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INSTITUTE OF FOOD SCIENCE AND BIOTECHNOLOGY
ÚSTAV CHEMIE POTRAVIN A BIOTECHNOLOGIÍ

PROCESSING OF SELECTED SPECIES OF EDIBLE INSECTS FOR FOOD PURPOSES
ZPRACOVÁNÍ VYBRANÝCH DRUHŮ JEDLÉHO HMYZU PRO POTRAVINÁŘSKÉ ÚČELY

MASTER'S THESIS
DIPLOMOVÁ PRÁCE

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Processing of selected species of edible insects for food purposes

Master's Thesis:

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* Characterization and composition of at least three pulverulent raw materials from crickets, mealworms and barn;
* Proposal of food product with use of insect powder;
* Analysis of selected components in samples;
* Processing of acquired data and evaluation of experiments.

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ABSTRACT

This diploma thesis is focused on nutritional evaluation of selected insect powders from house crickets, yellow mealworms and lesser mealworms and of one insect-based food product, which was also developed as one of the aims of this work. In addition to basic nutritional analyzes, also ICP-OES for mineral composition and GC-FID for fatty acid composition were used. Also, the crude protein content, using CHNSO analyzer and content of EAA was determined. Using PCA analysis, samples of individual insect powders were successfully distinguished, which confirmed the diversity of individual insect species, and thanks to the projection into the plane of the principal components, it is possible to see in terms of which nutritional parameters they differ the most. Sensory analysis of the newly developed insect-based product with addition of lesser mealworm powder was also performed in comparison to competing products. The work also includes an extensive consumer survey on a sample of 2,019 participants, concerning the perception of entomophagy in the Czech Republic and Slovakia, which was conducted online.

ABSTRAKT

Táto diplomová práca je zameraná na nutričné zhodnotenie vybraných práškov hmyzu zo svrčkov domových, z lariev múčiara obyčajného a potemníka stajňového a jedného potravinového produktu na báze hmyzu, ktorý bol tiež vyvinutý ako jeden z cieľov tejto práce. Okrem základných nutričných analýz boli použité aj ICP-OES pre zistenie obsahu minerálov a GC-FID pre zistenie zloženia mastných kyselín. Tiež bol stanovený obsah hrubej bielkoviny pomocou CHNSO analyzátora a stanovený obsah EAA. Pomocou PCA analýzy sa úspešne podarilo rozlíšiť vzorky jednotlivých hmyzích práškov, ktoré potvrdili rozmanitosť jednotlivých druhov hmyzu a vďaka projekcii do roviny hlavných komponent je možné vidieť, v ktorých nutričných parametroch sa najviac líšia. Bola tiež vykonaná senzorická analýza novo vyvinutého produktu na báze hmyzu s prídavkom prášku z potemníka stajňového v porovnaní s konkurenčnými produktmu. Súčasťou práce je aj rozsiahly spotrebiteľský prieskum na vzorke 2 019 účastníkov, týkajúci sa vnímania entomofágie v Českej republike a na Slovensku, ktorý sa uskutočnil online.

KEYWORDS

Edible insects, entomophagy, nutritional analysis, GC-FID, ICP-OES, CHNSO, PCA, sensory analysis, consumer survey

KĽÚČOVÉ SLOVÁ

Jedlý hmyz, entomofágia, nutričná analýza, GC-FID, ICP-OES, CHNSO, PCA, senozorická analýza, spotrebiteľský prieskum

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DECLARATION

I declare that the diploma/bachelor thesis has been worked out by myself and that all the quotations from the used literary sources are accurate and complete. The content of the diploma/bachelor thesis is the property of the Faculty of Chemistry of Brno University of Technology and all commercial uses are allowed only if approved by both the supervisor and the dean of the Faculty of Chemistry, BUT.

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In this way, I would like to thank the supervisor of my work, prof. Ing. Tomáš Svěrák, CSc., but also to several employees from the Institute of Food Science and Biotechnology at FCH BUT, and to my family and girlfriend for their help and support in writing this work.

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1. INTRODUCTION

Future of food sector on our planet is in recent decades increasingly oriented towards alternative sources of food and especially of protein. The main reasons are the inefficiency of current protein sources, rising costs of animal protein, the threat of future food and feed shortages, constantly growing world population, but also increasing demand for protein among the middle class. It is estimated, that in 2050, the human population will reach almost 10 billion and it will not be possible to feed all of them with the current way of food production [1, 2]. Therefore, various alternative protein sources need to be taken into consideration.

Alternative protein sources are not only a question of the future, but also of the present, as nearly 1 billion people worldwide nowadays suffer from hunger [3]. Alternative protein sources include various plants, cell-cultured meat, fermented sources or edible insects [4]. One of the main benefits why insects excel over other alternative options is the fact, that edible insects have always been part of human diet. Edible insects represent a highly nutritious and sustainable source of protein, but also of other valuable nutrients [1, 3]. It is estimated that insects form part of traditional diet of at least 2 billion people worldwide and that more than 2,100 species have reportedly been used as food [3, 5]. However, the entomophagy phenomenon, which represents the use of edible insect as food, is still minimal in developed countries. The main reason is the disgust factor, which is based mostly on social norms established in Western society [1, 4].

The importance of entomophagy as a novel food source is slowly being recognized by the whole society. Since the new Regulation (EU) 2015/2283 on novel foods entered into force on 1st January 2018, selected species of edible insects are considered a novel food and can be legally marketed as food [6]. This shift in legislation, together with other factors, had a significant impact on the still rising edible insect market in Europe, but also around the world [7].

However, edible insects sector is still in its infancy in the developed world and is likely to face many future challenges. Contribution to this sector was also the purpose behind writing this thesis. First part of this work is dedicated to literature research regarding edible insects and entomophagy in general. In the experimental part, this work deals with the nutritional analysis of powders from various types of edible insects, namely from house cricket (*Acheta domesticus*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Aplhitobius* diaperinus) and insect-based food supplement, the proposal of which was also one of the aims of this thesis. Also, the palatability and acceptability of this powder was evaluated using the sensory analysis and part of the work was also dedicated to the online survey to examine the consumer perception of edible insects on the Czech and Slovak market.

2. THEORETICAL PART

2.1 Insects as food

The practice of eating insects is known as entomophagy. Insects are thought to be part of the traditional diet of more than 2 billion people worldwide and more than 2,100 edible species have been cataloged as edible [3, 5]. In addition, insects are also important from an ecological point of view and are essential for human survival. They play an important role as pollinators of plants, thus ensuring their reproduction, improve soil fertility by bioconversion of waste, act as a biocontrol for various types of pests and are also a source of several valuable products of human consumption. This includes, for example, honey or silk. Some insect species, such as bees and silkworm caterpillars have a long history of domestication especially due to their valuable products. Among other things, insects are also used in medicine, for example in larval therapy in the treatment of chronic non-healing wounds. However, the concept of growing insects as food is relatively new, although practice of entomophagy has always existed and was probably an important source of nutrients in the past as it is today in some parts of the world. Another possible use of edible insects is not only the production of food or direct human consumption, but also the production of animal feed for aquaculture and livestock [3].

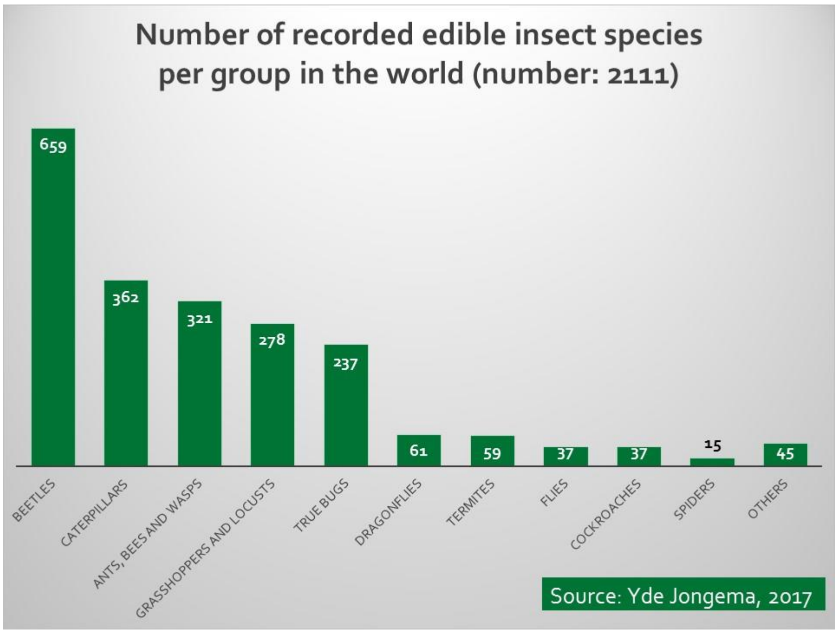

Figure 1 - Infographic about distribution of edible insect species [5]

Worldwide, the most frequently consumed insects include species of the order Coleoptera (beetles) which make up about 31 %, Lepidoptera (caterpillars) which represent about 18 %, Hymenoptera (bees, wasps and ants) with 14 %, Orthoptera (grasshoppers, locusts and crickets) make up about 13 % and the order Hemiptera (cicadas, leafhoppers, planthoppers, scale insects and true bugs) occupy approximately 10 %. Less represented species include insects of the order Isoptera (termites) with 3 % share, Odonata (dragonflies) with 3 % share, Diptera (flies) with 2 % share and the rest 5 % are made of other orders. [3]. Edible insects of different orders are consumed at different stages of their life cycle. For example, practically all butterflies and moths (Lepidoptera) are consumed in the larval stage known as catterpillars [8]. The species of Hymenoptera are largely used in their larval or pupal stages, the species from the order Coleoptera are consumed as both adult beetles and larvae and species from orders Orthoptera, Homoptera, Isoptera and Hemiptera are mainly consumed in their adult stages [3, 9].

Currently, most edible insects are harvested from the wild, although indoor rearing of insects and semi-domestication have increased the insect availability and the sustainability of their production. In traditional cultures, insects are usually incorporated into various recipes, which include processing techniques like roasting, drying, frying or steaming. In the Western cultures, edible insects are more commonly served in a non-recognizable form, due to the negative attitudes towards entomophagy. In a form of powder, insects are incorporated into foods like cookies, pasta or various snacks. Modern technologies of insects processing include also extraction of components like protein, fat or chitin which can be then used as food ingredients too [10].

2.1.1 History of entomophagy

Insects as a part of human diet are not something entirely novel. In publications dealing with hunting and meat eating by early humans, authors do not mention insect consumption often. In comparison to food plants and wild meat, entomophagy has been undervalued for a long time. It might be due to the view of insects as a fall-back food resource and the Western negative bias of insect consumption might have also played a role. Nevertheless there is ample evidence to show that insects have been part of the human diet since time immemorial [11].

One of the strongest pieces of evidence is the fact that insects are eaten virtually by all non-human primates. Altough the dietary contribution varies, almost ever record of wild primate diet contains insects to some extent. Another important reason is the fact that insects represent an important part of the diet of many traditional societies, even nowadays [11]. For example, the Aborigines, indigenous people in Australia, consume species like witchetty grubs, Bogong moths or termites. However, their practice of entomophagy has decreased in the past 200 years due to the influence of Western civilization [11, 12]. In Africa, around 250 edible insect species are consumed by many differenet ethnic groups. People in China consider insects as a food for at least 3000 years. Lastly, there is also an archaeological evidence of insectivory by hominids. For example, in South Africa, the practice of foraging termites as food by early hominids is evidenced by wear patterns on bone tools which were used to dig termites from mounds. Coprolites found in the Lakeside Cave in the Great Basin in USA in turn prove the consumption of migratory grasshoppers which were probably eaten in times of their outbreaks. Prehistoric human coprolites containing remains of insects were also found in other places in the US, but also in Mexico or in Peru. In northern Spain, there were found traces of insect fragments in the dental plaque of 1.2 million year old hominin which represents a direct evidence of insects as a food by Europe´s earliest human inhabitants [11].

People also used to manipulate the environment in spite of edible insect procurement which represent some form of semi-cultivation. For example, in Mexico, there is a popular dish called „Ahuauhtle“ or „Mexican caviar“ which is made from eggs of aquatic true bugs (Hemiptera: Neopomorpha) which are collected in higher amounts from prepared grass or reed bundles in marshes and ponds of Mexican Mesa Central. It is believed, that these insect eggs represented one of the prime protein sources for Aztecs, living in Mesoamerica, as their complex society with a high population density was developed without large domesticable animals. In the Amazon Basin, tropical Africa and New Guinea, indigenous people deliberately fell palm trees so that they can then collect the nutritious larvae of several species of palm weevils (Coleoptera: Curculionidae) and inhabitants of Sub-Saharan Africa, in turn, put young leaf feeding caterpillars (Lepidoptera) in trees near their home so they could collect them and eat after reaching maturity. These manipulations allow efficient collecting of larger numbers of insects and thereby increasing return rates. It represents an intelligent strategy for the procurement of valuable nutrients, especially of animal protein and fat [12].

Figure 2 - Witchetty grubs as a historical staple in the diet of Aboriginal Australians [13]

The practice of eating insects is also cited in a large amount of literature. For example in various religious literature – in the Christian Bible, or in Jewish and Islamic literature. There are also a lot of mentions about edible insects in the ancient literature, wether in the Middle East, China, or in Europe. In ancient Greece, for example, eating cicadas was considered a delicacy. In 1737, René Antoine Ferchault de Réaumur wrote: *„We could perhaps in time overcome our repugnance at eating insects and accept them as part of our diet, and then realize that there is nothing terrible about them and that they may perhaps even offer us agreeable sensations. We have grown accustomed to eating frogs, snakes, lizards, shellfish, oysters, etc. in the various provinces of France. Perhaps the first urge to eat them was hunger."* In 1885, Vincent M. Holt, a british entomologist, published a courageous booklet named „Why not to eat insects?" where he puzzled over the lack of acceptance of insects as a food, when the composition of these animals was nearly the same as the composition of other animals, like lobsters, which was and is still considered a delicacy [3].

The reason why entomphagy is not as widespread as it could have been is probably also the major influence of Europeans, conquesting the whole world, on food production. Their negative attitude towards eating insects for example managed to strongly suppress the practice of entomphagy in Northern America with aim of modernizing or „westernizing“ the indigenous people. It is probable that with more time and without European colonialization the semi-cultivation or domestication of edible insects would be more widespread nowadays [3].

Considering all these aspects together with fact, that insects provide key nutrients and provide lower risk compared to hunting, it is highly likely that insects comprised an important part of the diet of early humans and entomophagy is therefore considered an ancient habit.

2.1.2 Entomophagy today

As mentioned eariler, insects are currently thought to be part of the traditional diet of more than 2 billion people and more than 2,100 species of insects are considered edible. Practice of eating insects is commonplace mainly in tropical countries, predominantly in parts of Asia, Africa and Latin America. Approximately 113 countries around the world practice entomophagy [3, 5]. There are many reasons why entomophagy here is so popular. Insects tend to grow larger in tropics, which facilitates easier harvesting. Insects in the tropics often congregate in significant numbers, so harvesting is easier and more effective here. Large amount of edible insect species can be found year-round here, while in temperate zones they tend to hibernate to survive cold winters and therefore no active species can be found during these times. People in tropical countries also have better knowledge about harvesting insects while such knowledge has almost disappeared in westernized regions [3]. Insects are also consumed in countries partially or fully in temperate zones, such as Japan, China or Mexico [5].

Figure 3 - Edible insects at the Khong Hae Floating Market near Hat Yai in Thailand [14]

Generally said, people in temperate zones do not eat insect at all. In most Western countries, entomophagy is unfortunately viewed with feelings of disgust and people associate it with primitive behavior or consider it as a sign of poverty. This is also one of the factors why modern research has not addressed this topic too much. Despite many historical sources, where insects are mentioned as a source of livelihood, the topic of entomophagy has only received proper attention in the recent years [3]. Another important aspect is food neophobia, characterized as the tendency to refuse new or unknown food. When it comes to edible insects, neophobia is particularly high [15].

Entomophagy is strongly conditioned by culture or religion. For people from insect eating countries, insects have been and are an integral part of everyday life [3]. In Europe, insects are not consumed on a daily basis yet, but the popularity of this practice is slowly growing. In countries like Netherlands, Great Britain, Germany, but also Czech republic, there are already companies which produce and sell edible insects wether in whole or processed form. As the acceptance or rejection of entomophagy is mainly a question of culture, it is expected that insects will occupy a significant portion of the alternative protein sector in the future.

2.1.3 Reasons for eating insects

2.1.3.1 Nutritional aspects

Edible insects are a highly nutritious and healthy food source high in fat, protein, vitamins, minerals and fibre. Thanks to the huge diversity of edible insects species, the nutritional value is also highly variable. Even within a species, the nutritional value may vary, depending on the stage of life in which the insect finds itself, sex of the insect, the environment in which it lives and the food it consumes. Other important aspects, influencing nutritional composition, are also preparation and processing methods [3, 16].

Although, there are significant variations, generally the main components of insects are protein and fat, followed by fibre, nitrogen-free extract (NFE) and ash. Average contents for each insect Order can be seen in the *Figure* 4. Many edible insects provide satisfactory amounts of energy and quality protein, are high in mono- or poly-unsaturated fatty acids, and are often rich in some of the minerals, especially zinc, magnesium, iron, manganese, copper, phosphorus or selenium and vitamins like riboflavin, pantothenic acid, biotin or sometimes also folic acid. Often, the iron and calcium content is higher than in beef, pork or chicken [17]. Thanks to the high nutritional value of insects, entomophagy may be one of the key ideas to solve some of the world´s food problems like starvation and malnutrition, especially protein or mineral malnutrition in developing countries [18].

In this subchapter, the general nutritional composition of insects is described, while the nutritional composition of selected insects species, used in this thesis, is discussed in more detail in the following chapters.

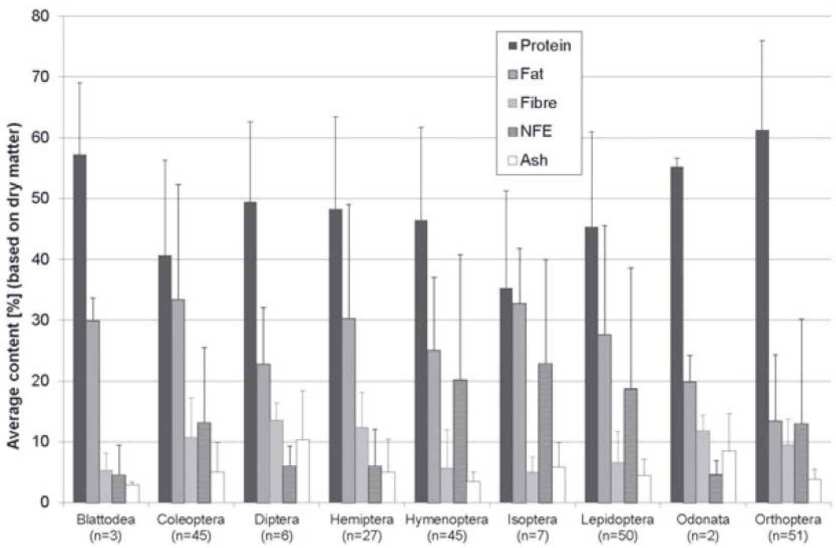

Figure 4 - Average nutrient contents of edible insects from various Orders [17]

Energy content

The total energy value of edible insects depends on their composition, mainly on the fat content. Compared to adults, larval or pupal stages tend to have higher fat and energy content. In contrast, species with a high protein content tend to have a lower energy content [16]. The mean energy contents of varioous edible insect orders range from 409,78 to 508,89 kcal per 100 g based on dry matter while the maximum energy contents may be as high as 776.85 kcal per 100g in some species and minimum contents as low as 216.94 kcal per 100 g of dry matter. Thanks to relatively high contents of protein and fat, most edible insects provide substantial amount of energy even in comparison to meat [17].

Protein content

The protein content of edible insects can vary from 4.90 % to 77.13 % of dry matter while the average protein content of various orders is in the range of 25.34 % for Isoptera and 61.32 % for Orthoptera [17]. Edible insects in general and especially species from the order Orthoptera, like crickets, locusts and grasshoppers, are rich in proteins and represent a valuable alternative protein source. Also, the quality of the insect protein is, with a few exceptions, at a high level. As can be seen in the *Figure* 5 below, most edible insects meet the amino acid requirements for adults published by WHO. The nutrient quality of insect protein could also be further improved by the removal of chitin [17, 19]. The digestibility of insect protein should be in the range of 76 to 96 %, which is on average a little smaller than values for egg protein (95 %) or beef (98 %), although higher than in the case of many plant proteins [16, 20]. Regarding the crude protein content, this parameter can often be overestimated in insects, since some nitrogen is also bound in their exoskeleton [21].

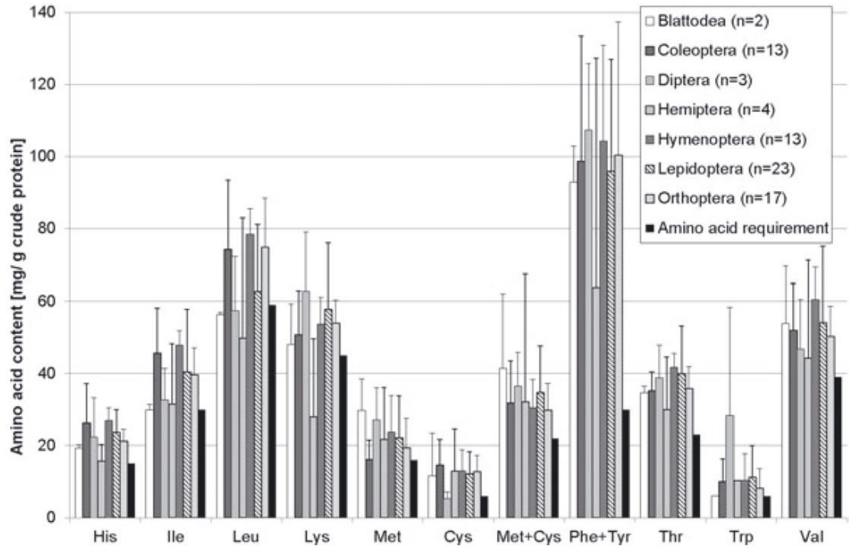

Figure 5 - Mean amino acid contents of edible insects from various Orders [17]

Regarding the amounts of protein in comparison to plants, the maximum protein yields of species Orthoptera is up to 77.13 % while maximum protein contents of plants (dry soy beans) is only 35.8 %, based on dry matter. Insects, especially grasshoppers, could therefore potentially represent an excellent alternative protein source [17].

Fat content

The average fat content of various insect orders, being the second most abundant nutrient in edible insects, ranges from 13.41 % for Orthoptera species to 33.40 % in Coleoptera species, based on dry weight. Larve of some Lepidopters species can contain up to 77.13 % of fat while larve of some species from Coleoptera order can contain only 0.66 % of fat based on dry matter. Generally, the fat content is higher in larval and pupal developmental stages of insects than in the adult stage and the fatty acid composition of insects is also dependent on the composition of their feed [17]. The lipids of insects are mainly present in the form of triacylglycerols which serve as an energy deposit for periods of high energy demand. Other types of lipids present in smaller amounts include cholesterol, partial glycerides, free fatty acids, phospholipids and wax esters [22].

Regarding the fatty acid composition, the average amount of saturated fatty acids in insects ranges from 30.83 % for Hymenoptera species to 41.97 % of total fat for species from Isoptera order. Main representatives of SFA in insects are usually palmitic acid and stearic acid. The average MUFA content ranges from 22.00 % for Isoptera species to 48.60 % for species of Hymenoptera order while the major representatives include palmitoleic acid and oleic acid. The mean fraction of PUFA amounts to between 15.95 % for Diptera species and 39.76 % for Lepidoptera order species. Especially species from the orders Lepidoptera and Orthoptera seem found to be comparably high in PUFA and polyunsaturated fatty acids like linoleic acid, α-linolenic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid or eicosapentaenoic acid can be found in the fatty acid spectra of edible insects [17]. The comparison of fatty acid profile of different insect orders can be seen in the *Figure* 6 below.

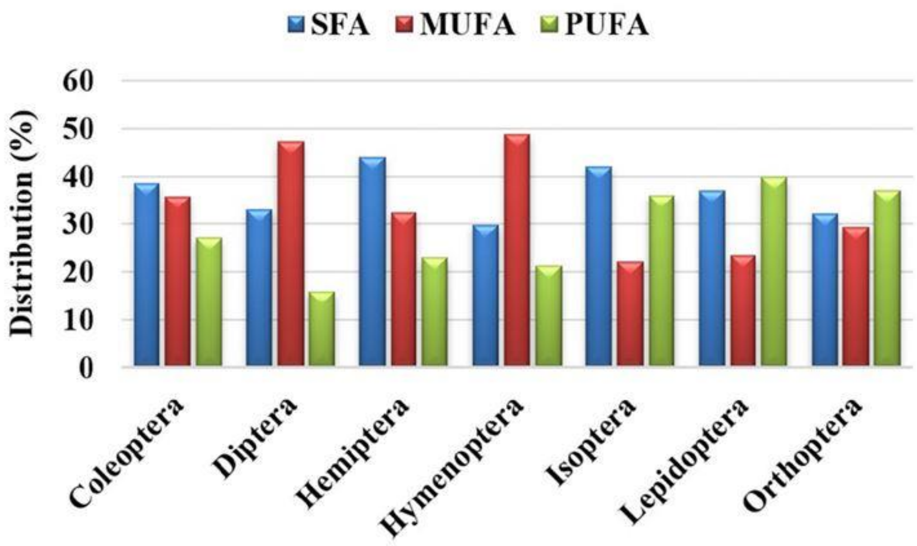

Figure 6 - Fatty acid profile of different insect Orders [17]

In general, the fatty acids of insects are comparable to the fatty acids of fish and poultry in their degree of unsaturation, except containing more PUFA [23]. In comparison, beef and pork contains only very little PUFA and MUFA make up the greatest portion of the present fatty acids. Overall, the unsaturated fatty acids predominate in the fatty acid spectra of edible insects and the cholesterol content is usually low too [17]. The incorporation of edible insects in the diet could therefore posses many positive health effects.

Dietary fibre content

Average fibre contents range from 5.06 % for Isoptera species to 13.56 % for Hemiptera species. Species with a maximum fibre content can reach up to 29 % of dietary fibre content and some species can contain only 0.12 % of dietary fibre based on dry weight [17].

Insoluble chitin is the most common form of fibre in edible insects, which is contained mainly in their exoskeleton [3]. In a commercially farmed insects the chitin content ranges from 11.6 to 137.2 mg per kg of dry matter [24]. Chitin is considered an indigestible fibre although the chitinase enzyme was found in human gastrointestinal tract and depending on its activity, chitin could be potentially digested by humans [16, 25].

It has been shown, that chitin functions as a coadjuvant, a substance that helps to induce adaptive immunity, although the mechanism of function as an adjuvant is still not well understood [26]. Although chitin is not commonly deemed a potential allergen, it can cause sensitization through frequent exposure [27].

Carbohydrate content

Carbohydrates other than fibre are commonly expressed as NFE. The average value in insects varies from 4.63 % for Odonata order species to 22.84 % for specied of the order Isoptera. The maximum amounts (85.30 %) were recorded in some Orthoptera species, while species like *Tenebrio molitor* from Coleoptera order can have carbohydrate content as low as 0.01 % [17].

Ash

Ash content is a good rough indicator for the nutritional value of food in terms of mineral content [28]. The average ash content of various insect orders ranges from 2.94 % for Blattodea order to 10.31 % for the order Diptera. Maximum amounts as high as 25.95 % can be found in some of the Lepidoptera species while minimum amounts as low as 0.34 % can be found in some Orthoptera species [17].

Vitamins and minerals

Vitamin and mineral content of insects is subject to an enormous variability. However, most edible insects can be interesting in terms of nutritional content of minerals like iron, zinc, potassium, calcium, phosphorus, magnesium, manganese or copper, and are generally low in sodium. For a 100 g of edible insects, in general, it can be concluded that even though the amounts of calcium and potassium are not sufficient, the amounts of copper, iron, magnesium, manganese, phosphorus, selenium and zinc are are significant and edible insects have a big potential to provide these micronutrients. Edible insects usually also contain much more iron and calcium than beef, pork and chicken [3, 17].

Regarding vitamins, 100 g of insects based on dry matter, is generally rich in riboflavin, pantothenic acid and biotin. Insects from Orthoptera and Coleoptera orders are also rich in folic acid. On the other hand, contents of vitamin A, vitamin C, niacin and in most cases thiamin, are pretty low in 100 g of dry insects and with a few exceptions, most edible insects would also be a poor source of vitamin E. All in all, insects can be a great source of vitamins but it is needed to specifically select species for the provision of desired vitamins [17].

The content of vitamins and minerals in widl edible insects is seasonal and in the farm bred species, it can be controlled via feed, which should be taken into account when rearing insects for food purposes [16, 17].

2.1.3.2 Environmental and socio-economic aspects

The population growth is also increasing the demand for protein sources while the amount of farmland is limited. In the 2050, world population is expected to reach almost 10 billion an it would not be possible to feed such amount of people with conventional protein sources due to their inefficiency [1]. Therefore, alternative protein sources, like edible insects, will be of great importance.

In comparison wih livestock, breeding insects seems to be more environmentally friendly as it emits less greenhouse gas but also NH_3 emissions, requires less water and uses less land [3, 29]. Also, thanks to the fact that insects are cold-blooded animals, their feed conversion effiency is much higher than in conventional livestock. For example, the feed conversion efficiency of house cricket (*Acheta domesticus*) is twice that of chicken, 4 times higher than in pigs and almost 12 times higher than in cattle (see Figure 7) [3, 30]. Findings were similar for mealworms (*Tenebrio molitor*) and superworms (*Zophobas morio*). The ecological costs related to raising larvae were essentially lesser than expenses related to the production of meat and milk. Also the overall land area for larvae production was lesser than land that is needed for the production of milk, chicken, pork and beef. Together with better feed efficiency index, high reproduction rates and very low reproductive ages, the farms for raising insect larvae can produce larger quantities of valuable food protein than traditional meat and dairy farms. Also, practices like genetic engineering and operational optimization, which is already reaching its limits in the meat and dairy industries, can render insects farming even more profitable [31].

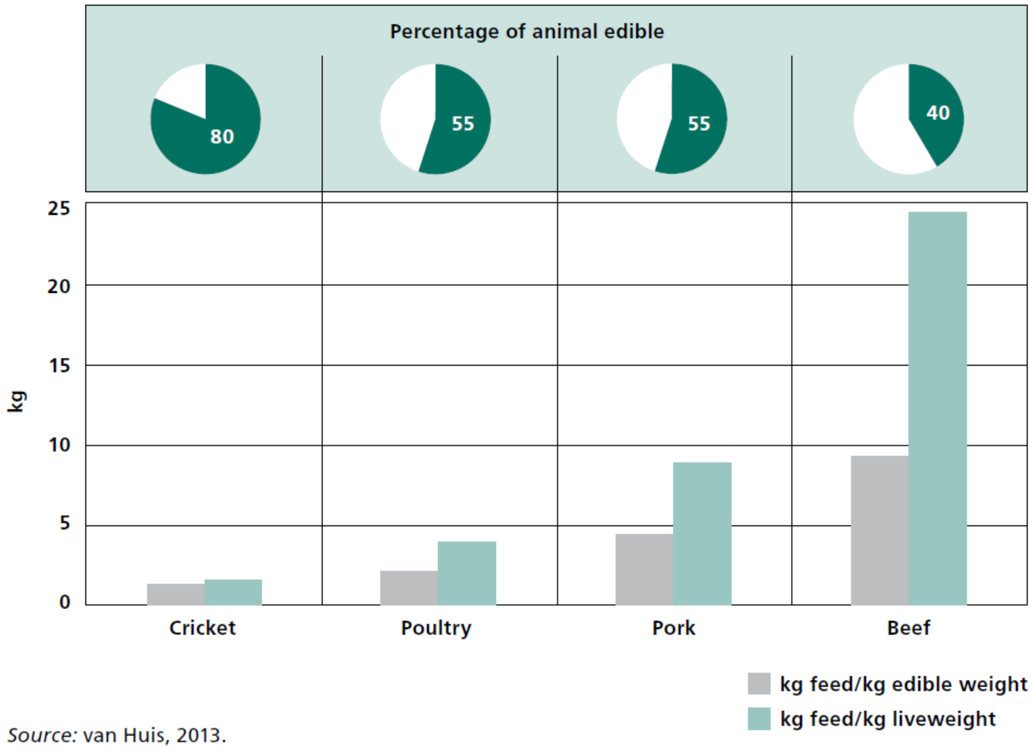

Figure 7 - Efficiency of production of conventional meat and crickets [3]

Compared to other food sources, other benefits of insect farming include the shorter life cycle of insects, their easier transportation, the simplicity of insect enterprise management, lower demands on staff training in insect farms and possibly fast and high investment returns. Also, products not consumed directly by humans can be used as livestock feed [32]. In addition, the waste product from insect rearing called frass, can be used as fertilizer or as feed for pond fish. Edible insects can also be fed on organic waste substrates and can help reduce environmental contamination, while adding value to waste through bioconversion. Edible insects farming could therefore represent a great sector for circular economy (see *Figure* 8) [3]. However, it is necessary to take into account the lower quality of the final product and be aware of potential health risks [33]. With good management, it can be a good solution for bioconversion of waste products and subsequent use, for example, for feed production or for insect protein synthesis [34].

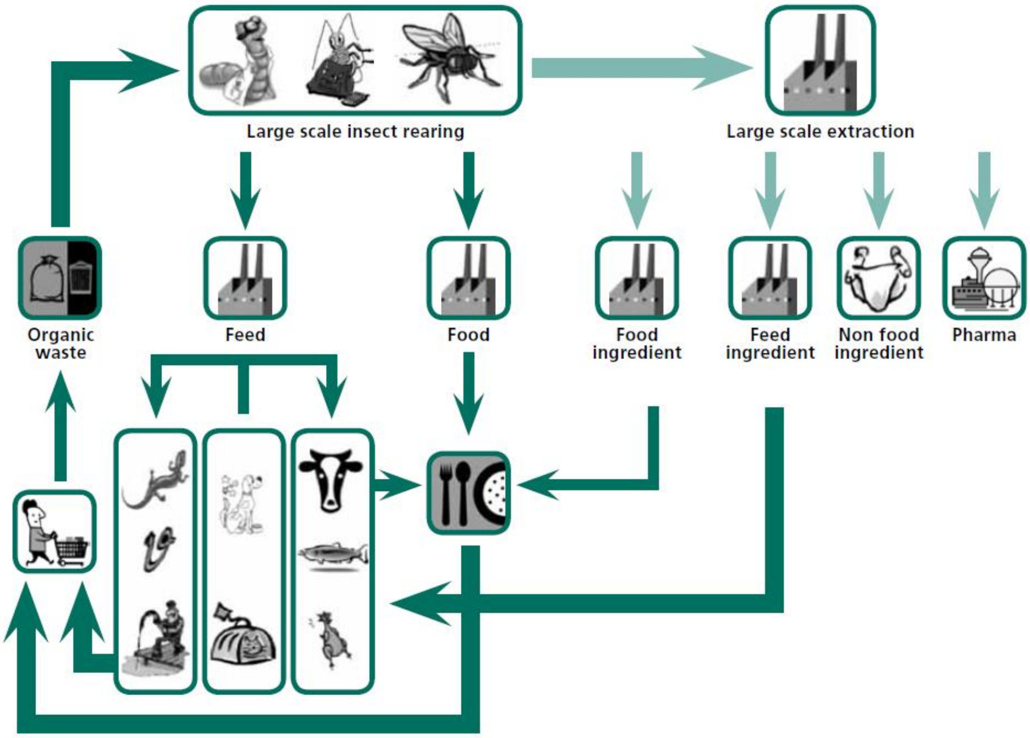

Figure 8 - Insects in the circular economy [3]

Another interesting environmental benefit of entomophagy might be reducing pesticide use. Collection of edible insects, which are considered pests in agriculture, can reduce the use of insecticides as has been proven in Mexico [35]. It is a paradox, that ceral crops, containing no more than 14 % of protein are being protected from „pests“ that can contain up to 77 % of higher-quality animal protein [36].

Also, the socio-economic beneftits of collecting insects itself should be taken into account in comparison to plant cultivation. When it comes to a global view of insect collection, breeding and consumption, this area also brings benefits in improving livelihoods, especially in developing but also in developed countries. In developing countries, it can improve the nutrition of the local population, it can also bring them a source of income or increase employment in the region without requiring costly initial investment [3].

2.1.4 Acceptance of entomophagy

In Western societies, there are large negative perceptions surrounding insects, usually entrenched in people since early childhood. Although people in western cultures shun eating insects, a significant number of people worldwide eat insects unwittingly in contamined food, like fruits, leaves or seeds, where remnant insects can be found [32]. There is a big misconception among the public that insects as food have their justification only in times of famine and are the food of the poor even though edible insects are consumed by a large part of the population of their own free will, mainly due to their palatability and commonplace in the culture. In the Western world, insects are often considered a nuisance and are associated with various diseases and inconveniences. Unfortunately, only a few people realize that most insects are beneficial and that very few are damaging. This irrational negative Western attitude also threatens the practice of entomophagy in Africa and had a great impact on the neglecting of edible insects in research in the previous decades [3]. Another factor that prevents people from eating insects is food neophobia, which is strongly associated with fear of the unknown for sanitary, cultural, or even religious reasons. Neophobia is also closely related to the emotion of disgust [10].

However, this negative attitude is only a prejudice, as many formerly unusual foodstuffs became traditional over the years. For example frogs, originating from France, or lobsters, which were a long time ago served to servants and prisoners as a punishment [37, 38]. Today, both of these foods are considered a delicacy and edible insects have similar potential. The attitude to entomophagy is determined by psychological, social, religious, anthropological and other factors. Food preferences are formed mainly in childhood and therefore the changes in perceptions can not be fast [30]. Fortunately, western attitudes are slowly but surely changing and also edible insect research has been progressing very rapidly recently.

The consumer acceptance of insects in the modern world can be increased in various ways. Educating and promoting the benefits of insect consumption, appropriate processing of insects to improve their sensory properties and economic efficiency and information transparency to customers are strategies that should help successful globalization of entomophagy [30]. Also tourism, recommendations from close relatives or friends and increasing media attention may encourage insect consumption, mostly by early-adopters [39].

Recent studies have shown that there is a greater willingness to eat insects if they are in an unrecognizable form. Processing methods are therefore of great improtance in promoting consumptions of insects. For example, dry insects can be crushed or pulverized and raw or boiled insects can be groung or mashed, and added in this form into various familiar foods [39, 40]. Also, the isolation of various components, like proteins or fats, is another option how to incorporate insects into diet [10]. These processing options are described in more detail in subchapter 2.4.

2.1.5 Risks of entomophagy

There are also some possible downsides and risks in the practice of eating insects. Just as it is the case with some plant or animal food products, also some insect species migh not be edible, or may even be dangerous for consumption. Liabilities of entomophagy include the possible content of allergenic and toxic substances, the presence of antinutrients or pathogens [17].

Regarding the allergenicity, it has been discovered that insects can similarly to other arthropods cause allergic reactions in certain people, caused predominantly by pan-allergenic proteins [41]. These allergens might be injectant (e.g. in bees, wasps or ants), contactant, inhalant (exuviae, excreta) or ingestant. The predominant allergens are mainly inhalant and contactant and therefore caution is advised for workers in the insect reating industry but also for people first time trying insect consumption [17]. It is advised that people allergic to to crustaceans, moluscs and dust mites should be aware of the potential allergenicity to edible insects too [3, 18]. Chitin is another component which could be problematic when consuming insects in some people as this polysaccharide can be difficult to digest. As mentioned in subchapter 2.1.3.1, chitin can potentially be digested as there was found chitinase in human GIT, however due to the lack of food containing chitin, there is a deficiency of this enzyme and in these people it could act as an allergen [42, 43]. For the great majority of people, however, eating and exposure to insects do not pose a significant risk of causing allergic reactions [3].

The microbial contamination and the native intestinal microbiota of insects might also be a threat in entomophagy. For example Vandeweyer (2017) found a microbial contamination in fresh mealworms and house crickets, consisting mainly of *Enterobaceriaceae*, lactic acid bacteria, yeast, moulds and aerobic bacterial endospores [44]. The lack of hygiene and inadequate processing and storage conditions are viewed as a more significant concern than the quality of the insects themselves [10]. Pathogenic bacteria may be present in non-processed insects depending on the rearing conditions and the feed used [42]. Therefore, it is crucial to implement appropriate post-harvesting technologies and decontamination methods for the conservation, transformation, distribution, and storage of edible insects, in order to provide products that are safe, wholesome and marketable [10, 17]. If currently allowed feed materials are used also to feed insects, the possible occurence of microbiological hazard in non-processed insects is expected to be comparable to other non-processed sources of protein of animal origin [42].

The risk of zoonotic infections is expected to be low as insects are taxonomically much more distant from humans than conventional livestock. However, this risk could increase with the use of waste products in production, the unhygienic handling of insects and with direct contact of farmed insects with wild insects outside the farm. More research in this area is therefore needed [3].

Some insect species might also contain toxins, either from feed or synthesized as a chemical defense mechanism against insectivores [17]. It is therefore important to have an adequate knowledge of insect species and also to know the correct preparation procedures for potentially dangerous species to eliminate the toxins [3]. In some species, the preparation procedures also include removing certain body parts. For example, consumption of grasshoppers or locusts without removing their feet can lead to intestinal blockage, which could have fatal consequences [16].

The production method through which the edible insects are produced, whether they are wild harvested or reared, is one of the main factors affecting safety of edible insects. Controlling the environment in which the edible insects are reared increases the edible insects safety. It is also easier to control food safety hazards in a controlled insect production e.g. via practising good hygiene practices, or by implementing Hazard Analysis and Critical Control Points systematic approach [45]. For example, insects harvested in the wild might contain pesticides if they have fed in pesticide-treated areas. That is why consumption of such insects cannot be recommended. In controlled insect rearing, the risk of pesticide contamination is very low [17]. Another possible contamination of wild harvested insects could be soil containing faecal matter from animals or heavy metals from the environment. Also, the consumption of contamined food by insects in wild can also increase the amount of microflora in the gut of insects [46].

Also processing methods like boiling, frying or roasting are also important as they greatly increase the safety of edible insects. The can lower the microbial load and the chemical hazards present in insects [47]. As cultural preferences and organoleptic aspects also play important roles in chosen processing or preservation method, specific measures may be required for differenet insect species to ensure safe and high quality food product, depending on the biological makeup of given insect [3].

Another important aspect of ensuring edible insects safety is legislation. In Europe, there already is a food safety regulation on edible insects. Other parts of the world should therefore follow this example and put in place appropriate food safety legislation including edible insects [42, 47].

2.2 Legislation of edible insects in the European Union

Insects as a food are still only an emerging value chain and the legislation and regulation of this entire sector is one of the main challenges hindering the development of this sector [3]. Insect producers must follow the same general rules that apply to any other food and feed business operators. These include the General Food Law legislation (Regulation (EC) No 178/2002), and legislation regarding the hygiene (Regulation No 852/2004 on the hygiene of foodstuffs and Regulation No 183/2005 laying down requirements for feed hygiene). In individual member states, in addition to complying with European legislation, it is also necessary to meet the conditions and comply with national legislation.

On the European market, in addition to these general requirements, the production and marketing of insects as food in Europe is governed by the Novel Foods legislation. Novel food is a type of food that had not been consumed to a significant degree by humans in the EU before 15 May 1997. This new Regulation (EU) 2015/2283 on novel foods entered into force on 1st January 2018 and from this time all insects and insect-derived products are subject to the novel foods approval procedure and must thus be authorised under the EU novel food system to be lawfully marketed within the EU. This evaluation serves to assess the potential safety risks implied by the consumption of the product, and thereby to substantiate the final decision to authorise or not its commercialisation at EU level. Only after their approval at EU level and inclusion in the Union list of novel foods, which will also specify the conditions under which the novel food may be used (i.e. food categories, maximum levels and, where appropriate, specific labeling requirements for such foods) the use of individual insect species as part of food or meals served in mass caterers will be allowed [6].

In some countries, like Czech republic or Belgium, it is possible to place selected insect species on the market as a food even before standard approval under Regulation (EU) 2015/2283. The list of such species can be found in Table 1 below. This regulation contained transitional measure (article 35.2) which allows European insect producers who have lawfully commercialised their products at a national level to continue to do so until EU novel food authorisations are being granted. The transitional measure ceases to be applicable once a decision on the application covering this food product has been taken by the European Comission.

However, this transitional measure must be implemented at a national level of each state, and in countries where this has not been the case, insects may only be placed on the market after successful novel food approval. Such countries include, for example, Slovakia, Spain or France [42, 48].

Table 1 - Insect species legalized on the EU market [48]

Latin name	Common name
Acheta domesticus	House cricket
Alphitobius diaperinus	Lesser mealworm
Gryllodes sigillatus	Tropical house cricket
Locusta migratoria	Migratory locust
Schistocerca gregaria	Desert locust
Tenebrio molitor	Yellow mealworm

Currently, following the first EFSA opinion covering an insect species from January 2021, dried yellow mealoworm (*Tenebrio molitor*) has been authorised on EU level as the first insect food product and the Novel Food Regulation entered into force in June 2021 [49]. At the beginning of July 2021, also dried and frozen migratory locust (*Locusta migratoria*) has received a positive opinion from EFSA and has been considered safe for human consumption [50]. Also, the risk profile for the house cricket (*Acheta domesticus*) has already been published earlier [51]. The other species, currently being evaluated by EFSA include lesser mealworm (*Alphitobius diaperinus*) or tropical house cricket (*Gryllodes sigillatus*) [52].

2.3 Characteristics of selected edible insect species

2.3.1 Insects in general

The word „insect“ comes from Latin „insectum“ which means „with a notched or derived body“ or „cut into sections“. This stems from a fact that insect bodies are made of three parts – head, thorax and abdomen [3]. Insects represent a class of animals within the phylum Arthropoda with chitinous exoskeleton, body derived into three parts, three pairs of jointed legs, two antennae and compound eyes [3, 53].

Insects are also amongst the most diverse groups of animals on Earth. There are more than 1 million species described which is more than half of all known living organisms. It is estimated that the total number of species is much higher than the amount described and that there should be less than 20 million of insect species. This class potentially represents more than 90 percent of the differing animals on the planet [3, 53]. Insects can be found in almost any environment and can be characterized by features like exoskeleton, quick reproduction and forming large populations. Insects are cold-blooded animals which undergo metamorphosis to be able to adapt to seasonal variations. Insects breath through tracheal tubes and often do not need parental care [3].

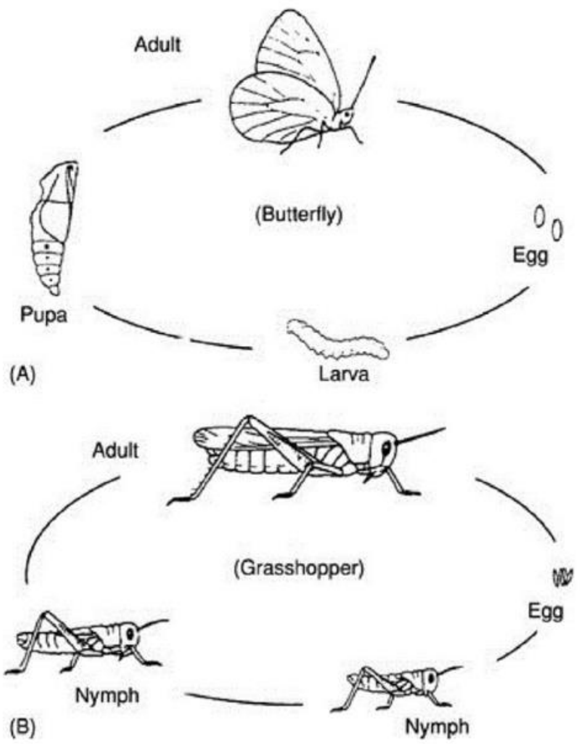

Figure 9 **-** *Life cycles of insects with complete (A) and incomplete (B) metamorphosis [53]*

Insects can be classified in the broader sense based on whether they have wings (pterygotan) or are wingless (apterygotan), and pterygotes can be further divided into two main groups – those that can fold their wings over the body (Neoptera) and those that cannot (Paleoptera). The neopterans can be further divided into two natural groups - on insects which undergo complete metamorphosis (endopterygotes) and those that undergo incomplete metamorphosis (exopterygotes) [53]. In insects, with incomplete metamorphosis, development takes place through nymphs. Nymphs look very similar in morphology to adults. They are characterized by their dysfunctional reproductive system and the absence of wings. The eggs develop into nymphs, which shred their exoskeleton several times during their growth [54]. The number of moultings and each new stage with a changed cuticle is called an instar. The process ends with the last moulting and the imago or adult develops. Endopterygotes, or holometabolous insects, are very different in the larval and adult stages. The larvae hatch from the eggs and then grow and eat until they pupate. The stage of the pupa, in which the development and organization of organs takes place, leads to the development of the adult individual. The larvae are fed a different source of food than adults [55]. Life cycles of both insects groups can be seen in the *Figure* 9 above.

2.3.2 House cricket (*Acheta domesticus*)

The house cricket belongs under the genus Acheta of the family Gryllidae and of the order Orthoptera. This species is hemimetabolous which means it undergoes an incomplete metamorphosis [56]. This species is probably native to southwestern Asia, from where it was widely distributed worldwide by man. House cricket is typically of yellowish-brown color and grows up to 16–21 mm in length (see Figure 10). Males and females of this species look very similar while females can be recognized by an approximately 12 mm long ovipositor, emerging from the rear. All adult house crickets also have hind wings, althought they sometimes shed them (in adulthood). In temperatures between 26–32 °C it takes about two to three months to complete their life cycle. Juvenile crickets resemble the adults except for being smaller and wingless. House crickets are also known for their calling song – a series of short chirps - which is made by males rubbing a scraper on the inner edge of the left wing against the teeth of a file that is beneath the right wing [57].

Figure 10 - House cricket [56]

The nutritional composition of a house cricket is one of the main advantages of this edible insect species. The protein content of adult house cricket ranges between 55.00–70.75 %, based on dry matter, which is very high compared to other edible insects [17]. The amino acid profile of house crickets and other species from the Order Orthoptera also meet the requirements of the WHO for amino acids for adults and therefore represent a valuable alternative protein source [17, 19]. The lipid content is in the range from 9.80 to 24.00 % while the major fatty acids are linoleic (41.39 %), oleic (20.18 %), palmitic (22.65 %) and stearic acid (8.54 %). Acheta domesticus lipids also have a P/S ratio (the polyunsaturated to saturated fatty acid ratio) close to one, which indicates their strong nutritional potential [17, 58]. House crickets are also a great source of fibre which is in the range of 6.20–22.08 %. The nitrogen free extract (NFE), representing carbohydrates other than fiber, of house crickets is in the range from 2.12 to 3.93 %. The total ash content is between 3.55 and 9.10 % and the total energy content is between 414.41 and 455.19 kcal kcal per 100g of dry matter [17].

Table 2 - Mineral content of house cricket compared to daily reference intake for adults [17, 59, 60]

Mineral	Amount [mg / 100 g]	Daily reference intakes for adults [mg / day]
Calcium	120.09-1290.00	800
Potassium	1126.62-1537.12	2000
Magnesium	80.00-160.00	375
Phosphorus	780.00-1100.44	700
Sodium	435.06-589.52	2000
Iron	6.27-19.68	14
Zinc	15.91-29.69	10
Manganese	2.97-5.28	2
Copper	0.85-2.23	1
Selenium	0.04-0.06	0.055

Table 3 - Vitamin content of house cricket compared to daily reference intake for adults [17, 59]

Vitamin	Amount per 100 g	Daily reference intakes for adults
Vitamin A	0.01-24.3 μg	800 μg
Vitamin E	2.81-5.43 mg	12 mg
Vitamin C	7.86-25.50 mg	80 mg
Vitamin B1	0.09-0.13 mg	1.1 mg
Vitamin B2	4.15-11.07 mg	1.4 mg
Vitamin B3	1.43-12.59 mg	16 mg
Vitamin B5	7.47-11.48 mg	6 mg
Vitamin B7	21.83-55.19 μg	50 μg
Vitamin B9	490-630 μg	200 μg

As can be seen from *Table* 2 and *Table* 3, crickets are also a valuable source of vitamins and minerals. Based on the daily reference intakes for adults, 100 g of crickets based on dry matter contains significant amounts of calcium, potassium, phosphorus, iron, zinc, manganese and copper. In terms of vitamins, 100 g of dry crickets contain significant amount of vitamin B2, B3, B5 and B7 [17, 59].

2.3.3 Yellow mealworm (*Tenebrio molitor*)

The term mealworm or yellow mealworm represents the larval form of the mealoworm beetle, a species of darkling beetle (family Tenebrionoidae) which belongs under the order Coleoptera. This species of insect has a complete metamorphosis which means it goes through all four life stages [61]. Development cycle from egg to the larvae takes about 10-12 days. After hatching, larvae reaches maturity typically after 3 – 4 months, but sometimes the larvae stage can take up to 18 months. The mature larva is about 2–3.2 cm long and weighs about 130–160 mg (see Figure 11). After final moulting it turns into pupal stage which lasts 7-9 days at room temperature. Final stage, an adult beetle, then lives for 2 to 3 months [62, 63]. The whole life cycle of *Tenebrio molitor* is shown in *Figure* 12.

Figure 11 - Yellow mealworm [61]

Mealworms are indigenous to Europe but nowadays can be found all over the world mainly due to globalization [61, 63]. Mealworms are often considered pests of the grain, flour and food stores and due to their easy breeding and valuable protein profile are also produced industrially as a feed for pets and other animals including birds, reptiles, small mammals and fish [62, 63]. In the recent years, also the production for food sector is growing with this insect species.

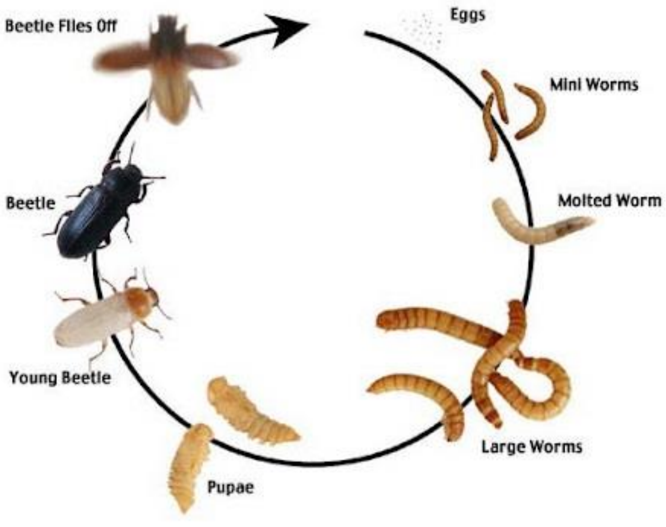

Figure 12 - Life cycle of yellow mealworm [64]

Based on dry matter, melaworms contain 47.18–49.43 % of protein, 35.17–43.08 % of fat, 5.00–14.96 % of fiber and the energy content of 539.63–577.44 kcal per 100 g. The ash creates 2.36–3.08 % of dry matter and the nitrogen free extract (NFE) is in the range of 0.26–7.10 % [17]. Also, the amino acid profile of mealworms meets the amino acid requirements for adults published by WHO, except some of the sulphur-containg amino acids which are considered limiting [17, 19, 65]. Mealworms therefore present a valuable energy-dense nutriton source with high amounts of quality protein and high fat content.

The fatty acid profile of mealworms is characterized by the major fatty acid – oleic acid (35.83 %). The other significantly represented fatty acids include linoleic (22.83 %), palmitic (21.33 %), stearic (7.92 %) and myristic acid (4.45 %) [58]. Compared to conventional foods, mealworms contain a similar composition of omega-3 and omega-6-unsaturated fatty acids as fish, and the protein content of vitamins and minerals is comparable to that of fish or meat [3].

Table 4 - Mineral content of mealworm compared to daily reference intake for adults [17, 59, 60]

Mineral	**Amount [mg / 100 g]**	**Daily reference intakes for adults [mg / day]**
Calcium	44.36-47.18	800
Potassium	761.54-895.01	2000
Magnesium	210.24-221.54	375
Phosphorus	697.44-748.03	700
Sodium	125.38-140.94	2000
Iron	5.41-5.51	14
Zinc	11.41-13.65	10
Manganese	0.92-1.36	2
Copper	1.60-1.64	1
Selenium	0.03-0.07	0.055

Table 5 - Vitamin content of mealworm compared to daily reference intake for adults [17, 59]

Vitamin	**Amount per 100 g**	**Daily reference intakes for adults**
Vitamin C	3.15-36.10 mg	80 mg
Vitamin B1	0.31-0.63 mg	1.1 mg
Vitamin B2	0.41-2.13 mg	1.4 mg
Vitamin B3	10.59-10.68 mg	16 mg
Vitamin B5	3.72-6.88 mg	6 mg
Vitamin B7	78.74-94.87 µg	50 µg
Vitamin B9	300-410 µg	200 µg

Mealworms also represent a good source of vitamins and minerals. The content of selected vitamins and minerals in a *Tenebrio molitor* larvae is shown in *Table* 4 and *Table* 5. It is clear, that 100 g of dried mealworms could provide significant amounts of potassium, magnsium, phosphorus, zinc, manganese, copper, vitamin B2, B5, B7 and B9 in the context of daily reference intakes for adults [17, 59].

2.3.4 Lesser mealworm (*Alphitobius diaperinus*)

Lesser mealworm or buffalo worm, are terms for the larval form of the darkling beetle *Alphitobius diaperinus*, sometimes also called litter beetle, from the family Tenebrionidae under order Coleoptera. This species is very similar to yellow mealworm (Tenebrio molitor) except being smaller (see Figure 13). For comparison, an adult beetle of mealworm reaches a size of 12 mm while adult beetle of lesser mealworm only 6 mm. This species has origins in Africa and Mediterranean regions but nowadays can be found nearly worldwide, mainly in places where poultry is mass-produced. It is considered a significant pest in the poultry industry as it consumes their feed, damage poultry barns and may carry pathogens. It is also known widely as a pest of stored food grain products like flour. On the other side, this insect species is also suitable for mass rearing and its larvae can be used as feed for livestock, but also in food sector [66, 67].

Figure 13 - Lesser mealworm [66]

Adult beetle is of shiny black color, or brown with reddish brown elytra. The larva resembles yellow mealworm, but is smaller, measuring only 11 mm in length in its final larval stage. It is whitish when newly emerged from the egg and it darkens to a yellow-brown. The larval stage has 6 to 11 instars [64]. Life cycle of the lesser mealworm is also similar to that of yellow mealworm, as both are holometabolous insects. The only exception is that it is a little bit shorter for lesser mealworm [67].

From the nutritional point of view, lesser mealworms contain about 59.30 % of protein, 26.66 % of fat, 7.58 % of fibre, 5.46 % of ash and 2.56 % of NFE. The total energy content of 100 gry lesser mealworms is approximately 502.46 kcal [68]. Regarding the amino acid composition, lesser mealworms contain all essential amino acids in sufficient quantities for humans, except the sulphur-containing ones, similarly to yellow mealworms [69]. The fatty acid profile is characteristic by high amounts of oleic acid (38.49 %), linoleic acid (23.28 %) and palmitic acid (25.18 %), and also smaller amounts of stearic acid (8.55 %) [70]. No studies, regarding the mineral and vitamin content of lesser mealworm were found in the scientific literature, however, it is expected that vitamin and mineral content will be similar as in other Tenebrionidae species.

2.4 Production and processing of edible insects for food purposes

Insects can be either wild harvested or reared at a specialized insect farm. Currently, most edible insects come from wild harvesting, although semi-domestication and indoor farming are becoming more popular while increasing the insect availablity and the sustainability of production [10]. Whether the insects are wild harvested or come from controlled production, might have a big impact on safety of such insects but also on their nutritional value. The safety concerns regarding the safety of wild harvested inects were further described in subchapter 2.1.5. However, the nutritional value is affected too as many conditions might have a strong influence on various nutritional parameters. In a study on mealworms from 2020, researchers have found significant differences in the crude protein content, amino acid composition, fat content or PUFA content, which were dependent on the rearing temperature and type of feed in a controlled production [71]. Controlled insect breeding is a relatively new issue in the food and feed sector, which has its own specifics and rules that must be respected for the safe production. Defined procedures, important points as well as recommendations in insect breeding are described in the Guide on Good Hygiene Practices, published by the non-profit organization IPIFF. An example of an insect production scheme is shown in Figure 14 below from the IPIFF guide [72]. Also FAO has published an extensive manual specifically focused on cricket farming in 2020 [73].

Insects can be then processed and consumed in several ways. After wild-harvesting or rearing in an insect farm, insects should be fasting for some time and after that are usually killed by freeze-drying, scalding or boiling. Some kind of heat treatment should always be present in order to prevent microbiological dangers [3, 35, 37]. They can then be processed and consumed as whole insects, in ground or paste form, or as an extract of protein, fat, chitin or other valuable component for fortifying food and feed products. Sometimes, insects are also fried live and consumed [3].

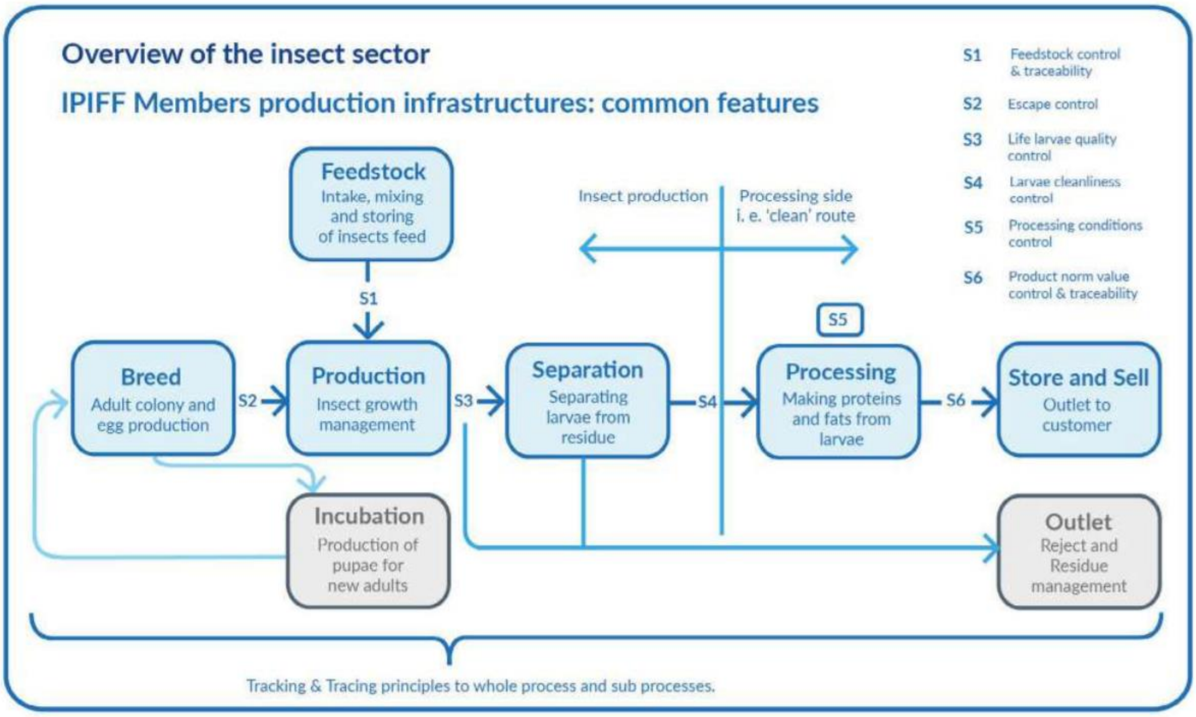

Figure 14 - General scheme of insect production [72]

The microbial contamination and the native intestinal microbiota of insects might also be a threat in entomophagy. For example Vandeweyer found a microbial contamination in fresh mealworms and house crickets, consisting mainly of *Enterobaceriaceae*, lactic acid bacteria, yeast, moulds and aerobic bacterial endospores [44]. The lack of hygiene and inadequate processing and storage conditions are viewed as a more significant concern than the quality of the insects themselves [10]. Pathogenic bacteria may be present in non-processed insects depending on the rearing conditions and the feed used [42]. Therefore, it is crucial to implement appropriate post-harvesting technologies and decontamination methods for the conservation, transformation, distribution, and storage of edible insects, in order to provide products that are safe, wholesome and marketable [10, 17].

2.4.1 Whole insects

Whole insects are most commonly consumed in tropical countries. Sometimes, additional step, like removal of certain body parts, is needed too. Fresh insects are then further processed accoridng to given recipe by a number of ways, for example by boiling, steaming, smoking, stewing, frying or roasting. This improves their sensory and nutritional qualities, but also their shelf-life. These techniques are preceded by blanching to reduce foodborne microorganism counts and to inactivate enzymes. From a nutritional perspective, cooking can ehnance the digestibility and bioactivity of protein in the digestive tract, although some nutrients could be lost through leakage, solubilization, biochemical reactions or by forming new byproducts [3, 10].

2.4.2 Ground or paste form

Ground or paste form might represent a more visually attractive and acceptable form of insects for people that are not used to insect consumption. Grounding or milling is a common food processing method that is applied in many conventional foods. Edible insects prepared this way can then be added to various foods to enhance their nutritional value. Standard procedure includes drying followed by grinding the insects into insect powders, also called insect flours [3]. Before the grinding step, insects are usually dried using various technologies like sun-drying, freeze-drying, oven-drying, fluidized-bed drying or microwave-drying [10].

Finished insect powders can then be incorporated into common food products, like bread, pastries, flour, pasta, biscuits, meat substitutes, salty snacks, candy or condiments [3, 10]. Also, high protein products are becoming more and more popular nowadays so products like protein bars or protein powders seem also like a good option for the addition of edible insects. Insects can also be processed into medicinal supplements to fortify the human immune system [3, 10].

2.4.3 Insect extracts

Modern methods of processing insects for food, feed, pharmaceutical and industrial purposes include extractions of various functional ingredients such as proteins, fats, chitin, bioactive peptides, vitamins, or minerals [10]. This could also be a useful way how to increase acceptability of entomophagy among more consumers. These extracts could then be used into various other foods for increasing their nutritional value, although this still needs futher research regarding the properties of the extracted components but also regarding the extraction effeciency in order to be competitive in the food and feed industries [3, 18]. It is very similar to other food commodities. Examples are the isolation of omega-3 fatty acids from fish or high-quality protein concentrates from dairy production.

The production, processing and storage of edible insects are achieved using a variety of conventional but also innovative approaches. The technologies used include drying, mentioned in the previous subchapter, but also new processing methods like ultrasound-assisted extraction, cold atmospheric pressure plasma, or dry fractionation which are designed mainly for protein, fat or chitin extraction [10]. As the two main constituents of insects are proteins and fats, a more detailed description of the insect protein and lipid extracts is described in the subchapters below.

2.4.3.1 Protein extracts

Regarding the extracts, studies are more directed to the protein fraction contained in insects and there are only a few studies dealing with the lipid and saccharide fraction [18]. As the protein concentration in the various species of insects is generally very high, the process of protein concentration from them is facilitated. Extraction and protein fractionation are necessary steps to produce ingredients derived from insect proteins. It was also confirmed that insect proteins have a good digestibility. The data shows that digestion also depends on the fraction of proteins considered [74].

The most frequently used method to obtain the protein fractions consists of alkaline extraction followed by acid precipitation of the protein at their isoelectric point [75]. However due to lack of standardized fractionation processing, there are not much studies on the technological functionality of insect proteins. This area therefore needs additional research and parameters like water and lipid retention capacity, thickening capacity, emulsifying capacity, foaming ability, gelling ability and structuring ability should be evaluated [18]. Enzymatic modification of proteins also represent a useful mechanism for improving their functionality in comparison to the native unhydrolyzed proteins. Insect protein hydrolysates could therefore represent another viable option how to take full advantage of this valuable raw material [76].

Protein hydrolysates are proteins digested into smaller fragments, peptides and amino acids using various proteolytic enzymes. These peptides contain about 2-20 amino acids and are inactive within the sequence of the original protein. After their release from the original protein, insect peptides could posses beneficial effects on human health or in food systems like antioxidant, antidiabetic, antihypertensive or antimicrobial properties and could also be used as functional food ingredients. The bioactivity of these peptides is influenced by various parameters, for instance source of protein, peptide structure, degree of hydrolysis, amino acid composition and type of protease used. However, the obtainment of bioactive peptides from insects also needs further research [18, 77].

2.4.3.2 Lipid extracts

During the isolation of proteins from insects, lipids represent a byproduct of this process which could be used for other applications. There was already investigated the extraction of lipids from insects for application as edible oils and the suitable solvents include carbon disulfide, petroleum naphtha, benzene, alcohol, pentane, trichloroethylene, supercritical carbon dioxide and commercial hexane. The type of extraction process does not influence the fatty acid composition much but have a great impact on the lipid extraction yield and the types of lipids extracted. The extraction process should be carefully selected according to the desired application of the insect lipids and to the costs of extraction process [78, 79].

Defatting an insect powder itself might also be advantageous for some uses as it increases the total protein content and lowers the overall energy value [80]. The removal of fat also reduces the stickiness of the protein concentrates and prevents the unwanted fatty acids oxidation processes [3, 18].

Insect lipids could be divided into two groups depending on their physical state. The liquid insect oils and solid insect fats. The insect oils are rich in essential PUFA and their physical properties make them ideal for use in mayonnaise, frying oils or in a food grade lubricants [81]. Such insect lipids can to the content of essential fatty acids represent a sustainable and health promoting alternative to the lipids traditionally used [82]. For example, mealworm oil shows a very similar physiochemical properties to vegetable oil and is also abundant in bioactive nutrients, especially γ-tocopherol, with only trace levels of cholesterol. It also posses antioxidant and anti-inflammation activities and its predicted shelf life is suitable for commercial use [80].

On the other hand, solid insect fats with high SFA content could be used for example in confectionery, margarines or pasta [81, 83] In a study from 2019, researchers were investigating a partial butter replacement in bakery products with fat from black soldier fly larvae. It was found out that 25% substition of butter with this insect fat did not change the overall food experience and liking. In some products even 50 % substitution did not influence consumer´s acceptance and it was found out that this insect fat has a similar properties to butter in bakery products [84]. Traditionally, the fat of some insect species is also used extensively for frying various food products [3].

2.5 Use of edible insects in sports nutrition

The field of sports nutrition is an ideal segment for edible insects, as they contain high amounts of protein and healthy fats which are key in this food segment. Whether the goal is muscle growth, weight loss, or just effort to live a healthier life, sports nutrition and various food supplements can be of great help in this regard.

Selected species of edible insects, which are legalized on the EU market, are characterized by relatively high protein content [17, 48]. This chapter is therefore specifically devoted to food supplements aimed at increasing intake of quality protein in the diet. High protein content makes insects an ideal raw material for use in high protein products, such as protein powders, protein bars, or powder mixtures of selected essential amino acids and that was also on of the key reasons for writing this thesis and development of own insect-based protein product.

The sports nutrition market is constantly growing and by 2026, compared to 2020, it is expected to almost double on a global scale as can bee seen in the *Figure 15*. Also the protein ingredients world market expects more than 158% growth in the same time period [85].

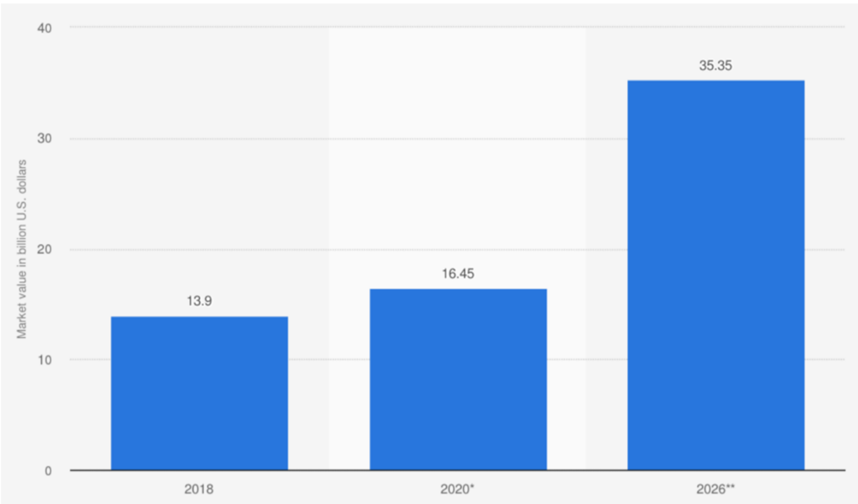

Figure 15 - Forecast of sports nutrition market worldwide from 2018 to 2026 [85]

2.5.1 Protein in nutrition

Proteins are among the essential macronutrients in human nutrition. They form a substantial part of skeletal muscle, part of organs, but also blood. They perform several functions in the body - structural, catalytic, transport, storage, or they serve as receptors. They are composed of individual amino acids, which are needed in the body for the synthesis of a number of its own proteins and other nitrogen molecules, necessary for life. Each amino acid (AA) has in its structure a central carbon atom (C), at least one amino group (-NH 2), at least one carboxy group (-COOH) and a side chain [86]. The general structure of the amino acids is shown in Figure 16 below.

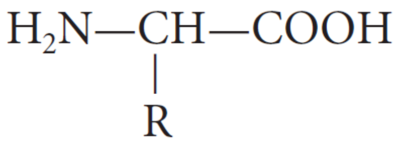

Figure 16 - General structure of amino acids [86]

In terms of nutrition, amino acids are distinguished into essential, semi-essential and non-essential. Amino acids overview is shown in Table 6. Essential amino acids are amino acids that the body cannot make on its own and is dependent on their external intake. The semi-essential AA, in turn, represent a kind of intermediate group. The body can usually create them itself, but in certain cases, e.g. in premature babies, or in some diseases (e.g. phenylketonuria) they become essential [86].

Table 6 - Classification of amino acids [86]

	Name	**3-Letter code**
Essential	Phenylalanine	Phe
	Methionine	Met
	Isoleucine	Ile
	Valine	Val
	Tryptophan	Trp
	Leucine	Leu
	Threonine	Thr
	Histidine	His
	Lysine	Lys
Semi-essential	Tyrosine	Tyr
	Cysteine	Cys
	Proline	Pro
	Arginine	Arg
	Glutamine	Gln
Non-essential	Alanine	Ala
	Asparagine	Asn
	Aspartate	Asp
	Glutamate	Glu
	Serine	Ser
	Glycine	Gly

2.5.2 Protein quality

Protein quality evaluation determines the capacity of food protein sources and diets to satisfy the metabolic demand for amino acids and nitrogen which predicts the overall efficiency of protein utilization. The protein utilization is determined by their digestibility and biological value. The protein digestibility is a measure of the dietary intake of proteins which is made availale to the organism after digestion and absorption and the biological value represents a measure of how well the amino acid profile matches the AA requirements. Net protein utilization (NPU) therefore reflects both digestibility and biological value of protein [19].

There have been developed various methods for assessing the protein quality. One of the approaches is called PDCAAS, which is an abbreviation of protein digestibility corrected amino acid score. This assay is based on measures of digestibility and amino acid composition an was proposed as a means of assessing the protein quality of both dietary mixtures and individual protein sources [19]. As this method still has its flaw, Food and Agriculture Organization has already proposed to replace it with another scale, called DIAAS (Digestible Indipensable Amino Acid Score). The PDCAAS might overestimate the protein´s bioavailability, as it also counts with losses of amino acids and peptides that are used by the microbiome in large intestine. The DIAAS overcomes this issue by analyzing how much protein was absorbed after it has left the small intestine [87].

Regarding the amino acid profile, in order for a protein to be complete, it must contain all the essential amino acids and contain them in sufficient amounts [19]. Typical example of complete proteins represent those from conventional animal sources, like meat, eggs or milk. On the contrary, most plant proteins are incomplete. For example, rice is relatively poor in lysine, soy and pea in methionine [88]. However, most edible insect species provide sufficient amounts of EAA and meet the amino acid requirements for the complete protein protein. Regarding the essential amino acid content, WHO has issued requirements which can be seen, together with an overview of the amino acid content of various protein sources in Table 7 below.

Table 7 - Amino acid requirements of adults and contents of AA in proteins [19, 88, 89]

Essential amino acid	mg / kg / day*	Complete	Milk	Pea	Rice	Soy	Whey
		[mg of AA per 1 g of a given protein]					
Leu	39	59	97	97	88	80	116
Ile	20	30	50	54	40	43	59
Val	26	39	58	81	55	44	58
Cys	4	6	9	14	17	12	20
His	10	15	30	17	24	26	21
Lys	30	45	80	83	33	60	102
Met	10	16	27	5	29	10	23
Met + Cys	15	22	30	8	39	14	36
Phe + Tyr	25	30	99	94	111	90	66
Thr	15	23	47	43	38	37	76
Trp	4	6	13	11	14	13	20
***mg / kg / day - daily requirement in mg of given AA per kilogram of body weight per day**							

Especially in sports nutrition intended for muscle growth, the amino acid availability is very important. Exercise has a profound effect on muscle growth, which can occur only if there is a positive muscle protein balance and muscle protein synthesis (MPS) exceeds muscle protein breakdown (MPB). After exercise, in the fasted state, both MPS and MPB are elevated while net muscle protein balance remains negative. To achieve a positive net balance, it is needed to increase the amino acid availabity, which in turn significantly increases MPS. Another important step is to inhibit MPB. Amino acids per se have only a small effect here, but the insulin, secretion of which is stimulated by glucose availability, has a profound effect on MPB inhibition. Ingestion of amino acids, combined with carbohydrates is thereofre important to increase the muscle protein anabolism after exercise [89]. The overall potential of a protein source to stimulate MPS depends on both digestion and AA absorption kinetics of the ingested food source as well as the AA composition with an emphasis on the essential ones, with the leucine content being of particular relevance [90].

2.5.3 Protein powder supplements

Protein powder is one of the most popular products in the food supplements industry. It is a powder with high protein content from animal or plant sources, often with added flavouring for better palatability. Protein powders are usually mixed with water and are generally consumed before, or after exercise or in place of meal [91, 92]. Active people or athletes usually consume protein supplements primarily to promote muscle growth, strength, and function [93]. The most common protein powders include whey protein powders, casein protein powders or soy protein powders. These powders usually come in three common forms - protein concentrates, isolates and hydrolysates [92].

Although the animal-based protein sources are great in terms of nutrition and muscle growth, the production of their sufficient amounts is not feasible to meet the growing global protein demand. Also, production of animal-source foods is more expensive and generates more GHG emissions than production of plant-based foods. Animal production seems to be of low efficiency and enviornmentally unsustainable. Therefore, the is a big pressure to reduce consumption of animal-source foods in global populations [94].

As a result, there is an increasing interest in the production of more sustainable and also alternative dietary protein sources [95]. Consumer market interest is increasingly directed toward the use of plant-based proteins as dietary components for increasing or preserving skeletal muscle mass. The use of various plant-based protein isolates in food formulations has become more popular recently because of greater sustainability and lower protein costs. In general, plant proteins posses lower muscle anabolic properties as opposed to animal-based proteins. This may be attributed to differences in protein digestion, AA absorption kinetics and AA composition. Most plant proteins have a relatively low leucine content. Therefore, various strategies for improving the MPS response after ingestion of plant-based protein have been developed. Those include the fortification of plant proteins with free AAs (especially methionine, lysine and/or leucine), the blending of various plant protein sources to achieve a more complete AA profile, selective breeding of plants to improve AA composition, or the consumption of greater amounts of plant proteins [90].

From the alternative dietary protein sources, edible insects represent a great option as they are protein-dense food source that may be produced more sustainably at a viable commercial scale for human consumption. In the latest study from this year, researchers from the Netherlands looked at whether insect protein is effective for muscle growth. In a double-blind, randomized study, they examined the effect of insect-derived protein ingestion on stimulation of proteosynthesis and also examined its digestibility and AA absorption kinetics. Specifically, it was a protein from the lesser mealworms (*Alphitobius diaperinus*). At the same time, they examined the same thing on milk-derived protein and compared the results. The participants had to do a certain amount of physical activity (strength training) and then received either 30 g of insect protein, obtained from 64 g of whole insect powder or 30 g of milk protein from 40 g of dried milk concentrate [95]. Insect protein has proven to be very digestible and usable by the body. No significant difference was found in this respect compared to milk protein. As can be seen in, it was also similar in stimulating MPS, whether at rest but also after exercise. Compared to milk protein, no differences were found. Ingestion of a meal-like amount of lesser mealworm protein is followed by rapid protein digestion and amino acid absorption and increases MPS rates both at rest and during recovery from exercise. As the postprandial protein handling of lesser mealworm in vivo in humans does not differ from ingesting the same amount of milk protein concentrate, considered a high-quality protein source, the insects can provide a viable high-quality protein source for human consumption [95].

In another study from 2018, researchers compared the posprandial AA availability and AA profile in the blood after digestion of protein isolate from the lesser mealworm, whey isolate and soy isolate. All three isolates increased the concentrations of EAA, BCAA and leucine over a 120 minute time period, while whey isolate having the greatest effect on change in AA profile. Insect protein induced blood AA concentrations similarly to soy protein. However, a tendency towards higher blood AA concentration after 120 minutes after ingestion was observed for insect protein, which indicates it can be considered a slower digestible protein source [96].

Insect protein can definitely serve as functional ingredients in food preparations. However, additional research is still needed to assess the functionality of various insect proteins in comparison to conventional proteins, regardless of processing method. More research is also needed to optimize processing methods and to obtain the best compromise between cost-effectiveness, tastiness, functionality and sustainability, while ensuring consumer safety. As the whole insects still have a pretty low acceptability in western cultures, insect protein concentrates could potentially represent a viable alternative protein source in food formulations [97].

The unsustainability of the current animal-based protein production, the often insufficient quality of their plant alternatives, together with the neglect of insects as a food were the main motives for writing this thesis and for the development of the unique food product which should help in solving these modern world problems.

3. EXPERIMENTAL PART

3.1 Materials

3.1.1 Materials for nutritional analysis

For selected nutritional analyses there were used 4 ground insect powders and 1 complex protein mix, consisting of plant proteins, insect powder and flavouring components (see *Figure* 17). A more detailed descripition of the samples is below:

Sample 1 (Cricket) – 100% cricket (*Acheta domesticus*) powder in the food grade quality from a manufacturer in the United Kingdom with particle size under 150 μm intended for human consumption.

Sample 2 (Cricket CoP) - 100% cricket (*Acheta domesticus*) powder with bigger particle size (> 150 μm) from the same UK manufacturer, which is produced as a by-product.

Sample 3 (Mealworm DeF) – 100% yellow mealworm (*Tenebrio molitor*) powder from a French manufacturer, which has undergone a defatting step, using the supercritical CO_2 extraction.

Sample 4 (Buffalo) – 100% lesser mealworm (*Alphitobius diaperinus*) powder from the Dutch manufacturer in the food grade quality intended for human consumption.

Sample 5 (Blend) – A mixture of lesser mealworm powder, pea protein, rice protein, defatted cocoa, cinnamon, guar gum, steviol glycosides and himalayan salt, prepared by mixing these powders together. Pea and rice protein were in ratio 1:1 and the total amount of the lesser mealworm powder used was 20 % of the total mixture.

Figure 17 – Samples of powders for nutritional analysis

3.1.2 Materials for sensory analysis

For sensory analysis, there were compared 3 protein powders. The conventional whey protein concentrate powder with chocolate flavour from Slovak manufacturer, the vegan protein powder mixture with chocolate-cinnamon flavour from Slovak manufacturer against the sample

5 from the previous subchapter 3.1.1. The aim of the sensory analysis was to compare the newly developed protein powder mixture, enriched with edible insects powder, with the competing products – with conventional whey protein concentrate and its vegan alternative.

Figure 18 - Prepared samples of protein shakes for sensory analysis

All powders were intended for use after mixing approximately 10 g of powder per 100 ml of water as a high-protein sports drink as can bee seen in *Figure* 18.

3.2 Chemicals and instrumentations

3.2.1 List of chemicals

- Ethanol 96% G.R., Lach-Ner, Czech republic
- Hexane p. a., Lach-Ner, Czech republic
- Hydrogen peroxide 30% G.R., Lach-Ner, Czech republic
- Methanol ≥ 99,9% CHROMASOLV®, Sigma-Aldrich, Germany
- Methyl butyrate, methyl capronate, methyl caprylate, methyl caprinate, methyl laurate, Merck, Czech Republic
- Mixed standard of fatty acid methyl esters (C14-C24), Restek, USA
- Multi-element aqueous certified reference material CZ 9091 MIX 011, Czech metrology institute, Czech republic
- Multi-element aqueous certified reference material CZ 9092 MIX 012, Czech metrology institute, Czech republic
- Nitric acid 67% ANALPURE® SD, Analytika, Czech republic
- Phosphorus – aqueous certified reference material CZ 9040, Czech metrology institute, Czech republic

- Sodium hydroxide G.R., Lach-Ner, Czech republic
- Sulfanilamide (4-Aminobenzenesulfonamide) G.R., Merck, Czech republic
- Sulphuric acid 96% G.R., Lach-Ner, Czech republic

3.2.2 Gases

- Air 5.0, SIAD, Czech republic
- Argon 5.0, SIAD, Czech republic
- Helium 5.0, SIAD, Czech republic
- Hydrogen 5.5, SIAD, Czech republic
- Nitrogen 5.0, SIAD, Czech republic
- Oxygen 5.0, SIAD, Czech republic

3.2.3 List of equipment

- Aluminium moisture dishes with lid
- Analytical digital balance GR-202, A&D Instruments Limited, United Kingdom
- CHNS-O Elemental Analyzer Euro Vector EA3000, Eurovector, Italy
- Compact balance, EK-1200i, A&D Instruments Limited, United Kingdom
- Computer with TRACE software
- Computer with Callidus™ 5.1 software
- Desiccator
- Drying oven UM400, Memmert GmbH + Co. KG, Germany
- Filter crucibles S2 SIMAX, KAVALIERGLASS, a.s., Czech republic
- Gas chromatograph TRACE GC, ThermoQuest Italia S. p. A., Italy, Flame Ionization Detector, Split/splitless injector, Capillary column DB WAX (30 m × 0,32 mm × 0,5 μm), Autosampler AI/AS 3000
- Heating mantle LTHS 500, Brněnská drutěva v.d., Czech republic
- High performance microwave digestion unit Milestone MLS 1200 Mega, Milestone Srl, Italy
- ICP-OES ULTIMA 2, HORIBA, Ltd., Japan
- LE laboratory chamber furnace, LAC, s.r.o., Czech republic
- Parafilm, Pechiney Plastic Packaging, Chicago, USA
- Porcelain crucibles
- PURELAB® Classic, ELGA LabWater – VWS Ltd., United Kingdom
- Reflux condenser

- Standard laboratory glass, plastics and equipment
- SOXTHERM Soxhlet Extraction System, C. Gerhardt GmbH & Co. KG, Germany
- Syringe nylon filters CHS Filterpure (25 mm / 0.45 μm), Chromservis s.r.o., Czech republic
- Vacuum filter flask PYREX®, Corning Inc., USA
- Vacuum rotatory evaporator, IKA®-Werke GmbH & Co. KG, Germany
- Vortex shaker Heidolph Reax Top, Heidolph Instruments GmbH & Co. KG, Germany
- Water-Bath Type W16, LHG GmbH, Germany

3.3 Determination of dry matter by drying

Around 4 grams of powdered sample (with 4 decimal place accuracy) were put into aluminum dish and dried out at 105 °C in a drying oven with open lid for 3 hours. Subsequently, the dish was closed and cooled down in desiccator. The sample was weighed. Another drying step lasted 30 minutes, following the cool down in desiccator and weighing. This step was repeated until reaching the constant weight (until the difference between the last two weighings was less than 2 mg). Before the whole procedure, also the empty aluminium dish with lid was weighed [98].

The difference between the weight of the sample before and after drying indicates the amount of water and volatile compounds. This difference represents moisture content (w_w). Dry matter (w_s) in % by weight was then calculated as a difference of 100 – w_w. The measurment was performed 3 times for each sample and the results are specified with two decimal place accuracy [98].

3.4 Gravimetric determination of ash

Approximately 1 g of sample was weighed with 4 decimal place accuracy in calcinated and weighed porcelain crucible. The samples in crucible was then carefully carbonized over a burner under the fume hood and calcined in a muffle furnace at the temperature of app. 750 °C for 3 hours. The crucible with ashes was subsequently cooled down in the desiccator and weighed [98].

The mass of ash (w_p) was calculated from the difference of the sample mass before and after calcination and is specified in the percentage by weight to 2 decimal places [98].

3.5 Determination of total nitrogen content by Dumas method

The samples were analysed using the CHNS-O Elemental Analyzer Euro Vector EA3000 based on the combustion (Dumas) method. The principle of this method is the catalytic combustion of a sample at a high temperature (~ 980 °C) in a stream of highly pure oxygen. The carrier gas is highly pure helium. After thermal decomposition of the sample, the gas mixture containing compounds C, H, N and S is converted to CO_2, H_2O, NO_X and SO_2 by the action of catalysts [99]. Other gases formed (halides, etc.) are removed by adsorption on phosphorus pentoxide and reaction with silver wool. The gases CO_2, H_2O, NO_X and SO_2 in the mixture are separated from each other on the basis of specific adsorption or desorption on a GC column. Their content is determined on a thermal conductivity detector in the order: NOX, H_2O, CO_2 and SO_2.

Calibration of the elemental analyzer was performed using the standard substance 4-aminobenzenesulfonamide (sulfanilamide). Samples weighing approximately 1 mg were burned in an oxygen atmosphere at a reactor operating temperature of 980 °C. At the time of combustion of the sample, this temperature is significantly higher, due to the strongly exothermic reaction of the oxidation of metallic tin to SnO_2. The samples were weighed in triplicate. The elemental composition of the samples was determined using the Callidus™ 5.1 evaluation program as the area under the curve.

After determining the total nitrogen content, the protein content of each sample was calculated using the protein factor 6.25. The total nitrogen content multiplied by 6.25 indicates the amount of total protein in percentage by weight [24, 99]. However, when using this factor, it is necessary to take into account the fact that the amount of protein may be overestimated, as insects have significant amounts of nitrogen stored in their chitinous exoskeleton [100].

3.6 Amino acid analysis

The analysis of the amino acid profile of selected samples was provided externally, in cooperation with the University of Veterinary Sciences Brno - Department of Animal Nutrition. Specifically, Cricket CoP, Mealworm DeF and Blend sample were analyzed in this manner. For Cricket and Buffalo sample, the amino acid profiles were available directly from the manufacturers.

3.7 Determination of total lipid content according to Soxhlet

Around 10 g of sample was weighed into extraction cartridge with 4 decimal place accuracy. The extraction cartridge was then wadded with cotton wool and put into the extraction chamber of Gerhardt SOXTHERM unit (see Figure 19) along with few pieces of pumice. There were added 100 ml of hexane and extraction was started using the SOXTHERM Manager program on a connected computer. The extraction was performed according to the conditions given in Table 8 below.

After extraction, the mixture of lipids and hexane was poured into a pre-weighed 50 ml distillation flask and the residual solvent was distilled off using a vacuum rotatory evaporator. The distillation flask with fat was then dried out out at 100°C for 30 minutes, cooled down in dessicator and weighed.

Table 8 - Conditions for SOXTHERM extraction

T-Classification	300 °C
Extraction temperature	170 °C
Reduction interval	3 min 30 s
Reduction pulse	3 s
Hot extraction	1 h 30 min
Evaporation A	5 X interval
Evaporation time	1 h 30 min
Evaportaion B	2 X interval
Evaporation C	10 min
Program length	3 h 4 min

Figure 19 - Gerhardt SOXTHERM Extraction Unit

The total lipid content in percentages by weight is then calculated by dividing the weight of extract by the weight of sample. Results were calculated with 2 decimal place eaccuracy [98].

3.8 Fatty acid analysis using Gas Chromatography

The GC-FID instrument was used to determine the individual fatty acids in the sample. The bound fatty acids in the triacylglycerols and the free fatty acids are converted into the fatty acid methyl esters (FAMEs), which are subsequently determined by gas chromatography. In gas chromatography of FAMEs with FID, hydrogen is used as the carrier gas (mobile phase). When using hydrogen, the separation can be performed in a shorter time with sharper peaks. The stationary phase is a microscopic layer of a thin liquid film on an inner solid surface made of steel, glass or fused silica. The analyzed volatile components react during their passage through capillary tubes with a stationary phase covering the inner surface of the column. Due to the different interaction of the different components (depending on the chain length, degree of (un) saturation and geometry and position of the double bonds), they leave the column at different times, called the component retention time, at a given setting of analysis parameters. Each sample was analyzed 3 times and the results were then processed on a computer using TRACE software. The measurement was performed after sample treatment, which is described in the following subchapter [101].

3.8.1 Sample preparation

10 mg of sample were weighed into a crimp vial. Subsequently, 1.8 ml of transesterification mixture (15 % (v/v) sulphuric acid in methanol) was added, vial was crimped and incubated in a thermoblock at 85 °C for 2 hours. After the transesterification, the mixture was cooled down and poured into 4 ml vials, containing 0.5 ml of 0.5 M NaOH solution (see Figure 20). Then, 1 ml of hexane was added, the vial was closed and shaked intensively using a vortex mixer. After phase separation, the upper hexane phase was transferred to a clean GC vial.

Figure 20 - Samples after transesterification

3.8.2 Conditions for the determination of FAMEs

- Gas chromatograph, TRACE GC, ThermoQuest Italia S. p. A., Italy
- Autosampler AI/AS 3000
- Capillary column DB WAX (30 m × 0,32 mm × 0,5 µm)
- Oven – temperature program
    - 50 °C 1 min
    - Ascending gradient 25 $°C \cdot min^{-1}$ do 200 °C with hold-up time 0 min
    - Descending gradient 3 $°C \cdot min^{-1}$ do 230 °C with hold-up time 30 min
    - Total analysis time: 47 min

- Inlet
    - Injector temperature: 250 °C
    - Splitless time: 1 min
    - Batch: 1 µl, splitless autosampler
- Carrier gas
    - Nitrogen flow: 1 ml·min^{-1}
- Flame ionization detector (FID)
    - Detector temperature: 250 °C
    - Air flow: 350 ml·min^{-1}
    - Hydrogen flow: 35 ml·min^{-1}
    - Nitrogen make-up: 30 ml·min^{-1}

3.8.3 Calculations

The concentration of fatty acid methyl ester in the extract (in the vial) is calculated according to the equation:

$$c_{FAME} = \frac{c_s \,.\, A_{FAME}}{A_s} \qquad (1.1)$$

The fatty acid concentration, calculated from the fatty acid methyl ester concentration, is calculated according to:

$$c_{FA} = \frac{c_{FAME} \,.\, Mr_{FA}}{Mr_{FAME}} \qquad (1.2)$$

The amount in the original volume in the vial is calculated according to:

$$w_{FA} = c_{FA} \,.\, V \qquad (1.3)$$

c_{FAME} – concentration of FAME in extract [mg.ml^{-1}]

A_{FAME} – peak area in extract [mV.s]

c_S – concentration of standrd [mg.ml^{-1}]

A_S - peak area of standard [mV.s]

c_{FA} – concentration of FA in extract [mg.ml^{-1}]

Mr_{FA} – molar mass of FA [g.mol^{-1}]

Mr_{FAME} – molar mass of FAME [g.mol^{-1}]]

w_{FA} – total mass of FA in vial [mg]

V – volume of hexane (1 ml)

3.9 Determination of dietary fibre according to Henneberg and Stohmann

Around 3 grams of sample were weighed into 500 ml distillation flask with 4 decimal place accuracy. Then, 200 ml of 5% sulphuric acid were added and the mixture was boiled using the heating mantle under the reflux condenser for 30 minutes with occasional stirring. The hot mixture was subsequently filtrated through the S2 filter crucible and washed with hot water several times. Residues from filter crucible were then quantitatively transferred back to the 500 ml distillation flask with 200 ml of 5% sodium hydroxide. The mixture was boiled under the reflux condenser again for 30 minutes. After that, the mixture was filtrated through dry weighed S2 filter crucible and washed with water and ethanol several times. The filter crucible with residues was then dried out at 105 °C, cooled down in desiccator and weighed. The last step included burning the filter crucible with residues in a muffle furnace at about 650 °C. After cooling in desiccator, the crucible with ash was weighed [98].

Weight of dietary fibre was then calculated as a difference of sample weight after drying and sample weight after burning [98].

3.10 Determination of total carbohydrates according to Dubois

Spectral methods using the visible light region are often used to determine carbohydrates. The Dubois method is a physico-chemical method based on the decomposition of sugars by sulphuric acid, followed by condensation of furfural with phenol. The result is colored reaction solution with absorption in the UV-VIS region. The absorbance of the solutions can be measured at a wavelength of λ = 490 nm [102].

Approximately 1 g of the sample was weighed (to 4 decimal places) into a plastic tube with a lid and 10 ml of distilled water and 28.4 µl of 35% HCl were added. The sample was left on a shaker for 1.5 hours. Subsequently, the sample was diluted 100 times and 0.5 ml of Carrez solution I and 0.5 ml of Carrez solution II were added. The solution was centrifuged at 5,000 rpm for 5 minutes. 1 ml of the clarified solution was taken into a test tube, 1 ml of 5% phenol and 5 ml of concentrated H_2SO_4 were added and the mixture was stirred. After 30 minutes, the absorbance at 490 nm was measured against a blank containing 1 ml of distilled water instead of a sample. The measurement was performed 3 times.

A glucose solution was used to prepare a calibration series with concentrations 0., 0.02, 0.04, 0.06, 0.08 and 0.1 g.l^{-1}.

3.11 Determination of mineral content by ICP-OES

Inductively coupled plasma optical emission spectrometry (ICP-OES) provides an excellent range for elementary qualitative, semi-quantitative and quantitative analysis. This is due to the very high temperatures (up to 10,000 K) of the plasma used to atomize the analyte present in the sample. Inductively coupled plasma is a partially ionized gas that is formed by a working gas in a high-frequency electromagnetic field induced by an induction coil. A carrier gas with a low flow rate can easily penetrate the ICP and evaporate and atomize and excite the sample. The excited atoms emit electromagnetic radiation at the wavelengths characteristic of the element. The emission intensity induces the concentration of the element in the sample, detected by a photomultiplier or a semiconductor detector. ICP-OES has multilevel analysis capability, wide linear range, low detection limit, good stability and reproducibility [103]. The determination of microelements and macroelements in samples was performed using the ULTIMA 2 ICP optical emission spectrometer from HORIBA Scientific which can be seen in the Figure 21 below.

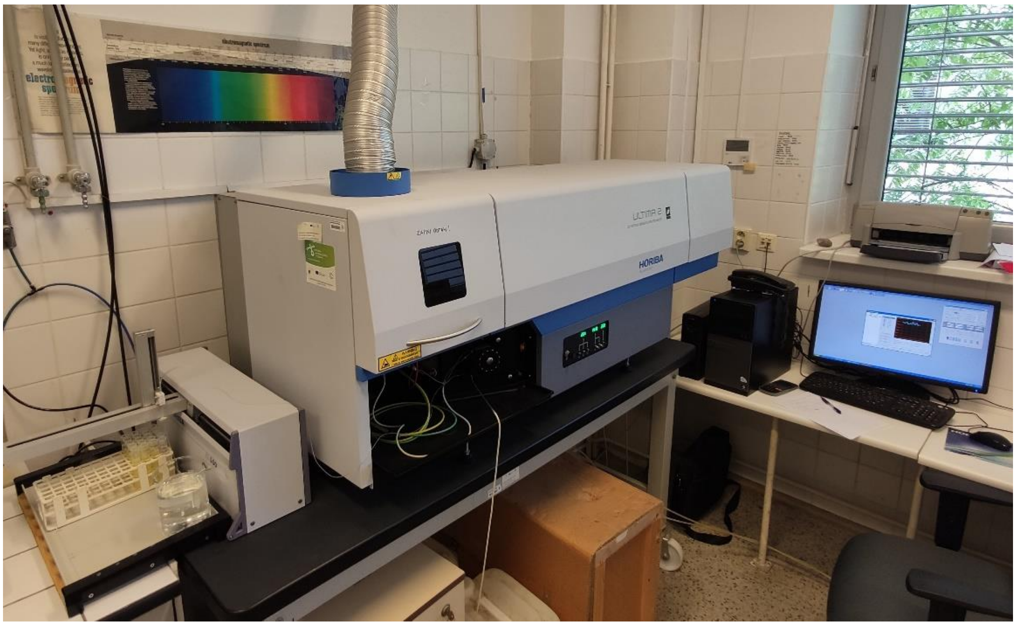

Figure 21 - ULTIMA 2 ICP-OES from HORIBA

The specification of the optical emission spectrometer is indicated in the Table 9 below.

Table 9 - Specification of ICP-OES

	ICP-OES Ultima 2 (Horiba Scientific Ltd.)
Generator frequency	40,68 MHz
Cooling system for generator and coil	GenCo type
Suction	direct connection to the plasma chamber
Working gas	argon
Purity of working gas	99,996%

Pump	3-channel peristaltic pump
Spray chamber	cyclonic
Nebulizer	Meinhard type
Pressure	3,02 bar
Plasma torch head configuration	radial
Wavelength	160 - 800 nm
Optical system	
Focal length	1 m
Optical grating	2400 scratches / mm
Optical resolution for 160 - 320 nm	< 5 pm
Optical resolution for 320 - 800 nm	< 10 pm
Detection	Dual PMT with HDD® system
Accessories	argon humidifier, autosampler AS-500

3.11.1 Sample preparation

Both determination of microelements and determination of macroelements included the pre-treatment of sample in the form of mineralization. Approximately 0.5-0.6 grams of sample were weighed into the mineralization tube with 4 decimal place accuracy. After that, 5 ml of 67% HNO_3 and 1 ml of 30% H_2O_2 were added. The tube was then inserted into the mineralization block of the Milestone MLS 1200 Mega High Performance Microwave Digestion Unit (see Figure 22) and the samples were mineralized according to the program given in Table 10.

Figure 22 - Milestone MLS 1200 Mega High Performance Microwave Digestion unit

After mineralization, each sample was quantitatively transferred into a volumetric flask and made up to 25 ml with distilled water. Subsequently, the solutions were filtered through nylon membrane syringe filters with a diameter of 25 mm and a pore size of 0.45 µm.

Table 10 - Mineralization program

Step	Time	Power
1	2 min	250 W
2	2 min	0 W
3	6 min	250 W
4	5 min	400 W
5	5 min	600 W

3.11.2 Preparation of the calibration series

Subsequently, a series of solutions were prepared to create a calibration curve separately for the determination of microelements and separately for the determination of macroelements.

For microelements, standard solutions for a mixture of copper, manganese, iron, zinc, cobalt, aluminum, cadmium, lead and chromium were prepared in 25 ml volumetric flasks. Solutions were prepared at concentrations of 0.5; 1; 2.5 and 5 mg / l and the volumes were made up with deionized distilled water.

For macroelements, standard solutions for a mixture of calcium, magnesium, sodium, potassium and phosphorus were prepared in 25 ml volumetric flasks. Solutions were prepared at concentrations of 5; 10 and 20 mg / l and the volumes were made up with deionized distilled water.

3.11.3 Measurement and its conditions

After the preparation of calibration series and their measurement, the measurement of the samples itself followed. Sample solutions were quantitatively transferred to plastic tubes and subsequently measured with an ICP-OES instrument under the conditions listed in the tables below. The measurement was performed separately for microelements and separately for macroelements. Sample solutions for the determination of macroelements were diluted 10-fold before the measurement. The measurement conditions for the determination of microelements are given in Table 11 and the measurement conditions for the determination of macroelements are in Table 12. Each sample was measured 3 times.

Table 11 - Conditions for the determination of microelements

Argon flow	
Carrier gas	0,254 L / min
Plasma gas	14,36 L / min
Auxiliary gas	0,2 L / min
Generator power input	
1300 W	

Table 12 - Conditions for the determination of macroelements

Argon flow	
Carrier gas	0,8 L / min
Plasma gas	14,36 L / min
Auxiliary gas	0,2 L / min
Generator power input	
1100 W	

3.12 Sensory analysis

In the sensory analysis, the effect of the addition of an insect powder, specifically a powder from the buffalo worm (larvae of *Alphitobius diaperinus*), on the sensory properties of a food supplement in the form of a protein drink prepared by mixing a powder mixture with water was investigated. For comparison, the evaluators were also presented with 2 competing products - the classic whey protein drink and the vegan alternative. A more detailed description with pictures of the samples used is in the subchapter 3.1.2. Samples were prepared by mixing the individual powders with water at a ratio of 10 g of powder per 100 ml of water and were administered in plastic cups of approximately 50 ml of each sample with coded labeling.

The sensory evalutaion was performed by filling in the prepared questionnaire. The first part of the questionnaire provided basic information about the evaluators and their relationship to similar products. Furthermore, the pleasantness of parameters like appearance and color, smell, taste and consistency was determined using hedonic graphic scales (the least pleasant / unacceptable -> the most pleasant / excellent). This was followed by a profile test of selected sample flavours (sweet, bitter and off-flavour) which was also determined using graphic scales (indistinguishable -> very strong). In the next step, participants were asked to rank the samples from the tastiest to the least tasty, and to add any comments to each sample. In the last part, the evaluators were asked to try to identify the sample with the addition of insect powder.

3.13 Online consumer survey

An online questionnaire was created to examine the consumer perception and acceptability of insects as a food in the population of the Czech Republic and Slovakia.

The questionnaire was created using the Google Forms online platform and contained a total of 28 questions with various answer possibilities. In the first part, basic information about the respondent was found out, as well as information about education, lifestyle but also attitudes towards insects as a food. It was also examined whether the respondent already has experience with the consumption of insects, or whether he would be willing to try insects out and if so, in what form. In the end, the respondent was asked what is in his opinion the biggest obstacle for entomophagy on the Czech and Slovak market, which product does he consider the best for the addition of insects in the form of powder and whether he would be willing to pay more for an insect-enriched product than for a conventional competing product with a higher environmental footprint.

4. RESULTS AND DISCUSSION

4.1 Determination of dry matter by drying

The dry matter in the samples was determined by drying and the results of the determination are shown in Table 13.

Table 13 - Results of dry matter determination

	Experimentally obtained data [%]	Data provided by the manufacturer [%]
Cricket	95.62 ± 0.06	97.95
Cricket CoP	95.90 ± 0.02	-
Mealworm DeF	93.22 ± 0.07	-
Buffalo	95.61 ± 0.05	> 95
Blend	93.84 ± 0.01	-

It is clear from the results that the dry matter content in the individual samples is very similar. The „Cricket“ sample and the „Buffalo“ sample were further compared with the data provided by the powder manufacturers. The dry matter content was comparable to the values stated by the manufacturer. The slightly lower dry matter content and thus the higher moisture content of the cricket powder can be attributed to the wetting of the powder during storage, because the package was not airtight closed.

The dry matter value for each powder was used in further analyzes, as the result was always recalculated to dry matter content.

4.2 Gravimetric determination of ash

The ash content of the samples was determined by the gravimetric method. The results were recalculated on dry matter and are shown in Table 14 and shown visually in Figure 23.

Table 14 - Results of ash determination (based on dry matter)

	Experimentally obtained data [%]	Data provided by the manufacturer [%]
Cricket	4.56 ± 0.05	6.53
Cricket CoP	3.80 ± 0.02	-
Mealworm DeF	6.62 ± 0.04	-
Buffalo	4.16 ± 0.12	-
Blend	3.23 ± 0.09	-

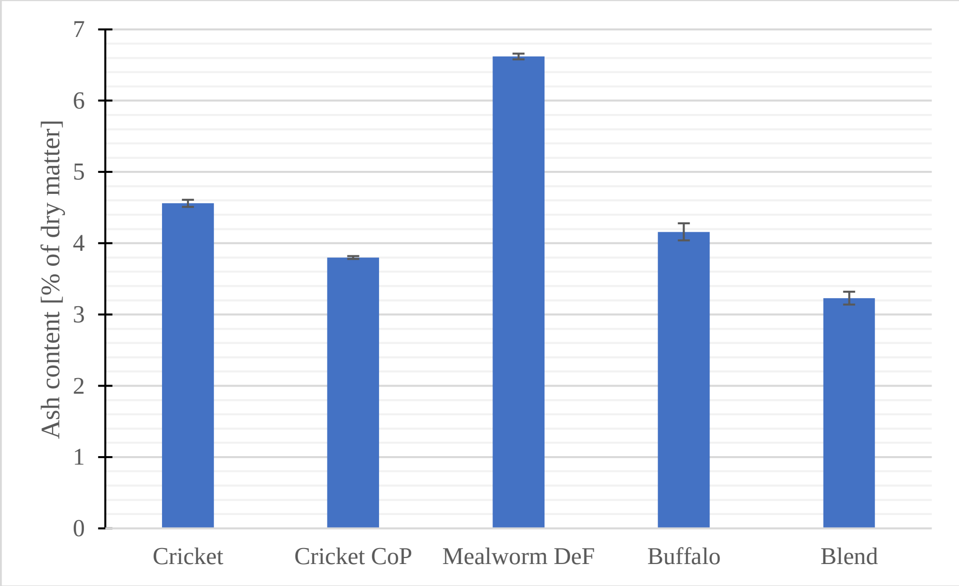

Figure 23 - Comparison of samples in terms of ash content

Ash refers to an inorganic residue that remains after ignition or complete oxidation of organic matter in a food sample. Inorganic residues consist mainly of minerals present in the food sample and the determination of the ash content is therefore a good rough indicator of the nutritional value of the food [28].

From the measured data it is clear, that the highest ash content was in the sample Mealworm DeF and the lowest in the sample Blend. The Mealworm DeF sample had the highest ash content probably due to the defatting step and thus had the content of all other ingredients increased per 100 grams. Overall, measured data were in accordance with data reported in the literature, with the exception of Mealworm DeF sample, where approximately two times higher content was measured. However, the already mentioned defatting step is probably to blame [17].

A more accurate determination of the mineral content was performed using ICP-OES, the results of which are given in the subchapter 4.9.

4.3 Determination of total nitrogen and crude protein content by Dumas method

By Dumas combustion method, firstly the total nitrogen content of the samples was determined. Subsequently, the crude protein content was calculated by multiplying the nitrogen content by a factor of 6.25 [24, 99]. The results are shown in Table 15 and in Figure 24.

As can be seen in the Figure 24, the Mealworm DeF sample showed the highest protein content (73.86 ± 0.49 % of dry matter). This is mainly due to the technological process of defatting, which has increased the content of all other nutrients. Yellow mealworm usually has a protein content only 47.18–49.43 % of dry basis [17].

Table 15 - Results of nitrogen and protein determination (based on dry matter)

	Total nitrogen content [%]	**Crude protein content [%]**
Cricket	10.09 ± 0.16	63.05 ± 0.98
Cricket CoP	10.90 ± 0.19	68.14 ± 1.17
Mealworm DeF	11.82 ± 0.08	73.86 ± 0.49
Buffalo	9.14 ± 0.36	57.14 ± 2.25
Blend	11.39 ± 0.16	71.17 ± 1.02

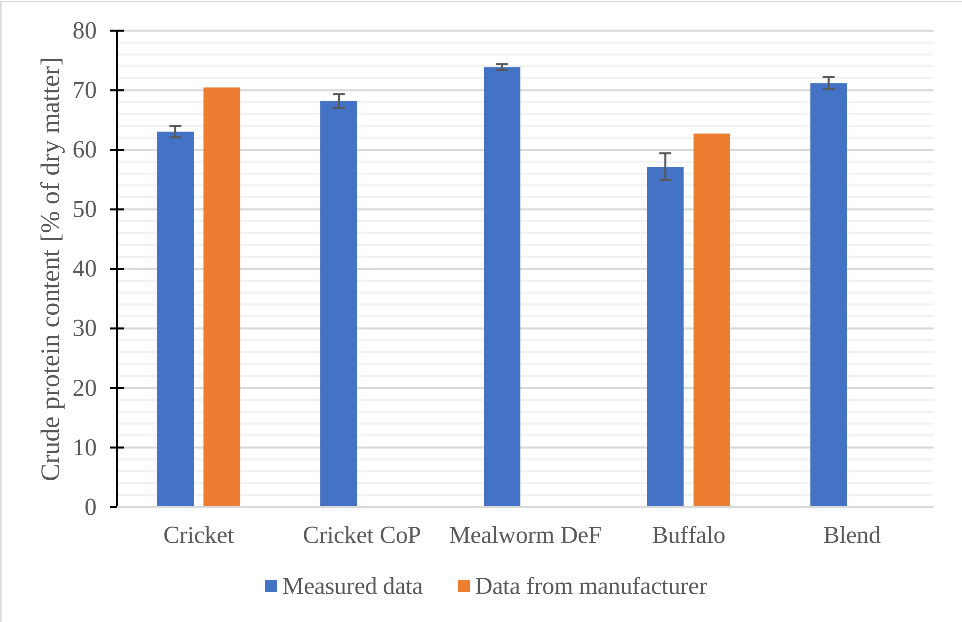

Figure 24 - Comparison of samples in terms of crude protein content

The second highest protein content (71.17 ± 1.02 %) was determined in sample „Blend“. Thus, by combining plant proteins together with lesser mealworm powder, a high-protein product cen be achieved. The protein content was similar to other protein powder products on the market.

The third in order of total protein content was the Cricket CoP sample with a value of 68.14 ± 1.17 % of dry matter which is in accordance with literature [17]. The coarser fraction of the powder from *Acheta domesticus* showed an even higher protein content than the fine cricket powder alone. It is therefore clear that, although it is only a by-product, it still has a high nutritional value. On the other hand, it is also needed to mention, that overally higher protein content in these insect powder samples, and especially in the Cricket CoP sample, with a larger share of exoskeletons, can be overestimated. There is also included the nitrogen from chitin which does not directly correspond with higher protein content. Therefore, other factors than 6.25 should be used to get a more precise protein content information [100].

In the Cricket sample, there was measured a protein content of 63.05 ± 0.98 %, which is in accordance with literature, however it is a little bit lower than the value stated by the manufacturer (70.44 %) [17].

The Buffalo sample had a protein content of 57.14 ± 2.25 % of dry matter, which is in accordance with literature and is also slightly lower than the value from manufacturer (62.74 %) [17]. This difference may be caused by using a different method for protein determination.

In general, however, all insect powder samples showed really high protein content which makes them an ideal food ingredient. Also, an insect-based product, the sample Blend, had significant protein content, similar to other protein powder products on the market.

4.4 Amino acid analysis

Cricket CoP, Mealworm DeF and Blend sample were analyzed externally in cooperation with the University of Veterinary Sciences Brno - Department of Animal Nutrition. For Cricket and Buffalo sample, the EAA contents were taken from the manufacturer.

Contents of selected EAA in 4 insect powder samples were compared with each other and also with respect to the amino acid requirements of complete protein. The results are shown in Table 16 and also visually in Figure 25.

*Table 16 - Selected EAA contents of insect powders compared to complete protein requirements (*data from manufacturer) [19, 88, 89]*

Essential amino acid	Cricket*	Cricket CoP	Mealworm DeF	Buffalo*	Complete
Leu	75	35	65	67	59
Ile	57	72	29	44	30
Val	89	71	47	57	39
His	34	29	14	34	15
Lys	64	43	44	65	45
Met	19	34	15	13	16
Phe	47	24	26	44	-
Thr	33	31	32	40	23

As can be seen from results, the selected EAA contents are pretty sufficient in all of the insect powders. The Cricket sample have even met all the AA requirements. Cricket Cop sample was not sufficient in the content of Leucine and Lysine, which shows, that different fractions of the same powder can have different nutritional value. Although the total protein content of this sample was higher compared to Cricket sample, the quality of protein is a slightly lower. This may also be a confirmation that the actual protein content of the sample is lower than the value calculated by a factor of 6.25 due to the nitrogen contained in chitinous exoskeleton.

The Mealworm DeF sample have also met all the AA requirements, except those for isoleucine, histidine, lysine and methionine, which were on the limits of requirements. The results for this powder are in agreement with the literature, which states that the mealworm may lag behind, especially in terms of sulphur amino acid content [65, 80].

The Buffalo sample has met all the AA requirements for complete protein too. The only limiting amino acid was methionine, which only confirms the similarity with the yellow mealworm.

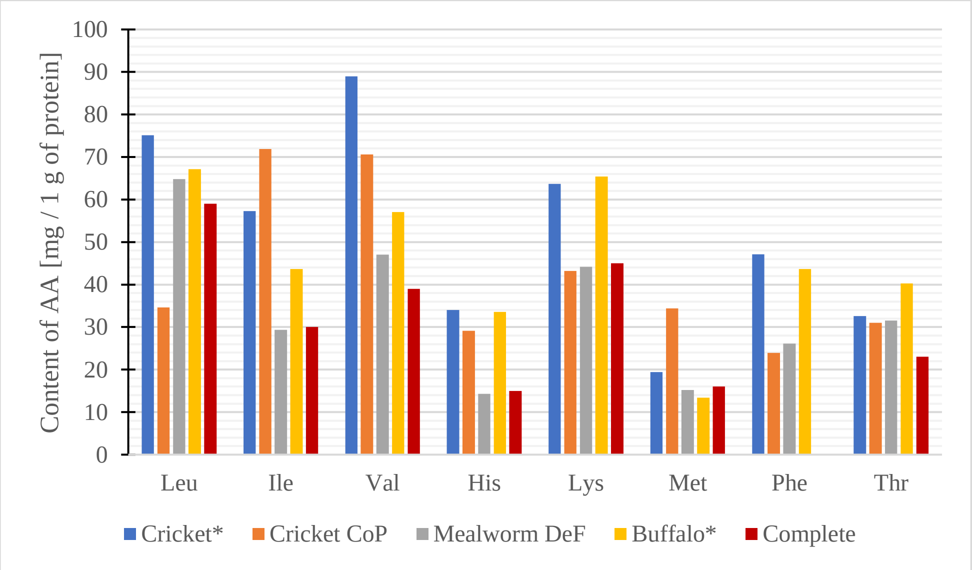

Figure 25 - Selected EAA content of insect powders compared to complete protein (Phe per se was not available in AA requirements) [19]

Also, the EAA Content of sample Blend was analysed and compared to the complete protein requirements and also to other protein concentrates or isolates from plant and dairy sources. The result are summarized in Table 17 the and in the Figure 26.

Table 17 - Selected EAA contents of the sample Blend compared to complete protein requirements and other protein sources [19]

Essential amino acid	Blend	Milk	Pea	Rice	Soy	Whey	Complete
Leu	64	97	97	88	80	116	59
Ile	37	50	54	40	43	59	30
Val	51	58	81	55	44	58	39
His	19	30	17	24	26	21	15
Lys	39	80	83	33	60	102	45
Met	3	27	5	29	10	23	16
Thr	32	47	43	38	37	76	23

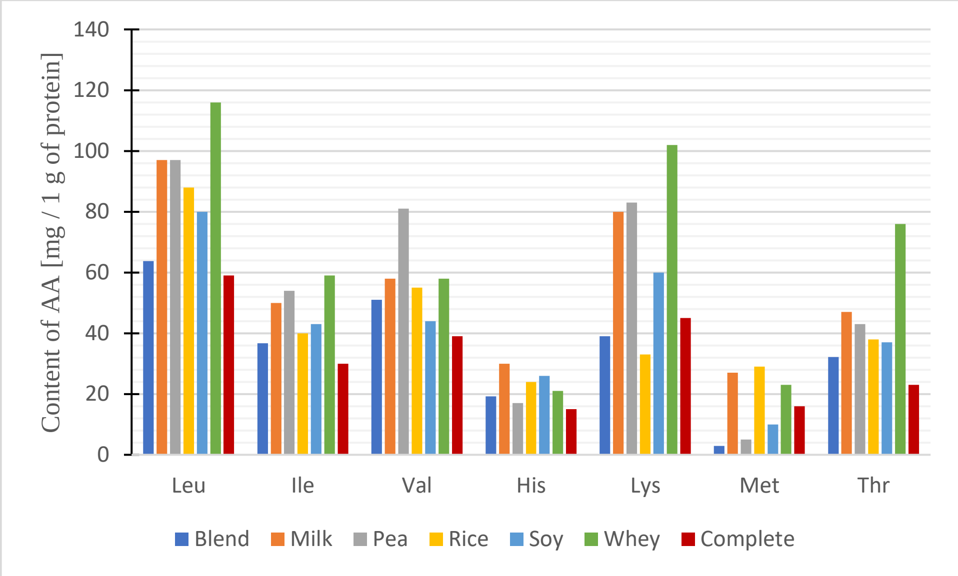

Figure 26 - Selected EAA content of the sample Blend compared to complete protein requirements and other protein sources [19]

It is clear from the results that the sample Blend meets almost all AA requirements for complete protein, except the requirements for lysine and methionine. This product was created using 20 % of the lesser mealworm powder, together with pea protein, rice protein and flavouring ingredients. Unfortunately, the amounts of lysine and methionine were not sufficient to meet the AA requirements. It might have been caused by using lower quality plant proteins (the AA profile of which was not available, the AA profile of plant proteins in Figure 26 is from literature), or by chosing the incorrect ratio of individual components in the mixture. Therefore some adjustments should be made to achieve a better AA profile. On the other hand, the Blend sample was higher in lysine than rice protein or higher in valine than soy protein.

It is also needed to say that amino acids were determined by acid hydrolysis using 6N HCl for 24 hours at 110 ° C. According to the mentioned method, the value of sulphur amino acids is lower than it actually is and thus the results for methionine may be underestimated.

4.5 Determination of total lipid content according to Soxhlet

The fat content of the samples was determined using the Soxhlet method. The results can be seen in Table 18 and in Figure 27 below. The highest fat content was measured in the Buffalo sample, namely 27.76 ± 0.47 % of dry matter, which is slightly lower than the data stated by the manufacturer (30.21 %) and similar to what is stated in literature [68]. The difference could be caused by losses that might have occured during the measuremnt but also because of unknown methodology for determining fat content by the manufacturer.

The second highest lipid content was measured in the Cricket sample (22.35 ± 0.44 %). The manufacturer's specification stated 17.87 % of dry matter which is in agreement with the literature [17]. The difference could be due to a different methodology for determining fat. In fact, all lipophilic compounds extractable with hexane and thus sterols, phospholipids, carotenoids and fat-soluble vitamins were determined by the Soxhlet method [98].

Table 18 - Results of total lipid content (based on dry matter)

	Experimentally obtained data [%]	**Data provided by the manufacturer [%]**
Cricket	22.35 ± 0.44	17.87
Cricket CoP	15.23 ± 0.38	-
Mealworm DeF	6.57 ± 0.12	-
Buffalo	27.76 ± 0.47	30.21
Blend	6.85 ± 0.03	-

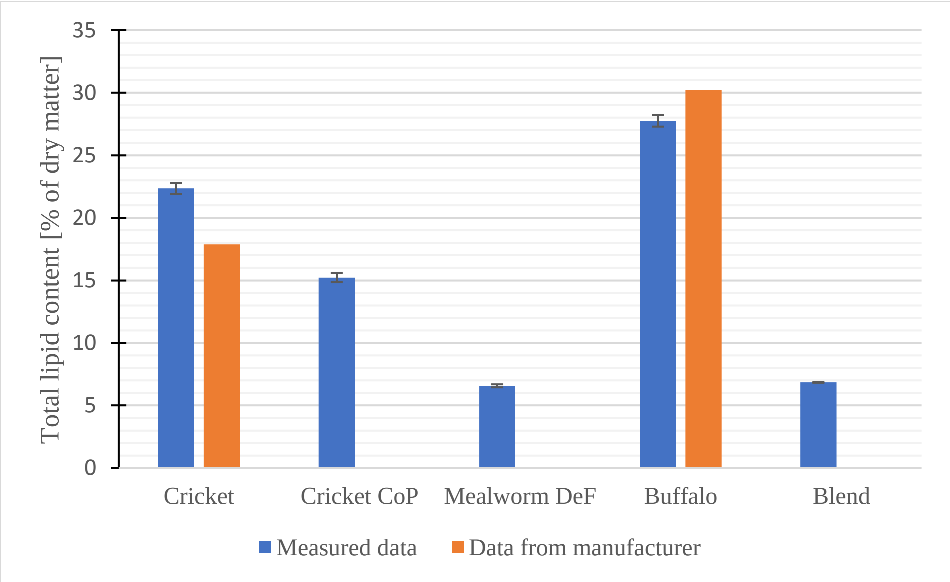

Figure 27 - Comparison of samples in terms of total lipid content

The lipid content of the Cricket CoP sample was determined at 15.23 ± 0.38 % and thus, although it is a by-product, it still has a pretty significant amount of fat, even though less than the original Cricket sample.

The Blend sample contained 6.85 ± 0.03 % of fat based on dry matter which is an acceptable value for a given type of product, i.e. a food supplement in the form of a high-protein powder for the preparation of a beverage.

The lowest lipid content was measured in the Mealworm DeF sample, exactly 6.57 ± 0.12 %. The measured fat content was very low as mealworms normally contain about 35.17–43.08 % of fat. However, it was a defatted version of the powder from mealworms. Therefore, during the defatting process, about 30 % of fat was lost. This kind of processing can be advantageous for certain applications, not only for mealworm powder but for insect powders in general. This step increases the proportion of other macronutrients and at the same time decreases the total energy value. Thus, for application in a variety of high protein or low fat products, it is ideal.

4.6 Fatty acid analysis using Gas Chromatography

Result of fatty acid analysis using GC-FID are summarized in Table 19 and visually presented in Figure 28.

Table 19 - Results of fatty acid analysis

Fatty acid	C12:0	C14:0	C16:0	C18:0	C18:1 (n-9)	C18:2 (n-6)	C18:3 (n-3)
	% of total fat						
Cricket	1.07 ± 0.91	0.74 ± 0.05	33.02 ± 3.47	14.69 ± 0.79	22.27 ± 1.37	26.96 ± 3.12	1.25 ± 0.20
Cricket CoP	2.89 ± 1.66	0.70 ± 0.07	33.31 ± 1.81	12.29 ± 1.01	20.54 ± 1.08	29.18 ± 1.00	1.10 ± 0.02
Mealworm DeF	5.17 ± 1.59	1.59 ± 0.09	37.39 ± 5.45	9.47 ± 0.98	28.70 ± 4.31	17.15 ± 1.62	0.52 ± 0.04
Buffalo	1.59 ± 0.67	0.68 ± 0.04	29.81 ± 1.20	10.47 ± 0.48	30.11 ± 0.87	25.65 ± 1.14	1.70 ± 0.07
Blend	2.13 ± 1.51	1.16 ± 0.64	31.35 ± 1.56	9.76 ± 0.76	28.77 ± 1.51	24.52 ± 0.21	2.33 ± 0.04

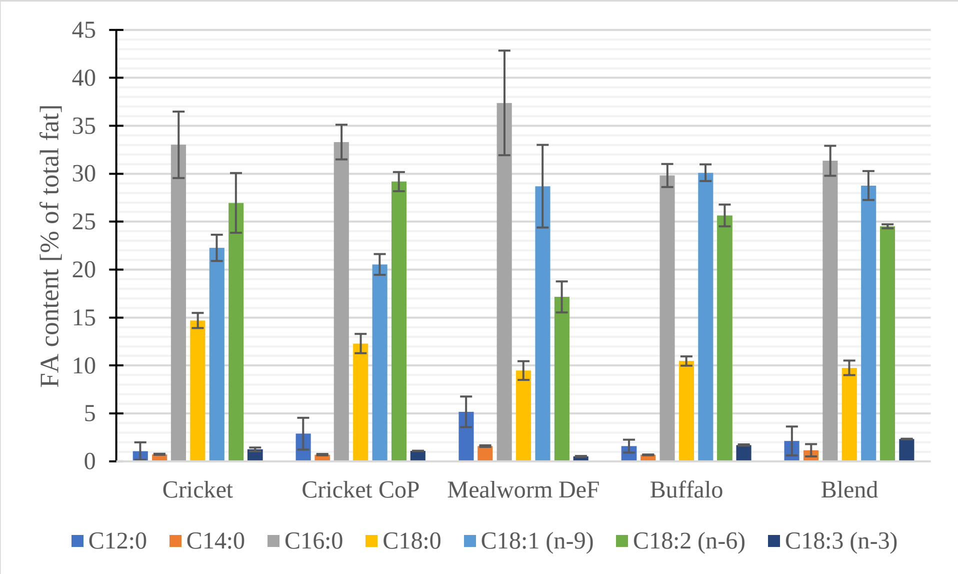

Figure 28 - Comparison of fatty acid contents in samples

The fatty acid contents of of all 4 insect powders were very similar. The most abundant FA in all samples, except the Buffalo sample, was palmitic acid (C16:0) which was present in quantities of 31.35 – 37.39 %. The second most abundant FA in Mealworm DeF sample was oleic acid (C18:1, n-9) with contents of 28.70 ± 4.31. In Buffalo sample, the order of two main FA was reversed. In these samples, the third most abundant FA was linoleic acid (C18:2, n-6), folowed by stearic acid (C18:0), lauric acid (C12:0) and myristic acid (C14:0). In the Cricket and Cricket CoP samples, the second most abundant FA was linoleic acid (C18:2, n-6), followed by oleic (C18:1, n-9), stearic (C18:0), lauric (C12:0) and myristic (C14:0) acid. The exact contents of FA can be seen in Table 19. In terms of main FA present, it can be concluded that the measured data is in accordance with literature, although there are some variations in the FA contents and their order.

The fatty acid contents in Blend sample were almost the same as in Buffalo powder, which is logical, and it also confirms the use of lesser mealworm powder in terms of autenticity, as almost all the fat in this sample comes from the lesser worm.

4.7 Determination of dietary fibre according to Henneberg and Stohmann

According to Henneberg and Stohmann, the dietary fibre content was analyzed in only 3 samples, namely in the Cricket CoP, Mealworm DeF and Blend sample. For the Cricket and Buffalo sample, data were available directly from the manufacturers of these powders. The results are shown in Table 20 and in Figure 29 below.

Table 20 - Results of dietary fibre determination

	Dietary fibre content [% of dry matter]
Cricket*	5.82
Cricket CoP	10.45 ± 0.54
Mealworm DeF	1.50 ± 0.22
Buffalo*	3.89
Blend	1.48 ± 0.05
*data from manufacturer	

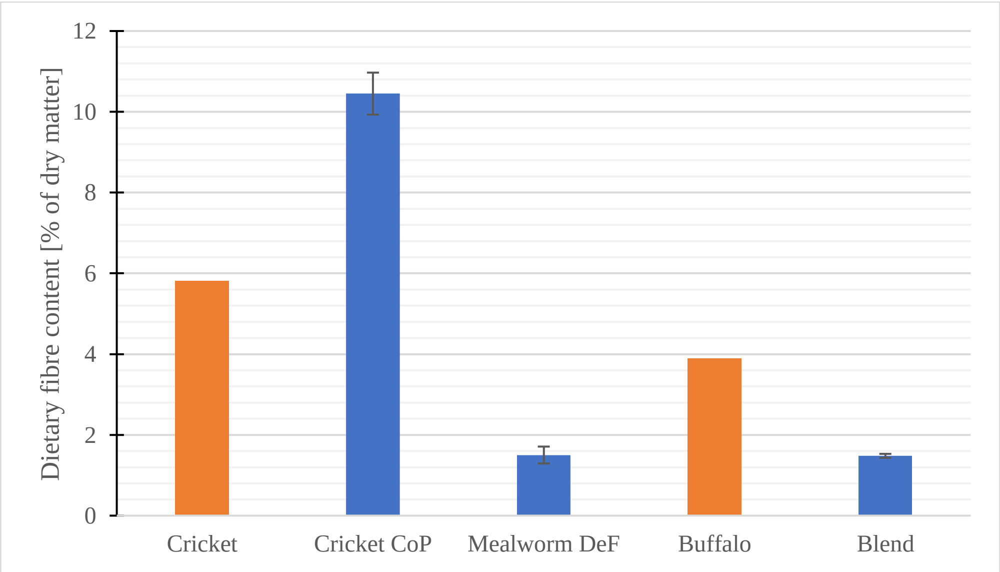

Figure 29 - Comparison of dietary fibre content of samples

From the results is clear, that the highest dietary fibre content (10.45 ± 0.54 %) was measured in Cricket CoP sample which is logical as it is a coarser fraction of Cricket sample, containing more chitin from insect exoskeletons. This by-product is therefore a significant source of dietary fibre and could thus find application in various foods to increase dietary fibre intake. The orange columns in Figure 29 represent data obtained from manufacturers. The second highest amount was measured in Cricket sample, followed by Buffalo sample. The lowest quantities were measured in Mealworm DeF and Blend sample. All the values, except those in Cricket and Cricket CoP sample, were lower than values stated in literature which may be caused by different methods used to determine the dietary fibre [17].

Either way, insects in the diet can definitely provide dietary fibre, which is beneficial for human health. Some of this fibre is made up of chitin, which can also have beneficial effects on human health [26].

4.8 Determination of total carbohydrates according to Dubois

The carbohydrate content of the samples was determined by the spectrophotometric method according to the procedure described in subchapter 3.10. The determined values in % of dry matter are given in Table 21 and are also shown visually in the Figure 30. A constructed calibration curve with the equation y = 9,5491x was used for the calculation of carbohydrate contents in samples.

Table 21 - Results of total carbohydrate content (based on dry matter)

	Carbohydrate content [% of dry matter]
Cricket	2.74 ± 0.99
Cricket CoP	1.41 ± 0.59
Mealworm DeF	2.58 ± 0.72
Buffalo	0.64 ± 0.39
Blend	2.08 ± 0.42

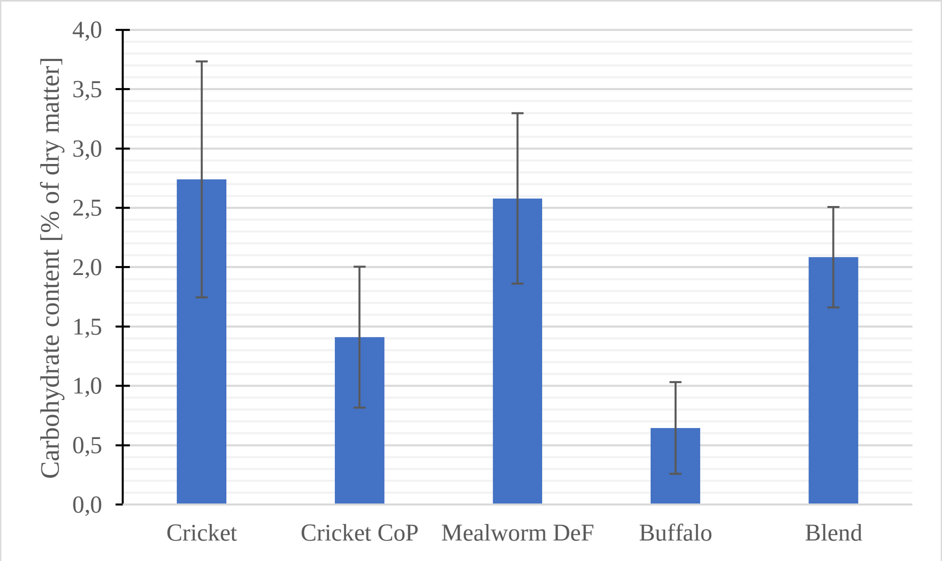

Figure 30 - Comparison of carbohydrate content of samples

The measured data are in accordance with literature, excpet the Buffalo sample, which had slightly lower amounts of carbohydrates [17, 68]. However, it should be mentioned that the analysis was subject to a relatively large deviation, caused by several factors to which the spectral analysis is prone.

In general, insects contain only small amounts of carbohydrates.

4.9 Determination of mineral conent by ICP-OES

The amounts of selected minerals in the samples were determined by the ICP-OES method. The results are clearly displayed in Table 22 and Table 23, together with the wavelengths at which the elements were measured and also with the recommended daily intakes for adults. The amounts of elements are expressed per 100 g of dry matter and were calculated using calibration equations, created for each element separately. The calibration curves of selected elements for illustration, together with linear regression equations can be seen in Figure 31 and Figure 32 below.

Table 22 - Results of mineral content determination - Heavy metals

	λ [nm]	Cricket	Cricket CoP	Mealworm DeF	Buffalo	Blend
		Amount [mg / 100 g of dry matter]				
Pb	220,35	ND	ND	ND	ND	ND
Cd	214,438	ND	ND	ND	ND	ND

Regarding the content of heavy metals, which are an important indicator of food safety, all samples passed very well. No lead or cadmium was found in any of the samples.

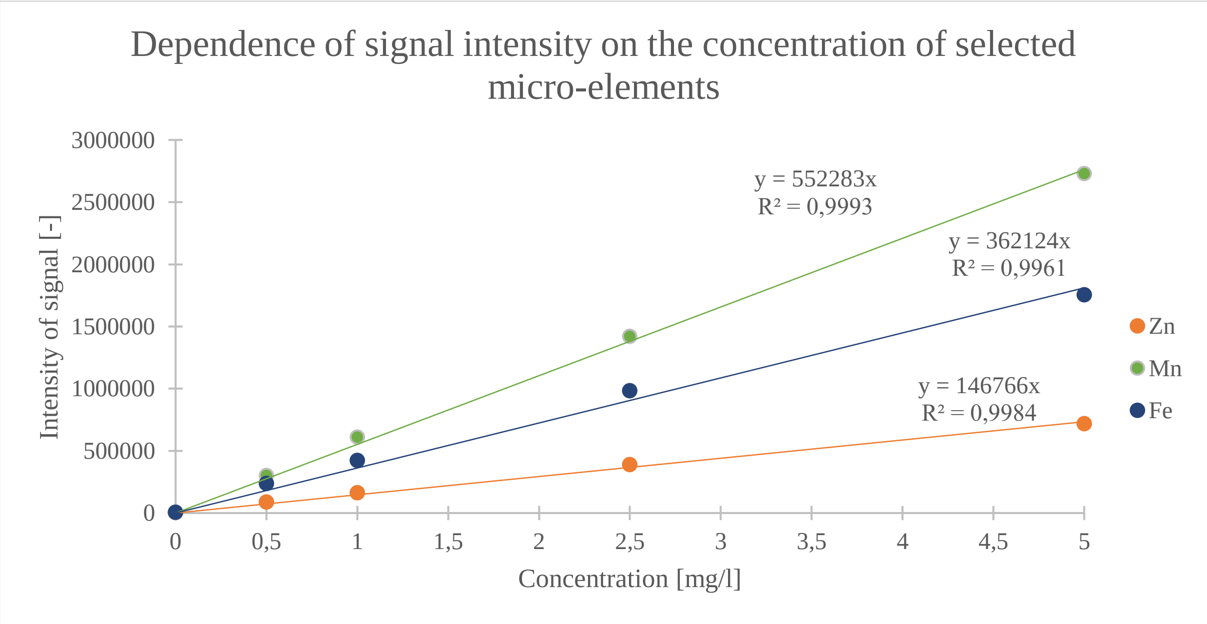

Figure 31 - Calibration curves of selected micro-elements

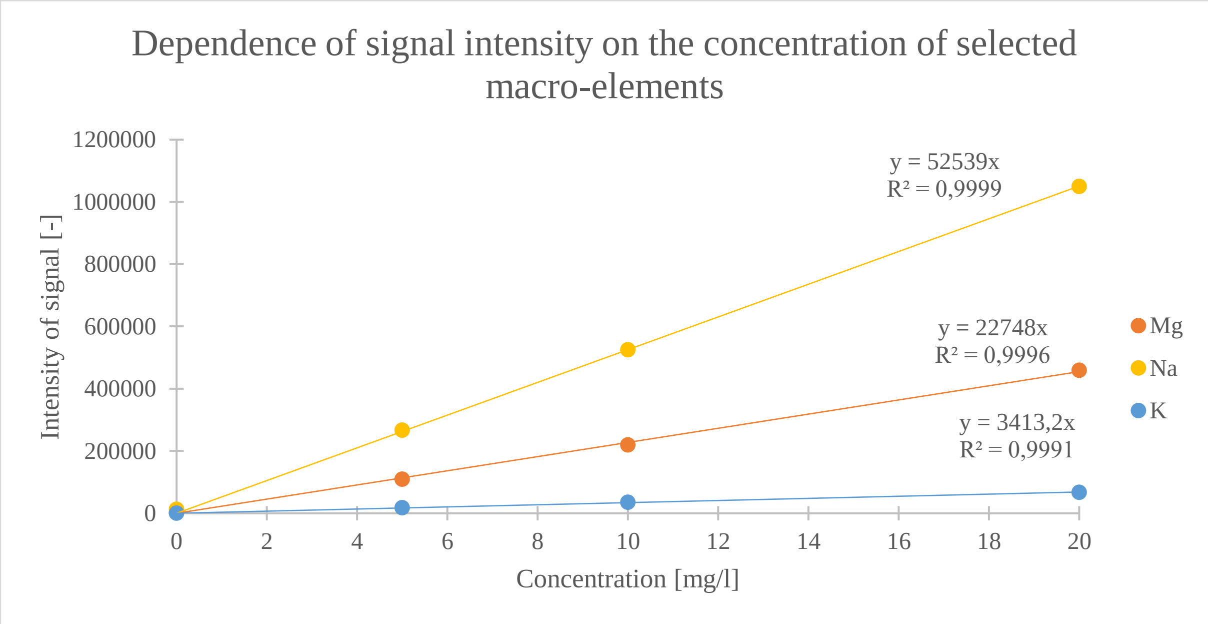

Figure 32 - Calibration curves of selected macro-elements

Table 23 - Results of mineral content determination (based on dry matter)

Mineral	λ [nm]	Cricket	Cricket CoP	Mealworm DeF	Buffalo	Blend	DRI* [mg / day]
		Amount [mg / 100 g of dry matter]					
Ca	393.366	175.38 ± 6.98	141.19 ± 10.11	595.69 ± 13.77	97.79 ± 0.96	122.98 ± 12.51	800
K	766.490	973.96 ± 16.19	890.65 ± 15.47	514.05 ± 1.73	897.54 ± 10.36	593.39 ± 25.17	2000
Mg	285.213	128.39 ± 1.69	98.85 ± 1.82	214.40 ± 4.14	125.48 ± 0.89	96.94 ± 3.44	375
P	214.914	1344.49 ± 36.78	1059.30 ± 37.33	613.39 ± 18.13	1169.33 ± 11.72	847.65 ± 6.34	700
Na	588.995	407.13 ± 2.35	373.48 ± 5.72	208.83 ± 5.74	215.91 ± 2.59	351.88 ± 31.03	2000
Fe	259.940	5.47 ± 0.24	4.28 ± 0.21	98.76 ± 19.74	4.67 ± 2.57	13.25 ± 0.27	14
Zn	206.191	17.19 ± 0.37	18.87 ± 0.38	8.98 ± 0.59	11.33 ± 0.32	9.06 ± 0.43	10
Mn	257.610	3.76 ± 0.10	2.82 ± 0.00	3.25 ± 0.12	0.14 ± 0.06	1.14 ± 0.06	2
Cu	327.396	2.86 ± 0.16	2.16 ± 0.06	1.50 ± 0.11	1.60 ± 0.06	1.28 ± 0.00	1
DRI* - Daily Reference Intakes for adults							

As can be seen from the results, all samples tested represent a significant source of several essential minerals. The Cricket sample seems like a very good source of potassium, phosphorus, zinc, manganese and copper and had all values exceedind or at least approching to DRI of those minerals. The Cricket CoP sample was very similar to Cricket sample, having all the minerals,except zinc, slightly lower. The Mealworm DeF sample contained the highest amounts of calcium and magnesium and the lowest amounts of phosphorus. The iron content of this sample was strangely high, which could be explained as a contamination from the production facility due to abrasion. This powder was only from a small-tonnage test grinding and inappropriate mill with high abrasion was probably used. The Mealworm DeF sample contained almost 100 % of DRI of zinc and exceeded the DRI for manganese and copper. Buffalo sample had significant amounts of phosphorus, zinc and copper, but also potassium content was on a pretty high level. The Blend sample can be a great source of potassium, iron, zinc, manganse and copper. Especially iron content was the highest from all samples, if the probably contamined Mealworm DeF sample is not taken into account.

All in all, insect powders and also the insect-based protein mixture can be a great source of various valuable minerals and for targeted supplementation of specific minerals, it is necessary to know specific insect species.

4.10 Principal component analysis – PCA

14 variables that are statistically significant for grouping according to differences were selected. These included basic nutritional parameters (ash, protein, fat, fibre, carbohydrate) together with mineral composition (P, Mg, Ca, Na, K, Zn, Mn, Fe, Cu). These 14 variables were used for evaluation and visualization using principal component analysis (PCA). The number of variables has been reduced to the main components that have an Eigenvalue greater than 1. The higher the Eigenvalue value, the more variables this component represents. According to the Kaiser criterion (Eigenvalue > 1), 3 main components were determined, which together described 97.68 % of the total variability. The components with the given Eigenvalue are listed in Table 24 and graphically processed in Figure 33 using the scree plot.

Table 24 - List of components with a given Eigenvalue and variability

Component	Eigenvalue	Total variability [%]	Cumulative variability [%]
1	8,81	62,94	62,94
2	3,59	25,61	88,55
3	1,28	9,13	97,68
4	0,24	1,74	99,42
5	0,03	0,25	99,66
6	0,02	0,18	99,84
7	0,01	0,10	99,94
8	0,01	0,05	99,98

9	0,00	0,01	99,99
10	0,00	0,01	100,00
11	0,00	0,00	100,00

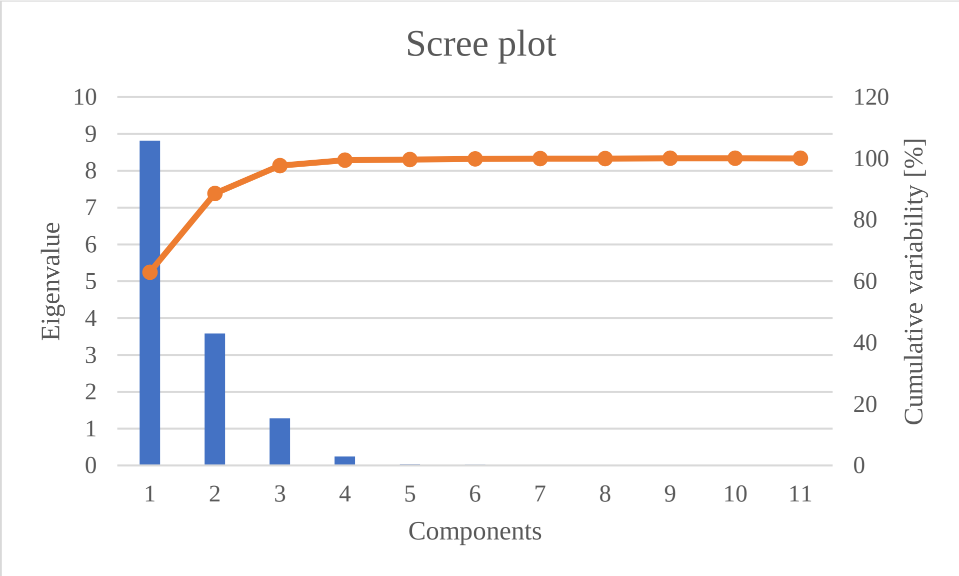

Figure 33 – Scree plot showing Eigenvalue size and achieved variability

Using selected main components, graphs were compiled that show individual projections on the factor plane coordinates. Component 1 and component 2 were selected for 2D projection. These components had the largest representation of variables (the sum variability of these two components was 88.55 %).

In Figure 34, the variables are projected in the 2D plane. From this graph it is possible to read which variables correlate the most given component. Component 1 is most positively correlated with fat content, phosphorus, potassium, fibre, zinc, copper and sodium content. Conversely, the most negatively correlated is the content of magnesium, iron, calcium and ash.

Component 2, on the other hand, is most positively correlated with fat content and negatively with the content of protein, carbohydrate and manganese, together with fibre, zinc, copper and sodium content.

Significant correlations can also be observed between variables themselves, such as correlation of protein with carbohydrate and manganese content, or correlation of ash with magnesium, calcium and iron. The other correlation can be found in case of phosphorus content with potassium and another one in case of fibre, correlating with the content of zinc, copper and sodium.

In Figure 35, the results are again projected into the plane of components 1 and 2. Using this visualisation, the distribution of samples can be seen and it is possible to distinguish the samples from different insect species. It is also possible to confirm the homogenity of samples, since 3 parallel measurements were performed for each tested parameter. It also confirms the quality of measurements.

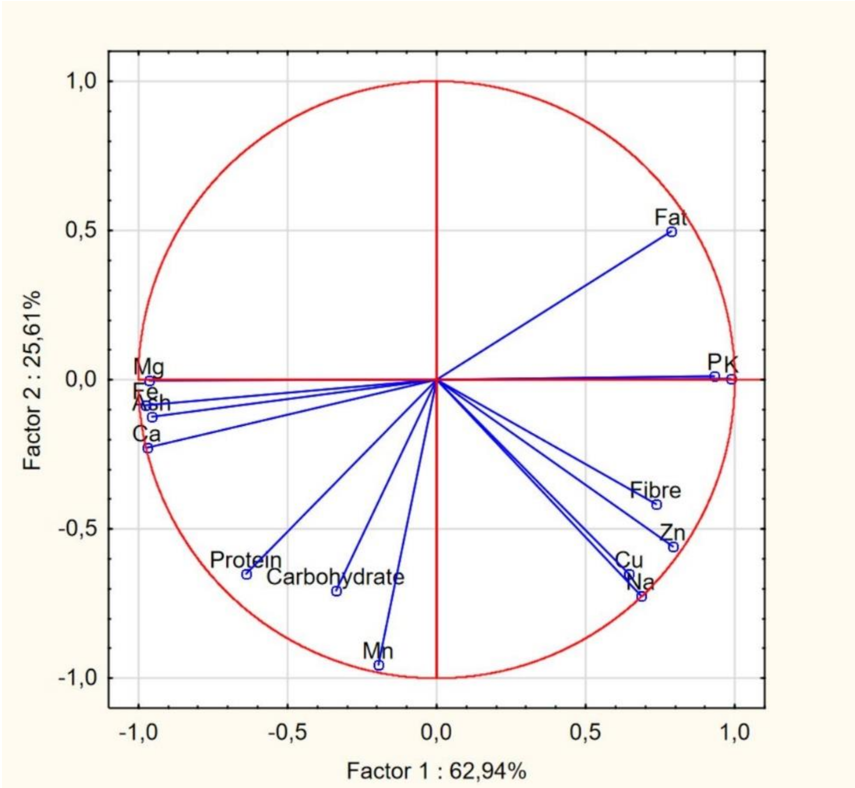

Figure 34 - Projection of the variables on the factor plane (1 X 2)

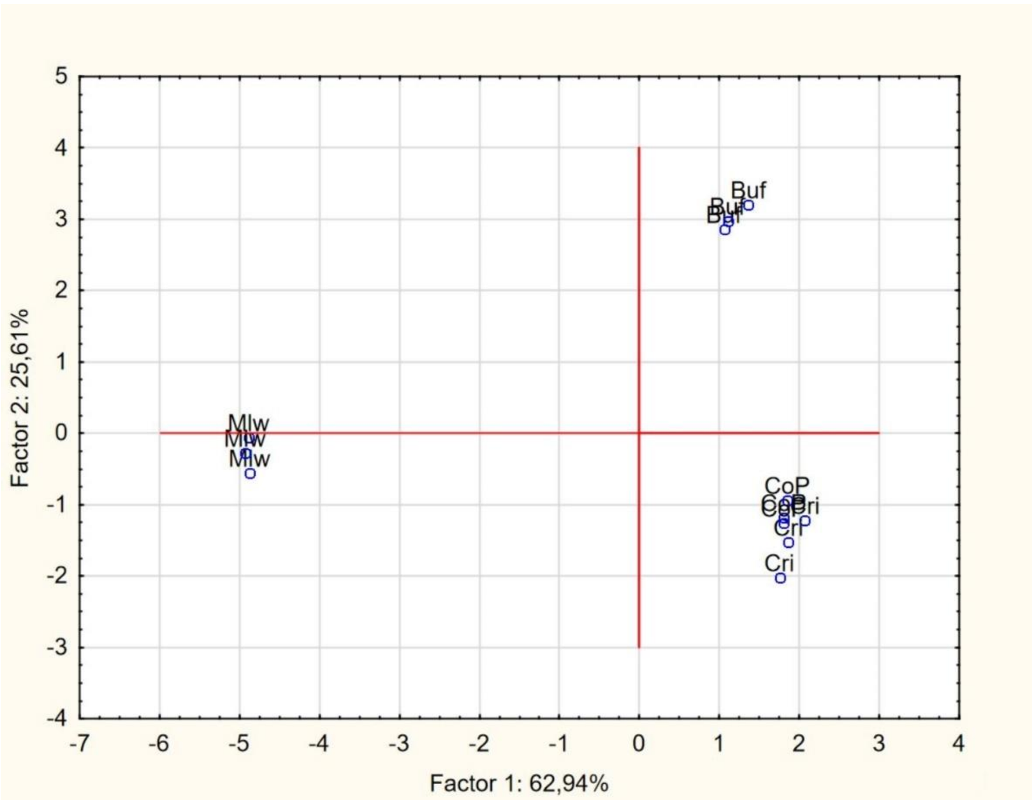

Figure 35 - Projection of the samples on the factor plane (1 X 2)

The Buffalo samples were projected into the 2nd quadrant, which means, they are characteristic by larger amounts of fat, but also of potassium and phophorus. On the other hand, they have lower protein, carbohydrate and manganese content.

The similarity of Cricket and Cricket CoP was confirmed, as these samples formed a cluster in the fourth quadrant. They are typically higher in fibre, zinc, copper and sodium and lower in ash, magnesium, iron and calcium content.

The Mealworm DeF samples created a cluster between the first and third quadrant. These samples are lower in fat content and are especially high in ash, magnesium, iron and calcium content. They are also higher in protein, carbohydrate and possibly manganese content. However, for this sample, it should be mentioned that it has undergone a defatting step and was probably contamined by iron form production equipment, as mentioned in the earlier chapters.

Using PCA analysis, samples of individual insect powders were successfully distinguished, which only confirmed the diversity of individual insect species, and thanks to the projection into the plane of the principal components, it is possible to see in terms of which nutritional parameters they differ the most.

4.11 Sensory analysis

Sensory analysis was performed on 30.6. (9 a.m. – 2 p.m) and on 2.7. 2021 (12 p.m. – 3 p.m.). A total of 24 evaluators participated in the sensory analysis. These were mostly students of the Faculty of Chemistry, Brno University of Technology, and a smaller part were also close relatives. All sensory evaluators were in good health conditions and were non-smokers. Most of them also had a positive relationship to protein preparations, as can be seen in the *Figure* 36 below.

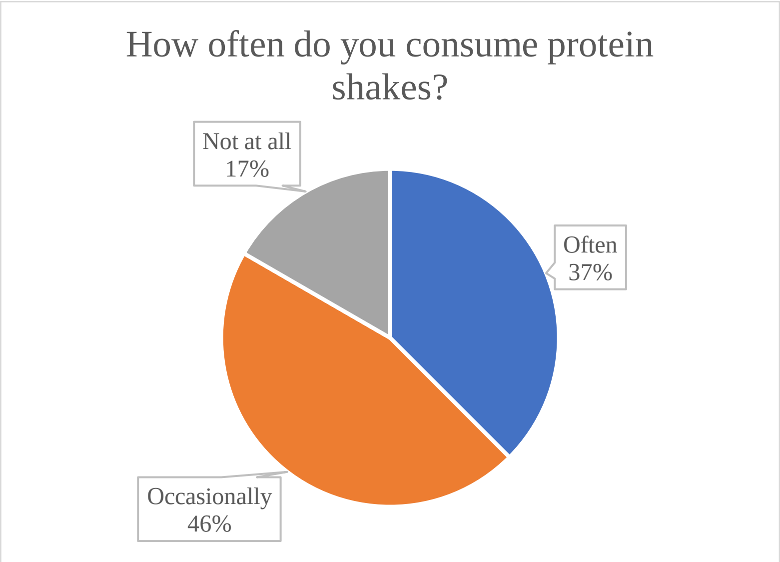

Figure 36 - Sensory analysis – relation to protein shakes

4.11.1 Pleasantness of selected parameters

For each sample and its ratings, the medians were calculated and the values were then plotted into a radar chart.

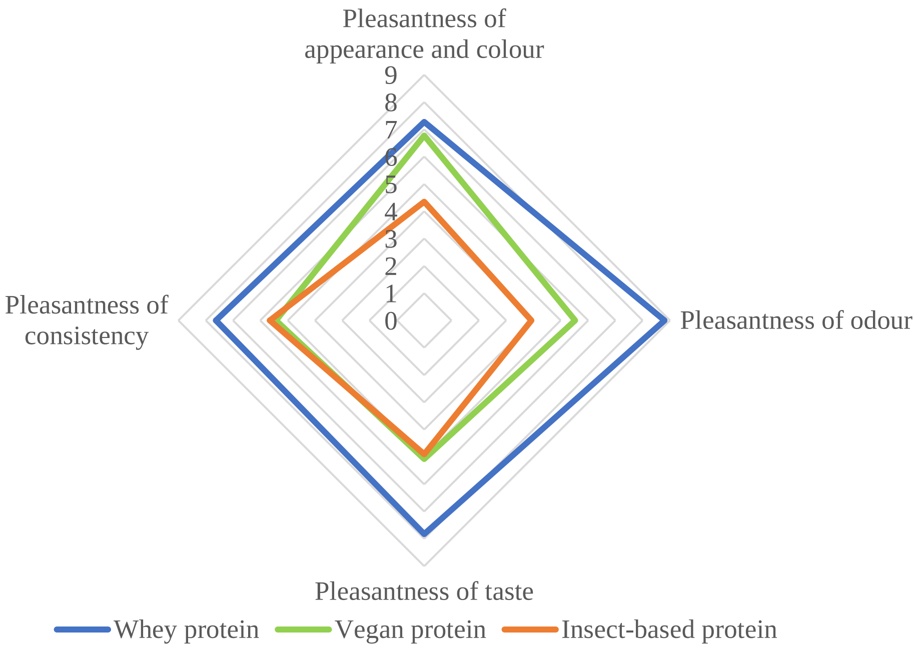

Figure 37 - Comparison of pleasantness of selected parameters

As can be seen in Figure 37, when comparing the individual samples in terms of the pleasantness of several parameters, the whey protein sample was rated as the most pleasant. This is probably due to the fact that most people are accustomed to the milky taste of protein shakes, as these are the most common on the market.

The vegan protein sample was comparable to whey protein in terms of pleasantness appearance and color. In terms of pleasantness of consistency and taste, this sample was comparable to insect-based protein and in terms of pleasantness of odour, it was somewhere between whey and insect-based protein.

The sample of insect-based protein seemed to be the least pleasant and lagged behind especially in terms of pleasantness of appearance and odour. This is mainly due to the fact that the insect powder used in the mixture had relatively coarse particles which were visible in the prepared drink and thus worsened the overall sensory perception. Therefore, for better acceptability, it would be appropriate to reduce its particle size. Regarding the odour, the insect based sample was also rated as the least pleasant. However, it should be emphasized that no aromas were used in it and therefore the chocolate-cinnamon odour was slightly weaker than in the other two competing samples. The product without the use of aromas has a less significant odour, but in the eyes of the customer it can often have a higher added value. Especially recently, there is a growing trend in the food industry to use less additives, flavorings, but also aromas, to reach as natural product as possible.

4.11.2 Profile test of selected flavours

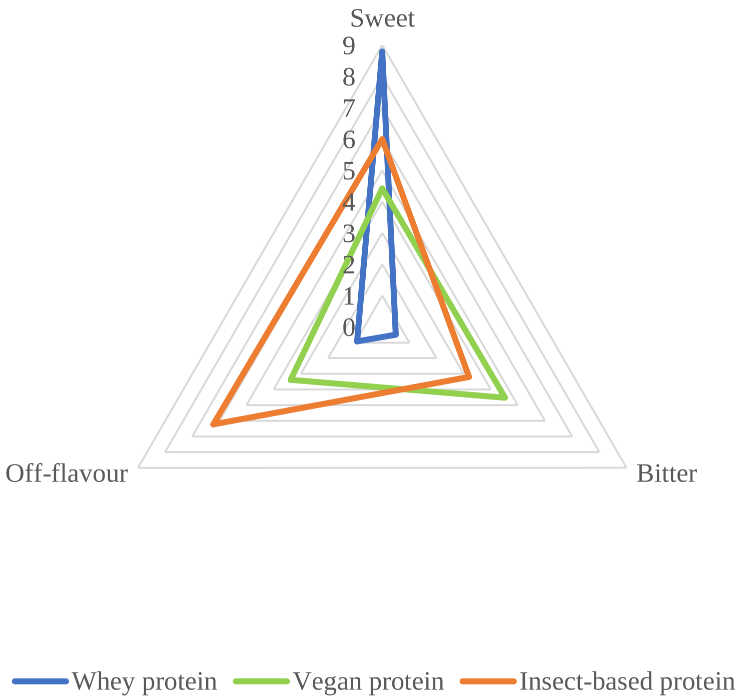

Figure 38 - Comparison of flavour profile

As can be seen in Figure 38, the whey protein sample was evaluated as the sweetest and also as the least bitter one. In this samples. The least off-flavours were also recorded in this sample.

The most bitter sample and also the least sweet sample turned out to be the vegan protein sample. In terms of off-flavours, this sample was evaluated somewhere between the whey protein and the insect-based sample. The off-flavours were most commonly referred to as grassy, astringent, cereal, or earthy.

The insect-based sample was evaluated as moderately sweet, less bitter than vegan protein sample and also as the sample with the most off-flavours. These off-flavours were most commonly described as foreign, unknown, earthy, mushroom-like, sawdust-like, legume-like, but also as nutty or dog-food-like.

4.11.3 Overall acceptability

In the ranking from the tastiest to the least tasty, the samples were scored and then sorted by the number of total points. For each first place the sample received 3 points, for the second place 2 points and for the third place 1 point. The total score for each sample was calculated and the samples were compared.

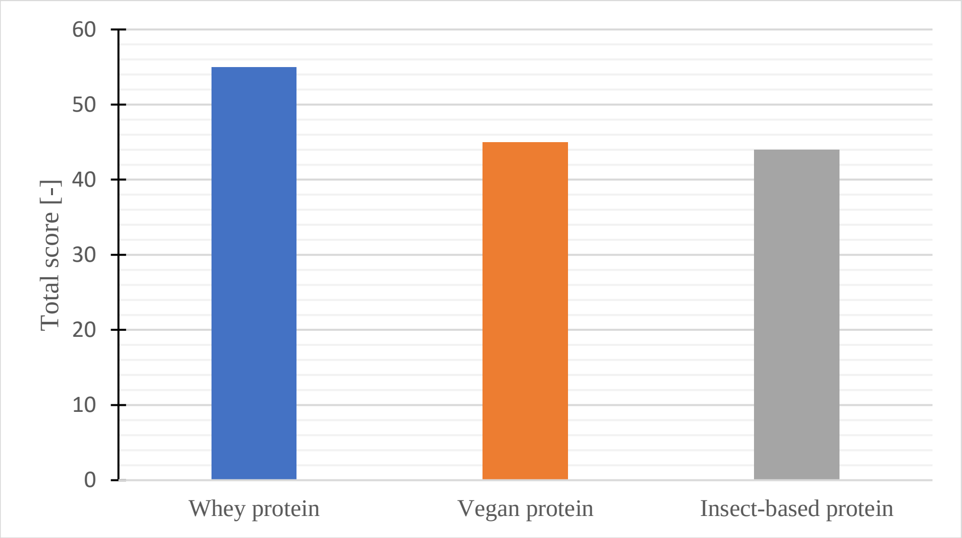

Figure 39 - Comparison of the overall acceptability score

As can be seen in Figure 39, the whey protein sample received the most points (55 in total), which means that it was rated as the most acceptable one.

In the second place, a sample of vegan protein was placed with 45 points and in the third place with only a small difference (44 points in total) was sample with the addition of insects.

Thus, it is clear that with regard to protein shakes, most evaluators like whey protein and are also accustomed to its milky taste. Alternative proteins such as the vegan or insect-based protein are slightly worse in terms of taste and consumer acceptance. However, they are comparable with one another, and in the field of protein beverages, therefore, mixtures containing insects will certainly have their place in the future. Their main advantage over plant proteins is mainly the higher quality of protein and, compared to conventional protein sources, they excel in terms of ecology and sustainability of their production.

4.11.4 Identification of the insect-based sample

In the end of the sensory evaluation, participants were asked to try to guess which sample was the one containing insects. The results are showin in Figure 40.

Up to 83.3 % of the participants identified the insect-based protein sample correctly. This was mainly due to the fact that the insect powder in this sample had a relatively large particle size, which adhered to the surface of the beverage and these particles were automatically considered insects. This was also confirmed in some of the comments participants made in the voluntary notes section, where they mentioned that floating particles were the reason why they thought so. Some participants (12.5 %) incorrectly marked the vegan protein sample as the one containing insects and only one participant marked incorrectly the whey protein sample.

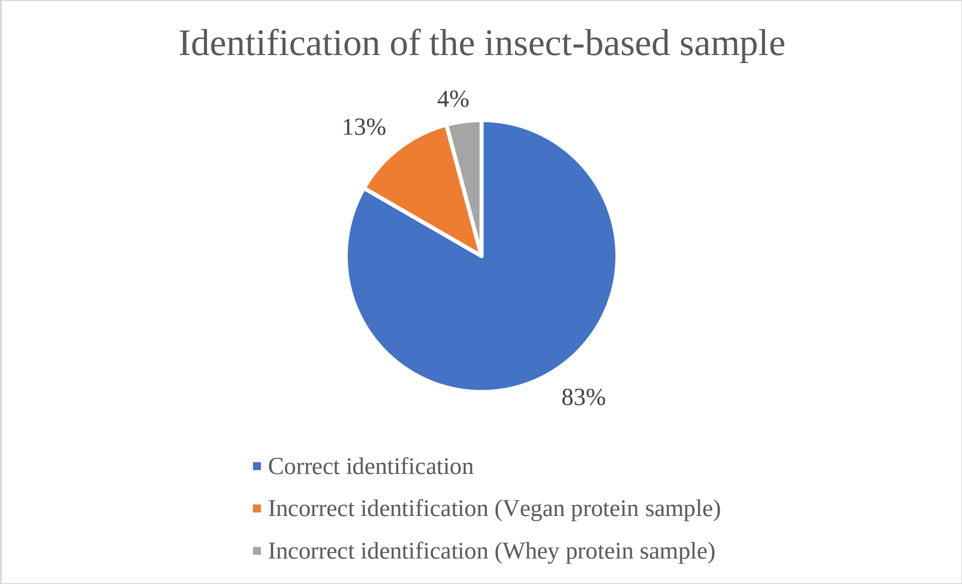

Figure 40 - Identification of the insect-based sample

4.12 Online consumer survey

The online questionnaire survey was conducted on 24.6. to 14.7.2021. In less than 3 weeks, a total of 2,019 responses were collected from the inhabitants of the Czech Republic and Slovakia. Respondents were obtained mainly through social networks.

4.12.1 Characterization of respondents

The majority of respondents were women (up to 78 %) and the average age of the respondent was approximately 24.11 years. The median was 22 years. Regarding the education of the respondents, most of them (59.1%) had a university degree or were at least university students. About 18 % of respondents had the highest level of education attained secondary school and 17.1% were still studying at secondary school. Only 5.8 % of respondents had only a primary school education. The redistribution of respondents in terms of the highest level of education attained is more accurately illustrated in Figure 41.

Regarding lifestyle, as many as 87.6 % of respondents said that they are interested in a healthy lifestyle and try to live a healthy life and about 56.1 % of respondents engage in regular physical activity (at least 4 times a week).

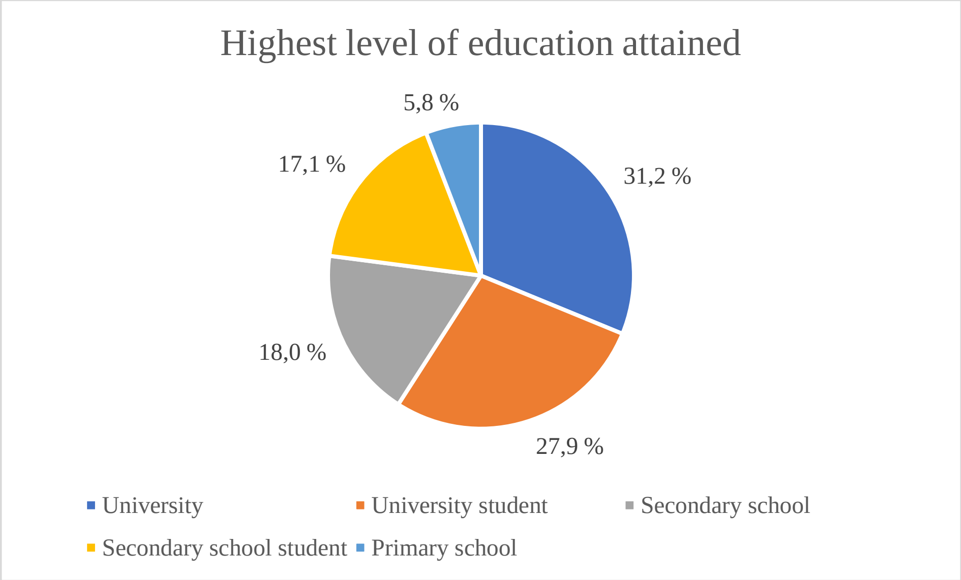

Figure 41 - Education of respondents

Furthermore, respondents were asked if they monitor their daily protein intake. The majority (55.4 %) stated that they do not have much overview of their protein intake, about 34.5 % of respondents monitor their daily protein intake at least approximately and 10.1 % of respondents record their diet and thus protein intake through various food tracking applications.

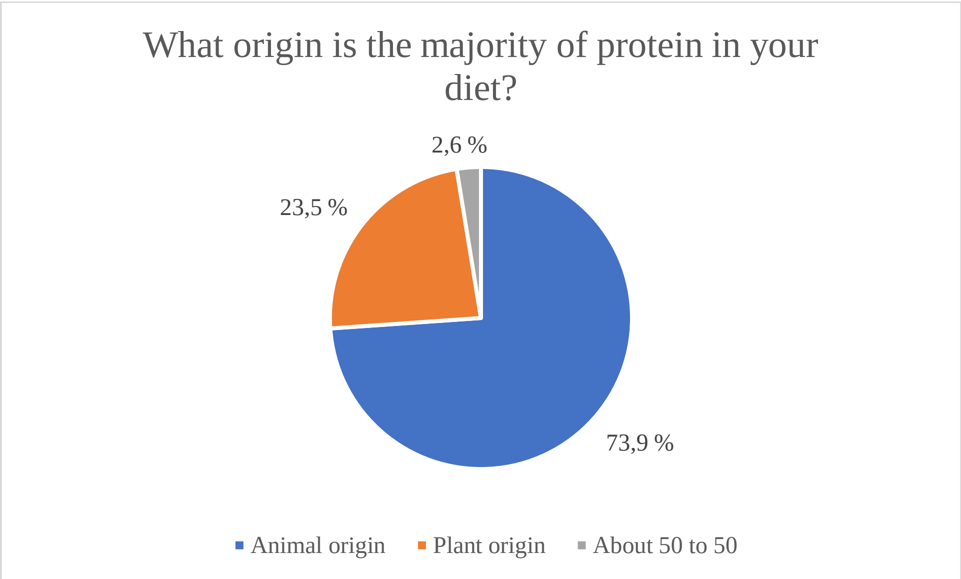

Figure 42 - Protein origin in the diet od respondents

The majority of respondents (up to 73.9%) stated that most of the protein in their diet is of animal origin. About 23.5% said that most came from plant sources and about 2.6% said that the protein in their diet was about half of plant and half of animal origin. The results are also shown visually in Figure 42.

There was another question related to this, which was "How often do you consume meat?". Up to 56.9 % of respondents consume meat a few times a week and up to 25.4 % of respondents even every day. 11.3 % stated that they consume meat a few times a month and 6.4 % do not consume meat at all. The results can also be seen in the Figure 43.

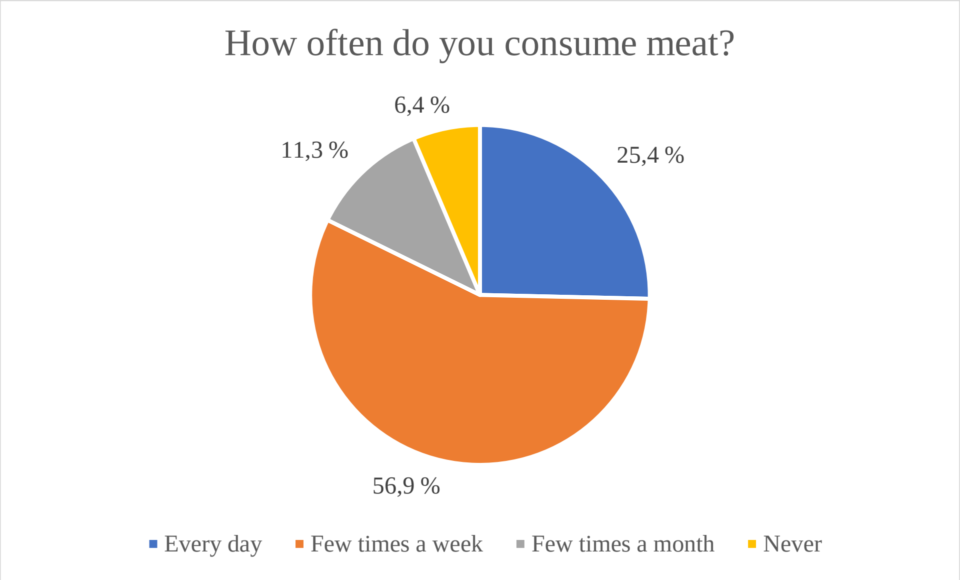

Figure 43 - Frequency of meat consumption

Another question was focused on perception of meat production (see Figure 44).

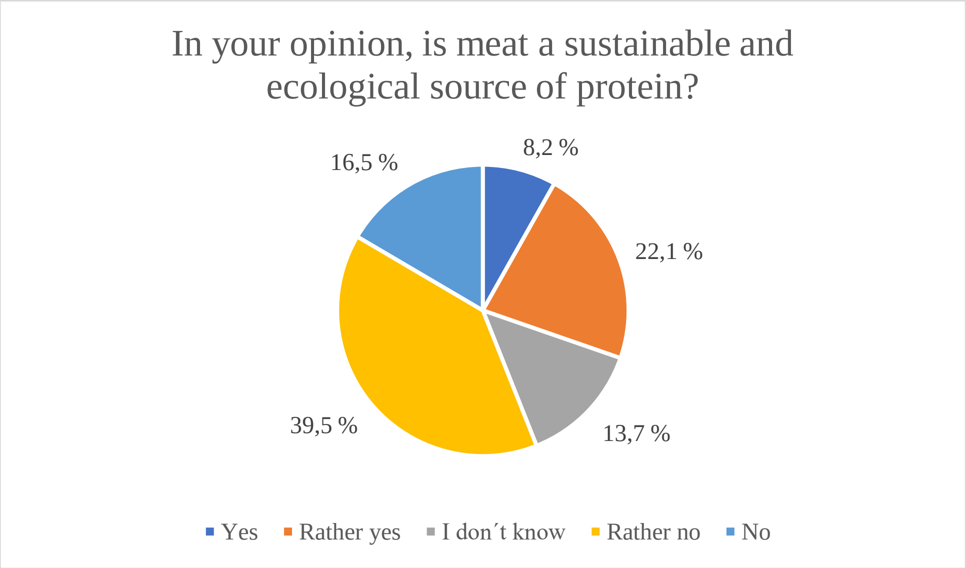

Figure 44 - Perception of meat production

The vast majority of respondents clearly perceive (16.5 %) meat as an enviornmentally unfriendly and unsustainable source of protein, or tend to take this view (39.5 %). About 13.7 % were undecided in this opinion. The remaining 22.1 % are more inclined to believe that the meat is okay in this respect and 8.2 % are convinced about that.

In terms of eating habits, it was also found out whether the respondents include food supplements, or other foods for increasing protein intake, in their diet and, if so, in what form. As can be seen in the *Figure* 45, about 40.7 % stated that they include such foods occasionally, 12.8 % quite often and 46.6 % do not include such foods at all.

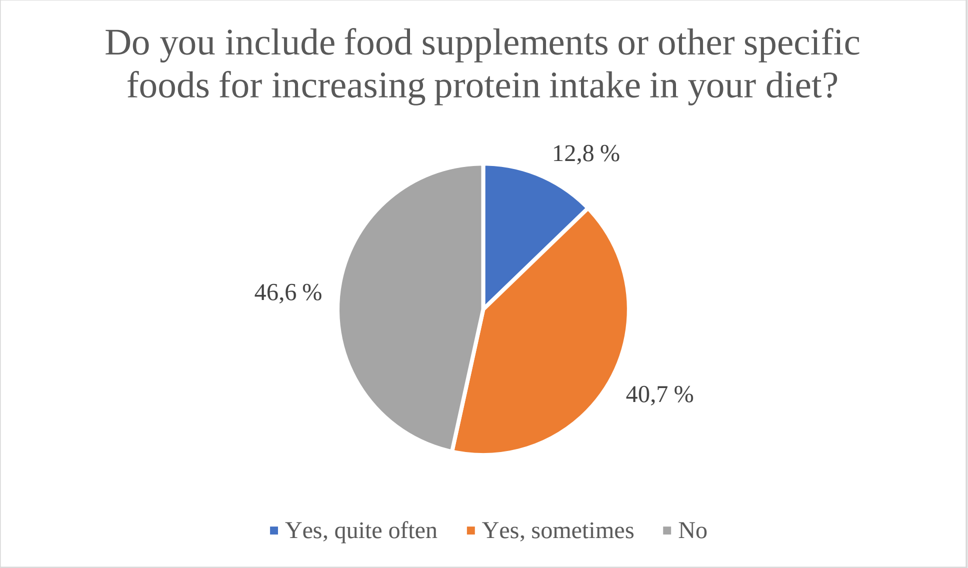

Figure 45 - Use of food supplements

The most common forms of such products included protein powders for beverage preparation (69 %), protein bars (64.9 %), protein puddings (41 %), protein yoghurts (40 %), protein nut butters (18.8%), or finished protein beverages in liquid form (17.1%) which can be seen in the Figure 46 below.

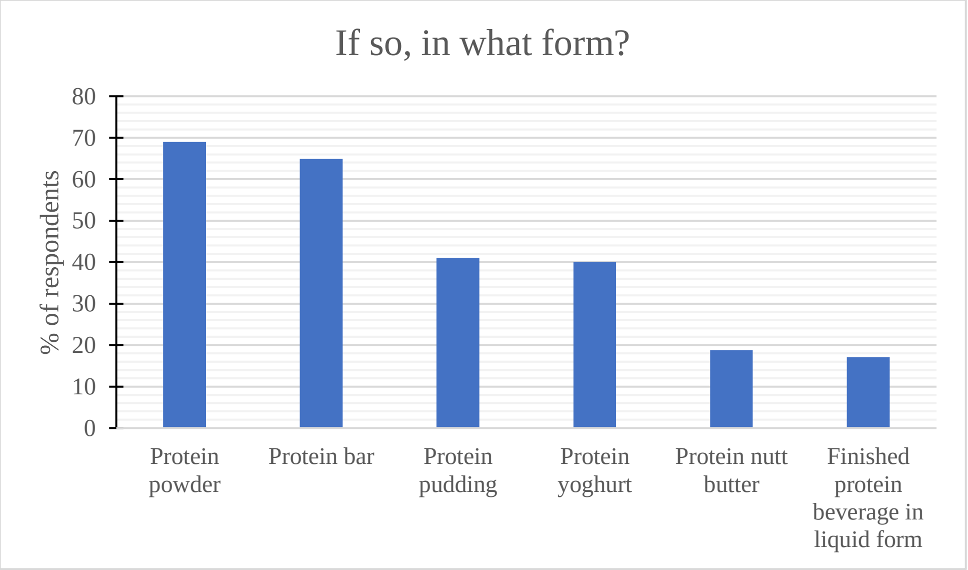

Figure 46 - Type of food supplement / high-protein product used

4.12.2 Knowledge of respondents on the issue of edible insects

The next part of the questionnaire examined the attitude of respondents towards insect consumption. In the first question, they were asked if they had ever heard of the use of insects as food.

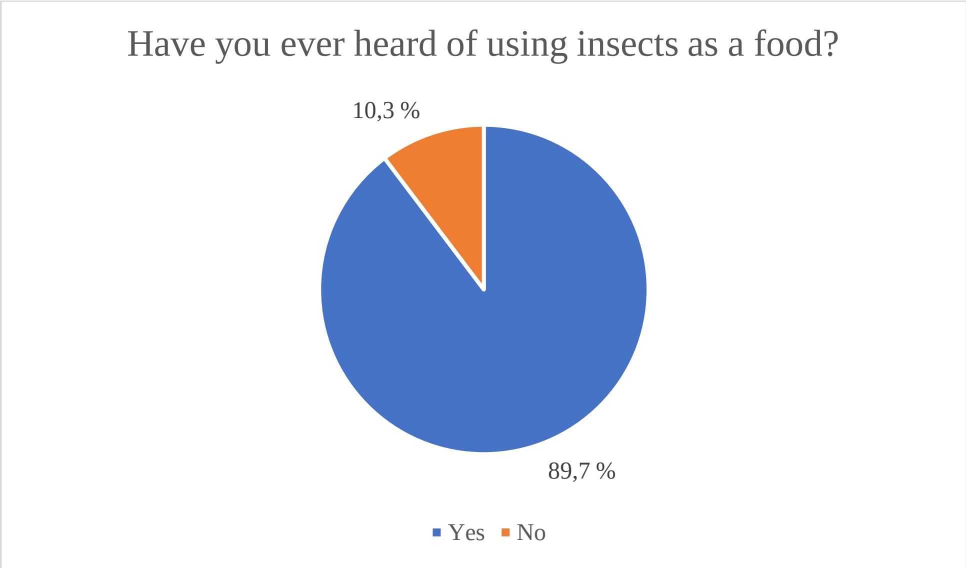

Figure 47 - Awareness of the existence of entomophagy

As many as 89.7 % of respondents said yes. In the following question the participants were asked if they knew that insects are commonly consumed by more than 2 billion people worldwide.

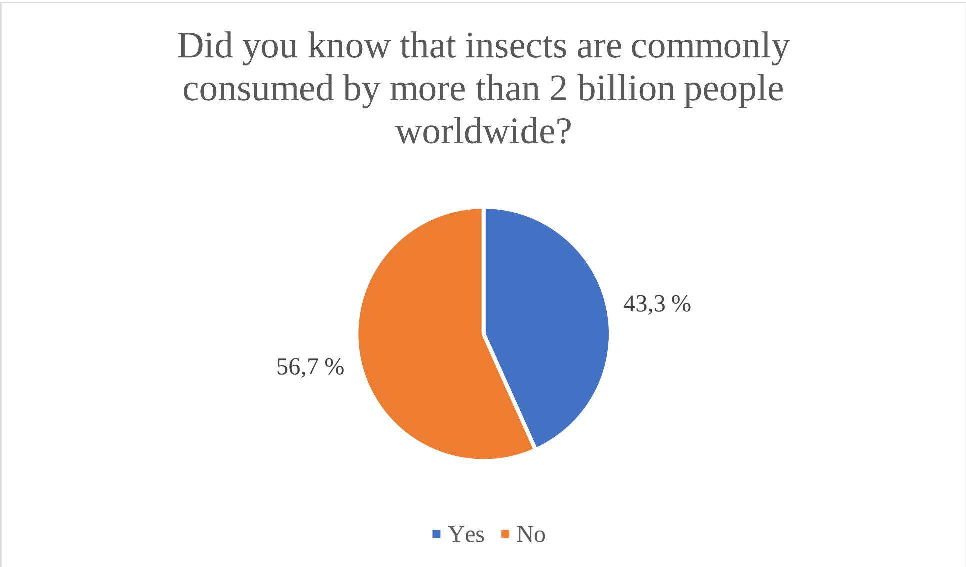

Figure 48 - Knowledge about the spread of entomophagy

As can be seen in Figure 48, as many as 56.7 % said they were not aware of this fact.

4.12.3 Attitude of respondents towards insects as a food

Regarding the attitude towards insect consumption, most of the respondents (44.2 %) stated that they have neutral attitude and do not have a strong opinion on this issue (see Figure 49). 17.3 % stated that they have a positive attitude towards insects and perceive insects as a food with many benefits. On the contrary, 38.5 % of the respondents stated that they have a negative attitude towards insects and that the idea of insect consumption is disgusting and unacceptable to them.

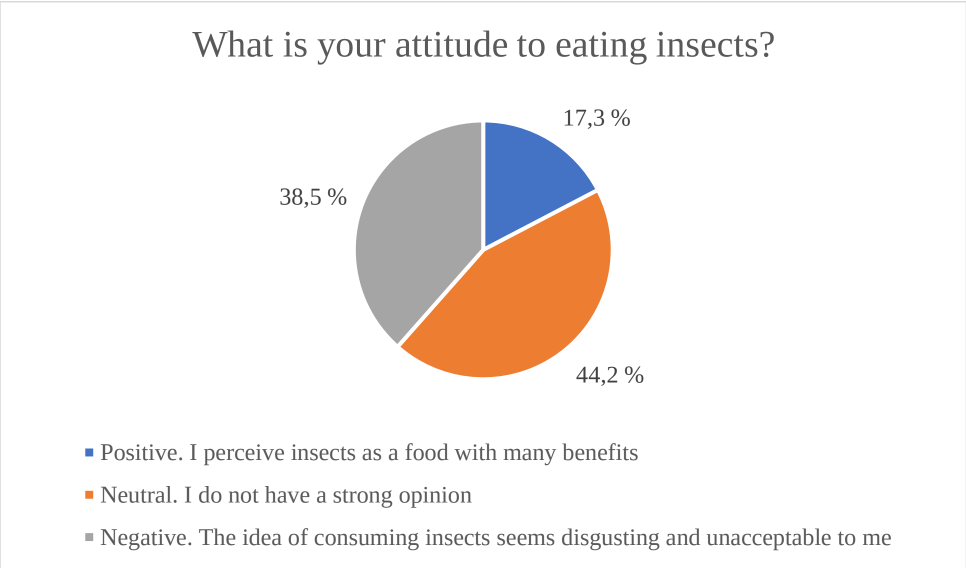

Figure 49 - Attitude towards eating insects

In the following question, participants could have describes in more detail how do they perceive edible insects. They could have checked more than one answer, or add their own. They most often indicated that they perceive it as a gastronomic curiosity (53.4 % of responses), but also as a food of the future (40.4 %). Approximately 37.9 % of people perceive it as something disgusting, 34.7 % of people perceive it as a nutritious food and 12.7 % as a food of the poor. Another important question was how they perceive insects in terms of safety.

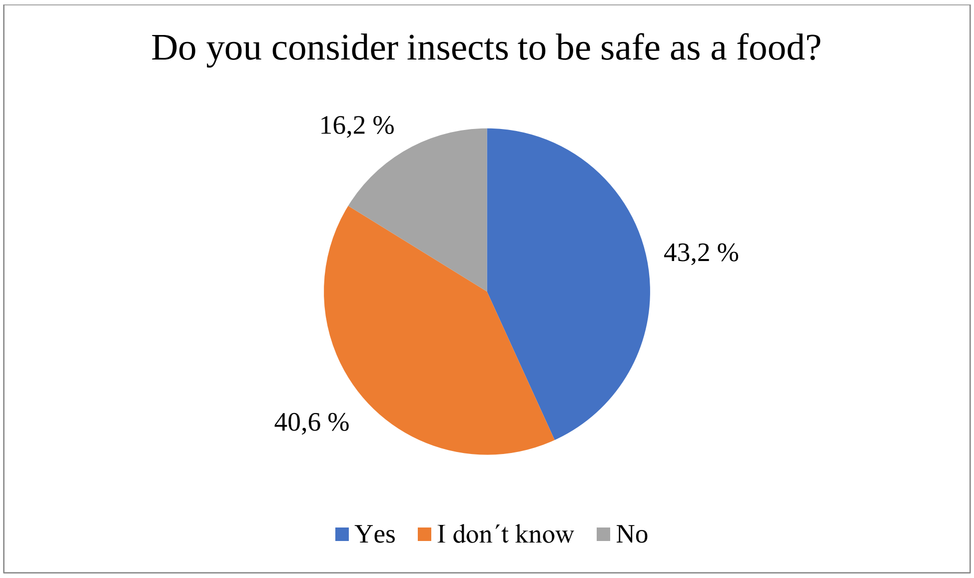

Figure 50 - Perception of safety of entomophagy

As can be seen from Figure 50, 43.2 % consider it a safe food, up to 40.6 % of respondents was not able to comment on the safety of edible insects and 16.2 % do not consider it a safe food at all. Regarding the nutritional value, as many as 69.7 % of respondents consider insects to be a nutritionally rich food, as can be seen in the Figure 51 below.

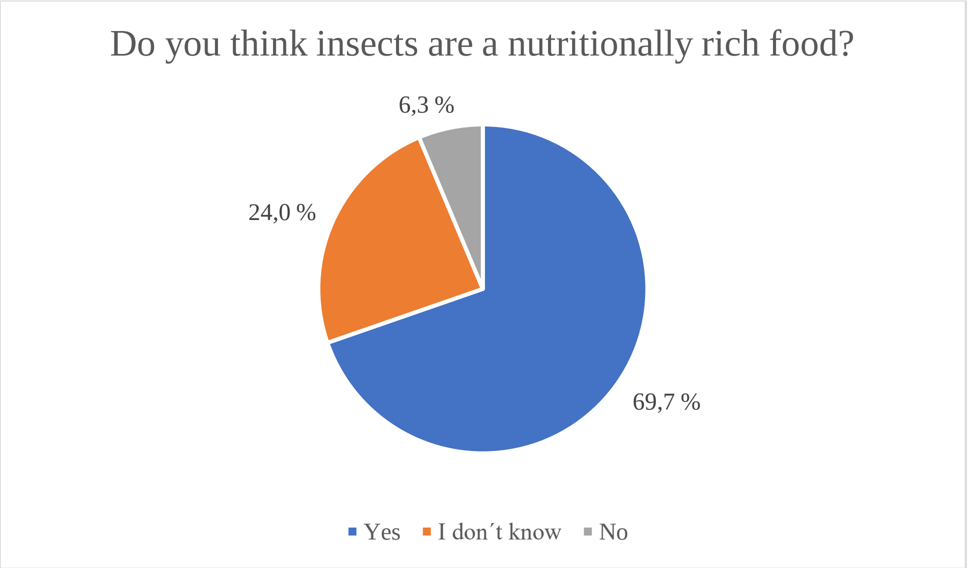

Figure 51- Perception of nutritional value of insects

The remaining 24 % do not know if the insect are nutritious and only 6.3 % of respondents do not consider the insect to be a nutritionally rich food. Another question concerned the perception of edible insect production for food purposes (see Figure 52).

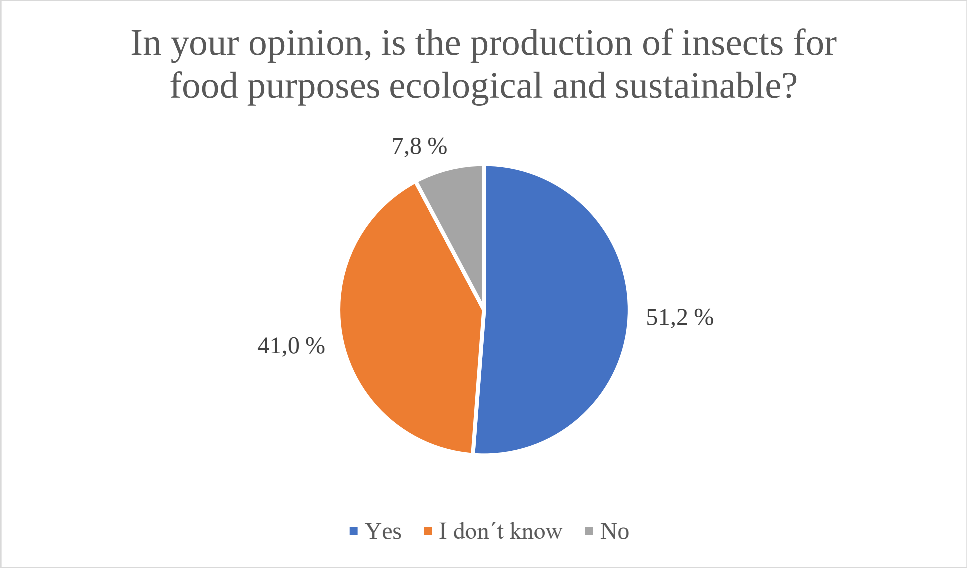

Figure 52 - Perception of ecology and sustainability of insects production

More than half of the respondents (51.2 %) perceive the production of edible insects as ecological and sustainable. 41 % do not know how insect production is in this respect and 7.8 % do not consider edible insect production to be environmentally friendly and sustainable.

4.12.4 Experience with insect consumption and willingness to try insects

In the first question of this section, respondents were asked if they had ever tasted insects in any form.

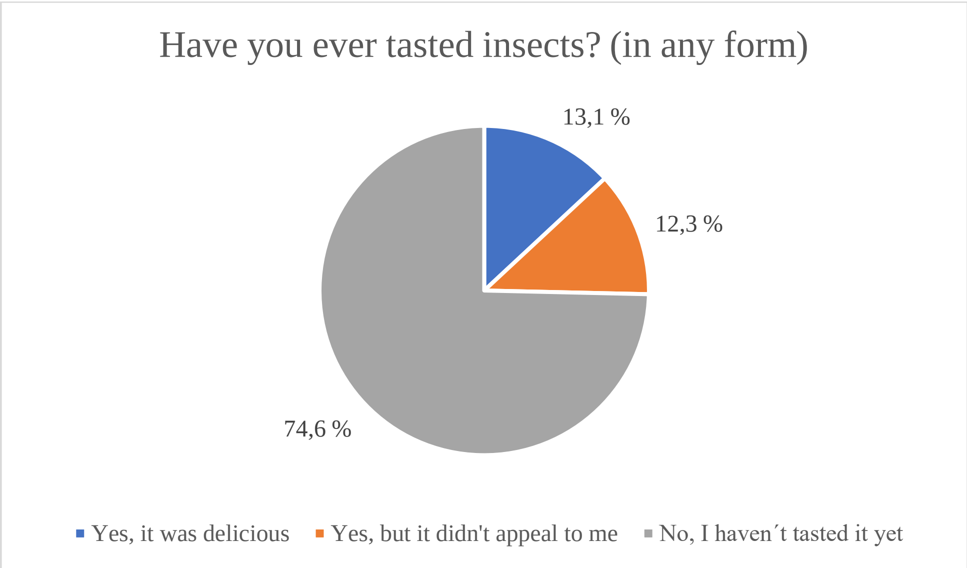

Figure 53 - Experience with tasting insects

As many as three-quarters (74.6 %) of respondents had never tasted insects before. Of the remaining respondents who had already tried insects, about half (13.1 %) considered it a tasty experience and the other half (12.3 %) said that it did not appeal to them too much. Those who had already tasted the insects had to answer the question in what form (see Figure 54).

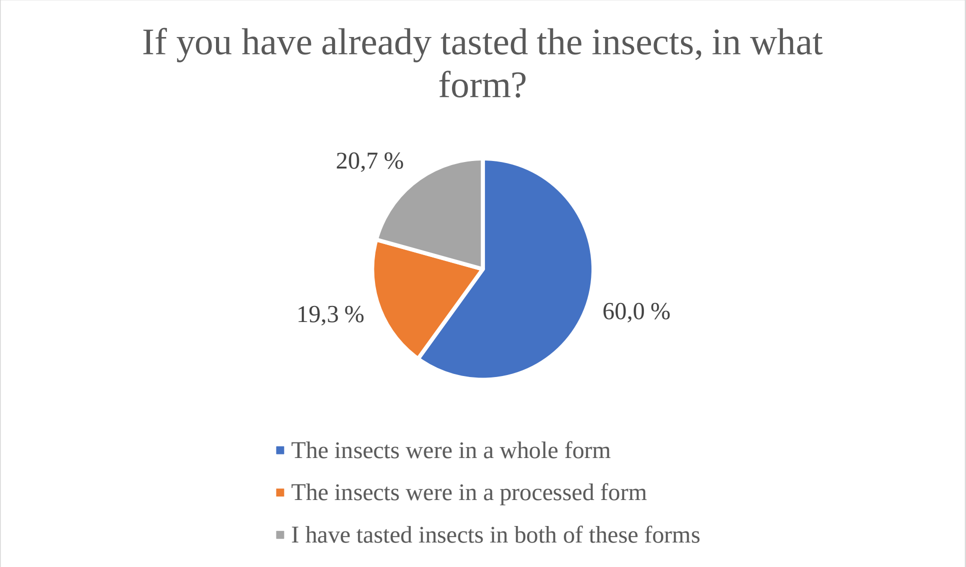

Figure 54 - Form of insects tasted

The majority (60 %) tasted the insects in a whole form. Approximately 19.3 % tasted insects in a processed form, for example incorporated in food in powder form, and 20.7 % had the honor of trying insects in both of these forms.

People who have not tasted insects yet were then asked how likely it is that they would try food containing edible insects sometimes in the future.

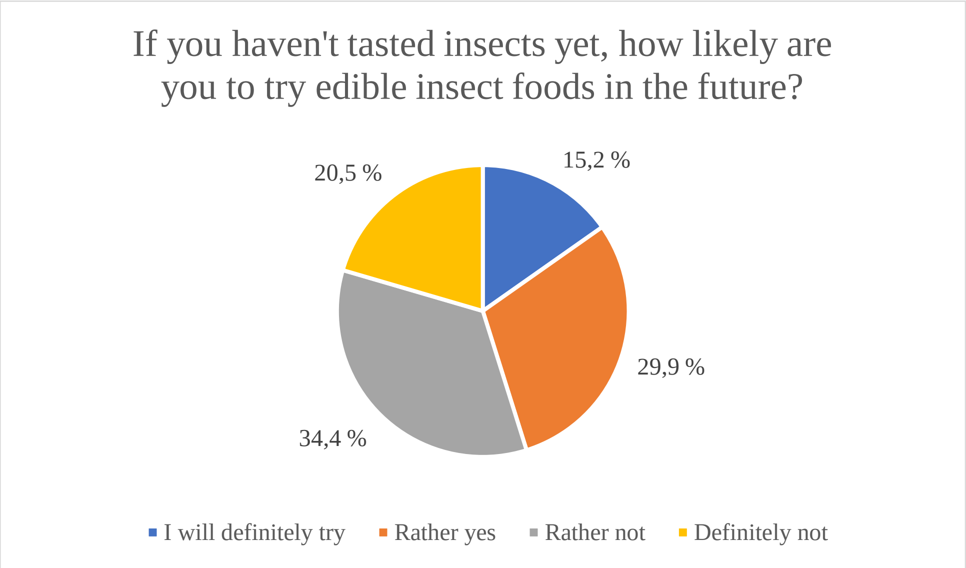

Figure 55 - Willingness to try insects

From Figure 55 is clear, that 15.2 % would definitely try insects in the future. Another 29.9 % also favoured this option. About 34.4 % of respondents said rather not and 20.5 % said certainly not. Another important question was in what form the consumption of insects seems to be the most acceptable to the respondents.

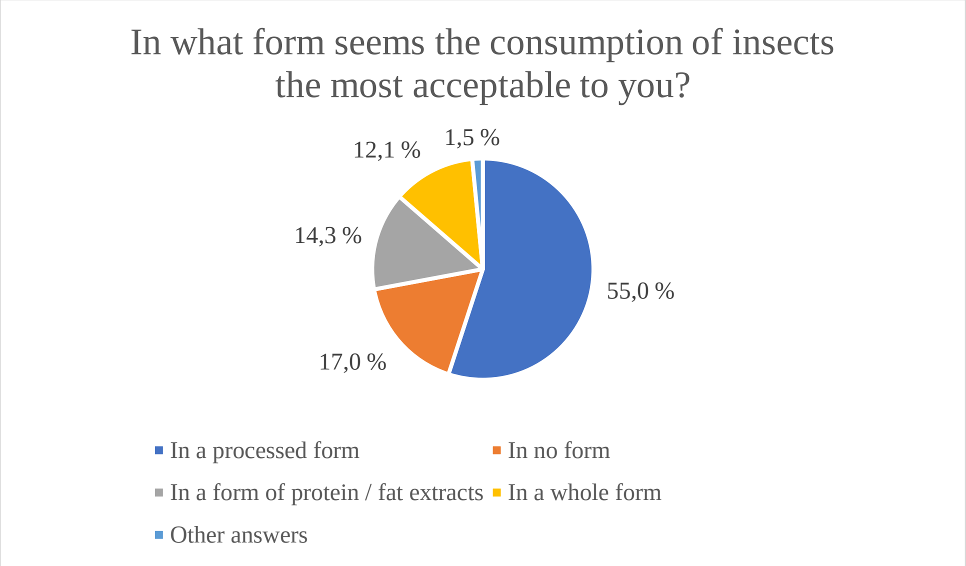

Figure 56 - Acceptability of the various forms of insects

More than half (55 %) indicated that they find the consumption of insects most acceptable in a processed form, for example in the form of powder, where individual parts of insects are not visible. 14.3 % of respondents see protein or fat extracts of insects as the most acceptable form and 12.1 % do not see a problem with insects in the whole form. However, as many as 17% of respondents did not perceive the consumption of insects as acceptable at all, and therefore marked the answer "in no form".

However, it is one thing to try insects as a curiosity, another thing is to consume them regularly like any other food. Another question, therefore, examined whether the survey participants could have imagined including insects in any form in their diet on a regular basis (see Figure 57).

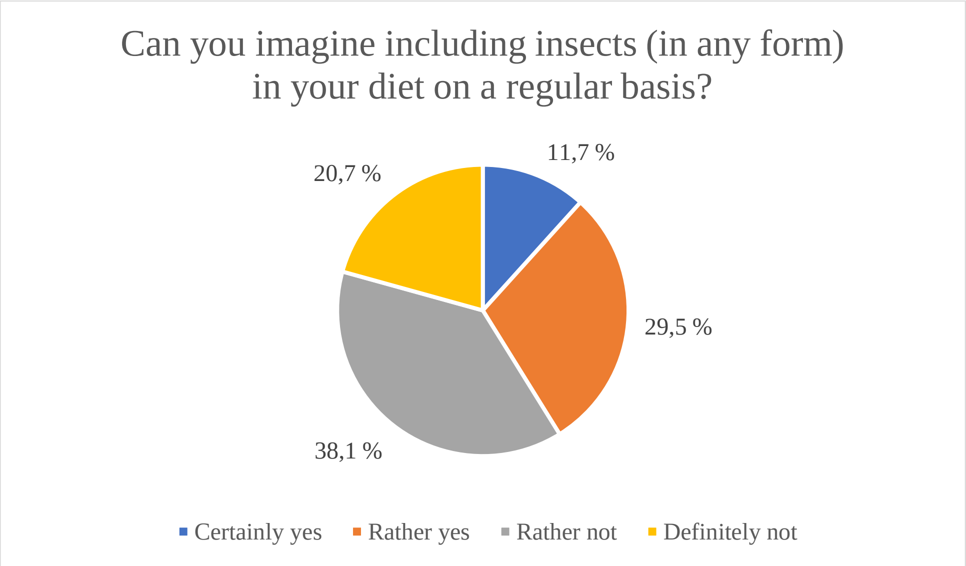

Figure 57 - The idea of regular consumption of insects

11.7 % of respondents said certainly yes and 29.5 % said rather yes. On the contrary, up to 38.1 % cannot imagine it much and 20.7 % said that they cannot imagine it at all.

There are many obstacles to insect consumption in areas where this practice is not typical. Respondents were therefore asked what they think are the biggest obstacles to trying insects as a food. It was possible to mark more than one answer, or add own answer.

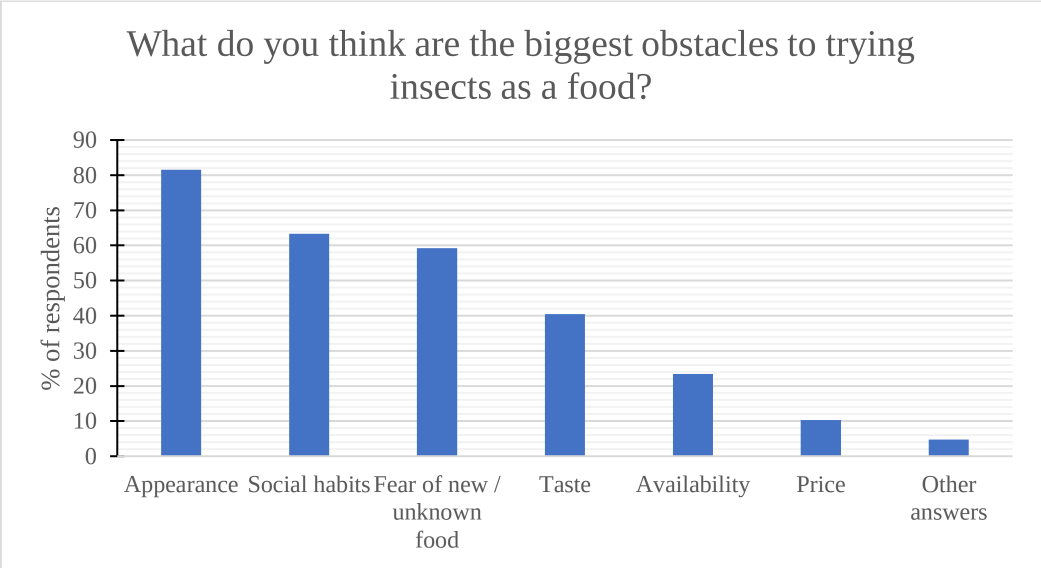

Figure 58 - Perception of obstacles in entomophagy

As can be seen in the Figure 58 above, the most common answers included appearance, social habits, fear of new or unknown food, taste, availability, but also price. Other responses included ethical reasons or concerns about the safety of insects as a food.

Then followed the question of which food product respondents perceive as suitable for the addition of insects in the form of powder. It was also possible to mark multiple answers or add own answer. As can be seen in Figure 59, the most common responses were protein powder, protein bar, pasta, salty snack, bread, cookies, granola or chocolate. Some of the respondents marked the answer "no food product" and in the other answers the most common products mentioned were meat substitutes.

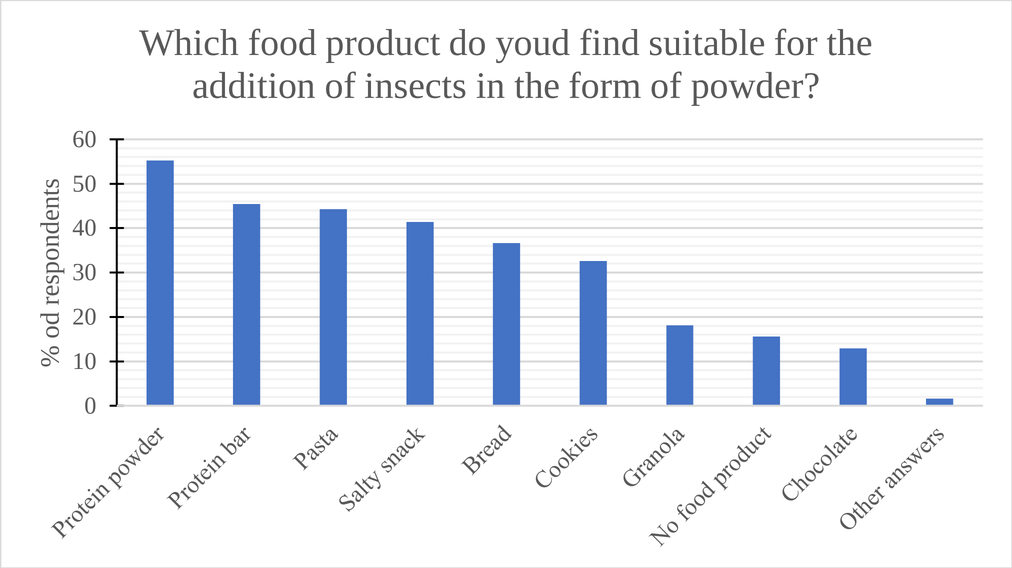

Figure 59 - Products suitable for addition of insect powders

In the end, participants were asked if they would be willing to try a protein product (a powder for making a high-protein drink) containing edible insects.

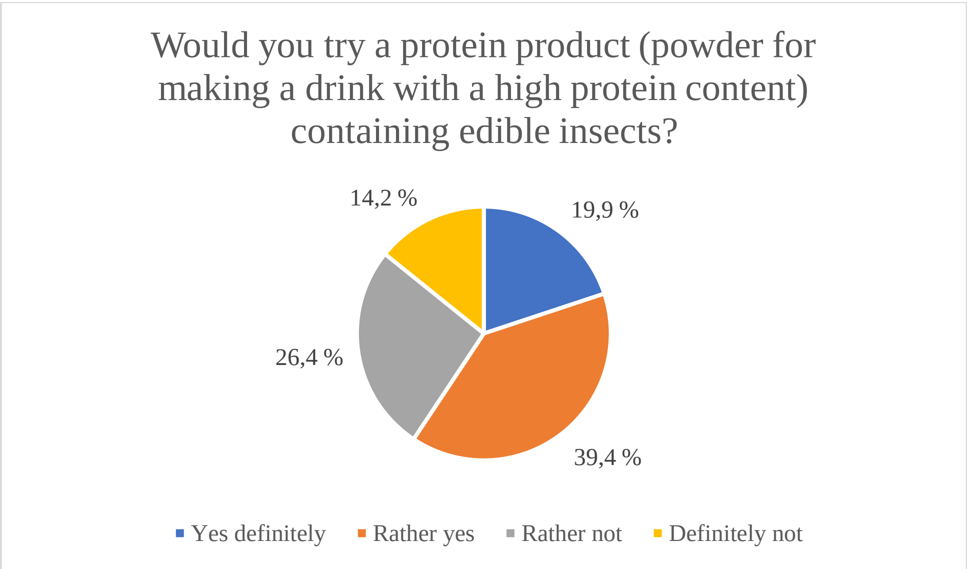

Figure 60 - Wilingness to try an insect-based protein product

As can be seen from Figure 60, almost two thirds of respondents were positive about this question and would like to try such an insect-containing product.

An important aspect in the insect food segment is also the price. As this food sector is still in its infancy, the price of insects as a raw food material is still relatively high, and respondents were therefore asked the last question about the acceptability of the higher price of a product containing edible insects.

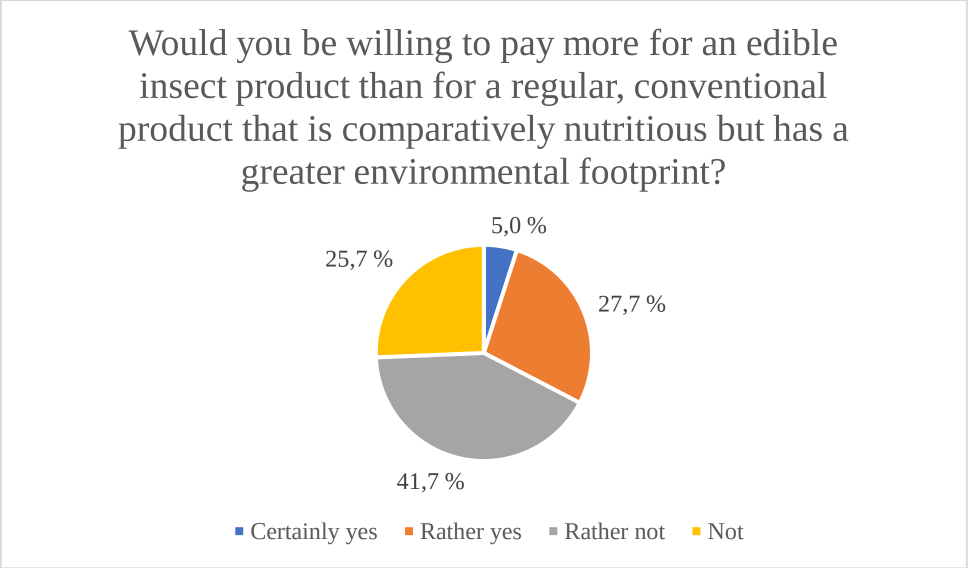

Figure 61 - Willingness to pay more for an insect-based product

Only about one third of respondents would be willing to pay extra for such a product compared to a conventional competing product. For the remaining two thirds, the price of the product is a more important factor, despite the greater ecological footprint of such product. The exact results can be seen in the Figure 61.

5. CONCLUSION

The aim of this thesis was to summarize the current state of edible insects in the food industry and to describe selected species of edible insects in more detail.

Another aim was the proposal of food product with the use of insect powder. A unique protein mixture with the addition of 20 % of the lesser mealworm powder was developed and was subjected to the analysis of several nutritional parameters together with 4 samples of insect powders. It was found out, that all insect powders and also product with the addition of insect powder, were high in protein and in several minerals. The protein quality, assessed in terms of EAA composition was also high in these powders, meeting almost all the AA requirements for complete protein from WHO [19]. Regarding the total lipid content, some insect powders contained also a significant amounts of fat which was typically high in palmitic (C16:0), linoleic (C18:2, n-6), oleic (C18:1, n-9) and stearic (C18:0) acid. The FA profile of analysed insect powders werer very similar. The Mealworm DeF sample was a defatted version of mealworm powder, which has lost about 30 % of the original fat. This defatting step can be advantageously used in several insect powders in order to reduce their total energy content and increasing all other nutritional parameters. Application of such powders into high-protein or low-fat food products might be a great option. The mineral content of all analysed powders was also interesting. Depending on the type of insect species, 100 g of edible insects could provide with sufficient daily amounts of phosphorus, iron, zinc, manganese or copper. Some powders can also be a great source of magnesium, potassium and calcium. For targeted supplementation of specific minerals, the certain edible insect species should be choosed. Regarding the safety of analysed powders, no lead or cadmium was found in them, which confirms their safety in terms of possible heavy metals contamination. Some powders has also been shown as a great source of dietary fibre, consisting mainly of chitin.

The Cricket CoP sample represented a by-product from the production of cricket powder (Cricket sample) with bigger particle size. This waste product was still very valuable in terms of nutrition and could be probably used in the feed sector or also as a food ingredient with high dietary fibre content but also protein content.

The Blend sample, representing a newly developed high-protein product with the addition of lesser mealworm powder, has been shown to have a great nutritional parameters to be used as a sport supplement with high protein content.

Using the PCA analysis, samples of individual insect powders were successfully distinguished, which only confirmed the diversity of individual insect species, and thanks to the projection into the plane of the principal components, it was possible to see in terms of which nutritional parameters they differ the most.

In the sensory analysis, the newly developed product, Blend sample, was compared to conventional high-protein products. It was found out, that most evaluators were accustomed to classic protein shake from dairy sources. Alternative vegan protein powder together with insect-based sample were rated slightly worse in terms of taste and overall acceptance. The main drawback that would need to be addressed specifically with this lesser mealworm powder used is the insect particles size, which has often been considered unpleasant. However, the insect-based and vegan sample were comparable with one another in terms of sensory analysis, and in the field of protein beverages, therefore, mixtures containing insects will certainly have their place in the future. Their main advantage over plant proteins is the higher quality of protein and, compared to conventional animal protein sources, they excel in terms of ecology and sustainability of their production.

In an online consumer survey on a sample of 2,019 participants, it has been found out, that inhabitants of Czech and Slovakia perceive the existence of entomophagy, although they still lack a lot of knowledge about this alternative protein and food source. Only a quarter of respondents already had a real experience with entomophagy. Regarding the form of edible insects, most of the respondent think that the processed form of insects is the most acceptable one and incorporation into food products like protein powders, protein bars, pasta, salty snacks, bread or cookies, seems like a best option. Also, the biggest obstacles to practice of entomophagy were identified. Specifically, this includes factors like appearance, social habits, fear of unknown food, but also taste, availability on the market and price.

All things considered, edible insects represent a valuable food material with many nutritional but also environmental benefits compared to conventional animal protein sources. Incorporation of edible insects into various food products can definitely improve their nutritional value.

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7. LIST OF ABBREVIATIONS

AA – Amino Acid

DIAAS – Digestible Indispensable Amino Acid Score

DRI – Daily Reference Intake

EAA – Essential Amino Acid

EFSA – Europe Food Safety Authority

EU – European Union

FA – Fatty Acid

FAME – Fatty Acid Methyl Ester

FAO – Food and Agriculture Organization of the United Nations

GC – Gas Chromatography

GC-FID – Gas Chromatography with Flame Ionizastion Detector

ICP-OES – Inducitvely Couples Plasma – Optical Emission Spectrometry

MUFA – MonoUnsaturated Fatty Acid

MPB – Muscle Protein Breakdown

MPS – Muscle Protein Synthesis

ND – Not Detected

NFE – Nitrogen-Free Extract

NPU – Net Protein Utilization

PCA – Principal Component Analysis

PDCAAS – Protein Digestibility Corrected Amino Acid Score

PUFA – PolyUnsaturated Fatty Acid

SFA – Saturated Fatty Acid

UV-VIS – UltraViolet-VIsible

WHO – World Health Organization