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NUTRITIONAL VALUE OF HERMETIA ILLUCENS FED WITH VEGETABLE BY-PRODUCTS

Valore nutrizionale di *Hermetia Illucens* nutrita con sottoprodotti di origine alimentare

TIPO TESI: Sperimentale

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Alla mia famiglia,
stella polare nel mare in tempesta, faro nella notte e
sole che hanno da sempre e per sempre illuminato il mio sentiero

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

ALA	α -LINOLENIC ACID
BSF	BLACK SOLDIER FLY
DHA	DOCOSAHEXAENOIC ACID
DS	DRY BASIS
EFA	ESSENTIAL FATTY ACIDS
EPA	EICOSAPENTAENOIC ACID
FAME	FATTY ACIDS METHYL ESTERS
FAO	FOOD AND AGRICULTURE ORGANISATION OF THE UNITED NATIONS
FC	FOLD CHANGE
GHG	ANTHROPOGENIC GREENHOUSE GAS
HI	HERMETIA ILLUCENS
MUFA	MONOUNSATURATED FATTY ACIDS
PAP	PROCESSED ANIMAL PROTEINS
PUFA	POLYUNSATURATED FATTY ACIDS
RH	RELATIVE HUMIDITY
USD	UNITED STATES DOLLAR

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO), is estimated that the human population will reach 8.5 billion in 2030 and 9.6 billion by 2050. Corresponding needs will require an increase in cultivation and livestock farming, which, in turn, will lead to further increase in food production, and generated by-products. In the EU**,** around 88 million tonnes of food waste are generated annually with associated costs estimated at 143 billion euros (FUSIONS, 2016). While an estimated 20% of the total food produced is lost or wasted**,** 36.2 million people cannot afford a quality meal every second day (Eurostat, 2020).

The production of animal feed is competing (for space and resources) with other fields related to the food and energy industries for resources such as land, water and fertilizers. This sector contributes approximately to 14.5% of all anthropogenic greenhouse gas (GHG) emissions (7.1 Gigatonnes of CO_2-equivalent per year) (Makkar et al. 2014).

Edible insects have the potential to increase food safety preserving environment. *Hermetia Illucens* (HI) is one such insect that is being studied (van Huis et al., 2013).

Therefore, the use of the larvae of HI as a feedstuff must be taken into consideration because it requires fewer resources and it is characterized by a lower environmental impact, such as less use of soil, water and less production of ammonia and gases (Jucker et al. 2021). The larvae of *Hermetia Illucens* are among the most promising insects due to their ability to convert a wide variety of organic wastes, such as vegetables, fruit, fish, municipal organic waste, and animal manure, into organic matter in the larval body (El-Dakar, Ramzy and Ji 2021; Truzzi et al. 2020). Their conversion into lipid and protein-rich biomass is excellent for use in animal feed (Leong et al. 2016).

In food production, high number of studies have been done to design the method, which could minimize food waste. For example, Tassoni et al., (2020) highlighted the use of pea, bean, and chickpea agro-industrial wastes in preparing rich in protein functional ingredients for the formulation of feed, food, cosmetic, and packaging products (Sayed-Ahmed et al., 2022). It has been found that waste from vegetable and legume processing are rich in bioactive compounds and can be used as insect feed (Kovalenko et al., 2021; van Huis et al., 2013;

Meneguz et al., 2018). BSF larvae by bioconverting agricultural by-products can contribute to recycling a biological matter. In fact, *Hermetia illucens* can reduce the volume of organic matter in substrates by 60- 90% and convert about 40% or more in the form of protein (Lopes et al., 2020; Luperdi et al., 2023). HI besides being rich in protein also contain a high number of beneficial compounds such as vitamins, minerals, and fatty acids. HI is used as a feeding material in aquaculture, livestock, and poultry. According to several studies, the fatty acid profile of insects can be modified using specific diets (Barroso et al., 2014; van Huis et al., 2014; St-Hilaire et al., 2007; Tomberlin & Sheppard, 2002). The purpose of this thesis was to study the bioconversion of fatty acids in industrial agro waste derived from fresh legumes and tomato by-products into edible insect biomass. Therefore, HI larvae were reared on diets formed on agro-industrial by products.

In this thesis project has been analyzed the fatty acid composition of six diets, prepared with chickpeas, peas, green beans, and tomatoes. HI larvae can bioconvert agricultural by-products in high-value fat and proteins, hence, are considered as animal feed (Giannetto et al. 2020; Belghit et al. 2019).

CHAPTER 1
BIBLIOGRAPHIC RESEARCH

1.1 *Hermetia Illucens*

Hermetia Illucens (Linnaeus, 1758), also known as "Black Soldier Fly" (BSF; Figure 1), belongs to the family Stratiomyidae, subfamily Hermetiinae, genus Hermetia. It is native of the tropical and warm climatic regions of the American continent, around 45° N to 40° S latitude (Purkayastha et al. 2017), but currently, it has a cosmopolitan distribution (Diener, Zurbrügg, et al. 2011). It was first reported in 1930 in the Hawaiian Islands (Gayatri R and Madhuri K 2013).

The species was originally described by Linnaeus as Musca Illucens in the tenth edition of the Systema Naturae. The Linnaean description was made on specimens that were part of the private collection of the Swedish entomologist Charles De Geer (Papavero, 1971). It can be distinguished from other Stratiomyidae for its size of 15-20 mm, and for its predominantly black color, with blue metallic highlights on the thorax and sometimes a reddish end of the abdomen (Figure 1). The antennae are about twice as long as the head. The wings are membranous and at rest are folded horizontally over the abdomen. Females have a longer abdomen, more white hairs on the head, and larger wing size compared to males, as well as a longer and translucent abdominal area from which the presence/absence of eggs can be seen (Marzouk 2016). Females begin oviposition after a further 2 days and each female may lay between 1200 and 1700 eggs in her lifetime and deposit them in dry cracks and crevices, in places adjacent to a food source (Wayne D. Lord et al., 1994).

Figure 1: Hemetia Illucens

The life cycle of the Soldier Fly consists of five main stages: egg, larva, prepupae, pupa and adult (Figure 2).

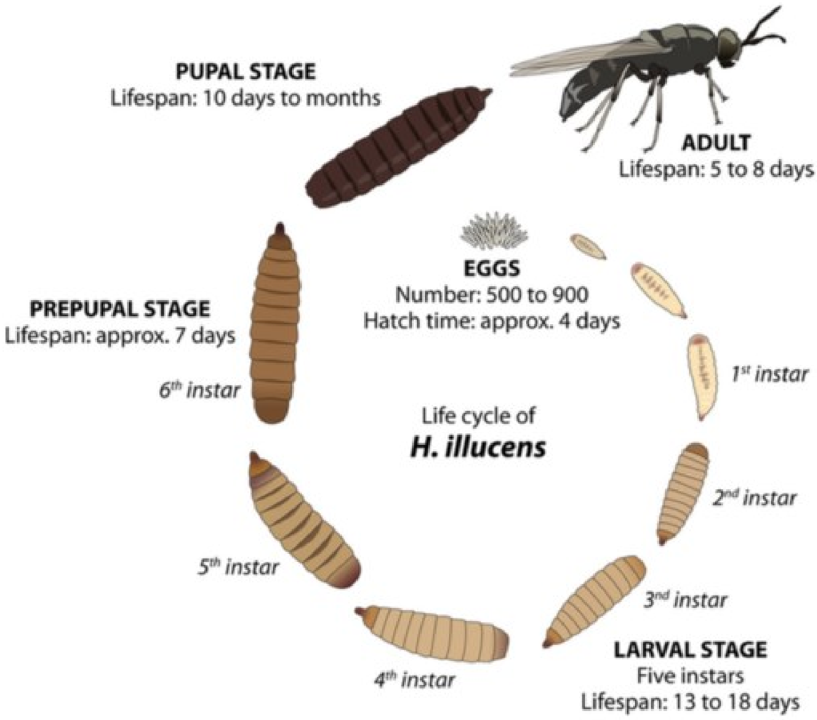

Figure 2: The life cycle of Hermetia Illucens

- Egg: The eggs, which are light yellow and about 1 mm long are laid on the organic substrate and hatch in about 4 days, if conditions are optimal.

- Larva: initially it is about 1-2 mm long until fully mature, and after three stages, which last 20-30 days, it reaches a length of about 2 cm. During these days it feeds continuously. They have a cylindrical, markedly segmented body, a protruding head containing chewing mouthparts and the color varies from white to very deep brown (Rozkosný, 1983;Figure 3).

- Pupa: having reached optimal maturity, the larva withdraws inside the last layer of skin, called the puparium, which, thanks to a process of keratinization hardens. At this point, the pupa enters a state dormancy waiting for optimal conditions for the transition to the adult stage.

- Adult: The adult can reach 2 cm in length; it is black and has wings with very dense veins covering the entire membrane. Adults are short-lived, they survive only 5-8 days, during which they mate about two days after flickering and do not feed because they exploit the fat stored in the larval stage (Diclaro and Kaufman, 2009). This aspect represents advantages compared to other insects, since less care is required and above all the lack of nutrition of the adult greatly reduces the risk of becoming a vector of disease. In nature, pupation, which is the transition from larva to pupa, can occur in a period ranging from 9 days to 5 months (Fernanda Oliveira et al., 2015).

State	Size (mm)	Color	Picture	Duration (days)
1 instar	5	White/ yellow		15
2 instar	12	Light brown		15
3 instar	19	Dark brown		8
Pupa	19	Dark brown		10
Fly	20	Black		7

Figure 3: Stages of Hermetia Illucens

This insect needs a warm environment for their food to degrade during reproduction, instead of using high energy and waste in cold or temperate environments. (Veldkamp et al., 2012). The important factors are temperature and humidity. Below 27°C and about ~70%

(RH) humidity, mating and oviposition are greatly reduced. (Holmes et al., 2012; Tomberlin and Sheppard, 2002). Therefore, controlling this stage is the most effective method of planning larval production. *Hermetia Illucens* is a species that has been extensively researched in recent years due to its potential to contribute to the circular economy, which is an approach to address two major global problems: waste reduction and sustainable resource management. The industrial breeding of this species has been found to offer numerous benefits, including economic, environmental, and food-related advantages. *Hermetia Illucens* is one of the best and efficient decomposers and has received considerable attention due to the ability of its larvae to grow on different substrates and convert a wide variety of organic sources. The residue from the degradation of organic material can be used as a fertilizer, (Choi et al., 2009).

1.2 Nutritional value of *Hermetia Illucens*

The chemical composition of insects is known to be influenced by many factors, including species, developmental stage, rearing technology, and diet composition (Weber et al., 2022). Therefore, studies have been focused on exploration of diet composition that caused increased level of protein and fatty acid profile of insects. (Mshayisa et al., 2022).

The larva stage is the most used from a technological point of view, due to its excellent nutritional composition, which makes it suitable for animal and human diets. On average, the larva of the BSF has protein and fat content from 37 to 63% and from 7 to 40% of dry matter, respectively (Zheng et al., 2012). Dry matter varies from 20 to 45% (Diener et al., 2009).

The body composition of larvae is strongly influenced by two main factors:

- The stage of larval development: for example, protein content decreases with advancing larval age, from 61% of dry matter after 5 days of development to 40-45% after 20 days (Rachmawati et al., 2010).
- The diet provided to the larvae: the type and amount of food provided to the larvae greatly influences the protein and fat content.

The crude protein content of HI can be compared to the soybean, which is the most widely used plant protein source in animal husbandry (Surendra et al. 2020).

The fatty acid content of *Hermetia Illucens* is characterized by 58-72% of saturated fatty acids and 19-40% of mono- and polyunsaturated fatty acids, with high levels of lauric acid (C12:0), (Caligiani et al, 2019; Meneguz et al., 2018) followed by oleic acid (C18:1n-9), palmitic acid (C16:0) (Barragan-Fonseca et al. 2017), and linoleic acid (C18:2n-6) (Caligiani et al., 2019).

HI has also been found to be a reliable source of minerals such as Ca, P, and K (Tschirner and Simon 2015; Cullere et al. 2018) and Mn (Wang and Shelomi 2017). Some other micronutrient content effective for animal development and welfare, such as Fe, Zn and vitamins, are also conteined (Rumpold and Schluter, 2013). *In vitro* studies have shown that Zn and Fe can be bioavailable in insects; hence, insects could be not only a good source of protein, fatty acids but also of micronutrients (Latunde-Dada et al., 2016).

HI larvae can be used in fish farming. It has been shown that, even with a limited feeding of HI meal the fish can still achieve similar growth and performance as they would with conventional feeds. (Barragan-Fonseca et al. 2017). HI larvae can be a sustainable and cost-effective source of protein for aquaculture (Kroeckel et al., 2012; Saeley et al., 2011; Aniebo et al., 2009). Studies have shown that they can be used as a total or partial alternative to conventional meals (Diener et al., 2009; Magalhaes et al., 2017; Bondari et al., 1981), even for shrimp and other aquatic invertebrates (Cummins et al., 2017).

1.2.1 Fatty acids

Fatty acids are monocarboxylic acids with a long hydrocarbon chain. They typically consist of a non-branched, non-cyclic chain of 4 to 30 carbon atoms.

The carboxylic group of the fatty acid represents the hydrophilic head, and the hydrocarbon portion of the fatty acid represents the hydrophobic tail.

Saturated fatty acids (SFA) such as palmitic acid (C16:0), stearic acid (C18:0) have no double bonds. Unsaturated fatty acids include monounsaturated fatty acids (MUFA) with one double bond (C=C) such as oleic acid (C18:1) and polyunsaturated fatty acids (PUFA) that contain two or more double bonds namely linoleic acid (C18:2), linolenic acid (C18:3), and arachidonic acid (C20:4). Based on the position of the first double bond from the last methyl group, FA are classified in ω3, ω6 and ω9 (Simopoulos, 2008; Hulbert, 2008; Kinsella & Andersson 1997).

Essential Fatty Acids (EFA) are necessary for the health and must be provided with food because these cannot be synthetized by the body. For example, PUFA such as linoleic acid

(AL), the parent of the omega 6 FA, and α -linolenic acid (ALA), the parent of omega 3 FA (Kaur et al., 2014).

Hl shows high lipid content (up to 500 g/kg), but the fatty acid composition is not always optimal for animal and human nutrition, because characterized by low amounts of MUFAs and PUFAs and high amounts of SFAs (Truzzi et al. 2020). HI larvae and prepupae contain about 58-72% of SFA, with high levels of lauric, palmitic and oleic acid, and about 19-40% of MUFA and PUFA (Barragan-Fonseca, Dicke, and van Loon 2017), instead they have a low content of Docosahexaenoic acid (DHA - C22:6) and Eicosapentaenoic acid (EPA -20:5) (El-Dakar et al. 2020).

1.3 Lipid profile of HI larvae influenced by the rearing substrate

Some studies demonstrated the changes in nutrient composition throughout the entire life cycle of HI. If an insect, like HI, are given certain foods, like brown algae and fish offal, they can get a type of fat such as eicosapentaenoic acid (EPA), which is usually not found in insects that live on land. Therefore, the fatty acid profile of insects can be changed by feeding them (Andreadis et al., 2022). Liu et al. (2017) found that the nutrient composition of HI changed dynamically from the larval stage to the adult stage. This study assessed metabolic changes in nutrition composition of HI from egg to adult. The result suggests a rapid increase of crude fat content was observed since the development of 4–14 days of larvae with its maximum level reaching 28.4% in dry mass. A sharp drop in crude fat was noticed from early prepupae to late pupae (24.2%, and 8.2% respectively) (Liu et al., 2017). For example, values ranging from 150-250 g/kg have been reported in larvae reared using poultry manure as a food substrate, to 420-490 g/kg in those fed oil-rich food waste (Makkar et al. 2014).

It was discovered that the lipid level of HI prepupae fed with fruit-based diets was significantly increased; in fact, the abundance of SFA was higher than control (Ducker et al. 2017).

1.4 Legislation on insects as livestock feeds

In Europe, the growing interest in insect breeding has provided the basis for the development of a new law. Therefore, on 8 October 2015, EFSA published a scientific opinion about risk profiles related to the production and consumption of insects such as food and feed ("Risk Profile Related to Production and Consumption of Insects as Food and Feed", 2015).

Since September 2016, the European Commission's Directorate-General for Health and Food Safety, together with EU Member States, has initiated a project to authorize insect meal as feed for fish reared in aquaculture (Müller, Wolf and Gutzeit, 2017).

Currently, from the EFSA report, only seven species have been authorized for use as aquaculture feed:

1. *Hermetia Illucens*

2. *Musca domestica*

3. *Tenebrio molitor*

4. *Alphitobius diaperinus*

5. *Acheta domesticus*

6. *Gryllodes sigillatus*

7. *Gryllus assimilis*.

EU Regulation 2017/893 lists *Hermetia Illucens* as one of the seven insect species currently farmed in the European Union that fulfils safety conditions.

1.5 Aquaculture and Aquafeed

Aquaculture is defined, by the Ministry of Health, as "the farming of aquatic organisms (fish, mollusks, crustaceans, and aquatic plants). This involves forms of human intervention through breeding in the growth processes, through seeding and control systems, feeding, protection or control from predators, etc. Breeding also implies ownership, in associated or individual form, of the breeding stock" (FAO, Circular 815).

Over the past 50 years, aquaculture has undergone rapid development, increasing its production compared to the fisheries sector, which has maintained a steady production trend. Modern intensive marine aquaculture began, only 35 years ago.

Nowadays, this sector is in continuous development, it has spread to several continents, exploiting different ecosystems and a wide range of species (Subasinghe, Soto and Jia, 2009). It constitutes as a major source of high-fat and high-protein food resources. This strong expansion is due to the continuous and growing demand for aquatic food products, which are an essential component for humans due to their nutritional profile.

World production of farmed fish exceeded that of meat for the first time in 2011. In 2009, aquatic products accounted for 20% of food consumption (Tacon and Metian. 2008). Fishing and aquaculture provided around 148 tons of fish in 2010 (with a total value of USD 217.5 billion) of which only 128 tons of fish were used as a source of human sustenance (FAO, 2016). Moreover, the consumption of aquatic products is estimated to increase by 60-70% by 2050 (Makkar, Tran, Heuzé, and Ankers, 2014a). Indeed, aquaculture plays a key role in the global fight against malnutrition (Subasinghe et al., 2009). Fish' products have a high protein content and a high concentration of omega 3, which is crucial in the prevention of cardiovascular diseases and the reduction of blood cholesterol levels. The aquaculture sector is, therefore, in continuous expansion due to the population's growing demand for fish products.

Global production of fishery resources reached 171 million tonnes in 2016, where 47% belong to the aquaculture sector (Sampantamit et al., 2020).

Nowadays aquaculture represents one of the most important sectors on international and global scale. It produces around 91 million tons of fish, of which 61% is for human consumption, while 28% is used for feed formulation (Tacon et al, 2006).

1.6 Pros and cons of *Hermetia Illucens* as aquafeed

Scientists have been studying the nutritional content of different insect species. They found that insect meal could be used as food for freshwater fish, but there is a gap in understanding of how it affects marine species like salmon. When insect meal is added to fish food, some of the other ingredients need to be taken out. Some studies showed that when insect meal is used in fish food, it could affect the type of fat that the fish can digest. One study found that when a certain type of insect meal was used in rainbow trout food, it did not affect their growth. But when too much insect meal was used, it made the fish eat less food. Another study found that insects can be a good source of protein to replace fishmeal, but too much of it might not be good for the fish. (Rumpold and Schluter, 2013; Barroso et al., 2014; Makkar et al., 2014; Sánchez-Muros et al., 2014; Henry et al., 2015).

1.6.1 Potential negative effects of using HI as a feed

Many studies have shown that diets with high HI meal content can cause an increase in saturated fatty acids (especially lauric acid) and a decrease in polyunsaturated fatty acids in Danio rerio. This can lead to severe lipid steatosis in the fish liver, likely due to a deficit of polyunsaturated fatty acids that limit the deposition of triglycerides in the liver. Other studies have reported stress-associated liver necrosis and intestinal damage after using excessive amounts of *Hermetia* meal (more than 75% in the diet) in *Cyprinus carpio var. jian*. In this case, the effects may be related not only to the high content of saturated fatty acids but also to the high content of chitin, which induces intestinal inflammation. Diets with lower levels of HI meal had no adverse effects on fish health. A study on *Lateolabrax japonicus* suggests that substituting up to 64% of the feed with HI meal did not lead to inflammatory events in their livers and intestines Diets with lower levels of HI meal had no adverse effects on fish health. (Wang et al. 2019). HI is recognized and permitted for feed purposes; it is important to consider the potential negative effects on animal welfare. This includes the elevated level of ash found in HI larvae and the high fat content of HI meal, which may affect the digestibility and liver integrity of animals. It is crucial to use HI meals in moderation and avoid excessive amounts to ensure the health and well-being of the animals being fed.

CHAPTER 2
AIM OF THE THESIS

The thesis was supported by the project BSFlyGreen, funded by the PSR-Marche 2014-2020 Measure 16.1 action 2 and aims to improve the environmental sustainability of agricultural and agro-industrial processes through the application of scientifically tested procedures (https://bioconversionemoscanera.it/). The aim of the project is to study the process of bio-convertion of the residue of fresh legume by-products into animal proteins and fats through *Hermetia illucens* or black soldier fly to be used in feed for fish, poultry and pets.

Fresh legumes represent 25% of the Italy harvest vegetable production and currently the residues are treated through anaerobic digestion processes. Vegetable by-products, apart from lipid and protein source, are also an alternative source of bioactive compounds such as fiber, mineral and vitamins, polyphenols, phytosterols.

HI composition can be modulated by the rearing substrate and most of studies are focused on fatty acids in edible insects for feed and food application.

HI larvae were reared on agro-industrial-based diets. The fatty acids composition of six diets, prepared from chickpea, pea, green bean, tomato was analysed and compared with the fatty acids content of HI reared on those diets to evaluate the entity of the bioconversion.

This study can help scientists in understanding of how to grow and process edible insects to be used in food and animal feed. They can use this information to apply it to other insects like crickets. It is important to note that some edible insects are only allowed to be used as animal feed, not for human consumption.

CHAPTER 3
MATERIAL AND METHODS

3.1 Agro-industrial fresh legumes and tomato by-products

Agro-industrial vegetable wastes such as fresh legumes by-products (pea, chickpea, and green bean) and tomato by-products were collected along the industrial at different points or steps of production (optical and manual sorting, washing, trimming, blanching, destemming). Agro-industrial wastes were provided by Covalm (Coltivatori Ortofrutticoli Valli delle Marche, Italy).

3.2 Diets preparation

The different fractions of waste (optical and manual sorting, washing, trimming, blanching, destemming) per each type of by-product were pooled together and used for the diets for HI preparation. Diets, as a substrate for HI, were prepared with a ratio 1:1 two different types of by-products with similar seasonality (pea with chickpea; chickpea with green bean, spinach with chickpea; and tomato with chickpea). A control diet was prepared from wheat bran (50%), flour of alfalfa (30%) and corn flour (20%).

- The diets discussed in this thesis were divided in 3 groups: Chickpea Diets (D1-D2-D3-D4);
- Tomato Diets (D2 and D6);
- Control Diet

Diets were kept at -20 °C before being used to as a feed on HI larvae and an aliquot was immediately freeze-dried, vacuum sealed, and stored in the freezer at -20°C.

3.3 Insects preparation

Hermetia Illucens prepupae were obtained after rearing phase performed at the D3A Department of the Marche Polytechnic University (Enthomology, Ancona, Italy). *Hermetia Illucens* larvae were reared on the control diet and diets based on legumes and tomato by-products in a climatic chamber at 27 ± 1 °C and relative humidity of 70%, in continuous darkness. At the end of the rearing phase, *Hermetia Illucens* prepupae were freeze dried, milled, and stored at -20°C until analysis.

3.4 Chemicals and reagents

Fatty acids standards (-98 %; tridecanoic and nonadecanoic acid methyil esters), 37 % boron trifluoride-methanol solution (BF_3-MeOH, 14% in methanol) were purchased by Merck (Darmstadt, Germany). HPLC grade Hexane, Anhydrous sodium sulphate was purchased by ITW Company (Darmstadt, Germany). Millia water was purified with Millipure System (Millford, SC, USA).

3.5 Extraction of total lipids and fatty acid profile

Total lipids were isolated as described by Folch, Lees, and Sloane Stanley (1957).

Minced freeze-dried insects (1g) were added of a solution of chloroform-methanol (2-1, v/v, 20 ml) agitated (5 min) and centrifuged (3000 rpm, 10 min, 4 °C). The organic phase was washed with distilled water (2.5 mL), filtered through Whatman filter paper (Grade 4, 90 mm, Merck KGaA, Darmstadt, Germany) over anhydrous sodium sulphate (3 g) and evaporated with rotary evaporator (30 °C), and the fat yield was calculated.

Fatty acid methyl esters (FAME) were obtained from total lipids through transmethylation by BF3-MeOH reagent (Medina et al. 1992). Briefly, for insects 20 mg of fat and for diets 100 mg of powder freeze-dried, were added of n-hexane (0.5 mL), BF3-MeOH solution (0.5 mL) at 4°C and vortexed for 30 sec. After 15 min at 100 °C, the reaction was stopped with distilled water (0.5 mL) and the mixture was centrifuged (3000 rpm, for 3 to 5 min). Taken the supernatant in conical tubes (only the hexane). Dried with speedvac, added 100 mL hexane and vortex for 30 sec. The organic phase (1µl) was injected and analyzed by capillary gas chromatography as reported by (Balzano, Pacetti, Lucci, Fiorini, Frega 2017).

3.6 Statistical analysis

Data are reported as mean values ± standard deviation (SD) of three replicates. Data were normalized using internal standard as a scaling factor and analysed by a one-way ANOVA and Tukey-Kramer post-hoc test at a significance level of $p < 0.05$ using in the Metaboalayst 5.0 software (Pang et al., 2022).

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Fatty acid profile in diets

In this study, we assessed the fatty acid profiles of diets based on vegetable by-products and in insects reared on those diets using a gas chromatography-mass spectrometry (GC-MS) approach. GC–MS is the most frequently used method for fatty acid analysis (Tiuca et al., 2015; Ecker, et al. 2012). We were able to detect 19 fatty acids in the diet samples and 18 fatty acids in the insect samples belonging to three groups: SFA, MUFA, and PUFA.

The predominant fatty acid class across the diets was SFA (n= 9), followed by MUFA (n= 8) and PUFA (n= 2) (Figure 4A). Among SFA, the most abundant was the palmitic acid (C16:0). These results are in agreement with previous studies (Pokorný & Dubská, 1986; Kamal-Eldin & Andersson, 1997; Rajah, 2002; El-Mallah et al., 2003; Alfawaz, 2004; Zambiazi et al., 2007; Fathi-Achachlouei & Azadmard-Damirchi, 2009; Kostik et al., 2013; Orsavova et al., 2015). Among MUFA, the most represented was the oleic acid (C18:1 n-9 trans), in agreement with a previous study (Orsavova et al., 2015). Among PUFA, the most predominant was the linolenic acid (C18:3 n-6). The less abundant fatty acids were represented by entadecanoic acid (C15:1) and by margaric Acid (C17:0) for MUFA and SFA, respectively.

Conducted principal component analysis (PCA) showed a clear separation of the diet composed of bean and chickpea from other diets as well as from the control diet (Figure 4B). Principal component one and two explains in total about 98% of the observed variation across the samples. The abundance of different classes of fatty acids was variable across tested diets.

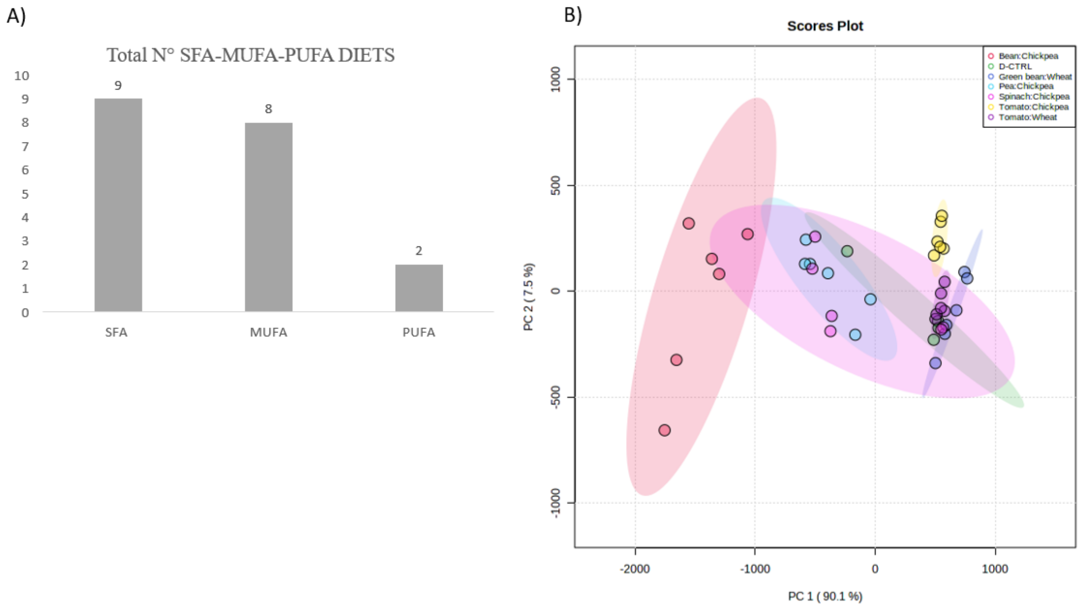

Figure 4: A general overview of the fatty acid profile of the diets based on the selected vegetable by-products used for the insect feed.
(A) Histograms represent the total number of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) identified by GC-MS in the different diets (B). Principal component analysis (PCA) of dietary fatty acids content. Each point represents a single sample replicate of a certain diet: Control diet (green dots), Bean: Chickpea diet (red dots), Tomato: wheat (purple dots), Tomato: chickpea (yellow dots), Pea: Chickpea (light blue dots), Spinach: Chickpea diet (fuchsia dots), Green bean: Wheat (blue dots).

Obtained heat map reflect fatty acid level among diets (Figure 5A). The diet based on bean and chickpea by-products showed significantly higher levels of SFAs and MUFAs. The diet based on spinach and chickpea as well as diet composed of chickpea and pea byproducts showed significantly higher levels of PUFAs compared to the other diets, including the control one (Figure 5B).

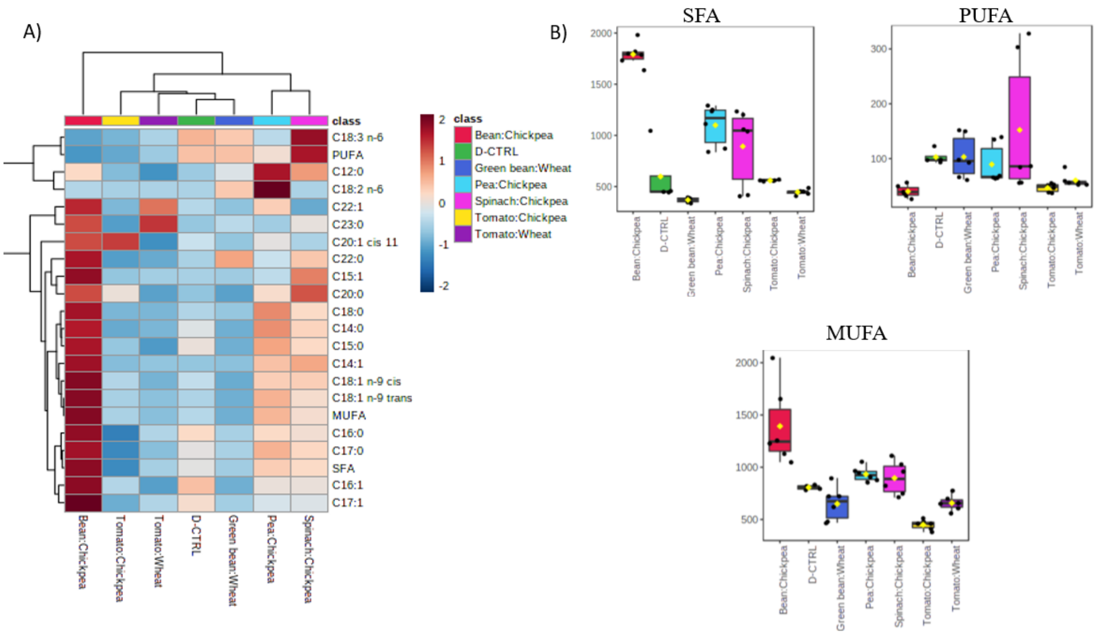

Figure 5: Representation of the differences in the fatty acid profile observed among the diets based on the selected vegetable by-products used for insects rearing.
(A). Hierarchical clustering heat-map of differential fatty acid compositions (fold change ratio) among different diets. Positive differential fold changed were in red color, while negative differential fold changed were in blue color scheme. (B). Boxplot for each fatty acid class according to the different diets. The black lines display the median, boxes cover interquartile range and error bars display the maximum and minimum values. Outliers are displayed as points.

4.2 Fatty acid profile in insects

A total of 18 fatty acids emerged from GC-MS profile analysis of the insects reared with a specific diet. The predominant fatty acid class in all larvae was SFA (n= 9), followed by MUFA (n= 7) and PUFA (n= 2) (Figure 6A), regardless of diet.

The finding that SFA was the main fatty acid class in larvae independent of the tested diet confirms previous studies (Meneguz et al., 2018, Jucker et al., 2017; Oonincx et al., 2020).

In particular, among SFA, the most predominant were the Lauric acid (C12:0) and the Palmitic acid (C16:0), while for the MUFA class, the most represented was the Oleic acid (C18:1 n-9 trans), in agreement with previous studies (Barroso et al., 2017; Liland et al., 2017; Meneguz et al., 2018; Spranghers et al., 2017; St-Hilaire et al., 2007).

Moreover, the Lauric acid (C12:0) was present at higher levels in all larvae, compared to the diets (0–2.3%) (Liu et al., 2017; Ewald et al., 2020), confirming the study of Spranghers et al. (2017) in which a synthesis of the acid by the larvae is suggested. Previous research has shown that BSF produces lauric acid exclusively de novo, using carbohydrates as a source of acetyl-CoA (Hoc et al., 2020).

Myristic acid (C14:0) also showed a similar trend, confirming its de novo synthesis by the insects. Myristoleic acid (C14:1), Palmitic acid (C16:0), and Stearic acid (C18:0) showed higher levels in diet composition than in larvae, according to Ewald et al., (2020).

Additionally, among the PUFA, the most predominant was the Linoleic acid (C18:2 n-6) in agreement with the literature (Ewald et al., 2020).

The principal component analysis showed the observed variation of fatty acids among insects. PC1 and PC2 explained 97.7 % of the total variance (PC1: 95.8% and PC2: 1.9 %) (Figure 6B).

The abundance of different classes of fatty acids was variable across tested insects (Figure 6B).

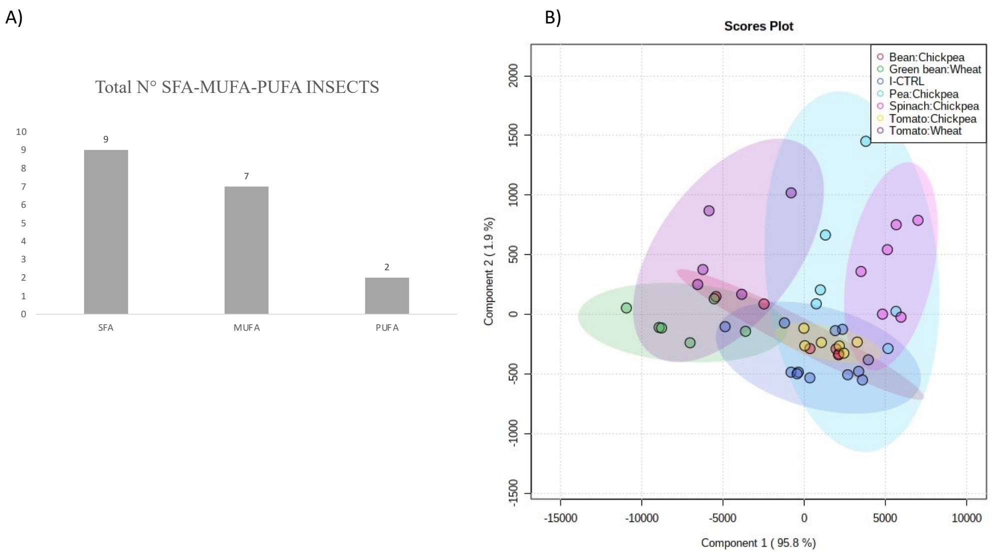

Figure 6: A general overview of the fatty acid profile of insects rearing with diets based on the selected vegetable by-products.

(A). Histograms represent the total number of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) identified by GC-MS among insects fed with different diets (B). Principal component analysis (PCA) clustering the different insect based on the fatty acid content of diets. Each point represents a single sample of a certain diet: Control diet (blue dots), Bean: Chickpea diet (red dots), Tomato: wheat (purple dots), Tomato: chickpea (yellow dots), Pea: Chickpea (light blue dots), Spinach: Chickpea (fuchsia dots), Green bean: Wheat (green dots).

Obtained heat map reflects fatty acid level among larvae (Figure 7A). Insects fed with Spinach and Chickpea showed significantly higher levels of MUFA compared to the control. Insect fed with Green bean: Wheat and Tomato: Wheat diets, showed significantly levels of SFA compared to the control (Figure 7B).

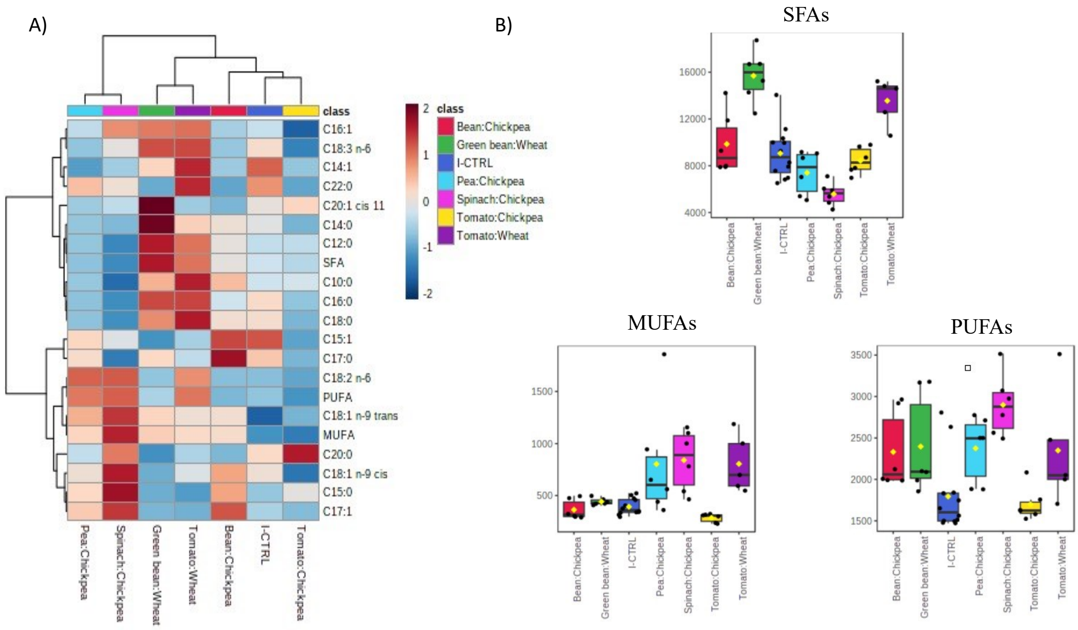

Figure 7: Representation of the differences in the fatty acid profile observed in insects rearing with diets based on the selected vegetable by-products.
(A). Hierarchical clustering heat-map of differential fatty acid compositions (fold change ratio). Positive differential fold changes were in red color, while negative differential fold changes were in blue color scheme. (B). Boxplot for each fatty acid class according to the different diets. The black lines display the median, boxes cover the interquartile range and error bars display the maximum and minimum values. Outliers are displayed as points.

4.3 Comparison of the fatty acid profile of the selected diets and insect samples reared on the selected diets

Based on the obtained fatty acid profile of the diets and insects, we selected three diet and insect samples reared on those diets for further analysis. (Figure 8). The highest levels of SFAs were observed in the Pea: Chickpea, Spinach: Chickpea, and Bean: Chickpea diets, compared to the control diet (Figure 8). Although the level of SFA was significantly higher in the selected diets, the FA profile of the insects reared on those diets was not affected (Figure

8). Obtained results were in agreement with a previous study in which the diets including agroindustry by-products did not significantly modify the level of SFA (Boukid et al. 2021).

The highest level of MUFAs were detected in the Bean and chickpea, followed by Pea and Chickpea and Spinach and Chickpea, compared to the control diet (Figure 8A). Furthermore, despite these significantly high levels, only the profile of fatty acids of the insects reared on the Bean: Chickpea diet was affected (Figure 8).

Finally, the highest level of PUFAs were observed in the Spinach: Chickpea, but this diet has no effect on the fatty acid profile of insects reared on this diet.

These results disagree with the work of Boukid et al. (2021) in which a decrease in unsaturated fatty acids in *Hermetia illucens* larvae was observed because of agro-industrial by-product diet.

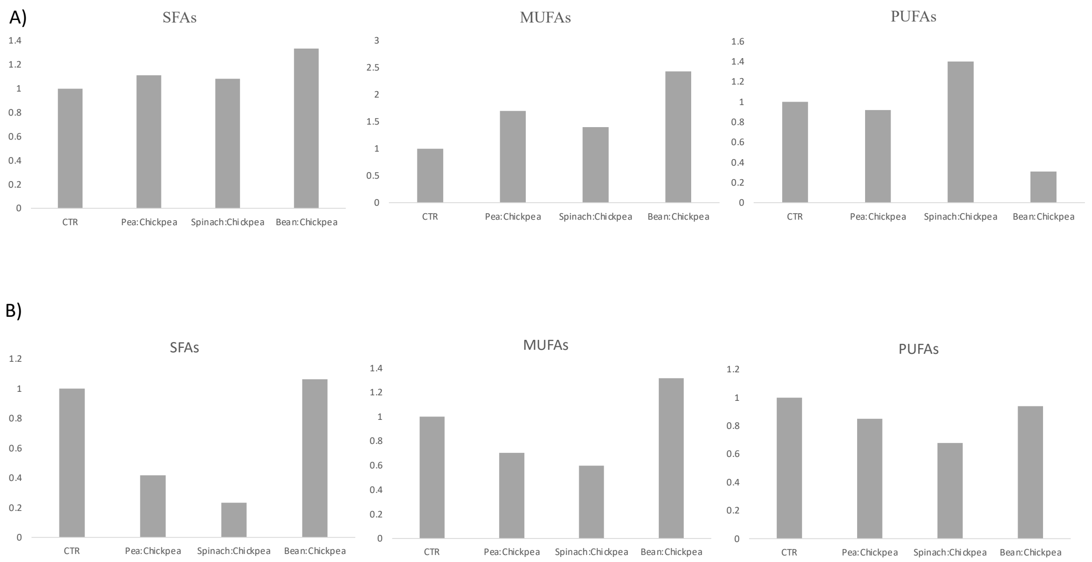

Figure 8: Comparison of the fatty acid composition between diet based on the selected vegetable by-products and insects reared on these diets.

(A) Fold change in abundance of fatty acids among both chickpea diets (Pea: Chickpea, Spinach: Chickpea, Bean: Chickpea) (B) and insects reared on these diets, relative to their controls.

CHAPTER 5
CONCLUSION

5.1 Conclusion

In this study, black solider fly larvae were reared on seven different diets. Larvae and diet fatty acids were analyzed to evaluate the influence of diet on the FAs profile of the insects. It can be summarized that the fatty acid profile of *Hermetia illucens* is high in saturated fatty acids (especially C12:0, C14:0, C16:0) and low in polyunsaturated fatty acids (PUFAs). These fatty acids are probably synthetized by insects.

Some fatty acids from diets such as C16:0, C18:1n-9 and C18:2n-6 could be partially taken up and accumulated in larvae bodies.

Our results showed that it is possible to improve the fatty acid profile of the larvae by modulating their diet. Insects display the ability to convert plant products into biomass with abundant lipids for feed and other food sources.

However further research is needed to determine this effect and extend these studies to other insects and diets as well, to identify a food production that preserves the environment.

This study shows that diet influences the fatty acid profile of the HI larvae. Considering the evidence, the chickpea diet has been shown to be high in unsaturated fatty acids and low in saturated fatty acid.

This fatty acid composition makes it a good candidate as a food substrate for HI larvae.

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