Literature search on sustainable production of insects for food

This literature search was written in context of the Interreg North-West Europe ValuSect project.

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Introduction

Today's society is facing enormous challenges. Due to the increasing world population and rising prosperity, the demand for alternative protein sources is growing. Demand for food is estimated to increase by 70-80% between 2012 and 2050 (Oonincx & De Boer, 2012; Pelletier & Tyedmers, 2010; Steinfeld, et al., 2006). It is estimated that there will be 9.7 billion people on Earth by 2050 (Guillou & Matheron, 2014). The demand for sustainable protein sources is as high as rising meat consumption and the choices for certain diets negatively affect greenhouse gas emissions. Current livestock farming is responsible for about 15% of the total greenhouse gas emissions caused by humans (Steinfeld, et al., 2006; Godfray, Pretty, Thomas, Warham, & Beddington, 2011).

Insects have the potential to help solving the previously described societal challenges (Ramos-Elorduy, 2005; van Huis, 2003; Meijer-Rochow, 1975; Wang & Shelomi, 2017; van Huis, et al., 2013; Bessa, Pieterse, Sigge, & Hoffman, 2017). After all, many insect species are able to convert low-value organic side streams into their own biomass consisting of high-quality substances such as proteins, fats and chitin. This makes the nutritional value of insects comparable to that of livestock.

Insects are believed to have a lower ecological impact than current livestock. Research has shown that insects produce less greenhouse gases and emit significantly lower ammonia emissions (Oonickx, et al., 2010). In addition, insects convert feed into biomass more efficiently: 2 to 3 times more efficiently than pigs and even 5 times more efficiently than cattle (van Huis, et al., 2013). This is because they are cold-blooded organisms and therefore use little energy to maintain their body temperature (Bjørge, Overgaard, Malte, Gianotten, & Heckmann, 2018). Furthermore, insects require less space because they can be stacked and they generally also require less water (Miglietta, De Leo, Ruberti, & Massari, 2015). Insects generally have (depending on the species) a higher slaughter yield and a higher protein content than chickens, pigs or cattle (Oonincx & De Boer, 2012; Oonickx, et al., 2010; Dobermann, Swift, & Field, Opportunities and hurdles of edible insects for food and feed, 2017).

At least 2 billion people worldwide already eat insects. In East Asia, South America and Africa, insects are even part of the traditional diet (van Huis, et al., 2013). Over 2000 edible insect species have been registered worldwide (Jongema, 2017). However, eating insects is a completely new concept for the Western population. Neophobia (aversion to new products) plays an important role in these countries: these people will be reluctant to consume insects. Consumer acceptance is currently low because insects are often associated with pests, pollution and health risks. However if the Western population becomes more familiar with entomophagy (eating insects) this attitude may change. People who know the concept are more inclined to include insects in their diet. In addition, insects will mainly have to be incorporated into products so that they are not visible. More emphasis should be placed on the taste and appearance of dishes with or products from insects so that they can't be distinguished from conventional meat products. Positive taste reactions will, of course, decrease negative reactions to entomophagy (Tan, van den Berg, & Stieger, 2016; Laureati, Proserpio, Jucker, & Savoldelli, 2016; Mancini, Moruzzo, Riccioli, & Paci, 2019).

This literature search is carried out as part of the Interreg NWE ValuSect project (work package 1: Quality improvement of the primary insect production process, activity 3: Literature search to clarify *questions from SMEs and companies regarding quality improvement of insect production process), in which all project partners of work package 1 are actively involved. The aim of this literature search is to obtain more knowledge about insect rearing on side streams and emissions related to insect rearing in order to be able to carry out applicable experiments during activity 1 (testing greenhouse gases of insect rearing) and activity 2 (testing side streams for insect rearing) of work package 1.*

The ValuSect project will contribute to the acceptance of insects in the human diet. This project focuses on 3 insect species, namely Tenebrio molitor (mealworm), Acheta domesticus (housecricket) and Locusta migratoria (migratory locust). For these insects a novel food dossier is submitted and under evaluation.

The aim of the project is, among others, quality improvement of the primary production process and the processing of insect products for food. This means that an attempt is made to obtain optimal rearing conditions for the insects in question, thereby demonstrating a positive environmental impact of these rearing conditions. This will lead to a sustainable and optimized insect production. Furthermore, efforts are being made to identify the nutritional value of insects and insect-based food products, as well as the factors that influence this. The project also focuses on the microbial load of insects and insect-based food products concerning food safety and shelf life. All this will initiate an insect-based food innovation.

Table of contents

1 Insect rearing for food

Rearing of insects for food is a relatively new business activity in the European Union (EU), consequently current procedures still need to be optimized. Research focuses on increasing insect yield, automation and sustainable insect production (e.g. reducing energy consumption, gas emissions, etc.). The Interreg NWE ValuSect project is working on the optimization of insect rearing with a focus on the production of sustainable insects.

Although numerous studies on insect rearing have been performed in recent years, differences in the experimental design hamper comparison among them. The conditions in which insects are reared greatly affect the results of the experiments (Morales-Ramos & Rojas, 2015; Barragan-Fonseca, Dicke, & van Loon, 2018). It is therefore important to define a standard rearing protocol per insect species, which is based on the needs of the insect (optimal conditions) and the type of experiment. However, certain regulations are imposed by law (European legislation) for the rearing of insects for food. It is possible that this is not taken into account during the rearing experiments for scientific purposes. These regulations are defined below so this can be taken into account if needed, for example during the production of insects for human food.

As part of the Horizon 2020 project Susinchain, an established group is already working on standard rearing protocols for conducting feed experiments with insects. These rearing protocols are used as basis for the experiments that are being carried out in the context of the ValuSect project. The basis will be further elaborated in this project to optimize insect rearing of *Tenebrio molitor*, *Acheta domesticus* and *Locusta migratoria*. It is important to note that the standardized rearing protocols will change as new expertise is obtained.

1.1 Standardized rearing protocol for feed experiments

1.1.1 Insect rearing conditions during the experiment

Any feed experiment is preferably performed with at least 4 replicates (but preferably 6), certainly when looking for differences between two groups (e.g. control versus experimental feed). This can be set-up in parallel or serial in time (Bosch, et al., 2020).

Parameters to be monitored during the feed experiment:

- climate conditions (temperature and relative humidity)
- feed conversion (diet provided and residue, fresh & dry matter)
- larval growth (total larval mass at the start & the end of the experiment, individual larval weight (by subsampling) throughout the experiment)
- survival of the larvae (number of larvae at the start and the end of the experiment)
- chemical composition (diet provided, residue and insects)

1.1.1.1 *Tenebrio molitor*

The suggested climate for a mealworm feed experiment is 27°C and 60% relative humidity (RH) in total darkness. The air temperature and humidity should be monitored at least daily throughout the experiment. Close contact of the objects to the walls and floor of the climate room should be avoided as they may have a different microclimate (Deruytter, Coudron, & Teerlinck, 2019).

1.1.1.2 *Acheta domesticus*

The suggested climate for a cricket feed experiment is 30°C and 50% RH with 14 hours of light. The air temperature and humidity should be monitored at least daily throughout the experiment (Clifford & Woodring, 1990). Close contact of the objects to the walls and floor of the climate room should be avoided as they may have a different microclimate.

1.1.1.3 *Locusta migratoria*

The suggested climate for a locust feed experiment is 30°C (optimum 30-35°C) and 50% RH with 12 hours of light (Hoste, et al., 2002; Hamilton, 1950; Hinks & Erlandson, 1994; Husain & Mathur, 1944; Kennedy, 1937; Tanaka, Hakomori, & Hasegawa, 1993). The air temperature and humidity should be monitored at least daily throughout the experiment. Close contact of the objects to the walls and floor of the climate room should be avoided as they may have a different microclimate.

1.1.2 Production of experimental population

1.1.2.1 *Tenebrio molitor*

To provide mealworms for experiments, wheat flour (particle size < 0.5 mm) is used as oviposition substrate for the beetles. The beetles are placed on a mesh (mesh size 2 mm) with a density of 0.1 g/cm², above the oviposition substrate. They are provided with agar cubes (25 g/L) of approximately 2*2*1 cm spaced no more than 10 cm in between, and some dry feed is placed on the mesh. The beetles are kept at 27°C and 60% RH.

Eggs are harvested after 24 hours or maximum 48 hours to minimize the age variability. Harvesting can be done by sieving the flour using a sieve with a 0.5 mm mesh size. The eggs should hatch after 7 to 8 days.

All collected eggs can be placed in a crate with 1 kg of wheat bran for every 100 gram of eggs. It is possible to have up to 200 gram of eggs in a 60*40 cm crate. Place the first half of the bran in the crate, then first and spread the eggs, evenly, on top before adding the other half of the bran. Place the box at 27°C and 60% humidity. The eggs should hatch after 7 to 8 days but leave the crate undisturbed for 2 weeks. After 2 weeks agar is provided as wet feed. This is provided as strips of the 1*1 cm with a length equal to the width of the crate. The strips should be spaced no more than 10 cm apart or 5 cm from the edge. Insufficient wet feed will reduce the growth rate and increase variability. The agar should be present at all times and should be replaced 3 times per week or when mould is growing.

After 4 weeks the larvae are ready to be used in the experiment.

1.1.2.2 *Acheta domesticus*

The adult crickets used for reproduction are kept at 30°C and 50% RH. As parental population young adults (1-2 weeks old) are preferably used since they provide the most eggs and probably of best quality. The oviposition substrate is placed in a tray (preferably 15 cm in width/diameter or more) and consists of material such as coconut choir or moist peat moss.

The oviposition box should be changed every day so the experiment can start with crickets of the same age. The oviposition box is closed at 30°C and stored until the eggs hatch. After hatching, one day old crickets are used for the experiment. The amount of crickets needed (depending on the density) can then be weighed.

1.1.2.3 *Locusta migratoria*

The parental population of locusts are kept at 30°C (optimum: 30-35°C) and 50% RH (Hoste, et al., 2002; Hamilton, 1950; Hinks & Erlandson, 1994; Husain & Mathur, 1944; Kennedy, 1937). Preferably young adults (1-2 weeks old) are used for reproduction since they provide the most eggs and probably of best quality. As oviposition substrate a mixture of 50% peat and 50% sand, moistened with water, is used. The oviposition substrate is placed in a plastic or glass jar of at least 10 cm deep (e.g. volume 1 liter). The locusts will only lay their eggs in a moist substrate to prevent the eggs from drying out (Kennedy, A preliminary analysis of oviposition behaviour by Locusta (Orthoptera, Acrididae) in relation to moisture. In Proceedings of the Royal Entomological Society of London, 1949; Lange, 2009).

The oviposition box should be changed every day so the experiment can start with locusts of the same age. The oviposition box is closed at 30°C and stored until the eggs hatch. After hatching, one day old locusts are used for the experiment.

1.1.3 Feed experiment

A distinction is made between rearing experiments and scientifically driven experiments. Experiments with a scientific approach will take into account physical and chemical factors (e.g. particle size, dry matter, density, etc.). Taking into account the characteristics of the control feed, the mass experimental feed to be given can be calculated. In rearing experiments, certain physical and chemical factors are mainly regarded as feed properties and do not necessarily have to be corrected.

It is advised to keep track of the growth of the larvae/nymphs during the experiment. By doing this on at least two occasions during the experiment and combined with initial and harvest weight a rudimentary growth curve can be constructed. This can provide information on growth speed on the experimental feed. However, assessing the growth on a weekly basis is advised.

Subsampling can be used to estimate the average weight of at least 100 well randomized individuals (see further). Due to the inherent size variability of the larvae a smaller subsample may result in large errors on the average weight estimate.

The end of an experiment could not be standardized as it depends heavily on the initial research question, but the 'time until the first observation of the last larval/nymphal molt' is not advised as this parameter is very variable. Other options are:

- fixed amount of time (e.g. 8 weeks)
- fixed average weight (e.g. 100 mg/larvae)
- 10% of the larvae have molted into pupa (in holometabolic insects) or 10% of nymphs have reached sexual maturity

1.1.3.1 *Tenebrio molitor*

As control diet for mealworm feed experiments wheat bran (dry feed) with a particle size smaller than 2 mm is used. Larger particles of dry feed reduce the growth rate. As source for moisture agar (wet feed) is used. It is known that mealworms thrive on this diet. Moreover, the composition and other parameters are also known. The control group larvae are fed 2.3 kg of wheat bran per 10 000 larvae. This should be enough to get an average larval weight of 100 mg at the end of the experiment. The agar is provided when the mealworms are 2 weeks old. This is supplied as strips of the 1*1 cm with a length equal to the width of the crate. These strips should be spaced no more than 10 cm apart or 5 cm from the edge. The agar should be presented *ad libitum* and should be replaced 3 times per week or whenever mould is growing.

The amount and distribution of the experimental feed should match the control feed. If the wet feed is to be investigated, wheat bran is used as experimental dry feed. If the dry feed is to be investigated, agar is used as experimental wet feed. The distribution of the experimental feed should match the distribution of the control feed.

1.1.3.2 *Acheta domesticus*

As control dry feed for crickets, chicken meal is suggested. The dry feed should be ground up in to a fine powder to avoid the crickets transporting the feed to other places in the crate (e.g. < 150 µm). As moisture source (wet feed), agar is used. The control feed can be placed in the crate by using petri dishes (6 petri dishes (80-100 mm)/crate: 3 are filled with agar, 3 are filled with dry feed). The agar should be replaced 3 times a week or whenever necessary (e.g. contamination, empty,…). The dry feed should be replaced at least every week.

The amount and distribution of the experimental feed should match the control feed. If the wet feed is to be investigated, chicken meal is used as experimental dry feed. If the dry feed is to be investigated, agar is used as experimental wet feed.

1.1.3.3 *Locusta migratoria*

As control feed for migratory locusts, fresh grass is suggested (moisture and feed source). An amount of 2 g fresh grass/adult locust should be provided daily. In addition to the grass to meet the needs of locusts, supplementary dry feed is provided. Oat flakes are used for this. These are placed on a petri dish (80-100 mm). The supplementary feed should be replaced at least every week and provided *ad libitum*.

The amount and distribution of the experimental feed should match the control feed. Experiments are usually carried out to investigate the fiber-rich moist feed, since locusts survive on this monostream and therefore do not always need the supplementary feed. The supplementary feed is offered to reduce mortality due to cannibalism.

1.1.4 Laboratory scale feed experiments

It can be interesting to first carry out experiments on a laboratory scale, to implement a systematic and efficient upscaling later in the experiment.

1.1.4.1 *Tenebrio molitor*

When conducting feed experiments with mealworms on a laboratory scale, it is highly recommended to use no less than 1000 larvae per replicate (or a density < 1,25 larvae/cm²). When a lower density is applied, the influence of metabolic heat produced by the insects is not included in the experiment.

1.1.4.2 *Acheta domesticus*

When conducting feed experiments with crickets on a laboratory scale, one gram of 1 day old crickets (approx. 1500 crickets) are placed in a 30*40 cm crate. In the middle 2 egg cartons of 20*25 cm are provided for the crickets. On the sides 6 petri dishes (left: first and third petri dish used for dry feed, second petri dish for wet feed; right: first and third petri dish used for wet feed, second petri dish for dry feed)) with an 80 mm diameter are placed.

1.1.4.3 *Locusta migratoria*

Because locusts can fly, they are kept in cages. A cage of 50*50*50 cm is used as standard. This makes it difficult to define experiments on a small scale. Smaller cages cannot be used, as only 100 adults fit in a 50*50*50 cm cage (density of 800 adults/m^3). Nymphs can be placed in the cage with a higher density than the adults, but this is not recommended because then they'll need to be moved to another cage (with a lower density) during the experiment. Moreover, locusts are cannibalistic. If the density is too high, the population will control itself with high mortality as a result (Hoste, et al., 2002; Hinks & Erlandson, 1994).

1.1.5 Pilot scale feed experiments

The density of the larvae may have an influence on the final result. This is due to differences in group dynamics, for example a few dozen mealworm larvae are not capable of increasing the substrate's temperature while this is the case for a few thousand larvae such as in an industrial setting. It is therefore relevant to up scale the experiments to pilot scale.

1.1.5.1 *Tenebrio molitor*

For pilot scale experiments with mealworms, the preferred crate size is 60*40 cm (inner surface approximately 2000 cm^2), other sizes can be used if needed. With a crate size of 60*40 cm, a density of 20 000 mealworms/crate is used.

1.1.5.2 *Acheta domesticus*

Due to a possible influence of the distance to the feed a standardized crate and number of crickets should be used.

For a pilot scale experiment with crickets, the preferred crate size is 60*40 cm (inner surface approximately 2000 cm^2), other sizes can be used if needed. In the middle of the crate, 4 egg cartons (20*25 cm) and 6 petri dishes (100 mm) on each side are provided. Ensure that the sides of the petri dishes touch the sides of the cardboard and each other to ensure easy access.

1.1.5.3 *Locusta migratoria*

To conduct experiments with locusts on a pilot scale, cages larger than 50*50*50 cm can be used. Depending on the dimensions of the cage, a density of 800 adults/m^3 is also applied here.

1.2 European legislation on insect rearing for food

The experiments that will be carried out in the scientific context of the ValuSect project, will not follow the European legislation for the rearing of insects for human food, because this is not the focus of the feed experiments with compound diets based on residual streams. However, when the insects are used for human consumption (e.g. taste and sensory experiments), this will be taken into account. The insects will then be purchased from an approved breeder/processor.

For the European legislation on the rearing of insects for food is referred to the Policy brief[1] 'Insects as feed and feed in the EU', that was written as part of the ValuSect project.

[1] https://www.nweurope.eu/media/11080/policy-brief-eu-legislation-on-insects-as-food-and-feed-2.pdf

2 Biomass side streams as substrate for insect production

2.1 Biomass side streams

This literature study provides a concise summary of biomass side streams within the EU and their valorisation potential through insect rearing. The intention of this project is to valorise these side streams to a greater extent than is already being done. Therefore, the current valorisation processes of these streams are also given.

2.1.1 Defining biomass side streams

Along with biomass production and consumption biodegradable side streams and residues are created. The ValuSect project will mainly focus on biomass side streams and residues from agricultural origin (both food and non-food).

Although several attempts have been made to clearly define biomass side streams, it is often not clear what is meant by the terms used. Agricultural side streams and residues are often defined as food waste. Generally, food waste and losses refers to plants and animals produced for human consumption but not ultimately consumed by people (Lipinski, Lomax, Kitinoja, Waite, & Searchinger, 2013). This excludes materials for "non-food" purposes. The point at which material becomes 'food' is when it is ready for harvest or slaughter, which means yield losses due to weather events or diseases are often excluded (Lipinski, Lomax, Kitinoja, Waite, & Searchinger, 2013). In this literature study pre harvest and slaughter side streams as well as residues from primary production for food and non-food will be included.

2.1.1.1 Food waste from the food supply chain

The food supply chain is the connected series of activities used to produce, process, distribute and consume food (Stenmarck, et al., 2016). In every sector of this food supply chain losses are created. The past few years several studies have tried to provide a quantitative overview of the losses that are created along the food supply chain within the EU (Stenmarck, et al., 2016).

In order to be able to account food waste from the food supply chain, it is imperative to properly define what is meant by this term. In this literature study the definition of food waste as defined by the FUSIONS definitional framework is used (Östergren, et al., 2014) i.e., food waste is any food, and inedible parts of food, removed from the food supply chain to be recovered or disposed. Besides a proper definition of food waste, the geographic scale and sectors implemented in the studies have to be clearly delimited. Here, data will be limited to the EU. If there is a deviation, this will be clearly indicated in the text. In order to be able to categorize the side streams, this literature study will distinguish between the following sectors: **primary production, processing, wholesale and logistics, combined with retail and markets, food service and households.**

Three central elements, i.e., food waste definition, food supply chain stages and the food waste destination, define the system boundaries. Figure 1 provides a schematic overview of these system boundaries. These boundaries exclude yield losses due to weather events or diseases as they start at the point at which material becomes 'food' i.e., when it is ready for harvest or slaughter.

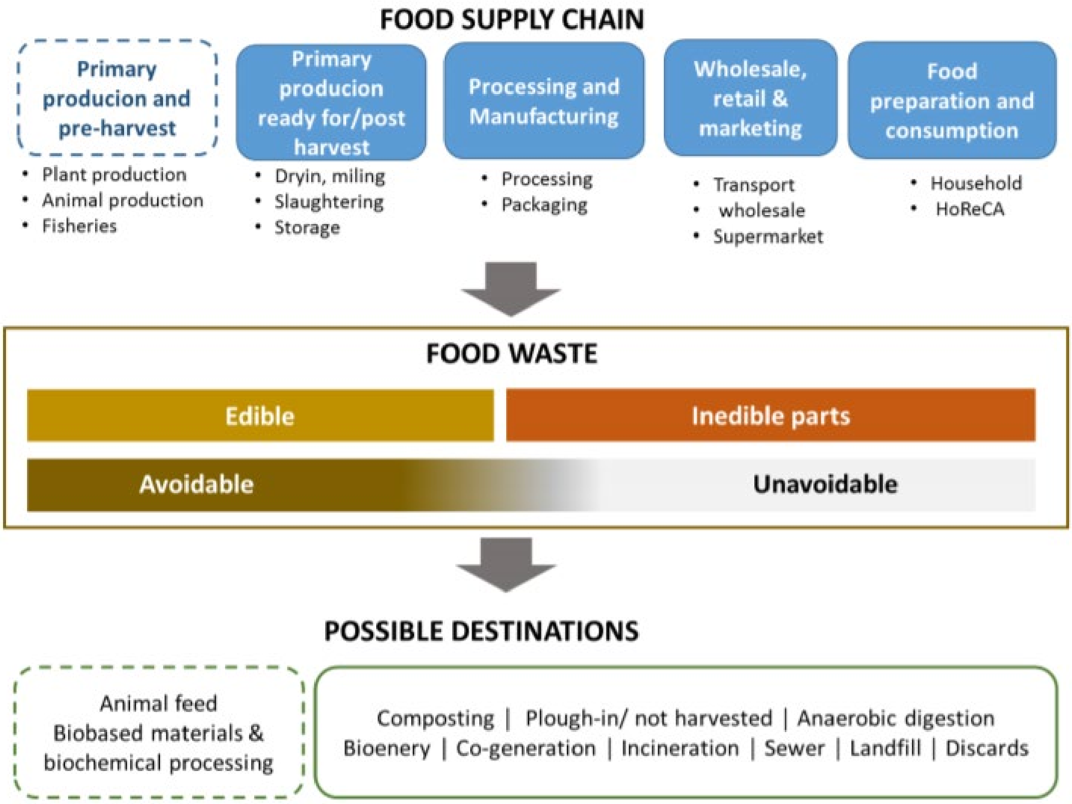

Figure 1: Framework defining the food supply chain and food waste adapted from Caldeira, et al. (2017) and Östergren, et al. (2014)

Caldeira, *et al.* (2017) collected several recent studies on food waste estimates in the EU. Figure 2 gives an overview of the total amount of food waste for each sector of the food supply chain reported in these studies. The total amount ranges from 173 kg per capita per year to 290 kg per capita per year. Also the percentage contribution of each sector varies between studies. In their study Caldeira, *et al.* (2017) could attribute most of the difference to dissimilarities in the definition of food waste & the system boundaries and data collection. In spite of the differences between the studies, they all indicate that around half of the food waste is created at the level of consumption.

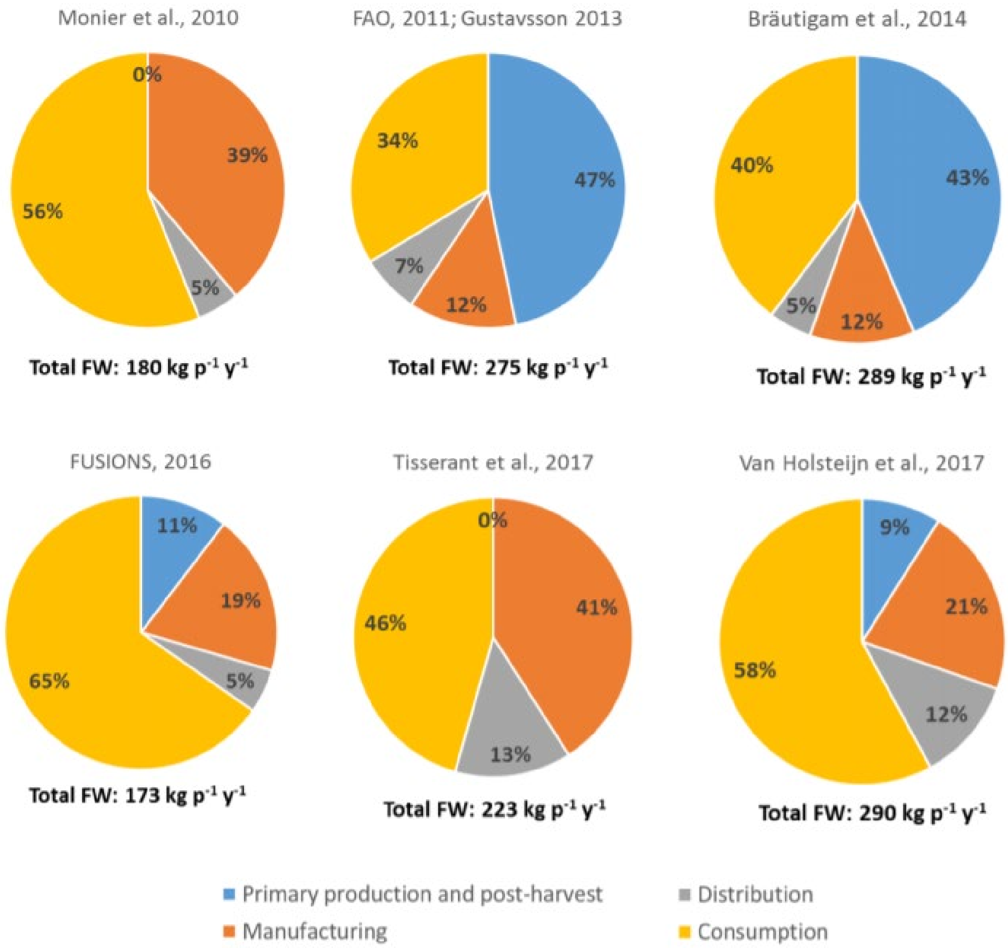

Figure 2: Total food waste quantification (in kg per capita per year) and share of food waste generated in each stage of the food supply chain (primary production, manufacturing, distribution and consumption) at European level reported in different studies (Caldeira, Corrado, & Sala, Food waste accounting - Methodologies, challenges and opportunities, 2017)

2.1.1.2 Side streams and residues from primary production

Losses considered at the stage of the primary production process include agricultural residues (e.g. roots and straw), unharvested crops and the losses during harvest. In agriculture the main sources of primary vegetal residues are non-edible plant parts that are left in the field, orchard or greenhouse after the main crop has been harvested. These residues mainly include straw, stover, stubble, stalks, sticks, leaves, haulms, roots, branches, twigs, brushes, trimmings and pruning; and they are produced from different sources including seeds, fruits, nuts, vegetables and energy crops.

2.1.2 Valorisation of biomass side streams

The demand for functional materials, fuel, food and animal feed are rising due to growing world population and increasing welfare. Biomass is expected to become an increasingly important factor in meeting these demands. To use the biomass efficiently, it is important that the side streams are used for an application as high as possible in value. The hierarchy of values for biomass applications is drawn up as follows: 1) food for human consumption, 2) feed for animals, 3) functional materials and products and 4) fuels and their applications (Bos-Brouwers, Langelaan, Sanders, van Dijk, & van Vuuren, 2012). It's important to note that when biomass is **valorised as food or feed**, it is **no longer classified as waste** according to the FUSIONS definition (Östergren, et al., 2014).

Mainly the use of organic side streams in animal feed (pig or cattle) is an application that has gained a lot of attention. Valorising food waste as animal feed is preferred by many sectors instead of sending it to waste processing because this distribution has a higher value and therefore may generate a financial advantage. The proportion food waste utilised as animal feed can be as high as 50-80% of the total food waste. In Table 1 the estimated amounts of food and by-products utilised as feed in the EU is presented. This list is incomplete, but it provides a picture of the current use of food waste in animal feed. The numbers shown must of course be considered in relation to the total amounts of food produced (Stenmarck, et al., 2016).

Table 1: Amounts of food and by- products utilised as feed in the EU-28

Country	Amount (tonnes)	Year	Reference
European Union	5 000 000	2015	(EFFPA)
UK	450 000	2011	(Whitehead, Parfitt, Bojczuk, & James, 2013)

According to the European Former Foodstuff Processors Association (EFFPA), 5 million tonnes of former food stuff is used as animal feed. They also claim that this can increase to 7 million tonnes until 2025. In the UK an estimation of 450 000 tonnes former food stuff is used in animal feed (2011).

Although complete data for the EU on distribution of valorised food waste could not be found, an example of Flanders can be presented (Figure 3). This figure also shows that animal feed is the most important destination for valorisation of food waste: 43% of all Flanders food waste is distributed to animal feed. Up to 92% of all Flemish food waste is valorised as animal feed, biobased materials or energy. Only 6% is incinerated with energy recovery (OVAM, 2017).

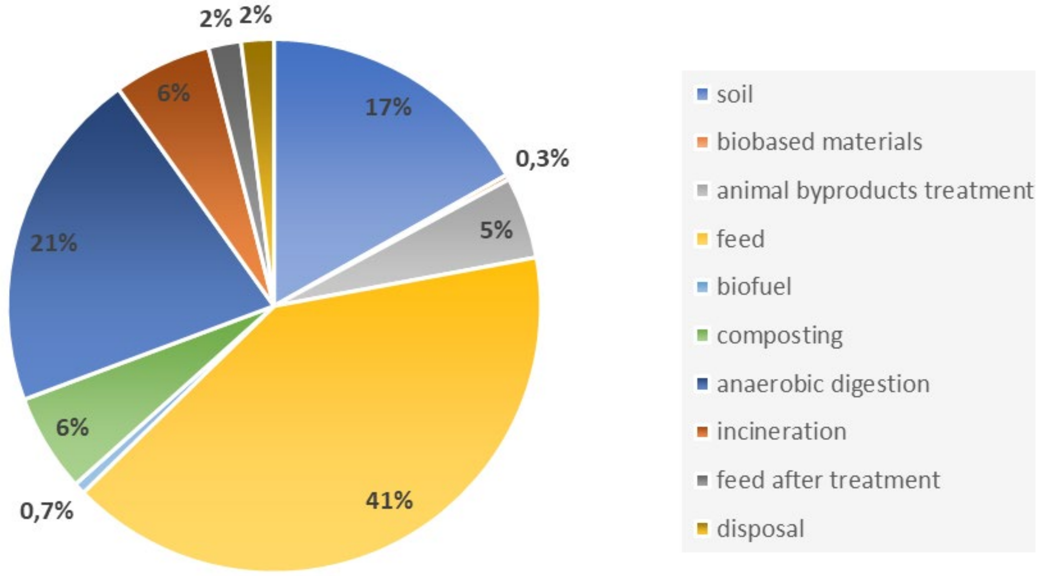

Figure 3: Distribution of destinations of food waste in Flanders in 2015 (OVAM, 2017)

However, integrated valorisation of biomass often faces some challenges. Therefore, the number of side stream based products available on the market is rather limited, despite a lot of valorisation projects, studies and patented processes. The most important challenge is to get the biomass collected. Often the (local) available biomass is limited and there is lack of continuous supply (time and volume) of the biomass. Moreover, transport and processing of biomass can lead to high production costs. Other challenges may include the composition of the side streams, legislation and also very important the perception of the society (Kips & Van Droogenbroeck, 2014).

As insects are considered as livestock by legislation, some biomass has potential as insect feed. However, it is best to check the current application(s) before investigating an organic side stream for insect feed. It is important to avoid competition with other sectors, as the availability of the biomass may depend on this. Therefore, the ValuSect project focusses on the investigation of non-valorised biomass or oversupplied biomass as potential insect feed. For example, it is interesting to consider side streams that are currently used for energy production as insect feed, as animal feed is more valuable than energy production.

2.1.3 Delineation of biomass side streams for the ValuSect project

Although a substantial share of food waste is created at the level of food services and households, these sectors will not be discussed in this project because most of the food waste created in these sectors may not be used as animal feed due to legal aspects (see 2.2). In this this paragraph examples of side streams and residues from wholesale and retail, processing and primary production will be discussed.

2.1.3.1 Wholesale and retail

According to Stenmarck, *et al.* (2016), approximately 5% of the total amount of food waste in the EU is generated in the wholesale and retail sector. This percentage refers to an amount of 4.6 million tonnes (± 1 million tonnes) of food waste at wholesale and retail level in 2012.

2.1.3.1.1 Auctions

The auction system that typically governs wholesale markets generates food losses and waste. Since consumers bid on both the quality and quantity of items, this means that products can be left unsold. Wholesale markets clear their inventory of perishable products at the end of the business day. This clearance is done to ensure that there is adequate space for the following day's auction items (Zhang R. J., 2019).

Fruit and vegetable auctions receive and sell fresh fruit and vegetables. The products sold vary from organisation to organisation and according to the season. Unfortunately, many products do not end up on auction or are not sold due to overproduction (incorrect timing). Other reasons for discarding crops include a deviating colour, shape or size that doesn't comply with the auction's criteria. Moreover, damaged crops, due to weather conditions or others, are also discarded (Roels & Van Gijseghem, 2017).

For example for Belgium, data on the number of received and unsold products at auctions was collected by the 'Verbond van Belgische Tuinbouwoperaties' (Table 2). This union comprises the major Belgian auctions, and represents 80% and 60% of the fresh markets vegetables and fruits, respectively (this excludes industrial vegetables). In 2018 and 2019 the Belgian auctions received more than 1 million tonnes of fresh fruit and vegetables. Only 2-3% is not sold. 96% of the losses are vegetables (tomato, leek, lettuce and courgette), 4% is fruit (mainly apple and pears) (Flemish Food Supply Chain Platform for Food Loss, 2017). Most of the loses go to feed. However, 4523 tonnes went to fermentation and 3040 was deposited on soil in 2018. In 2019 a total of 12528 tonnes was deposited for fermentation.

Table 2: overview of received products and their destination for Belgian fruit and vegetable auctions (represented in tonnes) (Verbond van Belgische Tuinbouwcoöperaties, 2019)

Year	2018	2019
Total received products	1039685	1145575
Total sold products	1011962	1116257
Total unsold products	24193	29016
Free distribution (e.g. food bank)	1491	1689
Free distribution others (e.g. marketing)	1626	1643
Feed	13512	13155
Fermentation	4524	12528
Composting	0	0
Soil	3040	0

2.1.3.2 Processing

When food is processed, large amounts of side streams are generated. According to a study of Caldeira, *et al.* (2019) 30.6 million tonnes of food waste is produced at processing and manufacturing level in the EU.

In the table below (Table 3), some streams from the processing industry are presented. The estimated quantities of the streams are given as well as their current use. The data are estimated on European level.

Table 3: Estimated quantities and current use of example streams of the processing industry (EU level)

Stream	Tonnes/year	Current use	Reference
sugar beet pulp	13 million	feed, bioenergy	(European Commision, 2020)
industrial onion waste*	> 500 000	-	(Waldron, 2001)
oil crops	10 million	feed, bioenergy	(Caldeira, De Laurentiis, Corrado, van Holsteijn, & Sala, 2019)
potato peels (steam, abrasive or lye peeling)	3 million	mostly used in feed	(Ćosić, et al., 2016)
vegetables industry	2.6 million	mostly used in feed	(Caldeira, De Laurentiis, Corrado, van Holsteijn, & Sala, 2019)
fruit industry	6.1 million	mostly used in feed	(Caldeira, De Laurentiis, Corrado, van Holsteijn, & Sala, 2019)
brewer's spent grain	3.4 million	feed, bioenergy	Eurostat data (Stojceska, Ainsworth, Plunkett, & İbanogˇlu, 2008)

cereals industry	2.5 million	mostly used in feed	(Caldeira, De Laurentiis, Corrado, van Holsteijn, & Sala, 2019)

* The main onion wastes include onion skins, two outer fleshy scales and roots generated during industrial peeling, and undersized, malformed, diseased or damaged bulbs.

It is important to note that the food processing industry is a very heterogeneous sector: many different types of products are produced. The food waste profile (type, amount) therefore varies greatly per food product processed. In addition, the food processing industries differ between (European) countries.

2.1.3.2.1 Meat co-products

Meat co-products are the non-meat components arising from meat processing/fabrication. Based on the information gathered from FAOstat, the number of porcine and cattle herds that have been slaughtered has varied in opposite directions within the European territory. While pork herds have increased a 106% from 2010 to 2018; in the same period of time the cattle herds have decreased to a 90% of the heads killed in 2010. On the other side, poultry production has been continuously growing over the past decade, which means that's there is an increasing production of poultry by-products as for example blood and feathers; being the last one the most relevant because of its high protein content.

When looking into detail what the situation is in the countries involved in this project (Belgium, Switzerland, Netherland, UK, and Ireland), the number of heads processed are presented in Table 4.

Table 4: Number of poultry (x1000) porcine and cattle heads in project member's states from 2010 to 2018. Livestock represented as number of heads. Variation represented as %

Country	Pork 2010	Pork 2018	Variation	Cattle 2010	Cattle 2018	Variation	Poultry 2010	Poultry 2018	Variation
Belgium	11900000	11230544	94.4	835198	888099	106.3	307950	304955	99.0
The Netherlands	13943600	15246163	109.3	2057000	1897989	92.3	479016	600951	125.5
Switzerland	2859112	2568789	89.8	651670	628644	96.5	53000	69863	131.8
UK	9454000	10938000	115.7	2710000	2811000	103.7	904000	1137000	125.8
Ireland	2657300	3446700	129.7	1716600	1896000	110.5	79500	86995	109.4
Total	40814012	43430196	106.4	10369198	10306077	99.4	1823466	2199764	120.6

These results indicate that in general terms the overall number of porcine and cattle heads had increased over time, although not significantly. In the same way, the production of offal and co-products is expected to increase in a similar percentage. On the other hand, chicken production has increased on average a 120% in that period of time.

Based on previous reports published by Southampton University and Ashtown Teagasc, (Angiestuff, 2020) the percentage of the animal live weight considered as offal, co-products or edible by-products can be as high as 25-30% for cattle (it does not include fat or Cat 1 and Cat 2 products); and around 20% for pork (excluding Cat 2 and fat). Finally, feathers can be up to the 10% of the body weight of the animal, although regular values range from 4 to 6%.

It means, that considering an average bodyweight of 1.5 kg for poultry, 100 kg for pork and 550 kg per cattle, the amount of non-meat products that are generated annually is quite large. Just for the partners involved in the project, the annual production in 2018 was of 1,417,085 tonnes of offal and co-products and around 175,600 tonnes of feathers. From which, the vast majority was used for rendering, land filling, pet-food and in a minor percentage for food.

Table 5: Tonnes of meat co-products and offal in the project partners countries in years 2010 and 2018

Country	Pork 2010	Pork 2018	Cattle 2010	Cattle 2018	Feathers 2010	Feathers 2018
Belgium	238000	224610	114839	122113	23096	22871
The Netherlands	278872	304923	282837	260973	35926	45071
Switzerland	57182	51375	89604	86438	3975	4620
UK	189080	218760	372625	386512	67800	85275
Ireland	53146	68934	236032	260700	5962	6524
Total	816280	868603	1425764	1417085	136760	164982

Mostly due to restrictions imposed by legislation, the valorisation of **meat co-products** in animal feed is quite limited with the remarkable exception of plasma from pork blood, which is used for broilers, piglets and dairy cows feeding and, more recently, for aquaculture purposes (Chahine, et al., 2019; Tapia-Paniagua, et al., 2020). However, the red cells fraction is still not exploited for these purposes. Other co-products, as for example, tongue, lungs, heart, kidneys or spleen have a limited market for human consumption, which is decreasing continuously, and is mostly employed as ingredient for pet food and very specific industrial applications (see Table 6); although not at great extend; since the most of them are used for land filling or composting.

Table 6: Animal co-products current uses: industrial products and pharmaceuticals

Co-product	Commercial product
Bile	Detergent, pharmaceuticals
Bones	Adhesives, animal feed, calcium and phosphorous source (bone meal), glycerine, glue, collagen
Blood	Spray dried plasma, iron supplement, functional ingredient, fat replacer
Brains and spinal cords	Steroid, cholesterol, lecithin, cephalin
Fats and fatty acids	Biodegradable detergents, animal feed, biodiesel, cosmetics, lubricants, plasticisers, emulsifiers, solvents
Glands	
• **Adrenal**	Cortisone, epinephrine, norepinephrine
• **Liver**	Heparin, vitamin B12, pet food, bile (detergent, pharmaceuticals)
• **Pancreas**	Chymotrypsin, insulin, pancreatin, trypsin, glucagon
• **Pituitary glands**	ACTH, prolactin
• **Spleen**	Ferritin
• **Thymus**	Thymosin
• **Thyroid**	TSH, hormones, …
Kidney	Pet food
Hides and skins	Gelatin, Collagen based adhesives, leather
Hairs, wool, skins, feathers, nails, horns, and hooves	Fibers, collagen, glue
Hearts	Pet food

Intestines	Sausage casings, strings, heparin, Small intestinal sub mucosa materials for clinical applications
Lungs	Pet food, heparin
Ovaries	Estrogen
Stomach and tripe	Pet food, glue, pepsin, rennin, lipase, trypsin
Trachea	Chondroitin sulphate

2.1.3.2.2 Fruit and vegetable processing industry

Fruits and vegetables are the most utilized commodities among all horticultural crops. They are consumed raw, minimally processed, as well as processed. Losses occur throughout all phases of the food supply chain from production throughout all postharvest stages before consumption, including during harvesting, transport to packinghouses or markets, classification and grading, storage, marketing but also processing (Sagar, Pareek, Sharma, Yahia, & Lobo, 2018; FAO, 2011). This section focusses on the residues from the fruit and vegetable processing industry.

Residues from the processing industry include losses due to spillage and degradation during industrial or domestic processing. Losses may occur when crops are sorted out if not suitable to process or during washing, peeling, slicing and boiling or during process interruptions and accidental spillage (FAO, 2011). Residues of fruit and vegetable industry are mainly composed of seed, skin, rind, and pomace, containing good sources of potentially valuable bioactive compounds, such as carotenoids, polyphenols, dietary fibres, vitamins, enzymes, and oils, among others (Sagar, Pareek, Sharma, Yahia, & Lobo, 2018). The current use of residues from fruit and vegetable processing industry mainly consists of animal feed (Table 3) (Caldeira, De Laurentiis, Corrado, van Holsteijn, & Sala, 2019).

According to a study of the FAO (2011), fruit and vegetable losses in industrialized countries occur mostly at retail and consumer levels, but in developing countries the losses occur mostly at post-harvest and processing levels (Figure 4). In Europe, an estimated 2% of fruits and vegetables gets lost at processing and packaging level (Figure 4, Table 7). According to a study of Caldeira, *et al.* (2019) 6,1 Mt (million tonnes) of fruit and 2,6 Mt of vegetables gets lost at processing and manufacturing level in the EU (total production: 67,9 Mt of fruit products and 68,5 Mt of vegetables) (Table 6).

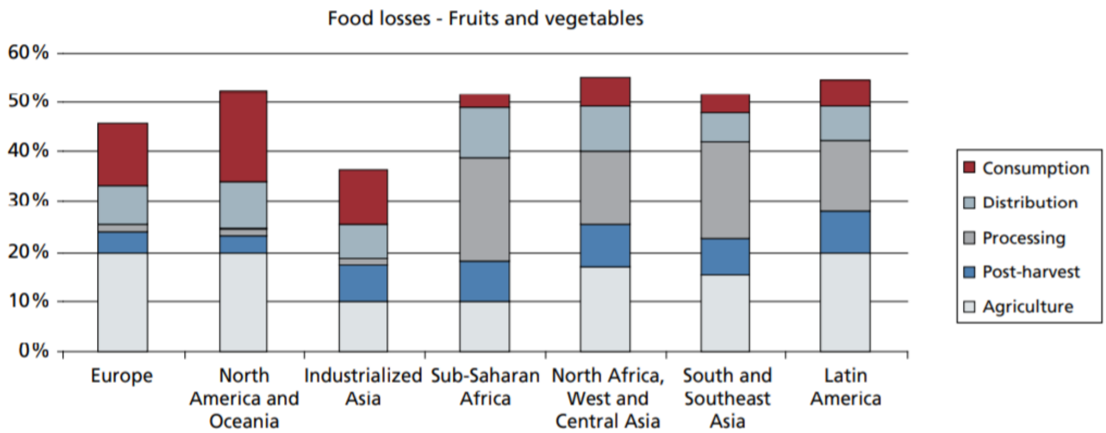

Figure 4: Part of the initial production lost or wasted at different stages of the food supply chain from fruits and vegetables in different regions (FAO, 2011)

Table 7: estimated waste percentages for fruit and vegetables in each step of the food supply chain for Europe inclusive Russia (FAO, 2011)

Agricultural production	Postharvest handling and storage	Processing and packaging	Distribution	consumption
20 %	5%	2%	10%	19%

2.1.3.2.3 Cereal processing industry

Cereal based food products cover over 20% of daily diet, making cereal production and processing one of the most important sectors of agri-food industry (Al-Thani, et al., 2017). Cereal based products are the basis of all Food Pyramid that were developed and proposed in different studies. Cereal products also increase the consumption of dietary fibre in the daily diet. Baiano (2014) estimated that about 12.9% of all food waste are generated from the cereal processing and manufacturing. Caldeira, *et al.* (2019) reported that 2.5 million tonnes cereal waste per year is produced at processing and manufacturing level in the EU.

Within the cereal chain some losses can be recovered, such as sesame husk and rice bran for obtaining dietary fibre (Nandi & Ghosh, 2015), oat waste for extraction of antioxidants (Serea & Barna, 2011) wheat bran for fructans (Verspreet, Dornez, Delcour, Harrison, & Courtin, 2015), and Brewers' spent grain for ferulic acid production (Mussatto, Dragone, & Roberto, 2006). Currently, cereal processing residues are mostly valorised in animal feed (Caldeira, De Laurentiis, Corrado, van Holsteijn, & Sala, 2019).

2.1.3.3 Primary production

In this section, side streams from primary production are subdivided according to application within the ValuSect project, namely grassy biomass and agricultural side streams. Grassy biomass greatly differs from agricultural side streams and is therefore discussed separately. Below, the subdivisions are defined and their availability is discussed.

2.1.3.3.1 Agricultural side streams

Agriculture is the most comprehensive word used to denote the many ways in which crop plants and domestic animals sustain the global human population by providing food and other products (Harris & Fuller, 2014). Agriculture implies both crop cultivation as raising domestic animals, but in this section the focus lays on agricultural crops as this is considered more relevant for the ValuSect project.

Agricultural side streams, or in this case crop residues, can be defined as the part of the plant that is left over after harvest (see 2.1.1.2). The residues vary greatly per crop in shape, structure, composition and decomposition rate (Lal, 2005). The residues mainly consist of foliage and stalks of plants and cannot be consumed as food (Searle & Malins, 2013).
Horticulture is considered a branch of agriculture dealing with garden crops, generally fruits, vegetables, and ornamental plants. Unlike the overall agriculture, horticulture does not include large-scale crop production or animal husbandry. In terms of scale, horticulture falls between domestic gardening and field agriculture, though all forms of cultivation naturally have close links (Synge, 2020). Horticultural side streams are also included in the ValuSect project. In fact, most of the side streams considered come from horticulture.

Globally, it is estimated that between 2003 and 2013, the production of agricultural residues increased by 33%, reaching 5 billion tonnes in 2013. The European continent produces 16% of the total crop residues (Cherubin, et al., 2018).

The agricultural residues mainly come from the pruning, harvesting, clearing and cleaning process of the crops. Some crops undergo a pruning process to get rid of shoots, sprouts, etc. because this costs the crop energy (e.g. tomatoes, strawberries). This way more energy is released for the fruit volume. Pruning is carried out several weeks before harvest, depending on the crop. For tomatoes, for example, this is done 5-6 weeks before harvesting the fruits. During cucumber cultivation, excessive fruits are removed, leaving more energy for the plant to be invested for the remaining fruits. Pruning will be carried out multiple times during the life cycle of the plants (several weeks before each harvest of the fruits) and mainly consists of foliage. The pruning residues are left in the greenhouse or on the field. In case of tomatoes and strawberries (greenhouse production), the pruning residues will be removed during the greenhouse clearing process. By then, the pruning residues will be dried out (non-fresh).
After the life cycle of the crops is complete (for example 9 months for tomatoes) the crops are completely removed to make room for new plants. The largest amount of agricultural residues thus arises during this clearing process. Foliage from agricultural crops is mostly left on the field (e.g. leek, sugar beet). However, in some cases it might be interesting to remove the foliage from the field; e.g. smell, too high nitrogen concentration.
During harvesting of the crops or fruits agricultural residues are also produced. Undersized, malformed, diseased or damaged crops or fruits are removed. Also for some crops (e.g. lettuce), the outer leaves are removed. Furthermore, during the cleaning process of the crops, agricultural residues are also produced.

Cellulose-rich agricultural side streams are often reused for agricultural applications, such as animal bedding. It is also used for the production of renewable energy (Searle & Malins, 2013). In fact, in 2017 18% of all biomass used for bioenergy production in Europe came from agriculture (Calderón, et al., 2019). Other agricultural side streams are often used in animal feed (Caldeira, De Laurentiis, Corrado, van Holsteijn, & Sala, 2019).

In the table below (Table 8), the estimated agricultural crop production and availability of crop residues is given. The estimated quantities are given for the UK, Belgium, The Netherlands, Germany, France, Ireland and Switzerland (partners of the ValuSect project), because the production quantities vary per country. Only the crops found relevant for the ValuSect project are presented in the table. Despite numerous studies attempting to estimate the level of production of agricultural residues in Europe and availability of crop residues, a series of shortcomings are clearly identifiable. In fact, hard data are not collected at EU level, estimations are based on different assumptions, and sparse data is collected from different crop commodities. The estimated crop production in Table 8 is based on data from different references, the estimated availability of crop residues is calculated using the study of Wirsenius (2000) and Ronzon, *et al.* (2015).

Table 8: Estimated agricultural crop production and availability of crop residues in tonnes/year for UK, Belgium, The Netherlands, Germany, France, Ireland and Switzerland. RTP ratio is residue-to-product ratio. Based on (FAO, 2020; European Commission, 2020; BioBoost, 2018; Jeannequin, Plénet, Carlin, Chauvin, & Dosba, 2015; Tridge, 2020; Wirsenius, 2000; Ronzon, Piotrowski, & Carus, 2015)

	United Kingdom			Belgium			The Netherlands			Ireland			Germany			France			Switzerland		
crop	**crop production**	**RTP-ratio**	**residue production**	**crop production**	**RTP-ratio**	**residue production**	**crop production**	**RTP-ratio**	**residue production**	**crop production**	**RTP-ratio**	**residue production**	**crop production**	**RTP-ratio**	**residue production**	**crop production**	**RTP-ratio**	**residue production**	**crop production**	**RTP-ratio**	**residue production**
apple	502700	0,20	100540	273950	0,20	54790	269000	0,20	53800	20100	0,20	4020	1198517	0,20	239703	1737412	0,20	347482	222431	0,20	44486
chillies & pepper	27398	0,20	5480	26040	0,20	5208	355000	0,20	71000	328	0,20	66	14658	0,20	2932	32183	0,20	6437	408	0,20	82
cabbage	223719	0,20	44744	122254	0,20	24451	208700	0,20	41740	26000	0,20	5200	604331	0,20	120866	162377	0,20	32475	42857	0,20	8571
cauliflower and broccoli	146841	0,20	29368	122344	0,20	24469	70100	0,20	14020	9600	0,20	1920	126097	0,20	25219	287333	0,20	57467	14057	0,20	2811
chicory*		0,30		50000	0,30	15000	52000	0,30	15600		0,30		14030	0,30	4209	168890	0,30	50667		0,30	
cucumber	55080	0,20	11016	24490	0,20	4898	410000	0,20	82000	1600	0,20	320	267589	0,20	53518	137849	0,20	27570	14550	0,20	2910
egg plant		0,20		10120	0,20	2024	55000	0,20	11000		0,20			0,20		31989	0,20	6398		0,20	
leek	37323	0,20	7465	154791	0,20	30958	82200	0,20	16440	3000	0,20	600	76984	0,20	15397	149816	0,20	29963	13681	0,20	2736
lettuce	107428	0,20	21486	47935	0,20	9587	101181	0,20	20236	6200	0,20	1240	350445	0,20	70089	217448	0,20	43490	73038	0,20	14608
potato	5028000	1,00	5028000	3045443	1,00	3045443	6029734	1,00	6029734	273000	1,00	273000	8920800	1,00	8920800	7870973	1,00	7870973	417156	1,00	417156
strawberry	131639	0,20	26328	43000	0,20	8600	64800	0,20	12960	5700	0,20	1140	141693	0,20	28339	56000	0,20	11200	9323	0,20	1865
tomato	66893	0,20	13379	258680	0,20	51736	910000	0,20	182000	3900	0,20	780	103266	0,20	20653	712019	0,20	142404	43243	0,20	8649
triticale	42350	1,18	49973	32132	1,18	37916	5450	1,18	6431		1,18		1935500	1,18	2283890	1381935	1,18	1630683	47342	1,18	55864
wheat	13555000	1,00	13555000	1652249	1,00	1652249	985297	1,00	985297	506800	1,00	506800	20263500	1,00	20263500	35798234	1,00	35798234	497250	1,00	497250

The production of agricultural residues often is seasonal and linked to the harvest and clearing period. This means the side streams are mostly not available year-round and most of the time a lot of biomass is released during a short period. The availability also differs per crop. For this reasons preserving of the residues needs to be considered. In the table below (Table 9), the period of availability of agricultural residues are presented. The table is based on crop production period (residues from harvesting process) and clearing period (bell pepper, tomatoes) in Northern and Western Europe.

*Table 9: Crop (residue) availability period of in Northern and Western Europe. High availability in dark grey, smaller production amounts in light grey and unavailable in white. * Includes only foliage from clearing greenhouses (foliage from pruning is left in the greenhouse until clearing) (aid infodienst Bonn , 2020).*

Crop	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
bell pepper*												
cabbage												
cauliflower												
forced chicory roots												
cucumber												
egg plant												
lettuce												
tomato*												
strawberry												
leek												

2.1.3.3.2 Grassy biomass

In every country there are a lot of verges, natural grass and parks. They are regularly cut for safety and/or to maintain the landscape. Grass from verges and nature parks is harvested about 2 times a year. Mostly a first time early in summer and a second time late summer/begin autumn. This to give the plants the possibility to grow and seed and house animals and insects. Sometimes verges are mowed more often if there's a security issue (nidirect, 2020; Informatie Vlaanderen, 2020). Grass clippings from verges, nature and parks are usually removed to impoverish the soil which gives flowers the possibility to grow. This generates large quantities of biomass that is often considered as waste. For the Netherlands it is estimated that 800-1200 ktonnes/year of verge cuttings are generated. Grass cuttings from waterways and nature are estimated to each account for 0.5-1 million tonnes/year (Oosterbaan, van Blitterswijk, Holshof, & de Jong, 2008; Brinkmann, 2014). Table 10 gives an overview of estimated verge grass clippings in The Netherlands, Belgium, United Kingdom, France and Switzerland.

Table 10: Overview of estimated verge grass cuttings in Netherland, Belgium, United Kingdom, France and Switzerland. Represented as fresh weight in ktonnes/year.

Country	Grass cuttings (ktonnes/year)	Reference
The Netherlands	800 - 1.200	(Brinkmann, 2014)
Switzerland	73	(OFROU, 2018) (OFS , 2020)
UK	657	(Brown, et al., 2020)
France	937	(Statista Research Department, 2020) (Ministère de la transation écologique, 2018)

		(Agr'eau, 2020)
Belgium	336 (Flanders) 303 (Wallonia)	(Velghe, Magielse, Moorkens, & Meester, 2014) (Van Meerbeek, Ottoy, De Meyer, Muys, & Hermy, 2016) (BdM, 2020)

The potential yield of grasses is dependent on many factors and is likely to vary significantly from year to year and site to site. Most of the verge grass is currently processed in green composting facilities.

Although there's a large availability of verge grass, farmers are not keen on using it as feed. Roadside grasses may be contaminated with litter and vehicle pollutants. Vehicles typically emit metals such as zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd) and organic contaminants including benzene, and polycyclic aromatic hydrocarbons (PAHs) (e.g., naphthalene, acenaphthylene, and anthracene) into the environment (Kaur & Katnoria, 2014; Mason, et al., 2020). Some of these contaminants may be accumulated by insects when fed on feed contaminated with these toxic elements (van der Fels-Klerx, Camenzuli, Belluco, Meijer, & Ricci, 2018; Van der Fels-Klerx, Camenzuli,, Van der Lee, & Oonincx, 2016).

2.2 Insect production on organic side streams

Because insects have the opportunity to convert (low-value) biomass into high-quality biomass, research has already focused on rearing insects on side streams. In this chapter, the potential of insect production on side streams as well as a summary of already tested substrates, their chemical analysis and the nutritional composition of the insects (*T. molitor*, *A. domesticus* and *L. migratoria*) produced on these substrates is given and discussed.

2.2.1 Insect nutritional requirements

Before side streams can be used in insect feed, it is important to understand the needs of the insect species in question. Below, the insect dietary requirements per species are discussed.

2.2.1.1 *Tenebrio molitor*

Tenebrio molitor needs a dry feed (e.g. bran), supplemented with a wet feed source will reduce development time and mortality. Depending on the amino acid compositions, the protein concentration can be as low as 10% (Davis & Sosulski, 1974), but in general they prefer a higher concentration (28%) (Morales-Ramos, Rojas, Shapiro-llan, & Tedders, 2013). The carbohydrate concentration should be high, 65% or higher and can consist entirely of starch. In all cases the diet should include vitamins of the B-complex (Ribeiro, Abelho, & Costa, 2018). A good standard diet can be: 80% wheat bran and 20% supplement consisting of 83% dry potato, 13% dry egg white, 2% soy protein and 2% peanut oil (Morales-Ramos, Rojas, Shapiro-llan, & Tedders, 2013). Beetles prefer a higher protein concentration compared to the larvae (Rho & Lee, 2014).

2.2.1.2 *Acheta domesticus*

Patton (1967) reported 4 diets that gave satisfactory results based on survival and growth rate. The protein content of these diets varied between 20 and 30% (on dry matter basis), carbohydrates from 32% to 47% and fat ranging between 3.2% and 5.2%. However they used several ingredients of animal origin. Morales-Ramos et al. (2020), designed several cricket diets through a self-selection process, concluding that Vitamin B and C, sterols and manganese had significant positive impact on live biomass production.

2.2.1.3 *Locusta migratoria*

Different attempts were made to construct artificial diets for *Locusta migratoria* especially by Dadd in the early 1960's (1960a, 1960b, 1960c, 1961a, 1961b), however none were as successful as their natural food sources. Despite that, a lot of insight can be gained about the nutritional needs of *L. migratoria*. For the nymphs to grow satisfactorily the diet had to contain protein (20% on DM basis), digestible carbohydrates (10%), linoleic acid (0.5%), cholesterol (0.5%), ascorbic acid (0.3%) and vitamins (0.2%). Also cellulose was required (66.1%), despite its indigestibility for locusts.

2.2.2 Potential of organic side streams for rearing edible insects

An overview of several studies on the use of organic side streams for rearing of *T. molitor*, *A. domesticus* and *L. migratoria* is given bellow. A distinction is made between side streams of vegetable origin and animal origin, as the properties of vegetable and animal material differ greatly from each other. Also, grassy biomass is discussed as potential feed for *L. migratoria*.

2.2.2.1 Vegetable co-products

The Interreg 2 Seas project BioBoost has given an indication of the use of horticultural residues as feed for insects. Horticultural residues were used as feeding substrates for mealworms and black soldier fly. The results of this project show that side streams have potential as a feeding substrate for insects. However, the mono streams do not seem to meet the nutritional needs of the insect species in question. The diet must be supplemented with high-quality products to ensure proper development of the insect. In other words, compound diets - which are a mixture of different ingredients - will have to be formulated. The advantage of mealworms is that they thrive on a dry substrate supplemented with a source of moisture. As long as the dry feed is nutritionally balanced, the nutritional value of the moisture source is irrelevant. This opens up more possibilities to use moist side streams as wet feed for mealworms. The same goes for crickets. However, it is not impossible to compose the dry feed of mealworms, crickets or grasshoppers with residual flows, here only a good nutritional composition must be taken into account to meet the needs of the insect.

Below, the results of the BioBoost project are given: residues as wet feed for the production of mealworms:

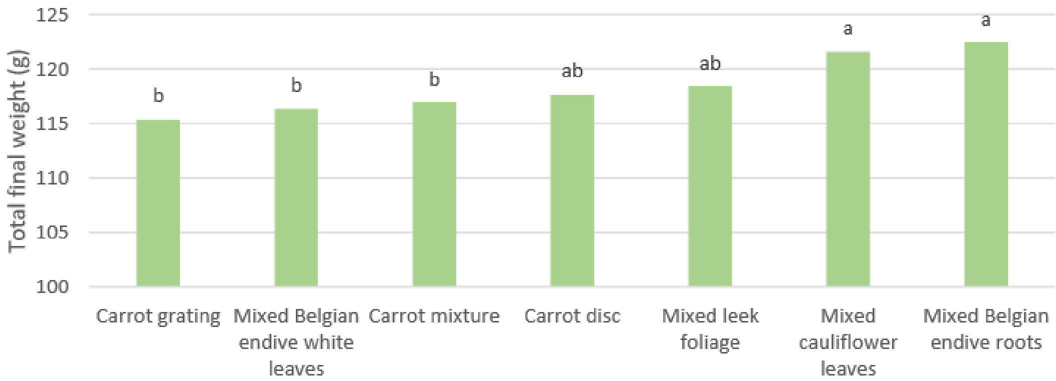

Figure 5: Total weight of mealworms harvested after 17 days on a diet of wheat bran and a daily supply of equal amounts of moisture source, the average value (n=3) (Coudron, Spranghher, Elliot, & Halstead, 2019)..

During the study, the residues were mixed into a pulp. Similar results of the different side streams as wet feed were obtained. This indicates that most residues can be used as mealworm wet feed without much trouble, as long as they are properly mixed. Mixed cauliflower leaves and mixed Belgian endive roots even outperformed carrots, which is often used as moisture source for rearing mealworms. Also, a recent PDPO project (2017-2019) 'Witloofwortels, ook meelwormen lutsen er wel pap van' investigated the potential of chicory roots and chicory leaves as a wet feed for mealworms: this also shows that this is a potential alternative wet feed for mealworm production.

Using side streams for mealworm rearing has also been reported by Oonincx, *et al.* (2015). They investigated, among others, a mixture of freeze-dried side streams (spent grains, beer yeast, and cookie remains) that supported good growth of *T. molitor*. Compared to the reference diet, a similar survival rate (84% for the reference diet and 79% for the side stream mixture) and decreased development time (116 days versus 145 days) was found. Similar findings were reported by Van Broekhoven, *et al.* (2015). In their study the lesser mealworm (*Alphitobius diaperinus*) was reared on a similar diet. They used a mixture of maize DDGS (Distillers Dried Grains and Solubles), beer yeast, bread remains, and potato peels. The mixture resulted in a higher larval survival (95%) compared to the control diet (79%–82%) with similar development time (44 days versus 42–66 days) and the same pupal weight (0.022 g). An even shorter development time (38 days) was obtained by replacing the potato peels by spent grains. Also Ramos-Elorduy, *et al.* (2002) successfully reared *T. molitor* on side streams. In their study a mixture of freeze-dried cereals, fruit, and vegetables was used. A larval weight varying between 0.07 and 0.11 g/larva on this mixture of side streams was obtained.
In the study of Gianotten, *et al.* (2020) the growth of *A. diaperinus* reared on six non-freeze-dried side-streams, namely corn DDGS, rice bran, wheat middlings, corn gluten feed, rapeseed meal and brewery grains, was investigated. Pure side streams as well as mixtures were tested. The lesser mealworm was able to survive on all tested side-streams, however the growth performance varied among side streams. Good results were obtained using the reference diet, brewery grains and wheat middlings. Replacement of 5% to 15% of wheat middlings in the reference diet by rice bran or rapeseed meal was found possible without negatively effecting larval growth or production costs.

Crop residues are often difficult to digest by animals because they contain 30-45% cellulose, 10-40% hemicellulose, and 5-25% lignin (Wadhwa & Bakshi, 2013), and high quantities of lignocellulose. Additionally, crop residues resist biodegradation, resulting in requirements of harsher pre-treatment (Mussoline, Esposito, Giordano, & Lens, 2013). This must be considered when rearing insects on cellulose-rich agricultural residues.

The use of crop waste residues as co-substrate for mealworms has been studied by Yang, *et al.* (2019). Five common lignocellulose-rich crop residues (wheat straw, rice straw, rice bran, rice husk, and corn straw) were tested as potential feedstock for rearing 6-7 instar mealworms. Prior to tests, all straws were pre-treated (cut into small pieces; washed; dried in a forced-air drying oven, 45 °C, 48 h; stored, clean polyethene bags, 4°C). Mealworms were divided into six feeding groups, each fed with wheat bran and one of the five crop residues. Over a 32-day period, 10.0 g of each feedstock was supplemented into the respective incubator every four days.
Yang, *et al.* (2019) concluded that mealworms can survive on the tested lignocellulose-rich crop residues as sole feedstock as well as they can when fed with normal wheat bran over a 32-day period. Except for wheat straw and rice husk, the residues supported mealworms' life activity and growth with consumption of the residues by 90% or higher and degraded lignin, hemicellulose and cellulose. Rice straw, rice bran and corn straw were found suitable as supplementary feedstock to rear mealworms.

In the study of Oonincx, *et al.* (2015) several side streams were investigated as feed ingredient for house crickets diet. From these ingredients (freeze-dried beet molasses, potato steam peelings , spent grains and beer yeast, bread remains, and cookie remains), four experimental diet were composed: 1) high protein, high fat; 2) high protein, low fat; 3) low protein, high fat; and 4) low protein, low fat. Oonincx, *et al.* (2015) concluded that survival rates were low on all diets and feed conversion was inefficient on most diets (range 2.3-6.1). Possibly, the culture was infected by a densovirus. On the high protein, high fat and their control diet the development time of house crickets was 4.5–11.5 weeks, but development was strongly prolonged on the other diets. Despite this, they also concluded that insects can be produced on diets composed of food by-products.
The study of Sorjonen, *et al*. (2019) also concluded that crickets (*A. domesticus* and *Gryllus bimaculatus*) can be successfully reared on feeds composed with by-products of food industry. In their study, 14 experimental diets containing by-products (potato protein, barley mash, barley feed, turnip rape and mix of broad bean and pea) were investigated. The by-product feeds were designed to meet the nutritional demand of crickets. Therefore, the survival of *A. domesticus* was relatively high in by-product diets (64–94%). Also, many of the tested diets produce enhanced growth, development and yield compared to the control diets. The overall best by-product diet for *A. domesticus* was medium-protein barley mash.
However, it is previously reported that diets composed mainly of organic waste or by-products (low value diets) may cause lower growth performance and survival of crickets (Dobermann, Michaelson, & Field, 2019; Lundy & Parrella, 2015). This indicates that diets including only by-products could lack nutritionally needed components for the development and growth of crickets. The study of Sorjonen, *et al.* (2019) shows that by-products can be used as a protein source for crickets when the diet is in balance with other nutritional components.

More information on the potential of side streams for insect production is discussed in 2.2.3, which also takes into account the nutritional composition of diets.

2.2.2.2 Grassy biomass

So far little is known about the potential of side streams as feed for locusts. Grasshoppers are known to be specialists, therefore side streams that resemble their natural diet, i.e. grassy biomass, will have to be considered.

The nutritional composition of grass highly depends on field and weather conditions, time, varieties and management. Dry matter content of fresh grass typically varies between 15-26%. The crude protein content of grass is 15-25% DM but depends on harvesting time. Water soluble carbohydrates content varies between 35% DM on a warm sunny summer day and 10% DM on a cool cloudy autumn day. Fibre content (measured as NDF (neutral detergent fibre), this being the insoluble fibre fraction (cellulose, hemicellulose, pectin and lignin)) is about 30-40% DM. Total fatty acid content of grass varies from about 2.5 to 5% of forage dry matter, with the PUFA component making up 65 – 78% of the total lipid content (Germinal, 2020).

2.2.2.3 Meat co-products

The use of meat by-products or meat co-products, including processing streams rich in proteins, as substrate for rearing insects, aimed to be used as food and feed, has not been widely explored; in spite of the use of these protein sources are included in the European legislation. According to an EFSA communication (EFSA, 2015) ; the risk of using animal by-products for insects rearing is considered as safe, or even safer, as any other currently authorized proteins, as long as they do not employ substances of ruminant or human (manure) origin. According to EU Regulation 2017/893; insects are considered "farmed animals" and therefore, rules for animal feeding must be observed following EC 1069/2009. In this regulation is specified that "the use of ruminant proteins, catering waste, meat-and-bone meal and manure as a feed for insects is prohibited." Nevertheless, other meat by-products or co-products are allowed to be used as for example those coming from poultry, pork, lamb or rabbit meat processing. Even more, in order to avoid cross-contamination, animal by-products shall come from 1) slaughterhouses which do not slaughter ruminants and which are registered by the competent authority as not slaughtering ruminants; or 2) cutting plants which do not bone or cut up ruminant meat and which are registered by the competent authority as not boning or cutting up ruminant meat; or 3) other establishments than those referred to in 1) or 2) which do not handle ruminant products and which are registered by the competent authority as not handling ruminant products.

The diet of the currently approved insects to be used in aquaculture: black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and field cricket (*Gryllus assimilis*)) varies remarkably. Among them, the most suitable candidates to be reared on a substrate including animal origin proteins are the black soldier fly (polyphagous), common housefly (which is a meat scavenger), yellow mealworm (able to eat meat and feathers) and lesser mealworm (usually feed on animal parts from chicken or pigeons). On the other hand, among the insects that will be soon deem for human consumption (locusts, mealworms, crickets, and grasshoppers), only the mealworms seem to be appropriate to be reared in a substrate including animal origin proteins. Nevertheless, using animal origin products as protein supplement has been already studied for crickets, as reported by Woodring et al., (1979), where a 2.5% of fish meal was employed as part of the diet.

Meat co-products and meat processing streams are an excellent source of proteins, minerals, vitamins and lipids as previously reviewed (View annexes) (Lynch, Mullen, O'Neill, Drummond, & Álvarez, 2018; Mullen, Álvarez, Pojić, Hadnadev, & Papageorgiou, 2015; Mullen & Álvarez, Offal: types and composition, 2016). However, depending on the species (beef, pork or lamb) and the type of co-product, proximate composition might differ remarkably. For example, fat content can be as low as 0.1% in blood, and as high as 70% in pork jowl. In the same way, protein content can range from 15 to 22% (wet weight), although this parameter is less variable than the fat. More relevant than overall protein content, is how high the fraction of collagen is compared to total protein. This might be of relevance for insect's substrate formulation because of the particular properties of this protein: low solubility, thermal resistance, and excellent as thickener, water retention and texturizer. In this case, skin, pork hands and lungs are a rich source of collagen; while blood, heart or liver possess less collagen content. The main impact of collagen is its amino acid profile, while is rich in proline, glycine and hydroxyproline; the amount of other amino acids, including the essential ones, is very low. This lack of essential amino acids might have to be compensated by adding other meat co-products to the mixture, with a better amino acid profile.

Studies carried out in 1970's (Davis, 1975) demonstrated that *Tenebrio molitor* larvae required 10 essential amino acids (His, Lys, Arg, Ile, Trp, Met, Leu, Phe, Val and Thr), which are quite similar to human needs. Based on such information, it can be concluded that blood is the most preferable source of essential amino acids, since practically the 60 % of its amino acid composition accounts for essential amino acids. On the contrary, offal rich in collagen will only provide around a 25% or 17% of essential amino acids, as for example lips or ear, respectively. Other common co-products as brain, kidney, liver or lung range from 40 to 48% content in essential amino acids.

Regarding vitamins, it has been reported (House, 1969) vitamins B1, B2, B3, B5, are essential for all insects' taxa (coleoptera, diptera, homoptera, lepidoptera and orthoptera); or at least found beneficial to obtain normal growth and development in captivity. Meanwhile, other as vitamin C, B8, B9 or choline are only essential for specific taxa. This is of special interest when formulating insect substrate including meat co-products, since the most of them are excellent source of vitamins from the B group; being kidney and liver the richest sources among them.

When considering minerals, those considered essential are calcium, chlorine, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulfur and zinc (Kraus, Monchanin, Gomez-Moracho, & Lihoreau, 2019). Based on the mineral profile of meat co-products, it can be stated that all these minerals are present in diverse amounts, that will help to compliment insect's diet. Specially, offal is a rich source of iron and zinc.

Finally, some lipids are regarded as essential in insects' diet, as for example cholesterol, linoleic acid and linolenic acid (Kraus, Monchanin, Gomez-Moracho, & Lihoreau, 2019). Some of the offal feasible to be used in substrate formulations are a source of both linoleic and linolenic acids; for instance, liver and kidney have been reported to have 0.04-0.05 and 0.01-0.10 g of linolenic acid per 100 grams; while linoleic was founds to be 0.15-0.19 and 0.15-0.47 g/100 g of kidney and liver respectively. Heart from pork, is also a source of these nutrients. However, when compared with fish oil, the values reported in the oil are between 4 and 10 times higher. It means, that depending of the requirements, diet could be supplemented with fish oil extracted from fish processing by-products; although other sources as plants and seeds can be considered.

More recent studies have been performed aiming to investigate the use meat-based diets on several insects, as for example *Macrolophus caliginosus* (Castañé & Zapata, 2005) or *Dicyphus tamainii* (Iriarte & Castañé, 2001). Regarding the first two species, there are carnivore and have a predatory behaviour, and conventionally are fed with live insects. In these two trials, live insects were replaced with a mixture of beef liver (25%), (50%) ground beef and some additives (sucrose, aspartate, ascorbate, casein, egg yolk and soybean oil). After seven generations reared on this substrate, it was observed that adults were smaller and lighter, and the nymphal development time was longer; however, these authors stated that is a common phenomenon found on artificial reared insects. It was also reported that in terms of reproduction no effect was observed.

Based on this information, there is a huge potential for including meat co-products as substrate for insect rearing in order to supply the required essential amino acids, minerals and vitamins; while collagen, can be used as a source of proteins but also as functional ingredient to provide texture and consistency to the substrate. However, there is a lack of research regarding the impact of using meat co-products as substrate for rearing insects, and the impact on production parameters as survival, weight, size and live cycle needs to be investigated.

2.2.3 Nutritional composition of insect rearing substrates

An overview of the available literature of tested substrates for insect production is given below. In Table 11 the tested substrates and its chemical analysis for *Tenebrio molitor* is given, in Table 12 this for *Acheta domesticus*. In Table 13 the tested substrates and chemical analysis for *Locusta migratoria* production is listed.

Most studies also examined the amino acid, fatty acid composition, etc. of the substrates. For this information is referred to the original publication.

The methods for processing the substrates that were used in the experiments in question are shown in the appendix.

Table 11: Nutritional composition of tested substrates for mealworm production

substrate	moisture (%)	ash (%)	lipids (%)	Crude protein (%)	fibre (%)	carbo (%calc)	starch (%)	NDF (%)	ADF (%)	ADL (%)	H-cellulose (%)	cellulose (%)	reference
mushroom spent corn stover	<10	6,9	12,8	3,9		76,4							(Zhang, et al., 2019)
spirit distillers grain	<10	15,0	6,9	13,9		64,2							
highly denaturated soybean	<10	11,6	4,8	43,2		40,5							
wheat bran	<10	5,0	10,1	17,0		67,9							
brewers' spent grain	22,0	3,8	7,3	22,5				57,95	22,94	8	35	15	(Melis, et al., 2019)
wheat bran	22,0	5,3	4,1	19,6				42,52	10,66	3,03	32	8	
fermented wheat straw	22,0							30,09		5,51	29	30	(Li, Zhao, & Liu, 2013)
wheat flour	9,7	0,8	1,2	13,2	0,6								(Ruschioni, et al., 2020)
wheat middlings	8,1	5,1	5,7	16,8	9,5								
middlings + olive pomace (75:25)	22,0	4,8	6,2	15,4	16,8								
middlings + olive pomace (50:50)	37,4	4,9	7,0	14,9	21,6								
middlings + olive pomace (25:75)	53,2	5,3	7,4	11,8	31,5								
byproducts high protein high fat	5,0		9,5	21,9									(Oonincx, van Broekhoven, van Huis, & van Loon, 2015)
byproducts high protein low fat	4,9		1,0	22,9									
byproducts low protein high fat	10,9		14,6	12,9									
byproducts low protein low fat	10,9		2,1	14,4									
byproducts high protein high starch			4,0	24,1			28,4						(van Broekhoven, Oonincx, van Huis, & van Loon, 2015)
byproducts high protein low starch			7,0	32,5			7,4						

byproducts low protein high starch	1,8	10,7	49,8
byproducts low protein low starch	6,2	20,0	19,4

Table 12: Nutritional composition of tested substrates for housecricket production

substrate	moisture (%)	ash (%)	lipids (%)	Crude protein (%)	fibre (%)	carbo (%calc)	reference
pure pride cricket feed	8,7	5,7	1,3	21,9	5,9	65,0	(Bawa, Songsermpong, Kaewtapee, & Chanput, 2020)
50% Pure pride + 50% Betagro chicken feed	1,1	6,7	3,4	18,9	5,6	65,3	
Betagro chicken feed	11,3	7,9	5,2	15,8	5,3	65,6	
pure pride +100 g fresh pumpkin pulp per day	31,5	5,7	1,7	17,5	6,0	69,1	
pure pride + 100 g dry pulp pumpkin powder per day	10,9	5,7	2,2	19,6	6,1	66,5	
byproducts high protein high fat	5		9,5	21,9			(Oonincx, van Broekhoven, van Huis, & van Loon, 2015)
byproducts high protein low fat	4,9		1	22,9			
byproducts low protein high fat	10,9		14,6	12,9			
byproducts low protein low fat	10,9		2,1	14,4			
chicken meal	10,1		4	17,1			

Table 13: Nutritional composition of tested substrates for migratory locust production

substrate	moisture (%)	ash (%)	lipids (%)	Crude protein (%)	fibre (%)	carbo (%calc)	sugars (%)	P (%)	Ca (%)	K (%)	Mg (%)	reference
fresh perennial ryegrass	80,3	9		19,1	47,6			0,24	0,45	1,59	0,23	(Oonincx & Van Der Poel, 2011)
wheat bran	11		4,5	18,0	46	54,3	1,9	1,3	0,2	1,5	0,5	
carrots	88,3		2,0	7,9	24	81,8	40,5	0,3	0,3	0,3	0,1	

2.2.4 Nutritional composition of the insects produced on side streams

Below, the chemical analysis of the insects produced on the tested substrates, which are defined above, is given. In Table 14 the chemical analysis of *Tenebrio molitor* is summarized, in Table 15 this for *Acheta domesticus* and in Table 16 the results for *Locusta migratoria* are shown.

Most studies also examined the amino acid, fatty acid composition, etc. of the insects. For this information is referred to the original publication.

The rearing technique used in these experiments may affect the results. However, the main purpose of the scientific studies referred to is usually chemical analysis, which often means that insect rearing conditions are not detailed. During this literature search, the rearing conditions described in the publications were taken into account.

Table 14: Nutritional composition of mealworms tested on different substrates

substrate	moisture (%)	ashes (%)	lipid (%)	protein (%)	carbohydrates (%)	reference
50% wheat 50% soya	52	3,9	39	50	19	(Alves, Sanjinez-Argandoña, Linzmeier, Cardoso, & Macedo, 2016)
25% wheat 25% soya 50% bocaiuva pulp	53	4,8	40	45	13	
mushroom spent corn stover	-	5,9	6	76	12	(Zhang, et al., 2019)
highly denaturated soybean meal	-	6,6	8	74	11	
spirit distiller's grain	-	7,7	12	70	10	
wheat bran	-	8,1	17	69	6	
wheat bran	63	3,4	34	36		(Melis, et al., 2019)
brewers' spent grain	66	3,7	19	43		
fermented wheat straw			6	76		(Li, Zhao, & Liu, 2013)
wheat flour	65	3,5	40	38		(Ruschioni, et al., 2020)
wheat middlings	61	3,9	34	50		
middlings + olive pomace (75:25)	62	3,9	32	48		
middlings + olive pomace (50:50)	63	3,9	35	39		
middlings + olive pomace (25:75)	68	4,6	36	38		
byproducts high protein high fat	59		27	54		(Oonincx, van Broekhoven, van Huis, & van Loon, 2015)
byproducts high protein low fat	63		23	54		
byproducts low protein high fat	62		27	44		
byproducts low protein low fat	62		29	48		
control	60		27	52		
byproducts high protein high starch	67		26	49		(van Broekhoven , Oonickx, van Huis, & van Loon, 2015)
byproducts high protein low starch	71		28	48		
byproducts low protein high starch	67		19	47		
control	73		25	45		

Table 15: Nutritional composition of housecricket adults and juveniles tested on different substrates

substrate	moisture (%)	lipid (%)	protein (%)	ashes (%)	carbohydrate (%)	fiber (%)	vitamins	minerals	ADF-N (%)	NDF (%)	reference
***Acheta domesticus* adults**											
pure pride cricket feed	70	9	76	4,6	10,2	3,7	x	x			(Bawa, Songsermpong, Kaewtapee, & Chanput, 2020)
50% Pure pride + 50% Betagro chicken feed	70	19	71	4,4	5,5	6,4	x	x			
Betagro chicken feed	68	15	70	4,5	10,3	7,5	x	x			
pure pride +100 g fresh pumpkin pulp per day	59	44	48	2,9	5,1	4,5	x	x			
pure pride + 100 g dry pulp pumpkin powder per day	71	13	76	4,5	6,8	6,6	x	x			
corn meal, wheat midds,soy bean hulls, meat meal,molasses, fish meal	73	23	60	5,1					0,7	19,1	(Barker, Fitzpatrick, & Dierenfeld, 1998)
***Acheta domesticus* juveniles**											
corn meal, wheat midds, soy bean hulls, meat meal, molasses, fish meal	67	9,8	51	9,1			x	x	0,6	16,4	(Barker, Fitzpatrick, & Dierenfeld, 1998)
byproducts high protein high fat	74	20,8	59,2								(Oonincx, van Broekhoven, van Huis, & van Loon, 2015)
byproducts high protein low fat	76	20,8									
byproducts low protein high fat	75	-									
byproducts low protein low fat	75	-									
chicken meal	76	17,4	57,8								
x = determined in the concerned study, but not included due to too much data											

Table 16: Nutritional composition of migratory locust adults and juveniles tested on different substrates

substrate	moisture (%)	lipid (%)	protein (%)	ashes (%)	carbohy-drate (%)	fiber (%)	energy (kcal)	Ca (%)	P (%)	K (%)	Mg (%)	Na (%)	reference
***Locusta migratoria* adults**													
fresh perennial ryegrass	69	19	65	4,0			509	0,08	0,65	1,03	0,09	0,20	(Oonincx & Van Der Poel, 2011)
wheat bran	66	23	58	3,8			538	0,06	0,64	0,931	0,12	0,16	
carrots	66	30	56	3,3			569	0,05	0,56	0,825	0,07	0,15	
***Locusta migratoria* juveniles**													
fresh perennial ryegrass	73	18	62	4,3			533	0,08	0,07	1,15	0,10	0,18	(Oonincx & Van Der Poel, 2011)
wheat bran	71	24	58	3,9			540	0,06	0,07	1,05	0,10	0,15	
carrots	71	25	56	3,7			557	0,06	0,07	1,00	0,10	0,15	

Below, general conclusions of the available literature regarding chemical composition of insects reared on side streams are given per insect species. For more details, the reader is referred to the original publication.

2.2.4.1 *Tenebrio molitor*

Given the large differences among the studies (different substrates, different parameters tested, different analyses performed, etc.), it is hard to make solid conclusions regarding parameters influencing mealworm rearing. However, some general points may hold for mealworms:

Mealworm growth and survival:

A source of water (like carrots or vegetable leaves) is necessary for decreased development time and increased survival. Lipid content in substrates of >30% may increase the mortality of beetles. High protein content substrates seem the most important factor for growth, survival and food conversion, but protein quality is important as well (denatured proteins are not good). Substrates that have a lack of N-content perform less well. Higher fiber content in substrates may increase larval growth and survival, but fiber contents above 20% should be digested.

Mealworm nutritional composition:

As reflected in Table 14, there is a large variability in lipid (range 6-40%) and protein (range 36-76%) levels between and within studies, indicating an influence of the substrate and /or rearing conditions on the proximate composition of the mealworms. However, the proximate composition of the substrate is not always reflected in the larvae. According to Alves, *et al.* (2016) higher protein content in the substrate may result in higher protein content in the larvae. Also data from Ruschioni, *et al.* (2020) suggests that larvae mirrored the proximate data of the substrates. However, this was not observed in Zhang, *et al.* (2019) where all mealworm larvae had higher protein and carbohydrate content compared to larvae bred on wheat bran despite regardless of the amount of proteins in the substrates. Perhaps the fiber fraction has an influence, as suggested in Melis, *et al.* (2019). Oonincx, *et al.* (2015) showed higher protein levels in larvae reared on high protein diets; the effect of lipids in the substrates was not clearly reflected in the larvae. Van Broekhoven, *et al.* (2015) indicate that the protein fraction in larvae was similar despite 2 to 3 fold differences in protein content in the substrate, suggesting that mealworms are able to regulate body protein content. A slight correlation was observed for fat content in substrate and larvae.

Mealworm lipid composition:

The main fatty acids include linoleic acid, oleic acid, palmitic acid and stearic acid. Mealworms contain good levels of PUFAs linoleic acid (n6) and α-linolenic acid (n3), however, the ratio of n6/n3 is larger than the optimal ratio of 5. It seems that the n6/n3 ratio is flexible and that yellow mealworms accumulate n6 fatty acids more efficiently than n3 fatty acids, resulting in a higher n6/n3 ratio in the yellow mealworm compared to their diet (Oonincx, et al., 2015). It is suggested by several authors that the fatty acid levels and the n6/n3 ratios can be altered by the diet, because there are differences observed in the fatty acid profiles of mealworm raised on different substrates (Melis, et al., 2019; Van Broekhoven, et al., 2015). This may be due to a selective fatty acid absorption from the diet and/or due to modulation of biosynthetic pathways by the dietary source. However, it is not clear to what extent this process can be influenced/steered, because the fatty acid profiles in larvae do not reflect the fatty acid profiles in the substrates (Ruschioni, et al., 2020; Oonincx, et al., 2015; Van Broekhoven, et al., 2015). As a note: carrot has a n6/n3 ration of 50:1 and thus may also have influence of the ratio in the larvae.

Mealworm protein composition:

According to Zhang, *et al.* (2019) the total amino acid content of mealworms is higher than FAO/WHO requirements. Several indispensable amino acids are however lower than the requirements. Diets are observed to affect the amino acid composition in the larvae, but there is no pattern in how the levels in the diet affect the levels in the larvae. Zhang, *et al.* (2019) suggest that protein denaturation may play a role. Ruschioni, *et al.* (2020) obtained a similar amino acid profile in larvae raised on different substrates with a total amount of essential amino acids ranging from 48.7-53.7%.

2.2.4.2 *Acheta domesticus*

Only limited information is available on rearing house crickets (Table 12, Table 15).

House cricket growth and survival:

Crickets fed on a diet containing 20-30% proteins have a high survival rate and a low FCR. Bawa, *et al.* (2020) added dry pulp pumpkin powder and fresh pumpkin pulp, which results in improved weight gain.

House cricket nutritional composition:

Bawa, *et al.* (2020) claim that if the protein, carbohydrate and fat ratio of the diet are not well matched, that the excess carbohydrates will be stored as fat. Additions of dry pumpkin powder or fresh pumpkin pulp has an influence on protein, fat, vitamin B3 and B12 and mineral amounts. On high protein diets, crickets had a high crude protein (58%) and a low lipid (17-21%) composition. The high variation in fat composition of the diets is not reflected in the composition of the crickets.

House cricket lipid composition:

The main fatty acid was C18:2 n6, although C16:0 and C18:1 n9 were also present in high concentrations. Large differences in C18:2 n6 and α-linolenic acid (C18:3 n3) concentrations were found due to dietary treatment. The n6/n3 ratio ranged from 15.3-29. C20:3 n3 and C22:6 n3 were not detected in the diet, but was present in the house crickets, this could suggest de novo synthesis. House crickets can elongate C18:3 n3 to C20:5 n3.

House cricket protein composition:

No information is available in the consulted reports.

2.2.4.3 *Locusta migratoria*

Only one study (Oonincx & Van Der Poel, 2011) is reported in which migratory locusts were fed a diet of fresh perennial ryegrass (FPR) or FPR + wheat bran or FPR + wheat bran + carrots. The nutrient contents of the substrates are shown in Table 10, however, these were not determined by the authors. No details on the proportions of the dietary mix are presented. Adding wheat bran decreased the protein content and increased fat content. Additionally adding carrots to the diet further decreased protein content and increased lipid content. These observations were done for both the juvenile as the adult locusts. Mineral concentrations of Ca, K, Mg, and Na, were significantly affected by diet. Concentrations of Ca, K, and Na decrease when wheat bran is provided. Wheat bran decreased the a-carotene content, which did not change by incorporating carrots in the diet. However, carrots did result in higher b-carotene concentrations. Retinol concentrations were increased by incorporating both wheat bran and carrots in the diet compared with the diet containing only grass.

3 Emissions

Emissions can be categorized into two groups, those with a global impact, such as greenhouse gas emissions, and those with a local impact, such as ammonia and particulate matter.

Some of the most potent greenhouse gases are CO_2, methane and nitrous oxide. To make it possible to calculate a cumulative greenhouse gas potential, the IPCC looks at the global warming potential of each individual gas and rates its gravity according to the GWP of CO_2, expressing it in CO_2 equivalents.

Table 17: Global Warming Potential (GWP) of CO_2, methane (CH_4) and nitrous oxide (N_2O) (Stocker, et al., 2013)

Chemical Formula	GWP 20-year	GWP 100-year
CO_2	1	1
CH_4	84	28
N_2O	264	265

3.1 Sources of agricultural greenhouse gas emissions

It is widely accepted that livestock agriculture (ruminant, pig and poultry) is a significant contributor to production of greenhouse gases. Although there are variations in estimates FAO has recently reported that total emissions from global livestock equate to 7.1 gigatonnes of Co2-equiv per year (FAO, 2020). This represents 14.5 percent of all anthropogenic GHG emissions which includes sources such as transport and energy. Because of the synergistic relationship with the rumen microbiota, ruminants can thrive on fibrous feed grown on land that is incapable of growing crops which can be eaten directly by humans. Thus pasture based agriculture is an important part of the food supply chain, supplying essential nutrients (iron, B vitamins) in a form easily absorbed by humans. However, rumen fermentation of fodder is a process generating large amounts of hydrogen which if not removed causes serious illness in the animal (ruminal acidosis). The evolutionary solution to this has been the role of the methanogenic archaea which reduce CO_2 in the rumen to CH_4 (methane). Methane has 20-30x global warming potential of CO_2 (IPCC, 2020) and hence decreased methane emission from ruminants is a key global target in tackling climate change. Ruminants are also inefficient in use of nitrogen. Of the forage protein they consume only about 30% is retained as milk or meat. This is due to inefficiencies in degradation and conversion into microbial protein in the rumen (MacRae & Ulyatt, 1974; Dewhurst, Mitton, Offer, & Thomas, 1996). The result is that significant amounts of ammonia and urea are excreted onto land, providing substrates for generation of nitrates (which pollute water) and nitrous oxide (N_2O), which has ~250 x global warming potential of CO_2. Therefore solutions to protein use efficiency will also contribute to mitigation of climate change. One potential route is the development of elite grass varieties with protein degradation better matched to the needs of the rumen offering a potential 25% decrease in ammonia production (Kamau, et al., 2020). Pig and chicken production accounts for 30 and 25% of global meat consumption respectively (MacLeod, et al., 2013). GHG emissions associated with pig production are relatively low, estimated to produce 0.7 gigatonnes CO_2-equivalents per annum representing 9% of the livestock sector's emissions. This associated mostly with N_2O arising from fertilisers used in feed production (60%) and CH_4 from manure storage (27%). It is a similar story for chickens which are estimated to emit 0.6 gigatonnes CO_2 equivalent, representing 8 percent of the livestock sector's emissions. Although currently low, the rapid increase in market and demand for pig and poultry products means that these emissions are likely to rise.

3.2 Emissions related to insect rearing

In this literature review the focus is on direct emissions, like these originating from animal born metabolic processes. The indirect emissions, originating during processes related to animal production (like feed production and transport or processing animal manure), will not be described.

It is not unreasonable that any new food product, including insect based products, would be expected to have a food conversion and GHG production lower than conventional livestock products. Key to this is accurate measurement of methane and ammonia in excreta, and a complete understanding of the emissions factors involved in insect feedstock production.

3.2.1 Greenhouse gas emissions

3.2.1.1 Carbon dioxide (CO_2)

As a by-product from cellular respiration, CO_2 emission is a common way to determine metabolic activity in insects. It has been of particular interest as a means to examine discontinuous gas exchange cycle (DGC) in insects. Respiration in insects is not a passive process, a cyclical pattern has been observed in over 50 insect species consisting of: 1) a closed phase with minimal CO_2 exchange, 2) a flutter phase with discontinuous gas exchange and 3) an open phase with rapid CO_2 release (Duncan, Krasnov, & McMaster, 2002). However little research was performed on CO_2 emissions per kg insect production.

Some numbers can be calculated for *Locusta migratoria* based on observations from Gouveia, *et al.* (2002). CO_2 production was continuously monitored and accordingly linked to the activity level of locusts. Especially during feeding CO_2 production peaked (5 ml CO_2/g/h or 216 g/kg BM/day), respiration at rest was much lower (averaging at 1.4 ml CO_2/g/h or 60 g CO_2/kg BM/day). Not only activity level of the insects play a role, ambient temperature (Bjørge, Overgaard, Malte, Gianotten, & Heckmann, 2018), the insect species (Duncan, Krasnov, & McMaster, 2002; Oonickx, et al., 2010) and development stage as well.

Table 18: CO_2 emissions according to different sources expressed per kg live weight (BM) per day and per kg mass gained

	CO_2 (g/kg BM/day)	CO_2 (g/kg mass gain)	References
Tenebrio molitor	61	1031	(Oonickx, et al., 2010)
Acheta domesticus	68	1468	(Oonickx, et al., 2010)
Locusta migratoria	110	734	(Oonickx, et al., 2010)
	60 – 216		(Gouveia et al., 2002)

3.2.1.2 Nitrous oxide (N_2O)

N_2O production is the result of microbial transformation (through nitrification and denitrification) of nitrogenous compounds, a process which takes place in soils, wastewater treatment plants, sediments and water bodies. During nitrification ammonia (NH_4^+) is oxidized in to nitrite (NO_2^-) and nitrate (NO_3^-), autotropic and heterotrophic pathways are known, both can be a source of nitrous oxide. During anaerobic denitrification NO_3^- is reduced in the presence of organic matter in to N_2, during which volatile intermediates such as N_2O are formed. These might dissipate before completing denitrification (Wrage, Velthof, Van Beusichem, & Oenema, 2001).

Ooninx, *et al.* (2010) determined nitrous oxide emissions of *Tenebrio molitor* (5th larval stage), *Acheta domesticus* (5th and 6th nymphal stage) and *Locusta migratoria* (3rd and 4th nymphal stage), all of which supplied with feed, over a 3 day period. Results are summarized in Table 19. Thévenot, *et al.* (2018) and Halloran, *et al.* (2017) observed similar results for *T. molitor* (0.87 mg/kg BM/day) and *A. domesticus* (3.5 mg/kg mass gain) respectively. Anaerobic conditions should be avoided in insect rearing, the presence of N_2O is probably due to nitrification of ammonia in the frass rather further denitrification (an anaerobic process).

Table 19: N_2O emissions according to different sources expressed per kg live weight (BM) per day and per kg mass gained

	N_2O (mg/kg BM/day)	N_2O (mg/kg mass gain)	References
Tenebrio molitor	1.5	25.5	(Oonickx, et al., 2010)
	0.87		(Thévenot, et al., 2018)
Acheta domesticus	0.1	5.3	(Oonickx, et al., 2010)
		3.5	(Halloran, et al., 2017)
Locusta migratoria	8	59.5	(Oonickx, et al., 2010)

3.2.1.3 Methane (CH_4)

Animals that feed on cellulose rich diets have formed a symbiosis with microorganisms such as bacteria, fungi and protozoa. They break down complex compounds by hydrolysis to produce volatile fatty acids (VFA), mainly acetate, propionate and butyrate. One of the end products of this anaerobic fermentation is hydrogen (H_2). To keep the partial pressure of H_2 low and direct fermentation toward production of acetate, methanogenesis takes place (Danielsson, et al., 2017). This reaction is catalysed by archaea and mediated by obligately anaerobic methanogens in the *Euryarchaeota* (Schmidt, 2019).

This process is most notable in ruminants, as described, however it is also present in other phylum. Hackstein & Stumm (1994) screened over 110 representatives of the taxa of terrestrial arthropods for methane production and discovered methane production in millipedes (Diplopoda), cockroaches (Blattaria), termites (Isoptera) and scarab beeltles (Scarabaeidae), while nothing was detected in 66 other arthropod species, including *Locusta migratoria*, *Acheta domesticus* and *Tenebrio* sp.. These results are confirmed by Ooninx, *et al.* (2010), methane emissions for 5 insect species were determined, including *L. migratoria*, *A. domesticus* and *T. molitor*, none of which showed traces of methane emissions. A similar experiment was conducted by Thévenot, *et al.* (2018) solely on *T. molitor*, they only detected traces of methane, around 1 mg/kg fresh weight/day.

Table 20: methane emissions according to different sources expressed per kg live weight (BM) per day and per kg mass gained.

	CH_4 (g/kg BM/day)	CH_4 (g/kg mass gain)	References
Tenebrio molitor	0	0.1	(Oonickx, et al., 2010)
	0.001		(Thévenot, et al., 2018)
Acheta domesticus	0	0	(Oonickx, et al., 2010)
		0.002	(Halloran, et al., 2017)
Locusta migratoria	0	0	(Oonickx, et al., 2010)

The insects of interest themselves show no signs of methanogenesis. However, a second source of methane could potentially be the substrate in which the larvae are reared. Anaerobic conditions are required for methanogenesis, while rearing conditions should be the following: a dry and granular substrate with sufficient air refreshment. Under these conditions methanogenesis is highly unlikely. The rearing conditions of other insect species, such as *Hermetia illucens* of which the larvae live in a wet feed, are more at risk for anaerobic conditions in their feed. However these species are out of the scope of this project.

3.2.1.4 Global Warming Potential (GWP)

Livestock emissions from respiration are part of a rapidly cycling biological system, where the plant matter consumed was itself created through the conversion of atmospheric CO_2 into organic compounds. Since the emitted and absorbed quantities are considered to be equivalent, livestock respiration is not considered to be a net source under the Kyoto Protocol (Steinfeld, et al., 2006). However, the ratio between growth and CO_2 production is an indicator of feed conversion efficiency and thereby a relevant indicator for the environmental impact (De Vries & de Boer, 2010).

It is important to notice that all insect data presented is based on measured emissions during a small part of the rearing cycle of these insects. No data is available on emissions during a full rearing cycle or from further processing of the frass (insect residue). When frass would be applied as a soil enhancer, microbial degradation of the frass could cause a new source of N_2O emissions.

Table 21: Estimated global warming potential for different livestock species expressed as CO_2 equivalents.

	CH_4 (g CO_2 eq. /kg live weight gain)	N_2O (g CO_2 eq. /kg live weight gain)	Total (g CO_2 eq. /kg live weight gain)
Tenebrio molitor	2.8	6.8	9.6
Acheta domesticus	0	1.4	1.4
Locusta migratoria	0	15.8	15.8
Poultry (MacLeod, et al., 2013)	0	146	146
Fattening pigs (Philippe & Nicks, 2015)	685	169	854
Cattle (Oonickx, et al., 2010)			2850

3.2.2 Ammonia emissions

Ammonia is a gaseous component that can react in the atmosphere to form secondary particulate matter (e.g. with nitrogen dioxide to form ammonium nitrate). If the amount of particulate matter increases, the air quality decreases. Ammonia has a short residence time in the air (a few hours to one day). Due to dry or wet deposition, it has an eutrophying and potentially acidifying effect on soil and surface and soil water. Acidification and eutrophication affect ecosystems (VMM, 2020).

Table 22: ammonia emissions expressed per kg live weight (BM) per day.

	NH_3 (mg/kg BM/day)	References
Tenebrio molitor	0	(Oonickx, et al., 2010)
Acheta domesticus	5.4	(Oonickx, et al., 2010)
Locusta migratoria	5.4	(Oonickx, et al., 2010)

3.2.3 Atmospheric particulate matter (dust)

Particles of all sizes may be deposited in the nose and pharyngeal region. However, only particles with an aerodynamic diameter of less than 15 µm can enter the tracheobronchial tree and only particles with an aerodynamic diameter of less than 7 µm can enter the alveoli. Approximately 50% of particles less than 5 µm aerodynamic diameter entering the respiratory system will reach the alveoli. Therefore, the fraction of dust including particles less than 5 µm aerodynamic diameter is the respirable fraction. The particle size range with the largest percentage of deposition in the lungs is 1–2 µm in aerodynamic diameter. Particles smaller than 0.5 µm in mean aerodynamic diameter are respirable, but it is more likely that they are exhaled and not deposited in the lungs. Therefore, interest lies in controlling respirable dust, 0.5–5 µm in mean aerodynamic diameter (Just, Duchaine, & Singh, 2009).

No scientific literature is available on dust production in insect rearing facilities.

3.3 Measurement of emissions

Direct measurements of emissions can take a variety of forms. The most reliable method for methane measurement is the use of gas exchange chambers. In this animals are housed in a sealed unit with controlled air flow. Inflow and outflow gas composition (methane, CO_2) is measured by infra-red gas analyzer (IRGA) and methane production rate can be calculated according to a number of parameters, usually feed intake, milk production etc. As methane is lost from the animal in belching laser based techniques have also been applied to direct measurement of methane production by individual animals housed in stalls, or over a wider area in the field. There are various limitations with this technique including the need to familiarize animals to the equipment and the inaccuracy of measurements (related to aiming across mouth) compared with chamber techniques. A more reliable measurement of methane in breath can be obtained from equipment such as the "Greenfeeds" units which measure gas composition as animals feed. A final option for whole animal measurements uses the proxy technique of sulphure hexafluoride (SF6) tracer (Berndt, et al., 2014). Basically a source of SF6 is placed in the rumen (the source of 95% of methane) and the concentration in exhaled breath samples determined on the assumption that the likelihood of detection of SF6 and methane originating from the rumen is the same, with calculations based on known release rate of SF6. Gas samples can also be analysed for methane by gas chromatography- mass spectrometry (GCMS).

It could be reasonably foreseen that the chamber technique could be easily adapted for measurement of methane production by a colony of insects. Equally gas headspace analysis could be undertaken by GCMS or IRGA.

Ammonia production by livestock is usually assessed in faecal or urine samples. Aqueous solutions are used in a colourimetric based assay. This is easily adaptable to an insect production situation. Similarly, urine and faeces are the main sources of nitrous oxide deposited on land. This is typically measured by a small chamber approach (Figure 6, Figure 7) where non-steady state measurements are made over a period of time then headspace sampled and analysed by gas chromatography (GC). This approach would be entirely consistent with sampling in an insect rearing situation (Chadwick, et al., 2018).

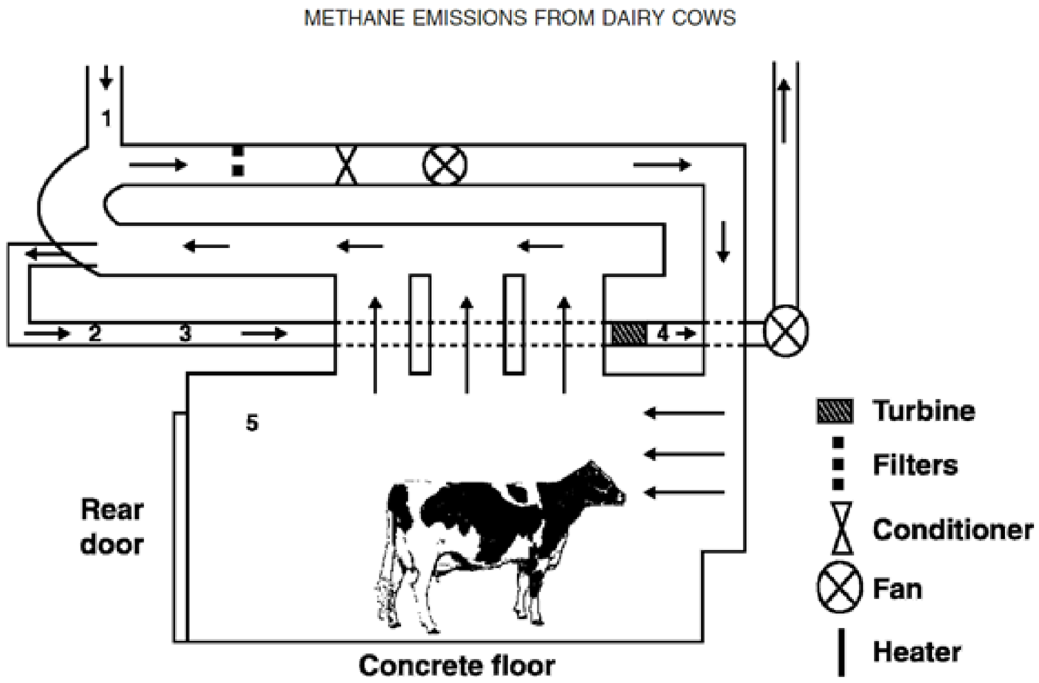

Figure 6: Schematic of the open-circuit respiration chambers, showing the airflow and conditioning, and release and sampling locations within the circulation system. Locations 1 and 2 are the intake and exhaust ducts sample points for noncalibration periods; location 3 is the injection point enabling the analytical system calibration; location 4 is the sample point for the system calibration; and location 5 denotes the chamber volume (Graigner, et al., 2007).

Figure 7: Emissions accumulation chamber with sheep (Daly, 2018)

4 EFSA requirements for human food

The European Food Safety Authority (EFSA) has published several Scientific Opinions on Dietary Reference Values for nutrients. In addition, they developed an interactive webpage[2] that allows looking up these values.

Dietary reference values (DRVs) is an umbrella term for the complete set of nutrient reference values which include population reference intakes (PRIs), the average requirements (ARs), adequate intakes (AIs) and reference intake (RIs) ranges for macronutrients. These values indicate the amount of a nutrient which must be consumed on a regular basis to maintain health in an otherwise healthy individual (or population).

- Energy: The AR for energy intake depend on age, gender, activity, … For adults the range is from 6.8 for a low physical activity level up to 14 MJ/day (high PAL) (1930 – 3973.6 kCal/day).

- Carbohydrates and dietary fibre: Data from dietary surveys show that average carbohydrate intakes in European countries varied between 45 to 60 E% (percentage of the energy intake) in adults. Average intakes of sugars varied between 16 to 36 E%. Average dietary fibre intake is 25 g per day in adults.

Reference intake for total fat for adults ranges from 20 to 35 E%. Saturated fat intake should be as low as possible. N-6 PUFA, linoleic acid has an adequate intake of 4 E% and for the n-3 PUFA alpha-linolenic acid an adequate intake of 0.5 E% is proposed. The ratio n6/n3 PUFAs is not relevant. Uptake of EPA and DHA can be beneficial and a combined intake of 250 mg/day appears to be sufficient.

For healthy adults (male and female) the average requirement is 0.66g protein/kg body weight per day. Based on the requirement distribution and assumptions around efficiency of utilization of dietary protein a Population Reference Intake of 0.83 g protein/kg body weight per day is proposed. For the indispensable amino acids, more data are needed to obtain values for requirements. (EFSA , 2017)

[2] https://www.efsa.europa.eu/en/interactive-pages/drvs

5 Discussion

The aim of this literature search is to obtain more knowledge about the potential of insects in solving societal challenges such as 1) valorizing residual streams that are currently regarded as 'waste' and 2) greenhouse gas emissions related to farmed animals. An overview of potential side streams is given and what has been tested so far for insect rearing. In order to assess the potential of side streams as a rearing substrate for insects, literature concerning the nutritional needs and rearing conditions of insects (*Tenebrio molitor*, *Acheta domesticus* & *Locusta migratoria*) are described. Based on this information, choices could be made regarding the streams to be investigated as insect diets in this project. The second part of the literature search focusses on emissions produced by farmed animals and determines which emissions might be relevant in insect production systems.

Based on the literature search, it can be concluded that the composition of insects can marginally be controlled by the composition of the substrate and the rearing conditions. Although previous studies demonstrated that the composition of the diet has an influence on the composition of the insect, so far, there is limited understanding of the relationship between diet and composition. This is presumably due to the difference in rearing conditions and the insect strain used in the research. To date, there is insufficient knowledge about this subject to steer the chemical composition of insects by diet (Zhang, et al., 2019; Ruschioni, et al., 2020; Oonincx, et al., 2015; Van Broekhoven, et al., 2015; Bawa, et al., 2020).

The selection of the side stream-based substrates will thus be based on the needs of the insect species in question and the supply of the side streams. This literature search has shown that most insects cannot be grown on mono streams, as these streams often have an unsuitable moisture content, texture or do not meet the nutritional needs of the insects. Therefore, compound diets will be made that are a mixture of side streams and possibly additional ingredients to increase the nutritional value or to make the physical properties more suitable for the insect species in question. Below, the needs per insect species are discussed. This information will be taken into account in order to formulate suitable experimental diets.

5.1 Dietary needs of insects

Below, the dietary needs per insect species included in this project are summarized.

Tenebrio molitor:

Mealworms need a dry feed, supplemented with a wet feed as a source of water for good development (low mortality, reduced development time). Mealworms prefer a high protein content (28%) (Oonincx, et al., 2015; Van Broekhoven, et al., 2015), but lower concentrations are possible if the amino acid composition is good (Morales-Ramos, Rojas, Shapiro-Ilan, & Tedders, 2013). Higher protein content in some cases seems to result in a higher protein content of the larvae. Anyway, the proteins must not be denatured, as the mealworms are probably not able to use these (Zhang, et al., 2019). In all cases the diet should include vitamins of the B-complex (Ribeiro, Abelho, & Costa, 2018).

As a control diet for feeding experiments with mealworms, wheat bran (dry feed) and agar (wet feed) is used, as proposed in the standard protocol. Since this is a suitable diet for rearing mealworms, the composition (and physical properties) of the experimental diet should at least match the control diet. However, in rearing facilities, carrots (or others, e.g. chicory roots) are often used as a moisture source. The moisture source has an influence on the insect growth and composition. Since mealworms (and crickets, see below) grow well on this moisture source, this can also be used to compare any experimental wet feed. However, the moisture source should be standardized to compare results between different experiments.

Based on literature and own research, wheat bran consists of 15-20% of proteins and a fat content below 6% (Melis, et al., 2019; Zhang, et al., 2019). Carrot consists of 7.9% proteins and 2% fat (Oonincx & Van Der Poel, 2011). Agar is mainly composed of water (2.5% dry matter (DM), ± 7% carbohydrates on a DM basis, ± 0.5% protein on a DM basis). However, the chemical composition of the control diet is often not consistent between different suppliers (and may vary throughout time), also the analytical method used to determine the chemical composition can be a cause of variation. Therefore chemical analysis of the feed ingredients during the experimental phase of the project will provide more insight.

Acheta domesticus:

Only limited knowledge is available on the nutritional needs of house crickets:

A protein content of the diet between 20-30% on a DM basis seems to ensure good survival and growth rates of crickets (high survival, low FCR) (Bawa, Songsermpong, Kaewtapee, & Chanput, 2020; Patton, 1967). Furthermore, research of Patton (1967) indicated that a carbohydrate content ranging from 32% to 47% and a fat content ranging between 3.2% and 5.2% is suitable for rearing house crickets. Morales-Ramos, Rojas, Dossey, & Berhow (2020) concluded that Vitamin B and C, sterols and manganese have a positive impact on live biomass production.

Like mealworms, the diet of crickets mostly consists of dry feed and a moisture source. Often, a water dispenser is used to replace the wet feed. In the laboratory, crickets are reared on a standard diet consisting of chicken meal (dry feed) and agar (moisture source), as described in the standard protocol for feeding experiments. However, in rearing facilities, carrot (or other) is often used as a moisture source (see above). The nutritional composition of chicken meal, as described by Oonincx, *et al.* (2015), consists of 17,1% protein content and 4% fat content. However, this may vary depending on the analysis method used and the supplier.

Locusta migratoria:

Research of Dadd (1960a, 1960b, 1960c, 1961a, 1961b) showed that none of the artificial diets used were as good as their natural food sources. This indicates that locusts are best reared on a diet based on their natural food sources, i.e. grassy biomass. In the laboratory (and rearing facilities), fresh grass is used as rearing diet for locusts. Oats are used as supplementary feed for reducing mortality by cannibalism. This diet will also be the control diet when conducting feed experiments.

Through his research, Dadd gained a lot of insights regarding the nutritional needs of *Locusta migratoria*. For the nymphs to grow sufficiently the diet had to contain 20% protein (DM basis), 10% of digestible carbohydrates, linoleic acid (0.5%), cholesterol (0.5%), ascorbic acid (0.3%) and vitamins (0.2%). Also cellulose seemed to be required (66.1%), despite it is indigestible for locusts.

5.2 Selected side streams

Besides the nutritional needs of insects, the supply of side streams was also reviewed. Although households contributes the most to food waste, this stream cannot be used as feed for insects due to legal restrictions. Therefore the focus will be on food waste coming from primary production, processing and wholesale, which are currently not valorised as feed. However, some of the side streams described below already have been valorized in feed. However, there is often an oversupply or they are of minimal interest as feed, which makes its use for insect production still interesting.

Taking into account the knowledge described above and availability from local partners, the following streams are currently suggested to be used as ingredients to formulate diets for rearing *Tenebrio molitor*, *Acheta domesticus* and *Locusta migratoria*:

wholesale	
	Unsold fruit and vegetables from auction
processing	
	Potato steam peels
	Red blood cell fraction
	Hydrolysed chicken feathers
	Grain middlings
primary production	
	Foliage from horticulture
	Forced chicory roots
	Grassy biomass

The insects included in the ValuSect project must be provided with a dry feed and a moisture source (wet feed), since they do not thrive on moist substrates. Therefore, a distinction is made between wet and dry feed. For wet feed it is especially important that these streams have a high moisture content, but still retain a certain texture so that no 'paste' is created when adding the dry feed.

Fruit and vegetables can serve as a moisture source for the insects, especially crickets and mealworms. Many fruits and vegetables from the auctions are not sold. These unsold products often end up in feed. However, some of these streams do not seem to be interesting for feed and are therefore not further valorised (composting). Based on the local supply options, certain fruits and vegetables are selected for the rearing experiments (e.g. tomato, cucumber, lettuce, cauliflower…), where they will serve as a moisture source for the insects. This stream can also be mixed and dried and then used as an ingredient for insect dry feed. It remains to be determined whether the pre-treatment can be carried out in a sustainable way.

Another suggested stream to be tested is **potato steam peels.** This is a starchy by-product that is released during the processing of potatoes into fries. This by-product can be used in feed, but there is a minimal interest for livestock farming, as a result there is an oversupply of this stream. Due to the local supply of the product, this could be used as a feed ingredient (starch addition) during the composition of the diets for the insects. However, this is a liquid product which requires pre-treatment if it is used as a dry feed ingredient. It is also a possibility to test this stream as wet feed for the insects (esp. crickets and mealworms), however, it must be taken into account that this has a paste-like structure and may therefore be less interesting.

Also, **foliage** from horticulture will be tested as a wet and dry feed stream for mealworms, crickets and locusts. However, it must be considered that some plants may have a negative effect on the insects, as seen in the BioBoost project. Plants from the Solanaceae family (tomato, bell pepper, …) had a negative effect on mealworms (survival, growth). Also, some crops may be treated with insecticides, which can obviously have a negative impact on insect survival. It must be taken into account that insects might accumulate insecticides or fungicides, which may cause certain maximum residue levels (MRLs) to be exceeded. To date, few studies have been done on the accumulation of resides of pesticides. The results in the literature so far indicate potential uptake of some of them depending on insect species and pesticide but often no bioaccumulation was found, or below MRL levels (Lalander, et al., 2016; Charlton, et al., 2015; Houbraken, et al., 2016; Gao, et al., 2014; Gao, et al., 2013; Lv, et al., 2013; Purschke, Scheibelberger, Axmann, Adler, & Jäger, 2017). The accumulation of pesticides can be analyzed when determining the insect quality for food applications. However, most horticulturists (esp. greenhouse horticulture) often use integrated pest management, which favors non chemical methods. When using streams from horticulture, pesticides should therefore not form a problem.

Grain middlings are high in fiber content and often contain interesting nutrients, e.g. mixture of plant material, but also unsuitable grains, etc. Provided that it is pretreated, this stream could potentially serve as a dry feed for the insect species in this project.

Previous projects have indicated that **forced chicory roots** are suitable as a wet feed for mealworms (BioBoost 2019; 'Witloofwortels: ook insecten lusten er wel pap van', 2019). This makes this stream interesting for further research, including the application in the production of the other insect species.

For locusts in particular, **grassy biomass** is suggested. For example grass from nature reserves or parks. However, it still is an issue on how this will be delivered as fresh grass. Therefore, rearing experiments with pretreated grass can be carried out (e.g. fermentation, drying, etc.). The same goes for roadside grass. Here it can also be a problem that it is contaminated with litter (negative impact on insects, not allowed by legislation). For locusts the following approach is proposed for the ValuSect experiments.
In the first step experiments can be carried out with grass that can be stored, i.e. pretreated grass (e.g. grass pellets, dried grass, hay (dried grass + other plants); whole or ground into powder and fermented grass). In a next step, grass-based side streams (e.g. grass from nature reserves, parks) can be used for these experiments; if pretreated grass was found successful. Also, straw (mainly stems of dried grain plants) can be tested as locusts dry feed. Anyway, it must be considered that when conducting rearing experiments with dried biomass, the locusts must be provided with a moisture source.

There is a lack of research done regarding the impact of using meat co-products as substrate for rearing insects. However, the potential has been described in this literature search. Based on the reviewed information, meat co-products have potential to provide the required essential amino acids, minerals and vitamins for insect rearing. Some examples are: blood, kidney, liver, heart, brain, lung and other offal of pork and cattle. Mealworms and locusts are cannibalistic, which indicates that they do eat animal products. However, not all animal products, e.g. offal, can be used for insect production according to the legislation (see further). However, blood products of non-ruminants are allowed, which makes this product a potential substrate for insect rearing, especially the **red blood cell fraction.** However, this substrate must be pretreated (e.g. dried) and made into a powder form since the insects included in the project do not thrive on moist substrates (see appendix 8: Blood processing).

Annually more than 1 million tonnes of feathers are produced as by-product from European poultry slaughterhouses. **Hydrolysed chicken feathers** have high protein content, which comprises 80–90% dry matter. More specifically chicken feathers are composed of crude lipid (0.83%), crude fiber (2.15%), crude protein (82.36%), ash (1.49%), NFE (1.02%) and have a moisture content of 12.33% (Tesfaye, Sithole, Ramjugernath, & Chunilall, 2017). When hydrolyzed they can be used as feed for insects according to EU Legislation. However, processing pressure and time during hydrolyzation influences feather meal protein quality (Moritz & Latshaw, 2001; Adler, Slizyte, Honkapää, & Løes, 2018). Also, due to the unbalanced amino acid composition of feather, e.g. smaller proportions of lysine, methionine, histidine, and tryptophan, feather meal may have to complemented with other protein sources (Yokote, et al., 2007; Bandegan, et al., 2010).

Most side streams will need to be pre-treated in order to make a diet more suitable for the insect species. A pre-treatment can consist of drying, grinding, pelleting, etc. Fermentation is also an example of a pre-treatment, this can be interesting to extend the shelf life of fresh side streams. It may be that the ensilage technique commonly used to preserve excess summer forage for winter feeding of ruminants can be utilized for insect feeding. This fermentation preserves protein and other important nutrients by a rapid drop in pH and can be undertaken from lab to field scale (academic or commercial). As there is a drive to remove livestock form pasture, but pressure to maintain landscapes, this may provide an alternative route to market for the forage offtake produced as part of management and contribute an income to rural communities. However, before pre-treatments can be applied to the side streams, it must be determined whether this can be carried out in a sustainable and cost-effective way. Potential changes in the properties of the side streams must also be identified. More information about pre-treatments (e.g. costs) is required.

Only limited information on the nutritional composition of the above described side streams could be gathered in this literature search and will therefore have to be determined before a final choice can be made. Before the rearing experiments can start, chemical analysis will have to be carried out on the side streams that have potential to be used for insect production. With this information, diets can be composed that meet the nutritional (and physical) needs of the insect species in question.

In the next step, rearing experiments will be performed using the described standard protocols for feed experiments as a basis. These protocols will be optimized during the ValuSect project using the obtained information and knowledge. In this way, the project can contribute to optimization of sustainable insect production. The following parameters will be monitored during the rearing experiments: feed conversion rate (FCR), insect growth rate, survival, chemical composition of the larvae and emissions.

5.3 Emissions related to insect rearing

Another approach to become a sustainable insect producer is optimizing the emissions during insect rearing. The emissions will be measured during the insect rearing experiments on the compound diets. In this literature search it is investigated which emissions are relevant and how they can be measured. The results of these trials will be a contributing factor in the selection process of compound diets. Diets with low overall emissions and good growth will be favoured over diets that produce more emissions.

There are no indications that **methane** is a relevant emission for the selected insect species, as they do not form a symbiosis with methanogenic microbiota. 4 different studies detected (almost) no methane emissions. A secondary source of methane might be from the substrate, however methanogenic activity requires anaerobic conditions. These conditions are unlikely to occur in a thin layer of dry granular substrate. **Nitrous oxide** was detected in all three insect species. **CO_2** was the most abundant produced gas, but is not considered to contribute to the GWP (global warming potential) (not when it is produced through metabolic activity). Also, it does not pose any immediate threats to the environment of an insect production facility. However, it is a clear indication of the FCR and it might pose health risks to staff when it can accumulate without proper ventilation. **Ammonia** emissions are a problematic gas due to its contribution to the production of particulate matter. It was detected in 2 of the 3 insect species. **Particulate matter** poses serious health risks, but has not been studied for insects in scientific literature. However allergies through contact with insect dust, is a known phenomenon in the insect industry.

Based on these findings, nitrous oxide, CO_2, ammonia and particulate matter are of interest for further research. CO_2 and ammonia sensors are common and will be used to determine these emissions continuously throughout larval/nymphal growth, which has never been done before. Nitrous oxide sensors are less common, so a discontinuous approach through air sampling might be a solution. Particulate matter is not only produced during insect rearing, but also while handling the insects. Measurements during growth and during harvest will be necessary here.

During the rearing experiments on the compound diets the following approach is suggested. The emissions are measured using specialized sensors. Within the ValuSect project there is room to optimize the measuring methods and certain sensors can be developed (e.g. nitrous oxide). In addition, samples will be taken so that emissions also can be measured using analytical methods (lab scale). In this way, other insights and more knowledge can be obtained. The measurement methods will be defined in detail during the preparation of the experimental design (activity 1).

5.4 Legislation

The ultimate goal of this project is to broaden the success of insects for food (products). When the insects are grown on side streams and can subsequently be used in human food, the cycle of sustainable production can be completed. However, when rearing insects for food, the European legislation needs to be taken into account. It is therefore important that at this stage streams are selected that can be used as feed for primary insect production.

Insects are considered as farmed animals in the EU and therefore producers of insects have to comply with the European 'food and feed legislation'. Together with the TSE legislation , the animal by-products legislation and the animal feed legislation , these impose, for example, the conditions with regard to the feed that may be used for insects.

More precisely, insects may only be fed with materials of vegetal origin, as well as some materials of animal origin such as fishmeal, blood products from non-ruminants, di- and tricalcium phosphate of animal origin, hydrolyzed proteins from non-ruminants, hydrolyzed proteins from hides and skins of ruminants, gelatin and collagen from non-ruminants, eggs and egg products, milk, milk based-products, milk-derived products, colostrum, honey and rendered fat. Feeding of catering waste, 'former foodstuffs', containing meat and fish (or manure/animal feces) to insects is prohibited. Furthermore, suppliers of the feed must comply with the requirements of EU feed hygiene legislation.

5.5 Nutritional value of insects

The nutritional value of the insects produced is of course also important when they are used in human food. For that reason, EFSA requirements for human food were listed in the literature search. However, it is very difficult to take this into account in the experimental phase because insects are often processed in products, which changes the nutritional value. This allows us to respond to the nutritional value to make the product suitable for human nutrition and thus to meet EFSA or WHO requirements. This will be included at a later stage of the project.

The literature search already provided insight into the nutritional value of produced insects. Again it is clear that insects are able to convert (low-value) biomass very efficiently into high-quality biomass. As indicated earlier, several studies show that the composition of insects is only marginally steerable, but that the diet does have an effect.

Tenebrio molitor

According to some studies, higher protein content in the substrate may result in higher protein content in the larvae (Alves, et al., 2016; Ruschioni, et al., 2020 ; Ooninckx, et al., 2015). However, this was not seen in all studies (van Broekhoven , Oonickx, van Huis, & van Loon, 2015; Zhang, et al., 2019). According to Zhang, *et al*. (2019) the total amino acid content of mealworms is higher than FAO/WHO requirements. Several indispensable amino acids are however lower than the requirements. Diets are observed to affect the amino acid composition in the larvae, but no clear pattern in how the levels in the diet affect the levels in the larvae could be observed (Zhang, et al., 2019).

The main fatty acids of mealworms include linoleic acid, oleic acid, palmitic acid and stearic acid. Mealworms contain good levels of PUFAs linoleic acid (n6) and α-linolenic acid (n3), however, the ratio of n6/n3 is larger than the optimal ratio of 5. It also seems that yellow mealworms accumulate n6 fatty acids more efficiently than n3 fatty acids. It is suggested by several authors that the fatty acid levels and the n6/n3 ratios can be altered by the diet. However, it is not clear to what extent this process can be influenced/steered, because the fatty acid profiles in larvae do not reflect the fatty acid profiles in the substrates (Ruschioni, et al., 2020; Melis, et al., 2019; van Broekhoven , Oonickx, van Huis, & van Loon, 2015; Ooninckx, et al., 2015).

Acheta domesticus

Bawa*, et al*. (2020) suggested that on high protein diets, crickets had a high crude protein (58%) and a low lipid (17-21%) composition. A high variation in fat composition of the diets was not reflected in the composition of the crickets. They also found that if the protein, carbohydrate and fat ratio of the diet are not well matched, that the excess carbohydrates will be stored as fat. The main fatty acid in crickets was C18:2 n6, although C16:0 and C18:1 n9 were also present in high concentrations. Large differences in C18:2 n6 and α-linolenic acid (C18:3 n3) concentrations were found due to dietary treatment.

Locusta migratoria

Only one paper concerning the nutritional composition of locusts was found relevant. This research indicated that the composition of locusts was affected by diet. For example adding wheat bran decreased the protein content and increased fat content. Additionally adding carrots to the diet further decreased protein content and increased lipid content. Also Mineral concentrations of Ca, K, Mg, and Na, and retinol concentrations were affected by diet. For further information, the reader is referred to 3.2.3 or the original publication.

6 General conclusion

This literature search summarises the side streams that can potentially be used as feed for rearing *Tenebrio molitor*, *Acheta domesticus* and *Locusta migratoria*. Based on the availability of non-valorised side streams that meet the nutritional requirements of the insects in scope, eight side streams (unsold fruit and vegetables from auction; potato steam peels; red blood cell fraction; hydrolysed chicken feathers; foliage from horticulture; grain middlings; forced chicory roots and grassy biomass) will be investigated for their potential in compound diets for insect rearing. To investigate the potential of these side streams, the chemical composition and physical characteristics of the side streams will be analysed. Based on this information, side streams will be pre-treated and mixed to formulate compound diets suited for insect rearing. In the next step, rearing experiments with these compound diets will be conducted.

Combining insect rearing on side streams with low emission of greenhouse gasses will eventually lead to a more sustainable production of insect based food products. Following emissions will be measured during the insect rearing experiments: nitrous oxide, CO_2, ammonia and particulate matter. These emissions will be measured with sensors, but will also be determined by analytical methods.

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Appendix

Appendix 1: Total protein content (%), individual essential amino acids (g/100 g protein) and total essential amino acids (%EAA) in selected offal across a number of species and fish by-products (Mullen & Álvarez, 2016).

Product	Specie	Prot. (%)	Leu	Ile	Lys	Met	Cys	Phe	Tyr	Trp	Thr	Val	His	% EAA
			Essential amino acid content (g/100 g protein)											
Blood	A	17-18	13.2	0.9	9.7	2.4	n.d.	10.7	1.4	-	4.8	8.7	8.8	60.6
	B	18.5	13.0	1.3	9.0	2.3	n.d.	9.7	2.9	1.5	3.7	9.0	5.6	58
Brain	A	10.5	7.5	3.9	6.0	2.1	1.8	5.0	3.6	4.7	4.7	4.9	2.5	42.8
	B	10.3	8.7	4.6	7.8	2.0	1.3	5.1	4.2	4.2	4.7	5.7	5.7	46.8
	C	12.3	7.8	4.0	6.4	2.0	1.1	4.8	3.7	1.1	4.5	4.8	4.8	42.8
Ear	B	22.3	5.2	2.2	4.7	0.6	1.3	3.2	1.8	0.2	2.8	3.7	1.2	25.6
Feet	B	21.2	4.2	1.6	4.3	1.0	-	2.7	1.5	0.2	2.6	2.3	1.2	21.6
Heart	A	17	8.8	4.4	8.2	2.6	1.3	4.5	3.6	1.1	4.7	5.2	2.7	47.1
	B	17	9.0	4.8	8.3	2.6	1.8	4.4	3.4	1.2	4.4	5.3	2.5	47.7
	C	18	8.5	4.3	7.5	2.2	0.8	4.3	3.1	1.1	4.7	5.0	2.3	43.8
Kidney	A	15.3	8.0	4.1	6.6	2.1	0.8	4.8	3.8	1.4	4.8	6.2	2.6	45.2
	B	15.4	9.0	5.3	7.2	2.1	2.2	4.7	3.6	1.3	4.1	6.0	2.5	48.0
	C	18.0	7.5	4.0	6.5	2.0	1.1	4.7	3.5	1.4	4.7	5.9	2.6	43.9
Lips	A	21.8	3.1	1.8	4.1	-	-	3.1	-	0.5	2.0	3.3	-	17.9
Liver	A	21	9.4	4.6	6.9	2.5	1.5	5.3	4.0	1.4	4.6	6.2	2.7	49.1
	B	19	8..9	5.1	7.7	2.5	1.9	4.9	3.4	1.4	4.2	6.2	2.7	48.9
	C	20.3	8.2	4.3	5.4	2.1	1.0	4.5	3.6	1.2	4.5	5.5	2.4	42.7
Lung	A	17	7.3	4.8	7.1	2.0	1.5	4.1	2.2	0.9	3.7	4.9	3.0	41.5
	B	15	7.8	4.0	7.3	1.6	-	4.2	-	0.9	3.5	6.0	2.5	37.8
	C	12.5	8.0	3.2	6.5	1.8	1.6	4.1	2.8	0.9	3.7	5.5	2.5	40.6
Spleen	A	19	8.8	3.8	7.2	1.8	2.9	4.0	5.5	1.0	3.9	6.0	3.6	48.0
	B	17.9	8.2	4.5	7.5	1.8	-	4.3	2.8	1.0	4.0	5.4	2.4	41.9
	C	17.2	8.9	6.3	7.7	1.9	1.3	4.5	2.9	1.1	4.1	6.5	3.3	48.5
Tongue	A	17.1	7.5	4.3	7.7	2.1	1.3	4.1	3.2	0.8	4.4	4.8	2.6	45.8
	B	16.3	8.0	4.6	8.2	2.2	-	4.1	-	1.2	4.2	5.2	2.5	40.2
	C	15.3	7.1	3.9	7.1	2.1	1.1	3.7	2.9	1.0	4.5	4.8	2.2	40.4

Fish by-product	D	15-30	9.1	5.3	10.1	6.9	1.6	4.0	1.8	0.8	2.8	6.4	5.2	54.0
	E	15-30	9.6	5.3	7.5	3.2	1.9	5.0	2.4	1.0	3.2	5.9	2.6	47.6

A: cattle, B: Porcine, C: Sheep, D: commercial saltwater fish waste, E: commercial freshwater fish waste.

Appendix 2: Fat, cholesterol and saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) per 100 g of raw material of several offal products. The content of linoleic and linolenic acid is showed in those products where data was available.

Product	Specie	Fat (%)	Cholesterol (mg)	SFA (g)	MUFA (g)	PUFA (g)	Linoleic (g)	Linolenic (g)
Blood[19]	A	0.4	90	0.1	0.1	0.1		
	B	0.4	40	0.1	0.1	0.1		
Brain	A	10.3	3010	2.3	1.9	1.6		
	B	9.2	2195	2.1	1.7	1.4		
	C	8.6	1352	2.2	1.6	0.9	0.01	0.0
Fat/Tallow	A	100	109	49.8	41.8	4		
	B	99	95	39.2	45.1	11.2		
	C	80	78	32.3	21.7	2.3		
	D	99	85	29.8	44.7	20.9		
	E	99	100	33.2	49.3	12.9		
Feet	B	22	82	6.5	9	2		
	D	14.6	84	3.9	5.5	3.0		
Heart	A	3.9	124	1.4	1.1	0.5		
	B	4.4	131	1.2	1.0	1.1	0.25	0.06
	C	5.7	135	2.2	1.6	0.6		
	D	9.3	136	2.7	2.4	2.7	0.308	0.09
Kidney	A	3.1	411	0.9	0.6	0.5	0.19	0.04
	B	3.2	319	1.0	1.1	0.3		
	C	3.0	337	1.0	0.6	0.6	0.15	0.05
Liver	A	3.6	275	1.2	0.5	0.5	0.29	0.01
	B	3.7	301	1.2	0.5	0.9		
	C	5.0	371	1.9	1.2	1.3	0.15	0.14
	D	4.8	345	1.6	1.1	0.8	0.47	0.01
Lung	A	2.5	242	0.9	0.6	0.3		
	B	2.7	320	1	0.6	0.3		
	C	2.6	n.d.	0.9	0.7	0.4		
Spleen	A	3.0	263	1.0	1.2	0.5		
	B	2.6	363	0.9	0.7	0.2		
	C	3.1	250	1.0	0.8	0.2		
Tongue	A	16.1	87	7.0	7.2	0.9	0.27	0.13
	B	17.2	101	6.0	8.1	1.8		
	C	17.2	156	6.6	8.5	1.1	0.34	0.27
Fish oil	Herrin	100	766	21.3	56.6	15.6	0.6-2.9	0.2-1.1

	Cod liver	100	570	22.7	46.7	22.5	0.8-2.1	0.9-1.1
	Salmon	100	485	19.9	29.1	40.3	1.2	0.5
DV		65 g	300 mg	<10%*	15-20%*	8-20%*		

A: cattle, B: Porcine, C: Lamb, D: chicken, E: duck. DV: daily value according to U.S. Food and Drug Administration. *: recommended amounts of fats based on the fact that it must supply 20-35% of total energy intake. Data from http://ndb.nal.usda.gov/ndb/

Appendix 3: Mineral content in raw offal expressed as mg/100g – with the exception of selenium which is expressed as µg/Kg

Product	Specie	Ca	Fe	Mg	P	K	Na	Zn	Cu	Mn	Se
Brain	A	43	2.5	13	362	274	126	1.02	2.9	0.03	21.3
	B	10	1.6	14	282	258	120	1.27	2.4	0.10	15.9
	C	9	1.75	12	270	296	112	1.17	2.4	0.04	9.0
Heart	A	7	4.31	21	212	287	98	1.70	4.0	0.03	21.8
	B	5	4.68	19	169	294	56	2.80	4.0	0.06	10.4
	C	6	4.6	17	175	316	89	1.87	4.0	0.05	32.0
	D	12	5.96	15	177	176	74	6.59	3.4	0.01	4.3
Kidney	A	13	4.6	17	257	262	182	1.92	4.3	0.14	141.0
	B	9	4.89	17	204	229	121	2.75	6.2	0.12	190.0
	C	13	6.38	17	246	277	156	2.24	4.5	0.12	126.9
Liver	A	5	4.90	18	387	313	69	4.00	9.8	0.31	39.7
	B	9	23.3	18	288	273	87	5.76	6.8	0.34	52.7
	C	7	7.37	19	364	313	70	4.66	7.0	0.18	82.4
	D	8	8.99	19	297	230	71	2.67	4.9	0.25	54.6
Lung	A	10	7.95	14	224	340	198	1.61	2.6	0.02	44.3
	B	7	18.9	14	196	303	153	2.03	0.8	0.02	17.8
	C	7	5.23	12	288	274	108	1.16	2.4	0.02	17.2
Spleen	A	9	44.5	22	296	429	85	2.11	1.7	0.07	62.2
	B	10	23.2	13	260	396	98	2.54	1.3	0.07	32.8
	C	9	42.0	21	280	358	84	2.84	1.2	0.05	32.4
Tongue	A	6	2.95	16	133	315	69	2.87	1.7	0.02	9.4
	B	16	3.35	18	193	243	110	3.01	0.7	0.01	10.4
	C	9	2.65	21	184	257	78	2.32	2.1	0.05	15.0

Fish by-products*	5800	10.0	170	2040	680	610	62.0	10	60	-
DV	1000 mg	18 mg	375 mg	1000 mg	3500 mg	2400 mg	15 mg	2 mg	2 mg	70 µg

A: beef, B: porcine, C: lamb, D: chicken, *: in dry basis[21]. DV: daily value according to U.S. Food and Drug Administration. Data from http://ndb.nal.usda.gov/ndb/

Appendix 4: Concentration of water-soluble vitamins in several types of offal. Units are given in mg/100 g, with the exception of vitamin B12 and folate which are presented as µg/100 g

Product	Specie	Thiamin	Riboflavin	Niacin	Pantothenic ac.	Vit. B6	Vit B12	Folate	Vit. C
Brain	A	0.092	0.199	3.550	2.010	0.226	9.51	3	10.7
	B	0.155	0.275	4.275	2.800	0.190	2.19	6	13.5
	C	0.130	0.300	3.900	0.920	0.290	11.30	3	16.0
Heart	A	0.238	0.906	7.530	1.790	0.279	8.55	3	2.0
	B	0.613	1.185	6.765	2.515	0.390	3.79	4	5.3
	C	0.370	0.990	6.140	2.630	0.390	10.25	2	5.0
	D	0.152	0.728	4.883	2.559	0.360	7.29	72	3.2
Kidney	A	0.357	2.840	8.030	3.970	0.665	27.50	98	9.4
	B	0.340	1.697	8.207	3.130	0.440	8.79	42	13.3
	C	0.620	2.240	7.510	4.220	0.220	52.41	28	11.0
Liver	A	0.189	2.755	13.175	7.173	1.083	59.30	290	1.3
	B	0.283	3.005	15.301	6.650	0.690	26.0	212	25.3
	C	0.340	3.630	16.110	6.130	0.900	90.05	230	4.0
	D	0.305	1.778	9.728	6.233	0.853	16.58	588	17.9
Lung	A	0.047	0.230	4.000	1.000	0.040	3.81	1	38.5
	B	0.085	0.430	3.345	0.900	0.100	2.75	3	12.3
	C	0.048	0.237	4.214	-	0.110	3.93	3	31.0
Pancreas	A	0.140	0.445	4.450	3.900	0.200	14.00	3	13.7
	B	0.105	0.460	3.450	4.555	0.460	16.40	3	15.3
	C	0.030	0.250	3.700	1.000	0.070	6.00	13	18
Spleen	A	0.050	0.370	8.400	1.081	0.070	5.68	4	45.5
	B	0.130	0.300	5.867	1.055	0.060	3.26	4	28.5

	C	0.047	0.348	7.895	-	0.110	5.34	4	23.0
Tongue	A	0.125	0.340	4.240	0.653	0.310	3.79	7	3.1
	B	0.490	0.485	5.300	0.641	0.240	2.84	4	4.4
	C	0.150	0.380	4.650	0.970	0.180	7.20	4	6.0
DV		1.5 mg	1.7 mg	20 mg	10 mg	2 mg	6 µg	400µg	60 mg

A: beef, B: porcine, C: lamb, D: chicken. DV: daily value according to U.S. Food and Drug Administration. Data from http://ndb.nal.usda.gov/ndb/

Appendix 5: Overview of pretreatment of tested substrates for the production of mealworms

substrate	pretreatment	reference
Acrocomia aculeata (palm tree)	45°C 48h + crushing + sieving 355µm	(Alves, Sanjinez-Argandoña, Linzmeier, Cardoso, & Macedo, 2016)
mushroom spent corn stover	air drying 30d untill <10% moisture + grinding + 2mm sieving	(Zhang, et al., 2019)
spirit distillers grain	air drying untill <10% moisture + grinding + 2mm sieving	
highly denaturated soybean	grinding + 2mm sieving	
Wheat bran		
brewers' spent grain	drying chamber untill 22% moisture (40h 60°C)	(Melis, et al., 2019)
Wheat bran		
Fermented wheat straw	crushing + 20-40mm mesh, 48h fermentation, air drying	(Li, Zhao, & Liu, 2013)
Wheat flour		(Ruschioni, et al., 2020)
wheat middlings		
middlings + olive pomace (75:25)	electric homogenized, mixed with substrate, 24h at 4°C	
middlings + olive pomace (50:50)	electric homogenized, mixed with substrate, 24h at 4°C	
middlings + olive pomace (25:75)	electric homogenized, mixed with substrate, 24h at 4°C	
byproducts high protein high fat	cut, freeze dried, mixing, -20° storage	(Ooninckx, van Broekhoven, van Huis, & van Loon, 2015)
byproducts high protein low fat	cut, freeze dried, mixing, -20° storage	
byproducts low protein high fat	cut, freeze dried, mixing, -20° storage	
byproducts low protein low fat	cut, freeze dried, mixing, -20° storage	

byproducts high protein high starch	freeze dried, grounding, mixing, -20°C storage	(van Broekhoven, Oonincx, van Huis, & van Loon, 2015)
byproducts high protein low starch	freeze dried, grounding, mixing, -20°C storage	
byproducts low protein high starch	freeze dried, grounding, mixing, -20°C storage	
byproducts low protein low starch	freeze dried, grounding, mixing, -20°C storage	

Appendix 6: Overview of pretreatment of tested substrates for the production of house crickets

Substrate	pretreatment	reference
Pure pride cricket feed		(Bawa, Songsermpong, Kaewtapee, & Chanput, 2020)
50% Pure pride + 50% Betagro chicken feed		
Betagro chicken feed		
Pure pride +100 g fresh pumpkin pulp per day		
Pure pride + 100 g dry pulp pumpkin powder per day	fresh pulp was dried at 600 W for 8 min at 2 min regular intervals, in a microwave oven. Pulp was then milled into powder.	
byproducts high protein high fat	cut, freeze dried, mixing, -20° storage	(Oonincx, van Broekhoven, van Huis, & van Loon, 2015)
byproducts high protein low fat	cut, freeze dried, mixing, -20° storage	
byproducts low protein high fat	cut, freeze dried, mixing, -20° storage	
byproducts low protein low fat	cut, freeze dried, mixing, -20° storage	
chicken meal		

Appendix 7: Overview of pretreatment of tested substrates for the production of migratory locusts

substrate	pretreatment	reference
fresh perennial ryegrass	grass of app. 15cm, freshly twice a day	(Oonincx & Van Der Poel, 2011)
wheat bran	wheat bran as whole bran in plastic container	

carrots	carrots cut into pieces of 8cm by 3cm, freshly once a day	

Appendix 8: Blood processing to obtain a dry powder

Blood production at global scale is not reported, to the best of our knowledge, by any official organization; but estimate calculations can be carried out based on the number of livestock slaughtered yearly and the volume obtained for each species. As a general rule, it can be said that 3.5 liters are generated per pork head slaughtered, 1.7 per sheep, 17.6 per cattle and around 0.15 per chicken. So, assuming that no blood is loss on collection, the total blood production in 2018 for the project partner members was of: 152 and 181 million liters for pork and cattle, respectively. It means, that after blood has been centrifuged, assuming an average yield of 40% red cells fraction and 60% plasma fraction and an average protein content of 35% and 7%; overall protein production can be estimated. Figures in Table 23.

Table 23: Volumes of blood and blodd fractions generated in 2018 in Belgium Netherlands, Switzerland, UK and Ireland

	Pork	Cattle
Litres of blood	152,005,686	181,386,955
Litres of plasma	91,203,411	108,832,173
Litres of red cells	60,802,274	72,554,782
Plasma proteins (kg)	6,384,239	7,618,252
Red Cell proteins (kg)	21,280,796	25,394,174

Current industrial practices to obtain a final dry powder are very straightforward and includes the next steps (Figure 8):

- Hygienically collection of blood (usually a drain closed system) and anticoagulant addition.
- Blood is stored at low temperatures under gentle stirring.
- Blood is transported to processing plants.
- Blood is centrifuged using a continuous separator where plasma and red cell fractions are obtained,
- Immediately after, plasma is concentrated using membrane units and then is dried using spray drying techniques and the resulting powder is packed. Red cells, can be either further processed (hydrolysis, for example) and then spray dried. After this, the product is stable at room temperature for long periods of time, since a_w is less than 0.5-06 units.

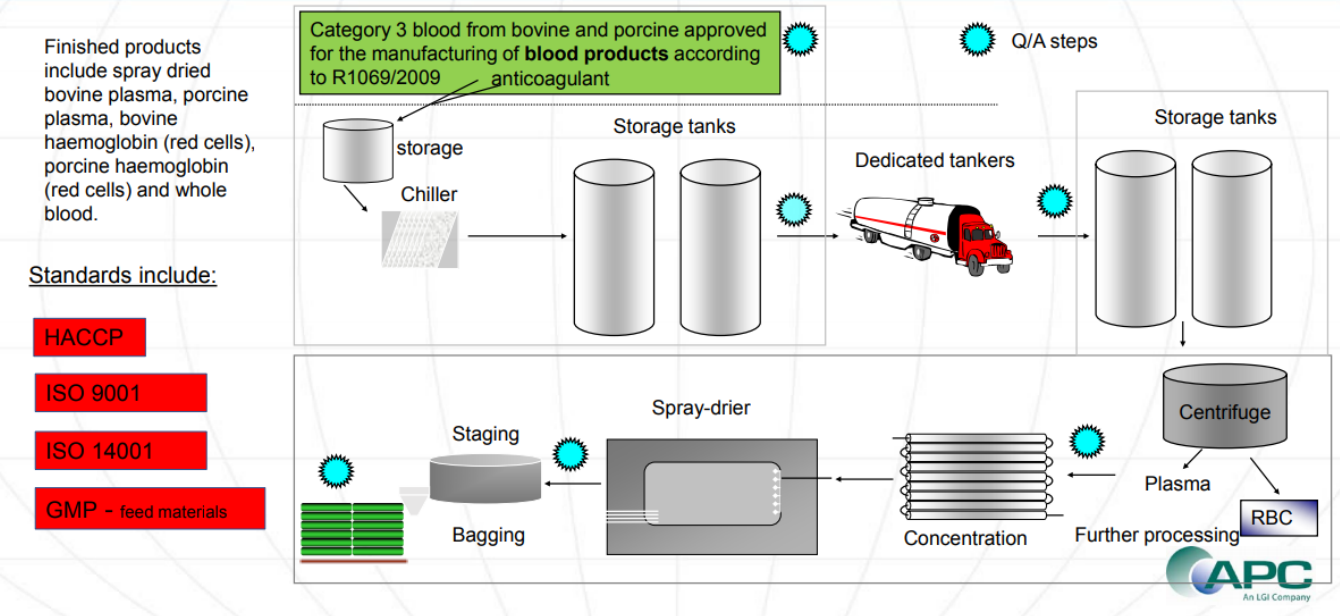

Figure 8: Schematic flow chart of blood processing at industrial scale

After this process has been completed, the resulting powder have a very high content in proteins; for plasma this content is around 80% (10 % ash, 5 % moisture) at industrial processing scale; while for the red cell fraction powder, the protein content can be as high as 95% in dry basis.

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