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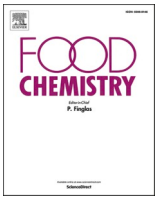

The distinctive effect of different insect powders as meat extenders in beef burgers subjected to cooking and *in vitro* gastrointestinal digestion

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ABSTRACT

Mealworm (MWP), migratory locust (LP), and house cricket (CP) are novel foods recently authorized by the European Commission. This work tested their powders as meat extenders at 5% inclusion in beef burgers. Insect powders were abundant in phenolics, recording the highest values in LP (1184.9 μg/g). The sensory analysis highlighted a higher visual and olfactory acceptability for MWP-burgers, followed by CP- and LP-burgers, whereas the texture of cooked burgers remained unaffected. Following pan-cooking, MWP-burgers and control exhibited comparable chemical profiles, while a significant down-accumulation of the heterocyclic amine 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline was observed in CP-burgers. In vitro gastrointestinal digestion highlighted metabolomic trends like control for MWP- and LP-burgers. In contrast, a reduced accumulation of lipids and increased content of dipeptides like glutaminylarginine (possibly acting as enzyme modulators) was observed for the CP-burgers.

1. Introduction

To date, animal proteins from meat represent some of the main concerns related to the sustainability of food production (Pintado & Delgado-Pando, 2020). From this perspective, partially replacing meat with non-meat substances with a high protein content confers interesting opportunities to reformulate meat products to make them more sustainable and promote human health (Rocchetti et al., 2023).

Insects are described as a promising source of proteins, as revealed by several recent studies (Rocchetti et al., 2023; Borges et al., 2022; Pintado & Delgado-Pando, 2020). Interestingly, some food authorities, such as the Food and Agriculture Organisation of the United Nations (FAO) and the European Food Safety Authority (EFSA), have investigated the possibility of using insects for food applications. At the European level, there are currently nine insect-based novel food applications undergoing safety assessment by EFSA and four which EFSA has authorised as novel foods: '*Tenebrio molitor*' larva (mealworm in frozen, dried and powder form; EFSA, 2021a), '*Locusta migratoria*' (migratory locust, grasshopper; EFSA, 2021b), '*Alphitobius diaperinus*' larva (frozen, freeze-dried formulations of lesser mealworm; EFSA, 2022a), and '*Acheta domesticus*' (dried, ground, frozen and partially defatted whole house cricket; EFSA, 2022b).

Relevant scientific knowledge gaps, however, still need to be filled, and several challenges remain (da Silva Lucas et al., 2020), such as those dealing with consumer acceptance in western societies (Megido et al., 2016) and food safety or toxicological and allergenicity issues (Borges et al., 2022; Leni et al., 2020). In the last years, two main research lines have been identified when considering insect-based foods. In one, a significant result has been reached on the fortification or supplementation of bakery products to increase their nutritional value (Cappelli & Cini, 2021); the other, concerning the reformulation of products aimed at the reduction or replacement of meat proteins (Rocchetti et al., 2023; Scholliers et al., 2020) is gaining interest.

In the meat-science area, the inclusion of insect proteins has resulted in a higher stability of meat emulsions, reduced cooking losses, and increased water-holding capacity, although conflicting results about the textural parameters of these products persist (Borges et al., 2022). It is also important to mention the limited number of studies evaluating the protein digestibility of edible insects added to food systems and the bioavailability of bioactive compounds and micro- and macro-nutrients. Few studies have comprehensively evaluated the behaviour of insect-based foods in the gastrointestinal tract or their effect on the gut

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microbiome and consumers' health. As noted by Ojha et al. (2021), future research activities should investigate the nutrient bioavailability of edible insects and their formulations based on *in vitro* and *in vivo* systems and their long-term effects resulting from controlled human trials.

As recently reviewed by Borges et al. (2022), although some advances in the development of meat products containing edible insects, it is necessary to further investigate their sensory, functional, and physicochemical properties with focused studies. To the best of our knowledge, the limits of incorporation of these new sustainable ingredients must not exceed 10% to avoid undesirable changes in meat products (Rocchetti et al., 2023; Borges et al., 2022; Pintado & Delgado-Pando, 2020). Therefore, we included 5% (w/w) of different commercial insect powders (from *T. molitor, L. migratoria*, and *A. domesticus*) in beef meat to formulate insect-fortified beef burgers with the final objective to determine, by using a multidisciplinary approach, the effect of insect powder incorporation on textural properties and those metabolites formed after cooking and *in vitro* gastrointestinal digestion process, thus potentially representing the biomarkers of insects' consumption. Also, sensory analyses have been used to support the metabolomics outcomes and translate differences at the metabolite level into consumers' perception.

2. Materials and methods

2.1. Insect powders

Cricket (*Acheta domesticus*) powder (CP; protein content: 76%, from boiled and dehydrated crickets) and locust (*Locusta migratoria*) powder (LP; protein content: 78%, from microwave-dried and milled locusts) were supplied by JR Unique Foods (Thailand), while dried yellow mealworm (*Tenebrio molitor*) powder (MWP; protein content: 60%, from oven-roasted mealworms) was supplied by Bug Bazaar (Finland). More information regarding the available nutritional facts of the commercial insect powders is reported in the supplementary material (Table S1). Before manufacturing the beef burgers, the insect powders were chemically characterised to evaluate their potential bioactive profile. Overall, 1 g of each insect powder (n = 3) was extracted in 10 mL of a hydro-alcoholic solution (80% methanol) acidified with 0.1% formic acid, according to a homogeniser-assisted extraction method, using an Ultra-turrax (Ika T25, Staufen, Germany) at 5000×*g* for 3 min. Following a centrifugation step (6000×*g* for 15 min at 4 °C), the extracts were filtered in amber vials through 0.2 μm cellulose syringe filters, then analysed by ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS). The next sections detail the UHPLC-HRMS chemical characterization of insect powders.

2.2. Manufacturing of fresh beef burgers fortified with insect powders

Fresh beef burgers were manufactured by the meat pilot plant at the Università Cattolica del Sacro Cuore (Cremona, Italy). For the trial, a meat batter of 4 kg was prepared, consisting of beef meat 98.8% (i.e., silver-side and top-side cuts containing approximately 5% fat), 1% of salt (NaCl), and 0.1% of ascorbic acid. The meat was prepared using an industrial meat mincer (Velati, Tribiano, Italy) with a perforated plate of 4 mm, and salt was added with an industrial mixer (Velati, Tribiano, Italy). The resulting mixture was subdivided into four equal batches following the indications of the study: (1) the control batch (CTR), with no additives (only 1% salt and 0.1% ascorbic acid), and three additional batches formulated with a 5% (w/w) of each insect powder under investigation, namely (2) MWP burgers, (3) LP burgers, and (4) CP burgers. Each batch was again individually mixed to obtain optimal uniformity of the mixture, then a manual burger maker was used to shape burgers into portions of 100 g in weight for the four batches described above. Overall, the experimental plan was designed considering three biological replicates (n = 3) for each burger type.

2.3. Proximate composition and amino-acid analysis of fresh beef burgers

Compositional analyses were performed on the formulated fresh beef-burger samples. All the minced samples were analysed for moisture, ash, and ether-extract contents. Next, the total amino acid analysis was done according to Leni et al. (2019) with slight modifications. Briefly, 0.5 g of each fresh burger sample were hydrolysed with 6 mL of HCl 6 N at 110 °C for 23 h, then the internal standard (7.5 mL of Norleucine 5 mM in water) was added. For cysteine and methionine, performic acid oxidation followed by acid hydrolysis was used, in order to determine them as cysteic acid and methionine sulphone. In this case, an amount of 0.5 g of each burger sample was added to 2 mL of performic acid freshly prepared (by mixing 9 volumes of formic acid with 1 vol of hydrogen peroxide). Samples were kept in an ice bath for 16 h at 0 °C and then 0.3 mL of hydrobromidric acid was added and the bromine formed was removed under nitrogen flow. The hydrolysed samples were analysed after derivatization of aminoacids with an AccQ Tag reagent (Waters Co., Milford, USA), as suggested by the supplier. Aminoacids were then analysed by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) using a Vanquish pump system and a TSQ Fortis triple-quadrupole as mass spectrometer (Thermo-Fisher Scientific, San Jose, CA, USA). The separation was achieved with a XBridge BEH C18 column (2.5 μm, 3.0 × 75 mm; Waters). The mobile phase consisted in water + 0.2% acetonitrile + 0.1% formic acid (eluent A) and acetonitrile + 0.1% formic acid (eluent B). Gradient elution was performed according to the following steps: isocratic 100% A for 1.8 min, from 100% A to 50% A by linear gradient in 11.4 min and 0.8 min at 50% A plus washing step at 0% A (100% B) and reconditioning. Flow rate was set at 0.2 mL/min, injection volume 5 μL and sample temperature 19 °C. Detection was performed by using Waters SQ mass spectrometer with the following conditions: ESI source in positive ionisation mode, source voltage 4.5 kV, source temperature 270 °C, desolvation temperature 200 °C. The acquisition was performed in SIM mode. As far as the analysis of tryptophan is concerned, this latter has been performed by hydrolysing 0.4 g of sample with 3 mL of 4 N NaOH at 100 °C for 4 h. Then the solution was neutralized, and the volume was brought to 100 mL with deionised water. The samples were then analysed with HPLC instrument, consisting of two PU-1580 chromatographic pumps, an AS 1555 sampling system, an FP 1520 fluorescence detector (Jasco Corporation, Tokyo, Japan). The separation was performed with C8-Select B column; the mobile phase was Methanol: Water + 2% acetic acid (10:90); the flow rate was 1 mL/min. The fluorimeter was set at 280 nm excitation and 340 nm emission wavelengths. Quantifications were performed against a set of standard solutions.

2.4. Microbiological, pH, and a_w analyses of insect powders and fresh beef burgers

Microbiological analyses based on standard ISO reference methods allowed to assess the overall acceptability of the commercial insect powders and formulated beef burgers. Mesophilic total microbial count (TMC), yeasts and moulds, *Enterobacteriaceae, Escherichia coli, Bacillus cereus*, and *Listeria monocytogenes* and *Salmonella* spp. were determined in duplicate as reported by Grabowski and Klein (2017). Sulfite-reducing *Clostridium* spp. were determined according to ISO 15213-1:2023. *Clostridium perfringens* and *Bacillus cereus* spores were determined according to ISO standards (ISO 7937:2005 and ISO 7932:2005), as reported by Rebecchi et al. (2015). Aerobic spores were enumerated using a TSA (tryptic soy agar) medium, following incubation at 30 °C for 2 days. After counts, mean and standard deviation values were calculated for each replicate (n = 3), and the results were expressed in Log CFU/g. The pH of insect powders and beef burgers was measured using the pH meter 127-m; 692 pH/Ion Meter (Metrohm, Laramie, Wyoming,

USA) following calibration. Furthermore, water activity (a_w) was measured at 25 °C using the a_w-meter AQUALAB Series 3 Model TE (Decagon Devices, Inc., Pullman, Washington, USA). The pH and a_w measurements were done in triplicate at each sampling.

2.5. Visual and olfactory analyses of fresh beef burgers

A five-point hedonic scale was used to assess the acceptability of the formulated fresh beef burgers. The sensory test was carried out at the sensory laboratory of the Università Cattolica del Sacro Cuore (Cremona, Italy). The ten sensory panellists (equally divided into males and females) recruited for the analysis comprised PhD students and university staff. The criteria used for the visual analysis included redness, colour uniformity, anomalous colorations, firmness, and overall acceptability. The criteria used for the olfactory analysis included intensity, fresh meat odour, negative aromas, vegetable and hay odours, roasted (nuts) aroma, other aromas, and overall acceptability. The hedonic scale range from 0 to 5, where 5 represented 'maximum perception' and 0 represented the 'absence' of the given parameters. Samples were blind coded with random three-digit numbers, and the order of serving samples was randomised so that each sample occurred equally. The participants gave their written consent to join the sensory test, after receiving full information. Finally, the subjects experienced no danger or discomfort as a result of the sensory test.

2.6. Cooking yield, colour, and texture of the formulated beef burgers

Beef burgers were cooked in a preheated frying pan. Four burgers (one for each formulation) were placed in each quadrant of the preheated pan to standardise the cooking conditions. Samples were cooked for 6 min at a hotplate power of 1400 W, with one turn at 3 min, until internal temperature reached 72 ± 1 °C. This procedure was repeated following a clockwise rotation until each formulation was cooked, and the burgers were then cooled and combined. Beef burgers were weighed before and after cooking, and cooking yield was determined as a percentage of final-to-initial weight (Akwetey & Knipe, 2012). Colorimetric analysis was then performed using a ColorFlex EZ spectrophotometer (HunterLab, Reston, Virginia, USA) to measure the Hunter scale parameters L, a*, and b*, as well as Chroma C* and hue H(°), as reported by Dordoni et al. (2019). Finally, texture analysis was done using the Perten 6700 TVT Texture Analyzer (Perten Instruments Srl, Rome, Italy). The hardness and fracturability of the cooked beef burgers were evaluated by a single-cycle cutting test. Additionally, texture profile analysis (TPA) based on a double-cycle compression determined firmness, springiness, cohesiveness, gumminess, and chewiness.

2.7. In vitro gastrointestinal digestion of cooked beef burgers

The INFOGEST harmonised *in vitro* digestion method detailed by Brodkorb et al. (2019) was used. The protocol involved three successive static *in vitro* digestion phases (i.e., oral: 2 min, 37 °C, pH 7.0; gastric: 120 min, 37 °C, pH 3.0; intestinal: 120 min, 37 °C, pH 7.0). All the *in vitro* conditions were described and justified by Minekus et al. (2014), and rabbit gastric lipase (60 U/mL in the final digestion mixture; RLG500; Lypolytech; France) was used in the gastric phase (Brodkorb et al., 2019). The *in vitro* conditions were scaled up for 50 g of sample. Liquid aliquots (n = 3) were carefully collected after each *in vitro* static digestion phase in 2 mL Eppendorf tubes and stored at −18 °C until further analysis.

2.8. UHPLC-HRMS untargeted metabolomics on cooked and digested beef burgers

The extraction of metabolites from cooked beef burgers was done as reported by Rocchetti et al. (2021). Burger samples (1 g) were extracted in ten volumes of a hydro-alcoholic solution (80% methanol, v/v) acidified with 0.1% formic acid using a homogeniser. The extracts were centrifuged (6000×*g* for 15 min at 4 °C), then filtered with 0.2 μm cellulose syringe filters in UHPLC vials. Additionally, the digestion (oral, gastric, and pancreatic phases) aliquots of each burger sample were centrifuged (6000×*g* for 15 min), filtered with 0.2 μm cellulose syringe filters, and finally collected in vials.

The UHPLC-HRMS untargeted metabolomics analyses were based on a Q Exactive™ Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Waltham, Massachusetts, USA) coupled to a Vanquish ultra-high-pressure liquid chromatography (UHPLC) pump equipped with heated electrospray ionisation (HESI)-II probe (Rocchetti et al., 2021). The chromatographic separation was achieved under a water-acetonitrile (both LC-MS grade from Sigma-Aldrich, Milan, Italy) gradient elution (6–94% acetonitrile in 35 min) using 0.1% formic acid as phase modifier, on a Waters BEH C18 column (2.1x100 mm, 1.7 μm). Briefly, the flow rate was 200 μL/min. For the full scan MS analysis, a positive ionization mode with a mass resolution of 70,000 at *m/z* 200 was used. The injection volume was 6 μL, using a *m/z* range of 70–1200. The automatic gain control target (AGC target) and the maximum injection time (IT) were $1e^6$ and 200 ms, respectively. Randomized injections of pooled quality control (QC) samples were acquired in a data-dependent (Top N = 3) MS/MS mode with full scan mass resolution reduced to 17,500 at *m/z* 200, with an AGC target value of $1e^5$, maximum IT of 100 ms, and isolation window of 1.0 *m/z*, respectively. The Top N ions were selected for fragmentation under stepped Normalized Collisional Energy (i.e., 10, 20, 40 eV). The HESI parameters for both MS and MS/MS were as follows: sheath gas flow 40 arb (arbitrary units), auxiliary gas flow 20 arb, spray voltage 3.5 kV, capillary temperature 320 °C. Prior to data collection, the mass spectrometer was calibrated using Pierce™ positive ion calibration solution (Thermo Fisher Scientific, San Jose CA, USA). To avoid possible bias, the sequence of injections was randomized.

Once collected, the raw data on the chemical profile of insect powders, together with data on the cooked and digested burger samples, were processed in the software MS-DIAL (version 4.90) (Tsugawa et al., 2015). Automatic peak finding, LOWESS normalization, and annotation via spectral matching against the comprehensive databases FooDB (https://www.foodb.ca, accessed: June 2023) and Mass Bank of North America (MoNA) coupled with the Phenol-Explorer (for MS1 annotations) were performed. The mass range 80–1200 *m/z* was searched for features with a minimum peak height of 10,000 cps. The MS and MS/MS tolerance for peak centroiding and accurate mass tolerance was set to 0.05 and 0.1 Da, respectively. The identification step was based on mass accuracy, isotopic pattern, and spectral matching. In MS-DIAL, these criteria were used to calculate a total identification score. The total identification score cut off was >40%, considering the most common (H)-ESI + adducts. Gap filling using peak finder algorithm was performed to fill in missing peaks, considering 5 ppm tolerance for *m/z* values. Under our experimental conditions, a level 2 of confidence in annotation (Salek et al., 2013; Blazenović et al., 2018) was used. Finally, the main phenolic classes identified, namely total flavonoids, total phenolic acids, and total other phenolics, were also semi-quantified according to calibration curves of luteolin, ferulic acid, and oleuropein, respectively, as previously reported (Moldovan et al., 2023). Calibration curves were built using linear fitting in the range of 10–1000 μg L^{-1}; the coefficient of determination $R^2 > 0.98$ was used as an acceptability threshold for calibration purposes.

2.9. Statistical analyses

The analysis of variance (one-way ANOVA; $p < 0.05$, Duncan's post-hoc test) was done in IBM PASW Statistics 26.0 (SPSS Inc.) for the chemical profile of insect powders, proximate composition parameters, technological and colour parameters, microbial counts, pH and a_w values, and amino acid content. Additionally, an unsupervised multivariate data analysis of metabolomics dataset was done in

MetaboAnalyst 5.0 based on hierarchical cluster analysis (HCA, Euclidean distance) and principal component analysis (PCA). Supervised modelling was carried out in SIMCA 13 (Umetrics, Malmo, Sweden) for orthogonal projections to latent structures discriminant analysis (OPLS-DA). The goodness-of-fit and prediction, together with permutation testing, cross-validation of the model, and absence of outliers, were checked in SIMCA 13. Finally, the variables importance in projection (VIP) approach was used to select discriminant compounds, considering a VIP score > 1.

3. Results and discussion

3.1. Chemical profile of the insect powders

This work investigated the chemical profile of MWP, LP, and CP powders through an untargeted metabolomics approach. Overall, the instrumental analysis allowed the putative annotation of 1071 compounds interpreted according to a chemical similarity enrichment approach. Amino acids and peptides were the most represented class of compounds, followed by isoprenoids, purines, indoles, fatty acids, fatty-acid derivatives, and phenolic compounds. The compounds annotated and structurally confirmed under our experimental conditions can be found in the supplementary material (Table S1), along with other annotation parameters. Next, the unsupervised PCA and hierarchical clustering multivariate approaches showed that the LP was characterised by a more distinctive chemical profile compared to CP and MWP (Table S1). Nonetheless, the heat map resulting from the hierarchical clustering analysis indicated that each powder possessed different profiles, likely because of the species, feeding systems, and technological treatment used to obtain the powders. OPLS-DA was then used to extrapolate the most discriminant compounds among the chemical profiles detected (Table S1). The model was good at predicting the orthogonal separation between LP and the other insect powders, showing a significant prediction ability: higher than 0.5 ($Q^2 = 0.991$). Among the 619 biomarker compounds of the differences observed (selected by the VIP approach), we found the phenolics (Z)-[(4-hydroxyphenyl)acetaldehyde oxime] (VIP score = 1.1718) and *trans*-2,3,4-trimethoxycinnamate (VIP score = 1.1716), followed by the dipeptide phenylalanyl-alanine (VIP score = 1.1707). All the marker compounds detected are reported in the supplementary material (Table S1). Reviewing the chemical profile of the insect powders, we successfully annotated and quantified the compound (13Z)-beta-Carotene in the LP powder (on average: 0.90 μg/g), and likely associated with the orange colour observed in its hydroalcoholic extract (Table S1). The semi-quantitative analysis of phenolic compounds by UHPLC-HRMS revealed that the best source was represented by LP (on average: 1184.94 μg/g), followed by CP (on average: 864.96 μg/g) and MWP (on average: 311.72 μg/g) (Table S1). Interestingly, the LP powder was abundant in phenolic acids (on average: 253.78 μg/g), mainly hydroxybenzoic acids, such as ellagic acid and its glycosylated derivatives.

From a microbiological point of view, the analyses (Table S1) revealed low values for anaerobic spores, spores from sulfite-reducing Clostridia, Enterobacteriaceae, moulds, and yeasts. Additionally, a total viable count of 4.8 Log CFU/g was recorded in MWP, followed by lower contents (i.e., 2.8 Log CFU/g) in the CP and LP (<2 Log CFU/g) samples. The microbiological results are coherent with the technological treatments used, namely oven roasting (MWP), microwaving and drying (LP), and boiling and dehydration (CP), likely contributing to reducing microbial counts.

3.2. Evaluation of the formulated fresh beef burgers fortified with insect powders

3.2.1. Proximal composition and amino-acid profile

The proximate and amino-acid composition of burgers after adding 5% of insect powders is summarised in the supplementary material (Tables S2 and S3, respectively). Overall, the addition of insect powders significantly reduced ($p < 0.05$) the moisture content of the burgers by on average 66.84 g/100 g (for insect-fortified burgers) vs 70.72 g/100 g (for the CTR sample). Interestingly, a significant variation was found in the crude lipid content for all insect-fortified burgers, recording the highest value for MWP-burger samples (10.99 g/100 g).

The addition of insect species in the burger formulations did not significantly affect total amino-acid content, recording numerically higher values for the LP burgers (21.62 g/100 g). No significative differences were observed in the essential amino-acid profile, with concentration values ranging from 7.45 g/100 g in the MWP burger to 9.95 g/100 g in the LP burger. On average, the amino-acid profile of insect-enriched burgers comprised about 46% of essential amino acids. Glutamic acid and glutamine were the most abundant amino acids detected, irrespective of the formulation considered (16.6 g/100 g of protein on average). Fortification with insects significantly increased alanine in the LP burgers (47% more than in the CTR burger), while glycine and proline increased in the LP and CP burgers (on average 36% more than in the CTR burger). Valine had the highest concentrations in the CP and LP burgers (1.01 and 1.08 g/100 g, respectively), with values significantly higher than the amount detected in the CTR burger (0.79 g/100 g). Aguilar-Miranda et al. (2002) incorporated 7% of *Tenebrio molitor* in a tortilla preparation, demonstrating a significant increase in essential amino acids. Severini et al. (2018) produced insect-enriched snacks by including 10% and 20% of *Tenebrio molitor* and demonstrated that the amount of histidine, lysine, sulphurated amino acids, threonine, and valine significantly increased after the addition. On the other hand, adding 10% of *Acheta domesticus* to bread formulation did not significantly affect the profile of essential amino acids (Cozmuta et al., 2022). Similar results were also obtained by Althwab et al. (2021), who substituted wheat flour with 5% of *Locusta migratoria* without identifying differences in the essential amino-acid profile of the produced bread. Our findings suggested a slight but different effect on the amino acid profile of the fortified burger samples as a function of the insect species included.

3.2.2. Microbiological, pH, and a_w analyses

The results from the microbiological evaluation of insect powders are reported in the supplementary material (Table S4). All microbiological results were within the limits reported by EFSA Regulation (2022), with MWP showing the highest TMC count (4.95 Log CFU/g). The microbiological results of fresh beef burgers fortified with insect powders and the pH and a_w values are reported in the supplementary material (Table S5). Statistically significant differences were observed for the levels of TMC, *Enterobacteriaceae*, moulds, yeasts, and spore-forming aerobic bacteria among the different burger samples analysed. MWP and LP burgers showed higher microbial counts than the CP and CTR burgers except for spore-forming aerobic bacteria. In all cases, adding insect powders to the beef burgers revealed very low increases in counts, showing TMC values within 5.66–6.08 Log CFU/g (Table S5). In all samples, *Salmonella* spp. and *Listeria monocytogenes* were absent in 25 g of the sample, as reported by other authors analysing edible dried and powdered insects (Grabowski & Klein, 2017). *E. coli* and sulfite-reducing clostridia bacteria and spores were undetectable for all samples (<1 Log CFU/g). This absence may be due to the technological process used to obtain the powders. Among spore-forming bacteria in edible insects, *Bacillus* is the most frequently detected genus, whereas *Clostridium* has only sporadically been detected (Osimani & Aquilanti, 2021). In our study, a reduced number of spores-forming aerobic bacteria and *B. cereus* spores were found (<2 and < 1 Log CFU/g, respectively) compared with other edible processed commercial insects (Fasolato et al., 2018). Therefore, considering the low level found in this study of the total viable count, total aerobic spores, and *B. cereus* count, these powder products could be defined as suitable from food-safety and microbial-quality points of view (Fasolato et al., 2018). Regarding the pH measurement of insect powders, LP revealed the lowest value (5.38),

followed by CP (5.92), while MWP reached a neutral value (Table S4). The same trend was found in the beef burgers; a slight decrease in pH was observed in the LP and CP samples (5.60 and 5.68, respectively) compared with the CTR (5.81) and MWP (5.79) samples (Table S5). Considering the water activity, a significant decrease was observed when adding insect powders to the beef burger, with MWP showing the highest a_w value (0.5096; Table S4).

3.2.3. *Sensory acceptability of the formulated fresh beef burgers*

Usually, neophobia is reported to affect people's willingness to eat insect-based food products (de Carvalho et al., 2019). Therefore, as reviewed by de Carvalho et al. (2019), a set of strategies could be used to make insect-based foods more familiar, e.g. providing more detailed sensory information about the fortified products. The results of sensory analysis of the fresh beef burgers fortified with insect powders are reported in Fig. 1. Concerning visual analysis (Fig. 1A), we found that the inclusion of insect powders significantly reduced ($p < 0.001$) the redness of the burgers, while at the same time increasing the presence of perceived anomalous colourations. Interestingly, no significant differences ($p > 0.05$) were outlined in terms of colour uniformity and firmness of the meat matrix. From an overall acceptability point of view, the CTR burger was the most appreciated by the panellists, followed by the MWP, CP, and LP burger samples (Fig. 1). Regarding olfactory perception (Fig. 1B), CP and LP burgers had the highest aroma intensity (mainly associated with negative aromas). The olfactory test revealed that LP burgers were the worst in this respect, showing a significant ($p < 0.001$) hint of vegetable/hay attributes. Moreover, the CP burgers presented marked roasted (nuts) attributes and lower vegetable/hay aromatic notes (Fig. 1B). Overall, the highest acceptability (in terms of both visual and olfactory perceptions) was obtained by the MWP burgers, which showed a lower perception of negative aromatic notes.

3.3. *Effect of cooking on colour and technological parameters*

CIELAB colour characterisation of the fresh burgers (Table 1) showed that the addition of insect powders significantly affected all the colour parameters. In terms of the changes in redness, the analysis agreed with panellists' perceptions, recording an overall and significant ($p < 0.001$) reduction of the a* value (redness), moving from 26.08 (for the CTR burger sample) to 12.56 (for the LP burger sample). Similar variations were detected for the b* (yellowness) parameter, with the highest value recorded for CTR (20.15), followed by MWP (18.38), CP (17.15), and LP

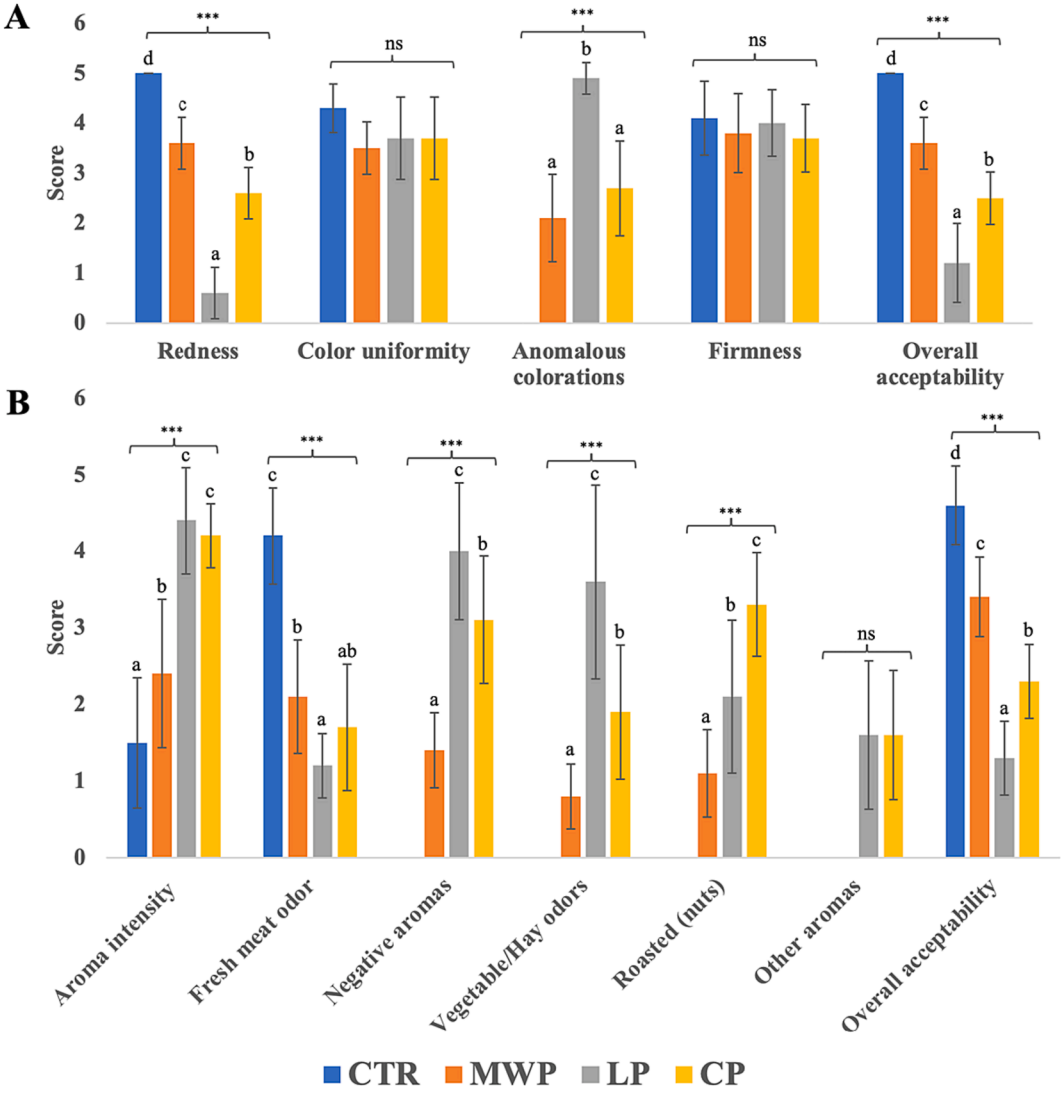

Fig. 1. Mean and standard deviation (n = 10) values of the sensory scores recorded for the formulated fresh beef burgers added with insect powders, when considering both visual (A) and olfactory (B) analyses. The different superscript letters above each bar (^{a,b,c,d}) indicate significant differences as resulting from ANOVA followed by Duncan's post hoc test ($p < 0.05$). *Abbreviations*: CTR (control burger); MWP (mealworm powder-burger); LP (locust powder-burger); CP (cricket powder-burger). *** = $p < 0.001$; ns = not significant ($p > 0.05$).

Table 1
Color parameters on both fresh and cooked burgers resulting from the CIELAB color space analysis. Additionally, on cooked insect-added beef burgers, the cooking yield (%) is also reported. The results are reported as mean values ± standard deviation (n = 6). Superscript letters (^{a,b,c,d}) within each row indicate significant differences as resulting from Duncan's post hoc test ($p < 0.05$).

Fresh burgers	**CTR**	**MWP**	**LP**	**CP**	**Sig.**
*L**	38.11 ± 1.49^{c}	38.74 ± 0.70^{c}	30.57 ± 0.61^{a}	35.26 ± 1.01^{b}	*p* < 0.001
*a**	26.08 ± 1.21^{d}	20.22 ± 1.01^{c}	12.56 ± 0.24^{a}	16.63 ± 0.61^{b}	*p* < 0.001
*b**	20.15 ± 0.94^{d}	18.38 ± 0.60^{c}	14.12 ± 0.38^{a}	17.15 ± 0.61^{b}	*p* < 0.001
*C**	32.96 ± 1.49^{d}	27.33 ± 1.09^{c}	18.90 ± 0.32^{a}	23.89 ± 0.79^{b}	*p* < 0.001
h	37.68 ± 0.67^{a}	42.28 ± 0.89^{b}	48.34 ± 0.95^{d}	45.87 ± 0.82^{c}	*p* < 0.001
Cooked burgers	**CTR**	**MWP**	**LP**	**CP**	**Sig.**
*L**	29.05 ± 2.39	26.22 ± 1.53	26.92 ± 1.16	27.65 ± 2.11	*p* = 0.087
*a**	8.39 ± 0.78	7.18 ± 0.96	7.93 ± 0.98	7.63 ± 0.72	*p* = 0.142
*b**	11.49 ± 1.28	10.29 ± 1.68	10.84 ± 1.09	11.92 ± 1.45	*p* = 0.227
*C**	14.24 ± 1.27	12.56 ± 1.89	13.45 ± 1.30	14.19 ± 1.11	*p* = 0.174
h	53.77 ± 2.37	54.95 ± 1.78	53.82 ± 2.93	57.17 ± 2.88	*p* = 0.304
Cooking yield (%)	68.00 ± 1.00^{a}	74.67 ± 2.08^{b}	78.67 ± 3.79^{b}	76.00 ± 2.65^{b}	*p* < *0.01*

Abbreviations: CTR (control burger); MWP (mealworm powder-burger); LP (locust powder-burger); CP (cricket powder-burger); Sig. (Significance).

(14.12). A different trend was recorded for lightness (L*), with MWP-burgers having values similar to those of CTR burgers, while the highest alteration was recorded for LP burgers (Table 1). Colour analysis was repeated on the cooked burgers, demonstrating that the prevalent effect of the cooking process resulted in non-significant statistical differences across burgers. The same trend was observed for the visual appearance of the fresh vs cooked beef burgers, as reported in the supplementary material (supplementary Fig. 1). Another interesting finding concerned the cooking yield (Table 1), which was improved in presence of all the insect powders tested, in agreement with the previous literature (Borges et al., 2022; Choi et al., 2017; Kim et al., 2017).

Regarding the effect of insect-powder addition on the technological properties of cooked beef burgers (Table S6), TPA analyses revealed no significant effect ($p < 0.05$) on fracturability parameters. Still, the insect-fortified burgers were characterised by numerically higher values of hardness (moving from 96.59 N for the CTR burger up to 114.85 N for the LP burger). A double-cycle compression was then used to determine firmness, springiness, cohesiveness, gumminess, and chewiness. Again, as shown in the supplementary material, no significant differences ($p < 0.05$) were detected for these textural parameters. As reported by Borges et al. (2022), the technological challenges of using insect powders as meat extenders are still present, with available studies showing significant and conflicting results. According to Scholliers et al. (2020), these conflicting results might be explained by the form of incorporation of the ingredient into the meat matrix, such as using fresh vs dried larvae or defatted vs hydrolysed flour.

3.4. Effect of cooking on the metabolomic profile of insect-fortified beef burgers

An untargeted-metabolomics approach was used to investigate the effect of pan cooking on the chemical profile of insect-fortified beef burgers. The HRMS analysis allowed the identification (according to a level 2 of confidence) of 416 compounds against the spectral database FooDB. The dataset is provided as supplementary material (Table S7), along with other annotation information (identification score, MS1-isotopic spectrum, MSMS spectrum, and relative abundance values). An unsupervised statistical approach was first used to discover similarities and differences driven by the cooking process. To this end, hierarchical clustering (HCA; Fig. 2A) and principal component analyses (PCA; supplementary Fig. 2) were used. Overall, the heat map resulting from the Fold-Change based distribution of each metabolite detected allowed for the discrimination of LP burgers from the other burger samples (Fig. 2A). Specifically, the most similar chemical profile was found when considering the MWP and CTR burger samples, thus outlining similar behaviour regarding the chemical modifications occurring in the meat matrix during the cooking process. Each insect-based inclusion, however, determined the up- or down-accumulation of particular groups of metabolites, as outlined by the heat map (Fig. 2A). Accordingly, the PCA score plot (Supplementary Fig. 2) showed that the first two principal components could explain a cumulative 68.3% of the total variability, thus confirming the ability of the metabolomics approach to capture the effect of cooking. Using an OPLS-supervised multivariate algorithm, we modelled the chemical behaviour following the cooking process. In particular, the OPLS-DA score plot provided good discrimination between two observation groups: i.e., LP burgers vs others (Fig. 2B). Looking at the within-group separation, the prediction model highlighted the difference of CP burgers from the MWP and CTR burger samples. The prediction model was characterised by acceptable and significant goodness parameters, with the goodness-of-fit (R^2Y) equal to 0.994 and the goodness-of-prediction (Q^2) equal to 0.962. In addition, the prediction model was cross-validated by CV-ANOVA ($p < 0.01$), while permutation testing (N = 100) and Hotelling's T^2 distribution were used to exclude overfitting and the presence of significant outliers, respectively (Table S7).

Therefore, the potential biomarker compounds of the distribution observed (Fig. 2) were extrapolated by a VIP selection method. Analysis revealed that the cooking process significantly affected mainly amino acids and peptides (61 metabolites), followed by 24 polyphenols, 19 fatty acids, and other compounds (including biogenic and heterocyclic amines, pyrazines, and ascorbic acid). A comprehensive list containing all the VIP marker compounds (organised in main classes) is reported in the supplementary material (Table S7), together with their VIP scores, cross-validation standard error (cvSE), and the significant Log Fold-Change (FC) comparisons for each insect-fortified burgers vs the CTR burger. Regarding the effect of the cooking process on the amino acids and peptides group, we found that the inclusion of insect powders provided the highest up-accumulation in the LP burgers (average LogFC = 1.95), followed by the CP burgers (average LogFC = 0.82) and MWP burgers (average LogFC = 0.27). Overall, the most discriminant amino acids outlined by the VIP analysis were proline (VIP score = 1.59), tryptophan (VIP score = 1.58), and dimethylglycine (VIP score = 1.52). Examining the LogFC variations against the CTR burger, proline and tryptophan were strongly up-accumulated in the cooked MWP burgers compared to the LP and CP burgers. On the other hand, the inclusion of the insect powders tested significantly reduced the distribution of dimethylglycine, recording down-accumulation values from −3.20 (for the LP burgers) up to −4.64 (for the MWP burgers). Regarding the other discriminant amino acids detected, arginine and alanine were markers of the CP burgers (LogFC values equal to 2.02 and 0.46, respectively), while tyrosine again characterised the MWP burgers (LogFC = 1.55). We also found several discriminant peptides containing branched-chain amino acids (i.e., isoleucine, leucine, and valine); interestingly, BCAA-containing peptides, such as isoleucyl-leucine (VIP score = 1.06; LogFC = 4.99), isoleucyl-valine (VIP score = 1.05; LogFC = 0.13), valyl-leucine (VIP score = 1.04; LogFC = 6.88), and leucyl-leucine (VIP score = 1.01; LogFC = 0.85), were strongly up-accumulated in the LP burgers compared with the CTR and the other insect-fortified burgers, thus representing potential biomarker compounds of LP inclusion following the cooking process.

Under our experimental conditions, we also identified two

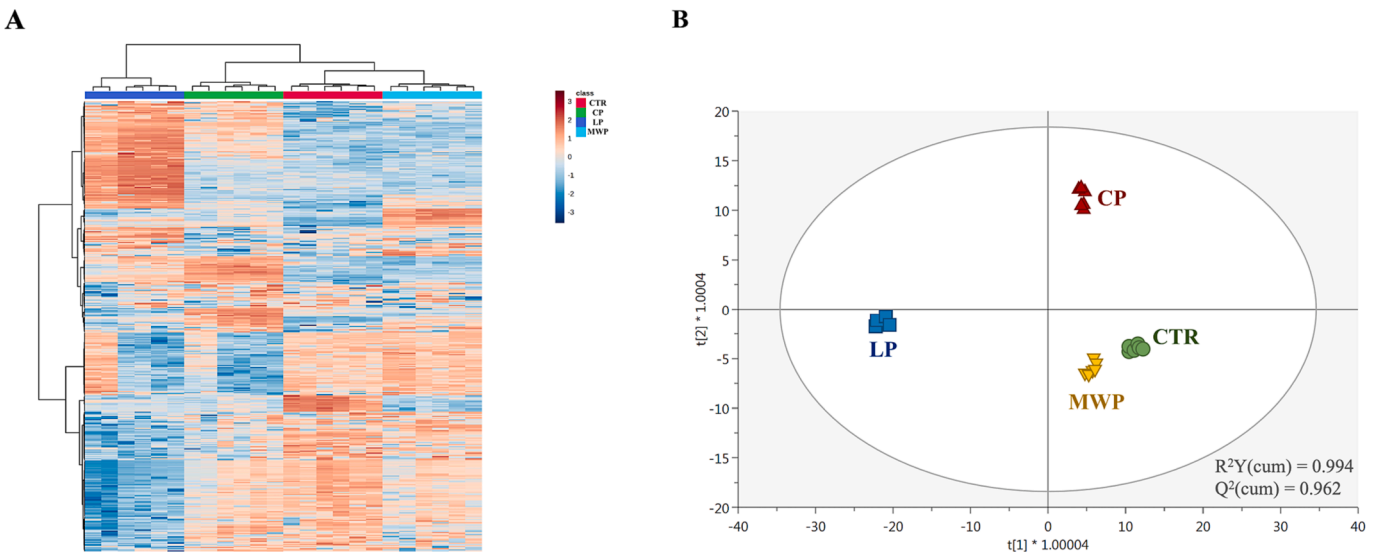

Fig. 2. Unsupervised hierarchical clustering (HCA) heat map (A) and supervised orthogonal projections to latent structures discriminant analysis (OPLS-DA) score plot (B) resulting from the untargeted metabolomic profile of control (CTR), locust powder (LP), mealworm powder (MWP), and cricket powder (CP)-burger samples following the cooking process.

heterocyclic amines: 1-Methyl-6-phenyl-1H-imidazo[4,5-*b*]pyridin-2-amine (PhIP) and 2-Amino-3,8-dimethyl-3H-imidazo[4,5-*f*]quinoxaline (MeIQx). The formation of heterocyclic amines (classified as carcinogenic compounds belonging to group 2A by IARC) during cooking depends on the type of meat, cooking temperature, the degree of browning, and the cooking time (Khan et al., 2022); the VIP analysis outlined that the LP and MWP burgers provided an overall up-accumulation of MeIQx (being the LogFC values equal to 0.43 and 0.87, respectively) compared with the CTR burger, while an opposite trend was recorded only in the CP burgers, which presented a LogFC value equal to −0.34. Regarding other compounds, ascorbic acid (VIP score = 1.16) showed an overall down-accumulation for each insect burger compared with the CTR sample, recording the lowest value following the addition of LP (LogFC = -5.19). Finally, fatty acids and phenolic compounds recorded a similar accumulation trend; in particular, these classes were significantly up-accumulated in the CP and LP burgers vs the CTR burger, while an overall down-accumulation was recorded for the comparison MWP burger vs the CTR burger. Among the discriminant phenolics, we listed several markers of LP inclusion, such as luteolin, isovitexin and vitexin derivatives, and chrysoeriol glycosides (Table S7), thus confirming that LP was also a potential source of phenolics following the cooking process. Finally, among the discriminant fatty acids, the highest prediction ability was held by oleic acid (VIP score = 1.39) and 9,10-epoxyoctadecenoic acid (VIP score = 1.37), both of which were strongly up-accumulated in the CP burgers.

3.5. *Effect of in vitro gastrointestinal digestion on the metabolomic profile of cooked burgers*

Following the cooking process, the harmonised INFOGEST *in vitro* gastrointestinal digestion protocol was used. To the best of our knowledge, no previous studies are available in the scientific literature on the effect of cooking and the *in vitro* simulated digestion of burgers manufactured with these insect powders. The HRMS metabolomics analysis annotated (according to a level 2 of confidence in the annotation) 558 compounds against the spectral database FooDB, as provided in the supplementary material (Table S8) along with other annotation information. To properly assess the efficacy of the *in vitro* gastrointestinal digestion process, we also incubated the beef burgers in the buffers with no enzymes and in the enzymes only (in the proper buffers) but with no burger samples. The correct progression of the *in vitro* digestion was evaluated through an unsupervised clustering approach (supplementary Fig. 3). Overall, two main clusters could be observed; the first included the burger samples incubated with no enzymes, while the second consisted of enzymatically digested burgers and only the enzymes. Therefore, the clustering obtained allowed us to confirm that the enzymes worked properly during the different digestion phases. Interestingly, the heat map resulting from the Fold-Change variation of each metabolite detected showed that the highest degree of chemical modifications could be observed following 120 h of intestinal digestion phase. A clear matrix effect, however, was noticed during each digestion phase, revealing that LP burgers had a chemical behaviour similar to the CTR burger during the oral and gastric phases. At the same time, the CP burgers were characterised by an exclusive profile during the intestinal phase and differed completely from the LP, MWP, and CTR burger samples (Supplementary Fig. 3).

Starting from these unsupervised findings, the supervised OPLS-DA was used next to investigate the chemical classes and discriminant metabolites driving the separation trends observed (Fig. 3). As shown, the model separated the oral and gastric phases from the intestinal phase, thus corroborating the results of the HCA (Supplementary Fig. 3). Overall, few differences could be highlighted within the oral groups, while more differences were found in the gastric and intestinal phases (Fig. 3). We then extrapolated the VIP discriminant compounds within each distinct digestion phase, as provided in the corresponding pie charts (Fig. 3). Notably, the amino acids and peptides presented a discriminant and exclusive weight mainly at the oral level (27 compounds), followed by the gastric and intestinal phases (15 and 6 compounds, respectively). Another challenging point was represented by the increased discriminant weight of some metabolites with the progress of the *in vitro* digestion, with terpenoids and phenolics becoming more relevant at the intestinal level.

Regarding the oral phase, 27 exclusive VIP compounds represented by amino acids and peptides showed a prediction score >1, with the highest score recorded for the dipeptide hydroxyprolyl-methionine (score = 1.48) presenting up-accumulation values for CP and LP burger samples, while being strongly down-accumulated for the MWP burger (LogFC vs the CTR = −3.60). At the oral-phase level, the group represented by exclusively amino acids and peptides showed an overall down-accumulation trend, moving from −0.16 (in the LP burgers) up to −0.99 (in the CP burgers) when compared with the CTR burgers. Interestingly, at the oral level, five peptides were identified as

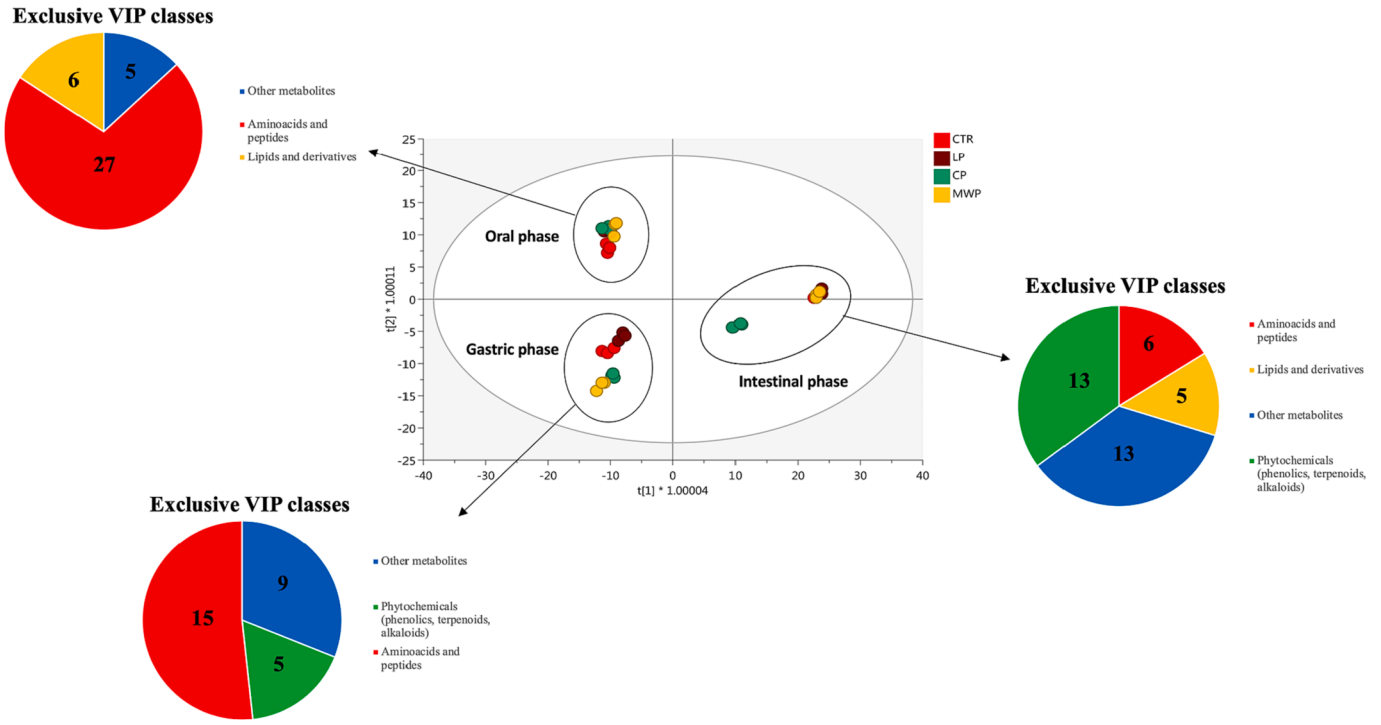

Fig. 3. Supervised orthogonal projections to latent structures discriminant analysis (OPLS-DA) score plot (B) resulting from the untargeted metabolomic profile of cooked control (CTR), locust powder (LP), mealworm powder (MWP), and cricket powder (CP)-burger samples following the *in vitro* gastrointestinal digestion process (i.e., oral, gastric, and intestinal phases).

biomarkers of the insect powders tested: glycylprolylhydroxyproline, valyl-leucine, aspartyl-isoleucine, isoleucyl-arginine, and tryptophyl-tryptophan, showing significantly high LogFC values when compared with the oral digested CTR burgers (Table S8). Regarding the discriminant and exclusive compounds reported at the gastric level (Fig. 3), the group represented by exclusively amino acids and peptides was again significant, although with a lower number of compounds. The highest VIP score at the gastric level was found for the dipeptide serylvaline (VIP score = 1.77), followed by L-phenylalanyl-L-proline (VIP score = 1.69), and L-tryptophan (VIP score = 1.63). Looking at the OPLS-DA score plot (Fig. 3), we found similar behaviour at the gastric level for the MWP and CP burgers due to a similarly high accumulation of the above-mentioned exclusive dipeptides together with other ones (such as leucyl-leucine) reported in the supplementary material (Table S8). Furthermore, the gastric phase of *in vitro* digestion highlighted a significant prediction ability for some chemicals, phenolics, terpenoids, and alkaloids, likely due to the addition of the insect powders (Table S8). Finally, at the intestinal level, the *in vitro* digestion process outlined a completely different behaviour for the CP burgers compared to the others (Fig. 3). The evaluation of the discriminant and exclusive VIP compounds (37 metabolites), although suggesting a higher prediction power exerted by some metabolites (mainly terpenoids), did not allow us to immediately understand why the CP burgers differed from the other digested burgers. Therefore, as the next step, we extrapolated all the VIP marker compounds of the OPLS-DA model exclusively built on the intestinal phase; the changes of the main classes of compounds detected are reported in Table 2.

The focus on the intestinal digestion step highlighted an overall and significant up-accumulation of amino acids and peptides in only the CP burgers compared to the other burger samples incubated under the same conditions. Accordingly, the most discriminant dipeptide detected, alanylvaline, was highly abundant in the CP burgers, showing a LogFC value equal to 2.86 (Table S8). Overall, dietary proteins naturally produce bioactive peptides during the gastrointestinal process in many organisms. In the last years, several attempts have been made to identify and characterise bioactive peptides from vegetable and animal sources. It is worth mentioning that increasing evidence supports bioactive peptides released by enzymatic hydrolysis from insects as being

Table 2
Most discriminant classes of compounds when considering control (CTR), locust powder (LP), mealworm powder (MWP), and cricket powder (CP)-burger samples during the intestinal phase of the *in vitro* INFOGEST gastrointestinal method. Each main chemical class is listed with its corresponding LogFC values, both averaged (avg) and cumulative (cum), for the comparison against the CTR-burgers, together with the most discriminant VIP marker outlined by OPLS-DA prediction modeling.

Class	CP vs CTR	LP vs CTR	MWP vs CTR	Most discriminant VIP
Aminoacids and peptides	LogFC (avg) = 0.27 LogFC (cum) = 7.90	LogFC (avg) = −0.16 LogFC (cum) = −4.68	LogFC (avg) = −0.48 LogFC (cum) = −13.98	Alanylvaline (VIP score = 1.28) Marker of CP-burgers
Lipids and fatty acids	LogFC (avg) = −1.66 LogFC (cum) = −44.73	LogFC (avg) = 0.82 LogFC (cum) = 22.24	LogFC (avg) = 0.03 LogFC (cum) = 0.69	(11R,12S,13S)-Epoxy-hydroxyoctadeca-*cis*-9-*cis*-15-dien-1-oic acid (VIP score = 1.65) Marker of LP-burgers
Prenol lipids	LogFC (avg) = −2.01 LogFC (cum) = −44.02	LogFC (avg) = 0.01 LogFC (cum) = 0.32	LogFC (avg) = 0.03 LogFC (cum) = 0.60	Alpha-Tocotrienol (VIP score = 1.72) Marker of CP-burgers
Polyphenols	LogFC (avg) = −0.39 LogFC (cum) = −3.89	LogFC (avg) = 0.43 LogFC (cum) = 4.29	LogFC (avg) = −0.57 LogFC (cum) = −5.66	5-(3′-Hydroxyphenyl)-gamma-valerolactone 3′-sulfate (VIP score = 1.50) Marker of CP- and LP-burgers

beneficial for human health, as they have antioxidant, antimicrobial, and antidiabetic properties, angiotensin I converting enzyme (ACE) inhibition activity, with potential application as functional food ingredients (Zielińska et al., 2020; Wu et al., 2018). As reviewed by da Silva Lucas et al. (2020), however, there remains little research on the release of bioactive peptides from insect protein hydrolysates, and no studies have comprehensively evaluated the release of potentially bioactive peptides following the inclusion of insect-based ingredients in a food matrix, such as meat. Furthermore, the production of *ad hoc* insect peptides with antioxidant, antimicrobial, and antihypertensive properties may motivate further research into the optimisation of extraction methods and food supplementation following hydrolysis. Notwithstanding this, the related bioactivity could be influenced by different parameters, such as the protein source, hydrolysis degree, and type of protease used to release a particular peptide structure and amino acids (de Castro et al., 2018).

As far as lipids discriminant ability is concerned (Table 2), the compound (11R,12S,13S)-Epoxy-hydroxyoctadeca-*cis*-9-*cis*-15-dien-1-oic acid (VIP score = 1.65) was highlighted as a marker of the LP-burger samples. As a general consideration, considering the untargeted nature of the metabolomics workflow used, future works (e.g., lipidomics based on ion mobility mass spectrometry) are recommended to strengthen lipid annotations and confirm their stereochemistry, especially for the discriminant lipids outlined by UHPLC-HRMS (Table S8). Overall, looking at the LogFC trends, a significant down-accumulation of lipids and derivatives for the CP burger samples was observed at the intestinal level compared with the other samples. Following the protein digestion of the CP burgers, as exerted by both pepsin (at the gastric level) and pancreatin (at the intestinal level), we postulated the generation of potential bioactive peptides able to modulate the pancreatic lipases, thus slowing lipid digestion and providing the down-accumulation trend observed. This hypothesis was strengthened by the significant presence, among the VIP discriminant metabolites of the intestinal-digested CP burgers, of the dipeptide glutaminylarginine (Glu-Arg), showing a VIP score > 1 and a LogFC value > 1.9 when compared with the CTR burgers (supplementary material). Our findings are consistent with a previous report by Zielińska et al. (2020) evaluating the ACE, alpha-glucosidase, and lipase inhibitory activities of peptides obtained by *in vitro* simulated gastrointestinal digestion of selected species of edible insects. In particular, the authors reported the highest lipase-inhibitory effect for two oligopeptides characterised by the same residue (Glu-Arg), which was associated with the strong and significant activity observed.

Notably, looking at the results in Table 2, it can be noticed that LP burgers provided the highest LogFC values for the accumulation of phenolic compounds, in agreement with the high phenolic content characterising the raw insect powder tested (Table S1). As far as the class of prenol lipids is concerned, we found a high discriminant power for the compounds alpha-Tocotrienol (VIP score = 1.72), being strongly down-accumulated in each digested insect-fortified burgers, recording the highest down-accumulation in the CP burgers (LogFC = −4.04), followed by the MWP burgers (LogFC = −1.72), and LP burgers (LogFC = −0.55). Alpha-Tocotrienol is a member of the vitamin E group, accumulating in cell membranes and then protecting against free radicals (Macho-González et al., 2021). Interestingly, we found a lower distribution of this metabolite in all insect-fortified burgers, thus postulating a possibly higher oxidative stress in these products compared to the CTR burgers. Future studies combining *in silico* modelling-based approaches and *in vitro* inhibition assays on enzymes of gastrointestinal interest (such as amylase, glucosidase, lipase, and others) could associate the profiling observed to a specific bioactive activity.

4. Conclusions

A multidisciplinary and comprehensive approach was used to highlight the effect of insect powders as meat extenders in the formulation of beef burgers when considering sensory, textural, and chemical properties, together with their gastrointestinal behaviour following a pan-cooking process. The commercial insect powders tested were a good source of potentially bioactive compounds (mainly phenolic compounds and terpenoids). Although conflicting results are reported in scientific literature, the 5% (w/w) inclusion of these powders did not affect the technological properties of the burgers, while a major effect was recorded at the sensory level, likely correlated to aromatic compounds associated with each insect powder tested. The insect extenders reduced the cooking losses following pan-cooking, and the metabolomics approach outlined a significant down-accumulation of the compound 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline exclusively related with the inclusion of CP. Regarding the simulated *in vitro* gastrointestinal digestion behavior, the most significant differences were recorded at the intestinal level again for the CP burgers, while similar metabolomic trends were observed for the control sample with MWP- and LP-burgers. Interestingly, a down-accumulation of lipid-like molecules was correlated to the up-accumulation of some dipeptides for the CP-burgers, including the compounds glutaminylarginine, arginylleucine, and alanylvaline. Some of the discriminant peptides detected are known to act as potential enzyme modulators, thus opening the way towards the characterization of the bioactive properties of the peptide fraction of these new sustainable ingredients. Taken together, our findings provide new metabolomic insights into the use of insects (as powdered forms) in meat reformulation. At the same time, our results suggest individual differences during the gastrointestinal digestion phase as a function of the insect powder considered, suggesting a clear matrix effect. Future works, based on targeted analytical approaches and/or higher-confidence annotation-based workflows (i.e., to better distinguish positional isomers or stereoisomers) are recommended to better elucidate the structure of some potential biomarkers of the consumption here proposed.

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CRediT authorship contribution statement

Gabriele Rocchetti: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Giulia Leni:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Annalisa Rebecchi:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Roberta Dordoni:** Methodology, Writing – review & editing. **Gianluca Giuberti:** Methodology, Writing – review & editing. **Luigi Lucini:** Methodology, Supervision, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.138422.

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