall, pasta samples formulated with insect protein extracts appeared different in colour compared to the control pasta, especially for the AD, as detected by the colour deviation (ΔE values). In the case of pasta samples enriched with insect protein extracts, the darker colour is likely due to enzymatic browning reactions (Yi et al., 2013).

Moreover, consumers consider the texture of the pasta to be important, in particular the firmness and adhesiveness of the cooked product. The firmness observed for AD and TM was significantly higher than that observed for CP pasta (Table 4), in agreement with the results obtained by Duda et al. (2019). These authors found that the firmness in pasta samples enriched in cricket powder was higher than that of the control sample when the level of wheat flour replacement was 15%.

Results presented in Table 4 also show that, at the optimum cooking time, the amount of water absorbed in AD pasta was significantly higher than that absorbed by the other two pasta formulations. This result was

Fig. 2. From left to right: visual appearance of the control pasta (CP), pasta with *Acheta domesticus* (AD), and pasta with *Tenebrio molitor* (TM) protein extracts.

Table 4
Colorimetric profile, cooking quality and texture analysis of control pasta (CP), pasta enriched with protein extract of *Acheta domesticus* (AD) or *Tenebrio molitor* (TM) (mean ± S.D.).

	CP	AD	TM
Raw pasta:			
L*	76.7 ± 2.3[a]	53.4 ± 0.7[c]	59.9 ± 0.2[b]
a*	1.51 ± 0.1[c]	10.1 ± 0.8[a]	3.5 ± 0.5[b]
b*	24.9 ± 0.4[b]	31.0 ± 1.3[a]	17.4 ± 0.6[c]
ΔE	0	25.5	13.2
Cooked pasta:			
Water absorption index (%)	148 ± 2.9[b]	178 ± 5.7[a]	150 ± 2.1[b]
Optimal cooking time (min)	11.5 ± 0.5[b]	12.5 ± 0.5[b]	13.0 ± 0.5[a]
Cooking loss (%)	4.1 ± 0.06[b]	7.8 ± 0.1[a]	8.1 ± 0.3[a]
Firmness (N)	12.4 ± 0.10[b]	13.7 ± 0.08[a]	13.8 ± 0.02[a]
Adhesiveness (N)	−0.01 ± 0.002	−0.01 ± 0.002	−0.008 ± 0.001

[a,b] Values in a row with different superscript letter are significantly different ($P \leq 0.05$).

probably attributable to the amino acid composition of AD, rich in hydrophilic amino acids which are able to form a structured gel during cooking. The well-defined gel can correspond to a protein network able to retain water during heating since it promotes protein denaturation (Gravel, 2020). This phenomenon has been observed by other authors when evaluating the gelation conditions in protein fraction from edible insects (Yi et al., 2013), also associated with improved texture (Aryee, Agyei, & Udenigwe, 2018). Increased firmness did not seem to limit cooking loss which was higher in AD and TM compared to CP (Table 4). This behaviour, already observed when using protein additives of plant origin (Kaur, Sharma, Nagi, & Ranote, 2013), can be related to the weakening of the protein matrix, associated with the gluten network whose dilution leads to an increased cooking loss, even though the adhesiveness performed similar in all samples and no significant differences were found.

4. Conclusions

Results of the present study indicate that powdered insect's protein can be used to replace a part of wheat flour for making pasta in order to improve the nutritional value. Insects protein fraction, given their aminoacidic composition, could be promising ingredients to improve the biological value of pasta protein, the latter being generally low in lysine. Besides the improvement of protein quantity and quality, also the texture of insect protein-made pasta was satisfactory in term of firmness and adhesiveness, despite a greater cooking loss than the control pasta. Nevertheless, a sensory evaluation of the final product should also be performed to assess the consumers' acceptability which, from previous researches, resulted critical in particular for the odour when raw insect flour was used. To this regard, it could be interesting to compare pasta enriched with insect flour and pasta enriched with insect protein extract, considering that these products could be one of the interesting prospects of the food industry. Moreover, the raw insect quality, the processing technique and proteins extraction will need to be standardised and developed to make them profitable and applicable for industrial use in the food sector.

CRediT authorship contribution statement

Gabriella Pasini: Conceptualization, Methodology, Resources, ValidationValiation, Data curation, Writing – original draft, Project administration, Funding acquisition. **Marco Cullere:** Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Mara Vegro:** Investigation, Data curation, Visualization. **Barbara Simonato:** Writing – original draft. **Antonella Dalle Zotte:** Conceptualization, Methodology, Resources, Writing – original draft, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

The research was financially supported by Project BIRD203914 of DAFNAE Department, BIRD funds of MAPS Department and DOR2021 funds of MAPS Department, University of Padova. The authors thank James Caon (INEF, Piombino Dese, Padova, Italy) for providing the insects.

References

AACC - American Association of Cereal Chemists. (2000). In *Approved methods of the AACC* (10th ed.). St. Paul, MN, USA: American Association of Cereal Chemists.
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
AOAC - Association of Official Analytical Chemists. (2000). In *Official methods of analysis* (17th ed.). Gaithersburg, MD, USA: The Association of Official Analytical Chemists.
Aryee, A. N. A., Agyei, D., & Udenigwe, C. C. (2018). Impact of processing on the chemistry and functionality of food proteins. In R. Y. Yada (Ed.), *Proteins in food processing* (pp. 27–45). Woodhead Publishing Series in Food Science, Technology and Nutrition. https://doi.org/10.1016/B978-0-08-100722-8.00003-6.
Azagoh, C., Ducept, F., Garcia, R., Rakotozafy, L., Cuvelier, M. E., Keller, S., et al. Lewandowski, R., & Mezdour, S. (2016). Extraction and physicochemical characterization of *Tenebrio molitor* proteins. *Food Research International, 88*, 24–31. https://doi.org/10.1016/j.foodres.2016.06.010
Azzollini, D., Derossi, A., Fogliano, V., Lakemond, C. M. M., & Severini, C. (2018). Effects of formulation and process conditions on microstructure, texture and digestibility of extruded insect-riched snacks. *Innovative Food Science & Emerging Technologies, 45*, 344–353. https://doi.org/10.1016/j.ifset.2017.11.017
Balzan, S., Fasolato, L., Maniero, S., & Novelli, E. (2016). Edible insects and young adults in a north-east Italian city an exploratory study. *British Food Journal, 118*, 318–326. https://doi.org/10.1108/BFJ-04-2015-0156
Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C., Paoletti, M. G., & Ricci, A. (2013). Edible insects in a food safety and nutritional perspective: A critical review. *Comprehensive Reviews in Food Science and Food Safety, 12*, 296–313. https://doi.org/10.1111/1541-4337.12014

Berggren, Å., Jansson, A., & Low, M. (2019). Approaching ecological sustainability in the emerging insects-as-food industry. *Trends in Ecology & Evolution, 34*(2), 132–138. https://doi.org/10.1016/j.tree.2018.11.005

Brogan, E. N. (2018). Protein and lipid characterization of *Acheta domesticus, Bombyx mori*, and *Locusta migratoria* dry flours. *Graduate Theses, Dissertations, and Problem Reports, 7498*. https://researchrepository.wvu.edu/etd/7498, 2018.

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

Bustos, M. C., Perez, G. T., & Leon, A. E. (2015). Structure and quality of pasta enriched with functional ingredients. *RSC Advances, 5*, 30780–30792. https://doi.org/10.1039/C4RA11857J

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 & Technology, 57*, 2750–2757. https://doi.org/10.1007/s13197-020-04315-7

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; CIE publication No. 15*. Paris, France: Bureau Central de la CIE. E-1.3.1), Suppl. 2.

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

Council of Europe. (2005). European Pharmacopoeia 5.0, 5th revised edition. 2.2.56, 5. In *Amino acid analysis – protein hydrolysis – methods, 1* p. 7). Strasbourg, France: Directorate for the Quality of Medicines, Council of Europe (pp- 87-92)-10: 9287152810; ISBN-13: 978-9287152817.

Dalle Zotte, A. (2021). Do insects as feed ingredient affect meat quality? *Theory and Practice of Meat Processing, 6*(3), 200–209. https://doi.org/10.21323/2414-438X-2021-6-3-200-209

De Marco, E. R., Steffolani, M. E., Martínez, C. S., & León, A. E. (2014). Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta. *LWT - Food Science and Technology (Lebensmittel-Wissenschaft und -Technologie), 58*, 102–108. https://doi.org/10.1016/j.lwt.2014.02.054

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

Elhassan, M., Wendin, K., Olsson, V., & Langton, M. (2019). Quality aspects of insects as food—nutritional, sensory, and related concepts. *Foods, 8*, 95. https://doi.org/10.3390/foods8030095

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

E.C.–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 Union, 41*, 14–28, 199.1998, L257/14 https://op.europa.eu/en/publication-detail/-/publication/856db9b7-6f1d-4d6e-a778-c766c9fa1776.

Fogang Mba, A. R., Kansci, G., Viau, M., Hafnaoui, N., Meynier, A., Demmano, G., et al. (2017). Lipid and amino acid profiles support the potential of *Rhynchophorus phoenicis* larvae for human nutrition. *Journal of Food Composition and Analysis, 60*, 64–73. https://doi.org/10.1016/j.jfca.2017.03.016

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. *Lebensmittel-Wissenschaft und -Technologie, 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 & 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

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

L'hocine, L., Boye, J. I., & Arcand, Y. (2006). Composition and functional properties of soy protein isolates prepared using alternative defatting and extraction procedures. *Journal of Food Science, 71*, C137–C145. https://doi.org/10.1111/j.1365-2621.2006.tb15609.x

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

Megido, R. C., Gierts, C., Blecker, C., Brostaux, Y., Haubruge, É., Alabi, T., et al. (2016). Consumer acceptance of insect-based alternative meat products in Western countries. *Food Quality and Preference, 52*, 237–243. https://doi.org/10.1016/j.foodqual.2016.05.004

Nebbia, S., Lamberti, C., Giorgis, V., Giuffrida, M. G., Manfredi, M., Marengo, E., & Rolla, G. (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

Oonincx, D. G., Van Itterbeeck, J., Heetkamp, M. J., Van Den Brand, H., Van Loon, J. J., & Van Huis, A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS One, 5*(12), Article e14445. https://doi.org/10.1371/journal.pone.0014445

Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02006R1924-20141213.

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. http://data.europa.eu/eli/reg/2015/2283/2021-03-27.

Regulation (EU) 2021/882 of the European Parliament and of the Council of 1 June 2021 on novel foods 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. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32021R0882&from=IT.

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. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R0188.

Ribeiro, J. C., Lima, R. C., Maia, M. R., Almeida, A. A., Fonseca, A. J., Cabrita, A. R. J., et al. (2019). Impact of defatting freeze-dried edible crickets (*Acheta domesticus* and *Gryllodes sigillatus*) on the nutritive value, overall liking and sensory profile of cereal bars. *Lebensmittel-Wissenschaft und -Technologie, 113*, Article 108335. https://doi.org/10.1016/j.lwt.2019.108335

da Rosa Machado, C., & Thys, R. C. S. (2019). Cricket powder (*Gryllus assimilis*) as a new alternative protein source for gluten-free breads. *Innovative Food Science & Emerging Technologies, 56*, Article 102180. https://doi.org/10.1016/j.ifset.2019.102180

Van Huis, A., & Oonincx, D. G. (2017). The environmental sustainability of insects as food and feed. A review. *Agronomy for Sustainable Development, 37*, 1–14. https://doi.org/10.1007/s13593-017-0452-8

Wu, R. A., Ding, Q., Yin, L., Chi, X., Sun, N., He, R., & Li, Z. (2020). Comparison of the nutritional value of mysore thorn borer (*Anoplophora chinensis*) and mealworm larva (*Tenebrio molitor*): Amino acid, fatty acid, and element profiles. *Food Chemistry, 323*, Article 126818. https://doi.org/10.1016/j.foodchem.2020.126818

Yi, L., Lakemond Catriona, M. M., Sagis Leonard, 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

Zhao, X., Vázquez-Gutiérrez, J. L., Johansson, D. P., Landberg, R., & Langton, M. (2016). Yellow mealworm protein for food purposes - extraction and functional properties. *PLoS One, 11*, Article e0147791. https://doi.org/10.1371/journal.pone.0147791