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Cricket and yellow mealworm powders promote higher bioaccessible fractions of mineral elements in functional bread

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ABSTRACT

The application of cricket and yellow mealworm powders to improve the mineral bioaccessibility of bread was investigated. Breads enriched with 10% cricket (CPB-10%) and 10% yellow mealworm (YMPB-10%) powders showed a 1.5-, 2.95-, and 1.22-fold increase in proteins, total lipids, and fibers, respectively, compared to the reference white wheat bread (WFB-100%). Compared to reference bread, a significant increase in the essential amino acids valine (9.72%) and tyrosine (1.86%) contents was observed in the CPB-10% and YMPB-10%. The MUFAs account for 35.22% in CPB-10%, 30.77% in YMPB-10%, and 32.34% in WFB-100%. *In vitro* digestion experiments showed a higher bioaccessibility of Na, K, Ca, Mg, P, Fe, Zn, Mn, and Li from insect bread than from white bread. Only Cu was more bioaccessible from WFB-100% than from insect bread. The results shed light on the possible contribution of insect bread consumption to mitigate deficiencies in several important macro- and microelements.

1. Introduction

Wheat-flour-based products are important components in the diets of a large part of the world's population. Flour from whole wheat grains is a rich source of valuable nutrients. However, bioactive compounds and minerals are not evenly distributed throughout the grain, with most of them concentrated in the outer part. As a result, whole grains are rich sources of K, Fe, Mg, Ca, Zn, and P but contain lower levels of trace elements, such as Zn, Cu, Mn, and Co (Heshe et al., 2016). Milling, a post-harvest process, separates the bran and retains the carbohydrate-rich endosperm. A large amount of nutrients, ranging between 10 and 80 %, are lost after milling (Oghbaei & Prakash, 2013). Valuable minerals are also removed in large quantities with the bran. Heshe et al. (2016) indicated 1.65-, 1.58-, and 2.34-fold lower amounts of Fe, Zn, and P, respectively, in hard white wheat flour compared to hard whole wheat flour, while another study reported that the level of Mn decreases by 90 %; Zn by 85 %; Mg, K, and Cu by 80 %; and Ca by 33 % (Oghbaei & Prakash, 2013). Due to the lack of large amounts of valuable phytochemicals, white flour bread is considered nutritionally inadequate. White flour fortification has proven to be effective in overcoming the lack of vitamins and proteins in white bread. Further, the addition of synthetic or natural compounds to bread has been practiced over the years to mitigate deficiencies. Edible insects are novel ingredients in the European food industry, with a high added value due to their large amounts of fat, protein, fiber, and minerals, and are considered a more sustainable nutritional alternative to conventional sources. The European Union's approval of the yellow mealworm, house cricket, and grasshopper species for human consumption has opened new pathways for functional food development. Significant nutritional enhancement of flour-based food products enriched with insect powders can be achieved, as shown in Duda et al.'s (2019) study, where pasta enriched with 5 % cricket powder displayed, beyond an increased protein and mineral content, improved culinary properties, thus becoming highly attractive to consumers. Another study reported the suitability of mealworm powder for manufacturing leavened rusks with enhanced protein, essential amino acid, and mineral contents as well as acceptable sensory characteristics (Roncolini et al., 2020).

Currently, few studies have focused on the bioaccessibility and

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bioavailability of nutrients in insect-based functional food. From a nutritional perspective, the digestibility and metabolism of nutrients in food, rather than their content, are key aspects (Ojha et al., 2021). Manditsera et al. (2019) revealed the effects of domestic processing on the protein digestibility and mineral bioaccessibility of two wild-harvested insect species, *Eulepida mashona* (beetle) and *Henicus whellani* (cricket). The study found that the raw insects displayed a higher protein *in vitro* digestibility than the boiled and roasted ones, with the maximum decrease in protein digestibility being approximately 25 % for boiling the beetles twice and for the boiled and roasted crickets. Regarding the minerals, boiling resulted in an approximately 50 % decrease in the bioaccessibility of Fe and Zn in both species. Azzollini et al. (2018) prepared extruded insect-enriched snacks and showed that the mechanical forces generated from extrusion were likely to improve the digestibility of *Tenebrio molitor* proteins, which are sclerotized and bound tightly to the exoskeleton. In another study, Igual et al. (2021) evaluated the amino acid release from bread enriched with *Alphitobius diaperinus*, *Tenebrio molitor*, or pea protein during *in vitro* gastrointestinal digestion. The results indicated that bread produced with 10 % *Alphitobius diaperinus* and 10 % *Tenebrio molitor* yielded the highest release values for glutamic acid, bread with 10 % *Tenebrio molitor* for histidine and proline, and bread with 10 % *Alphitobius diaperinus* for aspartic acid.

To the best of our knowledge, no studies have focused on the bioaccessibility of minerals from insect bread. In light of this, the main objective of this study was to comparatively investigate the bioaccessibility of mineral elements from insect and white bread, aiming to assess the functionality of insect bread in the mitigation of mineral deficiencies. We determined the proximate compositions of white wheat flour and the cricket and yellow mealworm powders and conducted physical–chemical investigations on white and 10 % insect bread, placing special emphasis on *in vitro* digestion to assess the bioaccessibility of minerals.

2. Materials and methods

2.1. Raw materials

Commercial white wheat flour was supplied by Baneasa (Bucharest, Romania). Salt (Salrom, Romania) and fresh yeast (Pakmaya, Romania) were purchased from the local market. Cricket (*Acheta domesticus*) powder (CP) was supplied by JR Unique Foods (Thailand). Dried yellow mealworm (*Tenebrio molitor*) powder (YMP) was supplied by Matina GmbH (Germany). HPLC grade methanol and chloroform were purchased from Sigma–Aldrich (Milan, Italy), and HPLC grade acetic acid (99–100 %) was bought from J.T. Baker B.V. (Deventer, the Netherlands). Hexane for residue analysis was supplied by Fluka-Riedel-deHaën (Milan, Italy). Anhydrous sodium sulfate, sodium chloride, and sodium hydroxide were purchased from Panreac Quimica SA (Barcelona, Spain). Deionized water (>18 MΩ·cm resistivity) was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). The standard mixture of fatty acid methyl esters (FAMEs), composed of 37 FAMEs (Supelco 37 Component FAME Mix), was purchased from Supelco (Bellefonte, PA, USA). L-amino acid analytical standards (alanine, glycine, valine, leucine, isoleucine, proline, methionine, serine, threonine, phenylalanine, aspartic acid, hydroxyproline, glutamic acid, arginine, asparagine, lysine, glutamine, histidine, tyrosine, and DL-norvaline as the internal standard, 98.5 % purity), a derivatization agent (*N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide, MTBSTFA, >99 % purity), HPLC grade solvents, hydrochloric acid (HCl, 37 %), and boron trifluoride-methanol solution (BF_3-MeOH, 14 % in methanol) were purchased from Merck (Darmstadt, Germany). The other reagents were purchased from Merck Company (USA). Milli-Q water was purified with the Millipore System (Millford, SC, USA).

2.2. Wheat flour and insect powder characterization and proximate composition analysis

Moisture content was determined by drying the sample in a drying oven (Binder GmbH, Germany) until a constant weight was achieved.

To avoid overestimation of the protein content, the non-protein nitrogen (NPN) content was first determined (as in Eq. 1), after the precipitation of proteins with trichloroacetic acid (DeVries et al., 2017). Briefly, 5 g of bread sample and water (10 % w/w) were centrifuged at 14,000*g* rpm for 1 min. 10 mL of supernatant were mixed with 10 g 24 % trichloroacetic acid (w/w), shaken vigorously for 30 s and allowed to rest for 10 min. After remixing, the solution was filtered and the total nitrogen in the filtrate ($N_{filtrate}$, %) was measured by using Kjeldahl assay (Velp Scientifica UDK 127, Italy, AOAC 945.18-B, 1995). The non-protein nitrogen (NPN, %) in the sample was calculated according to the next equation:

$$NPN(\%) = N_{filtrate} \times [(Ss + TCAs)/Ss] \times DF, \quad (1)$$

where $N_{filtrate}$ is the amount of nitrogen in the filtrate, %; *Ss* is the amount of sample solution, g; *TCAs* is the amount of trichloroacetic acid solution, g; and *DF* is the dilution factor, calculated as (g sample + g water)/g sample.

The protein content was determined by a Kjeldahl assay (Velp Scientifica UDK 127, Italy; AOAC 945.18-B, 1995) adapted to our study. The method is based on the digestion for 20 min at 420 °C of 2 g of sample dried at 105 °C until constant weight, in the presence of 7 g of K_2SO_4, 5 mg of Se powder, 12 mL 96 % H_2SO_4 and 5 mL 35 % H_2O_2. After cooling and dilution with 50 mL deionized water, the solution is distilled and the N-NH_3 is captured into 25 mL 4 % boric acid, and titrated with 0.2 N HCl in the presence of Tashiro's indicator. The results are calculated as in Equation 2:

$$\text{Protein (g/100 g on dry weight basis)} = (TN - NPN)*6.25, \quad (2)$$

where TN is the total nitrogen, %; NPN is the non-protein nitrogen, %; and 6.25 is the conversion factor of nitrogen in protein.

The total lipid content was determined by Soxhlet extraction (Velp Scientifica 148, Italy; AOAC 920.39.B, 1995). Accurate weighed 2 g of sample was subjected to preliminary acid hydrolysis at 80 °C for 40 min with 10 mL 8 N HCl. The lipids were extracted for 2 hrs in 60 mL petroleum ether. After solvent evaporation, the total lipid content was calculated on gravimetric basis, and expressed as g of lipids/100 g of dry sample.

The ash content was obtained by incineration of the samples for 24 h at 550℃ in a muffle furnace (Nabertherm Germany; AOAC 942.05, 1995) and calculated as g of ash/100 g of dry sample.

The crude fibers in the samples were quantified according to the method proposed by Commission Regulation (EC) No. 152/2009 (European Commission, 2009). It involves 5 min acid hydrolysis at boiling point for the extraction of sugars and starch from 1 g of sample, in the presence of 150 mL 0.13 mol/L H_2SO_4, followed by alkaline hydrolysis with 150 mL 0.23 mol/L KOH to remove the proteins and hemi-cellulose and lignin. The residue is filtrated, dried, weighed and ashed at 500 °C for 1 hr. The crude fibre content is calculated according to the expression:

$$\textit{Crude fibers (g/100 g)} = (m_0 - m_1)*100/m, \quad (3)$$

where *m* is the weight of the sample, g; m_0 is the loss of weight after washing, g; and m_1 is the loss of weight after ashing during the blank test, g.

The carbohydrate content was calculated by applying Equation 4 (Montowska et al., 2019):

$$\textit{Carbohydrates (g/100 g)} = 100 - (\textit{protein} + \textit{fat} + \textit{ash} + \textit{fiber}) \quad (4)$$

The energy provided by the samples (kcal/100 g) was calculated considering the conversion factors reported in Annex XIV of Regulation

(EU) No 1169/2011 for the protein, fat, carbohydrate, and fiber contents, as in Equation 5:

$$Energy\ value\ (kcal/100\ g) = 4{\cdot}carbohydrate(\%) + 4{\cdot}protein(\%) + 2{\cdot}fiber(\%) + 9{\cdot}fat(\%) \quad (5)$$

The mineral analysis involved microwave digestion (Berghof, MWS-2 method) and content quantification with a Perkin Elmer AAnalyst 800 spectrometer (Perkin Elmer, USA). 0.5 g of sample was digested in the presence of 6 mL 67 % HNO_3 and 1 mL 37 % HCl for 5 min at 145 °C and 60 % power, 10 min at 170 °C and 15 min at 200 °C at 80 % power (Berghof, MWS-2). The resulted solution was diluted to 100 mL with 67 % HNO_3 and submitted to Perkin Elmer AAnalyst 800 spectrometer (Perkin Elmer, USA) for the quantification of the minerals content. The results are expressed as mg of mineral/100 g of dry sample.

The photometric method was employed for the quantification of phosphorus level in samples (Commission Regulation (EC) No. 152/2009). Accurate weight sample of 1 g was mixed thoroughly with 20 mL of H_2SO_4 (1.84 g/mL), and keep at boiling point for 10 min. After a slightly cool, 2 mL of HNO_3 (1.38 g/mL) was added, solution was heat gently, leave to cool and after the addition of another 0.5 mL HNO_3 it was bring back to boiling point. The procedure was repeated until a colorless solution was obtained. After cooling, the solution was diluted with 5 mL of H_2O, filtered and brought up to 500 mL with hot water. 10 mL of filtrate is treated with 10 mL of molybdovanadate reagent and the optical density of the yellow solution was measured at 430 nm (UV/VIS Lambda 35 spectrophotometer, Perkin Elmer USA). The results are expressed as mg of phosphorus/100 g of dry sample.

The analysis of fatty acids was performed extracting lipids using the Folch method (Folch et al., 1957) and derivatization by transmethylation (Ichihara et al., 1996). Briefly, 1 g of sample was mixed with 20 mL of chloroform/methanol (2:1 v:v), and extracted for 3 min (Ultraturrax, IKA). The resulted mixture was filtered, mixed with 4 mL 0.73 % NaCl and shaken. After allowing the phases separation, the chloroform phase was collected, dried over sodium sulphate, filtered, and dried using a nitrogen stream. The extracts obtained from bread powder samples were dissolved in 0.5 mL of hexane, while the extracts from cricket powder and mealworm powder were dissolved in 5 mL of hexane. The transmethylation was performed on 0.5 mL extract hexane solution adding 0.1 mL 2 N methanolic solution KOH and vigorously stirring for 2 min. Aqueous acetic acid (1.5 mL, 0.15 M) was added to quench the reaction, and fatty acids methyl esters extracted with hexane. Hexane phase was separated by centrifugation, dried over sodium sulphate, filtered, and 1 μL was injected in a gas chromatograph coupled to a flame ionization detector (GC-FID, Agilent Technologies, Santa Clara, CA, USA) for analysing FAMEs. Chromatographic column was (50 %-cyanopropylphenyl)-dimethylpolysiloxane coated capillary column (DB-225, 30 m length, 0.25 mm film thickness, 0.25 mm internal diameter, from Agilent Technologies). The injection was performed in split mode (1:30) in a hot injector kept at 260 °C and the carrier gas (hydrogen) flow was 2.5 mL/min. The oven temperature programme was 40 °C for 3 min, then temperature was raised at 20 °C/min to 220 °C, held for 5 min and then raised at 20 °C/min to 240 °C that was held for 1.25 min, for a total run time of 19 min. The detector temperature was 260 °C, with hydrogen and air flows of 40 mL/min and 400 mL/min, respectively. Identification of FAMEs was performed by analysing a standard mixture composed by 37 FAMEs (Supelco 37 Component FAME Mix, from Supelco, Bellefonte, PA, USA). The results are expressed as the fatty acid % of the total fatty acids.

For the amino acids determination, an aliquot (20 mg) of freeze-dried and deffated sample was hydrolysed with 6 M HCl (500 μL) at 110 °C for 24 h. The hydrolysate was taken to dryness under speed vacuum for 1–3 h, reconstituted with 0.1 M HCl (500 μL) and centrifuged (4500 rpm, 3 min, 4 °C). Then, 50 μL of the extract was added to DL-norvaline as internal standard (50 μL of a 0.05 mg/mL solution in 0.1 M HCl), dried, added of dichloromethane (50 μL), dried again and derivatized as reported by Jiménez-Martín et al. (2012). All samples were injected (1 μL) in a GC/EI-MS (Thermo Scientific, USA) system, equipped with a split/splitless injector, single quadrupole, and a fused silica capillary column MDN-5 (30 m, 0.25 mm, 0.25 μm, Supelco, Bellefonte, PA). The chromatographic conditions were in accordance with Fico et al. (2018). Identification and quantification were carried as reported by (Pérez-Palacios et al., 2015). For the quantification, standard calibration curves of a mix amino acids (from 100 to 0.1 μg/mL) in 0.1 M HCl were prepared in function of concentration level of each amino acid and the ratio of each amino acid peak area/norvaline (internal standard) peak area (Jiménez-Martín et al., 2012). Good correlation coefficients were obtained (R^2 = 0.99–0.98). A standard stock solution (0.05 mg/mL) of norvaline internal standard was prepared in 0.1 M HCl. Then, 50 μL of each dilution was added of 50 μL of norvaline stock solution and dried under speed vacuum, added of 50 μL dichloromethane, dried and derivatized (Jiménez-Martín et al. 2012). The results are expressed as the amino acid % of the total amino acids.

2.3. Preparation and characterization of bread

Preliminary experiments were conducted by preparing white bread

Table 1
Formulae of white bread and insect-breads selected for the characterization.

Bread code	White flour, (%)	Cricket powder, (%)	Yellow mealworm powder, (%)	Yeast, (%*)	Salt, (%*)	Water, (%*)
100 % Wheat white flour bread WFB-100 %	100	0	0	1.4	0.6	75.0
Bread enriched with 5 % cricket powder CPB-5 %	95	5	0	1.4	0.6	75.6
Bread enriched with 10 % cricket powder CPB-10 %	90	10	0	1.4	0.6	76.2
Bread enriched with 15 % cricket powder CPB-15 %	85	15	0	1.4	0.6	76.8
Bread enriched with 5 % yellow mealworm powder YMPB-5 %	95	0	5	1.4	0.6	75.4
Bread enriched with 10 % yellow mealworm powder YMPB-10 %	90	0	10	1.4	0.6	75.6
Bread enriched with 15 % yellow mealworm powder YMPB-15 %	85	0	15	1.4	0.6	76.0

Yeast, salt and water are reported per 100 g of white flour and insect powder mixture (%*).
In all cases the ratio between dry matter and water was 1.1208 ± 0.0006.

and insect bread containing 5, 10, and 15 % CP and YMP (Table 1). The bread loaves were subjected to sensorial analysis, and those which received the highest scores for the overall assessment attribute were selected for further investigations. The sensory study of the bread loaves was conducted within 5 h after baking under conditions of room temperature (22 ∘C) and daylight, with a group of 68 panelists (34 women and 34 men, with ages from 18 to 60 years) who were regular bread consumers. The panelists were previously informed regarding the aim of the study, and provided written informed consent. Previous to sensorial study, the panelists were trained to develop a consensus of the descriptive vocabulary of the bread. The study was approved by the Ethics Committee of the Center for the Scientific Research into Environment, Food and Health Safety, Technical University of Cluj-Napoca (Romania), and complies with the principles of the Declaration of Helsinki as revised in 2013. The overall sensory score of each attribute was calculated as an average of the individual scores, and the final results represent the means of three independent experiments. The bread loaves containing insect powders with the highest scores assigned to the overall assessment attribute were selected for further investigations (details of the study are provided in the Supplementary Material). The white flour bread was also investigated as reference.

The moisture, protein, total lipid, ash, crude fiber, carbohydrate, mineral, energy, fatty acid, and amino acid contents or compositions were determined in the selected bread loaves, according to the methods described in Section 2.2. The rapeseed displacement method was used to measure the bread specific volume (V), calculated as in Equation 6:

$$V\ (\mathrm{cm^3/g}) = (m_1 + m_2 - m_3)*100/(m_1*\mathrm{d}), \quad (6)$$

where m_1 is the weight of the sample, g; d is the density of rapeseeds, where d = 1.02 g/cm^3; m_2 is the weight of the container filled with rapeseeds, g; and m_3 is the weight of the container with the sample and rapeseeds, g.

The crust firmness was evaluated on a 15-point scale at the minimum (Wagner FDK10 force dial penetrometer, Wagner Instruments, USA), and the average values were reported.

The height (H) and diameter (D) of each bread loaf were measured at five different positions of the bread using a caliper, and a H/D index was calculated based on the average values.

2.4. In vitro digestion for the bioaccessibility of mineral elements

In vitro digestion of the selected bread was carried out in triplicate considering the oral, gastric, and intestinal stages, according to the INFOGEST protocol described by Brodkorb et al. (2019). Details regarding the composition of simulated bodily fluids and the digestion procedure are provided in the Supplementary Material. Sampling was conducted at different time points during digestion. To ensure a higher reproducibility of the results, the digestion procedure was started with individual tubes for each phase and time point. Samples taken at different time points received heat shock treatment in boiling water for 5 min to ensure enzyme inactivation and were centrifuged at 6,000 × g for 30 min to separate the supernatant containing the bioaccessible fraction. Accurately measured 15-mL portions of the supernatant were diluted to 25 mL with distilled water and analyzed for mineral content, as outlined in Section 2.2. To exclude the contribution of minerals contained in the digestion fluids, blank digestions were run, and the mineral concentrations in the resultant fluids were measured at the same time points as in the case of bread digestion. The final results were calculated as the difference between the mineral amounts in the sample and blank digestion fluids.

The bioaccessibility of minerals was expressed both as (i) the amount of minerals released through *in vitro* digestion from 100 g of dried bread (mg/100 g) and (ii) the bioaccessible fractions measured as the percentage of minerals released from the total content in the bread samples (Eq. 7):

$$\text{Bioaccessible fraction}\ (\%) = (\mathrm{M_{SD}} - \mathrm{M_{SB}})*100/\mathrm{M_{UB}}, \quad (7)$$

where M_{SD} and M_{SB} are the amounts of minerals in the supernatant of the digested bread and the blank digestions, respectively, mg/100 g; and M_{UB} is the total amount of minerals in the undigested bread sample, mg/100 g.

The analysis protocols are detailed in the Supplementary Material.

3. Statistical analysis

At least three independent runs were performed in each experiment, and at least three measurements were conducted in each replication. The data are reported as the mean values ± standard deviation (SD). A one-way ANOVA (IBM SPSS Statistics 24) was used to assess the difference between the means, and the probability value of $p < 0.05$ was considered statistically significant.

4. Results and discussion

4.1. Proximate compositions of wheat flour and insect powders

Table 2 summarizes the proximate compositions of the wheat flour and insect powders. With respect to the protein and total lipid contents, the insect powders had significantly higher values ($p < 0.05$) than those measured in the white wheat flour (WF). The proteins in WF, mainly represented by gluten, are directly related to the functional properties of dough essential for the quality of bread in terms of strength, viscosity, elasticity, and specific volume. The protein content in CP is 30.75 % higher than in YMP, while the total lipid content in YMP is 48.14 % higher than in CP. In a previous study, CP was characterized by a significantly higher protein content (62.16 %) compared to other flours with protein contents, ranging from 11.52 % for WF to 55.67 % for buffalo worm flour (Kowalski et al., 2022). Mainly represented by free ammonia and amino nitrogen, the NPN in WF arises from the combined actions of protein or peptide breakdown mediated by native flour enzymes or microbial populations, the release of amino groups from the association with polysaccharides, or the loss of approximately-one amide group per molecule of gluten protein (Bell, 2006). NPN was not detected in the WF used in this study, while relatively equal amounts of NPN, related to the nucleic acids, chitin, phospholipids, and excretion products (e.g., ammonia) in the intestinal tract, were found in the insect powders (Janssen et al., 2017). The ash content in the CP (3.75 g/100 g) in this study is lower than the value of 4.41 g/100 g reported by Brogan et al. (2021), while that in the YMP (4.88 g/100 g) is comparable to the value of 4.25 g/100 g reported by Gonzales and Rosell (2019). The fiber content in the insect powders consists primarily of chitin and sclerotized proteins as components of the insect exoskeleton (Oonincx & Finke, 2021). Concentrated in wheat bran, the fibers are removed during the milling process, which explains their low quantities in the WF (0.56 g/100 g). Contrary to their higher levels of proteins, lipids, and minerals as compared to WF, the insect powders contain lower amounts of carbohydrates; their level in the YMP (8.89 g/100 g) is higher than the values ranging between 1 % and 7 % reported by Ramos-Elorduy et al. (2002), while that in the CP (7.52 g/100 g) is lower than the amount of 12.33 g/100 g obtained by González et al. (2019).

One can observe a higher level of minerals in the insect powders as compared to WF (Table 2), with the minerals in WF mostly removed with bran during milling. The levels of Ca, Mg, Na, and K in the CP are comparable with the values reported by Montowska et al. (2019). Higher levels of these minerals were found in the YMP than in the CP. Generally, the literature has reported Ca levels of <0.3 % in insects because of their lack of a mineralized skeleton (Oonincx & Finke, 2021). A higher amount of Ca could be related to the dietary Ca remaining in the gut. Reports have indicated a higher level of K than Na in insects (Oonincx & Finke, 2021), and our study confirmed this for both insect

Table 2
Proximate analysis of white wheat flour, cricket and yellow mealworm powders and bread loaves obtained by replacing 10% of white flour with cricket and yellow mealworm powders, respectively.

Parameter		Raw materials			Selected bread loaves		
		Wheat white flour WF	Cricket powder CP	Yellow mealworm powder YMP	100 % White wheat flour bread WFB-100 %	Bread enriched with 10 % cricket powder CPB-10 %	Bread enriched with 10 % yellow mealworm powder YMPB-10 %
Dry matter g/100 g		91.53 ± 2.74 (a)	97.96 ± 1.94 (a)	95.20 ± 3.49 (a)	64.05 ± 1.08 (a)	65.92 ± 4.02 (a)	63.05 ± 0.42 (a)
Protein, g/100 g		10.78 ± 0.30 (c)	67.21 ± 3.51 (a)	46.54 ± 2.23 (b)	10.62 ± 0.53 (c)	15.99 ± 1.01 (a)	13.76 ± 0.42 (b)
Non-protein nitrogen, g/100 g		nd	0.65 ± 0.03 (a)	0.64 ± 0.03 (a)	nd	0.03 ± 0.00 (a)	0.03 ± 0.00 (a)
Total lipids, g/100 g		0.92 ± 0.06 (c)	18.39 ± 0.82 (b)	35.46 ± 0.96 (a)	0.62 ± 0.01 (c)	1.82 ± 0.08 (b)	2.71 ± 0.11 (a)
Ash, g/100 g		0.71 ± 0.04 (c)	3.75 ± 0.20 (b)	4.88 ± 0.27 (a)	1.43 ± 0.08 (b)	1.71 ± 0.02 (a)	1.84 ± 0.09 (a)
Crude fiber, g/100 g		0.56 ± 0.09 (c)	3.14 ± 0.10 (b)	4.23 ± 0.15 (a)	0.74 ± 0.03 (b)	0.88 ± 0.05 (a)	0.90 ± 0.04 (a)
Carbohydrates, g/100 g		87.04 ± 0.52 (a)	7.52 ± 0.32 (b)	8.89 ± 0.52 (b)	82.40 ± 1.07 (a)	73.99 ± 2.75 (b)	74.46 ± 1.71 (b)
Energy, Kcal/100 g		400.69 ± 10.31 (c)	470.67 ± 7.81 (b)	549.30 ± 11.63 (a)	379.08 ± 5.73 (a)	378.04 ± 7.37 (a)	379.04 ± 4.24 (a)
Specific volume, cm^3/g		–	–	–	2.25 ± 0.06 (a)	2.08 ± 0.12 (ab)	1.89 ± 0.05 (b)
Hight/Diameter ratio (H/D)		–	–	–	1.03 ± 0.04 (a)	0.86 ± 0.03 (b)	0.79 ± 0.05 (b)
Failure force, Kgf		–	–	–	10.03 ± 0.62 (ab)	11.25 ± 0.46 (a)	9.14 ± 0.51 (b)
Mineral elements, mg/100 g	Na	185.18 ± 2.39 (c)	212.35 ± 2.20 (b)	255.42 ± 3.74 (a)	427.74 ± 9.57 (a)	433.20 ± 9.32 (a)	433.35 ± 4.07 (a)
	K	128.72 ± 2.65 (c)	566.94 ± 4.04 (b)	872.14 ± 7.08 (a)	151.50 ± 2.55 (c)	197.23 ± 12.95 (b)	226.13 ± 2.95 (a)
	Ca	45.82 ± 1.99 (c)	123.65 ± 3.16 (b)	225.07 ± 4.72 (a)	42.92 ± 1.85 (c)	50.85 ± 2.38 (b)	60.82 ± 1.73 (a)
	Mg	30.97 ± 2.22 (c)	51.88 ± 3.18 (b)	324.36 ± 3.55 (a)	31.44 ± 1.66 (b)	33.30 ± 1.65 (b)	60.04 ± 2.08 (a)
	P	103.46 ± 3.36 (c)	254.23 ± 12.69 (a)	216.32 ± 3.20 (b)	75.20 ± 2.54 (a)	78.96 ± 3.43 (a)	78.00 ± 2.03 (a)
	Cu	0.18 ± 0.01 (c)	2.25 ± 0.11 (b)	18.65 ± 0.55 (a)	0.18 ± 0.01 (c)	0.39 ± 0.02 (b)	1.98 ± 0.10 (a)
	Zn	0.51 ± 0.02 (c)	16.64 ± 1.00 (b)	53.05 ± 2.71 (a)	0.58 ± 0.03 (c)	2.25 ± 0.14 (b)	5.81 ± 0.16 (a)
	Mn	0.73 ± 0.05 (c)	3.26 ± 0.15 (b)	12.37 ± 0.23 (a)	0.70 ± 0.02 (c)	0.97 ± 0.01 (b)	1.86 ± 0.07 (a)
	Fe	1.99 ± 0.10 (c)	5.83 ± 0.18 (b)	27.24 ± 1.89 (a)	2.12 ± 0.09 (c)	2.53 ± 0.13 (b)	4.65 ± 0.12 (a)
	Li	0.26 ± 0.01 (c)	1.39 ± 0.09 (a)	0.88 ± 0.05 (b)	0.25 ± 0.01 (c)	0.36 ± 0.21 (a)	0.30 ± 0.01 (b)

Samples are coded as reported in the Table 1; Results are presented as mean values ± standard deviations ($n \geq 3$); nd- not detected.
Different letters within the same row indicate significant differences ($p < 0.05$) between mean values (Tukey test).

powders: in CP, K prevails, followed by P and Na; K is predominant in YMP, followed by Mg and Na. Significantly different ($p < 0.05$) values of P concentrations were measured in the insect powders, in agreement with Koutsos et al.'s (2019) study.

From the insect powders investigated, the YMP presented the richest source of Zn, Fe, Cu, and Mn, the amounts of which are significantly higher than the values reported by Koutsos et al. (2019) in mealworm larvae. Similarly, the concentrations of these minerals in the CP investigated herein are higher than those reported in the same study. The positive correlation between the concentrations of Fe and Zn in the investigated insect powders agrees with the study of Mwangi et al. (2018). The highest level of Zn in YMP (53.05 mg/100 g) is accompanied by the highest concentration of Fe (27.24 mg/100 g). Li, a trace element with a role in the transport and distribution of vitamin B12, was also identified in the WF and insect powders. As presented in Table 2, the WF is a poorer source of Li as compared to the insect powders: the Li content in the CP and YMP is 5.35- and 3.38-fold higher than in the WF, respectively.

The WF and insect powders differ significantly in their fatty acid composition (Table 3). The fatty acid profile in the WF is dominated by linoleic acid C18:2, *n*-6 (62.31 %), as Roncolini et al. (2020) have previously reported, followed by palmitic acid C16:0 (17.33 %); oleic acid C18:1, *n*-9 (12.88 %); and linolenic acid C18:3, *n*-3 (4.25 %). Fatty acids such as stearic C18:0; gondoic C20:1, *n*-9; caprylic C8:0; behenic C22:0; capric C10:0; myristic C14:0; palmitoleic C16:1, *n*-7; and pentadecanoic C15:0 acids are present in smaller amounts. Linoleic acid C18:2, *n*-6 also prevails in the CP (34.15 %) and in the YMP (40.28 %). In the insect powders, larger percentages of C14:0; C16:1, *n*-7; C18:0; and C18:1, *n*-9 were found as compared to the WF. In addition, arachidic acid C20:0, not detected in the WF, was identified in the CP and YMP. The polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) ratio is higher in the WF as compared to the insect powders; in CP, it was measured as 0.92, very close to the value of 0.89 reported by Kowalski et al. (2022), while in YMP, the value of 1.79 obtained herein is much higher than the value 0.59 reported by the same authors. The ratio *n*-6/*n*-3 exceeds the recommended range of 1:1 to 1:5, varying from 39.77 in CP to 21.40 in YMP and 14.66 in WF, suggesting relatively low amounts of *n*-3 fatty acids.

Table 4 shows the amino acid profiles in the WF and insect powders, with the WF showing a predominance of glutamic acid, leucine, and proline while the insect powders were richer in arginine, leucine, and alanine. Considering only essential amino acids, the CP displays higher percentages of valine and methionine, while the YMP is a richer source of methionine and tyrosine, as compared to the WF. In agreement with

Table 3
Fatty acids profiles in white wheat flour, cricket and yellow mealworm powders and breads obtained by replacing 10% of white flour with cricket and yellow mealworm, respectively powders.

Fatty acid % of total fatty acids ± SD							
Fatty acid		Wheat white flour WF	Cricket powder CP	Yellow mealworm powder YMP	White bread WFB-100 %	Bread enriched with 10 % cricket powder CPB-10 %	Bread enriched with 10 % yellow mealworm powder YMPB-10 %
Caprylic	C8:0	0.33 ± 0.01	nd	nd	0.39 ± 0.01 (b)	0.34 ± 0.00(c)	0.44 ± 0.02(a)
Capric	C10:0	0.24 ± 0.04	nd	nd	nd	nd	nd
Lauric	C12:0	nd	nd	0.26 ± 0.01	nd	nd	nd
Myristic	C14:0	0.19 ± 0.00 (c)	0.60 ± 0.02 (b)	2.26 ± 0.04(a)	nd	1.01 ± 0.00(a)	0.45 ± 0.03(b)
Pentadecanoic	C15:0	0.12 ± 0.00 (b)	0.07 ± 0.00 (c)	0.21 ± 0.00(a)	nd	nd	nd
Palmitic	C16:0	17.33 ± 0.05 (b)	27.04 ± 0.15 (a)	17.32 ± 0.15(b)	12.72 ± 0.07 (c)	14.26 ± 0.09(b)	19.89 ± 0.14(a)
Stearic	C18:0	1.30 ± 0.00 (c)	9.55 ± 0.01 (a)	3.37 ± 0.01(b)	4.78 ± 0.00 (b)	4.71 ± 0.02(c)	7.18 ± 0.02(a)
Arachidic	C20:0	nd	0.21 ± 0.00 (a)	0.1 ± 0.00(b)	0.35 ± 0.00 (a)	0.17 ± 0.24(a)	0.33 ± 0.01(a)
Behenic	C22:0	0.30 ± 0.03 (a)	0.21 ± 0.02 (b)	nd	0.90 ± 0.06 (a)	0.67 ± 0.01(b)	0.54 ± 0.04(c)
Myristoleic	C14:1, *n*-5	nd	nd	0.08 ± 0.00	0.30 ± 0.01 (a)	0.13 ± 0.19(a)	nd
Palmitoleic	C16:1, *n*-7	0.19 ± 0.00 (c)	0.76 ± 0.02 (b)	1.61 ± 0.03(a)	0.43 ± 0.01 (c)	0.79 ± 0.00(a)	0.57 ± 0.00(b)
Oleic	C18:1, *n*-9	12.88 ± 0.02 (c)	26.56 ± 0.10 (b)	32.38 ± 0.08(a)	31.28 ± 0.02 (b)	34.30 ± 0.31(a)	29.96 ± 0.05(c)
Gondoic	C20:1, *n*-9	0.57 ± 0.01 (a)	nd	0.24 ± 0.00(b)	0.33 ± 0.02 (a)	nd	0.25 ± 0.02(b)
Linoleic	C18:2, *n*-6	62.31 ± 0.01 (a)	34.15 ± 0.01 (c)	40.28 ± 0.13(b)	47.70 ± 0.01 (a)	42.89 ± 0.15(b)	39.59 ± 0.12(c)
Linolenic	C18:3, *n*-3	4.25 ± 0.05 (a)	0.86 ± 0.05 (c)	1.88 ± 0.01(b)	0.82 ± 0.02 (a)	0.74 ± 0.01(a)	0.81 ± 0.06(a)
ΣSFA		**19.80 ± 0.02 (c)**	**37.67 ± 0.14(a)**	**23.52 ± 0.19(b)**	**19.14 ± 0.01 (c)**	**21.15 ± 0.27(b)**	**28.83 ± 0.21(a)**
ΣMUFA		**13.64 ± 0.02 (c)**	**27.32 ± 0.08(b)**	**34.32 ± 0.05(a)**	**32.34 ± 0.04(b)**	**35.22 ± 0.12(a)**	**30.77 ± 0.03(c)**
ΣPUFA		**66.57 ± 0.04 (a)**	**35.01 ± 0.06(c)**	**42.17 ± 0.14(b)**	**48.52 ± 0.03(a)**	**43.63 ± 0.14(b)**	**40.40 ± 0.17(c)**
***n-6/n-3* ratio**		**14.66 ± 0.17 (c)**	**39.77 ± 2.23(a)**	**21.40 ± 0.06(b)**	**58.53 ± 1.26(a)**	**57.63 ± 1.01(a)**	**49.19 ± 3.26(b)**
PUFA/SFA ratio		**3.36**	**0.92**	**1.79**	**2.53**	**2.06**	**1.40**
AI index		**–**	**–**	**–**	**0.23**	**0.47**	**0.35**
TI index		**–**	**–**	**–**	**0.37**	**1.12**	**0.53**
h/H index		**–**	**–**	**–**	**4.53**	**2.23**	**3.81**

Results are presented as means ± standard deviations of triplicate independent experiments. Samples are codified as reported in Table 1. Results are presented as mean values ± standard deviations ($n \geq 3$); nd – not detected; SFA- Saturated Fatty Acids; MUFA – Monounsaturated Fatty Acids; PUFA – Polyunsaturated Fatty Acids.
AI (atherogenic index) = (C12:0 + 4 × C14:0 + C16:0)/(Σn-6 + Σn-3 + ΣMUFA); TI (thrombogenic index) = (C14:0 + C16:0 + C18:0)/[0.5 × ΣMUFA + 0.5 × Σn-6 + 3 × Σn-3 + (Σn-3/Σn-6)]; h/H (hypocholesterolemic/hypercholesterolemic ratio) = (C18:1 + C18:2 + C18:3 + C20:2 + C20:4 + C20:5 + C22:5 + C22:6)/(C14:0 + C16:0).
Different letters within the same row indicate significant differences ($p < 0.05$) between mean values (Tukey test).

Boulos et al. (2020), mealworms and crickets had similar amino acid profiles, where the authors found true protein contents of 51 and 55 g/100 g (dry weight basis), respectively. In our study, the protein contents were 46.54 (mealworm) and 67.21 (cricket) g/100 g powder. This difference falls within the framework of the large variability that can occur in the proximate composition of edible insects, depending on the insect species, developmental stage, and feed composition (Oonincx & Finke, 2021).

4.2. Characterization of white bread and selected insect bread

The sensory analysis (Section 3.1.SM in the Supplementary Material) of the prepared bread indicates that the insect bread containing 5 and 10 % CP and YMP received the same scores for the overall assessment. As a result, the 10 % insect bread loaves were selected for further investigation as they showed a higher concentration of nutrients in comparison with the 5 % insect bread. Bread made entirely from WF (WFB-100 %) was also investigated as a reference.

4.2.1. Proximate analysis of selected bread

The results of the proximate analysis carried out on the selected breads are displayed in Table 2. The inclusion of insect powders led to higher amounts of proteins, lipids, and fibers in the obtained bread as compared to white bread. On the other hand, the amount of carbohydrates is significantly reduced in the insect bread, lower by 10.21 % in the 10 % cricket powder bread (CPB-10 %) and 9.64 % in the 10 % yellow mealworm powder bread (YMPB-10 %), as compared to WFB-100 %. Notably, no significant differences in the fiber and carbohydrate contents between the two insect breads were observed. A reduction in the specific volumes of the insect bread was expected, considering the dilution of the gluten network that alters the gas-retention ability of dough. However, no significant difference ($p < 0.05$) exists between the specific volumes of the WF and cricket breads, or between the two insect breads. With the addition of insect powders, the maximum height (*H*) of

Table 4
Amino acids profiles in white wheat flour, cricket and yellow mealworm powders and bread loaves obtained by replacing 10% of white flour with cricket and yellow mealworm, respectively powders.

Amino acid % of total amino acids ± SD						
Amino acid	Wheat white flour WF	Cricket powder CP	Yellow mealworm powder YMP	White bread WFB-100 %	Bread enriched with 10 % cricket powder CPB-10 %	Bread enriched with 10 % yellow mealworm powder YMPB-10 %
Essential amino acids (EAA) % of total amino acids						
Valine	8.26 ± 0.45 (b)	11.42 ± 1.10 (a)	9.74 ± 1.75 (ab)	7.94 ± 0.44 (b)	9.72 ± 0.38 (a)	8.80 ± 0.49 (ab)
Leucine	16.23 ± 0.56 (a)	19.83 ± 1.01 (a)	17.65 ± 0.18 (a)	16.91 ± 0.69 (a)	16.68 ± 2.69 (a)	16.60 ± 1.08 (a)
Isoleucine	7.82 ± 0.60 (a)	9.76 ± 0.61 (a)	8.38 ± 1.26 (a)	8.00 ± 0.41 (a)	8.71 ± 1.24 (a)	8.27 ± 0.92 (a)
Methionine	0.05 ± 0.04 (b)	0.28 ± 0.07 (a)	0.23 ± 0.09 (a)	0.06 ± 0.03 (a)	0.02 ± 0.00 (a)	0.02 ± 0.00 (a)
Threonine	1.67 ± 1.03 (a)	0.58 ± 0.12 (b)	1.75 ± 0.41 (a)	2.25 ± 0.08 (a)	2.59 ± 0.22 (a)	2.53 ± 0.19 (a)
Phenylalanine	5.66 ± 1.00 (a)	4.62 ± 0.23 (a)	5.39 ± 0.14 (a)	6.53 ± 0.25 (a)	5.86 ± 1.20 (a)	5.99 ± 0.20 (a)
Lysine	0.02 ± 0.02 (a)	0.01 ± 0.00 (a)	0.06 ± 0.05 (a)	0.03 ± 0.03 (a)	0.13 ± 0.19 (a)	0.05 ± 0.03 (a)
Histidine	0.03 ± 0.03	nd	nd	0.05 ± 0.07 (a)	0.30 ± 0.50 (a)	0.05 ± 0.04 (a)
Tyrosine	2.22 ± 1.29 (b)	2.48 ± 2.16 (b)	12.30 ± 0.95 (a)	1.27 ± 0.08 (b)	1.57 ± 0.23 (ab)	1.86 ± 0.31 (a)
total EAA	**41.99 ± 0.34 (b)**	**49.01 ± 3.99 (ab)**	**55.54 ± 3.59 (a)**	**43.07 ± 1.38 (a)**	**45.61 ± 5.24 (a)**	**44.21 ± 2.12 (a)**
Nonessential amino acids (NEAA) % of total amino acids						
Alanine	5.45 ± 1.45 (c)	12.51 ± 0.90 (a)	9.83 ± 0.10 (b)	4.83 ± 0.28 (a)	6.66 ± 1.09 (a)	6.91 ± 0.30 (a)
Glycine	1.66 ± 0.25 (a)	1.91 ± 0.38 (a)	2.22 ± 0.72 (a)	1.99 ± 0.19 (a)	2.32 ± 1.10 (a)	2.17 ± 0.25 (a)
Proline	11.30 ± 0.37 (a)	4.01 ± 0.41 (b)	4.81 ± 0.76 (b)	14.15 ± 3.52 (a)	11.41 ± 2.73 (a)	12.6 ± 1.18 (a)
Serine	2.54 ± 1.24 (a)	1.35 ± 0.09 (b)	1.69 ± 0.92 (ab)	3.10 ± 0.26 (ab)	3.25 ± 0.84 (a)	3.26 ± 0.57 (a)
Aspartic acid	2.03 ± 0.19 (a)	2.90 ± 0.64 (a)	2.82 ± 1.26 (a)	1.77 ± 0.06 (a)	2.41 ± 0.42 (a)	2.34 ± 0.29 (a)
Hydroxyproline	0.07 ± 0.05 (a)	0.11 ± 0.02 (a)	0.06 ± 0.01 (a)	0.03 ± 0.01 (a)	0.05 ± 0.01 (a)	0.04 ± 0.01 (a)
Glutamic acid	30.69 ± 0.03 (a)	4.79 ± 1.12 (b)	5.83 ± 2.23 (b)	25.89 ± 0.70 (a)	22.17 ± 0.54 (b)	24.03 ± 2.09 (ab)
Asparagine	0.01 ± 0.01 (a)	0.01 ± 0.00 (a)	0.01 ± 0.00 (a)	0.01 ± 0.00 (a)	0.08 ± 0.00 (a)	0.01 ± 0.01 (a)
Glutamine	nd	nd	nd	nd	nd	nd
Arginine	4.20 ± 0.16 (b)	23.37 ± 4.72 (a)	17.14 ± 0.58 (a)	5.11 ± 0.34 (ab)	6.03 ± 0.53 (a)	4.38 ± 0.17 (b)
total NEAA	**57.98 ± 0.35 (a)**	**50.99 ± 3.69 (b)**	**44.46 ± 5.72 (b)**	**56.91 ± 1.38 (a)**	**54.39 ± 2.98 (a)**	**55.78 ± 2.11 (a)**

Results are presented as means ± standard deviations of triplicate independent experiments. Samples are codified as reported in Table 1.
Results are presented as mean values ± standard deviations (n ≥ 3);
Different letters within the same row indicate significant differences ($p < 0.05$) between mean values (Tukey test).

the resultant bread decreased while the diameter (*D*) increased, leading to ratios of $H/D < 1$. The crust hardness of insect bread is not significantly different from that of WF bread, supported by the absence of significant differences between the dry matter of breads.

Table 3 reports the fatty acid profiles of the investigated breads. As expected, linoleic acid C18:2, *n*-6 (47.70 %) is the most abundant acid in the white bread; PUFAs and MUFAs account for 48.52 % and 32.34 %, respectively, while SFAs represent 19.14 % of the total fatty acids. Linoleic acid also prevails in the cricket (42.89 %) and yellow mealworm (39.59 %) bread; the PUFAs decreased to 43.63 % and 40.40 %, while the MUFAs increased to 35.22 % and decreased to 30.77 %, respectively, as compared to the white bread. The SFAs increased to 21.15 % and 28.83 %, respectively, mainly due to the increase in the palmitic and stearic acid contents. Higher amounts of PUFAs in white bread are correlated with lower oxidative stability. The white bread herein is characterized by higher *n*-6/*n*-3 and PUFA/SFA ratios than the insect bread, with a consequently different lipid nutritional quality. In all the investigated loaves of bread, the ratio *n*-6/*n*-3 exceeds the recommended range of 1:1 to 1:5. Insect bread displays higher atherogenic (AI) and thrombogenic (TI) indices than white bread. AI and TI values higher than 1.00, and PUFA/SFA ratios >0.4 are considered appropriate for a healthy dietary oil/fat intake (Roncolini et al., 2020). In our study, the cricket bread shows a TI value (1.12) higher than the recommended value.

Regarding the protein content, as one may expect, differences were found in the breads obtained from the enrichment of white bread with insect powder. The production of bread based entirely on CP or YMP exhibits 15.99 % and 13.76 % protein levels, respectively, in contrast to the 10.62 % protein represented in WFB-100 %. While the amino acid profile in bread enriched with CP and YMP shows slight variations between the samples (Table 4), a significant difference is observed for some amino acids in relation to the addition of insects to bread. The level of essential amino acids (EAAs) reaches 43.07 % in WF, while CP and YMP show higher percentages of 45.61 % and 44.21 %, respectively. However, WF shows a higher level (56.91 %) of non-essential amino acids (NEAAs) in comparison to 55.78 % and 54.39 % in YMP and CP, respectively. Similar trends in the EAA and NEAA profiles of WFB-100 %, CPB-10 %, and YMPB-10 % can be observed, but these are not

statistically significant. Current studies have revealed that the addition of 10 % insect powder, independent of the insect species, is not sufficient to cause a significant difference in the amino acid profile. In our study, the exceptions occur in the cases of valine and tyrosine (EAAs) which increase in CBB-10 % and YMPB-10 %, after the addition of CP and YMP, respectively. The glutamic acid (a NEAA) decreased in the YMPB-10 %. Osimani et al. (2018) found that the addition of 10 % and 30 % CP enhanced the nutritional value of bread in terms of EAAs (threonine, tyrosine, valine, methionine, and lysine). Such differences in the literature could be due to the variability in bread preparation (i.e., leavening, WF composition, and cooking parameters), insect powder variability (species, developmental stage, and feeding), and amino acid determinations, which, in this study, was performed with gas chromatography coupled with mass spectrometry rather than the HPLC method. For instance, the differences between the amino acid contents of the WF and insect breads could depend on the combination of the proteolytic activity of the microbiota of the dough, and the activation of proteases in the flour (Gobbetti et al., 2019).

The addition of insect powders enriches the insect breads with regard to some mineral elements (Table 2). Among the macrominerals, Na displays the highest levels in all investigated breads. The levels of K and Ca are significantly higher in the insect breads compared to white bread, and also notably higher in yellow mealworm bread relative to cricket bread. Although the insect powders are richer sources of P than WF, their incorporation (10 %) into dough did not lead to insect bread with significantly increased amounts of P as compared to white bread. According to European Food Safety Authority (EFSA, 2015), the effective absorption of Ca in Caucasian adults requires a Ca:P molar ratio ranging between 1.4:1 and 1.9:1. The Ca:P molar ratios of 1:2.26 in the white bread, 1:2.00 in the cricket bread, and 1:1.65 in the yellow mealworm bread do not fall within the reference range, suggesting the difficult absorption of Ca. The WFB-100 % and CPB-10 % display almost equal contents of Mg, while YMPB-10 % contains double the amount. The WFB-100 % contains lower concentrations of the microelements Cu, Zn, Mn, Fe, and Li than the insect breads, and between the latter, CPB-10 % contains a higher amount of Li while YMPB-10 % has larger amounts of Cu, Zn, Mn, and Fe.

4.2.2. *Bioaccessibility of mineral elements in selected bread*

Considering the mineral element concentrations, one can assume that CPB-10 % and YMPB-10 % are richer sources of K, Ca, Cu, Zn, Mn, Fe, and Li compared to WFB-100 %. Their functionality in the mitigation of mineral deficiencies depends on the minerals' bioaccessibility. Table 5 presents the amounts of mineral elements released from the investigated breads after *in vitro* digestion. The results were calculated as the difference between the amount of minerals in the sample and blank digestion fluids (Tables 3.SM and 4.SM in the Supplementary Material). Values associated with oral digestion are not displayed, as the short contact time between the breads and the simulated salivary fluid (SSF) resulted in insignificant differences between the amount of minerals in the sample saliva and SSF.

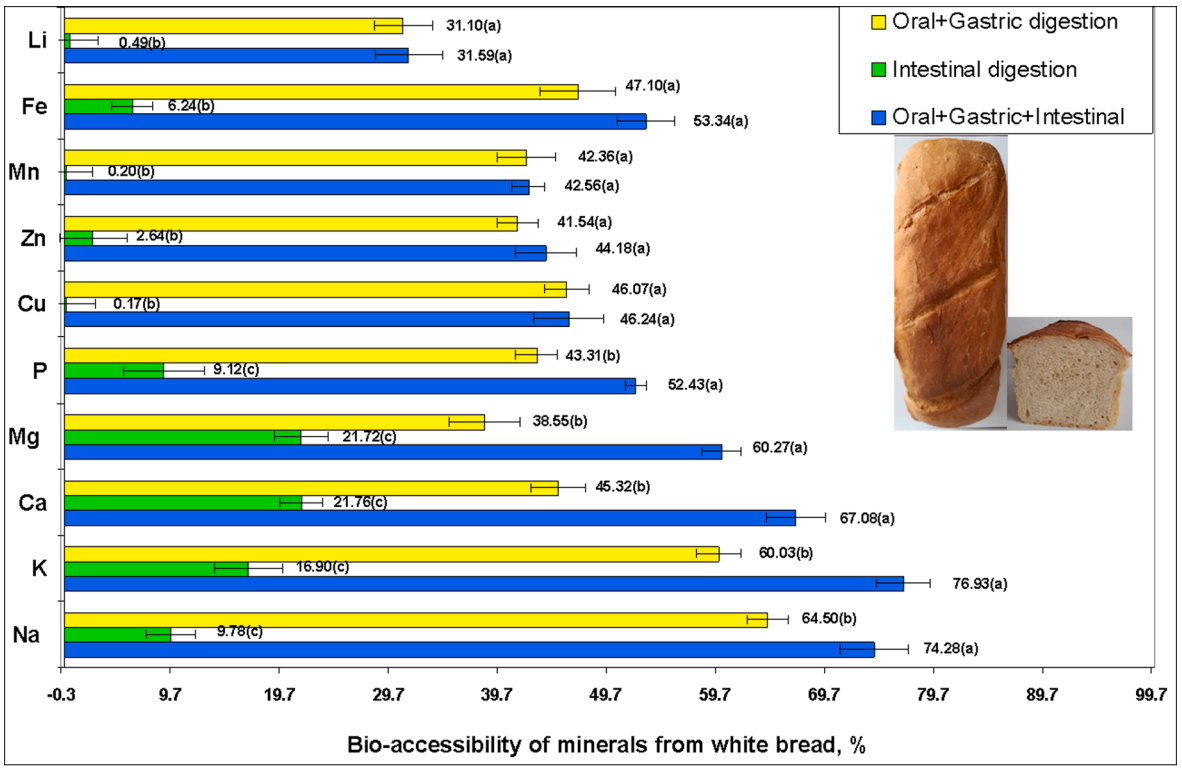
During the *gastric digestion*, CPB-10 % released the highest amounts of Na and P (355.80 mg/100 g and 44.15 mg/100 g, respectively), while WFB-100 % solubilized the lowest amounts (275.89 mg/100 g and 32.57 mg/100 g, respectively). The bioaccessible K, Ca, and Mg contents in YMPB-10 % (175.77 mg/100 g, 39.61 mg/100 g, and 36.70 mg/100 g, respectively) were higher than in CPB-10 % (147.96 mg/100 g, 36.53 mg/100 g, and 20.62 mg/100 g, respectively) and WFB-100 % (90.95 mg/100 g, 19.45 mg/100 g, and 12.12 mg/100 g, respectively). The levels of the soluble microminerals of Cu, Zn, Mn, and Fe were highest in YMPB-10 % and lowest in WFB-100 %, and the released amounts of Li were similar in CPB-10 % and YMPB-10 % and lower in WFB-100 %. With the exception of Cu, during the *intestinal digestion*, additional bioaccessible mineral amounts were released. In the intestinal liquid, the amounts of Na, K, and Ca increased respectively by 15.16 %, 28.15 %, and 48.02 % in WFB-100 %; 15.66 %, 23.87 %, and 33.51 % in YMPB-10 %; and 4.68 %, 9.28 %, and 33.82 % in CPB-10 %. Approximately equal increases in Mg were obtained in WFB-100 % (56.32 %) and CPB-10 % (56.96 %), with a lower increase in YMPB-10 % (44.96 %). The P and Li levels in the intestinal liquid rose the most in CPB-10 % (23.35 % and 61.34 %, respectively), and increased to a lesser extent in YMPB-10 % (17.28 %) and WFB-100 % (1.28 %). Larger amounts of Mn were released from the insect breads during intestinal digestion than in gastric digestion. Cu is the only mineral for which reductions in concentration occurred after the intestinal digestion of insect bread, suggesting a significant precipitation of Cu ions, with the largest reduction (-90 %) in the case of YMPB-10 %. In the case of WFB-100 %, a slight increase of 5.81 % in bioaccessible Cu was noticed.

The *bioaccessible fractions* of the investigated minerals, displayed in Fig. 1 a to d, indicate that the total amounts of minerals in bread are not released in an absorbable form during digestion. Since the solubility of mineral compounds from food is facilitated by an acidic medium with the release of metallic cations, the acid released during the gastric stage improves the bioaccessibility of minerals. The mineral level at the end of the intestinal phase is the result of two simultaneous processes occurring due to the rise in pH from the buffering effect exerted by the meal,

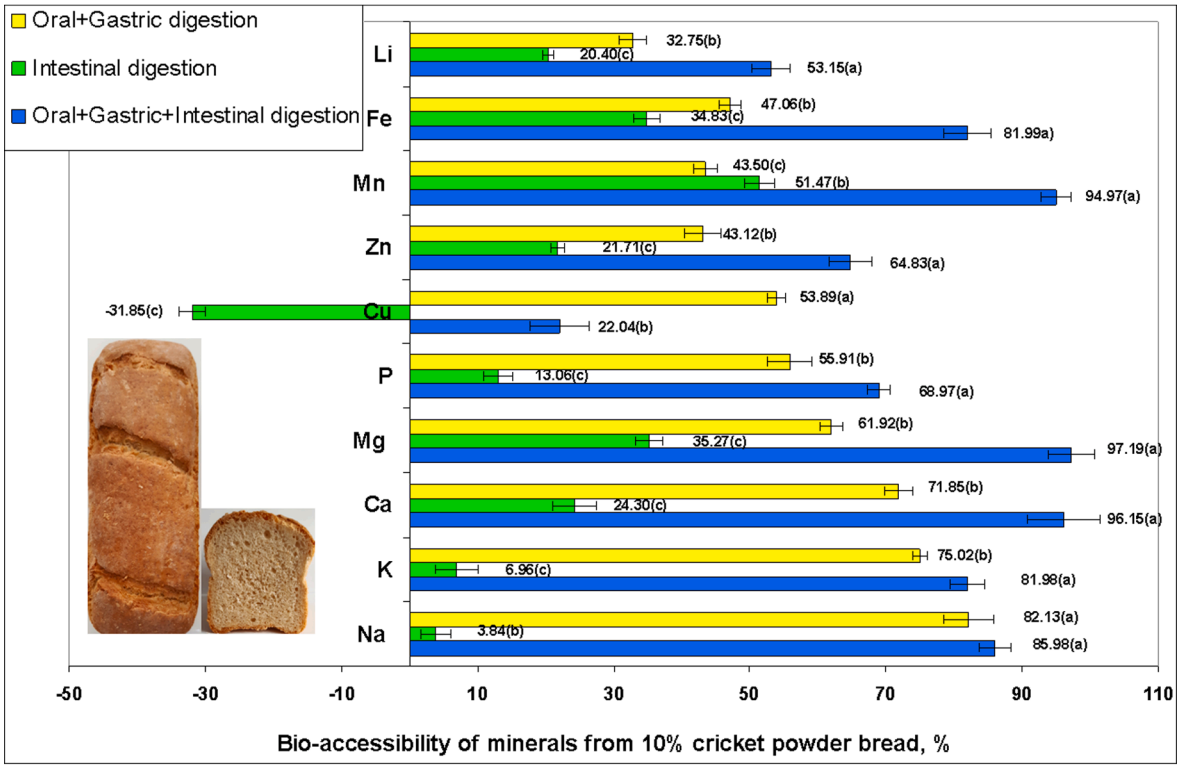
Table 5
Bioaccessibility of mineral elements from white bread and breads enriched with 10% cricket and yellow mealworm powders during *in vitro* digestion.

Mineral composition, mg/100 g	Bioaccessible minerals resulted from *in vitro* digestion of white bread WFB-100 %		Bioaccessible minerals resulted from *in vitro* digestion of cricket-bread CPB-10 %		Bioaccessible minerals resulted from *in vitro* digestion of yellow mealworm-bread YMPB-10 %	
	Oral + Gastric stage	Oral + Gastric + Intestinal stage	Oral + Gastric stage	Oral + Gastric + Intestinal stage	Oral + Gastric stage	Oral + Gastric + Intestinal stage
Na	275.89 ± 5.63(b)	317.72 ± 4.63(b)	355.80 ± 18.64 (a)	372.45 ± 14.94(a)	337.73 ± 10.72 (a)	390.60 ± 2.20(a)
K	90.95 ± 4.51(c)	116.55 ± 3.77(c)	147.96 ± 7.01(b)	161.69 ± 3.47(b)	175.77 ± 9.18 (a)	217.72 ± 7.94(a)
Ca	19.45 ± 0.35(b)	28.79 ± 0.45(b)	36.53 ± 0.36(a)	48.89 ± 2.25(a)	39.61 ± 2.13(a)	52.88 ± 2.68(a)
Mg	12.12 ± 0.26(c)	18.95 ± 0.54(c)	20.62 ± 0.52(b)	32.36 ± 0.79(b)	36.70 ± 2.09(a)	53.20 ± 0.63(a)
P	32.57 ± 1.32(b)	39.43 ± 0.72(c)	44.15 ± 2.43(a)	54.46 ± 0.68(a)	42.27 ± 2.15(a)	49.57 ± 2.26(b)
Cu	0.09 ± 0.00(c)	0.09 ± 0.02(a)	0.21 ± 0.01(b)	0.09 ± 0.01(a)	0.98 ± 0.04(a)	0.10 ± 0.01(a)
Zn	0.24 ± 0.05(c)	0.26 ± 0.01(c)	0.97 ± 0.02(b)	1.46 ± 0.05(b)	2.70 ± 0.06(a)	4.30 ± 0.12(a)
Mn	0.30 ± 0.02(c)	0.30 ± 0.00(c)	0.42 ± 0.01(b)	0.92 ± 0.02(b)	0.90 ± 0.03(a)	1.81 ± 0.03(a)
Fe	1.00 ± 0.03(c)	1.13 ± 0.04(c)	1.19 ± 0.07 (b)	1.40 ± 0.01 (b)	2.31 ± 0.02(a)	2.75 ± 0.11(a)
Li	0.08 ± 0.01(a)	0.08 ± 0.01(b)	0.12 ± 0.03(a)	0.19 ± 0.00(a)	0.11 ± 0.01(a)	0.18 ± 0.03(a)

The results are calculated as difference between the minerals amount in the sample digestion fluids and blank digestion fluids (mineral amounts in the blank digestion fluids and sample digestion fluids are presented in the Supplementary Material).
Results are presented as mean values ± standard deviations ($n \geq 3$);
Different letters within the same row indicate significant differences ($p < 0.05$) between mean values (Tukey test).

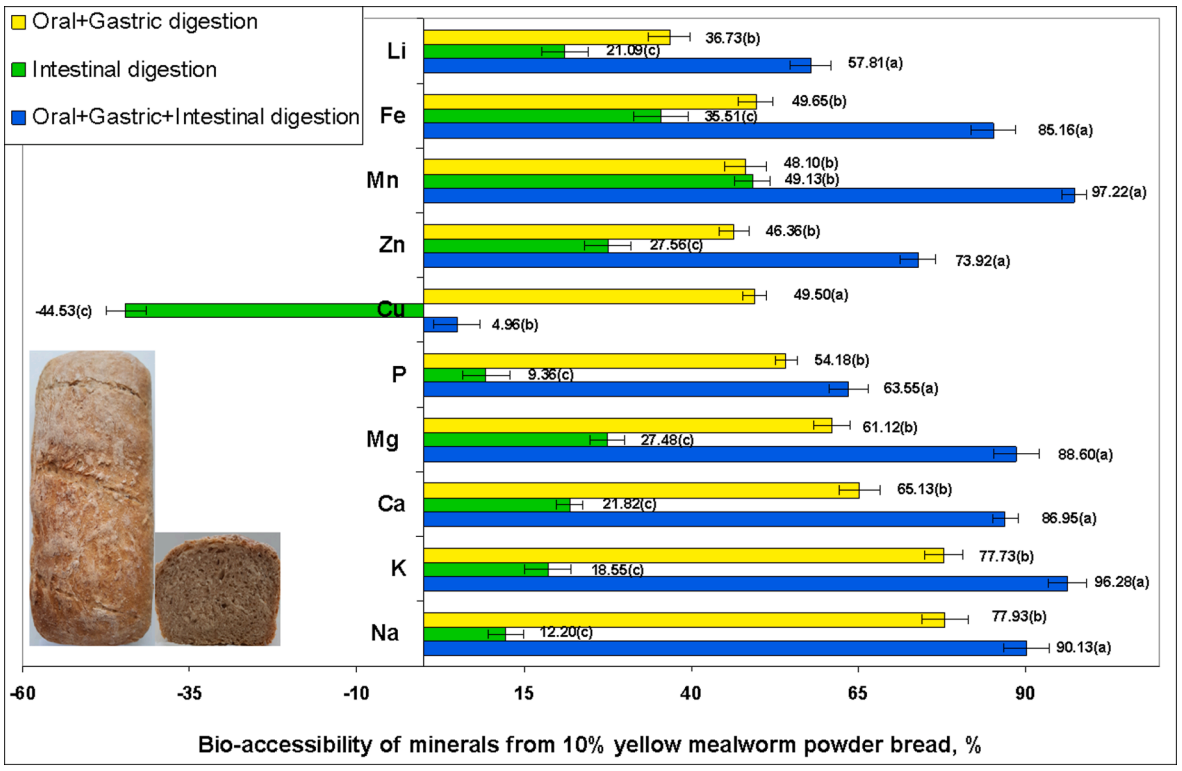


(a)

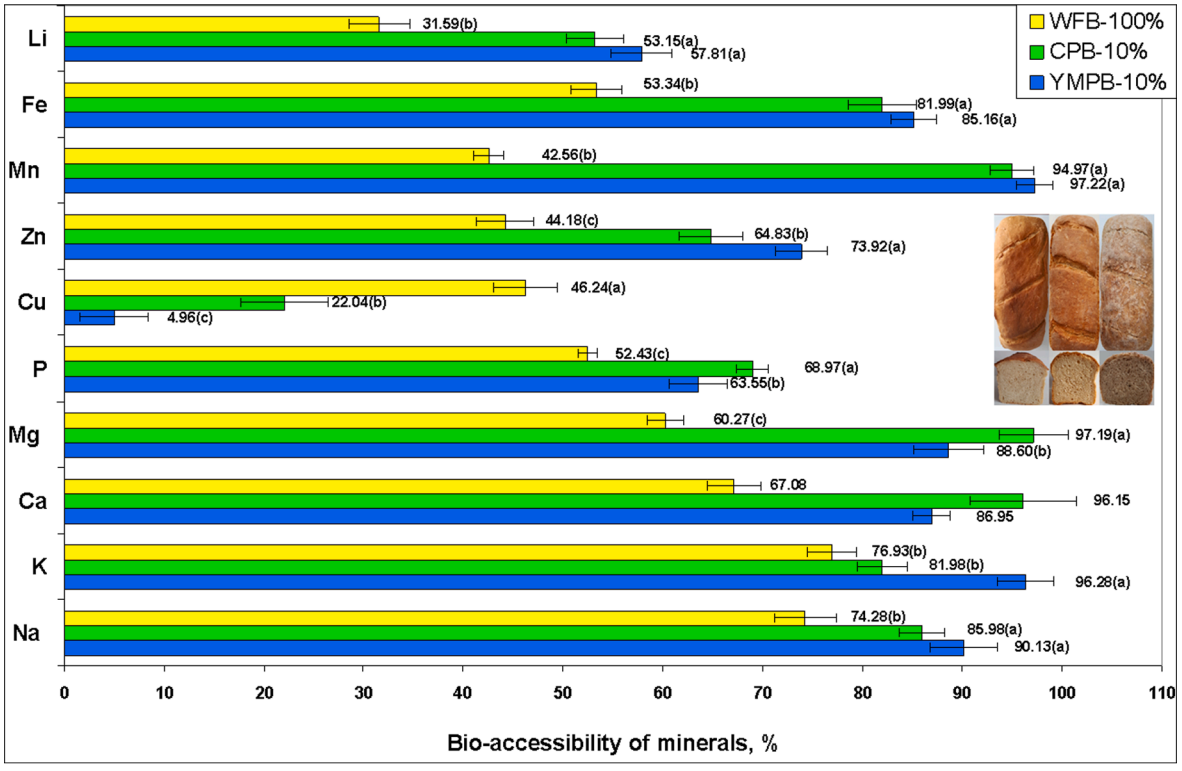


(b)

Fig. 1. Bioaccessible fractions of mineral elements from investigated breads: a – white bread; b – 10 % cricket powder bread; c – 10 % yellow mealworm powder bread; d – comparative analysis of overall bioaccessible mineral fractions from investigated bread. Different letters within the same digestion stage for the same mineral elements indicate significant differences ($p < 0.05$) between mean values (Tukey test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



(c)



(d)

Fig. 1. (*continued*).

pancreatic, and biliary secretions: (i) the further solubilization of minerals from undigested gastric chyme, and (ii) the presence of anti-nutrients that can precipitate mineral ions from gastric liquid, rendering them less bioaccessible or impeding their absorption. Reports have indicated that phytic acid and its salts, oxalates, tannins, and fibers in WF strongly bind the divalent cations and precipitate them as anti-nutritional mineral complexes that are unavailable as nutritional factors (Akter et al., 2012). Chitin, tannins, alkaloids, and saponins fall in the

category of antinutrient compounds introduced by edible insects (Ojha et al., 2021), which hinder food digestibility.

As Fig. 1 a to d displays, most of the Na and K from the investigated breads were released as soluble fractions in the gastric phase, with a bioaccessibility higher than 60 %. Na was released to the largest extent from CPB-10 % (82.13 %), while YMPB-10 % released the highest bioaccessible fraction of K (77.73 %) and Na (77.93 %). The WFB-100 % revealed the lowest bioaccessible fractions of Na and K. The release of these two minerals continued during the intestinal phase, where the pH change did not induce their precipitation (Figure 3.SM in the Supplementary Material). Although CPB-10 % releases Na with the maximum yield (82.13 %) in the gastric phase, it releases Na in the lowest yield (3.84 %) in the intestinal phase. K from YMPB-10 % shows the highest bioaccessible fraction (18.55 %) in the intestinal stage. As a result of gastric and intestinal digestion, the bioaccessible fractions of Na vary from 90.13 % in YMPB-10 % to 85.98 % in CPB-10 % and 74.28 % in WFB-100 %. The same trend is observed for K, with total bioaccessible fractions of 96.28 %, 81.98 %, and 76.93 % released from YMPB-10 %, CPB-10 %, and WFB-100 %, respectively. Of all the investigated minerals, Na and K were released to the highest extent (90.13 and 96.28 %, respectively) from YMPB-10 %.

<50 % of the Ca and Mg amounts from WFB-100 % were released in the gastric stage (Fig. 1 a–d). The overall bioaccessible fraction of Ca (45.32 %) from the white bread in our study is lower than the value of 71.25 % reported by Rebellato et al. (2017). Larger amounts of Ca and Mg were leached from the insect breads, with bioaccessible fractions higher than 60 %. In WF, Ca and Mg are mainly stored as phytate salts embedded in protein-rich globoid structures with strong cell walls (De Brier et al., 2015), which protect them, to some extent, from the attack of gastric acid. The addition of insect powders reduces the concentration of phytate salts in the insect breads, and mineral release is favored. During intestinal digestion, relatively similar values were obtained for the bioaccessible fractions of Ca from YMPB-10 % (21.82 %) and WFB-100 % (21.76 %), with a slightly higher value in the case of CPB-10 % (24.30 %). Mg has the highest bioaccessible fraction in CPB-10 % (35.27 %), followed by YMPB-10 % (27.48 %) and WFB-100 % (21.72 %). Precipitation of Ca and Mg as Ca– and Mg–phytate complexes with the pH increase during the intestinal stage can partially explain the low amounts of Ca and Mg ions in the intestinal mixture. Overall digestion led to the release of Ca and Mg in the highest yields from CPB-10 % (96.15 and 97.19 %, respectively) among the minerals assessed.

During the gastric phase, Fe is released in equal percentages from WFB-100 % (47.10 %) and CPB-10 % (47.06 %), and at a slightly higher level from YMPB-10 % (49.65 %). Additional increases of 34.83 % and 35.51 % were obtained during the intestinal digestion of CPB-10 % and YMPB-10 %, resulting in the overall bioaccessibility levels of 81.99 % and 85.16 %, respectively. In WFB-100 %, a small increase of 6.24 % was observed in the intestinal phase, with the overall bioaccessibility of Fe as 53.34 %. Differences between the intestinal solubilization of Fe in the white and insect breads can be explained based on the form of the Fe and the acidity of the digestion fluids. WF contains non-heme iron, mostly in the form of ferric ions and mainly localized in small intracellular bodies (Balk et al., 2019). Its absorption involves the reduction of ferric ions by the apical membrane-bound enzymes of enterocytes to ferrous iron before co-transportation with a proton across the cellular membrane. In the insect powders, Fe predominates as the non-heme molecules of ferritin and holoferritin. Ferritin functions as a storage protein for Fe, with each molecule capable of binding thousands of Fe ions, typically in the ferrous state (Mwangi et al., 2018). The low pH of gastric juice promotes the release of Fe from protein complexes and provides weak chelators (peptides, amino acids, and sugars) that allow the Fe to remain soluble (Bohn et al., 2008). The rise in pH in the intestinal stage affects the stability of ferrous and ferric ions differently and controls the number of ions in the intestinal fluid. At a pH above 2, the ferric ions become insoluble and precipitate as a hydroxide. It appears that during the intestinal digestion of WFB-100 %, due to the rise in pH, the precipitation of ferric ions increases in parallel with a slowdown in the release of Fe from gastric chyme, with the result of these two opposing processes being a small increase in the level of bioaccessible Fe. On the other hand, the ferrous ions remain dissolved over a wider pH range as compared to the ferric ions, with their precipitation starting at a pH of approximately 8 (Figure 3.SM). Thus, higher amounts of bioaccessible Fe were gained from insect bread as compared to white bread in the intestinal phase, resulting in the higher overall bioaccessibility of Fe in the former. The level of overall bioaccessible Fe in WFB-100 % (53.34 %) is higher than the value of 49.89 % reported by Rebellato et al. (2017) for unfortified French bread.

The bioaccessible fractions of Mn and Zn are comparable in the gastric phase for CPB-10 % (43.50 % and 43.12 %, respectively) and WFB-100 % (42.36 % and 41.54 %, respectively), while a larger bioaccessible fraction of Mn (48.10 %) than Zn (46.36 %) was found in YMPB-10 %. Of the investigated minerals, the Mn from insect bread is the only mineral with a bioaccessibility percentage higher in the intestinal than in the gastric phase. In the intestinal stage, the bioaccessible fraction of Mn is 51.47 % in CPB-10 % and 49.13 % in YMPB-10 % (Fig. 1 a–d), as compared to 43.50 % and 48.10 %, respectively, in the gastric stage. The high pH of approximately 9 for the precipitation of Mn (Figure 3.SM) in association with the higher affinity of antinutrients for macrominerals rather than microminerals can explain such behavior. Zn presents lower release yields in the intestinal stage of CPB-10 % (21.71 %) and YMPB-10 % (27.56 %). Slight increases in the release of Mn (0.2 %) and Zn (2.64 %) from WFB-100 % occurred in the intestinal phase, suggesting the predominance of the precipitation or coprecipitation of Zn and Mn as Zn–Mn–phytate complexes or other insoluble salts. The overall digestion resulted in bioaccessible fractions of 94.97 % (Mn) and 64.83 % (Zn) in CPB-10 %, 97.22 % (Mn) and 73.92 % (Zn) in YMPB-10 %, and 42.56 % (Mn) and 44.18 % (Zn) in WFB-100 %. Our value of 44.18 % for the bioaccessibility of Zn in WFB-100 % is higher than the value of 36 % obtained by Agrahar-Murugkar (2020).

Among the investigated bread loaves, CPB-10 %, with a total Cu content of 0.387 mg/100 g, displays the highest gastric digestibility of Cu (53.89 %). The WFB-100 %, with a total Cu content of 0.178 mg/100 g, shows a lower bioaccessible fraction of Cu (46.07 %) than YMPB-10 % (49.50 %), which displays a significantly higher total Cu content of 1.980 mg/100 g (Table 2). The results indicate that the bioaccessibility of Cu is not directly correlated with its level in bread. Kumari and Platel (2017) also reported the highest bioaccessibility of Cu in rice, with a low concentration of Cu, while finger millet, with a higher Cu content, showed a lower bioaccessibility. As with Ca and Mg, Fe, Cu, and Zn are also stored in the grains as phytate salts protected by the strong cell walls of the protein-rich globoid structures in which they are embedded (De Brier et al., 2015). Cu is present in the circulatory fluids of insects as the nucleus of hemocyanin respiratory metalloproteins in which oxygen is bound for transportation in the ratio of one oxygen atom to two Cu atoms. Higher bioaccessibility ratios of Cu in the insect breads during gastric digestion suggest that the sulfur and oxygen bridges that link Cu to the hemocyanin and oxyhemocyanin in insects are destabilized to a larger extent as compared to the strong intra- and intermolecular bonds that chelate the metal with the phosphate groups of the phyhtic acid molecule. A significant decline in the bioaccessibility of Cu during the intestinal phase can be observed in the insect breads, indicating a negative balance between the dissolution of Cu in gastric chyme and the precipitation of Cu ions with increases in pH. Thus, the bioaccessible Cu decreases by 44.53 % in YMPB-10 % and 31.85 % in CPB-10 %, with the overall fractions lowered to 4.96 % in YMPB-10 % and 22.04 % in CPB-10 %. A possible explanation involves their complexation by chitin, a major component of the exoskeleton of insects, present in the insect powders. The tendency for the precipitation of chitin at a high pH value, combined with its higher affinity for Cu^{2+} relative to other divalent cations (such as Zn^{2+} or Fe^{2+}), can explain the significant precipitation of Cu^{2+} during the intestinal phase. The precipitation of Cu^{2+} at a pH of approximately 4.5, lower as compared to pH values above 6 for Zn^{2+},

Fe^{2+}, and Mn^{2+} (Figure 3.SM), also favors Cu^{2+} precipitation, rendering it less bioaccessible. In contrast, the Cu released from gastric chyme in the case of WFB-100 % seems to predominate over the precipitation of Cu ions and results in a slight increase of 0.17 % in the bioaccessibility of Cu. This result is in line with Champagne and Fisher's (1990) study, which demonstrated that at a pH of 7 and a high phytic acid:copper molar ratio (10:1), Cu–phytate complexes remain soluble.

P has the highest gastric digestibility in CPB-10 % (55.91 %). It is present in insects in various biological molecules, such as phospholipids, ATP, DNA, or RNA. The strong walls of the phytin-based components in which P is mainly present in WFB-100 % slow down its release during gastric digestion and explain the low bioaccessible fraction of 43.31 %. Further, in the intestinal phase, the largest amount of P is released from CPB-10 % (13.06 %), while YMPB-10 % and WFB-100 % show comparable, lower bioaccessible fractions. Although no significant differences were found between the total amounts of P in the insect and white breads (Table 2), the bioaccessible fractions of P are higher in the former than in the latter, with the highest fractions of 68.97 and 63.55 % displayed by CPB-10 % and YMPB-10 %, respectively. The high affinity of the negatively charged phytate for mineral cations, present to a larger extent in WFB-100 %, led to the precipitation of metal–phytate species as the pH increased during the intestinal phase.

Li is the element with the lowest bioaccessibility in the gastric phase. Comparable soluble fractions were released from WFB-100 % (31.10 %) and CPB-10 % (32.75 %), whereas YMPB-10 % displayed a slightly higher Li release signature (36.73 %). Such low values indicate that only a small portion of the Li is bonded to molecules prone to digestion by the gastric fluid. Lower bioaccessible fractions of Li are released in the intestinal phase than in the gastric phase. Since the $Li^{\pm}$ ions remain as soluble forms over a pH range exceeding the approximate pH of 9 achieved in intestinal digestion (Figure 3.SM), one can assume that the increase in the bioaccessibility of Li in the intestinal phase mainly results from its release from bread, while the precipitation of Li-containing compounds is negligible. A discrepancy between the Li released from the insect and white breads can be observed in the intestinal phase compared to the gastric phase. Thus, Li release yields are similar in CPB-10 % and YMPB-10 % (20.40 and 21.09 %, respectively) and much lower in WFB-100 % (0.49 %). A possible explanation is related to the tannic acid present in wheat that coprecipitates Li and Ca as tannins. Due to gastric and intestinal digestion, more than half of the Li content in insect bread is bioaccessible, with the highest value (57.81 %) obtained for YMPB-10 %.

A complete picture of the composition of insect powders also points the risks associated to the human entomophagy, beyond the promising nutritional profile and high bioaccessibility of valuable mineral elements. Poisonous compounds (such as cyanogenic glycosides) in the insects originating from consumed plants (Zagrobelny et al., 2009), intestinal discomfort induced by consuming the cricket feet (Bouvier, 1945), allergies caused by chitin to sensitive person, human foodborne pathogens (*Staphylococcus aureus*, *Clostridium* spp., *Bacillus cereus* group), or other biological contaminants (fungi, viruses, protozoa and prions) carried by insects, are among the risks indicated by the literature (Magara et al., 2021; Vandeweyer et al., 2021). Appropriate rearing conditions in insect farms, heat treatment of insect powders, labelling of allergenic components, are essential steps to ensure the health and safety of consumers.

5. Conclusions

The study aimed to test the suitability of cricket and yellow mealworm powders in the development of functional bread. With the addition of 10 % cricket and yellow mealworm powders, the nutritional values of insect bread are enhanced in terms of protein, lipid, fiber, and mineral content as compared to white flour bread. Other valuable benefit of insect powders-bread includes a significant increase in the essential amino acids valine and tyrosine contents, with valine enhanced reaching up to 9.72 % in the cricket bread, and tyrosine level reaching 1.86 % in mealworm bread. The fatty acids profile of insect breads is dominated by the polyunsaturated fatty acids, although they are at lower ratio compared to the reference bread. On the other hand, the monounsaturated fatty acids were at highest level in the cricket bread (35.22 %), while they account for 32.34 % in the white bread, and 30.77 % in the mealworm bread. The study is the first to present data on the bioaccessibility of mineral elements in insect bread containing 10 % cricket and yellow mealworm, respectively powders. The highest bioaccessibility of P (68.97 %), Mg (97.19 %), and Ca (96.15 %) was found in cricket bread, while Li (57.81 %), Fe (85.16 %), Mn (97.22 %), Zn (73.92 %), K (96.28 %), and Na (90.13 %) were more accessible from yellow mealworm bread. Contrarily, only copper was found to be more bioaccessible from white bread (46.24 %) than from cricket bread (22.04 %) and mealworm bread (4.96 %), probably due to its complexation at a larger extent by chitin present in the insect powders.

Due to insights gained in relation to the bioaccessibility of mineral elements, the study presents an important contribution to the field of nutrition, helping in the mitigation of mineral deficiencies. Further analyzes are needed to assess the bioavailability of mineral elements in light of the well-known observation that, a high nutrient solubility in digestion fluids does not necessarily correspond to a subsequent high absorption rate.

CRediT authorship contribution statement

A. Mihaly Cozmuta: Conceptualization, Methodology, Investigation, Writing – original draft. **C. Nicula:** Formal analysis, Investigation, Validation. **A. Peter:** Investigation, Resources, Writing – review & editing. **L. Mihaly Cozmuta:** Writing – review & editing. **A. Nartea:** Formal analysis, Methodology. **A. Kuhalskaya:** Formal analysis, Software. **D. Pacetti:** Writing – review & editing. **S. Silvi:** Writing – review & editing. **D. Fiorini:** Investigation, Formal analysis, Writing – review & editing. **L. Pruteanu:** Validation.

Declaration of Competing Interest

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

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2022.105310.

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