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CHEMICAL ANALYSIS OF FATTY ACIDS AND VOLATILE COMPOUNDS IN PROCESSED EDIBLE INSECTS

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled “Chemical Analysis of Fatty Acids and Volatile Compounds in Processed Edible Insects” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 2023

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Abstract

With the current rise in population, meat consumption is continually increasing. This results into intensification of livestock production. This practice is deemed to be unsustainable due to its negative impacts on the environment as the livestock industry continues to be a significant contributor to the global greenhouse gas (GHG) emissions. To address this problem, edible insects have been studied as a potential alternative to meat in the human diet. To broaden the knowledge regarding edible insects, this study aimed to determine the volatile compounds and fatty acid composition of edible insects, namely honeybee larvae, crickets, and superworms, upon application of food processing techniques, specifically oven-drying, roasting and freeze-drying. The results showed that all three food processing techniques are effective in lowering water activity values to acceptable levels and pH values were observed to not be significantly different across the techniques. The fatty acid profile of the insects showed to be promising as it is comparable with the fatty acids found in meat, and freeze-drying showed to be effective in retaining high content of fatty acids. Volatile compounds profile for different insects showed that volatile compounds characterized with pleasant odours have been detected. Results also showed that for all three insects, freeze-drying showed to have the least number of volatile compounds present.

Keywords: entomophagy; edible insect; fatty acids; volatile compounds; chemical composition; GC/MS

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List of abbreviations

GHG	Greenhouse gas
EU	European Union
WHO	World Health Organization
PUFA	Polyunsaturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
EPA	Eicosapentanoic Acid
DHA	Decohexaenoic Acid
ARA	Arachidonic Acid
AMD	Age-related Macular Degeneration
VOC	Volatile Organic Compounds
SPME	Solid-Phase Microextraction
SDE	Simultaneous Distillation Extraction
GC	Gas Chromatography
MS	Mass Spectrometry
pH	Potential Hydrogen
TAG	Triacylglycerides
FAME	Fatty Acid Methyl Ester
MW	Molecular weight
RI	Retention Index
RT	Retention Time

1. Introduction

In general, the growth of the global population is slowing. However, in some countries, population growth is still on the rise. Asia and Africa were estimated to have 9 billion people, out of the 11 billion projected to inhabit the planet by 2100 (FAO 2017). As the population rises, the livestock sector would have to continually increase at a rate that would keep up with the growing demand for protein source (Delgado et al. 2001). Since the 1960's, meat consumption has been increasing and this has been more noticeable in the diets of developed countries, with foods sourced from animals accounting for a quarter of the protein consumed and provides the 18 % of total calories consumption globally (Mottet et al. 2017; González et al. 2020). There are different types of animal meat available to the present, but the high increase in consumption was shown to be in pig and poultry meats (Milford et al. 2019). To support this growing demand for animal protein, intensification of livestock production has been continually practiced. This action is deemed to be unsustainable due to its negative impacts on the environment as the livestock industry continues to be a significant contributor to the global greenhouse gas (GHG) emissions, ranging from 12 % to 18 % of total GHG emissions (Henchion et al. 2017; Prosekov & Ivanova 2018; González et al. 2020). Aside from that, the livestock industry can also put biodiversity at risk, such as in case of the Amazon where extensive cattle production is the main driver of land acquisition and the leading cause of deforestation in the region (Bonaudo et al. 2021). A large area of land is needed for livestock production, and currently, over 70 % of global agricultural land is used for this purpose (Mottet et al. 2017). Seeing the effects of extensive livestock production to the environment, research has been done to address the unsustainability of extensive food production. Research on a plant-based diet, ways to increase efficiency in agriculture, and looking for an alternative protein source that is more sustainable long-term has been focused for the past several years.

Among the potential alternative sources of protein, edible insects have been studied significantly in recent years due to the lower environmental and economic cost it takes to cultivate them compared to livestock (Payne et al. 2016). Rearing insects for

human and animal consumption would require less land and water, and it would also emit fewer greenhouse gases comparing with the current practice of intensive cattle raising (Raheem et al. 2019; Lange & Nakamura 2021). They are also comparable to livestock in terms of nutritional content as they can have protein content that ranges from 45-70 grams per 100 grams of dry weight depending on the species (Bukkens 1997). Despite an increase in the number of studies on edible insects and their nutritional and ecological benefits (e.g. (Kaya et al. 2015; Zielińska et al. 2015; Oibiokpa et al. 2018; Mishyna et al. 2019)) and a rise in insect consumption is observed, a large portion of the population is still skeptical about including edible insects in their diet due to the disgust factor that a lot of people associate with entomophagy despite its nutritional benefits (Murefu et al. 2019). As a result, promoting the consumption of insects has been quite challenging.

To address this problem, it is necessary to fill the knowledge gap regarding edible insects and food processing techniques that can be used to make them more appealing to consumers. The protein content of insects is one of the main arguments to promote insect consumption, but it is also important to look into other aspects such as fat and volatile compounds profile. Volatile compounds contribute significantly to the aroma of the food, which influences the overall acceptability of the final food product and determining the fat content in edible insects can emphasize the nutritional benefits of consuming insects. Focused in this paper are three edible insects, specifically crickets (*Acheta domesticus*), which is one of the insects included in the approved list of novel foods in EU (European Commission 2023), superworm (*Zophobas morio*), and bee larvae (*Apis mellifera*), both of which has a huge potential as edible insect as they are both accessible.

2. Literature Review

2.1. Insects in Human Diet

Entomophagy refers to the consumption of insects, it has been practiced since ancient times. Humans initially had insects as part of their diet, but the continuous evolution led to a change in the diet where insects were replaced by meat, and fruit consumption as they settle in communities (Ramos-Elorduy 2009). Evidence related to entomophagy were even observed to be written in the sacred books of some religions such as Christian and Islamic. The use of insects such as grasshoppers, locust and crickets as food were mentioned from Leviticus 11:22 of the Holy Bible. While in Judaism, some species of locusts are considered as kosher, which signifies that these insects are allowed for consumption (Govorushko 2019).

The region of Fertile Crescent, which includes the fertile lands from Middle east to the Nile Delta, is considered as the cradle of civilization where innovations, and even agriculture are believed to have started. From there, food production has been practiced wherein large herbivore and omnivore mammals were domesticated, and this practice has been widely spread throughout Europe. The animals that were domesticated served many roles such as mode of transportation, warmth, wool, and mainly, as a significant source of meat. These multiple roles of large domesticated mammals in early civilizations have been thought to be a great factor as to why insects, which could not offer the same benefits, have failed to be integrated more into agriculture (Van Huis A, Van Itterbeeck J, Klunder H 2013). Nevertheless, entomophagy is still practiced in many parts of the world, especially in Asia, Africa, and South America. Insects are considered as an important source of nutrient in these places and are usually considered as a traditional delicacy (Raheem et al. 2019). According to Yen (2005), aboriginal people in Australia has a low fat diet where a high portion of it are polyunsaturated fatty acids and insect is a common food to be consumed. Honey ants, grubs, and moths are among the well-known insects to be consumed, and most of the ones that are consumed feed on roots or wood. However, this practice has declined since the settlement of Europeans in

Australia. Consuming insects have also been used as a solution to combat poverty and hunger, especially when enough yield in crop production were not met. This was the case in Benin and the *Pedi* tribe from South Africa (Gahukar 2020). Insects such as grasshoppers, larvae, and crickets are also commonly consumed by the tribal people of Nagaland, while red and termites are eaten by tribal people of Mayurbhanj, Sundergarh, Koraput districts of Orissa in India. Certain native American tribes in the United States also consume insects. Grasshoppers, mealworms, crickets, and ants are the typical insects consumed in this region, where the common method of preparation is deep frying the insects prior to consumption. For instance, a dish called *Koo-tsabe* in which the pupae of the fly *Ephvdra hians* are used and consumed (Srivastava et al. 2009). Meanwhile in China, there are traces of insects being consumed 3,000 years ago. Some of the edible insects were even given to the emperor and high-ranking officials as gifts and served during banquets, but even now, entomophagy is still practiced in many parts of China (Chen et al. 2010). It is evident that consuming insects is not a new concept for some regions. Globally, beetles (Coleoptera) make up 31 % of the total percentage for the most commonly consumed insect. This is followed by caterpillars (Lepidoptera) and bees, wasps, and ants (Hymenoptera) which cover 18 % and 14 %, respectively. Insects from the order Orthoptera, Hemipteran, Odonata, and Blattodea make up the rest of the consumed insects worldwide (Zielińska et al. 2015).

For most countries in Europe, entomophagy is not widely practiced, but with the recent rise of research regarding the benefits of insect consumption, an increased interest on entomophagy were also observed (Murefu et al. 2019). Insect farms had been established in some parts of Europe where insects such as house cricket (*Acheta domestica*), yellow mealworm beetle (*Tenebrio molitor*), and desert locust (*Schistocerca gregaria*) are bred and consumed (Kouřimská & Adámková 2016).

2.2. Nutritional value of insects

Besides being part of the traditional diet, the nutritive value of edible insects has intrigued the scientific community. Generally, insects are considered as a great source of proteins, fats, and vitamins. However, this depends on the life stage, the species of the insects consumed, and the feed composition (Rumpold & Schlüter 2013; Zielińska et al. 2015). Due to their nutritional value, edible insects have been used to address the

problem of malnutrition in some regions. In some cases, children were given pulp mixed with flour that is made up from caterpillar to combat malnutrition. Termites and red ants, which were found to be high in iron and calcium, respectively, were given to people who feel weak and have anemia (Srivastava et al. 2009).

Protein is the predominant component of edible insects. The high protein value in insects typically ranges between 40 % to 70 % on dry weight basis. Another advantage of proteins from insects is that they were found to be more digestible compared to plant-based proteins with 76-98 % and 52 % digestibility, respectively (Gravel & Doyen 2020). The high-quality insect proteins also contain essential amino acids in amounts that can meet the recommended ratios set by World Health Organization (WHO) (Chen et al. 2010; Belluco et al. 2013; Raheem et al. 2019; Baiano 2020). According to Chen et al. (2010), the average amino acid in edible insects can range from 13 % - 66 % on a dry weight basis after an assessment of 100 insect species (Table 1). However, not all insect species contain all the essential amino acids. Insects such as flies and mosquitoes which belong to the Diptera order were found to be lacking in leucine and cystine, while insects such as caterpillars and bed bugs from order Hemiptera were deficient in tyrosine, phenylalanine, valine, and lysine (Raheem et al. 2019).

Table 1. Average Protein and Amino Acid Content of edible insects (% dry weight)

Order	Ave. Protein	Ave. Amino Acid
Ephemeroptera	66.26	65.97
Odonata	58.83	46.03
Orthoptera	44.10	38.87
Homoptera	51.13	42.45
Hemiptera	55.14	48.72
Coleoptera	50.41	13.27
Magaloptera	56.56	53.31
Lepidoptera	44.91	32.88
Hymenoptera	47.81	45.18

Source: (Chen et al. 2010)

The second most abundant component of edible insects is fats. Fats have an important function in the human body such as energy storage. Fats are also important

in digestion, especially for the absorption and transportation of fat-soluble vitamins in the human body (Mahan & Escott-Stum 2008).

The fat content of insects may range from 10 % to 50 % (Table 2) and generally, insects in larvae and pupae stages have higher fat content than the adult stages (Table 3) (Chen et al. 2010). The feeding diet can also influence the fat composition of insects (Rumpold & Schlüter 2013). Insects are composed of fatty acids with high nutritional value such as saturated, monounsaturated, and polyunsaturated fatty acids (Chen et al. 2010; Ordoñez-Araque & Egas-Montenegro 2021). Lipids in insects contain more Polyunsaturated Fatty Acid (PUFA) than other meat consumed at present such as pork and beef which contains small amount of PUFA. Monounsaturated Fatty Acid (MUFA) instead makes up the biggest percentage of fatty acids available in these meat (Zielińska et al. 2015). There has been great interest in PUFA, such as eicosapentaenoic (EPA), and decosahexaenoic (DHA) and arachidonic acids (ARA), over the years. This is due to the influence of such molecules on health, especially in decreasing the cardiovascular risks in an individual and the risk of diabetes by lowering the insulin resistance (González-Fernández et al. 2017). It is also suggested that marine-based PUFA decosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the retina can reduce the risk of age-related macular degeneration (AMD). These effects are attributed to the anti-inflammatory and anti-oxidative properties of these molecules (Swanson et al. 2012).

Table 2. Fat content of selected edible insect orders (%dry weight)

Order	Fat		
	High	Low	Average
Odonata	41.28	14.23	25.38
Orthoptera			2.2
Homoptera	30.60	24.85	27.73
Hemiptera	44.30	9.73	30.43
Coleoptera	35.86	14.05	27.57
Lepidoptera	49.48	5.0	24.76
Diptera			12.61
Hymenoptera	55.10	7.99	21.42

Source: (Chen et al. 2010)

Table 3. Fatty Acids of selected edible insects (%)

Species	Saturated Fatty Acids		Unsaturated Fatty Acids		
Macrotermes annandalei (Silvestri)	18.54	9.98	51.14	13.01	0.65
Macrotermes subhyalinus	33.0	1.4	9.5	43.1	3.0
Oxya chinensis (Thunberg)	25.0	26.1	27.1	2.3	
Locuta migratoria migratorioides (R. & F.)	25.5	5.8	47.6	13.1	6.9
Melanoplus sanguinipes (Fabricius)	11.0	4.0	19.0	20.2	43.0
Schistocerca gregaria (Forska) male adult	40.3	6.7	31.7	7.5	3.6
Schistocerca gregaria (Forska)female adult	34.6	5.8	37.6	10.2	6.2
Rhynchophorus phoenicis (Fabricius)	36.0	0.3	30.0	26.0	2.0
Tenerbrio molitor L.	23.6	1.4	44.7	24.1	1.5
Antheraea pernyi Guerin-Meneville pupa		2.37	27.81	24.74	24.87
Dendrolimus houi Lajonquiere pupa	3.04	4.40	29.77	9.96	22.24
Dendrolimus houi Lajonquiere adult	36.64	7.84	32.82	6.0	8.79
Galleria mellonella L.	39.6	3.1	47.2	6.5	
Musca domestica L. larva	12.7	2.3	18.2	32.5	3.3
Polyrhachis dives Smith	21.14	2.29	62.44	1.39	1.21

Source: (Chen et al. 2010)

Aside from protein and fats, insects also contain carbohydrates but in lower amounts than protein and lipids. Carbohydrates play an important function in the human body such as providing energy, contribution to the normal metabolism of fat, and its protein-sparing action, where proteins are spared for energy metabolism and are used instead in repairing and building body tissues (Roth 2011). Carbohydrates in insects vary ranging from 1-10% of dry weight depending on the type of insect (Table 4).

Many of the insect's exoskeleton is composed of chitin, an amino polysaccharide polymer (Doucet & Retnakaran 2012). The range of chitin content in organism ranges from 5 % to 40 % of dry mass of the cuticle, and the organic matrices in honeybees were reported to have 23 % to 32 % of chitin content (Berezina 2016). As the second most abundant polymer in nature, chitin has been looked into for the past few years because of its being a potential source of bioactive materials. This polymer was also reported to have several properties such as antimicrobial behaviors and biocompatibility which makes it useful in different industries (Achinivu et al. 2022).

Table 4. Carbohydrate content in some insect orders (% dry weight)

Order	Carbohydrate		
	high	low	average
Odonata	4.78	2.36	3.75
Orthoptera			1.20
Homoptera	2.80	1.54	2.17
Hemiptera	4.37	2.04	3.23
Coleoptera	2.82	2.79	2.81
Lepidoptera	16.27	3.65	8.20
Diptera			12.04
Hymenoptera	7.15	1.95	3.65

Source: (Chen et al. 2010)

2.3. Consumer acceptability of edible insect

The issue with food insecurity has been a growing concern in recent years. Hence, there is a growing interest in research about entomophagy and commercializing insect-based foods, using it for human and animal consumption as an alternative protein source (Gahukar 2020). However, a lot of people has an ingrained perception since childhood that insects are unclean creatures and for most people, that is the main reason why they reject the idea of using insects as food. In contrast to this idea, majority of the insects used in entomophagy, such as grasshoppers, weevils, beetles, and larvae of moths, butterflies, and bees are mostly herbivores, so they mostly feed on fresh plant leaves or wood material (House 2016).

Currently, the majority of the scientific articles that investigate insect acceptability and consumption in western society look into the sensory aspect or psychological issues related to the subject. The studies looked into the willingness of people to consume insects or insect-based foods, the inclination of consumers to adopt insects in their diets, which typically includes the attitudes related to food acceptability such as food neophobia, disgust factors on insect consumption, and other factors such as demographic, culture and other food-based practices (House 2016). The literature and studies have shown that people who have less disgust sensitivity and neophobia are those who are familiar or more receptive to the idea of insects as food (Chen et al. 2009; Verbeke 2015; Hartmann et al. 2015), and those with experiences of insect consumption or have entomophagy as part of the social norm (Hartmann et al. 2015; Gahukar 2020). The interest in edible insects was also found to be higher in people who believes that insects are a better alternative as protein source compared to meat in terms of impacts on the environment, which suggest that people recognize the negative effects of meat production (Verbeke 2015). However, according to Hoek et al. (2011), "providing information and increasing the awareness on the environmental benefits of eating meat substitutes is not likely to be very effective". The ethical aspects of using meat substitutes in the diets of non-users and light-users of meat substitute are not the first thing that motivates them to replace meat in the diet, but rather the sensory quality of the meat substitute. Also, the health benefits with regards to food choices are not a significant motivating factor for people to consume insects, suggesting that the reported potential health benefits of edible insects are still not enough to persuade people to substitute meat with insects (Verbeke 2015).

2.4. Western Honey Bee (*Apis mellifera)*

European honey bees (*Apis mellifera L.*) are one of the most important insects that humans utilize as they are essential to agriculture, playing a crucial role in the plant life cycle as approximately 70 percent of crop species worldwide are pollinated by bees (*Apoidea*). However, pollinating is not specific only to bees and insects from other orders can be efficient pollinators as well, such as Diptera and Lepidoptera insects (Kunc et al. 2019). Nevertheless, humans have been taking advantage of bees since ancient times,

through high valued and profitable activity such as beekeeping and in farming. Thus, their significance extends beyond the realm of ecology and extends into the realm of economics as well (Mortensen et al. 2013).

In some countries around the world such as Mexico, Thailand and China, the consumption of honey bees, specifically the brood, is a culturally acceptable and common practice. They are used in a variety of culinary preparations or as ingredients, and their processing involves a variety of operations and cooking techniques, including drying and frying. Lyophilization is also an alternative method for processing honey bee broods in Asia, where the resulting powder is marketed for use in healthy foods and beverages (Guiné et al. 2022). The current knowledge regarding the nutrient composition and functional properties of honey bee broods indicates that it can be a potential source of nutrition for humans and as animal feeds (Ghosh et al. 2021). Honey bee drone broods in particular can be a promising bee product as some beekeepers remove them from the colony as anti-swarm precaution, and to prevent the spread of Varroa mites (Borkovcová et al. 2022).

2.5. House Cricket (*Acheta domesticus*)

The *Grillidae* family is one of the taxonomic order that contains some of the most popular edible insects globally, and insects under this family are collectively referred as crickets. Among the species of crickets under *Grillidae*, *Acheta domesticus*, commonly called as House cricket, is the most reared species (Kemsawasd et al. 2022). House crickets are found to be high in protein, and compared to other animal meat consumed by humans as a protein source, crickets take up less resources, such as land and water, to be produced and has a shorter production cycle. The high potential of crickets for human consumption and as an animal feed made it popular among insect eaters, leading to the development of insect cricket farms for subsistence and commercial purposes, even in Europe where entomophagy is not commonly practiced (Magara et al. 2021). As of February 2022, frozen, dried and powdered forms of *Acheta domesticus* were authorized to be marketed as a novel food by the Commissions of the European Union (European Union 2022). The increased interest in crickets for human consumption in western society has led to the formulation of various cricket-based products such as cricket flours and protein bars (Kemsawasd et al. 2022).

2.6. Superworm (*Zophobas morio*)

Despite the increasing interest in the nutritional value of insects for human consumption and animal feeds, there are still scarce information regarding the other insects that are viable for human consumption, such as the *Zophobas morio* (Andrade et al. 2021). *Zoophobas morio*, or more commonly known as Superworm or Giant mealworm, belong to the species of darkling beetles from the order Coleoptera which are commonly reared as feed for birds, fishes, and reptiles. This insect is currently present in many parts of Europe and Asia as it has been introduced to these regions as animal feeds, but its origin can be traced back to the tropical regions of Central and South America, where several ethnic groups in Mexico have also been reported to consume this insect (Ramos-Elorduy 2009; Andrade et al. 2021). Recent studies have shown that *Z. morio* has a good potential in food application and inclusion for insect-based products in the future (Scholliers et al. 2019; Scholliers et al. 2020)

2.7. Food Processing Techniques

2.7.1. Drying

Since ancient times, various preservation techniques have been used to extend the shelf life of foods. One of these techniques is drying, which is unquestionably one of the most widely used food preservation methods in use today. It is the process of removing water from food. This water removal is accomplished through either vaporization or sublimation. Vaporization converts a solid or liquid into a gaseous (vapor) state, while sublimation directly converts a solid into a gaseous (vapor) state. Several factors, including air velocity and temperature, initial moisture content and moisture diffusion in the food sample, and the surface area of the food sample exposed to drying, can influence the drying rate of food (Guiné 2018).

This processing technique removes excess moisture in food samples leading to less chances of degradation through any chemical, enzymatic or microbiological reactions. The principal reason for this is that by removing the excess moisture in the food sample, the enzymes that can cause degradation and the microorganisms that may cause food spoilage and food poisoning will have no available water to access limiting their growth

and multiplication in the food sample. Aside from extending the shelf life of food, drying is also advantageous in reducing space and costs for the transport and storage of foods (Ahmed et al. 2013). On the other hand, drying can lead to changes in the nutritional content of the food sample affecting the quality of the final product. Vitamins such as Vitamin C and some B-vitamins are heat sensitive thus, pretreatment is sometimes applied to food prior to drying. Other sensory properties that are affected by drying are the texture and aroma. Shrinkage is a common occurrence in dried products along with the loss of colors. Drying can also cause the loss of volatile compounds in food, which can alter the aroma and flavor of the finished product (Sagar V R & Suresh Kumar 2010).

Drying food items can be energy-intensive and time consuming, especially over large amounts similar to commercial applications. Hence, there have been a lot of advances in the technology used for removing moisture in food. Aside from solar drying, which remains to be the cheapest way to remove moisture from food, oven drying and freeze-drying are being increasingly used especially in industries.

Oven Drying is a drying technique prevalent in foodstuffs where the air passes over the food product. The capacity of the air to remove the moisture in the food product is dependent on the temperature and air humidity. When the food is placed inside the dryer, it reaches a humid temperature and the drying process continues at a constant rate (constant drying rate), which equalizes the removal and absorption of moisture from food (Mazandarani et al. 2014).

Another drying technique practiced especially in industries is Freeze-drying, or also called lyophilization. It works by converting the frozen water in the food sample directly into vapor state by reducing the surrounding pressure. The temperature and pressure of the chamber where substances are freeze-dried are kept under the water's triple point, the point where the three phases of a substance can coexist in equilibrium (Peruzzi et al. 2015). Before the food undergoes the drying process in the freeze-drier, it undergoes freeze concentration pretreatment, where the samples freeze, forming crystalline ice and a glassy layer from the moisture. Sublimation in a vacuum follows freezing in the primary drying stage. The food shrinks and converts the crystalline ice into vapor, leaving a porous shell that is partially dried. The primary drying phase ends with the frozen layer gone. After primary drying, desorption occurs in secondary drying.

The substance is gradually heated at low pressure which leads to removing moisture as much as possible from the glass layer (Oyinloye & Yoon 2020). This drying method is a suitable method for heat sensitive substances. However, there is the disadvantage of energy intensive and long processing times, and the equipment for freeze-drying being expensive (Guiné 2018).

2.7.2. Cooking

Various food processing techniques have been practiced since ancient times, with cooking being the most widely used throughout history, allowing humans to diversify their knowledge. Aside from drying, this food preservation technique extends the shelf life of the food by destroying and inactivating microorganisms and reducing the enzymatic activities in the food, increases the bioaccessibility of nutrients in food, and it can also improve food's sensory qualities and palatability (Joardder & Masud 2019). Heat and mass transfer are the two most important phenomena that occur during cooking. Heat energy is transferred between two regions with a temperature difference using a heat source. In general, heat moves from a high temperature area to a low temperature area and is transferred to the food via conduction, convection, or radiation. Meanwhile, mass transfer can refer to the movement of compounds such as volatile compounds and nutrients from food to the cooking medium or from water to and from the food sample (Masud et al. 2019).

Cooking can refer to six different types of heating that differ in duration and how heat energy is applied. Boiling, stewing, baking, broiling, roasting, and frying are examples of these methods. Boiling and stewing are moist-based methods of cooking in which heat is transferred through the use of water or any liquid, whereas baking, broiling, and roasting are dry methods of cooking that may require a relatively high temperature (around 100 °C or higher). Meanwhile, frying requires the use of oil and temperatures that can sometimes exceed 100 °C (Whitney & Rolfes 2011).

Several studies (Van Huis A, Van Itterbeeck J, Klunder H 2013; Hartmann et al. 2015; van Huis 2017) have recommended that promoting insect consumption to the general public through psychological and culinary approaches may be more effective than informational approach that focuses the nutritional and environmental benefits of entomophagy (Chow et al. 2021). This suggests that cooking and other food processing

techniques applied to insects can increase the willingness of people who are not familiar with entomophagy and would be beneficial in promoting insects as part of diet (Hamerman 2016).

2.8. Volatile Compounds

The term volatile organic compound (VOC) refers to a large group of organic chemical compounds that readily evaporate and are found in numerous products (United States Environmental Protection Agency 2022). As a result of their high volatility, mobility, and resistance to degradation, VOCs can be transported over great distances in the environmental medium once they are released. Aromatic hydrocarbons (benzene, toluene, ethyl benzene, and xylene) and halogenated hydrocarbons such as chloroethylene and trichloroethylene are the most common VOCs (Pandey & Yadav 2018). In food, volatile compounds induce odour and aroma. It can also be used to check ripening, senescence and decay, as well as tracking the changes that occur in food during food processing, such as cooking and preservation, and storage (Aprea 2020). It is fundamentally essential to know the volatile compounds present in food as it goes through the different process as this can help in developing and applying a suitable product design (Perez-Santaescolastica et al. 2022).

2.8.1. Volatile Compounds Isolation

There are different kinds of techniques used to isolate organic compounds from samples and only a few have been utilized in studies analyzing volatile compounds in edible insects. The solid-phase microextraction (SPME) is the most commonly applied method in extracting volatile compounds in foods, including edible insects (Hook et al. 2002). This relatively new extraction method is a fast and simple method that proved to be efficient in the isolation of compounds from dilute solutions from complex matrix composition, including those in the liquid or gaseous state. The SPME apparatus (Figure 1) resembles a modified syringe that consists of a fiber holder that contains the SPME fiber, which is a thin silica-fused optical fiber coated with a polymer film (Sajid et al. 2019). Direct, headspace, and membrane SPME extraction modes exist. In direct mode, the fiber is placed in the sample and analytes are adsorbed onto or absorbed into the fiber coating directly from the sample matrix. In headspace mode, the SPME fiber is suspended above the sample, and analytes segregate from the sample matrix to the

fiber coating. The air in the vial prevents high molecular weight compounds and other non-volatile interferences from fouling the SPME fiber. The third mode protects SPME fibers from heavily contaminated samples with a membrane (Mottaleb et al. 2014).

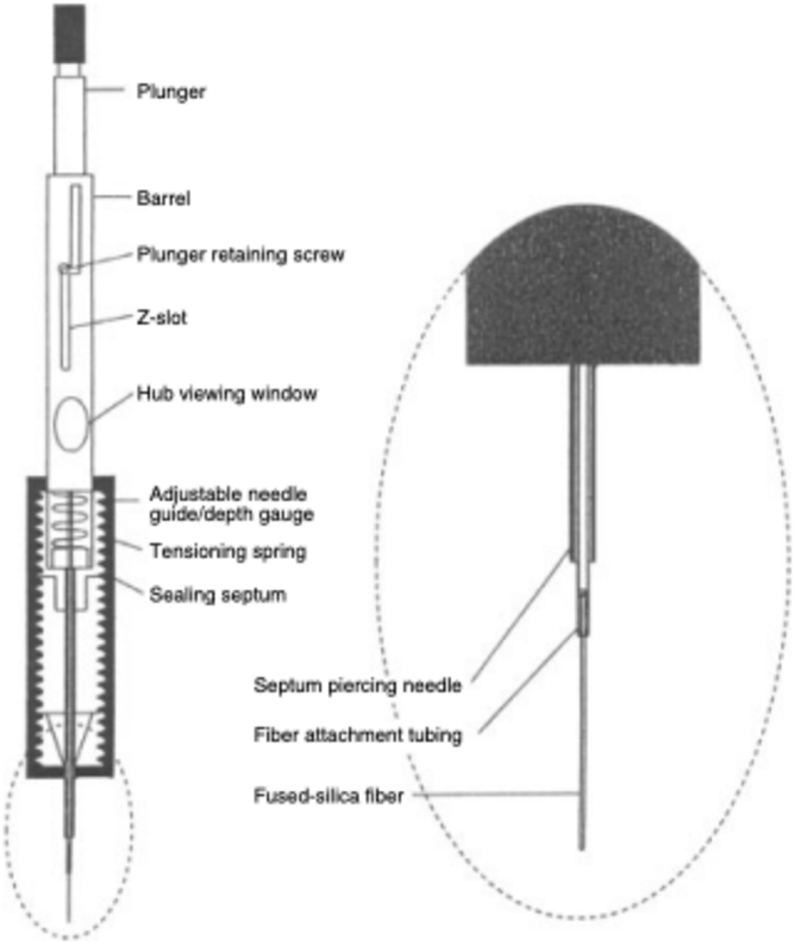

Figure 1. SPME Apparatus (Source: Vas & Vékey 2004)

A recently developed technique called HP-SPME Arrow is also utilized for volatile compound extraction. The apparatus used in this technique closely resembles that of SPME apparatus, except that the sorbent materials lines an inner metal rod and is protected by an outer metal tube which makes it more robust than the usual SPME fibers. The apparatus used in this technique contains more sorbent phase allowing more compounds to be extracted compared to traditional SPME apparatus (Šikuten et al. 2021). Currently, there are just few studies that applied this technique in extracting

volatile compounds in foods, and only Lee et al. (2022) applied the technique on oils taken from freeze-dried edible insects.

Traditionally, volatile compounds in foods, including edible insects, are extracted by steam distillation followed by extraction (Perez-Santaescolastica et al. 2022). The technique called Simultaneous Distillation Extraction (SDE) is utilized where a steam distillate is condensed and extraction of an immiscible solvent occurs at the same time and is done using the Likens and Nickerson apparatus. This technique remains to be common in volatile compound extraction as it proves to be quite effective in achieving a good recovery amount of compounds in samples (Gu et al. 2009).

2.8.2. Gas Chromatography-mass spectrometry

Gas Chromatography coupled with mass spectrometry (GC-MS) is a widely used technique to analyze pre-extracted volatile compounds. Mass spectrometry is an analytical technique which distinguishes ionized particles such as atoms and molecules based on the ratios of their charges to their respective masses (masses/charge; m/z) and can be used to calculate the molecular weight of the particles (Murayama et al. 2009; Kogler et al. 2023). Because of its low cost, stability, capability of reproducing the same results and ease of processing data, GC-MS has been commonly used in analysis compared to other methods such as nuclear magnetic resonance or liquid chromatography-mass spectrometry (Putri et al. 2022).

Samples are introduced at the column head via a sample port where a microsyringe injects the sample through a rubber septum into the vaporization chamber (Figure 2). Commercial gas chromatographs alternate between packed and capillary columns using split and splitless injections. The vaporization chamber is heated 50 °C above the lowest boiling point of the sample and mixed with the carrier gas, which can be helium, nitrogen, argon or hydrogen gas to transport the sample into the column (Perez-Santaescolastica et al. 2022). This technique separates compounds based on their varying affinity for the polymer coating of a capillary column (Zuo et al. 2013). After compound detection comes compound identification, where he use of at least two identification methods requisite, which includes the use of reference compounds (Cheseto et al. 2020; Khatun et al. 2021), literature comparison (Ssepuuya et al. 2020; Nissen et al. 2020; Żołnierczyk & Szumny 2021), or the use of different databases

available such as Wiley (Haber et al. 2019; Nissen et al. 2020; Lee et al. 2021), and NIST (Tzompa-Sosa et al. 2019; Mishyna et al. 2020; Alagappan et al. 2021).

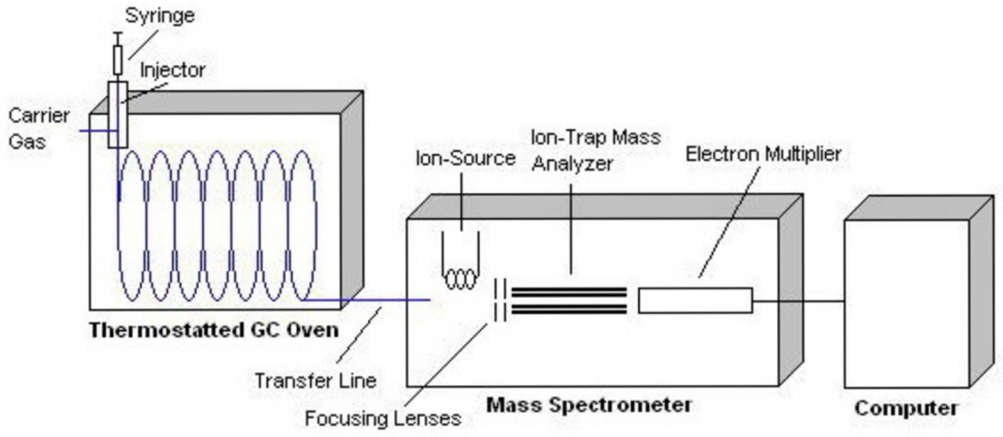

Figure 2. Schematic diagram of GC-MS (Source: Thet & Woo 2020)

3. Aims of thesis

3.1. Statement of the Problem and Objectives

This paper aims to determine the volatile compounds and fatty acid composition of processed edible insects, specifically honeybee larvae, crickets, and superworms. Identifying the fatty acid composition and volatile compounds in the insect can be useful as an added value for making edible insects appealing to possible consumers, and this study first asks:

Do the food processing techniques applied affect the pH, water activity, fatty acid and volatile compounds composition in the edible insects?

Based on the research questions, the main objective of this study is to identify the fatty acid component and volatile compound profile of the edible insect after it undergoes different processing techniques, specifically oven-drying, roasting, and freeze-drying. Given the goal of checking the fatty acid and volatile component profile of edible insect, the specific objectives are;

1.) To determine the fatty acid and volatile compounds composition of the insects in different processing methods.
2.) To compare the pH, water activity, fatty acid and volatile compound profiles in each food processing methods.

4. Methodology

4.1. Sample Collection

The study is focused on the edible insect Honeybee larvae (*Apis mellifera*), Crickets (*Acheta domesticus*), and Superworms (*Zophobas morio*). Honeybee larvae was obtained from local beekeepers from Pardubice region in the Czech Republic. The bee colony were fed in nature, collecting pollen and nectar. The crickets and worms were bought from a local shop in Prague. All the samples were immediately frozen after collection and the samples were transferred to the laboratory in the Faculty of Tropical Agrisciences at the Czech University of Life Sciences in Prague and frozen until further use.

Figure 3. Bee larvae (*Apis mellifera*), House Cricket (*Acheta domesticus*), and Superworm (*Zophobas morio*) samples (from left to right)

4.2. Processing of insect samples

The samples underwent three food processing techniques, specifically oven-drying, roasting, and freeze-drying (Figure 3). Pre-tests were conducted to determine the duration of processes for oven-drying and freeze-drying techniques. For oven drying, 50 g samples underwent drying where the samples were placed in the oven dryer

(Memmert SF30, Schwabach, Germany) maintained at the temperature of 60 °C for 7 hours, which was when the constant weight was achieved (Nyangena et al. 2020). The samples were removed from the drying chamber and were cooled at room temperature for 10 minutes. The dried samples were then grounded into powder with a grinder with 5000 rpm speed (Retsch Grindomix GM 100, Germany), and stored for further tests. For roasting, 50 g raw insect samples were placed in a stainless pan and placed over a hot plate heater without the addition of any cooking oil for the duration of 10 minutes with the occasional turning of samples in the pan using a spatula to avoid the sticking of samples in the pan. The samples were then allowed to cool at room temperature for 10 minutes and then grounded into powder with a grinder and then stored for further testing (Nyangena et al. 2020). Lastly, freeze-drying was applied to 50 g of each sample using freeze-dryer chamber (SP VirTis AdVantage Pro Freeze Dryer, USA). Frozen samples were placed in the metal trays and placed in a way that the samples were separated do not overlap with each other and sensors were attached to the samples to be able to monitor the temperatures. The process started by setting the pre-treatment temperature into -20 °C and the freeze dryer was sealed with a vacuum to also set the pressure within the chamber. This allowed the samples to be in the same temperature prior to the drying process. After attaining the ideal temperature for the samples, the drying thermal treatment started with the set temperature of -16 °C with a pressure of 774 mTorr (103.192Pa) with the duration of 95 hours.

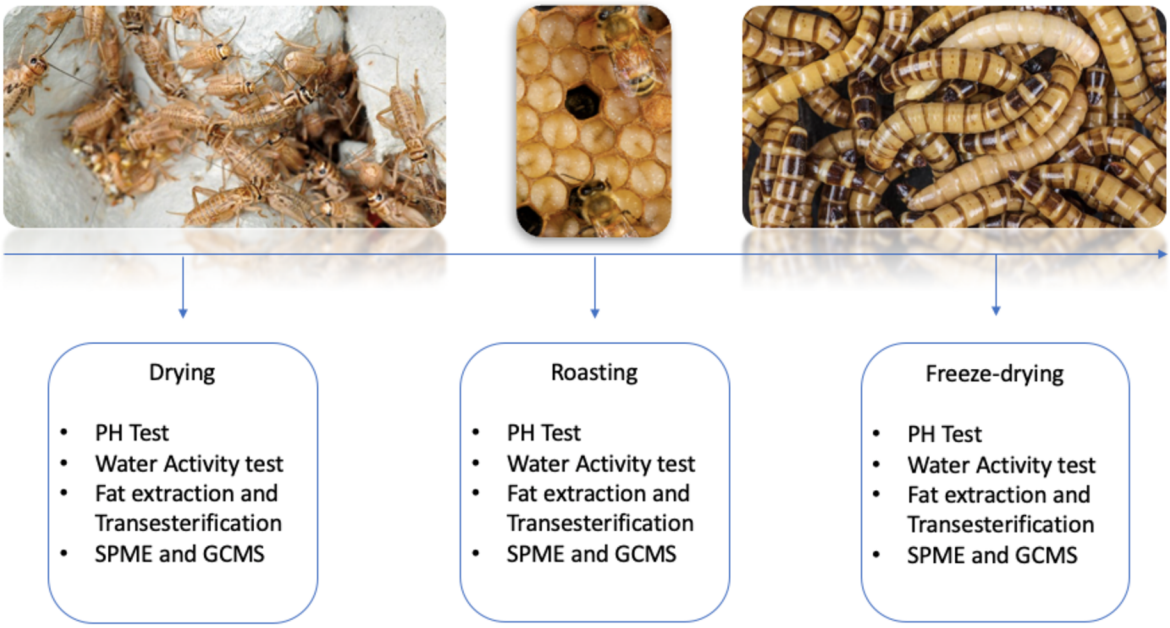

Figure 4. Summary of Food Processing Techniques and tests that were conducted on the samples (AZ Animals 2023)

Figure 5. Grinded Bee larvae (*Apis mellifera*), House Cricket (*Acheta domesticus*), and Superworm (*Zophobas morio*) samples (from left to right)

4.3. pH test

pH Test was conducted on the grinded samples using the pH meter (Orion Star A211 Benchtop pH meter, USA). The pH meter was firstly calibrated where the pH electrode probe was immersed in 25 ml aliquot of pH buffer 7 for neutral samples until

the reading was finished. The same process was done for acidic and basic samples using pH buffer 4 and 10, respectively while rinsing the electrode with distilled water in between calibrations. To test the pH of the grounded samples, 1:10 dilution was followed where 1 gram of sample was mixed to 10 grams of distilled water to create a slurry from each sampled insect. The electrodes were in the electrode holder clamps so that, when lowered into the beaker, the glass electrode was immersed just deep enough into the solution. The electrodes were soaked in the sample solution and the pH reading was initialized and the pH for each sample was recorded after each reading. The test was done in triplicates for each sample and electrodes were rinsed with distilled water after each testing (United States Environmental Protection Agency 2004).

4.4. Water Activity (A_w)

Water Activity test was conducted to the grinded insect samples using water activity meter (Novasina LabTouch-aw, Lachen, Switzerland). The sample cup of the equipment was filled enough to cover the bottom of the cup with the grinded samples without compressing the samples. The measurement was initialized in the equipment and the water activity level was recorded.

4.5. Fatty Acid Analysis

4.5.1. Fat Extraction

To determine the fat component in the insect samples, fat extraction was conducted to the grounded insect samples following Randall method of extraction using a Solvent Autoextractor (Velp Scientifica SER 158 Series Automatic Solvent Extractor, Italy). Samples (6 g) were weighted and placed inside a weighted cellulose cup, which was then placed inside a glass cup. 50 ml of n-hexane was added to the cup and then placed into the autoextractor. Modified method of fat extraction in oily seeds and nuts was the used program in the equipment, where the immersion time was set at 1 hour, followed by a removal stage with a set duration of 10 minutes. The washing stage followed with a duration set to 50 minutes and finally, the recovery period of 30 minutes. The whole process allows for the extraction of triacylglycerides (TAGs) with the solvent hexane from the sample. To separate the solvent n-Hexane from the fat, the final product was

stored at room temperature for at least a day to allow the evaporation of the solvent (Yanti et al. 2021).

4.5.2. Transesterification

In order to analyze the TAGs extracted from the samples, transesterification was conducted to prepare fatty acid methyl ester (FAME) from TAGs. Boron trifluoride/methanol reagent is a widely used reagent. However, for this test, sodium methoxide/methanol (0.5 M) was used as the boron triflouride is toxic and unstable during storage (AOAC -28:057). Samples weighing 0.1 g were put inside vials and were mixed with 1 ml of toluene, which was added as a solubilizer. Once the samples were dissolved, 5 ml of the reagent (sodium methoxide/methanol) was added. The mixture was heated to 50 °C for 15 minutes to allow the reaction to take place. After heating, the sample was allowed to cool then 5 ml of 5% aqueous acetic acid was added, followed by 4 ml of hexane. The mixture was shaken thoroughly and allowed to separate. Once the layers were visibly separated, the top layer was transferred to a small vial and used for GCMS analysis (Hammond E.W. 2003).

4.5.3. Fatty Acid Methyl Ester (FAME) Analysis

Fatty acid composition was analyzed based on the method used by Khatun et al. (2021) where GC-MS (GC 7890B/5977A MSD Agilent Technologies, USA) equipped with HP-5 column 0.25 mm x 60 m x 0.25 μm column (Sigma Aldrich, USA) was used and helium was utilized as the carrier gas with a rate of 1 ml/min. The initial temperature was set at 70 °C for 5 minutes and was gradually increased to 280 °C at the rate of 10 °C/min and the run time was 28 minutes. Data were processed using MassHunter Workstation Software Qualitative Analysis B.07.00. The peak area was obtained from electronic integration. The identification of the substances was based on the comparison of the mass spectra of the detected substances with the mass spectra of the NIST/EPA/NIH version 2.2 library. The identification was confirmed by comparing the measured RI with the database of the National Institute of Standards and Technology (NIST, USA).

4.6. Volatile Compounds

4.6.1. GC-MS Analysis

Volatile compound analysis was conducted by headspace solid-phase microextraction coupled with GC-MS (Haber et al. 2019). A blank measurement was run at the start of the day prior to any analysis to condition the equipment and make sure that contaminations between samples were avoided. 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, PA, USA) was used for volatile compound extraction. Grinded sample weighing 0.5 g was placed in a vial capped with a polytetrafluoroethylene septum and heated at 50 °C for 15 minutes while being agitated at 250 rpm. The extraction was performed by inserting the SPME fiber into the headspace, carefully avoiding contact between the fiber and the samples, while the samples were continuously heated and agitated with a magnetic stirrer for 30 minutes. After the extraction process, the fiber was transferred to the GC-MS injector.

The GC-MS analysis was performed on GC 7890B/5977A MSD (Agilent Technologies, USA) equipped with HP-5 column where the injector temperature was set at 250 °C and helium was used as carrier gas with the rate of 1 ml/min. The oven temperature was set at 40 °C for 5 minutes, then increasing it to 200 °C at a rate of 5 °C/min, followed by an increase to 250 °C degrees at a rate of 10 °C (Haber et al. 2019). The identification of constituents was based on the comparison of their retention indices (RI) and spectra with the National Institute of Standards and Technology Library ver. 2.2.f (NIST,USA), together with the authentic standards and literature (Adams 2007). Data were processed using MassHunter Workstation Software Qualitative Analysis B.07.00. The peak area was obtained from electronic integration. The identification of the substances was based on the comparison of the mass spectra of the detected substances with the mass spectra of the NIST/EPA/NIH version 2.2 library. The identification was confirmed by comparing the measured RI with the database of the National Institute of Standards and Technology (NIST, USA). The RI were calculated using the retention times of n-alkanes series ranging from C7 to C40 (Sigma-Aldrich, Prague, CZ).

4.7. Data Analysis

All tests were performed in triplicates and statistical analysis was carried out in IBM SPSS version 18.3 (IBM, New York, NY, USA). One way analysis of variance (ANOVA) was performed with Duncan's test as a post-hoc with 95 % level of confidence to evaluate the significant difference.

5. Results

5.1. pH Test

Generally, the pH of all insect samples ranged from 5.60 to 6.60 across the three different food processing techniques applied (Table 5). In bee larvae, the pH observed in roasted and freeze-dried larvae was not significantly different with a pH value of 6.32 for both food processes, while oven-dried bee larvae showed to have significantly lower pH value of 5.60 compared to roasting and freeze-drying. Meanwhile, in crickets, oven-dried and freeze-dried pH values were not significantly different from each other, with pH values of 6.10 and 6.17, respectively. However, roasted crickets showed a significantly higher pH value compared to oven-drying and freeze-drying, with a value of 6.59. Lastly, superworms were observed to have a significantly different pH value across three food processing techniques. Oven-dried worms showed the lowest pH value of 5.67, followed by freeze-dried worms then roasted worms with 6.06 and 6.60 pH values respectively.

Table 5. pH values of edible insects processed by different techniques

pH	Food Processing Technique			p-value
	Oven-dried	Roasted	Freeze-Dried	
Bee Larvae	5.60^{a}	6.32^{b}	6.32^{b}	0.003
Crickets	6.10^{a}	6.59^{b}	6.17^{a}	0.002
Superworms	5.76^{a}	6.60^{c}	6.06^{b}	0.000

The mean difference is significant at the 0.05 level
Values in the same row having different superscripts letters differ significantly ($p < 0.05$)

5.2. Water Activity (A_w) Test

The water activity test results showed no significant difference between the different processing techniques for all three insect samples (Table 6). In bee larvae, the water activity values ranged from 0.20, which was observed in oven-drying, to 0.26 observed from roasting. For crickets, the water activity values ranged from 0.21, which was observed in oven-drying, to 0.30 that was observed in freeze-drying. Meanwhile, in superworms, the water activity value ranged from 0.20 that was observed in roasting to the water activity value of 0.41, which was observed in freeze-drying.

Table 6. Water activity(a_w) values of edible insects processed by different techniques

a_w	Food Processing Technique			p-value
	Oven-dried	Roasted	Freeze-Dried	
Bee Larvae	0.20	0.26	0.23	0.757
Crickets	0.21	0.24	0.30	0.575
Superworms	0.24	0.20	0.41	0.260

The mean difference is significant at the 0.05 level

5.3. Fat Analysis

The fatty acid composition of the insect samples was determined after the transesterification of fats with methanol and analysis in GC-MS. Retention Indices of fatty acid methyl esters were calculated and compared to the literature. A total of 23 fatty acids were identified from the corresponding fatty acid methyl esters detected during the fatty acid analysis of the three insect samples (Table 7).

Table 7. Retention Indices of FAME detected in all three insect samples

Corresponding Fatty acids	Retention Index calc.	Retention Index lit.
Octanoic acid	1124	1129
Decanoic acid	1328	1327
Dodecanoic acid	1528	1524
(*Z*)-Tetradec-9-enoic acid	1705	1703
Tetradecanoic acid	1730	1725
10,13-Dimethyltetradecanoic acid	1731	*
12-Methyltetradecanoic acid	1801	*
Pentadecanoic acid	1831	1826
Hexadeca-7,10-dienoic acid	1903	*
(*Z*)-Hexadec-9-enoic acid	1910	1890
Hexadecanoic acid	1928	1921
15-Methylhexadecanoic acid	1995	1996
14-Methylhexadecanoic acid	2004	*
(*Z*)-Heptadec-10-enoic acid	2011	*
Heptadecanoic acid	2032	2028
(9Z,12Z)-octadeca-9,12-dienoic acid	2107	2101
(*Z*)-Octadec-9-enoic acid	2116	2100
Octadecanoic acid	2136	2135
Nonadecanoic acid	2234	2230
Eicos-11-enoic acid	2312	*

Continuation of Table 7

Corresponding Fatty acids	Retention Index calc.	Retention Index lit.
Eicosanoic acid	2336	2339
Docosanoic acid	2535	2531

*indicates that the retention index of fatty acid is not available in the literature used for comparison

5.3.1. Bee Larvae

Analysis of Fatty Acids revealed that the majority of fatty acids detected in bee larvae are saturated fatty acids. Specifically, 11 saturated fatty acids, followed by 3 monounsaturated fatty acids (MUFAs) and 1 polyunsaturated fatty acid (PUFA) were detected across all three food processing techniques applied (Table 8). Roasting showed to have the highest number of fatty acid composition, where 1 polyunsaturated, 3 monounsaturated, and 8 saturated fatty acids were detected. This was followed by freeze-drying with 2 monounsaturated, and 8 saturated fatty acids. Oven-drying yielded the least fatty acid composition with only 1 monounsaturated, and 4 saturated fatty acids.

Fatty acids such as Oleic acid, Myristic acid, Palmitic acid, Stearic acid, and Arachidic acid were observed to be present in all three food processing techniques, and results showed that the quantity of these fatty acids in freeze-drying was significantly higher compared to both oven-drying and roasting (Table 8). Palmitoleic acid and 14-methylhexadecanoic acid were both detected in roasted and freeze-drying, where the quantity for both FAs was observed to be significantly higher in freeze-drying compared to roasting. Meanwhile, some fatty acids were observed in just one food processing technique. Hexadecadienoic acid, (*Z*)-heptadec-10-enoic acid, Pentadecylic acid, Margaric acid, and Nonadecylic acid were detected only in roasting, and Lauric acid, 15-methylhexadecanoic acid, Behenic acid were detected only in freeze-drying.

In terms of the fatty acids that are most abundant in each food processing technique, the content of Oleic acid was found to be the highest in oven-drying, followed by Palmitic acid, then Stearic acid. Roasting followed the same trend, where Oleic acid, Palmitic acid, Stearic acid showed as the top three fatty acids in the same order. However, Palmitic acid was found to be in higher content than Oleic acid in freeze-drying. This was followed by Stearic acid.

Table 8. Fatty Acids (area value x 10^5) in bee larvae applied with different food processing techniques

Fatty acids	Common Name	Food Processing Techniques			p-values
		Oven-dried	Roasting	Freeze-dried	
PUFA					
7,10-Hexadecadienoic acid	Hexadecadienoic acid	-	6.52	-	
MUFA					
(*Z*)-Hexadec-9-enoic acid	Palmitoleic acid	-	26.61[a]	46.82[b]	0.046
(*Z*)-Heptadec-10-enoic acid	-	-	3.77	-	
(*Z*)-octadec-9-enoic acid	Oleic acid	108.22[a]	493.78[a]	2,104.00[b]	0.000
Saturated Fatty Acids					
Pentadecanoic acid	Pentadecylic acid	-	2.41	-	
Dodecanoic acid	Lauric acid	-	-	2.39	
Tetradecanoic acid	Myristic acid	3.96[a]	7.04[a]	297.26[b]	0.000
Hexadecanoic acid	Palmitic acid	105.60[a]	252.71[a]	2,390.35[b]	0.000
14-Methylhexadecanoic acid	-	-	0.88[a]	4.07[b]	0.041
Heptadecanoic acid	Margaric acid	-	6.00	-	
15-Methylhexadecanoic acid	-	-	-	5.95	
Octadecanoic acid	Stearic acid	46.33[a]	77.64[a]	812.53[b]	0.001
Nonadecanoic acid	Nonadecylic acid	-	0.95	-	
Eicosanoic acid	Arachidic acid	1.45[a]	3.56[a]	31.55[b]	0.004
Docosanoic acid	Behenic acid	-	-	12.06	

PUFA – Polyunsaturated fatty acid; MUFA – Monounsaturated fatty acid
The mean difference is significant at the 0.05 level
Values in the same row having different superscripts letters differ significantly ($p < 0.05$)

5.3.2. Crickets

In fat analysis of crickets, results showed that the majority of the fatty acids detected were saturated fatty acids. The analysis for all three food processing techniques detected 17 fatty acids, which is composed of 12 saturated fatty acids, followed by 4 monounsaturated fatty acids and 1 polyunsaturated fatty acid (Table 9). Compared to other food processing techniques, freeze-drying showed to have the highest number of fatty acid composition, where 10 saturated, 3 monounsaturated and 1 polyunsaturated fatty acid. This was followed by roasting, where 8 saturated, 2 monounsaturated, and 1 polyunsaturated fatty acid were detected. Oven-drying showed the least fatty acid composition, where 4 saturated, 2 monounsaturated, and 1 polyunsaturated fatty acid were observed.

Fatty acids such as Linoleic acid, Palmitoleic acid, Palmitic acid, and Stearic acid were detected in all three food processing techniques, where the contents of Linoleic acid, Palmitic acid, and Stearic acid were significantly higher in freeze-drying compared to oven-drying and roasting. However, the content for Palmitoleic acid across all three food processing techniques were shown to be significantly different from each other, where the lowest content was observed in oven-drying and the highest content was observed in freeze-drying (Table 9).

Lauric Acid, Myristic acid, Pentadecylic acid, 14-methylhexadecanoic acid, Margaric acid, and Arachidic acid were detected in both roasting and freeze-drying. However, it is only the contents of Margaric acid and Arachidic acid in freeze-drying that were found to be significantly higher compared to roasting. On the other hand, Oleic acid was detected only in oven-drying and roasting, and it is shown to have significantly higher content in the latter. Other notable fatty acids detected were (*Z*)-Heptadec-10-enoic acid, Gondoic acid, 12-methyltetradecanoic acid, and 15-methylhexadecanoic acid, which were observed only in freeze-drying.

In terms of the fatty acids that are most abundant in each food processing technique, it was also observed in oven-drying that Oleic acid was in the highest quantity, followed by Linoleic acid, then Palmitic acid. However, in roasting, Palmitic acid was found to have the highest quantity, followed by Oleic acid, then Linoleic acid. In freeze drying, Linoleic

acid showed the highest quantity, followed by Palmitic acid, then Stearic acid, which was also observed to be the fourth most abundant fatty acid in roasting.

Table 9. Fatty Acids (area value x 10^5) in crickets applied with different food processing techniques

Fatty Acids	Common Name	Food Processing Techniques			p-values
		Oven-dried	Roasting	Freeze-dried	
PUFA					
(9*Z*,12*Z*) -octadeca-9,12-dienoic acid	Linoleic acid	9.12[a]	248.83[a]	1,378.86[b]	0.016
MUFA					
(*Z*)-Hexadec-9-enoic acid	Palmitoleic acid	0.22[a]	14.57[ab]	57.52[b]	0.031
(*Z*)-Heptadec-10-enoic acid	-	-	-	6.21	
Eicos-11-enoic acid	Gondoic acid	-	-	12.98	
(*Z*)-octadec-9-enoic acid	Oleic acid	18.23[a]	273.38[b]	-	0.001
Saturated Fatty Acids					
Dodecanoic acid	Lauric acid	-	1.11	2.54	0,058
10,13-Dimethyltetradecanoic acid	-	0.05	-	-	
Tetradecanoic acid	Myristic acid	-	10.68	29.43	0.051
12-Methyltetradecanoic acid	-	-	-	2.95	
Pentadecanoic acid	Pentadecylic acid	-	2.01	6.62	0.070
Hexadecanoic acid	Palmitic acid	8.94[a]	295.58[a]	942.36[b]	0.007
15-Methylhexadecanoic acid	-	-	-	3.04	
14-Methylhexadecanoic acid	-	-	0.66	3.71	0.119
Heptadecanoic acid	Margaric acid	-	5.44[a]	23.26[b]	0.011
Octadecanoic acid	Stearic acid	4.61[a]	92.51[a]	420.04[b]	0.003
Eicosanoic acid	Arachidic acid	-	1.55[a]	13.25[b]	0.029

PUFA – Polyunsaturated fatty acid; MUFA – Monounsaturated fatty acid
The mean difference is significant at the 0.05 level
Values in the same row having different superscripts letters differ significantly ($p < 0.05$)

5.3.3. Superworms

In fat analysis of superworms, results showed that majority of the fatty acids detected were saturated fatty acids. Overall, 15 saturated, 6 monounsaturated, and 1 polyunsaturated fatty acids were observed across all three food processing techniques (Table 10). Both oven-drying and freeze-drying were detected to have similar number of fatty acid composition (18). 12 saturated, 5 monounsaturated, and 1 polyunsaturated fatty acids were observed in oven-drying, while 12 saturated, and 6 monounsaturated fatty acids were observed in freeze-drying. On the other hand, 8 saturated and 2 monounsaturated fatty acids were found in roasting.

Some fatty acids such as Oleic acid, Palmitoleic acid, Lauric acid, Arachidic acid, Stearic acid, Myristic acid, and Palmitic acid were observed to be present in all three food processing techniques. For Oleic acid and Palmitoleic acid, the contents of both fatty acids from freeze-drying were significantly higher compared to both oven-drying and roasting, while the contents of Lauric acid, Arachidic acid, Stearic acid, and Myristic acid from oven-drying were found to be significantly lower compared to both roasting and freeze-drying. Contents of Palmitic acid were observed to be significantly different across all three food processing techniques, where the lowest value was observed in oven-drying while the highest value was observed in freeze-drying. Meanwhile, some fatty acids were observed to be present only in both oven-drying and freeze-drying, and among those, only trans-16-octadecenoic acid was observed to have no significant difference in values (Table 10). Other notable fatty acids detected were Gondoic acid, which was present only in freeze-drying, and Hexadecadienoic acid, which was the only polyunsaturated fatty acid detected and present only in oven-drying.

In terms of fatty acids that are most abundant in each food processing technique, Oleic acid, Palmitic acid, and Stearic acid were found to be in the highest quantity across all three food processing techniques. In oven-drying, Oleic acid was observed to have the be the most abundant fatty acid, followed by Palmitic acid, then Stearic acid. Freeze-drying followed the same trend where Oleic acid was the most abundant, followed by Palmitic acid, then Stearic acid. However, in roasting, Palmitic acid showed to be in the highest quantity, followed by Oleic acid, then Stearic acid.

Table 10. Fatty Acids (area values x 10^5) in superworms applied with different food processing techniques

Fatty Acids	Common Name	Food Processing Techniques			p-values
		Oven-dried	Roasting	Freeze-dried	
PUFA					
Hexadeca-7,10-dienoic acid	Hexadecadienoic acid	20.05	-	-	
MUFA					
Eicos-11-enoic acid	Gondoic acid	-	-	21.14	
(*Z*)-Heptadec-10-enoic acid	-	8.65[a]	-	66.75[b]	0,005
(*Z*)-Tetradecenoic acid	Myristoleic acid	3.49[a]	-	26.09[b]	0,000
(*Z*)-Octadec-9-enoic acid	Oleic acid	1,125.13[a]	1,203.41[a]	3,153.72[b]	0,000
(*Z*)-Hexadec-9-enoic acid	Palmitoleic acid	76.57[a]	33.42[a]	429.22[b]	0,000
Saturated Fatty Acids					
Decanoic acid	Capric acid	1.45[a]	-	7.87[b]	0,031
Docosanoic acid	Behenic acid	-	5.16	-	
Dodecanoic acid	Lauric acid	1.63[a]	12.54[b]	9.70[b]	0,001
Eicosanoic acid	Arachidic acid	5.67[a]	17.45[b]	18.31[b]	0,001
Heptadecanoic acid	Margaric acid	15.66[a]	-	77.05[b]	0,012
14-Methylhexadecanoic acid	-	2.38[a]	1.80[a]	21.49[b]	0,000
15-Methylhexadecanoic acid	-	-	3.10	-	
Nonadecanoic acid	Nonadecylic acid	1.29[a]	-	9.51[b]	0,050
Octanoic acid	Caprylic acid	6.77[a]	-	27.38[b]	0,010
Hexadecanoic acid	Palmitic acid	618.89[a]	1,299.92[b]	2,298.70[c]	0,000
Pentadecanoic acid	Pentadecylic acid	7.66[a]	-	45.25[b]	0,021

Continuation of Table 10

Fatty Acids	Common Name	Food Processing Techniques			p-value
		Oven- dried	Roasting	Freeze-dried	
Octadecanoic acid	Stearic acid	205.39[a]	512.62[b]	578.56[b]	0,010
12-Methyltetradecanoic acid	-	1.68[a]	-	9.35[b]	0,045
Tetradecanoic acid	Myristic acid	29.21[a]	140.41[b]	153.22[b]	0,001

PUFA – Polyunsaturated fatty acid; MUFA – Monounsaturated fatty acid
The mean difference is significant at the 0.05 level
Values in the same row having different superscripts letters differ significantly ($p < 0.05$)

5.4. Volatile Compounds

5.4.1. Bee Larvae

In total, 38 compounds were identified across all three food processing techniques. The highest number of volatile compounds composition were observed in roasting with 24 compounds. In oven-drying, 22 compounds were detected while only 9 compounds were detected in freeze-drying (Table 11). In terms of volatile compound quantity in the food processing techniques, the most abundant volatile compound observed in oven-drying was γ-Terpinene representing 27.62 % . This is followed by m-Cymene with 25.61 % and α-Terpinene with 12.61 %. In roasting, the most abundant volatile compound observed was 2,6-Dimethyl-5-aminopyridine with 15.78 %, and it was only present in roasting. This was followed by 2,5-Dimethylpyrazine with 15.54 % and 2-Ethyl-3,6-dimethylpyrazine with 9.63 %. Other notable compounds detected in roasting were Ocimene, and D-Limonene, representing 8.62 %, and 7.60 % respectively. Meanwhile, only γ-Terpinene was present in high amount for freeze-drying representing 37.45 %. Some of the volatile compounds such as m-Xylene, p-Xylene, 3-Thujene, β-Pinene, β-Myrcene were present in all three food processing techniques.

Table 11. Volatile Compounds identified in bee larvae applied with different food processing techniques

Volatile compounds	MW	RI calc.	RI lit.	Area Sum %		
				Oven-drying	Roasting	Freeze-drying
Dimethyl disulphide	94	726	727	0.29	-	-
Methyl benzene	92	751	746	0.25	-	0.68
Diallyl sulphide	114	855	862	0.16	-	
m-Xylene	106	858	866	0.20	0.27	0.72
p-Xylene	106	867	888	0.33	1.12	0.29
n-Nonane	128	898	899	0.38	-	-
2,5-Dimethylpyrazine	108	916	915	-	15.54	-
Allyl methyl disulfide	120	917	922	0.50	-	-
3-Thujene	136	933	931	0.11	0.24	0.10
1R-α-Pinene	136	934	939	0.15	-	-
1-Butylpyrrole	123	949	*	-	1.15	-
Propylbenzene	120	956	955.3	0.46	-	-
p-Ethyltoluene	120	972	965.3	1.78	0.73	0.76
β-Pinene	136	978	980	0.58	0.59	0.97
β-Myrcene	136	993	991	0.93	0.55	0.24
Mesitylene	120	997	998	1.52	-	-
2,6-Dimethyl-5-aminopyridine	122	1008	*	-	15.78	-
α-Terpinene	136	1019	1018	12.61	-	0.47
5-Ethyl-m-xylene	134	1029	1058	-	0.83	-
m-Cymene	134	1031	1030	25.61	-	-
D-Limonene	136	1033	1031	5.30	7.60	-
Ocimene	136	1052	1041	-	8.62	-
1-Pentylpyrrole	137	1058	*	-	0.44	-
γ-Terpinene	136	1063	1062	27.62	1.65	37.45
2-Ethyl-3,6-dimethylpyrazine	136	1084	1082	-	9.63	-
Allyl disulphide	146	1085	1085	0.70	-	-
2-Ethyl-3,5-dimethylpyrazine	136	1092	1090	-	1.95	-
p-Cymenene	132	1095	1095	0.14	-	-
n-Nonaldehyde	142	1108	1102	-	1.30	-
4-Amino-3-(1-methylethyl) phenol	151	1126	*	-	0.73	-

Continuation of Table 11

Volatile compounds	MW	RI calc.	RI lit	Area Sum %		
				Oven-drying	Roasting	Freeze-drying
Allo-Ocimene	136	1134	1131	-	0.22	-
2-Methyl-5,6-diethylpyrazine	150	1161	1151	-	0.30	-
2-Methyl-3,5-diethylpyrazine	150	1164	1156	-	1.27	-
Pyrazine, 3-isobutyl-2,5-dimethyl-	164	1208	1208	-	0.78	-
1-Nitroso-2,4-dimethylamino-benzene	165	1232	*	-	0.31	-
2-Methyl-6-isopentylpyrazine	164	1258	1242	-	0.25	-

*indicates that the retention index of volatile compound is not available in the literature used for comparison

Contaminants and compounds with area sum % less than 0.10 were excluded from the table

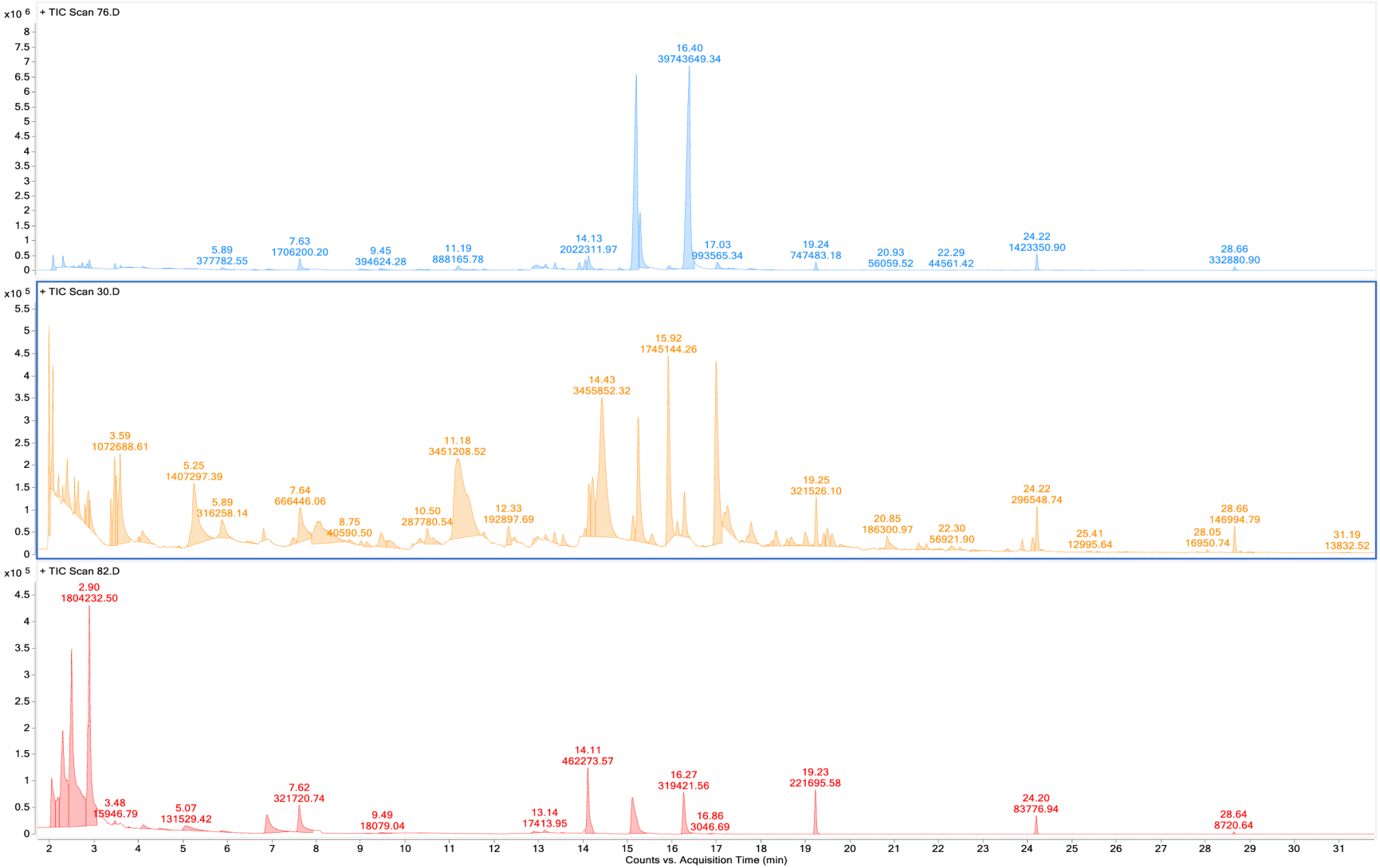

Figure 6. Gas Chromatography of oven-dried, roasted, and freeze-dried bee larvae (from top to bottom)

5.4.2. Crickets

The volatile compound analysis in crickets across all three food processing techniques detected 32 compounds in total. The highest number of volatile compounds were detected in oven-drying, where 22 compounds were observed. This was followed by roasting, with 12 compounds and freeze-drying where 5 compounds were detected (Table 12). In terms of the volatile compounds present in each processing technique, γ-Terpinene was found to have the highest quantity in oven-drying, representing 36.09 %. This was followed by m-Cymene with a value of 34.97 % and D-Limonene with 7.66 %. The rest of the compounds were present with values of 1.50 % or less. Meanwhile, in roasting, D-Limonene was observed to be the highest in quantity, with 54.05 %, followed by m-Cymene with a value of 10.18 % and γ-Terpinene representing 9.22 %. β-Pinene was also observed with 4.36 %. In freeze-drying, m-Cymene was found to have the highest quantity showing 9.25 %. This was followed by γ-Terpinene with a value of 5.87 %. Unlike oven-drying and roasting, D-Limonene was not observed in freeze-drying. Instead, n-hexanal was detected as third most abundant volatile compound in freeze-drying.

Table 12. Volatile Compounds identified in Crickets applied with different food processing techniques

Volatile compounds	MW	RT calc.	RT lit.	Area Sum %		
				Oven-drying	Roasting	Freeze-drying
Methyl benzene	92	749	746	0.92	-	-
n-Caproaldehyde	100	791	802	0.38	-	-
n-Hexanal	100	792	802	-	-	4.85
Diallyl sulfide	114	854	862	0.33	-	-
m-Xylene	106	858	866	0.10	-	-
1,3-Cyclopentadiene, 5-isopropylidene-	106	859	858	-	1.57	-
1,2-Dimethylbenzene	106	866	850	0.55	-	-
Dimethylfulvene	106	869	858	-	-	0.16
2-Heptanone	114	889	891	0.40	-	-
2,5,6-Trimethyldecane	184	898	*	-	0.72	-
Methyl 2-propenyl disulfide,	120	916	922	1.50	-	-

Continuation of Table 12

Volatile Compounds	MW	RI calc.	RI lit.	Food Processing Techniques		
				Oven-drying	Roasting	Freeze-drying
4,5-Dimethylpyrimidine	108	918	*	-	1.51	-
2-Thujene	136	926	920	0.14	-	-
1R-α-Pinene	136	933	937	0.26	1.00	-
n-Propylbenzene	120	955	955,3	0.11	-	-
o-Ethyl-toluene	120	964	973,3	0.89	-	-
Allyl 4-methylbenzyl ether	162	971	*	-	-	0.65
psi-Cumene	120	972	976	0.55	-	-
β-Pinene	136	978	980	0.76	4.36	-
β-Myrcene	136	993	991	0.94	-	-
1,3,5-Trimethylbenzene	120	996	1002	1.17	-	-
2,6-Dimethyl-5-aminopyridine	122	1007	*	-	2.20	-
α-Terpinene	136	1019	1018	0.32	-	-
m-Cymene	134	1030	1030	34.97	10.18	9.25
D-Limonene	136	1031	1031	7.66	54.05	-
γ-Terpinene	136	1063	1062	36.09	9.22	5.87
2-Ethyl-3,6-dimethylpyrazine	136	1085	1082	-	1.32	-
Allyl disulfide	146	1085	1085	1.34	-	-
2-Methoxy-5-methylbenzaldehyde	150	1164	*	-	0.29	-

*indicates that the retention index of volatile compound is not available in the literature used for comparison

Contaminants and compounds with area sum % less than 0.10 were excluded from the table

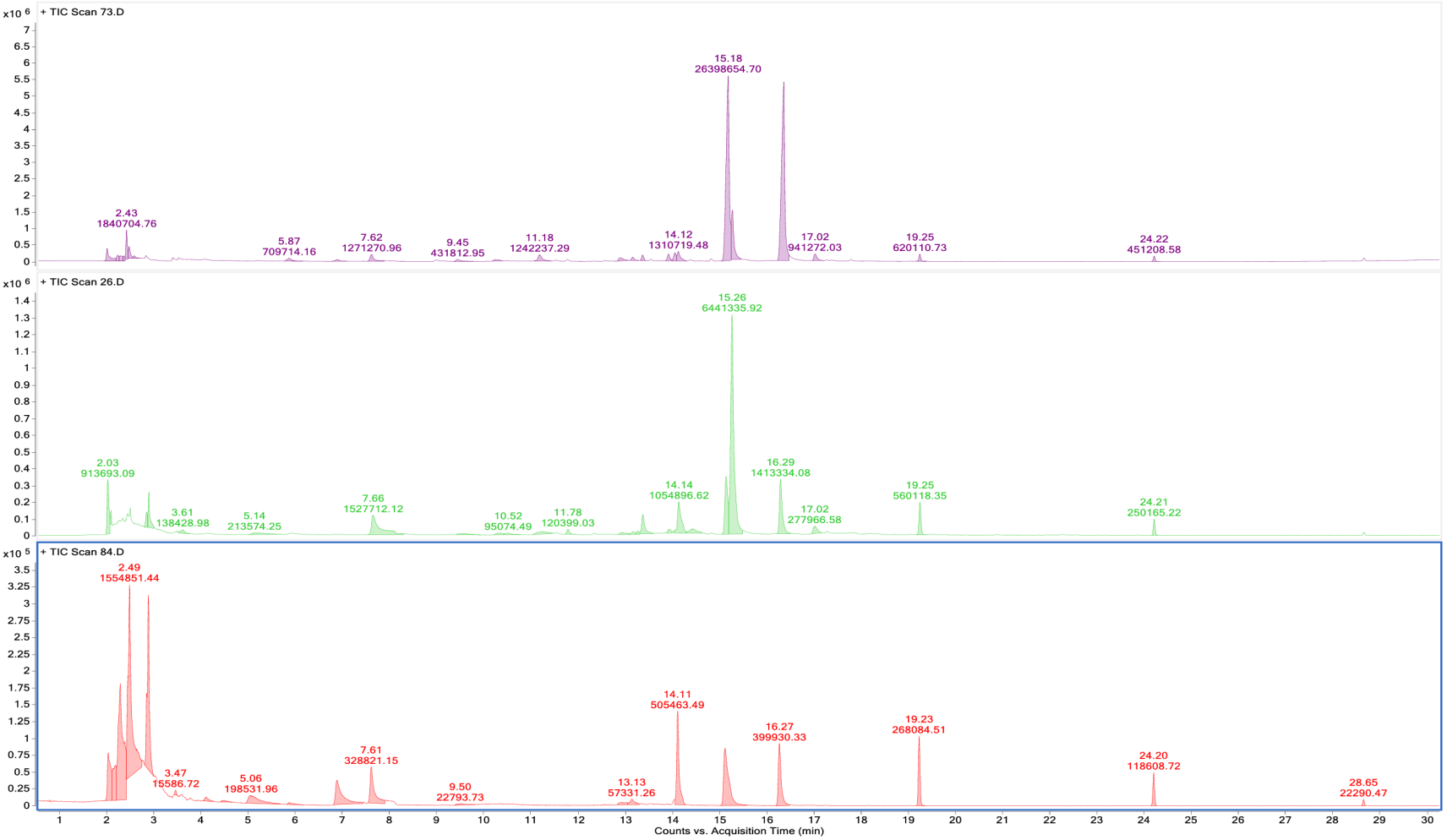

Figure 7. Gas Chromatography of oven-dried, roasted, freeze-dried crickets (from top to bottom)

5.4.3. Superworms

In the analysis of volatile compounds in superworms across all three food processing techniques, 40 volatile compounds were detected in total. Roasting showed to have the highest number of volatile compounds, with 23 compounds. This was followed by oven-drying, then freeze drying, where 20 and 14 volatile compounds were detected, respectively (Table 13). In terms of the volatile compounds present in each processing technique, m-Cymene was observed to have the highest percent quantity in oven drying, with 16.98 %. This was followed by γ-Terpinene with a value of 15.21 % and n-Octanoic acid isopropyl ester showing 7.98 %. Octanoic acid, methyl ester and Octanoic acid, ethyl ester were also observed with a value of 2.06 % and 1.60 %, respectively. In roasting, 1-butyl-pyrrolidine was observed to have the highest percent with a value of 12.17 %. 2-Pipecoline was the second compound with the highest percent value with 7.04 %, followed by D-Limonene with a percent value of 4.85 %. Meanwhile, n-Hexanal was found to have the highest percent in freeze-drying, with a value of 14.63 %. This was followed by γ-Terpinene with a percent value of 13.88 % and m-Cymene 10.60 %.

Table 13. Volatile Compounds identified in superworms applied with different food processing techniques

Volatile Compounds	MW	RT calc	RT lit	Area sum %		
				Oven-drying	Roasting	Freeze-drying
N,N-Dimethyl-2-aminoethanol	89	704	*	-	3.67	-
2,3-Dithiabutane	94	726	747	-	0.82	-
2-Pipecoline	99	743	*	-	7.04	-
Methyl benzene	92	749	749	-	-	0.25
n-Hexanal	100	789	802	-	0.35	14.63
n-Caproaldehyde	100	792	802	-	1.09	-
Diallyl sulfide	114	854	862	0.61	-	-
p-Xylene	106	858	860	0.36	-	-
m-Xylene	106	866	866	0.90	-	0.34
1-Butylpyrrolidine	127	887	*	-	12.17	-
2-Heptanone	114	890	889	1.14	-	-
Heptanal	114	900	896	-	-	0.22
2,6-Dimethylpyrazine	108	912	915	-	1.50	-

Continuation of Table 13

Volatile Compounds	MW	RI calc.	RI lit.	Food Processing Techniques		
				Oven-drying	Roasting	Freeze-drying
Methyl 2-propenyl disulfide,	120	916	922	1.60	-	-
2-Thujene	136	926	920	0.20	-	-
1R-α-Pinene	136	933	937	0.30	-	-
1-Butylpyrrole	123	949	*	-	1.60	-
1-Ethyl-3-methylbenzene	120	963	963.9	0.69	-	0.38
1,3,5-trimethyl-benzene	120	972	970.1	0.34	-	-
β-Pinene	136	977	980	0.96	0.57	0.25
psi.-Cumene	120	996	993.9	0.81	0.93	0.60
n-Caprylaldehyde	128	1004	1001	-	-	0.20
4-Amino-2,6-lutidine	122	1005	*	-	0.93	-
m-Cymene	134	1029	1030	16.98	0.22	10.60
D-Limonene	136	1032	1031	3.79	4.85	-
γ-Terpinene	136	1063	1062	15.21	2.02	13.88
2-Ethyl-3,6-dimethylpyrazine	136	1083	1082	-	1.48	-
2-Ethyl-3,5-dimethylpyrazine	136	1091	1090	-	0.43	-
(E)-2-Undecene	154	1093	1104	0.50	-	-
n-Nonaldehyde	142	1108	1102	0.34	-	0.20
Octanoic acid, methyl ester	158	1128	1125	2.06	2.23	-
2-Butyl-3-methylpyrazine	150	1137	*	-	0.24	-
Octanoic acid, ethyl ester	172	1199	1193	1.60	-	2.39
d-Mannose	180	1210	*	0.15	-	-
n-Octanoic acid isopropyl ester	186	1222	*	7.98	-	-
2-Adamantanone oxime	165	1232	*	-	2.41	-
n-Caprylic acid	144	1210	1192	-	0.11	6.44
Decan-1-ol	158	1280	1275	4.68	2.10	3.63

*indicates that the retention index of volatile compounds is not available in the literature used for comparison

Contaminants and compounds with area sum % less than 0.10 were excluded from the table

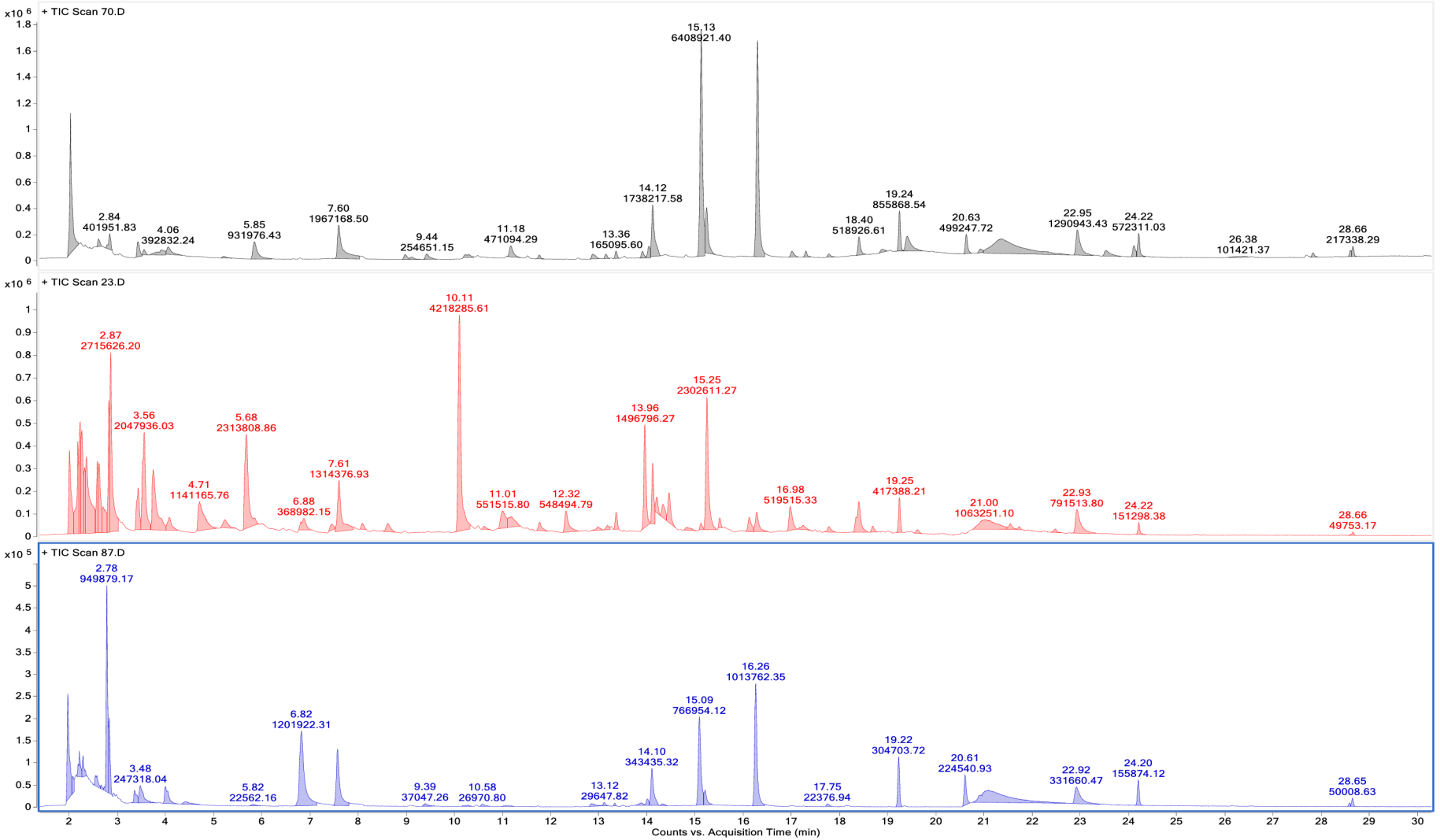

Figure 8. Gas Chromatography of oven-dried, roasted, and freeze-dried superworms (from top to bottom)

6. Discussion

6.1. pH and water activity

The results in pH showed that the pH values for the three insect samples ranged from 5.60 to 6.60. It can also be observed in the results that there were higher pH values in roasting. However, even though significant differences in values were observed between different food processing techniques, the overall pH results for the insect samples in this study would indicate that they can be considered as low acid foods since the pH values observed from the samples showed to be higher than 4.50 and less than 7. Low acid foods are one of the main concerns in terms of food safety since the pH values in these foods, such as the ones observed in this study, are beneficial for growth of microorganisms such as *Clostridium botulinum*, and other pathogenic microorganisms (Lewis 2023). The results in this study are comparable to the study conducted by Fombong et al. (2017) on a different insect sample showing that the drying methods applied did not influence the quality, which includes pH, in the insect.

In the water activity results, it was observed that the values across the three insect samples across different processing techniques ranged from 0.20 to 0.41 and no significant difference was observed between different food processing techniques. Generally, foods characterized by water activity lower than 0.6 inhibits the growth of microorganism due to the lack of water available for their biological processes (Fellows 2009). This indicates that all methods are equally effective in reducing the water activity in insects to an acceptable level.

With the different processing techniques applied to the insects showing no drastic difference in pH and water values in this study, it would be useful to look into which food processing technique could be less energy consuming and can be applied for edible insects, which are perceived to be a much more environment and resource-friendly alternative to meats. Among the three food processing techniques applied, freeze-drying is the most time-consuming and it requires significantly more energy consumption compared to oven-drying and roasting (Oyinloye & Yoon 2020). Hence, it might not be the ideal technique to use in certain circumstances.

6.2. Fatty Acid Composition

This study showed that saturated fatty acids are predominantly present in all the insect samples subjected to different food processing techniques, followed in order by monounsaturated fatty acids then polyunsaturated fatty acids. The results showed to be in coherence with the studies investigating the lipid profile in edible insects (Lenaerts et al. 2018; Udomsil et al. 2019; Riekkinen et al. 2022). Intakes of saturated fatty acids that are over the recommended levels for the human diet have been linked to high levels of cholesterol and increased mortality rates of coronary heart diseases. However, some studies stated that saturated fatty acids might have beneficial and specific biological roles in the body (Rioux & Legrand 2007; Gershuni 2018; Liu et al. 2020; Lemaitre & King 2022). Meanwhile, unsaturated fatty acids are known to be healthy fats that can improve blood cholesterol levels and improve muscle health (Marcus 2013). However, unsaturated fats are less stable compared to saturated fats due to its structure. Hence, they are more prone to rancidity and oxidation. The thermal heating application, such as drying and roasting, in this type of fats were also observed to produce some lipid oxidation products, which could have negative effects on the body (Zhuang et al. 2022). According to the results of the study, freeze-drying appears to have more diversity in the fatty acid profile compared to roasted and oven drying in crickets and superworms. This supports the idea that oxidation could play a part in the presence of different fatty acids in edible insects and that application of thermal heating might decrease the content of some fatty acids in foods (Mancini et al. 2021). On the other hand, results in bee larvae showed to be in contrast with the findings in cricket and worms, where roasting showed to have more diverse fatty acid profile in bee larvae (Table 8). Different factors could have influenced this result. The duration of the food processing technique that is applied to the insect could have played a role in the resulting fatty acid profile since oven-drying and freeze-drying were conducted for a significantly longer duration compared to roasting. This has not been observed in crickets and worms and it might be due to the presence of hard exoskeleton in these insects. Hence, it is recommended to consider which processing technique is the most suitable for specific insects.

However, the fatty acid profile is not the only important factor in selecting the most suitable food processing technique applied to insect, but also the fatty acid abundance.

In terms of the amounts of fatty acid present in the insects, it was observed that in many fatty acids that were present in different food processing techniques, freeze drying had significant higher amounts compared to roasting and oven-drying (Tables 8, 9, 10). This implies that freeze-drying is the best technique among the three to use in conserving amounts of fatty acid in the insect. The result of higher content of some fatty acids in the freeze-dried insects could be attributed to the fact that the change in the state of the food during the sublimation phase in freeze-drying can minimize the possibility of macronutrient loss due to drag from the cell interior (Khatun et al. 2021). Comparing with the other food processing methods applied to foods where moisture content could be present in a liquid state within the food, thus, increasing the likelihood of cell structure damage such as the case in dehydration (Castañeda-Saucedo et al. 2014). Another reason for the lower content of some fatty acids in the other processing techniques could be attributed to the oxidation of fats during oven-drying and roasting, which was previously mentioned to affect more the unsaturated fatty acids (Mancini et al. 2021).

Examining the most abundant fatty acids, Oleic acid, Palmitic acid, and Stearic acid appear to be the most abundant fatty acid in bee larvae (Table 8). Similarly, in the study conducted by Ghosh et al. (2016), results showed Oleic acid to be the most abundant fatty acid, followed by Palmitic acid and Stearic acid after the application of oven-drying. These fatty acids were also detected in other studies investigating the nutrition content of honeybees (Haber et al. 2019; Choi 2021). The fatty acid composition can be explained by the influence of their diet. Aside from nectar, which is the bee's main source of carbohydrates, honeybees obtain the other main nutrients, mainly protein and fats, from pollens. Manning (2001) described in his study how lipids in pollens affect the development and health of honeybee colony and showed fatty acids such as Oleic acid, Palmitic acid and Stearic acid as quite common in pollens from different plants used as a food source by honeybees. Oleic, Palmitic, and Stearic fatty acids were also found to be predominant in superworms (Table 8). These results are comparable to the fatty acid composition reported by Rumbos & Athanassiou (2021). However, in the studies conducted by Rumbos & Athanassiou (2021), aside from the three fatty acids that were observed in our study, Linoleic fatty acid was found to be predominant in the fatty acid

composition of superworms. However, Linoleic acid was not detected in our results of fatty acid analysis. Linoleic acid is one of the essential fatty acids and an importance fatty acid in human health, especially for normal growth and development (Whelan & Fritsche 2013). The same phenomenon, where one of the main fatty acids detected in other studies did not appear in our results, was also observed in the crickets. For crickets, the most abundant fatty acids were found to be Oleic acid, Linoleic acid, Palmitic acid, and Stearic acid (Table 9). Other studies that investigated the fatty acid composition in crickets also observed the similar fatty acids as the most abundant in quantity (Tzompa-Sosa et al. 2019; Udomsil et al. 2019; Khatun et al. 2021). However, the essential fatty acid α-linolenic acid, was detected to be one of the main fatty acids on dried crickets on the study conducted by Udomsil et al. (2019) and Riekkinen et al. (2022). Linolenic acid is known to be important in promoting the heart health in the human body (Fan & Chapkin 1998). This fatty acid was not observed in the fatty acid composition of the crickets used in our study. The occurrence of not being able to detect some of the fatty acids that were detected in other studies could have been influenced by the substrates used in the insects during rearing. As reported by Fischer et al. (2021) and Riekkinen et al. (2022), the substrates used in insect rearing have an influence on the fatty acid composition of the insects, where the dietary lipids of substrates may reflect in the fatty acid content in the insects, but not identical as it could be utilized differently in insects. It would be beneficial to investigate the effect of diets on the fatty acid composition of insects.

Examining the predominant fatty acids in the three insects and comparing their quantity across different food processing techniques, it can also be observed that the predominant fatty acid contents are significantly higher in freeze-drying compared to oven-drying and roasting. The same results were observed in the study conducted by Lenaerts et al. (2018), where some of the fatty acids, such as Oleic, Palmitic, Stearic, and Palmitoleic acid, were present in higher amounts in freeze-dried insects than in the insects that were applied with microwave drying. This emphasizes the effectivity of freeze-drying at retaining amounts of fatty acids in insects as mentioned. On the contrary, in other studies that looked into the fatty acid profile of processed insects, it has been noted that freeze drying has not significantly affected the fatty acids in the

insects compared to oven-drying (Selaledi & Mabelebele 2021; Khatun et al. 2021). The temperatures and duration of the food processing techniques applied or the difference in insects that have been used for these studies might have played a role in the difference in results. It would be recommended in future studies to investigate the most suitable drying procedures to apply to insects.

Looking at the fatty acid profile of crickets (Table 9), it was also shown that Oleic acid, which was one of the main fatty acids present in the insect after oven-drying and roasting, was not detected during freeze-drying. However, this fatty acid is still detected in freeze-drying for other insects. The reason could be attributed to the fact that crickets that were used for the test in freeze-drying were from different batches compared to the one used in oven-drying and roasting due to the limitation in the research procedure. Hence, factors such as difference in diet or rearing could have influenced the results. It would be recommended to utilize insects reared in the same environment and diet for future studies.

Generally, the fatty acid composition of the bee larvae, crickets, and superworms demonstrates to be diverse and present in good quantities across different processing techniques. The predominant fatty acids in the three insects are found to be comparable to those in different types of consumed meat, which predominantly contains palmitic, stearic, oleic, and linoleic among others (Belluco et al. 2013; Raheem et al. 2019). This makes insects a valuable alternative to meats for human consumption.

5.3. Volatile Compounds

Examining the result of the volatile compound analysis in this study, it showed that roasting exhibits the most number of volatile compounds in the bee larvae and superworm samples followed by oven drying (Table 8, 10). On the other hand, oven-drying exhibited the most volatile compounds in crickets, followed by roasting. Lastly, freeze-drying appeared to have the least number of volatile compounds in all three insects. One factor that may have played a role in the generation of high numbers of volatile compounds in roasting and drying is the higher temperatures applied to these techniques. In foods, numerous complex reactions generate volatile compounds. As stated by Perez-Santaescolastica et al. (2022), fatty acids, amino acids, and peptides serve as substrates for subsequent reactions such as oxidation, Strecker and Maillard

processes, which are greatly influenced by processing, particularly thermal treatments. These reactions are responsible in the generation of different volatile compounds in food.

In bee larvae, it can be observed that the predominant volatile compounds present in the insect are different in each food processing technique. γ-Terpinene, m-Cymene, and α-Terpinene are found to be the main volatile compounds in oven-drying. The predominant compound in oven-drying, γ- Terpinene, is a monoterpene hydrocarbon that is described to have a lemon odour and is used in foods, cosmetics and confectionery (Pyka & Bober 2002). This compound was also observed to be the main volatile compound in freeze-drying. While the other monoterpene present, α-Terpinene, is a known to be present in many plants, such as wood of the pine (P*inaceae*), as a component of essential oil (Pyka & Bober 2002; Qi et al. 2018). The second most predominant volatile compound, m-Cymene, is known to be present in some plant sources such as myrtle berries and shell ginger and is described to have a woody, and earthy odour (Feng et al. 2021). In roasting, the predominant volatile compounds were observed to be 2,6-Dimethyl-5-aminopyridine, 2,5-Dimethylpyrazine, and 2-Ethyl-3,6-dimethylpyrazine. These compounds are part of pyrazine group and mostly responsible for the roasted flavors in insects and they are described to have a cocoa roasted, and nutty odour. These compounds are naturally present in heat-treated food products and are commonly used as a flavor and odour-enhancing compound in food products (Mortzfeld et al. 2020). D-limonene and Ocimene were also detected in roasting. These compounds were described to have a green-flowery, and lemon-like odour. The Ocimene compound was also found to be the predominant volatile compound, aside from diacetyl and nonanal, based on the study done on freeze-dried bee larvae conducted by Haber et al. (2019). However, this is not the case in our findings. Based on our results, we could state that the volatile compounds in the oven-drying and freeze-drying are mostly based on compounds found in plants and could be more related to sweet and citrus smell-like odours. The different compounds detected could have been influenced by the diet of the bees. Meanwhile, compounds found in roasting could be more attributed to the heat treatments applied and gives a roasted and nutty odour.

In crickets, volatile compounds such as m-Cymene and γ- Terpinene, which were previously described to have a lemon-like odour, were present in high quantity across the three food processing techniques. Additionally, D-limonene, another volatile compound described to have a lemon-like odour, was found to be predominant in the processing techniques except in freeze-drying. Similarly, this compound is also not detected in freeze-dried cricket conducted by (Khatun et al. 2021). Instead of D-limonene, n-Hexanal was detected to be the third most abundant volatile compound present in freeze-drying. This compound is described as having a fruity, and grassy odour. Similarly, n-Hexanal was also detected in another study that investigates the volatile compound of house crickets and field crickets (Khatun et al. 2021). Based on the results, the predominant volatile compounds present in each food processing technique observed in crickets do not drastically differ from each other in terms of composition.

For superworms, m-Cymene and γ- Terpinene were also found to be abundant in oven-drying. It can also be observed that fatty acid esters of octanoic acid were detected in oven-drying. These compounds were described to have a fruity and flowery odour (Liu et al. 2011; Zhao et al. 2022). The presence of fatty acid esters in oven-dried worms might be caused by some reactions that occurred during the thermal treatment. The volatile compounds detected to be predominant in roasting were different compared to oven-drying, where 1-Butyl-pyrrolidine, 2-Pipecoline, and D-limonene were observed to be the predominant volatiles in this process. No odour characterization has been found to describe the 1-Butyl- pyrrolidine. However, 1-Butyl-pyrrolidine was also detected to be present in roasted and fried mealworms conducted by Seo et al. (2020). The presence of pyrrolidine could be attributed to the complex series of thermal reactions that occur during food processing. Meanwhile, 2-Pipecoline is a volatile compound that was described to have ammoniacal and unpleasant odour and is part of an organic compound group called piperdine, which is naturally present in some plants such as black pepper (*Piper nigrum*) (Ojima Iwao & Iula Donna 1999; Aziz-ur-Rehman et al. 2017). The results in this study differ from the volatile compounds found to be present in roasted superworms from another study conducted by Żołnierczyk & Szumny (2021), where pyrazines were the main volatile compounds that was detected. Pyrazines were also detected in our results (Table 13). However, it does

not seem to be the principal volatiles that can be found in our results. This could be due to the difference in the roasting procedure done between the two studies wherein the insects from Żołnierczyk & Szumny (2021) were subjected to higher temperatures during food processing. In freeze-drying, volatile compounds such as n-Hexanal, γ- Terpinene, and m-Cymene that were present in other food processing technique were also observed to be the predominant volatile compounds. Hence, it could be described that fruity, earthy and lemon-like odours would be predominant in freeze-dried worms. Based on the results, the main odours that could be observed in oven-drying and freeze-drying are almost similar and processed superworms are predicted to have a pleasant odour, while roasting might not give off a good odour during roasting.

In general, some volatile compounds that are characterized by pleasant odours were seen in all food processing techniques. However, it is dependent on the specific insect which food processing technique would be suitable to use. Food processing techniques such as oven-drying and roasting give off more volatile compounds, some of which are described to have positive odours. Pyrazine compounds could also be predominant in insects processed by roasting. Natural sources of pyrazines are continuously sought out as they are common to use in food production as flavor and odour enhancers (Żołnierczyk & Szumny 2021). For this purpose, roasted insects could be as used as an additional ingredient in other food products since they are observed to contain some pyrazine compounds. However, roasting could also result in unpleasant odours such as the case in superworms. Meanwhile, freeze-dried insects, which appeared to have the least volatile compounds than the other processing techniques applied, could be more appealing to people who are new to entomophagy. The presence of certain odours in insects could be unpleasant for some people who are not used to edible insects (House 2016). Different factors such as the insect diet, food processing technique procedures, and chemical reactions that occur, could also affect the volatile compound profile of the edible insects.

7. Conclusion

The study aimed to determine the volatile compounds and fatty acid composition of edible insects, specifically honeybee larvae, crickets, and superworms after the application of food processing techniques, such as oven-drying, roasting, and freeze-drying. Identifying the fatty acid composition and volatile compounds in the insect can be useful as an added value for making edible insects appealing to possible consumers, and knowing the effects of different food processing techniques on edible insects could be beneficial in knowing the most suitable processing technique to apply. The analysis of pH values in insects showed to have different values across different food processing samples. However, all three insects could still be considered as low-acid foods, which are considered to be very susceptible to microbial growth, regardless of the food processing technique applied. Thus, it is important to limit the other factors that could help microbial growth in edible insects. The results of the water activity analysis showed no significant difference in water activity values between the food processing techniques applied. Oven-drying, roasting and freeze-drying were found to decrease the water activity values to an acceptable level that could help hinder the microbial growth. Considering the results of these two tests, it can be concluded that the three food processing techniques effectively improve the intrinsic factors that influence food safety.

In the determination of fatty acid profile, results showed that the three insects contain fatty acids that are comparable to meat consumed by humans. The predominant fatty acids present in different food processing techniques also remain to be nearly the same. In the overall fatty acid profile of each, freeze-drying is shown to be more effective in maintaining the high fatty acid content of the insects compared to oven-drying and roasting.

The volatile compounds analysis showed that the application of thermal processing, such as oven-drying and roasting, in edible insects would result in a higher number of volatile compounds present. The freeze-drying technique resulted in the least number of volatile compounds across the three insects. Volatile compounds characterized by pleasant odours were detected in all three insect samples across the different food processing techniques. However, some processing techniques might also produce

volatile compounds with unpleasant odours, such as the observed result in the roasting of superworms.

Generally, different factors such as insect diet and different chemical reactions that occur can also influence the fatty acid profile and volatile compounds produced in the insects.

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