UNIVERSITA' DEGLI STUDI DELLA BASILICATA

PhD Programme
Applied Biology and Environmental Safeguard

Use of organic materials to obtain valuable products through bioconversion

Scientific disciplinary sector
General and Applied Entomology – AGR/11

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Co-Tutor
Prof. Laura SCRANO

Cycle XXXIII

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In the framework of the financial initiative
"Innovative PhD agreement with specialization in enabling technologies in Industry 4.0" - Basilicata Region

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ABSTRACT

Bioconversion is a biological process by which organic materials are converted in products with higher biological and commercial value. During its larval stage, the Black Soldier Fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is extremely voracious and able to feed on a wide range of organic materials, ranging from fruits and vegetables to animal manure. This ethological characteristic is particularly interesting for an innovative waste management treatment. The bioconversion process is highly efficient, as the organic substance the larva feeds on is reduced up to 65%-70% and converted into larval biomass. At the end of bioconversion of the waste, the larvae are suitable for pet food as they are rich in proteins and lipids. High energy content meal, deriving from appropriately dried and ground larvae can also be used as feed for select species by the aquaculture industry, according to recent European regulation. Resulting larval frass can be used as soil conditioner for crop fertilization. Furthermore, chitin and its derivative, chitosan, are relevant for medical, pharmaceutical, agricultural fields. In order to study the effect of different substrates from the agri-food chain on several parameters such as insect growth, final larval yield, protein and lipid content, substrate reduction and bioconversion values by the BSF, 10000 larvae were reared on 7,0 kg of six substrates (watermelon, kiwi, a mix of cabbage/savoy cabbage, strawberry, tangerine, orange) and on a standard diet, used as control. The results highlight that BSF is able to successfully feed on all the tested diets, although development time, growth rate, final larval biomass and larval components (ashes, proteins and lipids) impacted by different substrates. Although the standard diet is considered balanced in all nutrients and the best diet for BSF development, strawberries turned out to be more suitable substrate, among the analysed substrates, that are by-products from the agri-food chain. Although larvae fed on a mix of cabbage/savoy cabbage achieve the worst growth and bioconversion performances, BSF consumed this substrate, still accumulating high protein content, and confirming the BSF extraordinary ability to feed and reduce different kinds of substrates. Preliminary research was carried out also on zootechnical wastes (dairy mature and fresh manure). BSF is able to correctly feed also on manure, favouring their disposal and total recovery. This research project shows that waste management through the BSF bioconversion processes can represent a new economically important resource for companies belonging to agri-food and zootechnical fields and opens new perspectives for a sustainable and environmentally friendly industrial development in which by-products and wastes from these industries could be disposed and enhanced in this unconventional way.

1. INTRODUCTION

1.1 General background

Recently, the fast population increase, and urbanization have raised two relevant issues: food supply, in particular concerning proteins, and waste management. According to the United Nations global population will grow by almost 50% from 2000 to 8,5 billion in 2030, 9,7 billion in 2050, and 10,9 billion in 2100 (UN, 2019). Consequently, the demand for animal proteins, especially meat and milk, is expected to increase by 70% in 2050 compared to the levels in 2010 (FAO, 2011). The increase of animal-based protein claim will have a negative environmental impact: increase of gas emissions, exploitation of not-renewable resources such as water and land, soil acidification, erosion, compaction, overgrazing and nitrification (Grossi *et al*., 2019). Moreover, aquaculture uses fish for fishmeal production and therefore diverts a food source that could be consumed by humans (Huntington and Hasan, 2009). For this reason, more sustainable production of both existing sources of protein and alternative one for livestock feeds and direct human consumption need (Henchion *et al*., 2017). Currently protein supply is provided by vegetables (57%), meat (18%), dairy (10%), fish (6%) and other products of animal origin (9%) (FAO, 2010).

Recently, insects have been identified as an alternative source of protein both for feed and for food, with a lower environmental impact (Oonincx and de Boer 2012; Van der Spiegel *et al*., 2013; Kim *et al*., 2019; Rodríguez-Miranda *et al*., 2019). Insect farming, indeed, require less water and emit lower levels of greenhouse gases and NH_3 compared to classical livestock (Figures 1.1, 1.2).

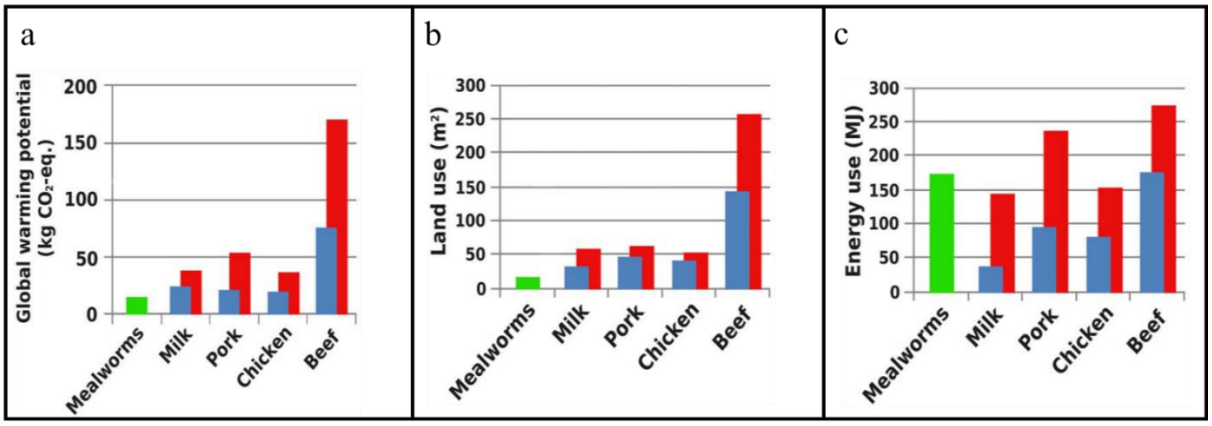

Figure 1.1: Environmental impact (global warming potential (a), land usage (b), energy usage (c) of mealworms compared to other animal products. All these parameters are evaluated in the production of one kg of edible protein (modified from Oonincx and de Boer 2012).

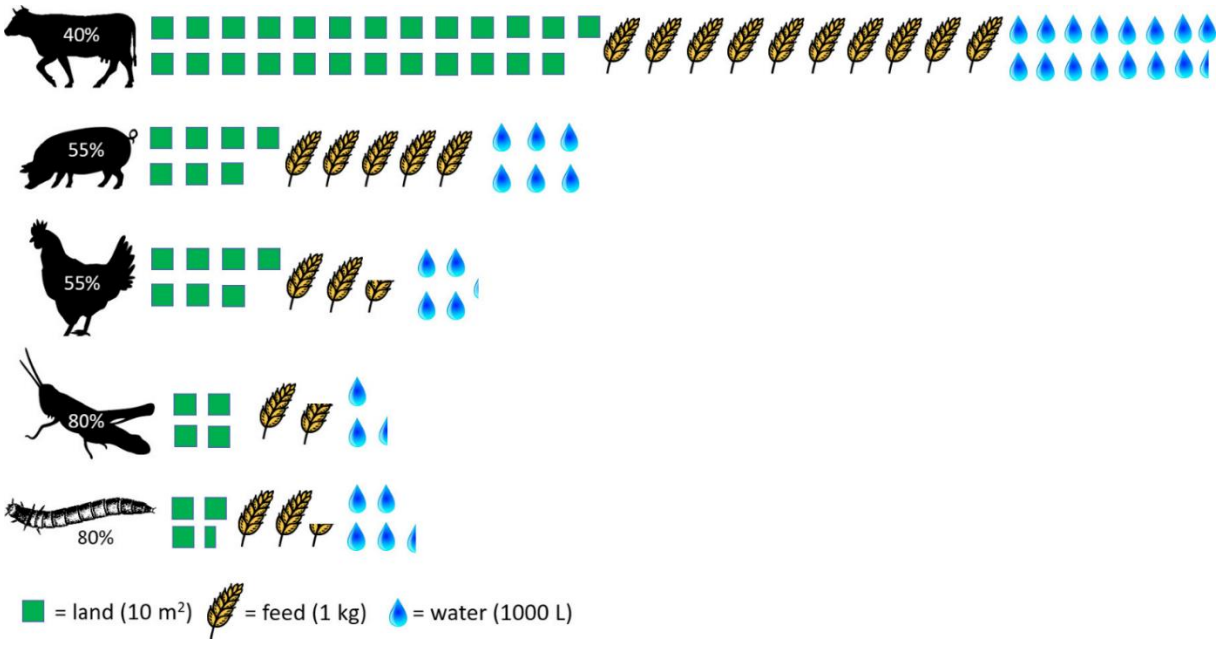

Figure 1.2: Amount of land, feed and water needed to produce 1 kg of live animal and percent of the animal which is edible (Dobermann *et al.*, 2017).

Insect can be reared on organic waste (Figure 1.3), creating valuable products, and reducing waste (Van Huis, 2013; Smetana *et al.*, 2016; Henchion *et al.*, 2017).

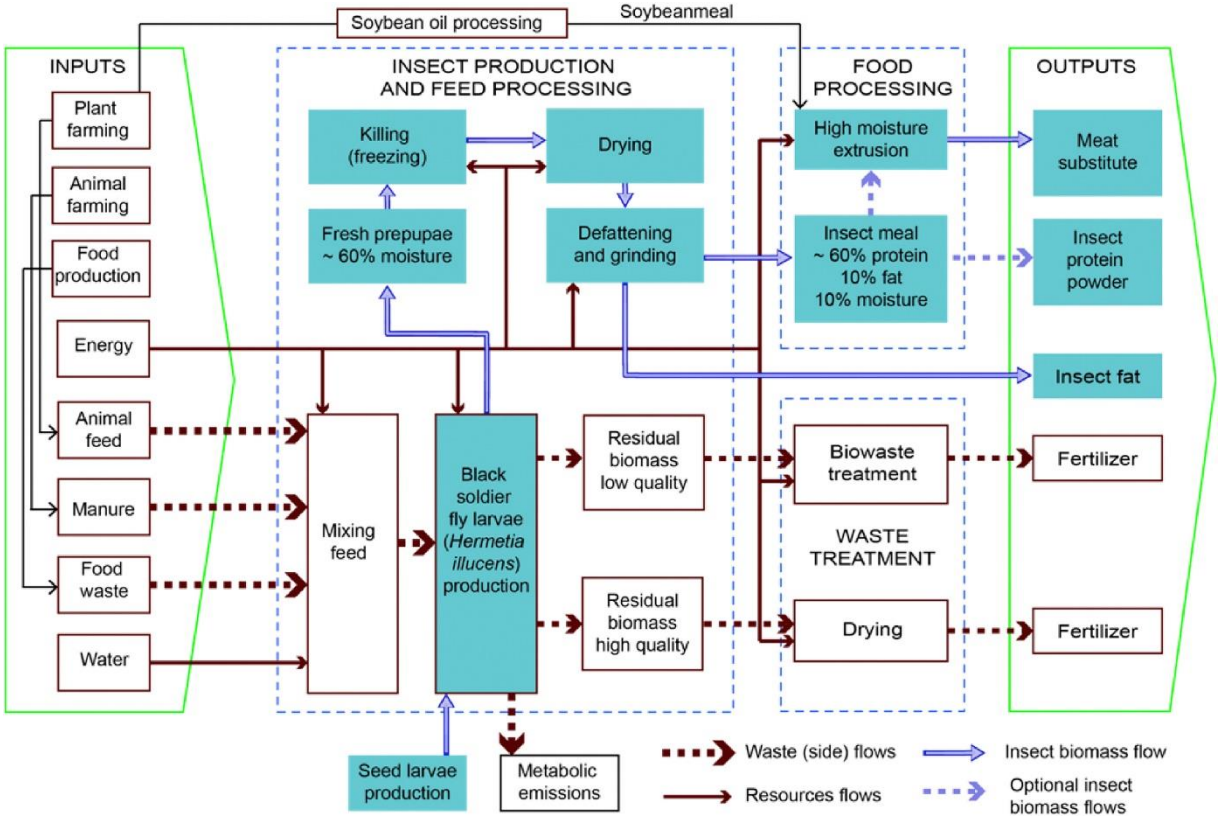

Figure 1.3: Schematic representation of insects fed on different organic material/waste, obtaining valuable products (Smetana *et al.*, 2016).

Another advantage of the usage of insects as food and feed is linked to the high percentage of edibility and digestibility of the insect body (Kinyuru *et al.*, 2010). Many insects have a

favourable nutritional profile for humans (Table 1.1): published data report indicate in this case insects have high digestibility (77–98%), high protein content (40–75% on a dry weight basis) and high essential amino acids, vitamins B1, B2 and B3 and minerals, such as iron and zinc profile (Belluco *et al*., 2013, Kouřimská and Adámková, 2016). Some insect species are rich in lysine, threonine, and tryptophan while some other insects are deficient in these amino acids (Sogbesan and Ugwumba,2008; Kouřimská and Adámková, 2016). Moreover, insects vary considerably in fat, from 2% to 77% of dry matter (Table 1.1), resulting in high variations of energy levels (Kouřimská and Adámková, 2016; Elhassan *et al.,* 2019). Insect digestibility is also strictly influenced by chitin, a polymer of N-acetyl-D-glucosamine, main component of arthropod exoskeleton, metabolized by chitinase enzyme (Tabata *et al*., 2018; 2019). The possibility to remove chitin improves insect digestibility (Finke, 2007).

Insect order	**Protein (% dry matter)**	**Lipid (% dry matter)**	**Fibre (% dry matter)**	**Energy (Mj/100g)**
Blattoidea	57.30	29.90	5.31	-
Coleoptera	40.69	33.40	10.74	2,05
Hemiptera	48.33	30.26	12.40	2,01
Hymenoptera	46.47	25.09	5.71	2,03
Isoptera	35.34	32.74	5.06	-
Lepidoptera	45.38	27.66	6.60	2,13
Odonata	55.23	19.83	11.79	1,81
Orthoptera	61.32	13.41	9.55	1,78

Table 1.1: Average content of protein, fat, and energy of specific insect orders (modified from Rumpold and Schlüter, 2013).

In addition to all these positive aspects related to insect edibility and environmental safety, a lot of insect species can reproduce quickly within a short period of time, so that ensuring large quantities of potential food/feed in a short time: insects mature within weeks to a month and generally can lay thousands of eggs (Van Huis, 2015; Gullan and Craston, 2014).

Entomophagy, that is insect consumption by humans, has occurred for thousands of years. Approximately 2000 species of insects have been used as food and they are part of the traditional diets of at least 2 billion people (Figure 1.4) especially in Asia, Africa, and South America (Jongema, 2017).

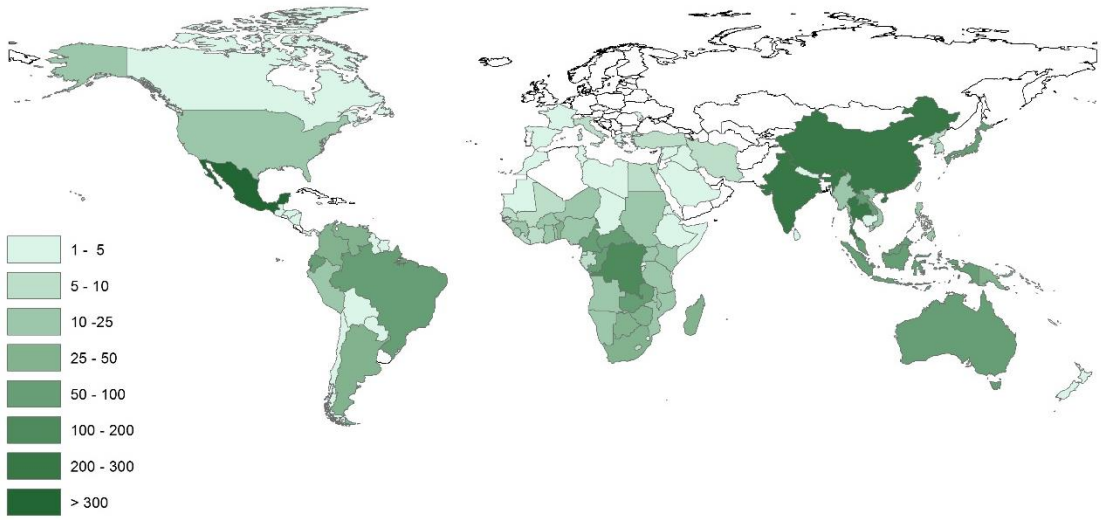

Figure 1.4: Recorded edible insect species, by country (Jongema, 2017).

According to Jongema (2017), Coleoptera are the most consumed insects (31%), followed by Lepidoptera (18%); Hymenoptera (bees, wasps, and ants) (14%); Orthoptera (grasshoppers, locusts, and crickets) (13%); Rhynchota (cicadas, leafhoppers, planthoppers), Hemiptera (scale insects, and true bugs) (10%); Isoptera (termites) (3%); Odonata (dragonflies) (3%); Diptera (flies) (2%); and others (5%) (Figure 1.5).

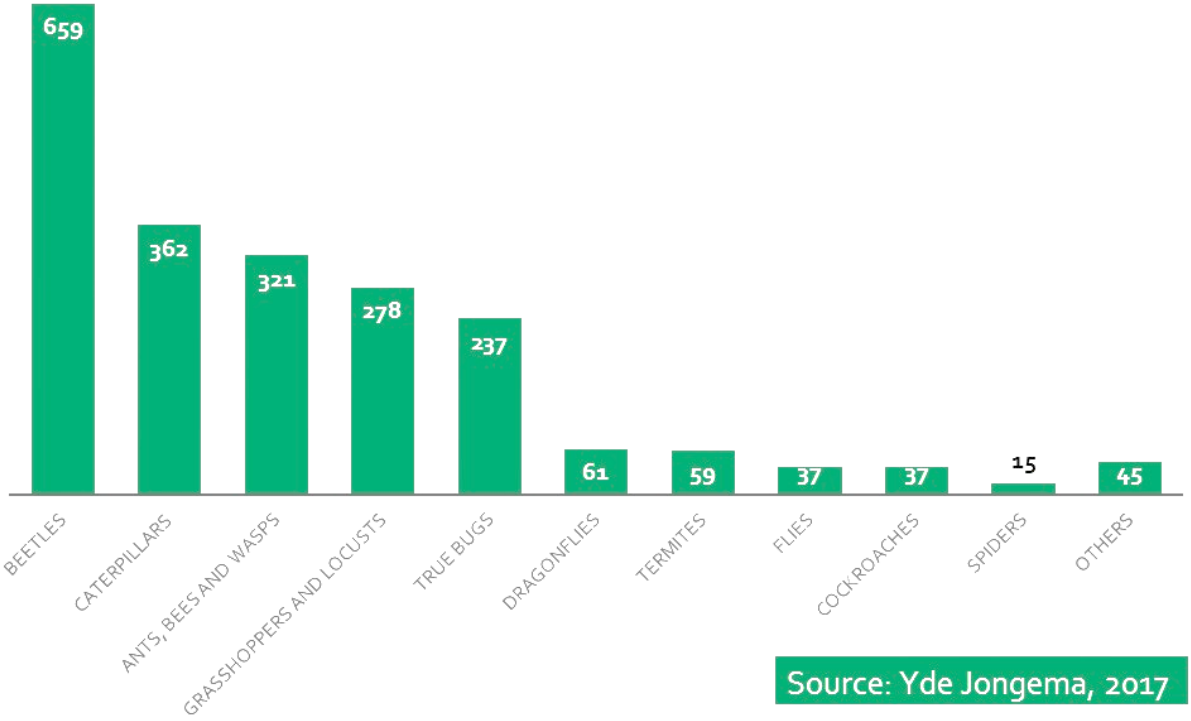

Figure 1.5: Number of recorded edible insect species per group in the world (Jongema, 2017).

While insects are an established part of food culture in some countries, they are not generally well accepted in Western world, in which insects are often considered as pests and a source of contamination, and thus to be avoided (Looy *et al.*, 2014; Dobermann *et al*., 2017). In last few years, attitude towards insects is also changing thanks to new legislation (European Regulation 2015/2283) on novel food that has entered into force from 01/01/2018: the law provides for the production and marketing of insects or food containing insects for human nutrition (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32015R2283). To date, most of regulations enacted concerned insect as animal feed: the larval and the pre-pupal biomass obtained from the bioconversion process, both rich in nutritive components, can be used for pet food for game, reptiles, fur animals and other insectivorous animals (European Regulation 68/2013 - https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02013R0068-20190220, European Regulation 142/2011 - https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02011R0142-20191214), or can be transformed into animal proteins (PAPs, processed animal proteins) and then into high nutritional content meal, replacing or supplementing the protein and lipid levels present in conventional feeds without any specific restrictions on insect species that may be used. On the contrary, according to European Regulation 999/2001 (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02001R0999-20191214), insects PAPs are forbidden as feed for farmed livestock animals (i.e., ruminant and monogastric animals). Also the specific conditions of processing, production, storage, transport and use of insect meal in aquaculture for fish farming was regulated by the European Regulation 2017/893 (https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32017R0893&from=IT), which allows the use of proteins derived only from seven different species of insects (Figure 1.6): *Gryllodes sigillatus* (Walker, 1869) (Orthoptera, Gryllidae), *Gryllus assimilis* (Fabricius, 1775) (Orthoptera, Gryllidae), *Acheta domesticus* Linnaeus, 1758 (Orthoptera, Gryllidae), *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera, Tenebrionidae), *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera, Tenebrionidae), *Musca domestica* (Linnaeus, 1758) (Diptera, Muscidae), *Hermetia illucens*.

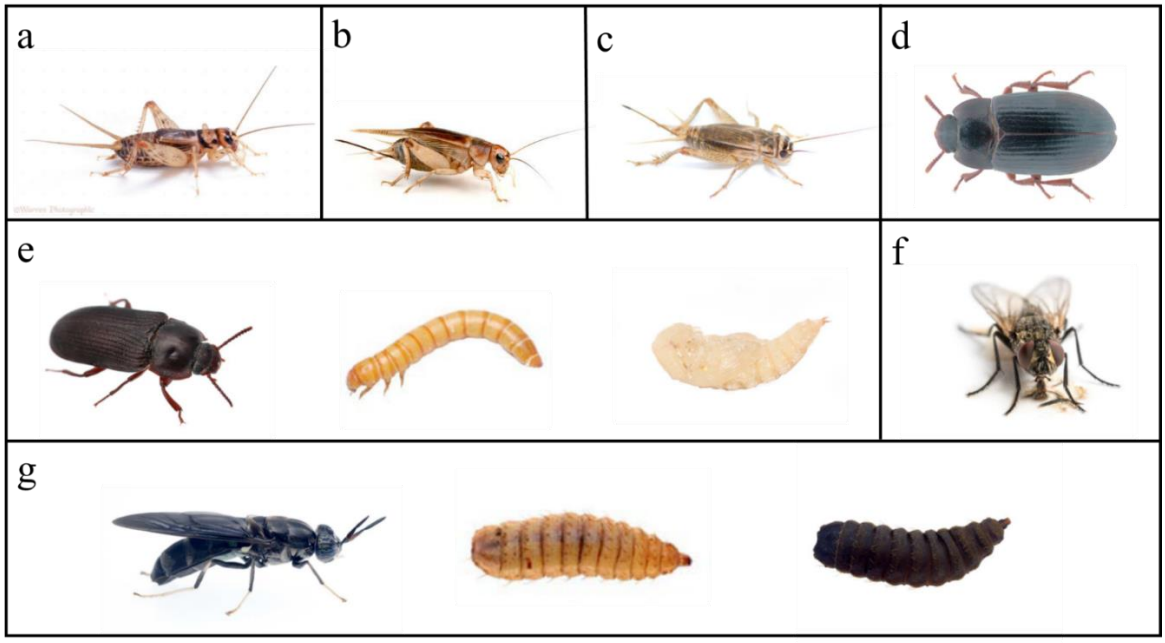

Figure 1.6: seven different species of insects which proteins are allowed for aquaculture: *Gryllodes sigillatus* (a), *Gryllus assimilis* (b), *Acheta domesticus* (c), *Alphitobius diaperinus* (d), *Tenebrio molitor* (e – adult, larva, pupa), *Musca domestica* (f), *Hermetia illucens* (g – adult, larva, pupa) (IPIFF Guide on Good Hygiene Practices).

Moreover, insect fats and hydrolysed proteins are authorized and for pet food and fur animals, without restrictions as to the insect species. The feeding of live insects to farmed and pet food animals are not subject to limitations at European level but are regulated at the national level, as these products are traditionally used as feed for niche markets such as birds, reptiles, or zoo animals. Regulation of this market is not simple and often in individual countries there are no clear guidelines. In figure 1.7 some parameters regarding current dispositions on insect treatment are summarized: allowed and denied substrates on which is possible to feed insects, with respective regulations; type of insect (alive, PAPs or insect fats and proteins) as feed for different kinds of target animals, with respective regulations.

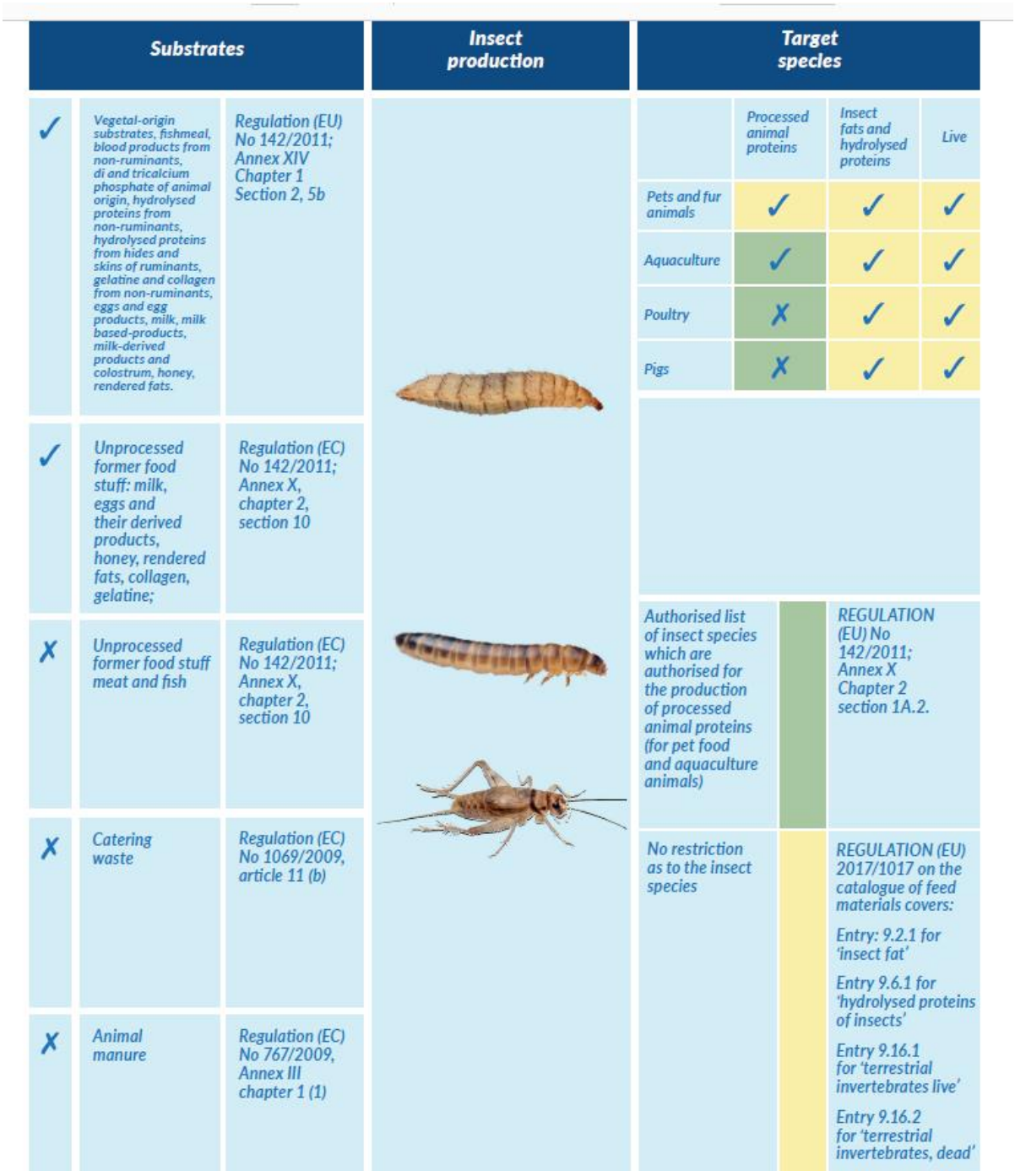

Figure 1.7: summary of current dispositions on insect treatment. In the table are listed the allowed and denied substrates on which is possible to fed insects, with respective regulations, and different type of insect or part of it (alive, PAPs or insect fats and proteins) allowed or denied as feed for different kind of target animals (pets, fur animals, poultry, pig, aquaculture), with respective regulations (IPIFF Guide on Good Hygiene Practices).

1.2 *Hermetia illucens* (Diptera: Stratiomyidae)

Hermetia illucens (Linnaeus, 1758) (Diptera: Stratiomyidae), also known as the Black Soldier Fly (BSF), native of the south-eastern United States, spread throughout warm temperate regions and tropics (McCallan, 1974; Newton *et al.*, 2005). Although evidence suggested that BSF could be brought to Europe around 500 years ago, in recent decades, climate change and human activities facilitated its spreading in the old continent and to other countries around the world such as Asia and Australia (Benelli *et al.*, 2014; Marshall *et al.*, 2015). Nowadays, BSF can be considered a natural insect of almost 80% of the world between latitudes 46°N and 42°S (Martínez-Sánchez *et al.*, 2011). BSF could be considered a pest insect because of its aptitude to colonise several organic wastes, including manure (Tomberlin and Van Huis, 2020). However, its extraordinary ability to dispose, recycle and enhance these wastes and its valuable usage in forensic entomology, allow to overcome this negative concern and to consider it a greatly beneficial insect. Nowadays BSF can be considered a pest insect, only if not properly reared and managed (Tomberlin and Van Huis, 2020).

BSF life cycle consists of four stages: egg, larva (six instars), pupa and adult (Figure 1.8).

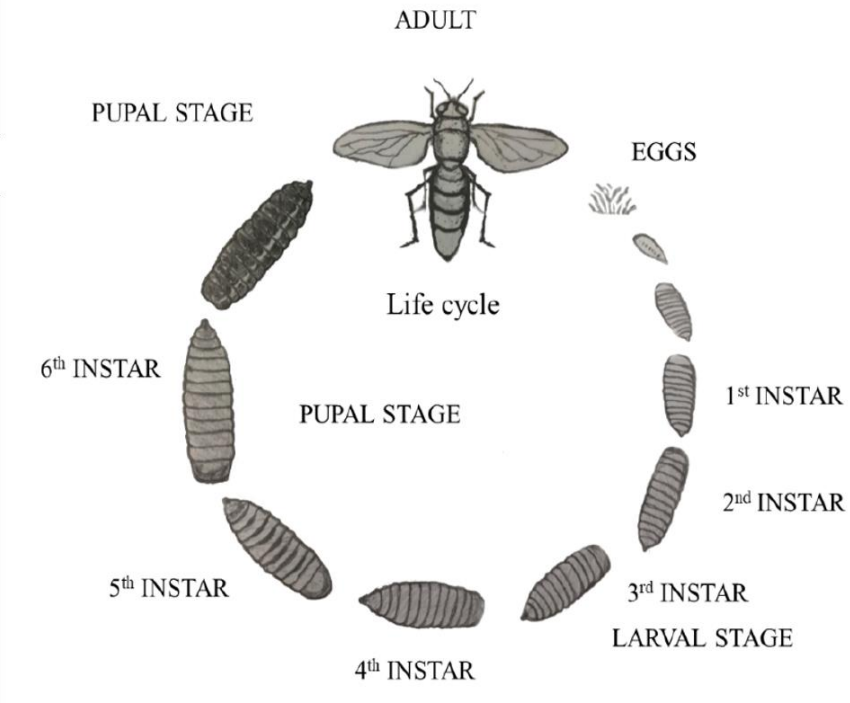

Figure 1.8: BSF (Linnaeus 1758) (Diptera: Stratiomyidae) life cycle.

BSF has several sexually dimorphic traits such as body size (females are usually larger than males), features on the head, abdominal spots (reddish for females and bronze-silvery for male) and the shape of their abdominal terminalia and sexual organ (in females is long and scissor-

shaped and in male is short and fan like); females have more whitish hairs than males on their face and only four black frontal tubercles (Rozkosný, 1983). BSF adults can reach and 27 mm in length, Adult specimens are black or blue in colour, their length range 6 mm in width and from 15 to 27 mm in length, with average wing length of 14.24 mm for male and about 14.67 mm for female. The antennae are elongated with three segments and the end of each leg is white (Rozkosný, 1983; Sheppard *et al*., 2002; Diener *et al.*, 2009; Gobbi *et al.*, 2013). Unlike other Diptera such as *Musca domestica* (L.), *Calliphora vomitoria* (L.) (Diptera: Calliphoridae) or *Chrysomya spp*. (Robineau-Desvoidy) (Diptera: Calliphoridae), BSFs are considered non-dangerous insects. Indeed, they do not transmit pathogens and despite the larval attitude of feeding on decomposing organic matter, adult fly does not assume this kind of food, but they succeed in surviving with reserves gathered during larval stages (5-8 days) (Tomberlin *et al*., 2002, Diener *et al*., 2009; van Huis *et al.,* 2013). Moreover, they are not attracted to human habitation, foods or faeces and they do not transmit faeces-borne diseases to humans (Newton *et al.,* 2005; da Silva and Hesselberg, 2020). Sheppard (1983) have also demonstrated that BSF can successfully reduce *M. domestica* populations, avoiding *M. domestica* oviposition: for this reason, disease transmission caused by this dipteran can be considerably reduced in areas colonized by BSF. Also, the larval stages can compete with other infesting flies by massively feeding on the food substrate, subtracting resources, and limiting their presence (Furman *et al.*, 1959).

Mating is a real ritual: males try to find spots in the lekking area when it is time to mate. Males are not extremely competitive, indeed if another male comes in the area, the two will fight only if a female is coming and the winner will take the territory. Even if adults are generally weak fliers, mating occurs specifically during flight (Tomberlin and Sheppard, 2001). Environmental conditions influence mating: direct sunlight encourages the copula, instead, rain or snow are not optimal contexts because of the low level of the sunlight (Zhang *et al.,* 2010). Beside light, temperature and humidity, also organic volatile compounds (VOCs) deriving from decomposing organic substances influence insect attitudes. It was hypothesized that VOCs strongly influence the whole life cycle behaviour of BSFs, also underlying how much all stages are linked to each other: BSF larvae perceive volatile compounds emitted by decaying organic matter or rotten food and this affected the adult choice to oviposit eggs near the most abundant food resource, that will be used as nourish for the progeny (Tomberlin *et al.,* 2002; Sripontan *et al.,* 2017; Hoc *et al.,* 2019). Moreover, for *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) it has been demonstrated that VOCs perceived during the larval stages could

also influence the adult tendency to search for different oviposition sites (Jaenike 1983). This attitude could also be typical of BSF.

BSF larval stages are dull, whitish with a small, projecting head containing chewing mouthparts. Larval development, divided in six instars, requires, in optimal environmental conditions, approximately 14 days (Hall and Gerhardt, 2002; Rozkosný, 1983). As previously said, only larvae can feed and they consume decaying organic material, both of vertebrate and vegetable origin (Nguyen *et al.*, 2015; Spranghers *et al.*, 2017).

After larval stages, BSF turns into a prepupa, which is the last larval stage before pupating. A prepupa can be considered a mature larva and it does not feed, like the adult stage. When larvae are ready to pupate, they move to a dry and hidden area to bury in and turn black thanks to the releasing of melanin that not only darkens the exoskeleton, but also increases skin durability (Tomberlin and Sheppard, 2001; Tomberlin *et al.,* 2013). In this habitat, pupae go into a sleeping mode for at least two weeks at the end of which an adult fly emerges (Sheppard *et al.*, 2002). At all stages, BSFs can live in a range of temperatures between 27-30° Celsius (Tomberlin *et al.,* 2009). After oviposition, the eggs take about 3-4 days at 27° Celsius to hatch, the larval stage takes and the pupal stage take about two weeks respectively, but this time can increase up to five months in cases of limited resources (Furman *et al.* 1959, Oliveira *et al.*, 2015). Adults have a lifespan of 12-17 days; during this period, they mate, and females lay eggs (Tomberlin *et al.*, 2009). Under the rearing conditions of 28°C and 75% relative humidity, the total larval cycle lasts 20–35 days (Zhou *et al.*, 2013). The number of eggs per female ranges 500–1000 eggs, depending on the fertility level of the female, which in turn depends on diets and rearing conditions at the larval stage (Furman *et al.*, 1959; Tomberlin *et al.*, 2005). For this reason, the larval stage can be considered the most important one, because it determines and influences the longevity of other stages and the productivity of the adult stage.

1.3 Bioconversion process mediated by BSF

Bioconversion is the biological process by which organic materials are converted into available products or energy sources. Many insects naturally feed on organic waste, reducing them, and simultaneously gaining nutrients (Fowles and Nansen, 2020). BSF is one of the most important bioconverter insect in the world (Wang and Shelomi, 2017). It is a saprophagous insect, feeding on a lot of decomposing organic materials, both of animal and vegetable origin (Bava *et al.*, 2019; da Silva and Hesselberg, 2020) such as manure (Sheppard 1983, Newton *et al.*, 2005; Myers *et al.*, 2008; Zhou *et al.*, 2013, Bortolini *et al.*, 2020), waste from agri-food chain (Nguyen *et al.*, 2015; Supriyatna *et al.*, 2016; Jucker *et al.*, 2017; Scala *et al.*, 2020), distillers grains (Webster *et al.*, 2016; Chia *et al.*, 2018a; Bava *et al.*, 2019; Grossule *et al.*, 2020; Jucker *et al.*, 2020a; Scala *et al.*, 2020) and many other organic matters. Its attitude of scavenger animal is clear since the oviposition: females, oviposit on or near decaying organic matter so that larvae feed immediately after eggs hatching (Tomberlin *et al.*, 2002). The efficiency of bioconversion is strictly related to environmental conditions and substrate characteristics: the optimal moisture content of substrate is about 60% (Diener *et al.*, 2009) and optimal temperatures range from 27 to 32 °C (Tomberlin *et al.*, 2009). Literature data show that feeding larvae on different decomposing substrates can reduce the substrate to 50–80% and convert into larval biomass within 14 days (Diener *et al.*, 2011; Zhou *et al.*, 2013; Barragan-Fonseca *et al.*, 2017; Jucker *et al.*, 2020b). Simultaneously to bioconversion process, BSF is also able to reduce bacterial load in the substrate, such as *Escherichia coli* and *Salmonella enterica* (Liu *et al.*, 2008) in chicken and cattle manure (Erickson *et al.*, 2004) and human faeces (Lalander *et al.*, 2013). This ability (Park *et al.*, 2015) is probably related to the production of antimicrobial peptides (AMPs).

AMPs are small molecules composed of 10-100 amino acid residues and are key components of the humoral innate response of insects (Wu *et al.*, 2018). They are perfect candidates for the design of new antibiotics because of their natural antimicrobial properties and a low propensity for the development of resistance (Hollmann *et al.*, 2018). Moreover, evidence suggests that they can display anticancer activities (characterized by a strong selectivity and efficacy on cancer cells), antifungal and antiviral activity (Kingsolver *et al.*, 2013; Faruck *et al.*, 2016).

At the end of the larval development, that means at the end of the bioconversion process, it is possible to obtain many products of high biological and commercial value. First, it is possible to obtain larvae rich in nutrients (proteins, lipids, and vitamins) that could be used as pet food or transformed into meals for aquaculture uses, according to European regulation (see paragraph 1.2 “General background”). Indeed, nowadays BSF is considered as a potential environmentally sustainable alternative to conventional poultry, pigs, and aquaculture feed, as

the nutritional values are comparable or even better than that of the soybean meal used (Veldkamp and Bosch, 2015; Barragan-Fonseca *et al.*, 2017; Liu *et al.*, 2017).

As the usage of insects as an alternative environmentally friendly sustainable source of food for a wide range of animals and for human diet is increasing, further and continuous studies need to deepen the knowledge of the BSF nutrient values. The possibility to use BSF larvae as a nutritional source is strictly linked to the ability of considerably increasing their biomass while they are eating. Indeed, larvae are able to transform the nitrogen in the substrate and the low-quality proteins into high quality proteins that can range from 37 to 63% of their dry weight (Barragan-Fonseca *et al.*, 2017) and into saturated (lauric, myristic, palmitic and stearic acid) and unsaturated (hexadecenoic and octadecenoic) fatty acids, which can reach up to 39% of larvae total weight (Ushakova *et al.*, 2016). It is important to underline that all parameters related to the larval growth, such as time to reach the pupal stage, weight gain and nutritional composition of larvae, but also the reduction of the organic waste are strictly linked to the substrate (Nguyen *et al.*, 2013; Rehman *et al.*, 2017; Liland *et al*., 2017; Jucker *et al.*, 2017; Liu *et al.*, 2017; Meneguz *et al.*, 2018; Julita *et al.*, 2019). Many studies have been carried out, feeding BSF larvae on different substrates, often with opposing results. Spranghers *et al.* (2017), for example, reared BSF larvae on vegetable waste, chicken feed, biogas digestate and restaurant waste highlighting that fed BSF on vegetable waste provided high-quality output with high potential for incorporation in animal feed. In this work, it has been demonstrated that protein content and amino acid profile is not substrate dependent, on the contrary fat and ash contents are influenced by initial diet. Indeed, larvae reared on energy-rich substrates, such as restaurant wastes or biogas digestate, showed high fat content (Spranghers *et al.*, 2017). These larvae/pupae could be used as high energy-rich food in comparison to conventional feed resources. On the contrary, Nguyen *et al.* (2013, 2015) found a great fluctuation in protein and fat acid content, depending on the different organic substrate on which larvae were fed. Significant variations in these two parameters were found in all substrates administered: control poultry feed, liver, manure, kitchen waste, a mixture of fruits and vegetables and fish. Also, in Jucker *et al.*, 2017, nutritional analysis revealed that different diets, vegetables, fruit and a mixture of fruits and vegetables, strongly affected the chemical composition of BSF prepupae: the fruit-based diet increased the total fat content and affected the specific fatty acid composition of prepupae, resulting in a high percentage of long and medium chain saturated fatty acids. Protein content, on the contrary, was not really influenced by different diet composition, even if vegetables food provided the larvae with the highest protein yield (Jucker *et al.*, 2017).

All these findings point out how the characteristics of original organic waste really influence the bioconversion process and how much it is important to analyse as much substrates as possible, in order to define the best bioconversion ratio and the best quality of BSF larvae, pre-pupae and pupae. Indeed, also during the different stages of the BSF life cycle it is possible to observe changes concerning nutritional values. Indeed, analysing all BSF stages, from eggs to adults, Liu *et al.*, (2017) detected important changes in amino acid, fat, vitamin, and mineral contents among all developmental phases.

1.4 Secondary products of bioconversion process

At the end of the bioconversion process, beside the nutrient-rich larval biomass, it is possible to obtain also other products with high biological and economical value.

The bioconversion residue, made of BSF frass and not converted organic matter, is comparable to organic soil conditioner. This bioconversion residue has a great potential for improving soil fertility and can be used for crop fertilization, as a valid alternative to chemical fertilizers (Choi *et al.,* 2009; Quilliam *et al.,* 2020; Schmitt and de Vries, 2020). Many examples of BSF frass as a soil conditioner were provided: Choi *et al*. (2009) observed that the growth rate and chemical composition of cabbage grown using BSF frass were identical to commercial fertilizers; Setti *et al*. (2019) focused their attention to baby leaf lettuce, basil, and tomato potted plants: all investigated crops showed chemical, microbiological, and agronomic characteristics values significantly greater than the same plants supplied with synthetic solid fertilizer. Moreover, insect frass compost usage induces reduction of pathogens and pesticides (Lalander *et al.* 2016). These encouraging findings may be due to the added ammonia ($NH4^+$) from nitrogen in insect frass, which has been shown to increase fivefold relative to the non-fertilized plants (Green and Popa, 2012) and the presence of chitin. The usage of this biopolymer as additive to common fertilizers, is widely used in agriculture as improver of crop growth and enhancer of the plant defence mechanisms (Sharp, 2013¸ De Tender *et al.,* 2019). The positive effect of chitin can depend on different elements (Sharp, 2013; El Hadrami *et al.,* 2010) such as concentration, degree of acetylation, viscosity, and the applied formulation (soil amendment, foliar application; chitin alone or in association with other treatments). Many studies reported that chitin or chitosan application induce host defence through different mechanism: lignification (Ali *et al.,* 2014), ion flux variations, cytoplasmic acidification, membrane depolarization, and protein phosphorylation (Felix *et al.,* 1993, 1998; Kikuyama *et al.,* 1997; Kuchitsu *et al.,* 1997), phytoalexin biosynthesis (Yamada *et al*., 1993), biosynthesis of jasmonic acid (Nojiri *et al.,* 1996), and the expression of unique early responsive and defence-related genes (Pusztahelyi, 2018).

Beside the usage of chitin as biofertilizer, this secondary product of the bioconversion process can be used in many other applications, together with chitosan, its deacetylated derivative. Chitin and chitosan, have interesting properties such as high biocompatibility, biodegradability, and non-toxicity, making them particularly suitable for use in medical, pharmaceutical, alimentary, and other fields (Singh *et al.,* 2017; El Knidri *et al.,* 2018). It exhibits anticoagulant activities (Chang and Huang, 2012; Subhapradha *et al.,* 2013); it seizes many free radicals and metal ions, so it can be considered a potential natural antioxidants (Ngo and Kim, 2014;

Avelelas *et al.*, 2019); it has hypocholesterolemic activity, as it can form digestive complexes with dietary fat, in neutral pH environments such as the intestine (Ramya *et al.*, 2012), and a low level of immunogenicity, that makes it useful as a biofilm component in drug release and in tissue replacement (Balagangadharan *et al.*, 2017; Tonda-Turoa *et al.*, 2017; Li *et al.*, 2018; Safdar et *al.*, 2019). Nowadays, thanks to its characteristics (delay the qualitative and nutraceutical alteration, extend the shelf life, antioxidant activity), chitosan biofilm finds applications also in food industry (Figure 1.9), as an excellent alternative to classic food packaging (Cazón and Vázquez 2019; Hu and Gänzle, 2019).

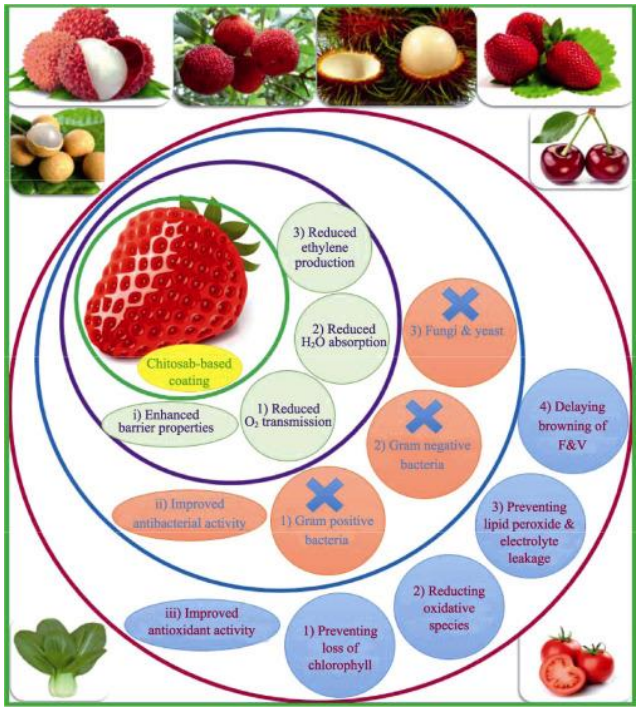

Figure 1.9: different effect of chitosan coating on fruits and vegetables (Duan *et al.*, 2019).

Beside the opportunity to extract chitin from BSF pre-pupae, pupae or their exuviae (Purkayastha and Sarkar, 2020), it is also possible obtaining lipids from larvae to use them as component in diet for pet food (see paragraph 1.2 General background), as alternative source of biodiesel or in applications in cosmetic fields (Li *et al.*, 2011; Leong *et al.*, 2016; Verheyen *et al.*, 2018). The possibility of extracting lipids from larval biomass to produce biofuel is a good alternative to the use of animal lipids or classical raw materials such as starch, vegetable oils (rape, sunflower, or others) especially since the usage of agricultural ground exclusively for fuel production, rather than for food industry, is not sustainable any longer (Li *et al.*, 2011). Moreover, the high concentration of medium chain saturated fatty acids and the low concentration of the polyunsaturated fatty acids in BSF prepupae, makes the biodiesel derived

from BSFs an high-quality biofuel (Surendra *et al.*, 2016). In BSF prepupae oil derived from larvae fed on a mix of organic waste the concentration of the medium chain saturated fatty acids (67% of total fatty acids) was higher compared to the soybean (11% of total fatty acids) and palm oil (37% of total fatty acids); and that the concentration of unsaturated fatty acids (28% of total fatty acids) was lower than in the soybean oil (85%) and palm oil (55%) (Surendra *et al.*, 2016). High concentrations of long chain saturated fatty acids turn into biodiesel with poor cold flow property, with high oxidative stability, which increases storability of the biodiesel (Ramos *et al*., 2009). BSF fatty acid profile is similar to coconut oil and palm kernel oil, frequently used in cosmetics applications, such as creams for skin care (Dubois *et al.*, 2007; Verheyen *et al*., 2018). Despite lipidic composition of BSF is strictly stage and diet dependent the most abundant lipid is lauric acid (Ushakova *et al.*, 2016; Liu *et al.*, 2017; Rabani *et al.*, 2019; Ewald *et al.*, 2020). This characteristic, together with the saponification value of the refined oil, allow the usage of BSF lipids as antimicrobial agents in cosmetics, especially in soaps and hand washing detergents (Mai *et al.*, 2019).

1.5 Regional partner - Apofruit Italia

On the international scene for over 50 years, Apofruit Italia (Figure 1.10) is a cooperative company that operates with its own structures and producer members from north to south Italy.

Figure 1.10: Official Apofruit brand (Apofruit, fruits and vegetables made in Italy).

The cooperative deals with collection, storage, packaging and marketing of fresh fruits and vegetables, supplied by the producer, members of the cooperative. On the national territory, it has 16 structures for the collection and storage of products and 15 structures for processing products. This company has the "mission" of acquiring the maximum specialization on the main Italian fruits and vegetables. This aim is pursuit through the high standard of the offer, the intense activity and the product and process innovation. Indeed, Apofruit Italia promotes agricultural research and experimentation programs, conversion, production rationalization and modernization of its member companies in order to safeguard the variety of products and ensure high quality levels. Moreover, the Cooperative, has as objective the use of cultivation practices, production techniques and waste management practices that respect the environment. Apofruit Italia, aware of the importance and limited nature of natural resources, promotes production policies compatible with sustainable development objectives. Therefore, the aim of the Cooperative aim is to limit production and consumption with high polluting potential, opening to the introduction of technological innovations, capable of limiting negative aspects. The Cooperative, therefore, has a strong interest in developing valuable products from by-products of the agri-food chain. During the whole year, the Cooperative receives different types of fruit from its associates. Thanks to both manual work and cutting-edge equipment, Apofruit Italia selects between the truly marketable fruit and the fruit that should be considered a waste or a by-product. Firstly, all fruits are subjected to multiresidue analysis, a particular kind of analysis through which it is possible to search and quantify most of the pesticides allowed in products for human or animal nutrition. Following this safety analysis, fruits must respect specific standards to be considered marketable:

- not have altered organoleptic characteristics;
- have specific colour, index of being slightly or too ripe;

- have specific dimension;
- not being in a state of partial or total decomposition.

When these standards are not respected, the fruit is considered a waste or by-product. To date, these by-products are destined for two different companies: fruit juice companies or energy producers from biomass. The European Directive, indeed, defines biomass as "the biodegradable fraction of products, wastes and residues of biological origin from agriculture (including plant and animal substances), forestry and related industries, including fishing and aquaculture, as well as the part biodegradable of industrial and urban waste ".

In this research project “Apofruit Italia” provided some by-products, which it generally disposes of in the modality above described, to feed BSF.

1.6 Aim of the research

The exponential growth of the world population has led, in recent years, to an increase in the consumption of food resources and, consequently, the intensification of the usage of the resources available in the environment. A direct consequence of this problem is the increasing waste. A great opportunity to give solutions to both problems is linked to bioconvertor insects. Bioconversion is the transformation of organic materials, such as plant or animal waste, into usable products or energy sources by biological processes. One of the best insects able to convert organic matter is *Hermetia illucens* (L.) (Diptera: Stratiomyidae), commonly called Black Soldier Fly (BSF). BSF farming, that utilizes organic waste as substrates, has the great potential to offer a solution to waste management and protein deficiency problems. This research project, supported by Basilicata Region in the program "Innovative PhD agreement with specialization in enabling technologies in Industry 4.0", aims to study and deepen the bioconversion process of organic waste or by-products deriving from the agri-food and zootechnical chain, mediated by BSF, in order to obtain valuable products. Different types of agri-food products and zootechnical wastes were used, some of them deriving from the regional partner, the Cooperative "Apofruit Italia", with the aim of evaluating the efficacy of bioconversion process, through the characterization of the organic components (proteins, lipids, ashes) of the larvae fed on these wastes. This analysis will allow to optimize the bioconversion process, favouring the recovery of agri-food by-products, otherwise to be disposed of in conventional ways. Furthermore, it will be possible to develop new production processes related to the food industry (animal feed and eco-compatible food packaging), energy (biodiesel production) and medical-pharmaceutical (through the different uses of chitosan) fields, which will have high quality products of biological and economic value (proteins, lipids, and chitin) deriving from the bioconversion process, as result. The expected results are part of a bigger project, in order to optimize the company's production processes, with a considerable reduction in the impact on the environment and the costs of managing the disposal of the chain's waste, which will be converted into valuable products that can be marketed. These products will be highly sustainable, as they derive from a completely eco-friendly process. The impact of the results expected from the process innovation proposed here will be relevant, as it will offer a highly innovative, alternative, and valid solution to the problem of the disposal of organic substances of agri-food and zootechnical origin. In addition, wastes from the agri-food chain could be enhanced, through the bioconversion process, obtaining many products of high biological and commercial value: larvae rich in nutrients that could be used as pet food or transformed into meal for aquaculture uses, larval frass that can be used as soil conditioner for

crop fertilization, and chitin and exploitable in medical, pharmaceutical, agricultural fields. The current European regulations forbid the usage, for food and feed applications, of insects reared with animal manure. For this reason, the BSF biomass obtained from bioconversion process on manure wastes can be used to obtain other product, including biofuel from its lipid component. The agri-food and zootechnical waste valorisation, is part of a circular economy perspective, that wants to demonstrate the feasibility of organic waste management by BSF, reared on by-products and wastes at industrial scale.

2. MATERIAL AND METHODS

2.1 Insect rearing

Black soldier fly eggs were collected from a colony maintained in the Laboratory of Physiology and Molecular Biology of Insect, at University of Basilicata. They were stored in 500 ml clean glass jars in an environmental chamber at 27,0 ± 1,0 °C, 70,0 % relative humidity (RH) until hatching. After hatching, about 10.000 neonate larvae for each of three replicates, were placed in 500 ml plastic container in the same environmental condition above described and fed 500 g of standard Gainesville diet consisting of 30% alfalfa, 50% wheat bran, 20% corn meal (Hogsette, 1992), at 70% moisture, for four days. On the fifth day, larvae, frass, and the left dry Gainesville diet were transferred into plastic boxes (56 cm x 40 cm x 13 cm) containing 7,0 kg of each substrate used in this experiment. The substrate was also surrounded by dry Gainesville diet (50 g for each day of experiment), in order to prevent larvae from escaping. Similarly, a control group was formed with 10.000 neonate larvae, again transferred into plastic boxes (56 cm x 40 cm x 13 cm) but containing 7,0 kg of Gainesville diet only. Plastic boxes with larvae were placed in the previously described environmental chamber and conditions. Hence, subsequent experiments were carried out for testing six diets from fruits and vegetables collected from the agri-food chain: 1) watermelon 2) kiwi 3) cabbage and savoy cabbage mixed in 1:1 ratio (w/w) 4) strawberry 5) tangerine 6) orange.

Below are reported the nutritional value of each substrate (Table 2.1), as published at the USDA, the U.S. Department of Agriculture (https://fdc.nal.usda.gov/). Nutritional value of standard diet was analysed through the methodologies described in the following paragraphs:

Substrate	Protein	Lipid	Ash	Fibre
Standard diet	13,90%	4,00%	4,50%	12,60%
Watermelon	0,61%	0,15%	0,25%	0,40%
Kiwi	1,06%	0,44%%	0,71%	3,00%
Cabbage + savoy cabbage	1,28%+2,00%	0,1%+0,1%	0,64%+0,80%	2,5%+3,1%
Strawberry	0,67%	0,30%	0,40%	2,00%
Tangerine	0,81%	0,31%	0,38%	1,80%
Orange	0,94%	0,12%	0,60%	2,40%

Table 2.1: Nutritional value of each substrate, as published at the USDA, the U.S. Department of Agriculture (https://fdc.nal.usda.gov/). Nutritional value of standard diet was analysed by the Kjeldahl method (protein content), the chloroform extraction (lipid content), the incineration in a Muffle Furnace (ash content), the boiling in a neutral and acid detergent (fibre).

2.2 Dry matter and water content

Moisture of the organic diets was calculated at the beginning of the experiment. An amount equal to 10 g of each organic diet in replicates was weighed, placed into an aluminium dish, and dried for 24 h at 55 °C in a Gallenkamp Hotbox Oven (London, UK, Europe). Dry matter and water content were determined according to the following formulas:

$$\text{Dry matter (\%)} = \frac{\text{Final weight (g)}}{\text{Initial weight (g)}} * 100$$

$$\text{Moisture content (\%)} = 100 - \text{Dry matter (\%)}$$

2.3 Larval analyses

2.3.1 Growth curves, Growth Rate, and Index of Growth by Time

At the beginning of the experiment a sample of ten 5-day-old-larvae for each trial were collected, weighed on an analytical balance (Sartorius analytic, Goettingen, Germany) and used as a starting point for the following measures. Six groups of ten larvae from each box were randomly selected on a daily base, weighed, and returned to their respective boxes. Each experiment was considered concluded when a decrease in larval weight was registered, showing that larvae stopped feeding and they are ready to pupate. The mean larval weight recorded on the date that larvae stopped gaining weight defined the final larval weight. Growth rate was then calculated following the methods described by Leong *et al.* (2016), applying the following formula:

$$\text{Growth rate (g d -1)} = \frac{\text{10 larvae average final weight (g) - 10 larvae average initial weight (g)}}{\text{days of experimental trial (d)}}$$

The Index of Growth by Time was calculated applying the following formula:

$$\text{Index of growth by time (g/d)} = \frac{\text{Total larval biomass (g)}}{\text{days of experimental trial (d)}}$$

At the end of the trials, BSF larvae were separated and collected manually after sieving the frass. Larvae and frass were weighed separately.

2.3.2 Ash and mineral content

In order to determine the ash content, that means mineral and metal content, an amount of 10 g of larvae from each experimental trial was dried for 48 h at 80 °C in a Gallenkamp Hotbox Oven (London, UK, Europe), followed by incineration at 550 °C for 3 h in a Muffle Furnace (Gefran 1001, Provaglio d'Iseo, Italy). The ash content was calculated according to the following formula:

$$\text{Ash content (\%)} = \frac{\text{final weight}}{\text{initial weight}} * 100$$

In larvae fed on standard diet, strawberry, tangerine and orange, mineral content was then determined by atomic absorption spectrometry in an atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan) after digestion in sulfuric acid and selenium powder. Calcium, potassium, magnesium, and sodium were measured at wavelengths of 422.7, 766.5, 285.2, 589.0, respectively. Phosphorus was determined using a UV–visible spectrophotometer (Shimadzu, Japan) at a wavelength of 880 nm (Irungu *et al.*, 2018).

2.3.3 Protein and amino acid content

In order to determine the protein content, an amount of 10 g of larvae from each experimental trial was dried for 24 h at 55°C in a Gallenkamp Hotbox Oven (London, UK, Europe).

Total nitrogen was measured using the Kjeldahl method in accordance with method No. 984.13 of AOAC International. To estimate more accurately BSF protein content, in addition to the conventional nitrogen-to-protein (N-factor) conversion factor of 6.25, a conversion factor of 4.76 was used, as suggested by Janssen *et al.* (2017).

In addition to protein content, in larvae fed on standard diet, strawberry, tangerine and orange, also specific amino acid content was determined by HPLC performed on oxidised and hydrolysed samples, following the procedure in 2009/152/EC. In addition, tryptophan was determined separately, since this amino acid is destroyed during acid hydrolysis (European Commission, Commission Regulation (EC) No. 152/2009).

2.3.4 Lipid and fatty acid content

In order to determine the lipid content, an amount of 10 g of larvae from each experimental trial was dried for 24 h at 55 °C in a Gallenkamp Hotbox Oven (London, UK, Europe).

Following modified methods contained in Loveridge (1973), larvae were first placed into an aluminium dish and dried for 24h at 55°C in a Gallenkamp Hotbox Oven (London, UK, Europe) to deprive of water content. Then, they were weighed on an analytical balance (Sartorius analytic, Goettingen, Germany), to evaluate the initial dry weight. To extract the fat body, larvae were immersed in changes (24 h each) of cold chloroform in round glass tubes. The first changes of chloroform became yellowish, while the last remained colourless indicating that the fat extraction was complete. Larvae were then dried for 2 h in the afore-mentioned oven and reweighed. This second weighing gave the dry mass deprived by chloroform-soluble substances (final dry weight). This residual dry matter was used to calculate the percentage of lipids in each group of larvae fed on the different diets, using the following formula:

$$\text{Lipid content (\%)} = \frac{\text{larval initial dry weight (g) - larval final dry weight (g)}}{\text{larval initial dry weight (g)}} *100$$

In larvae fed on standard diet, strawberry, tangerine and orange, also specific fatty acid composition was evaluated by infrared spectroscopy following the procedure described by Christy and Egeberg (2006).

2.3.5 Fibre content

In order to determine the fibre content, an amount of 10 g of larvae fed on standard diet, strawberry, tangerine and orange, was dried for 24 h at 55°C in a Precision Scientific Gallenkamp Hotbox Oven.

The neutral detergent fibre was determined by boiling a 0.5 g sample for 1 h in 100 mL of neutral detergent plus 0,05 mL of heat-stable α-amylase (ANKOM Technology, NY, USA) and 0,5 g of analytical grade sodium sulphite (Sigma-Aldrich, Darmstadt, Germany) (Van Soest *et al.*, 1991). The acid detergent fibre was determined as described by Goering and Van Soest (1970).

2.4 Frass analysis

2.4.1 Dry matter and water content

Dry matter and water content of the frass of each experiment were calculated at the end of the experiment. An amount of frass equal to 10 g deriving from each experiment was weighed, placed into an aluminium dish, and dried for 24 h at 55 °C in a Gallenkamp Hotbox Oven (London, UK, Europe). The weight measured after drying was used to calculate the percentage of frass dry mass, using the two formulae in paragraph "2.2 Dry matter and water content".

2.4.2 Ash, mineral, and fibre content

Ash, mineral, and fibre content of frass derived from larvae fed on standard diet were detected through the methodologies above described.

2.4.3 Chemical analysis

An amount of 300 g of frass derived from larvae fed on standard diet was sent to an external laboratory and total nitrogen, organic carbon content and organic substance were detected according to the following Regulations: UNI EN 13654–1:2001, UNI EN 13137:2002 and DM 13/09/1999.

2.4.4 Microorganism analysis

Larval frass, derived from larvae fed on the standard diet, was analysed on microbiological content, in particular microorganisms at 30 °C, Enterobacteriaceae and specifically *Salmonella* spp.

Bacterial count at 30 °C was evaluated by homogenizing 10 g of sample in 90 ml Maximum recovery diluent (Biolife Italiana S.r.l, Monza, Italia). The solution was then distributed into a Petri dish with the Tryptic Glucose Yeast Agar (Plate Count Agar) medium (Biolife Italiana S.r.l, Monza, Italia) and incubated at 30 °C for 72h (UNI EN ISO 4833-1).

Enterobacteriaceae count was evaluated by homogenizing 10 g of sample in 90 ml Maximum recovery diluent (Biolife Italiana S.r.l, Monza, Italia). The solution was then distributed into a Petri dish with the Violet Red Bile Glucose Agar medium (Biolife Italiana S.r.l, Monza, Italia) and incubated at 37 °C for 24h (ISO 21528-2).

Salmonella spp research was evaluated by homogenizing 25 g of sample in 225 ml of Buffered Peptone water (Biolife Italiana S.r.l, Monza, Italia). The suspension was incubated for 20 h at 37 °C (pre-enrichment phase). Hence, 0.1 ml of the pre-enriched solution was diluted into 10 ml of Rappaport Vassiliadis Soy Broth (Biolife Italiana S.r.l, Monza, Italia) and incubated for

24 h at 41 °C (enrichment phase) and 1 ml of the pre-enriched solution was diluted into 10 ml of Muller Kauffmann Tetrathionate Broth Base (Biolife Italiana S.r.l, Monza, Italia) and incubated for 24 h at 41 °C (enrichment phase). Samples from the enrichment phase were taken with an inoculation loop and a smear was performed on XLD agar and Brilliant Green Agar (Biolife Italiana S.r.l, Monza, Italia) in petri dishes. After the incubation at 37 °C for 24 h, the presence of *Salmonella* spp colonies are pointed out by black colonies (UNI EN ISO 6579-1).

2.5 Bioconversion parameter analysis

2.5.1 Substrate reduction

Substrate reduction was calculated following modified methods contained in Tschirner and Simon, 2015, applying the following equation:

$$\text{Substrate reduction (\%)} = \frac{\text{initial substrate (g) - frass(g)}}{\text{initial substrate (g)}} * 100$$

2.5.2 WRI and ECD

For each experimental essay, different parameters were calculated:

- the WRI (Waste Reduction Index), which indicates the ability of the larvae to reduce the amount of administered food;

- the ECD (Efficiency of Conversion of Digestive Feed), which indicates the efficiency of the larvae in converting the food taken into larval biomass.

The WRI was calculated using the following formula (Leong *et al.*, 2016):

$$\text{WRI} = \frac{\text{substrate reduction}}{\text{days of experimental trial}} * 100$$

The ECD was calculated according to the following formula (Leong *et al.*, 2016):

$$\text{ECD} = \frac{\text{larval biomass}}{\text{initial substrate - frass}}$$

2.6 Larval meal

In order to obtain an integral insect meal, BSF pre-pupal larvae, fed on standard diet, were dried for 24 h at 55 °C in a Gallenkamp Hotbox Oven (London, UK, Europe). Larvae were then grinded with a Waring Commercial Blender.

Dry matter and water content, protein, amino acid, lipid, fatty acid, fibre, ash, mineral, and microbiological characteristics were detected through the methodologies above described.

2.7 Statistical analysis

Dry mass and water content, growth rate and index of growth by time, larval total biomass, ash and mineral content, protein and amino acid content, lipid and fatty acid content, fibre content, frass total weight, substrate reduction, WRI and ECD were presented as mean ± SE (standard error) of three independent biological replicates and were compared by analysis of variance (ANOVA) and Bonferroni *post-hoc* test using GraphPad Prism 6.00 software for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com).

3. RESULTS

3.1 Substrate dry matter and water content

At the beginning of the experiment, the water content of each substrate was verified. The seven different substrates used in the experiment slightly differ in moisture content. Water content of standard diet (70,86% ,84%), kiwi (74,05% ± 1,10%), and the mix of cabbage/savoy cabbage (75,34% ± 1,13%) differed from water content of watermelon (89,35% ± 0,29%), strawberry (88,63% ± 0,87%), tangerine (85,84% ± 1,23%), and orange (87,13% ± 0,49%) (Tables 3.1, 3.2, Fig. 3.1, 3.2).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	28,49	10,21	27,50	23,76	9,85	12,91	13,03
2	28,14	11,19	26,53	23,32	11,40	12,96	13,63
3	30,80	10,56	23,81	26,90	12,85	16,62	11,95
Mean	29,14	10,65	25,95	24,66	11,37	14,16	12,87
SE	0,84	0,29	1,10	1,13	0,87	1,23	0,49

Table 3.1: Dry matter (%) of different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Dry matter of each substrate was measured at the beginning of the experiment.

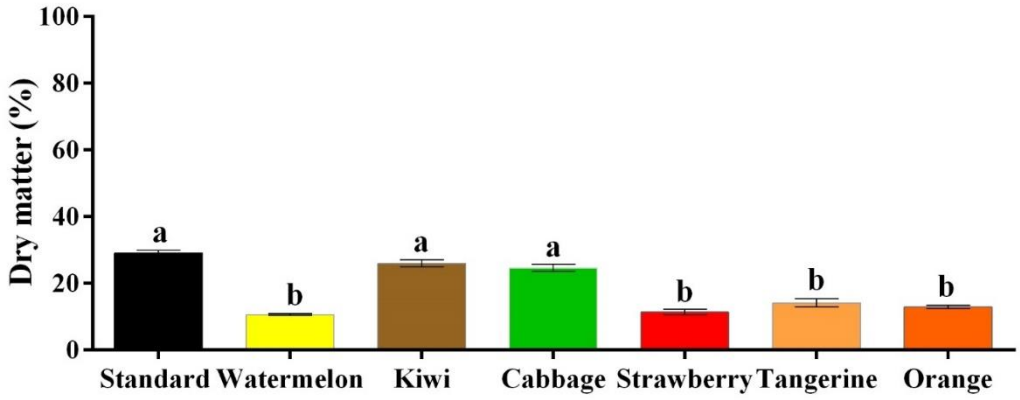

Figure 3.1: Dry matter (%) of different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Dry matter of each substrate was measured at the beginning of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	71,51	89,79	72,49	76,238	90,15	87,09	86,97
2	71,86	88,80	73,47	76,68	88,60	87,04	86,38
3	69,20	89,44	76,19	73,10	87,15	83,38	88,05
Mean	70,86	89,35	74,05	75,34	88,63	85,84	87,13
SE	0,84	0,29	1,10	1,13	0,87	1,23	0,49

Table 3.2: Water content (%) of different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Moisture content of each substrate was measured at the beginning of the experiment.

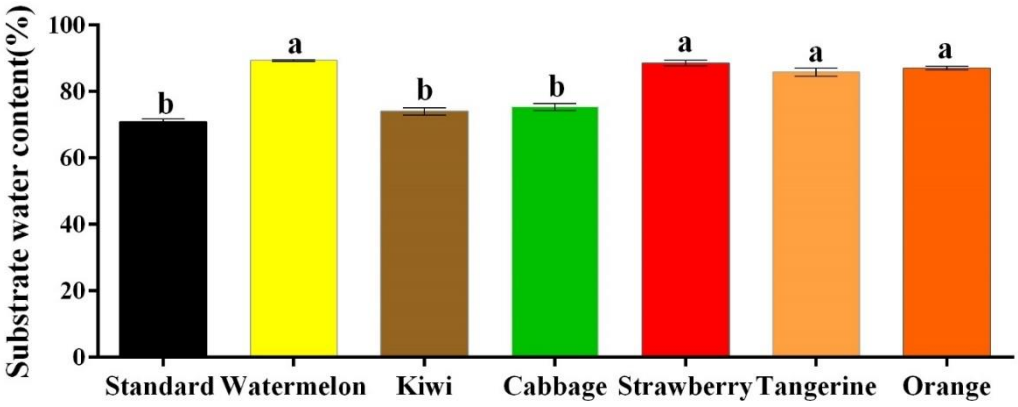

Figure 3.2: Water content (%) of different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Water content of each substrate was measured at the beginning of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

3.2 Growth curves, Growth Rate, and Index of Growth by Time

Control larvae reached their maximum weight (1517,28 mg ± 66,73 mg) in 7 days, completing the larval stage in 12 days after eggs hatching (Table 3.3, Fig. 3.3) with a growth rate of 143,78 mg/d ± 5,90 mg/d (Table 3.10, Fig. 3.4) and an index of growth by time of 0,10 kg/d ± 0,004 kg/d (Table 3.11, Fig. 3.5). Larvae fed on different substrates from the agri-food chain regularly increased their weight over the course of the experiment, completing larval stage in a range of 16-18 days after eggs hatching, reaching the mature larval stage with delay respect to control larvae: 16 days for larvae fed on watermelon (maximum weight: 984,64 mg ± 27,50 mg) (Table 3.4, Fig. 3.3) with a growth rate of 70,47 mg/d ± 1,46 mg/d (Table 3.10, Fig. 3.4) and an index of growth by time of 0,07 kg/d ± 0,002 kg/d (Table 3.11, Fig. 3.5), 17 days for larvae fed on kiwi (maximum weight: 1110,36 mg ± 40,05 mg), strawberry (maximum weight: 1966,88 mg ± 27,59 mg) and tangerine (maximum weight: 1384,41 mg ± 42,28 mg) (Tables 3.5, 3.7, 3.8, Fig. 3.3), with a growth rate of 74,05 mg/d ± 1,18 mg/d, 129,57 mg/d ± 1,43 mg/d and 97,43mg/d ± 7,90 mg/d respectively (Table 10, Fig. 3.4) and an index of growth by time of 0,07 kg/d ± 0,002 kg/d, 0,11 kg/d ± 0,001 kg/d and 0,08 kg/d ± 0,004 kg/d respectively (Table 3.11, Fig. 3.5), 18 days for larvae fed on the mix of cabbage/savoy cabbage (maximum weight: 713,16 mg ± 29,28 mg) and orange (1429,49 mg ± 46,27 mg) (Tables 3.6, 3.9, Fig. 3.3) with a growth rate of 43,99 mg/d ± 0,43 mg/d, and 91,56 mg/d ± 10,13 g/d, respectively (Table 3.10, Fig. 3.4) and an index of growth by time of 0,039 kg/d ± 0,001 kg/d and 0,06 kg/d ± 0,001 kg/d respectively (Table 3.11, Fig. 3.5). Larvae fed on standard diet and strawberry showed the highest growth rate and the highest index of growth by time, that returns a better evaluation of the best diet, that is the one on which BSF larvae can convert the higher biomass in the shorter time. Larvae fed on the mix of cabbage/savoy cabbage showed the lowest growth rate and the lowest index of growth by time. Statistical differences were recorded between standard diet/strawberry and other substrates, in particular with the mix of cabbage/savoy cabbage that revealed differences also with watermelon, kiwi, tangerine and orange (Fig. 3.4).

a) Standard diet –replicate 1

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
1	41,00	100,70	234,00	316,00	608,60	912,20	1478,10	1697,90	1996,90
2	40,00	110,80	270,30	295,90	605,20	1177,60	1538,80	1554,30	1455,40
3	35,90	110,50	222,20	336,20	545,80	1113,10	1411,80	1596,90	1275,80
4	40,00	80,80	280,70	314,90	591,30	1121,10	1381,60	1668,40	1442,00
5	81,50	120,40	300,40	327,30	634,90	987,50	1512,30	1730,50	1370,70
6	60,00	118,00	189,90	336,30	621,10	899,90	1498,00	1636,50	1110,80
Mean	49,70	106,90	249,60	321,10	601,20	1035,20	1470,10	1647,40	1442,40
SEM	7,20	5,90	16,90	6,30	12,60	48,10	24,90	26,60	122,40

b) Standard diet –replicate 2

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
1	61,40	100,80	200,00	280,80	665,80	907,70	1232,00	1541,90	1998,70
2	55,30	120,10	200,10	286,30	671,70	1074,50	1105,10	1538,10	1148,00
3	65,70	115,10	225,20	320,10	612,30	970,00	1403,10	1504,20	1103,50
4	65,30	97,10	214,40	304,50	611,00	928,90	1398,70	1546,20	1452,50
5	41,50	83,20	222,10	302,30	600,20	1120,00	1411,20	1532,00	1286,30
6	56,30	148,00	183,90	305,80	659,70	1001,10	1248,20	1585,80	1207,20
Mean	57,60	110,70	207,60	299,9	636,80	1000,40	1299,70	1541,40	1336,00
SE	3,70	9,20	6,40	5,8	13,20	33,90	51,00	10,80	136,11

c) Standard diet –replicate 3

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
1	41,40	103,40	203,70	290,10	665,40	1061,30	1171,80	1403,60	1241,50
2	65,20	115,10	200,90	317,80	659,70	1040,60	1111,30	1432,20	1393,90
3	66,50	124,50	233,40	328,40	666,50	915,20	1285,10	1503,00	1023,30
4	61,30	126,30	222,20	365,00	623,80	958,00	1282,30	1441,00	1367,30
5	44,50	147,30	195,10	300,80	655,10	1009,0	1274,30	1531,00	1170,00
6	35,30	142,00	233,10	325,20	687,90	990,30	1298,30	1492,90	1383,80
Mean	52,40	126,40	214,70	321,20	659,70	995,70	1237,10	1467,30	1263,30
SE	5,50	6,70	6,90	10,60	8,50	21,90	31,40	19,90	60,30

Table 3.3: Biological replicates (a-b-c) of BSF larvae fed on standard diet. For each day of the biological assay, the weight in milligrams of ten larvae are reported; the mean and the standard error of the 6 technical replicates (6 groups made of 10 larvae) are also reported.

a) Watermelon –replicate 1

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
1	86,00	86,20	133,80	187,00	456,20	590,70	741,10	821,50	913,90	977,80	939,00	1097,30	1008,40
2	71,90	82,30	142,50	190,70	296,50	705,40	757,50	889,30	893,90	846,70	1001,50	1101,10	967,10
3	78,70	85,10	197,10	188,70	279,80	386,30	717,40	920,00	920,00	1074,00	1571,00	1122,20	996,60
4	74,10	95,00	110,00	134,90	143,80	310,00	677,80	886,50	886,50	953,40	1004,60	1073,70	1170,80
5	80,10	81,50	97,00	157,40	224,00	490,80	739,80	832,00	834,00	830,60	958,80	1083,20	1081,50
6	65,10	92,30	169,30	141,00	221,00	670,80	795,80	813,00	918,90	828,20	916,60	1005,90	950,90
Mean	76,00	87,10	141,60	166,60	270,20	525,70	738,20	860,40	894,50	918,50	1065,30	1080,60	1029,20
SE	3,00	2,20	15,20	10,40	43,20	64,50	16,10	17,90	13,30	40,80	102,10	16,40	33,80

b) Watermelon –replicate 2

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
1	73,30	85,10	170,00	181,10	432,10	512,40	548,50	850,70	721,10	927,20	985,10	1089,60	1004,90
2	76,10	86,30	195,80	258,60	461,20	731,30	723,40	830,90	843,60	941,30	1003,50	1051,30	863,50
3	64,20	86,70	124,60	194,10	374,30	641,30	743,60	832,80	918,10	1029,70	1007,80	1029,50	979,50
4	54,80	80,70	97,50	183,50	236,70	392,00	729,90	846,30	996,00	933,50	1003,30	1051,40	931,40
5	69,10	92,40	92,50	166,60	402,20	538,90	793,10	721,40	800,00	1000,20	1059,90	1087,40	1003,70
6	63,30	86,70	142,20	218,30	585,90	603,50	713,20	638,80	861,00	935,90	951,00	1078,40	963,20
Mean	66,80	86,30	137,10	200,40	415,40	569,90	708,60	786,80	856,60	961,30	1001,80	1064,60	957,70
SE	3,10	1,50	16,60	13,60	46,60	47,70	34,00	35,50	38,70	17,50	14,50	9,900	21,90

c) Watermelon –replicate 3

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
1	50,20	85,10	170,00	181,10	432,10	512,40	548,50	850,70	720,10	927,20	986,10	1089,60	1071,90
2	70,20	99,30	96,10	253,30	561,00	712,30	725,40	863,30	682,60	933,30	1006,30	1075,40	1003,50
3	74,20	91,70	212,70	377,90	418,80	676,40	721,30	833,10	941,30	843,60	957,40	1051,40	917,50
4	54,60	93,30	97,50	183,50	546,60	392,00	729,10	846,60	996,00	933,50	1003,30	1051,40	907,50
5	61,80	91,20	233,70	227,20	594,10	741,80	732,90	836,50	911,90	1006,50	1101,00	1087,40	914,20
6	66,50	85,20	132,50	838,20	412,20	685,50	772,50	848,20	852,20	786,50	1004,90	1171,40	987,40
Mean	62,90	91,00	157,10	343,50	494,10	620,10	705,00	846,40	850,70	905,10	1009,80	1087,80	967,00
SE	3,80	2,20	23,80	103,20	33,40	56,10	32,20	4,40	51,10	31,70	19,80	18,10	26,79

Table 3.4: Biological replicates (a-b-c) of BSF larvae fed on watermelon. For each day of the biological assay, the weight in milligrams of ten larvae are reported; the mean and the standard error of the 6 technical replicates (6 groups made of 10 larvae) are also reported.

a) Kiwi –replicate 1

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	61,60	97,40	133,10	216,00	340,00	525,60	661,50	771,40	813,80	805,00	920,30	1057,10	1277,70	1144,30
2	71,20	88,70	113,20	140,60	283,00	533,60	772,50	694,40	892,80	965,00	1036,60	1019,40	1194,80	1030,30
3	62,20	72,40	135,70	225,50	344,30	477,00	646,00	606,60	850,20	948,20	974,40	1104,40	1274,40	1128,40
4	80,10	95,90	195,30	226,30	360,00	562,60	606,20	700,10	835,90	954,40	977,70	1022,50	1258,00	1132,50
5	83,10	184,80	163,30	264,60	591,00	484,00	599,00	734,60	813,50	945,80	963,30	1073,70	1286,30	1085,50
6	73,70	154,20	159,70	244,70	564,20	632,80	611,00	708,60	823,70	811,20	948,80	1144,00	1283,30	1079,80
Mean	72,00	115,50	150,10	219,60	413,80	535,90	649,40	702,60	838,30	904,90	970,20	1070,20	1262,40	1100,10
SE	3,60	17,90	11,80	17,30	53,00	23,40	26,60	22,40	12,30	30,80	15,80	19,70	14,10	17,60

b) Kiwi –replicate 2

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	81,10	91,70	153,80	271,90	503,30	535,70	686,20	736,20	944,40	846,70	952,40	1031,40	1228,30	1052,80
2	61,60	95,00	160,40	252,90	310,30	399,00	619,50	603,30	829,90	976,90	1004,10	1066,30	1516,60	1294,40
3	80,40	85,00	189,00	254,40	468,80	473,80	534,40	713,50	936,10	1001,70	902,70	1075,10	1241,40	1059,80
4	63,60	84,00	283,80	322,20	576,80	623,50	623,80	821,30	918,10	911,20	1008,20	1102,10	1328,30	1204,40
5	72,20	130,20	262,00	391,70	596,00	686,80	575,30	632,40	896,00	936,40	1002,40	1081,80	1302,10	1233,30
6	87,00	93,30	261,60	344,40	519,30	624,90	519,30	624,00	719,60	870,00	1002,10	1180,60	1286,90	1012,50
Mean	74,30	96,50	218,40	306,30	495,80	557,30	593,10	688,50	874,00	923,80	978,70	1089,60	1317,30	1142,90
SE	4,20	7,00	23,40	22,90	41,80	44,10	25,50	34,20	35,10	24,50	17,40	20,50	42,70	47,20

c) Kiwi –replicate 3

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	69,10	98,50	136,40	353,60	556,30	532,00	570,00	858,50	815,60	960,40	1005,80	1045,20	1296,60	1194,30
2	71,10	155,20	219,30	248,00	484,40	571,10	511,70	734,70	771,10	734,00	945,10	1047,30	1487,10	1295,60
3	74,10	84,40	287,10	357,40	487,50	511,00	535,00	738,60	715,40	918,70	1000,80	1081,40	1284,30	965,10
4	69,30	146,30	267,30	205,60	480,30	484,90	590,70	689,90	808,70	977,70	1009,50	1009,90	1481,00	964,30
5	82,50	131,40	296,50	284,50	573,10	484,30	715,60	619,00	867,00	953,40	1066,60	1077,50	1287,40	1103,40
6	83,30	70,40	177,80	286,50	319,40	440,00	624,80	739,30	700,20	776,00	953,00	1032,40	1296,70	1005,80
Mean	74,90	114,40	230,70	289,20	483,50	503,90	591,30	730,00	779,70	887,30	996,80	1049,00	1355,50	1088,10
SE	2,60	14,20	26,20	24,20	36,60	18,40	29,70	32,00	26,00	42,90	18,00	11,10	40,70	55,30

Table 3.5: Biological replicates (a-b-c) of BSF larvae fed on kiwi. For each day of the biological assay, the weight in milligrams of ten larvae are reported; the mean and the standard error of the 6 technical replicates (6 groups made of 10 larvae) are also reported.

a) Cabbage/Savoy Cabbage –replicate 1

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1	41,30	81,30	136,40	136,20	257,20	246,10	396,50	423,10	480,00	536,8	528,00	644,00	641,70	726,40	791,10
2	61,70	72,10	116,20	116,50	246,10	271,20	393,30	432,20	431,00	579,7	551,00	613,60	653,40	724,00	716,80
3	43,40	88,40	115,60	172,10	271,20	281,80	439,70	418,00	415,00	595,7	534,80	628,70	785,40	750,00	644,00
4	51,60	83,10	173,20	139,50	264,00	284,10	335,70	495,30	472,80	555,3	561,70	688,00	643,70	844,00	637,70
5	47,60	80,40	149,20	166,50	272,90	238,10	337,30	412,70	415,40	598,7	541,00	693,40	661,40	763,10	748,20
6	54,70	84,00	166,50	167,00	240,60	239,20	373,60	430,90	486,60	517,9	752,00	623,40	879,20	729,10	647,60
Mean	50,10	81,60	142,90	149,60	258,70	260,10	379,40	435,40	450,10	564	578,10	648,50	710,80	756,10	697,60
SE	3,10	2,20	10,00	9,10	5,40	8,70	16,10	12,40	13,60	13,4	35,10	13,90	40,40	18,70	26,20

b) Cabbage/Savoy Cabbage –replicate 2

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1	44,70	72,10	128,50	188,80	295,90	260,40	393,00	482,90	428,80	543,40	635,80	615,00	693,20	791,00	795,70
2	52,80	71,40	95,50	133,90	210,10	217,30	490,60	471,90	432,90	510,00	452,10	542,30	680,50	727,80	621,30
3	43,10	53,30	197,10	218,70	277,00	393,00	494,70	625,00	433,50	639,50	679,10	623,80	614,20	722,90	794,00
4	58,10	98,00	88,70	164,30	244,70	256,30	293,20	454,30	478,20	555,30	561,70	688,00	643,70	844,00	789,90
5	52,80	80,40	149,50	139,50	272,90	238,10	337,30	473,40	490,30	598,00	587,60	634,40	647,50	763,90	705,10
6	67,70	91,00	173,40	190,30	181,00	388,00	464,00	441,70	448,40	573,90	530,40	522,40	784,60	799,0	629,10
Mean	53,20	77,70	138,80	172,60	246,90	292,20	412,10	491,50	452,00	570,00	574,40	604,30	677,28	774,80	722,50
SE	3,70	6,50	17,50	13,40	18,00	31,70	34,50	27,40	10,70	18,40	32,60	25,10	24,34	18,80	33,80

c) Cabbage/Savoy Cabbage –replicate 3

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1	59,00	93,30	124,30	120,30	152,00	245,60	251,90	265,00	380,50	475,80	541,60	647,40	617,90	868,00	729,80
2	45,60	75,70	73,10	177,50	249,40	243,40	287,00	308,90	456,80	472,10	489,80	577,60	597,20	844,70	728,00
3	45,20	77,60	172,00	203,90	238,40	260,40	449,10	524,80	488,70	487,70	574,00	577,60	786,60	844,30	679,60
4	65,80	76,50	91,60	165,70	234,80	375,20	343,30	356,20	397,30	410,10	484,80	499,40	637,70	778,00	607,80
5	65,40	89,00	87,00	195,90	248,30	286,50	363,40	434,60	518,00	527,80	614,50	617,60	783,00	753,40	774,70
6	58,90	91,10	15,70	184,70	253,10	274,00	343,10	378,40	437,40	515,40	594,30	671,10	717,70	742,80	796,40
Mean	56,70	83,90	94,00	174,70	229,30	280,90	339,60	378,00	446,50	481,50	549,80	598,50	690,00	805,20	719,40
SE	3,80	3,40	21,30	12,10	15,70	20,00	27,70	37,80	21,40	16,90	22,10	25,00	34,90	21,90	27,80

Table 3.6: Biological replicates (a-b-c) of BSF larvae fed on a mix of Cabbage/Savoy Cabbage (1:1). For each day of the biological assay, the weight in milligrams of ten larvae are reported; the mean and the standard error of the 6 technical replicates (6 groups made of 10 larvae) are also reported.

a) Strawberry –replicate 1

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	61,60	119,50	264,70	410,00	986,30	926,60	760,00	1255,00	1343,80	1503,60	1952,90	1793,50	1957,20	1717,80
2	63,20	156,70	275,30	533,00	980,10	948,00	1038,40	1061,80	1632,20	1801,30	1538,30	1847,30	2004,50	1822,60
3	62,20	128,20	337,20	459,20	879,30	967,10	1045,50	1045,30	1464,10	1595,20	1903,80	2007,30	1989,90	1834,30
4	72,20	139,70	276,20	525,70	986,30	779,80	951,90	1017,20	1593,70	1562,70	1787,00	1911,20	1902,50	1907,70
5	73,40	143,60	256,10	513,10	689,70	847,00	1340,40	1236,40	1669,60	1512,70	1725,60	1854,60	2001,40	1917,00
6	73,70	163,70	373,10	405,70	692,30	943,60	1141,30	1370,00	1465,70	1666,60	1812,70	2005,20	1953,20	1842,70
Mean	66,10	141,90	297,10	474,40	869,00	902,00	1046,30	1164,30	1528,20	1607,00	1786,70	1903,20	1968,10	1840,30
SE	3,50	6,80	19,20	23,60	58,70	29,80	78,80	58,30	50,70	45,80	59,80	36,00	15,90	29,30

b) Strawberry –replicate 2

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	60,70	126,10	249,30	575,10	785,20	906,30	1117,30	1304,90	1516,00	1306,80	1703,50	1920,6	2018,70	1939,30
2	71,10	236,20	264,30	404,60	855,80	957,00	988,10	1053,00	1294,70	1792,40	1970,90	1923,8	1937,40	1887,60
3	61,40	127,10	367,10	500,80	828,40	861,20	1074,60	1096,30	1282,40	1641,20	1743,20	2069,4	2061,60	1934,30
4	71,90	215,50	355,50	508,50	780,90	813,20	1400,50	1113,30	1598,10	1674,10	1590,90	1938,6	2118,00	1930,00
5	77,70	149,10	385,10	439,10	680,10	875,70	1242,20	1222,20	1355,70	1482,90	1614,10	1825,8	1898,80	1856,80
6	77,00	208,30	371,90	500,30	973,20	998,30	1389,00	1363,60	1148,60	1482,90	1844,30	1889,5	1939,90	1893,00
Mean	69,90	177,10	332,20	488,10	817,30	902,00	1202,00	1192,20	1365,90	1563,40	1744,50	1928	1995,70	1906,80
SE	3,00	19,90	24,20	24,30	39,60	27,40	69,60	50,90	67,30	70,60	58,70	32,7	34,50	13,40

c) Strawberry –replicate 3

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	61,90	156,00	344,40	564,80	591,00	719	83,80	1089,30	1179,30	1662,20	1632,50	1937,90	1801,70	1933,00
2	63,90	189,20	266,10	497,90	621,30	925,8	1164,10	1379,00	1449,50	1667,60	1906,10	1793,90	1964,10	1983,00
3	79,30	216,70	319,80	567,50	907,60	766,8	1117,20	1279,00	1245,40	1659,30	1939,50	1823,80	1963,30	1964,60
4	78,60	208,90	318,50	428,00	620,60	764,5	981,60	1181,50	1536,80	1494,20	1605,90	1755,70	2018,80	1844,90
5	67,40	122,50	285,30	499,50	857,20	961,6	1303,00	1188,80	1512,70	1444,60	1698,30	1884,60	1986,60	1849,00
6	78,10	208,40	321,50	545,20	753,10	909,8	889,60	1081,00	1421,10	1704,80	1812,00	1972,10	1886,20	1840,00
Mean	71,50	183,60	309,30	517,20	725,10	841,2	923,20	1199,80	1390,80	1605,50	1756,60	1861,30	1936,70	1902,40
SE	3,20	15,10	11,60	21,80	55,10	41,9	177,90	46,60	59,60	44,00	57,70	34,50	32,30	26,80

Table 3.7: Biological replicates (a-b-c) of BSF larvae fed on strawberry. For each day of the biological assay, the weight in milligrams of ten larvae are reported; the mean and the standard error of the 6 technical replicates (6 groups made of 10 larvae) are also reported.

a) Tangerine –replicate 1

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	43,70	97,00	219,50	500,00	626,90	605,50	660,50	768,50	842,20	984,60	1194,10	1493,40	1659,30	1693,10
2	71,10	81,90	256,70	393,10	587,60	616,20	623,70	688,50	962,40	945,40	1392,00	1339,00	1556,10	1660,30
3	41,70	88,60	228,20	301,40	552,90	625,60	828,40	893,00	772,60	1463,80	1271,30	1403,20	1604,40	1386,50
4	77,00	67,10	239,70	736,80	720,70	904,30	791,20	850,60	811,00	1338,20	1040,60	1765,00	1593,10	1551,70
5	90,00	90,10	243,60	379,10	651,20	769,80	712,80	693,30	766,10	989,20	1120,10	1536,00	1415,10	1543,40
6	42,00	80,50	263,70	793,00	703,10	689,10	682,40	751,70	862,90	868,80	1240,60	1303,10	1509,30	1631,50
Mean	60,90	84,20	241,90	517,20	640,40	701,80	716,50	774,30	836,20	1098,30	1209,80	1473,20	1556,20	1577,80
SE	8,60	4,20	6,80	82,80	26,50	47,70	32,20	33,90	29,60	98,70	45,00	68,60	34,80	45,20

b) Tangerine –replicate 2

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	84,40	63,30	144,30	307,20	472,70	585,90	531,80	588,10	785,00	880,70	1059,50	1398,60	1590,60	1368,30
2	90,60	96,00	228,20	230,30	557,20	586,40	611,10	620,50	773,00	834,00	1183,20	1413,90	1555,90	1623,40
3	40,70	74,20	260,60	274,00	595,00	599,70	368,40	801,20	752,80	939,60	1123,10	1152,00	1461,70	1460,60
4	75,30	98,00	295,00	223,50	638,30	694,50	486,50	865,40	921,30	1144,40	1350,00	1403,20	1687,50	1649,30
5	34,40	60,90	158,50	213,90	658,40	675,10	677,30	585,50	780,10	1087,90	1341,60	1130,30	1582,90	1394,40
6	44,40	84,40	95,50	248,60	698,80	543,60	325,80	640,50	910,80	1010,90	1185,50	1267,30	1622,50	1430,20
Mean	61,60	79,50	197,00	249,60	603,40	614,20	500,20	683,50	820,50	982,90	1207,10	1294,20	1583,50	1487,70
SE	10,00	6,50	31,20	14,40	33,00	23,80	55,60	48,80	30,60	49,10	47,70	53,20	30,50	48,80

c) Tangerine –replicate 3

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	82,60	60,20	127,10	187,00	416,30	413,50	844,20	526,50	845,70	845,80	1044,40	1239,10	1578,40	1227,40
2	45,00	50,20	193,40	118,00	457,50	488,60	531,30	711,20	754,90	944,50	988,60	1073,40	1653,70	1120,60
3	58,00	85,20	146,50	380,10	393,00	596,00	590,80	629,30	726,40	990,90	1213,30	1138,40	1510,30	1229,30
4	68,90	54,00	120,10	211,80	435,00	462,60	742,90	556,30	746,40	988,80	1234,70	1203,90	1620,40	1251,50
5	63,50	65,80	186,20	165,40	431,20	406,10	698,70	714,90	730,20	867,90	983,30	1376,40	1579,90	1127,60
6	50,50	65,50	152,00	183,10	457,80	526,00	717,80	643,30	691,50	906,00	1148,30	1687,90	1557,80	1306,50
Mean	61,40	63,40	154,20	207,60	431,80	482,10	687,60	630,20	749,10	924,00	1102,10	1286,50	1583,40	1210,50
SE	5,50	5,00	12,20	36,80	10,20	29,30	45,60	31,70	21,30	25,00	45,60	90,50	20,30	29,70

Table 3.8: Biological replicates (a-b-c) of BSF larvae fed on tangerine. For each day of the biological assay, the weight in milligrams of ten larvae are reported; the mean and the standard error of the 6 technical replicates (6 groups made of 10 larvae) are also reported.

a) Orange –replicate 1

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1	61,30	68,40	190,40	226,80	334,00	443,60	502,20	842,20	722,40	758,40	834,70	845,00	1270,50	1545,40	1711,90
2	86,80	44,40	200,40	295,70	495,50	397,10	617,80	713,80	713,50	816,64	878,40	947,00	1522,10	1803,00	1700,20
3	45,40	44,10	216,30	308,60	316,80	433,50	549,30	640,80	834,00	858,00	935,60	1047,00	1227,60	1785,80	1678,40
4	42,00	56,90	196,60	220,10	327,20	378,10	211,70	691,00	798,00	754,70	895,40	867,60	1198,80	1700,400	1755,50
5	45,20	51,30	217,40	274,20	468,20	407,70	518,50	723,20	823,20	756,50	889,10	1153,00	1238,10	2121,20	1843,10
6	65,50	46,40	197,90	290,30	307,40	458,40	751,20	700,80	880,30	855,10	901,00	1207,00	1152,30	2243,40	1712,10
Mean	57,70	51,90	203,20	269,20	374,80	419,70	525,10	718,60	795,20	799,90	889,00	1011,10	1268,20	1866,50	1733,50
SE	7,00	3,80	4,50	15,20	34,20	12,40	72,80	27,40	26,80	20,30	13,40	61,10	53,30	107,70	24,10

b) Orange –replicate 2

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1	51,60	69,00	235,40	265,00	443,70	625,20	729,30	925,30	872,20	820,30	972,50	1107,50	1364,00	1348,10	1228,60
2	44,10	64,10	228,30	246,80	277,30	423,50	523,70	741,20	915,30	850,20	893,30	735,20	1205,60	1388,70	1314,20
3	49,60	31,60	210,10	270,00	458,80	536,20	559,20	605,30	754,00	840,00	983,10	820,30	1037,80	1312,70	1439,00
4	69,10	48,10	240,50	266,50	295,10	403,50	689,80	7270,00	776,70	793,30	956,20	1045,20	951,30	1389,30	1021,20
5	48,80	49,50	217,00	267,90	348,50	405,00	713,70	722,00	788,30	802,40	889,70	874,40	936,10	1367,00	1145,10
6	42,80	56,60	210,10	258,30	311,10	526,00	509,40	632,60	820,10	834,40	930,40	784,50	956,20	1594,00	1332,30
Mean	51,00	53,10	223,60	262,40	355,80	486,60	620,90	725,60	821,10	823,40	937,50	894,50	1075,20	1400,00	1246,70
SE	3,90	5,40	5,30	3,50	31,70	36,90	41,10	45,90	25,10	9,10	16,30	61,00	70,90	40,50	60,70

c) Orange –replicate 3

Larvae	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1	54,50	60,80	204,50	271,20	395,00	487,90	820,80	796,30	852,30	791,80	1012,30	753,00	1359,30	1349,20	1067,70
2	55,50	50,90	225,70	377,80	373,50	470,80	650,70	699,50	852,70	855,30	987,70	1029,50	1180,70	1402,90	1461,30
3	41,70	72,50	293,80	280,50	401,50	464,60	473,60	708,80	912,40	871,80	978,30	941,00	1278,30	1275,60	1336,00
4	80,20	71,40	224,30	269,30	361,00	549,60	700,20	692,60	872,30	884,70	928,70	764,80	1065,50	1274,60	1361,10
5	65,10	60,30	258,10	285,70	370,30	435,60	593,70	724,90	794,40	937,30	997,50	1202,10	1033,20	1274,10	1276,70
6	61,10	59,80	190,50	275,90	407,50	574,40	792,30	712,80	820,10	949,70	965,50	896,40	1294,70	1155,20	1346,40
Mean	59,70	62,60	232,80	293,40	384,80	497,20	671,90	722,50	850,70	881,80	978,30	931,10	1202,00	1288,60	1308,20
SE	5,20	3,30	15,40	17,10	7,80	21,90	52,70	15,40	16,70	23,50	11,90	69,20	53,80	34,20	53,90

Table 3.9: Biological replicates (a-b-c) of BSF larvae fed on orange. For each day of the biological assay, the weight in milligrams of ten larvae are reported; the mean and the standard error of the 6 technical replicates (6 groups made of 10 larvae) are also reported.

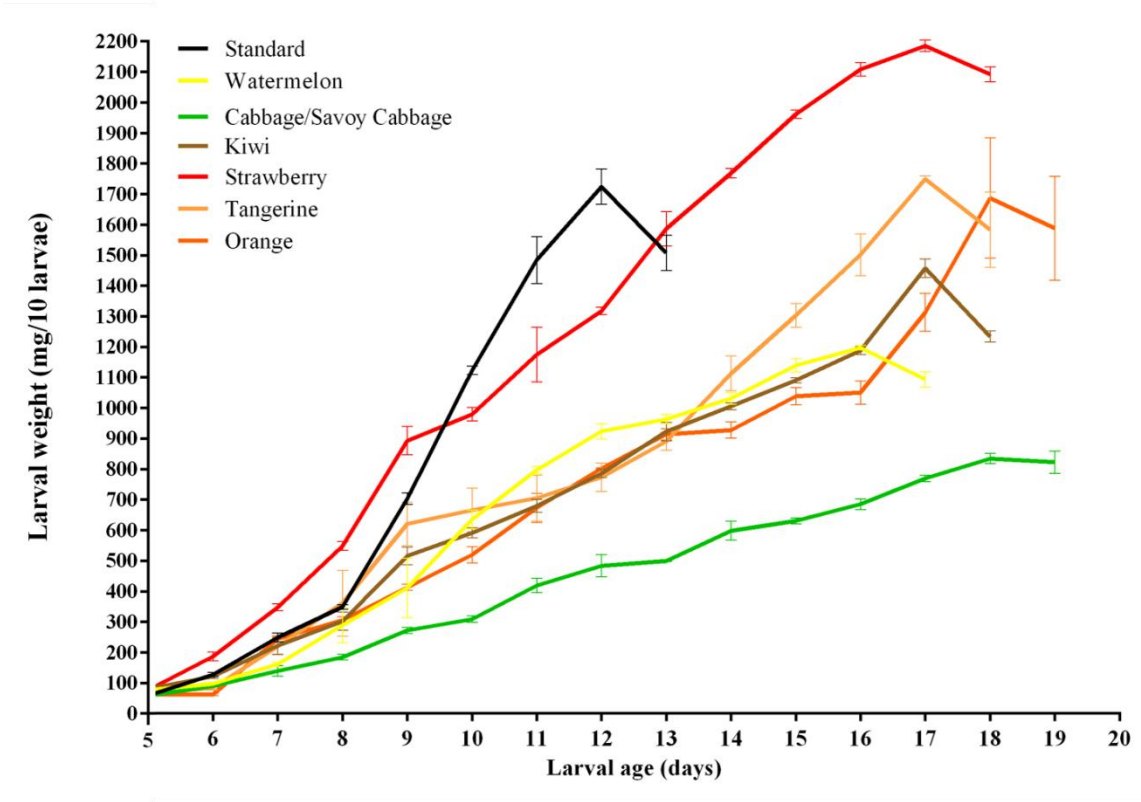

Figure 3.3: Growth curves of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Data are presented as mean ± SE of the 3 biological replicates and for each day the mean weight of 10 larvae, presented in milligrams.

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	154,74	73,32	73,44	43,17	126,73	108,35	111,72
2	142,04	68,53	76,33	44,62	131,21	101,86	79,71
3	134,54	69,55	72,37	44,18	130,78	82,08	83,23
Mean	143,78	70,47	74,05	43,99	129,57	97,43	91,56
SE	5,90	1,46	1,18	0,43	1,43	7,90	10,13

Table 3.10: Growth rate (mg/d) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Growth rate was calculated considering the initial and final larval weight and the days of bioassay and showed the daily increase of larval weight.

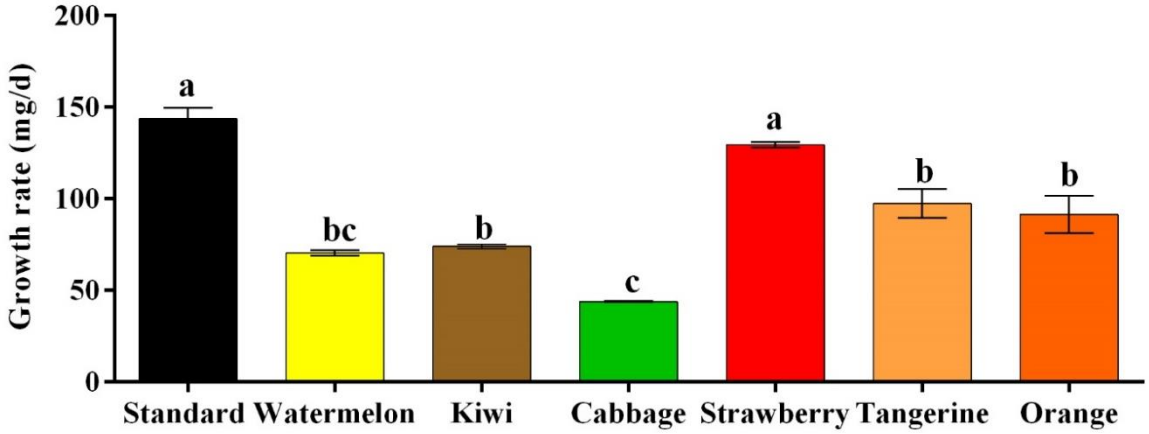

Figure 3.4: Growth rate (mg/d) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Growth rate was calculated considering the initial and final larval weight and the days of bioassay and showed the daily increase of larval weight. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	0,110	0,074	0,072	0,035	0,112	0,082	0,066
2	0,094	0,071	0,070	0,041	0,112	0,082	0,062
3	0,108	0,065	0,066	0,040	0,108	0,066	0,062
Mean	0,104	0,070	0,071	0,039	0,111	0,077	0,063
SE	0,004	0,002	0,001	0,001	0,001	0,004	0,001

Table 3.11: Index of growth by time (kg/d) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Index of growth by time was calculated considering the final larval total biomass and the days of bioassay. Data are presented as the mean ± SE (n = 3).

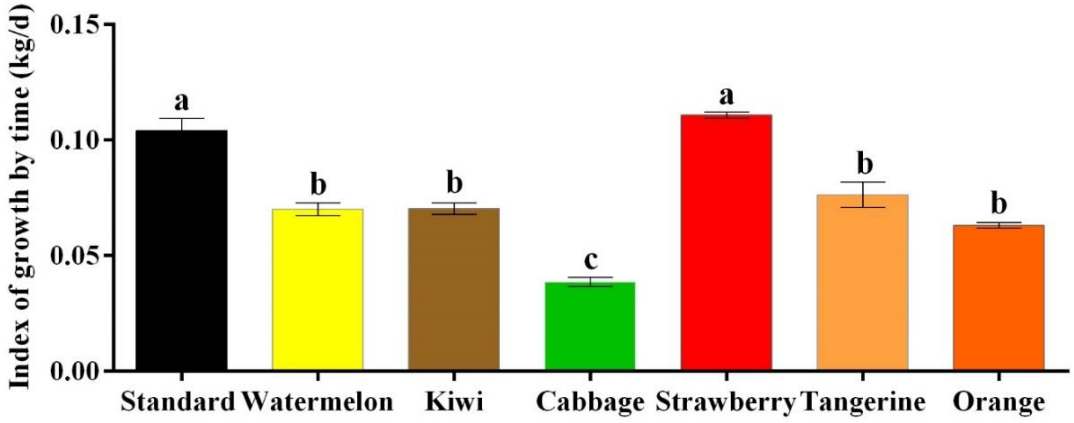

Figure 3.5: Index of growth by time (kg/d) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Index of growth by time was calculated considering the final larval total biomass and the days of bioassay. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

3.3 Analyses on larvae

3.3.1 Dry matter and water content

Larval dry mass is between 24,98% ± 0,71 % of larvae fed on cabbage and 33,69 % ± 1,11% of larvae fed on standard diet (Table 3.12, 3.13, Fig. 3.6, 3.7).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	31,47	24,64	28,59	23,60	28,49	29,13	30,28
2	34,91	26,64	28,65	25,96	30,34	26,16	25,90
3	34,70	29,54	31,02	25,39	26,11	28,57	25,88
Mean	33,69	26,94	29,42	24,98	28,31	27,95	27,35
SE	1,11	1,42	0,80	0,71	1,22	0,91	1,46

Table 3.12: Dry matter (%) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Dry matter of larvae fed on each substrate was measured at the end of the experiment.

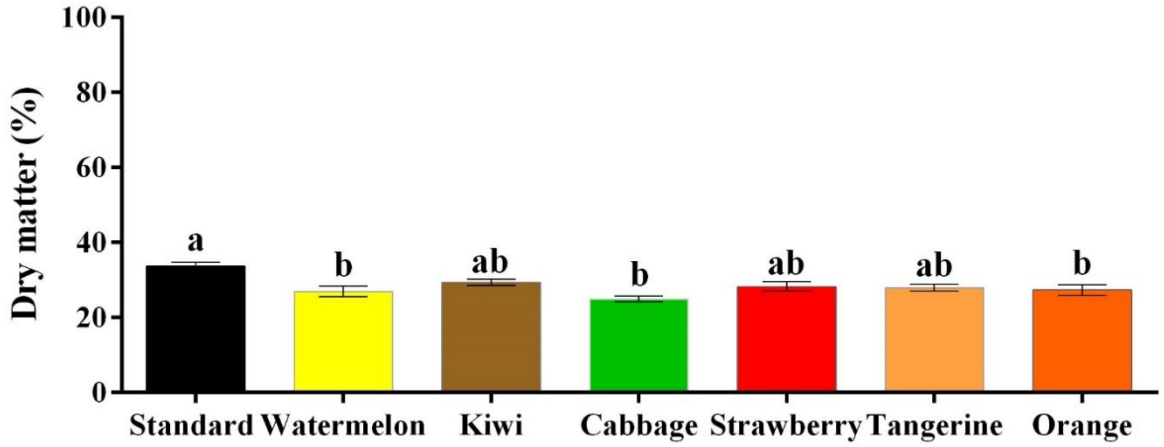

Figure 3.6: Dry matter of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Dry mass of BSF larvae was measured at the end of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and a Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,032).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	68,53	75,36	71,41	76,39	71,51	70,87	69,72
2	65,09	73,36	71,35	74,04	69,67	73,84	74,10
3	65,30	70,46	68,98	74,61	73,89	71,079	72,36
Mean	66,31	73,06	70,58	75,02	71,69	71,93	72,06
SE	1,11	1,42	0,80	0,71	1,22	0,96	1,27

Table 3.13: Water content (%) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Water content of larvae fed on each substrate was measured at the end of the experiment.

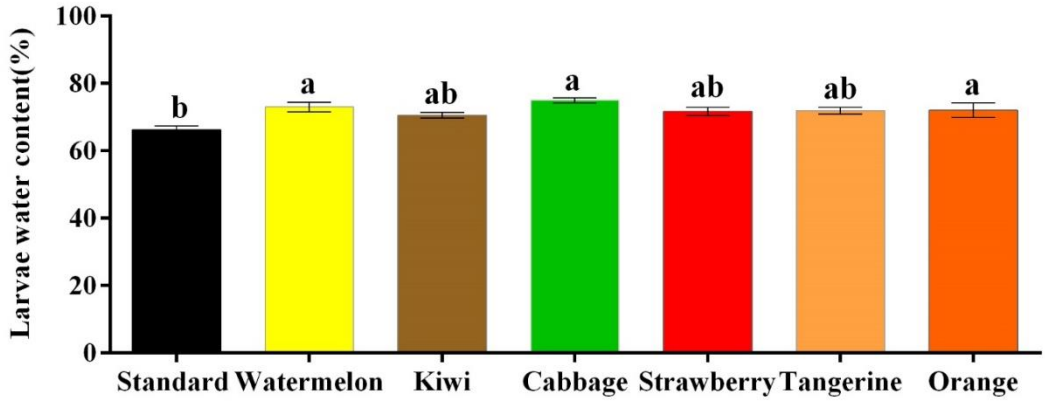

Figure 3.7: water content of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Water content of BSF larvae was measured at the end of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,029).

3.3.2 Total biomass

The larval total biomass differed among different substrates (Table 3.14, Fig. 3.8). The lowest larval total weight, represented by larvae fed on the mix of cabbage/savoy cabbage (0,58 kg ± 0,03 kg), significantly differed from the other weights. The highest larval total weight represented by larvae fed on strawberry (1,53 kg ± 0,02 kg) differed from all other samples.

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	1,38	0,97	1,00	0,52	1,52	1,15	0,98
2	1.28	0,92	1,04	0,62	1,57	1,15	0,92
3	1,20	0,85	0,92	0,60	1,51	0,92	0,93
Mean	1,29	0,91	0,99	0,58	1,53	1,07	0,95
SE	0,05	0,04	0,04	0,03	0,02	0,08	0,02

Table 3.14: Larval total biomass (kg) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Total biomass of BSF larvae was measured at the end of the experiment.

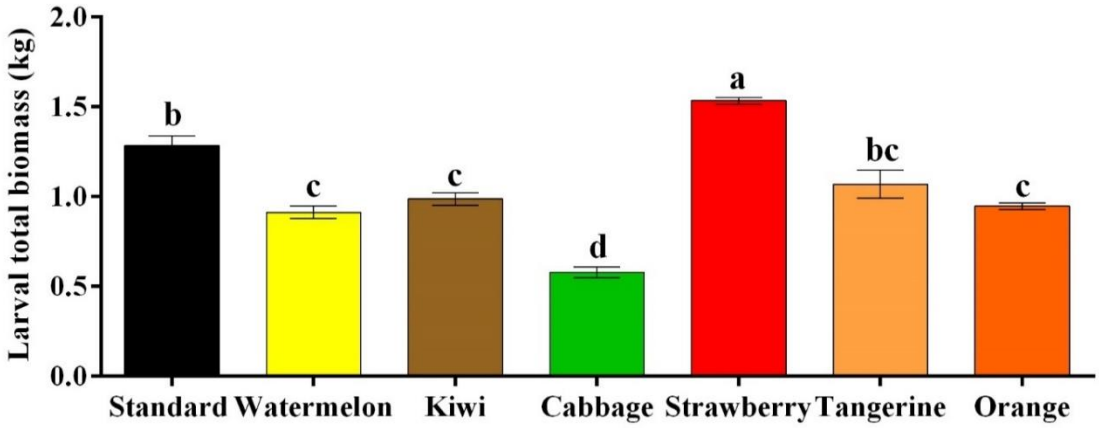

Figure 3.8: Larval total biomass (kg) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Total biomass of BSF larvae was measured at the end of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences (p value < 0,0001).

3.3.3 Ash and mineral content

Larvae fed on cabbage contain the highest ash content (14,71% ± 0,51%), while the lowest content is contained in larvae fed on tangerine (8,92 % ± 0,16 %) and orange (8,85 % ± 0,09%) (Table 3.15, Fig. 3.9).

The mineral composition was investigated in larvae fed on standard diet, strawberry, tangerine, and orange. The most abundant minerals, among the analysed substances, were calcium and potassium, while the lowest was sodium (Table 3.16). Differences were detected concerning calcium on larvae fed on orange, phosphorus on larvae fed on strawberry and magnesium among all the analysed samples.

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	12,99	12,17	10,48	15,73	10,10	9,19	8,87
2	13,29	13,97	11,46	14,18	10,39	8,92	8,72
3	12,39	12,14	11,44	14,25	10,32	8,65	9,04
Mean	12,89	12,76	11,13	14,72	10,27	8,92	8,85
SE	0,26	0,60	0,32	0,51	0,09	0,16	0,09

Table 3.15: Ash content (%) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Ash content of larvae fed on each substrate was measured at the end of the experiment, after incineration at 550 °C for 3 h in a Muffle Furnace.

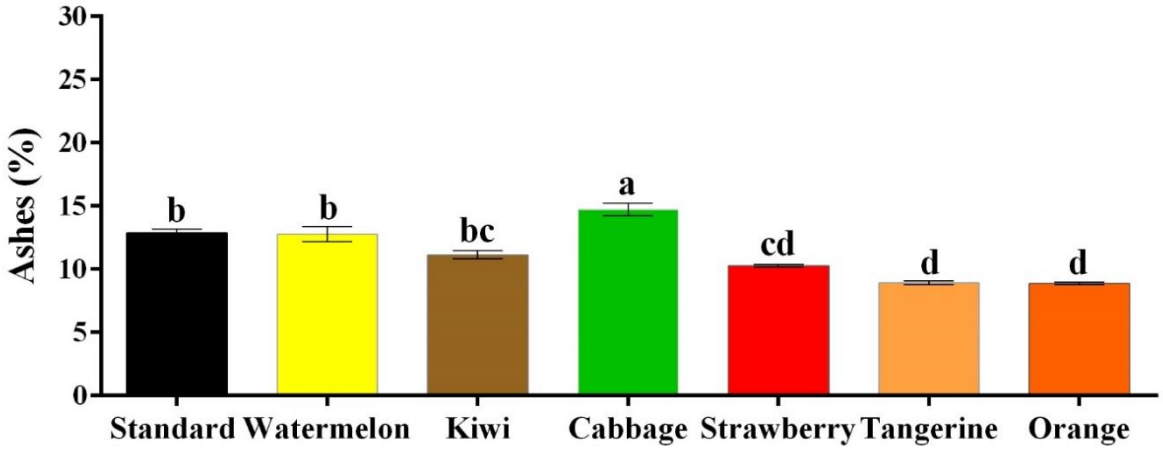

Figure 3.9: Ash content (%) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Ash content of BSF larvae was measured at the end of the experiment, after incineration at 550 °C for 3 h in a Muffle Furnace. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

Mineral	**Standard (Mean ± SE)**	**Strawberry (Mean ± SE)**	**Tangerine (Mean ± SE)**	**Orange (Mean ± SE)**
Calcium	14,36 ± 0,15^{a}	15,12 ± 0,95^{a}	16,18 ± 1,15^{a}	11,95 ± 0,13^{b}
Potassium	10,55 ± 0,55^{a}	9,02 ± 0,11^{a}	11,60 ± 1,17^{a}	9,24 ± 0,36^{a}
Phosphorus	5,16 ± 0,21^{a}	3,62 ± 0,22^{b}	6,32 ± 0,49^{a}	5,74 ± 0,23^{a}
Magnesium	3,91 ± 0,15^{a}	4,02 ± 0,05^{ab}	4,46 ± 0,11^{b}	3,32 ± 0,06^{c}
Sodium	0,68 ± 0,05^{a}	0,97 ± 0,07^{a}	1,11 ± 0,04^{a}	0,83 ± 0,15^{a}

Table 3.16: Mineral content (g/kg) of BSF larvae fed on standard diet, strawberry, tangerine, and orange. Mineral content was determined by atomic absorption spectrometry in an atomic absorption spectrophotometer after digestion in sulfuric acid and selenium powder. Calcium, potassium, magnesium, and sodium were measured at wavelengths of 422,7, 766,5, 285,2, 589,0, respectively. Phosphorus was determined using a UV–visible spectrophotometer (Shimadzu, Japan) at a wavelength of 880 nm. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (Calcium p value = 0,0219, Potassium p value = 0,0827, Phosphorus p value = 0,016, Magnesium p value = 0,0004, Sodium p value = 0,0431).

3.3.4 Protein and amino acid content

Protein content is about 38%-40% for all the samples, without statistically significant differences, except for larvae fed on cabbage, in which protein content is the highest value (48,04%) (Table 3.17, Fig. 3.10). Amino acid contend was analysed in larvae fed on standard diet, strawberry, tangerine, and orange. Although differences in amino acid concentration were detected, the less abundant amino acid was Tryptophan, while the most abundant were Leucine, Valine, and the couple Phenylalanine/Tyrosine, both characterized by a benzyl group (Table

3.18). No statistically significant differences were detected among larvae fed on strawberry, tangerine, and orange.

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	39,45	40,67	40,27	48,72	38,57	38,35	38,43
2	37,83	41,94	39,15	48,15	39,07	37,11	39,39
3	38,68	39,25	40,79	47,26	37,75	39,92	39,05
Mean	38,66	40,62	40,07	48,04	38,46	38,46	38,96
SE	0,47	0,78	0,48	0,42	0,39	0,81	0,28

Table 3.17: Protein content (%) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Protein content of larvae fed on each substrate was measured at the end of the experiment, using the Kjeldahl method.

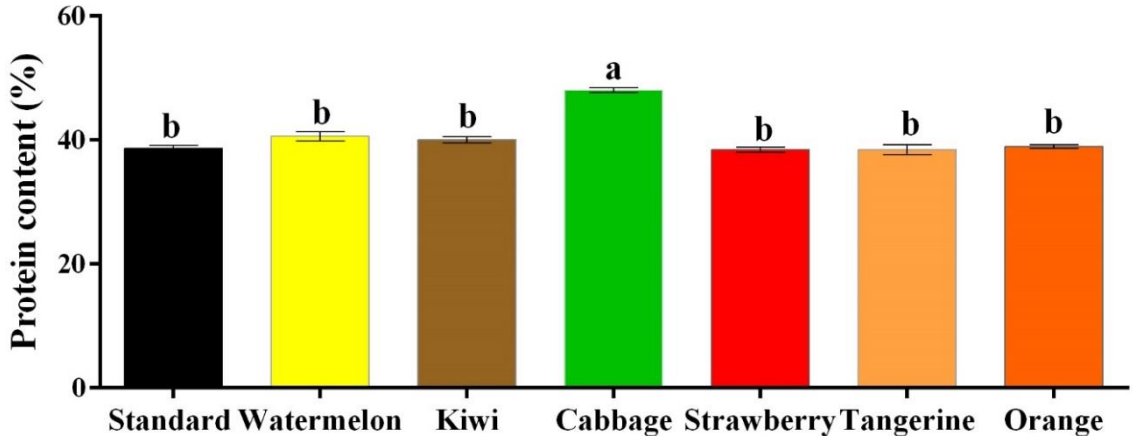

Figure 3.10: Protein content (%) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Protein content of BSF larvae was measured at the end of the experiment, using the Kjeldahl method. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

Amino acid	Standard (Mean ± SE)	Strawberry (Mean ± SE)	Tangerine (Mean ± SE)	Orange (Mean ± SE)
Lysine	3,37 ± 0,21[a]	2,67 ± 0,04[b]	2,65 ± 0,04[b]	2,63 ± 0,04[b]
Methionine	1,13 ± 0,01[a]	0,73 ± 0,01[b]	0,74 ± 0,01[b]	0,74 ± 0,01[b]
Methionine + Cysteine	1,72 ± 0,07[a]	0,99 ± 0,01[b]	0,93 ± 0,01[b]	0,98 ± 0,01[b]
Tryptophan	0,67 ± 0,01[a]	0,33 ± 0,01[b]	0,31 ± 0,01[b]	0,33 ± 0,003[b]
Threonine	2,65 ± 0,19[a]	1,68 ± 0,03[b]	1,35 ± 0,02[b]	1,31 ± 0,02[b]
Leucine	5,68 ± 0,21[a]	2,88 ± 0,05[b]	2,96 ± 0,04[b]	2,91 ± 0,04[b]
Isoleucine	3,07 ± 0,001[a]	1,83 ± 0,03[b]	2,21 ± 0,33[b]	1,96 ± 0,03[b]
Valine	4,13 ± 0,15[a]	3,14 ± 0,05[b]	2,89 ± 0,04[b]	2,85 ± 0,04[b]
Histidine	2,26 ± 0,07[a]	1,38 ± 0,02[b]	1,36 ± 0,02[b]	1,40 ± 0,02[b]
Arginine	3,36 ± 0,13[a]	2,11 ± 0,03[b]	2,18 ± 0,03[b]	2,11 ± 0,03[b]
Phenylalanine + Tyrosine	8,03 ± 0,20[a]	4,30 ± 0,08[b]	4,40 ± 0,06[b]	4,46 ± 0,06[b]

Table 3.18: Amino acid composition (%) of BSF larvae fed on standard diet, **strawberry, tangerine, and orange.** Specific amino acid composition was detected through HPLC analysis performed on oxidised and hydrolysed samples, following the procedure in 2009/152/EC. Tryptophan was determined separately, following the procedure N° 152/2009. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001, except Phenylalanine + Tyrosine p value = 0,0001, Lysine p value = 0,036).

3.3.5 Lipid and fatty acid content

It is possible to divide larvae in three groups, according to lipid content: larvae fed on standard diet and kiwi, with about 35%-37% of lipid, larvae fed on watermelon, strawberry, tangerine, and orange, with about 30% of lipids and larvae fed on cabbage, with the lowest amount of lipids (21, 49% ± 0,76%). Statistical differences were recorded among the three groups (Table 3.19, Fig. 3.11). Fatty acid content was analysed in larvae fed on standard diet, strawberry, tangerine, and orange. Although in larvae fed on standard diet and other samples differences in fatty acid concentration were detected, saturated fatty acids were the most abundant (Table 3.20).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	35,04	30,94	37,12	20,01	33,20	30,68	29,01
2	33,60	30,50	36,24	22,53	32,43	30,59	30,94
3	36,57	30,50	38,16	21,93	32,80	31,35	30,10
Mean	35,07	30,65	37,17	21,49	32,81	30,88	30,02
SE	0,86	0,15	0,55	0,76	0,22	0,24	0,38

Table 3.19: Lipid content (%) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Lipid content of larvae fed on each substrate was measured at the end of the experiment, through the lipid extraction in chloroform.

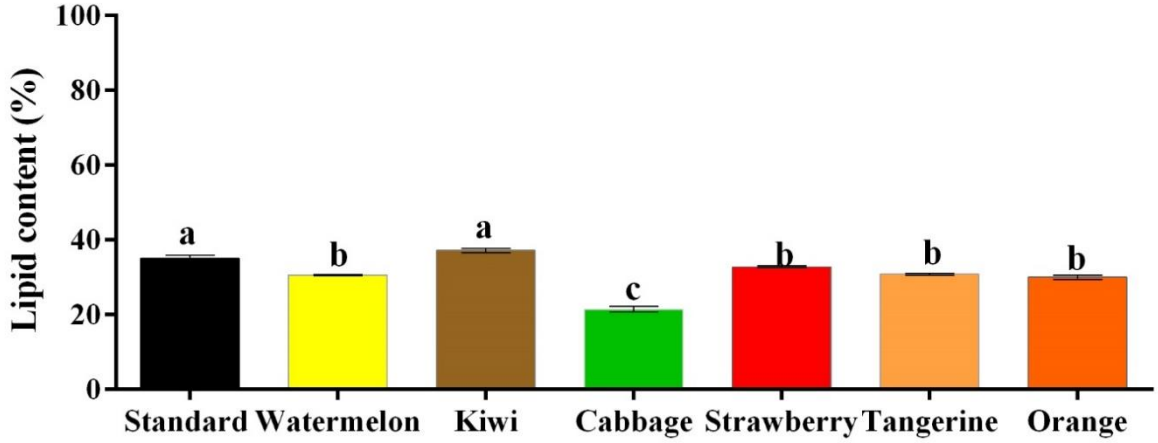

Figure 3.11: Lipid content (%) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Lipid content of BSF larvae was measured at the end of the experiment, through the lipid extraction in chloroform. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

Fatty acid	**Standard (Mean ± SE)**	**Strawberry (Mean ± SE)**	**Tangerine (Mean ± SE)**	**Orange (Mean ± SE)**
Saturated	$53,12 \pm 0,75^{b}$	$60,07 \pm 0,68^{ac}$	$60,82 \pm 0,79^{a}$	$59,13 \pm 0,19^{c}$
Monounsaturated	$26,02 \pm 0,09^{a}$	$21,11 \pm 0,34^{b}$	$21,35 \pm 0,29^{b}$	$21,21 \pm 0,23^{b}$
Polyunsaturated	$21,52 \pm 0,22^{a}$	$18,81 \pm 0,34^{b}$	$18,65 \pm 0,22^{b}$	$19,67 \pm 0,28^{c}$

Table 3.20: Fatty acid composition (%) of BSF larvae lipids fed on standard diet, strawberry, tangerine, and orange. Fatty acid composition was evaluated by infrared spectrometry. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

3.3.6 Fibre content

Fibre content was analysed in BSF larvae fed on standard diet, strawberry, tangerine, and orange (Table 3.21, Figure 3.12). No statistical differences were detected among different samples.

Replicate	Standard	Strawberry	Tangerine	Orange
1	35,04	34,05	33,33	31,70
2	33,60	34,20	34,47	33,20
3	36,57	32,83	35,20	35,53
Mean	34,13	33,69	34,33	33,48
SE	0,74	0,43	0,54	1,11

Table 3.21: Fibre content (%) of BSF larvae fed on standard diet, strawberry, tangerine, and orange. Fibre content was evaluated boiling each sample in a neutral and acid detergent.

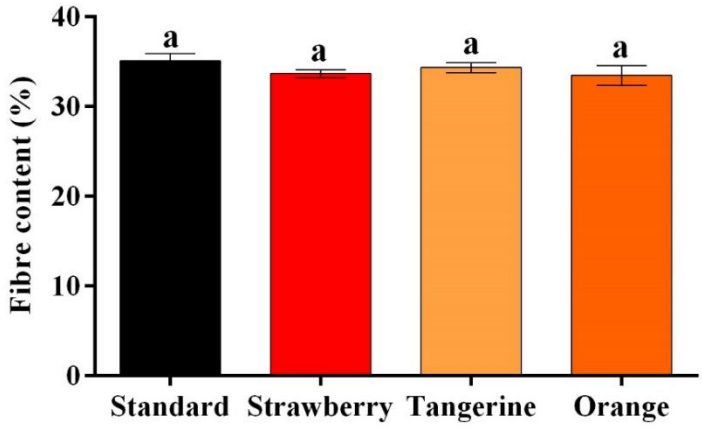

Figure 3.12: Fibre content (%) of BSF larvae fed on standard diet, strawberry, tangerine, and orange. Fibre content was evaluated boiling each sample in a neutral and acid detergent. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,5113).

3.4 Frass analysis

3.4.1 Dry matter and water content

At the end of the bioassay dry matter and water content of a frass derived from the bioconversion process of each substrate were evaluated. These two parameters slightly differed among the different experimental conditions: the highest water content was found in frass derived from larvae fed on strawberry (78,04% ± 1,54%), while the lowest derived from larvae fed on standard diet (61,81% ± 0,32%) (Tables 3.22, 3.23, Fig. 3.13, 3.14).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	38,81	38,17	35,53	31,82	24,63	32,11	32,02
2	38,08	33,12	38,54	32,01	21,95	31,91	31,34
3	37,70	23,50	32,13	31,02	19,29	28,97	35,34
Mean	38,20	31,60	35,40	31,62	21,96	31,00	32,90
SE	0,32	4,30	1,85	0,30	1,54	1,01	1,24

Table 3.22: Dry matter (%) of frass derived from BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Dry mass of frass was measured at the end of the experiment.

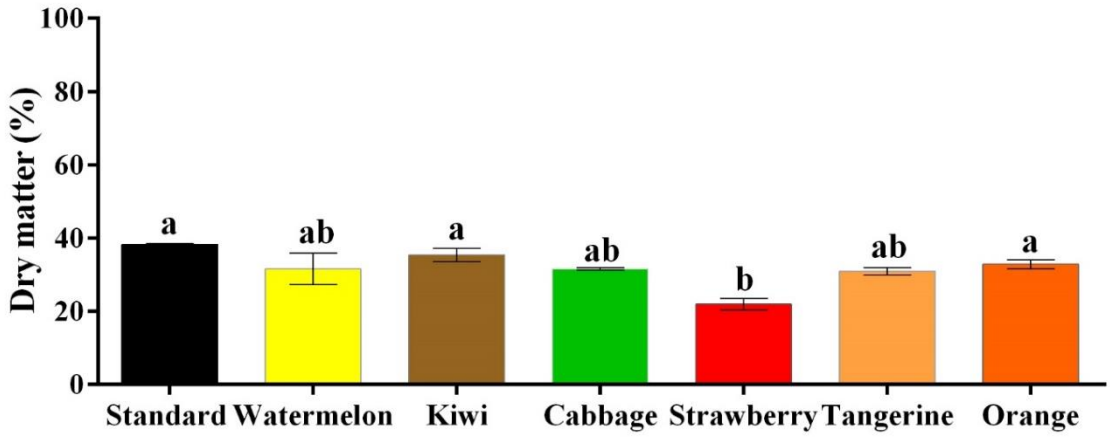

Figure 3.13: Dry matter (%) of frass derived from BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Dry matter (%) of frass was measured at the end of the experiment. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,0018).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	61,19	61,83	64,47	68,18	75,37	67,89	67,98
2	61,92	66,88	61,46	67,99	78,05	68,088	68,66
3	62,30	76,50	67,87	68,98	80,71	71,03	64,66
Mean	61,80	68,40	64,60	68,38	78,04	69,00	67,10
SE	0,32	4,30	1,85	0,30	1,54	1,01	1,24

Table 3.23: Water content (%) of frass derived from BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Water content of frass was measured at the end of the experiment.

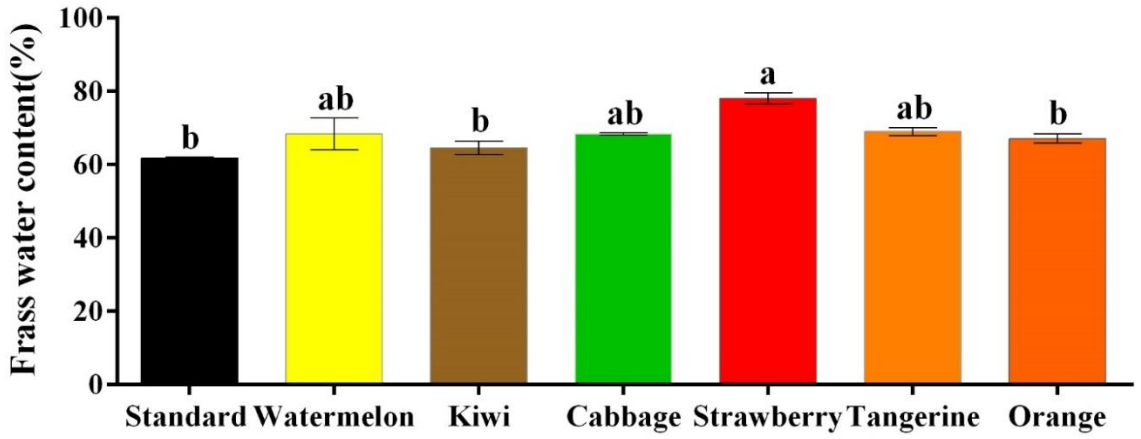

Figure 3.14: Water content (%) of frass derived from BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Water content (%) of frass was measured at the end of the experiment. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,0018).

3.4.2 Total weight

The weight of the frass, measured after the bioconversion process, slightly differed among treatments (Table 3.24, Fig 3.15).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	1,66	1,96	2,29	2,07	1,68	1,77	1,90
2	1,95	1,88	2,19	2,13	1,66	1,96	1,78
3	1,83	2,04	2,14	2,02	1,95	1,90	1,97
Mean	1,81	1,96	2,21	2,07	1,77	1,88	1,88
SE	0,08	0,05	0,04	0,03	0,09	0,06	0,06

Table 3.24: Frass total weight (kg) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Frass total weight was measured at the end of the experiment.

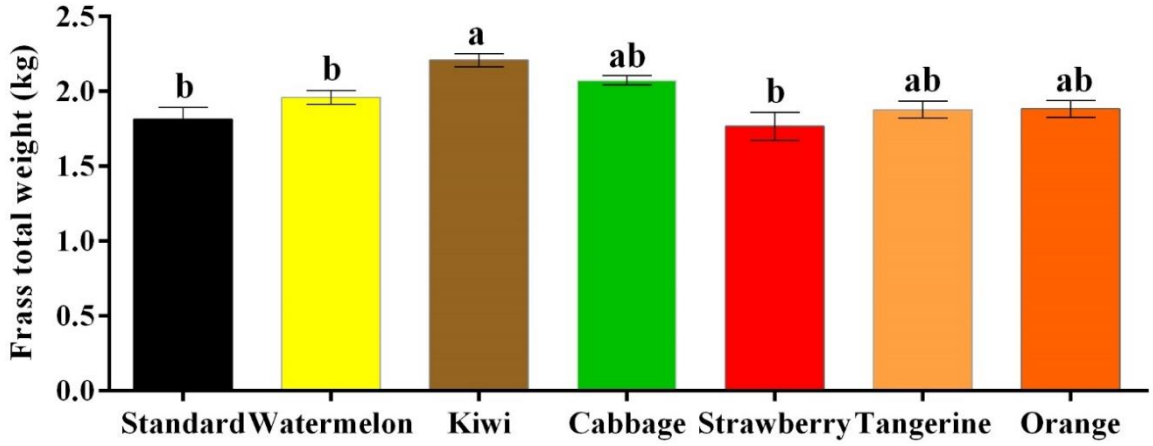

Figure 3.15: Frass total weight (kg) of BSF larvae fed on different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange). Frass total weight was measured at the end of the experiment. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and a Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,023).

3.4.3 Ash, mineral, and fibre content

Frass derived from BSF larvae fed on standard diet was also more in depth analysed, evaluating ash (Table 3.25), mineral (Table 3.26) and fibre content (Table 3.27).

The most abundant mineral, among the analysed substances, was potassium, while the lowest was sodium.

Ash content	Replicate1	Replicate2	Replicate3	Mean	SEM
	3,92	3,89	3,81	3,87	0,03

Table 3.25: Ash content of frass derived from BSF larvae fed on standard diet. Ash content of frass was measured at the end of the experiment, after incineration at 550 °C for 3 h in a Muffle Furnace.

Mineral	Replicate1	Replicate2	Replicate3	Mean	SEM
Calcium	1,58	1,60	1,43	1,53	0,05
Potassium	11,48	10,72	10,69	10,97	0,26
Phosphorus	5,44	5,35	5,34	5,38	0,03
Magnesium	3,05	3,11	2,98	3,05	0,04
Sodium	0,47	0,48	0,46	0,47	0,01

Table 3.26: Mineral content (g/kg) of frass derived from BSF larvae fed on standard diet. Mineral content of frass was determined by atomic absorption spectrometry in an atomic absorption spectrophotometer after digestion in sulfuric acid and selenium powder. Calcium, potassium, magnesium, and sodium were measured at wavelengths of 422,7, 766,5, 285,2, 589,0, respectively. Phosphorus was determined using a UV–visible spectrophotometer (Shimadzu, Japan) at a wavelength of 880 nm.

Fibre content	Replicate1	Replicate2	Replicate3	Mean	SEM
	14,50	14,75	14,79	14,68	0,09

Table 3.27: Fibre content (%) of frass derived from BSF larvae fed on standard diet. Fibre content of frass was evaluated boiling the sample in a neutral and acid detergent.

3.4.4 Chemical analysis

Frass derived from BSF larvae fed on standard diet was also more in depth analysed, evaluating organic substance, total nitrogen, organic carbon and ratio between carbon and nitrogen (Table 3.28).

Parameters	Replicate1	Replicate2	Replicate3	Mean	SEM
Total Nitrogen	0,53	0,57	0,58	0,56	0,018
Organic Carbon	2,89	2,81	2,76	2,82	0,034
Carbon/Nitrogen	5,46	4,91	4,73	5,03	0,22
Organic Substance	4,92	4,79	4,87	4,85	0,034

Table 3.28: Total nitrogen (%), organic carbon (%), carbon/nitrogen, and organic substance (%) of frass derived from BSF larvae fed on standard diet. Total nitrogen, organic carbon, and organic substance were detected according to the following Regulations: UNI EN 13654–1:2001, UNI EN 13137:2002 and DM 13/09/1999.

3.4.5 Microorganism analysis

Microbiological analysis was performed on frass derived from BSF larvae fed on standard diet, detecting high general microbial and Enterobacteriaceae count, without *Salmonella* spp colonies (Table 3.29).

Microorganism	Replicate1	Replicate2	Replicate3
Microbial count at 30 °C [CFU/g]	> 300000	> 300000	> 300000
Enterobacteriaceae count [CFU/g]	> 150000	> 150000	> 150000
Salmonella spp	absent	absent	absent

Table 3.29: Microorganism analysis of frass derived from BSF larvae fed on standard diet. Bacterial count at 30 °C, Enterobacteriaceae count, and *Salmonella* spp were detected according to the following Regulations UNI EN ISO 4833-1, ISO 21528-2, and UNI EN ISO 6579-1.

3.5 Bioconversion parameter analysis

3.5.1 Substrate reduction

The percentage of substrate reduction slightly differed among substrates (Table 3.30, Fig 3.16)

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	76,23	72,00	67,29	70,43	75,94	74,63	72,84
2	72,17	73,14	68,71	69,71	76,23	71,94	74,64
3	73,90	70,86	70,00	71,14	72,11	73,30	71,81
Mean	74,10	72,00	68,67	70,43	74,76	73,29	73,10
SE	1,18	0,66	0,78	0,41	0,94	0,55	0,58

Table 3.30: Substrate reduction (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange) after BSF feeding. Substrate reduction was calculated considering the initial substrate (7 kg) and the final product, made of frass and substrate residue.

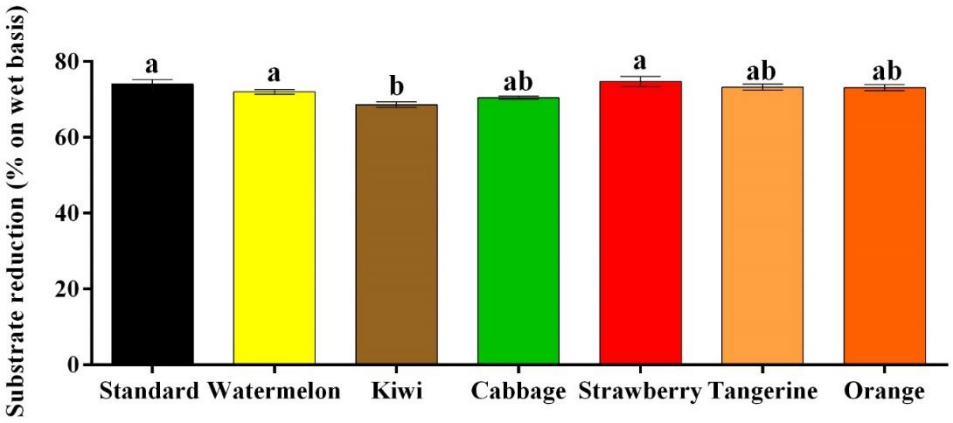

Figure 3.16: Substrate reduction (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange) after BSF feeding. Substrate reduction was calculated considering the initial substrate (7 kg) and the final product, made of frass and substrate residue. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,034).

3.5.2 WRI and ECD

For each experimental condition different parameters, strictly related to bioconversion, were calculated: the WRI (Waste Reduction Index), which indicates the ability of the larvae to reduce the amount of administered food; and the ECD (Efficiency of Conversion of Digestive Feed), which indicates the efficiency of the larvae in converting the food taken into larval biomass.

WRI slightly differed among different substrates, except for the standard diet in which WRI had the highest value (8,23 ± 0,13) (Table 3.31, Fig. 3.17).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	8,45	5,54	4,81	4,70	5,42	5,33	4,86
2	8,02	5,63	4,91	4,64	5,44	5,14	4,98
3	8,21	5,45	5,00	4,74	5,15	5,24	4,79
Mean	8,23	5,54	4,90	4,70	5,34	5,24	4,88
SE	0,12	0,05	0,05	0,03	0,09	0,05	0,06

Table 3.31: Waste reduction index (WRI) of different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange) after BSF feeding. Waste reduction index was calculated comparing the percentage of substrate reduction and time of bioassay.

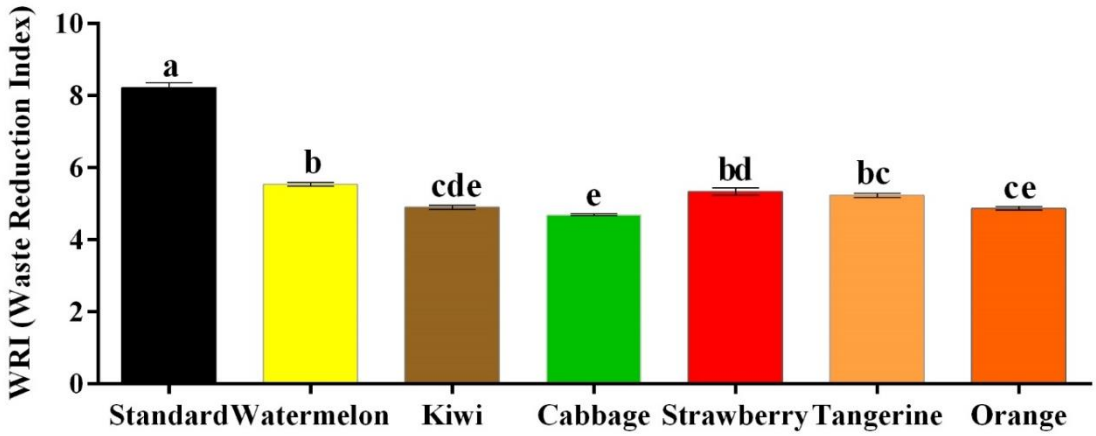

Figure 3.17: Waste reduction index (WRI) of different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange) after BSF feeding. Waste reduction index was calculated comparing the percentage of substrate reduction and time of bioassay. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

ECD slightly differed among different substrates, except for strawberry substrate diet in which ECD had the highest value (0,30 ± 0,001) and the mix of cabbage/savoy cabbage in which ECD had the lowest value (0,12 ± 0,004) (Table 3.33, Fig. 3.18).

Replicate	Standard	Watermelon	Kiwi	Cabbage	Strawberry	Tangerine	Orange
1	0,26	0,19	0,21	0,10	0,29	0,22	0,19
2	0,25	0,18	0,22	0,13	0,29	0,23	0,18
3	0,23	0,17	0,19	0,12	0,30	0,18	0,19
Mean	0,25	0,18	0,20	0,12	0,30	0,21	0,19
SE	0,009	0,004	0,01	0,004	0,001	0,01	0,003

Table 3.32: Efficiency of Conversion of Digestive Feed (ECD) of different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange) after BSF feeding. ECD was calculated comparing larval biomass and intake food, as the difference between initial substrate and the final frass.

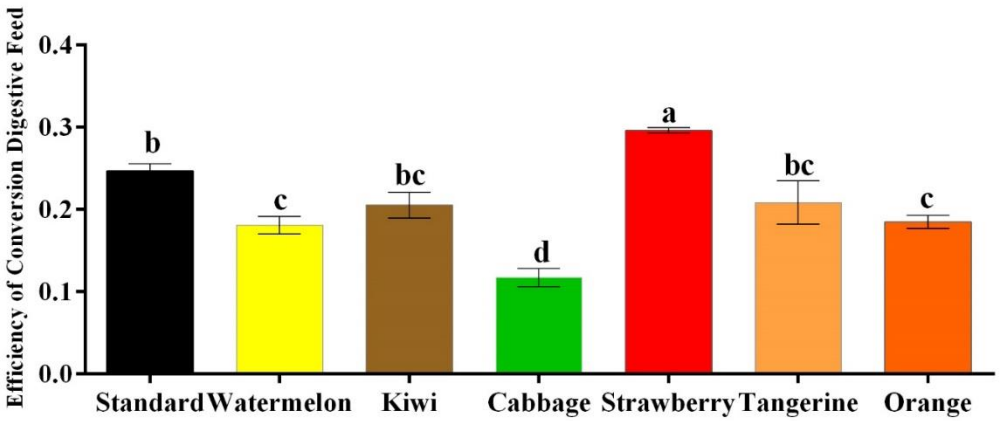

Fig 3.18: Efficiency of Conversion of Digestive Feed (ECD) of different substrates (standard diet, watermelon, kiwi, mix of cabbage/savoy cabbage, strawberry, tangerine, orange) after BSF feeding. ECD was calculated comparing larval biomass and intake food, as the difference between initial substrate and the final frass. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

3.6 Larval meal

Preliminary data were also provided for integral meal derived from BSF larvae fed on standard diet. Integral meal analysed parameters are reported in Table 3.33.

Parameters	Replicate 1	Replicate 2	Replicate 3	Mean	SEM
Water content	11,97	11,26	11,05	11,43	0,28
Dry mass	88,03	88,74	89,95	88,91	0,56
Proteins	41,97	42,71	42,46	42,38	0,22
Lysine	3,12	4,00	4,05	3,72	0,30
Methionine	1,30	1,24	1,15	1,23	0,05
Methionine + Cysteine	1,80	2,28	1,60	1,89	0,20
Tryptophan	0,75	0,81	0,66	0,74	0,0
Threonine	3,08	2,90	2,90	2,96	0,06
Leucine	6,13	6,02	6,53	6,23	0,16
Isoleucine	3,05	3,56	3,53	3,38	0,16
Valine	4,77	3,92	4,97	4,55	0,32
Histidine	2,20	2,89	2,00	2,46	0,26
Arginine	3,49	4,40	3,26	3,72	0,35
Phenylalanine + Tyrosine	8,72	8,60	9,12	8,81	0,16
Lipids	19,93	18,64	18,67	19,08	0,43
Saturated fatty acid	43,54	46,43	45,76	45,24	0,87
Monounsaturated fatty acid	35,35	28,19	30,99	31,51	2,08
Polyunsaturated fatty acid	21,11	25,37	23,24	23,24	1,23
Fibres	11,72	10,68	12,54	11,65	0,54
Ashes	11,92	12,88	10,53	11,78	0,68
Calcium	19,20	23,29	21,86	21,45	1,20
Potassium	9,33	8,12	9,89	9,12	0,52
Phosphorus	6,50	5,10	5,37	5,66	0,43
Magnesium	3,45	4,39	3,71	3,85	0,28
Sodium	0,64	0,78	0,75	0,72	0,04

Table 3.33: Dry mass (%), protein (and specific amino acid) content (%), lipid (and specific fatty acid) content (%), ashes (%) and mineral content (g/kg) and fibres (%) of BSF integral meal derived from larvae fed on standard diet.

4. DISCUSSION

The aim of this PhD project was to study the bioconversion process of organic by-products from the agri-food process mediated by *Hermetia illucens* (Diptera: Stratiomyidae) larvae. Many insects consume organic waste materials and convert them into various resources (Fowles and Nansen, 2019). The advantage of the bioconversion process is triple: the reduction of wastes, the gain of nutrients from insects and the possibility to obtain final products of high economic and biological value. *H- illucens*, also known as Black Soldier Fly (BSF), is one of the most important bioconverter insect and many studies has been carried out in order to deeply understand this process and the effect of the substrates on which this Diptera feed. Indeed, larval growth rate, total weight at the end of the process of bioconversion and nutrient composition (protein, lipid, and mineral contents) are strictly connected to the starting substrate (Nguyen, 2013; Newton, 2005; Rehman *et al.*, 2017; Liland *et al.*, 2017; Jucker *et al.*, 2017; Liu *et al.*, 2017; Tschirner and Simon, 2015; Meneguz *et al.*, 2018; Fadhillah and Bagastyo, 2020).

This study, in which six different substrates from the agri-food chain were analysed, also confirm that many parameters connected to larval growth, larval composition and to the bioconversion processes are influenced by the substrate, as it is reported in many previous studies (Nguyen *et al*., 2013; Rehman *et al*., 2017; Liland *et al.*, 2017; Jucker *et a*l., 2017; Liu *et al*., 2017; Spranghers *et al*. 2017 Meneguz *et al.*, 2018; Julita *et al.*, 2019). The six substrates from the agri-food chain were also compared to a standard diet (Gainesville diet) composed of 30% alfalfa, 50% wheat bran and 20% corn meal, corresponding to about 13,9 % protein, 4% of lipids, 12,6% fibres. Nutritional values of substrates from the agri-food chain, on the contrary, are different and range from 0,40% to 1,50% for proteins, from 0,08% to 0,3% for fats and from 0,92% to 4,5% for fibres (USDA). The percentage of moisture content ranging from 70,9% of standard diet to 89,3% of watermelon, assuring the right degree of humidity for BSF development.

BSF was able to feed and correctly grow on all analysed substrates, although some differences are observed, especially comparing the standard diet and the diets made up exclusively of vegetable or fruit. The reaching of prepupal stage, identified as larvae stop feeding and lose weight, was faster in larvae fed on control larvae (14 days after eggs hatching) and slower in larvae fed on substrate from the agri-food chain: 17/19 days after eggs hatching, with delay comparing to control larvae. Larvae fed on standard diet, tangerine and orange reach an average mean of maximum weight similar to each other, but larvae fed on fruit-based diet have a delay in time to reach it.

Growth rate, calculated comparing development time and the gained weight, was the highest in larvae fed on standard diet and strawberry, while the lowest in larvae fed on the mix of cabbage/savoy cabbage. Also, the index of growth by time, calculated as the ratio between the total larval biomass and the time needed to achieve this biomass, was the highest in larvae fed on standard diet and strawberry, while the lowest in larvae fed on the mix of cabbage/savoy cabbage. The highest growth rate and the highest index of growth by time in these two substrates is given by the balance in development time and achieved weight: indeed, control larvae reached a minor weight compared to larvae fed on strawberry but in a faster time, on the contrary larvae fed on strawberry reached a heavier weight, but in slower time. Other substrates show a similar growth rate, except for larvae fed on cabbage.

Standard diet, that is accurately balanced from a nutritional point of view, is the best diet for BSF development, associated with the gained weight and the short development time, as previously reported by Cammack and Tomberlin (2017), affirming that larvae reared on a balanced diet of protein and carbohydrates developed faster. On the other hand, the possibility to correctly rear BSF on "simplified" diet, based exclusively on fruits or vegetables, that are by-products or wastes from the agri-food chain, even if with delay in time, is a good opportunity to reduce and dispose wastes obtaining high value products (larvae rich in proteins and lipids).

Among fruit diets, the strawberry-based diet was the best one in terms of larval weight and time of growth, while the cabbage-based diet was the worst.

It is possible to hypothesize that plant materials such as herbs and leaves cause a delay in growth and in development time, due to the inability of the larvae of BSF to digest the high content of lignin and cellulose in these substrates. Although cellulose-, chitin-, and lignin-degrading enzymes have been characterized in BSF gut microbiota (Muller *et al.*, 2017) and these enzymes are determinant for the BSF ability to feed on vegetable substrates (Karagodin *et al.*, 2017), the use of substrates rich in cellulose or lignin slows down both the growth and weight gain of BSF larvae. Our results concerning cabbage diet (prolonged time of feeding and scant growth rate) confirms data in the literature in which the use of a mix of plant substrates, such as beet pulp, lettuce, green beans, or banana peel limit and make more difficult BSF development, but they do not completely prevent it (Jucker *et al.*, 2017; Karagodin *et al.*, 2017; Isikiba *et al.*, 2019).

Also, the final larval yield is strictly related to substrate, with a maximum yield in larvae fed on strawberry and a minimum yield in larvae fed on cabbage. Although the maximum weight gained is quite different among substrates, concerning the total final biomass it is important to consider that when larvae stop feeding, they empty their gut and lose weight, preparing

themselves to pupate (Ghaly and Alkoaik, 2009). For this reason, the final total biomass of larvae reared on each substrate is nearly directly dependent from the average final weight and the initial number of larvae: the lack of differences detected among substrates could be due to this physiological weight loss.

This study confirms that, beside the growth rate, also the final composition of nutrients highly depends on the growing substrate, that differs in nutritional composition. Standard diet, as previously said, is a nutritionally proportionate substrate, while fruits and vegetables are really unbalanced. In comparison to larvae fed on substrates rich in proteins, many examples of prolonged feeding phase to reach their critical weight are reported: larvae fed on substrates exclusively composed by fruit could be strongly influenced by the low quantity of nutrients (Jucker *et al.*, 2017; Meneguz *et al.*, 2018). It is well known that, in insects, one of the most important variables influencing time of development and larval body weight is the quantity and the quality of nutrients in the feeding substrate (Friend, 1958; House, 1961; Oonincx *et al.*, 2015; Tschirner and Simon, 2015).

From these first data it is possible to assert that BSF larvae are able to feed on different kinds of organic substrates from the agri-food chain. Even if development time on all substrates considerably differs from standard diet, the possibility to correctly feed larvae on these substrates and the suitable larval growth are the first valuable information obtained from this study. Moreover, BSF after reaching the final larval stage, correctly pupate and finally emerge to adult instar.

Many studies, at laboratory scale, reported that BSF can correctly fed on substrates from the agri-food chain, to the best of our knowledge, this is one of the first research carried out at industrial scale, in order to understand the real possibility to dispose of large number of by-products using them as substrate for BSF. Laboratory scale research is characterized by lower larval number and substrate amount: hundred larvae on grams of substrate vs thousand larvae on kilograms of substrate for industrial scale. Comparison between mobilization from laboratory to the industrial scale could produce significant change, could be not linear, as in this context, and different factors could influence BSF production and performances.

Many example of BSF reared al laboratory scale on fruits are reported: in Nguyen *et al.* (2013), 150 larvae were fed on a mix of fruits and vegetables (28 °C, 65 ± 10% RH, from 6 g to 11 g of diet each day) reaching the pupal stage in a wide range from 21,67 ± 0,333 to 40,33 ± 1,542 days and with an average weight of about 130 mg, with huge developmental delay; in Meneguz *et al.* (2018), 100 larvae fed on a mix of fruit, containing also strawberries, oranges, tangerines,

and kiwis (27 ± 0,5° C, 70 % ± 5 % RH, approximately 50 g of diet each day), reached the pupal stage in 28,2 ± 0,98 days with an average weight of about 0,006 g. In Cappellozza *et al.* (2019), a diet composed of a mix of fruit and vegetables, including orange and kiwi, was tested in laboratory and mass rearing conditions (27 ± 1°C, RH 50 ± 0.5%): 200 larvae vs 5000, with 40 g and 600 g of diet for each day of experimental trial, respectively. BSF larvae reached the pre-pupal stage in 45 and 31 days respectively, with a final individual weight of 183,3 ± 6,6 mg and 230,8 ± 26,9 mg, respectively.

Although the slower development time in laboratory scale experiments could be related to different rearing conditions, such as lower temperature and relative humidity (Chia et al., 2018b), in similar rearing conditions, industrial scale really improve growth performance, as it is also possible to observe in our experiments, in which development time is indeed shorter compared to literature data. As concerns final larval weight, it is possible to assert that mass rearing experiment broadly guarantees a major final weight, except for larvae fed on the mix of cabbage/savoy cabbage. Moreover, as is known, larvae reared individually or in small groups grow more slowly than larvae reared in large groups, because the large number of larvae generates heat that positively influences BSF development performances (Long, 1953; Gere, 1956; Slone and Gruner, 2007; Parra *et al.*, 2015). The environmental heat (rearing condition) and the heat generated by BSF larvae themself, can also influence the regulation of larval gut enzymes, inducing a faster digestive activity, which optimum is about 45 °C (Bonelli *et al*., 2019).

Although larval dry matter does not differ substantially between the various diets, some differences can be evaluated in nutrient composition, such as ash, protein, and lipid content.

Ash content, ranging from 8,87% to 14,72%, can be considered slightly higher to that found in previous research: some studies, indeed, reported that ash content could be around 4% for larvae fed on different kind of spent grain, 8-9% for larvae fed on supermarket waste and around 10-13,5% for larvae fed on chicken feed (Liu *et al.*, 2017; Spranghers *et al.*, 2017; Bava *et al.*, 2019; Smets *et al.*, 2020). For larvae fed on by-products of agri food chain, as in our study, ash content is around 5,6% for fruit wastes, much lower than our findings, and from 9,6% to 14,3% for vegetable wastes, consistent with ash content in larvae fed on cabbage (Jucker *et al.*, 2017; Spranghers *et al.*, 2017). Also mineral content was strictly connected to substrate: larvae fed on chicken feed has higher calcium content compared to our finding, while phosphorus and sodium are similar (Liu *et al.*, 2017); larvae fed on chicken manure, kitchen waste and spent grain have a lower calcium, potassium, phosphorus, and magnesium content than our samples (Shumo *et*

al., 2019). Sodium content, on the contrary, is higher, except for larvae fed on spent grain in which it is similar to our findings (Shumo *et al.*, 2019). For larvae fed on supermarket wastes, calcium and sodium content are higher than our findings, magnesium content is similar, while potassium and phosphorus are lower (Smets *et al.*, 2020). Comparing larvae fed on other vegetable wastes, calcium is lower in our finding, while some other minerals are similar or higher (potassium, phosphorus, magnesium, and sodium) (Spranghers *et al.*, 2017). In addition, Makkar *et al.* (2014) reports that calcium content in BSF larvae reared on animal manure ranged between 5,0% and 8,6%, a higher value compared to ours.

Protein content does not statistically differ among substrates, except for larvae fed on cabbage, in which protein composition is about 48%. This data is important, as the comparison between standard diet and substrates from the agri-food chain highlights that, although the original protein content is low in these substrates, larvae are able to convert them into high values. Similar values are found by Spranghers *et al.*, (2017) and Meneguz *et al.*, (2018) on vegetable wastes. On the contrary, our results show a greater protein content compared to other studies: for example, in Jucker *et al.* (2017) and in Nguyen *et al.*, (2015), protein content of larvae fed on vegetable and fruit wastes are around 10% and 13%. Comparing the specific amino acid content of larvae fed on standard diet and fruit wastes with that of larvae fed on other substrates it is possible to notice differences: all analysed amino acids have lower concentration compared with amino acid profile described in Smets *et al.*, (2020), for larvae fed on supermarket wastes. Lysine, isoleucine (except for larvae fed on strawberry), arginine, valine and threonine (except for larvae fed on tangerine and orange) have higher concentration in our samples compared to larvae fed on chicken feed, vegetable and restaurant wastes (Liu et al., 2017; Spranghers *et al*., 2017); isoleucine has higher concentration also compared to larvae fed on kitchen wastes, chicken manure and spent grain, while valine has higher concentration compared to larvae fed on kitchen manure and arginine compared to larvae fed on chicken manure (Shumo *et al.*, 2019). Similar amino acid content is found for methionine in larvae fed on chicken feed, vegetable and restaurant wastes (Liu et al., 2017; Spranghers *et al*., 2017) and leucine in larvae fed on chicken feed, chicken manure, spent grain, vegetable, restaurant, and kitchen wastes (Liu et al., 2017; Spranghers *et al*., 2017; Shumo *et al.*, 2019). In this last case, leucine content in the standard diet is even higher. Regarding the profile of the selected and analysed amino acids, in larvae fed on supermarket wastes (Smets *et al*., 2020), chicken feed, restaurant and vegetable wastes (Spranghers *et al.*, 2017) is very similar to our result, with tryptophan as less abundant amino acid and leucine, valine and phenylalanine/tyrosine as the most concentrated ones. This complex picture clearly indicates that substrates significantly affect protein and amino acid

content and the choice of by-products on which feed BSF is critical for obtaining larvae of high-quality level (Lalander *et al.*, 2019). Previous studies showed that the amino acid profile of several edible insects is comparable to that of soybean meal, so they can be used as alternative feed according to each country's regulations (Veldkamp and Bosch, 2015).

Instead of proteins, the lipid content statistically differs among substrates. Results can be divided in three groups: standard diet and kiwi, with the higher fats content (35-37%), watermelon, strawberry, tangerine, and orange (29-31%) and larvae fed on cabbage, with the lower fats content (21,49%). Literature data asses in a wide range, too: in Liu *et al.*, (2017) lipid content in larvae fed on chicken feed is about 28%, in Spranghers *et al.*, (2017) is 37,1%, 40,7 % for fruit wastes and 26% for vegetable wastes in Meneguz *et al.*, (2018). Fats content is around 20% for fruit wastes and almost totally absent for vegetable wastes in Jucker *et al.*, (2017), similarly to Nguyen *et al.*, (2015), in which fats are 2,2%. Generally, fed BSF larvae on vegetable wastes rather than on fruits wastes (as on cabbage in our experiment) induces a minor concentration of lipid content, for this reason diet can be correctly balanced according to specific need. Also the specific fatty acid composition can vary according to substrate, and related to literature data concerning other substrates, our results highlighted a similar tendency in saturated and unsaturated fatty acid ratio, with saturated as the most abundant ones (Juker *et al.*, 2017; Spranghers *et al.*, 2017; Meneguz *et al.*, 2018; Smet *et al*., 2020).

Fibre content, analysed on larvae fed on standard diet, strawberry, tangerine, and orange, was higher than larvae fed on other vegetable and fruit wastes (Meneguz *et al.*, 2018).

All these results about protein, amino acid, lipid, fatty acid, ash, and mineral content highlight and confirm that the original substrate strongly influences the final larval composition. Consequently, the possibility of feeding larvae on different organic substrates allows to obtain a final product (larval biomass) highly diversified for the needs of the market. Indeed, depending on the nutritional needs, it will be possible to choose on which waste from the agri-food chain feed the larvae. For example, if larvae with high protein content are requested, BSF can be fed on vegetable mix, cabbage/savoy cabbage, in our case; if larvae with high lipid content BSF can be fed on fruit by-products, kiwi in our case. Balanced protein and lipid content are also detected in all the other substrates, that can be used alternatively as substrates to obtain larvae of high nutritional value for pet food or aquaculture, according to European Regulations (142/2011, 999/2001, 68/2013, 2283/2015, 893/2017).

Preliminary data were also obtained for integral meals derived from BSF larvae fed on standard diet, in order to use it in aquaculture. Protein content was higher than mature larvae, ash and mineral content are similar, while lipid content is lower. As BSF meal is complete and rich in all nutrients it is possible to use it in aquaculture.

The bioconversion residue, made of BSF frass and not converted organic matter, is another secondary product of high biological and economic value, as it is comparable to organic soil conditioner. Frass amount, composed of larval faeces and not consumed food, ranged between 1,77 kg and 2,21 kg. It can be a good source of nitrogen and phosphorus, but also other micronutrient, including calcium, potassium and magnesium and the high contribution on chitin content, derived from BSF larval and pre-pupal exuviae (Schmitt and Vries, 2020). Although several studies use BSF frass in crop fertilization, data on frass compositions are rarely reported and it is not so easy to understand how the composition of the insect frass changes in according to the original substrate (Choi *et al.*, 2009; Alattar *et al*., 2016; Wu *et al*., 2020). Our analysis differs from results obtained in a similar study conducted by Setti *et al.*, (2019) on frass derived from BSF fed on standard diet: total nitrogen, organic carbon, the ratio carbon/nitrogen, and some micronutrients, such as calcium, magnesium and sodium are lower in our experiment. Another factor influencing the quality of the fertilized crops is the presence of microorganisms in frass. In our experiment CFUs (colony-forming unit) of microbial count at 30°C and Enterobacteriaceae count are higher, compared to Setti *et al.* (2019) trial. This could be related to the different rearing condition: in Setti *et al.* (2019) a laboratory scale experiment, with 500 larvae fed on about 2 kg of substrate, was carried out, on the contrary in our mass rearing experiment 10.000 larvae were fed on 7 kg of substrate. A significant result is linked to the total absence of Salmonella spp in both the experiments: BSF activity, indeed, especially through the AMPs production significantly decreases the presence of pathogenic bacteria, such as *Escherichia coli*, *Salmonella* spp., and other Gram-negative bacteria (Erickson *et al*., 2004; Lalander *et al.*, 2013; Auza *et al*., 2020). Further studies need in order to assess these differences in nitrogen, carbonic and mineral content and evaluate the effect of frass as a biofertilizer with a higher economic value, compared to conventional compost materials.

Beside these secondary products of high biological and economic value obtained at the end of the bioconversion process, it is not to underestimate the high ability of BSF larvae to reduce the organic substance on which they feed. The high percentage of substrate reduction highlights the high bioconversion ability of BSF, which is able to reduce the initial substrates from 68,66% (of kiwi substrate) to 74,76% (of strawberry substrate), showing the great potential of BSF

larvae in the consumption and decomposition of vegetable and fruit wastes (Nguyen *et al.*, 2013; Nguyen *et al.*, 2015). Except for kiwi, the percentage of substrate reduction slightly differed among substrates, showing that BSF larvae can easily reduce products from the agri-food chain, both wastes and standard diet. Many studies confirm the efficiency of substrate reduction: around 60-70% for vegetable substrate, around 65% for fruit substrate or fruit and vegetable mix (Saragi and Bagastyo, 2015; Meneguz *et al*., 2018; Giannetto *et al.*, 2019; Mentari *et al.*, 2020). This percentage begins to decrease when other components, for example fish, were added to vegetable and fruit (around 50%) and it is even lower for exclusively fish waste (around 40%) (Saragi and Bagastyo, 2015). Lower values were also recorded for chicken manure substrate and faecal sludge (37%-55%) (Oonincx *et al*., 2015; Rehman *et al.*, 2017). Generally, substrates from the agri-food chain are extremely reduced, but it is important to evaluate each potential food, as it cannot be perfectly reduced by BSF larvae: cassava peel, for example can be reduced to a maximum of 37% (Supriyatna *et al*., 2016), grinded rice straw to a maximum of 32% (Manurung *et al*., 2016) and cucumber, tomato, and mustard green around 40%, if they are used as individual substrates (Mentari *et al.*, 2020). Substrate reduction can reach high percentages not only with organic substances from the agri-food chain, but also with other kinds of wastes, for example of restaurant origin, that can reach up to 82% (Nyakeri *et al*., 2017). These findings clearly display that BSF larvae are able to feed on organic matter from the agri-food chain, with differences among substrates: for this reason, if on one hand it is possible to take this ability for granted, on the other hand it is worthy to test every single substrate.

In addition to substrate reduction, other parameters, strictly related to the bioconversion process were taken into account: the WRI (Waste Reduction Index), which indicates the ability of the larvae to reduce the amount of administered food and it is related to substrate reduction and day of experimental trail; and the ECD (Efficiency of Conversion of Digestive Feed), which indicates the efficiency of the larvae in converting the taken food into larval biomass and is related to substrate reduction and the final larval biomass. Standard diet, as it is balanced in proteins and carbohydrates, has the highest WRI and ECD. The WRI values of the other substrates do not differ much from each other, indeed both the percentage of substrate reduction and the development time are quite similar between the various experimental conditions. On the contrary, significant differences are detected on ECD value: the highest value is related to larvae fed on strawberry, the lowest value is related to larvae fed on cabbage. Higher WRI and ECD indicates that the larvae are efficient at reducing waste. Literature data reported that WRI values of agri-food substrates range between 2,8 and 3,2, while ECD values of agri-food

substrates range between 0,07 and 0,18 (Leong *et al.*, 2016; Meneguz *et al.*, 2018; Giannetto *et al*., 2019) but, as previously said, different kind of substrates influence BSF performances, so also in this case WRI and ECD value can be higher or lower. For example, a study carried out on cocoa pod in different conditions, alone or with other wastes, fresh or composted, showed that both WRI and ECD can substantially vary among experimental conditions, showing that many parameters, including substrate moisture and specific protein and lipid content are determining in BSF nourishment and its ability to reduce substrate and digest high percentage of administered food, correctly and quickly (Yusup *et al.*, 2020). Lower WRI values (1,4 and 1,5) were recorded for coconut testa and banana peel. This might be caused by the low water content of these substrates. The moisture content of substrate, whose optimum is about 60% (Diener *et al.*, 2009), plays an important role in BSF ability of feeding and food absorption (Putra *et al.*, 2020). Another factor that can influence WRI, and especially ECD, is the high cellulose component, requiring specific enzyme for the digestion process, already found in BSF gut microbiota, as previously said (Lee *et al*., 2014; Taggar, 2015; Muller et al., 2017). An interesting study was performed by Jucker *et al.* (2020b), in which wastes from other insect (*Schistocerca gregaria*, *Gryllus bimaculatus* and *Gryllodes sigillatus*) wastes are used to feed BSF larvae. BSF are able to correctly feed also on these particular substances with a substrate reduction ranging from 28% to 72%, a WRI and ECD index equal to 4,5 and 0,19 for cricket waste, respectively, and 1,8 and 0,47 for locust. Higher ECD value, found in locust waste, shows a good efficiency in the conversion of the growing substrate into larval biomass (Jucker *et al.*, 2020b). Our results, with higher values compared to literature (from 0,18 of watermelon and orange substrates, to 0,29 of strawberry) show the great potential of BSF larvae in degradation of this specific fruit and vegetables wastes. Waste reduction is directly proportional to BSF larval growth (Saragi and Bagastyo, 2015): indeed, the higher the growth rate of larvae fed on standard diet and on strawberry corresponds to the higher value of WRI and the amount of waste reduction in the shorter development time. Indeed, larvae fed on standard diet took less time to reach the prepupal stage compared to the others, while larvae fed on strawberry are the faster larvae among the tested substrates from the agri-food chain. The same situation, even if at the opposite side, is observed on larvae fed on cabbage: to the lesser growth rate and the extended time of development correspond a lower value of WRI.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Bioconversion processes mediated by BSF larvae are widely studied, analysing the ability of feeding on different organic matter, including manure, fruits, and vegetables. The analysis performed on this study on some by-products from the agri-food chains (watermelon, kiwi, a mix of cabbage/savoy cabbage, strawberry, tangerine, orange) allow to highlight the high ability of BSF to feed on these substrates, favouring both their disposal and their recovery. The usage of by-products or wastes provided by companies of the agri-food sector as nourishment for BSF can represent an excellent strategy for companies to optimize their production processes: the disposal mediated by BSF larvae has a considerable reduction in the environmental impact and on the costs of managing the waste disposal in conventional ways. In order to accomplish this objective and offer these companies a mean for the disposal of their wastes, we realize a mass BSF rearing, with 7 kg of each substrate. All the studies that have been carried out so far have been on a laboratory scale and mobilization of these results to an industrial setting is not linear and it is necessary to verify this BSF ability empirically. In our experiment each substrate was successfully used, demonstrating that organic waste of the agri-food chain can be used for BSF feeding at an industrial scale. At the end of bioconversion process, which corresponds to the end of larval development, it is possible to obtain high quality products of biological and economic value: larvae that can be used in pet food or aquaculture, as they are rich in proteins and lipids and frass that can be used in agriculture as soil conditioner for crop fertilization. Although BSF is able to correctly feed on all the tested substrates, development time, growth rate, final larval biomass and larval composition (ashes, proteins and lipids) were not equal among the tested substrates; hence, it is possible to conclude that substrate really influences BSF performances. In addition to the standard diet, balanced in all nutrients and therefore the more suitable for BSF development, strawberry was the best substrate in terms of time of development, growth rate, larval biomass, and bioconversion parameters, while the mix of cabbage/savoy cabbage was the worst. Further studies have already been planned, in order to figure out which provides the larvae and the frass with the best characteristics for market need, but also to understand which substrate can be actually disposed of with this innovative and ecological process. Moreover, it would be of great interest studying the effects of the differently produced BSF meal on animal nutrition and performances. To this regard, it may be an opportunity starting with the use of animal models such as mice or, even better, zebrafish. Indeed, the use of these animals might speed up the knowledge production process while containing the research costs. This approach might be useful for finding new market opportunities for both the waste disposal industry and the feed industry.

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Appendix – Preliminary results on manure

A1. Substrate dry matter and water content

At the beginning of the experiment, the moisture content of each substrate was verified. Water content of standard diet (70,86% ± 0,84%) and mature manure (69,34% ± 0,21%) slightly differ, on the contrary fresh manure moisture content was higher (84,48 % ± 0,31%) (Tables A1, A2).

Replicate	Standard	Mature	Fresh
1	28,49	30,58	14,92
2	28,14	30,35	15,88
3	30,80	31,05	15,78
Mean	29,14	30,66	15,52
SE	0,84	0,21	0,31

Table A1: Dry matter (%) of standard diet, mature manure, and fresh manure. Dry matter of each substrate was measured at the beginning of the experiment.

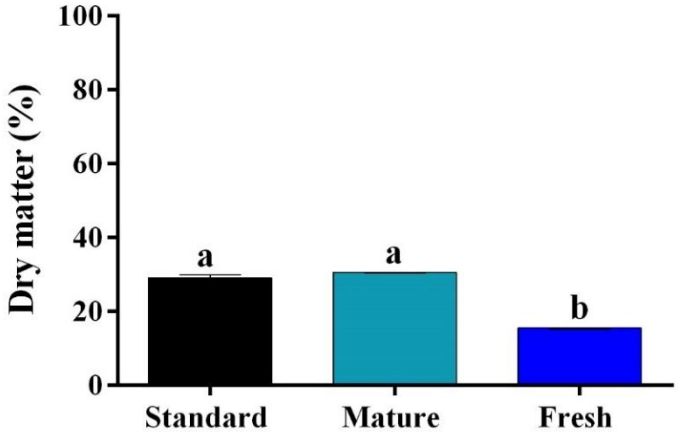

Figure A1: Dry matter (%) of standard diet, mature manure, and fresh manure. Dry matter of each substrate was measured at the beginning of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

Replicate	Standard	Mature	Fresh
1	71,51	69,42	85,08
2	71,86	69,65	84,12
3	69,20	68,95	84,22
Mean	70,86	69,34	84,48
SE	0,84	0,21	0,31

Table A2: Water content (%) of standard diet, mature manure, and fresh manure. Water content of each substrate was measured at the beginning of the experiment.

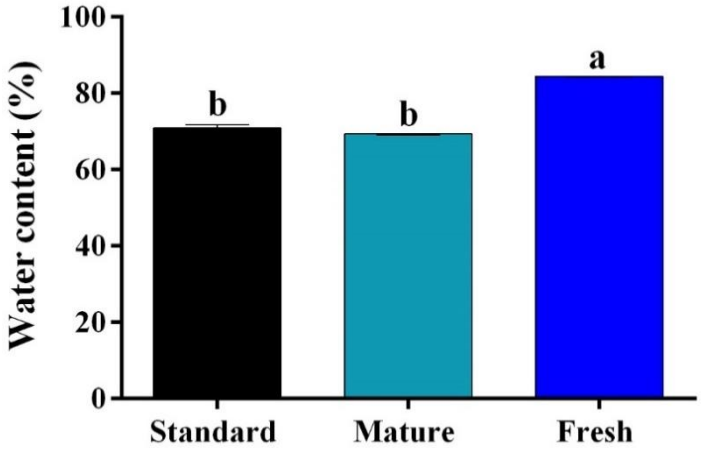

Figure A2: Water content (%) of standard diet, mature manure, and fresh manure. Water content of each substrate was measured at the beginning of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,001).

A.2 Larval developmental time and Index of Growth by Time

Control larvae reached the completed larval stage in 13 days after eggs hatching with an index of growth by time of 0,104 g/d ± 0,004 g/d (Table A3, Fig. A3). Larvae fed on manure regularly increased their weight reaching the mature larval stage with delay respect to control larvae: 30 days for larvae fed on mature manure with an index of growth by time of 0,029 g/d ± 0,0005 kg/d (Table A3, Fig. A3), 25 days for larvae fed on fresh manure with an index of growth by time of 0,032 kg/d ± 0,001 kg/d (Table A3, Fig. A3).

Replicate	Standard	Mature	Fresh
1	0,110	0,030	0,034
2	0,094	0,028	0,032
3	0,108	0,028	0,030
Mean	0,104	0,029	0,032
SE	0,004	0,0005	0,001

Table A3: Index of growth by time (kg/d) of BSF larvae fed on standard diet, mature manure, and fresh manure. Index of growth by time was calculated considering the final larval total biomass and the days of bioassay. Data are presented as the mean ± SE (n = 3).

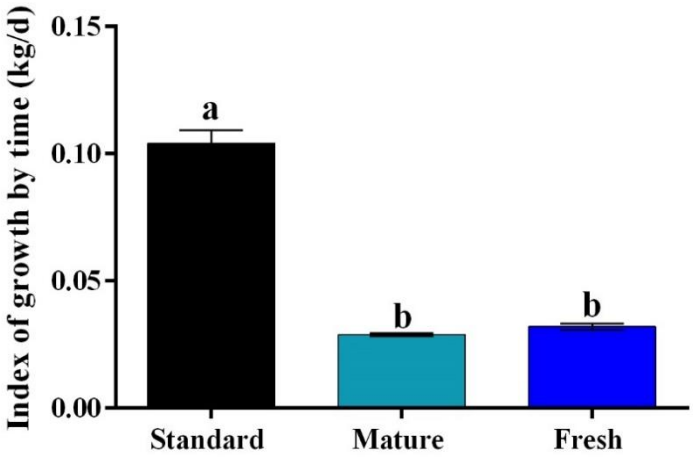

Figure A3: Index of growth by time (kg/d) of BSF larvae fed on standard diet, mature manure, and fresh manure. Index of growth by time was calculated considering the final larval total biomass and the days of bioassay. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,4051).

A.3 Larval dry matter and water content

Larval dry matter is between 30,96% ± 0,13%of larvae fed mature manure and 35,46 % ± 0,49% of larvae fed fresh manure (Table A4, Fig. A4).

Replicate	Standard	Mature	Fresh
1	31,47	30,71	34,69
2	34,91	30,92	35,11
3	34,70	31,24	36,57
Mean	33,69	30,96	35,46
SE	1,11	0,13	0,49

Table A4: Dry matter (%) of larvae fed on standard diet, mature manure, and fresh manure. Dry matter of larvae was measured at the beginning of the experiment.

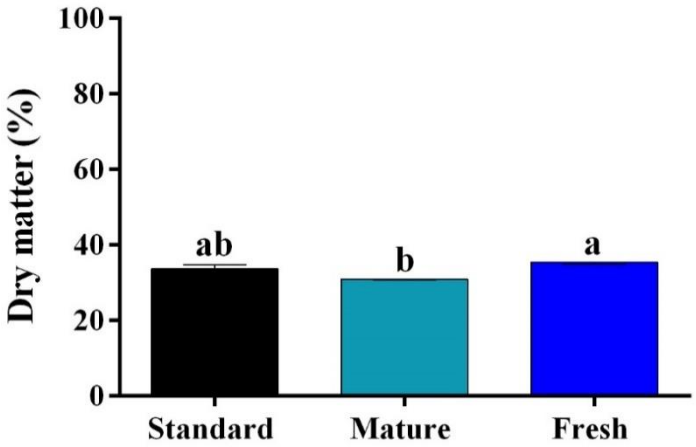

Figure A4: Dry matter (%) of larvae fed on standard diet, mature manure, and fresh manure. Dry matter of larvae was measured at the beginning of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,0131).

Replicate	Standard	Mature	Fresh
1	68,53	69,29	65,31
2	65,09	69,08	64,89
3	65,30	68,76	63,43
Mean	66,31	69,04	64,54
SE	1,11	0,13	0,49

Table A5: Water content (%) of larvae fed on standard diet, mature manure, and fresh manure. Dry matter of larvae was measured at the beginning of the experiment.

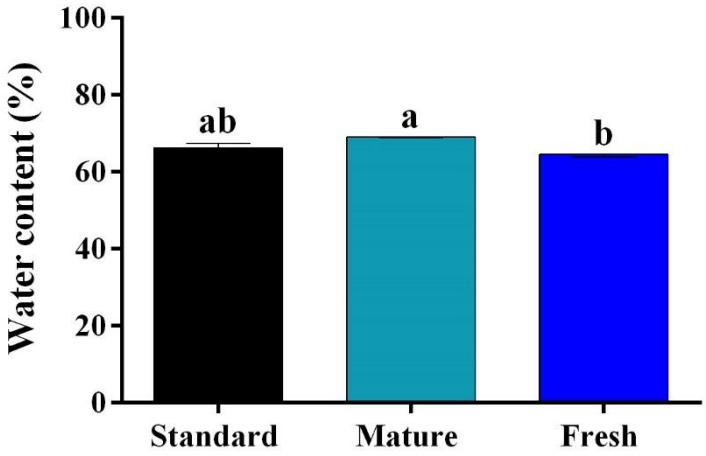

Figure A5: Water content (%) of larvae fed on standard diet, mature manure, and fresh manure. Water content of larvae was measured at the beginning of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,0131).

A.4 Larval total biomass

The larval total biomass differed among standard diet and manure samples, but not between the two different kinds of manure (Table A6, Fig. A6).

Replicate	Standard	Mature	Fresh
1	0,99	0,90	0,85
2	0,84	0,85	0,80
3	0,97	0,85	0,75
Mean	0,94	0,87	0,80
SE	0,05	0,014	0,025

Table A6: Larval total biomass (kg) of BSF larvae fed on standard diet, mature manure, and fresh manure. Total biomass of BSF larvae was measured at the end of the experiment.

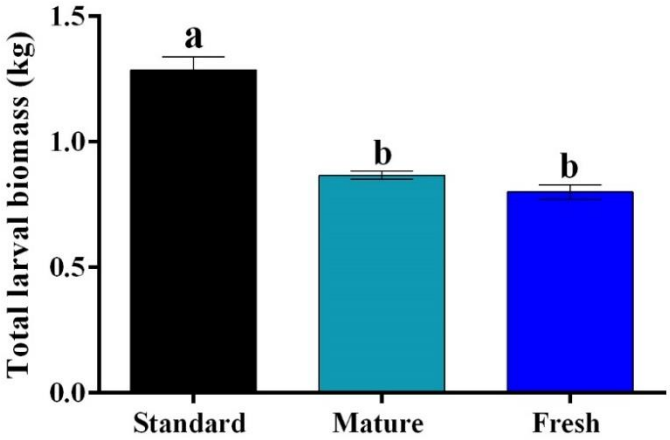

Figure A6: Larval total biomass (kg) of BSF larvae fed on standard diet, mature manure, and fresh manure. Total biomass of BSF larvae was measured at the end of the experiment. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences (p value = 0,0001).

A.5 Larval ash and mineral content

Larvae fed on cabbage standard the highest ash content (12,89% ± 0,23%), no differences were detected between the two kinds of manure (Table A7, Fig. A7).

The most abundant minerals, among the analysed substances, were calcium and potassium, while the lowest was sodium (Table A8). Differences were found in the concentration of almost all minerals, except for potassium among all samples.

Replicate	Standard	Mature	Fresh
1	12,99	11,10	9,00
2	13,29	10,40	10,00
3	12,39	10,30	10,40
Mean	12,89	10,60	9,80
SE	0,23	0,22	0,36

Table A7: Ash content (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Ash content of larvae fed on each substrate was measured at the end of the experiment, after incineration at 550 °C for 3 h in a Muffle Furnace.

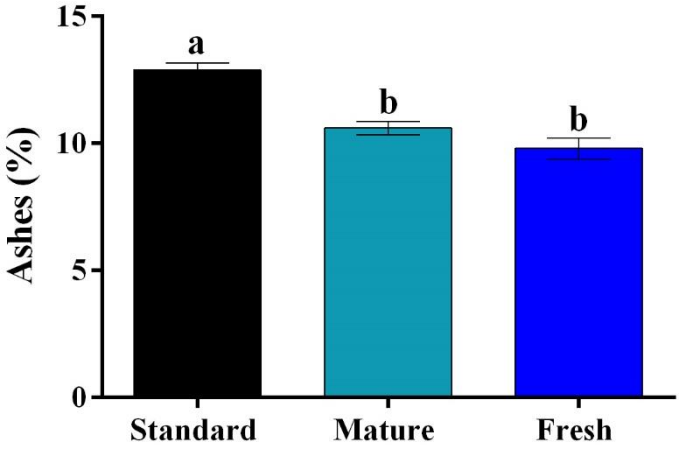

Figure A7: Ash content (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Ash content of BSF larvae was measured at the end of the experiment, after incineration at 550 °C for 3 h in a Muffle Furnace. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,012).

Mineral	Standard (Mean ± SE)	Mature (Mean ± SE)	Fresh (Mean ± SE)
Calcium	$14,36 \pm 0,15^{b}$	$25,22 \pm 0,12^{a}$	$26,19 \pm 0,5^{a}$
Potassium	$10,55 \pm 0,55^{a}$	$9,05 \pm 0,04^{a}$	$9,23 \pm 0,06^{a}$
Phosphorus	$5,16 \pm 0,21^{ab}$	$4,53 \pm 0,02^{b}$	$5,46 \pm 0,14^{a}$
Magnesium	$3,91 \pm 0,15^{b}$	$5,17 \pm 0,02^{a}$	$4,52 \pm 0,07^{c}$
Sodium	$0,68 \pm 0,05^{b}$	$1,29 \pm 0,01^{a}$	$1,66 \pm 0,01^{c}$

Table A8: Mineral content (g/kg) of BSF larvae fed on standard diet, mature manure, and fresh manure. Mineral contents were determined by atomic absorption spectrometry in an atomic absorption spectrophotometer after digestion in sulfuric acid and selenium powder. Calcium, potassium, magnesium, and sodium were measured at wavelengths of 422,7, 766,5, 285,2, 589,0, respectively. Phosphorus was determined using a UV–visible spectrophotometer (Shimadzu, Japan) at a wavelength of 880 nm. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (Calcium p value < 0,0001, Potassium p value = 0,0277, Phosphorus p value = 0,0113, Magnesium p value = 0,0003, Sodium p value < 0,0001).

A.6 Larval protein and amino acid content

Protein content is about 41% for larvae fed on manure, with statistically significant differences with larvae fed on standard diet (Table A9, Fig A8). Although in larvae fed on standard diet and larvae fed on manure differences in amino acid concentration were detected, the less abundant amino acid was Tryptophan, while the most abundant were Leucine, Valine, and the couple Phenylalanine/Tyrosine (Table A10). No statistically significant differences were detected between larvae fed on the two kinds of manure.

Replicate	Standard	Mature	Fresh
1	39,45	41,01	41,99
2	37,83	41,14	41,37
3	38,68	41,00	39,58
Mean	38,65	41,05	40,98
SE	0,41	0,03	0,63

Table A9: Protein content (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Protein content of larvae fed on each substrate was measured at the end of the experiment, using the Kjeldahl method.

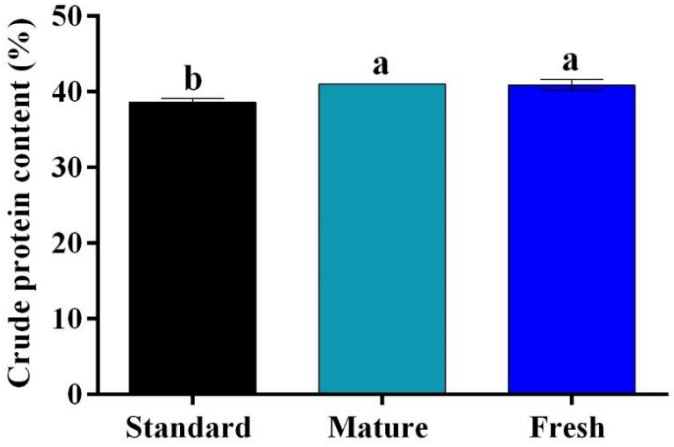

Figure A8: Protein content (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Protein content of BSF larvae was measured at the end of the experiment, using the Kjeldahl method. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,0252).

Amino acid	**Standard (Mean ± SE)**	**Mature (Mean ± SE)**	**Fresh (Mean ± SE)**
Lysine	3,37 ± 0,21[a]	2,74 ± 0,28[a]	2,72 ± 0,24[a]
Methionine	1,13 ± 0,01[a]	0,78 ± 0,01[b]	0,76 ± 0,11[b]
Methionine + Cysteine	1,72 ± 0,07[a]	0,94 ± 0,20[b]	0,93 ± 0,17[b]
Tryptophan	0,67 ± 0,01[a]	0,32 ± 0,07[b]	0,33 ± 0,07[b]
Threonine	2,65 ± 0,19[a]	1,31 ± 0,03[b]	1,31 ± 0,02[b]
Leucine	5,68 ± 0,21[a]	3,06 ± 0,22[b]	3,04 ± 0,17[b]
Isoleucine	3,07 ± 0,001[a]	1,96 ± 0,19[b]	1,95 ± 0,16[b]
Valine	4,13 ± 0,15[a]	2,95 ± 0,41[a]	2,93 ± 0,37[a]
Histidine	2,26 ± 0,07[a]	1,36 ± 0,27[a]	1,34 ± 0,24[a]
Arginine	3,36 ± 0,13[a]	2,23 ± 0,15[b]	2,22 ± 0,12[b]
Phenylalanine + Tyrosine	8,03 ± 0,20[a]	4,66 ± 0,43[b]	4,63 ± 0,38[b]

Table A10: Amino acid composition (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Specific amino acid composition was detected through HPLC analysis performed on oxidised and hydrolysed samples, following the procedure in 2009/152/EC. Tryptophan was determined separately, following the procedure N° 152/2009. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (Lysine p value = 0,1850, Methionine p value = 0,0113, Methionine + Cysteine p value = 0,0183, Tryptophan p value = 0,0016, Threonine p value = 0.0002, Leucine p value = 0,0001, Isoleucine p value = 0,0022, Valine p value = 0,0691, Histidine p value = 0,0354, Arginine p value = 0,0014, Phenylalanine + Tyrosine p value = 0.0006).

A.7 Larval lipid and fatty acid content

Lipid content statistically differed among all the samples (Table A11, Fig. A9). Although differences in the concentration of fatty acids were found in larvae fed the standard diet and other samples, in all cases the most abundant are saturated fatty acids (Table A12).

Replicate	Standard	Mature	Fresh
1	35,04	19,27	27,45
2	33,59	19,73	28,33
3	36,57	19,29	28,86
Mean	35,07	19,43	28,21
SE	0,86	0,13	0,36

Table A11: Lipid content (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Lipid content of larvae fed on each substrate was measured at the end of the experiment, through the lipid extraction in chloroform.

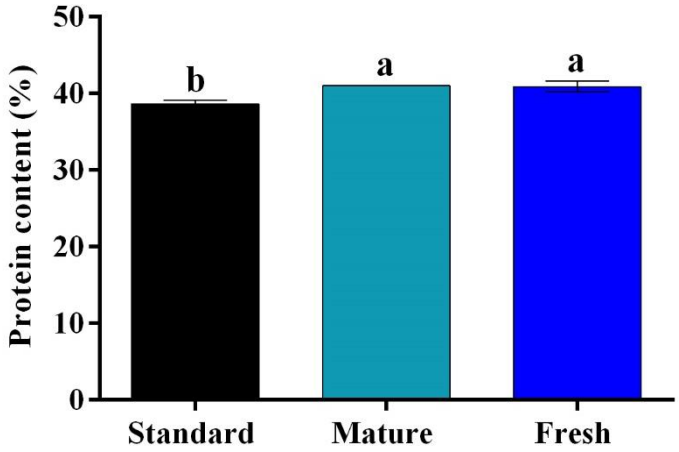

Figure A9: Lipid content (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Lipid content of BSF larvae was measured at the end of the experiment, through the lipid extraction in chloroform. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

Fatty acid	Standard (Mean ± SE)	Mature (Mean ± SE)	Fresh (Mean ± SE)
Saturated	$53,12 \pm 0,75^{b}$	$59,12 \pm 0,37^{a}$	$61,15 \pm 0,81^{a}$
Monounsaturated	$26,02 \pm 0,09^{a}$	$21,97 \pm 0,18^{b}$	$21,80 \pm 0,37^{b}$
Polyunsaturated	$21,52 \pm 0,22^{a}$	$18,91 \pm 0,46^{ab}$	$16,81 \pm 0,86^{b}$

Table A12: Fatty acid composition (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Fatty acid composition was evaluated by infrared spectometry. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (Saturated and Monounsaturated p value < 0,0001, Polyunsaturated p value = 0,0064).

A.8 Larval fibre content

Statistical differences were detected among larvae fed on standard diet and larvae fed on manure (Table A13).

Replicate	Standard	Mature	Fresh
1	34,17	23,51	24,31
2	35,39	22,76	25,11
3	32,82	23,77	25,52
Mean	34,13	23,35	24,98
SE	0,74	0,26	0,31

Table A13: Fibre (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Fibre content was evaluated boiling each sample in a neutral and acid detergent.

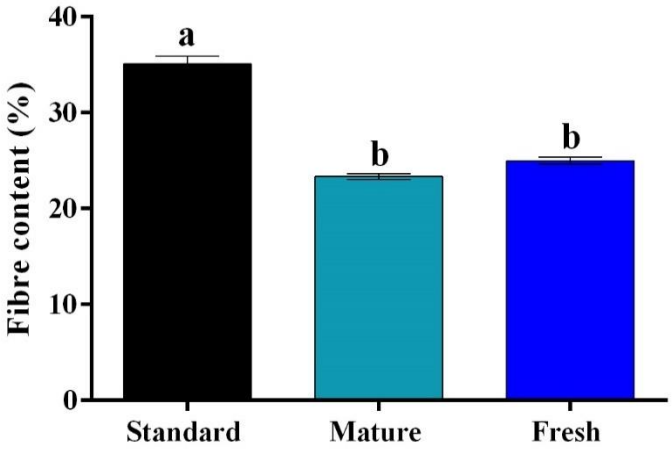

Figure A10: Fibre (%) of BSF larvae fed on standard diet, mature manure, and fresh manure. Fibre content was evaluated boiling each sample in a neutral and acid detergent. Data are presented as mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

A.9 Frass total weight

The weight of the frass, measured after the bioconversion process, statistically differed between larvae fed on standard diet and larvae fed on manure (Table A14, Fig A11).

Replicate	Standard	Mature	Fresh
1	1,66	0,90	0,85
2	1,95	0,85	0,80
3	1,83	0,85	0,75
Mean	1,81	0,87	0,80
SE	0,08	0,01	0,03

Table A14: Frass total weight (kg) of BSF larvae fed on standard diet, mature manure, and fresh manure. Frass total weight was measured at the end of the experiment.

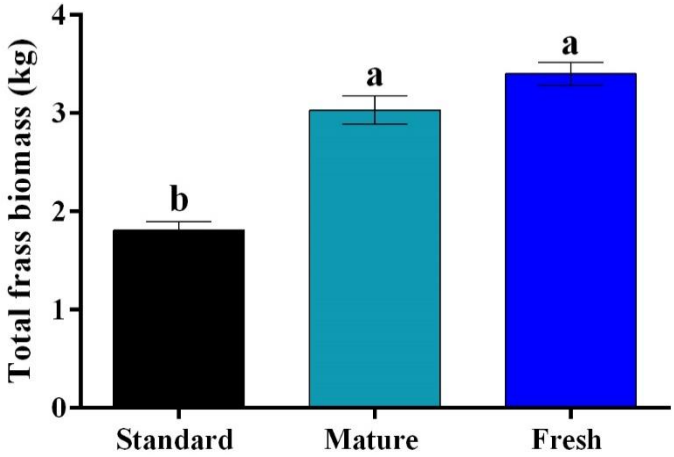

Figure A11: Frass total weight (kg) of BSF larvae fed on standard diet, mature manure, and fresh manure. Frass total weight was measured at the end of the experiment. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and a Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,0002).

A.10 Substrate reduction

The percentage of substrate reduction statistically differed between larvae fed on standard diet and larvae fed on manure (Table A15, Fig A12).

Replicate	Standard	Mature	Fresh
1	76,23	60,00	54,29
2	72,17	57,14	48,57
3	73,90	52,88	51,57
Mean	74,10	56,67	51,48
SE	1,18	1,79	1,43

Table A15: Reduction of standard diet, mature manure, and fresh manure after BSF feeding. Substrate reduction was calculated considering the initial substrate and the final product, made of frass and substrate residue.

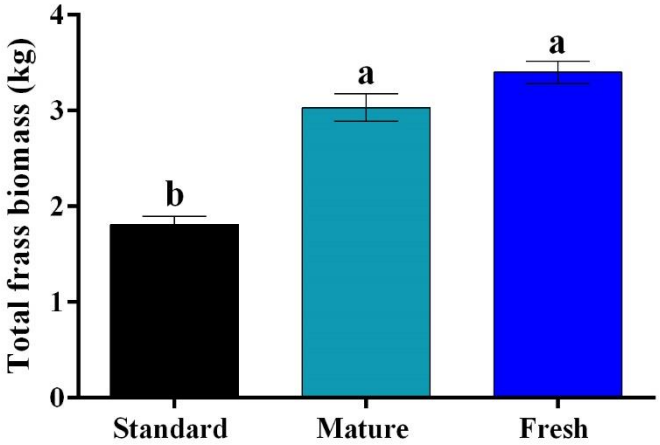

Figure A12: Reduction of standard diet, mature manure, and fresh manure after BSF feeding. Substrate reduction was calculated considering the initial substrate and the final product, made of frass and substrate residue. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,0002).

A.11 WRI and ECD

For each experimental condition different parameters related to bioconversion, were calculated: the WRI (Waste Reduction Index), which indicates the ability of the larvae to reduce the amount of administered food; and the ECD (Efficiency of Conversion of Digestive Feed), which indicates the efficiency of the larvae in converting the food taken into larval biomass. WRI statistically differed between larvae fed on standard diet and larvae fed on manure (Table A16, Fig A13), while ECD value does not differ among substrates (Table A17, Fig. A14).

Replicate	Standard	Mature	Fresh
1	8,45	2,00	2,16
2	8,02	1,87	1,92
3	8,21	1,77	2,08
Mean	8,23	1,88	2,05
SE	0,13	0,06	0,06

Table A16: Waste reduction index (WRI) of standard diet, mature manure, and fresh manure after BSF feeding. Waste reduction index was calculated comparing the percentage of substrate reduction and time of bioassay.

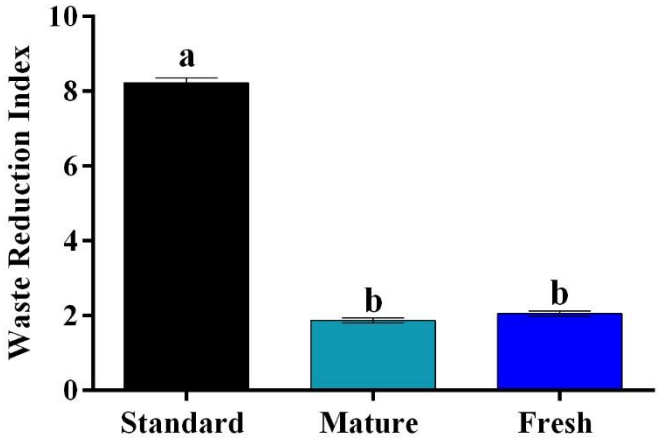

Figure A13: Waste reduction index (WRI) of standard diet, mature manure, and fresh manure after BSF feeding. Waste reduction index was calculated comparing the percentage of substrate reduction and time of bioassay. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value < 0,0001).

Replicate	Standard	Mature	Fresh
1	0,19	0,21	0,22
2	0,17	0,21	0,24
3	0,19	0,23	0,21
Mean	0,18	0,22	0,22
SE	0,004	0,005	0,007

Table A17: Efficiency of Conversion of Digestive Feed (ECD) of standard diet, mature manure, and fresh manure after BSF feeding. ECD was calculated comparing larval biomass and intake food, as the difference between initial substrate and the final frass.

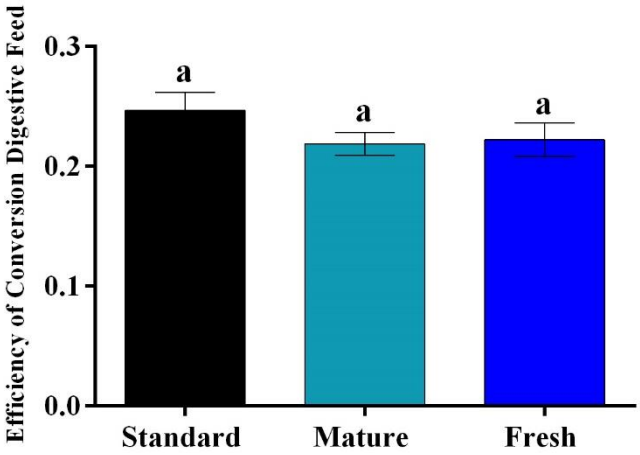

Figure A14: Efficiency of Conversion of Digestive Feed (ECD) of standard diet, mature manure, and fresh manure after BSF feeding. ECD was calculated comparing larval biomass and intake food, as the difference between initial substrate and the final frass. Data are presented as the mean ± SE (n = 3). Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post-hoc* test. Different letters indicate significant differences among groups (p value = 0,0776).

A.12 Discussion

Dairy manure, containing undigested organic matter, is considered as a pollutant for the environment (Aillery *et al*., 2005). Untreated livestock wastes cause damage to water due to the eutrophication process, to air due to ammonia and greenhouse gas emissions and to soil due to nutrient accumulation (Ann *et al*., 2002, Martinez *et al.*, 2009). Specifically, manure has noxious characteristics that could be a problem in a long composting process, such as unpleasant odour, low C/N ratio, high humidity content, phytotoxic compounds, pathogens, and attraction of vector borne diseases (da Silva and Hesselberg, 2020; Bortolini *et al.,* 2020).

One of the most promising methodologies for the simultaneous disposal and valorisation of organic wastes, including dairy manure, is related to their usage as substrate for mass-rearing insects (Diener *et al.*, 2009; van Huis 2013; Salomone *et al.*, 2017). BSF, in addition to substrate reduction, is also able to reduce odours (Sheppard 1983; van Huis *et al.,* 2013) and bacterial load (Erickson *et al*., 2004; Liu *et al*., 2008), thanks to the AMP production (Park *et al.*, 2015).

In the second field of research, we started to analyse the performances of BSF larvae fed on a substrate of animal origin, bovine mature manure (preserved for few days) and fresh manure (just harvested). BSF larvae correctly fed on both the substrates, reaching the mature larva instar with a delay compared to the standard diet ranging from 12 to 17 days, even much more slowly than the previously described substrates of vegetable origin. The slow development time, the low index of growth by time and the low total biomass could be related to the high fibre content of dairy manure: cellulose, hemicellulose and lignin content could reach the 80% in fresh manure and 65% in mature manure (Li *et al*., 2011; Rehman *et al.,* 2017). Despite the higher content in fibres, fresh manure highlighted better performances compared to mature manure, so it could be more suitable for BSF development, increasing the manure processing capacity, as already reported in Oonincx *et al.* (2015). Substrate reduction, about 51- 56%, was similar to results reported in literature. For dairy manure substrate reduction is around 33-57% (Li *et al*., 2011; Myers *et al*., 2008; Zhou *et al.*, 2013), and 36-60% for poultry manure (Lalander *et al*., 2019; Rehman *et al*., 2017).

Comparing our results with other similar studies, it is clear that also in this case BSF nutritional composition is influenced by substrate and that even little changes in substrate characteristics have a strong effect on larval composition. In Wang *et al.*, (2020), although BSF was reared on cow manure, some differences with nutritional component are noticeable: for example, ash content is higher in our experiment (10% vs 5%), protein content is similar, while lipid content is lower in our trial (19% - 28% vs 36%) (Wang *et al.,* 2020). Substrate significantly affected

the levels of minerals and amino acids: calcium, that is in both cases the most abundant mineral, has higher concentration in Wang *et al.* trial, while potassium, phosphorus, magnesium, and sodium (that is the less abundant mineral in both cases) are highly concentrated in our samples (Wang *et al*., 2020). Calcium levels have a great variability in BSF reared on different materials, from 1 g/kg to 66 g/kg (Spranghers *et al.*, 2017). The high potassium, phosphorus and magnesium content in our larvae is fundamental for the next step, in which these larvae will be used as pet food or in aquaculture, when the European law will allow the usage of these larvae in human and animal nourishment. Concerning amino acid content, some of them has similar concentration (methionine, cysteine, histidine, and arginine), some other has lower concentration in our experiment (tryptophan and threonine) and some other has higher concentration (lysine, valine, isoleucine, and cysteine) (Wang *et al*., 2020). Differences were also detected with pig, swine, sheep, and horse manure (Julita *et al*., 2018; Wang *et al*., 2020). BSF reared on pig and poultry manure have lower ash content than ours, but similar protein content (Wang *et al.,* 2020). Similar protein content was also detected in larvae fed on sheep manure, while a lower protein content was detected in larvae fed on horse manure (Julita *et al*., 2018); in these two kinds of manure, ash content is higher than our values, 15% and 19% respectively (Julita *et al*., 2018). A particular comparison should be done for lipid content, as it is around 15-35% for larvae fed on poultry manure (Arango Gutierrez *et al*., 2004, Newton *et al.*, 2005), 28-34% for those fed on swine manure (Newton *et al*., 2005, Yang *et al*., 2016), 14% for larvae fed on horse manure (Julita *et al.,* 2018) and 10 % for larvae fed on sheep manure (Julita *et al.,* 2018). These data demonstrated that the fat content is highly variable and strictly depending on the type of substrate.

Our preliminary work demonstrated that BSF is able to correctly feed on manure and it represents a useful tool, never used so far in our region, to reduce zootechnical wastes, favouring their total recovery. The current feed safety regulations forbid in the European Union the usage, for food and feed applications, of insects (includes BSF) reared with animal manure or restaurant, catering, and supermarket wastes (European Commission, 2001, 2017; IPIFF, 2019, 2020). For this reason, the earned BSF biomass can be used to obtain, for example, biofuel from its lipid component, and larval frass, made of leftover substrate and real frass, can be used as fertilizer. The zootechnical waste valorisation, together with agri-food chain product enhancement, is part of a circular economy perspective, that wants to demonstrate the feasibility of organic waste management by BSF, reared on by-products and wastes at industrial scale.

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Appendix - Publications

Part of the work of the first year of PhD course, in addition to data obtained by Dr. Andrea Scala (XXXI cycle of PhD in "Applied Biology and Environmental Safeguard"), allowed the publication of a scientific paper entitled "Rearing substrate impacts growth and macronutrient composition of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae produced at an industrial scale" (Scala *et al*., 2020). In this paper apple, banana, and spent grain from a brewery was used as substrates. In this paper, for the first time BSF was reared at industrial scale of 10,000 larvae. For each diet treatment different parameters were evaluated: substrate reductions, larval development time, index of growth by time, final biomass, and nutritional values (protein and lipid content). Differences were recorded across all variables, except substrate conversion, for larvae fed on fruit and spent grain (alone or with fruit). The goal of this study was to demonstrate the ability of the BSF to recycle organic waste at an industrial scale.

Moreover, during the PhD program I had the opportunity to work on other projects with my team.
My contribution in these research activities resulted in the publication of other scientific manuscripts in international journals.

Two of them are focused on post-embryonic development and moulting in insects: in the first manuscript an in depth analysis of the cellular signalling cascade of ecdysone secretion in prothoracic glands after the prothoracicotropic hormone (PTTH) stimulation in *Heliothis virescens* (Lepidoptera: Noctuidae) was carried out (Scieuzo *et al.*, 2018); in the second paper the effects of a home-made recombinant PTTH hormone of *H. virescens* was analysed, comparing them to the effects of the natural brain extract (Nardiello *et al.*, 2019).

One manuscript concerned a bioinformatic study on new potential antimicrobial peptides (AMPs) identified in *H. illucens* in which their putative antimicrobial, anticancer, antiviral, and antifungal activity have been evaluated by using different bioinformatic tools (Moretta *et al.*, 2020). In this paper also preliminary data concerning the inhibition activity on *Escherichia coli* of four peptides, chemically synthetized, was evaluated.

In the other four publications the host-parasitoid interaction in three different systems was analysed.

In the first manuscript, we demonstrate that PI3K/AKT/TOR cellular signalling in *H. virescens* is one of the targets of *Toxoneuron nigriceps* (Hymenoptera: Braconidae) Polydnavirus, *T. nigriceps* Bracovirus *Tn*BV, able to block ecdysteroidogenesis through alterations of the PI3K/Akt/TOR pathway at the transcriptional level (Salvia *et al.*, 2018). Moreover, we investigated the role of *T. nigriceps* ovarian proteins, involved in the induction of precocious symptoms in the host immune system alteration in *H. virescens* haemocytes, explaining the high-level of the loss of haemocytes functionality and mortality (Salvia *et al.,* 2021). In the second host-parasitoid system, it was investigated the unconventional mechanism by which teratocytes dissociated from the serosal membrane of *Aphidius ervi* (Hymenoptera, Braconidae) in *Acyrthosiphon pisum* (Homoptera, Aphididae) body release Ae-FABP and Ae-ENO in the extracellular space (Salvia *et al.*, 2019). In the third host-parasitoid system, the main proteins of the venom of *Torymus sinensis* (Hymenoptera: Torymidae), parasitoid of Dryocosmus kuriphilus (Hymenoptera: Cynipidae) were identified, through an integrated transcriptomic and proteomic approach (Scieuzo *et al.,* 2021).