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# Effects of formulation and process conditions on microstructure, texture and digestibility of extruded insect-riched snacks

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ABSTRACT

Extruded cereals made of wheat flour and grinded Yellow mealworm larvae (*Tenebrio molitor*) were produced to investigate the effect of insect inclusion (0%, 10%, 20%) and processing conditions (barrel temperature and screw speed) on their nutritional content, microstructure, texture and digestibility. Snacks enriched with 10% mealworm powder shifted their macronutrient composition towards a protein content high enough to claim the food as "source of protein" according to European food regulation. At 10% of enrichment, the adoption of high barrel temperature and screw speed significantly improved the microstructure, in terms of expansion and pore structure, delivering acceptable textural qualities. At 20% substitution, snacks showed poor expansion properties, mainly due to the presence of fat in the larvae. Starch and protein digestibility of were correlated with microstructure properties as a function of porosity, pore size and wall thickness. Interestingly, mechanical forces generated in extrusion likely improved the digestibility of *T. molitor* proteins which are tightly bound and sclerotized to the exoskeleton. Tailoring processing conditions and formulation insect ingredients can be successfully incorporated into extruded cereal snacks.

*Industrial relevance:* This study evaluated the nutritional and technological properties of extruded cereal snacks enriched with an edible insect powder (*T. molitor*). Results suggested that edible insects can be used as novel ingredient in extruded snacks and pointed out how processing conditions can modulate snack digestibility.

## 1. Introduction

Insects are highly valued as tasty and nutritious food by over 2 billion people mostly living in Asia, Africa and South America (FAO, 2013). Given the wide range of edible insect species, over 2000 reported by Jongema (2013), their nutritional composition vary largely, displaying a broad range of protein, fat and carbohydrate concentrations (Raubenheimer, Rothman, Pontzer, & Simpson, 2014). Consequently, some species containing high amounts of specific nutrients represent a promising, yet underexploited food source. For instance, species of the order Orthoptera (grasshoppers, locusts, crickets) can contain up to 77% protein (dry basis) while a fat content of about 77% fat is reported for larvae of *Phassus triangularis*, Lepidopteran (moths and butterflies) (Ramos-Elorduy, Pino-M, & Cuevas-Correa, 1998). Furthermore, insects are also rich in important minerals, including copper, selenium, iron, zinc, magnesium, manganese, phosphorous, and vitamins like biotin, riboflavin, pantothenic acid, and folic acid (Rumpold & Schlüter, 2013).

Recently, insects have gained much interest in Western societies, especially as an alternative source of protein. One of the main advantages over other protein sources is the low environmental costs of production, which becomes essential to satisfy the rise in the global proteins demand (van Huis, 2013). Nevertheless, for distribution of edible insects on industrial scale different challenges need to be addressed, covering the development of automated rearing facilities, safe hygienic processing practices, and the establishment of international food regulations (Rumpold & Schlüter, 2013).

Westerners show a great aversion towards the consumption of insects. To date, rejection towards insects has been mainly attributed to cultural and psychological barriers (Harris, 1985) and association with unhygienic or rotten foods (Caparros Megido et al., 2016). However, several studies show that presenting insects invisibly within familiar preparations may be effective to reduce negative perceptions and to increase their acceptance (Hartmann & Siegrist, 2016; Looy, Dunkel, & Wood, 2014; Tan et al., 2015; Tan, van den Berg, & Stieger, 2016). Recently, Le Goff and Delarue (2015) reported that consumers reject the idea of tasting chips made by an invisible insect based flour, but they accept it after the first bite, suggesting that processing insects to

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create appealing products may be a good strategy to vehicle insect into food designed for Western consumers.

Ready-to-eat expanded snacks are very popular among Western consumers principally due to their convenience, attractive appearance and unique texture attributes. In fact, the consumption of expanded products has increased tremendously in recent years and their global market is projected to reach 31 billion dollars by 2019 (marketsandmarkets, 2014). Expanded snacks are produced through the extrusion-cooking technology, which is an efficient manufacturing process. By combination of mechanical shear, high temperature and high pressure, starch solid materials are converted into a viscoelastic fluid that is pushed by a screw through a die. The sudden decrease in pressure vaporizes the water embedded in the fluid, leading to the formation of a solid foam with specific structural characteristics (pore wall size and thickness, porosity, density) and mechanical properties (Guy, 2001). Although starchy materials are the most suitable to deliver good technological features for production of acceptable snacks, they are often low in protein and dietary fiber being unable to satisfy the needs of an increasing number of health-conscious consumers (Brennan, Derbyshire, Tiwari, & Brennan, 2013).

Improving the nutritional value of these types of snacks is a twofold opportunity either for consumers' health and food industries. Up to now the focus of the nutritional improvement was on whole grains, addition of legumes and other functional ingredients, which cause a decrease of sectional expansion and density, thereby increasing hardness of extruded products (Chanvrier, Desbois, et al., 2013; Lazou, Michailidis, Thymi, Krokida, & Bisharat, 2007; Ramos Diaz et al., 2015; Robin et al., 2012; Sumargo, Gulati, Weier, Clarke, & Rose, 2016; Yu, Ramaswamy, & Boye, 2013). To date, the use of edible insects in expanded extruded snacks has not yet been investigated. Besides the foreseen progress of insects' fractionation systems to obtain protein, fat and chitin, the incorporation of insects invisibly into familiar foods like snacks may be a valid strategy to speed their adoption among consumers.

This paper investigates the effects of Yellow mealworm larvae added to extruded snacks. The enrichment in insect powder has been studied at different operating conditions with two main aims: (i) to assess the microstructure and texture properties of the extrudates; (ii) to evaluate the digestibility of the obtained final products.

## 2. Materials and methods

### 2.1. Materials

Wheat flour of type 0 was supplied by Molino Taramazzo (Pezzolo Valle Uzzone, Cuneo, Italy). Larvae of Yellow mealworms were supplied in dry form by from HaoCheng Mealworm Inc. (Xiangtan, Hunan, China). Larvae were microwave dried by the provider maintaining the temperature below 80 °C. Dry larvae were ground for 60 s at 6000 rpm in a knife mill (Grindomix GM 200, Retsch, Germany) to pass through 900 μm sieves. Three blends of wheat flour and mealworm powder were formulated in mass ratios of 100:0, 90:10 and 80:20 on a dry matter basis (Table 1). Before use, the blends were mixed through a planetary mixer (cooking chef, Kenwood Ltd. UK).

### 2.2. Extrusion processing

The extrusion trials were conducted using a co-rotating twin-screw extruder (BC-21 extruder, Clextral, Firminy, France). The barrel consisted of nine independent zones with a total barrel length of 900 mm. The screw had a distance between shafts of 21 mm (L/D = 36:1), and a circular die opening of 5 mm. Further specifications on the screw configuration are described by De Pilli, Carbone, Derossi, Fiore, and Severini (2008). The extruder was operated at a constant feed rate of 10 kg/h and 18% moisture dry bases. The decision of the hydration regime was based on preliminary trials. More specifically, higher moisture values (20%) led to very poor expansion at all processing conditions, and the use of lower moisture (16%) caused clogging of the feed material in the barrel.

**Table 1**
Formulation and extrusion conditions used for the processing of Exp.1 and Exp.2 and moisture loss of extrudates at the die. Temperature of the last three zones are reported.

| Samples | Wheat: mealworm ratio | Barrel temperature (°C) | Screw speed (rpm) | SME (kJ/kg) | Moisture loss (%) |
|---|---|---|---|---|---|
| Experiment 1 | | | | | |
| i0 | 100:0 | 120, 150, 160 | 400 | 485 | 40.3 ± 0.3 |
| i10 | 90:10 | 120, 150, 160 | 400 | 339 | 32.3 ± 0.3 |
| i20 | 80:20 | 120, 150, 160 | 400 | 166 | 9.9 ± 0.4 |
| Experiment 2 | | | | | |
| i1 | 10 | 100, 110, 120 | 240 | 155 | 17.8 ± 0.3 |
| i2 | 10 | 100, 110, 120 | 320 | 244 | 27.0 ± 0.2 |
| i3 | 10 | 100, 110, 120 | 400 | 445 | 26.9 ± 0.2 |
| i4 | 10 | 110, 130, 140 | 240 | 127 | 18.7 ± 0.1 |
| i5 | 10 | 110, 130, 140 | 320 | 197 | 27.4 ± 0.2 |
| i6 | 10 | 110, 130, 140 | 400 | 392 | 28.8 ± 0.1 |
| i7 | 10 | 120, 150, 160 | 240 | 104 | 32.4 ± 0.1 |
| i8 | 10 | 120, 150, 160 | 320 | 170 | 35.6 ± 0.1 |
| i10 | 10 | 120, 150, 160 | 400 | 339 | 32.3 ± 0.3 |

SME: specific mechanical energy.

Blends were extruded in two sets of experiments as reported in Table 1 and herein referred to as Exp.1 and Exp.2. In the first experiment three different formulations containing a mass fraction of 0, 10 and 20% of grinded mealworms were extruded as reported above, while in the second experiment the formulation containing 10% grinded mealworms was processed with 9 different combination of extruder screw speed and barrel temperature. The rationale for choosing the formulation of 10% was to fully study the effect of temperature and screw speed, and build a complete design of experiments with these factors while still maintaining an average level of insect enrichment.

In all experiments, temperature profile of the first six zones was set at 30, 30, 50, 60, 80 and 100 °C in forward order, while temperature of the last three zones and screw speed were set as reported in Table 1. Specific mechanical energy (SME) was computed according to De Pilli et al. (2008). After extrusion, samples were dried at 50 °C for 6 h, sealed in plastic bags and stored at − 20 °C till the experiments.

### 2.3. Nutritional analysis of extruded products

Extruded snacks were milled to pass through 90 μm sieves and analysed for total starch by Megazyme total starch kit K-TSTA 09/14 (AOAC, 2005); lipid by Soxhlet extraction (AOAC, 1920); ash by gravimetric method (AOAC, 1923); protein content, analysed by Dumas method of combustion (AOAC, 1996) using a Nitrogen/Protein analyser (model FP-528, Leco St. Joseph, USA). EDTA was used as standard (Elemental Microanalysis Ltd., Okehampton, UK). Different nitrogen-protein conversion factors were used for each formulation. Proteins in wheat flour and mealworm powder were calculated by using respectively 5.71 and 6.25 N conversion factor. For blends, the N-conversion factors were computed as weight average between single N-factors and protein content.

### 2.4. Expansion characterization

#### 2.4.1. Moisture analysis

Moisture loss corresponds to the moisture content of extruded snacks at the die exit and it was computed by subtracting the residual moisture from the in-barrel moisture. Moisture was measured according to AOAC (1999) on six replicates.

#### 2.4.2. Microstructure

Microstructural features of the extruded products were analysed

using X-ray microtomography. Three pieces of snacks from each treatment were stuck on a sample holder and scanned by a micro-CT scan (SkyScan 1174, Brüker, Kontich, Belgium) under the following settings: voltage 50 kV, current 800 μA, sample rotation 360°, rotation step 0.6°, average frame 3, magnification 28.5 μm, exposure time 1600 ms. Scans were performed with the Skyscan software (version 2), reconstruction with the Skyscan NRecon software (version 1.6.2.0) and 3D image analysis with CTAn software (version 1.12.0.0). A number of 300 cross sectional greyscale images were reconstructed and converted into binary images by applying Otsu's method. The volume of interest was selected by the shrink-wrap function and the volume was eroded automatically to adjust to the surface of the sample. Product diameter was computed from the average area of extruded products as obtained by 2D analysis assuming samples had a circular shape. From that, expansion ratio was computed as the ratio between the square of product diameter and the square of die diameter. The pore size distribution (PS) was obtained by the structure separation function and the pore wall thickness distribution (PWT) was obtained by the structure thickness function. To reduce the error from noise and artefacts only voxels larger than $3 * 3 * 3$ pixels were considered pores. Porosity was calculated as the ratio of the volume of pores and the total volume of the object. Pore density was expressed as ratio among number of pores per cubic millimetre. The total volume was subdivided into two regions, each of 300 images from bottom up.

### 2.5. Texture

Mechanical properties of the snacks were measured with a texture analyser (model TA-XTplus) equipped with a 50.00 kg load cell and operating with the software Exponent 6.1.4.0 software (Stable Micro Systems, Surrey, UK). Six dried pieces obtained from each extrusion condition were compressed to 70% of their initial diameter using a 75 mm compression plate (test speed of 1.0 mm/s). From the force-displacement curves, the maximum peak of the curve corresponded to the maximum compression force (Fmax) (N). The area under the curve ($S$) and the number of peaks ($n$) were used to calculate the spatial frequency of ruptures ($N_{sr}$) as in Eq. (1), the average crushing force ($F_{cr}$) as in Eq. (2) and crispness work ($W_c$) as in Eq. (3) (Agbisit, Alavi, Cheng, Herald, & Trater, 2007; Karkle, Keller, Dogan, & Alavi, 2012).

$$N_{sr} = n/d \ (\mathrm{mm}^{-1}) \quad (1)$$

$$F_{cr} = S/d \ (\mathrm{N}) \quad (2)$$

$$W_c = F_{cr}/N_{sr} \ (\mathrm{N\ mm}) \quad (3)$$

where $d$ corresponds to the probe travel distance.

### 2.6. Static in vitro gastrointestinal digestion

To evaluate the digestibility of extruded products, the standardised static *in vitro* digestion protocol developed within the COST FA1005 INFOGEST Network was adopted (Minekus et al., 2014). The protocol holds an international consensus and it is based on human gastrointestinal physiological conditions. Before digestion, samples from Exp.1 and Exp.2 were obtained using a laboratory mill (model A 11, IKA, Staufen, Germany) and sieved through stainless steel sieves to obtain a particle size between 0.9 and 1.4 mm. The reasoning for choosing this range of particle size was to retain most of the snack microstructure while being consistent with the particle size reduction upon mastication (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007). Additional digestion was performed on snack samples with a particle size below 0.09 mm in order to investigate digestibility in a condition in which the effect of the pore segments on digestibility is reduced. Stock solutions of concentrated simulated salivary (SSF), gastric (SGF) and duodenal fluids (SDF) were prepared according to Minekus et al. (2014). An amount of 0.5 g of ground snacks were hydrated with 2.0 mL of deionized water in centrifuge tubes. Subsequently, 1.75 mL of SSF were added, the pH was adjusted to 7.0, followed by the addition of a solution of $Ca_2Cl$ to achieve 0.75 mM in the final mixture and the necessary amount of deionized water to reach 50:50 ratio (w/v) between the sample solution and SSF. The solution was agitated for 2 min at 37 °C to reproduce the oral phase. In this study salivary α-amylase was not added since the salivary hydrolysis of starch is negligible respect to the amount degraded by pancreatin in the intestinal phase (Woolnough, Bird, Monro, & Brennan, 2010). Bolus derived from the oral phase was mixed with 3.75 mL of SGF supplemented with pepsin (P6887 from Sigma, CAS n. 9001-75-6) to achieve 2000 U $ml^{-1}$ in the final digestion mixture. The solution was adjusted to pH 3.0, $Ca_2Cl$ was added (to achieve 0.075 mM in the final mixture), followed by deionized water as indicated above. The digestate was kept at 37 °C for 2 h followed by a gentle agitation at 20 rpm with an overhead shaker. Subsequently, 9.25 mL of SDF containing pancreatin (100 U trypsin activity/mL of digestate) and bile (10 mM in the total digesta) was added to the gastric chyme, the pH was adjusted to 7.0 followed by the addition of $Ca_2Cl$ to achieve 0.3 M in the final mixture and deionized water. Samples were then incubated for 120 min as indicated above. Trypsin activity of pancreatin (P1750 4 × USP, from Sigma) was assayed using the *p*-toluene-sulfonyl-L-arginine methyl ester (TAME) assay as recommended by Minekus et al. (2014). The intestinal phase was stopped by adding 4 μL of 5 mM the protease inhibitor (Pefabloc® SC – 76307) and immediately centrifuged at 2000 g for 5 min at 4 °C to remove unreacted starch (Warren, Zhang, Waltzer, Gidley, & Dhital, 2015). Thereafter the supernatant was filtered through a 0.45 μm Phenex-PTFE filter, snap-freezed in liquid nitrogen and stored at − 20 °C until analysis. The digestion was performed in triplicate and the degree to which protein and starch were digested was determined according to the methods described respectively in Sections 2.6.1 and 2.6.2.

#### 2.6.1. Determination of protein digestibility

The degree of protein hydrolysis after *in vitro* digestion was analysed by determining the content of free α-amino groups ($NH_2$) in the digestate supernatant. Free α-amino groups were determined after reaction with ortho-phthalaldehyde (OPA) following the method of Nielsen, Petersen, and Dambmann (2001). Complete hydrolysis of samples was performed in 6 N HCl at 110 °C for 24 h for obtaining the number of total $NH_2$ groups. For quantification of $NH_2$ groups, a calibration curve of L-serine (0.1 to 1.6 mM) was made and values of digestibility were computed as in Eq. (4)

$$\text{Protein digestibility:}[(\text{free } NH_2 \text{ groups after INFOGEST digestion}) / (\text{free } NH_2 \text{ groups after acid digestion})] \times 100 \quad (4)$$

#### 2.6.2. Determination of starch digestibility

The degree of starch hydrolysis, after *in vitro* digestion, was assessed by determining the content of glucose. To this purpose, sugars in the supernatant, released from the pancreatic phase, were hydrolysed to glucose. Hydrolysis was performed with excess of amyloglucosidase from *Aspergillus niger* (800 U/mL of supernatant; 10113, Sigma) in 10 mM Na-acetate buffer, pH 4.8, at 60 °C for 3 h. Glucose was quantified using a glucose oxidase kit (GOPOD format, Megazyme Wicklow, Ireland) and converted to starch by a multiplication factor of 0.9. Starch digestibility was computed as in Eq. (5)

$$\text{Starch digestibility:}[(\text{Starch after INFOGEST digestion}) / (\text{Total starch in extruded snacks})] \times 100 \quad (5)$$

were total starch was measured as reported in the nutritional analysis section.

**Table 2**
Nutritional composition of snacks and ground Yellow mealworms. Data are expressed as g/100 g (dry basis). Values in brackets (%) correspond to the percentage of energy provided by the nutrient in the formulation.

| Nutritional composition | i0 | i10 | i20 | Yellow mealworms | AMDR (%)*** |
|---|---|---|---|---|---|
| Starch | 81.1[a] (85.5) | 73.3[b] (75.5) | 66.0[c] (67.0) | 5.3 (4.4) | 45–65 |
| Protein | 11.8[a] (12.5) | 15.9[b] (16.4) | 20.4[c] (20.7) | 54.2 (44.8) | 10–35 |
| Lipid | 0.9[a] (2.1) | 3.5[b] (8.1) | 5.4[c] (12.3) | 27.4 (50.9) | 20–35 |
| Ash | 0.7[a] | 1.1[b] | 1.4[c] | 4.0 | – |
| Other* | 5.5 | 6.2 | 6.8 | 9.1 | – |
| Energy value (kcal/100 g)** | 379 | 387 | 394 | 484 | – |

Means (n = 3) with different subscript within the same row are significantly different ($p < 0.05$).

* Includes fiber, other carbohydrates and vitamins.

** Heat of combustion considered: starch (4 kcal/100 g), protein (4 kcal/100 g), lipid (9 kcal/100 g).

*** Acceptable macronutrient distribution ranges of food (Food and Nutrition Board, 2002).

### 2.7. Statistical analysis

The data analysis was aimed at understanding the product characteristics (structure, texture and digestibility) as a function of formulation and process parameters. Analysis of variance (ANOVA) was performed to identify the main effect (Statistica, Statsoft, Inc., USA). Post-hoc analyses (Turkey HSD) were performed to assess the significance of difference at 95%.

## 3. Results and discussion

### 3.1. Composition of extruded snacks

The proximate composition of the extruded snacks obtained with different formulations is provided in Table 2. By increasing the percentage of mealworms, the protein content increased from 11.8 to 20.4 g/100 g and the lipid content from 0.9 to 5.4 g/100 g with a corresponding decrease in starch content from 81.1 to 66.0 g/100 g. Snacks enriched with mealworm powder improved their macronutrient composition towards the recommended ratio of nutrients for human diet as reported in the Acceptable Macronutrient Distribution Ranges of Food and Nutrition Board (2002). The high concentration of protein and lipid in mealworm powder, respectively of 54.2 g/100 g and 27.4 g/100 g, led to a considerable increase in the same components in the extruded snack. For samples i10 and i20 the protein accounts for respectively 16 and 21% of the total energy value, enough to claim the food respectively as “source of protein” and “high protein” according to European food law n. 1924/2006. Furthermore, the lipid content of 5.4% as present in i20 was still lower than those found in many extruded commercial products, in which lipid content may reach 35% of total weight (Ilo, Schoenlechner, & Berghofe, 2000).

### 3.2. Microstructure of extruded snacks

The representative cross-sectional X-ray images of the extruded products from Exp.1 and Exp.2 reported in Fig. 1 showed that the extruded product obtained from only wheat flour (i0) was the largest and the most aerated one. Generally, the addition of grinded mealworms progressively reduced the size of extruded snacks, the overall porosity as well as the size of the pores. Similarly, for Exp.2, it seems that the increase in screw speed promoted the expansion and the porosity fraction of the extrudates. However, with the aim to precisely analyze the effect of both formulation and processing conditions on microstructure, Fig. 2 shows the main microstructure properties on 3D structure of the extruded snacks. From the first set of experiments, expansion ratio of snacks, shown in Fig. 2b, changed from 2.95 to 2.42 and 1.45 respectively for i0, i10 and i20, indicating a negative linear relationship with the level of mealworm enrichment ($r^2$ 0.97). Also, as well as the expansion ratio, porosity fraction of snacks decreased with added Yellow mealworm. Interestingly compared to i0, the drop in porosity fraction by 12% for i10 was significant but limited if compared to i20, whose porosity fraction was almost halved. Furthermore, a decrease in average pore size (PS) and an increase pore wall thickness (PWT) was observed by increasing mealworms content from 10 to 20%.

On the other hand, when a fixed amount of mealworm powder of 10% was used (Exp.2) the results showed that the increase of barrel temperature and screw speed induced a higher expansion ratio and increased the pore size. These results perfectly matched the micro-tomographic images of Fig. 1 where a progressive increase of the overall diameter and pore size of the extruded snacks were reported. In addition, from the cross sectional images of Fig. 1, a series of knot-like formations were observed, especially in products obtained with mealworms and, for the Exp.2, for low processing temperature and low screw speed. Such knots are likely the results of poor product expansion, caused by limited starch conversion and the increase in protein and lipid content after insect enrichment (Ramos Diaz et al., 2015). For instance, snacks extruded at 160 °C and 400 rpm (i10) showed a porosity fraction of 76%, while exhibited a porosity fraction of 33% when operating at 120 °C and 240 rpm (i1) (Table S.1 in Supplementary material).

These observations reinforce intuitive expectations upon addition of oily and protein rich ingredients to an extruded product (Ilo et al., 2000; Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011). Structural properties are the result of viscoelastic properties of the melt, which depend on the characteristics of the raw materials (Moraru & Kokini, 2003). Looking at results of Exp.1, changes in microstructural properties could be mainly attributed to the lipid content. Concentrations of lipids below 1% lubricate and stabilize the extrusion process and favour the snack expansion, while higher concentrations reduce the melt viscosity causing a loss in mechanical energy (SME) conveyed to the melt (Ilo et al., 2000). This is in accordance with the SME values reported in Fig. 2. For blends in Exp.1 the SME decreased from 485 kJ/kg to 166 kJ/kg for sample i0 and i20 respectively, reflecting the effect of lipids in reducing the viscosity of the melt and hindering the hydration of starch, which is necessary for its gelatinization and expansion. Furthermore, the parallel increase in protein concentration may also have had a negative effect on expansion (Moraru & Kokini, 2003). Similar findings on extrusion of spirulina-corn (Joshi, Bera, & Panesar, 2014) and soy-rice (Yu et al., 2013) enriched extruded products were previously reported. During extrusion, proteins affect water distribution in the matrix and create a network through covalent and nonbonding interactions that changes extensional properties of the melt, limiting its expansion (Moraru & Kokini, 2003). Furthermore, although the observed changes can be mainly attributed to the increase of lipids and proteins, the lower expansion may also partially arise from the lower starch content in the recipes.

The mealworm level also affected the pore density in the extruded snacks from 2.2 to 10.3 pores per cubic millimetre in i0 and i20 respectively (Fig. 2). Of course, this effect is strictly related with the reduction in average PS above discussed. Microstructural attributes of extruded products from Exp.1 were further characterized in terms of volumic frequency of pore wall thickness and pore size. Results are presented in Fig. 3 and showed a clear decrease in pore size as mealworm concentration increased. In detail, the largest 20% of the pores were between 2.1 and 3.0 mm, 1.5 and 2.5 mm, 0.3 and 1.2 mm, in i0, i10 and i20, respectively. Similarly, the PWT increased as mealworm increased. Product having 20% grinded mealworm displayed the broadest spread as compared to those having 10 and 0% of mealworm.

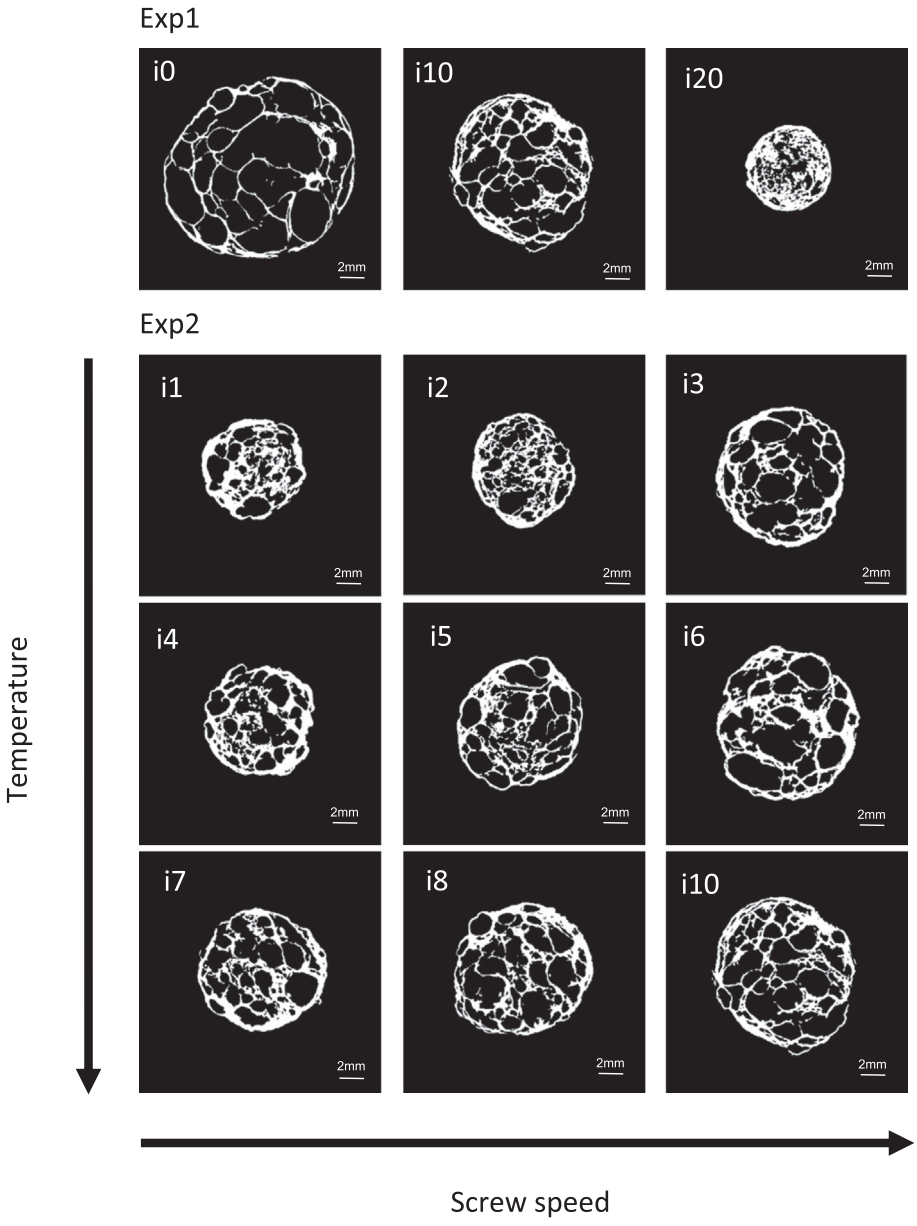


**Fig. 1.** Representative cross-sectional X-ray tomography images of extrudates from Exp.1 and Exp.2. Coded values are reported in Table 1.

Our results confirm that thicker walls and smaller pore sizes were the result of limited expansion at the die caused by poor viscoelastic properties of the melt (Chanvrier, Jakubczyk, Gondek, & Gumy, 2013) and low specific mechanical energy (SME) (Chanvrier et al., 2007).

Taking into account Exp.2 the results showed a dependency of microstructure on processing conditions (Fig. 2). The raise in temperature from 120 to 160 °C increased the porosity from 56 to 72% and decreased pore density from 5.4 to 3.9 pores per cubic millimetre. Porosity was positively and linearly correlated with mean pore size ($r^2$ = 0.78), while an inverse relationship was observed with mean pore wall thickness ($r^2$ = 0.95). The measured SME decreased with increasing temperature. More specifically, a drop in SME 280 to 204 kJ/kg was recorded when temperature raised from 120 to 160 °C. These results are in line with those of Altan, McCarthy, and Maskan (2008) and Moraru and Kokini (2003), who found that an increase in temperature led to a lower melt viscosity and to a consequent reduced SME. Although a reduced SME upon increasing temperature may suggest a loss in expansion, as above discussed, this effect was not observed in Exp.2. Bhattacharya (1997) justified this behaviour with two hypotheses. At first, high temperatures could increase the extent of starch gelatinization, improving the melt homogeneity and its extensional properties. Secondly, a higher input of thermal energy enhances the amount of superheated steam. Hence, moisture evaporated more easily, providing a more expanded and porous structure to snacks. According to this hypothesis, as from the data of Table 1, by increasing barrel temperature a significant increase in moisture loss was observed, particularly between samples extruded at 140 and 160 °C.

However, considering the effect of mealworm addition, only the 9.9% of initial moisture was lost in 20% mealworms snacks, compared with a loss of 40.3% for samples having 0% mealworms. That means a significant effect of formulation on water evaporation during the extrusion process.

As for barrel temperature, similar observation can be made for increasing screw speed, which exhibited a positive effect on product expansion as also reported from Moraru and Kokini (2003). Robin et al. (2012) observed a similar trend and they attributed the effect of screw speed on SME to a higher mechanical stress and shear rate transmitted from the screws to the melt, allowing a better hydration of starch and improved expansion. This is in line with our study, in which the increase in screw speed from 240 to 400 rpm increased porosity fraction

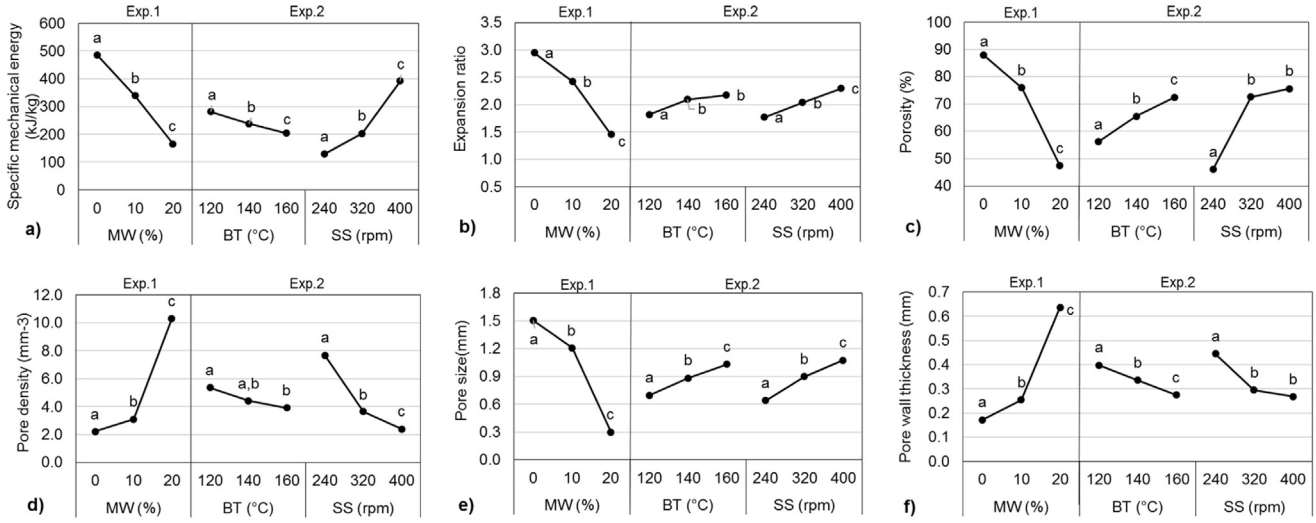


**Fig. 2.** Main effects of formulation and process conditions on specific mechanical energy (SME) and structural characteristics of extrudates from Exp.1 and 2. MW (mass fraction of mealworm), BT (barrel temperature), SS (screw speed). Points with different letters within the same independent variable are significantly different ($p < 0.05$).

from 46% to 75% and SME from 128 kJ/kg to 377 kJ/kg. On the other hand a negative effect between screw speed and pore density, which decreased from 7.7 to 2.4 pores per cubic millimetre respectively, was observed, as previously shown in Fig. 2.

### 3.3. Mechanical properties of extruded snacks

Textural attributes are summarized in Table 3. The maximum compression force of extruded products is usually associated with the sensory perception of hardness during chewing (Roudat et al., 2002). Considering insect powder addition (Exp.1), our experiments showed an increase of compression force with level of Yellow mealworm, while a decrease was observed with barrel temperature and screw speed (Exp.2). However, for a better understanding of the relationship between microstructure and texture properties, the compression forces were plotted against the total porosity (Fig. 4a). The graph clearly suggested that for both the different formulation and processing conditions, the compression force is negatively correlated with porosity fraction, following the expected solid foam model (Gibson & Ashby, 1997). In Exp.1 the samples i20 with porosity fraction below 50% exceeded a hardness of 400 N, while snacks corresponding to i0 and i10 showed lower Fmax. A similar behaviour was reported by Chanvrier, Desbois, et al. (2013) who showed that beside porosity, protein content further increased hardness. This was reasonably related to the high compactness of the extruded snacks obtained from the formulation richest in Yellow mealworm which was associated with modification of the pore wall composition and their morphology. However, the effect of protein cannot be stated in our case, probably due to the co-presence of proteins and lipids within the formulation.

**Table 3**
Textural characteristics of snacks from Exp.1 and Exp.2. Values with different letters within the same column indicate significant difference ($p < 0.05$).

| Sample | Texture | | | |
|---|---|---|---|---|
| | Fmax (N) | $N_{sr}$ ($mm^{-1}$) | $F_{cr}$ (N) | $W_c$ (N mm) |
| Exp.1 | | | | |
| i0 | $116^{a}$ | $12.51^{h}$ | $64^{a}$ | $5.1^{a}$ |
| i10 | $171^{b}$ | $10.56^{g}$ | $85^{a}$ | $8.0^{a}$ |
| i20 | $493^{f}$ | $4.23^{a}$ | $219^{f}$ | $52.0^{f}$ |
| Exp.2 | | | | |
| i1 | $561^{g}$ | $4.37^{a}$ | $221^{f}$ | $50.8^{f}$ |
| i2 | $379^{e}$ | $6.12^{c,d}$ | $177^{c}$ | $29.2^{d}$ |
| i3 | $281^{c,d}$ | $7.70^{e,f}$ | $144^{b}$ | $18.8^{b,c}$ |
| i4 | $469^{f}$ | $4.87^{a,b}$ | $200^{d,e}$ | $41.3^{e}$ |
| i5 | $391^{e}$ | $6.71^{d}$ | $179^{c,d}$ | $27.0^{d}$ |
| i6 | $274^{c}$ | $7.00^{d,e}$ | $145^{b}$ | $20.7^{c}$ |
| i7 | $312^{d}$ | $5.53^{b,c}$ | $169^{c}$ | $30.6^{d}$ |
| i8 | $250^{c}$ | $8.66^{f}$ | $133^{b}$ | $15.4^{b}$ |
| i10 | $171^{b}$ | $10.56^{g}$ | $85^{a}$ | $8.0^{a}$ |

Fmax: maximum compression force; $N_{sr}$: spatial frequency of ruptures; $F_{cr}$: average crushing force; $W_c$: crispness work.

Moreover, taking into account the main microstructure properties of the extruded snacks as reported in Table S.1, the lowest porosity product (i20) also exhibited the lowest spatial frequency of ruptures ($N_{sr}$)

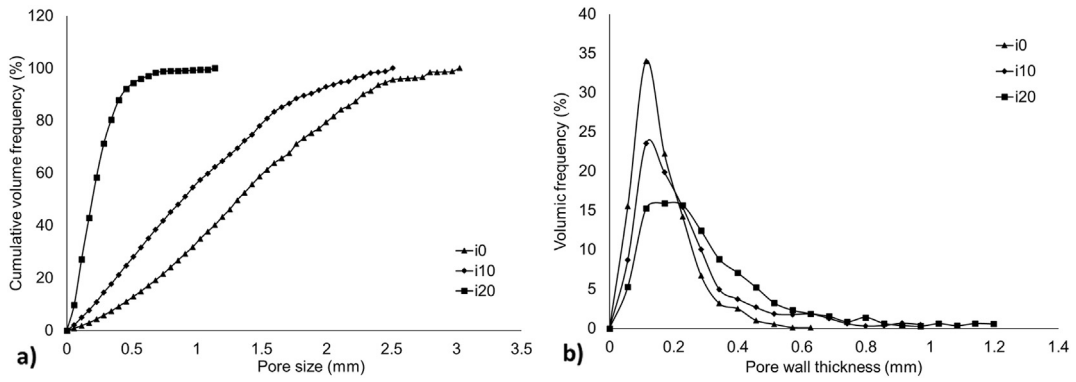


**Fig. 3.** a) Cumulative cell size-volumetric distribution; b) cell wall thickness volumetric-distribution of extruded snacks from Exp.1.

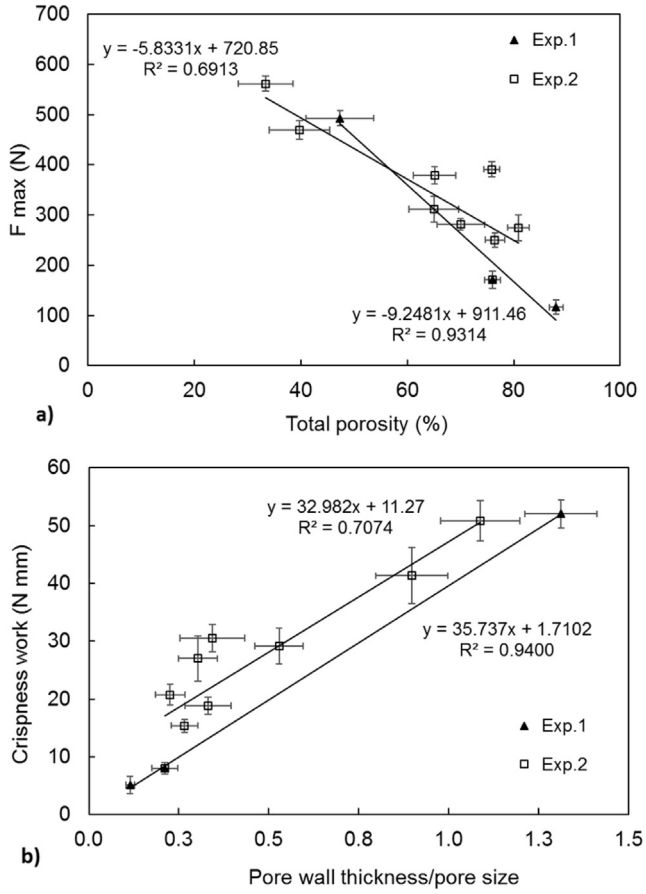


**Fig. 4.** Mechanical properties of extrudates from Exp.1 and Exp.2: (a) maximum compression force (Fmax) *versus* total porosity and (b) crispness work *versus* PWT/PS. Error bars represent one standard deviation from the mean.

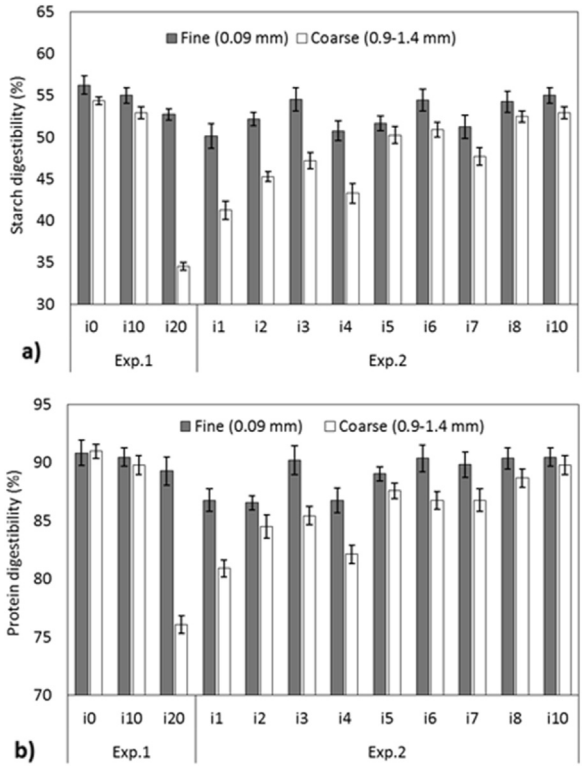


**Fig. 5.** Comparison of a) starch and b) protein digestibility between extruded snacks at fine (< 0.09 mm) and coarse (0.9–1.4 mm) particle size. Error bars represent one standard deviation from the mean.

which describes the number of fracture events occurring during compression. We also found that $N_{sr}$ was positively correlated with pore size of the extruded snacks (data not shown). This is in line with findings of Chanvrier, Valle, and Lourdin (2006), who related this behaviour to the propagation of fractures at the phase interface, so that large pores exhibit a higher interfacial area and give more peaks. Crispness work ($W_c$), can be interpreted as the sensory parameter of fracturability and describes the work required to fracture one pore or a group of pores. From Table 3, both processing conditions and mealworm enrichment had an impact on $W_c$. In particular, crispness work increased with mealworm addition from 5.1 to 52 N mm for sample i0 and i20, respectively. Also, the snacks processed at lower barrel temperature and screw speed showed the higher $W_c$ with maximum value of 50.8 N mm for sample i1 obtained at 120 °C and 240 rpm. Again, crispness work data were plotted against the pore wall thickness/pore size with the aim to better understand the relationship between microstructure and texture properties (Fig. 4b). A strong relationship was found showing as the average force required to break a pore decreased when the extruded snacks were larger with thinner pore walls. Furthermore, the samples of Exp.1 fell in the right hand of the figure showing a higher pore wall thickness/pore size at constant $W_c$ values than those of Exp.2. It should be remembered that snacks were obtained at the highest screw speed and barrel temperature, which may explain these values.

### 3.4. *In-vitro digestibility of extruded snacks*

Digestion of extruded snacks was performed with the standardised *in vitro* INFOGEST protocol. To properly mimic the oral processing occurring during human consumption, it is important that also particle size of the digestate is considered. During mastication, food is broken into small particles. For snacks, the average particle size before swallowing is in the range between 0.9 and 1.4 mm (Jalabert-Malbos et al., 2007). On these basis and in order to highlight the potential impact of microstructure properties, the determination of starch and protein digestibility was performed on both fine (below 0.09 mm) and coarse (0.9–1.4 mm) extruded snacks. Therefore, to improve readability, the results on digestibility of snacks with different particle sizes will be discussed in two different sections.

#### 3.4.1. *In-vitro starch and protein digestibility of the fine particle of the extruded snacks*

Results of fine particle starch digestibility (FSD) are reported in Fig. 5a. In Exp.1 values of FSD ranged from 55.9 to 52.7% with the highest FSD measured for snack obtained without insects. The addition of 10% insects did not influence the FSD ($p > 0.05$), while a significant decrease to 52.7% was observed at 20% addition. The degree of starch digestibility in extruded products is mainly linked to its gelatinization (Altan, McCarthy, & Maskan, 2009). The reduction of FSD with insect addition is likely due to the effect of lipids on the mechanical energy input and the consequent limited starch transformation. Lin, Hsieh, and Huff (1998) reported that the presence of 5% fat, similar to that found in i20, reduced the SME and the melt temperature, causing the decreasing of starch gelatinization from 100% to 82%. Furthermore, fat was also hypothesized to prevent absorption of moisture and gelatinization by forming a hydrophobic layer outside starch granules. According to Guha, Ali, and Bhattacharya (1998) and Altan et al. (2009), protein-starch networks, developed during extrusion, may also decrease starch digestibility by trapping starch granules and reducing their susceptibility to enzymatic attack. In Exp.2, FSD increased significantly ($p < 0.05$) as screw speed increased, while no significant effects were observed for changes in barrel temperature. Snacks processed at 240 rpm had an average FSD of 50.7%, while increasing the screw speed at 320 and 400 rpm the FSD raised by 3.9% and 7.8%

respectively (Fig. 5a). Screw speed is known to play an important role in starch gelatinization (Lin et al., 1998; Moraru & Kokini, 2003). As previously discussed, a faster screw speed generates a higher friction, which tears starch granules apart and makes them more easily digestible. In our case an overall linear relationship was found between SME and FSD ($r^2$ = 0.62). These results are in agreement with Lin et al. (1998), who demonstrated that a high screw speed significantly increased SME and starch gelatinization.

The values of protein digestibility for fine particles (FPD) were not significantly affected by snack formulation, remaining approximately stable at the average value of 90.2% (Fig. 5b). This was in disagreement with literature, where a significant loss in protein digestibility for formulation containing insects was reported. For instance, Yi, Van Boekel, Boeren, and Lakemond (2016) found that in Yellow mealworm larvae, only 54% of undenatured Yellow mealworm proteins were digestible after duodenal tract, while Marono et al. (2015) identified at 66% the level of protein digestibility. In addition, the low protein digestibility reported in literature could be due the presence of amino acids highly sclerotized or bound to chitin and therefore difficult to digest (Finke, 2007). In our case the higher protein digestibility could be caused by the denaturation of proteins. Reasonably, as for other plant-based proteins (Day & Swanson, 2013), the shear forces in the extrusion may have mechanically ruptured, these links and improved the digestibility. This hypothesis finds confirmation in the data coming from samples of the Exp.2 for which FPD increased for samples extruded at elevated screw speed and barrel temperature.

However, when the fractions of digestible proteins were multiplied with the amount of protein in the respective formulation, a substantial increase in net digestible proteins was calculated. In this case, snacks obtained with only wheat flour had the lowest net digestible protein content of 10.3 g 100 $g^{-1}$, while in those enriched at 10% and 20% mealworms, net digestible protein significantly increased to 13.7 and 14.8 g 100 $g^{-1}$ respectively.

### 3.4.2. *In-vitro digestibility of the coarse particle of the extruded snacks*

Fig. 5a and b also reported the values of starch (CSD) and protein (CPD) digestibility of snacks at this specific particle size. Considering the different formulation, both starch and protein digestibility of i0 and i10 did not significantly differ each other. Interestingly, the situation is different for the samples prepared with 20% grinded mealworm for which CSD and CPD were significantly lower. Particularly, in i20, the average values of CSD and CPD were of ~34% and ~75% while average values of ~52% and ~90% were observed for i0 and i10, respectively.

Chanvrier et al. (2007) hypothesized that microstructure may govern accessibility of the digestive enzymes towards macromolecules, and therefore their digestion. In our case the similarities observed for i0 and i10 were probably due to the effect of the high porosity and the small wall thickness (*i.e.* microstructure properties), which produces a high surface contact area and so a higher diffusion of enzymes. In addition, it is reasonable to suppose that samples i0 and i10, having a higher fracturability (Table 3), resulted in a more homogeneous particle size in the range considered, consequently leading to a similar digestibility. Contrarily, the lower digestibility of particles obtained from i20 was likely due to a higher compactness, which leads to a decrease in enzyme accessibility.

This hypothesis finds confirmation when the digestibility data of both Exp.1 and Exp.2 were analysed with respect to microstructural properties and an inverse correlation was found (Fig. 6).

For the same formulation (Exp.2), CSD decreased from 54.4% to 41.3% while CPD decreased from 89.8% to 80.9% in i10 and i1, respectively. According to the above discussion i10 showed both the highest porosity and mean pore size (Table S.1).

Considering samples as i2 and i7 with a similar porosity (Table S.1), protein digestibility was significantly different at 84.6 and 86.8% respectively only at $p < 0.1$. When looking at their pore structure, the sample i7 had larger pores that may justify its higher digestibility, while a similar PWT was observed. Our results are in line with Karkle et al. (2012), who proved the influence of microstructure on digestibility of extruded products and confirm the possibility of combining microstructural properties to control digestibility.

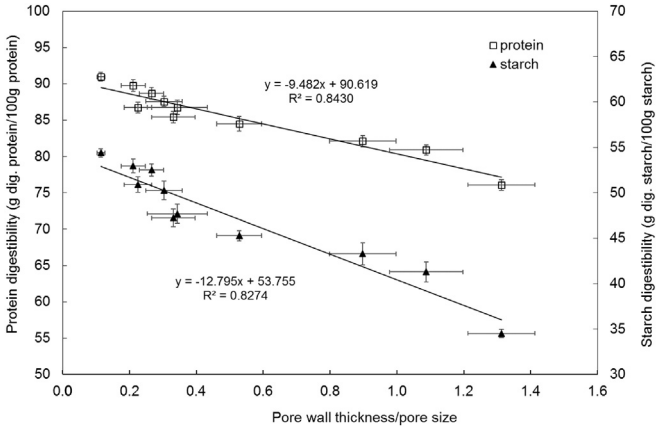


**Fig. 6.** Protein and starch digestibility of coarse particle snacks obtained from both Exp.1 and Exp.2 *versus* ratio of pore wall thickness to pore size. Error bars represent one standard deviation from the mean.

The use of an *in vitro* digestion procedure to assess the digestibility is surely a limitation of this work. However INFOGEST is now considered the most reliable procedure for *in vitro* assessment of digestibility. Due to the novelty of the INFOGEST protocol, our results are difficult to compare with data from the literature. The only record on adoption of the same digestive procedure is found in Stuknytė et al. (2014). In their work, a value of digested starch of about 15.9 g 100 $g^{-1}$ was found in durum wheat pasta. This amount was lower than 45.8 g 100 $g^{-1}$ (d.m.) of digestible starch found in snacks produced with only wheat flour (i0), probably due to different raw material and processing condition.

## 4. Conclusions

The addition of insect powder (*T. molitor*) in cereal-based extruded snacks significantly affected their physical, microstructural and nutritional properties. Data obtained by *in vitro* digestion highlighted the role of formulations and processing conditions in controlling protein and starch digestibility of extruded products. Overall, snacks obtained with only wheat flour had the lowest net digestible protein content of 10.3 g 100 $g^{-1}$, while in those enriched at 10% and 20% mealworms, net digestible protein significantly increased to 13.7 and 14.8 g 100 $g^{-1}$ respectively. Both temperature and screw speed improved the digestibility of starch and proteins of extruded snacks.

Microstructural and textural properties of snacks were also affected by formulation. At an inclusion of 20%, Yellow mealworm snack showed poor expansion properties because of the higher content in lipid reduced the viscosity and mechanical energy, while slight differences where observed between not enriched samples and those enriched at 10%. The adoption of high barrel temperature and screw speed significantly changed microstructure by increasing expansion, porosity, and pore size while a reduction of the pore wall thickness and pore density was obtained.

Textural properties well matched the changes in microstructure of the extruded snacks. Enriched snacks at 20% of insects were the most compact exhibiting the highest compression force, the lowest spatial frequency of ruptures and the lowest fracturability. Also, extruded conditions were able to modify the texture of the snacks. The temperature and screw speed reduced the crispness work and the maximum compression force indicating a better fracturability of the snacks. The relationships between microstructural properties, textural properties and digestibility should be considered to design high quality extruded

snacks. Our results pave the way for designing of innovative food microstructures with tailored digestibility characteristics. To date, ingredients derived from insects fractionation are not allowed in EU. Insect protein isolate would probably overcome technological issues due to the high lipid content, however their market adoption is currently denied. The addition of grinded mealworm significantly improved the nutritional profile of the extruded product and the protein concentration of snacks added with 10% mealworm allows to use the claim “source of protein” according to European food law.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2017.11.017.

## Author contributions

Azzollini, D., and Derossi, A., contributed to the study design, running experiments, data analyses and paper writing; Fogliano, V. and Severini, C. contributed to the study design and paper writing; Lakemond C.M.M. contributed to the paper revision.

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