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In Italian Food and Wine

Entomophagy: an experimental case for food production and by-products valorisation

Supervisor

Prof. Angela Trocino

Co-supervisor

Dr. Sara Bellezza Oddon

Submitted by

Bartolomeo Intranò

Student n. 2058711

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Abstract

In the last few years insect-based food received a great attention by media and politics. The growing interest among consumers about environment and animal welfare, together with concern for the consequences of climate change, further prompted the demand toward new protein source: ethical, safe, capable to ensure food for a growing population. These factors have brought to the fore novel foods, that aim to counteract some of the listed issues with innovative products. The attention of this work is focused on insects, one of the most controversial foods actually in the market. Indeed, along the history insects can boast many different gastronomic traditions, that have been analysed, in some cultures and some places. Among these we have focused on *Tenebrio molitor*, or yellow mealworm, a coleoptera belonging to the family of Tenebrionid. The present thesis evaluated the possibility of raising it on some by-products and wastes coming from the feed and food industrial district of the city of Turin, in order to produce a meal, suitable to be included in some food products to fortify the protein content. At the same time the response of consumer and their preferences have been taken into account to assess the future trends of these products in the Italian and European market.

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1. Introduction

Global warming, climate change, the erosion of water resources and the emission of pollutants are some of the most discussed issues between governments from all above the world and the scientific community (United Nations, 2023).

Over time, it seems increasingly clear that the ways in which we produce and consume food are factors that can affect the variables listed. In fact, according to the estimation, in 2017 the agricultural contribution to the CO_2eq total emission was about the 20%. This share includes an 11% from the crop and livestock activities within the farm gate (FAO, 2020). For this reason, the agri-food industry is increasingly being asked to offer ethical, safe, environmentally sustainable products, following market trends.

Among these needs in the last years the demand for alternative protein sources has grown (Malek and Umberger, 2023), as opposed to meat, reflecting the will to find resources less impacting on the environment (Gonzàlez et al., 2020) capable to provide the right protein supply to a growing population, expected to reach 9.7 billion people in 2050 (United Nation, 2022). Various foods have been proposed as a sustainable alternative to the animal proteins currently consumed (Kim et al., 2019), but in the last period in particular insects have been the focus of attention (Patel et al., 2019).

Furthermore, the recent regulation n. 2023/5 of the European Commission, about the authorization for the placing on the market of the *Acheta domesticus* defatted powder, ignited the interest among consumers about insect consumption all over the European Union. In the Italian context, public opinion has been suspicious, consequently also the political parties strongly took a stand about this topic. The government so campaigned against this decision, claiming that these products threaten Italian typical food productions (Alliva, 2023).

However, insect consumption is a complex subject which has its roots in many different gastronomic cultures, so it is essential to explore this issue with appropriate tools, avoiding simplifications and clichés, analysing in deep all the elements we have at the moment.

2. Objectives

The present thesis has multiple targets: in the first part it defines the entomophagy across different western countries and habits, with a specific focus in the Italian country. Then, it tries to identify the reason of the growing interest on insect farming and consumption, in the perspective of sustainability and valorisation of by-products for both food and feed. In order to better understand the phenomenon, it is accurately explained the actual regulatory framework, set by institution at multiple levels, making comparisons between different geographical areas, and retracing the approval itinerary for new products, according to the law.

In this way we arrive to the core of this work, based on a stage experience, conducted in the experimental centre Tetto Frati, located in Carmagnola (Italy) belonging to the Agricultural, Forest and Food Sciences Department (University of Turin). Here a trial was performed using *Tenebrio molitor*, a beetle already approved by EU (Reg. 2022/169) within the Advagromed project, which involves multiple partners in the Mediterranean basin: the University of Turin and the ISPA[1] in Italy, but also foreign institutions from Spain, Morocco, Germany, Portugal, Greece. All of them promote circularity in the agricultural sector.

The goal of this project is the creation of a sustainable and reproducible agroecological model, using insects as a bio-converter capable to valorise local wastes and by-products in order to produce a valuable resource, such larvae are, for zootechnical use, feeding local poultry breeds. Furthermore, the combination of the digested substrate of insects with chicken manure allows to produce a fertilizer for the farming activity based on local crops. The whole structure of this project is based and managed on circular economy basis as shown in Figure 1.

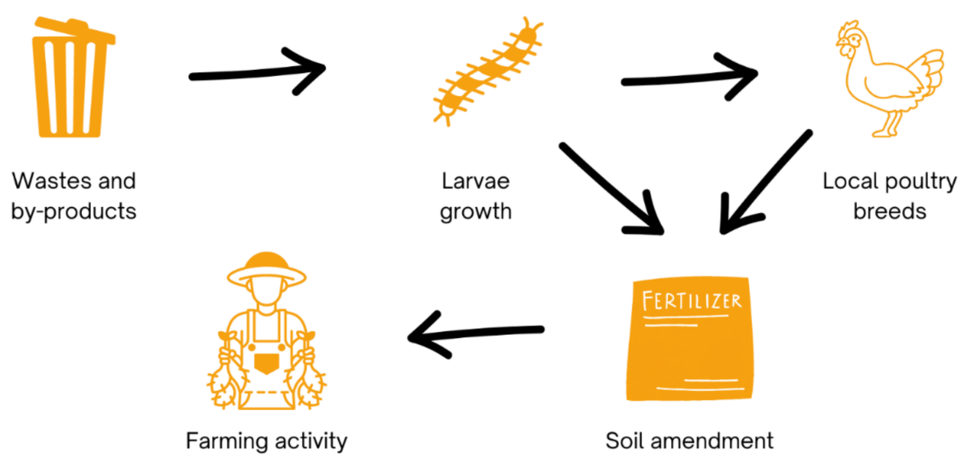


Figure 1. Layout of the Advagromed project.

[1] Istituto di Scienze per le Produzioni Animali

The thesis project was carried out under the supervision of the Professor Laura Gasco, from the University of Turin, in collaboration with the staff of the experimental centre of Carmagnola.

The trial allowed us to evaluate the growing performance of insects farmed on different substrates, composed by some by-products available in the specific geographic area in which the centre is located.

In order to reach this goal, different parameters of our larvae were monitored during the farming, such as the larvae weight, recorded week-by-week, by taking samples. At the end of the growth phase, the chemical composition of an insect meal produced by *Tenebrio molitor* has been determined.

The last part of the thesis will discuss the possibility of using this meal for different purposes and preparations, but also the safety issue and consumer perception, considering some strategies seek to minimize consumer’s repulsion toward insect products.

3. Definitions

Entomophagy: It is a word composed from the Greek ἔντομα, term coined by Aristotle to catalogue insects, and φαγεῖν, that is the verb *to eat*. The greek author says: "Καλῶ δ’ ἔντομα ὅσα ἔχει κατὰ τὸ σῶμα ἐντομάς" (I call "notched" all those that have notches in their body). Talking about this term we refer to the practice of using insect as a food, eating them in different stages of development, different ways, collecting them from their natural environment, or other suitable habitats through the farming activity, as we can see by the following definitions:

- “The eating of insects as food” (Collins, 2023);
- “The practice of humans eating insects as food” (Cambridge, 2023);
- “Si dice di insetti o altri organismi che si nutrono di insetti […]” (Treccani, 2023);
- “Entomophagy is the technical term for eating insects. Humans have harvested the eggs, larvae, pupae and adults of certain insect species from forests and other suitable habitats to eat for thousands of years. […] Insects as food are an excellent source of proteins, vitamins, fats and essential minerals. […]” (CISR.UCR, 2023);

These definitions contribute to explain the size of this phenomenon that can’t be reduced to a transitory trend, considering that it is well rooted in the history. Furthermore, FAO in 2013 estimated that, the practice of eating insect is followed by around 2 billion people in the world (van Huis et al., 2013).

There are around 2,037 different species of insects actually consumed, belonging to the group of Coleopter mainly (634), followed by the group of Lepidoptera (359) then Hymenoptera (302) Orthoptera (279), Hemiptera (220) and finally Isoptera (63), dragonflies (60) e Diptera (35) (Jongema, 2015).

In 1974, Julieta de Conconi asked to 12,000 people in Mexico City their opinion about attitudes to eat insect. The 93% of the questioned people thought that insect foods were 'in the future' and commercial insect food products should be developed (Vane-Wright, 1991). This is not surprising, although it is a survey carried out several years ago, Mexico even today seems to be one of the countries where there is the largest consumption of insects. According to the Meat Atlas report there are over 300 species of insects registered in Mexico as consumed by humans (Heinrich Böll Stiftung, 2021).

However, the perception of consumers with respect to the issue of edible insects is not homogeneous between the various continents and countries, such for each dish. As reported by the anthropologist Marvin Harris: “But there are also many substances that men do not eat while being perfectly edible from the biological point of view: this is demonstrated by the fact that in some places certain groups eat, even finding it delicious just what other groups disdain and detest [...] One may well suspect that there is something beyond the pure and simple physiology of digestion to affect the definition of what is good to eat. This something are the gastronomic traditions of people, their food culture.” (Harris, 2006).

So, food culture of a land affect choices with regard to food: appreciate or not a dish is not just a matter of personal taste or nutrients' availability. It is a process linked to the historical roots of a country. For this reason, it is fundamental to analyse the links between entomophagy and gastronomic traditions in order to understand and overcome the stigma of insect consumption.

FAO, about this issue, suggested that with evolution and development of western culture, people based more and more the breeding activity, and so food security, on the big-size mammals, instead of insects (FAO, 2013) avoiding the natural unpredictable variation of their presence and development.

This hypothesis anyway is not enough to justify the repulsion, based much more likely on the mechanism of competition for resources that is established with invasive species, such as rodents and insects, capable of causing enormous damage to the foodstuffs stored, of reproducing effectively thanks to their great fertility (Mariani, 2020) causing disease transmission (Gałęcki et al., 2023). However, the stigma against this food has not always existed. We find in history several testimonies which suggest that in different periods this resource has been widely used to meet the needs of consumers.

4. History of entomophagy

In the book *Un insetto nel piatto* (Maffei and Tacchini, 2016) the authors trace the origins of entomophagy: in the prehistoric period traces of insects were found during bone analysis of *Australopitecus*; instead, after the discovery of fire, we find traces of insects in ashes and humans' feces. However, this is not the only evidence we have.

In the *Grotte des Trois-Frères*, one of the most important caves in south-west France, located in Montesquieu-Avantès, discovered in 1914 and known for its cave paintings, we can look at several engravings depicting animals and human subjects. Of all these images, the most striking is the image of a grasshopper (Figure 2). Although it is not possible to be sure that this insect was part of the diet of the cave's inhabitants, its presence impressed on the walls is curious (Paoletti, 2005).

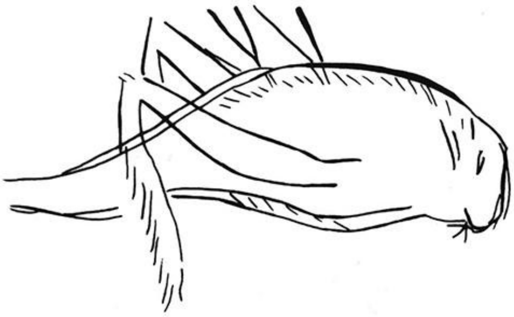


Figure 2. A cricket in Grotte des trois frères (Bégouën, 1929)

In the bas-relief that decorates a long corridor of the palace of Nineveh, we can find another depiction of insects in a banquet, precisely the inauguration of the court of Ashurbanipal. The servants are portrayed carrying long skewers of what look like crickets or grasshoppers (Figure 3) (Lanfranchi, 2005).

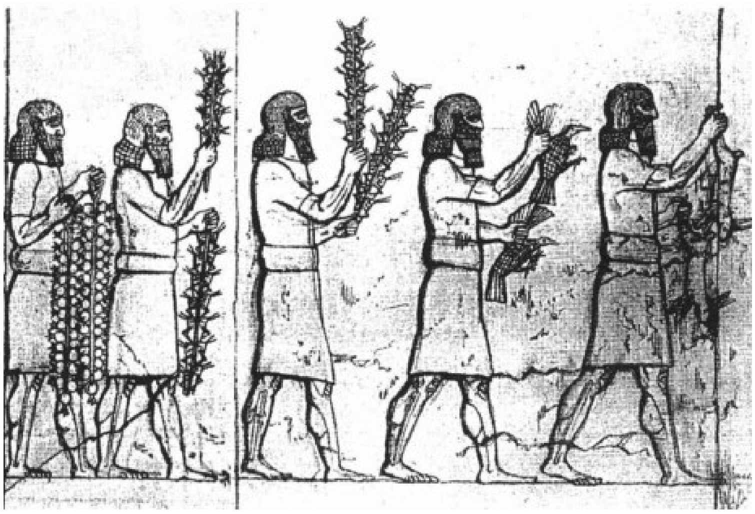


Figure 3. Slaves bringing insects in the Southwest Palace of Sennacherib, Nineveh (702-692 B.C.) (Layard, 1853)

In sacred texts there are several passages that mention the consumption of insects belonging to orthoptera, especially. In the Old Testament we find a reference in Leviticus, where explicit reference is made to which insects can be eaten and which instead make unclean. "So, you can eat the following: every species of grasshopper, every species of locust, every species of acrydes and every species of cricket. Every other winged bug that has four feet shall be a disgrace to you; for they make you unclean: whosoever touches their corpse shall be unclean until the evening, and whosoever carries their corpses shall wash his clothes and be unclean until the evening" (Leviticus XI: 20-25). In the New Testament, instead, in the Gospel of Matthew, the character of Saint John the Baptist (San Giovanni Battista), who lived in the desert, is described by referring to his diet: "John dressed in a camel-hair habit tied at the waist by a leather strap. He lived on a diet of locusts and wild field honey" (Matthew 3: 4-6).

In the Fifth Book of the Work of Aristotele, *Historia Animalium*, in addition to accurately cataloguing the morphology and establishing a series of definitions still of great importance today, the scholar speaking of cicadas said: "The grub, on attaining full size in the ground, becomes a tettigometra (or nymph), and the creature is sweetest to the taste at this stage before the husk is broken" (Smith et al., 1910).

This sentence shows that this kind of insects were used as food during the Greek period. We have reasons to think that also among romans insects were integrated in the diet. Cossus was a typical dish made of larvae of *Lucanus cervus* mixed with flour and wine (Ballarini, 2018). Probably it is the same dish mentioned by Plinio il Vecchio as stated by Linneo and Ray (Bargagli, 1887).

Traces of the use of insects dating back to the Middle Ages are also the production of the liqueur Alkermes of Florence, a liqueur coloured with a dye produced by the dried and powdered body of the females of cochineal (MiPAAF, 2016). The beverage, today registered as PAT[2] by the Tuscany Region, contains an interesting historical reconstruction in the registration document.

"But the history of alkermes is much more ancient: according to historical sources, this liqueur, considered a medicinal specialty, was already produced as long-lived elixir by the Sisters of the Order Santa Maria dei Servi of Florence, founded in 1233. At the beginning of the sixteenth century, we have news of the preparation also by the friars of Santa Maria Novella and the Carthusians (alkermes of Florence or rosolio). It became famous thanks to Caterina de' Medici who made it known in France" (Regione Toscana, 2023).

Also, the naturalist Ulysses Aldrovandi in 1602 in *De Animalibus Insectis Libri Septem* which emphasized how German soldiers in Italy frequently ate fried silkworms (Panetto, 2013) habit also confirmed by the book *Un insetto nel piatto* (Maffei Tacchini, 2016).

[2] PAT (Prodotti Agrolimentari Tradizionali) are food products, characterized by a rooted tradition of production, included in regional-based lists implemented by the Ministry of Agriculture

Another testimony tells us about the consumption of *Melolontha* cockchafer (Scarabaeidae). It seems that these insects were particularly abundant in Europe before pesticides took hold. They grew in the earth by eating the roots of plants.

Friedrich Simon Bodenheimer, an Israeli biologist, explain that in 1688 in Ireland, because of the famine, people would eat these beetles (Bodenheimer, 1951). But cockchafer soup is also a European dish. It was a delicacy in Germany and France until the mid-1900s (van Huis, 2020). Anyway, it seems also to be consumed in Czechia, up to the first decades of the 20th century (Kulma, 2023). Yet, at the turn of the 18th and 19th centuries, in 1875, the American continent was overrun by massive swarms of locusts. This phenomenon was reduced until 1890 and then completely extinguished. During this time a committee was set up to fight this insect, headed by the entomologist C.V. Riley. About 5 pages of the first report of this commission focused on the possibilities of consuming the insect as food (Lockwood, 2004).

During the 19^{th} century it is important to mention a speech, pronounced in front of the Italian Society of Entomology by Pietro Bargagli, an Italian entomologist specialized on coleoptera, in 1877, then reported in *La Rivista Europea.* The author tries to take stock about the edible insect consumed in the world, but reveal the disgust and aversion of the modern society toward this habit, premising to his article that "*non intendo far l'apologia della commestibilità degli insetti* – I don't want to make the apology of the insects' edibility" (Bargagli, 1877). This explain that, the stigma we described before, during this period was already rooted among consumers and those working in the sector too. However, this will not prevent entomophagic traditions from continuing to spread in the following centuries, as happened near Tramonti di Sotto, in Friuli Venezia Giulia, a region in the north of Italy, where people traditionally used to eat raw the abdomen rich in sugar of some species of butterfly of the genera *Zygaena* and *Syntomis;* but it is also reported they consumed *Tenebrio molitor* and *Bombyx mori* (Paoletti and Dreon, 2005; van Huis, 2020).

A last testimony to be thinned is that of Casu Frazigu, a pecorino cheese of ancient origins typical of Sardinia, an Italian region. The cheese is obtained thanks to the development of small larvae of *Piophila casei* during the maturing phase. This product, although today its consumption is not allowed by European Union rules, is registered as PAT at the appropriate register of the Sardinia region (Laore, 2021). Similar cheeses are typical of different regions all along the Italian country: such as Pecorino Marcetto in Abruzzo (ONAF, 2018) or Formaggio Punto in Puglia (Ceriani, 2013).

5. The regulatory framework

Despite the history of insect consumption that we have described, these products are classified today as novel food. The law establishes that this kind of products are foods or food ingredients for which it is not demonstrable a "significant" consumption until the 15 May 1997, day of entry into force of the Regulation, within the European Union (Reg. (EU) 258/97). This legislation is updated by the recent Regulation (EU) 2015/2283, which replaced the previous norm, defining more precisely procedures and introducing a centralised assessment and authorisation procedure for a food belonging to this category, in order to be consumed and placed on the market within the European Union. This differs from the previous rules because the requests are directly submitted to the European Commission, instead of to the member states, as it was before.

However, even before the authorization request, in order to understand if the product is covered by this Regulation, a specific procedure is set for the food operator by the article 4 of the Regulation (EU) 2018/456. This is a request made by a food operator, eager to recognize and authorize a product as novel food, presented to the belonging Member State.

A consultation request is transmitted to the Member State involved, accompanied by a cover letter, a technical dossier, supporting documentation and finally an explanatory note, clarifying the purposes and relevance of the documentation. Where the food operator submits a request with inadequate information, the recipient Member State should ask for further documents.

The Member State is responsible for determining the validity of the request and inform the food operator, the other Member States and the Commission about the outcome. If the request is considered not valid the Member State should provide justifications about the result. Within 4 months by the validation of the request, the Member State should conclude if the submitted product can be considered or not a novel food, informing all the involved parties mentioned above and justifying the decision.

The notification includes the name and the description of the food, a statement indicating whether the food concerned is novel, the reason why it is or not. If the outcome of the assessment is positive it must be specified also the most appropriate category under which the food-stuff falls in, according to the Article 3 of Regulation (EU) 2015/2283. Thus, the Commission should make publicly available this information on their official website. At this point the authorization procedure starts.

According to the law, a novel food must receive authorization from the European Commission by submission of an application document, described at the article 10 of the Regulation 2015/2283, including the name of the products, a brief description, the chemical composition, the methodologies and techniques aimed to obtaining it, accompanied by scientific evidence that the product cannot produce harmful effects on consumers by the safety perspective, the planned analysis methodologies and a labelling proposal.

This Act identifies as applicant a member state, a third country or any interested party, also composed by different actors involved in the submission of the request. Their names and domiciles must be reported on the application document too. The Commission may, if needed, also ask for the intervention of the European Food Safety Authority (EFSA) to obtain an opinion on food safety about the applicant product.

Also, the Commission establishe that a product defined as novel food should not mislead the consumer, especially if the food is intended to replace an existing one and the nutritional values are modified. In any case the modification in the nutritional values should not differ from that food in such a way that the consumption would be nutritionally disadvantageous for the consumers.

Finally, the Regulation focuses also on the label requirement for novel foods, which are added to the rules about labels already existing.

Within 7 months from the opinion of the competent authority (EFSA), the Commission shall submit an execution proposal to authorize the placing on the market of the product and update the list of European Union, to the Standing Committee on Plants, Animals, Food and Feed, set up at the article 58 of Regulation (EU) 178/2002.

Where a third country wishes to place on the European market a product which has been previously traditionally consumed inside the third country, a simplified authorisation procedure is codified by the Article 14 of the Regulation (EU) 2015/2283, including a document related to a safe use tradition of that product and a proposal related to the labelling requirements.

Currently, the insect products that have completed this complex approval process are 6, but come from 4 different species of insects:

1. REGULATION (EU) 2021/1975 of 12 November 2021 authorising the placing on the market of frozen, dried and powder forms of *Locusta migratoria.*
2. REGULATION (EU) 2022/169 of 8 February 2022 authorising the placing on the market of frozen, dried and powder forms of yellow mealworm (*Tenebrio molitor* larva).
3. REGULATION (EU) 2021/882 of 1 June 2021 authorising the placing on the market of dried yellow mealworm (*Tenebrio molitor* larva)
4. REGULATION (EU) 2022/188 of 10 February 2022 authorising the placing on the market of frozen, dried and powder forms of *Acheta domesticus*.
5. REGULATION (EU) 2023/5 of 3 January 2023 authorising the placing on the market of *Acheta domesticus* (house cricket) partially defatted powder.
6. REGULATION (EU) 2023/58 of 5 January 2023 authorising the placing on the market of the frozen, paste, dried and powder forms of *Alphitobius diaperinus* larvae (lesser mealworm).

Once the product has been approved, the applicant has exclusive rights to market about the authorised product for five years from the entry into force of the Regulation, unless a new approval process takes place for the same product - but which cannot use the protected data submitted by the applicant entity in the Article 2 of all the aforementioned regulations – or alternatively the entity's approval. In addition to the listed novel food already on the market, there are 8 more products actually under assessment by EFSA (European Commission 2023).

Anyway, the commission may, in accordance with the opinion of the authority, also decide to monitor the product post-authorisation, including, where appropriate, the identification of the operators of the sector. Operators shall, for their part, be obliged to inform the commission:

1. of any new scientific or technical information which may affect the safety assessment of the use of novel food;
2. any prohibition or restriction imposed by a third country where the novel food is placed on the market.

The Commission shall make this information available to all the Member States (Reg. 2015/2283).

These Regulations are applied in Italy, without the need of any national legislation.

Under European legislation indeed, regulations are binding throughout the European Union from the date of entry into force. Therefore, contrary to the Directives, they must not be transposed by the Member States (EUI, 2021).

Despite this, the Italian government has reached an agreement on a draft decree at the points 11, 12, 13 and 14 of the state-regions conference of 22 March 2023, about insect meal's commercialization. The agreement provides a common position of the Ministry of Agriculture, Food Sovereignty and Forestry with the Ministry of Industry and Made in Italy, in order to set further mandatory guidance for the placing in the market of foods containing powder of *Acheta domesticus, Alphitobius diaperinus, Tenebrio molitor and Locusta migratoria*. The indications are aimed to involving the Ministry of public Health in the discussed decrees, but also to place these products in dedicated shelving (Presidenza del Consiglio dei Ministri, 2023).

During the presentation press conference, it was specified that these decrees are inspired by principles of transparency, aimed to increase the awareness of consumers about the composition of the purchased products and preserve them from unwanted food-stuff. Nevertheless, the Minister of Health Orazio Schillaci reassured: "We will be vigilant thanks to the strict control of the NAS[3] both in the use of these meals and in compliance with the obligations of transparency and health protection. This Government

[3] Nuclei Antisofisticazione e Sanità, is a section of the Italian corp of Carabinieri, responsable of monitor the hygienic regulation of the production, marketing and sale of foodstuffs and beverages, in order to protect public health.

wants to defend consumers from possible mixing, with the use of flours from insects in our typical products such as pasta and pizza" (MASAF, 2023).

This probably refers to the two judgments of the Constitutional Court in 1980 and 1997 (Cappellini, 2022), which concerned a dispute about the use of different types of wheat flour and not the use of additional ingredients, which are granted by the norm as we will analyse. Therefore, also changes in regulations are not taken into account.

The minister's statement, first of all, would be contrary to European law, since neither simple pasta nor pizza are registered as GI[4] products, even if there are some specialities, like Pasta di Gragnano PGI, or Pizza Napoletana TSG those are protected by a Geographical Indication and therefore protected by the European Union for reasons of uniqueness, protection of the gastronomic heritage and local savoir faire (MiPAAF, 2013; MiPAAF, 2010).

Instead, as clarified in 1994 by the TRIPS Agreement, it is not possible to register as GI a common term, used in the language as customary, such as in the case for pizza and pasta, which, although universally traceable to the Mediterranean diet, cannot be linked to a specific geographical origin.

Consequently, no limits can be placed to the use of the term *pasta* in relation to the raw materials utilized, if the product composition is regularly displayed on the label, if the consumer is informed about the allergens in the product and the process respects the minimum hygienic requisites.

Furthermore, in Italy, according to the Presidential Decree n. 187 of the 9th February 2001 the production of special pasta is allowed at the article 7, intended as pasta containing raw materials different from wheat. Moreover, the use of Tenebrio meal as ingredient for pasta, biscuits, bread and other snacks and food products is allowed and explicitly provided for by the aforementioned Regulation 2022/169. Therefore, for a principle of hierarchy of sources, the principles of inspiration of the four decrees proposed by the Italian ministries are not feasible.

Also, from an economic point of view, considering that the cost of this product is currently quite high, because of the resources needed for the development of a production plant aimed at transforming larvae into meal, about 30 times the cost of wheat flour according to some producers (Gabrielli, 2023), it is unlikely that a novel food marketing company would have an interest in placing on the market a product containing this powder without a statement to inform the consumers about the composition: on one hand to justify the high price for sale, on the other hand also to give the right recognition to its research and development team, which before the product launch has certainly worked hard for the design, the analyses and the authorization procedure as described in this chapter.

[4] "Indications which identify a good as originating in the territory of a Member, or a region or locality in that territory, where a given quality, reputation or other characteristic of the good is essentially attributable to its geographical origin" (WTO, 1994).

Finally, with regard to the regulatory framework, it should be stressed that the current legislation does not allow to feed the larvae of *Tenebrio molitor* on any waste substrate if our intention is to use it for human consumption. Indeed, the permitted substrates are cereal bran and flours, enriched of fruits and vegetables (Kooh et al., 2020). The larvae must also do a fast of 24 hours before processing so that the intestinal tract is empty (Reg. (EU) 2021/882). For insects used in animal feed, Regulation (EC) 882/2004 for the feeding of productive animals is followed by the companies.

6. Material and methods

6.1 *Tenebrio molitor* description

The species that will be the case study of the present thesis is *Tenebrio molitor*. It is a coleoptera belonging to the family of tenebrionid. As a pest, it is widespread in animal feed and grains, causing economic losses and damages to the nutritive value of these foods. Insects not only eat the stored products, but can also contaminate them with the exuviates, excrements and dead individuals (Siemianowska et al., 2013). However, *Tenebrio molitor* is also one of the most popular insects used as feed and food, considering the sustainability concerns (Oonincx et al., 2012) aimed to the production of proteins for the next generations, but also looking to the versatility offered by the species in view of valorising by-products from the feed and food industry converting them into biomass (Ramos-Elorduy et al., 2002; Hong et al., 2020).

The life cycle ranges from 280 to 630 days in a temperature range of 22-27 °C; the eggs hatch after 10-12 days and the larvae become mature in a period of 3 to 4 months depending on temperature, humidity, photoperiod, oxygen concentration, population density, parental age and food quality. Anyway, according to the literature optimal conditions for the rearing of these insects are at 28°C and 60% of RH (Sönmez and Koç, 2019; Loreto, 2019).

The larva newly emerged (Figure 4.a) measures 3 millimetres and is white (Siemianowska et al., 2013); then, once reached the maturity phase (Figure 4.b) it is of a yellow-brown colour, measures between 20 and 32 mm and weights between 130 and 160 mg (Loreto, 2019). The number of instars of this species stands at 20 (Kröncke et al., 2023). The pupa (Figure 4.c), 12-18 mm long, is of creamy white colour (Siemianowska et al., 2013); this stage lasts from 7 to 9 days, but pupation can occur at different ages, considering the variability in development and number of instars. During this period, it is very common to observe a cannibalistic behaviour of the larvae in the last instars with the new pupae (Loreto, 2019; Morales-Ramos et al., 2015). Adults (Figure 4.d) grow about 12 to 15 mm and are characterized by a dark brown colour and an elongate shape. Females can lay up to 600 eggs during their lifetime, but it is important to note that males prefer mature females to immature ones, that is because immature females tend to have underdeveloped oocytes and fertility is thus compromised. The egg deposition starts 4-17 days after copulation. (Loreto, 2019; Adámková et al., 2019; Siemianowska et al., 2013). Eggs are white and elongated (Siemianowska et al., 2013), characterized by a sticky consistency (Morales-Ramos et al., 2012; Frooninckx et al., 2022) capable to adhere to the substrate and thus to hide their appearance, this is due to a waxy layer enveloping them. In particularly dry air conditions, the eggs transpiring can lose moisture and this results in a loss of weight and a desiccation (Punzo and Mutchmor, 1980).

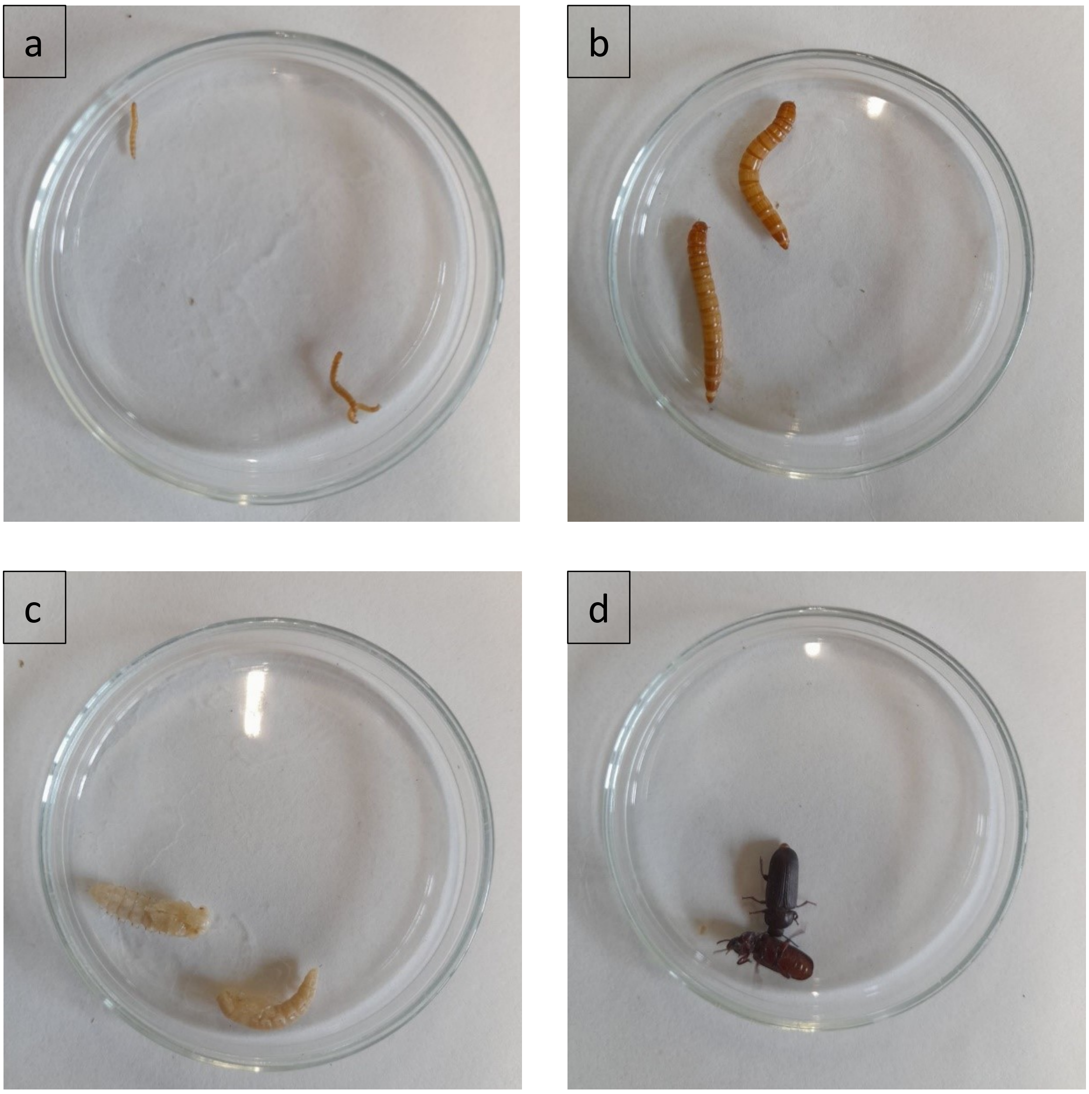


Figure 4. Stage of growth: a) 5-week larvae b) 10-week larvae c) 13-week pupae d) 15-week adults

The reason why this species is considered suitable for the use in food production is the excellent protein content, between 41% and 66% on a dry matter basis (Gkinali et al., 2022), rich in leucine, isoleucine, lysine, tyrosine, valine and methionine (Zielinska et al., 2015). The fat content on a dry basis is 28-30%, divided in saturated (24.8%), monounsaturated (48.8%, such as oleic and palmitic acids), and polyunsaturated (26.4%, such as linoleic acid) fatty acids. Finally, the fibre content is around 5%, with chitin being the most abundant source of dietary fibre (EFSA, 2021).

6.2 Experimental facilities and management of *Tenebrio molitor*

The trial has been conducted in the facilities of the Department of Agriculture, Forest and Food Sciences of the University of Turin (UNITO) in Carmagnola (TO), Italy, where a colony of Tenebrio molitor is kept and maintained.

The life cycle of Tenebrio can vary according to the housing and farming conditions. In the experimental centre of Carmagnola (University of Torino), the farming activity of *Tenebrio molitor* is based on a period of 16 weeks. All the operations, aimed to mainten the colony, are executed between Monday and Friday. The colony is organized in boxes disposed in columns and each column is made of 7 boxes which are transportable by a trolley in order to make easier the operations. The whole colony is located in a climatic chamber, where temperature and humidity are set and checked at an optimal point of 29 °C and 60% RH. A mix of wheat bran and soy, respectively in percentages of 70% and 30%, has been chosen as food source for the first 5 weeks of farming, then wheat bran is supplied for the rest of the cycle.

Water is provided by cubes of agar, a polysaccharide used as natural gelling agent. Each one is about 1x1 centimetre and weighs 5 g. The quantity of water grows along the insect cycle and the provision starts from the 4th week of life. As regard the life cycle, the 1st week is considered as the oviposition stage. A group of 250 g of adults is placed in a 5 mm gauged grid for 7 days (Figure 5). The grid is laid in a box filled with the aforementioned mix of wheat bran and soy meal, placed in 2 layers of 750 g and 350 g, respectively.

Figure 5. Box with adults placed on the grid.

The group of adults is composed of 66% of mature adults and a 33% quote of replacement made of new young adults, just emerged from the pupation. This arrangement allows to standardize and keep constant the number of eggs produced, compensating continuously for the adult loss at the end of the cycle and whereas the number of eggs laid is positively affected by the presence of a grid, which avoids cannibalistic behaviour by adults, and negatively affected by the age of insects, which show a lower production when too young or too old (Frooninckx et al., 2022).

Thus, during this week, the adult mating and the egg deposition within the substrate take place. According to the literature mentioned before, the eggs adhere to the bran, making difficult their isolation and quantification, so the estimation will be performed later, directly on the larvae.

On Thursday the group of adults, after the oviposition, is separated from the substrate and replaced with a new group, composed according the percentage described before, and prepared for the following week deposition. The grid full of adults is raised and shaken, so as to drop on the bottom of the box as much as possible of eggs, which pass through the mesh of the grid, while adults are retained. Then, the removal of old adults is carried out eliminating all those dead individuals, or of poor vitality, thanks to the use of a craftsmanship machine (Figure 6). It is a pending grilled belt, that moves around rollers, manually activated by a crank. While the crank is mobilized the adults are dropped on the belt, right on the grilled part, so as to take advantage of the inherent characteristics of this insect, able to cling to the jagged surfaces when alive, letting the dead adults instead slide along the belt into a waste box. On the other hand, the live insects, clinging, are dragged up to a brush that, acting on the surface of the tape; insects that are harvested in a collection box. In order to minimize the adult loss, this operation in performed twice, processing again the discard.

Old adult mortality is calculated weekly as the difference between the weight of the whole adults before the separation of deaths (WAW), and that observed after separation (SAW), times a hundred, divided by the whole adults. Usually, its value is around 10%.

$$Mortality = (WAW-SAW)100 / WAW$$

The quantity of old adults used to compose 7 new deposition tanks, with groups of 250 g of insects, is 1170 g (around 66% of the total needed, 1750 g). The same machine is also used to separate dead pupae and exuviates from new adults. In this case, the weight of the waste material is subtracted from the initial weight to monitor the progress of pupal maturation. The quantity of new adults used to compose 7 new deposition tanks, with groups of 250 g of insects, is 580 g (around 33% of the total needed, 1750 g). This replacement gives the green to the farming week.

Figure 6. Manufactured separation machine.

Following this, the isolated substrate, full of eggs, is incubated until the 5th life week. The larvae estimation is performed on Friday, at the 5th life-week, when the new-born larvae are visible and countable by the operator. The substrate rich of larvae is transferred from the 7 boxes to a vat where is weighted (Kern & Sohn GmbH, GAB 12K0.1N, Balingen, Germany) and mixed five time using another vat, in order to homogenise the content. After the mixing up, four samples are taken from the vat using a measuring cup, then samples are weighted and larvae are counted using a clicker, displacing the larvae in another little cup, in order to weight them. This procedure is summarized in Figure 7.

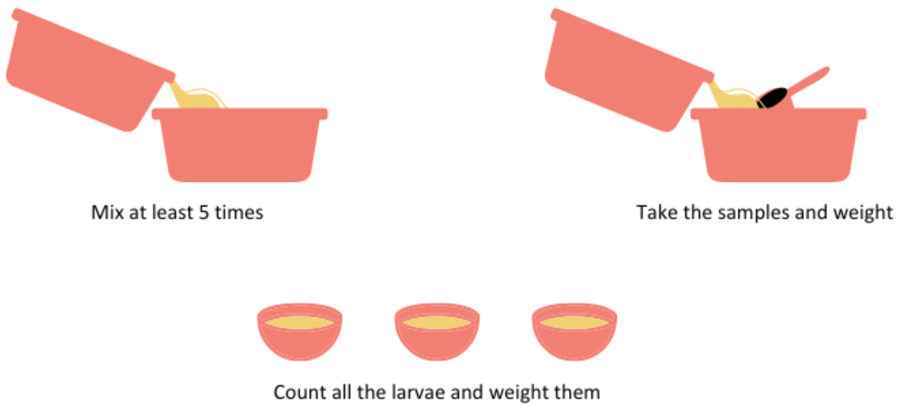


Figure 7. Mixing and sampling procedure adopted to count and estimate larvae in a batch.

Based on the batch and samples weight, the average larvae weight is calculated and the larvae content of the vat is estimated as follows:

Average larva weight = Sample weight / Larvae counted in the sample

*Whole larvae estimation = Larvae counted in the sample * Batch weight / Sample weight*

Thanks to this information, 7 groups of 5,000 larvae are isolated to be inoculated for the maintenance. If, used for a trial, the inoculum must contain 10,000 larvae.

Normally, the food supplied to the colony at this stage is 2.5 kg of wheat (0.5 gram/larva). In the case of a trial, the quality and quantity of diet are variable according to the specific purposes of the trial.

Once the number of larvae is standardized among the 7 boxes, each batch is incubated until the 12th life-week, providing daily water as described before. On Tuesday of the twelfth week, the larvae undergo a process of separation from the pupae using a vibrating sieve with a grid, with rectangular meshes measure 0.3 cm x 0.7 cm (Flexiever Garden 17-0383-141; SMO bvba, Eeklo, Belgium). The machine vibrates while insects with their substrate and frass are overturned on the grid. This process allows to retain on the grid all the pupae, curved and thicker, while last instars larvae, long and slander, fall down in a discard box, avoiding the cannibalistic behaviour typical of this stage of the growth. The inclination of the machine let the pupae slide down in a collection box (Figure 8). The sieving is also repeated in the 13th, 14th and 15th weeks, so as to remove all possible pupae from the substrate.

Figure 8. Vibrating sieve for pupae separation.

The weight of the pupae is recorded to monitor colony production according to the development. The whole pupae harvested on Tuesday are left to ripen on a light bran bed, divided in groups of 400 g (Figure 9), until Thursday of the following week, when the newly emerged adults will be used to replace the 33% old adults belonging to the 1st life-week. This takes us back to the beginning of the cycle described.

Figure 9. A box containing 400 grams of pupae with some agar.

All these tasks are repeated on a weekly-base. Thus, in the climatic cell where the colony is housed, the growth of 16 columns of 7 boxes (total of 112 boxes) is managed contemporary, with one life-week difference from each other.

6.3 Design of the experimental trial

The trial took place in a climatic chamber at 28°C (±0.6°C) and 73 RH% (±3.7%), optimal conditions for this insect (Bjørgea et al., 2018). For the whole trial, the substrate temperature was recorded by a datalogger (LogTag TRIX-8) every 30 minutes, in 3 replicates per treatment. Another datalogger detected the chamber temperature for the whole duration of the experiment. The twenty boxes containing the diet and the larvae were placed on the shelves in a random order and their positioning was changed each week at the end of the sampling activities.

The strain of *Tenebrio molitor* used for the trial was of Belgian origin and it is usually reared in large scale (4-week-larvae: 350,000/week). The eggs to start the experiment had been collected for 4 days, instead of the usual 7, to homogenise the larval age. To harvest them 12 boxes (from 7 to 9/60 x 40 cm) were used which were filled with 350 g wheat flour; the boxes contained a 3-mm grid, capable of retaining the adult insects and letting pass eggs and flour at the bottom of the box; other 750 g of wheat flour with 250 g of adults were placed on the grid. During the deposition about 20 g of agar for box were provided as water source, every day, in 1 cm cubes.

The eggs obtained were sieved with a 0.2 mm sieve and placed in boxes with additional flour for the hatching period (1 g of eggs every 20 g of substrate). At 2 weeks of life, all the larvae were sieved using a sieve of 0.2 mm to separate them from the flour. Their number was estimated using 3 samples with a coefficient of variation <10%, then weighted.

Three experimental diets, prepared using by-products from the industrial district in the nearness of the experimental centre, were tested and compared to a control diet made of wheat bran. Each box contained 3 kg of diet and an inoculum of 10,000 larvae (feed ratio: 0.3 g/larva) (Figure 10), 5 replicates per dietary treatments were used for a total of 20 boxes (5 boxes x 4 diets).

Figure 10. 10,000 larvae inoculated in one of the replicates of the trial.

As a water source, cubes of agar were provided to the larvae (about 1 cm x 5 g). Agar was provided on a weekly and a daily basis, every day the residues remaining in the box were removed from the box and weighted in order to trace the water intake. Finally, water consumption was calculated as percentage of worn-out cubes of agar, compared to those inserted in the trays.

6.4 In vivo recordings

Every 7 days, starting from the beginning of the trial, the box content and a sample for each box - containing > 100 larvae - were weighted and counted to monitor the larval growth performances. Weekly data were used to formulate a larval growth curve for each diet, designed by the sequence of average larva weight recorded. The coefficient of variation (<10%) was used to assess the consistency of the data collected. The curve predicts from the initial weight an exponential growth, before reaching a peak, beyond which a lag phase is observed that lasts until the curve is inverted and the weight of the larvae decreases, while they are preparing to reach the pupal phase.

The growth curves for the different diets were compared to assess the differences. The end of the trial was considered reached when the percentage difference between two week-weight measurement was equal or lower than +50% in 3 replicates for treatment, at least. Then, the weight of each box and the sampling-day were recorded.

6.5 Final sampling and chosen indices

At the last sampling corresponding to the end of the trial, whole larvae were sieved and the total weight of the residue substrate and that of the larval biomass and residual frass were recorded. Mealworms were sampled three times – the minimum larvae number was at least equal to 100 individuals - and the number of larvae and their weight were recorded. The coefficient of variation was used to validate the data among the 3 samples and, if more than 10%, an additional sample was evaluated.

The survival rate (%) was calculated in each box, comparing the estimated larvae number, based on the samples, at the end of the trial, to the number of larvae inoculated at the beginning.

The Feed Conversion Ratio (FCR) was calculated to assess the efficiency of the biomass transformation starting from the by-products as follows:

FCR = Diet provided / Biomass Weight

6.6 Chemical analysis

For each replicate, 200 g of clean larvae were accurately clean and inactivated at -20°C and then stored for the chemical analyses, while all the digested substrates were stored at room temperature in a dry place for further analysis.

To perform the chemical analysis, the larvae were minced (Retsch GM200, Verder Scientific s.r.l., Pedrengo -BG-) and stored at -80°C. Then, the samples were freeze-dried and ground.

The larvae dry matter (DM; AOAC #934.01), crude protein (CP; AOAC #984.13; conversion factor for larvae N x 4.47) and the ash (AOAC #942.05) were determined by the International AOAC (AOAC, 2000), while the ether extract (EE; AOAC #2003.05) by the International AOAC (AOAC, 2003). The amylase neutral detergent fibre organic matter (aNDFom) content was determined according to Mertens (2002).

6.7 Statistical analysis

Data was analysed using the IBM SPSS Statistics software (V28.0.0.). For the growth performance, water consumption, temperature trend and larval composition the statistical unit was the replicate box. The assumptions of residuals normality and equal variances were determined using Shapiro-Wilk and Levene's homogeneity of variance tests, respectively. Data were analysed by one-way ANOVA test (post-hoc test: Tukey). The results were expressed as mean and pooled standard error of the mean (SEM) and the level of significance considered was ≤ 0.05.

6.8 Waste and by-products

By-products utilized for the diet preparation were selected according to two different criteria: all the companies involved were in the radius of 100 km from the facilities of Carmagnola (Figure 11) to valorise materials coming from the productive area considered. The content of dry matter of the wastes was assessed, considering the eating habits of yellow mealworm. Sixteen wastes were approved for their suitability to compose the diet: the (1) Colomba and (2) Panettone, two typical Italian desserts, prepared during Easter and Christmas period, respectively, but characterized by a similar dough composition; then (3) groats, by-products derived from the wheat meal production, usually exploited for the feed production; (4) Wafer, (5) cookies and (6) overcooked wafer wastes, coming from the same company producing the aforementioned desserts, were expired products or wastes; the (7) coffee silvery film and (8) hazelnut film were by-products obtained after the roasting process of these products; the (9) dry vinasse and the (10) filtering waste derived from wheat processing. Three by-products derived from the processing of rice: (11) rice husk, (12) rice chaff and (13) middlings. Another product was the (14) breading waste, a breadcrumb remnant from the industrial production of some breaded products. The (15) feed waste came from the initial and final part of feed production for rabbit, poultry and cow. Finally, the (16) rice crackers were expired products.

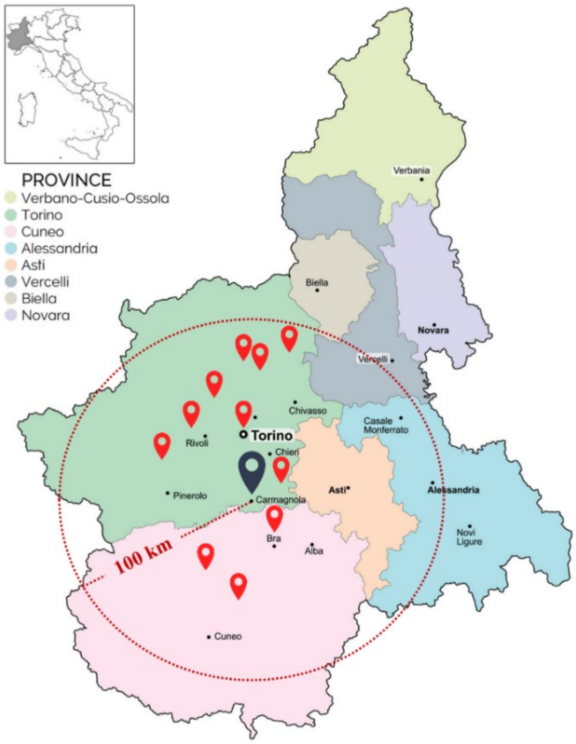


Figure 11. Marked as red points, all the producers involved in the recovery of wastes, maximum distance from the experimental centre is bordered by a dotted line, marked as black Carmagnola facilities.

Not all the by-products mentioned were ready-to-use for the insect diet. Some of these wastes were further transformed in order to make the matrix suitable for the Tenebrio consumption. Colomba (Figure 12.1) and Panettone (Figure 12.2) were manually separated from their wrappers, then ground with a meat-grinder (Rheninghaus Factory, LABOR 32; San Mauro Torinese -TO- Italy) and dried for 48 h at 35°C before being ground again to dust with a cutting mill (Fimar, CUCL823050M; Villa Verucchio -RN- Italy). The expired wafer (Figure 12.4) and cookies (Figure 12.5) were previously broken in smaller parts by hand and then ground with the same cutting mill aforementioned. The overcooked wafers (Figure 12.6) were dried for 48 h at 35°C, then ground by hammer and blade mill (Ceccato, Lucme 90; San Giorgio delle Pertiche -PD- Italy). The coffee silvery film (Figure 12.7), the hazelnut film (Figure 12.8) and the rice husk (Figure 12.11) were grinded by jaw crusher (Retsch GmbH 5657; Haan, Germany). The breading waste (Figure 12.14) was just sieved with a 0.8 mm sieve. The fractions of the feed waste (Figure 12.15), of different origin (cow, rabbit and poultry feed), were just homogenized together. The rice crackers (Figure 12.16) were ground using the aforementioned cutting mill.

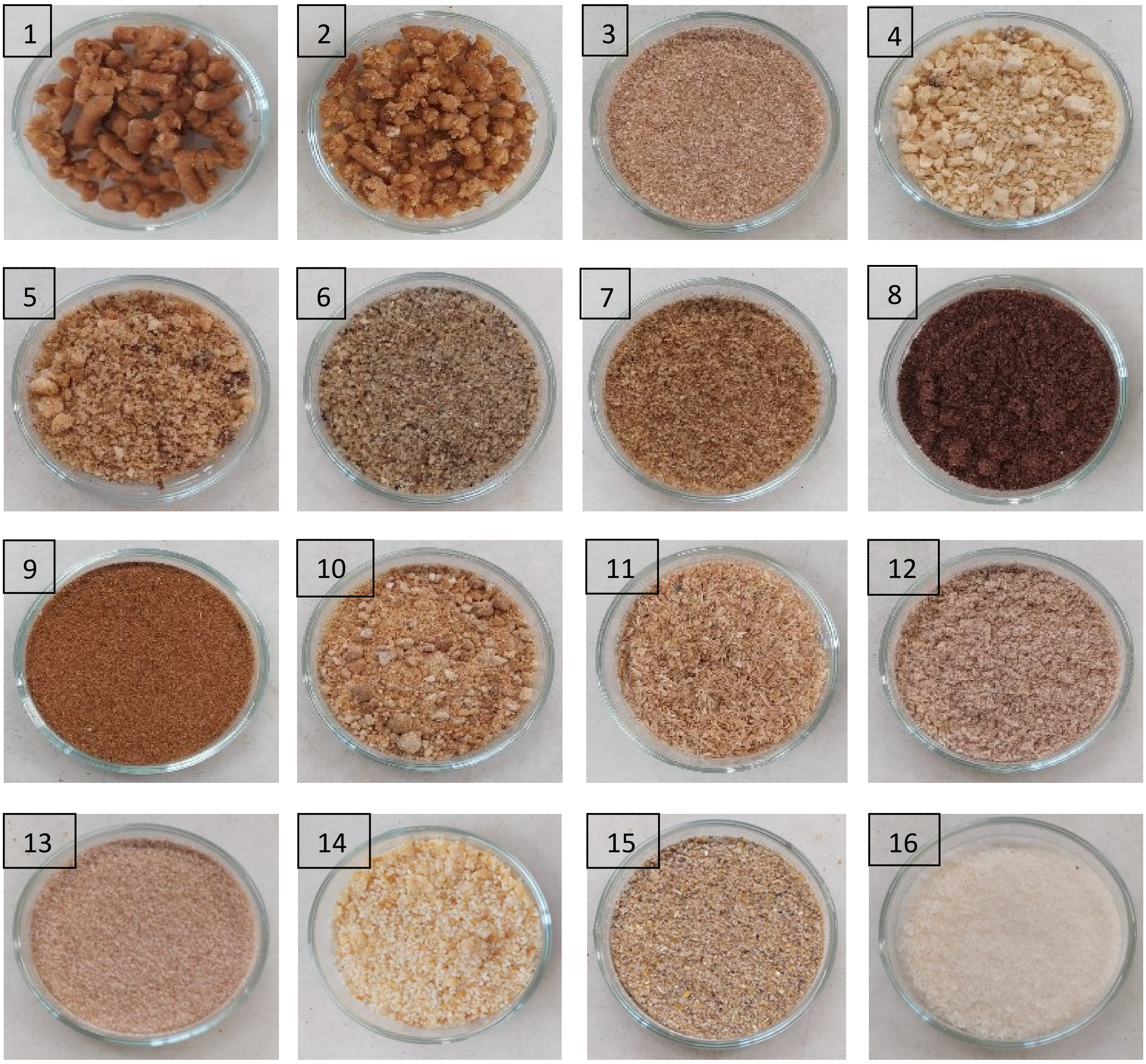


Figure 12. All the by-products and wastes processed.

All the by-products described were sampled and analysed in order to determine the composition of each ingredient for the diet formulation. The same methodologies described for the analysis of larvae were utilized, with the only difference of a different conversion coefficient used for nitrogen - 6.25 instead of 4.47. The chemical composition - as it is - of the collected waste and by-products is shown in Table 1.

Table 1. Chemical composition (as is) of the waste and by products (%), cost (€/ton).

Items	Dry matter, %	Ash, %	Crude Protein, %	Ether Extract, %	Neutral Detergent Fibre, %	Cost, €/ton
Panettone (1)	91.83	1.53	1.66	9.57	10.42	110
Colomba (2)	94.23	1.43	1.51	9.13	9.69	110
Wheat groats (3)	86.75	4.56	4.40	5.25	5.07	330
Wafer (4)	94.92	2.11	2.22	9.58	10.09	130
Cookies (5)	86.90	4.05	4.66	17.05	19.62	150
Cooked wafer dough (6)	73.91	0.99	1.34	9.45	12.78	130
Coffee silvery film (7)	91.24	6.71	7.36	15.21	16.67	0
Dry vinasse (8)	91.36	3.02	3.31	34.96	38.27	400
Vinasse filtering waste (9)	71.58	25.26	35.29	9.60	13.41	0
Rice husk (10)	90.50	16.40	18.13	2.13	2.36	110
Rice chaff (11)	87.65	6.14	7.00	13.03	14.87	250
Rice middlings (12)	87.51	7.37	8.43	14.62	16.70	305
Breading waste (13)	83.62	3.67	4.39	11.69	13.98	0
Hazelnut film (14)	95.79	2.75	2.87	9.42	9.84	0
Feed waste (15)	86.61	8.71	10.05	17.00	19.62	0
Rice crackers waste (16)	91.85	1.41	1.54	9.00	9.80	0

Then, 6 different diets were formulated made of different by-products or wastes, aimed to balance the macronutrients content and forming diets characterized by a similar content in lipids, crude protein and energy having a percentage difference of less than 4% between these values, but with different costs. Among the 6 diets only 3 of them were selected for the experiment, as shown in Table 2, according to criteria of homogeneity and different ranges of cost. The exact composition of diets cannot be disseminated for reasons of confidentiality. When an ingredient is present it is marked with an asterisk, otherwise the bar indicates that the ingredient has not been used for the preparation of the diet. The fourth diet used only wheat bran (100%), as usual in the maintenance of the species, thus representing the control group (CTRL).

Table 2. Formulation of the experimental and control diets, analysis of their composition (%).

Ingredients	**CTRL**	**TM2**	**TM3**	**TM6**
Wheat Groats	-	*	-	-
Cookies	-	-	-	-
Wafer	-	-	-	-
Wafer dough	-	*	*	-
Filtering waste	-	-	-	-
Vinasse	-	*	-	-
Coffee silvery film	-	*	-	-
Rice Husk	-	-	-	*
Rice Chaff	-	-	-	*
Rice middlings	-	-	-	*
Breading waste	-	*	*	*
Hazelnut film	-	-	-	-
Feed waste	-	*	*	*
Crackers	-	-	-	-
Colomba	-	-	-	-
Panettone	-	-	*	-
Wheat bran	100.00	-	-	-
Dry matter, %	86.30	86.50	86.30	86.40
Ether extract, %	2.50	3.00	3.00	3.00
Crude protein, %	15.80	15.80	15.80	15.80
Ashes, %	5.90	5.80	7.50	8.30
NDF %	48.50	17.10	14.50	17.50
Cost, €/ton	237.00	87.80	83.30	95.50

7. Results

The trial ended at the 9th farming week (16 May 2023). On that day, the weekly growth rate, intended as the percentage difference detected from the previous measurement, was on average ≤50% (TM2=38.5%; TM3=49.7%; TM6=44.2%; CTRL=49.9%). Growth performance were assessed over the whole farming period.

The analysis demonstrated that insects fed diet TM6 had the highest growing performance as overall ($p<0.001$; 0.134 g) (Figure 13a) and also at the 9th week of farming ($p<0.001$; 0.127 g) (Figure 13 b), showing a statistically significant difference in both the cases among the treatments.

The administration of diet TM3 (0.126 g) and diet TM2 (0.130 g; $p>0.05$) produced similar results when the weight of the larvae was considered as an average of the different recordings detected during the trial. On the other hand, results differed between insects fed diets CTRL and TM6 ($p<0.001$).

At the end of the trial, at 9th weeks of age, insects fed diet TM3 had a significant higher weight compared to those fed diet TM2 (0.118 g vs.0.113 g) and all the other treatments ($p<0.001$). Finally, insects fed the CTRL diet showed the worst growth performance as overall ($p<0.001$; 0.109 g) and on the last farming week ($p<0.001$; 0.087 g) compared to insects fed the experimental diets (Figure 13, 13b).

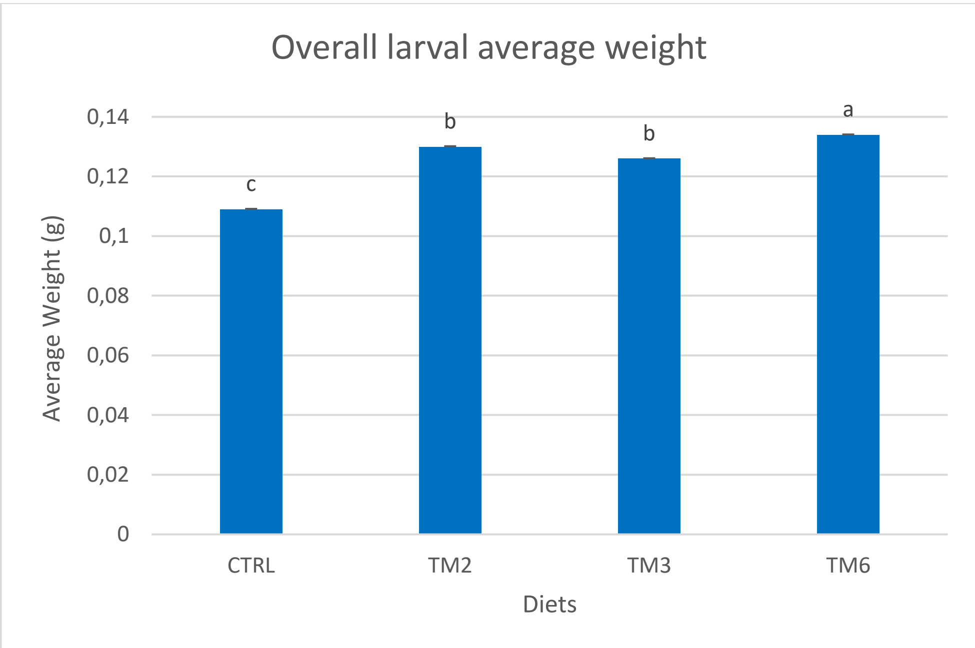


Figure 13a. Bar chart with the average weight of each treatment as overall of the whole farming period (TM2, TM3 and TM6 containing respectively wafer dough, wheat and coffee by products, breading and feed waste; wafer dough, breading and feed waste, panettone; rice by-products, breading and feed waste) and insects fed the control diet (wheat bran).

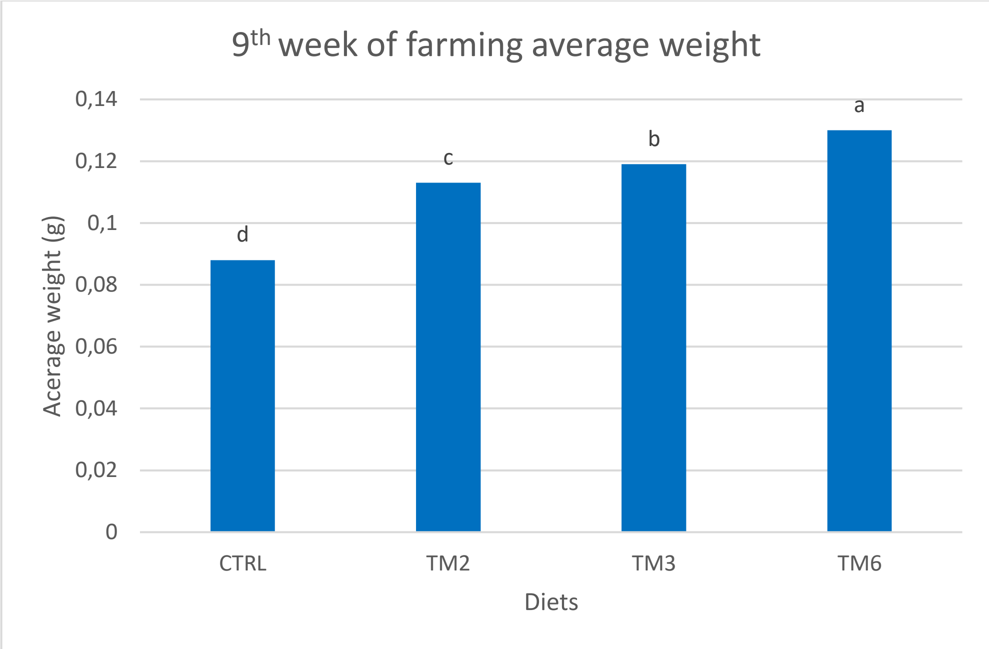


Figure 13b. Bar chart with the average weight of each treatment measured at the last week of farming.

Looking at the growth curve (Figure 14), at the beginning of the trial, larval weight was homogeneous between all the treatments (0.008 g; p>0.05); after 5 weeks of farming, no significant differences were yet reported, whereas after the growth pattern differed according to the feeding substrate. During the 6^{th} week, insects fed diet TM2 recorded the highest weight (0.0174 g) when compared to insects fed diets TM6 and TM3 (0.0162 and 0.0152 g, respectively; p<0.001), which did not differ from each other (p>0.05). The CTRL group already registered the lowest value (0.0132 g; p<0.001). During the 7^{th} week of farming, insects fed diet TM6 reached the highest weight (0.043 g) compared to insects fed diets TM2 and TM3 (0.039 and 0.038 g; p<0.001, respectively), which were similar between them (p>0.05), while insects fed the CTRL diet confirmed the lowest weight (0.030 g; p<0.001). During the 8^{th} week of farming, once more, insects fed diet TM6 again were those with the highest weight (0.088 g) compared to insects fed diets TM2 and TM3 (0.079 g and 0.082 g, respectively; p<0.001) and those fed the CTRL diet got, which gave the worst result (0.058 g; p<0.001).

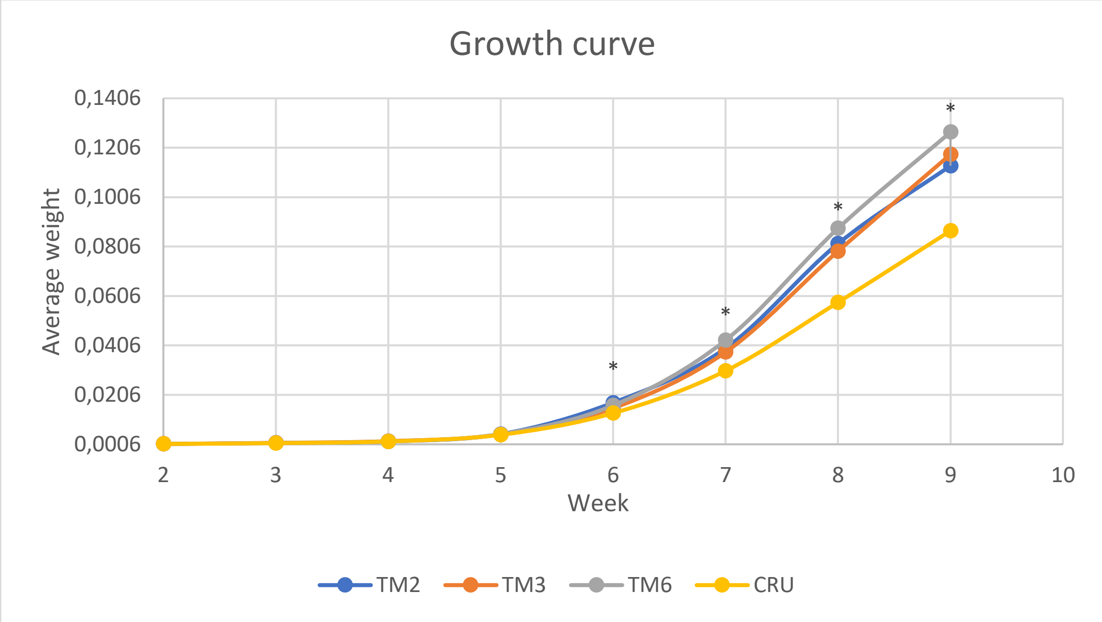


Figure 14. The growth curve of insects fed the three experimental diets (TM2, TM3 and TM6 containing respectively wafer dough, wheat and coffee by products, breading and feed waste; wafer dough, breading and feed waste, panettone; rice by-products, breading and feed waste) and insects fed the control diet (wheat bran).

The achievement of the final stage of larval development, in the experimental diets, was also highlighted by the appearance of the first pupae within them, the stage after commercial maturity for this insect, useful for reproduction but not for transformation. At the end of the trial, 42 pupae per sample were recorded among insects fed the diet TM2 (2.3%), 59 pupae in those fed diet TM3 (3.2%) and 99 pupae in those fed diet TM6 (6.1%). Among the larvae fed on the control diet, no pupae appeared after nine weeks of growth (0%).

As regard the FCR index, low values were recorded for insects fed diets TM3 and TM6, which were statistically similar each other ($p>0.05$) but different from all the other treatments ($p<0.001$) (Table 3). Then, insects fed diet TM2 showed intermediate results, statistically different from the other experimental diets ($p<0.001$). Finally, insects fed diet CTRL group registered the highest FCR ($p<0.001$).

Table 3. Feed conversion ratio (FRC) in insects fed diets containing wafer dough, wheat and coffee by products, breading and feed waste; wafer dough, breading and feed waste, panettone; rice by-products, breading and feed waste; wheat bran (TM2, TM3, TM6 and CTRL diet, respectively) (SEM, standard error of the mean).

	Experimental diets					
Items	TM2	TM3	TM6	CTRL	SEM	p-value
Feed conversion ratio	5.54^{b}	5.01^{a}	4.96^{a}	6.35^{c}	0.069	0.000

Within the boxes with insects fed diet TM3 the lowest temperature was recorded, with significant differences with insects fed the diet CTRL and diet TM2 ($p<0.05$) (Table 4). Moreover, temperature in boxes fed CTRL, TM6 and TM2 diets did not differ among them ($p>0.05$). Lastly, temperature in boxes with insects fed TM3 and TM6 diets did not differ ($p>0.05$).

Table 4. Temperature (°C) recorded in 3 boxes per each treatment all along the trial (average of 9 weekly recordings).

	Experimental diets					
Items	TM2	TM3	TM6	CTRL	SEM	p-value
Temperature (°C)	28.46[a]	27.81[b]	28.25[ab]	28.38[a]	0.111	0.017

As for to water consumption (Table 5), the insects fed with the CTRL diet required the lowest quantity of water, with significant differences from all the other diets (p<0.001). Then, insects fed diet TM2 showed a value comparable to those fed diet TM3 (p>0.05) and lower than what requested by insects fed diet TM6 (p<0.001). Insects fed diet TM3 recorded an intermediate result. Finally, insects fed diet TM6 showed a water intake similar to insects fed diet TM3 (p>0.05) with significant differences compared to all other treatments (p<0.001).

Table 5. Water consumption expressed as percentage of the consumed agar on the total provided.

	Experimental diets					
Items	TM2	TM3	TM6	CTRL	MSE	p-value
Water intake, % of agar consumption	87.86[b]	89.32[bc]	90.80[c]	79.68[a]	0.544	0.000

The analysis performed on the insect meal showed that the insects fed the CTRL diet showed the lowest dry matter content (p<0.001), but guaranteed a higher protein level as well as a lower ether extract when compared to the other diets (p<0.001) (Table 6). On the other hand, insects fed diets in TM2, TM3 and TM6 had similar results as regard dry matter, crude protein and ether extract content (p>0.05). Insects fed diet TM2 showed the lowest content of ash compared to all other experimental groups(p<0.001), with the exception of insects fed diet TM6 (p>0.05). Then, TM6 showed an intermediate value, similar also to TM3 (p>0.05), which instead had mutual differences with TM2 (p<0.001). Finally, the ash content of the CTRL group was the highest one (p<0.001). As regard chitin, no significant differences were observed among treatments (p>0.05).

Table 6. Analysis on insects fed experimental diets made of by-products and a wheat bran diet, dry-matter basis.

	Experimental diets					
Items	TM2	TM3	TM6	CTRL	SEM	p-value
Dry matter %	35.46[a]	35.04[a]	35.11[a]	32.10[b]	0.161	0.000
Ash %	3.14[a]	3.29[b]	3.23[ab]	3.93[c]	0.026	0.000
Crude protein %	37.00[b]	37.69[b]	38.09[b]	40.39[a]	0.288	0.000
Ether extract %	35.65[b]	34.79[b]	34.73[b]	25.34[a]	0.329	0.000
Chitin %	5.41	4.94	5.04	5.56	0.575	0.1

Finally, as regard the survival rate (Table 7), insects fed the CTRL diet had the best results ($p<0.001$) greater than all the experimental diets. Insects fed diet TM3 registered the highest value among the experimental diets with values different from insects fed diet TM2 ($p<0.001$), but comparable to those fed diet TM6 ($p>0.05$). The survival rate of insects fed diet TM2 was similar to those fed diet TM6 ($p>0.05$), but lower than the survival rate of insects belonging to all the other treatments ($p<0.001$).

Table 7. Survival rate expressed as percentage of the estimated larvae at the end of the trial on the total inoculated.

	Experimental diets					
Items	TM2	TM3	TM6	CTRL	MSE	p-value
Survival rate, %	53.58[c]	58.58[b]	56.42[bc]	64.19[a]	0.746	0.000

8. Discussion

8.1 Case study: wastes for feed formulation for *Tenebrio molitor*

The trial took 9 weeks of farming, 7 of which were spent on the specific feeding substrate. In general, the reported survival values, all under 65%, were much lower than those found in the literature, which report in the larval phase a survival value close to 98% (Toviho & Bársony, 2022). Such a low survival rate can be linked to the protocol, which prescribed to feed on wheat flour the larvae for the first two weeks, probably generating a condition of nutrient deficiency in the early stages of development. Also, the prolonged and early stimulation to which the larvae have been subjected can be a cause, having carried out the first sampling activities at 2 weeks of life. Also, the control group obtained the best survival, while the experimental diets obtained the worst results.

Assessing the growth, all the experimental diets showed a better growth performance than the control group, from the sixth week onwards, in all the sampling-weeks up to the end. Moreover, among the by-product substrates, the best weight was reached by diet TM6 (rice by-products, breading and feed waste) at the end of the trial, followed by diet TM3 (wafer dough, breading and feed waste, panettone) and diet TM2 (wafer dough, wheat and coffee by products, breading and feed waste).

The development time in 9 weeks is rather consistent with the literature (Bordiean et al., 2020), even if the criteria to stop the trial were different. We used the variation in growth rate, while more often the appearance of the first pupae is the selected element (Melis et al., 2019). In another research, 0.070 g larvae were obtained in 9 weeks, using wheat-bran as feeding substrate (Bordiean et al., 2022). Thus is slightly less than the 0.087 g reported in our work, but should be noted that the starting number of larvae and their weight were different, furthermore the feed was provided weekly in different quantities. In another study performed in Pakistan, the average weight of the larvae fed on wheat bran after 9 weeks of life was 0.065 g, under similar condition of temperature and humidity, but the feed ratio was 10 times higher than that found in our case (Riaz et al., 2023). Instead, another study in Poland which used always wheat bran as control but a lower feeding rate - 0.5 per larva - gave more similar to ours with 9-weeks larvae having an average weight of 0.134 g, starting from 4-weeks larvae fed ad libitum on chicken feed (Bordiean et al., 2020). It is also important to emphasise that in our trial a diet containing residues from rice processing performed best in terms of growth, when compared to wheat bran one. In fact, other works had investigated this kind of wastes in the past. In Greece a diet made exclusively of rice bran was compared to a control diet made of wheat bran achieving better results with the control diet in terms of survival and final weight in *Tenebrio molitor* (Andreadis et al., 2022). Surely, the result obtained in our

research may be related also to the other ingredients included in TM6 diet, such as feed waste or breading waste.
Feed Conversion Ratio had been utilised to assess the bioconversion of wastes where the lower the value, the greater is the edible weight, and so the better is the conversion. Considering the results of the present trial, the experimental diets showed better results when compared to the CTRL diet with the diets TM6 and TM3 giving the best output. Referring to the FCR values found in another study (Bordiean et al., 2022) - ranging between 3.0 and 4.0 - they are all lower than those found in this trial. The differences are linked to the high mortality observed at the end of the farming period in our trial.
As regard the rearing conditions, the trial took place at 28°C (±0.6°C), a temperature consistent with those reported by some articles (Ramos-Elorduy et al., 2002; Bordiean et al., 2020; Bordiean et al., 2022) or slightly higher according to other sources, where the set temperature was 25°C (±1°C) (Melis et al., 2019; Peng et al., 2019). While the humidity - 73 RH% (±3.7%) - was higher than almost all the afore-mentioned sources of few percentage points, nevertheless it was within an optimal range for the insect (Bjørgea et al., 2018). The temperatures recorded in the boxes in the presen trial always remained above the set point, with the only exception of those recorded by the dataloggers in the TM3 boxes. However, the values obtained were still close to the optimum one.
Moving on the results of the chemical analysis performed on the larvae, the experimental diets recorded the best performance. In fact, generally, the higher is the dry matter content the greater is the performance during the transformation process. So, in order to produce an insect meal, experimental diets were all better and gave homogenous results. The control diet, on the other hand, produced a lower dry matter content of larvae, with a likely worse yield to the transformation. Literature report values around 37%, a value slightly higher than what we got in experimental diets and markedly higher than what obtained with the control diet (Toviho & Bársony, 2022).
The ashes were between 3 and 4% in all four the treatments, but the value recorded for the control group was significantly higher than all the groups fed the experimental diets. This result appears to be slightly lower than a study conducted in Norway that estimated ash at 5.4 % on a dry matter basis (Khanal et al., 2023). In the meal-production perspective it is difficult to interpret this data. Some researches sustained that within the ash some important minerals could be included for human health (Gantner et al., 2022), so further analysis would be carried out in order to characterize them.
As regard crude protein, the best result was that of CTRL group, with a markedly higher value than all the experimental diets. A high content of protein is in fact an excellent factor for the production of a protein insect meal, to be added as an ingredient in food.
Anyway, the registered values are slightly lower than some publications, that found protein at 45.6% (Costa et al. 2020). This was especially true for insects fed the experimental diets in our trial, where crude protein

was around 37-38% are quite below the aforementioned level. For larvae farmed on wheat bran, instead, we can find identical results on the protein content (Khanal et al., 2023).

The ether extract content found in all larval samples fed on experimental diets was high whereas larvae of the CTRL group showed a low-fat content. A favourable condition for the production of an insect-based meal rich in protein is precisely a low-fat content. The value we measured are perfectly in line with the ether extract contents found in a research work few years ago, based on larvae fed on wheat bran (Jajić et al., 2019).

Concerning chitin, which is not a positive factor in food production but can have further applications in the pharmaceutical and cosmetic fields (Satitsri & Muanprasat, 2020), non-significant differences between larvae fed on different diets were observed in our trial and values were similar to those recorded in another article (Son et al., 2021).

As regard water consumption, the source used - agar cubes - allowed to standardize the amount of water provided to the larvae, but it was pretty expensive. So, insects of the CTRL group, characterized by a low water consumption, showed the best results, followed by insects fed diet TM2 among the experimental diets. There are currently no accurate indications of how to interpret this data. However, we can assume that the increased consumption of water in by-product diets is due to the composition of the same ingredients, instead the alleged ability of bran to retain moisture from the environment. These facts can justify the lower need of fresh agar cubes.

Furthermore, it is really important to mention the economic factor, in the perspective of a future large-scale production. The estimated costs/ton of diets TM2, TM3 and TM6 were lower than that for the wheat bran control diet, resulting in a faster and higher growing performance at a lower cost for the experimental diets.

Beyond the present thesis, the assessment for the *Advagromed* project will include differences in weight, cost, composition, development time to obtain ready-to-use larvae. A series of elements wider from those that must be considered in order to produce a protein insect meal.

Based on the information that we have recorded, if the mortality rate is contained by managing the breeding protocol differently, the diets TM6 would be able to produce up to 446 g of protein per box. A result far exceeding the control, which would produce up to 279 g. The diets TM3 and TM2 would lead to lower quantities - 402 g and 413 g, respectively.

In light of the results obtained, it is interesting also to understand how an insect meal, in particular one of *Tenebrio molitor*, can be used in the food industry, assessing territorial specificities and traditions and how novel food should be introduced within the gastronomic heritage of a European country, starting from their size and shape as developed in the following chapters.

8.2 Consumer acceptance

The literature, in addition to considering the mentioned sustainability and regulatory aspects, as well as the nutritional value, must duly understand which is the position of the consumers towards insects as food. Actually, food neophobia, defined as the tendency of the individual to avoid unfamiliar foods, is one of the main obstacles for insects-food widespread into the diet of European people (Sogari et al., 2019). Therefore, it is the task of the research to investigate which are the novel foods that can more easily be introduced into the diet, in which form and through which preparations.

A recent study carried out in Italy investigated consumer behaviour towards seaweed, jellyfish and insects. An element of particular interest in this research concerns the association that insects induce among the consumers towards the idea of a healthy and at the same time ecological product (Palmieri et al., 2023). It should also be noted that the category of consumers most likely to taste this food, as well as to introduce it in their diet, was that of young people (18-30 years).

Finally, a key element emerged is the format in which consumers would prefer to introduce this food into the diet. Respondents would prefer to consume it in powder form and especially as an ingredient in processed foods, such as sweet bakery products, snacks, energy bars and cereals.

The age element is confirmed by another research performed in Spain, in which according to the respondents the group of consumers more willing to accept insects as food is that of adolescents. Furthermore, in this case, the 69.82% of the sample considers that in order to make insect food more attractive the natural appearance of insects cannot be seen (Ros-Baró et al., 2022).

Comparing the results obtained from these research projects with another one from Greece, focused on Gen Z subjects, divided in 3 smaller groups of age, we can make further considerations. In fact, just the 41.4% of the respondents stated to be familiar with what entomophagy is, while no one of them had prior experience with insect-based products, and just a few of them (6.3%) would like to participate to a sensory test (Kamenidou et al., 2023). This result stands as really low when compared to other similar studies. A study conducted in Belgium showed that 71.5% of participants had previously heard of entomophagy (Verbeke, 2015), while one conducted in Quebec claimed that 96% of respondents had knowledge of what entomophagy was, and 67% had previous experiences of tasting with insect-based foods (Hénault-Ethier et al., 2020).

These differences among Countries seem to be mainly linked to the strong attachment of the families from this area to the Mediterranean diet. Indeed, the 40% of the respondents from the Greek study declared to still follow this pattern of diet (Kamenidou et al., 2023).

Furthermore, the place of production is an important element to consider as well. Research conducted in Brazil showed as a Country-of-origin-label (COOL) attached on the product is able to change consumer perception about quality. In fact, the intention to buy and also quality expectations were higher for the cricket flour produced in the United States rather than the same product from China (Kuff et al., 2023).

8.3 Insect-based products

On the basis of these results, we can define some aspects that should be carefully considered approaching insect-based novel food and designing new products:

1. It seems that the size and format in which insect-based foods are offered to consumers is of paramount importance. The appearance of insects, in fact, is capable of catalysing food neophobia and so the literature shows that consumers would be willing to eat them, if it were possible not to distinguish them in food. Indeed, flours seem to be the preferred way for many to take these products, even integrating a percentage of insect flour in recipes as ingredients.
2. Nationality seems to be another important factor to consider among consumers. Therefore, within the formulation of a product made from insect meal, intended for a market in a Member State, it is necessary to assess the implications due to the gastronomic culture of the territories. For different countries, having different eating habits, different products can be designed and proposed.
3. Finally, it should be considered that the age range of consumers is an additional factor. In general, the literature suggests that younger population groups are more inclined to taste new foods and that therefore, food neophobia is less developed among young people.

Starting from these assumptions, we can decline them with respect to the geographical area of reference and therefore formulate a food proposal aimed at the use of a flour of *Tenebrio molitor* that can be produced in Italy and used in the creation of Italian products.
In general, people from the Mediterranean basin follow the pattern of the Mediterranean diet, with some peculiarities related to the nation of origin. In Italy, wholegrain meal and the various products associated with it (Ruggiero et al., 2019), pasta (Coldiretti, 2021), and pizza (Coldiretti, 2017) are some of the products that can never be missing in the tables of Italians and are consumed in large quantities. Differently, in the North Europe countries, where habits are different, rye bread is popular among consumers (Hasselbbalch et al. 2012), even more than wheat bread.
A recent publication highlighted how cricket and yellow mealworm can be used in the production of pasta. Small percentage of inclusion (14%) of Acheta *domesticus* and *Tenebrio molitor* meal had been tested replacing semolina to improve the protein content of pasta. The fortified product has been tested in order to evaluate the technological and quality aspects, such as colour, cooking quality and texture analysis, also compared to a control pasta, made only of semolina. The Tenebrio-meal, as well as the cricket one, have

been partially defatted in order to avoid the interaction lipid-protein, capable to decrease the protein solubility (Lam et al., 2018). Furthermore, the protein extract has been analysed to determine the aminoacid profile, so the essential aminoacid content.
As regard the colour, both two the insect-enriched pasta shows a different appearance, assessed through the colour deviation: darker and redder in the cricket one due to some browning reactions, whereas the mealworm pasta reported lower values for the yellow indicator. Both two products take on a colouring reminding the whole wheat pasta, a product already known and widespread among the Italian consumers.
As regard the cooking quality, the index considered has been the water absorption, higher in the cricket pasta, due to the high content in hydrophilic aminoacids which produce a structured protein gel capable to retain water during the cooking process. This feature is associated with an improved texture, resulting in a firmer pasta, while the adhesiveness seems to remain more or less unchanged between the experimental and the control pastas, consequently the optimal cooking time for these products is longer than for the control pasta.
These characteristics, however, fail to limit the cooking losses, which are higher in experimental pasta likely because of the weakening of the protein matrix due to the dilution of the gluten network (Pasini et al., 2022).
Ultimately, it seems that these ingredients have features suitable for the use in this sector, presenting technological characteristics that meet the needs of the manufacturer and the demands of the consumer. At the same time, these products offer the possibility of improving the essential amino acid content of pasta, in a general context where the demand for a protein source is growing (Berggren et al., 2019) and where, according to FAO, over one million people suffer from a protein deficiency in their diet (FAO, 2020). However, it should be noted that a sensory analysis should be carried out, as observed by the authors themselves, in order to evaluate the consumer acceptance of a similar product, considering the smell emitted by insect flour. Moreover, a series of parameters should be made stable and standardized, starting from the removal of the fat fraction, if we want to imagine an industrial development for the production of this food.
In this sense, the London company *Sens Food* has already moved, which among its products, all based on insect flours, began the sale of a pasta made of red lentils and cricket flour in two formats: fusilli and penne (Sens Food, 2022). This decision should be seen in the context of a broad campaign to promote similar products, accompanied by marketing and ambassadors.
The Thai company *Bugsolutely* has already launched a pasta on the market some years ago, the result of a long research and development process, with a 20% inclusion of cricket flour, making use, again, of a promotion campaign in which have joined chefs and industry experts such as Christophe Mercier, but also a video showing the making-off process (Busolutely, 2016). Also, the French company *Micronutris* has

started the production of a pasta containing the 10% of a *Tenebrio molitor* meal, plus other ingredients of vegetal origin (21 Bites, 2023).

However, pasta is not the only product of the Mediterranean cuisine for which the inclusion of insect meal has been tested. Recent researches have studied the possibility of producing bread with variable inclusion rates of *Tenebrio molitor* meal, assessing the impact of this addition on the technological and sensory aspects through some laboratory analysis combined with a panel test made with trained assessors, besides assessing the microbiological quality of the product. It emerged that *Tenebrio molitor* meal is suitable to fortify bread enhancing the protein and lipids level, but also minerals such as potassium, calcium, iron, and magnesium (Gantner et al., 2022). This product has been integrated in different replacement percentage instead of wheat flour: 5%, 10% and 15% of inclusion have been investigated. The substitution of wheat flour with insect powder did not affect significantly the features of the raw dough, yellow mealworm meal inclusion just shows a greater viscosity, but this did not affect the machinability of the dough and consequently loaf formation. The acidifying capacity basically was unchanged, instead dough strength instrumentally measured was reduced, probably due to the lower gluten content.

Anyway, consumer choices are affected mainly by bread's colour, texture and volume. These parameters showed significant difference compared to the value of the control bread as regard the browning index, the lightness and also the red and yellow level, both in the crust and the crumb of bread. These differences, more marked in the 10% and 15% insect meal inclusion, depend on the powder colour, but are also linked to the different protein content of the final product, so to the Maillard reaction that takes place into the baking process and brings to the sugars' reduction.

As regard the texture only in the 15%-inclusion bread was noted an enhanced hardness of slices, no differences have been observed among the other samples, as confirmed during the sensory analysis. Also, no differences have been perceived about the mouthfeel characteristics describing texture such as porosity.

About odour and flavour, the bread smell dominated the assessment in all the preparation, but decreased in the insect-bread, making space for sweet and nutty smell especially in the 15%-replacement bread. Similarly, the predominant flavour was the typical bread one, decreased in the modified recipes, characterized by an enhanced bitterness and a nutty flavour. According to the data collected the 5% of inclusion group collected the best results in almost all the considered parameters, imposing as the best even for the total perceived quality.

Finally, the water activity level remained constant in all the preparations, also no significant differences have been noted in the Total Viable Count of microbes in the enriched recipes compared to the traditional one. Anyway, we must consider that bread belong to the easy-spoilage food products, due to the moisture

loss and the possibility to develop yeast, bacteria and moulds. The recipe's modification does not affect this propensity.
In Italy the Baker Enrico Murdocco, famous for his brand *Tellia*, opened in Turin with 3 different stores, purposed for a small audience of customers a cricket-bread, made with a small percentage of inclusion of insect meal, however, did not disclose the percentage nor started a large-scale production of the bread, his choice is probably linked to the reaction of some groups of users on social networks, who have lashed out at the chef for his innovative idea (Cresci, 2023).
Furthermore, the Milanese restaurant *Pane e Trita* started to sell this year a cricket-burger. The main difference is that, in this case, the insect powder is not included in the bread, contrary to the expectations linked to the green colour of the loaf but within the burger, always in small percentage – just 1.6% - whereas the other ingredients are mainly beans, potatoes and bread crumb (Spaccini, 2023).
However, is important to highlight that, typical specialities like pasta or bread, are not the only products tested by the researchers to include insect meals. We should mention protein bars, chips, other insect-based snacks, but also many insects dried and consumed as they are. Nevertheless, in the first case, these are relatively new products, whose history of consumption is not linked to a particular ingredient and I decided to focus my research work on some product already belonging to the Mediterranean cuisine; while in the second case, the appearance, which does not conceal the presence of legs, antennae and in general the anatomical parts of the insect, makes it difficult for the consumer to accept these products. For these reasons I would not deepen other products.

8.4 Application of the HACCP method for the hazard analysis

In the designing process of a new food or a new ingredient, the formulation of an efficient and safe HACCP plan is a crucial part, capable to guarantee food safety and minimize food-born disease among consumers. This kind of approach has been introduced in Europe with the adoption of the "Hygiene Package", including two Regulations of absolute importance. Regulations (EC) 852/2004 - general hygiene requirements - and 853/2004 - hygiene requirements for products of animal origin - completed by the Regulation (EC) 854/2004 - requirements for the official control.
However, the current legislation has been repealed and updated with the introduction of Regulation (EU) 382/2021 and the Regulation (EU) 625/2017. Actually, HACCP is the most effective instrument in order to maximize safety (Wallace & Mortimore, 1998).
The approach is based on a risk-analysis procedure, capable to identify any dangerous step along the designing, productive and distribution chain and prescribe a detailed behavioural pattern, in order to reduce the contamination's possibility in accordance with the principles of this method.

These rules apply to all foods, including insects, for this reason it is interesting to imagine how a plant for the production of an insect meal should operate in order to guarantee food safety. About this, should be noted that it is a potentially dangerous product: its intestinal track could be a vector for the spread of pathogens.

As always, the plan is based on the GMP (Good Manufacturing Practice) and GHP (Good Hygiene Practice) which set the basic optimal conditions in order to produce safe food and keep the environment suitable for food production. Moreover, it is generally structured on 7 principles and 12 tasks (Wallace & Mortimore, 1998).

Principles: 1. Hazard detection and analysis; 2. Identification of Critical Control Points; 3. Determination of critical limits for all critical control points; 4. Definition of the monitoring system; 5. Identification of any necessary corrective actions; 6. Definition of verification procedures; 7. Registration and collection of all the necessary documentation to implement the plan.

Tasks: 1. Assemble and train the HACCP team; 2. Describe the products and processes; 3. Identify intended users; 4. Construct a flow diagram; 5. Validate the flow diagram (from the 6^{th} to the 12^{th} point each task corresponds to the principles mentioned above).

Considering that insect farming and processing is an emerging business, a recent project of research has focused on the creation of a HACCP plan for the production of a *Tenebrio molitor* meal, and therefore the processing, using the insect powder for some food products intended for certain well-defined categories of consumers. Along the lines of this work, we can build a new model based on our experience (Kooh et al., 2020):

Task 1. The first step is the creation of a HACCP team, in this phase it is fundamental that the skills offered are multiple, therefore criteria of multidisciplinarity and heterogeneity must be central in the establishment of the working group. Expertise in insect farming is appreciated, together with specific expertise in the design of a HACCP plan, as well as some experience gained in the field, and a figure with microbiological and epidemiological skills, whereas there is little literature available on this subject.

Task 2. So, a characterization of the product that we want to obtain, or insect meal. Based on our chemical analysis, the composition of this powder mainly includes 38.09% crude proteins and 34.73% ether extract[5]. Then ashes (3.93%) then fibre - mainly chitin (5.56%) carbohydrates and the residual moisture, of course. According to the article, pH must range between 6.5 and 7.0, a_w[6] must be <0.50

[5] The mentioned values refer to the TM6 diet.

[6] Water activity.

and moisture content <6%. The processing that we have applied to the larvae to obtain the powder for the analysis is consistent with a use in the food industry and represents one of the possible working layouts for this product, also mentioned in the considered article, even if it is not the most convenient.

A) Mealworms receiving, visual inspection of the batch in order to identify any anomalies. Larvae must be yellow-brown in colour, alive. The presence of black larvae, therefore of dead insects, in concomitance also with strong and pungent odours are indicative of a bad state of conservation, bringing us to destroy the batch.
B) According to the law subsequently must be performed a fasting period of at least 24 hours, in order to obtain larvae with a clean intestinal tract.
C) Sieving is performed with the aim of remove all the frass residues from the batch, water can be used for a rinsing process.
D) Inactivation and slaughtering of all the larvae can be performed with hot or cold temperatures, in our case we choose the second option. Are needed 4 hours at -18°C. The layer should not exceed 5 cm in order to have an optimal cold diffusion.
E) Whole insects are subjected to freeze-drying, a process in which water is eliminated passing directly from solid (ice) to gaseous (steam) state. The process involves the preparatory phase, in which the substrate is homogenised, and subsequently a freezing phase at -60/-80 °C - or less rigid temperature -20/-30°C - and then the product is subjected to high vacuum.

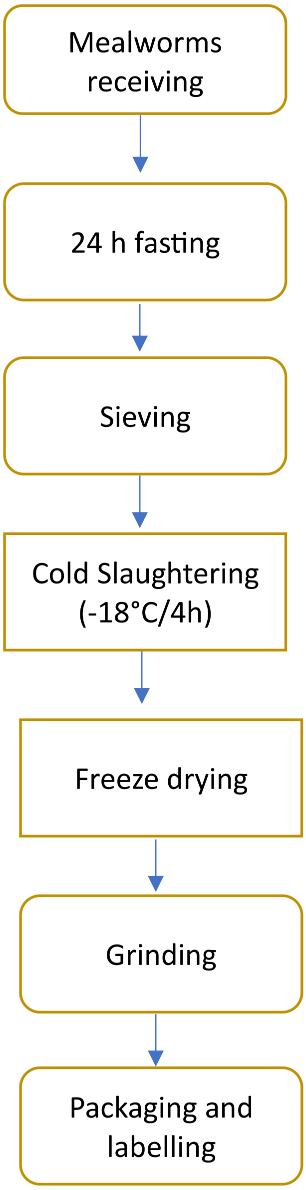
   Once the container reaches the designed pressure value - between 5 and 20 Pascals or more - a first heating is carried out between -10°C and -30°C - sometimes more, if the starting temperature was more rigid - to remove the water. This part is called primary drying and last until the ice is totally eliminated.

   Then, with a second heating - about 40°C - since the matrix is rich in protein, should not be higher than this - we move away the steam, thus reducing the a_w up to 0.5, counteracting the microbial spoilage and increasing the shelf-life to several months.

   Although this is the basic operation, it is subject to great variability: the pressure and temperature values can be coupled differently with different results in terms of cost and process times (Nowak & Jakubczyk, 2020).
F) Grinding the dried insects until a fine powder is obtained using an industrial mill.
G) Packaging of the product in a multilayer plastic bag that allows to preserve its properties. The powder must be stored in a dry place at room temperature. According to these procedures, best if used within 6 months from manufacturing date.

Task 3. The intended users are infant, children, adults, elderly people. No limits are set by the two authorizations for the marketing of dried, frozen larvae or powder of *Tenebrio molitor* (Reg. (EU) 2022/169; Reg. (EU) 2021/882). The products that contain meal are several: including porridges, biscuits, protein drinks. They adapt to different age groups.

Task 4. Construction of a flow diagram



1. Visual inspection of the batches
2. In order to obtain a clean intestinal tract
3. Elimination of residual frass
4. Slaughtering of larvae using cold temperatures
5. Cold temperature + high-vacuum to reduce the microbial load.
6. Grind the product obtaining a fine powder
7. Multilayer packaging

Task 5. Validation of the flow diagram on-site comparing the chart to the productive plant.

Task 6. Hazard analysis. In this step the chemical, physical and biological hazard for this product are analysed. It immediately emerges with great evidence that there are no heat treatments in the production layout that we have identified, in a product where the pH is neutral. This, although it can avoid protein denaturation due to thermal damage, increasing the nutritional quality of the preparation, is a negative aspect for food safety. It would be a good idea, for an industrial production, to create a layout that foresees high temperatures, to ensure a reduction of the microbial load, perhaps with high temperatures in a short time, in order to avoid as much as possible thermal damage (Kooh et al., 2020). Moreover, energy costs and the environmental impact of such a method are unsustainable and contradict the very purpose of breeding and processing insects for food, namely to produce a sustainable protein matrix.

According to the literature is reported a list of the biological hazards (ANSES, 2015; Garofalo et al., 2019; Kooh et al., 2019) associated to some scores (Table 8).

First the relevance of their natural reservoir in relation to the insect breeding (R). 5 points for telluric microorganism, 3 points for ubiquitous microorganism and 1 point for microorganism with a really specific reservoir. Then the persistence of each microorganism or metabolic product to resist in the process condition described before (P). 5 Points are attributed to the sporulated microorganism, 3 points to all the other elements considering that no thermal treatment has be done – so also viruses and histamine represent a relevant hazard. Likelihood Index, based on the product of two aforementioned parameters (R*P) (Li). Severity index based on the results of the aforementioned official reports (ANSES, 2015; Garofalo et al., 2019; Kooh et al., 2019) (S). Finally, the Risk (Rk), based on the product of Likelihood and the Severity indices mentioned (Li * S) (Kooh et al., 2020).

Table 8. Establishment of the Likelihood and Risk indices based on the R, P and S parameters.

Biological hazards	R	P	Li	S	Rk
B. cereus	5	5	25	1	25
Campylobacter spp.	1	3	3	3	9
C. botulinum	5	5	25	5	125
C. perfringens	5	5	25	1	25
Cronobacter spp.	5	3	15	5	75
HAV	1	3	3	3	9
Histamine	3	3	9	1	9
L. monocytogenes	3	3	9	5	45
Norovirus	1	3	3	1	3
Salmonella spp.	3	3	9	3	27
S. aureus	*3*	*5*	*15*	*1*	*15*
STEC	1	3	3	5	15
Yersinia spp.	1	3	3	3	9

From this analysis we have 6 most important biological risks in the production of this food product: *B. cereus, C. botulinum, C. prefringens, Cronobacter spp., L. Monocytogenes and Salmonella* spp. If a hot treatment is added in the production layout the scores must be adjust reducing the Risk.

Task 7, 8, 9. For the fine-tuning of this novel food, it is necessary to implement control measures to minimize the risks related to the consumption of the product at an acceptable level. This step is possible through the identification of the Critical Control Points (CCP) step of the production process in which the control is necessary to ensure the healthiness of the meal. For each CCP it is also necessary to set critical limits that must not be exceeded to ensure food safety. However, the proposed production layout does not present this step, because it does not provide heat treatments. The prevention mechanism is therefore based on the correct functioning of GHP and GMP. Attention would focus on the hygiene of the environment, the hygiene of all the equipment and machines used and the training of staff, in order to follow all the guidelines with the aim of minimize risks.

In addition, should be paid attention to pressure and temperature parameters, as well as to operating times, during the freeze-drying phase. As we have said, the variables can be combined in different ways in order to ensure an effective reduction of microbial load and an optimal lowering of water activity. Much will depend, certainly, also on the initial microbial load, so it would be necessary to carry

out random checks to monitor and verify the presence of microorganisms beyond the alert thresholds, keeping track of the results obtained.
If a heat treatment is inserted - for example, the slaughter of insects by boiling, or a process of cooking the insect-paste before carrying out a hot drying - the CCP would be all the steps that require the use of high temperatures. Then the maintenance of 100°C to boil whole insects in hot water for 10 minutes. Or a cooking process at 140 °C for 30 minutes.

Task 10. This task requires identifying corrective actions to remedy the situation when an anomaly is detected. This task is central to the formulation of the control plan. Corrective action should normally allow - if a CCP has lost control - to bring it under control. According to the procedure there are no steps to correct, considering the absence of CCP. However, a batch showing discrepancies or whose chemical analyses give positive results for some microorganisms above the alert threshold must be destroyed.

Task 11. This step involves the development of HACCP plan verification procedures, in addition to monitoring, aimed at verifying that the plan complies with the law and is effective.
The task includes the validation of the plan, the development of a HACCP audit system, so an on-site verification made by experts based on observations, interviews and record analysis. Then also, the calibration of the equipment used to measure the various parameters, a series of sampling and analysis.
In addition, the scientific relevance of the data on which the hazard detection was based and their actuality must be demonstrated.

Task 12. Finally, the last step includes the organization and preparation of documents and evidence. Which can be used as evidence. It is important that documents are sorted and updated continuously. There are various kinds:

- Basic documents utilized in order to design the HACCP plan (system documentation).
- Documentation related to the methods and procedures utilized to obtain the product but also as regard the description and monitoring activities for CCP (working documents).
- Monitoring records and verification records of process (dynamic documents).
- Information about the staff training based on hygiene concerns. Contents and evaluations must be archived.

In our case must be collected the a_w values reached, measure of water content, results of the microbial analysis.

9. Conclusions

We can conclude that insects consumption has undeniable historical roots in Western culture and is coming back into vogue for a growing need for protein sources. Among insects, *Tenebrio molitor* is a suitable species for many industrial purposes.

It is possible to breed it on waste substrates with good results in terms of growth, when compared to larvae fed on wheat bran; moreover, is possible to establish interesting models of circular economy on a local scale. Attention must be paid to the management of the colony in relation to the survival rate, which in our case had an unsatisfactory outcome. Parameters to take care of are also a water resource and the values of temperature and humidity.

Yellow mealworm also has excellent nutritional characteristics, as regard the protein and lipid content, as well as the micronutrients. Different levels of macronutrients have been obtained as the diet changes, this in the future may be an interesting aspect to deepen.

The powder of the insect can be utilized in different products of the Mediterranean cuisine. This process allows us to increase the nutritional value of foods, but also to circumvent the problem of insects' acceptance, which is particularly marked in southern Europe. If the appearance of the bugs in fact is a great obstacle to its spread among consumers, the reduction in meal seems to be a possible solution.

The prospects of expansion of this work are also the amino acids characterization, analysis on the distribution of fatty acids and analysis on the micronutrients such as minerals, which would allow to obtain detailed information about the properties of this product.

Then, although the data collected are encouraging, the growth of the insect for human nutrition is limited to a strict number of substrates, so further approval process should be needed for the use of larvae bred in these conditions. Anyway, all the elements collected are useful to trace the possible expansion phase of a market currently niche, but with great commercial potential.

Ultimately, it is clear by Italian perspective that chasing public opinion is an approach that has not brought the desired effects. Moreover, great benefits can be derived from a path designed by the same productive companies, in cooperation with costumers, in order to design new products capable to meet the specific needs of consumers.

In fact, the awareness is that if Italian companies delay in organizing an efficient and competitive sector, the market will not be suppressed, but simply handed over to foreign companies and investors, throwing away a golden opportunity for the entire food compartment.

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