EXTRACTION AND PHYSICOCHEMICAL CHARACTERIZATION OF INSECT OILS OBTAINED FROM *ACHETA DOMESTICUS & TENEBRIO MOLITOR*

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

AHMET ERDEM UĞUR

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

JANUARY 2019

Approval of the thesis:

EXTRACTION AND PHYSICOCHEMICAL CHARACTERIZATION OF INSECT OILS OBTAINED FROM *ACHETA DOMESTICUS & TENEBRIO MOLITOR*

submitted by **AHMET ERDEM UĞUR** in partial fulfillment of the requirements for the degree of **Master of Science in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Serpil Şahin
Head of Department, **Food Engineering** _____

Prof. Dr. Hami Alpas
Supervisor, **Food Engineering, METU** _____

Examining Committee Members:

Prof. Dr. Alev Bayındırlı
Food Engineering, METU _____

Prof. Dr. Hami Alpas
Food Engineering, METU _____

Prof. Dr. Rahmi Ertan Anlı
Food Engineering, Ankara University _____

Assoc. Prof. Dr. Mecit Halil Öztop
Food Engineering, METU _____

Assist. Prof. Dr. Emin Burçin Özvural
Food Engineering, Çankırı Karatekin University _____

Date: 25.01.2019

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Surname: Ahmet Erdem Uğur

Signature:

ABSTRACT

EXTRACTION AND PHYSICOCHEMICAL CHARACTERIZATION OF INSECT OILS OBTAINED FROM *ACHETA DOMESTICUS* & *TENEBRIO MOLITOR*

Uğur, Ahmet Erdem
M.S., Department of Food Engineering
Supervisor: Prof. Dr. Hami Alpas

January 2019, 133 pages

Edible insects have become one of the most attracted and attention-grabbing alternative food sources in recent years due to the constituents that include proteins, oils, carbohydrates, minerals and vitamins.

The goal of this study is to explore insect oils in terms of physicochemical properties to help their utilization in future against the possible scarcity of the resources in the world, and the most important point is to help to enable these valuable edible insect species as one of the main nutrient sources of the human being.

In this study, oil portion of the two edible insect species, *Tenebrio molitor* (yellow mealworm) and *Acheta domesticus* (house cricket) were focused and it was

investigated how the physicochemical properties of the oil changed with different extraction conditions.

The oil content of *Tenebrio molitor* and *Acheta domesticus* are >34 and >28% respectively and they include significant amount of Ω-3 and Ω-6 fatty acids that are important for the diet and they are also rich in antioxidants and phenolics that help to fight against health problems. In this study, High Hydrostatic Pressure (HHP) was used and the effect of HHP on extraction was compared with the conventional methods. Following the extraction of oil, fatty acid composition, peroxide value, crystallization and melting points, total phenolic content and the antioxidant activities were determined. Besides, it was examined how the HHP affected the composition of insect oils obtained from the two species. Oil yield was found in the range of %22.75-24.22 and %16.17-18.09 for mealworm and cricket, respectively. It was also found that the amounts of myristic acid, palmitoleic acid and linolenic acid in mealworm and cricket oils were relatively high, although the most abundant fatty acids found in both insects were palmitic acid, stearic acid, oleic acid and linoleic acid. Moreover, the difference between crystallization and melting point of mealworm were found to be higher than the cricket. The amount of unsaturated fatty acids in mealworm oil was almost two fold of saturated fatty acids, whereas this ratio was less in cricket oil.

Keywords: Yellow Mealworm, House Cricket, Oil, High Hydrostatic Pressure, Temperature, Entomophagy

ÖZ

ACHETA DOMESTICUS & TENEBRIO MOLITOR BÖCEK TÜRLERİNDEN ELDE EDİLEN YAĞLARIN EKSTRAKSİYONU VE FİZİKOKİMYASAL KARAKTERİZASYONU

Uğur, Ahmet Erdem
Yüksek Lisans, Gıda Mühendisliği Bölümü
Tez Yöneticisi: Prof. Dr. Hami Alpas

Ocak 2019, 133 sayfa

Yenilebilir böcekler içerdikleri protein, yağ, karbonhidrat, mineral ve vitamin gibi temel yapı taşları sayesinde son yıllarda en çok ilgi ve dikkat çeken alternatif gıda kaynaklarından biri haline gelmiştir. Bu çalışma, iki yenilebilir böcek türü olan Tenebrio molitor (un kurdu) ve Acheta domesticus (çekirge)'in içerdiği yağ kısmına yoğunlaşmış ve yağların fizikokimyasal özelliklerinin, biri yenilikçi biri konvansiyonel metot olmak üzere iki farklı extraksiyon yöntemi ile nasıl değiştiğini araştırmaktadır.

Bu çalışmanın amacı, dünyadaki besin kaynaklarında oluşabilecek olası bir kıtlığa karşı bir gelecek yaratmak ve güçlü bir alternatif oluşturmaktır; en önemli nokta ise bu değerli yenilebilir böcek türlerini insanların kendileri için gerekli olan temel yapı taşlarının temel kaynaklarından biri haline getirmektir.

Tenebrio molitor ve *Acheta domesticus*'ta bulunan yağ miktarları sırasıyla %34 ve %28 civarında olup, esansiyel olarak tüketilmesi gerekli olan Ω-3 ve Ω-6 yağ asitlerinden kayda değer miktarda içermektedir. Ayrıca, bu yağlar içerdikleri antioksidan ve fenolikler sayesinde serbest radikal kaynaklı ciddi sağlık problemleriyle savaşmada kullanılan önemli savunma mekanizmalarıdır. Yenilikçi bir metot olarak Yüksek Hidrostatik Basınç (YHB) kullanılmış ve YHB'nin yağların ekstraksiyonu ve fizikokimyasal özellikleri kapsamında konvansiyonel metotlara karşı etkisi incelenmiştir. İki farklı ekstraksiyon metoduyla optimum performansta yağ elde edildikten sonra verim, yağ asidi kompozisyonu, peroksit değeri, kristalleşme ve erime noktaları, toplam fenolik miktarı ve antioksidan aktivitesi gibi yağların fizikokimyasal özellikleri araştırılmıştır. Bununla birlikte, YHB'ın bu iki böcek türünde bulunan yağlar ve kompozisyonları üzerinde nasıl bir etkisi olduğu belirlenmiştir. Yağ miktarı un kurdu ve çekirgede sırasıyla %22.75-24.22 ve %16.17-18.09 aralığında bulunmuştur. Her iki böcekte de baskın miktarda bulunan yağ asitleri palmitik, stearik, oleik ve linoleik olmasına rağmen, miristik, palmitoleik ve linoleik asit de yüksek miktarda bulumaktadır. Bunun dışında, un kurdundaki kristalleşme ve erime noktaları çekirgeden yüksek bulunmuştur. Un kurdundaki doymamış yağ asidi miktarı doymuş yağ asitlerinin neredeyse iki katı iken çekirgede bu oran daha düşük seviyelerdedir.

Anahtar Kelimeler: Un Kurdu, Çekirge, Yağ, Yüksek Hidrostatik Basınç, Sıcaklık, Entomofaji

To my beloved family

ACKNOWLEDGMENT

It was quite exciting to end the long journey by fulfilling all the responsibilities related to this work. Some people gave undeniable support behind in both this road and my life.

I am grateful to my supervisor, Prof. Dr. Hami Alpas, for his valuable support, trust and positive approach in whole steps of the study. I also appreciate to my 'out-of-record' co-supervisor, Assoc. Prof. Dr. Mecit Halil Öztop, for being a loadstar during the long path, for being enthusiastic in every step of the work, for providing full support and for mentorship.

I owe a debt of gratitude to my parents, Nihal Uğur and Adnan Uğur. Until today from the day I was born, they are all I have. They are the most important people that provided me to come to these days. I believe that every positive attitude and behaviors that I have is thanks to them. These priceless people are my guiding lights for my life and for my future. I offer thanks to have such a valuable, special and interested parents. Besides, I would like to salute to my elder brother who is completely different with me, Emre, his wife and my nephews. And the special thanks goes to my elder sister, Eda, her husband and again my nephews; also, she is the female version of me whether she does not accept or not.

Here comes the person that changes my life, Berkay Bolat. She is my life, my love, my future and the person that gives the meaning of everything in my world since she became a part of it. This educational level that I reached is thanks to her, and the real effort on this work was belong to her even if she does not accept. I hope that her life will be full of success, happiness, beauty and peace. The our adventure is just beginning.

This life and this work would not have been possible without those people.

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CHAPTER 1

INTRODUCTION

1.1. Edible insects

1.1.1. General information

Insects are in the class of invertebrate animals as a simple definition and they are the largest groups of organisms with at least 1 million species; in other words, among the whole known species in the world, the insects comprise of 75% of them (Halloran et al., 2018). The existence of insects is based on before 400 million years; they are the oldest land animals (Tiencheu & Womeni, 2017). The origin of the name of the insect comes from an *Insectum*, a Latin word (Delong, 1960). The insects are classified into 25 different groups according to their properties with some well-known types of beetles (*Coleoptera*); moths and butterflies (*Lepidoptera*); true flies (*Diptera*); ants, bees and wasps (*Hymenoptera*); crickets, grasshoppers and locusts (*Orthoptera*) (Halloran et al., 2018). It is expected that the population of the world will approach to 9 million at 2050 according to Food and Agricultural Organization (FAO) (Tao & Li, 2018). The increased population directed people to consume alternative products in their diet due to shortage in the traditional sources like animals besides of the necessity of water and land for farming. The small amount of the insects, approximately 5,000 species, among the 1 million species is thought as hazardous for corps, livestock or human beings (Huis et al., 2013).

In today's world, more than 2000 insect species are known as edible and consumed in 300 ethnic groups in 113 countries all over the world (Kourimska & Adamkova, 2016; Tiencheu & Womeni, 2017). The beetles, wasps, bees, ants and caterpillars are the most frequently used insect species for consumption around the world and crickets, grasshoppers, cicadas, bugs, dragonflies and such species follows them (List of edible insects of the world, 2017). The European countries do not prefer to consume insect too much in their Western diet compared to the Africa, Southern Asia and northern part of Latin America countries, who are the largest consumers of edible insects (Kourimska & Adamkova, 2016). If the farming conditions are taken into the consideration, house cricket (*Acheta domesticus*), yellow mealworm beetle (*Tenebrio molitor*), superworm (*Zophobas morio*), African migratory locust (*Locusta migratoria*), Jamaican field cricket (*Gryllus assimilis*), western honey bee (*Apis mellifera*) and wax moth *(Galleria melonella*) species can be bred and consumed in the Europe thanks to the suitable farming conditions (Kourimska & Adamkova, 2016).

The edible insects with the existence as huge number in nature become significant and popular in terms of for not only personal health but also the health and future of the planet Earth. The edible insects are the important source of the primary macronutrients like proteins, oils and carbohydrates and micronutrients like minerals and vitamins in the way of nutrition for human consumption as in livestock. However, the eco-friendly lifestyle and the environmentally friendly breeding conditions in terms of lower emission of greenhouse gases like CO_2, CH_4, NO_2, and NH_4; lower pollution level; lower usage of water and land; less nutrients requirement and easy storage conditions compared to the livestock production and also the higher feed conversion ratio makes edible insects significant and favorable for the future of the planet Earth (Huis et al., 2013).

1.1.2. Entomophagy

The origin of the word of 'Entomophagy' that describes the practice of eating insects comes from the Greek words of éntomon and phagein with the meanings of insect and eating, respectively (Evans et al., 2015). The archeological studies show that the entomophagy is not new for human beings and its past is based on older times in history whether it seems as new trend (Dobermann et al., 2017). The human kind was omnivorous in prehistoric times, and the insects constituted the significant part of human diet before hunting or farming. The evidence of entomophagy was found by fossil analyzing from the caves in USA and Mexico; besides, the paintings that belongs to the years of 9000-3000 BC in the caves in northern Spain are also the evidence of existence of entomophagy in prehistoric times (Kourimska & Adamkova, 2016).

In many countries, especially the developing countries such as African, Asian and Latin American, the edible insects constitute the major parts of their diet due to the problems of hunger and malnutrition (Huis et al., 2013). The hunger causes undernourishment in people in terms of proteins, fats, carbohydrates, vitamins and minerals, and it is known that the insects are significant sources of these macronutrients and micronutrients. The scarcity in livestock and other resources makes people to tend the indigenous food resources. In Central Africa, almost 50% of the dietary protein is supplied from the edible insects (Dobermann et al., 2017). In Africa and Latin America, the insects are consumed as roasted and fried to provide building blocks such as protein, fat, vitamins and minerals to people for continuity of life. The rapid growth, enormous biomass and sustainability of the insect population makes attractive them in diet (Tiencheu & Womeni, 2017).

For many people in Western societies, eating insects is a taboo and is thought as a primitive and disgusting behavior in tropical countries, because the insects seem as pests and an alien habit (Huis & Dunkel, 2017). Besides, the cultural bias plays an important role in the attitude towards to entomophagy and the acceptance of

insects as food source becomes difficult (Huis & Dunkel, 2017). However, the interest has increased in recent times thanks to the opinion of some reliable organizations such as Food and Agricultural Organization (FAO) and European Food Safety Authority (EFSA) on the edible insects (Huis et al., 2013). The possible shortage of the nutrition increases with irregular increase in world population, so it is necessary to consider the alternative food sources. Whether the bias becomes moderate against the edible insects, it is difficult to overcome completely due to the effect of environment, history, the structure of community and moral judgments (Huis et al., 2013).

According to a study among the Italian consumers, the people have a tendency to consume the edible insects prefers to consume them as hidden form in the meal such as powder or additive (Buβler et al., 2016). In another study between the German and Chinese consumers show that Chinese, who are familiar to entomophagy, tends to eat the both processed and unprocessed insect-based products while the Germans are uncompanion able to unprocessed products (Hartmann, 2015). According to another study, the participants from Netherlands and Australia have positive towards about the insect consumption, and the common idea is that the interest in consuming the insects increases if they are served in unrecognizable form (Lensvelt & Steenbekkers, 2014; Huis & Dunkel, 2017). Besides, the question of why insects consumed has different answers for people from Europe and Asia. Dutch people are influenced by entomophagy due to being sustainable while people from Thailand prefer insect consumption because of being familiar to insects and their taste (Dunkel, 1996). The studies provide insights about the acceptance of the insect-based product consumption. If the familiarity and reliability of edible insects' increases, and if the sensory attributes satisfy the expectations, the people may prefer to consume them in their diet as processed form. However, there are some gaps in the knowledge about the usage of insect products in diet as either whole form or additive, so it is necessary to continue the investigation of all possible functional properties and effect on

health in detailed way (Risk profile related to production and consumption of insects as food and feed, 2015).

1.1.3. Benefits of insect production and consumption

1.1.3.1. Health benefits

Edible insects provide high nutritional value whether they seem as tiny; however, the amount of the nutrients and composition of nutrients differs from one specie to another. In fact, the same specie with different metamorphic stage has a different content of compounds along with the different feeding conditions (Huis et al., 2013). It is not a surprise that the edible insects have significant health benefits when looking at the macronutrients and micronutrients contained. Whether the composition of nutrients depends on the metamorphic stage, diet and the applied process, the consumption of many of the edible insects provides energy and protein; includes high amount of monounsaturated and polyunsaturated fatty acids; also contains high amount of micronutrients like copper, magnesium, phosphorus, zinc, iron, manganese and selenium (Rumpold & Schlüter, 2013).

Insects are significant source of proteins and the amino acid composition and the digestibility of proteins determines their quality (Dobermann et al., 2017). The studies show that the content of protein and the content of the amino acids, especially essential amino acids, reveals the treasury that edible insects have. According to a study of Mexicans, the average protein content of random types of edible insects are in the range of 15 to 81%, and the range of digestibility is between the 76 and 96% (Ramos-Elorduy et al., 1997). Besides of that, the edible insects include significant amino acids. Some of the important amino acids which are quite high in edible insects are phenylalanine and tyrosine, the essential amino acids (Kourimska & Adamkova, 2016). Moreover, the essential amino acids of lysine, tryptophan and threonine takes part in high amounts in some edible insects.

It is known that all of three amino acids are deficient in the cereals and the cereal based diet is popular worldwide (Kourimska & Adamkova, 2016; Bukkens, 2005). The analysis reveals that the amount of the essential amino acids included in the edible insects are in the range of 46 to 96% of the total amino acids (Xiaoming et al., 2008).

The second highest macronutrient group of insects included is fats with the constituents of triglycerides that contains glycerols and fatty acids (Huis et al., 2013). The content of fat differs from 10 to 60% in dry matter according to the metamorphic stage; the larval stages has higher fat content than the adult forms (Kourimska & Adamkova, 2016). Triglycerides consists of the about 80% of the fat, and the remain part includes phospholipids with a percentage of maximum 20% (Tzompa-Sosa et al., 2014). The high amount of the triglyceride, which serves as an energy reserve, can be explained with the high-energy necessity for longer flights (Kourimska & Adamkova, 2016). The oils of insects are rich in polyunsaturated fatty acids; the content of C18 fatty acids such as oleic, linoleic and linolenic, frequently the essential linoleic and α-linolenic acids, are higher in the edible insects (Huis et al., 2013; Tzompa-Sosa et al., 2014). Linoleic (Ω-6) and α-linolenic acids (Ω-3) are quite important for the development of the children and infants (Michaelsen et al., 2009). The content of the desired unsaturated fatty acids in insects is almost same with the poultry and fish products, and the amount of polyunsaturated fatty acids is higher than the poultry and red meat products (Dobermann et al., 2017).

The insects contain high amount of oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), which are necessary to consume in diet due to the importance on health; besides, they may be converted into the body to arachidonic acid (C20:4) and eicosapentaenoic acid (C20:5) which are also important for health (Dobermann et al., 2017). Apart from these, the insects also include high amount of palmitic acid (C16:0), a saturated fatty acid (Kourimska & Adamkova, 2016). In addition to the triglycerides and phospholipids, the other constituents of

insect oils are sterols, especially cholesterol, and glycolipids (Gilby, 1965). The important point that is necessary to take into the consideration is that the oil composition of the insects depends on their diet, especially the plants (Huis et al., 2013).

Another important group that the insects included is fibers and the most abundant part of the fiber is chitin derived from the exoskeleton of insects (Huis et al., 2013). Chitin is a long chain polymer of N-acetyl glucosamine, a derivative of glucose (Paoletti et al., 2007). Besides it acts like cellulose, a polysaccharide, in the human; that is, whether the chitin is thought as indigestible fiber for human, the enzyme of chitinase is found in the gastric juices of human (Paoletti et al., 2007; Kourimska & Adamkova, 2016). Moreover, chitin is a defender against the parasitic infections and allergic problems along with the antivirally activity against the tumor formation (Huis et al., 2013; Lee et al., 2008). The chitosan, a derivative of chitin, provides people resistance against the pathogenic bacteria and viruses (Kourimska & Adamkova, 2016).

Insects also includes vitamins and minerals that are the primary micronutrients. Vitamins are quite significant for metabolic processes stimulation and immune system functions' enhancement and the amount of vitamins, which are water soluble or lipophilic, is considerable in insect species (Huis et al., 2013; Kourimska & Adamkova, 2016). It is found that the vitamin B_1 (thiamine) with the function of co-enzyme that metabolizes carbohydrates into energy; vitamin B_2 (riboflavin) with the function of metabolism; vitamin B_5 (pantothenic acid); and vitamin B_7 (biotin) are in the high level in most of the insect species (Bukkens, 2005; Rumpold & Sclüter, 2013). Vitamin B_{12} (cobalamin), an animal-based vitamin, is found most in the *Tenebrio molitor* (yellow mealworm) larvae and *Acheta domesticus* (house cricket) among the insect species (Huis et al., 2013). Besides, some studies show that retinol and β-carotene (Vitamin A) may also found in insects but not as a best source (Huis et al., 2013). Vitamin E (α-tocopherol) may be also found in some types on insects as high level (Huis et al.,

2013). In general, the insects are not good sources of vitamin A, vitamin C and vitamin B_3 (niacin) (Kourimska & Adamkova, 2016).

Insects also contain some significant minerals such as iron and zinc. Most of the edible insects have extremely higher iron content than beef; besides, it is thought that the most of the insects are good source of zinc as higher than the beef again for some types (Bukkens, 2005). The other significant minerals that insects include are potassium, sodium, calcium, phosphorus, magnesium, manganese and copper (Kourimska & Adamkova, 2016).

In addition to the valuable nutrients above, the insect also includes some bioactive compounds such as antioxidants and phenolic compounds; thus, the consumption of the insects may reduce the health risks and increase the strength of immune system (Roos & Huis, 2017).

1.1.3.2. Environmental and economic benefits

The eco-friendly lifestyle of the insects makes them important for environment in terms of greenhouse gas emissions, feed conversion ratio, usage of land and water, organic side streams, animal welfare and zoonotic infections.

Greenhouse gases (GHG) and ammonia (NH_3) emissions for livestock production corresponds to 18% of the human based emissions (Steinfeld, 2006). CH_4 (methane) with 37% and N_2O (nitrous oxide) with 65% have the highest potential for global warming when compared to CO_2 (carbon dioxide) with 9% (Huis et al., 2013; Huis, 2013). A study shows that the highest negative impact for 1 kg product is in order of beef (14.8 kg), pork (3.8 kg) and chicken (1.1 kg) as CO_2 equivalents (Finke, 2002). Livestock production causes environmental pollution with the wastes including ammonia, and this causes eutrophication of water, soil acidification and nitrification (Steinfeld, 2006; Huis et al., 2013). The production

of ammonia and greenhouse gas also occurs due to the insects but in very low level compared to livestock and the only insects that produces methane are cockroaches, termites and scarab beetles (Huis, 2013). Especially, yellow mealworm and house cricket are more preferably than livestock animals in terms of both low greenhouse gas emissions and ammonia (Huis, 2013).

The demand of meat, grains and feed of protein increases day by day and the highest demand is for meat among the others (Huis, 2013). Besides, obtaining high-quality 1 kg of protein from the livestock requires 6 kg of plant protein that livestock feeds and obtaining 1 kg weight of animal requires 2.5 kg feed for chicken, 5 kg feed for pork and 10 kg feed for beef (Huis et al., 2013; Smil, 2002). Whether the necessity of 1 kg weight of insects, in this case crickets, is 1.7 kg; the edible and digestible parts of the insects (80%) is higher than the chicken and pig (55%) and cattle (40%). This shows that the feed conversion ratio of cricket is 2 times higher than chicken, 4 times higher than pig and 12 times higher than cattle (Huis et al., 2013).

According to FAO, a scarcity about water is expected at the countries all over the world with the total population of 1.8 billion people by 2025 (Huis, 2013). In terms of the future of the planet Earth, the water and land are the key factors. Almost 70% of the water source is consumed in the agricultural area (Oonincx & Boer, 2012). The amount of water that is necessary to produce 1 kg of chicken is 2,300 liters, 1 kg of pork is 3,500 liters and 1 kg of beef is 22,000 liters (Pimentel et al., 2004). As water, the increased demand to meat increases the amount of land cropped and thereby increases the deforestation and usage of fertilizer (Dobermann et al., 2017). Insects have much lower feed, land and water necessity compared to the traditional sources.

Another positive effect of insect for environment is that they reduce the bio-waste by consuming them as their food source. They are capable to reduce 1.3 million

tons of bio-waste in a year; especially yellow mealworm is one of the most significant bio-converter insect (Huis et al., 2013).

1.1.4. Insects oils in literature

In literature, there is little research about the oil portion of the edible insects and it is necessary to stick to this concept because of the importance of fatty acids and antioxidants inside the oil.

*Tenebrio molitor (*yellow mealworm) is a significant source of polyunsaturated fatty acids (PUFA) (especially Ω-6 and Ω-3). These types of fatty acids have quite positive effect on the many pathological condition prevention, especially cardiovascular diseases, so it was focused on the possibility of modification of the fat content and fatty acid composition of *Tenebrio molitor* with different feeding conditions and habitats. In larval stage of the yellow mealworm it contains at least 34% fat. The main fatty acids in the fat portion of the yellow mealworm are PUFA (Ω-6 and Ω-3 which are α-linoleic and α-linolenic acids), palmitoleic acids (monounsaturated fatty acids, MUFA) and myristic, palmitic and stearic acids as saturated fatty acids. According to their diet, the amount of saturated fatty acids decreases significantly and the content of PUFA, especially α-linolenic acids increase; thus, the fat obtained from the yellow mealworm become more prone to prevent the cardiovascular diseases and appropriate for the human consumption (Dreassi et al., 2017).

In a research, Barroso et al (2017), studied on the modification of fatty acid composition of house cricket larvae with different larval feed composition to enrich the diet with Ω-3 very long chain PUFA. As a result, the nutritional value of the house cricket was found to increase almost three times (Barroso et al., 2017).

The effect of diets of different Ω-3 and Ω-6 ratio and PUFA and fatty acid concentration of mealworm larvae due to the importance in the Western human diet was observed in another. The increased concentration of fatty acids in larva diets favored the PUFA accumulation to the monounsaturated and saturated fatty acids' detriment (Fasel et al., 2017).

The lipid of the house cricket was investigated and it was found that the amount of fatty acids did not change between the 3rd and 11th week of postembryonic life of crickets. Lipids had the highest amount of linoleic (30-40%), oleic (23-27%), palmitic (24-30%) and stearic acids (7-11%). The composition of fatty acids of house cricket is almost the same with the dietary lipids. The triglyceride amount of cricket increased from 2nd to 8th week, then dropped and the other classes of lipids like simple esters, hydrocarbons, mono and diglycerides, sterols almost remained constant (Hutchins & Martin, 1968).

In another work, it was drawn attention to edible insects due to their highly rich amount of lipid content. In dry basis, the content of crude fat was nearly 77% with high calorie and fatty acid profiles; also, they included significant amount of essential fatty acids. It was observed that the key point to get high quality and high amount of fat was the extraction method. In this work, supercritical CO_2 as well as conventional hexane extraction on mealworm was used and the fatty acid composition, profile and their physicochemical properties was investigated. Consequently, it was found that the fatty acids involved in these species were mainly even-numbered and the major fatty acids were oleic (40%) and lauric acid (45%) (Irungu et al., 2018).

The dry fractionation of mealworm was also performed by obtaining the oil using Soxhlet extraction under the effect of three different cooling temperatures which were 0, 2 and 4°C. Before fractionation, to eliminate crystals in fat, the oil was melted and placed into the water bath. Afterwards, fatty acids were analyzed with

Gas Chromatography (Tzompa-Sosa et al., 2016). Moreover, the effect of extraction methods on insect lipids' chemical characteristics were examined. For this purpose, *Tenebrio molitor, Acheta domesticus* and other two species were extracted with aqueous and Soxhlet extraction (industrial method) and Folch extraction (laboratory method). As a result, the highest lipid content was found in *T. molitor* as 13% using Folch extraction while the lowest recovery was obtained with aqueous method. Besides, the amount of Ω-6 fatty acids was the most abundant with Folch extraction. Finally, it was also found that C16:1 (palmitoleic acid) and C18:1 (oleic acid) had been reported in lipid of insects (Tzompa-Sosa et al, 2014).

In a regional study, the total lipid content and PUFA composition of six different species Thailand were investigated and determined. The highest PUFA content was found in the ground cricket as 2,883 mg/100 g and the major PUFA was C18:2 Ω-6. The range of total monounsaturated fatty acid content was found as 714 mg/100 g in giant water bug and the major MUFA was C18:1 in all insect species. As a result, it was found that the aquicolous edible insects could be a significant alternative source of C20 long-chain polyunsaturated fatty acids (Yang et al., 2006).

1.1.5. Current status of insects in food market

Insects are very popular in rural countries in Africa, Asia and South America because of their high nutrition value in terms of protein, fat, vitamin and mineral. Those communities prefer to consume insect as whole and snacks (Tiencheu & Womeni, 2017). While the crickets and grasshoppers are consumed as seasonal as snack, the beetles decorate the salads (Srivastava & Gupta, 2009).

In several African countries, people earn their livelihood by selling insects gathered from the bushes and farms. Some of the gathered insects are processed and sold to the shops and restaurants in both cities and out of the cities (Tiencheu & Womeni, 2017; Fasoranti & Ajiboye, 1993; Chavunduka, 1975).

There are also some companies in USA and some European countries producing insect-based products. Exo and Chapul take the lead in USA with the production of bars containing cricket flour. In the European part of the insects, there is works in production of energy bars with cricket flour in Iceland; some restaurants in Copenhagen serve insects to human consumption and a company in Sweden presents insect products to human consumption. Besides of those, there is an initiative work in Sweden aiming to produce 'Bug Burger' by using insects instead of meat. It is also started an enterprise in Sweden by an architect firm with the purpose of integration the condos and rearing of crickets and bees (Jansson & Berggren, 2015).

There is also increased interest in the media so as to draw attention to entomophagy. The insect-based product of Exo company ranks among the top ten in the category of innovative companies in 2015 according to the magazine Fast Company; besides, insect bars are seen as trend of 2015 in Time magazine (Jansson & Berggren, 2015).

According to report on the edible insects, it is expected that the market value of edible insects will reach to 1,181.6 million USD by 2023 (Ltd, 2018). The report shows how much the market size of insects is big explicitly.

1.1.6. *Acheta domesticus* (House cricket)

House cricket is the species called as *Acheta domesticus*, and it belongs to the *Animalia* kingdom, *Insecta* class, *Orthoptera* order and *Grylliade* family according to the taxonomic hierarchy (Acheta Linnaeus 1758, n.d.). The origin of the *Acheta domesticus* is the Southeastern Asia; however, it is seen in different parts of almost all over the world such as Europe, Northern Africa, Western Asia, Indian Subcontinent, Australasia, Mexico, Canada and United States of America (Mariod et al., 2017). In Thailand and Laos, *Acheta domesticus* is used for food production (Oonincx et al., 2015).

The life cycle of house cricket is completed in two to three months under the rearing conditions of 27 to 32°C (Featured Creatures, n.d.). The stages of the house cricket, which is hemimetabolous, during metamorphosis are egg, nymph and adult (Hackewitz, 2018; Rumpold & Sclüter, 2013). The eggs hatch after 11 to 15 days and the nymphs start to grow slowly in terms of size; besides, the nymps molt 7 to 10 times until become an adult at the 6th to 8th weeks (Hackewitz, 2018). After 1 to 3 days of maturation, they start to mate; and after 1 to 2 days of mating, the females lay eggs (Murtaugh & Denlinger, 1985). A single female may lay an average of 728 eggs on her own (House Cricket, n.d.). The length of adult house cricket reaches to 16 to 21 mm with the color of light yellowish-brown (Featured Creatures, n.d.).

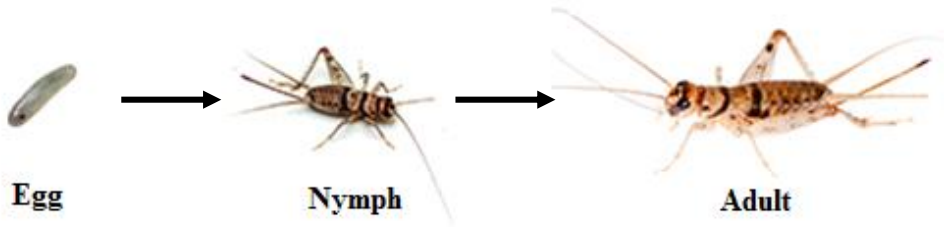

Figure 1.1. Life cycle of house cricket

Whether the house crickets are known as important source of protein, they include undeniable amount of high-quality lipids (Ω-3 and Ω-6 fatty acids), vitamins and minerals (Hackewitz, 2018). The protein content of an adult house cricket differs in the range of 64.4 to 70.8%; the fat content is in the range of 18.6 to 22.8% and the fiber content is in the range of 16.4 to 19.1% (Hackewitz, 2018). In addition to those macronutrients, there are also high amount of vitamin A, vitamin B complex, vitamin B12, vitamin C and vitamin E as the vitamins; and calcium, potassium, magnesium, phosphorus, sodium, iron, zinc, manganese, copper and selenium as minerals (Mariod et al., 2017).

The major fatty acids that adult house cricket includes are linoleic (30 to 40%), oleic (23 to 27%), palmitic (24 to 30%) and stearic (7 to 11%) acids (Hutchins & Martin, 1968). Besides of those, the smaller amount of linolenic, palmitoleic and myristic acids are also found in the lipid part of the house cricket (Hutchins & Martin, 1968). While the amount of fatty acids does not change between the 3rd and 11th week of postembryonic life, there is a steady increase in the amount of triglycerides between the 2nd and 7th or 8th week of postembryonic life of house cricket (Hutchins & Martin, 1967). The compositions may differ according to the diet of the house crickets.

The house cricket is generally consumed as deep-fried snack and is sold and used in powder form (Mariod et al., 2017). In Thailand, house cricket consumption is popular among the other types of insects due to its soft body (Huis et al., 2013). For example, 10 tons of house crickets are produced by the 2 villages with 400 families in the period of maximum production so as to export and sell in their domestic markets (Raubenheimer & Rothman, 2013). In addition to this, lots of countries in Thailand have several amounts of cricket farms and farmers (Huis et al., 2013).

1.1.7. *Tenebrio molitor* (Yellow mealworm)

Yellow mealworm is the species called as *Tenebrio molitor*, and it belongs to the *Animalia* kingdom, *Insecta* class, *Coleoptera* order and *Tenebrionidae* family according to the taxonomic hierarchy (*Tenebrio molitor* Linnaeus 1758, n.d.). The homeland of the *Tenebrio molitor* is Europe; however, it is seen in different parts of almost all over the world (Mariod et al., 2017). The consumption of *Tenebrio molitor* is very popular in Africa, Asia, America and Australia (Alves et al., 2016).

The life cycle of yellow mealworm is completed in three to six months according to the rearing conditions (Makkar et al., 2014). The stages of the yellow mealworm, which is holometobolous, during metamorphosis are egg, larvae, pupa and adult (Rumpold & Sclüter, 2013; Makkar et al., 2014). The eggs hatch after 10 to 12 days near 20°C and the larvae is born. The larvae molt 10 to 20 times until it become pupa. In general, the larvae stage lasts 3 to 4 months with some maturation stages at ambient temperature; however, they could maintain their life as larvae up to 18 months. The length and weight of mature yellow mealworm larvae reaches to 25 to 35 mm and almost 200 mg respectively with the color of light yellow-brown. (Finke, 2002; Makkar et al., 2014; Feng, 2018). The stage of pupa (cocoon) lasts 7 to 9 days and the cocoon opens at the end of this period; thus, the adult mealworms (beetles) are born and lives for two to three months (Makkar et al., 2014). The beetles start to mate, and after 4 to 17 days of mating, the females lay eggs (Feng, 2018). A single female may lay 500 eggs on her own (Aguilar-Miranda, 2002). Besides, there is a problem about the mealworms cruelty. The big mealworms may eat smaller ones, and the beetles may eat whole stages of metamorphosis like eggs, larvae and pupa (Kvassay, 2017). This is because they must be placed in separate places.

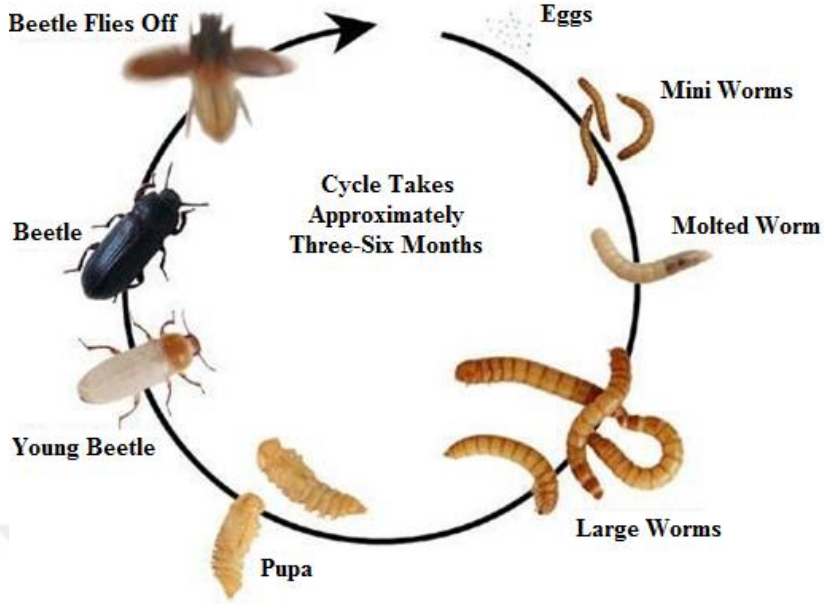

Figure 1.2. Life cycle of mealworm

Mealworms are full of the nutritious substances such as protein, lipids, vitamins and minerals. The protein content of yellow mealworm differs in the range of 47.76 to 53.13%, the fat content is in the range of 27.25 to 38.26% and the average fiber content is 6% (Bovera et al., 2015). The freeze-dried yellow mealworm larvae include 33% of fat, 51% of crude protein and 43% true protein in dry basis (Zhao et al., 2016). Another study shows that the amount of crude protein is lower in the larval form of mealworm with the 46.44% than in the adult form with the 63.34%; besides, the amount of crude fat is higher in the larval form of mealworm with the 32.7% than in the adult form with the 7.59% (Ravzanaadii et al., 2012). In addition to those macronutrients, there are also high amount of minerals and vitamins in mealworm larvae. The amount of minerals such as phosphorus, magnesium, zinc, iron, cupper and manganese that fresh and powdered mealworm larvae includes are higher than the meat and eggs. Nevertheless, the amount of the all compounds that mealworm larvae includes depends on the climate, habitation and feed (Siemianowska et al., 2013).

The major fatty acids that yellow mealworm includes are oleic (37.7 to 43.17%), linoleic (27.4 to 30.23%) and palmitic (16.72 to 21.1%) acids (Ravazanaadii et al., 2012; Makkar et al., 2014). Besides of those, the smaller amount of linolenic, palmitoleic, stearic and myristic acids are also found in the lipid part of the yellow mealworm (Makkar et al., 2014).

The mealworms, which are omnivorous insects, are harvested, processed and consumed in the larvae stage (Feng, 2018). In general, they consumed as live; however, the canned, dried and powder forms are also popular consumption types (Veldkamp et al., 2012).

1.2. Important nutrient constituent of insect oils

1.2.1. Lipids

The lipid family contains triglycerides, phospholipids and sterols (Whitney & Rolfes, 2018).

Fats are important in terms of energy supply and have several roles in health. There is confusion between the structures of fats and oils. Both fats and oils belong to the group of triglycerides (triacylglycerols) of lipids, and there exists the three fatty acid group joined to glycerols in their esters (Whitney & Rolfes, 2018; Fats and Oils, 2018). The simple difference between two of them is that if a triglyceride is solid at 25°C, it is called as fat; and if a triglyceride is liquid at the same temperature, it is called as oil. It is known that animal fats become mainly solid due to the obtained triglycerides from animal sources, that is the amount of saturated fatty acids is high in animal-based oils; and vegetable oils become generally liquid due to the obtained triglycerides from plant origin which means that the plant based oils include significant amount of unsaturated fatty acids (Whitney & Rolfes, 2018; Fats and Oils, 2018).

The fatty acids and triglycerides consist of carbon, hydrogen and oxygen atoms and the fatty acids are divided into two groups that are unsaturated fatty acids and saturated fatty acids (Whitney & Rolfes, 2018). Fatty acids include even number of carbon between 4 and 24, the most common and important fatty acids in terms of nutrition includes 18 carbons in their chains (Whitney & Rolfes, 2018). In room temperature, the unsaturated fats are in liquid form while the saturated ones are solid. Besides, the fats obtained from plant origin includes higher amount of unsaturated fatty acids (Fats: their functions and good food sources, 2013).

Among the several types of unsaturated fatty acids, two of them are called essential fatty acids, which are linolenic acids, an omega-3 fatty acid (Ω-3) and linoleic acids, an omega-6 fatty acid (Ω-6), due to the necessity to supply them in diet. It is necessary to ingest the essential fatty acids from the foods, because they are not made in the human body. They are quite important for life; Ω-3 lowers the coronary heart disease and stroke risk, reduces inflammation and has an important role in growth and development, especially in brain function and eyes (Fats: their functions and good food sources, 2013). Some sources of the monounsaturated fatty acids are canola oil, olive oil, nuts, avocado and some seeds like sesame and pumpkin while some polyunsaturated fatty acids are sunflower oil, corn oil, soybean oil, walnuts and especially fish which most of it includes high amount of Ω-3 fatty acids. Besides of the unsaturated fatty acids, it is possible to find the saturated fatty acids, which are made in the human body, in many animals and some plants. The effect of the saturated fatty acids differs in the body in terms of health. The high uptake of some of them may cause several health problems unlike unsaturated fatty acids, so it is important that their amount should be controlled in the diet. They are mainly found in animal origin products like beef, cheese, butter and whole milk while the coconut and red palm oil are plant origin and both of them do not cause serious health risks (Fats: their functions and good food sources, 2013).

In this study, an alternative type of oil shows up as an edible insect oil. Whether the both cricket and mealworm oils are animal origin, they are in liquid form in the room temperature; that is, they are rich in unsaturated fatty acids.

The house crickets include undeniable amount of high-quality lipids (Ω-3 and Ω-6 fatty acids) (Hackewitz, 2018). The oil content is in the range of 18.6 to 22.8%. The major fatty acids that adult house cricket includes are linoleic (30 to 40%), oleic (23 to 27%), palmitic (24 to 30%) and stearic (7 to 11%) acids (Hutchins & Martin, 1968). Besides of those, the smaller amount of linolenic, palmitoleic and myristic acids are also found in the lipid part of the house cricket (Hutchins & Martin, 1968). While the amount of fatty acids does not change between the 3rd and 11th week of postembryonic life, there is a steady increase in the amount of triglycerides between the 2nd and 7th or 8th week of postembryonic life of house cricket (Hutchins & Martin, 1967). The compositions may differ according to the diet of the house crickets.

The oil content of the yellow mealworm is in the range of 27.25 to 38.26%. (Bovera et al., 2015). The major fatty acids that yellow mealworm includes are oleic (37.7 to 43.17%), linoleic (27.4 to 30.23%) and palmitic (16.72 to 21.1%) acids (Ravazanaadii et al., 2012; Makkar et al., 2014). Besides of those, the smaller amount of linolenic, palmitoleic, stearic and myristic acids are also found in the lipid part of the yellow mealworm (Makkar et al., 2014).

1.3. Road to characterization

1.3.1. Conventional extraction

The high lipid bioavailability of insects makes them significant lipid source, so it is required to choose the best extraction method to protect both the lipids without destructing their structure and the other compounds that are important in terms of nutrition. The lipids are isolated thanks to the effective extraction (Choi et al., 2017).

Selection of the appropriate solvent during the extraction process is important, because the aim is not only the isolation of lipids but also to protect their structure from negative effects. For edible insects, the better yield is obtained as minimum 96% with the usage of n-hexane as a solvent. There are also some extraction methods like ethanol-based oil extraction, aqueous extraction and supercritical CO_2 extraction; however, the highest effectiveness in terms of yield and protection of the structure is provided with the usage of n-hexane (Ricochon & Muniglia, 2010). The findings also showed that hexane was quite efficient to obtain lipid part of the insect samples (Choi et al., 2017). The other important point in selection hexane as solvent is being easy to remove due to its high volatility; moreover, the hexane has the ability to mix with the oil aggressively without affecting the other nutrients like protein (Anderson, n.d.).

Hexane, a non-polar molecule with the boiling point of 69°C, is a universally accepted solvent with its greater ability in the oil extraction; in other words, the aim of existence of hexane is to act as a solvent to extract the edible oil from their sources; in this case the sources are two insect species which are yellow mealworm and house cricket (Toxicological Profile for n-Hexane, 1999). The significant communities like Environmental Protection Agency and World Health Organization classifies the hexane as non-carcinogenic; that is, there is not

negative effect in long term on human health (Toxicological Profile for n-Hexane, 1999).

1.3.2. High Hydrostatic Pressure (HHP)

In history, the application of high hydrostatic pressure (HHP) dates back to old times until the earlier 19th century (Balasubramaniam et al., 2015). In the year of 1882, the starch conversion into the glucose was investigated under the effect of the pressure and Certes did a scientific research about the relation of the high hydrostatic pressure on the organisms in 1883; however, the researcher that stepped high hydrostatic pressure to the new age in terms of food industry by revealing the high hydrostatic pressure effect on foods was Bert Hite and his co-workers with the aim of milk preservation from the food borne microorganisms applying the pressure up to 650 MPa and the remarkable reduction in the amount of spoilage bacteria is obtained in 1889 (Soxhlet, 1881; Elamin et al., 2015; Chawla et al., 2010).The engineering aspect of the HHP was worked comprehensively by Percy W. Bridgman by focusing on the compressibility, polymorphic transformations, phase change and thermal conductivity between the years of 1909 and 1959 (Bridgman, 1909; Bridgman, 1912; Bridgman, 1914; Bridgman, 1923). Even the research of Bridgman was based on the water phase diagram (Balasubramaniam et al., 2015). The applications of the HHP continued gradually with some processes like treatment on fruits and vegetables with the aim of preservation; investigation the effect of pressure on bacterial spores and research on the high pressure effect on chemical reactions until the years of 1980 (Hite et al., 1919; Larson et al., 1918; Brown, 1920). The significant milestone of the high pressure was realized in the year of 1992 with the production of first pressure treated product, jam, in Japan; after the renascent demand and popularity of high hydrostatic pressure as an alternative method to traditional thermal processing treatment methods in the middle of the 19th century (Elamin et al., 2015; Knorr, 1993). After that uprising against the thermal treatment, the usage of

high hydrostatic pressure continued increasingly up to today, and it seems that the advancing will be continue in food industry.

The application area of HHP has increased in today's world with the increasing health consciousness and advanced technologic development (Chawla et al., 2010; Ginsau, 2015). The dairy products, meat products, the fishery industry, fruits and vegetables need a treatment that do not destroy their characteristics and functionalities; HHP is quite suitable in terms of treatment of those products by preserving and increasing their organoleptic characteristics. In addition to the organoleptic properties, eliminates or reduces the pathogenic and spoilage microorganisms and microbial load according to the level of applied pressure by inactivating the enzymes, harming to DNA and ribosomes and destroying their cell walls (Ginsau, 2015). There are several application areas of HHP in food industry like pasteurization, sterilization, homogenization, freezing and thawing, dense phase CO_2 (Balasubramaniam et al., 2015). If it is thought specifically, there are some proved works in terms of the effect of high hydrostatic pressure on food products. For example, ripening of the cheese is accelerated by pressure application by providing desirable quality and preventing or reducing the possible microorganism problem (Chawla et al., 2010). However, whether the microorganism is reduced or eliminated, the spores of molds and bacteria, and also viruses are resistant to the HHP (High Pressure Processing of Foods, 2015). The safety and shelf life of foods can be enhanced by destruction of food borne-microorganisms with hydrostatic pressure. The effectivity of hydrostatic pressure depends on magnitude of pressure, pressurization time and temperature, microbial types, cell growth phase, suspending media, and the presence of antimicrobial substances (Alpas et al., 1999).

The application of HHP has innumerable advantages and benefits such as inactivating the enzymes, microorganisms and spores, protein modification and denaturation, decreasing the freezing point of water. Thanks to being non thermal

application, HHP prevents the thermal degradation of compounds of the food (Parekh et al., 2017). In HHP, the pressure is transmitted uniformly and instantly to the food compounds whatever the shape and size is. On the contrary to the conventional treatment applications; the color, aroma, nutritional value, flavor and the texture characteristics like desired attributes of the food compound is preserved and is enhanced in HHP treated food compounds thanks to not being a physical and chemical changing; besides, the shelf life is also extended 2 to 3 folds of by preserving the antimicrobial system and reducing the number of microorganism without modifying the sensory and nutritional properties (Parekh et al., 2017; Chawla et al., 2010). The one another important benefit of the HHP is that the covalent bonds are preserved under the effect of pressure and the original essence of the food compounds is preserved (Yaldagard et al., 2008). HHP is also an eco-friendly technology and produces almost no waste (Ginsau, 2015).

HHP is an increase in the pressure of a liquid isostatically via the liquid compression, and is considered as a non-thermal treatment method in food processing with the application of the pressure in the range of 100 to 1000 MPa (Orlien, 2017; Elamin et al., 2015; Liepa et al., 2016). During the process, the temperature and time is also specified in the range of 1°C to 95°C and a few seconds to 20 minutes respectively according to the type of food compounds and the type of the process (Yaldagard et al., 2008; Erkmen & Bozoğlu, 2016). The pressure is distributed rapidly and quasi-instantaneous uniformly through the sample of both liquid and water-containing solid (Balasubramaniam, 2015; Liepa et al., 2016). The one of the significant attention-grabbing feature of the HHP method is that the treatment causes no damage and no distortion on the food compound so long as the product does not include any emptiness inside (Elamin et al., 2015).

The standard components of a typical HHP system comprises of pressure vessels, pressure generator vehicle, process control system, a handling system for product

assembly and removal, yoke which is used for reining in to cover the pressure vessel, intensifier and two end closures covering the vessel (Elamin et al., 2015; Ting, 2011). The pressure vessel is one of the most significant parts of the HHP system due to the aim of usage, and the pressure vessel is designed among of the three design techniques which are autofrettage technique, heat-shrink technique (multilayer vessel) and wire-wounded vessel so as to provide longer shelf life and durability, maximum pressure and less weight of the shell: besides, the thickness of the vessel is determined according to the maximum pressure, diameter of the vessel and cycle number (Mathur, 2009; Koutchma, 2014; Elamin et al., 2015; Mertens, 1995). The pressure generator vehicle is used with the purpose of generation of high-pressure levels in the pressure vessels with the types of indirect (piston) compression and direct (pump) compression; the indirect compression is used in the food industry due to the ability to achieve much higher pressure levels (Elamin et al., 2015; Martin et al., 2002).

The pressure transmitting fluid is also quite significant in the HHP process and acts as pressurizing medium, because the pressure is transmitted quasi-instantaneous uniformly to the products thanks to this fluid (Balasubramaniam et al., 2015). The transmission is independent from volume, size, shape and type of pressure vessel (Farr, 1990; Torres et al., 2009). The most commonly used fluid is water in industry besides of the alternatives of the glycol, glycol-water, silicone oil, sodium benzoate ant castor oil (Balasubramaniam et al., 2015). The parameters in the selection of the pressure transmitting fluid are viscosity under the effect of pressure based upon the prevention from the corrosion during HHP, heat of compression and thermal characteristics (Hogan et al., 2005; Balasubramaniam et al., 2015; Buzrul et al., 2008).

The impact of HHP on the oils that the mealworm and cricket species include is not investigated widely, so the exact effect and mechanism is not known. There are many works about the relation between protein and HHP and carbohydrate

and HHP; however, the works are limited in terms of oils (Povedano et al., 2014). The temperature of the sample increases during the HHP processing due to the compressive work that is generated by adiabatic heating. The highest compression heat value belongs to the fats and oils as 0.08 K per MPa among the all food compounds where the compression heat of water is 0.02 K per MPa (Rasanayagam et al., 2003; Varma et al., 2010). In literature, it is found that the unsaturated fatty acids did not affect from the HHP significantly (Povedano et al., 2014). For the liquid unsaturated fatty acids, the conformational changes become reversible under the effect of pressure; however, the unsaturated fatty acids which are solids at room temperature are subjected to an irreversible conformation change (Povedano et al., 2014). In a case of milk lipids, the fat fraction and proteins are affected from HHP while vitamins, amino acids and flavor compounds are not affected (Chawla et al., 2010). The free fatty acid content increases due to alteration in the membrane of milk fat globules under the effect of HHP. The amount of free fatty acids does not increase with the treatments in the range of 100 to 500 MPa at the temperatures of 4, 25 and 50°C; however, that content decreases above 50°C (Liepa et al., 2016). In other words, the modifications of the raw ewe milk in the distribution and the size of the milk fat globules is catalyzed by the pressure up to 500 MPa (Gervilla et al., 2001). The reason of this modification is not because of the damage on the milk fat globule membrane; it is because of the aggregation and disintegration of the membranes of fat globules (Chawla et al., 2010).

It is possible to retain or improve the bioavailability and bioaccesibility of antioxidant and other compounds which are highly nutritious by applying the HHP (Vazquez-Gutierrez et al., 2013). Besides, the antioxidant activity of essential oils obtained from plants and the availability of total phenolic compounds improves with the application of high pressure (Cherrat et al., 2013; Lee et al., 2015). Phenolic compounds are directly related with the sensory and nutritional quality of both fresh and processed foods (Lattanzio et al., 1994). HHP may cause a change in the distribution and aggregation of the phenolic

compounds due to the cellular walls' disruption and hydrophobic bonds' disruption in the cell membrane of the phenolic compound included products such as fruits with the pressure application by increasing the influx of solvent and contact with solvent during the extraction (Casquete et al., 2015). For this reason, the amount of total phenolic compounds may increase due to the increased extractability of some types of antioxidants, which includes phenolic hydroxyl groups (Cao et al., 2011). The effectiveness of HHP on the antioxidant capacity depends on the compounds subjected to pressure and the parameters of HHP (Campos & Aguilera-Ortiz, 2017). For example, while there is an increased tendency to the higher antioxidant activity of the blackberry puree after pressure treatment, there is no change in the strawberry puree; besides, the pressure at 400 MPa decreases the total phenolic content in the strawberry pulps while the amount of total phenolic content increases at 500-600 MPa (Patras et al., 2009; Cao et al, 2011). Moreover, it is found that HHP has a positive effect on tomato and carrot puree (Campos & Aguilera-Ortiz, 2017). If it is necessary to rephrase, the retaining and the improving ability on the antioxidant activity is higher in HHP treatment between 400-600 MPa than the conventional thermal treatments in terms of pressure treated phenols, anthocyanins and ascorbic acid in the strawberry puree and pressure treated blackberry puree compared to the thermal treated ones (Patras et al., 2009). As mentioned above, the total phenolic content and antioxidant activity depends on the time, temperature and the pressure level, but the levels tend to remain same as with the untreated sample and tend to increase.

1.4. Objectives

The combination of the increasing population, increasing demand and possible scarcity of the food sources canalizes people to find alternative sources in terms of the essential macronutrients and micronutrients that the humanity needs for their healthy future. The traditional sources like livestocks and poultry husbandry may not be adequate and meet the demand of huge population. The insects come into play at this point as an alternative nutritional source with their amazing nutritional value in the sense of macronutrients and micronutrients. The environmentally friendly lifestyle of insects provides less greenhouse gases emmisions, water and land usage and more feed conversion ratio during the breeding period accompanied by several health benefits when compared to traditional methods. Even though the entomophagy is a problematic issue for lots of people, the solution is to serve the insects to consumers in processed form such as additives so as to prevent the recognition by consumers; because there is a big difference in consumption insects as a whole and consumption them as unrecognized form. The key point is that they will know what they eat and why they eat, so the people must keep informed about the possible future trend and possible food source with the aim of answering those questions unambigiously by experiencing the entomophagy.

In the scope of this study, mealworm and cricket oil were extracted with conventional and HHP assisted extraction to investigate how HHP and temperature affected the physicochemical characteristics of extracted oil. In the first part of the study, oils were extracted from yellow mealworm and house cricket with hexane with the proportion of 1:15 (w/v) with or without application high hydrostatic pressure at 500 MPa at 30°C and 40°C.

The focus of this study is the oil obtained from the insect types of *Acheta domesticus* (House cricket) and *Tenebrio molitor* (Yellow mealworm). It is aimed

to determine the effect of HHP assisted extraction on the lipid content and composition against the conventional methods first. After then, the physicochemical properties of insect oils were investigated by focusing on the peroxide value, crystallization and melting points, total phenolic content and antioxidant activity. Oil yield was found in the range of %22.75-24.22 and %16.17-18.09 for mealworm and cricket, respectively. It was also found that with Gas Chromatography the amounts of myristic acid, palmitoleic acid and linolenic acid in mealworm and cricket oils were relatively high, although the most abundant fatty acids found in both insects were palmitic acid, stearic acid, oleic acid and linoleic acid. Moreover, the difference between crystallization and melting point of mealworm is higher than the cricket as results of Differential Scanning Calorimetry. The amount of unsaturated fatty acids in mealworm is almost two fold of saturated fatty acids, whereas this ratio is nearly one and a half in cricket.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Freeze dried larvae of yellow mealworm and house cricket powder were supplied from Tasty Worms Nutrition Inc. (Florida, USA) and JR Unique Foods Ltd. (Udon Thani, Thailand), respectively. The main focus of the project, the oil portion of the mealworm and house cricket, was obtained from these powders using hexane procured by Sigma Aldrich Chemical Cooperation (St. Louis, MO, USA). Furthermore, there were several reagents supplied from Sigma Aldrich Chemical Cooperation (St. Louis, MO, USA) used during the experimental procedure such as copper chloride ($CuCl_2$), neocuproine (2,9-Dimethyl-1,10-phenanthroline), ammonium acetate ($CH_3CO_2NH_4$), trolox (TR), ethanol (C_2H_5OH), acetic acid (CH_3CO_2H), $DPPH^{\cdot}$(2,2-diphenyl-1-picrylhydrazyl), methanol (CH_3OH), Folin-Ciocalteu's phenol reagent, chloroform ($CHCl_3$), starch, sodium thiosulfate ($Na_2S_2O_3$), potassium idodide (KI), pure nitrogen (N_2), capric acid ($C_{10}H_{20}O_2$), indium, n-dodecane, methanol sodium hydroxide, boron trifluoride methanol.

2.2. Methods

2.2.1. Extraction of oil from insect powders

Two different extraction methods which are conventional and novel extraction by application of HHP with different parameters were used to observe their effects on physicochemical characterization of insect oils.

2.2.1.1. Conventional extraction

The conventional extraction procedure was performed as explained in two studies with some alternations and different parameters (Buβler et al., 2016; Zhao et al., 2016). In the extraction process; the powder of yellow mealworm and house cricket were separately mixed with hexane at the proportion of 1:15 (w/v), and the extraction was performed at 30°C for 15 minutes under hot plate magnetic stirrer (Daihan Scientific Co., Ltd., Korea); then, the centrifugation was applied at 9,500 rpm for 20 minutes at 30°C to obtain the oil-hexane mixture (Sigma 2-16PK, SciQuip Ltd., UK). Hexane in the oil-hexane mixture was removed with the evaporation for 24 hours in a drying oven at 40°C; thus, the pure oil portion of the two edible insects were obtained in liquid form.

2.2.1.2. High Hydrostatic Pressure

2.2.1.2.1. High Hydrostatic Pressure equipment

High Hydrostatic Pressure (HHP) application was performed with 760.0118 type pressure equipment (SITEC-Sieber Engineering AG, Zurich, Switzerland). The equipments that HHP included were a pressurization chamber, two end closures, a means for restraining the end closures, a hydraulic unit, a pressure pump and a temperature controller. The properties of pressurization chamber were a volume of vessel of 100 ml with internal diameter of 24 mm and length of 153 mm. A built-in heating-cooling system was used with the purpose of keeping the system at the

constant temperature (Huber Circulation Thermostat, Offenburg, Germany). Besides, the distilled water was used as the transmitting medium. The rate of pressurization was 300 MPa/min for 500 MPa and the pressure releasing times were less than 20 s.

Figure 2.1. HHP Equipment

2.2.1.2.2. High Hydrostatic Pressure asssisted extraction

As in the conventional extraction, the powder of yellow mealworm and house cricket were separately mixed with hexane with the proportion of 1:15 (w/v) and high hydrostatic pressure at two different temperatures which are 30ºC and 40ºC were applied to the hexane-insect powder mixture at 500 MPa. The products obtained from the HHP were centrifuged at 9,500 rpm for 20 minutes at both 30ºC and 40ºC. The evaporation was carried out as in the conventional extraction and the pure oil portion of the two edible insects were obtained in liquid form.

2.2.2. Physicochemical characterization of insect oils

2.2.2.1. Determination of fatty acid composition with Gas Chromatography

To determine the fatty acid composition, the Gas Chromatography (GC) method described by Jeon et.al. (2016) was used with some modifications. 0.25 g of oil and 6 ml of 0.5 N of methanol sodium hydroxide were mixed to produce methyl esters and heated in water bath at 80°C for 10 minutes. After cooling the oil on ice for 3 minutes, 7 ml of 14% boron trifluoride methanol was added to the solution and the mixture was heated 80°C for 2 minutes; then the solution was cooled in ice for 3 minutes before the 5 ml of n-hexane addition. After preparation of the solution; the oil was heated for 1 minutes and the layer on the top was separated and transferred to a vial. The GC system included a capillary column and ionization detector (260 flames). As the carrier gas, Helium was used at a rate of 1.3 ml/min. 1 µl of the solution was injected with a split ratio of 50:1. The fatty acid composition was determined as a relative percentage of the total peak area (Jeon, et al., 2016).

2.2.2.2. Determination of crystallization and melting point with Differential Scanning Calorimeter

With the purpose of determination of the freezing point of both conventional extracted oils and HHP assisted extracted oils, the Differential Scanning Calorimeter analysis described by Tomaszewska-Gras (2016) was used with some modifications. Perkin Elmer DSC 4000 differential scanning calorimeter (Perkin Elmer, Turkey) operating with Pyris software was used as the instrument. Pure nitrogen was used as the purge gas with a flowing rate of 19.8 ml/min and the calibration of DSC was done using indium (melting point: 156.6^{o}C, ΔH_f = 28.45 J/g) and n-dodecane (melting point: -9.65^{0}C, ΔH_f = 216.73 J.g^{-1}); Capric acid melting with the melting point of 31.6^{o}C controlled the calorimeter's calibration. The oil samples were weighed around 10-11 mg into aluminum pans and

hermetically sealed. An empty hermetically sealed aluminum pan was used as the reference. During an operation for each sample, two replicates were analyzed. The pan with the sample was located into the calorimeter at 25°C and the following time-temperature program was applied;

i. Heating from 25°C to 60°C at 5°C/min to melt all crystals and nuclei,
ii. Cooling at 5°C/min to -40°C and keeping for 3 minutes at -40°C,
iii. Heating at 5°C/min from -40°C to 60°C

By following the temperature program above, melting point of the 1st peak (T^1) which is the melting of the low melting fraction (LMF), temperature of 2nd melting peak (T^2) of medium melting fraction (MMF), the final melting temperature (T^{end}) which is the clarification temperature, and the enthalpy ΔH (J/g) is specified as the area between the base line and melting curve are determined (Tomaszewska-Gras, 2015).

2.2.2.3. Determination of peroxide value

The peroxide value of each sample was determined by using the method described in the journal of IFRA Analytical Method: Determination of the Peroxide Value (2011) with some modifications to get optimum results. 1 g of each insect oil was weighted and mixed with 10 ml of chloroform. The mixture was agitated rapidly so that the oil was dissolved in the chloroform. 15 ml of acetic acid and 1 ml of potassium iodide were added the chloroform-oil mixture respectively, then the mixture was agitated for a minute and the solution remained at room temperature in a dark place for 5 minutes. After the waiting period, 75 ml of distillate water and 1 ml of starch were added to the solution. The mixture was titrated with 0.002 N of sodium thiosulfate ($Na_2S_2O_3$) solution if the expected peroxide value was less than 12.5 or 0.01 N of sodium thiosulfate ($Na_2S_2O_3$) solution if the expected

peroxide value was 12.5 or more. The value of peroxide was calculated with the equations below (IFRA Analytical Method: Determination of the Peroxide Value, 2011);

For 0.01 N $Na_2S_2O_3$; the peroxide value = $\frac{10x(V_2 - V_1)xF}{m}$ mEq g O_2/kg (Eq. 2)

For 0.002 N $Na_2S_2O_3$; the peroxide value = $\frac{2.8x(V_2 - V_1)xF}{m}$ mEq g O_2/kg (Eq. 3)

2.2.2.4. Determination of total phenolic content (Folin-Ciocalteu Assay)

Folin-Ciocalteu reagents were used to determine the total phenolic content (TPC). The oil portion obtained from the both conventional extraction and HHP assisted extraction were analyzed in terms of their total phenolic content. The method described by Al-Rimavi et al. (2016) was based on in this study. The insect powders were dissolved in ethanol: water: acetic acid mixture (50:42:8) with the ratio of 1:10 (ml/ml). The agitation of the mixtures was done using Vortex for 30 seconds. To assure that the extraction was fully completed, the extraction of phenolic compounds was performed for 60 minutes. After the elapsed time; the mixture was syringed and filtered through a 0.45 µm micro filter. 40 µl of sample was mixed with the 1.8 ml of Folin-Ciocalteu reagent (0.1 M); after the vortex, the mixture was left stand at the room temperature in dark for 5 minutes. Then, 1.2 ml of sodium bicarbonate ($NaHCO_3$) (7.5% w/v) was added to the mixture and vortexed again; later, the mixture was kept at room temperature in dark for 1 hour. Afterwards, the absorbance values were measured at 760 nm using the UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. LTD, Korea).

The blank was prepared by mixing 40 µl of ethanol: water: acetic acid solution (50:42:8), 1.8 ml Folin-Ciocalteu reagent (0.1 M) and 1.2 ml of $NaHCO_3$.

The calibration curve was prepared as gallic acid equivalent (GAE) with the gallic acid concentration of 20, 40, 60, 80, 100 ppm in ethanol: water: acetic acid solution (50:42:8). Thus, the total phenolic content was found as 'mg GAE/ml sample' (Al-Rimavi et al., 2016).

2.2.2.5. Determination of antioxidant activity (AA)

2.2.2.5.1. DPPH assay (Free Radical Scavenging Activity)

To determine the antioxidant activity (AA) of the insect oils the samples were extracted with some modifications mentioned by Brand-Williams et al. (1995). 0.1 ml of insect oils were weighted and dissolved in the solution of ethanol: water: acetic acid (50:42:8) a ratio of 1:10 (ml/ml) and the agitation of the mixtures was done using Vortex for 30 seconds. To assure that the extraction was fully completed, the extraction of phenolic compounds was executed for 60 minutes. After the elapsed time; the mixture was syringed and filtered through the 0.45 µm micro filter.

3.9 ml of the 0.0634 mM (25 ppm) DPPH in methanol (95%) was added to each extract which is 100 µl in an aluminum foil covered glass tube. After 1 hour, the mixtures were vortexed and the absorbance values of all samples were measured at 517 nm using UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. LTD, Korea).

The formula to determine the percentage inhibition of DPPH was;

$$\% \text{ inhibition of DPPH} = \frac{A^o - A^f}{A^o} \times 100 \text{ (Eq. 4)}$$

In the above equation A^o and A^f represents the absorbance of a solution of 3.9 ml DPPH and 100 µl methanol (95 %) at 517 nm and absorbance of the oil extracts after 60 minutes, respectively. Furthermore, percentage inhibition of DPPH of the trolox (TR) samples were also determined with the above equation where A^f represents the absorbance of a solution of 3.9 ml DPPH and 100 µl methanol (95 %) at 517 nm and absorbance of the oil extracts after 60 minutes.

The calibration curve was determined by the relation between percentage inhibition and ppm trolox samples. AA of oil extracts was expressed as mg TR/ml sample by using the calibration curve. (Al-Rimavi et al., 2016)

2.2.2.5.2. CUPRAC assay (Cupric Reducing Antioxidant Power)

As in the DPPH Assay, the antioxidant extraction was applied to the samples prepared for CUPRAC Assay. After all of the antioxidants were extracted, the method of Apak et al. (2008), which was the cupric ion reducing antioxidant capacity, was applied. 0.1 ml of oil extracts with 1 ml of the distilled water, 1 ml of 10 mM cupper chloride solution, 1 ml of 7.5 x 10^{-3}M neocuproine solution prepared with ethanol (99.9%) and 1 ml of ammonium acetate (1 M) were mixed and 4.1 ml solution was prepared. To prepare blank, it was added to 0.1 ml distilled water instead of oil extracts unlike the previous solution. The absorbance values of each samples were determined at 450 nm after 30 minutes. The calibration curve was prepared using TR in different concentrations. AA of oil

extracts was expressed as µmol TR/ml sample by applying the absorbance values to the calibration curve. (Al-Rimavi et al., 2016)

2.2.2.6. Statistical analysis

The statistical analysis was done for all experiments by using MINITAB (Version 16.2.0.0, Minitab Inc., Coventry, UK). The effect of the different oil extraction conditions, methods and the type of insects were investigated by analyzing with the ANOVA (analysis of variance). The comparison of the results was done by applying Tukey's multiple comparison test with at confidence interval of 95%.

2.3. Summary of Experimental Design

Table 2.1. *Summary of experimental design*

Experiments	Factors	Levels	
GC DSC Peroxide Value Total Phenolics DPPH Assay CUPRAC Assay	Insect Type	*Acheta domesticus* *Tenebrio molitor*	
	Extraction Type	Conventional extraction	30°C
			40°C
		HHP assisted extraction	30°C
			40°C

CHAPTER 3

RESULTS AND DISCUSSION

The oil portion of the yellow mealworm and house cricket was obtained by HHP assisted extraction at 500 MPa for 15 min and conventional extraction at 30°C and 40°C. During the extraction processes, it was aimed to find the effect of pressure on oil content, fatty acid composition, peroxide value, crystallization and melting points, total phenolic content and antioxidant activity.

3.1. Oil content

The initial stage of characterization was the oil extraction from the mealworm and cricket powders. Conventional and HHP assisted extraction processes were applied to obtain oil and it was investigated how HHP affected the quantity of extracted oil. Oil contents of insect powders obtained by different extractions were shown in Figure 3.1.

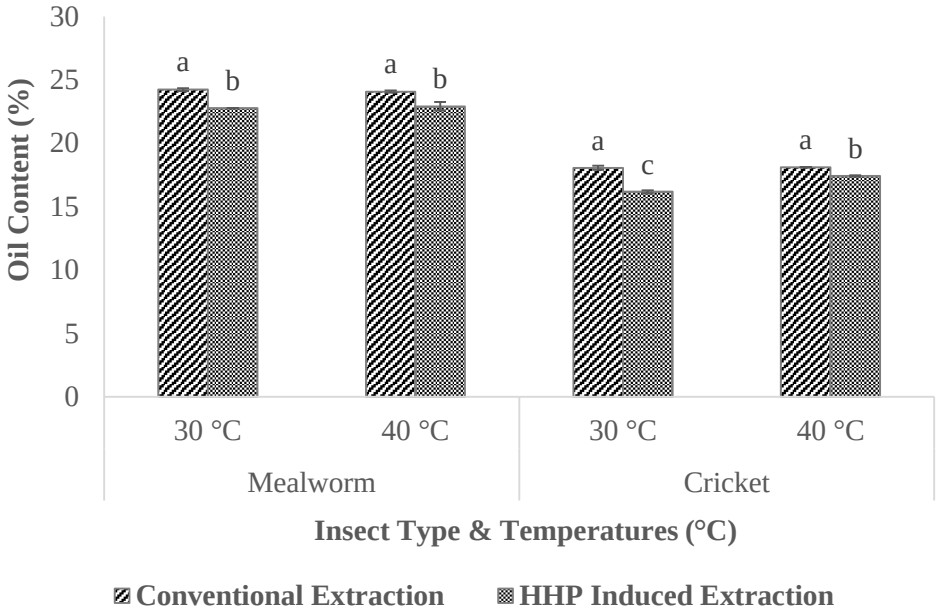

Figure 3.1. Oil content of insects. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

In mealworm, the oil content was in the range of 22.9 and 24.2% with different extraction methods. The results showed that the oil content decreased with HHP. In the conventional method, the highest oil content was obtained at 30°C while the highest content was obtained at 40°C with HHP assisted oil extraction. According to the statistical analyses, there was significant difference between pressurized and non-pressurized samples ($p<0.05$), while the temperature was not a significant factor ($p>0.05$).

In cricket, the oil content was in the range of 16.2 and 18.1% with different extraction methods. The results showed that oil content decreased with the application of HHP as in the mealworm. In conventional method, the highest oil content was obtained at 40°C while the highest content was obtained at 40°C with HHP assisted oil extraction. According to the statistical analyses, there was significant difference between pressurized and non-pressurized samples ($p<0.05$), and the difference was also significant at different temperature values ($p<0.05$).

In general, it can be said that HHP might have disrupted the structures of triglycerides and some fatty acids that the mealworm and cricket include by affecting the bonds (Bovera et al., 2015) . Besides, the highest oil content in mealworm is 24.2% and in cricket is 18.1%; however, those contents were low according to the literature values. In literature, the oil content of mealworm is in the range of 27.2 to 38.3%; the oil content of cricket is in the range of 16.4 to 19.1% (Bovera et al., 2015; Hackewitz, 2018). The reason of this difference may be the duration of the extraction in this study. In this study, the effect of HHP against the conventional method were investigated with the same extraction time and temperature. Normally conventional extraction could take 30 min to 60 min and it could be applied as twice (Zhao et al., 2016 & L'hocine et al., 2006). However 15 min was used as the processing time in this study. Extending the extraction time more than 15 min does not make HHP as a feasible alternative. That is why for comparison purposed the durations were kept same. It was expected that HHP would increase the yield because of its ability of charge groups' deprotonation and disruption of hydrophobic bonds and salt bridges; thus, the more solvent could penetrate into the cells thanks to the high pressure (Prasad et al., 2007; Ahmed & Ramaswamy, 2006). In this case, the processing time was less even the pressure was in high value as 500 MPa. The lower processing time may be inadequate to complete penetration of the solvent into the cell, so the yield of oil extracted with HHP was lower than the conventional extraction.

In addition to the effect of HHP processing time on the lipid extraction, temperature was also another important parameter, and was is expected to have a positive effect on the lipid yield with the increasing temperature (Islam et al., 2014). However, it was found that there was no significant difference in conventional extraction of both species ($p>0.05$). Temperature range was kept low for obtaining both good quality protein rich powder and lipids. If temperature value was used in the range of 70 to 120°C, the content of the lipid may be higher than the range of 30 to 40°C (Islam et al., 2014).

3.2. Fatty acid composition

Fatty acid composition of insect oils obtained by different extractions were shown in Table 3.1 and Table 3.2.

Table 3.1. *Experimental results of fatty acid composition of insect oils*

Fatty Acids	Common Name	Extraction Type		Insect Type	
		Pressure (MPa)	Temperature (ºC)	Mealworm (%)	Cricket (%)
C12:0	Lauric	**0.1**	**30**	0.215 ± 0.007[a]	-
			40	0.210 ± 0.000[a]	-
		500	**30**	0.210 ± 0.000[a]	-
			40	0.210 ± 0.000[a]	-
C13:0	Tridecanoic	**0.1**	**30**	0.050 ± 0.000[a]	-
			40	-	-
		500	**30**	0.050 ± 0.000[a]	-
			40	-	-
C14:0	Myristic	**0.1**	**30**	2.195 ± 0.050[a]	0.590 ± 0.000[a]
			40	2.180 ± 0.028[a]	0.590 ± 0.000[a]
		500	**30**	2.160 ± 0.056[a]	0.575 ± 0.007[b]
			40	2.180 ± 0.014[a]	0.600 ± 0.000[a]
C14:1	Tetradecanoic	**0.1**	**30**	0.175 ± 0.007[a]	-
			40	0.170 ± 0.000[a]	-
		500	**30**	0.170 ± 0.000[a]	-
			40	0.170 ± 0.000[a]	-
C15:0	Pentadecanoic	**0.1**	**30**	0.225 ± 0.007[a]	0.070 ± 0.000[a]
			40	0.215 ± 0.007[a]	0.070 ± 0.000[a]
		500	**30**	0.215 ± 0.007[a]	0.065 ± 0.007[a]
			40	0.210 ± 0.000[a]	0.070 ± 0.000[a]
C16:0	Palmitic	**0.1**	**30**	17.120 ± 0.226[a]	23.675 ± 0.191[ab]
			40	17.110 ± 0.127[a]	23.545 ± 0.219[ab]
		500	**30**	16.885 ± 0.304[a]	23.070 ± 0.184[b]
			40	16.985 ± 0.007[a]	23.930 ± 0.141[a]
C16:1	Palmitoleic	**0.1**	**30**	2.165 ± 0.021[a]	1.250 ± 0.014[a]
			40	2.140 ± 0.014[ab]	1.245 ± 0.007[a]
		500	**30**	2.120 ± 0.042[ab]	1.230 ± 0.014[a]
			40	2.060 ± 0.000[b]	1.250 ± 0.014[a]
C17:0	Margaric	**0.1**	**30**	0.600 ± 0.014[a]	0.145 ± 0.007[a]
			40	0.600 ± 0.000[a]	0.140 ± 0.000[a]
		500	**30**	0.590 ± 0.014[a]	0.145 ± 0.007[a]
			40	0.600 ± 0.000[a]	0.140 ± 0.000[a]
C17:1	Heptadecanoic	**0.1**	**30**	0.210 ± 0.000[a]	0.110 ± 0.000[a]
			40	0.195 ± 0.007[a]	0.105 ± 0.007[a]
		500	**30**	0.200 ± 0.000[a]	0.110 ± 0.000[a]
			40	0.195 ± 0.007[a]	0.110 ± 0.000[a]

Different small letters indicate significant differences between extraction conditions ($p<0.05$)

Table 3.2. *Experimental results of fatty acid composition of insect oils (cont'd)*

Fatty Acids	Common Name	Extraction Type		Insect Type	
		Pressure (MPa)	Temperature (ºC)	Mealworm (%)	Cricket (%)
C18:0	Stearic	**0.1**	**30**	12.030 ± 0.905[a]	13.970 ± 0.170[a]
			40	9.235 ± 0.233[ab]	13.015 ± 0.120[a]
		500	**30**	11.095 ± 0.502[a]	10.985 ± 0.007[b]
			40	9.435 ± 0.092[a]	10.435 ± 0.587[b]
C18:1	Oleic	**0.1**	**30**	24.060 ± 0.947[a]	23.695 ± 1.633[a]
			40	27.460 ± 1.103[a]	24.680 ± 1.612[a]
		500	**30**	24.000 ± 1.258[a]	24.710 ± 0.099[a]
			40	27.300 ± 0.480[a]	27.305 ± 1.100[a]
C18:2	Linoleic	**0.1**	**30**	36.255 ± 0.064[a]	31.465 ± 0.063[a]
			40	35.915 ± 0.191[a]	31.510 ± 0.085[a]
		500	**30**	35.610 ± 0.085[a]	30.610 ± 0.014[b]
			40	36.000 ± 0.297[a]	31.510 ± 0.085[a]
C18:3	Linolenic	**0.1**	**30**	1.960 ± 0.000[a]	0.390 ± 0.000[a]
			40	1.895 ± 0.078[a]	0.390 ± 0.000[a]
		500	**30**	1.880 ± 0.056[a]	0.395 ± 0.035[a]
			40	1.890 ± 0.085[a]	0.385 ± 0.007[a]
C20:0	Eicosanoic	**0.1**	**30**	0.120 ± 0.000[a]	0.750 ± 0.000[a]
			40	0.120 ± 0.000[a]	0.750 ± 0.000[a]
		500	**30**	0.120 ± 0.000[a]	0.730 ± 0.014[a]
			40	0.120 ± 0.000[a]	0.750 ± 0.000[a]
C20:3	Eicosatrienoic	**0.1**	**30**	0.060 ± 0.000[a]	0.175 ± 0.007[b]
			40	0.060 ± 0.000[a]	0.175 ± 0.007[b]
		500	**30**	0.060 ± 0.000[a]	0.230 ± 0.014[a]
			40	0.060 ± 0.000[a]	0.165 ± 0.007[b]
C20:4	Arachidonic	**0.1**	**30**	0.060 ± 0.000[a]	0.105 ± 0.007[a]
			40	0.060 ± 0.000[a]	-
		500	**30**	0.065 ± 0.007[a]	0.105 ± 0.007[a]
			40	0.070 ± 0.000[a]	-
C20:5	Eicosapentaenoic	**0.1**	**30**	0.065 ± 0.007[a]	-
			40	0.060 ± 0.000[a]	0.175 ± 0.007[a]
		500	**30**	0.070 ± 0.000[a]	-
			40	0.075 ± 0.007[a]	0.180 ± 0.000[a]
C24:0	Tetracosanoic	**0.1**	**30**	-	0.130 ± 0.000[a]
			40	-	0.135 ± 0.007[a]
		500	**30**	-	0.135 ± 0.007[a]
			40	-	0.130 ± 0.000[a]

Different small letters indicate significant differences between extraction conditions ($p<0.05$)

Figure 3.2 and Figure 3.3. represents the example of Gas Chromatogram of mealworm and cricket

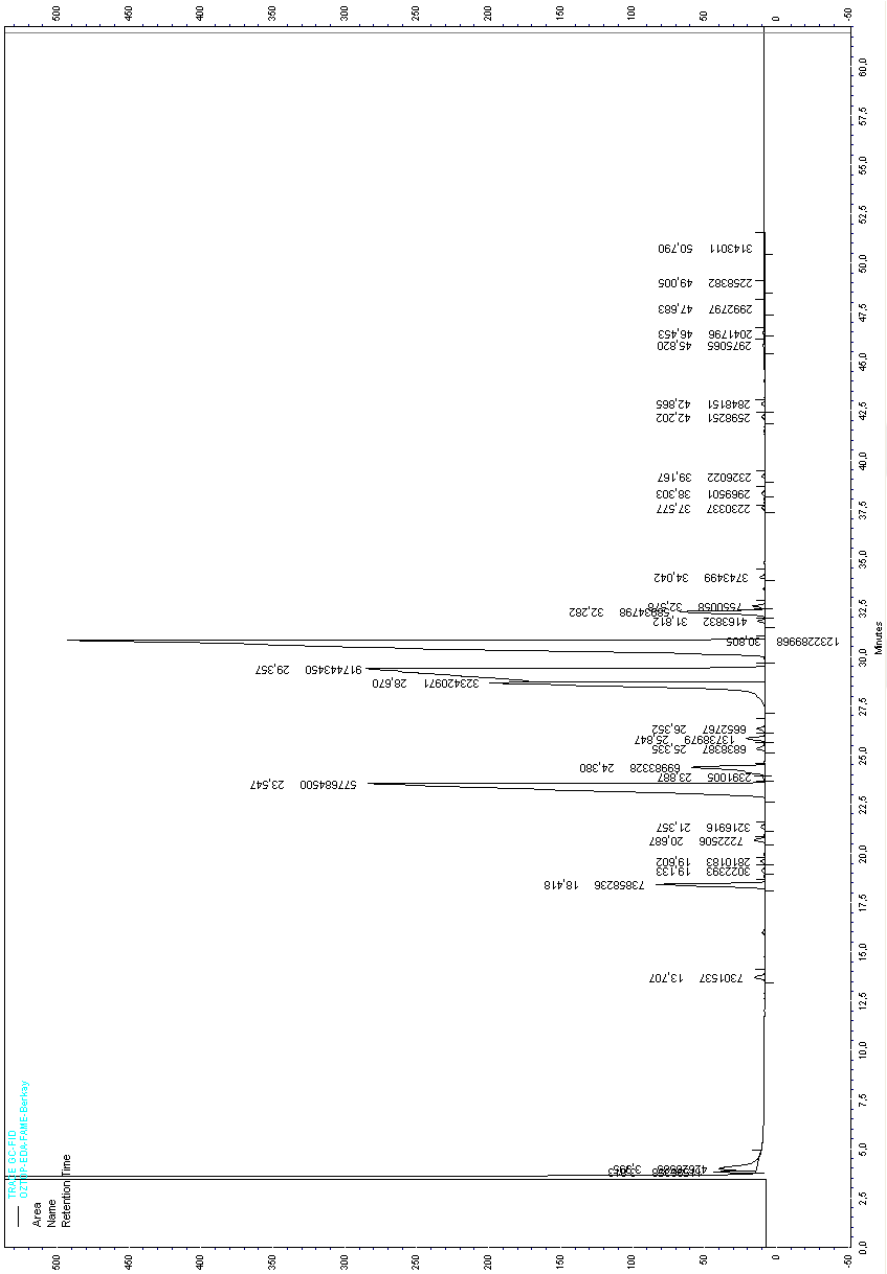

Figure 3.2. Gas chromatogram of cricket

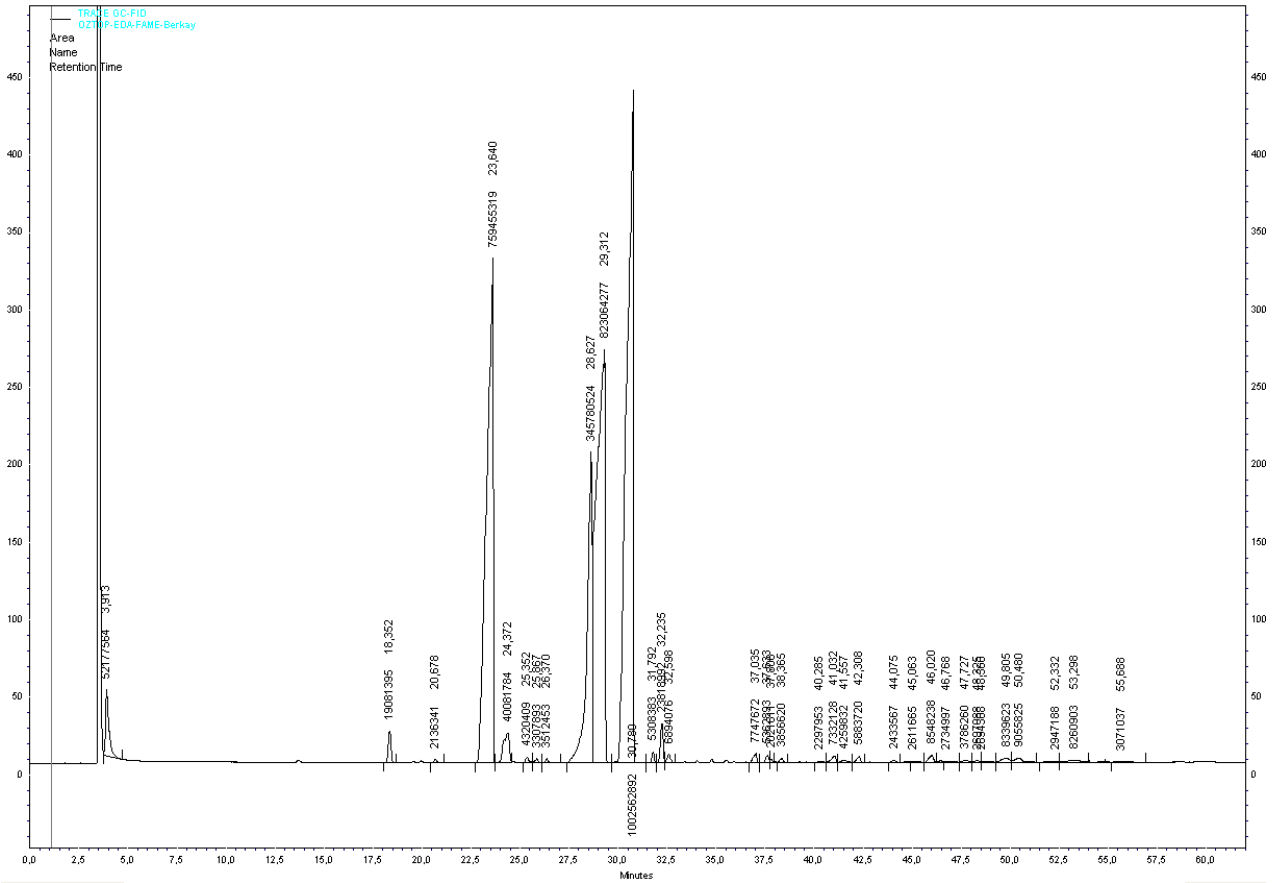

Figure 3.3. Gas chromatogram of mealworm

The composition of fatty acids that the insect species include were determined by Gas Chromatography for all parameters. It was detected that mealworm had lauric acid, tridecanoic acid and tetradecanoic acid which did not exist in cricket; besides, cricket had tetracosanoic acid which did not exist in mealworm.

The dominant fatty acids in both species were palmitic acid, stearic acid, oleic acid and linoleic acid; also, there were a considerable amount of myristic acid, palmitoleic acid and linolenic acid in both mealworm and cricket.

In mealworm, the combined effect of pressure and temperature was not significant for most of the fatty acids like lauric, myristic, tetradecanoic, pentadecanoic, palmitic, margaric, heptadecanoic, oleic, linoleic, linolenic, eicosanoic, eicosatrienoic, arachidonic and eicosapentaenoic acids; however, there was significant difference in tridecanoic, palmitoleic and stearic acids as combination of pressure and temperature. The amount of palmitic acid decreased with the application of pressure; however, the situation was different in tridecanoic acid, because it existed only at 30°C for both pressurized and non-pressurized samples. tridecanoic acid may be disrupted at 40°C or may be converted into a different fatty acid due to the increasing temperature.

In cricket, the combined effect of pressure and temperature was not significant for most of the fatty acids such as pentadecanoic, margaric, heptadecanoic, oleic, linolenic, eicosanoic and tetracosanoic acids. Moreover, the amount of stearic acid decreased significantly with HHP according to the statistical analyses ($p<0.05$). While the pressure at 30°C decreased the amount of linoleic acid significantly, it increased the amount of eicosatrienoic acid. Interestingly, arachidonic acid existed in both conventional and HHP assisted extraction at only 30°C, while eicosapentaenoic acid existed in both methods at only 40°C. It can be concluded that arachidonic acid might have been disrupted at 40°C or be converted into a different fatty acid due to the increasing temperature and also it was possible to say that eicosapentaenoic acid was formed from different fatty acid by changing

structure, its existence may be explained like this. For example, the arachidonic acid that existed in both conventional and HHP assisted extraction at only 30ºC can be converted into the eicosanoids which are the small lipids, so it is possible to say that the increasing temperature may cause a formation of eicosanoids by the conversion of arachidonic acid (Norris & Carr, 2013). There may be a conversion some fatty acids as in the case of arachidonic acid with the effect of temperature.

Table 3.3 represents the experimental results of total saturated, monounsaturated and polyunsaturated fatty acid composition of insect species

Table 3.3. *Experimental results of total saturated, monounsaturated and polyunsaturated fatty acid composition of insect species*

Insect Type	**Extraction Type**		**Mealworm (%)**	**Cricket (%)**
	Pressure (MPa)	**Temperature (ºC)**		
∑SFA	0.1	30	32.555	39.330
		40	29.670	38.245
	500	30	31.325	35.705
		40	29.740	36.055
∑MUFA	0.1	30	26.610	25.055
		40	29.965	26.030
	500	30	26.490	26.050
		40	29.725	28.665
∑PUFA	0.1	30	38.400	32.135
		40	37.990	32.250
	500	30	37.685	31.340
		40	38.095	32.240

Different small letters indicate significant differences between extraction conditions ($p<0.05$)

*SFA, MUFA & PUFA indicates that saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids, respectively

Table 3.4. represents the experimental results of total fatty acid composition of insect species

Table 3.4. *Experimental results of total fatty acid composition of insect species*

Insect Type	Extraction Type		Mealworm (%)	Cricket (%)
	Pressure (MPa)	Temperature (ºC)		
∑FA	0.1	30	97.565	96.520
		40	97.625	96.525
	500	30	95.500	93.095
		40	97.560	96.960
∑Others*	0.1	30	2.435	3.480
		40	2.375	3.475
	500	30	4.500	6.905
		40	2.440	3.040

Different small letters indicate significant differences between extraction conditions ($p<0.05$)

*Indicates that there is slight amount of fatty acids with unknown standards

In mealworm; the highest total amount of saturated fatty acids was obtained at the 30ºC for both conventional and HHP assisted extractions, the highest total amount of monounsaturated fatty acids was obtained at the 40ºC for both conventional and HHP assisted extractions and the highest total amount of polyunsaturated fatty acids was obtained at the 30ºC with conventional extraction. In cricket; the highest amount of saturated fatty acids was obtained at 30ºC with conventional extraction, the highest total amount of monounsaturated fatty acids was obtained at the 40ºC with HHP application; however, the amount of polyunsaturated fatty acids did not change significantly for all combinations of parameters ($p>0.05$).

All in all, it was found that the highest percentage of the unsaturated fatty acids is obtained at 40ºC for both conventional and HHP assisted extractions for mealworm, and the highest percentage of the unsaturated fatty acids is obtained at 40ºC with the application of HHP for cricket. The total amount of fatty acids

decreased significantly at 30ºC with the application of pressure for both insect species while the other combinations of parameters did not cause a significant difference.

Lewis (1967) investigated the marine animals in terms of their fatty acid composition at different depths under the water to determine the effect of pressure occured with the increased depth of water, and he was found that both the medium chain saturated fatty acids (C16:0 and C18:0) and long chain unsaturated fatty acids (C20 and C22) decreased with depth of the water except oleic acid; that is, the increasing pressure caused a decrease on the medium chain saturated and long chain unsaturated fatty acids of fish white muscle (Lewis, 1967). In this study, there was not a significant difference between pressurized and non-pressurized fatty acids with regard to fatty acid composition ($p>0.05$). The reason of this may be the processing time; in other words, the fishes were affected high pressure for a long time when compared to the 15 min pressure treatment.

The unsaturated fatty acids like linoleic acid that is liquid at the room temperature can change conformationally as reversible under the effect of extreme pressure conditions around 700 MPa; however, the change in the unsaturated fatty acids that are solid at room temperature are irreversible (Povedano et al., 2014). In this study, the pressure conditions were mild compared to the 700 MPa, so there was no significant difference between pressure-treated fatty acids and non-treated fatty acids ($p>0.05$). Besides, Kamimura et al. (1992) found that the amount of the unsaturated fatty acids of C16:1, C17:1 and C18:1 of the deep-sea bacterium did not change with pressure treatment in the range of 0.1 to 40 MPa (Kamimura et al., 1992). The phase transformations and conformational changes may occur in the extreme pressure conditions, so it could be indicated that the combination of high pressure and high temperature with longer processing time caused an increase the content of unsaturated fatty acids (Povedano et al., 2014).

3.3. Crystallization and melting points

Table 3.5. represents the experimental results of crystallization and melting point of insect oils

Table 3.5. *Experimental results of crystallization and melting point of insect oils*

Insect Type	Extraction Type		Crystallization Point (ºC)	Melting Point (ºC)
	Pressure (MPa)	Temperature (ºC)		
Mealworm	0.1	30	-15.37 ± 0.23[a]	-10.56 ± 0.12[a]
		40	-15.13 ± 0.11[ab]	-10.76 ± 0.06[a]
	500	30	-15.80 ± 0.11[bc]	-10.33 ± 0.33[a]
		40	-16.13 ± 0.12[c]	-10.69 ± 0.06[a]
Cricket	0.1	30	-2.25 ± 0.05[b]	1.11 ± 0.11[a]
		40	-2.43 ± 0.04[b]	0.03 ± 0.00[b]
	500	30	-0.96 ± 0.00[a]	0.20 ± 0.01[b]
		40	-1.21 ± 0.12[a]	-1.09 ± 0.18[c]

Different small letters indicate significant differences between extraction conditions ($p<0.05$)

A pure substance has the same freezing and melting points and they indicate the same transition of matter, which is transition of solid to liquid stage. However, the freezing point is lower than the melting point for some organic compounds, such as mixtures and oils. When a mixture freezes, the solid that is formed first has a composition which is generally different from the liquid, and the formation of the solid changes the composition of the remaining liquid, so that it usually reduces the freezing point steadily. (Britannica, 2016). The difference between melting and freezing points in insect oils can originate from different amounts of saturated and unsaturated fatty acids in their composition. In addtion to that information, the difference between crystallization point and melting point of mealworm is higher than the cricket. The reason of this can be explained by the ratio of saturated and unsaturated fatty acids that the insect species included. The amount of unsaturated fatty acids in mealworm is almost two fold of saturated fatty acids, whereas this

ratio is nearly one and a half in cricket. The energy required to crystallize unsaturated fatty acids is higher than the saturated fatty acids like olive oil and sunflower oil which are rich in unsaturated fatty acids (Povedano et al., 2014).

According to the statistical analyses, there is significant difference in crystallization point of mealworm oil under different extraction conditions ($p<0.05$). The application of HHP caused a decrease in the crystallization point of mealworm oil while there was no significant difference in the melting points for all extraction conditions ($p>0.05$). It can be explained with the amount of the saturated and unsaturated fatty acids that oil includes. The higher unsaturated fatty acids may cause lower crystallization temperature, so the lowest crystallization temperature was obtained at 40°C with pressure application with the existence of highest percentage of unsaturated fatty acids among others.

In cricket, there is significant difference for both crystallization and melting points when looking at the combined effect of pressure and temperature ($p<0.05$). The oil extracted with conventional extraction comes to the forefront in terms of lower crystallization point when compared to HHP assisted extracted oils. Besides, it was found that the highest melting point was achieved at 30°C without pressure and the lowest was obtained at 40°C with pressure.

Lipid systems are known as the most sensitive biological components under the effect of pressure (Rivalain et al., 2010). The reason of this is that the hydrophobic interactions between the lipids are quite sensitive to pressure and the lipids that are liquid at the room temperature form crystals under the effect of pressure by increasing the melting point of triglycerides (Cheftel & Culioli, 1997). Even though the melting point of mealworm oil decreased with pressure, for cricket oil increase on melting and crystallization was observed. The hydrophobic interactions between the lipid groups of cricket might have been more sensitive than the mealworm.

The example DSC curve of insect oils obtained by different extractions were shown in Figure 3.4 and 3.5.

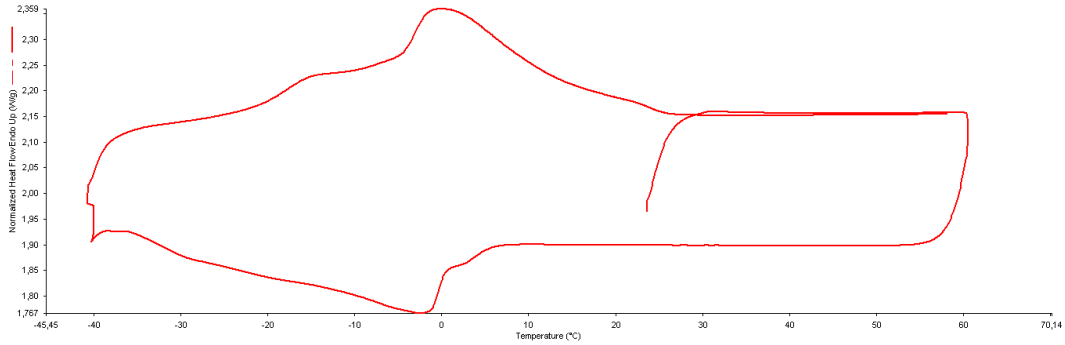

Figure 3.4. DSC curve of cricket

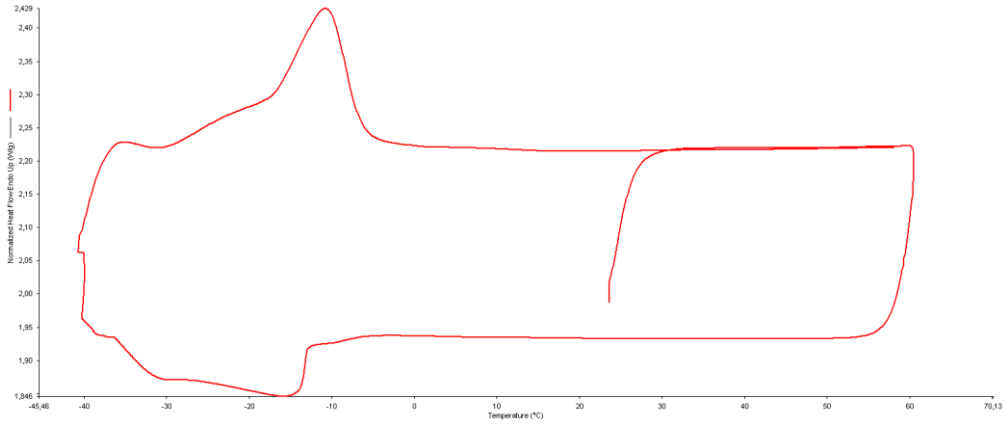

Figure 3.5. DSC curve of mealworm

3.4. Peroxide value

Peroxide value of insect oils obtained by different extractions were shown in Figure 3.6.

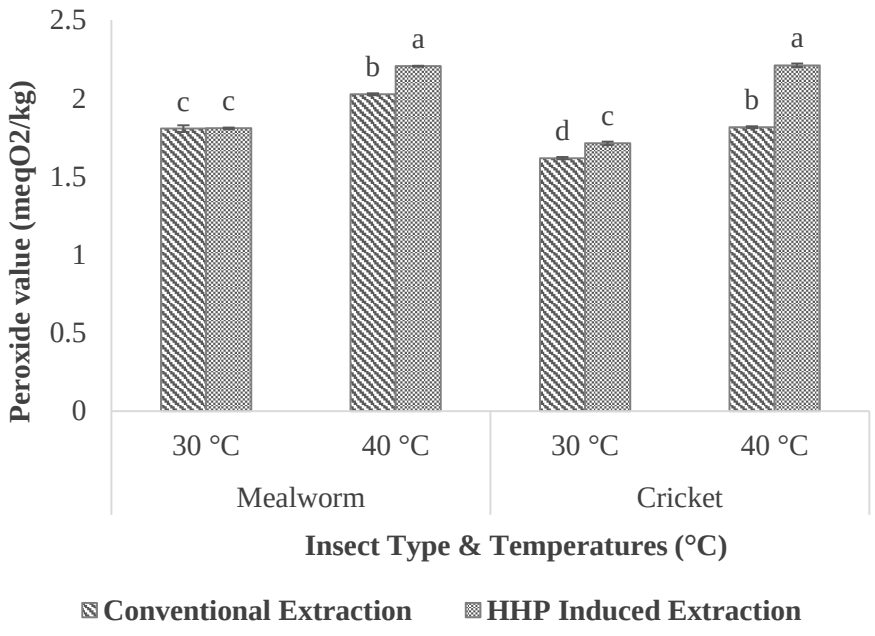

Figure 3.6. Peroxide value of insect oils. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

Peroxides, which are the compounds of primary oxidation, are formed in the oxidation of oils in early stages; besides, they have a part in the generation a variety of both volatile and nonvolatile secondary products by possible subjection to succeeding oxidation reactions (Dobarganes & Velasco, 2002)

Actually, it is possible to investigate the secondary oxidation with TBARS (Thiobarbituric acid reactive substances). According to the Codex General Standard for Fats and Oils (1999), the upper limit of peroxide value for fats and oils is 10 milliequivalents of active oxygen / kg oil. In this study, the peroxide values are in the range of 1,61 and 2,21 meqO_2/kg oil for both insect species; that is, they are far from the upper limit and safe to consume. Therefore, it is not

required to study on secondary oxidation of insect oils. However, the decomposition of the hydroperoxides (ROOH) releases the secondary products like alcohols, lactones, ketones, aldehydes, esters and hydrocarbons; in addition to this, the threshold value of those secondary products are very low in terms of taste and smell, so degradation happens quickly (Gardner, 1989; Matthäus, 2010). The key factor that stabilizes both primary and secondary oxidation is the existence of significant amount of antioxidants that the edible insects included. Antioxidants scavenge the free radicals such as lipid alkyl hydroxyl or lipid peroxyl radicals; also, they quench singlet oxygen. Besides of that antioxidants can donate hydrogen hydrogen atoms to free radicals; thus, free radicals converts into more stable nonradical products. All in all, the existence of antioxidants limits the oxidation in the fats and oils, so it is expected lower secondary oxidation values (Choe & Min, 2006).

According to the statistical analyses, the lowest oxidation amount of mealworm was obtained at the samples treated at 30°C for both conventional and HHP assisted extraction methods. The results showed that the increasing temperature caused an increase in the peroxide value; that is, the increasing temperature causes oxidation in the mealworm oil samples. Besides, it was possible to say that the pressure had negative effect for mealworm oil in terms of the peroxide value. It can be seen from the experimental results that the peroxide value was higher in pressure treated samples when compared to the conventionally extracted oils at same temperature.

In cricket, there was also significant difference for all different extraction conditions. The lowest oxidation value was obtained for the samples extracted conventionally at 30°C. As in the mealworm oil, the peroxide value was higher in pressure treated samples when compared to the conventionally extracted oils at same temperature; that is, pressure has negative effect on the oxidation status of cricket oils.

Lipid systems are known as the most sensitive biological components under the effect of pressure (Rivalain et al., 2010). Also, the products rich in high unsaturated fat fractions such as meat and fish show high sensitivity to the degradation of lipids (Medina-Meza et al., 2013). The effect of pressure under the 300 MPa has a slight effect on lipid oxidation, but the oxidation increases above 300 MPa in pork fat (Medina-Meza et al., 2013). Besides, it was found that the hydrostatic pressure at 800 MPa for 20 min caused an increase in the peroxide values of rendered pork fat (Cheah & Ledward, 1995). In this case, both the mealworm and cricket contains high amount of unsaturated fatty acids, the pressure of 500 MPa caused an increase in oxidation; in other words, causes an increase in the peroxide values.

3.5. Total phenolic content

Total phenolic content of insect oils obtained by different extractions were shown in Figure 3.7.

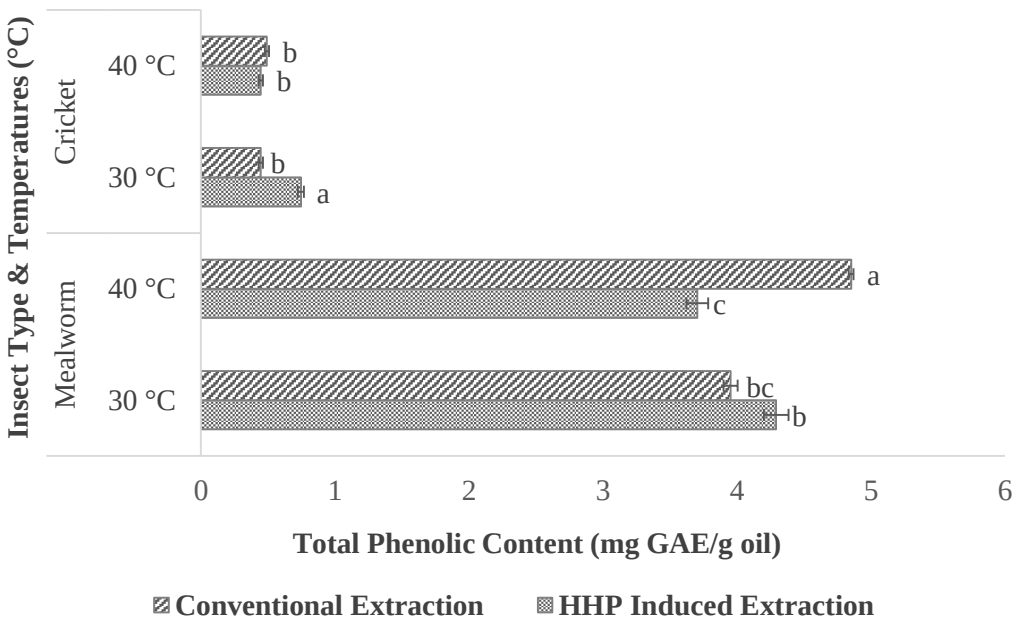

Figure 3.7. Total phenolic content of insect oils. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

According to the statistical analyses, it was found that there was significant difference in mealworm oil in terms of total phenolic content. Temperature was not significant ($p>0.05$); however, the pressure was ($p<0.05$). The combined effect of pressure and temperature gave clue about the sensitivity of phenolic compounds that mealworm oil included. In 30ºC, the total phenolic content increased significantly with the pressure; however, it decreased at 40ºC. It may be concluded that pressure had negative effect on phenolic content when the temperature also increased.

In cricket, there was also significant difference in pressure and temperature separately ($p<0.05$). The individual increase in temperature caused a decrease in total phenolic content while the pressure caused an increase. Moreover, there was significant difference in combined effect of pressure and temperature except for HHP assisted cricket oil extraction at 30ºC. There was significant increase in phenolic content at 30ºC with the pressure application. The phenolic compounds that the cricket oil included were resistant to 40ºC and became more active at this temperature when compared to 30ºC.

Folin-Ciocalteu reagent is a useful tool that determines the total phenolic content of the samples, but the main target of this reagent is hydrophilic groups; that is, it is not good at measuring the lipophilic antioxidants because of the high susceptibility of Folin-Ciocalteu chromophore against water (Berker et al., 2013). In this case, the total phenolic content of mealworm oil was higher than the cricket oil; thus, it is possible to say the amount of the hydrophilic phenolic compounds in the mealworm oil is higher and cricket may be rich in lipophilic phenolic compounds.

In general, the phenolic compounds may not affected with the heat and pressure treatment (Patras et al., 2009). The temperature did not cause a significant difference in the mealworm oil ($p>0.05$), while it caused a significant difference in cricket oil ($p<0.05$). The mealworm oil fit to the situation that Patras et al.

(2009) explained. However, cricket had a reverse situation. The phenolic compounds that cricket include might have oxidized easily with increase in temperature (Reblova, 2012). It could be said that the phenolic compounds that mealworm oil included are more heat resistant compared to the cricket oil.

The treatment of pressure might have resulted in cellular walls' disruption and hydrophobic bonds in the membrane; thus, the distribution and aggregation of phenolic compounds might have changed and the interaction between solvent and phenolic compounds increased (Prasad et al., 2009). Besides, this increase might be related with the increased extractability of some antioxidant compounds (Cao et al., 2011). Actually, it was expected an increase in the total phenolic content; however, the pressure caused a decrease, because, the hydrophilic groups in mealworm was thought to be higher in concentration and the Folin-Cicocalteu was good at determining the hydrophilic phenolic compounds. This meant that the hydrophilic groups contacted with solvent under the effect of pressure and were removed from the hexane-oil complex. That was thought to be the reason of why pressure had a negative effect on the total phenolic content in mealworm oil.

3.6. Antioxidant activity

3.6.1. Antioxidant activity with DPPH assay

Antioxidant activity with DPPH assay of insect oils obtained by different extractions were shown in Figure 3.8.

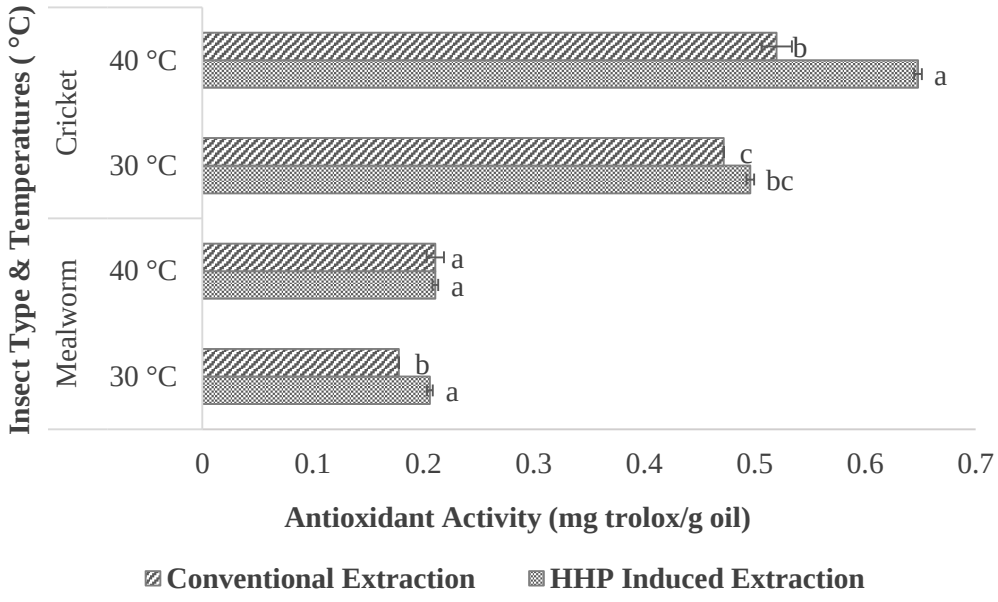

Figure 3.8. Antioxidant activity of insect oils with DPPH. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

The statistical analyses showed that pressure and temperature caused significant difference on the antioxidant activity in mealworm oil separately ($p<0.05$). However, the combined effect of pressure and temperature did not cause significant difference in different extraction conditions except conventionally extracted mealworm oil at 30ºC. The difference between conventionally extracted and HHP assisted extracted mealworm oil at 30ºC was that the antioxidants might have stayed in the protein rich side during the extraction stage without pressure, and the compounds showing antioxidant property might have been extracted with oil portion thanks to the high pressure application.

In cricket, there was also significant difference in terms of pressure and temperature both separately and combinely ($p<0.05$). The statistical analyses showed that the amount of antioxidants increased with pressure application at same temperature. As in the mealworm oil case, the antioxidants might have stayed in the protein rich side during the extraction stage without application of pressure, and the antioxidant might have be embedded in oil portion due to the pressure during extraction.

The pressure might have released the antioxidant compounds into the extracellular environment by disrupting the cell walls due to the change in the matrix of the tissue (Briones-Labarca et al., 2011). This coulde be the reason of the increase in the antioxidant activity with pressure treatment. Besides, the different antioxidants show different antioxidant activity and the effect of pressure on them may be differ from one to another (Briones-Labarca et al., 2011).

Moreover, the effect of temperature on the antioxidant activity of both species wassignificant ($p<0.05$). The increase could have occured because of the equilibrium principle; that was, the increased temperature could cause increase in the rate of extraction and that provided higher recovery of antioxidant compounds (Garcia-Marquez et al., 2012). In this case, the range of the temperature was mild as 30 and 40°C. Higher temperature under the extraction might have caused degradation to the heat sensitive antioxidant compounds (Liyana-Pathirana & Shahidi, 2005). It could be concluded that that the heat stability of the antioxidant compounds of both mealworm and cricket oil were not that low.

3.6.2. Antioxidant activity with CUPRAC assay

Antioxidant activity with CUPRAC assay of insect oils obtained by different extractions were shown in Figure 3.9.

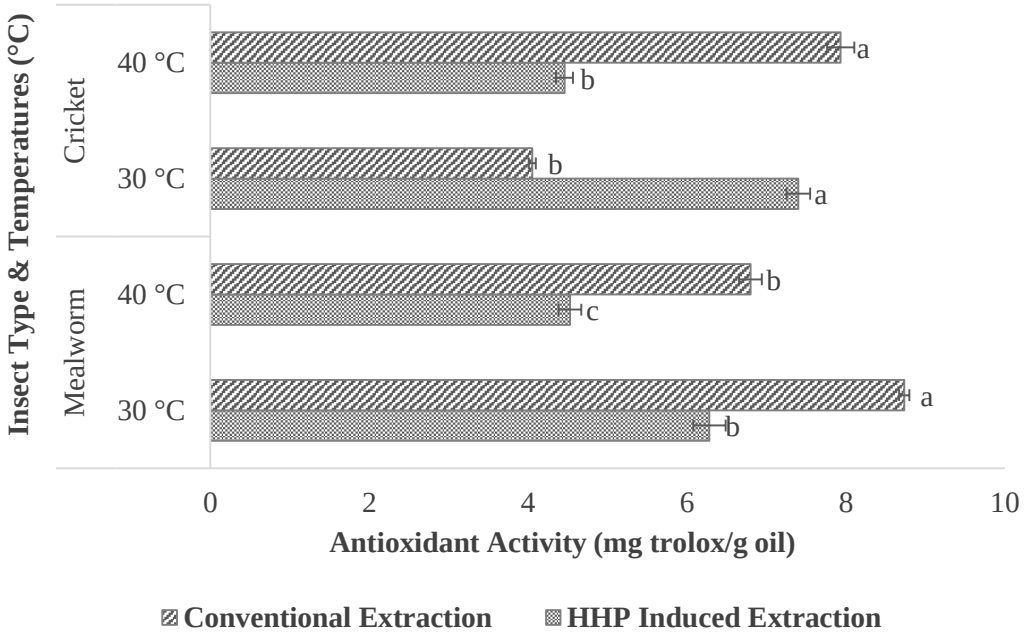

Figure 3.9. Antioxidant activity of insect oils with CUPRAC. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

In mealworm oil, the increasing temperature and increasing pressure created a significant difference in the antioxidant activity ($p<0.05$). The amount of antioxidants, could have been negatively affected with increasing pressure and temperature in the extraction step or the antioxidants might have been entrapped in the protein rich side at the end of extraction with respect to the applied parameters. They might be embedded in the insect powders due to the pressure application or increased temperature or may be degraded the antioxidant compounds.

In cricket oil, the situation in terms of antioxidants was different than the mealworm oil. The increased temperature caused an increase in the antioxidant

activity in conventional extraction, while the increase temperature caused a decrease in the antioxidant activity with the existence of high pressure. At 30 ^{o}C, the pressure application increased the antioxidant activity, but the antioxidant activity decreased at 40°C with pressure. That significant difference might have been explained due to pressure forcing the antioxidants to embed in insect powder side instead of oil at slightly higher temperature values, while the lower temperatures provided high yield antioxidants extraction in addition to oil extraction.

The increased temperature may be the main reason of the natural antioxidants' depletion (Abdul Rahim et al., 2010). The significant difference in the temperature values of both mealworm and cricket oil might be explained from this deterioration ($p<0.05$). In this case, the natural antioxidants that the species included are sensitive to the temperature of 40 ^{o}C. Besides, the thermal treatment may decrease the content of micronutrients in insect species; it may also increase the bioavailability of some bioactive compounds (Abdul Rahim et al., 2010).

With the application of pressure, an increase in the antioxidant activity is expected; however, the being hydrophilic of the antioxidants that the mealworm oil included could have caused a decrease in the antioxidant activity. As in the total phenolic content, the hydrophilic groups contact with solvent under the effect of pressure and were removed from the hexane-oil complex. This was the reason of why pressure had negative effect on the antioxidant activity in both mealworm and cricket oil. According to the Briones-Labarca et al. (2011), the pressure may release the antioxidant compounds into the extracellular environment by disrupting the cell walls due to the change in the matrix of the tissue; however, the hydrophilic property of released antioxidants kept them out of the non-polar solvent in this case.

CHAPTER 4

CONCLUSION AND RECOMMENDATION

In the scope of this study, mealworm and cricket oil were extracted with conventional and HHP assisted extraction to investigate how HHP and temperature affected the physicochemical characteristics of the extracted oil. In the first part of the study, oils were extracted from yellow mealworm and house cricket with hexane at a proportion of 1:15 (w/v) with or without high hydrostatic pressure at 500 MPa at 30°C and 40°C for 15 minutes.

Results showed that HHP affected the amount of extracted oil in both insect species. The highest amount of oil was obtained with conventional extraction at 30°C or 40°C for each species, similarly. According to the results, fatty composition of insects were significantly affected from both HHP treatment and variation in temperature. However, desired extraction condition for different fatty acids changed with insect type and application parameters. Although amounts of myristic acid, palmitoleic acid and linolenic acid in mealworm and cricket oils were relatively high, the most abundant fatty acids found in both insects were palmitic acid, stearic acid, oleic acid and linoleic acid.

In mealworm, fatty acids like lauric, myristic, tetradecanoic, pentadecanoic, palmitic, margaric, heptadecanoic, oleic, linoleic, linolenic, eicosanoic, eicosatrienoic, arachidonic and eicosapentaenoic acids were not significantly influenced by the combined pressure and temperature effect ($p>0.05$). But, the

combined effect of pressure and temperature caused a difference in tridecanoic, palmitoleic and stearic acids. Statistical results showed that, application of HHP increase the amount of arachidonic and eicosapentaenoic acids whereas, significant decrease was obtained in palmitic acid. On the other hand, amount of oleic acid significantly increased by increase in temperature from 30ºC to 40ºC, margaric acid, heptadenoic acid and strearic acid were negatively affected from temperature rise. Also, tridecanoic acid was only detected only at 30ºC in both pressurized and non-pressurized samples.

In cricket, significant decrease in the amount of stearic acid was obtained with the application of HHP. The pressure at 30ºC significantly reduced the amount of linoleic acid and increased the amount of eicosatrienoic acid. However, arachidonic acid was detected only at 30ºC in both conventional and HHP-induced extraction, whereas eicosapentaenoic acid is only found at 40ºC in both extraction. Additionally, HHP caused a decrease in the crystallization point of mealworm oil, while it did not show a significant difference in melting points in case of all extraction conditions. In cricket, highest melting point was achieved at 30ºC without application of HHP. Besides, HHP and increase in temperature caused an increase in the peroxide value.

The results of the study indicated that mealworm and cricket oil are potential sources of essential fatty acids and antioxidants. Also, oil extraction with high hydrostatic pressure can be an alternative method to conventional extractions when the optimum parameters are chosen. However, for industrial applications, it may be required to do a feasibility study between two extraction methods. Additional and extensive research are required to explore the properties of insect oils with different parameters.

The most abundant fatty acids found in both insects were palmitic acid, stearic acid, oleic acid and linoleic acid. The functional properties and antioxidant

activities of mealworm and cricket powders were significantly affected from both HHP treatment and variation in temperature. The optimum extraction conditions varied depending on insect type and functional properties. Besides, it was found that HHP and increase in temperature caused an increase in the peroxide value.

All in all, the experiments in this study touched in an important matter in terms of the insect oils. Edible insects provide high nutritional value in terms of their oil portion. Whether they seem as tiny, their rapid reproduction makes them a strong rival aginst the conventional oils with the treasure that they included such as unsaturated fatty acids, especially Ω-3 and Ω-6 fatty acids, antioxidants and phenolic compounds. Besides, the extraordinary oxidative stability increase the significance of insect oils among the conventional oils. In the light of the aforementioned points, whether they seem as tiny, undoubtedly that the insect oils will be the huge part of the food industry in near future.

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APPENDICES

A. CALIBRATION CURVES

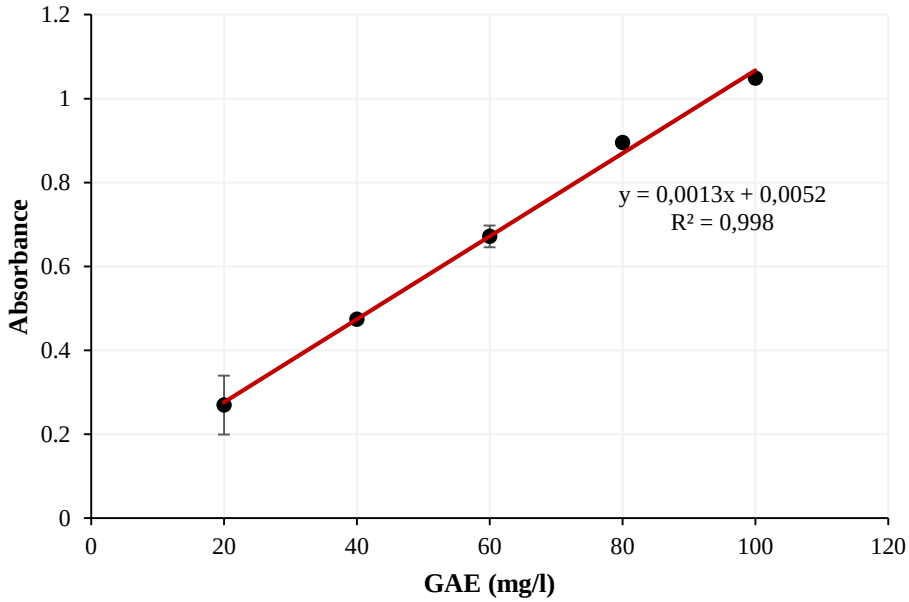

Figure A.1. Calibration curve for Gallic Acid Equivalent

Abs (at 760 nm) = 0.0013 * (mg trolox/l) + 0.0052 where R^2 = 0.998

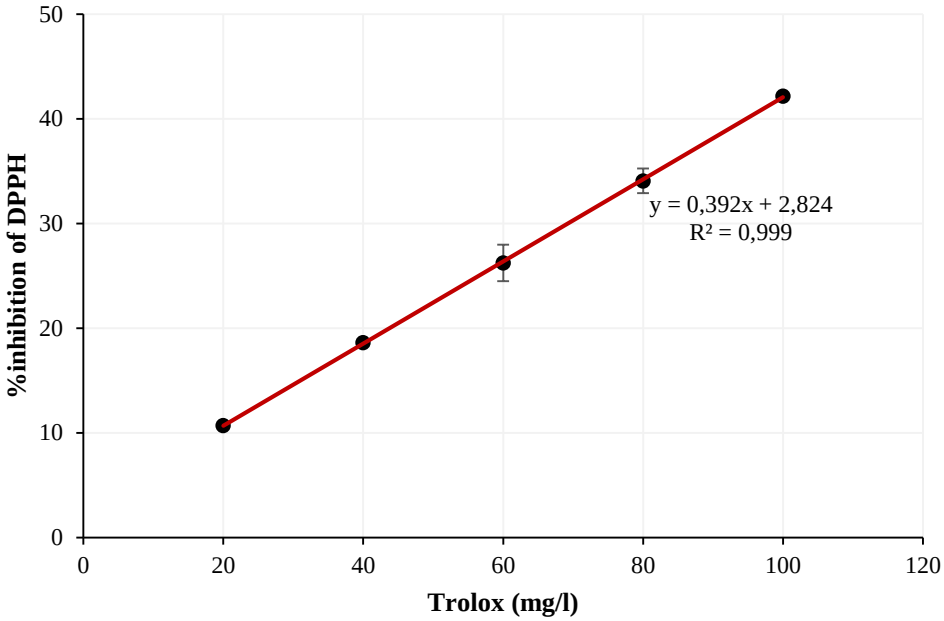

Figure A.2. Calibration curve for DPPH method

% inhibition of DPPH is calculated with the equation below to find the antioxidant activity at 517 nm;

$$\% \text{ inhibition of DPPH} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} * 100$$

% inhibition = 0.8478 * (mg trolox/l) + 5.7609 where R^2 = 0.9991

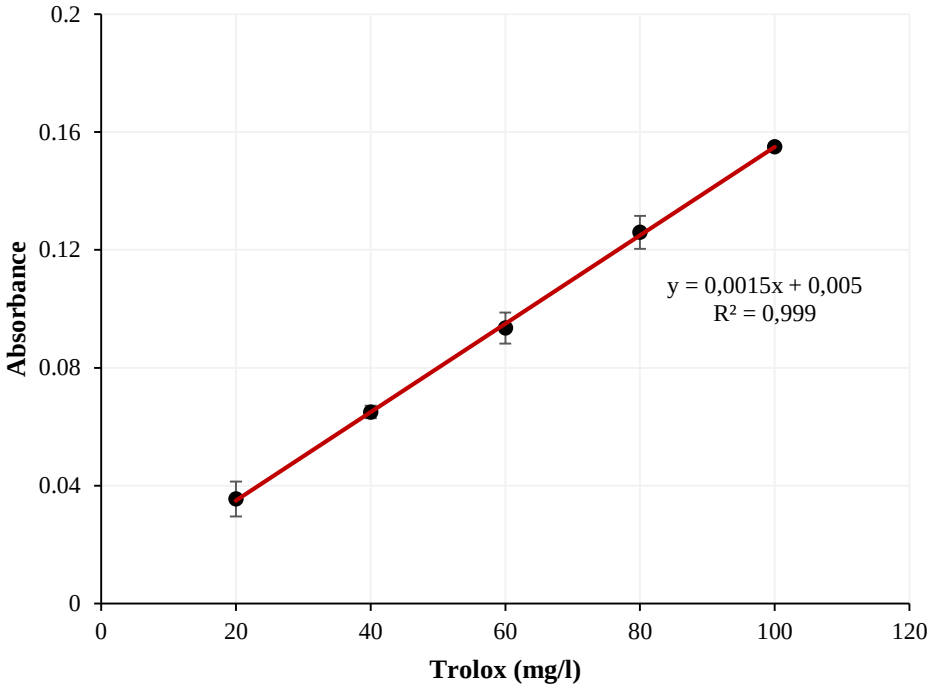

Figure A.3. Calibration curve for CUPRAC method

Abs (at 450 nm) = 0.0015 * (mg trolox/l) + 0.005 where R^2 = 0.9996

B. COMPARATIVE TABLES

Table B. 1. *Experimental results of oil content, peroxide value, crystallization and melting point*

Insect Type	Extraction Type		Oil Content (%)	Peroxide Value ($meqO_2/kg$)	Crystallization Point (ºC)	Melting Point (ºC)
	Pressure (MPa)	Temperature (ºC)				
Mealworm	0.1	30	24.220 ± 0.115^{a}	1.806 ± 0.022^{c}	-15.375 ± 0.233^{a}	-10.560 ± 0.127^{a}
		40	24.066 ± 0.078^{a}	2.026 ± 0.006^{b}	-15.130 ± 0.113^{ab}	-10.765 ± 0.064^{a}
	500	30	22.754 ± 0.012^{b}	1.808 ± 0.006^{c}	-15.800 ± 0.113^{bc}	-10.335 ± 0.332^{a}
		40	22.902 ± 0.333^{b}	2.205 ± 0.003^{a}	-16.135 ± 0.120^{c}	-10.695 ± 0.064^{a}
Cricket	0.1	30	18.054 ± 0.173^{a}	1.617 ± 0.007^{d}	-2.255 ± 0.049^{b}	1.110 ± 0.113^{a}
		40	18.090 ± 0.024^{a}	1.815 ± 0.006^{b}	-2.430 ± 0.042^{b}	0.030 ± 0.000^{b}
	500	30	16.168 ± 0.119^{c}	1.712 ± 0.011^{c}	-0.960 ± 0.000^{a}	0.205 ± 0.007^{b}
		40	17.409 ± 0.055^{b}	2.211 ± 0.012^{a}	-1.215 ± 0.120^{a}	-1.095 ± 0.176^{c}

Different small letters indicate significant differences between extraction conditions ($p<0.05$)

Table B. 2. *Experimental results of total phenolic content and antioxidant activity*

Insect Type	Extraction Type		Total Phenolic Content (Folin-Ciocalteu) (mg GAE/g oil)	Antioxidant Activity (DPPH) (mg trolox/g oil)	Antioxidant Activity (CUPRAC) (mg trolox/g oil)
	Pressure (MPa)	Temperature (ºC)			
Mealworm	0.1	30	3.952 ± 0.052^{bc}	0.178 ± 0.000^{b}	8.734 ± 0.064^{a}
		40	4.852 ± 0.017^{a}	0.211 ± 0.008^{a}	6.799 ± 0.144^{b}
	500	30	4.292 ± 0.092^{b}	0.206 ± 0.003^{a}	6.282 ± 0.203^{b}
		40	3.704 ± 0.081^{c}	0.211 ± 0.003^{a}	4.527 ± 0.144^{c}
Cricket	0.1	30	0.446 ± 0.015^{b}	0.472 ± 0.000^{c}	4.053 ± 0.043^{b}
		40	0.492 ± 0.015^{b}	0.520 ± 0.014^{b}	7.934 ± 0.171^{a}
	500	30	0.746 ± 0.023^{a}	0.496 ± 0.003^{bc}	7.401 ± 0.149^{a}
		40	0.446 ± 0.015^{b}	0.647 ± 0.003^{a}	4.458 ± 0.107^{b}

Different small letters indicate significant differences between extraction conditions ($p<0.05$)

C. STATISTICAL ANALYSES

Table C. 1. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of oil content of mealworm oil*

General Linear Model: Mealworm Oil Content versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Mealworm Oil Content (%), using Adjusted SS for
Tests

Source                DF  Seq SS  Adj SS  Adj MS      F      P
Pressure               1  5,1827  5,1827  5,1827  53,13  0,000
Temperature            1  0,0000  0,0000  0,0000   0,00  0,987
Pressure*Temperature   1  0,0681  0,0681  0,0681   0,70  0,428
Error                  8  0,7804  0,7804  0,0976
Total                 11  6,0313


S = 0,312335   R-Sq = 87,06%   R-Sq(adj) = 82,21%


Unusual Observations for Mealworm Oil Content (%)

     Mealworm Oil
Obs   Content (%)      Fit  SE Fit  Residual  St Resid
 12       22,2515  22,9022  0,1803   -0,6507     -2,55 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         6  24,1  A
1         6  22,8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           6  23,5  A
40           6  23,5  A

Means that do not share a letter are significantly different.
```

```
Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         30           3  24,2  A
0         40           3  24,1  A
1         40           3  22,9    B
1         30           3  22,8    B

Means that do not share a letter are significantly different.
```

Table C. 2. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of oil content of cricket oil*

General Linear Model: Cricket Oil Content versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Cricket Oil Content (%), using Adjusted SS for
Tests

Source               DF  Seq SS  Adj SS  Adj MS       F      P
Pressure              1  4,9416  4,9416  4,9416  138,15  0,000
Temperature           1  1,2239  1,2239  1,2239   34,22  0,000
Pressure*Temperature  1  1,0901  1,0901  1,0901   30,47  0,001
Error                 8  0,2862  0,2862  0,0358
Total                11  7,5417


S = 0,189129   R-Sq = 96,21%   R-Sq(adj) = 94,78%


Unusual Observations for Cricket Oil Content (%)

     Cricket Oil
Obs  Content (%)      Fit  SE Fit  Residual  St Resid
  1      17,7126  18,0539  0,1092   -0,3413     -2,21 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         6  18,1  A
1         6  16,8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           6  17,7  A
30           6  17,1    B
```

```
Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
Pressure  Temperature  N  Mean  Grouping
0         40           3  18,1  A
0         30           3  18,1  A
1         40           3  17,4    B
1         30           3  16,2      C
Means that do not share a letter are significantly different.
```

Table C. 3. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Lauric Acid of mealworm oil*

General Linear Model: Lauric C12:0 MW Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Lauric C12:0 MW Oil, using Adjusted SS for Tests

Source               DF     Seq SS     Adj SS     Adj MS     F      P
Pressure              1  0,0000125  0,0000125  0,0000125  1,00  0,374
Temperature           1  0,0000125  0,0000125  0,0000125  1,00  0,374
Pressure*Temperature  1  0,0000125  0,0000125  0,0000125  1,00  0,374
Error                 4  0,0000500  0,0000500  0,0000125
Total                 7  0,0000875


S = 0,00353553   R-Sq = 42,86%   R-Sq(adj) = 0,00%


Unusual Observations for Lauric C12:0 MW Oil

     Lauric C12:0
Obs        MW Oil       Fit    SE Fit   Residual  St Resid
  1      0,210000  0,215000  0,002500  -0,005000     -2,00 R
  2      0,220000  0,215000  0,002500   0,005000      2,00 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   0,2  A
1         4   0,2  A

Means that do not share a letter are significantly different.
```

```
Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   0,2  A
40           4   0,2  A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         30           2   0,2  A
1         40           2   0,2  A
1         30           2   0,2  A
0         40           2   0,2  A
Means that do not share a letter are significantly different.
```

Table C. 4. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Tridecanoic Acid of mealworm oil*

General Linear Model: Tridecanoic C13:0 MW Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Tridecanoic C13:0 MW Oil, using Adjusted SS for
Tests

Source                DF     Seq SS     Adj SS     Adj MS        F      P
Pressure               1  0,0000000  0,0000000  0,0000000     0,00  1,000
Temperature            1  0,0050000  0,0050000  0,0050000  5000,00  0,000
Pressure*Temperature   1  0,0000000  0,0000000  0,0000000     0,00  1,000
Error                  4  0,0000040  0,0000040  0,0000010
Total                  7  0,0050040


S = 0,001   R-Sq = 99,92%   R-Sq(adj) = 99,86%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   0,0  A
0         4   0,0  A
Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   0,0  A
40           4   0,0    B

Means that do not share a letter are significantly different.
```

```
Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         30           2   0,0  A
0         30           2   0,0  A
0         40           2   0,0    B
1         40           2   0,0    B
Means that do not share a letter are significantly different.
```

Table C. 5. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Myristic Acid of mealworm oil*

General Linear Model: Myristic C14:0 MW Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Myristic C14:0 MW Oil, using Adjusted SS for
Tests

Source                DF    Seq SS    Adj SS    Adj MS     F      P
Pressure               1  0,000613  0,000613  0,000613  0,37  0,577
Temperature            1  0,000012  0,000012  0,000012  0,01  0,935
Pressure*Temperature   1  0,000612  0,000612  0,000612  0,37  0,577
Error                  4  0,006650  0,006650  0,001662
Total                  7  0,007887


S = 0,0407738   R-Sq = 15,69%   R-Sq(adj) = 0,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   2,2  A
1         4   2,2  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   2,2  A
30           4   2,2  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         30           2   2,2  A
1         40           2   2,2  A
0         40           2   2,2  A
1         30           2   2,2  A
Means that do not share a letter are significantly different.
```

Table C. 6. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Tetradecanoic Acid of mealworm oil*

General Linear Model: Tetradecanoic C14:1 MW Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Tetradecanoic C14:1 MW Oil, using Adjusted SS
for
     Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0000125  0,0000125  0,0000125  1,00  0,374
Temperature            1  0,0000125  0,0000125  0,0000125  1,00  0,374
Pressure*Temperature   1  0,0000125  0,0000125  0,0000125  1,00  0,374
Error                  4  0,0000500  0,0000500  0,0000125
Total                  7  0,0000875


S = 0,00353553   R-Sq = 42,86%   R-Sq(adj) = 0,00%


Unusual Observations for Tetradecanoic C14:1 MW Oil

     Tetradecanoic
Obs   C14:1 MW Oil       Fit    SE Fit   Residual  St Resid
  1       0,170000  0,175000  0,002500  -0,005000     -2,00 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   0,2  A
1         4   0,2  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   0,2  A
40           4   0,2  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         30           2   0,2  A
1         40           2   0,2  A
1         30           2   0,2  A
0         40           2   0,2  A
Means that do not share a letter are significantly different.
```

Table C. 7. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Pentadecanoic Acid of mealworm oil*

General Linear Model: Pentadecanoic C15:0 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Pentadecanoic C15:0 MW Oil, using Adjusted SS for
Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0001125	0,0001125	0,0001125	3,00	0,158
Temperature	1	0,0001125	0,0001125	0,0001125	3,00	0,158
Pressure*Temperature	1	0,0000125	0,0000125	0,0000125	0,33	0,595
Error	4	0,0001500	0,0001500	0,0000375		
Total	7	0,0003875				

S = 0,00612372 R-Sq = 61,29% R-Sq(adj) = 32,26%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	0,2	A
1	4	0,2	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
30	4	0,2	A
40	4	0,2	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0	30	2	0,2	A
1	30	2	0,2	A
0	40	2	0,2	A
1	40	2	0,2	A

Means that do not share a letter are significantly different.

Table C. 8. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Palmitic Acid of mealworm oil*

General Linear Model: Palmitic C16:0 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Palmitic C16:0 MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,06480	0,06480	0,06480	1,62	0,272
Temperature	1	0,00405	0,00405	0,00405	0,10	0,766
Pressure*Temperature	1	0,00605	0,00605	0,00605	0,15	0,717
Error	4	0,15990	0,15990	0,03997		
Total	7	0,23480				

S = 0,199937 R-Sq = 31,90% R-Sq(adj) = 0,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	17,1	A
1	4	16,9	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	17,0	A
30	4	17,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0	30	2	17,1	A
0	40	2	17,1	A
1	40	2	17,0	A
1	30	2	16,9	A

Means that do not share a letter are significantly different.

Table C. 9. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Palmitoleic Acid of mealworm oil*

General Linear Model: Palmitoleic C16:1 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Palmitoleic C16:1 MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0078125	0,0078125	0,0078125	12,76	0,023
Temperature	1	0,0036125	0,0036125	0,0036125	5,90	0,072
Pressure*Temperature	1	0,0006125	0,0006125	0,0006125	1,00	0,374
Error	4	0,0024500	0,0024500	0,0006125		
Total	7	0,0144875				

S = 0,0247487 R-Sq = 83,09% R-Sq(adj) = 70,41%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	2,2	A
1	4	2,1	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
30	4	2,1	A
40	4	2,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0	30	2	2,2	A
0	40	2	2,1	A B
1	30	2	2,1	A B
1	40	2	2,1	B

Means that do not share a letter are significantly different.

Table C. 10. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Margaric Acid of mealworm oil*

General Linear Model: Margaric C17:0 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Margaric C17:0 MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0000500	0,0000500	0,0000500	2,00	0,230
Temperature	1	0,0002000	0,0002000	0,0002000	8,00	0,047
Pressure*Temperature	1	0,0000500	0,0000500	0,0000500	2,00	0,230
Error	4	0,0001000	0,0001000	0,0000250		
Total	7	0,0004000				

S = 0,005 R-Sq = 75,00% R-Sq(adj) = 56,25%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	0,2	A
1	4	0,2	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping	
30	4	0,2	A	
40	4	0,2		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0	30	2	0,2	A
1	30	2	0,2	A
1	40	2	0,2	A
0	40	2	0,2	A

Means that do not share a letter are significantly different.

Table C. 11. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Heptadecanoic Acid of mealworm oil*

General Linear Model: Heptadecanoic C17:1 MW Oil versus Pressure;Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Heptadecanoic C17:1 MW Oil, using Adjusted SS
for
     Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0000500  0,0000500  0,0000500  2,00  0,230
Temperature            1  0,0002000  0,0002000  0,0002000  8,00  0,047
Pressure*Temperature   1  0,0000500  0,0000500  0,0000500  2,00  0,230
Error                  4  0,0001000  0,0001000  0,0000250
Total                  7  0,0004000


S = 0,005   R-Sq = 75,00%   R-Sq(adj) = 56,25%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   0,2  A
1         4   0,2  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   0,2  A
40           4   0,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         30           2   0,2  A
1         30           2   0,2  A
1         40           2   0,2  A
0         40           2   0,2  A

Means that do not share a letter are significantly different.
```

Table C. 12. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Stearic Acid of mealworm oil*

General Linear Model: Stearic C18:0 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Stearic C18:0 MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,2701	0,2701	0,2701	0,95	0,384
Temperature	1	9,9235	9,9235	9,9235	35,00	0,004
Pressure*Temperature	1	0,6441	0,6441	0,6441	2,27	0,206
Error	4	1,1341	1,1341	0,2835		
Total	7	11,9719				

S = 0,532482 R-Sq = 90,53% R-Sq(adj) = 83,42%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	10,6	A
1	4	10,3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
30	4	11,6	A
40	4	9,3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0	30	2	12,0	A
1	30	2	11,1	A B
1	40	2	9,4	B
0	40	2	9,2	B

Means that do not share a letter are significantly different.

Table C. 13. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Oleic Acid of mealworm oil*

General Linear Model: Oleic C18:1 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Oleic C18:1 MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0242	0,0242	0,0242	0,02	0,883
Temperature	1	22,4450	22,4450	22,4450	22,84	0,009
Pressure*Temperature	1	0,0050	0,0050	0,0050	0,01	0,947
Error	4	3,9300	3,9300	0,9825		
Total	7	26,4042				

S = 0,991211 R-Sq = 85,12% R-Sq(adj) = 73,95%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	25,8	A
1	4	25,6	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping	
40	4	27,4	A	
30	4	24,0		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0	40	2	27,5	A
1	40	2	27,3	A
0	30	2	24,1	A
1	30	2	24,0	A

Means that do not share a letter are significantly different.

Table C. 14. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Linoleic Acid of mealworm oil*

General Linear Model: Linoleic C18:2 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Linoleic C18:2 MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,15680	0,15680	0,15680	4,62	0,098
Temperature	1	0,00125	0,00125	0,00125	0,04	0,857
Pressure*Temperature	1	0,26645	0,26645	0,26645	7,84	0,049
Error	4	0,13590	0,13590	0,03397		
Total	7	0,56040				

S = 0,184323 R-Sq = 75,75% R-Sq(adj) = 57,56%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	36,1	A
1	4	35,8	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	36,0	A
30	4	35,9	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0	30	2	36,3	A
1	40	2	36,0	A
0	40	2	35,9	A
1	30	2	35,6	A

Means that do not share a letter are significantly different.

Table C. 15. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Linolenic Acid of mealworm oil*

General Linear Model: Linolenic C18:3 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Linolenic C18:3 MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,003612	0,003612	0,003612	0,88	0,402
Temperature	1	0,001512	0,001512	0,001512	0,37	0,577
Pressure*Temperature	1	0,002812	0,002812	0,002812	0,68	0,455
Error	4	0,016450	0,016450	0,004112		
Total	7	0,024387				

S = 0,0641288 R-Sq = 32,55% R-Sq(adj) = 0,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	1,9	A
1	4	1,9	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
30	4	1,9	A
40	4	1,9	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0	30	2	2,0	A
0	40	2	1,9	A
1	40	2	1,9	A
1	30	2	1,9	A

Means that do not share a letter are significantly different.

Table C. 16. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Eicosanoic Acid of mealworm oil*

General Linear Model: Eicosanoic C20:0 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Eicosanoic C20:0 MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0000000	0,0000000	0,0000000	0,00	1,000
Temperature	1	0,0000000	0,0000000	0,0000000	0,00	1,000
Pressure*Temperature	1	0,0000000	0,0000000	0,0000000	0,00	1,000
Error	4	0,0002000	0,0002000	0,0000500		
Total	7	0,0002000				

S = 0,00707107 R-Sq = 0,00% R-Sq(adj) = 0,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
1	4	0,1	A
0	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	0,1	A
30	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	40	2	0,1	A
1	30	2	0,1	A
0	40	2	0,1	A
0	30	2	0,1	A

Means that do not share a letter are significantly different.

Table C. 17. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Eicosatrienoic Acid of mealworm oil*

General Linear Model: Eicosatrienoic C20:3 MW Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Eicosatrienoic C20:3 MW Oil, using Adjusted SS
for
     Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0000125  0,0000125  0,0000125  1,00  0,374
Temperature            1  0,0000125  0,0000125  0,0000125  1,00  0,374
Pressure*Temperature   1  0,0000125  0,0000125  0,0000125  1,00  0,374
Error                  4  0,0000500  0,0000500  0,0000125
Total                  7  0,0000875


S = 0,00353553   R-Sq = 42,86%   R-Sq(adj) = 0,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   0,1  A
0         4   0,1  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   0,1  A
30           4   0,1  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         40           2   0,1  A
1         30           2   0,1  A
0         40           2   0,1  A
0         30           2   0,1  A

Means that do not share a letter are significantly different.
```

Table C. 18. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Arachidonic Acid of mealworm oil*

General Linear Model: Arachidonic C20:4 MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Arachidonic C20:4 MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0001125	0,0001125	0,0001125	9,00	0,040
Temperature	1	0,0000125	0,0000125	0,0000125	1,00	0,374
Pressure*Temperature	1	0,0000125	0,0000125	0,0000125	1,00	0,374
Error	4	0,0000500	0,0000500	0,0000125		
Total	7	0,0001875				

S = 0,00353553 R-Sq = 73,33% R-Sq(adj) = 53,33%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping	
1	4	0,1	A	
0	4	0,1		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	0,1	A
30	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	40	2	0,1	A
1	30	2	0,1	A
0	40	2	0,1	A
0	30	2	0,1	A

Means that do not share a letter are significantly different.

Table C. 19. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Eicosapentanoic Acid of mealworm oil*

General Linear Model: Eicosapentaen C20:5 MW Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Eicosapentaenoic C20:5 MW Oil, using Adjusted SS
for
     Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0002000  0,0002000  0,0002000  8,00  0,047
Temperature            1  0,0000000  0,0000000  0,0000000  0,00  1,000
Pressure*Temperature   1  0,0000500  0,0000500  0,0000500  2,00  0,230
Error                  4  0,0001000  0,0001000  0,0000250
Total                  7  0,0003500


S = 0,005   R-Sq = 71,43%   R-Sq(adj) = 50,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   0,1  A
0         4   0,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   0,1  A
30           4   0,1  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         40           2   0,1  A
1         30           2   0,1  A
0         30           2   0,1  A
0         40           2   0,1  A

Means that do not share a letter are significantly different.
```

Table C. 20. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Myristic Acid of cricket oil*

General Linear Model: Myristic C14:0 C Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Myristic C14:0 C Oil, using Adjusted SS for
Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0000125  0,0000125  0,0000125   1,00  0,374
Temperature            1  0,0003125  0,0003125  0,0003125  25,00  0,007
Pressure*Temperature   1  0,0003125  0,0003125  0,0003125  25,00  0,007
Error                  4  0,0000500  0,0000500  0,0000125
Total                  7  0,0006875


S = 0,00353553   R-Sq = 92,73%   R-Sq(adj) = 87,27%


Unusual Observations for Myristic C14:0 C Oil

        Myristic
Obs  C14:0 C Oil       Fit    SE Fit   Residual  St Resid
  5     0,570000  0,575000  0,002500  -0,005000     -2,00 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   0,6  A
1         4   0,6  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   0,6  A
30           4   0,6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         40           2   0,6  A
0         40           2   0,6  A
0         30           2   0,6  A
1         30           2   0,6    B

Means that do not share a letter are significantly different.
```

Table C. 21. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Pentadecanoic Acid of cricket oil*

General Linear Model: Pentadecanoic C15:0 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Pentadecanoic C15:0 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0000125	0,0000125	0,0000125	1,00	0,374
Temperature	1	0,0000125	0,0000125	0,0000125	1,00	0,374
Pressure*Temperature	1	0,0000125	0,0000125	0,0000125	1,00	0,374
Error	4	0,0000500	0,0000500	0,0000125		
Total	7	0,0000875				

S = 0,00353553 R-Sq = 42,86% R-Sq(adj) = 0,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	0,1	A
1	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	0,1	A
30	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	40	2	0,1	A
0	40	2	0,1	A
0	30	2	0,1	A
1	30	2	0,1	A

Means that do not share a letter are significantly different.

Table C. 22. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Palmitic Acid of cricket oil*

General Linear Model: Palmitic C16:0 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Palmitic C16:0 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,02420	0,02420	0,02420	0,70	0,450
Temperature	1	0,26645	0,26645	0,26645	7,71	0,050
Pressure*Temperature	1	0,49005	0,49005	0,49005	14,17	0,020
Error	4	0,13830	0,13830	0,03457		
Total	7	0,91900				

S = 0,185944 R-Sq = 84,95% R-Sq(adj) = 73,66%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	23,6	A
1	4	23,5	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	23,7	A
30	4	23,4	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	40	2	23,9	A
0	30	2	23,7	A B
0	40	2	23,5	A B
1	30	2	23,1	B

Means that do not share a letter are significantly different.

Table C. 23. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Palmitoleic Acid of cricket oil*

General Linear Model: Palmitoleic C16:1 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Palmitoleic C16:1 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0001125	0,0001125	0,0001125	0,69	0,452
Temperature	1	0,0001125	0,0001125	0,0001125	0,69	0,452
Pressure*Temperature	1	0,0003125	0,0003125	0,0003125	1,92	0,238
Error	4	0,0006500	0,0006500	0,0001625		
Total	7	0,0011875				

S = 0,0127475 R-Sq = 45,26% R-Sq(adj) = 4,21%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	1,2	A
1	4	1,2	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	1,2	A
30	4	1,2	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	40	2	1,3	A
0	30	2	1,3	A
0	40	2	1,2	A
1	30	2	1,2	A

Means that do not share a letter are significantly different.

Table C. 24. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Margaric Acid of cricket oil*

General Linear Model: Margaric C17:0 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Margaric C17:0 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0000000	0,0000000	0,0000000	0,00	1,000
Temperature	1	0,0000500	0,0000500	0,0000500	2,00	0,230
Pressure*Temperature	1	0,0000000	0,0000000	0,0000000	0,00	1,000
Error	4	0,0001000	0,0001000	0,0000250		
Total	7	0,0001500				

S = 0,005 R-Sq = 33,33% R-Sq(adj) = 0,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
1	4	0,1	A
0	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
30	4	0,1	A
40	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	30	2	0,1	A
0	30	2	0,1	A
1	40	2	0,1	A
0	40	2	0,1	A

Means that do not share a letter are significantly different.

Table C. 25. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Heptadecanoic Acid of cricket oil*

General Linear Model: Heptadecanoic C17:1 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Heptadecanoic C17:1 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0000125	0,0000125	0,0000125	1,00	0,374
Temperature	1	0,0000125	0,0000125	0,0000125	1,00	0,374
Pressure*Temperature	1	0,0000125	0,0000125	0,0000125	1,00	0,374
Error	4	0,0000500	0,0000500	0,0000125		
Total	7	0,0000875				

S = 0,00353553 R-Sq = 42,86% R-Sq(adj) = 0,00%

Unusual Observations for Heptadecanoic C17:1 C Oil

Obs	Heptadecanoic C17:1 C Oil	Fit	SE Fit	Residual	St Resid
3	0,110000	0,105000	0,002500	0,005000	2,00 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
1	4	0,1	A
0	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
30	4	0,1	A
40	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	40	2	0,1	A
1	30	2	0,1	A
0	30	2	0,1	A
0	40	2	0,1	A

Means that do not share a letter are significantly different.

Table C. 26. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Stearic Acid of cricket oil*

General Linear Model: Stearic C18:0 C Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Stearic C18:0 C Oil, using Adjusted SS for Tests

Source                DF   Seq SS   Adj SS   Adj MS       F      P
Pressure               1  15,4846  15,4846  15,4846  159,74  0,000
Temperature            1   1,1325   1,1325   1,1325   11,68  0,027
Pressure*Temperature   1   0,0820   0,0820   0,0820    0,85  0,410
Error                  4   0,3877   0,3877   0,0969
Total                  7  17,0869


S = 0,311348   R-Sq = 97,73%   R-Sq(adj) = 96,03%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4  13,5  A
1         4  10,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4  12,5  A
40           4  11,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         30           2  14,0  A
0         40           2  13,0  A
1         30           2  11,0    B
1         40           2  10,4    B

Means that do not share a letter are significantly different.
```

Table C. 27. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Oleic Acid of cricket oil*

General Linear Model: Oleic C18:1 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Oleic C18:1 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	6,625	6,625	6,625	2,74	0,173
Temperature	1	6,408	6,408	6,408	2,65	0,179
Pressure*Temperature	1	1,296	1,296	1,296	0,54	0,505
Error	4	9,688	9,688	2,422		
Total	7	24,017				

S = 1,55624 R-Sq = 59,66% R-Sq(adj) = 29,41%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
1	4	26,0	A
0	4	24,2	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	26,0	A
30	4	24,2	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	40	2	27,3	A
1	30	2	24,7	A
0	40	2	24,7	A
0	30	2	23,7	A

Means that do not share a letter are significantly different.

Table C. 28. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Linoleic Acid of cricket oil*

General Linear Model: Linoleic C18:2 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Linoleic C18:2 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,36551	0,36551	0,36551	78,39	0,001
Temperature	1	0,44651	0,44651	0,44651	95,77	0,001
Pressure*Temperature	1	0,36551	0,36551	0,36551	78,39	0,001
Error	4	0,01865	0,01865	0,00466		
Total	7	1,19619				

S = 0,0682825 R-Sq = 98,44% R-Sq(adj) = 97,27%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping	
0	4	31,5	A	
1	4	31,1		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping	
40	4	31,5	A	
30	4	31,0		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping	
1	40	2	31,5	A	
0	40	2	31,5	A	
0	30	2	31,5	A	
1	30	2	30,6		B

Means that do not share a letter are significantly different.

Table C. 29. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Linolenic Acid of cricket oil*

General Linear Model: Linolenic C18:3 C Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Linolenic C18:3 C Oil, using Adjusted SS for
Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0000000  0,0000000  0,0000000  0,00  1,000
Temperature            1  0,0000500  0,0000500  0,0000500  0,15  0,715
Pressure*Temperature   1  0,0000500  0,0000500  0,0000500  0,15  0,715
Error                  4  0,0013000  0,0013000  0,0003250
Total                  7  0,0014000


S = 0,0180278   R-Sq = 7,14%   R-Sq(adj) = 0,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   0,4  A
0         4   0,4  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   0,4  A
40           4   0,4  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         30           2   0,4  A
0         40           2   0,4  A
0         30           2   0,4  A
1         40           2   0,4  A

Means that do not share a letter are significantly different.
```

Table C. 30. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Eicosanoic Acid of cricket oil*

General Linear Model: Eicosanoic C20:0 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Eicosanoic C20:0 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0002000	0,0002000	0,0002000	4,00	0,116
Temperature	1	0,0002000	0,0002000	0,0002000	4,00	0,116
Pressure*Temperature	1	0,0002000	0,0002000	0,0002000	4,00	0,116
Error	4	0,0002000	0,0002000	0,0000500		
Total	7	0,0008000				

S = 0,00707107 R-Sq = 75,00% R-Sq(adj) = 56,25%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	0,8	A
1	4	0,7	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	0,8	A
30	4	0,7	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	40	2	0,8	A
0	40	2	0,8	A
0	30	2	0,8	A
1	30	2	0,7	A

Means that do not share a letter are significantly different.

Table C. 31. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Eicosatrienoic Acid of cricket oil*

General Linear Model: Eicosatrienoic C20:3 C Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Eicosatrienoic C20:3 C Oil, using Adjusted SS
for
     Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0010125  0,0010125  0,0010125  11,57  0,027
Temperature            1  0,0021125  0,0021125  0,0021125  24,14  0,008
Pressure*Temperature   1  0,0021125  0,0021125  0,0021125  24,14  0,008
Error                  4  0,0003500  0,0003500  0,0000875
Total                  7  0,0055875


S = 0,00935414   R-Sq = 93,74%   R-Sq(adj) = 89,04%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   0,2  A
0         4   0,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   0,2  A
40           4   0,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         30           2   0,2  A
0         40           2   0,2    B
0         30           2   0,2    B
1         40           2   0,2    B

Means that do not share a letter are significantly different.
```

Table C. 32. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Arachidonic Acid of cricket oil*

General Linear Model: Arachidonic C20:4 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Arachidonic C20:4 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0000000	0,0000000	0,0000000	0,00	1,000
Temperature	1	0,0220500	0,0220500	0,0220500	882,00	0,000
Pressure*Temperature	1	0,0000000	0,0000000	0,0000000	0,00	1,000
Error	4	0,0001000	0,0001000	0,0000250		
Total	7	0,0221500				

S = 0,005 R-Sq = 99,55% R-Sq(adj) = 99,21%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
1	4	0,1	A
0	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping	
30	4	0,1	A	
40	4	0,0		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping	
1	30	2	0,1	A	
0	30	2	0,1	A	
0	40	2	0,0		B
1	40	2	0,0		B

Means that do not share a letter are significantly different.

Table C. 33. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Eicosapentaenoic Acid of cricket oil*

General Linear Model: Eicosapentaenoic C20:5 C Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Eicosapentaenoic C20:5 C Oil, using Adjusted SS
for
     Tests

Source                DF    Seq SS    Adj SS    Adj MS        F      P
Pressure               1  0,000012  0,000013  0,000013     1,00  0,374
Temperature            1  0,063012  0,063012  0,063012  5041,00  0,000
Pressure*Temperature   1  0,000013  0,000013  0,000013     1,00  0,374
Error                  4  0,000050  0,000050  0,000012
Total                  7  0,063087


S = 0,00353553   R-Sq = 99,92%   R-Sq(adj) = 99,86%


Unusual Observations for Eicosapentaenoic C20:5 C Oil

     Eicosapentaenoic
Obs       C20:5 C Oil       Fit    SE Fit  Residual  St Resid
  4          0,180000  0,175000  0,002500  0,005000      2,00 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   0,1  A
0         4   0,1  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   0,2  A
30           4   0,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         40           2   0,2  A
0         40           2   0,2  A
0         30           2   0,0    B
1         30           2   0,0    B
Means that do not share a letter are significantly different.
```

Table C. 34. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Tetracosanoic Acid of cricket oil*

General Linear Model: Tetracosanoic C24:0 C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Tetracosanoic C24:0 C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,0000000	0,0000000	0,0000000	0,00	1,000
Temperature	1	0,0000000	0,0000000	0,0000000	0,00	1,000
Pressure*Temperature	1	0,0000500	0,0000500	0,0000500	2,00	0,230
Error	4	0,0001000	0,0001000	0,0000250		
Total	7	0,0001500				

S = 0,005 R-Sq = 33,33% R-Sq(adj) = 0,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
1	4	0,1	A
0	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
40	4	0,1	A
30	4	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	30	2	0,1	A
0	40	2	0,1	A
1	40	2	0,1	A
0	30	2	0,1	A

Means that do not share a letter are significantly different.

Table C. 35. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Crystallization Point of mealworm oil*

General Linear Model: Crystallization Point MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Crystallization MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	1,02245	1,02245	1,02245	43,28	0,003
Temperature	1	0,00405	0,00405	0,00405	0,17	0,700
Pressure*Temperature	1	0,16820	0,16820	0,16820	7,12	0,056
Error	4	0,09450	0,09450	0,02362		
Total	7	1,28920				

S = 0,153704 R-Sq = 92,67% R-Sq(adj) = 87,17%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0	4	-15,3	A
1	4	-16,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
30	4	-15,6	A
40	4	-15,6	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0	40	2	-15,1	A
0	30	2	-15,4	A B
1	30	2	-15,8	B C
1	40	2	-16,1	C

Means that do not share a letter are significantly different.

Table C. 36. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Melting Point of mealworm oil*

General Linear Model: Melting Point MW Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Melting MW Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,07411	0,07411	0,07411	2,20	0,212
Temperature	1	0,11281	0,11281	0,11281	3,35	0,141
Pressure*Temperature	1	0,03001	0,03001	0,03001	0,89	0,399
Error	4	0,13475	0,13475	0,03369		
Total	7	0,35169				

S = 0,183542 R-Sq = 61,68% R-Sq(adj) = 32,95%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
1	4	-10,5	A
0	4	-10,7	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
30	4	-10,5	A
40	4	-10,7	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
1	30	2	-10,3	A
0	30	2	-10,6	A
1	40	2	-10,7	A
0	40	2	-10,8	A

Means that do not share a letter are significantly different.

Table C. 37. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Crystallization Point of cricket oil*

General Linear Model: Crystallization Point C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Crystallization C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	3,1500	3,1500	3,1500	673,81	0,000
Temperature	1	0,0924	0,0924	0,0924	19,78	0,011
Pressure*Temperature	1	0,0032	0,0032	0,0032	0,68	0,455
Error	4	0,0187	0,0187	0,0047		
Total	7	3,2644				

S = 0,0683740 R-Sq = 99,43% R-Sq(adj) = 99,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping	
1	4	-1,1	A	
0	4	-2,3		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping	
30	4	-1,6	A	
40	4	-1,8		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping	
1	30	2	-1,0	A	
1	40	2	-1,2	A	
0	30	2	-2,3		B
0	40	2	-2,4		B

Means that do not share a letter are significantly different.

Table C. 38. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Melting Point of cricket oil*

General Linear Model: Melting C Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Melting C Oil, using Adjusted SS for Tests

Source                DF  Seq SS  Adj SS  Adj MS       F      P
Pressure               1  2,0605  2,0605  2,0605  186,89  0,000
Temperature            1  2,8322  2,8322  2,8322  256,89  0,000
Pressure*Temperature   1  0,0242  0,0242  0,0242    2,20  0,213
Error                  4  0,0441  0,0441  0,0110
Total                  7  4,9609


S = 0,105   R-Sq = 99,11%   R-Sq(adj) = 98,44%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   0,6  A
1         4  -0,4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   0,7  A
40           4  -0,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         30           2   1,1  A
1         30           2   0,2    B
0         40           2   0,0    B
1         40           2  -1,1      C

Means that do not share a letter are significantly different.
```

Table C. 39. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Peroxide Value of mealworm oil*

General Linear Model: Peroxide Value MW Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Peroxide Value MW Oil, using Adjusted SS for
Tests

Source                DF    Seq SS    Adj SS    Adj MS        F      P
Pressure               1  0,016290  0,016290  0,016290   115,23  0,000
Temperature            1  0,190653  0,190653  0,190653  1348,56  0,000
Pressure*Temperature   1  0,015753  0,015753  0,015753   111,43  0,000
Error                  4  0,000566  0,000566  0,000141
Total                  7  0,223262


S = 0,0118901   R-Sq = 99,75%   R-Sq(adj) = 99,56%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   2,0  A
0         4   1,9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   2,1  A
30           4   1,8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         40           2   2,2  A
0         40           2   2,0    B
1         30           2   1,8      C
0         30           2   1,8      C

Means that do not share a letter are significantly different.
```

Table C. 40. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Peroxide Value of cricket oil*

General Linear Model: Peroxide Value C Oil versus Pressure; Temperature

Factor	Type	Levels	Values
Pressure	fixed	2	0; 1
Temperature	fixed	2	30; 40

Analysis of Variance for Peroxide Value C Oil, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	0,12079	0,12079	0,12079	1390,34	0,000
Temperature	1	0,24325	0,24325	0,24325	2800,04	0,000
Pressure*Temperature	1	0,04515	0,04515	0,04515	519,71	0,000
Error	4	0,00035	0,00035	0,00009		
Total	7	0,40954				

S = 0,00932068 R-Sq = 99,92% R-Sq(adj) = 99,85%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping	
1	4	2,0	A	
0	4	1,7		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping	
40	4	2,0	A	
30	4	1,7		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping			
1	40	2	2,2	A			
0	40	2	1,8		B		
1	30	2	1,7			C	
0	30	2	1,6				D

Means that do not share a letter are significantly different.

Table C. 41. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Total Phenolic Content of mealworm oil*

General Linear Model: MW Oil Phenolic Content versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Mealworm Oil Phenolic Content, using Adjusted SS
for
     Tests

Source                DF   Seq SS   Adj SS   Adj MS       F      P
Pressure               1  0,32618  0,32618  0,32618   36,16  0,004
Temperature            1  0,04853  0,04853  0,04853    5,38  0,081
Pressure*Temperature   1  1,10776  1,10776  1,10776  122,81  0,000
Error                  4  0,03608  0,03608  0,00902
Total                  7  1,51855


S = 0,0949735   R-Sq = 97,62%   R-Sq(adj) = 95,84%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   4,4  A
1         4   4,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   4,3  A
30           4   4,1  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         40           2   4,9  A
1         30           2   4,3    B
0         30           2   4,0    B C
1         40           2   3,7      C

Means that do not share a letter are significantly different.
```

Table C. 42. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of Total Phenolic Content of cricket oil*

General Linear Model: Cricket Oil Phenolic Content versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Cricket Oil Phenolic Content, using Adjusted SS
for
     Tests

Source                DF    Seq SS    Adj SS    Adj MS      F      P
Pressure               1  0,032219  0,032219  0,032219  51,86  0,002
Temperature            1  0,032219  0,032219  0,032219  51,86  0,002
Pressure*Temperature   1  0,059911  0,059911  0,059911  96,43  0,001
Error                  4  0,002485  0,002485  0,000621
Total                  7  0,126834


S = 0,0249259   R-Sq = 98,04%   R-Sq(adj) = 96,57%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   0,6  A
0         4   0,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   0,6  A
40           4   0,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         30           2   0,7  A
0         40           2   0,5    B
1         40           2   0,4    B
0         30           2   0,4    B

Means that do not share a letter are significantly different.
```

Table C. 43. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of DPPH of mealworm oil*

General Linear Model: Mealworm DPPH versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Mealworm (mg trolox/g sample), using Adjusted SS
for
     Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0004054  0,0004054  0,0004054  11,00  0,029
Temperature            1  0,0007538  0,0007538  0,0007538  20,45  0,011
Pressure*Temperature   1  0,0004054  0,0004054  0,0004054  11,00  0,029
Error                  4  0,0001474  0,0001474  0,0000369
Total                  7  0,0017120


S = 0,00607068   R-Sq = 91,39%   R-Sq(adj) = 84,93%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   0,2  A
0         4   0,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   0,2  A
30           4   0,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         40           2   0,2  A
0         40           2   0,2  A
1         30           2   0,2  A
0         30           2   0,2    B

Means that do not share a letter are significantly different.
```

Table C. 44. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of DPPH of cricket oil*

General Linear Model: Cricket DPPH versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Cricket (mg trolox/g sample), using Adjusted SS
for
     Tests

Source                DF    Seq SS    Adj SS    Adj MS       F      P
Pressure               1  0,011531  0,011531  0,011531  107,56  0,000
Temperature            1  0,020036  0,020036  0,020036  186,89  0,000
Pressure*Temperature   1  0,005360  0,005360  0,005360   50,00  0,002
Error                  4  0,000429  0,000429  0,000107
Total                  7  0,037356


S = 0,0103542   R-Sq = 98,85%   R-Sq(adj) = 97,99%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   0,6  A
0         4   0,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   0,6  A
30           4   0,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
1         40           2   0,6  A
0         40           2   0,5    B
1         30           2   0,5    B C
0         30           2   0,5      C

Means that do not share a letter are significantly different.
```

Table C. 45. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of CUPRAC of mealworm oil*

General Linear Model: Mealworm Oil CUPRAC versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Mealworm Oil Antioxidant Activ., using Adjusted
SS for
     Tests

Source                DF   Seq SS   Adj SS   Adj MS       F      P
Pressure               1  11,1533  11,1533  11,1533  257,01  0,000
Temperature            1   6,8072   6,8072   6,8072  156,86  0,000
Pressure*Temperature   1   0,0163   0,0163   0,0163    0,38  0,573
Error                  4   0,1736   0,1736   0,0434
Total                  7  18,1504


S = 0,208316   R-Sq = 99,04%   R-Sq(adj) = 98,33%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   7,8  A
1         4   5,4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   7,5  A
40           4   5,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         30           2   8,7  A
0         40           2   6,8    B
1         30           2   6,3    B
1         40           2   4,5      C

Means that do not share a letter are significantly different.
```

Table C. 46. *Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of CUPRAC of cricket oil*

General Linear Model: Cricket Oil versus Pressure; Temperature

```
Factor       Type   Levels  Values
Pressure     fixed       2  0; 1
Temperature  fixed       2  30; 40


Analysis of Variance for Cricket Oil Antioxidant Activ., using Adjusted
SS for
     Tests

Source                DF   Seq SS   Adj SS   Adj MS       F      P
Pressure               1   0,0082   0,0082   0,0082    0,25  0,641
Temperature            1   0,4402   0,4402   0,4402   13,63  0,021
Pressure*Temperature   1  23,2838  23,2838  23,2838  721,13  0,000
Error                  4   0,1292   0,1292   0,0323
Total                  7  23,8613


S = 0,179689   R-Sq = 99,46%   R-Sq(adj) = 99,05%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   6,0  A
1         4   5,9  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4   6,2  A
30           4   5,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  Temperature  N  Mean  Grouping
0         40           2   7,9  A
1         30           2   7,4  A
1         40           2   4,5    B
0         30           2   4,1    B

Means that do not share a letter are significantly different.
```