DEPARTMENT OF AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCES

DEGREE COURSE: FOOD AND BEVERAGE INNOVATION AND MANAGEMENT

EVALUATION OF THE VOLATILE FRACTION AND COLORIMETRIC CHARACTERISTICS OF INSECT ENRICHED FLATBREAD

VALUTAZIONE DELLA FRAZIONE DEI VOLATILI E DELLE CARATTERISTICHE COLORIMETRICHE DI FLATBREAD ARRICCHITO CON FARINA DI INSETTI

TYPE OF DISSERTATION: Research

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1 – INTRODUCTION

1.1 – Introduction to edible insects

Over 2000 species of insects are eaten worldwide, where the majority are beetles (31%), caterpillars (18%), wasps, bees and ants (15%), crickets, grasshoppers and locusts (13%), and others (Jongema et al., 2015). Although in the Western world, until recently, insects were never considered as food, because of several factors:

- Insects in temperate zones are smaller than in the tropics
- Occurrence of insects is less clumped
- Unavailability in wintertime
- Western negative attitude towards insects (Looy et al., 2014)

The negative attitude towards insects is not justified, indeed less than 0.2% of the total estimated insect species in the world are harmful for plants, man and animals (Van Lenteren et al., 2006). However, the demand for animal protein is expected to increase globally by 76% from 2005 to 2050 (Alexandratos & Bruinsma, 2012), while the utilizable agricultural area for livestock production is already more than two-thirds of all agricultural land (68%) as reported on FAOSTAT, therefore actions must be taken to produce more animal proteins. People attitude is gradually changing, partly due to the emphasis on sustainable diets (Burlingame et al., 2012). More and more people are questioning the consumption of meat, with high criticisms towards the sustainability of its production (Tilman et al., 2014) and with great preoccupation towards greenhouse gas emissions and ammonia emissions (Gerber et al., 2013). In order to meet the 2°C temperature-increase target set by the United Nations Framework Convention on Climate Change, mitigation strategies in livestock production will not be sufficient, but what will be needed are dietary changes (Eisler et al., 2014; UNFCCC 2010; Hedenus et al., 2014). Insects are an interesting alternative considering the low emission of greenhouse gases, the small land area needed to produce 1 kg of protein, their efficient feed conversion efficiencies, and their ability to convert organic side streams in high value protein products (Oonincx et al., 2010; Abbasi et al., 2015). Apart from insects for human consumption, also important are insects as feed for pets, livestock and fish, where for this aim the most studied species are the black soldier fly *Hermetia illuscens*, the common housefly *Musca domestica* and mealworms, locusts/grasshoppers/crickets and silkworms. The advantage is that these insects for feed could be reared on organic side streams of other productions (FAO, 2011). In 2013, for the first time in history, more fish for human consumption have originated from farms than from wild gathering and the production of fish is expected to grow by 24% from 2010 to 2030 (Msangi et al., 2013). This rapid growth of aquaculture means that the sector requires growing volumes of feed, indeed soya-based feed isn't appropriate for many reasons including high land use, limited aminoacidic profiles, anti-nutritional factors and competition with use for human consumption. Therefore, using insects for aquafeed could be an effective solution to meet the increase in demand of fish feed (Lock et al., 2015). Even though most insects in tropical countries are collected from nature, efforts are made to farm the insects. In Thailand, 20000 domestic cricket farms produce an average of 7500 metric tons of insects per year for home consumption and for the market. Indeed, farming insect is an activity that's growing quite a lot in Thailand, offering significant income and livelihood opportunities for tens of thousands of Thai people (Hanboonsong et al., 2013). In a similar way, to improve the health status of people in Cambodia, the cricket *Teleogryllus testaceus* is mass produced as a sustainable, cost-effective and high-quality alternative source of protein to traditional livestock. To do this, residues of typical Cambodian production are being employed as feed for the crickets (Caparros et al., 2016).

1.2 – The circular economy

According to the International Platform of Insects as Food and Feed (IPIFF), 1 billion US dollars have been invested in the European insect industry with exciting results. For example, in 2019, its members produced more than 6000 tons of insect protein (https://ipiff.org/). Previsions say that the worldwide insect market will increase at a compound annual growth rate of 26.5% from 2020 to 2027 to reach 4.63 billion US dollars by 2027. Apart from the common economic advantages associated with insect mass production, the insect production ties well with the circular economy concept. The circular economy concept can offer tools to enhance and optimize the sustainability of a food system. A sustainable food cycle can have five stages that are, food production, processing, distribution, food consumption and food waste management. If each of these steps is managed properly, we can achieve overall sustainability in the food cycle (Wunderlich et al., 2018). The circular economy concept aims to overcome the linear pattern of production and consumption by adopting strategies of a circular or "closing the loop" system in industrial production systems (Maina et al., 2017). Using the food waste for rearing insects provides an attractive key for closing the loop of the food value chain (Image 1).

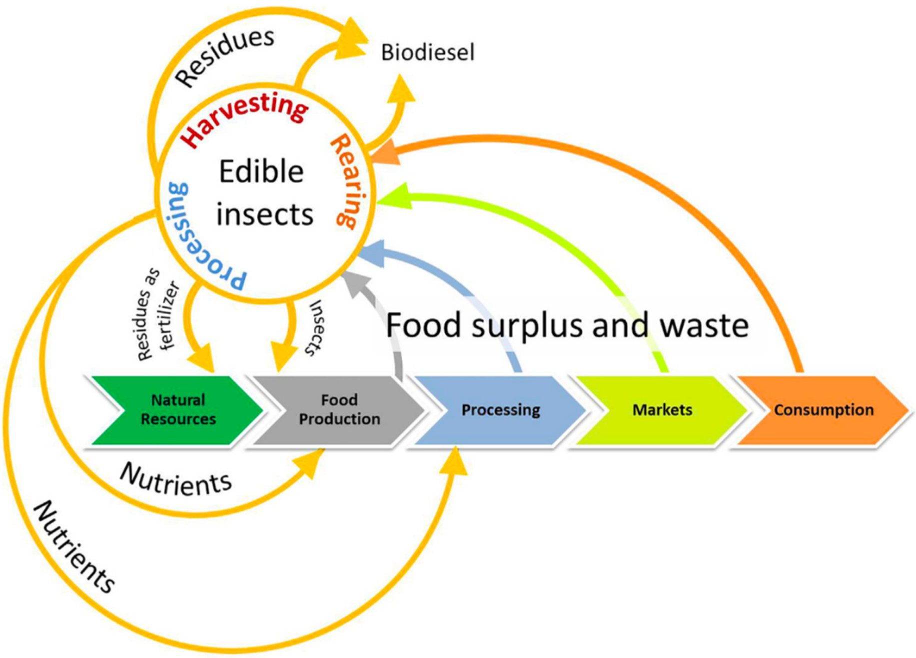


Image 1 - Circular economy in edible insect production

Currently there are three types of insect farms, farms that purchase eggs or small larvae from a supplier and rearing them to the harvestable stage, farms covering the entire production process, from eggs to dried insects, and farms that cover both production and processing, such as extracting proteins or the chitin (Niyonsaba et al., 2021). Advantage of farming insects is the possibility to utilize organic side streams of other productions to feed the insect. Also, manure could be possibly used to feed insects, but this is not currently approved in the EU, United States, or other Western nations, even though the potential is high (Cammack et al., 2021). Discussions are also arising in relation to the possibility to use insects, in combination with microbes, to recycle other waste streams than those commonly used and not yet considered as substrates, such as plastics (Zhang et al., 2021). It has been demonstrated for example that *Tenebrio* species and their gut microbes are able to biodegrade polystyrene (Peng et al., 2019). The insects mass produced is just one of the primary products manufactured. The other is the digested waste mixed with insect frass that remains. These wastes have high similarities to inorganic fertilizers, however, unlike traditional inorganic fertilizers, the waste resulting from mass producing insects contains valuable additives, mainly chitin. Chitin, a key component of the exoskeleton of insects, could promote plant health by reducing the likelihood of disease. But not all insect waste is the same, differences between species, but also diets provided, influence the characteristics of the waste. Also, the levels of bioavailable phosphorous in insect frass are high, which is important considering the limited availability of phosphorous for agriculture in the future (Chavez et al., 2021). The concept of the land application of insect frass enables the reintroduction of insect rearing side stream back into the food production chain, thus is consistent with the circular economy's principles. Further, a new opportunity for the inclusion of insect frass in the production of biogas has also shown promising results in a cost efficient and sustainable manner. Also, taking advantage of the high fat content of some insect larvae, insect fat has successfully been used for production of biodiesel of similar qualities to oilseeds derived fuel (Bulak et al., 2020).

1.3 – Legislation

Insect producers must conform with the same general rules that apply to operators in other sectors. Therefore, insect producers must follow the General Food Law with Regulation 178/2002 and the Hygiene Package regulations, in particular Regulation 852/2004 on the hygiene of foodstuffs and Regulation 183/2005 laying down requirements for feed hygiene. Relating to the feed, insects may only be fed with materials of vegetal origin, with some exceptions however permitted for materials of animal origin such as milk, eggs and their products, honey, rendered fat or blood products from non-ruminant animals. The feeding of insects with other slaughterhouse or rendering derived products, manure, or catering waste is prohibited. Furthermore, obligations lie with insect producers to ensure that their animals are kept in good health so as to prevent the spreading of diseases among their production flock. To this end, EU policy makers have established the responsibilities of animal breeders in the area of health and biosecurity in the EU Animal Health Law with the Regulation 429/2016 on transmissible animal diseases. European insect producers must conform with the EU environmental legislation. Notably, Regulation 1143/2014 on the prevention and management of the introduction and spread of invasive alien species, restricts the insect species that are eligible for farming purposes. Today, the only species prohibited by this Regulation is the Asian predatory wasp, yet this list shall be updated on a regular basis. Directive 98/58 concerns the protection of animals kept for farming purposes, but EU policymakers have left out invertebrate animals, and thus insects, from the scope of the EU animal welfare legislation. Today insect producers are exempted from any EU legal obligations in the area of animal welfare. In any case good welfare practices manuals have been developed by associations such as the IPIFF. In addition to the general food hygiene requirements the production and marketing of insects as food in Europe is governed by the Regulation 2283/2015 on novel foods. This legislation applies to all categories of foods that "*were not used for human consumption to a significant degree*" within the European Union before 15 May 1997. At the moment four novel food authorizations have entered into force for edible insects. In January

2021 EFSA developed its first opinion on an edible insect, on the dried yellow mealworm *Tenebrio molitor*, that has been authorized on EU level as the first insect food product according to the novel food regulation. The second EFSAs positive opinion regarded the authorization of dried and frozen migratory locust *Locusta migratoria* as novel food. Finally, the dried and powdered yellow mealworm and ground and frozen cricket *Acheta domesticus* were authorized respectively the 8th and 11th of February 2021. In several EU countries, insect producers may continue to commercialize their products, even in the absence of EU novel food authorization. Indeed, article 35.2 of Regulation 2283/2015 provides for a transitional measure that aims to ensure that products which were lawfully commercialized in a EU Member State before 1st January 2018, the date of application of the new novel food legislation, may remain on the market of this particular country for a given period of time, subject to certain conditions. To commercialize their products across the European Union, insect producing companies must receive an a priori authorization, granted by the European Commission. The European Food Safety Authority carries out a complete review of the documentation provided by the company and compiled in the so called "Novel Food application". EFSAs evaluations serves to assess the potential safety risks implied by the consumption of the product. Novel Food applications must be submitted to the European Commission services through the e-submission system available on the DG SANTE website (Image 2).

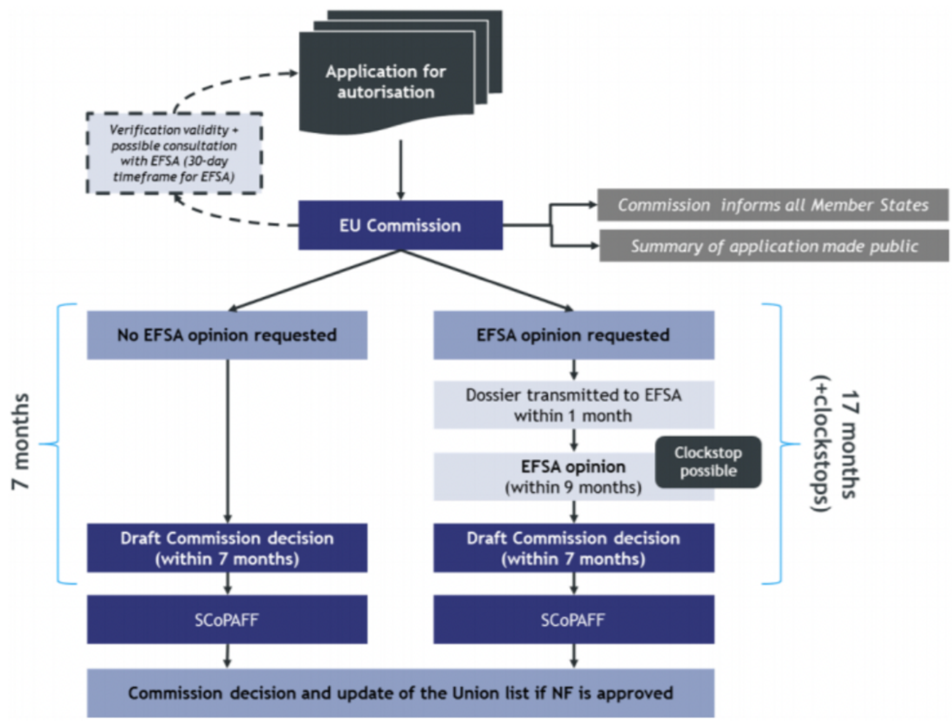


Image 2 – EU Novel Food authorization procedure

In article 10.2 of Regulation 2283/2015 the main pieces of information to be included in the novel food application are set out, whereas the required format for the organization and presentation of this information is defined in the implementing Regulation 2469/2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation 2283/2015. Two distinct procedures are open to insect producers, the “standard” procedure and the “notification procedure for traditional foods from third countries”. Although insect producing companies must receive an a priori authorization, all authorizations then become generic. This means that subsequent operators producing previously authorized insect species are entitled to place the concerned product freely on the EU market. An exception to this is however admitted when the prior authorization is “data protected” according to article 26 of Regulation 2283/2015. In such a case, its producer benefits from a market exclusivity of 5 years following the authorization of the product (IPIFF.org, 2022; Reg. 2283/2015; Reg. 178/2002; Reg. 852/2004; Reg. 183/2005; Reg. 429/2016; Reg. 1143/2014; Reg. 2469/2017; Belluco et al., 2015).

1.4 – Edible insect tradition in Italy

While the historical consumption of insects in Europe is minimal, when compared to other regions of the world (FAO, 2013), there are some traditional delicacies which have remained part of regional food cultures. An example of a particular delicacy is *casu marzu*, a sheep cheese found in Sardinia (Image 3).

Image 3 - Casu marzu

The traditional production method of this cheese is characterized by a ripening period in which the cheese is allowed to be infested by eggs of *Piophila casei*. The larvae that originate from the eggs laid on the cheese will feed on the cheese, causing its consequent chemical modification and conferring typical organoleptic characteristics. The major concern about this cheese is the uncontrolled infestation by uncontrolled insects, but to address this problem producers developed a technique to produce the cheese using *Piophila casei* reared in captivity, allowing the traditional cheese to be produced in more hygienic conditions (Mazzette et al., 2010). The Regional Government of Sardinia produced a document to obtain the Traditional Specialty Guaranteed (TSG) certification for the *casu marzu* in 2014. The legal function of the TSG is to certify that a particular agricultural product objectively possesses specific characteristics which differentiate it from all others in its category, and that its raw materials, composition or method of production have been consistent for a minimum of 30 years. Thus, TSG food denominations are registered trade signs with a distinctive function (Tosato, 2013). To date, despite the efforts, *casu marzu* is still not listed in the EU Database of Origin and Registration as a TSG, but the cheese was added to the list of traditional products of the Region of Sardinia on 18 July 2000. However, there is no recognition of the cheese outside the Region, and therefore, it cannot be exported.

1.5 – Consumer acceptance

In the Western world, insects have never been on the menu, and there is a strong rejection of insects as human food. Even in the tropics there is a decreasing trend in insect consumption, because of the westernization that is reaching the major cities (Mcgranahan et al., 2014). The disgusting reaction in the Western world appears to be entirely acquired, arising in the period between the age of 2 and 5 years. It’s not primarily based on the sensory properties of potential foods, but rather on knowledge of the nature or history of a potential food. Disgust has been identified as the main reason for persons totally rejecting insects as food (Ruby et al., 2015). To facilitate insect consumption some measures were proposed:

1. Taste experiences, the so called “bug banquets”
2. Providing information about the benefits of edible insects
3. Processing insects into familiar products
4. Using role models, for example the head chef of Noma in Copenhagen that is considered one of the best restaurants in the world
5. Showing how close insects and crustaceans are
6. Providing information about the safety of eating insects

7. Making delicious insect-based food
8. Providing available and affordable products

To adopt insect successfully into human consumption patterns, a good availability of insect-based food products in grocery stores is required. Low availability and a poor selection of products leads to passive rejection and decreases the likelihood of consumers buying the products more frequently (Schäufele et al., 2019). Although it has been suggested that people should be encouraged to eat insects on a regular basis, edible insects should find their own place in the food sector rather than being used as a substitute for meat or hidden in other food. Insect integration into Western food culture could be done using a transitional phase, in which ground insects are included in familiar ready to eat meals. The adaptation of this strategy should reduce neophobic reactions and increase the willingness to eat insects (Caparros et al., 2016; Hartmann et al., 2015). The degree of processing that insect-based food undergoes may be a key factor in enhancing consumer acceptance, and the incorporation of insects in processed foods may be seen as an acceptable way to serve insects (Orkusz et al., 2020; Mishyna et al., 2020). In Mexico, tortillas supplemented with yellow mealworm powder had excellent consumer acceptance. The powder contained 58% protein rich in essential amino acids and had a fatty acid composition of 20% oleic acid and linoleic acids as determined by GC-MS (Aguilar-Miranda et al., 2002). In the process of making people come close to the edible insects important was the release of books through the years. Already in 1885, there was a release of a book called "*Why not eat insects"* (Holt et al., 1995). In 1951 Bodenheimer reviewed insect eating from all over the world in his book "*Insects as human food; a chapter of the ecology of man"*. Gene DeFoliart published "*The Food Insects Newsletter*" from 1988 to 2000 (DeFoliart et al., 2009). High interest in the world was created with the publication of the Food and Agricultural Organization book "*Edible insects: future prospects for food and feed security*" which was downloaded more than seven million times and has been translated in Korean, French and Italian. Another boost was the conference "*Insect to feed the world*" jointly organized by the Food and Agriculture Organization and Wageningen University in the Netherlands which attracted 450 participants from forty-five countries. Relating to the gastronomy aspect, several cookbooks have been produced, some with recipes from insects from all over the world and some with insects that are locally available (Ramos-Elorduy, 1998; Van Huis et al., 2014) (Image 4).

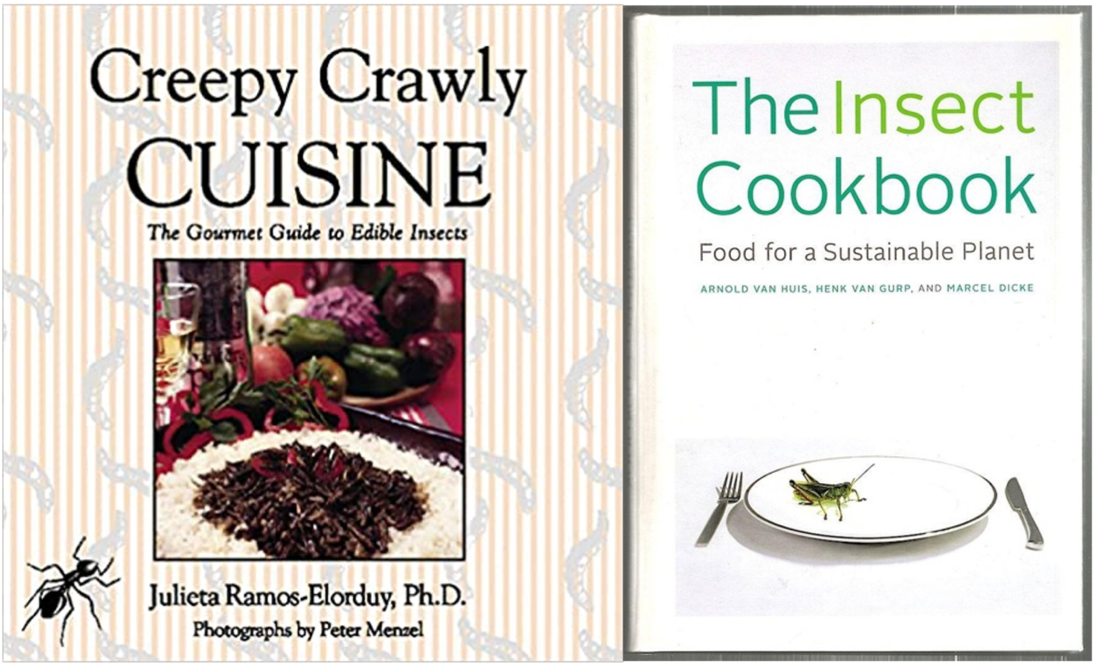


Image 4 – Creepy Crawly Cuisine and The Insect Cookbook

The environmental aspect is of paramount importance when convincing people about the benefits of eating insects, indeed people who seem interested in eating insects in Western countries are young men with a high educational level who are open to trying novel foods and are interested in the environmental impact of their food choices (Verbeke et al., 2015; Mancini et al., 2019; Zielinska et al., 2020). Vegetarians have the most positive attitude towards edible insects, and both vegetarians and omnivores consider entomophagy as "wise and a solution to the world's nutrition problems", whereas vegans describe insect eating as "immoral and irresponsible" (Elorinne et al., 2019).

1.6 – Ethical issues of edible insects

As more insect-based products enter the Western market, it is necessary to assume that more questions will be raised regarding their production. Different studies highlight the consumers' concern on animal welfare of typical livestock production, where their concerns are reflected towards their purchase decisions (Klink-Lehmann et al., 2019). The International Platform of Insects for Food and Feed has been trying to promote insect welfare by applying Brambell's Five Freedoms to insect farming and encouraging producers to follow them. However, except for the Finnish Food Safety Authority, which specifically mentions animal welfare regulations for insects, the Thai National Bureau of Agricultural Commodity and Food Standards and FAO, which both published cricket farming guidelines, and the Dutch Animal Act, which lists some insect species as ‘production animals’, there are currently few official species-specific regulations regarding insect welfare (IPIFF, 2019; Brambell, 1965; Hanboonsong et al., 2020). Brambell’s Five Freedoms (Image 5) state that all farmed animals should have freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury, or disease, freedom to express normal behavior, freedom from fear and distress.

Five Freedoms	How to Achieve Them
Freedom from Hunger and Thirst	by ready access to fresh water and a diet to maintain full health and vigour.
Freedom from Discomfort	by providing an appropriate environment including shelter and a comfortable resting area
Freedom from Pain, Injury or Disease	by prevention or rapid diagnosis and treatment
Freedom to Express Normal Behavior	by providing sufficient space, proper facilities and company of the animal’s own kind
Freedom from Fear and Distress	by ensuring conditions and treatment which avoid mental suffering

Image 5 – Brambell’s Five Freedoms

The European Commission’s animal welfare regulations for example are designed to reflect the Five Freedoms. But, while regulations for the animal welfare of conventional livestock are clearly defined, there are a few official standards for insect farming and several guidelines regarding the production of different insect species exist. However, these are not regulations, which means that insect farmers work in a “legislative grey zone” and must actively seek out information. In addition, few guidelines specifically mention animal welfare as an aspect to consider when breeding insects. Consequently, insect farmers often must guess and trial and error the best practices (Bear et al., 2019; Bear et al., 2021). Based on differences in their biology, it appears plausible to assume that insect animal welfare might differ from vertebrate welfare. For example, even though specific space requirements vary between species, high stocking density is often associated with poorer animal welfare for prevalent livestock. For industrial insect farming, in contrast, high stocking density has been claimed to not be problematic because “many insect species naturally live in large groups in small amounts of space” (Dossey et al., 2016). The optimum density of an insect farming container, however, needs to be considered on a species level as some species, such as *Hermetia illucens* larvae, may overheat in high densities. And regarding the cricket *Gryllus bimaculatus*, population density has been shown to drastically influence behavioral, morphological, and physiological aspects with high population density being linked to suppressed growth and development as well as different behavior, such as decreased aggressiveness, increased activity and response to tactile, visual, or olfactory stimuli (El-Damanhouri et al., 2011). These examples highlight that insect welfare regulations need to be species-specific. Different entities are suggesting that the insect welfare should be based on the Five Freedoms, where the benefit of this approach is that the Five Freedoms are well-established set of principles that incorporate the different aspects of animal welfare. Yet, a potential problem with the Five Freedoms is their general view on farmed animals without defining species-specific aspects. Indeed, insect welfare standards should aim to provide tangible recommendations for specific species in the same way current regulations differentiate between different species such as cow and chicken. The "freedom from fear and distress" raises the question of how to measure fear and distress in insects and whether insects are negatively affected by stress. Some studies suggest that just like mammals, hormone responses to environmental stresses might be seen in insect, but these responses are species specific (Lubawy et al., 2020). In crickets, high stocking densities result in smaller insects, therefore less growth hormone stimulation (Wey et al., 2019). Parallel to the ambiguities regarding insects' stress response, the "freedom from pain, injury and disease" links to the discussion of whether insects can experience pain. While insects have been shown to have sensory neurons so-called nociceptors that respond to injury and damaging stimuli, this does not necessarily imply awareness of their nociceptive response (Elwood et al., 2019). In contrast to nociception, pain perception involves the subjective experience of "an aversive sensation and feeling associated with actual or potential tissue damage" (Broom, 2001). Thus, the experience of pain is linked to the ability to experience subjective states (Adamo, 2016). Given that insects cannot communicate verbally, investigating whether they subjectively experience pain is not straightforward. To this point, however, there is no clear consensus whether insects have the capacity to feel, perceive or experience subjectively (van Huis, 2019). In any case, the absence of proof should not be misunderstood as proof of absence when talking about experience of pain for insect (Gjerris et al., 2016), therefore insect farmers ought to act in line with the precautionary principle, which postulates that we should avoid any actions which are likely to cause pain in insects when this avoidance doesn't (or does only minimally) affect our own welfare. Any actions that could cause harm to an insect should thus be avoided (Lockwood, 1987). Because insect species are so many, it would be unthoughtful to try developing general standards for insect animal welfare, but there should be a focus on the most commonly farmed insects for human consumption, such as crickets, grasshoppers and mealworms (Bopprè et al., 2019) (Image 6).

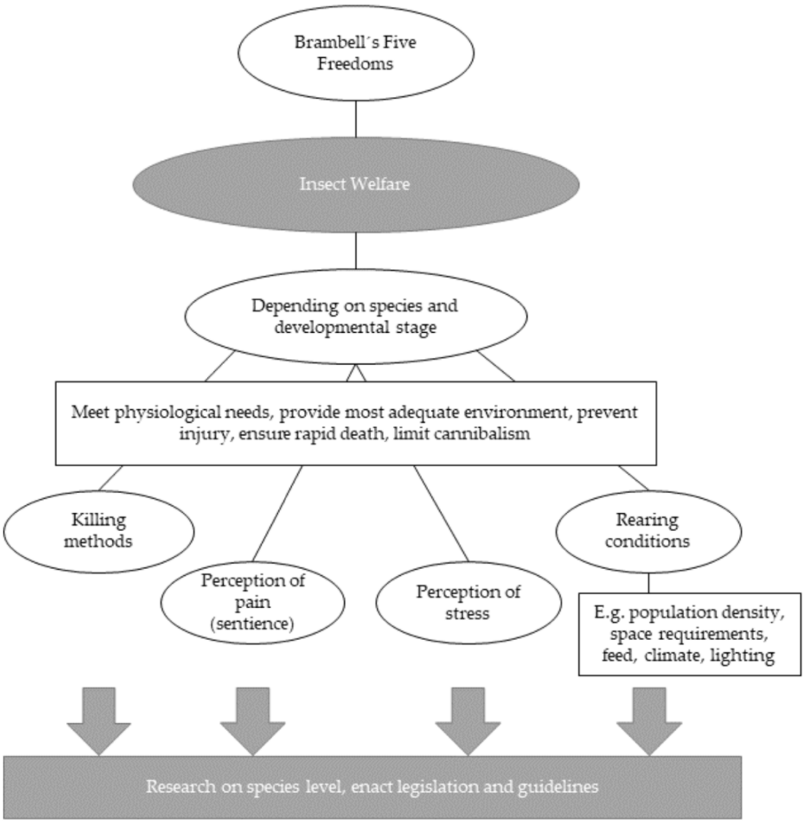


Image 6 - Summary of insect welfare aspects based on the Five Freedoms

In the Western world, consumers commonly base their understanding of animal welfare on an anthropomorphic perspective (Klink-Lehmann et al., 2019), where anthropomorphism is defined as "the tendency to assign human characteristics, including emotions and cognitions, to animals and to objects" (Amiot et al., 2015). Therefore, animals that are closer to humans regarding their morphology and behavior are more anthropomorphized (Urquiza-Haas et al., 2015). Such factor is highly relevant to consumers' perspective of insect welfare, as the phytogenetical distance of humans to insects is much larger than to most prevalent livestock. Linked to anthropomorphism, assigning sentience to animals significantly affects their welfare and moral status. Specifically, living beings who are perceived to have more mental capacities are attributed higher moral standing. Thus, people show more moral concern for animals whom they perceive as having mental states, such as the capacity to experience pain. Relating to the sentience, the UK government formally recognized animals as "sentient beings", but in the definition of sentient animals only vertebrates are included, meaning that invertebrates are excluded from the list of sentient beings. People in fact commonly perceive invertebrates as less sentient as shown in the results of a Finnish study where people were asked to rate the mental capacities of different animal species and the participants attributed the lowest scores to shrimp that was the only invertebrate in the list of animal species (Kupsala et al., 2016). In general people do not even consider insects as animals, indeed they are often referred to as "products, biomass, raw material, ingredient" and the killing of insects is called "harvesting", a term used for crops, such as cereals. Parallel to insects not being described as animals, the term "entovegan diet" suggests that the consumption of insects may be compatible with plant-based diets. As such insects are not perceived as an immoral food source such as meat (Santaoja et al., 2019). Supporting this idea, a qualitative study investigating consumers' perspective of edible insects found that insects are perceived as more "ethical" (referring to both sustainability and animal welfare) than conventional meat not only by meat-eaters but also vegetarians. Thus, even though vegetarians reported concern for animal welfare, this concern seemed to exclude insects (House, 2016). Regarding the ethicalness of insect consumption, it is assumed to be ethical by default, because of "the allegedly smaller environmental impact as a central argument for ethicalness". From this perspective, consumers may not question the ethical standards of edible insect production, as the consumption of insects is perceived as a sustainable choice itself (Santaoja et al., 2019). There also appears to be a difference in perception between different types of insects. Specifically, insects such as butterflies or bees, are differentiated from other types of insects towards which many people feel disaffection. Honeybees, for example, have received extensive media coverage and are commonly portrayed positively. While other insect species are often perceived as "alien" and many species commonly evoke a disgust response in humans. From a psychological perspective, disgust has been linked to dehumanization and moral exclusion, which might be why people struggle to empathize with insects and one way to potentially overcome these negative attitudes is experience (Mikhalevich et al., 2020).

1.7 – Nutritional composition of insects

The nutritional composition of edible insects is highly variable, not only for the wide variety of species present. Even within the same group of edible insect species, values may differ depending on the metamorphic stage of the insect, on the habitat, on the diet and on the preparation and processing methods. Although significant variation is found, many edible insects provide:

- Satisfactory amounts of **energy** (Table 1)
- Satisfactory amounts of **protein** (Table 2; Table 3; Table 4)
- **Amino acid** requirements for humans
- **Monounsaturated** and/or **polyunsaturated fatty acids** (Table 5)

- **Micronutrients**
- **Vitamins**

Location	Common name	Scientific name	Energy content (kcal/100 g fresh weight)
Australia	Australian plague locust, raw	*Chortoicetes terminifera*	499
Australia	Green (weaver) ant, raw	*Oecophylla smaragdina*	1 272
Canada, Quebec	Red-legged grasshopper, whole, raw	*Melanoplus femurrubrum*	160
United States, Illinois	Yellow mealworm, larva, raw	*Tenebrio molitor*	206
United States, Illinois	Yellow mealworm, adult, raw	*Tenebrio molitor*	138
Ivory Coast	Termite, adult, dewinged, dried, flour	*Macrotermes subhyalinus*	535
Mexico, Veracruz State	Leaf-cutter ant, adult, raw	*Atta mexicana*	404
Mexico, Hidalgo State	Honey ant, adult, raw	*Myrmecocystus melliger*	116
Thailand	Field cricket, raw	*Gryllus bimaculatus*	120
Thailand	Giant water bug, raw	*Lethocerus indicus*	165
Thailand	Rice grasshopper, raw	*Oxya japonica*	149
Thailand	Grasshopper, raw	*Cyrtacanthacris tatarica*	89
Thailand	Domesticated silkworm, pupa, raw	*Bombyx mori*	94
The Netherlands	Migratory locust, adult, raw	*Locusta migratoria*	179

Table 1 - Energy content of different insect species

From this table developed by FAO in 2012 it's possible to notice how some species have particularly high kcal/100g of fresh product, such as *Oecophylla smaragdina* with 1272 kcal/100g FW, the Australia locust with 499 kcal/100g FW, the field cricket with 120 kcal/100g FW, *Tenebrio molitor* larvae with 206 kcal/100g FW.

Animal group	Species and common name	Edible product	Protein content (g/100 g fresh weight)
Insects (raw)	Locusts and grasshopppers: *Locusta migratoria, Acridium melanorhodon, Ruspolia differens*	larva	14–18
	Locusts and grasshopppers: *Locusta migratoria, Acridium melanorhodon, Ruspolia differens*	Adult	13–28
	Sphenarium purpurascens (chapulines – Mexico)	Adult	35–48
	Silkworm (*Bombyx mori*)	Caterpillar	10–17
	Palmworm beetles: *Rhynchophorus palmarum, R. phoenicis, Callipogon barbatus*	Larva	7–36
	Yellow mealworm (*Tenebrio molitor*)	Larva	14–25
	Crickets	Adult	8–25
	Termites	Adult	13–28
Cattle		Beef (raw)	19–26
Reptiles (cooked)	Turtles: *Chelodina rugosa, Chelonia depressa*	Flesh	25–27
		Intestine	18
		Liver	11
		Heart	17–23
		Liver	12-27
Fish (raw)	Finfish	Tilapia	16–19
		Mackerel	16–28
		Catfish	17–28
	Crustaceans	Lobster	17–19
		Prawn (Malaysia)	16–19
		Shrimp	13–27
	Molluscs	Cuttlefish, squid	15–18

Table 2 - Protein content of insects, reptiles, fish and mammals

This table produced by FAO in 2012 compares the protein content of different edible products. It's possible to notice that protein values of insect species are really close, if not higher than the most commonly available meat protein sources. For example, the protein content of raw cattle beef is 19-26g/100g, of mackerel 16-28g/100g, of lobster 17-19g/100g and of shrimp of 13-27g/100g. The protein content of locust and grasshoppers is 13-28g/100g FW, of the yellow mealworm 14-25g/100g FW, of crickets 8-25g/100g FW and of *Sphenarium purpurascens* 35-48g/100g FW.

Insect order	Stage	Range (% protein)
Coleoptera	Adults and larvae	23 – 66
Lepidoptera	Pupae and larvae	14 – 68
Hemiptera	Adults and larvae	42 – 74
Homoptera	Adults, larvae and eggs	45 – 57
Hymenoptera	Adults, pupae, larvae and eggs	13 – 77
Odonata	Adults and naiad	46 – 65
Orthoptera	Adults and nymph	23 – 65

Table 3 - Crude protein content by insect order

As said the nutritional values among insects is highly variable. In Table 3 are reported various insect orders with their range of protein values. Orthoptera 23-65% protein, Coleoptera 23-66% protein, Lepidoptera 14-68% protein (Xiaoming et al., 2010).

Insect stage	Gram protein/100 g fresh weight
Instar:	
First	18.3
Second	14.4
Third	16.8
Fourth	15.5
Fifth	14.6
Sixth	16.1
Adult	21.4

Table 4 - Protein content variation in different metamorphosis phases of the variegated grasshopper

Within the same species, variability in the protein content can be found depending on the different metamorphosis phases. For example, in this table are reported the protein values of the variegated grasshopper in its various metamorphosis phases. The higher protein content can be found in the adult phase with 21.4g of protein/100g FW. This type of information is important considering that insect farms must know the best time to harvest insects (Ademolu et al., 2010).

Edible insect species	Fat content (% of dry matter)	Composition of main fatty acids (% of oil content)	SFA, MUFA or PUFA1
African palm weevil (*Rhynchophorus phoenicis*)	54%	Palmitoleic acid (38%)	MUFA
		Linoleic acid (45%)	PUFA
Edible grasshopper (*Ruspolia differens*)	67%	Palmitoleic acid (28%)	MUFA
		Linoleic acid (46%)	PUFA
		α-Linolenic acid (16%)	PUFA
Variegated grasshopper (*Zonocerus variegates*)	9%	Palmitoleic acid (24%)	MUFA
		Oleic acid (11%)	MUFA
		Linoleic acid (21%)	PUFA
		α-Linolenic acid (15%)	PUFA
		γ-Linolenic acid (23%)	PUFA
Termites (*Macrotermes* sp.)	49%	Palmitic acid (30%)	SFA
		Oleic acid (48%)	MUFA
		Stearic acid (9%)	SFA
Saturniid caterpillar (*Imbrasia* sp.)	24%	Palmitic acid (8%)	SFA
		Oleic acid (9%)	MUFA
		Linoleic acid (7%)	PUFA
		α-Linolenic acid (38%)	PUFA

Table 5 - Fat content and main fatty acids of common edible insect species

Edible insects are a considerable source of fat. Oils extracted from several insects are rich in polyunsaturated fatty acids and frequently contain the essential linoleic and α-linolenic acids (ω-6 and ω-3 fatty acid), similarly to fish and crustaceans. Unsaturated fatty acids are considered better for human health compared to saturated fatty acids, but higher is the presence of unsaturated fatty acids and higher will be the rapidity of the oxidation, causing the insect food products to become rancid quickly (Womeni et al., 2009). Relating to the micronutrients, edible insects have equal or higher iron contents than beef, 8-77mg/100g compared to 6mg/100g of beef. This is important considering that iron deficiency is the world's most common and widespread nutritional disorder. Zinc deficiency is another core public health problem, especially for child and maternal health. Indeed, not sufficient zinc quantities can cause growth retardation, skin lesions, alopecia, increased susceptibility to infections. Most insects are good sources of zinc. Beef averages 12.5mg/100g FW, while insects can contain 26.5mg/100g FW (WHO, 2008; Bukkens et al., 2005).

1.8 – Crickets

Among the Orthopteras, crickets stand as the most consumed insects across the globe. Both the nymph and adult stages of crickets are consumed as food. The most common species usually reported include *Brachytrupes membranaceus, Gryllus similis, Gryllus bimaculatus,* *Gryllotalpa orientalis* and *Acheta domesticus* (Magara et al., 2019). Crickets have been consumed as food in Asia, Latin America, Africa as far back as prehistoric times. In Biblical scriptures, cricket consumption is recommended to the Israelites by God to be fit for their consumption. In the Leviticus 11:22 it's written "*these you may eat any kind of locust, cricket or grasshopper"*. In recent years, consumption of edible crickets has become more appreciated in Europe, America and Australia with the recognition of its nutritional benefits and food security. The greatest species abundance is found in the tropics where temperatures are warm and suitable for their faster development compared to cold regions (Fuah et al., 2016). Edible crickets are excellent sources of proteins, lipids, carbohydrates, minerals, and vitamins (Table 6). However, the nutritional composition varies across the different species and within the same species can vary depending on stage of development, habitat, climate, sex and the food substrate fed on by the cricket. The nutritional value can also be influenced by the way they're processed (Musundire et al., 2016).

Cricket species	Stage	Protein (g/100 g dry weight)	Lipid (g/100 g dry weight)	Fiber (g/100 g dry weight)	Ash (g/100 g dry weight)	Carbohydrates (g/100 g dry weight)	Energy value (kcal/100 g dry matter)
Acheta domesticus	Nymph and Adult	62.41–71.09 NR	9.80–22.8 19.20–29.58	10.20 NR	5.10–9.10 NR	NR NR	455.19 NR
Gryllus assimilis	Adult Nymph	56.00 ± 3.10 55.60 ± 1.10 65.52 ± 1.39 71.04 ± 0.01 56.4	21.80 ± 2.65 11.90 ± 0.50 7.00 ± 0.12 NR 34.00	8.28 8.00 7.00 NR NR	6.40 NR NR NR 4.08 ± 0.43	12.46 ± 0.16 8.60 ± 1.49 NR NR NR	397.00 ± 1.69 NR NR NR 537.50
Gryllus bimaculatus	Adult	57.49–70.10	14.93–33.44	9.53 ± 0.46	NR	NR	120.00
Brachytrupes spp	Adult	65.35 ± 0.36	11.76 ± 0.63	13.29 ± 1.61	4.88 ± 0.23	2.50 ± 0.85	536.42 ± 0.47
Gryllus testaceus	Adult	58.30 ± 0.91	10.30 ± 0.31	10.40	2.96 ± 0.09	NR	18.10
Tarbinskiellus portentosus	Adult	58.00 ± 0.05	23.70 ± 0.05	1.16 ± 1.01	7.93 ± 0.04	NR	460.82
Gryllodes sigillatus	Nymph	56.00	NR	NR	NR	NR	
Teleogryllus emma	Adult	55.65 ± 0.28	25.14 ± 0.11	10.37 ± 0.19	10.37 ± 0.19	NR	
Brachytrupes membranaceus	Adult Nymph	53.4 ± 0.19	15.80 ± 0.23	6.30 ± 0.14 5.0 ± 0.30	6.00 ± 0.12 3.23 ± 0.01	15.10 ± 0.22	454.7 ± 2.25
Brachytrupes portentosus	Adult	48.69 ± 0.25 NR	20.60 ± 0.60 NR	11.61 ± 0.20 0.5–8.3	5.40–20.50 9.36 ± 0.34	NR NR	90.06–134.0 NR
Gryllotalpa africana	Adult	22.0 ± 0.86	10.80 ± 1.24	7.4 ± 0.24	12.60 ± 0.97	47.20 ± 0.32	362.3 ± 2.34
Acheta testacea	Adult	18.6	6.00	NR	NR	NR	133.00
Acheta confirmata	Adult	NR	21.14	NR	NR	NR	NR
Animal tissue							
Goat, roasted		27	3	0	NR	0	143
Broiler		24	14	0	NR	0	165
Pork		27	6.00	0	NR	1.5	242

Table 6 - Nutritional composition of different species of edible crickets and selected animal tissues

Most of the edible crickets supply adequate energy and proteins to the consumer diet, at the same time meeting the amino acid requirements. Crickets also possess a high value of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). Besides, these insects are rich in micro-nutrient elements such as calcium, potassium, magnesium, phosphorus, sodium, iron, zinc, manganese, and copper as well as vitamins like folic acid, pantothenic acid, riboflavin, and biotin, which are the most deficient nutrients in humans (Ghosh et al., 2017; Akullo et al., 2018). Relating to proteins, compared to the protein content of the common meat sources listed in the table, most of the edible crickets have a higher protein content than that of the roasted goat, broiler chicken and pork. The protein digestibility of some crickets was also investigated and was 50.2% for *Brachytrupes* species and 83.9% for *Acheta domesticus.* These protein digestibility values for the crickets are slightly lower compared to values for eggs (95%), beef (98%) and cow milk (95%). On the other hand, protein digestibility values for the crickets are higher than those of many plant proteins, such as maize (73%), wheat (81%) and rice (66%) (Finke, 2004; Klunder et al., 2012). Edible crickets contain, on average, 4.30-33.44% of lipids in dry matter basis. In some cricket species, the lipids content are higher in the nymphal stages than in adults, while in other species they are lower in nymphs compared to the adult stage. *Gryllus bimaculatus* and *Acheta domesticus* are among those cricket species with the highest lipid content (Barker, 1997). Crickets are richer in lipid content when compared to goat, chicken and pork meats. Crickets contain a significant quantity of fiber that ranges between 0.5 and 13.4%. The insoluble chitin in the exoskeleton of the edible crickets forms a major part of fiber. In commercially farmed crickets, the chitin ranges from 2.7 to 49.8mg per kg of fresh weight and 11.6 to 137.2mg per kg of dry weight. People from tropical countries can digest chitin by the help of a bioactive chitinase enzyme, which has developed in their gastric juices as a result of consuming edible crickets in their regular diet unlike people from outside the tropics. To enable people from outside the tropics to consume crickets without any complication, the chitin must be removed (Paoletti et al., 2007; Finke, 2007; Muzzarelli, 2010). Chitosan from the chitin of the edible cricket species exoskeleton has been identified to be a possible intelligent and biodegradable bio-based polymeric material for packaging of various foods. Such natural packaging using the "exoskeleton" of crickets can change the internal conditions of the food product, thereby protecting the food product from spoiling. This is possible because chitosan has antioxidant and antimicrobial properties (Zielinska et al., 2017; Portes et al., 2009; Cutter, 2006). Edible crickets are also rich in amino acids. Glutamic acid is the most abundant amino acid in *T. portentosus, G. assimilis, G. testaceus, A. testacea, G.bimaculatus* and *A. demesticus*. The most abundant essential amino acids in these crickets are valine, leucine and lysine (Bednarova et al., 2014). Compared to amino acids from livestock meats, crickets like *T.portentosus, G. sigillatus* and *G. assimilis* have more valine amino acid than pork and broiler chicken and similar content of all other amino acids (Strakova et al., 2006). *T. portentosus, G. testaceus* and *A. domesticus* have a higher content of phenylalanine than chicken, but similar content to that of pork (Cai et al., 2010; Zlender et al., 2000).

Fatty acid	Cricket species										Animal tissue	
	Tarbinskiellus portentosus	*Gryllus testaceus*	*Gryllus assimilis*	*Acheta domesticus*	*Gryllus bimaculatus*	*Teleogryllus emma*	*Brachytrupes* sp.	*Brachytrupes portentosus*	*Acheta testacea*	*Acheta confirmata*	Pork loin	Broiler
Lauric acid (C12:0)	1.16 ± 0.16	0.54 ± 0.04	0.12 ± 0.00	0.10 ± 0.00	0.04	0.02	NR	NR	NR	NR	0.21	NR
Tridecanoic acid (C13:0)	NR	NR	0.02 ± 0.01	NR	0	0	NR	NR	NR	NR	NR	NR
Myristic acid (C14:0)	6.74 ± 0.47	0.39 ± 0.02	1.28 ± 0.01	0.44 ± 0.00	0.05	0.18	0.96 ± 0.01	Nd	NR	26.10	1.3–1.4	0.45–0.69
Pentadecanoic acid (C15:0)	16.74 ± 1.33	NR	0.37 ± 0.01	0.11 ± 0.00	0.01	0.02	NR	Nd	NR	NR	4.1–4.7	NR
Palmitic acid (C16:0)	NR	10.18 ± 0.20	25.85 ± 0.06	22.65 ± 0.37	2.16	3.06	21.31 ± 0.49	1.61 ± 0.05	NR	5.50	23.2–27.3	23.8–24.9
Heptadecanoic acid (C17:0)	NR	NR	0.57 ± 0.01	0.12 ± 0.00	0.03	0.04	NR	0.13 ± 0.02	NR	NR	0.2–0.3	NR
Stearic acid (C18:0)	NR	2.63 ± 0.09	14.07 ± 0.03	8.54 ± 0.00	0.76	0.07	12.24 ± 0.24	35.79 ± 0.02	NR	1.20	12.2–16.1	5.7–5.9
Arachidic acid (C20:0)	NR	NR	0.56 ± 0.01		0.12	0.09	0.49 ± 0.01	Nd	NR	NR	NR	NR
Heneicosanoic acid (C21:0)	NR	NR	0.03 ± 0.00	0.24 ± 0.00	0.04	0.04	NR	NR	NR	NR	NR	NR
Behenic acid (C22:0)	2.34 ± 0.27	NR	0.57 ± 0.00		0.03	0.01	NR	NR	NR	NR	NR	NR
Tricosanoic acid (C23:0)	NR	NR	0.22 ± 0.01	0.02 ± 0.04	0	0.07	NR	NR	NR	NR	NR	NR
Lignoceric acid (C24:0)	NR	NR			0.01	0.01	NR	NR	NR	NR	NR	NR
Myristoleic acid (C14:1)	NR	NR	0.06 ± 0.01	0.44 ± 0.00	0	0.02	NR	NR	NR	NR	NR	NR
Palmitoleic acid (C16:1)	NR	3.11 ± 0.10	1.92 ± 0.01	0.34 ± 0.00	0.17	0.91	0.96 ± 0.00	0.71 ± 0.03	NR	2.40	2.1–2.8	7.1–7.4
Heptadecenoic acid (C17:1)	NR	NR	0.19 ± 0.00	0.24 ± 0.00	0.01	0.03	NR	NR	NR	NR	NR	NR
cis Oleic acid (C18:1n-9)	NR	29.58 ± 0.20	25.03 ± 0.11	20.18 ± 0.02	2.91	6.98	38.27 ± 0.67	3.4 ± 0.03	NR	31.10	32.8–43.7	40.3–40.9
Eicosenoic acid (C20:1)	NR	NR	0.24 ± 0.00	NR	0.03	0.04	NR	NR	NR	NR	NR	NR
Erucic acid (C22:1n-9)	NR	NR	0.05 ± 0.01	0.52 ± 0.01	0.01	0.04	NR	NR	NR	NR	NR	NR
cis Linoleic acid (C18:2n-6)	18.94 ± 0.02	37.82 ± 0.20	26.13 ± 0.18	41.39 ± 0.29	4.15	9.61	22.14 ± 0.59	NR	NR	32.20	10.7–14.2	16.2–17.5
Eicosadienoic acid (C20:2)	NR	NR	1.60 ± 0.01	0.00	0.04	0.02	NR	NR	NR	NR	NR	NR
Eicosatrienoic (C20:3n-3)	NR	NR	0.01 ± 0.00	NR	NR	NR	NR	NR	NR	NR	NR	NR
Eicosatetraenoic (C20:4n-3)	NR	NR	0.21 ± 0.02	NR	NR	NR	NR	NR	NR	NR	NR	NR
Docosadienoic acid (C22:2n-6)	NR	NR	0.03 ± 0.02	0.11 ± 0.01	0.02	0.01	NR	NR	NR	NR	NR	NR
Linolenic acid (C18:3n-6)	NR	10.12 ± 0.10	NR	1.11 ± 0.00	0.01	0	2.55 ± 0.18	NR	NR	NR	NR	NR
Alpha-linolenic acid (C18:3n-3)	NR	NR	NR	NR	0.08	0.22	NR	Nd	NR	1.70	1.0–1.1	0.77–0.85
Eicosatrienoic acid (C20:3n-6)	NR	NR	NR	0.01 ± 0.02	0.02	0.01	NR	7.94 ± 0.04	NR	NR	NR	NR
Arachidonic acid (C20:4n-6)	0.55 ± 0.28	NR	NR	0.01 ± 0.02	0.01	0.27	NR	50.43 ± 0.55	NR	NR	0.1–0.2	0.76-0.97
Eicosapentaenoic (C20:5n-3)	NR	NR	NR	0.01 ± 0.02	0	0.01	NR	Nd	NR	NR	0.2–0.4	0.05–0.07
SFA	50.58	13.74	43.72	32.22	3.25	3.61	34.99 ± 0.24	37.54 ± 0.08	36.5	32.80	40.7	30.9–32.2
MUFA	28.98	32.69	27.49	21.72	3.13	8.02	39.23 ± 0.66	4.11 ± 0.06	30.1	33.50	47.2	48.0–49.1
PUFA	20.32	47.94	28.80	42.64	4.33	10.15	24.68 ± 0.77	58.37 ± 0.59	31.1	33.90	11.7	19.1–20.4
TUFA	49.30	80.63	56.29	64.36	7.46	18.17	63.91	62.48	61.20	67.40	58.90	67.10–69.50
PUFA/SFA ratio	0.40	3.49	0.66	1.32	1.33	2.81	0.71	1.55	0.86	1.03	0.6	0.61–0.66
n-3	NR	NR	1.99	0.01	0.08	0.23	NR	NR	NR	NR	1.2–1.5	0.82–0.93
n-6	19.49	47.94	26.81	42.63	4.25	9.92	24.69	58.37	NR	NR	10.8–14.4	17.8–19.1
EFA	18.94 ± 0.02	37.82 ± 0.20	26.13 ± 0.18	41.39 ± 0.29	4.23	9.83	22.14 ± 0.59	NR	NR	33.90	11.70–15.30	16.20–18.35

Table 7 - Fatty acid composition (g/100g) of different species of edible crickets

As compared to other fatty acids, the edible crickets possess higher contents of oleic, linoleic, linolenic, stearic and palmitic acid (Ghosh et al., 2017; Akullo et al., 2018) (Table 7). Linoleic acid, ranging from 4.15 to 41.39 g/100g of dry matter, is the most abundant fatty acid in *T. portentosus, G. testaceus, G. assimilis, A. domesticus, G. bimaculatus, T. emma* and *A. confirmata.* On the other hand, oleic and arachidonic acid, respectively 38.27 g/100g DM and 50.43 g/100g DM, are the most abundant fatty acids in *Brachytrupes* species and *B. portentosus.* The second and third most abundant fatty acid in various crickets varies. In *T. portentosus*, they are pentadecanoic and myristic acid. In *G. testaceus* they're oleic and palmitic acid, in *G. assimilis* palmitic and oleic acid, in *A. domesticus* palmitic and oleic acid, in *G. bimaculatus* oleic and palmitic acid, in *T. emma* oleic and palmitic acid, in *A. confirmata* oleic and myristic acid, in *Brachytrupes* species linoleic and palmitic acid and in *B. portentosus* stearic and eicosatrienoic acid. (Orinda et al., 2018; Barker, 1997). The different cricket lipids are highly unsaturated, with either linoleic and oleic or linoleic and pentadecanoic acid or arachidonic and eicosatrienoic acid being the most abundant unsaturated fatty acids and palmitic, myristic and stearic acids being the most abundant saturated fatty acids (Rumpold et al., 2013). *A. domesticus, G. testaceus, G. assimilis, A. confirmata* and *B. portentosus* have higher content of PUFA compared to pork and broiler chicken meat, but *Gryllus bimaculatus* and *T. emma* have a lower content of PUFA compared to pork and broiler chicken meat. Crickets generally have more unsaturated fatty acids compared to saturated fatty acids, but a notable exception occurs in *T. portentosus*, which has more SFA compared to UFA (Mlček et al., 2018; Tang et al., 2019; Narzari, 2020). Crickets gathered from the wild or the one raised in the farms must be processed before they are consumed. The processing consists in starving the crickets for 1-3 day, so that their digestive tract gets emptied, and then killing them by using hot water, or freezing, or employing microwaves. After killing them, they're then cooked, smoked, fried, toasted, dried, or processed into cricket products, such as crackers (van Huis et al., 2013; Borkovcova et al., 2009). Processed crickets and their products have diverse taste, color and flavor. The flavor of the cricket depends on their surface odor. The flavor of crickets also depends on the diet they eat, indeed diet choices for crickets can be adapted depending on how we want them to taste. During cooking, the cricket color may change from the initial shades of gray or brown to red, or its color may be retained, especially if the cricket is black (Ramos-Elorduy et al., 1998). Crickets containing a considerable amount of oxidized fat, or improperly dried crickets, may be black, which is a color that may discourage consumers. Properly dried crickets are golden or brown and can be easily crushed by the fingers (Borkovcova et al., 2009).

2 – MATERIALS AND METHODS

Flatbread samples were prepared in Poland and sent to UNIVPM for the analysis of volatiles and sensorial characteristics (Table 8). Also, microbiological analysis will be performed in the microbiology district.

CODE	SAMPLE
FWB	Wheat flatbread + baker's yeast
FIB	Insect (20%) flatbread + baker's yeast
FITAB	Italian buckwheat-enriched (18%) insect (20%) flatbread + baker's yeast
FPOLB	Polish buckwheat-enriched (18%) insect (20%) flatbread + baker's yeast
FWS	Wheat flatbread + wheat sourdough
FIS	Insect (20%) flatbread + wheat sourdough
FITAS	Italian buckwheat-enriched (18%) insect (20%) flatbread + Italian buckwheat sourdough
FPOLS	Polish buckwheat-enriched (18%) insect (20%) flatbread + Polish buckwheat sourdough

Table 8 – Flatbread samples

RECIPES:

FWB – Wheat flatbread + Baker's yeast

- 238g of soft wheat flour
- 102g of durum wheat flour
- 32g of chickpea flour
- 20g of oat flour
- 8g of barley malt
- 4g of salt
- 2g of baker's yeast
- 200g of water

FIB – Insect (20%) flatbread + Baker's yeast

- 182g of soft wheat flour

- 78g of durum wheat flour
- 80g of cricket powder
- 32g of chickpea flour
- 20g of oat flour
- 8g of barley malt
- 4g of salt
- 2g of baker’s yeast
- 200g of water

FITAB/FPOLB – Italian/Polish buckwheat-enriched (18%) insect (20%) flatbread + Baker’s yeast

- 168g of soft wheat flour
- 72g of durum wheat flour
- 80g of cricket powder
- 72g of buckwheat flour
- 8g of barley malt
- 4g of salt
- 2g of baker’s yeast
- 200g of water

FWS – Wheat flatbread + Wheat sourdough

- 238g of soft wheat flour
- 102g of durum wheat flour
- 32g of chickpea flour
- 20g of oat flour

- 8g of barley malt
- 4g of salt
- 133g of wheat sourdough
- 156g of water

FIS – Insect (20%) flatbread + wheat sourdough

- 182g of soft wheat flour
- 78g of durum wheat flour
- 80g of cricket powder
- 32g of chickpea flour
- 20g of oat flour
- 8g of barley malt
- 4g of salt
- 133g of wheat sourdough
- 156g of water

FITAS/FPOLS – Italian/Polish buckwheat-enriched (18%) insect (20%) flatbread + Italian/Polish buckwheat sourdough

- 168g of soft wheat flour
- 72g of durum wheat flour
- 80g of cricket powder
- 72g of buckwheat flour
- 8g of barley malt
- 4g of salt
- 133g of buckwheat sourdough
- 156g of water

Solid phase microextraction (SPME) was used to collect the volatile components from the headspace of 10ml glass vials. 2 grams of each sample was taken and reduced to a powder, which was inserted into vials. The vials were heated at 45°C for 10 minutes in a thermostatic bath. After the 10 minutes the BLUE SPME fiber was exposed in the vial head space for 20 minutes, after which the injection for the GC run was performed. The gas chromatograph (Trace 1300) equipped with a capillary column (30 m x 0,25 mm) Zebron ZB-5ms with a film thickness of 0,25 µm (Phenomenex, Torrance, CA, USA) paired with the ISQ 7000 single quadruple mass spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) uses helium as mobile phase that's injected with a constant flow of 1ml per minute. As reported by Foligni et al., 2022, the operative parameters for the run were a temperature of 250°C set for the injection while for the oven a starting temperature of 40°C was set, with 6°C increment per minute and kept constant every 5 minutes till the reaching of 220°C. Volatile compounds identification was made according to Mozzon et al., 2020. For the colorimetric assay each sample was analyzed three times using the Minolta Chroma Meter CR-200 and the reading was performed using the D65 lamp. L*, a* and b* parameters from CIELAB scale were measured. L* indicates the lightness, while a* and b* are the chromaticity coordinates, where +a* is for the red and -a* for the green, while b* ranges from yellow, +b*, to blue, -b*. Starting with the L*, a* and b* parameters collected, it is possible to process further information, such as determining the ΔE. ΔE is an equation to describe the difference between two colors and it's defined by the equation:

$$\Delta E=\sqrt{(L1-L2)^2+(a1-a2)^2+(b1-b2)^2}$$

Generally, a ΔE equal to 1 is barely perceptible, and a ΔE greater than 3 means that we have different colors. Another factor that was possible to calculate was the Browning Index, with the formula:

$$(BI)=[100(x - 0.31)]/0.17, \text{ where } x=(a^*+1.75L^*)/(5.645L^*+a^*-0.3012b^*)$$

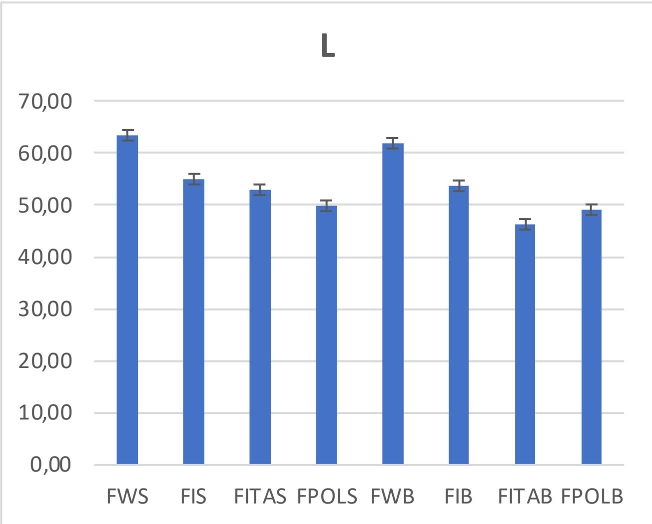
The Hue was also calculated thanks to the formula applied in the excel spreadsheet:

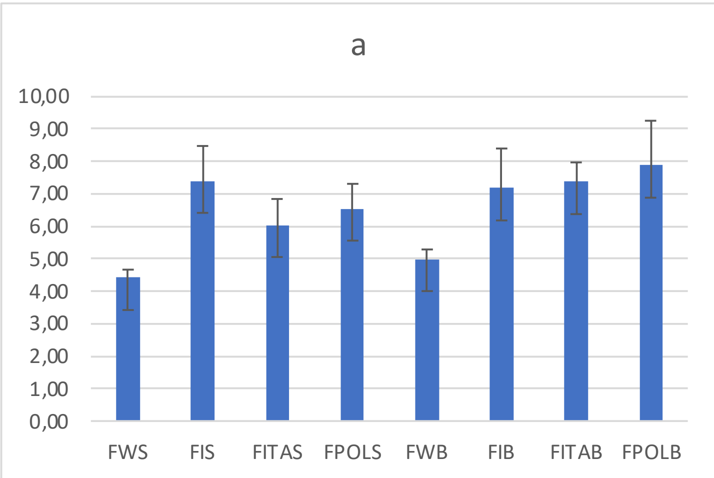
Hua=(ARCTAN(@b/@a)/6,28832)*360

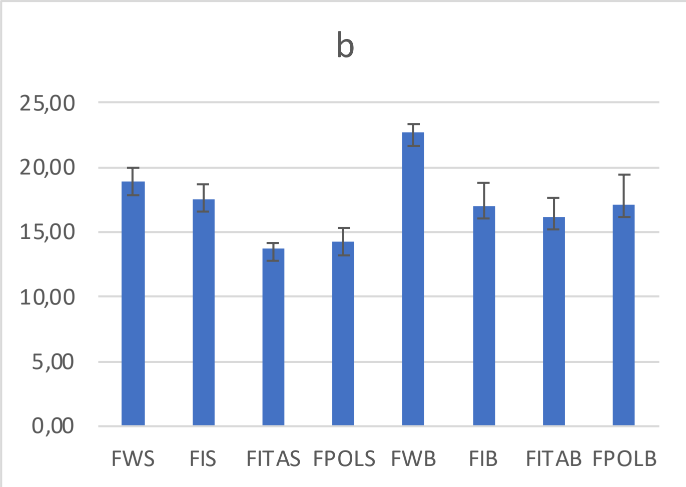
3 – RESULTS

Colorimetric assay

Color is an important feature of food products, especially considering that the product in question is an insect-based product. Therefore consumers, already susceptible towards eating insects, need to be attracted by the product, to simplify their approach to it. Indeed, it is the sight that transmits to consumers the first stimulus and response of attraction or avoidance (Porretta, 2021). Flatbread samples enriched with cricket flour should have a typical bakery product color so that the consumers will immediately associate it to a normal flatbread product, simplifying the acceptance process. The results of the colorimetric assay are here reported (Graph 1).

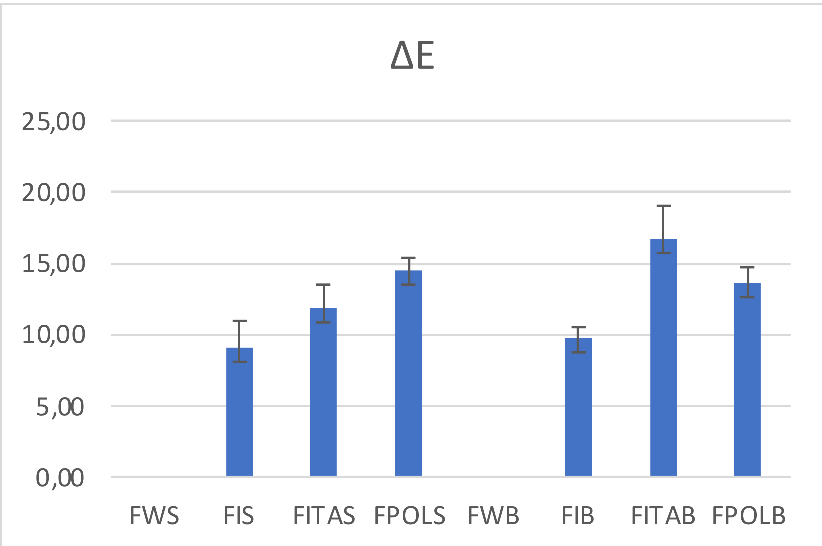






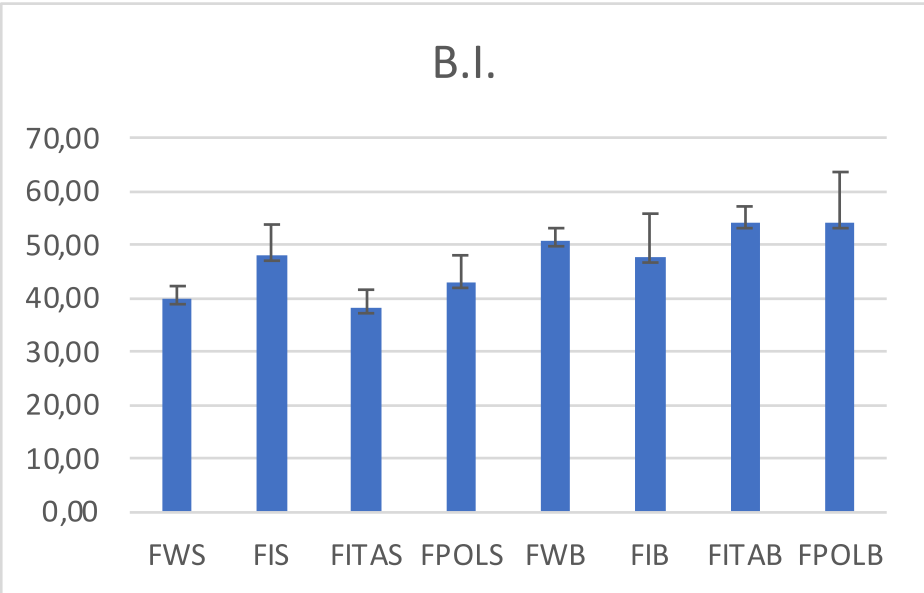
Graph 1 – Results of the colorimetric assay

The highest L* parameters are detected in the FWS and FWB samples without insect flour enrichment (63,35 – 61,86), while the lower L* values are identified in the samples presenting insect flour and buckwheat. Comparing the sample with baker's yeast not enriched with cricket flour FWS, with the other samples with baker's yeast enriched with cricket flour we obtain the ΔE values of 9,13 for FIS, 11,8 for FITAS and 14,48 for FPOLS. For the flatbread samples obtained with wheat sourdough not enriched with cricket flour FWB and the ones enriched with the cricket flour we obtain the ΔE values of 9,76 for FIB, 16,7 for FITAB and for 13,6 FPOLB (Graph 2).



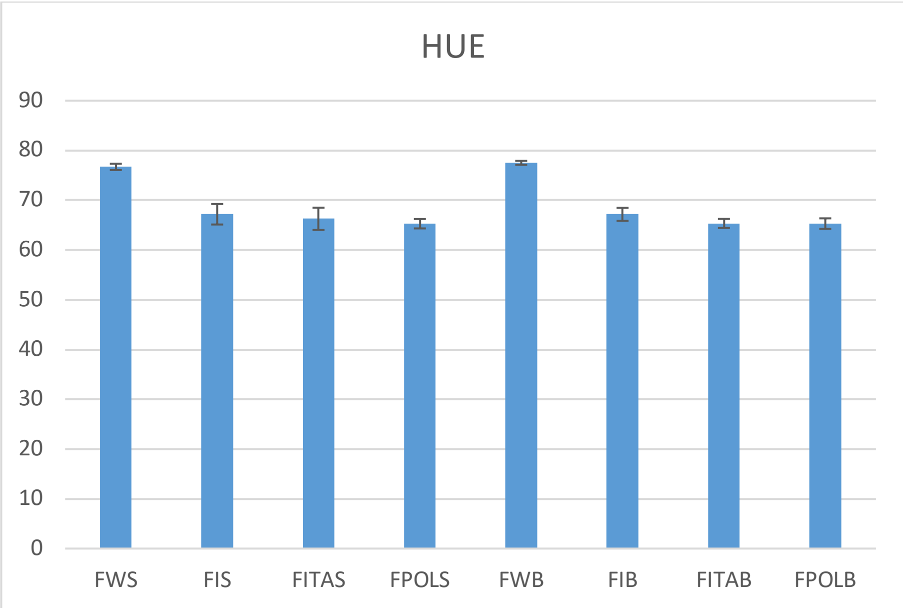
Graph 2 – ΔE values of flatbread samples – FIS, FITAS and FPOLS are compared with FWS, produced using baker's yeast. FIB, FITAB and FPOLB are compared with FWB, produced using sourdough

It's possible to notice that the samples FITAS and FPOLS compared with FWS, and the samples FITAB and FPOLB compared with FWB show a higher ΔE than respectively FIS and FIB. This might be due to the formulation of the flatbread where FITAS, FPOLS, FITAB and FPOLB were obtained using buckwheat. The higher ΔE values, pointing out the fact that the samples obtained with buckwheat are browner than the other samples, is wanted considering that the FITAS, FPOLS, FITAB and FPOLB samples can be considered whole wheat products, and consumers will expect a brown appearance. The results of the browning index calculations are the following (Graph 3):



Graph 3 – Browning Index results

Higher browning index values are identified in the samples obtained using sourdough when compared with the ones produced using baker's yeast. FITAB and FPOLB have the highest BI values (53,99 – 54,24), probably due to the combination of cricket flour, sourdough, and buckwheat. Finally, the Hue angle was calculated (Graph 4; Image 7).



Graph 4 – Hue angle results

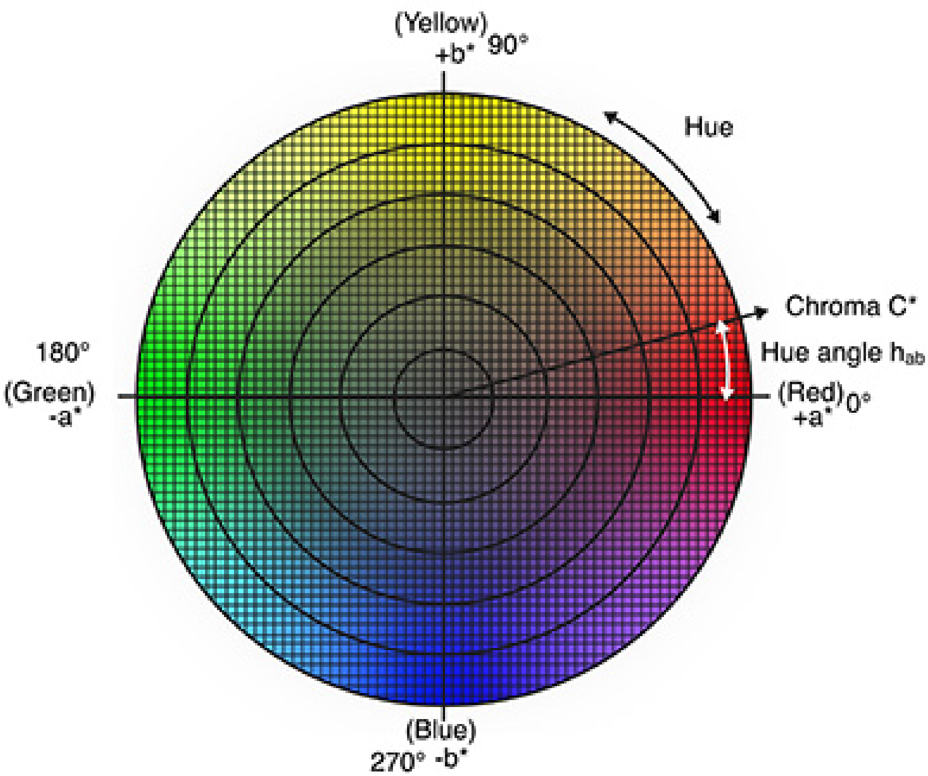


Image 7 – HUE angle color wheel

When considering the Hue angle number, it's important to also consider the L* value. FWS and FWB have the highest Hue angle number of 77, this means that the color of these samples is more tending to yellow, as expected considering they were produced without cricket flour and with soft wheat and durum wheat flour. FIS and FIB have slightly higher Hue angle value compared to FITAS, FPOLS and FITAB, FPOLB. This might be due to the presence of the buckwheat flour. Results of the color measurement showed that even small amounts of insect enrichment influence the color of the samples, as all color measurement values changed with the adding of cricket flour. With the adding of insect flour the L* value is expected to decrease, while the a* value to increase, and this occurs in all samples when compared with the FWS and FWB samples. These results are in line with literature data, as the L* value is also decreased and a* value is also increased in the case of cockroach enriched bread products and locust enriched rice cakes (De Oliveira et al., 2017; Indriani et al., 2020).

Volatiles characterization

The analysis of the volatile component of the flatbread samples has allowed to identify and quantify 23 volatile compounds belonging to 7 different classes. In detail **acids** (octanoic acid, propanoic acid, butanoic acid), **alkanes** (dodecane, nonane,2,3-dimethyl-), **alcohols** (ethanol, ethenone,1-(2-furanyl)), **aldehydes** (2,4-decadienal, nonanal, benzeneacetaldehyde, benzaldehyde, 2-heptenal, heptanal, hexanal, pentanal, butanal-2-methyl, butanal-3-methyl), **ketones** (acetophenone, 2-heptanone, acetoin), **terpenes** (limonene), **aromatics** (pyrazine,2-ethenyl-6-methyl, pyrazine,2,6-dimethyl) have been identified. Hexanal, butanal-3-methyl, butanal-2-methyl are the most represented volatile compounds (Table 9; Table 10).

Name	**CAS-NUMBER**	**Category**	**Flovour note**	**FWB Mean ± SD**	**FIB Mean ± SD**	**FITAB Mean ± SD**	**FPOLB Mean ± SD**
Ethanol	64-17-5	Alcohols	Alcohol, mild	365,9 ± 19,2^{c}	161,6 ± 7,6^{d}	200 ± 29,2^{d}	204,8 ± 25,2^{d}
Butanal-3-methyl	590-86-3	Aldehydes	Suffocating to apple like	221,45 ± 8^{c}	353 ± 10,8^{bc}	256,1 ± 16,4^{c}	101,7 ± 3,1^{c}
Butanal-2-methyl	96-17-3	Aldehydes	Dark chocolate, malt, green	222 ± 13^{bc}	424,3 ± 9^{bc}	305,1 ± 3,1^{bc}	93,3 ± 1,6^{c}
Pentanal	110-62-3	Aldehydes	Bready, fruity, nutty				
Acetoin	513-86-0	Ketones	Buttery	56,3 ± 5,7^{a}	59,2 ± 1,2^{a}	51 ± 1,5^{a}	28,7 ± 4,9^{b}
Butanoic acid	107-92-6	Acids	Parmesan, body odor	5,95 ± 0,92^{c}	17,6 ± 1^{b}	33 ± 1,6^{a}	6,9 ± 2,4^{c}
Hexanal	66–25-1	Aldehydes	Grassy	1262 ± 35^{a}	541 ± 41^{bc}	255,8 ± 0,5^{cd}	143,3 ± 1^{a}
Propanoic acid	79–09-4	Acids	Pungent				

2-heptanone	110–43-0	Ketones	Fruity, waxy, green	25,45 ± 0,78^{b}	50,7 ± 4,5^{a}	16,8 ± 0,3^{bcd}	6 ± 1,4^{cd}
Heptanal	111–71-7	Aldehydes	Fruity	62,3 ± 7^{a}	39 ± 6^{b}	19,6 ± 0,8^{c}	12,5 ± 2,9^{c}
Ethanone, 1-(2-furanyl)	1192-62-7	Alcohols	Balsamic, nutty	27,7 ± 2,4^{a}	13,2 ± 1,2^{bcd}	21 ± 5,7^{ab}	6,7 ± 1,2^{cd}
Pyrazine, 2,6-dimethyl	108-50-9	Aromatics	Chocolate	60,5 ± 10^{bc}	96,2 ± 6,7^{b}	160,7 ± 8,7^{a}	65,5 ± 12,3^{bc}
2-heptenal	18829-55-5	Aldehydes	Green	37,6 ± 3,1^{b}	20,2 ± 1,2^{d}	13,3 ± 2^{e}	10,7 ± 0,1^{e}
Benzaldehyde	100-52-7	Aldehydes	Almond	44,8 ± 1,8^{ab}	42,6 ± 4,8^{ab}	33,8 ± 4,8^{bc}	18,3 ± 4,1^{d}
Nonane,2,3-dimethyl-	2884-06-2	Alkanes	Petroleum			8,2 ± 1,3^{a}	2,6 ± 0,9^{bc}
Pyrazine,2-ethenyl-6-methyl	13925-09-2	Aromatics	Nutty, baked potato		7,7 ± 0,8^{c}	15,3 ± 1,2^{b}	4,7 ± 0,5^{cd}
Limonene	138-86-3	Terpens	Lemon, sour	21 ± 0,7^{cd}	30 ± 2,1^{ab}	27,8 ± 2,4^{abc}	12,2 ± 2,7^{ef}
Benzeneacetaldehyde	122-78-1	Aldehydes	Green, floral		5,4 ± 0,8^{c}	4,2 ± 0,1^{cd}	4,7 ± 0,7^{cd}
Dodecane	112-40-3	Alkanes	Musk				
Acetophenone	98-86-2	Ketones	Floral			110,2 ± 1,4^{b}	143 ± 16,5^{a}
Nonanal	124-19-6	Aldehydes	Rose, orange	78,1 ± 2,6^{b}	62 ± 14,4^{bc}	42,1 ± 4,4^{c}	32,4 ± 5,2^{c}
Octanoic acid	124-07-2	Acids	Pungent		6,2 ± 1,7^{bc}	1,5 ± 0,9^{bc}	1,3 ± 0,2^{bc}
2,4-decadienal	25152-84-5	Aldehydes	Fatty	15,3 ± 2,5^{a}			

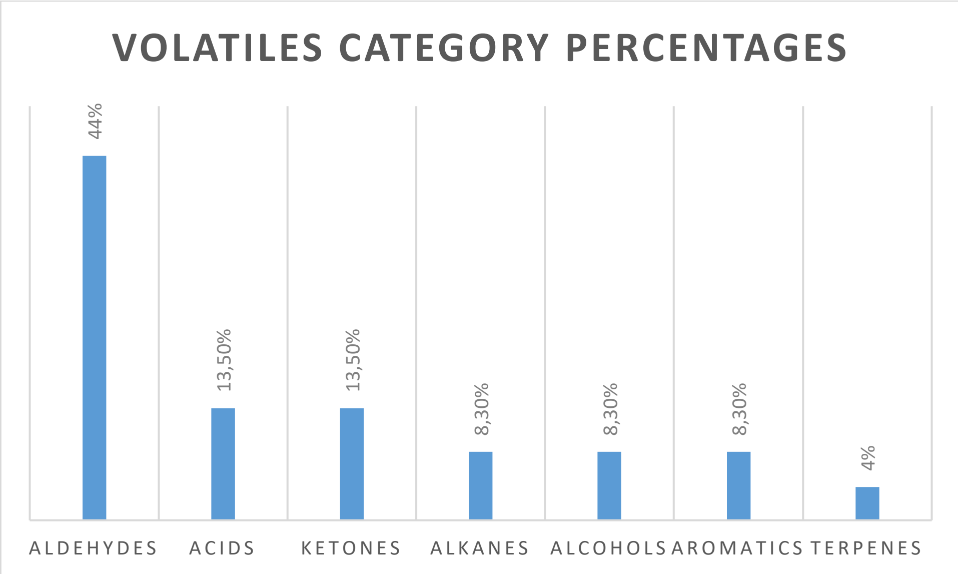
Table 9 – Volatiles table of samples obtained with baker's yeast

Name	**CAS-NUMBER**	**Category**	**Flovour note**	**FWS Mean ± SD**	**FIS Mean ± SD**	**FITAS Mean ± SD**	**FPOLS Mean ± SD**
Ethanol	64-17-5	Alcohols	Alcohol, mild	928,8 ± 9,4^{a}	558,7 ± 43,3^{b}	43,7 ± 4,1^{e}	28,8 ± 5,8^{e}
Butanal-3-methyl	590-86-3	Aldehydes	Suffocating to apple like	655,1 ± 129^{ab}	749,1 ± 60,1^{a}	94,6 ± 4,3^{c}	847,8 ± 177^{a}
Butanal-2-methyl	96-17-3	Aldehydes	Dark chocolate, malt, green	509,4 ± 72,2^{b}	927,5 ± 116,5^{a}	81,6 ± 1,4^{c}	972,1 ± 231^{a}
Pentanal	110-62-3	Aldehydes	Bready, fruity, nutty	44,6 ± 1,3^{a}			
Acetoin	513-86-0	Ketones	Buttery		5,2 ± 1,9^{c}	19,7 ± 5,2^{b}	4,7 ± 0,5^{c}
Butanoic acid	107-92-6	Acids	Parmesan, body odor			7,9 ± 0,5^{c}	
Hexanal	66–25-1	Aldehydes	Grassy	1217,2 ± 214^{a}	734,1 ± 107,4^{b}	204,4 ± 24^{cd}	408,6 ± 54^{bcd}
Propanoic acid	79–09-4	Acids	Pungent		24,7 ± 1,8^{a}		
2-heptanone	110–43-0	Ketones	Fruity, waxy, green	26,8 ± 4,6^{b}	59,6 ± 10,4^{a}	4,8 ± 0,3^{d}	22,6 ± 2,8^{bc}
Heptanal	111–71-7	Aldehydes	Fruity	46,7 ± 4,4^{b}	37,5 ± 0,5^{b}	12,2 ± 0,7^{c}	14,6 ± 0,7^{c}

Ethanone, 1-(2-furanyl)	1192-62-7	Alcohols	Balsamic, nutty	$21,9 \pm 1,2^{ab}$	$16,6 \pm 2,6^{bc}$	$4,1 \pm 0,1^{d}$	$6,9 \pm 0,7^{cd}$
Pyrazine, 2,6-dimethyl	108-50-9	Aromatics	Chocolate	$39 \pm 0,2^{c}$	$171,7 \pm 7,5^{a}$	$44 \pm 7,5^{c}$	$84 \pm 14,14^{b}$
2-heptenal	18829-55-5	Aldehydes	Green	$62,4 \pm 2,6^{a}$	$28,6 \pm 0,07^{c}$	$26,9 \pm 0,4^{cd}$	$26,6 \pm 1^{cd}$
Benzaldehyde	100-52-7	Aldehydes	Almond	$53,3 \pm 2,6^{a}$	$50,3 \pm 0,5^{a}$	$25,7 \pm 3,4^{cd}$	$33,4 \pm 1,4^{bc}$
Nonane,2,3-dimethyl-	2884-06-2	Alkanes	Petroleum		$6,8 \pm 1,3^{ab}$	$2,3 \pm 0,5^{bc}$	$8,5 \pm 2,7^{a}$
Pyrazine,2-ethenyl-6-methyl	13925-09-2	Armomatics	Nutty, baked potato	$5,8 \pm 1,5^{cd}$	$22,9 \pm 1^{a}$	$3 \pm 0,3^{de}$	
Limonene	138-86-3	Terpens	Lemon, sour	$22 \pm 2,1^{bcd}$	$33,8 \pm 2,8^{a}$	$8,2 \pm 0,7^{f}$	$18,5 \pm 2,1^{de}$
Benzeneacetaldehyde	122-78-1	Aldehydes	Green, floral	$41,5 \pm 0,8^{a}$	$8,5 \pm 0,9^{bc}$	$10,7 \pm 0,6^{b}$	$7,3 \pm 2,9^{bc}$
Dodecane	112-40-3	Alkanes	Musk				$133,8 \pm 20,7^{a}$
Acetophenone	98-86-2	Ketones	Floral				
Nonanal	124-19-6	Aldehydes	Rose, orange	$113,6 \pm 14,1^{a}$	$59,7 \pm 4,5^{bc}$	$38,9 \pm 0,2^{c}$	$58,3 \pm 2,1^{bc}$
Octanoic acid	124-07-2	Acids	Pungent	$7,8 \pm 0,7^{b}$	$42,8 \pm 5,1^{a}$	$1 \pm 0,0^{bc}$	
2,4-decadienal	25152-84-5	Aldehydes	Fatty				

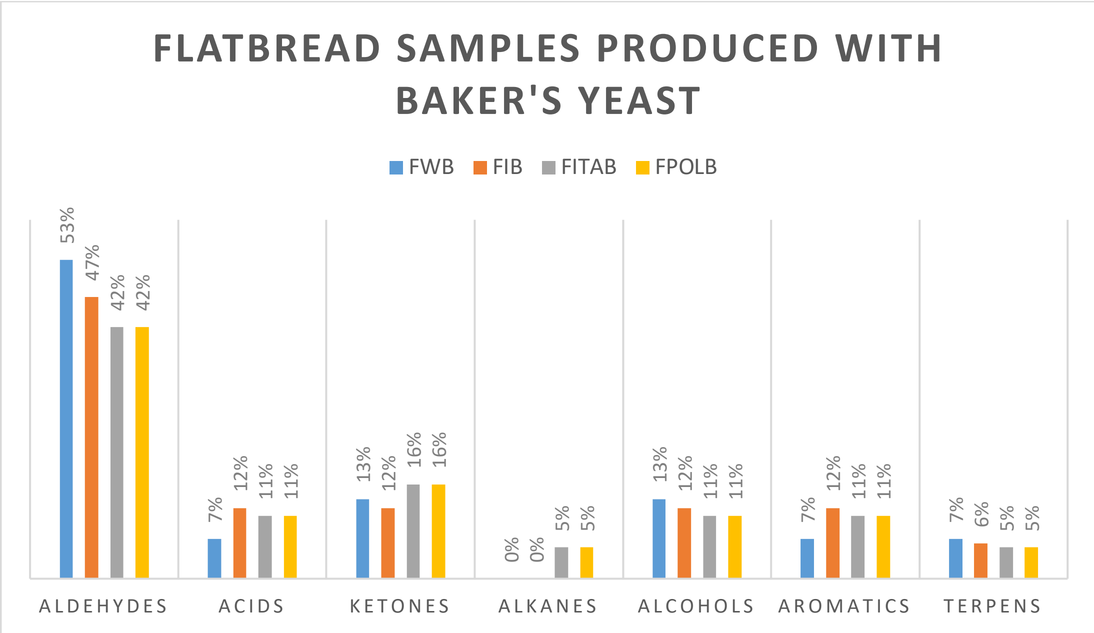
Table 10 – Volatiles table of samples obtained with sourdough

Among the volatile components 44% are aldehydes that in general provide a green, floral, nutty flavor note, 13,5% acids that provide a pungent flavor note, 13,5% ketones that provide floral and green flavor notes, 8,3% alkanes, 8,3% alcohols, 8,3% aromatics that provide a roasted aroma, 4% terpenes (Graph 5).

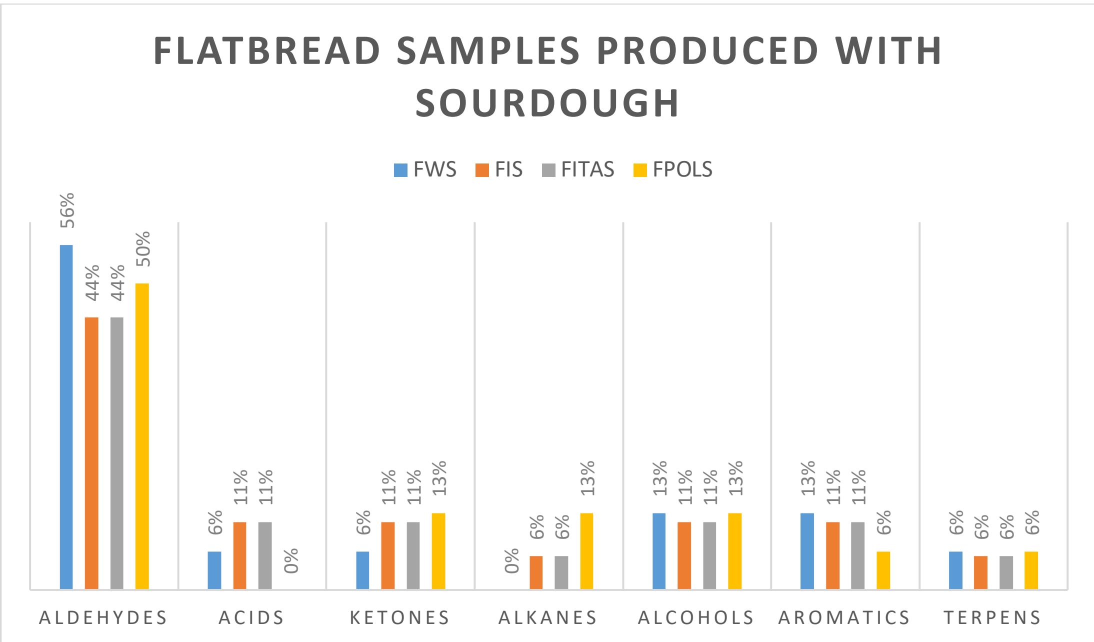


Graph 5 – Volatiles category percentages

Volatiles category percentages for the flatbread samples produced with baker's yeast and for the flatbread samples produced with sourdough are reported in the following graphs (Graph 6; Graph 7).

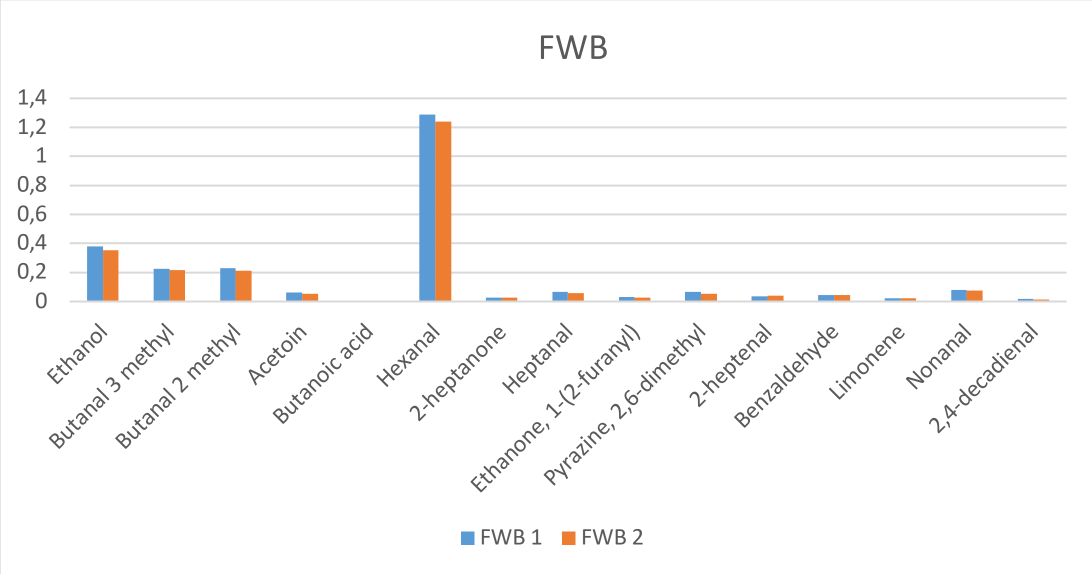


Graph 6 - Flatbread samples produced with baker's yeast



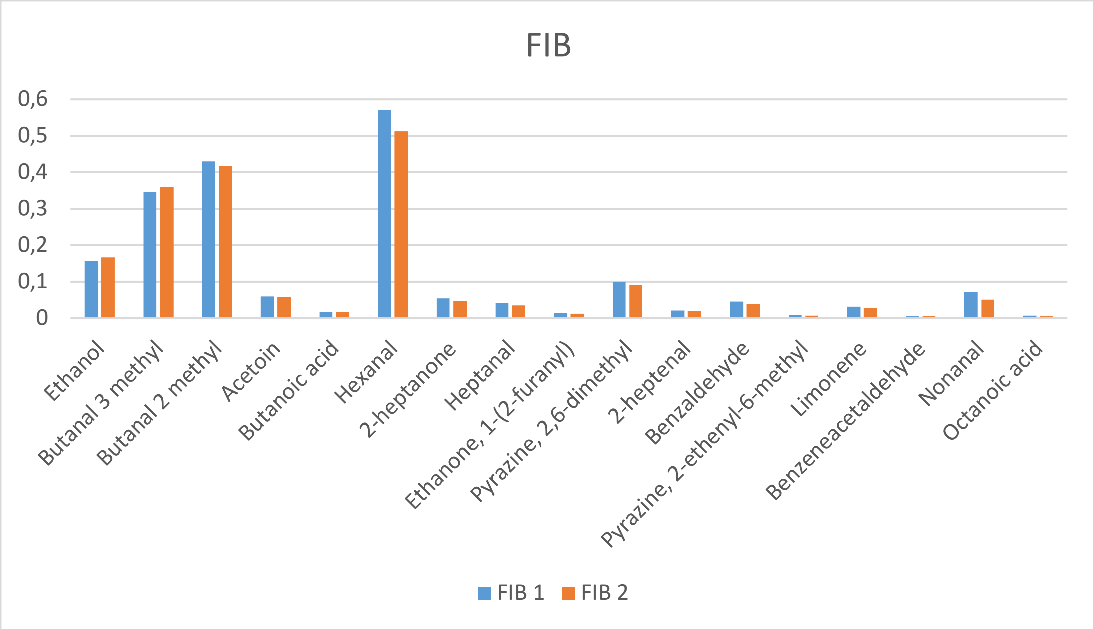
Graph 7 - Flatbread samples produced with sourdough

The chromatographs were analyzed to find out the major volatiles present in the flatbread samples. In the **FWB** sample 15 relevant peaks were identified (Graph 8), and the molecules found are ethanol, butanal-3-methyl, butanal-2-methyl, acetoin, butanoic acid, hexanal, which is the most abundant, 2-heptanone, heptanal, ethenone,1-(2-furanyl), pyrazine,2,6-dimethyl, 2-heptenal, benzaldehyde, limonene, nonanal and 2,4-decadienal. 2,4-decadienal is an aldehyde with fatty type odor that was found only in this sample.



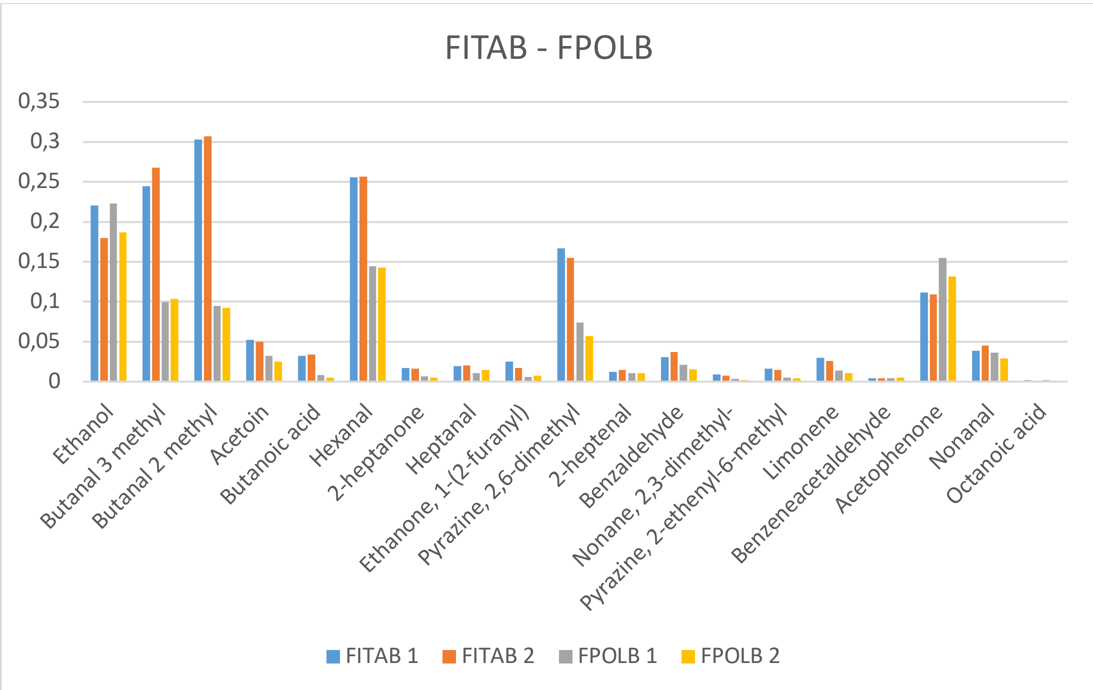
Graph 8 – FWB volatiles

In the **FIB** sample 17 volatiles were identified (Graph 9), the same of FWB, without 2,4-decadienal, and pyrazine,2-ethenyl-6-methyl, benzeneacetaldehyde and octanoic acid. Hexanal is the most present volatile.



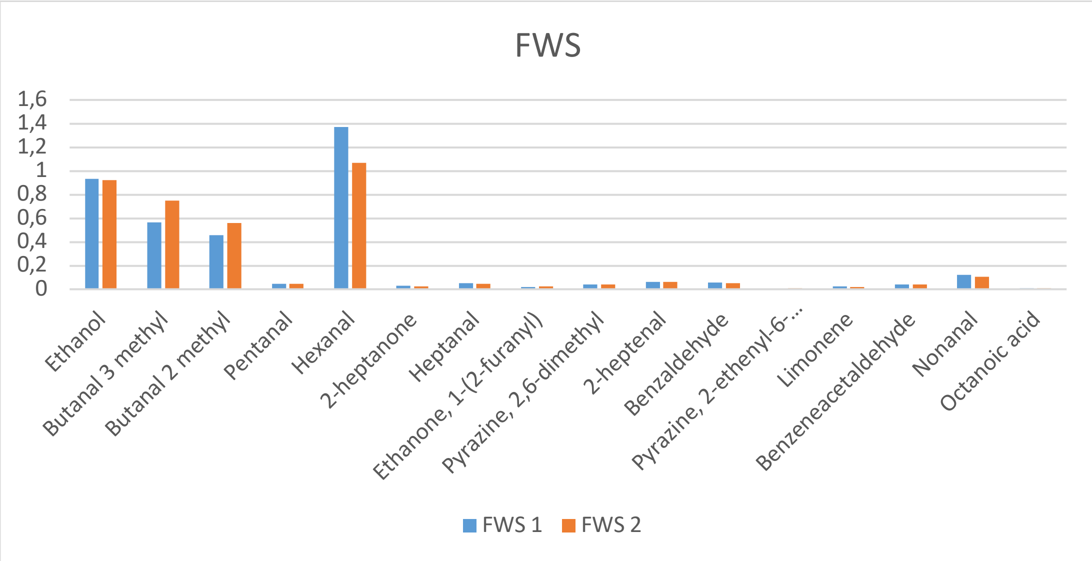
Graph 9 – FIB volatiles

FITAB and **FPOLB** present the same volatiles which are the same of FIB and added to them nonane,2,3-dimethyl and acetophenone (Graph 10). FITAB presents mainly butanal-2-methyl and butanal-3-methyl, while FPOLB presents mainly ethanol and acetophenone.



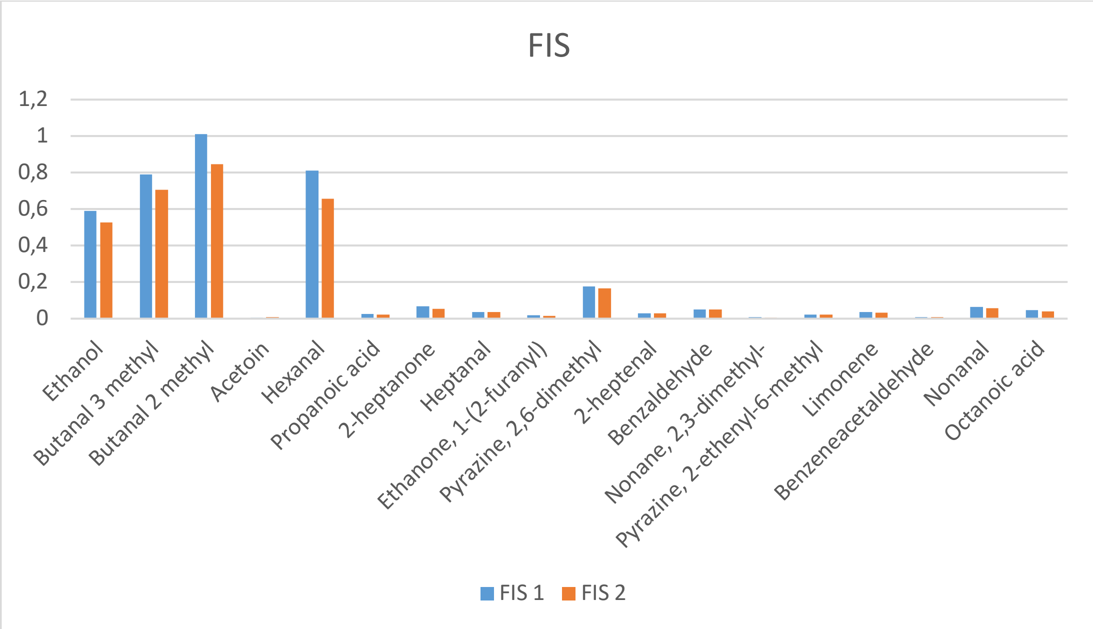
Graph 10 – FITAB and FPOLB volatiles

The sample **FWS** presents 16 volatiles (Graph 11) that are ethanol, butanal-3-methyl, butanal-2-methyl, pentanal, hexanal, 2-heptanone, heptanal, ethenone,1-(2-furanyl), pyrazine,2,6-dimethyl, 2-heptenal, benzaldehyde, pyrazine,2-ethenyl-6-methyl, limonene, benzeneacetaldehyde, nonanal and octanoic acid. Hexanal is the most abundant volatile found. Pentanal was identified only in the FWS sample, providing a bready, fruity, and nutty odor.



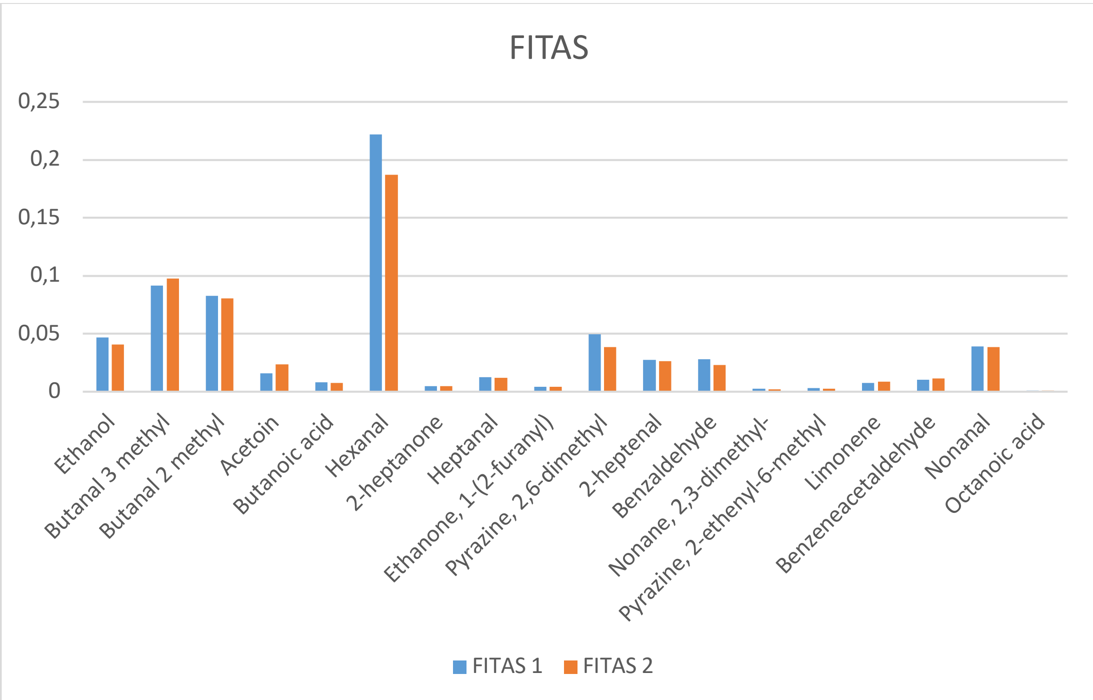
Graph 11 – FWS volatiles

FIS presents ethanol, butanal-3-methyl, butanal-2-methyl, which is the most abundant, acetoin, hexanal, propanoic acid, 2-heptanone, heptanal, ethenone,1-(2-furanyl), pyrazine,2,6-dimethyl, 2-heptenal, benzaldehyde, nonane,2,3-dimethyl, pyrazine,2-ethenyl-6-methyl, limonene, benzeneacetaldehyde, nonanal and octanoic acid (Graph 12).



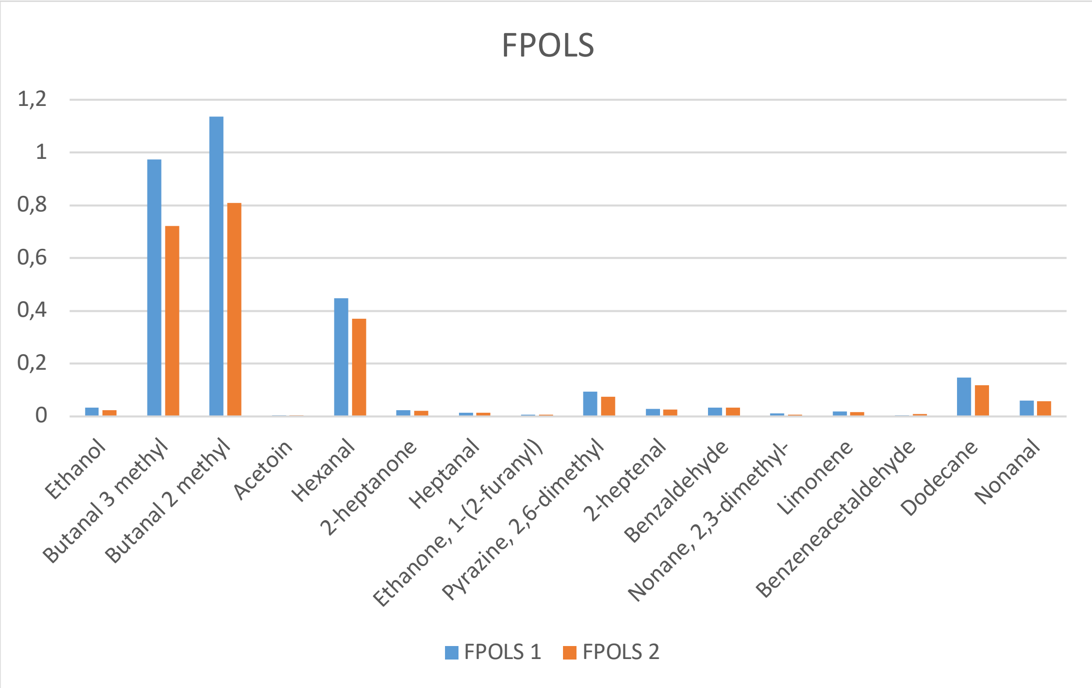
Graph 12 – FIS volatiles

FITAS presents butanoic acid and doesn't present propanoic acid compared to FIS, and the most present compound is hexanal (Graph 13).



Graph 13 – FITAS volatiles

FPOLS presents ethanol, butanal-3-methyl, butanal-2-methyl, acetoin, hexanal, 2-heptanone, heptanal, ethenone,1-(2-furanyl), pyrazine,2,6-dimethyl, 2-heptenal, benzaldehyde, nonane,2,3-dimethyl, limonene, benzeneacetaldehyde, dodecane, nonanal (Graph 14). The most abundant compound is butanal-2-methyl.



Graph 14 – FPOLS volatiles

Nonane,2,3-dimethyl is found in all samples containing insect flour, except from FIB. Acetophenone is a ketone that confers floral odors and is found only in the samples FITAB and FPOLB that are made with baker's yeast, buckwheat, and insect flour. Each volatile found will confer to the flatbread samples different odor characteristics, where the odor characteristics of each volatile found is reported in the following summary table (Table 11).

	Odor descriptor
Ethanol	Vinous odor
Butanal 3 methyl	Suffocating to apple like odor
Butanal 2 methyl	Cocoa type odor
Pentanal	Bready, fruity, nutty
Acetoin	Buttery type odor
Butanoic acid	Parmesan cheese, body odor
Hexanal	Grassy odor
Propanoic acid	Pungent odor
2-heptanone	Fruity, waxy and green odor
Heptanal	Fruity odor
Ethanone, 1-(2-furanyl)	Balsamic type, nutty odor
Pyrazine, 2,6-dimethyl	Chocolate type odor
2-heptenal	Green type odor
Benzaldehyde	Almond odor
Nonane, 2,3-dimethyl-	Petroleum like odor
Pyrazine, 2-ethenyl-6-methyl	Nutty odor
Limonene	Lemon like, sour odor
Benzeneacetaldehyde	Green, floral odor
Dodecane	Musk type odor
Acetophenone	Floral odor
Nonanal	Rose, orange odor
Octanoic acid	Pungent odor
2,4-decadienal	Fatty type odor

Table 11 – Odor descriptors

Propanoic acid, conferring a pungent aroma was only found in the FIS sample. 2,3-dimethyl-nonane was identified in the FITAB, FPOLB, FIS, FITAS and FPOLS samples. Its detection might be due to the presence of the cricket flour into the formulation, and it might be related to the utilization of buckwheat. Dodecane, providing a musk flavor note, is found only in the FPOLS sample, that is produced using polish buckwheat and sourdough. Acetophenone was detected only in the FITAB and FPOLB samples, therefore its presence might be due to the formulation of these flatbreads, consisting of cricket powder, buckwheat, and baker's yeast. Acetophenone, from the ketone group, is a volatile compound with implication in the overall flavor of flour products which could be formed during Maillard reactions (Chai D. et al., 2019). Its odor perception is pleasant, having musty, flowery, and almond characteristics. Among the volatiles, hexanal is the most abundant, providing pleasant grassy notes. Also relevant is the presence of butanal-2-methyl that confers chocolate, malt, and green flavor notes. Pyrazines are responsible of the roast aroma of the insects (Zolnierczyk et al., 2021).

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