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Effect of replacing durum wheat semolina with *Tenebrio molitor* larvae powder on the techno-functional properties of the binary blends

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ABSTRACT

Tenebrio molitor (TM) larvae, due to their high nutritional value, are gaining growing attention in food and feed sectors. Although few studies dealt with wheat-based products functionalized with TM larvae powder, there is a lack of comprehensive characterization of the raw materials to optimize the formulations for end-product recommendation.

This study aimed at investigating the effects of partial replacement of durum wheat semolina with increasing amounts of TM larvae powder (5–30%) on the techno-functional properties of the binary blends. Color, granulometry, hydration properties, pasting characteristics, spectral characteristics (FTIR), reducing sugar content, and bioactivity in terms of total phenolic content (TPC) and antioxidant activity (FRAP, DPPH, ABTS) were assessed in the resulting blends.

The increasing insect powder decreased the lightness (L*) and yellowness (b*) but increased the redness (a*) of the samples. In turn, the addition of insect powder did not negatively alter the hydration properties, which were comparable to those detected for semolina. Higher amounts of insect powder led to increased protein and lipid contents, as corroborated by the FTIR spectra, and decreased pasting parameters, with stronger starch granule stability detected when 20% and 30% of insect powder were added to the formulation.

Significant increases in TPC and antioxidant activity were observed with increasing amount of insect powder (up to 87%, 78%, 2-fold, 67%, for TPC, FRAP, DPPH, and ABTS, respectively, compared to semolina).

Therefore, these promising results have highlighted the possibility of using TM larvae powder as an unconventional ingredient for wheat-based products, by enhancing the nutritional and health-promoting values.

1. Introduction

To face population growth and the increasing demand for animal proteins by the consumers, world meat supply should almost double by 2030 (FAO, 2013). In the last years, the potential use of non-conventional protein sources has been investigated to find alternatives to animal protein sources that are environmentally impacting, expensive, and whose consumption is associated with a high risk for chronic diseases (FAO, 2013).

The consumption of edible insects as non-conventional protein sources, has been suggested as a potential solution to face the aforementioned issues, and to ensure food security (EFSA, 2015) with a lower environmental footprint than other protein sources (Oonincx et al., 2010).

Despite the several challenges associated with edible insects, including acceptability, allergenicity, and processing (Gravel and Doyen, 2020), in line with the current worldwide growing interest in this novel protein source, the global insect market is poised to quadruplicate by 2027, and its second-largest application sector, after animal nutrition, is expected to be Food and Beverages (Kbv research, 2023).

The principal components of edible insects, that are consumable in every stage of their life cycle (Benes et al., 2022), are proteins (13%–77% on dry basis) and fats (9%–67% on dry basis) (FAO, 2013). Among insects, the yellow mealworm (*Tenebrio molitor*) has been identified as

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one of most acceptable insect species by the consumers (Gkinali et al., 2022a, b) and highly suitable for industrial-scale production (Khanal et al., 2023).

Larvae of *T. molitor* compared to other edible insects have well-balanced nutrient profiles, providing about 206 kcal/100 g (on a fresh weight basis) (Gkinali et al., 2022a, b; Grossmann et al., 2021). These insects possess high-quality protein content (45–66% on dry basis), fulfilling the demands for essential amino acids, lipid content (19–43% on dry basis) rich in monounsaturated fatty acids, and fiber (2–19% on dry basis) that commonly involves chitin (Gkinali et al., 2022a, b). Researchers were pushed to investigate previously unexplored pathways to enhance the potential of *T. molitor* as a novel food. This interest blew up when mealworms went in the spotlight following the positive scientific opinion on the safety assessment of dried *T. molitor* larvae published by EFSA (European Food Safety Authority) in 2021 (Turck et al., 2021) and the authorization for placing this product on the market as a novel food (Regulation (EU) 2021/882). In terms of biological activity, recent studies have reported that *T. molitor* larvae and its oil inhibited the growth of hepatocarcinoma cells (Wu et al., 2020; Lee et al., 2015) and the activity of beta-secretase enzyme (Youn et al., 2014), showed anti-inflammatory effects (Oh et al., 2022), and mitigated in vitro and in vivo obesity (Seo et al., 2017). Moreover, recent findings also supported that *T. molitor* larvae, rich in hydrophilic and lipophilic antioxidants, may promote antioxidant activity preventing oxidative stress (Keil et al., 2022; Baek et al., 2019).

However, despite the nutritional, biological, and environmental benefits of these insects, their consumption is still very limited in Western countries due to cultural and psychological barriers. Research studies have proved that people are willing to change their opinion when fully informed about their health and environmental benefits, considering more acceptable insects in savory foods than sweety foods (Lombardi et al., 2019). In a society where consumers are not used to eat whole insects, the most promising strategy to increase insects acceptability and reduce negative perceptions, is using them in powder form as ingredients for different staple foods such as bakery products (Belleggia et al., 2023; Cappelli et al., 2020; Garciá-Segovia et al., 2020; González et al., 2019; Roncolini et al., 2020), pasta (Çabuk and Yılmaz, 2020; Pasini et al., 2022), extruded snacks (Azzollini et al., 2018; Garciá-Segovia et al., 2020), and meat products (Choi et al., 2017; Kim et al., 2016). Moreover, some researchers are paying attention to the development of wheat-based snacks enriched with *T. molitor* powder with the use of 3D printing to improve their appeal (Caporizzi et al., 2018). Recent studies focusing on the use of *T. molitor* in bread making (Cappelli et al., 2020; Garciá-Segovia et al., 2020; González et al., 2019; Roncolini et al., 2020) revealed that a wheat flour replacement of 5 and 10% resulted in doughs with increased stability and breads with improved nutritional profiles. Recently, muffins prepared by replacing 15% of wheat flour with mealworm powder received very good liking scores (Çabuk, 2021) and exhibited enhanced biological activity (Zielińska et al., 2021). The partial substitution of wheat flour with *T. molitor* larvae flour led to biscuits with much darker appearance and enhanced free radical scavenging capacities (Zielińska and Pankiewicz, 2020). In turn, incorporating yellow mealworm (15%) into egg pasta negatively impacted the cooking performance and sensory acceptability (Çabuk and Yılmaz, 2020). Likewise, the insect protein inclusion (14%) into pasta resulted in a darker and firmer pasta, with a higher water absorption index (Pasini et al., 2022).

However, although few studies on incorporating *T. molitor* larvae flour into food products exist, due to the novelty of this topic, there is still a knowledge gap in the fundamental and comprehensive characterization of the raw materials used to formulate and prepare new food products, whose results are of utmost importance to understand the effects of their incorporation as ingredients into foods, as well as the influence of the processing steps on the quality of the food products. Among the raw materials used for wheat-based foods, such as bread and pasta, durum wheat semolina, a cereal product typical of the Mediterranean region, is considered the best for its characteristic yellow color, high protein and gluten content (Ficco et al., 2023; Bresciani et al., 2022). In fact, looking at pasta, in many European countries, it is mandatory by law to use 100% durum wheat semolina.

Therefore, this study is addressed to investigate the effect of the addition to durum wheat semolina of edible insects' flour, namely *T. molitor* larvae flour, at different concentration (5%–30%, *w/w*) on the techno-functional properties of the binary blends. A comprehensive characterization of the mixtures, including water and oil absorption capacity, water solubility, swelling, colorimetric profile, rheological parameters, phenolic compounds, antioxidant activity and reducing sugars content, was carried out to further assess their potential application in the formulation of wheat-based foods, promoting healthier diet without requiring consumers to completely change their eating habits.

2. Materials and methods

2.1. Raw materials and chemicals

Durum wheat semolina (*Triticum durum*) was provided by Gran Mugnaio Molino Spadoni (Ravenna, Italy) and its composition per 100 g of product, as reported on the label, was: 1.2 g of fats, of which 0.4 g saturated fatty acids, 70.5 g of carbohydrates, of which 1.9 g sugars, 12.0 g of proteins, 2.2 g of fiber, and 0.02 g of salt. Gluten content of semolina, determined according to the method reported by Tateo (1980), was 10.3 ± 0.6 g per 100 g of product.

Dried larvae of yellow mealworm (*Tenebrio molitor*) in powder form were provided by Nimavert (Harelbeke, Belgium). 100 g of *T. molitor* larvae powder contained 30.8 g of fats, of which 7.62 g saturated fatty acids, 6.7 g of carbohydrates, of which 2.0 g sugars, 50 g of proteins, 3.3 g of fiber, and 4 g of salt. Both the raw materials were stored in sealed bags at dry and refrigerated conditions until use. Solvents, all reagents and standards used in the analyses were purchased from Pol-Aura, Zabrze, Poland.

2.2. Preparation of the binary blends

T. molitor larvae powder and durum wheat semolina mixtures (5%, 10%, 20%, 30% of insect flour and 95%, 90%, 80%, 70% of semolina) were prepared in a rotary drum mixer (TM100, Vevor, China) operated for 10 min. 100% of *T. molitor* larvae powder and 100% of semolina were also analyzed as control samples. The final weight of each sample, namely 0IN100S (durum wheat semolina), 5IN95S (5% insect powder - 95% semolina), 10IN90S (10% insect powder - 90% semolina), 20IN80S (20% insect powder - 80% semolina), 30IN70S (30% insect powder - 70% semolina) and 100IN0S (insect powder), was 550.87 g, 549.74 g, 548.61 g, 546.35 g, 544.08 g, 528.25 g, respectively.

The moisture content of each sample, determined according to the AACC 44-19.01 method, was 9.24%, 8.88%, 7.65%, 7.56%, 6.85%, and 4.37%, respectively.

2.3. Colorimetric parameters

Lightness (L*), redness (a*), and yellowness (b*) parameters of durum wheat semolina and *T. molitor* larvae mixtures were determined by using a CR-310 colorimeter (Konica Minolta CR-310 chroma meter, Ramsey, NJ, USA), according to the official method CIELab. The color difference (ΔE*) between each sample and durum wheat semolina (0IN100S) was calculated according to Eq. (1). The chroma (C^*_{ab}), that indicates the color purity, and intensity or saturation, and the hue angle (h^*_{ab}), that describes the relative amounts of redness (0°/360°) and yellowness (90°) of the sample, were evaluated according to Eq. (2) and Eq. (3), respectively.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$h^*_{ab} = \arctan\left(\frac{b^*}{a^*}\right) \quad (3)$$

2.4. Particle size distribution, bulk and tapped density

Particle size distribution of durum wheat semolina and *T. molitor* larvae powder mixtures was determined by sieving, according to the standard method AACC 66-20.01 with slight modifications. Briefly, a vibratory shaker LPzE-2e (Multiserw Morek, Brzeźnica, Poland) equipped with five sieves with different opening size (500, 355, 250, 180, and 150 μm) was used. Data were reported as the weight of the sample remaining on a specified sieve after sieving at 0.65 mm vibration amplitude for 10 min, expressed as a percentage of the original weight of the sample (10 g).

Bulk and tapped density of durum wheat semolina, *T. molitor* larvae flour, and their mixtures were determined according to the methods described by De Barros Fernandes et al. (2013).

2.5. Hydration properties

Water absorption capacity (WAC), which reveals how samples absorb and maintain water when forces like mixing and centrifugation are applied, oil absorption capacity (OAC) and hydrophilic/lipophilic index (HLI) of the samples were determined according to the method described by Villanueva et al. (2018) with minor modifications. 1 g of sample (w_S) was mixed with 30 mL of distilled water, for WAC determination, and with 30 mL of oil, for OAC determination, in centrifuge tubes (w_T). The dispersions were vortexed (Heidolph Reax, Schwabach, Germany) for 30 s and let to rest for 10 min. This procedure was repeated twice. The dispersions were then centrifuged for 25 min at 3000×*g* (Thermo Fisher Scientific, Waltham, USA) and the supernatants discarded. The tubes were placed, with an angle of 15–20° with respect to the vertical axis, in an oven (Vindon Scientific, Rochdale, UK) at 50 °C for 25 min, to remove the residual liquids, and then weighed (w_{TS}). WAC (g of water/g of dry solids) and OAC (g of oil/g of dry solids) were determined using Eq. (4), while HLI was calculated using Eq. (5):

$$WAC;\ OAC = \frac{w_{TS} - w_T}{w_S} \quad (4)$$

$$HLI = \frac{WAC}{OAC} \quad (5)$$

Water absorption index (WAI), which shows how samples gelatinize in the cooling–heating cycle and if contain any small molecules which can be entrapped within the matrix or released into supernatant, water solubility index (WSI), which reflects the number of compounds released from the gel, and swelling power (SP) were determined following the method reported by Harasym et al. (2020) with slight modifications. 1 g (w_S) of each sample was dispersed in 30 mL of distilled water in centrifuge tubes (w_T, weight of the empty tube) and heated for 10 min at 90 °C in a water bath (MLL147, AJL Electronics, Kraków, Poland). Samples were cooled to room temperature and centrifuged at 3000×*g* for 10 min. The sediment was weighed (w_{WS}, weight of the sediment plus tube) and the supernatant was poured into a pre-weighed stainless steel Petri dish (w_{PD}) and loaded in an oven (SML, Zalmed, Łomianki, Poland) at 110 °C for 24 h to determine the solid content (w_{SS}). WAI (g of water/g of dry solid), WSI (g of water/100 g of dry solid), and SP (g of water per g of dry solid) were calculated using Eqs. (6)–(8):

$$WAI = \frac{w_{WS} - w_T}{w_S} \quad (6)$$

$$WSI = \frac{w_{SS} - w_{PD}}{w_S} * 100 \quad (7)$$

$$SP = \frac{w_{WS} - w_T}{w_S - (w_{SS} - w_{PD})} \quad (8)$$

To determine the water holding capacity (WHC), which reveals how samples absorb water when gravity is the only force acting, 2 g (w_S) of each sample were dispersed in 20 mL of distilled water in centrifuge tubes (w_T) and left at room temperature for 24 h. After 24 h the supernatant was carefully discarded, and the tubes weighed (w_{TS}). WHC (g of water/g of dry solid) was calculated as follows (Eq. (9)):

$$WHC = \frac{w_{TS} - w_T}{w_S} \quad (9)$$

2.6. Pasting properties

The determination of rheological properties of durum wheat semolina and *T. molitor* larvae flour mixtures were carried out with a Rapid Visco Analyser (RVA 4500, Perten Instruments, Massachusetts, USA), according to the official method AACC 76-21.01 (AACC, 1999). Dynamic and isothermal ramps were run as follows. A holding ramp at 50 °C was applied for 2 min, then, a dynamic heating ramp from 50 °C to 95 °C was applied at a heating rate of 5 °C/min. The sample was kept at 95 °C for 5 min, afterward cooled down at a rate of 10 °C/min back to 50 °C, and finally held at 50 °C for 4 min.

The measured parameters included peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), setback viscosity (SV), peak time, and pasting temperature.

2.7. Fourier Transform Infrared spectroscopy (FTIR) analysis

The FTIR spectra of the samples were recorded using a Fourier transform infrared (FTIR) spectrophotometer (Nicolet 6700 FT-IR, Thermo Fisher Scientific, Massachusetts, USA) equipped with a diamond crystal cell for attenuated total reflection (ATR) operation. The spectra were acquired with 64 scans per sample in the wavelength range of 4000–500 cm^{-1} at a nominal resolution of 4 cm^{-1}. The spectra were corrected using the background spectrum of air.

2.8. Bioactivity of durum wheat semolina and T. molitor larvae powder mixtures

2.8.1. Samples preparation

Total phenolic compounds from *T. molitor* larvae and durum wheat semolina mixtures were extracted following the methodology described by Crizel et al. (2015) with slight modifications. Briefly, 1.5 g of each sample were homogenized with 5 mL of a methanol/water (80:20 v/v) solution acidified with 1% HCl for 1 min in a vortex (MX-S, Chemland, Stargard, Poland). Then, the tubes were agitated using a laboratory scale rotary shaker (MX-RD PRO, Chemland, Stargard, Poland) at room temperature for 2 h, and centrifuged at 3500×*g* and 4 °C for 10 min (MPW-350, MPW MED. INSTRUMENTS, Warsaw, Poland). The collected supernatants were stored at −8 °C until use.

The same procedure, but using water as extracting solvent, was followed for the determination of reducing sugars *(2.8.5)*.

All the spectrophotometric analyses were performed by the methodologies previously described by Olędzki et al. (2022) with slight modifications.

2.8.2. Total phenolic content (TPC)

TPC of the samples was determined using the Folin-Ciocalteau assay. Briefly, 0.1 mL of Folin–Ciocalteu reagent and 1.58 mL of H_2O were added to 0.02 mL of sample. After 5 min, 0.3 mL of saturated sodium carbonate solution was added. The absorbance of the reacting mixture was measured at 765 nm using a UV/Vis spectrophotometer (SEMCO S91E, EMCO, Poland) after 30 min of incubation at 38 °C. Gallic acid was used as a standard to generate the calibration curve (100–600 mg/mL). The TPC values were expressed as mg of gallic acid equivalent

(GAE)/g of sample dry weight (g_{DW}).

2.8.3. Ferric Reducing Antioxidant Power (FRAP assay)

To determine the Ferric Reducing Antioxidant Power of the samples, each sample (0.0345 mL) was added to 0.998 mL of freshly prepared FRAP solution (Olędzki et al., 2022). The absorbance of the reacting mixture was measured at 593 nm after 15 min of incubation. Ferrous sulphate ($FeSO_4$) was used as a standard to generate the calibration curve (100–800 μmol/L). The FRAP values were expressed as mg of $FeSO_4$ equivalent/g of sample dry weight (g_{DW}).

2.8.4. DPPH assay

The antioxidant capacity of the samples was measures through DPPH essay. Briefly, 0.0345 mL of sample were added to 1 mL of (0.1 mM) methanolic DPPH (2,2-diphenyl-1-picrylhydrazyl radical) solution. The absorbance of the reacting mixture was measured at 517 nm after 20 min of incubation. Trolox was used as a standard to generate the calibration curve (100–800 μmol/L). The antioxidant capacity was expressed as mg Trolox equivalent (TE)/g of sample dry weight (g_{DW}).

2.8.5. ABTS assay

The antiradical capacity of samples was determined by ABTS+ (2,2-azo-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay. In brief, 0.0204 mL of each sample were added to 1.0 mL of the diluted ABTS + solution, prepared according to Olędzki et al. (2022). The absorbance of the reacting mixture was recorded at 734 nm, 10 s after mixing the sample with the ABTS + solution. Trolox was used as a standard to generate the calibration curve (100–800 μmol/L). The antiradical capacity was expressed as mg Trolox equivalent (TE)/g of sample dry weight (g_{DW}).

2.8.6. Reducing sugar content

The addition of edible insects to wheat-based products could lead to increased interactions between starch and protein by reducing the content of rapidly digested starch (RDS) associated with the generation of lower reducing sugars and blood glucose spikes (Zielińska et al., 2020). To measure the reducing sugar content of the samples 0.25 mL of DNS reagent (3,5-dinitrosalicylic acid) was added to 0.5 mL of the sample and mixed thoroughly. The resulting mixture was then incubated for 5 min in a boiling water bath and then cooled to 50–60 °C. Afterward, 3 mL of distilled water were added to the mixture, and the absorbance detected at 530 nm. Glucose was used as a standard to generate the calibration curve (100–800 μg/mL). The content of reducing sugars was expressed as mg of glucose equivalent/g of sample dry weight (g_{DW}).

2.9. Statistical analysis

The preparation of the samples to determine the bioactivity of the mixtures (section 2.8) and all the analyses were performed in triplicate. The results reported as means ± standard deviations. Differences among mean values were analyzed by one-way variance (ANOVA) using SPSS 20 (SPSS IBM, Chicago, USA) statistical package. Tukey test was carried out to determine statistically significant differences ($p < 0.05$).

3. Results and discussion

3.1. Colorimetric profile

Color is one of the main sensory attributes that mostly affects the consumers' perception of food quality (Sant'Anna et al., 2013). Therefore, the effect of replacing durum wheat semolina (0IN100S) with increasing concentrations of *T. molitor* larvae powder (100IN0S) on the colorimetric profile of the resulting mixtures was investigated.

Results reported in Table 1 show that the substitution of semolina with insect powder influenced the colorimetric profile of the semolina itself. Specifically, all the mixtures showed positive values of lightness (L*), in the range of 71–85, indicating that this component was predominant in all cases. However, due to the dark color of 100IN0S sample (33.7 ± 1.53), significantly lower L* values were detected for all the mixtures with respect to 0IN100S sample (85.54 ± 1.12). The lowest L* value, with a 20% reduction compared to the pure semolina, was shown by the mixture with 30% of insect powder (30IN70S).

Moreover, with increasing the concentrations of *T. molitor* larvae powder an increased level of redness (a*) was observed, even though no statistically significant changes were detected among the mixtures except for 30IN70S (+65% in redness compared to 0IN100S).

High-quality semolina, which is rich in xanthophylls, show yellowness (b*) values greater than 25 (Sissons et al., 2012). A reduction of the b* values up to the 28% was detected on the mixture containing the highest amount of insect flour (30IN70S).

Therefore, consistently with previous findings, the addition of *T. molitor* larvae powder, induced a substantial decrement in the whiteness of the mixtures, characterized by a darker and redder color (Choi et al., 2017; González et al., 2019; Pasini et al., 2022).

These changes are attributable to the darken and yellowish-brown color displayed by the shells of *T. molitor* larvae (González et al., 2019) and the presence of natural pigments, namely β-carotene and melanin, as predominant pigments of mealworm larvae (Iaconisi et al., 2017; Kim et al., 2016).

Overall, the mixtures formulated with insect powder showed color differences with respect to semolina, as confirmed analyzing the trend of ΔE values (Table 1). Consistently with the color parameters reported previously, ΔE significantly increased (3.73–18.38) with increasing the concentration of insect flour in the mixture from 5% to 30%, with the highest ΔE value (55.22 ± 2.14) detected for pure *T. molitor* larvae powder (100IN0S).

Regarding the chroma, which provides information on the vividness of a color, and the hue angle, which is the attribute according to which

Table 1
Color coordinates (L*, a*, b*), color difference (ΔE*) between each mixture and durum wheat semolina (0IN100S), hue angle, and chroma (C*) of the samples investigated.

Sample	0IN100S	5IN95S	10IN90S	20IN80S	30IN70S	100IN0S
						
L*	87.54 ± 1.12^{f}	84.69 ± 1.09^{e}	79.83 ± 1.41^{d}	77.14 ± 1.05^{c}	70.82 ± 1.17^{b}	33.7 ± 1.53^{a}
a*	1.44 ± 0.10^{ab}	1.1 ± 0.19^{a}	1.23 ± 0.30^{ab}	1.54 ± 0.27^{b}	2.38 ± 0.29^{c}	7.31 ± 0.55^{d}
b*	26.91 ± 0.42^{e}	24.68 ± 1.35^{d}	21.61 ± 0.58^{c}	21.61 ± 1.18^{c}	19.38 ± 0.64^{b}	16.26 ± 1.06^{a}
ΔE*	/	3.73 ± 1.65^{a}	9.41 ± 1.96^{b}	11.76 ± 1.31^{b}	18.38 ± 1.63^{c}	55.22 ± 2.14^{d}
Chroma	26.94 ± 0.42^{e}	24.70 ± 1.34^{d}	21.65 ± 0.59^{c}	21.67 ± 1.19^{c}	19.52 ± 0.65^{b}	17.82 ± 1.18^{a}
Hue angle	1.52 ± 0.00^{d}	1.53 ± 0.01^{d}	1.51 ± 0.01^{c}	1.50 ± 0.01^{c}	1.45 ± 0.01^{b}	1.15 ± 0.01^{a}

Values with different lowercase letter within the same row are significantly different ($p \leq 0.05$).

colors have been defined as reddish (0°), yellowish (90°), greenish (180°) and blueish (270°) (Sant'Anna et al., 2013), the addition of insect flour to semolina led to a slight reduction in their values. The chroma was reduced up to 28% for 30IN70S sample compared to pure semolina, highlighting the lower color intensity perceived for mixtures made with insect powder. Likewise, the values of the hue angle were slightly reduced (5%) shifting towards the reddish region, consistently with the increasing redness (a*) values detected upon the addition of insect powder.

Overall, the mixtures formulated with *T. molitor* larvae flour appeared different in color compared to durum wheat semolina, as detected by the color deviation. The addition of insect flour induced different brownish intensities to the mixtures which became similar to the color of the flours to be used in whole wheat- and lentil-based foods (Pasini et al., 2022; Barbana and Boye, 2013). Indeed, consumers associated the darkened color of fortified products with the appearance of "healthy" high-fiber foods, as reported by Cecchi et al. (2019).

3.2. Particle size distribution, bulk and tapped density, and hydration properties of the mixtures

The particle size distribution of flours is an important parameter determining the effectiveness of the heat and mass transfer processes occurring during the formulation and transformation of powdery mixtures, as well as their hydration, gelling and rheological properties (Garciá-Segovia et al., 2020). The particle sizes of the investigated samples measured by sieving are reported in Table 2.

The preferred particle size distribution in durum wheat semolina ranges from 250 to 350 μm for commercial applications (Carpentieri et al., 2022). In line with these consideration, 0IN100S sample showed the highest fractions with particle sizes ranging between 250 μm (35.71%) and 355 μm (35.20%).

T. molitor larvae powder (100IN0S) was characterized by the highest fraction of particles with sizes above 500 μm, while in pure semolina and the mixture with 5% of insect powder (5IN95S) the most abundant fraction of particles was that with particle size below 150 μm. Samples with 20% and 30% of insect powder, and 100IN0S possessed the lowest fraction of small particles with no significant differences between them. As expected, the increasing trend of the fraction with particle sizes higher than 500 μm was observed when the amount of insect flour was increased.

Therefore, the partial replacement of durum wheat semolina by 5% *T. molitor* larvae flour resulted in the most comparable mixture to the semolina itself in terms of particle size distribution, with no significant differences in the fractions with particle sizes of 250–150 μm.

The particle size distribution also influences the bulk and tapped density of powders. The values of bulk density detected in this study ranged from 408.16 to 695.65 kg m^{-3} (Table 2). Durum wheat semolina showed the highest bulk density (695.65 kg m^{-3}), in line with previous findings (670–790 kg m^{-3}) (Abecassis et al., 2012; Yüksel et al., 2017). The finer the particle size, the more the amount of powder that can be accommodated in the same volume, therefore the bulk density increases with compaction (Changmai and Purkait, 2021). Indeed, it was verified that bulk density decreased with the increase of particle size of the mixtures which may be also a result of reduction in starch content.

Tapped density of a powder represents its random dense packing and is related to packaging requirements, transport, handling and applications (Amidon et al., 2017). Tapped density increased compared to bulk density due to the reduced sample volume during the tapping procedure, and decreased when increasing the amount of insect flour in the mixtures. This may be due to less void space available in the composite flour (Raihan and Saini, 2017). Indeed, the increase of tapped density over bulk density decreases with the increased amount of insect flour, indicating reduced compaction after tapping.

The lower the bulk and tapped densities, the higher the number of particles that can stay together making these flours valuable for food applications, and offering also packaging advantages (Dereje et al., 2020).

The particle size distribution is of utmost importance in determining the proper hydration of flour mixtures. Small particles tend to over-hydrate, resulting in a sticky dough, while large particles tend to under-hydrate, resulting in a stiff dough (Carpentieri et al., 2022). A decrease in particle size was usually associated with an increase in density, reduction in water holding capacity (WHC), water absorption capacity (WAC) and oil absorption capacity (OAC) (Elleuch et al., 2012).

WHC and WAC indicate the total amount of water that can be absorbed per gram of a protein powder. These properties depend on the characteristics of individual compounds, starch, fiber, protein type and physical structure, the degree of association to form hydrogen bonds, and availability of water binding sites (Harasym et al., 2020), which may be damaged by excessive grinding which, consequently, reduces the capacity of binding water of mixtures with smaller particle sizes (Elleuch et al., 2012). Indeed, a positive correlation between the increase in particle size and the water holding, and water and oil absorption capacities was also observed in the present study, as reported in Table 3. Even though the investigated concentrations of insect powder did not induce significant changes in the hydration properties of the mixtures, *T. molitor* larvae powder (100IN0S) showed the highest WHC and WAC values (+37% and +33%, respectively, compared to semolina). The fact that the replacement of durum wheat semolina with insect flour at the investigated amounts (5–30%) did not significantly impact the hydration properties of mixtures makes these results promising for potential applications of these mixtures in the preparation of wheat-based foods.

According to Zielińska et al. (2018) *T. molitor* powder has WHC values (1.29 g/g) higher than pulse protein flours and comparable to those of soy and milk protein concentrates, suggesting that this novel protein source could be used as a functional ingredient in food formulation.

Oil absorption capacity (OAC) refers to the amount of lipids that can be absorbed by a defined amount of protein powder and is highly correlated with emulsifying properties (Gravel and Doyen, 2020). Fats and oils are usually absorbed and retained by small, low-density, and hydrophobic proteins.

Interestingly, the substitution of semolina (5–30%) with *T. molitor* larvae powder did not induce any statistically significant changes in OAC for all the mixtures investigated. However, the insect flour

Table 2
Particle size distribution (g/100 g), bulk density and tapped density (kg m^{-3}) of the mixtures.

Sample	0IN100S	5IN95S	10IN90S	20IN80S	30IN70S	100IN0S
500 μm	1.80 ± 0.14^{aA}	6.95 ± 1.34^{bA}	9.25 ± 0.63^{bB}	14.25 ± 0.64^{cB}	18.45 ± 0.07^{dC}	99.3 ± 0.14^{eC}
355 μm	35.20 ± 0.14^{dD}	33.80 ± 0.57^{bcC}	32.90 ± 0.28^{bD}	33.30 ± 0.28^{bcC}	34.40 ± 0.28^{cdD}	4.5 ± 0.28^{aB}
250 μm	35.71 ± 0.01^{bcD}	35.15 ± 0.78^{bC}	38.75 ± 0.35^{cE}	42.75 ± 1.63^{dD}	47.25 ± 0.49^{eE}	0 ± 0.00^{aA}
180 μm	12.00 ± 0.14^{bC}	18.60 ± 0.14^{cB}	16.25 ± 1.77^{cC}	12.55 ± 0.21^{bB}	2.45 ± 0.21^{aB}	0 ± 0.00^{aA}
150 μm	7.05 ± 0.21^{cB}	6.90 ± 1.27^{cA}	3.95 ± 0.64^{bA}	0.30 ± 0.14^{aA}	0.15 ± 0.07^{aA}	0 ± 0.00^{aA}
Bulk density (kg/m^3)	695.65 ± 2.11^{eA}	666.67 ± 3.01^{dA}	645.16 ± 2.43^{cA}	588.24 ± 3.67^{bA}	588.24 ± 0.57^{bA}	408.16 ± 2.75^{aA}
Tapped density (kg/m^3)	842.11 ± 0.79^{aB}	740.74 ± 1.98^{bB}	714.29 ± 3.06^{cB}	641.03 ± 2.89^{dB}	632.91 ± 1.52^{eB}	487.80 ± 0.77^{fB}

Values with different lowercase letter within the same row are significantly different ($p \leq 0.05$).
Values with different uppercase letter within the same column are significantly different ($p \leq 0.05$).

Table 3
Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), Hydrophilic/Lipophilic Index (HLI), Water Holding Capacity (WHC), Water Solubility Index (WSI), Water Absorption Index (WAI), Swelling Power (SP) values of the mixtures.

Sample	0IN100S	5IN95S	10IN90S	20IN80S	30IN70S	100IN0S
WAC (g/g)	1.75 ± 0.13^a	1.54 ± 0.10^a	1.58 ± 0.15^a	1.42 ± 0.14^a	1.72 ± 0.01^a	2.60 ± 0.17^b
OAC (g/g)	1.61 ± 0.01^a	1.66 ± 0.04^a	1.67 ± 0.01^a	1.83 ± 0.16^a	1.93 ± 0.02^a	2.84 ± 0.32^b
HLI (-)	1.09 ± 0.09^b	0.93 ± 0.04^{ab}	0.95 ± 0.09^{ab}	0.78 ± 0.01^a	0.89 ± 0.01^{ab}	0.92 ± 0.04^{ab}
WHC (g/g)	2.47 ± 0.18^a	2.67 ± 0.11^{ab}	2.75 ± 0.05^{ab}	2.88 ± 0.11^{ab}	3.11 ± 0.43^{ab}	3.39 ± 0.21^b
WSI (g/100 g)	9.18 ± 0.30^a	9.71 ± 0.25^a	10.17 ± 0.94^a	11.03 ± 0.89^a	11.04 ± 2.05^a	17.04 ± 0.73^b
WAI (g/100 g)	5.70 ± 0.50^b	6.12 ± 0.00^b	5.58 ± 0.38^b	5.72 ± 0.10^b	5.47 ± 0.40^b	3.54 ± 0.12^a
SP (g/g)	6.27 ± 0.57^b	6.77 ± 0.02^b	6.22 ± 0.49^b	6.43 ± 0.18^b	6.16 ± 0.59^b	4.26 ± 0.11^a

Values with different lowercase letter within the same row are significantly different ($p \leq 0.05$).

(100IN0S) showed the highest OAC value (2.84 ± 0.32 g/g). These findings are appealing for potential use of these mixtures in the formulation of wheat-based foods.

It is also interesting to notice that all the samples analyzed showed a hydrophilic/lipophilic index ranging from 0.78 ± 0.01 to 1.09 ± 0.09 indicating a good balance between WAC and OAC values. In line with these findings, Zielińska et al. (2018) and Borremans et al. (2020) demonstrated that *T. molitor* larvae powder possessed HLI values of 0.75 and 1.19, respectively. Likewise, Zhao et al. (2016) found that the protein extract from *T. molitor* larvae had an HLI equal to 0.80, attributable to the presence of hydrophilic amino-acids, such as tyrosine, and high fat content (30%) (Zhao et al., 2016). The ability of the blends to retain water or oil was similar to that reported for flours coming either from *T. molitor* or other insect species (1.29–2.82 g/g) as well as typical food protein concentrates and legume flours (Ettoumi, 2015; Gkinali et al., 2022a, b; Zielińska et al., 2018).

The water solubility index (WSI) is an indicator of the type and amount of compounds released from the matrix and is affected by the amino acid composition, protein size and structure, as well as ionic strength, and temperature (Harasym et al., 2020). Indeed, the presence of negatively charged and polar amino acids at the protein surface favors interactions with water increasing the solubilization (Villanueva et al., 2018). *T. molitor* larvae powder (100IN0S) showed the highest WSI value (17.04 ± 0.73 g/100g) due to the presence of water-soluble proteins with molecular weights usually between 10 and 25 kDa, including hemolymph proteins (MW of ~12 kDa), cuticle proteins (MWs between 14 and 30 kDa) or chymotrypsin-like proteinase (24 kDa) (Borremans et al., 2020; Bußler et al., 2016; Gkinali et al., 2022a, b). However, the obtained results (Table 3) demonstrated that the addition of insect powder to durum wheat semolina did not significantly affect the WSI values of the analyzed mixtures.

Coherently with the functional parameters discussed so far, adding insect powder to the semolina affected only slightly the water absorption index (WAI) and swelling power (SP) of the investigated blends. The WAI gives insights on the capacity of a sample to absorb and keep water through gel formation upon the reorganization of solubilized amylose and amylopectin fragments (Harasym et al., 2020). The SP provides information on the water holding capacity of starch (Villanueva et al., 2018). As expected, in line with the previously discussed hydration properties, *T. molitor* larvae powder 100IN0S showed the lowest WAI and SP values, 35% on average lower than the other analyzed samples. Nevertheless, no differences were detected among the SP values of binary blends, that were comparable to that of durum wheat semolina.

Interestingly, replacing durum wheat semolina with *T. molitor* larvae powder (5–30%) did not significantly alter the hydration properties of semolina, allowing us to foresee the behavior of these insect-based blends during wheat-based foods production process.

3.3. Pasting properties

Pasting properties give information on the interactions occurring between the different components of a food system, and on the effect of any modification of its functionality when applying heat in the presence of water (Meares et al., 2004).

Therefore, the peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV) and setback viscosity (SV) of the analyzed systems have been determined and reported in Table 4, while the corresponding pasting curves are depicted in Fig. 1.

The peak viscosity (PV) indicates the maximum viscosity that occurs at the equilibrium point between swelling and polymer leaching (Punia et al., 2021). In the present study, a significant decrease in the PV values of the mixtures when increasing the amount of insect powder was observed (Table 4). Despite no significant differences in PV between blends containing 5% and 10% of *T. molitor* larvae flour, and between blends with 20% and 30% of insect powder were detected, PV values of the mixtures were statistically significantly lower than that observed for durum wheat semolina, with a decrease of 27% on average for 5IN95S and 10IN90S samples, and 64% on average for 20IN80S and 30IN70S samples.

The decrease in PV values might be attributed to the progressively reduced amount of starch replaced with insect powder, as well as the interactions occurring between amylose, phospholipids and proteins that compete with starch for water binding, restricting the swelling of granules and increasing hydrogen bonding and stability within granules (Gull et al., 2018; Sayar et al., 2005).

These findings are in agreement with those reported in previous studies which confirmed that the reduction of starch due to the replacement with proteins caused lower PV values in food systems (Gull et al., 2018; Ronda et al., 2014).

The breakdown viscosity (BV), defined as the difference between the peak and trough viscosity values, reflects the degree of starch granule disintegration during the holding time and is positively correlated with the peak viscosity (Rani et al., 2019).

Blends with increasing amount of insect powder showed much lower breakdown viscosities with respect to the semolina (Table 4), decreasing from 152.5 cP for semolina, to 53.7 cP on average for 5IN95S and 10IN90S samples, and −4.5 cP on average for 20IN80S and 30IN70S samples. Reduced BV exhibited by the mixtures is suggestive of their higher resistance to withstand breakdown toward shear thinning and heating process, and is related to granule rigidity, high lipid content, and surrounding protein matrix (Singh et al., 2003). Similarly, Villanueva et al. (2018) demonstrated that the stability of starch increased, and the BV value decreased significantly when higher protein amounts were added to the systems.

The results achieved were also confirmed by the pasting curves reported in Fig. 1. In particular, the semolina and the samples with 5% and 10% of insect powder, despite the lower values of pasting viscosities, showed similar pasting profiles. Conversely, the blends composed of 20% and 30% of insect powder showed no breakdown viscosities, being the trough viscosity values slightly higher than the peak viscosity values. Therefore, by replacing durum wheat semolina with an amount of *T. molitor* insect powder higher than 20%, the starch became less competitive in binding to water, being the quantity of semolina not sufficient and water in excess.

As indicated in Table 4 and depicted in Fig. 1 the final viscosity (FV) values of the systems after subsequent cooling decreased when increasing *T. molitor* larvae powder. Consistently, Villanueva et al.

Table 4
Pasting properties of the mixtures.

Sample	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time (min)	Pasting Temperature (°C)
0IN100S	1482 ± 55.2^{d}	1329.5 ± 20.5^{e}	152.5 ± 34.6^{c}	2885 ± 15.6^{d}	1555.5 ± 4.9^{d}	5.44 ± 0.05^{ab}	88.03 ± 0.04^{c}
5IN95S	1170 ± 12.7^{c}	1112.5 ± 21.9^{d}	57.5 ± 9.2^{b}	2443.5 ± 51.6^{c}	1331 ± 29.7^{c}	5.34 ± 0.09^{ab}	87.58 ± 0.60^{c}
10IN90S	$975.5 \pm 99.7_{c}$	925.5 ± 67.2^{c}	50 ± 33.5^{b}	2064.5 ± 109.6^{c}	1139 ± 42.4^{c}	$5.27{\pm}0^{ab}$	91.53 ± 4.91^{c}
20IN80S	585.5 ± 78.5^{b}	590 ± 79.2^{b}	-4.5 ± 0.7^{a}	1393.5 ± 204.4^{b}	803.5 ± 125.2^{b}	$5.20{\pm}0^{a}$	87.35 ± 3.32^{c}
30IN70S	469.5 ± 19.1^{b}	471 ± 18.4^{b}	-1.5 ± 0.7^{a}	1187 ± 22.6^{b}	716 ± 4.2^{b}	$5.13{\pm}0^{a}$	70 ± 7.07^{b}

Values with different lowercase letter within the same column are significantly different ($p \leq 0.05$).

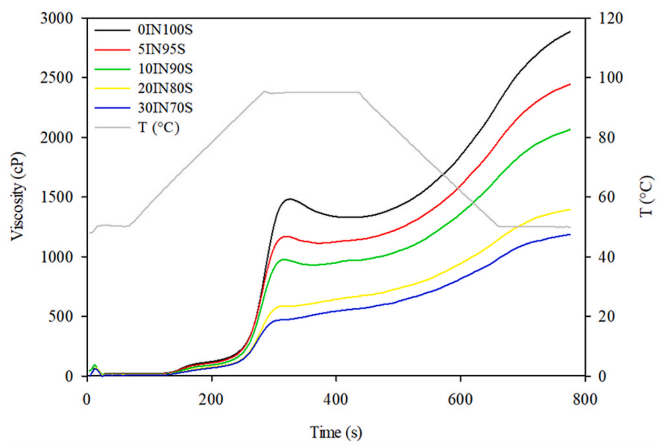

Fig. 1. Pasting curves of *T. molitor* larvae and durum wheat semolina blends.

(2018) stated that FV values of the samples investigated decreased in the presence of proteins.

Similar decreasing tendencies were observed for the setback viscosity (SV) values, calculated by subtracting trough viscosity from final viscosity, which are related to the retrogradation tendency and reordering of starch after gelatinization and cooling (Patil et al., 2020). SV decreased from 1555.5 cP for semolina, to 1232 cP on average for 5IN95S and 10IN90S samples, and 759.8 cP on average for 20IN80S and 30IN70S samples. Lower setback values indicated that the substitution of semolina with increasing amounts of insect flour led to lower starch retrogradation rates and syneresis (Rani et al., 2019).

Peak time indicates the time required to swell the starch granules until their rupture (Yildiz et al., 2013). Although, the peak time values of the mixtures slightly decreased upon the addition of insect powder, no significant differences were observed among them.

Pasting temperature (PT) is an indicator of the bonding strengths between chains within the starch granule (Ocheme et al., 2018). No statistical differences in PT were observed among the investigated samples, except for the mixture with 30% of insect powder, which showed a significant decrease in PT.

Therefore, the findings achieved in the present work suggested a potential correlation between the pasting properties and the proximate composition of the mixtures, assuming a direct relationship between the pasting properties and the starch content, and an inverse relationship between the pasting properties and the protein content. It could be speculated that the pasting parameters decrease when the starch content in the mixtures decreases, while as the protein content increases, due to the increased amount of *T. molitor* larvae powder, the pasting parameters decrease. Mixtures with 5% and 10% insect powder showed pasting profiles comparable to that of semolina. In turn, insect powder higher than 20% may help maintain the structure of gelatinized starch granules, enhance their ability to withstand shear thinning and heating processes, and improve dough stability in a potential preparation of wheat-based products.

3.4. Fourier Transform Infrared spectroscopy (FTIR)

In Fig. 2 are reported the FTIR spectra of all durum wheat semolina and *T. molitor* larvae powder mixtures including the 0IN100S and 100IN0S samples. The spectral characteristics of durum wheat flour, of which proteins and starch are the main components, have been described by several authors (De Girolamo et al., 2019; Song et al., 2018). Conversely, few studies have been conducted on the spectral analysis of *T. molitor* larvae powder or on products based on this protein source (Benes et al., 2022; Gkinali et al., 2022a, b).

Consistently with previous findings, all spectra (Fig. 2) showed a high-intensity peak in the region between 3000 and 3600 cm^{-1}, which are attributed to the hydroxyl group and reflect the interactions between water molecules and the semolina components through hydrogen bonds. Garcia-Valle et al. (2021) reported that the band centered at 3270 cm^{-1} indicates the prevalence of bonding of water molecules and OH functional groups of starch. Likewise, the spectra of *T. molitor* larvae powder showed a wide peak in the same wavelength range slightly shifted in position due to changes occurring in the electron distribution in the molecular bonds (Gkinali et al., 2022). Moreover, since the durum wheat semolina used in the present study contains 1.2% of lipid content, it showed a small peak at 2930 cm^{-1}, corresponding to the C–H stretching of aliphatic group and lipid-amylose complexes. Due to the composition of the insect flour, which consists mainly of proteins, fats, and carbohydrates, absorption peaks associated with these compounds appeared. New peaks and differences in intensity were observed. In particular, the insect flour (30.8% of lipid content) and the mixtures investigated exhibited a double peak, not detected in semolina, at about 2921 and 2850 cm^{-1}, corresponding to the symmetric and asymmetric vibrations of the aliphatic-CH_2 bonds of lipids (Garcia-Valle et al., 2021; Gkinali et al., 2022a, b). The intensity of this doublet increased with increasing the amount of insect flour in the mixtures. *T. molitor* larvae powder and the mixtures investigated showed also a peak at 1745 cm^{-1}, corresponding to the carbonyl group of the esters of lipid triacylglycerols

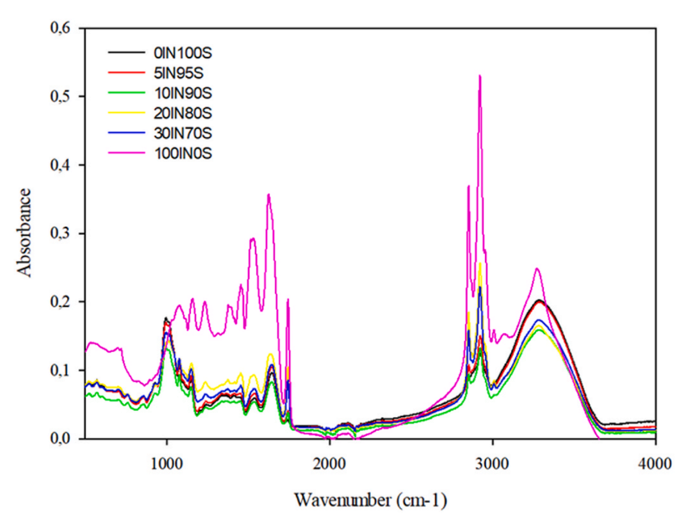

Fig. 2. Fourier Transform Infrared (FTIR) spectra of the mixtures.

(Garcia-Valle et al., 2021; Gkinali et al., 2022a, b).

The bands at about 1640 and 1540 cm^{-1} are typical of proteins and were attributed to Amide I, reflecting 80% C=O stretch and minor contribution of C–N stretch, and Amide II, reflecting 60% N–H, 30% C–N, and minor contribution of C–C stretches. The low-intensity peak at 1240 cm^{-1}, more visible in the insect powder, was previously ascribed to Amide III (N–H stretch) (Gkinali et al., 2022a, b).

The spectral region of polysaccharides, mainly starch in the case of semolina and the mixtures investigated, is located in the range of 800–1200 cm^{-1} and reflects the vibrations of CO, CN, CC and CH groups, while the peak at 1080 cm-1, more distinct in the insect powder, was attributed to the asymmetric stretching of the C–O–C structure in chitosan present in *T. molitor* larvae (Song et al., 2018).

The band characteristic of starch is composed of different overlapping contributions, such as the band at 1047 cm^{-1}, indicative of the amount of ordered structures, and the band at 1022 cm^{-1}, characteristic of amorphous structures. Therefore, the ratio 1047/1022 ($R_{1047/1022}$), reported in Table 5, indicates the intermolecular short-range order in starch granules. Interestingly, in line with Garcia-Valle et al. (2021), who investigated the addition of different concentrations of chickpea flour in durum wheat pasta, this ratio increased with the content of protein-rich insect flour, indicating that the starch chains were better organized with the addition of the non-conventional flour, due to the interference of insect powder's proteins with the internal organization and gelatinization of starch granules.

3.5. Bioactivity of durum wheat semolina and T. molitor larvae powder mixtures

The total phenolic content (TPC) and the antioxidant activity of the samples from durum wheat semolina, *T. molitor* larvae powder and their mixtures were determined, and the results achieved were reported in Fig. 3.

It is known that durum wheat semolina has important levels of functionality due to its composition, with a detected TPC of 0.15 ± 0.02 mg GAE/g_{DW} of semolina, in agreement with the TPC ranges found elsewhere (Gull et al., 2018; Spinelli et al., 2019).

Likewise, in terms of biological activity, recent studies have reported that in *T. molitor* larvae are present phenolic compounds with antioxidant activity (Baek et al., 2019; Song et al., 2019; Wu et al., 2020). Interestingly, the insect powder showed the highest level of TPC (0.69 mg GAE/g_{DW}) and antioxidant activity (0.29 mg $FeSO_4$/g_{DW}, 0.31 mg Trolox/g_{DW}, 0.61 mg Trolox/g_{DW} for FRAP, DPPH, and ABTS assays, respectively) compared to the other samples investigated. Even though the TPC and antioxidant activity levels detected in *T. molitor* lavae flour depend on several factors (raw materials, equipment and experimental protocols), the TPC achieved in the present work is in line with that found by Keil et al., (2022) for methanolic samples of yellow mealworms (0.21–0.85 mg GAE/g_{DW} larvae).

Several authors also found that mealworms yielded good antioxidant capacity (Keil et al., 2022) and scavenging activities similar to the activity level of 40–60 μM tocopherol (Baek et al., 2019). Hydrophobic and aromatic amino acid content of mealworm proteins increase their antioxidant properties due to the electron-donating capacity of the amino acids, and the radical scavenging ability of lipids (Oh et al., 2022). This suggests that mealworms have great potential as a source of bioactive compounds which could be used in food, pharmaceutical, and cosmetic fields (Kim et al., 2018).

Table 5
FTIR 1047/1022 ratio ($R_{1047/1022}$) associated to the hydrated and ordered starch structures in the samples investigated.

Sample	0IN100S	5IN95S	10IN90S	20IN80S	30IN70S
$R_{1047/1022}$	0.766 ± 0.01^{ab}	0.738 ± 0.02^{a}	0.802 ± 0.02^{ab}	0.836 ± 0.03^{b}	0.817 ± 0.02^{b}

Despite the increasing popularity of insects and the commercial relevance of durum wheat, studies on the effect of replacing durum wheat semolina with *T. molitor* larvae powder on the bioactivity of the mixtures or wheat-based products fortified with the same insect powder are still limited.

However, as shown in Fig. 3, the substitution of semolina with *T. molitor* larvae insect powder significantly increased the bioactivity of the semolina itself in terms of phenolic compounds and antioxidant activity.

Compared to semolina, the mixtures showed significantly higher TPC (Fig. 3a), with increments ranging from 20% to 87% when increasing the amount of insect powder from 5% to 30%.

In line with Zielińska et al. (2020), who demonstrated that the antioxidant activity levels of shortcake biscuits increased when adding mealworm flour in the recipe, a similar trend was observed for the antioxidant activity of the mixtures that, regardless of the method used, showed average values significantly higher than those detected for semolina (+78% for FRAP, + 2-fold for DPPH, and +67% for ABTS). Most antioxidants contain hydroxyl groups that could interact with starch and proteins via non-covalent bonds, such as hydrogen bonds and electrostatic interactions, to form complexes (Ngo et al., 2022; Zhu, 2015) which may influence the recovery of antioxidant compounds from the mixtures, and their bioaccessibility. Nevertheless, bonding between bioactive compounds and macromolecules may provide adequate protection against their deterioration during the transformation process and preparation of the potential food while improving its nutritional value (Seczyk et al., 2019).

The values of the reducing sugar content measured in the aqueous samples obtained from durum wheat semolina, insect powder and their mixtures are reported in Fig. S1. Durum wheat semolina and *T. molitor* larvae powder had similar amounts of reducing sugars in their proximate composition. Therefore, since the starch and the complex sugars of the semolina are usually hydrolyzed during mixing, dough-making and leavening, producing reducing sugars (Hidalgo and Brandolini, 2010), no significant differences in reducing sugars content in the raw mixtures investigated could be expected.

4. Conclusions

Results of the present study represent valuable keylines for the technological exploitation of *T. molitor* larvae flour in food formulations.

As expected, the substitution of durum wheat semolina with increasing amounts of insect flour leads to visible color changes. In turn, no differences are observed among the hydration properties of mixtures and semolina, suggestive of negligible undesired changes in the final products.

T. molitor larvae flour enhances the bioactivity of the mixtures in terms of phenolic content and antioxidant activity, and could also improve the stability of doughs, as suggested by the trend of the pasting parameters.

Therefore, owing to its remarkable techno-functional characteristics, *T. molitor* larvae flour could be used as a novel ingredient for food products formulation as an alternative to traditional proteins, bringing environmental, economic, and nutritional advantages.

Although there have been advances in the development of food products functionalized with edible insects, it is needed to further investigate their functional, physicochemical, and sensory attributes, the mechanisms of interaction of bioactive compounds with the food components and their stability during the transformation processing steps.

In this scenario, the potential addition of *T. molitor* larvae flour to low-cost staple foods with long shelf life, as a vector of compounds that are not daily consumed in sufficient quantities, could be an opportunity to promote healthier and more sustainable lifestyles without drastically altering consumers' habits.

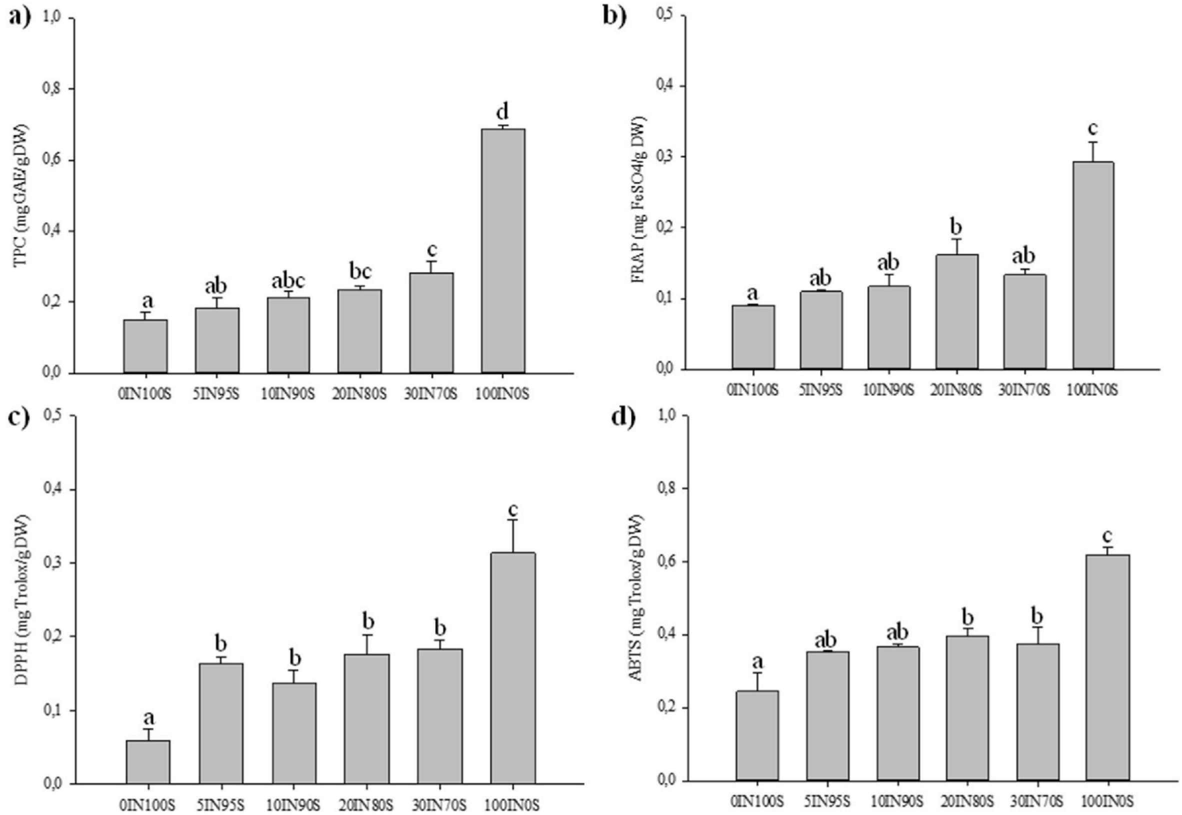

Fig. 3. Total phenolic content (TPC) (a), Ferric Reducing antioxidant Power (FRAP) (b), Total antioxidant activity (DPPH) (c), Total antioxidant capacity (ABTS) (d) of the samples obtained from the mixtures.
Values with different lowercase letter are significantly different ($p \leq 0.05$).

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Unlisted references

Fernandes et al. (2013); AACC, 1999; Zielińska et al., 2020; .

CRediT authorship contribution statement

Serena Carpentieri: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Agnieszka Orkusz:** Conceptualization, Methodology, Investigation, Formal analysis, Supervision. **Giovanna Ferrari:** Conceptualization, Data curation, Writing – review & editing, Supervision. **Joanna Harasym:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – review & editing, Funding acquisition, Supervision, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

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

Data availability

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.crfs.2023.100672.

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