INVESTIGATION OF TECHNO-FUNCTIONAL PROPERTIES OF PROTEIN RICH INSECT POWDERS OBTAINED FROM *ACHETA DOMESTICUS* & *TENEBRIO MOLITOR*

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ABSTRACT

INVESTIGATION OF TECHNO-FUNCTIONAL PROPERTIES OF PROTEIN RICH INSECT POWDERS OBTAINED FROM *ACHETA DOMESTICUS* & *TENEBRIO MOLITOR*

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Edible insects have become one of the most attractive food sources and the most striking concept in recent times all over the world especially in Europe as a potential nutrient supply for the near future. Recently, researches on edible insects have been increasing since they are considered as an environmentally friendly option to livestock. This study is carried out with a purpose of investigating the physical and functional characterization of the protein extracts of two edible insect species; *Acheta domesticus* and *Tenebrio molitor*. The insect species include 69.80 and 52.50 % crude protein in granulated form respectively. In the study, the fat content was reduced primarily by defatting procedure with hexane. While defatting, insect powders were also exposed to the high hydrostatic pressure at 500 MPa for 15 minutes at 30^{o}C or 40^{o}C to observe changes in their antioxidant capacities and functionality of protein fractions. Protein content of protein rich

insect powders was determined with Kjeldahl method and the results were found as in the range of 72.80-74.50 % and 79.05-81.50 % for mealworm and cricket powders, respectively. Protein solubility in distilled water was determined with Lowry method to get knowledge about hydrophilicity mechanism of insects. Results indicated that, temperature increase from 30 to 40 ^{o}C caused a decrease in solubility of both insect proteins. For characterization of gelling ability, NMR T_2 relaxometry was used to interpret the relation between polymer and water retention. $CaCl_2$ showed salting out salt property for mealworm proteins while it behaved as salting in salt in the presence of cricket proteins. According to FTIR spectroscopy, the strongest absorbance peaks were absorbed mainly in amide I, amide II and amide III regions in both species.

This study aimed to provide a comprehensive solution for more nutritious foods by searching on the functional properties of insect proteins because of the possible food shortage and increasing demand for the nourishing foods in all around the world.

Keywords: Mealworm, Cricket, Protein, Temperature, High Hydrostatic Pressure

ÖZ

ACHETA DOMESTICUS & *TENEBRIO MOLITOR* BÖCEK TÜRLERİNDEN ELDE EDİLEN PROTEİNCE ZENGİN TOZLARIN TEKNO-FONKSİYONEL ÖZELLİKLERİNİN İNCELENMESİ

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Yenilebilir böcekler, yakın geçmişe yönelik potansiyel bir besin kaynağı olarak, özellikle Avrupa'da son zamanlarda en çekici gıda kaynaklarından ve en çarpıcı konseptlerden biri haline gelmiştir. Son zamanlarda yenilebilir böcekler üzerindeki araştırmalar artmaktadır çünkü besi hayvancılığına karşı çevre dostu bir seçenek olarak kabul edilmektedirler. Bu çalışma, iki yenilebilir böcek türü olan *Acheta domesticus ve Tenebrio molitor*'dan elde edilen protein ekstraktlarının fiziksel ve fonksiyonel karakterizasyonunun araştırılması amacıyla gerçekleştirilmiştir;.

Böcek türleri, toz haline getirilmiş formda sırasıyla %69.08 ve %52.50 protein içermektedir. Çalışmada ilk olarak, yağ içeriği hekzan kullarak yağ ayrıştırma işlemi ile azaltılmıştır. Yağı giderme sırasında, böcek tozlarının antioksidan kapasiteleri ve protein fraksiyonlarının işlevselliğini gözlemlemek için yüksek

hidrostatik basınca maruz kalmıştır. Protein bakımından zengin böcek tozlarının protein içeriği, Kjeldahl yöntemiyle belirlendi ve sonuçlar, yemek kurdu ve kriket tozları için sırasıyla %72.80-74.50 ve %79.05-81.50 aralığında bulunmuştur. Saf sudaki protein çözünürlüğü, böceklerin hidrofilik mekanizmaları hakkında bilgi edinmek için Lowry yöntemiyle belirlenmiştir. Sonuçlar, 30°C'den 40°C'ye kadar olan sıcaklık artışının her iki böcek proteininin çözünürlüğünde bir azalmaya neden olduğunu göstermiştir. Jelleşme kabiliyetinin belirlenmesi için NMR T_2 relaksometresi polimer ve su tutma arasındaki ilişkiyi yorumlamak için kullanılmıştır. $CaCl_2$, un kurdu proteinleri için salting-out etkisi gösterirken, çekirge proteinlerinin mevcudiyetinde salting-in işlevi göstermiştir. FTIR spektroskopisine göre, her iki böcek türünde de en güçlü absorbans noktaları amid I, amid II ve amid III bölgelerinde gözlemlenmiştir.

Anahtar Kelimeler: Un kurdu, Çekirge, Protein, Sıcaklık, Yüksek hidrostatik basınç

To the ones that deserve best, especially my beloved family

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INTRODUCTION

1.1. Edible insects

1.1.1. General view

Edible insects have been consumed traditionally in around 113 countries worldwide and more than 2000 insect types are considered as an edible food source (Kouřimská & Adámková, 2016). From those species, beetles, bees, ants, wasps and caterpillars are the most preferred ones and other species that are relatively consumed are crickets, cicadas, dragonflies, leafhoppers, grasshoppers, locusts, bugs, termites, flies (Jongema, 2015). These species are mostly consumed in Asia, Africa and Latin America but they are not preferred in Western countries in an excessive amount (Jongema, 2015).

Nowadays, the potential role of insects as a sustainable protein source for the rapidly growing world population has been debated increasingly. Scientists have been focusing on the edible insects and their related studies since their CO_2 and greenhouse gas emissions are lower than that of cattle and small cattle, they require less nutrients and water, their storage is easy and secure on large scales and they have an ecofriendly lifestyle (Dobermann et al., 2017). It is also thought that, people are struggling with the troubles regard to limited food supplies that result in malnutrition and health problems in some regions (Adámková et al.,

2017). Therefore, edible insects can be the possible solution as easily discoverable food sources and nutritional food supplies.

1.1.2. Entomophagy

Entomophagy, the word used to describe the action of insect eating, has been practiced by human kind over the centuries and insects still keep a remarkable place as the traditional food in several countries, especially in developing ones. Consumption of ants and beetle larvae by African and Australian tribes, and popularly consumed fried beetles and locusts in Thailand show that insect consumption is not a new concept. However, acceptance is relatively low in some Western nations because entomophagy is generally not promoted and Western dietary patterns are not easy to be abandoned (Van Huis, 2013). Since insect consumption has been thought as a rural and primitive behavior, insects have never drawn an interest among Western consumers (Megido et al., 2016).

According to the research conducted on 32 Italian consumers about their behavior towards insect consumption, most consumers have indicated that they are willing to try; only a few of them said they would definitely not eat insects since their appearance appeared to be a primary factor on deciding not to eat insects (Balzan et al., 2016). The research concludes that, minimizing the extent of perceptibility increases the acceptability of entomophagy. Some researches among Western consumers show that consuming insects in a whole form brings to their mind that the animal was alive before it becomes ready for the consumption and this idea provokes negative attitudes towards insects (Hartmann et al., 2015). Therefore, it is a good way to serve insects in a masked form as insect flour or protein extract to consumers. However, it is necessary to have an extensive data on their functional properties before the addition of insect-based hemi products/ingredients and protein extracts to the food products (Bußler et al., 2016). Moreover, there are too many research gaps and uncertainness about the usability of insect species in

the food and feed industry (EFSA, 2015). In order to create a global culture that approve entomophagy, efforts must be made not only to inform consumers about the nutritional and sustainable advantages of insect consumption, but also to make consumption desirable (Tan et al., 2016).

1.1.3. Benefits of insect consumption

1.1.3.1. Health aspect

In nature, diversity among insect species and difference in their size result in various nutritional profile of insects. Also, their origins, eating habits, metamorphosis stage and environment are the important factors that play roles in different nutritional composition (Morales-Ramos et al., 2014). Apart from these, different preparation and processing steps like frying, cooking or drying may influence the nutritional value (Van Huis et al., 2013).

The research conducted (Payne et al., 2015) to compare the nutrient value scores (NVS) of six different edible insects with traditional meat sources demonstrated that all insect species used in the study were statistically healthier than chicken and beef. Among those species, cricket and honey bees were the only ones that had higher nutrient value score than pork and the mealworm's score was found so close to this meat source (Payne et al., 2015). The difference between nutrient value score of edible insects and pork was due to the high fat portion of pork which also explained why mealworm and pork's scores were close to each other.

From a nutritional point of view, insects are considered as protein rich alternative meat sources since the largest part of the insects consist of proteins and the contents vary from 20 to 76 g on a dry matter basis (DM) depending on the effecting factors (Kouřimská & Adámková, 2016). As a result, the recommended

dietary protein intake of average human adult (50 g/day) can be provided from 100 g of dried insects between the rates of 24.2-148 %. This means, most edible insects provide adequate energy and protein need for human consumption while also meeting the amino acid requirement. The report published by World Health Organization (WHO) about daily requirements of essential amino acids for the average healthy adult, infants and children revealed an information that most of the insect species involved all the essential amino acids for growth (Joint WHO/FAO/UNU Expert Consultation, 2007). Therefore, insect consumption may be beneficial for poor societies especially in Africa, where there is an essential amino acids deficiency (Bukkens, 1997).

According to the research conducted by Rumpold and Schlüter (2013), edible insects are mostly rich in phenylalanine + tyrosine among the essential amino acids. Moreover, some insects involve high amounts of tryptophan, lysine and threonine, which are insufficient in some cereal proteins. Amino acid content of insects also varies from species to species and can be linked to feed, climate and habitat variations same as protein content.

Generally, edible insects have a high oil content which is mostly found more in larval stages than in adults and contain around 10 to 60% oil in the dry matter (Xiaoming, Ying & Hong, 2010). Since lipids are important biomolecules having high caloric values that fulfill the most of the energy reserves and contain essential fatty acids, they are also considered as the main energy source to struggle against hunger and malnutrition in undeveloped or developing countries (Smit, Muskiet, & Boersma, 2004). Insect lipids are constituted of considerable amount of unsaturated fatty acids which are most importantly oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) (DeFoliart, 1991). On the other hand, certain types of insects contain high amount of saturated fatty acids such as palmitic acid (C16:0) (Tzompa-Sosa et al., 2014). In general, it is known that the

majority of insect lipids are triacylglycerols which constitutes approximately 80% of the lipid content. Yet, they also consist of significant amount of phospholipids, sterols and glycolipids (Gilby, 1965).

Furthermore, edible insects include substantial amount of fibers. Exoskeleton of edible insects are made up from chitin and estimated chitin content in insects are between 11.6-137.2 mg/kg in dry matter basis (DM) (Finke, 2007). The chitin amount in insects makes contribution to the insoluble fiber content because it is formed by a long chain polymer (van Huis et al., 2013). Chitin is known as an indigestible fiber, despite the presence of chitinase enzyme in gastric juices of human (Paoletti et al., 2007). Therefore, decrease in chitin content enhances the digestibility of insects (Finke, 2007).

Insect species are also rich in variety of minerals and vitamins. From those, iron, zinc and calcium, potassium, sodium, copper and manganese are the minerals present in insects in specific amounts (van Huis, 2013). Also, amount of calcium and iron in edible insects is in higher quantities when compared with traditional animal sources (Payne et al., 2015; Bukkens, 1997). Therefore, supplementation of these minerals with the consumption of edible insects can be a possible solution to fight against mineral deficiencies in several regions.

Vitamins found in insects vary depending on the types of insects and feed composition. Riboflavin, pantothenic acid and biotin are the most common vitamins exist in these species (Rumpold & Schlüter, 2013). On the other hand, they also include some amount of B12, retinol and β-carotene (Kouřimská & Adámková, 2016).

Despite the need for a more comprehensive investigation on nutrient compositions, researches on insect species up to now has demonstrated that these species are nutrient rich alternative meat sources that can meet the demand of growing population and have high potential to deal with starvation and malnutrition in all around the world.

1.1.3.2. Environmental and economical aspect

Insects are the animals that have a green life style in comparison with conventional meat sources since insect farming results in less greenhouse gas emissions, land usage and water pollution (Van Huis et al., 2013). Moreover, insects have a rapid growth rate which means, they increase in large numbers in a short span of time (Premalatha et al., 2011). Additionally, they have a high feed conversion efficiency because they do not spend energy in order to regulate the temperature of their body and this feature plays an important role in their use for breeding purposes (Van Huis et al., 2013; Premalatha et al., 2011). For example, *Acheta Domesticus* known as the house cricket has a higher feed conversion rate which is around twice of chicken, 4 times higher than pigs and 12 times more than cattle (Van Huis et al., 2013).

Another positive aspect of entomophagy is to minimize the use of pesticide. Since insects are considered as a pest, picking them up from environment for human consumption makes remarkable contribution to decrease in insecticides usage (Cerritos, 2009). For instance, collection of insects for eating purposes in Mexico has decreased the farmers' financial burden by decreasing the amount of pesticides used in the production of agricultural products (Cerritos, 2009; Cerritos and Cano-Santana, 2008).

Insect breeding has a great chance to create a cheap, sustainable and environmentally friendly protein source alternative to traditional animal farming. Because, small-scale farming systems existing in some countries have a contribution to rural subsistence by also supporting the green economy (Halloran, Roos & Hanboonsong, 2016).

In recent times, insect farming has gained an importance and generation of insect cells in suspensions in bioreactors have been researched (Verkerk et al., 2007). In bioreactors, there is a good opportunity to produce insect proteins with a high reproducible capacity and serial production rate since they are closed and controlled systems (Mitsuhashi, 2002). In addition, contamination risk is very low in these systems, and the cells' composition and biomass can be changed and controlled with the help of certain types of tissues. Thus, cells can be produced at optimum conditions and undesirable components in insect body like chitin can easily be minimized (Verkerk et al., 2007; Mitsuhashi, 2002). Furthermore, production of insect cells in bioreactors do not necessitate tools to control temperature or carbon dioxide as opposed to produce livestock cells such as cattle. As a result, these systems have provided an effective and easy production and even created a chance to generate insect cells in unfamiliar environments like space (Bessa et al., 2018).

1.1.4. Insects in literature

In the literature, there are limited scientific researches and datum related with nutrient composition and protein characterization of insects. Ghaly and Alkoaik (2009) searched on yellow mealworms which were in different sizes and cultivated in two growing places (wheat flour and brewer's yeast) to observe the protein quality. The growth behavior of young and old larvae was investigated with respect to their weights. As a result, the best efficient production level of larvae was determined as 100-110 mg. Besides, it was found that, mealworm larvae included significant amount of essential amino acids, minerals and fat, so

this made them a valuable food source in terms of these constituents; even so, the further research should be done and the composition must be investigated in detailed way alongside of the environmental parameters such as temperature, heat and humidity.

Siemianowska et al. (2013) worked on the mealworms in both fresh and powdered form to determine its composition and 3-month-old larvae of mealworm was used as the raw material in the research. While the composition of fresh larvae was determined as 56% water, 18% total protein, 22% total fat and 1.55% ash, the minerals found inside the larvae were found as Mg (87.5/100g), Zn (4.2mg/100g), Fe (3.8mg/100g), Cu (0.78mg/100g) and Mn (0.44mg/100g). Besides, it was found that, there is high proportion of Ω-6 and Ω-3 fatty acids. On the other hand, the larvae powder included twice as much protein, fat, minerals and ash content. As a result, authors claimed that powder of mealworm larvae could be used as supplements due to its valuable and rich composition.

Del Valle et al. (1982) carried out a research about protein extraction from Mexican fruit fly (*Anastrepha ludens*). It was found that the larvae of *Anastrepha ludens* contains 9.8 % protein in wet matter basis with high amounts of lysine and methionine but included trace amount of valine, isoleucine and tryptophane. According to the results, the protein solubility was minimum at pH 5 which was the isoelectric point and maximum at pH 10 with a percentage of 8 % and 95 % respectively.

A different research related with edible insects was about the investigation of the techno-functional properties of proteins from five insect species: *Tenebrio molitor* (larvae), *Zophobas morio* (larvae), *Alphitobius diaperinus* (larvae), *Acheta domesticus* (adult) and *Blaptica dubia* (adult). (Yi et al, 2013). After performing aqueous protein extraction, the range of protein purity was found as 50-75 % on

dry basis. Besides, it was also found that the insect proteins had gelling ability depending upon the concentration and pH and this ability enabled them to be used as gelling agents and texturizers (Yi et al, 2013).

In another research, two defatted, dried and ground Sudanese beetles (*Aspongubus viduatus* & *Agonoscelis pubescens*) proteins were extracted to compare their gelation properties with the commercially available gelatin as a stabilizer in ice cream and panelist evaluated the gelatin extracted from insects as agreeable. (Mariod et al, 2011). Mariod (2013) also proved that there was no significant difference between the insect gelatin and commercial gelatin on ice cream production.

Zhao, et al. (2016) stated that, in order to utilize proteins cost-effectively from the larvae of yellow mealworm for human beings, it was necessary to optimize protein extraction yields and conditions and determine protein purity and functionality. For that purpose, protein extraction from yellow mealworm larvae was conducted and the resulting protein extract was characterized according to its functionality. As a result, the isoelectric point of proteins that showed the lowest solubility in distilled water was detected between pH 4 and 5 and protein solubility increased with increasing or decreasing pH. Rheological tests showed that the elastic modulus was affected by salt and enzyme addition, temperature, concentration, incubation and pH. These results pointed out that the functional properties of these protein extracts could be altered for their usage in different food applications.

Buβler et al. (2016) examined the extractability of protein from insects and the techno-functional properties of the flours of *T. Molitor* (60% crude protein) and *H.illucens* (36% crude protein). In the study, the crude fat (20% in both) was reduced with defatting procedure to 2.8% (*T.molitor*) and 8.8% (*H.illucens*); thus,

the amount of crude protein was increased to 68% in *T.molitor* and 47% in *H.illucens*. The solubility of proteins was adjusted by changing the extraction temperature, ionic strength and pH of the solvent with the purpose of getting optimum percentage of proteins from the flours. This study emphasized the necessary steps to develop sustainable and microbiologically safe insect processing technologies by underlining the significance of process design and defatting step.

Another research about investigation of the composition and techno-functional properties of protein concentrate obtained from *L.migratoria* was carried out by Purschke et al. (2018). According to the results, the maximum solubility of protein concentrate was obtained at pH 9. When the functionality was compared with the egg white protein, a considerable emulsifying activity at pH 5, foamability at pH 3 and 3% NaCl was found. Also, higher foam stability was obtained at pH 9. This study proved that protein concentrate of *L.migratoria* could be used in food industry because of having high protein content and considerable functional properties.

Study performed by Zielińska, Karaś, and Baraniak (2018) investigated the functional properties of *Tenebrio molitor, Schistocerca gregaria* and *Gryllodes sigillatus*. The water and oil holding capacity, solubility, and emulsifying ability and foamability were examined in both insect flours and protein fractions. According to the results, at pH 5, the protein solubility was the lowest among all species and there was a recognizable water and oil holding capacity. Moreover, the highest emulsion stability was found in the *T.molitor* protein preparation (51.31%) whereas the *G.sigillatus* protein fraction exhibited the highest emulsion activity, foaming capacity, foam stability (72.62%, 99.0% and 92.0%). As a result, it was found that insect flours or protein fractions could be a good source for development of novel food products.

González, Garzón and Rosell (2018) aimed to identify the potential usability of three insect flours obtained from *H. illucens*, *A.domesticus* and *T.molitor* in bakery products with the purpose of protein enrichment. To evaluate rheological properties, insect flours were added to replace 5% wheat flour in bakery goods. As a result of the study authors concluded that, flour of those insects could be a significant alternative to wheat flour in doughs and breads by improving their nutritional quality with high protein and fiber content, without remarkably changing the rheological properties.

Azzollini et al. (2018) also researched on the determination of the influence of adding mealworm larvae to the extrudes with the purpose of evaluating microstructure and texture properties and also analyzed the digestibility of attained final products at different insect substitutions and processing conditions (barrel temperature and screw speed). Authors reveal that, digestibility of starch and proteins of snacks increased with the increase of insect addition and also with both temperature and screw speed. As a result, it was concluded that the edible insects might be used as novel ingredient for extruded snacks.

1.1.5. Insects in food industry

As research on usability of insects in food industry have increased, production of insect-based products have also increased day by day. However, consumer acceptance is still the biggest obstacle for marketing of insect products to the customers.

Insects are already consumed as a snack or traditional food in several countries such as Asia, Africa and South America. However, the Western world still has a negative attitude towards insect consumption although they are getting closer to

the idea of entomophagy thanks to the various insect products that exist in the food industry.

In the industry, there are several companies that have come up with a great way of serving insects to the costumers as tasty food alternatives. For example, Chapul Company from USA produces tasty bars that include cricket flour and these bars are offered to the customers with different flavors like chocolate-peanuts and Matcha tea-banana-organic tahini (Chapul Inc., n.d.). Similar with Chapul Company, Exo Company in USA also produces cricket flour based protein bars with different flavors such as apple-cinnamon and blueberry-vanilla (Exo Inc., n.d.). Moreover, Bitty Foods and Six Foods Companies in USA, offers Cricket chips to the customers with different aromas (Bitty Foods, n.d.; Six Foods, n.d.). In addition to insect based food producers in the United States, various products have been started to be produced from edible insects in Europe. In Belgium, Goffard sisters produce wheat pasta enriched with a flour of mealworms (*Tenebrio Molitor* or *Alphitobius Diaperinus*) with a brand name of Aldento (Goffard sisters, n.d.). Also, Micronutris within Eap Group established the first French insect farm to grow mealworms and crickets and they started to produce biscuits, crackers, pasta, chocolate and macarons from those edible insects (Micronutris, n.d.). Additionally, Proti-Farm in the Netherlands produces insect ingredients in high quantities and qualities in order to use in the food and pharmaceutical industry (Proti-Farm, n.d.)

Figure 1.1. Examples of insect products from food industry

1.1.6. *Acheta domesticus* as a nutritional food source

Acheta domesticus is an insect which belongs to the Orthoptera order and generally referred as house cricket. Its origin is based on the Southwestern Asia, but widely available all over the world (Walker, 2007). It is produced in high amounts in Thailand for eating purposes and especially preferred due to its good texture quality and taste. According to the study conducted in Thailand in 2002, there were cricket farms located in 53 of 76 provinces (Yhoung-Aree and Viwatpanich, 2005). Additionally, in the study performed in 2012, it was found that there were around 20,000 cricket farmers living in North-eastern Thailand (Hanboonsong, Jamjanya and Durst, 2013). Since, *Acheta domesticus* has a short life cycle, its farming is also cost-efficient (Van Huis, 2013).

In an adult *Acheta domesticus*, the protein content changes between 64.4 % - 70.8 %, the lipid content is ranged from 18.6 % - 22.8 % and fiber content varies between 16.4% and 19.1% in a dry matter. Linoleic, oleic, palmitic and stearic acids are the most abundant fatty acids found in an adult cricket. Yet, there are trace amounts of palmitoleic, myristic, and linolenic acids existing in these species. Furthermore, they are mostly rich in potassium and phosphorus and include high amount of vitamin A, B complex, C and E (Mariod et al., 2017).

Figure 1.2. Acheta domesticus

Because of their high protein content, adult house crickets are also rich in essential and non-essential amino acid. They contain around 396.8 mg /g protein, essential amino acids, 412.8 mg/g protein, non-essential amino acids. Also, number of sulfur containing amino acids which are Met + Cys, isoleucine,

leucine, lysine in house crickets are about 228.9 mg/g crude protein (Rumpold & Schlüter, 2013). The detailed amino acid composition of adult cricket is shown in Table 1.1.

Currently, house cricket adults are consumed as protein powder or protein extracts, as well as mostly being enjoyed as deep-fried snacks.

Table 1.1. *Aminoacid content of mealworm (larvae) and cricket. (Finke, 2002)*

Type of amino acid	Amino acid (g/kg)	Mealworm (larvae)	Cricket (adult)
Hydrophobic	Glycine	10.4	10.4
	Alanine	15.4	18
	Valine	11	10.7
	Leucine	19.9	20.5
	Isoleucine	9.4	9.4
	Methionine	2.4	3
	Proline	13	11.5
	Phenylalanine	6.6	6.5
	Tryptophan	1.5	1.3
Hydrophilic	Tyrosine	13.7	10
	Serine	9.6	10.2
	Threonine	7.7	7.4
	Cysteine	1.6	1.7
	Glutamic acid	21.1	21.5
	Aspartic acid	15.2	17.2
	Lysine	10.2	11
	Histidine	5.9	4.8
	Arginine	9.7	12.5

1.1.7. *Tenebrio molitor* as a nutritional food source

Tenebrio molitor is an insect which belongs to the order of Coleoptera and commonly called as mealworm or yellow mealworm. It is the larvae of darkling beetles and nutrient composition differs from other metamorphosis stages as it is in other insect types. Effectiveness of yellow mealworms in biological transformation of organic wastes and their ability of converting about 1.3 billion tons of bio-waste annually, have played a significant role in recent years. (Veldkamp et al., 2012). It has been suggested that, insect farming can be more lucrative if feeds used for growing insect species are replaced with those organic side streams. (Offenberg, 2010). Yet, this movement is not allowed by the food and feed legislation for now.

The larval stage is the most variable stage in the life cycle of these species and it varies between 3 and 18 months. Also, development time of larval stage is highly influenced by temperature and feed. Therefore, feeding the mealworms not only with wheat bran but also with variety of vegetables is very important in order to increase the possibility of survival of larvae and feed conversion rate and decrease the larval development time. (Makkar et al., 2014; Cortes Ortiz et al., 2016)

Figure 1.3. Life cycle of mealworms (Life Cycle, n.d.)

Like other insect species, mealworm is also known as a valuable protein source because it contains essential amino acids like, leucine, isoleucine and lysine (Ravzanaadii et al., 2012). Also, its essential amino acids like isoleucine, leucine, valine, tyrosine, and alanine are found in higher amounts, even more than beef.

(Sun-Waterhouse et al., 2016). In Table 1.1, amount of amino acids found in cricket and their comparison with mealworm larvae is demonstrated in detail.

Paul et al. (2017) states that mealworms can also be counted as a novel lipid source since lipids are the second important and supreme part of yellow mealworms and it covers around 33% of dry matter. (Paul et al. 2017). The most abundant fatty acids found in mealworms are oleic, linoleic and palmitic acids. (Ravzanaadii et al., 2012).

In addition, there are significant amount of calcium, zinc, and magnesium in *Tenebrio molitor* (Nowak et al., 2016). However, they are not rich in calcium since there is no internal skeleton in these species. (Hunt, Ward & Ferguson, 2001).

1.2. The way forward for characterization of insect powder

1.2.1. Solvent-based fat extraction

Fat extraction is a prior step before the processing of insect flour. Removal of fat portion of insects during investigation of protein properties and production of insect-based food products decrease the sticky structure of the protein concentrate and protect against undesirable oxidation of fatty acids (Van Huis et al., 2013). Defatting process is not only used to characterize extracted lipids but also used to enhance functional properties and characteristics of proteins (L'Hocine et al., 2006).

Despite the presence of several fat extraction choices such as super critical CO_2 or aqueous extraction, defatting with hexane is the best alternative with 96 % rendement or more (Ricochon and Muniglia, 2010).

Solubility of protein is also an important factor for the selection of solvent used for fat extraction. Pace et al. (2004) stated that most of the proteins became unstable and slightly soluble in polar solvents such as ethanol. On the other hand, proteins tend to make folded structure which made them mostly insoluble in non-polar solvents like cyclohexane. Since hexane is also a non-polar organic solvent, it was used in this project to minimize protein loss during fat extraction.

1.2.2. A non-thermal novel method for processing insect powders: High Hydrosatic Pressure (HHP)

In 1883, it is found that there may be an effect of high pressure on organisms by Certes, but the first work that displays the effect of high hydrostatic pressure (HHP) on foods was at the end of the 19th century and done by Bert Hite (1899) and his associates with the purpose of the preservation of milk for shelf life extension by increasing the value of pressure up to 650 MPa at West Virginia University (Chawla et al., 2010 & Elamin et al., 2015). The engineering perspective, especially the compressibility, of the HHP was studied by Percy W. Bridgman (Bridgman, 1909). Bridgman also investigated the thermal conductivity, phase change and polymorphic transmission (Bridgman, 1923; Bridgman, 1914 & Bridgman, 1912). Later on, the pressure was used on fruits and vegetables with the intent of preservation in 1914. However, the development and utilization of the method was not considerable until 1980s (Elamin et al., 2015). After some time, the HHP treatment reappeared with the increasing seeking to an alternative preservation technique to thermal processing in food industry and with the successful development of commercial HHP in middle of the 1980s. Later, 1992 became the year when HHP technology made a revolutionary breakthrough in Japan with the presentation of the first pressurized product, jam, into the market (Knorr, 1993). Since then, the HHP technique has been developing day by day and being used effectively in food industry.

In today's world, HHP application has become popular and preferred especially in the products with high water content such as fishery, meat and dairy products, fruit juices, vegetable juices, jams, smoothies and dips. The degree of application depends on the purpose; spoilage control, pathogen control, organoleptic preservation, product reformulation, product foaming or shellfish shucking. HHP reduces the microbial load and eliminates the pathogens like *Listeria monocytogenes* to the safe levels (Alpas et al., 1999). On the contrary to the thermal treatment techniques, HHP preserves the organoleptic properties and improves the characteristics of products (High Pressure Processing of Foods, 2015). Moreover, HHP is applied on antigens and antibodies to inactivate the viruses and tumors in vaccine development (Murchie et al, 2005). Briefly, the common usage of HHP in food industry is for pasteurization, pressure assisted thermal processing, pressure ohmic thermal sterilization, high pressure freezing and thawing, high pressure homogenization and dense phase CO_2 (Balasubramaniam et al., 2015).

The main benefits of the HHP application are minimizing thermal degradation while retaining the natural antimicrobial systems, nutritional value, aroma and flavor in significant degrees because HHP does not break the covalent bonds up to 1000 MPa, enable uniform and instantaneous transmission and can create new functional properties, texture and taste (Huang et al., 2017 & Yaldagard et al., 2008). Other significant advantages of HHP application are being a clean technology and independent of time and mass and needing less process time (Parekh et al., 2017 & Yaldagard et al., 2008). Besides these advantages, the pressure resistant enzymes and bacterial spores and high installation cost could be the possible disadvantages of HHP (Yaldagard et al., 2008).

The principle behind HHP is the isostatic pressure, that is the equal application of pressure to the all the parts of sample, and the pressure increases in the sample

thanks to the compression of the surrounding liquid (Orlien, 2017). The application of HHP is uniform and the ultra-high pressure is applied in a few minutes through the all sides of product without any damage and distortion on food samples (Elamin et al., 2015). The main purposes of HHP, or cold isostatic pressure, are extending the shelf life by reducing the number of microorganisms and retarding or stopping the enzyme activity (Lee et al., 2011). Besides these, the degradation of vitamins and the formation of possible off flavors in thermal treatment are removed by using high pressure (Rastogi, 2013). During the process, the food samples are exposed to high pressure in the range of 100 – 1000 MPa which is higher than the pressure in deep seas (Ginsau, 2015 & Parekh et al., 2017). The temperature is adjusted from -20^{o}C to 100^{o}C depending on the process and the nature of food, and the time may range from a couple of seconds to more than 20 minutes (Yaldagard et al., 2008 & Parekh et al., 2017).

The pressure transmitting fluid is used with the purpose of transmission of pressure to the samples in the pressure vessel uniformly and instantaneously and the most common one is water for industrial usage. Besides water; glycol, mixture of glycol and water, silicone oil, castor oil or sodium benzoate oil may be used for pilot scale equipment as alternatives. The selection of the fluid is done according to its properties of corrosion prevention, viscosity change under the effect of pressure, heat compression, and the endurance to seal under the effect of pressure (Balasubramanian & Balasubramaniam, 2003; de Heij et al., 2003; Matser et al., 2004; Otero & Sanz, 2003).

The compression against the intermolecular forces causes an increase in temperature of both product and fluid for every 100 MPa in the pressure vessel; which also known as adiabatic heating;

$$\frac{dT}{dP} = \frac{T\alpha_p}{\rho C_p} \quad \text{(Eq. 1)}$$

In Eq. 1, temperature increase (dT) depends on the volumetric expansion coefficient (α_p), density (ρ), initial temperature (T) and the heat capacity of the sample (C_p) (Juliano et al., 2009; Denys, Van Loey, & Hendrickx, 2000). The temperature of all materials, which are compressible, changes under the effect of compression (Patazca et al., 2007 & Ting et al., 2002).

Both the pressure and heat are the thermodynamic variables; that is, the temperature effect cannot be considered without the pressure effect. While the pressure affects the product volume first, thermal effects influence both volume and energy change during the processing (Balasubramaniam et al., 2015). The combination of both thermal and pressure effects can be considered as antagonistic, synergistic and additive (Gupta et al. 2011). The relation between pressure (P) and temperature (T) is determined with Gibbs equation where S and H are entropy and enthalpy, U is internal energy and V is volume (Balasubramaniam et al., 2015);

$$G \equiv H - TS$$

$$H \equiv U + PV$$

$$d(\Delta G) \equiv \Delta V dP - \Delta S dT$$

The behavior of the food sample under the influence of high pressure is explained with the principles of Le Chatelier, isostatic pressing and microscopic ordering (Elamin et al., 2015 & Balasubramaniam et al., 2015). The principle of Le Chatelier supports the idea that in chemical reactions under equilibrium condition,

the phase transition or the molecular configuration change is accompanied by a volume decrease which is amplified by pressure (Chawla et al., 2010 & Elamin et al., 2015). The isostatic pressing principle is that the pressure transmittance is instantaneous and uniform regardless of the size and geometry (Ramaswamy et al., 2004). The microscopic ordering principle is explained as the degree of molecules ordering increases with increasing pressure at a constant temperature, so the effect of temperature and pressure show converse behavior on chemical reactions and molecular structure (Balny & Mason, 1993).

The equipment of a standard HHP system are pressure vessel (thick-wall cylinder), two end closures to cover the cylindrical pressure vessel, pressure relief valve, yoke (structure for restraining end closures under the effect of pressure), high pressure pump and intensifier used so as to target pressure generation, process control and instrumentation, and a system to handle the load and product removal (Balasubramaniam et al., 2015).

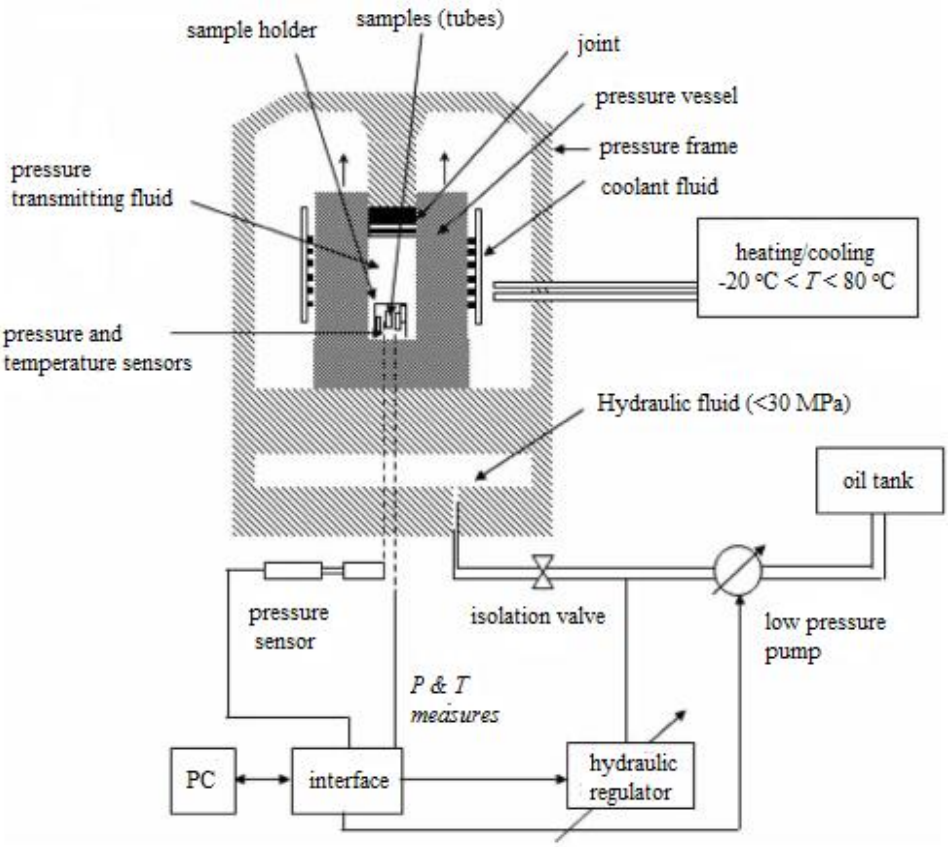

Figure 1.4. Schematic diagram of high pressure equipment (Buzrul et al., 2007)

The mechanism or operation of the HHP is investigated under three different systems; batch, continuous and semi continuous. In batch systems, the food product, either solid or liquid, is loaded to the pressure vessel and the pressure transmitting fluid is pumped to the vessel isostatically with the aim of air removal (Elamin et al., 2015). Then, the pressure relief valve is closed and pressure is allowed to apply; after the desired pressure is achieved, the pressure relief valve is opened to remove compression on pressure transmitting fluid (Chawla et.al, 2010 & Elamin et al., 2015). In continuous systems, only the liquid food products are treated with high pressure (Balasubramanium et al., 2015). The products are pressurized 100 MPa or more via the high pressure intensifiers in an open ended tube system, in the end, the product is depressurized and decompressed. While the fluid is passing through the pressure release component, significant amount of

heat is generated due to the frictional effects, shearing and cavitation, so it is difficult to analyze the effect of thermal process and pressure in terms of microbial inactivation (Cavender, 2011). Because of this dilemma caused by the high shear forces and frictional heating, the continuous system is substituted by semi continuous system (Elamin et al., 2015). In semi continuous systems, two or more pressure vessels, which contain a free moving divider piston so as to separate product and pressurizing fluid, are adapted to the system (Balci & Wilbey, 1999; Ting, 2011). The system provides continuous product output; when one vessel discharges the product, it passes the second system and is pressurized, and continues with up to the number of vessels (Balasubramaniam et al., 2015 & Elamin et al., 2015). The process ends up with the decompressing of the food products by depressurizing of the vessel (Hogan et al., 2014).

The effect of HHP treatment on proteins may be both beneficial and adverse. The proteins are stabilized by covalent bonds, hydrogen bridges, electrostatic interactions and hydrophobic interactions. All the stabilization mechanisms mentioned may be affected by pressure in different ways. It is hard to break the covalent bonds in proteins by HHP; in other words, the primary structure of proteins stays durable under the effect of high pressure (Mozhaev et al., 1994). However, the secondary structure consisting of electrostatic interactions, the tertiary structure including hydrogen and hydrophobic bonding and the quaternary structure through hydrophobic interaction are affected from high pressure treatment (Goyal et al., 2013). The dissociation of oligomeric structures, unfolding of monomeric structures, aggregation of proteins and gelation of protein can be observed if the pressure is higher than 100-200 MPa (Cheftel, 1995). The most sensitive structures of proteins are tertiary and quaternary under the effect of high pressure beyond 100 MPa (Chapleau et.al, 2003). The secondary structure is also affected from pressure but not much as the tertiary and quaternary, for example β sheets, which are more stable than α helices, remain unchanged under the effect of HHP (Winter & Dzwolak, 2005). In milk proteins, the changes

between 100 MPa and 300 MPa are reversible, but the denaturation occurs above 300 MPa as irreversible (Rastogi et.al, 2007). Whether the pressure is in the levels above 700 MPa, the secondary structures are destroyed irreversibly (Balny & Masson, 1993). The tertiary structure including the hydrophobic and ionic interactions is easily disrupted at about 200 MPa, while the distortion of quaternary structures including non-covalent bonds occurs at about 150 MPa (Hendrickx et.al, 1998; Balci & Wilbey, 1999). In brief, the most of the proteins denatures at the pressure above 400 MPa; however, some small molecules such as vitamins, flavors and amino acids may survive due to having simple structures (Balci & Wilbey, 1999). Due to being non-disrupted on covalent bonds of small molecules, it may guarantee the conservation of nutrients and provide high quality products (Tedford et al., 1998). The change under the high pressure of the functional properties of proteins, which are hydrogen related and surface related, usually depends on the conformation (Morr & Ha, 1993).

1.3. Characterization of insect powders

1.3.1. Determination of protein content

Estimation of protein content is done by using various methods including Kjeldahl method and Lowry method which are the main focus of the study. Both methods were discussed in the following sections in detail.

1.3.1.1. Kjeldahl Method

The Kjeldahl method is used to determine the nitrogen content in both organic and inorganic samples; thus, the protein content is found from the content of nitrogen in the sample. (Sáez-Plaza et al., 2013; Nitrogen Determination by Kjeldahl Method, 2018). The royalty of the method belongs to a brewer lived in 18th century, Johann Kjeldahl, the developer of the Kjeldahl method (Kjeldahl, 1883).

The method has been used since that time, 1883, in order to determine the nitrogen amount in any food samples such as drinks, meat, cereals and feeds; also wastewaters and soils (Sáez-Plaza et al., 2013; Nitrogen Determination by Kjeldahl Method, 2018). A sample, in this case is food, is digested with a strong acid; thus, the sample releases nitrogen and the amount of nitrogen is determined by a titration procedure (Analysis of Proteins, n.d.). Afterwards, the protein content is determined with the related equation improved attentively. The method is still used nowadays with only some improvements in terms of increasing the speed of the procedure and obtaining the more accurate results (Sáez-Plaza et al., 2013). The Kjeldahl method is accepted as standard method for determination of the nitrogen content, so the protein content of any sample (Analysis of Proteins, n.d.). The only deficit of the method is that the Kjeldahl method does not measure directly the protein content, so a conversion factor (F) is necessary to find the protein content via the nitrogen content (Moore et al., 2010).

The procedure of Kjeldahl method consists of three main steps; digestion, distillation and titration (Saha et al., 2012). All of the nitrogen bonds in the sample are broken and the organically bonded nitrogen converts into ammonium ions (NH_4^+) during digestion steps; thus, the organic material carbonizes and seems as foam. In the end, the foam decomposes and the chemical reaction is completed with obtaining the clear liquid. The sample is mixed with sulfuric acid between 350^{o}C and 380^{o}C to achieve this purpose. The higher temperature means the faster digestion; also, the digestion process is speeded up by salt and catalyst addition. The potassium sulfate has a role in increasing boiling point of the sulfuric acid in the mixture and the catalyst has a role in increasing the digestion step's efficiency by increasing the speed (Nitrogen Determination by Kjeldahl Method, 2018).

$$\text{Protein (-N)} + H_2SO_4 \longrightarrow (NH_4)_2SO_4 + CO_2 + H_2O$$

The digestion step is done and the sample is allowed to cool; later on the Kjeldahl method continues with the distillation step. During this step, the conversion of the ammonium ions (NH_4^+) into the ammonia (NH_3) takes place with the addition of alkali (NaOH), and ammonia is obtained by the steam distillation in a receiving vessel which is full of an absorbing solution to capture the dissolved ammonia gas (Nitrogen Determination by Kjeldahl Method, 2018).

$$(NH_4)_2SO_4 + 2NaOH \longleftrightarrow 2NH_3\,(gas) + Na_2SO_4 + 2H_2O$$

The absorbing solution commonly involves aqueous boric acid, $B(OH)_3$ at the concentration of 2-4%. Besides, the precise dose of sulfuric acid or hydrochloric acid can be used with this purpose instead of aqueous boric acid (Nitrogen Determination by Kjeldahl Method, 2018);

$$B(OH)_3 + NH_3 + H_2O \longleftrightarrow NH_4^+ + B(OH)_4^-$$

$$H_2SO_4\,(total) + 2NH_3 \longrightarrow SO_4^{2-} + 2NH_4^+$$

After distillation step is done, the concentration of the captured ammonium ions is determined with two different titration ways. If the boric acid is used as the absorbing solution, the acid-base titration is applied by using the sulfuric acid or hydrochloric acid's standard solutions and indicator. The concentration of the titrant varies in the range of 0.01N to 0.5N according to the amount of ammonium ions present in the solution; that procedure is called as direct titration (Nitrogen Determination by Kjeldahl Method, 2018);

$B(OH)_4^- + HX \longleftrightarrow X^- + B(OH)_3 + H_2O$, where HX is strong acid

If the sulfuric acid or hydrochloric acid is used as the absorbing solution, the non-reacted sulfuric acid is titrated with NaOH standard solution, and the difference gives the ammonia amount; that procedure is called as back titration (Nitrogen Determination by Kjeldahl Method, 2018);

$$H_2SO_4 \text{ (total)} + 2NH_3 \longrightarrow SO_4^{2-} + 2NH_4^+$$

After all steps, crude protein content is determined with the specified formula and conversion factor.

1.3.1.2. Lowry Method

The development of Lowry method is described by the Lowry in 1951 (Lowry et.al, 1951). It is one of the most accurate, valuable and sensitive methods that determines the protein content (Waterborg, 2009). The advantage of the Lowry method is the detection of the absolute protein concentrations (Sapan et.al, 1999). Besides, the method is a strong alternative to others in the circumstances of determination the protein mixtures or crude extracts whether the sensitivity of method is reasonably steady from protein to protein or not (Lowry et al., 1951). The Lowry method is a colorimetric assay. It is based on the reaction between copper source, sodium carbonate, sodium potassium tartarate and Folin Ciocalteu's phenol reagent. (Lowry et al., 1951). A modification of Lowry method is defined to maximize the sensitivity and decrease the incubation time by using more concentrated reagents (Frolund et al., 1995).

The copper ions interact with protein through the peptide bonds, and then the addition of Folin Ciocalteu's phenol reagent triggers the Folin–Protein binding

under alkaline conditions. At the complex, reduction occurs slowly; thus, it appears a color change as yellow to blue (Hansen, 2001);

$$Cu^{2+} + \text{Protein (Peptide Bonds)} \rightarrow (Cu^{1+}\text{-Peptide Bond Complex})$$

$$\text{Folin-Phenol Reagent} + (Cu^{1+}\text{-Peptide Bond Complex}) \rightarrow \text{Reduced Folin Phenol Reagent}$$

Then, the absorbance of complexes is measured at 660 nm using the spectrophotometer against control that do not include any protein inside. After that, the value of absorbance converted into the percentage of the protein amount by using bovine serum albumin as the reference sample (Niamke et al., 2006).

As one of the most used method, Lowry determines the protein amount in both biological and chromatographic solutions as mentioned; nevertheless, the distribution of the other molecules of that kind of habitats or environments is heterogeneous, and they may react with proteins or reagents. It is considered as the only weakness of the Lowry method, because the results are affected in protein content estimation if a reaction occurs in those conditions (Niamke et al., 2006).

1.3.2. Water binding capacity

One of the significant property of proteins is their interaction ability with the surrounding water due to effect of the water on gel formation, dissolving, swelling and action as stabilizers in emulsions, so it is necessary to understand the interaction between water and proteins (Chou & Morr, 1979; Zayas, 1997). The water binding capacity (WBC) of proteins determines the quantity of water that

proteins holds; in other words, the ability to hold water against the gravitational force physically or physicochemically (Zayas, 1997).

The importance of the WBC amount is that, the higher values of WBC, the higher maintenance on the moisture content of product, because the moisture content has a significant effect on the product's quality (Köhn et al., 2014; Wang et al., 2006).

WBC also plays a significant role in the texture of the food; besides, the WBC of the protein additives in various foods affects the finished product's quality characteristics. The rate of binding water can help to determine the usage form of protein such as powder and rehydrated before added to the mixture; thereby, the composition of developed packaging material is affected from the interaction between moisture and protein (Zayas, 1997).

The considerable amount of water, thanks to its dipolar characteristics, interacted with the proteins is retained by H-bonds via the polar hydrophilic groups of proteins and the H-bonds holds the structural water and polypeptide groups of proteins together. The amount of polar groups in polypeptide chain specifies the protein capacity in terms of retaining moisture; besides, the composition of amino acid of a protein give an idea about the WBC (Zayas, 1997).

There are various methods used in industry to determine the WBC of proteins in literature. The most common method that used with the purpose of WBC determination of proteinaceaus particles is that to make a dispersion of those particles in excess water and centrifuge them (Berghout et al., 2014; Ige et al., 1984; Wang & Kinsella, 1976; Yu et al., 2007). At the end of the centrifugation period, the supernatant is discarded, the retained water is quantified by weighting

the remained pellet and the WBC of the pellet is determined with the Eq. 2 (Peters et al., 2016);

$$\text{WBC (g } H_2O\text{/g dry matter)} = \frac{m_{wet} - m_{dry}}{m_{dry}} \quad \text{(Eq. 2)}$$

m_{wet} and m_{dry} describe the pellet weight after centrifugation and the weight of dry sample, respectively; thus, the specific meaning of the water binding capacity appears as a protein sample's ability to bind water under the subjection of external force (Peters et al., 2016).

1.3.3. Oil binding capacity

The interaction between oil and protein is a significant functional property due to the effect on the texture and the quality of the finished food products. The significance of the ability of protein on oil absorption or oil retention and interaction with lipids in foods creates a relation in terms of emulsification, flavor absorption and dough preparation. The oil binding can be expressed as the physical entrapment of oil by proteins (Zayas, 1997). There must be a relation to establish a bond between two sides which are oil and protein, and that bond is directly related with the protein content. The primary interaction is the physical entrapment of the oil because of the proteins' chemical modifications resulting with increased bulk density. The smaller particle size of protein powders with low density provides more oil adsorption or entrapment than the high density ones (Zayas, 1997).

The mechanism of oil binding is based on the protein material microstructure at first (Kinsella, 1979). Thereafter, it is explained that the connection between oil

and proteins depends on the nonpolar site chains of proteins. The protein molecules' nonpolar site chains are the first connected sites of oil-protein interaction, so the protein concentration and the amount of nonpolar sites directly affect the oil-protein interaction (Kinsella, 1979; Sathe et al., 1982).

Oil binding capacity (OBC) is higher if the protein is insoluble and hydrophobic and there is a correlation between the protein surface hydrophobicity and protein solubility; in other words, it is difficult to remove the tightly bound oil from protein surface or hydrophobic site (Zayas, 1997).

The determination of the amount of oil bound to the protein is done by addition of the oil to the protein, and continues with the centrifugation period. After the centrifugation, the supernatant is separated. The retained oil is quantified by weighting the remained pellet and the OBC of the sample is determined with Eq. 3 (Schwenke, 1981).

$$\text{OBC (g oil/g dry matter)} = \frac{m_{oily} - m_{initial}}{m_{initial}} \quad \text{(Eq. 3)}$$

m_{oily} and $m_{initial}$ describes the pellet weight after centrifugation and the weight of dry sample, respectively.

1.3.4. Determination of total phenolic content

The Folin-Ciocalteu (F-C) assay is one of the methods used for the measurement the total phenolic content (TPC) in products and is the simplest method used for this purpose (Agbor et.al, 2014). However, the development of this method is

based on the early 19th century. Folin and Denis aimed to determine the tyrosine and tryptophan in proteins to measure the protein concentration in 1927 (Sánchez-Rangel et al., 2013). The composition of the Folin-Denis (F-D) reagent consists of sodium molybdate and sodium tungstate. When there is interaction between phenols and F-D reagent, blue color appears which absorbs at 765 nm, and it is thought that the blue color appears because of the existence of Mo (W) species (Walker et al, 2010). While both F-C and F-D are based on the reaction between oxidant reagent and the amino acids of tyrosine and tryptophan; the difference between them is the existence of Mo (Molybdate) in the reagent composition in F-D assay. Due to the existence of white precipitates in F-D assay, Folin and Ciocalteu prevents that precipitation by increasing the amount of Mo, so the sensitivity and reproducibility of F-C assay is much more than the F-D assay (Sánchez-Rangel et al., 2013). Later, a modification applied on the F-D assay is used to measure the protein concentration, which is Folin-Lowry (F-L) assay. Tryptophan is non-phenolic and reactive approaching the reagent and it contributes approaching the absorbance of protein in F-L assay (Walker et al, 2010).

F-C phenol reagent includes a mixture of the heteropoly acids, phosphotungstic and phosphomolbytic acids where the Mo (molybdenum) and W (tungsten) are in the 6+ oxidation state; when the reaction occurs between reagent and a reductant, the Mo blue and W blue are formed and the metals' mean oxidation state become between 5 and 6 (Agbor et.al, 2014);

$$Na_2WO_4/Na_2MoO_4 \text{ yellow} \rightarrow (\text{Phenol-}MoW_{11}O_{40})^{-4} \text{ blue}$$

$$Mo^{6+} \text{ (yellow)} + e^{-1} \rightarrow Mo^{5+} \text{ (blue)}$$

$$Mo^{5+} + e^{-1} \rightarrow Mo^{4+} \text{ (blue)}$$

$$ØOH \rightarrow ØO^{\cdot} + H^{1+} + e^{-1}$$

$$ØO^{-1} \rightarrow ØO^{\cdot} + e^{-1}$$

The above mechanism is slow at acidic pH and fast when alkaline and is highly sensitive and exact, but the specificity is insufficient. This is why; an improved method is used with a reagent named as molybdotungstophosphoric heteropolyanion ($3H_2O$-P_2O_5-$13WO_3$-$5MoO_3$-$10H_2O$) that reduces the polyphenols in a way and makes the method being more specific. (Singleton & Rossi, 1965)

1.3.5. Determination of antioxidant activity

Estimation of the antioxidant activity is done by using various different methods including CUPRAC (Cupric Ion Reducing Antioxidant Activity) assay and DPPH (2,2-di(4-tert-octyl-phenyl)-1-picrylhydrazyl) Radical Scavenging Methods. Both methods will be discussed in the following sections in detail.

1.3.5.1. Antioxidant activity with DPPH˙ Radical Scavenging Method

The method called as DPPH˙ (2,2-diphenyl-1-picrylhydrazyl) is used with the aim of determination of the antioxidant activity (AA) and total antioxidant capacity (TAC) by using a stable free radical α, α-diphenyl-β-pipcrylhydrazyl. The method is rapid, simple, accurate, economic and widely used to evaluate radical scavenging activity of antioxidant and antioxidant activity in foods, and to measure the ability of compounds to act as free radical scavengers (or hydrogen/electron donors) (Kedare & Singh, 2011).

DPPH is a stable radical in the solution and the appearance of the color is purple absorbing at 515 nm in methanol. The mechanism of action of DPPH is based on the principle that 2,2-diphenyl-1-picrylhydrazyl (DPPH˙) on accepting H (hydrogen) atomfrom scavenger molecule, in this case the scavenger molecule is

antioxidant. The interaction results with the reduction of DPPH˙to DPPH2; thus, the purple color turns to yellow with accompaniment decrease in absorbance at 515 nm (Mishra et al., 2012). The color change is observed by the spectrophotometry.

The efficiency of antioxidants is measured at ambient temperature; hence, it eliminates the thermal degradation risk of molecules. However, the reaction between DPPH and antioxidant also depends on the antioxidants' structural conformation; while some compounds react rapidly with DPPH, some react slowly (Bondet et al., 1997). The reaction of DPPH reacted with antioxidant follows this manner;

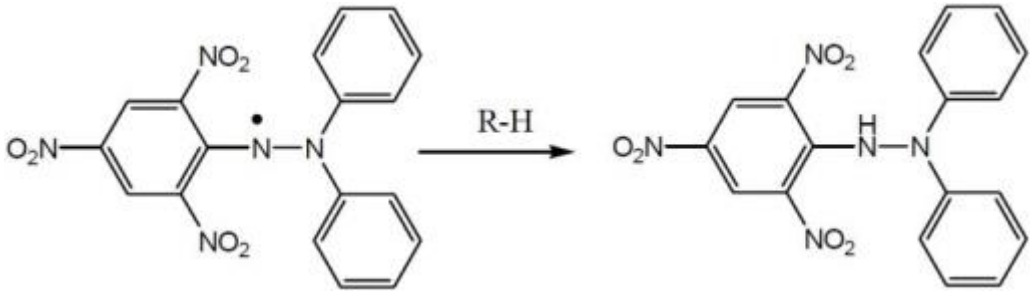

Figure 1.5. Reaction of DPPH with antioxidant

1.3.5.2. Antioxidant activity with Cuprac Assay

CUPRAC method is one of the novel methods used for determination of the total antioxidant capacity (TAC) based on an electron transfer mechanism called as cupric ion reducing antioxidant activity. It is a simple and versatile method which is useful for determination of the polyphenols comprising phenolic acids, flavonoids, carotenoids, hydroxycinnamic acid, anthocyanins, vitamin C and E (Özyürek et al., 2011). In addition to being a simple method, CUPRAC is advantageous over the similar methods like FRAP (ferric reducing antioxidant power) due to the promising kinetics and selectivity in the redox chemistry of copper (II) (Armstrong, 2008).

Cu(II)-Nc (the bis (neocuproine) copper (II) cation) is used as chromogenic oxidizing reagent and acts as an outer sphere electron transfer agent while the Cu(I)-Nc (the bis(neocuproine) copper (I) cation) is the CUPRAC chromophore which is formed by the reduction of the Cu(II)-Nc. The Cu(I)-Nc activity is optimum at pH 7 and the chelate of Cu (I) is formed with the reducing polyphenols, in this case antioxidants by redox reaction; and is measured spectrophotometricallyat 450 nm. The reaction of antioxidant and the reagent of Cu(II)-Nc follows this manner (Özyürek et al., 2011);

n $Cu(Nc)_2^{2+}$ + n-electron reductant (AO) $\longleftrightarrow$ n $Cu(Nc)_2^{+}$ + n-electron oxidized product + n H^+

$$n\ Cu(Nc)_2^{2+} + Ar(OH)_n \longrightarrow n\ Cu(Nc)_2^{+} + Ar(=O)_n + n\ H^+$$

As seen in the reaction above, polyphenolic antioxidants are oxidized to the corresponding quinone and the chelate of Cu(I) is formed as the reduction product which shows maximum absorption at 450 nm (Apak et al., 2007).

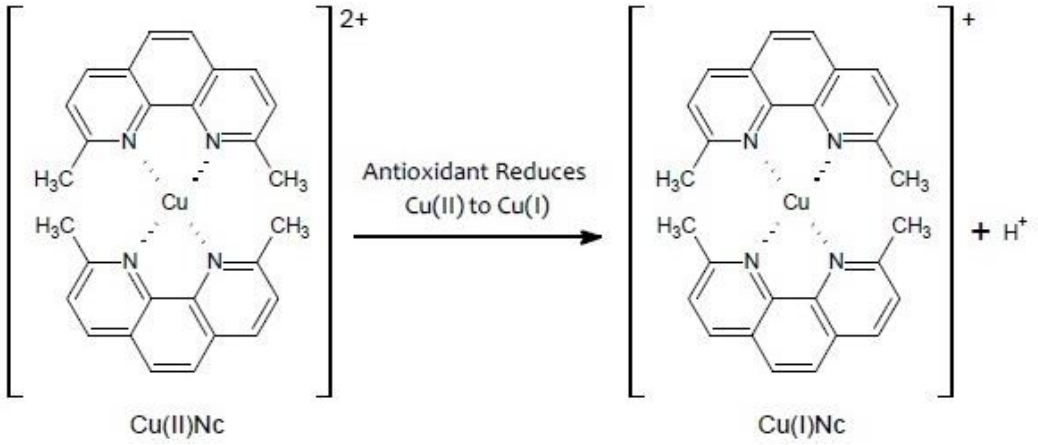

Figure 1.6. Reduction of neocuproin-copper (II) in the presence of antioxidant

1.3.6. Gelling Behavior with Nuclear Magnetic Resonance (NMR) Relaxometry

The method of nuclear magnetic resonance (NMR) was discovered in the 20th century by using simple compounds with the principle of the magnetic properties of certain nuclei exploitation after the World War II (Bloch et al., 1946; Purcell et.al, 1946; Ronczka & Müller-Petke, 2012). Before the discovery of NMR method, it was thought on the permanent magnetic moment of atomic nucleus, but the only studies were done on molecular beam in 1937 by Rabi (Hoffmann, 2012). With the development of the first borehole, the detection and quantification of water directly became possible due to the association NMR signals' initial amplitude to the exiting protons amount (Brown & Gamson, 1960; Seevers, 1966). The experiments on NMR has been applied in continuous wave mode until 1950s; the principle of continuous wave mode is that the irradiation of a sample with radio frequency field at constant intensity which is also the basis of Fourier transform NMR spectroscopy (Hoffman, 2012). The years between 1950 and 1980, the improvements on NMR technology came with the developments on high resolution NMR spectroscopy, dipolar spin-spin coupling in solids by fast spinning (Andrew et al., 1958; Lowe et al., 1959; Hartman & Hahn, 1962); Fourier transform NMR in liquid (Ernst & Anderson, 1966) and 2-D spectroscopy (Aue et al., 1976). The determination of spatial structure of large molecules was realized with the application of 2-D NMR spectroscopy in the beginning of 1980s (Wütrich, 1986; Cavanagh et al., 1996). The evolution of NMR has continued until the discovery to today's science.

NMR is based upon the atomic nucleus's intrinsic magnetic dipole moment, which is produced by a spin due to the finite angular momentum of most atomic nucleus in the ground states (Hoffmann, 2012). Many biophysical studies such as protein folding are done with the modern multidimensional and multinuclear NMR spectroscopy techniques (Krishna et al., 2004; Englander et al., 2007). The

structure of the complex food systems is determined by NMR, which is the most influential technique in structure determination; besides, the changes between the untreated and heat treated foods, the change in water mobility and water distribution in foods are detected by NMR without destructing to food (Karakıs et al., 2011; Parlak & Güzeler, 2016)

NMR relaxometry; a noninvasive, nondestructive, sensitive, informative and promising method, is used with the purpose of obtaining information on mobile protons and is a significant tool for pore size distributions of porous media, water uptake and content and redistribution (Ersus, et al., 2010; Oztop et al., 2012; Bayer et.al, 2010). For the physical and dynamical properties of a sample, NMR Relaxometry is the one with the primary attention on measuring nuclear spin relaxation times among two other subgroups of NMR, which are spectroscopy and imaging. The significant advantage of the NMR relaxometry is being convenient by itself; in other words, the relaxation analyses is done without using NMR spectroscopy or NMR imaging with the key attributes of simplicity, portability and cost (Cistola & Robinson, 2016). Unlike spectroscopy, the principle of relaxometry application to sample is based on using low field permanent magnets with relatively inhomogeneous fields (Cistola & Robinson, 2016).

The relaxation is that the initially hot nuclear spins cool down by releasing some magnetic energy to surroundings (Gerothanassis et al, 2002). The effectivity of relaxation between rotational (or vibrational) levels is high due to the fact that the rotational (or vibrational) states change via the molecules distortion during collision in general spectroscopic techniques, but the orientation change of nucleus is a difficult task because of the slower relaxation between nuclear spin levels in NMR. This is why the relaxation times of vibrational and rotational states are generally in the level of 10^{-4} and 10^{-9} seconds respectively whereas the

relaxation times of seconds are standard in nuclear magnetic resonance (Harris, 1994).

There are two considerable relaxation mechanisms which are both first order rate by their own time constant. First is T_1 relaxation (spin-lattice relaxation or longitudinal relaxation) which occurs due to the energy exchange between states of spin and surrounding medium, and the second one is T_2 relaxation (spin-spin relaxation) which occurs due to the energy exchange between separate nuclear spins. The T_2 relaxation affects the spin levels' relative energies while the T_1 affects the population lifetimes of spin energy levels (Gerothanassis et al., 2002). In other words; energy exchange between proton and sample lattice is determined by T_1 relaxation and while the T_1 value of the pure water is nearly 2.5 seconds long, the T_1 relaxation time of solids are shorter; the adjacent spins are determined with T_2 relaxation, so the T_2 relaxation is exceedingly interested with the sample microstructure in preference to lattice (Kirtil & Oztop, 2015). While the range of T_1 is between the tens of milliseconds and several seconds, the T_2 range is in between tens of microseconds and hundreds of milliseconds (Hoffmann, 2012).

The spin-spin relaxation, NMR signal and diffusion in inhomogeneous magnetic fields are determined efficiently by the Carr-Purcell-Meiboom-Gill (CPMG) sequence (Song, 2002). The CPMG sequence is attention-grabbing due to the production of long train echo signals enabling spin-spin relaxation (T_2) and diffusion constant efficiently by maximizing the signal/noise ratio (Carr & Purcell, 1954; Meiboom & Gill, 1958; McDonald, 1997; Eidmann et.al, 1996). CPMG measurements includes thousands of electromagnetic pulses to both record relaxation process densely and avoid diffusion dependent relaxation processes (Ronczka & Müller-Petke, 2012). The effect of the magnetic gradient, in which inhomogeneities influence significantly the relaxation process in x-y plane, is minimized by CPMG conduction of 180^0 pulses (Ronczka & Müller-Petke, 2012).

In industry, there is a tendency to higher field of NMR because of the higher ratio of signal to noise at higher fields; however, the studies on low field NMR have been started and lasted by several researchers in recent years (Volegov et al., 2010). The low field NMR has become feasible, effective, practical, applicable and preferred to perform magnetic resonance on the occasion of utilization of enclosures shielded magnetically, pre-polarization of magnetic field and advancement in SQUID (Superconducting Quantum Interference Devices) sensor technology which are magnetic flux detectors independent from frequency and permits the sensitive detection in pulsed field environment (Greenberg, 1998; McDermott, 2002; Volegov et al., 2010). The efficacy of the low field NMR on imaging increases with shifting to higher fields (Vaughan et.al, 1994). The static magnetic field is 1 T or less and operation frequencies are in the range of 10 MHz and 50 MHz for relaxation in low field NMR while the static magnetic field in the high field NMR is much higher than 1 T with the much higher frequencies (Low-field NMR, Fraunhofer LBF, n.d.).

The importance of water is that it exists as both bound and free state in a material (Kuntz & Kauzmann, 1974). Besides, if a strongly gel formation occurs, the water cannot release from the gel system. The mobility and structural properties of water molecules' different fractions that are partially immobilized by protein in the gel system can be estimated due to the fact that T_2 relaxation like molecular motion sensitive quantities are measured by low field pulsed NMR relaxometry (Kuntz & Kauzmann, 1974; Yasui et al., 1979). The longer relaxation time of water molecules causes more flabbily bounds to the macromolecules than the species including the shorter relaxation times (Yasui et al., 1979).

The dynamics and structure of the proteins are determined by the application of NMR (Kay, 2005; Cavanagh et al., 2006). In proteins, the NMR active nuclei are the spin 1/2 isotopes of ^{1}H, ^{13}C, ^{15}N and ^{31}P displaying magnetic dipole moments;

besides, the spin 1 of ^{2}H displaying magnetic dipole moments and quadrupole moments together. The isotopes mentioned here are in a non-disturbing mode at coveted locations in the protein; therefore, they provide local structure's and dynamics' site specific probes (Ohki & Kainosho, 2008; Ruschak & Kay, 2009).

NMR is an impressive method on studying the gelation mechanism and gel structure (Ahmad et al., 2007). The mobility of water and mobility of macromolecules are indicated by the spin-spin relaxation and spin-lattice relaxation times of water in food based systems through via the chemical exchange between proton of water and macromolecules' exchangeable proton (Baianu et al., 1982; Richardson et al., 1985; Matsukawa et al., 1999; Cornillon & Sallim, 2000; Hills et al., 2000; Okada et al., 2002; Choi & Kerr, 2003). The observed water proton spin-spin relaxation time (^{1}H T_2) decreases rapidly first, then increases with temperature decrease in temperature induced gelation systems. The initial decrease mirrors the polymers' motional restriction with the conformational change via the chemical exchange while the reason of the increase in latter is the temperature decrease due to the retardation of the chemical exchanging rate (Ablett et al., 1976; Zhang et.al; 2002). In a case of gelation, the protein surface contacted with water, where chemical exchange involves exchangeable proton, increases in raw samples including ordered protein structure at the denaturation time on heating and the protein surface is decreased by the aggregation of protein on subsequent heating. The number of exchangeable protons alters in terms of the change in protein surface; also the observed water ^{1}H T_2 is changed in terms of the change in protein surface. This reveals the importance of water ^{1}H T_2 on gelation mechanism (Ahmad et al., 2007). The water mobility and the structure of the protein is affected with the heat treatment; besides, the relaxation times are temperature dependent as mentioned. The water loss is because of the T_2 decrease, while the gain of water is because of T_2 increase, and heating the proteins in solution cause thermal denaturation, and so does protein unfolding (Lambelet et al., 1989; Han et al., 2014). Whether the ionic

strength and protein concentration is precise, the gelation occurs (Bertram et al., 2006). The value of T_2 decreases with gelation and this shows that the outer hydration layers is immobilized by gelation; hence, T_2 shortens, and water with shorter T_2 means lower mobility (Raun et al., 1999). Nevertheless, it was proposed in a study that for lower concentrations (1-10 g/L) of β-lactoglobulin (β-LG) solutions, there was no change in T_2 even after heating to 90°C. However, for higher protein concentrations (>10 g/L), heating to 70°C and above has caused a decrease in T_2 (Indrawati et al., 2007).

Paramagnetism is a key feature on the forward step of NMR Relaxometry experiments due to the possible occasions offered for proteins in terms of the paramagnetic metal ions such as Mn^{+2}, Fe^{+2}, Cu^{+2}, Ni^{+2}, Co^{+2} and several amount of lanthanide ions. The origin of paramagnetism comes from the unpaired electrons which are found in organic radicals and metal ions. Mn^{+2} and Fe^{+2} like paramagnetic ions are neutral in oxidation states in the reaction with water and amino acids (Otting, 2010). Paramagnetic ions include unpaired electrons that modify the relaxation times of proton, so they are probable intracellular contrast reagents.

The simple mechanism of paramagnetism nuclear relaxation is explained by two mechanism which are Solomon mechanism and Curie spin mechanism (Solomon, 1955; Gueron, 1975; Weiner, 1986). The contribution of Solomon mechanism with nuclear relaxation rates is dipolar mechanism that allows relaxation operated by electronic spin states' limited lifetime. In molecules that are slowly tumbling with electronic spin states' long lifetime, Solomon relaxation is mostly prevailing. If the electronic relaxation is much faster than rotational tumbling of molecules, the Curie relaxation becomes significant. The rotational tumbling of molecule reveals nuclear relaxation. The net magnetic moment is coincided with the Curie spin (Otting, 2010).

Divalent manganese (Mn) ions have the ability of shortening the both spin-spin relaxation time (T_2) and spin-lattice relaxation times (T_1) thanks to the effectiveness of their unpaired electrons (Zhang et al. 2009). It enhances the decay rates of relaxation in magnetic resonance imaging (Oztop et al, 2012). The increment of the relaxation with the divalent cation of Mn^{+2} is higher in solutions containing protein than in water alone (Barnhart & Berk, 1986). Besides, Mn^{+2} can be used as a tool to understand the behavior of one another divalent cation which is Ca^{+2} (Getz et al, 1979).

Except for paramagnetic ion manganese; divalent calcium (Ca) ions, which are non-paramagnetic, are also effective on the strength of gelation degree and significant for gel formation and functionality, and plays a significant role in gelation (Getz et al., 1979; Yang et al., 2013). In presence of Ca^{+2} ions, it is expected that the gel formation is governed by a mechanism called as 'egg-box' (Dobies et al., 2005). The ultimate mechanism could also be observed with Magnetic Resonance Imaging (MRI).

1.3.7. Fourier Transform Infrared (FTIR) Spectroscopy analysis

The history of the chemical infrared spectroscopy (CIS) is based on the 18th century. As an initiative invention, the interferometer was invented in this century by A.A. Michelson to observe the speed of light; however, the chemical infrared spectroscopy was not progressed until the earlier 1940s. The usage of the CIS has been started to become popular in industry after the optical null dispersive spectrophotometer was developed. Peter Fellgett, an astrophysicist, was the producer of the first Fourier transform infrared (FTIR) spectra in 1949 by measuring the light from celestial bodies with interferometer, but it had taken sometime to use this device effectively. The availability of commercial FTIR was realized with the ability of microcomputers on Fourier transform in 1960s. The

Cooley-Tukey algorithm's development in 1966 made it possible to do Fourier transform quickly. Over years, the cost of technology decreased and availability of technology increased, and the FTIR spectra technology, which is the third generation infrared spectrometer, has become widely used with the enhancing ability and capacity (Hsieh, 2008).

The vibrational properties of amino acids and cofactors are examined thoroughly with the help of Fourier transform infrared (FTIR) spectroscopy. This technique provides directly probing to the vibrational properties of water molecules, amino acid side chains and cofactors which are extremely sensitive to structural changes (Berthomieu & Hienerwadel, 2009).

The aim of the usage of FTIR spectroscopy is based on the complementation of the molecular level and total carbon measurements of an organic matter due to the simplicity in providing all chemical details like functional groups and the bonds that constitutes the entire aerosol (Blando et al., 2001).

FTIR that is used for qualitative and quantitative analysis of organic compounds in terms of molecular structure and chemical bonding has been applied for many years to study proteins, enzymes, lipids, glycolipids, nucleic acids and photobiological systems (Gerwert & Kötting, 2010).

FTIR is a time saving, accurate, nondestructive and rapid technique used with the purpose of detection of the functional groups in terms of chemical composition and physical state (Amir et al., 2013). FTIR Spectroscopy gives information about the all functional groups as mentioned in terms of the infrared (IR) signatures or wavelengths.

The IR signatures in chemical groups of the specific residues are identified with several strategies such as isotope labeling, hydrogen/deuterium exchange and site-directed mutagenesis by using the reaction-induced FTIR difference spectroscopy (Berthomieu & Hienerwadel, 2009).

The absorption bands of the functional groups are determined according to their vibrations. The functional groups with permanent dipole have strong infrared absorptions such as polar bonds. The bond strength and atomic mass affects the frequency of vibrations. For example, double and triple bonds have higher vibration frequency than the single bonds (Berthomieu & Hienerwadel, 2009).

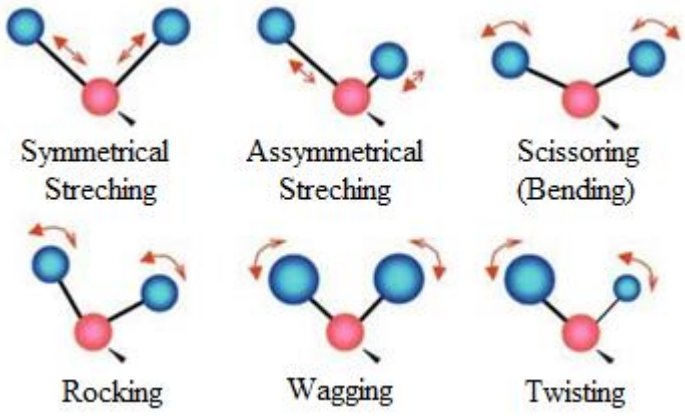

Figure 1.7. Vibration modes of molecules

The electromagnetic radiation interacting with the sample or substance is absorbed, reflected or transmitted; thus, the reaction provides information on the structure of the molecule and energy transition level. At the end of the FTIR spectroscopy application, functional groups and chemical bonds in a molecule are determined according to the peak absorbance intensity (Munajad et al., 2018).

The advantages of the FTIR over the old fashioned dispersive systems are high sample throughput, improvement in sensitivity which is almost 100 folds, increased energy throughput, auto calibration of wavelength, elimination of stray light, low maintenance, constant resolution, freedom from spectral discontinuities and positive compound identification (Bhanot, 2014).

Standard FTIR spectrophotometers include a source, sample compartment, amplifier, interferometer, computer, analog to digital (A/D) convertor and detector. The radiation is generated from the source and the radiation passes the sample through the interferometer and reaches detector. After the amplified signal passes through the A/D converter and transferred to computer, where the Fourier transform is applied, as digital with the purpose of decoding (Libretexts, 2016). In the end**,** the spectral information is mapped and the spectrum graph appears on the monitor. The quite small samples like 20 microns can be analyzed through the microscope attachment. Determination of the unknown materials and additives in polymers is done by following this path (Mathias, 2015).

The infrared spectrum is placed in the x-axis or horizontal axis of the graph while the y-axis or vertical axis represents the frequency or absorbance which is the absorbed or transmitted infrared light amount. The range of the IR region is 12800~10cm^{-1} and categorized as near infrared region (12800~4000cm^{-1}), mid infrared region (4000~200cm^{-1}) and far infrared region (200~10cm^{-1}) (How an FTIR Spectrometer Operates, 2015). If the range is wide, the IR spectra of organic compounds are more specific and include two regions; the functional group region (4000~1500cm^{-1}) and fingerprint region (1500-400cm^{-1}). The peaks in the functional group region are used to determine if there is a specific functional group according to their characteristics of specific kind of bonds. In fingerprint region, the reason of the peaks is the molecular deformation due to the multiple bond deformation (Infrared Spectroscopy: Identifying Functional Groups, 2015).

Each peak at that range determines which functional group is in the sample with its intensity.

The IR spectrum of some important functional groups are 3000~3100 cm^{-1} for aromatic CH bonds, 3020~3080cm^{-1} for alkenes CH group, 1640~1680cm^{-1} for C=C group and 1690~1760cm^{-1} for aldehydes C=O group (Singh et.al, 2010). The region of some another functional groups is that acids (1650~1690cm^{-1}), esters (1740~1750cm^{-1}), ketones (1680~1720cm^{-1}), amides (1650~1715cm^{-1}) for C=O bonding; moreover, the region of O-H (alcohol) group is 3300~3600cm^{-1}, O-H (acids) is 2400~3000cm^{-1}, C≡C and C≡N groups are 2100~2200 cm^{-1}, C-O group is 1200~1300 cm^{-1} and N-H group is 3400 cm^{-1}. For N-H group; two peaks indicate NH_2 and single peak indicates NH which is secondary amines/amides (Infrared Spectroscopy: Identifying Functional Groups, 2015). Moreover, the molecules are affected by the vibrations, so their regions and positions are. For example, while the stretching and intermolecular bonded alcohol (O-H) molecule is in the range of 3300~3600 cm^{-1} by giving a broad and strong peak, the stretching and free alcohol is in the frequency range of 3600~3700cm^{-1} by giving a sharp and strong peak; furthermore, while the stretching molecule of alcohol (O-H) is in the range of 3300~3700 cm^{-1} together with both intermolecular bonded and free, the bending alcohol molecule (O-H) is in the range of 1395~1440$cm^{-1}$with a medium peak (Libretexts, 2016). This information demonstrates the sensitivity and accuracy of the FTIR Spectroscopy.

Amide I and Amide II bands from amide bonds that bind amino acids are found among the characteristic bands in the infrared spectra of proteins and polypeptides. The absorption associated with the amide I band causes stretching vibrations of the amide C=O bond, whereas the bending vibrations of the N-H bond are linked with the Amide II band. Thanks to the roles of C=O and the N-H bonds in the hydrogen bonding between the different elements of secondary

structure of proteins, Amide I and Amide II bands are directly linked with the secondary structure of these polymers. However, Amide II band is not as good a predictor for secondary structure content of proteins whereas amide I band gives a good correlation with the quantity of secondary structure. (Surewicz et al., 1988; Byler and Susi, 1986). It was claimed that the frequency range between 1500 cm−1 and 1700 cm−1 named as Amide I/II region. To be more specific, Amide I band generally shows up in the frequency range of 1600–1700 cm−1 and Amide II band occurs in the frequency range between 1480–1600 cm−1(Kong & Yu, 2007; Naumann et al., 1991). The low intensity peaks between 1200 cm^{-1} and 1400 cm^{-1} are generally linked to amide III bonds representing the vibrations in the C−N plane and N−H groups of the peptide bond. (Kong & Yu, 2007; Muyonga et at., 2004; Ozel et al., 2018). Also, the bonds mainly occur in carbohydrates observed due to the C−O stretching vibrations at frequency between 900–1200 cm^{-1} and 700 to 900 cm^{-1} (Naumann et al., 1991). The vibrations of O - H group stretching is observed in the range between 3000 and 3600 cm - 1 as a broad peak (Lozano-Vazquez et al., 2015).

1.4. Objectives

In an increasing population, there will be an additional demand for food worldwide since it is predicted that the population will be at least 9 billion by 2050. (Kouřimská & Adámková, 2016). Also, traditional animal protein sources like beef, chicken and pork may not be adequate enough to meet the demand and people may seek to find a new nutritious sources (Akhtar & Isman, 2018). Thanks to their eco-friendly life styles, edible insects show an excellent potential to be used for an alternative, nutritious food supply. Therefore, it is essential to search for beneficial ways in order to utilize insects as a sustainable food source by considering their nutritional value. Since entomophagy is still a taboo in several regions in the world, it is also possible to increase consumer acceptability by serving them to the consumers in less recognizable forms like protein rich insect

powders. This study provides additional, extensive knowledge about protein functionality of two main insect species; *Acheta domesticus* and *Tenebrio molitor*, in addition to the existing research.

In this thesis, the main objective is to obtain high quality protein rich insect powders from *Acheta domesticus* & *Tenebrio molitor* after conventional (30 or 40^{o}C/15 min/0.1 MPa) and HHP induced extraction (30 or 40^{o}C/15 min/500 MPa) with a high efficiency and investigate the functionality of insect proteins by measuring their crude protein content, solubility, water binding capacity, oil binding capacity, gelling ability, phenolic content and antioxidant capacity.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

In the study, freeze-dried mealworm was supplied from Tasty Worms Nutrition Inc. (Florida, USA) and freeze-dried cricket powder was procured from JR Unique Foods Ltd. (Udon Thani, Thailand). Fat portion separated insect powders were used as the base material in the experiment. Hexane, gallic acid ($C_7H_6O_5$), sodium carbonate (Na_2CO_3), neocuproine (2,9-Dimethyl-1,10-phenanthroline), $DPPH^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl), Copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$), potassium sodium tartrate tetrahydrate ($KNaC_4H_4O_6.4H_2O$), sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), Folin-Ciocalteau's phenol reagent, trolox (TR), ethanol (C_2H_5OH), methanol (CH_3OH), acetic acid (CH_3CO_2H), hydrochloric acid (HCl), boric acid (H_3BO_3), methyl red and Bovine Serum Albumin (BSA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Çotanak brand rape seed oil used for the determination of oil binding capacity was supplied from Altaş Group (Altınordu, Ordu).

2.2. Methods

2.2.1 Preparation of protein rich insect powders

For defatting of fat, two different extraction methods were used to observe the changes in functional properties, antioxidant activities and phenolic compounds of protein rich insect powders.

2.2.1.1. Conventional extraction

In this method, conventional extractions were done by using 2 extraction temperatures. (30^{o}C and 40^{o}C) . Extraction was performed with 15:1 ml/g hexane to insect powder ratio at 30^{o}C/40^{o}C for 15 minutes stirring under the hot point magnetic stirrer (Daihan Scientific Co., Ltd., Korea). After extraction, centrifugation was done at 9500 rpm for 20 min at 30^{o}C/40^{o}C to remove hexane-oil part and obtain fat-reduced insect powder (Sigma 2-16 PK, SciQuip Ltd., UK). Residual hexane that remained in both powder was removed with evaporation overnight in a drying oven at 40^{o}C.

2.2.1.2. HHP equipment and HHP assisted extraction

2.2.1.2.1. HHP equipment

High Hydrostatic Pressure (HHP) treatment was performed with 760.0118 type pressure equipment (SITEC-Sieber Engineering AG, Zurich, Switzerland). The HHP equipment includes a pressurization chamber, two end closures, a means for restraining the end closures, a hydraulic unit, a pressure pump and a temperature control device. Pressurization chamber is 24 mm in internal diameter, 153 mm in length and it has 100 ml capacity. A built-in heating-cooling system (Huber Circulation Thermostat, Offenburg, Germany) was used to maintain and control required temperature, which is measured by a thermocouple type K in the vessel.

The vessel was filled with a pressure transmitting medium consisting of distilled water. Pressurization rate was 300 MPa/min for 500 MPa. Pressure release times were <20 s. Pressurization time reported in this study excluded the pressure increase and release times.

Figure 2.1. HHP equipment

2.2.1.2.2. HHP assisted extraction

Similar with conventional extraction, HHP induced extraction was performed with 15:1 ml/g hexane to insect powder ratio with 15 min application of 500 MPa at two different temperatures; 30ºC and 40ºC. HHP was applied prior to conventional extraction to explore the effect of pressure on extraction. This time both temperature and HHP were the parameters that are thought to influence the properties of protein portion of insects. After application of HHP, centrifugation was carried out at 9,500 rpm for 20 minutes at 30ºC and 40ºC depending on the temperature of HHP application. The residual hexane was evaporated with the same procedure.

2.2.2. Characterization of insect powders

2.2.2.1. Determination of protein content

2.2.2.1.1. Kjeldahl Method

Crude protein content of insect flours were determined by the Kjeldahl method according to the guideline of ASTM E258 – 07 (2015).

To determine the nitrogen content, each of 1 g samples were weighted and put into the Kjeldahl tubes. Kjeldahl catalyst tablet was added to the tubes and three of the boiling chips were put into each tube. For 1 g sample, 25 ml of H_2SO_4 was added to the tubes. After addition antifoam agent tablet to prevent the foaming, the solution was boiled in the pre-firing device until become transparent. The boiled and clarified solution in Kjeldahl tubes was remained to cool.

The Kjeldahl tubes was mounted on the distillation unit one by one and the device added the NaOH solution (40%) automatically according to the amount of H_2SO_4 in the sample. 50 ml of the H_3BO_3 solution (4%) and 3 drops of indicator which was methyl red were put into an Erlenmeyer and mixed.

After distillation was finished, the composed NH_3 was added to the H_3BO_3 solution and the mixture was titrated with the 0.1 N HCl. The turning point of the titration was observed when the yellow color turned to the pink color and the amount of HCl consumed during the titration was recorded.

With the purpose of obtain the blank of the Kjeldahl method procedure, the whole procedure was applied to the tube that was prepared without using sample.

The amount of crude protein content was found by using the following formula;

$$\% \text{ Nitrogen} = \frac{(V_1 - V_0) \times N \times 0.014}{m} \times 100 \quad \text{(Eq. 4)}$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times f \quad \text{(Eqn. 5)}$$

In the Eq. 4, V_1 represents the consumption of HCl during titration, V_0 is the amount of HCl consumed for blank, N is the normality of the HCl used as titrant, m is the weight of sample. In the Eq. 5, f represents the factor of the sample (f = 6.25).

2.2.2.1.2. Lowry Method

The protein content of the powders were determined according to the Lowry method which is based on the reaction between proteins and copper ions at alkali environment (Lowry et al., 1951). In the experiment, insect powders were dissolved in distilled water by mixing them for 30 min under magnetic stirrer. After stirring for 30 min at room temperature, the solutions were centrifuged at 2500 rpm for 15 min and the supernatant was decanted and diluted with a ratio of 1:200 with distilled water. The other reagents necessary for the experiment are given in Table 2.1;

Table 2.1. *Reagents of Lowry Method*

Reagent A:	2% Na_2CO_3 dissolved in 0.1 N NaOH
Reagent B:	2% $CuSO_4.5H_2O$, Copper source
Reagent C:	2% Na-K Tartarate
Lowry Reagent:	Mix of Reagent A:B:C with a ratio of 100:1:1
Folin and Ciocalteu's Phenol Reagent:	Diluted solution with a ratio of 1:1 with distilled water
Bovine Serum Albumin:	20 ml 1 mg/ml BSA stock solution

Additionally, 1mg/ml BSA stock solution was diluted to 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml and 0.03125mg/ml with distilled water to construct a calibration curve for determination of protein concentration in protein samples.

After preparation steps, the experiment started with adding 0.5 ml of diluted BSA standards, distilled water and protein samples in to the different tubes. Then, 2.5 ml of Lowry reagent was added to each tube. Each tubes were vortexed and waited for 10 min in the dark. 0.25 ml of Folin reagent was put in the tubes, mixed well and waited at room temperature for 30 min in the dark. After all, absorbance values were read at 660 nm with UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. LTD, Korea). Calibration curve was constructed as absorbance vs mg/ml BSA solution (y=1.7894x+0.1054) and the protein concentration of protein in the samples was determined using this curve (See Appendix A). All experiments were carried out in duplicates.

2.2.2.2. Determination of water binding capacity

Water binding capacity of insect powders were measured according to the method described by Quinn and Paton (1979) with some modifications. Firstly, 1 g of insect powders was put into the 25 ml centrifugation tubes and weight of centrifugation tubes that includes insect powder was noted. After that, 5 mL of

distilled water was added into tubes and samples were mixed for 30 seconds to mix solid and liquid parts thoroughly. Solutions were also mixed with an orbital shaker (Daihan Scientific Co., Ltd., Korea) for 5 minutes at 300 rpm to ensure complete water hydration. Then, tubes were centrifuged for 20 min at 3914 g and the weight of tubes were measured after decanting the supernatant and putting the beaker as downward on filter paper for 60 min. The water binding capacity of insect powders were calculated with the formula below (Bußler et al., 2015; Reinkensmeier et al., 2015).

$$WBC = \frac{(m_i - m_f)}{m_{DM}} \quad \text{(Eq. 6)}$$

In the equation, m_i and m_f indicated the initial weight of the tube that contained insect powder and the final weight of the tube including insect powder, respectively. Also, m_{DM} represented the weight of dry sample.

2.2.2.3. Determination of oil binding capacity

For the measurement of fat binding capacity, the method described by Schwenke et al. (1981) was taken as the reference. Similar to water binding capacity, 1 g of insect powders was added into the 25 ml centrifugation tubes and the weight of centrifuge tubes containing insect powder was noted. Then, insect powders and 10 mL of rape seed oil were mixed for 1 min with vortex. Solutions were also mixed with an orbital shaker for 5 minutes at 300 rpm and it was done twice with giving a 5-minute intermission between each to be certain about complete oil absorption. Following centrifugation for 20 min at 3914 g, the weight of samples was measured after decanting the supernatant and oil binding capacity of powders were calculated with the same formula explained in water binding capacity procedure.

2.2.2.4. Determination of total phenolic content

Total phenolic content of insect powders obtained with either conventional extractions or HHP induced extractions were determined by the Folin-Ciocalteu method with some modifications in the procedure (Krawitzky et al., 2014). In the experiment, 100 mg of insect powders were dissolved in 1 ml of ethanol:acetic acid:water mixture (50:8:42) at a ratio of 1:200 (g/ml). The mixtures were agitated using a vortex mixture (Daihan Scientific Co., Ltd., Korea) for 30 seconds and extraction of the phenolic compounds was performed for 1 hour to ensure complete extraction. Then, the mixture was filtered through a micro filter (Gema Medical Filter, Spain) with a pore size of 0.45 μm. 500 μl of sample was taken from each mixture and added into the glass tubes covered with aluminum foil to prevent light exposure. 2.5 ml of Folin-Ciocalteu reagent (0.1 N) was added to each tubes, mixtures were vortexed and remained at room temperature for 5 min at dark. Then, 2 mL of sodium carbonate (7.5%, w/v) was added to each tubes followed by vortex and mixtures were allowed to stand at room temperature for 60 min at a dark place. Absorbance values at 760 nm were recorded with UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. LTD, Korea) to quantify the phenolic compounds.

The mixture of 2.5 ml diluted Folin-Ciocalteu reagent, 2 ml sodium carbonate solution and 500 μl of ethanol:acetic acid:water mixture (50:8:42) was used as a blank.

The calibration curve (y=0,0013x-0,0052) was prepared as the absorbance vs ppm gallic acid equivalents (GAE) at different concentrations of gallic acid (20, 40, 60, 80, 100 ppm) in ethanol:acetic acid:water mixture (50:8:42) and total phenolic content was determined as mg gallic acid equivalents (GAE)/g sample (See Appendix A).

2.2.2.5. Determination of antioxidant activity

2.2.2.5.1. Antioxidant activity with DPPH˙ Radical Scavenging Method

Antioxidant activity of insect powders obtained from two specified extraction procedures were determined by the method described by Brand-Williams et al. (1995) with some modifications. 100 mg of insect powders were accurately weighted and dissolved in ethanol:acetic acid:water mixture (50:8:42) with a ratio of 1:12 (g/ml) in 2.5 ml tubes. The mixtures were agitated by using vortex for 30 seconds and allowed to stand for 1 hour to ensure complete extraction of antioxidants. After that, the mixtures were filtered with a micro filter.

In the experiment, 3.9 ml of 25 ppm DPPH solution in methanol was added into the 100 µl of each extracts in glass tubes covered with aluminum foil. The mixture was vortexed and absorbance of samples were measured at 517 nm after 1 hour waiting period with using UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. Ltd., Korea). In order to find the percentage inhibition of DPPH of the different protein extracts, absorbance of 100 µl methanol and 3.9 ml of DPPH solution was measured at 517 nm and methanol was used as a blank. Then, percentage inhibition of DPPH of samples were found using the formula given below.

$$\% \text{ inhibition of DPPH} = \frac{A^o - A^s}{A^o} \times 100 \quad \text{(Eq. 7)}$$

In the equation, A^o indicates the absorbance of a solution prepared by 3.9 ml of DPPH and 100 µl methanol (95%) measured at 517 nm and A^s represents the absorbance of protein extracts measured at 517 nm after 1 hour. Also, percentage inhibition of DPPH of trolox samples at different concentrations were calculated

with the same formula where A^s indicated the absorbance of a solution prepared by 3.9 ml of DPPH solution and 100 µl trolox samples at different concentrations. After measurement was done, a calibration curve (y=0,3922x + 2,8244) was prepared as the percentage inhibition vs ppm trolox samples. Antioxidant activity of protein extracts were stated as mg Trolox/g sample by using this standard curve (See Appendix A).

2.2.2.5.2. Antioxidant activity with Cuprac Assay

Similar to DPPH assay, extraction of antioxidants was done by dissolving 100 mg of insect powders and they were accurately weighted and dissolved in ethanol:acetic acid:water mixture (50:8:42) with a ratio of 1:200 (g/ml). The mixtures were agitated with vortex for 30 seconds and remained 1 hour to ensure complete extraction of antioxidants. Following that, the mixtures were filtered with a micro filter.

After extraction was completed, the method of Apak et al. (2008) was performed. 4.1 ml solution was prepared by mixing 100 µl of protein extracts with 1 ml of distilled water, 1 ml of 10 mM of cupper chloride solution, 7.5×10-3 M neocuproine solution prepared with ethanol (99.9%) and 1 ml of 1 M ammonium acetate buffer solution. Blank was prepared by mixing 1.1 ml of distilled water with 1 ml of 10 mM of cupper chloride solution, 1 ml of 7.5×10-3 M neocuproine solution prepared with ethanol (99.9%) and 1 ml of 1 M ammonium acetate buffer solution and absorbance values of protein extracts was measured at 450 nm against blank after 30 min waiting period. Standard curve (y=0,0015x + 0,005) was prepared using different concentrations of Trolox. The results were expressed as mg Trolox/g sample (See Appendix A).

2.2.2.6. Determination of gelling behavior with Nuclear Magnetic Resonance (NMR) Relaxometry

Gelling behavior of insect powders was examined by preparing solutions at different concentrations (25%, 20%, 15%, 10%, 5%) with distilled water. To begin with, 5 g of insect powders which were either pressurized or conventionally extracted were mixed with 20 ml of distilled water to prepare a stock solution at 25% concentration. The mixture was well-mixed by mixing with an orbital shaker (Daihan Scientific Co., Ltd., Korea) for 2 hours at 300 rpm to ensure total dissolution. Then, centrifugation was done and supernatant was separated from the solid part to obtain a mixture that consists of soluble proteins. After that, supernatants were diluted to 20%, 15%, 10% and 5% concentrations with distilled water. The prepared solutions at different concentrations were put into tubes and NMR T_1 (longitudinal relaxation times) and T_2 (spin-spin relaxation time) values were measured.

With the purpose of creating gel-structured material, the temperature of the final solutions was first raised to 90°C with a rate of 1°C/min in a water bath (Daihan Scientific Co., Ltd., Korea) and remained at that temperature for 30 min as conducted in the research of Zhao et al. (2016). Then, solutions were cooled down to 20°C and kept for 30 min again. Final solutions were poured into the tubes and brought to room temperature of 25^{o}C and NMR T_1 and T_2 relaxation times were measured.

Spin-spin relaxation time experiments (T_2), were measured on 0.5 T NMR spectrometer working at a frequency 22.34 MHz with an equipment having radio frequency coil with 10-mm diameter (SpinCore Inc., Gainsville, FL, USA). The T_2 relaxation data were recorded with the CPMG (Carr-Purcell-Meiboom-Gill) pulse

sequence with an echo time of 1000 ms, 3s repetition time, 16 scans and number of echoes changed between 512 - 4000 depending on concentration and insect type.

T_1 and T_2 values were found using the following monoexponential relations using MATLAB (R2017a, The MathWorks Inc., USA) Monoexponetnial assumes that relaxation occurs just to one component. Monoexponential T_1 and T_2 values could be considered as relaxation times of a weighted sum of individual components having different relaxation behavior.

The spin-lattice (longitudinal) relaxation time experiments (T_1) were also conducted on the same instrument using an Inversion Recovery (IR) pulse sequence. The data were recorded with 5 scans, 3s repetition time and the inversion time between 10000 - 35000 ms.

Mn^{+2} is a paramagnetic ion as stated before and is known to affect the relaxation behavior of proteins even at lower concentrations. To explore its effect, these protein solutions were also prepared in $MnCl_2$ solution. As Mn^{+2} being a divalent ion, a non paramagnetic divalent ion $CaCl_2$ was also studied as the control. The presence of divalent ions also effects gelling behavior. $MnCl_2$ and $CaCl_2$ were used as the salts to explore gelation.

2.2.2.7. Fourier Transform Infrared (FTIR) Spectroscopy analysis

Determination of the molecules and the bonds that constitutes the structure of the protein rich insect powders was measured using the IR Affinity-1 Spectrometer with Attenuated Total Reflectance (ATR) attachment (Shimadzu Corporation, Kyoto, Japan). Three replicates of each sample were analyzed in the region of

4000-500 cm^{-1} at a resolution of 4 cm^{-1} for 32 scans. The protein rich insect powder samples were dried before analysis to obtain constant weight.

2.2.2.8. Statistical analysis

Statistical analysis was carried out in all steps of the experiment with the help of MINITAB (Version 16.2.0.0, Minitab Inc., Coventry, UK).

To see if different temperatures and HHP application caused a difference on functional properties, analysis of variance (ANOVA) was conducted. Tukey's comparison test was also performed with 95 % confidence interval as the multiple comparison test. Pearson correlation was also performed in relevant sections.

2.3. Summary of experimental design

Tablo 2.1. *Experimental design parameters*

Experiments	Factors	Levels	
1. Total protein content 2. Soluble protein content 3. Water binding capacity 4. Oil binding capacity 5. Total phenolic content 6. Antioxidant activity 7. Nuclear Magnetic Resonance (NMR) Relaxometry 8. Fourier Transform Infrared (FTIR) Spectroscopy analysis	Insect Type	*Acheta domesticus* *Tenebrio molitor*	
	Extraction Type	Conventional extraction	30ºC 40ºC
		HHP induced extraction	30ºC 40ºC
	Salt Type (only for NMR experiment)	No salt $MnCl_2$ $CaCl_2$	
	Concentration (only for NMR experiment)	5, 10, 15, 20, 25 %	

CHAPTER 3

RESULTS AND DISCUSSION

Defating of mealworm and cricket powders were practiced with or without HHP treatment at 30 and 40^{o}C with the purpose of removing fat portion of powders. HHP treated samples were named as '*Pressurized samples*' and the conventionally extracted ones are named as '*Non pressurized ones*' through the remaining of the text.

3.1. Protein content

3.1.1. Total protein content with Kjeldahl Method

Protein content of the original freeze dried non-defatted insect powders was measured with Kjeldahl method to observe if there is an effect of temperature and high hydrostatic pressure on the final protein concentration with different defatting conditions. The protein content of non-defatted insect powders were found for cricket and mealworm as 69.08 ± 0.04 % and 52.50 ± 0.09 % respectively.

Defatted powders' protein content are given in Figure 3.1. Results show the effect of both HHP and temperature on the protein concentration of two insect flours.

Moreover, all the statistical results at different temperature and HHP applications can be found in Appendix C in the ANOVA outputs. The order follows the same order with the text.

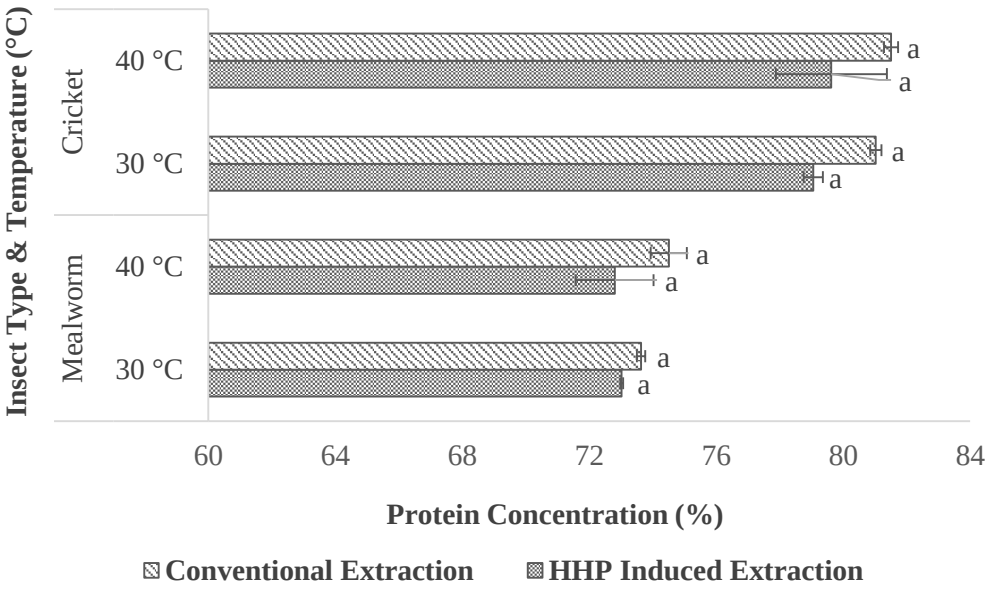

Figure 3.1. Experimental results of protein concentration of mealworm and cricket powders with Kjeldahl Method. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

ANOVA results showed that, there are no significant effects of temperature and high hydrostatic pressure on the protein content of both protein rich insect powders ($p>0.05$).

ANOVA was also performed for each type of insect to find if there is a difference in percentage increase of protein content of mealworm and cricket powders according to the initial protein content of both species which are non treated. Results showed that, effect of both temperature and HHP were not significant in percentage increases ($p>0.05$). Between two insect species, the percentage increase in protein content was around 38-42 and 14-18% for mealworm and cricket, respectively. Higher increase in protein content of mealworms in dry matter could be attributed with their higher lipid content than crickets. (Paul et al. 2017; Mariod et al., 2017).

In any circumstances, the combined effect of both temperature and pressure had no effect on the protein concentration of each insect separately. In other words, the temperature change in constant pressure and the pressure change in constant temperature had no effect on the protein concentration, so there was not a significant difference ($p>0.05$).

It is possible to say that the amount of protein stays almost same with the application of pressure and different temperature values. Whether the proteins start to become denaturated with increase in temperature and pressure, there was no significant change in the range between 30 and 40 ^{o}C and at 500 MPa. Even denaturation could have accored in an extent with pressure, no significant change was expected since Kjeldahl method measured only the nitrogen content.

3.1.2. Soluble protein content with Lowry Method

Amount of soluble proteins found in insect powders was determined with Lowry Method after application of different defatting parameters. Results are shown in Figure 3.2. Detailed experimental results and ANOVA tables are provided in Appendix. (See Appendix B & C).

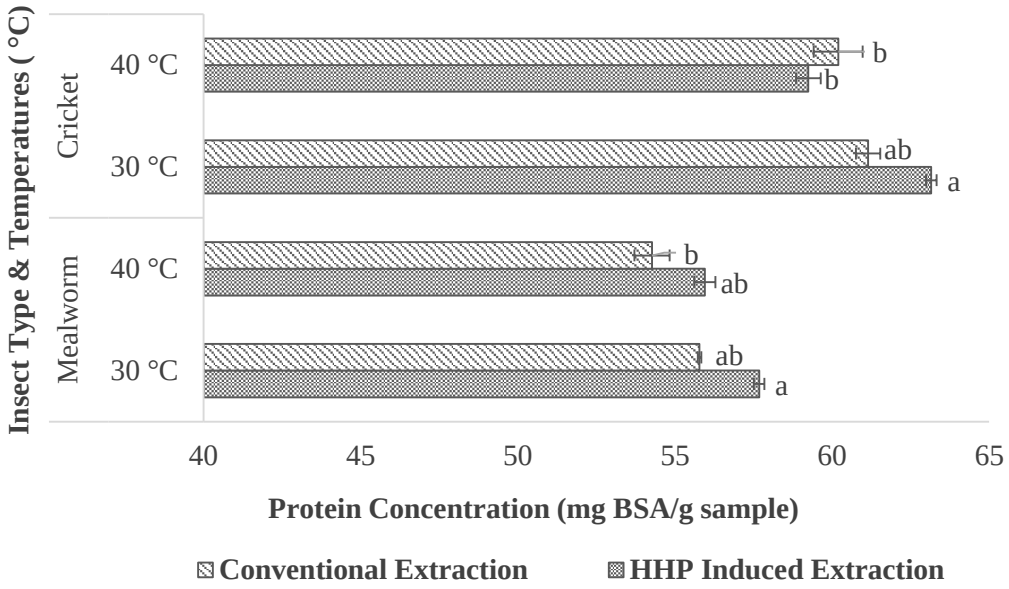

Figure 3.2. Experimental results of protein concentration of mealworm and cricket powders with Lowry Method. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

According to the statistical analyses, effect of pressure on cricket protein solubility was not significant ($p>0.05$). Both pressurized and non-pressurized crickets had almost same mean values; that is, there is no solo effect of pressure in protein solubility in cricket powder. On the other hand, pressure caused significant difference on protein solubility in mealworm ($p<0.05$). Rise in protein content after HHP application was explained by the amount of the tertiary and quaternary structures existed in the insect types. It has been known that, the high pressure has no effect on the primary structure of the proteins and has almost no effect on secondary structure (Goyal et al., 2013). On the other hand, proteins tertiary and quaternary structures are known to be highly sensitive to HHP (Goyal et al., 2013). Change on these structures could definitely cause changes on the solubility. Results depicted in Fig 3.2. indicated that, the tertiary and quaternary structures of mealworm proteins are affected more than the cricket proteins and HHP result in much more increase in solubility by opening the structures and the releasing the soluble proteins. Covalent type interactions could be higher in

cricket proteins compared to mealworm and that may explain the insignificant change due to pressure.

Results revealed that, temperature also caused significant difference on protein solubility of both cricket and mealworm ($p<0.05$). Solubility of both proteins decreased with increasing extraction temperatures ($p<0.05$). The reason of that may be considered as the temperature-denaturation relation. Proteins start to unfold as temperature increases and consequently interaction between hydrophobic groups cause a decrease in water binding and hence result in coagulation and precipitation (Pelegrine & Gasparetto, 2005). It has been known that the solubility of protein generally increases with temperature up to denaturation temperature of each specific protein (Pelegrine & Gasparetto, 2005). After denaturation, protein solubility reduces when it is compared with the native protein and this results in aggregation of proteins. The denaturation is usually caused by the effect of temperature on non-covalent interactions such as hydrogen bonds, and hydrophobic and electrostatic interactions. Therefore, increase in temperature may cause destruption in the structure of the proteins and this may cause a decrease on the protein solubility. Another reason for the decrease in protein solubility due to temperature increase may be the increase in the extraction of other non-protein substances by temperature rise. Bußler et al. (2016) claimed that the presence of non-protein impurities must be taken into account because it may form complexes with lipids or nucleic acids that can limit protein solubility. Therefore, non-protein substances that was increased as a result of lipid extraction may react with protein and decrease the solubility. Fennema (1996) also stated that, proteins which included highly hydrophobic groups might show a negative relationship with temperature although protein solubility mainly increased with temperature increase from 0^{o}C to 40^{o}C. If the results obtained in this study were considered, the solubility of proteins decreased with increasing temperature. The percentage decrease in the solubility of cricket (~4 %) was higher compared to mealworm (~2.8 %). That might be due to the amount of hydrophobic groups in

cricket being higher than the mealworm species as it was shown in Table 1.1. Results of this study confirmed that insect proteins contained high amount of hydrophobic amino acids (Finke, 2002).

Additionally, correlation analysis was conducted between two protein content determination method for both insect species individually and results were found not correlated ($p>0.05$) for both mealworm and cricket with r equals to 0.69 and 0.17 respectively. The correlation results was not unexpected since Lowry method was performed to determine only the amount of soluble insect proteins in distilled water. Although the total protein content did not change after different extraction conditions, applied HHP and temperature definitely affected the solubility behavior of insect proteins. Since different proteins become soluble at different pH levels, amount of protein may differ in distilled water at pH 7 after different treatments.

ANOVA was also performed considering the insect type as the sole factor and protein content of cricket was found higher than mealworm for both methods as expected ($p<0.05$).

3.2. Water binding capacity

The results of water binding capacity of mealworm and cricket powders were given in Table 3.1.

Table 3.1. *Experimental results of water binding capacity of mealworm and cricket powders*

Insect Type	Extraction Type		Water Binding Capacity
	Pressure (MPa)	Temperature (°C)	
Mealworm	0.1	30	1.999 ± 0.055[a]
		40	1.967 ± 0.092[a]
	500	30	2.019 ± 0.017[a]
		40	1.974 ± 0.050[a]
Cricket	0.1	30	1.735 ± 0.071[a]
		40	1.747 ± 0.095[a]
	500	30	1.796 ± 0.005[a]
		40	1.822 ± 0.037[a]

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

ANOVA results showed that, individual effects of both pressure and temperature were not significant on both mealworm and cricket powder ($p>0.05$). Additionally, the combined effect of temperature and pressure on insect types shows no significant difference ($p>0.05$). It is possible to say that the effect of both temperature and pressure did not affect the water binding capacity of protein rich insect powders.

Between the two insect species, the water binding capacity of mealworm was found to be higher than that of the cricket for all different parameter combinations ($p<0.05$), possible indicating that mealworm was more capable of retaining

moisture inside so the amount of water interacted with proteins was also higher in the mealworm powder.

This observation could be explained by the oil extraction. Hexane used in the experiment for extraction is a non-polar solvent and it is very important that the amphilic phospholipids could not be effectively extracted by this solvent (Li et al., 2014). As, the water binding capacity of mealworm is quite higher than cricket, it could be hypothesized that the amount of phospholipids remained in cricket powder was more than that in mealworm powder. When these phospholipids interacted with water, they tend to form micelle structures due to their amphipathic nature and these micelles may act a barrier for hydration resulting in lower water holding capacities as was stated by Berg et al. (2002).

Finally, dietary fiber content of insects can be an another reason of the difference between water binding capacity of insects. Since the defatted powders are not the protein isolates, amount of fiber inside the insects can also play a role in water binding capacity. Therefore, higher dietary fiber content of the mealworm may result in higher water binding capacity (Finke, 2015). Robertson and Eastwood (1981) claimed that dietary fibres have a water holding capacity (WHC) which mostly varies with fibre source and measurement method and water can contact with fibre either as trapped water or bound water.

3.3. Oil binding capacity

The results of oil binding capacity of mealworm and cricket powders are given in Table 3.2.

Table 3.2. *Experimental results of oil binding capacity of mealworm and cricket powders*

Insect Type	Extraction Type		Oil Binding Capacity
	Pressure (MPa)	Temperature (°C)	
Mealworm	0.1	30	1.799 ± 0.014[a]
		40	1.756 ± 0.004[a]
	500	30	1.569 ± 0.010[b]
		40	1.537 ± 0.019[b]
Cricket	0.1	30	1.309 ± 0.023[ab]
		40	1.214 ± 0.016[b]
	500	30	1.430 ± 0.032[a]
		40	1.321 ± 0.002[ab]

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

According to the ANOVA results, HHP caused a significant difference for both mealworm and cricket powder ($p<0.05$). On the other hand, temperature caused a significant difference on mealworm only ($p<0.05$).

Results demonstrated that, high hydrostatic pressure caused a decrease in oil binding capacity of cricket while oil binding capacity of mealworm powder was significantly increased after pressure treatment. On the other hand, temperature caused a decrease in oil binding capacity of mealworm.

Between two of the insect spices, the oil binding capacity of mealworm was found to be higher than the cricket for all possible scenarios. Although hydrophobic groups found in cricket might be higher than mealworm as it was claimed in Lowry method, other compounds in powder like fibers might also contact with lipids and resulted in an increase in oil binding capacity. Hamaker (2008) stated that many dietary fibers can effectively bind oil while some of them are fat or oil dispersible.

Mealworm was also affected more from the temperature as in the case of protein. It is probable that temperature and pressure exposed the amino acids that tended to bind with oil much more compared to cricket flour.

3.4. Total phenolic content (TPC)

Figure 3.3. shows the total phenolic content of defatted powders obtained through different extraction procedures. A more detailed comparative table is also provided in Appendix B.

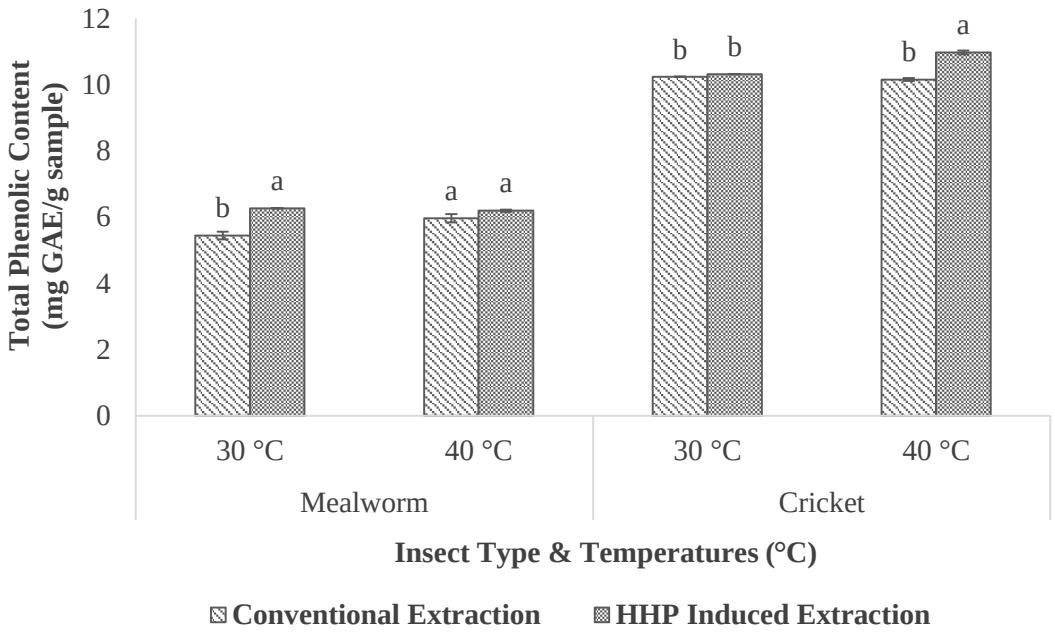

Figure 3.3. Effect of temperature and HHP application on phenolic content of cricket and mealworm powders. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

Results showed that, temperature did not cause a significant difference in mealworm while HHP assisted extraction increased the total phenolic content of mealworm powders ($p<0.05$). Therefore, the phenolics in mealworm were not to be effected from temperature rise form 30 to 40ºC. Interaction of temperature and pressure on the other hand was found to be significant on TPC ($p<0.05$). On the other hand, in cricket powders, effect of both temperature and pressure were significant ($p<0.05$). Although ANOVA was conducted separately for each powder; it was obvious that the amount of phenolic compounds were higher in cricket when compared to mealworm.

Results showed that increasing pressure caused increase in total phenolic content of insect powders. It was reported that, HHP could result in deprotonation of charged groups and deterioration of salt bridges and hydrophobic bonds (Jun et

al., 2011). These destruptions could end up with conformational changes and protein denaturation that cause cell wall, membranes and organelles to collapse (Jun et al., 2011). Therefore, these HHP stimulated changes could provide the release of both hydrophilic and lipophilic phenolic compounds from cells. The raise in total phenolic content can be attributed to increase in extractability.

In addition, the effect of phenolics from temperature was severe in the case of cricket compared to mealworm. In general, phenolic compounds are known to be temperature sensitive; however, the temperature increase from 30^{o}C to 40^{o}C showed an adverse effect. Application of heat treatment from 30^{o}C to 40^{o}C resulted in the release of the phenolic compounds, and thus increased the amounts of phenolic compounds in powder extracts. Increase in total phenolic content may be related with the formation of Maillard reaction products or releasing the glycosylated and esterified bonds after increase in temperature as in the case of onion powder reported in literature (Maillard et al., 1996; Sharma et al., 2015). Duh et al. (2001) found that the roasting conditions above 200^{o}C causes a reduction in the phenolic content of barley while the bounded phenolic acids are released due to the reasonable increase in the temperature.

3.5. Antioxidant activity

3.5.1. Antioxidant activity with DPPH˙ Radical Scavenging Method

Antioxidant activity of cricket and mealworm powders were determined with DPPH˙ Radical Scavenging Method and effect of HHP and temperature on antioxidant activity were demonstrated in Figure 3.4.

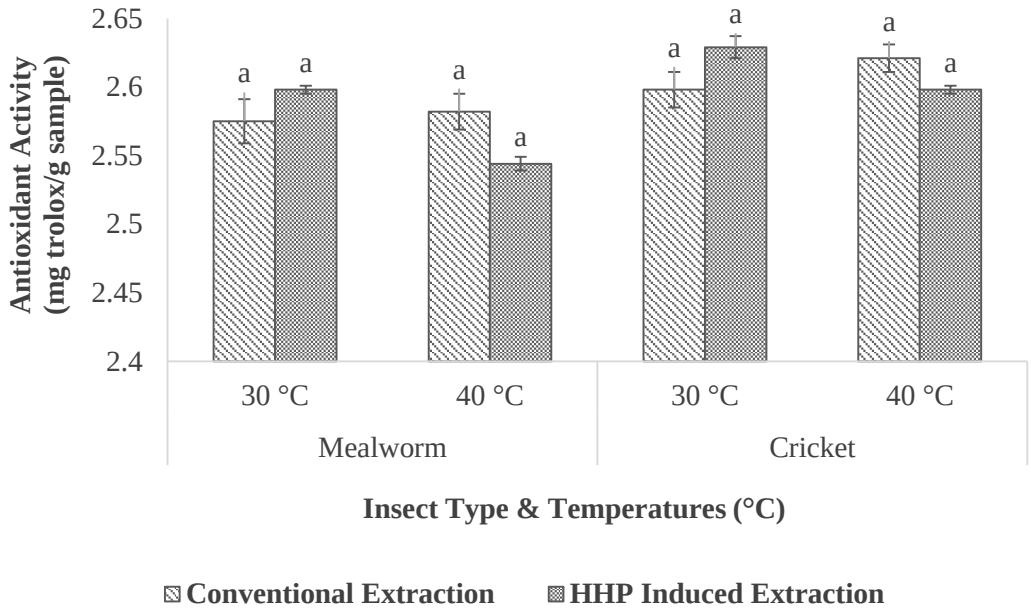

Figure 3.4. Effect of temperature and HHP application on antioxidant activity of cricket and mealworm powders with DPPH method. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

Results showed that, HHP had no significant effect on the antioxidant activity of both cricket and mealworm powders ($p>0.05$). Similar with HHP effect, no significant difference was obtained for the antioxidant activity of both insect species with different temperature conditions ($p>0.05$). In any circumstances, the combined effect of both temperature and pressure had no effect on the antioxidant activity of each insect separately.

For both types of insects, the antioxidant activity did not change with respect to temperature and pressure. When just the insect type is considered antioxidant activity was found a little bit higher in mealworm powder than cricket powder with both temperature and HHP application ($p<0.05$).

Phenolics of mealworm and cricket were affected from pressurization and temperature but these effects were not reflected on antioxidant results. Although

the phenolic compounds that are bounded the untreated sample may be released with the effect of pressure and temperature, they may show lower antioxidant activity or they may be inhibited by the different compounds that the extracts included (Maillard et al., 1996).

The different type of antioxidants shows different properties with the pressure treatment due to their different matrices; besides, the evidence in the literature proves that the antioxidant activity varies according to the time and matrix (Keenan et al., 2012; Oey et al., 2008). The level of pressure and the duration of the pressure treatment affects the antioxidants; that is, they may increase or maintain the activity of antioxidants (Di Scala et al., 2013). Also, the phenolic compounds that are responsible for increasing total phenolic content may not be completely detected by DPPH Method, even though there is an increase in phenolic content after temperature raise and application of HHP.

Pearson correlation analysis between TPC and DPPH results were also conducted and the correlation coefficient was found to be -0.01 and -0.41 for mealworm and cricket powders, respectively ($p>0.05$).

3.5.2. Antioxiant activity with Cuprac Assay

Antioxidant activity is usually tested through with various methods. In this study , as an additional study to the DPPH˙ Radical Scavenging Method, antioxidant activity of cricket and mealworm powders were also detected by Cuprac Assay. Theory of this assay was discussed in the first chapter of the thesis.

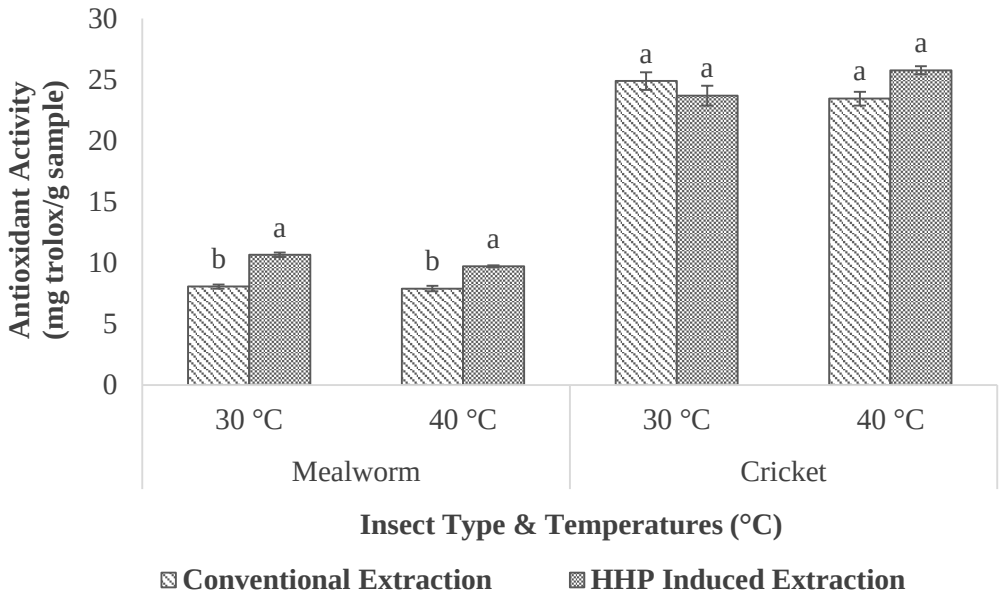

Figure 3.5. Effect of temperature and HHP application on antioxidant activity of cricket and mealworm powders with Cuprac method. Different small letters indicate significant differences between extraction conditions ($p<0.05$)

Statistical results showed that, temperature and HHP have no significant effect on antioxidant acitivity of cricket ($p>0.05$). On the other hand, effect of temperature and pressure significantly changed the antioxidant activities of mealworm powder ($p<0.05$).

Similar with total phenolic content (TPC) results, an increase in the amount of antioxidants in mealworm powders was observed in the Cuprac method after HHP treatment. However, the amount of antioxidants decreased with an increase in temperature from 30ºC to 40ºC.

Increase in antioxidant activity may be associated with increase in extractability, with removal of charged groups and disruption of salt bridges and hydrophobic bonds as a result of HHP application (Jun et al., 2011).

With temperature change from 30 to 40^{o}C, TPC remained constant on mealworm powders, but probably some other heat sensitive antioxidant compounds that were not phenolics were also degraded. Pokorný (1986) claimed that, initiation reactions could be fastened with an increase in temperature and this acceleration results in a decrease in the activity of antioxidants.

Similar with DPPH method, there was no change in antioxidant activity of cricket powder after different extractions. The reason might be the same with the reasons of DPPH method and their activity might display lower antioxidant activity or might be inhibited by the different compounds they contain, despite the possible release of phenolic compounds under the influence of pressure and temperature (Maillard et al., 1996). Also, some phenolics compounds in cricket powder that caused an increase in total phenolic content with temperature increase and HHP treatment could not have been detected with Cuprac Method as mentioned in DPPH Method.

Correlation analysis was also done between the methods. A significant correlation between TPC and Cuprac Method for mealworm powder and correlation coefficient was found as 0.745 ($p<0.05$). However, no signicant correlation was found for cricket powder with the correlation coefficient of 0.673 ($p>0.05$). On the other hand, correlation coefficient between DPPH and Cuprac Method were found to be 0.136 for mealworm powders ($p>0.05$). In case of cricket flour, negative correlation was detected between the methods with the correlation coefficient of -0.742 ($p<0.05$).

3.6. Exploring gelling behavior with Nuclear Magnetic Resonance (NMR) Relaxometry

Gelling experiments were conducted in the absence and presence of salts. Since relaxation times are good indicators of the mobile protons; gelation was aimed to be explored using these times and the protein solutions were exposed to heating as explained in Chapter 2 (Section 2.2.2.6).

The relaxation times of the protein solutions , particularly the T_1 measurements take long time due to the nature of Inversion Recovery pulse sequence. A usual way to decrease relaxation times and shorten the experiment time is to use paramagnetic ions. Thus, to decrease relaxation times, assuming that the salts would not have any significant effect on proteins at that low concentration, the defatted insect protein solutions (after centrifugation) was first prepared using 0.0001 N $MnCl_2$ solution.

In Table 3.3. and 3.4, monoexpontial T_1 and T_2 values of both defatted mealworm and cricket powders at 5, 10, 15, 20, 25 % concentrations prepared in $MnCl_2$ solution are given.

Table 3.3. *Experimental results of NMR Relaxometry of mealworm powders includuing $MnCl_2$ salts*

Insect Type	Extraction Type		Concentration (%)	Before Heating	After Heating	Before Heating	After Heating
	Pressure (MPa)	Temperature (ºC)		T_1		T_2	
Mealworm	**0.1**	**30**	**25**	315.276 ± 1.085	684.580 ± 2.083	157.148 ± 0.727	303.252 ± 0.221
			20	270.851 ± 0.705	464.178 ± 0.524	134.897 ± 1.455	222.902 ± 2.619
			15	239.823 ± 0.443	488.058 ± 5.194	115.039 ± 1.364	280.144 ± 2.554
			10	213.010 ± 0.437	434.768 ± 1.335	102.441 ± 1.899	299.751 ± 0.866
			5	198.032 ± 1.379	469.068 ± 2.891	89.728 ± 2.374	234.701 ± 1.282
		40	**25**	402.849 ± 4.931	539.973 ± 2.582	199.534 ± 0.864	268.093 ± 0.476
			20	322.558 ± 3.674	516.902 ± 2.168	156.881 ± 1.414	296.202 ± 4.951
			15	273.071 ± 1.933	441.976 ± 2.025	129.469 ± 1.492	236.915 ± 1.209
			10	239.271 ±2.835	352.846 ± 2.046	104.996 ± 0.664	214.324 ± 0.379
			5	235.915 ± 0.105	429.764 ± 4.040	90.907 ± 0.763	281.245 ± 0.839
	500	**30**	**25**	365.529 ± 1.133	472.652 ± 0.129	181.222 ± 0.063	251.868 ± 0.566
			20	299.275 ± 1.610	406.024 ± 1.493	150. 316 ± 0.576	231.847 ± 0.990
			15	251.903 ± 1.403	281.900 ± 2.691	123.915 ± 0.389	165.687 ± 0.675
			10	222.253 ± 1.043	261.229 ± 0.855	104.786 ± 0.679	156.104 ± 1.451
			5	202.943 ± 1.281	270.537 ± 1.107	90.263 ± 0.764	183.335 ± 1.906
		40	**25**	424.680 ± 0.105	704.385 ± 6.081	207.306 ± 1.308	343.115 ± 1.734
			20	337.814 ± 2.289	460.098 ± 3.241	159.720 ± 2.037	254.132 ± 0.100
			15	274.599 ± 1.406	351.508 ± 0.395	125.459 ± 0.411	202.654 ± 0.306
			10	243.987 ± 1.595	357.847 ± 2.575	106.088 ± 0.539	231.538 ± 0.395
			5	206.385 ± 3.102	388.701 ± 0.009	91.442 ± 0.590	287.441 ± 1.432

Table 3.4. *Experimantal results of NMR Relaxometry of cricket powders including $MnCl_2$ salt*

Insect Type	Extraction Type		Concentration (%)	Before Heating	After Heating	Before Heating	After Heating
	Pressure (MPa)	Temperature (ºC)		T_1		T_2	
Cricket	**0.1**	**30**	**25**	323.733 ± 1.462	360.256 ± 2.060	168.021 ± 0.997	185.681 ± 0.534
			20	283.911 ± 0.019	312.479 ± 0.820	143.161 ± 0.630	162.995 ± 1.861
			15	253.808 ± 0.213	271.687 ± 1.069	122.962 ± 0.453	136.747 ± 1.345
			10	231.960 ± 0.605	251.103 ± 1.168	107.236 ± 0.369	123.966 ± 0.899
			5	220.820 ± 0.340	235.271 ± 0.238	95.981 ± 0.644	103.951 ± 0.305
		40	**25**	322.457 ± 0.159	374.004 ± 0.042	169.573 ± 0.441	195.133 ± 1.359
			20	281.500 ± 0.855	313.848 ± 1.785	142.692 ± 0.099	157.922 ± 0.777
			15	251.130 ± 2.489	272.072 ± 1.848	123.107 ± 0.391	133.643 ± 0.460
			10	231.138 ± 2.243	250.640 ± 0.657	109.490 ± 0.182	121.115 ± 0.524
			5	220.753 ± 0.720	238.264 ± 0.866	95.138 ± 0.625	103.485 ± 0.338
	500	**30**	**25**	341.806 ± 0.492	364.472 ± 0.538	180.906 ± 0.528	187.884 ± 0.987
			20	293.902 ± 1.088	324.309 ± 1.337	152.955 ± 0.642	167.993 ± 1.189
			15	264.795 ± 0.758	275.461 ± 1.515	130.301 ± 0.489	138.603 ± 0.453
			10	239.766 ± 0.312	255.605 ± 0.075	113.733 ± 0.116	129.713 ± 1.297
			5	227.238 ± 0.589	246.664 ± 1.079	98.387 ± 0.091	111.802 ± 0.773
		40	**25**	351.837 ± 0.176	370.911 ± 0.030	173.215 ± 0.440	188.575 ± 0.692
			20	299.570 ± 0.396	317.395 ± 0.094	149.238 ± 1.098	160.446 ± 0.548
			15	264.033 ± 1.341	277.078 ± 1.076	129.420 ± 0.293	139.416 ± 0.260
			10	238.845 ± 1.622	252.412 ± 0.156	113.025 ± 0.198	121.189 ± 0.435
			5	225.659 ± 0.229	239.076 ± 0.749	98.105 ± 0.319	105.735 ± 0.113

According to ANOVA results, T_1 of both cricket and mealworm proteins in the distilled water containing $MnCl_2$ were significantly affected by both gelling process and concentration ($p<0.05$). However, results revealed that, pressure and temperature had no significant effect on T_1 values ($p>0.05$). In the case of T_2, the same results were also obtained for both insect species and it was proven that there were no significant effect of both pressure and temperature ($p>0.05$).

However, an interesting result was obtained after these experiments. Normally, as soluble protein concentration increases, mobility of water protons in the solution is expected to decrease which can reflect itself as a decrease in relaxation times. However this was not the case observed for the insect powders prepared by the paramagnetic ion $MnCl_2$. As protein concentration increased relaxation times increased as well. That was not an expected trend.

Another interesting result was the increase of relaxation times after heating it is expected to induce gelation and decrease the mobility thus decrease the relaxation times of the proteins (Indrawati et al., 2007). However that was not also not the case observed.

To resolve this unexpected behavior, gelling experiments were also conducted in the absence of $MnCl_2$ and in the presence of another divalent ion, that is $CaCl_2$ to understand whether it was the salt effect or the paramagnetic effect. These experiments were only conducted for the non pressurized samples that were extracted at 30 ^oC.

Table 3.5. *Experimantal results of NMR Relaxometry of mealworm powders with no salt, MnCl2 and CaCl2 addition*

Insect Type	Salt Type	Concentration (%)	Before Heating	After Heating	Before Heating	After Heating
			T_1		T_2	
Mealworm	**w/o salt**	**25**	975.596 ± 1.802	940.886 ± 6.193	600.657 ± 7.805	492.682 ± 13.574
		20	1143.453 ± 4.180	1067.836 ± 3.854	728.867 ± 6.938	575.779 ± 2.864
		15	1385.890 ± 5.016	1247.433 ± 0.094	911.037 ± 7.425	752.769 ± 7.036
		10	1731.072 ± 5.365	1502.653 ± 0.311	1136.976 ± 6.894	959.323 ± 10.499
		5	2242.703 ± 5.997	1935.343 ± 2.310	1573.178 ± 6.761	1418.243 ± 10.138
	$MnCl_2$	**25**	315.276 ± 1.085	684.580 ± 2.083	157.148 ± 0.727	303.252 ± 0.221
		20	270.851 ± 0.705	464.178 ± 0.524	134.897 ± 1.455	222.902 ± 2.619
		15	239.823 ± 0.443	488.058 ± 5.194	115.039 ± 1.364	280.144 ± 2.554
		10	213.010 ± 0.437	434.768 ± 1.335	102.441 ± 1.899	299.751 ± 0.866
		5	198.032 ± 1.379	469.068 ± 2.891	89.728 ± 2.374	234.701 ± 1.282
	$CaCl_2$	**25**	1297.616 ± 6.046	1320.774 ± 1.783	610.826 ± 4.809	577.281 ± 3.278
		20	1451.720 ± 6.422	1500.274 ± 11.411	770.250 ± 4.927	696.955 ± 12.825
		15	1645.276 ± 6.764	1733.304 ± 1.576	954.698 ± 11.695	915.887 ± 2.762
		10	1866.348 ± 9.514	1983.696 ± 8.279	1203.278 ± 6.164	1168.381 ± 12.825
		5	2164.342 ± 7.038	2252.784 ± 6.452	1560.879 ± 4.869	1487.836 ± 10.109

Table 3.6. *Experimental results of NMR Relaxometry of cricket powders with no salt, $MnCl_2$ and $CaCl_2$ addition*

Insect Type	Salt Type	Concentration (%)	Before Heating	After Heating	Before Heating	After Heating
			T_1		T_2	
Cricket	**w/o salt**	**25**	599.143 ± 2.998	673.096 ± 1.194	354.347 ± 1.535	373.588 ± 1.951
		20	715.171 ± 0.201	770.241 ± 1.810	428.837 ± 1.686	435.495 ± 2.002
		15	887.461 ± 2.607	928.190 ± 0.044	537.503 ± 4.826	546.271 ± 2.715
		10	1138.398 ± 1.380	1195.264 ± 3.286	713.578 ± 3.263	707.689 ± 14.594
		5	1568.563 ± 6.852	1649.325 ± 6.035	1077.263 ± 5.811	1085.443 ± 19.683
	$MnCl_2$	**25**	323.733 ± 1.462	360.256 ± 2.060	168.021 ± 0.997	185.681 ± 0.534
		20	283.911 ± 0.019	312.479 ± 0.820	143.161 ± 0.630	162.995 ± 1.861
		15	253.808 ± 0.213	271.687 ± 1.069	122.962 ± 0.453	136.747 ± 1.345
		10	231.960 ± 0.605	251.103 ± 1.168	107.236 ± 0.369	123.966 ± 0.899
		5	220.820 ± 0.340	235.271 ± 0.238	95.981 ± 0.644	103.951 ± 0.305
	$CaCl_2$	**25**	797.463 ± 0.120	799.126 ± 1.902	321.984 ± 0.334	282.440 ± 1.304
		20	950.951 ± 0.572	907.846 ± 7.235	382.906 ± 1.418	341.587 ± 4.839
		15	1156.065 ± 5.071	1076.265 ± 1.866	480.772 ± 1.653	438.228 ± 4.747
		10	1444.320 ± 0.725	1503.304 ± 4.362	649.603 ± 0.876	585.789 ± 17.083
		5	1876.518 ± 0.316	1923.106 ± 2.553	1075.256 ± 0.305	986.982 ± 3.813

In the absence of $MnCl_2$ as protein concentration decreased relaxation times increased which was the normal behavior expected from the proteins. Moroever, in the presence of $CaCl_2$, initial T_1 values of both mealworm and cricket and T_2 values of mealworm were even found to be higher than their 'no salt' counter parts.

Results indicated that there is definitely a salt effect on proteins even at those low concentrations. While preparing the protein soluttions for gelation, the procedure involved dissolving the powder in the solution containing the salts and centrifuging afterwards and letting the supernatant to form gel with heating. In other words soluble proteins were remaining in the solution. However in the presence of salts, divalent ions were thought to bind to the protein and decreased the proteins solubility and after centrifugation the amount of soluble protein in the supernatant decreased and this case became more pronounced as protein concentration increased. Basically, the paramagnetic ion, Mn^{+2} bound to the proteins and decreased their solubility and the amount of Mn^{+2} in the supernatant decreased with increased protein concentration due to this bonding. As a result, with less paramagnetic ion concentration remaining in the solution, relaxation times increased with increased protein concentration. With heating due to proteins being denatured and Mn^{+2} was interacting with them, the paramagnetic ion concentration on the free water decreased and relaxation times increased. Effect of protein concentration on T_1 values was similar on heated cricket samples but for mealworm samples an unexpected increase was observed at the very low protein concentration values (5%). The unexpected increase of T_1 values at the very low protein concentration for mealworm could be explained by the insufficient protein concentration to form a gel network. However for T_2 values for both protein types the same trend followed.

In summary it could be concluded that $MnCl_2$ acted as salting out salt for the mealworm and cricket proteins. Salting out salts are known as the salts that promote protein denaturation.

To isolate the the paramagnetic effect of Mn^{+2} and see whether it was the divalent ions that is causing the salting out effect, for the non pressurizes samples extracted at 30^{o}C, gelation experiments were also conducted using $CaCl_2$ as the main salt. Results were compared with 'no salt' case and with $MnCl_2$ results.

3-Way ANOVA was conducted to understand the effect of salt addition, concentration and heating on the relaxation times of T_1 and T_2 separately for both powders. ANOVA Tables are given in Appendix part (See Appendix C).

Results showed that, heating, salt addition and concentration and their interactions had a significant effect on T_1 and T_2 values of mealworm and cricket proteins ($p<0.05$).

As stated before the effect of concentration on the samples prepared with $CaCl_2$, increase in protein concentration resulted in a decrease on the relaxation times. So this trend was smilar to 'no salt' samples.

Effect of gelation is usually better explained by the changes in T_2 so from this point on discussion will more be based on the changes in T_2 (Ozel et al., 2017). With increasing heating, due to gelation T_2 values are expected to decrease (Indrawati et al., 2007). And that was the case for the mealworm proteins both for 'no salt' and $CaCl_2$ cases. However the change in relaxation times in the presence of $CaCl_2$ was not as high as the 'no salt case'. Morever relaxation times before heating were also higher in the case of $CaCl_2$ compared to 'no salts' solutions . The longer relaxation times and smaller changes in relaxation times with heating also indicated that $CaCl_2$ could be salting out salt for mealworm proteins as well.

However the situation was different for cricket proteins. In the absence of $CaCl_2$ relaxation times were longer before heating. $CaCl_2$ did not increase the T_2 times before heating but decreased them. It is possible that it could have resulted in more protein solubility thus could have decreased the mobility and consequently T_2 times. In fact, that was also reflected with the heating experiments. In the absence of $CaCl_2$, T_2 values increased with heating indicating that protein unfolded and tried to isolate itself from the solution letting more mobile protons and more free water. On the other in the presence of $CaCl_2$ T_2 values decreased with heating indicating that it helped to form a gel network. Thus $CaCl_2$ salts could be considered as salting in for the cricket proteins.

As can bee seen NMR experiments not only provided insight on gelling behavior but also helped to show either salt and the protein had favorable interaction or not.

3.7. Fourier Transform Infrared (FTIR) Spectroscopy analysis

FTIR analysis was performed on defatted insect powders. Figure 3.6 and 3.7 show the compounds of mealworm and cricket powders obtained after conventional and HHP assisted extraction at 30 ºC and 40 ºC.

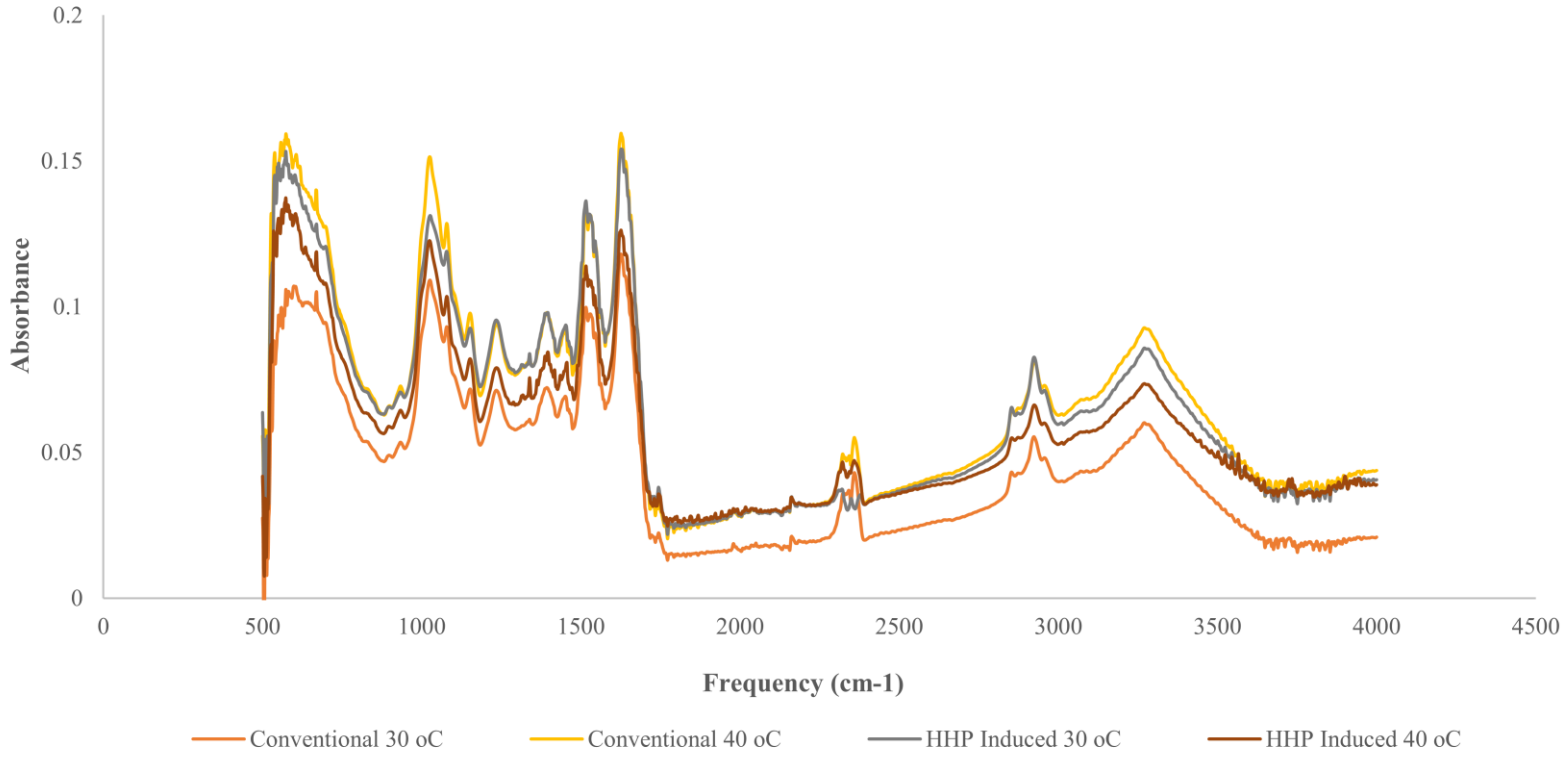

Figure 3.6. Compounds of mealworm powder at different extraction conditions

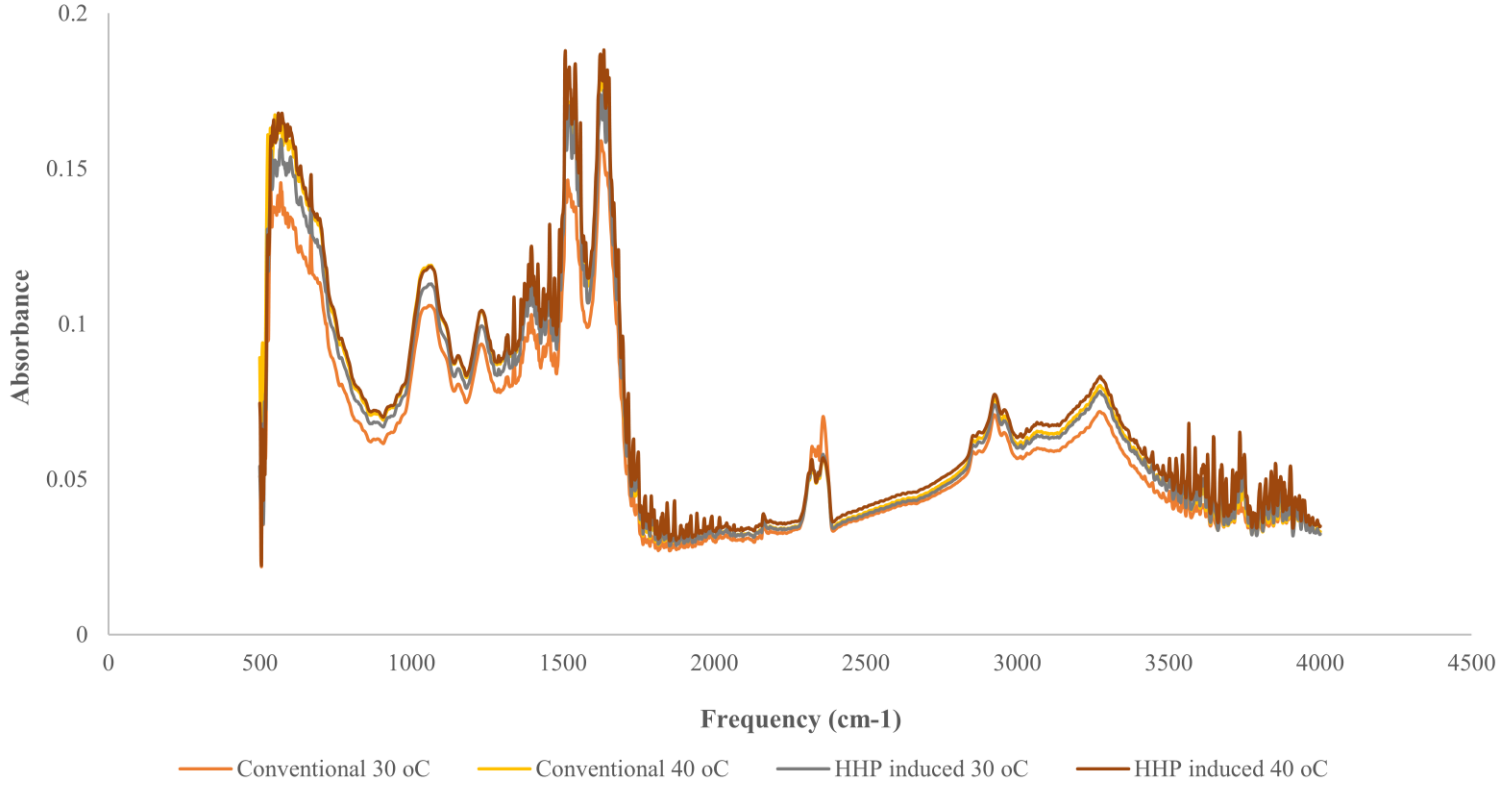

Figure 3. 1. Compounds of cricket powder at different extraction conditions

Analysis demonstrated different absorption bands at specific wavenumbers. The graphs showed that spectrum changed according to the type of insect and treatments.

Table summarizes the bonding groups found in high amounts in mealworm and cricket.

Table 3.7. *Bond interactions in mealworm and cricket powders*

Interactions	**Absorbance peak (cm^{-1})**	
	Mealworm	**Cricket**
C-O stretch	1026	1064
N-H bend, C-N stretch	1234, 1392	1230, 1396
N-H bend, C-N stretch	1516	1508
C=O stretch	1625	1624
C≡N stretch	2358	2358
C-H stretch	2924	2924
O-H stretch	3267	3271

Studies showed that, Amide I band generally arises in the frequency range of 1600–1700 cm^{-1} and Amide II band is found in the wavelength between 1480–1600 cm^{-1} (Kong & Yu, 2007; Naumann et al., 1991). Therefore, the strong absorbance peaks observed due to the C=O stretching at around 1624 cm^{-1} and 1625 cm^{-1} for cricket and mealworm powders are the proof of the amide I band. The results were consistent with the FTIR results found by Ozel et al. (2018) and amide I band gave a peak at the wavelength around 1633 cm^{-1} in whey protein isolate. Another strong absorbance peaks at wavenumbers of 1508 cm^{-1} and 1516 cm^{-1} are related with amide II band and obtained as a result of C-N stretching and N-H bending. Also, broad absorbance range with low spectral density in the range of 1200-1400 cm^{-1} was related with the amide III band (Muyonga et al., 2004). The results of mealworm and cricket powders were matched with the existing

researches and the two peaks in this spectral region were observed due to the N-H bending and C-N stretching of peptide bonds in this range. Naumann et al. (1991) declared that C−O stretching vibrations at frequency between 900–1200 cm^{-1} and 700 to 900 cm^{-1} are mainly caused by the bonds within the carbohydrates. Ozel et al. (2018) also stated that peak around 1040 cm^{-1} as a result of C−O stretching in hydrogels and dry powders emphasizes the presence of carboxylic acid units. (Tonyalı et al., 2018; Ebrahimi et al., 2016; Yang et al., 2015). It was also claimed that, this band is commonly observed in polysaccharides and can be the demonstration of galacturonic acid, glucuronic acid and etc (Blanco-Pascual et al., 2014). Thus, the peaks arised in the wavelength around 1026 cm^{-1} and 1064 cm^{-1} are the proof of carbohydrate related compounds in mealworm and cricket powders, respectively. The weak absorbance peak observed at the 2358 cm^{-1} as a result of C≡N stretching for each insect type was attributed with the presence of nitrile compound (Libretexts, 2016). The search conducted by Tonyalı et al. (2018) indicated that, the weak peak between 2800 and 3000 cm^{-1} is observed in whey protein based films because of the stretching vibrations of C-H group. The absorbance range between 2850-2980 cm^{-1} especially indicated the stretching groups of $–CH_2$ and $–CH_3$ (Ebrahimi et al., 2016; Yang et al., 2015). It can be stated that the absorbance peak in 2924 cm^{-1} can be related with C-H stretching in this range. The research on whey protein isolate also had small absorbance peak at the same spectral range (Ozel et al., 2018). According to the research, the broad peak observed in the range between 3000 cm^{-1} and 3600 cm^{-1} was the results of the vibrations of O-H group stretching (Lozano-Vazquez et al., 2015). Therefore, the peaks observed in the 3267 cm^{-1} and 3271 cm^{-1} for insect powders were considere as an outputs of O-H group stretching. The results were also supported by other researches (Ozel et al., 2018).

Results showed that, absorbance peaks of the both pressurized and non-pressurized powders from the same insect species arised at around the same wavenumbers. However, it was necessary to do statistical analysis to determine

the effect of temperature and pressure on the absorption peaks of powders after each extraction.

Anova results revealed that effect of temperature and pressure had no significant effect on the intensity of absorption peaks of mealworm powder and hence intensity of the mealworm compounds. On the other hand, it was observed that temperature and pressure caused a significant difference in some absorption peaks in case of cricket powder.

In cricket, the difference was found in the protein related bands which are amide I, amide II, amide III and intensity of compounds increased with the temperature change from 30^{o}C to 40^{o}C. On the other hand, temperature increase result in decrease of the intensity of nitrile compound. In the other frequencies, no significant change was observed. Besides, it was detected that intensity of amide II band in cricket flour significantly increased with the pressure application of 500 MPa. The results of FTIR experiment has a relation with solubility behavior of insect powders. According to the results of Lowry method, solubility of insect proteins decreased when extraction temperature was increased from 30^{o}C to 40^{o}C. Also, the decrease in solubility was higher in cricket powder than mealworm powder. Bußler et al. (2016) stated that the presence of non-protein impurities must be concerned since they may form complexes with lipids or nucleic acids that can result in the reduction of protein solubility. The reason of the decrease in the protein solubility may be related with the interactions between non-protein impurities and proteins, and these interactions occurred due to the increasing temperature were observed as bonds in amide I, amide II and amide III regions in the spectra analysis with higher intensity. Since the decrease in solubility was lower in mealworm than cricket when temperature was raised from 30 to 40^{o}C, no significant change was observed for mealworm powders in these regions.

Anova results that are conducted by considering insect type as a sole factor showed that significant increases were found in the absorbance peak of the protein related compound bands which are amide I, amide II, amide III and nitrile. If the compounds and bonds related mainly with protein are compared, their intensities were much higher in cricket than mealworm. Since the protein content of cricket was also higher than the mealworm after each extraction according to the results of Kjeldahl method, it was possible to say that the intensity of protein related compounds as a result of N-H bending, C-N stretching and C≡N stretching increase with increase in protein content.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In the current study, the influences of different extraction parameters with or without HHP treatment and temperature variations (30^{o}C & 40^{o}C) on techno-functional properties of protein rich mealworm and cricket powders were investigated. In order to characterise the protein properties and determine the antioxidant activities of the insect powders, the amount of oil was reduced with 15 minutes application of conventional and HHP assisted extraction at 30^{o}C and 40^{o}C with hexane.

Results showed that functional properties and antioxidant acitivities of mealworm and cricket powders were significantly affected from both HHP treatment and variation in temperature. However, the optimum extraction condition varied depending on insect type and functional properties.

According to the results, effect of temperature and pressure on the total protein concentration of each insect were not significant ($p>0.05$). This meant that the amount of protein remained almost the same with different extraction conditions. On the other hand, Lowry results showed that pressure at around 500 MPa caused an increase on the protein solubility in the mealworm while there was no change in the solubility of cricket after HHP treatment. Also, considering that the denaturation was accelarated with raise in temperature, the solubility of both insect proteins decreased as temperature increased. Results revealed that, both temperature and pressure had no effect on the water binding capacity of protein-rich powders. On the contrary, HHP (500 MPa, 15 min) caused a significant difference for both insect powders and increased the oil binding capacity of

mealworm while oil binding capacity of cricket powder significantly decreased. Additionally, temperature only showed a significant effect on mealworm and caused a decrease in oil binding capacity.

In this research, total phenolic content and antioxidant activity of insect powders were also investigated deeply. While increase in temperature did not cause any effect on total phenolic content, amount of phenolic content raised with temperature change from 30^{o}C to 40^{o}C. On the other hand, increase in pressure caused an increase in total phenolic content of both insect species. While antioxidant activity of insects did not change with variations in temperature and HHP application at 500 MPa for 15 min according to DPPH Method, significant changes occurred in Cuprac method.

Besides, NMR experiments gave a detailed idea about the gelation behavior of insect powders and showed whether the salts and proteins interacted favorably. Statistical analysis indicated that, $CaCl_2$ shows salting out salt property for mealworm proteins whereas it behaved as salting in salt in the presence of cricket proteins.

Study revealed that application of high hydrostatic pressure at 500 MPa for 15 minutes for effective extraction of antioxidant compounds and alteration of functional properties may be an alternative method to traditional extraction methods. However, for commercial use, it may be necessary to compare the operating costs between HHP induced extraction and conventional extraction and to carry out further research with application of different parameters.

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APPENDICES

A. CALIBRATION CURVES

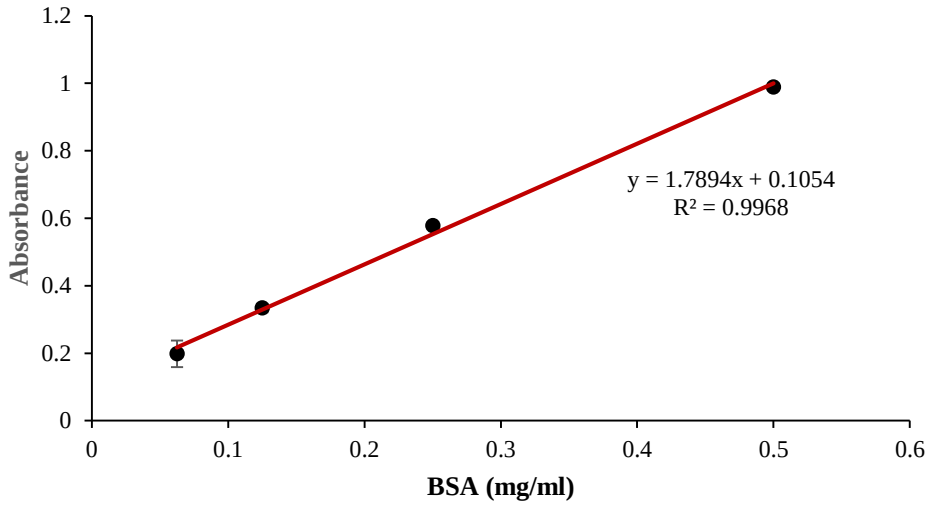

Figure A. 1. Calibration curve for Lowry Method prepared by Bovine Serum Albumin (BSA) to determine soluble protein contents in insect powders

Absorbance (at 660 nm) = 1.7894 * (mg BSA/ml) + 0.1054 where $R^2 = 0.9968$

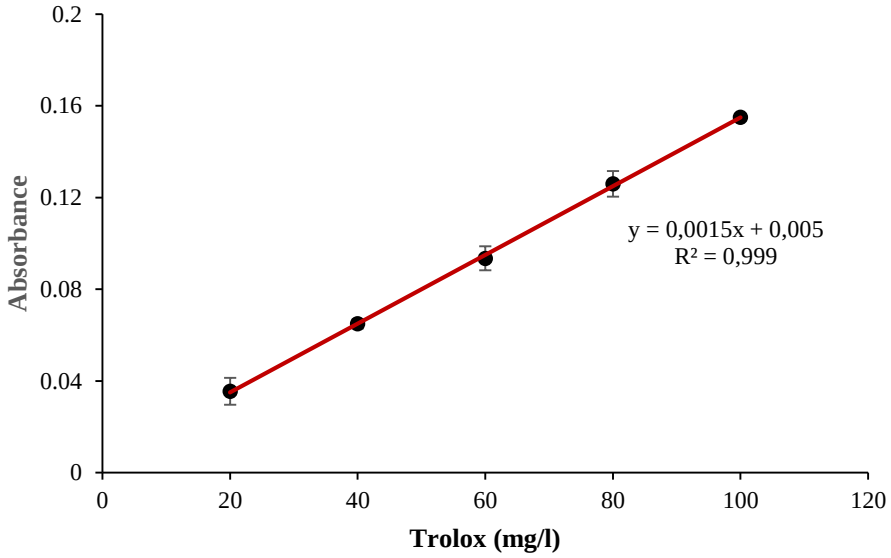

Figure A. 2. Calibration curve for Cuprac assay prepared by Trolox to determine antioxidant activity in insect powders

Absorbance (at 450 nm) = 0.0015 * (mg trolox/L) + 0.005 where $R^2 = 0.9996$

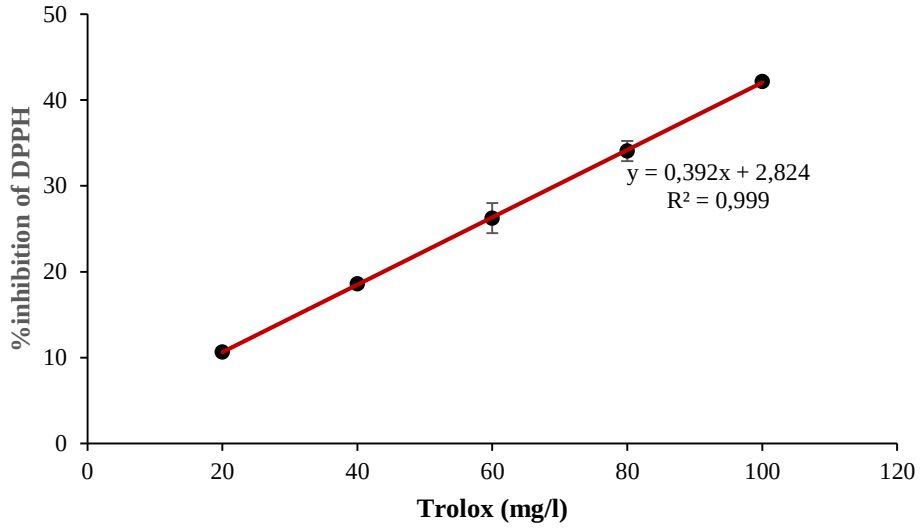

Figure A. 3. Calibration curve for DPPH assay prepared by Trolox to determine antioxidant activity in insect powders

Initially, % inhibition of DPPH should be calculated by the formula given below in order to find the antioxidant activity of samples.

$$\text{\% inhibition of DPPH for insect powders:}$$

$$\frac{\text{absorbance of the control (at 517 nm)-absorbance of the sample (at 517 nm)}}{\text{absorbance of the control (at 517 nm)}} * 100$$

$$\text{\% inhibition} = 0.3922 * (\text{mg trolox/L}) + 2.8244 \text{ where } R^2 = 0.9999$$

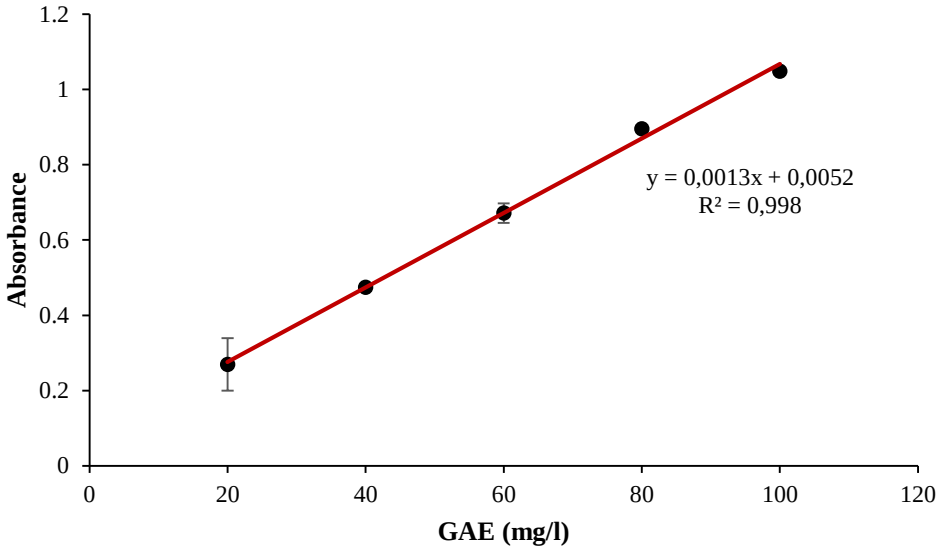

Figure A. 4. Calibration curve prepared by Gallic acid to determine total phenolic content in insect powders

Absorbance (at 760 nm) = 0.0013 * (mg trolox/L) - 0.0052 where R^2 = 0.9975

Table B. 1. *Experimental results of protein concentration, water and oil binding capacity of mealworm and cricket powders*

Insect Type	Extraction Type		Protein Concentration (%) (Kjeldahl Method)	Protein Concentration (mg BSA/g sample) (Lowry Method)	Water Binding Capacity	Oil Binding Capacity
	Pressure (MPa)	Temperature (ºC)				
Mealworm	0.1	30	73.631 ± 0.131^{a}	55.784 ± 0.056^{ab}	1.999 ± 0.055^{a}	1.799 ± 0.014^{a}
		40	74.506 ± 0.569^{a}	54.275 ± 0.559^{b}	1.967 ± 0.092^{a}	1.756 ± 0.004^{a}
	500	30	73.019 ± 0.044^{a}	57.684 ± 0.168^{a}	2.019 ± 0.017^{a}	1.569 ± 0.010^{b}
		40	72.800 ± 1.225^{a}	55.952 ± 0.335^{ab}	1.974 ± 0.050^{a}	1.537 ± 0.019^{b}
Cricket	0.1	30	81.025 ± 0.175^{a}	61.149 ± 0.391^{ab}	1.735 ± 0.071^{a}	1.309 ± 0.023^{ab}
		40	81.506 ± 0.219^{a}	60.199 ± 0.782^{b}	1.747 ± 0.095^{a}	1.214 ± 0.016^{b}
	500	30	79.056 ± 0.306^{a}	63.161 ± 0.168^{a}	1.796 ± 0.005^{a}	1.430 ± 0.032^{a}
		40	79.625 ± 1.750^{a}	59.249 ± 0.391^{b}	1.822 ± 0.037^{a}	1.321 ± 0.002^{ab}

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

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

Insect Type	Extraction Type		Total Phenolic Content	Antioxidant Activity (DPPH)	Antioxidant Activity (Cuprac)
	Pressure (MPa)	Temperature (°C)	mg GAE/g sample	mg trolox/g sample	mg trolox/g sample
Mealworm	0.1	30	5.446 ± 0.115[b]	2.575 ± 0.016[a]	8.058 ± 0.180[b]
		40	5.965 ± 0.127[a]	2.582 ± 0.013[a]	7.898 ± 0.220[b]
	500	30	6.265 ± 0.012[a]	2.598 ± 0.003[a]	10.657 ± 0.180[a]
		40	6.196 ± 0.035[a]	2.544 ± 0.005[a]	9.718 ± 0.080[a]
Cricket	0.1	30	10.235 ± 0.012[b]	2.598 ± 0.013[a]	24.876 ± 0.720[a]
		40	10.154 ± 0.046[b]	2.621 ± 0.010[a]	23.437 ± 0.560[a]
	500	30	10.315 ± 0.000[b]	2.629 ± 0.008[a]	23.676 ± 0.800[a]
		40	10.973 ± 0.058[a]	2.598 ± 0.003[a]	25.756 ± 0.320[a]

* 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 protein content of mealworm powder with Kjeldahl Method.*

General Linear Model: Mealworm Protein versus Pressure; Temperature

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


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

Source                DF  Seq SS  Adj SS  Adj MS     F      P
Pressure               1  2,6883  2,6883  2,6883  2,92  0,163
Temperature            1  0,2153  0,2153  0,2153  0,23  0,654
Pressure*Temperature   1  0,5981  0,5981  0,5981  0,65  0,466
Error                  4  3,6865  3,6865  0,9216
Total                  7  7,1883


S = 0,960011   R-Sq = 48,72%   R-Sq(adj) = 10,25%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4  74,1  A
1         4  72,9  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  73,7  A
30           4  73,3  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  74,5  A
0         30           2  73,6  A
1         30           2  73,0  A
1         40           2  72,8  A

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 protein content of cricket powder with Kjeldahl Method.*

General Linear Model: Cricket Protein versus Pressure; Temperature

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

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

Source                DF  Seq SS  Adj SS  Adj MS     F      P
Pressure               1   7,411   7,411   7,411  4,58  0,099
Temperature            1   0,551   0,551   0,551  0,34  0,591
Pressure*Temperature   1   0,004   0,004   0,004  0,00  0,964
Error                  4   6,470   6,470   1,617
Total                  7  14,436


S = 1,27176   R-Sq = 55,18%   R-Sq(adj) = 21,57%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4  81,3  A
1         4  79,3  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
40           4  80,6  A
30           4  80,0  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         40           2  81,5  A
0         30           2  81,0  A
1         40           2  79,6  A
1         30           2  79,1  A

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 percentage increase in protein content of mealworm powder with Kjeldahl Method*

General Linear Model: % Increase (Meal versus Pressure; Temperature

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


Analysis of Variance for % Increase (Mealworm Protein), using Adjusted SS
for
     Tests

Source                DF  Seq SS  Adj SS  Adj MS     F      P
Pressure               1   9,753   9,753   9,753  2,92  0,163
Temperature            1   0,781   0,781   0,781  0,23  0,654
Pressure*Temperature   1   2,170   2,170   2,170  0,65  0,466
Error                  4  13,375  13,375   3,344
Total                  7  26,080


S = 1,82859   R-Sq = 48,72%   R-Sq(adj) = 10,25%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4  41,1  A
1         4  38,9  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  40,3  A
30           4  39,7  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
0         40           2  41,9  A
0         30           2  40,3  A
1         30           2  39,1  A
1         40           2  38,7  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 percentage increase in protein content of cricket powder with Kjeldahl Method*

General Linear Model: % Increase (Cric versus Pressure; Temperature

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


Analysis of Variance for % Increase (Cricket Protein), using Adjusted SS
for
     Tests

Source                DF  Seq SS  Adj SS  Adj MS     F      P
Pressure               1  15,212  15,212  15,212  4,58  0,099
Temperature            1   1,131   1,131   1,131  0,34  0,591
Pressure*Temperature   1   0,008   0,008   0,008  0,00  0,964
Error                  4  13,279  13,279   3,320
Total                  7  29,630


S = 1,82201   R-Sq = 55,18%   R-Sq(adj) = 21,57%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4  17,5  A
1         4  14,7  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  16,5  A
30           4  15,7  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
0         40           2  17,8  A
0         30           2  17,1  A
1         40           2  15,1  A
1         30           2  14,3  A

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 protein content of mealworm powder with Lowry Method.*

General Linear Model: Mealworm (mg BSA versus Pressure; Temperature

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


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

Source                DF   Seq SS  Adj SS  Adj MS      F      P
Pressure               1   6,3961  6,3961  6,3961  28,05  0,006
Temperature            1   5,2530  5,2530  5,2530  23,04  0,009
Pressure*Temperature   1   0,0250  0,0250  0,0250   0,11  0,757
Error                  4   0,9119  0,9119  0,2280
Total                  7  12,5861


S = 0,477479   R-Sq = 92,75%   R-Sq(adj) = 87,32%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4  56,8  A
0         4  55,0    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  56,7  A
40           4  55,1    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
1         30           2  57,7  A
1         40           2  56,0  A B
0         30           2  55,8  A B
0         40           2  54,3    B

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 protein content of cricket powder with Lowry Method.*

General Linear Model: Cricket (mg BSA/ versus Pressure; Temperature

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


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

Source                DF   Seq SS   Adj SS   Adj MS      F      P
Pressure               1   0,5637   0,5637   0,5637   1,19  0,336
Temperature            1  11,8194  11,8194  11,8194  24,98  0,008
Pressure*Temperature   1   4,3864   4,3864   4,3864   9,27  0,038
Error                  4   1,8926   1,8926   0,4731
Total                  7  18,6621


S = 0,687858   R-Sq = 89,86%   R-Sq(adj) = 82,25%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4  61,2  A
0         4  60,7  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  62,2  A
40           4  59,7    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         30           2  63,2  A
0         30           2  61,1  A B
0         40           2  60,2    B
1         40           2  59,2    B

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

Table C. 7. *One way ANOVA and Tukey's Comparison Test with 95% confidence level for comparison of protein content with Kjeldahl Method considering insect type as the sole factor.*

General Linear Model: Protein Content (Kjeldahl) versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for Protein Content (Kjeldahl), using Adjusted SS
for
     Tests

Source       DF  Seq SS  Adj SS  Adj MS       F      P
Insect Type   1  185,73  185,73  185,73  120,24  0,000
Error        14   21,62   21,62    1,54
Total        15  207,35


S = 1,24281   R-Sq = 89,57%   R-Sq(adj) = 88,83%


Unusual Observations for Protein Content (Kjeldahl)

        Protein
        Content
Obs  (Kjeldahl)      Fit  SE Fit  Residual  St Resid
 15     77,8750  80,3031  0,4394   -2,4281     -2,09 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
C       8  80,3  A
M       8  73,5    B

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

Table C. 8. *One way ANOVA and Tukey's Comparison Test with 95% confidence level for comparison of protein content with Lowry Method considering insect type as the sole factor.*

General Linear Model: Protein Content (Lowry) versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for Protein Content (Lowry), using Adjusted SS for
Tests

Source       DF  Seq SS  Adj SS  Adj MS      F      P
Insect Type   1  100,63  100,63  100,63  45,08  0,000
Error        14   31,25   31,25    2,23
Total        15  131,88


S = 1,49399   R-Sq = 76,30%   R-Sq(adj) = 74,61%


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
C       8  60,9  A
M       8  55,9    B

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 water binding capacity (WBC) of mealworm powder.*

General Linear Model: Mealworm WBC versus Pressure; Temperature

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


Analysis of Variance for Mealworm WBC, using Adjusted SS for Tests

Source                DF    Seq SS    Adj SS    Adj MS     F      P
Pressure               1  0,000374  0,000374  0,000374  0,10  0,763
Temperature            1  0,002915  0,002915  0,002915  0,81  0,418
Pressure*Temperature   1  0,000085  0,000085  0,000085  0,02  0,885
Error                  4  0,014305  0,014305  0,003576
Total                  7  0,017679


S = 0,0598023   R-Sq = 19,08%   R-Sq(adj) = 0,00%


Grouping Information Using Tukey Method and 95,0% Confidence

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

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 water binding capacity (WBC) of cricket powder.*

General Linear Model: Cricket WBC versus Pressure; Temperature

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


Analysis of Variance for Cricket WBC, using Adjusted SS for Tests

Source                DF    Seq SS    Adj SS    Adj MS     F      P
Pressure               1  0,009214  0,009214  0,009214  2,40  0,196
Temperature            1  0,000735  0,000735  0,000735  0,19  0,684
Pressure*Temperature   1  0,000104  0,000104  0,000104  0,03  0,877
Error                  4  0,015349  0,015349  0,003837
Total                  7  0,025403


S = 0,0619451   R-Sq = 39,58%   R-Sq(adj) = 0,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   1,8  A
0         4   1,7  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   1,8  A
30           4   1,8  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   1,8  A
1         30           2   1,8  A
0         40           2   1,7  A
0         30           2   1,7  A

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

Table C. 11. *One way ANOVA and Tukey's Comparison Test with 95% confidence level for comparison of water binding capacity considering insect type as the sole factor.*

General Linear Model: WBC versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for WBC, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS   Adj MS      F      P
Insect Type   1  0,18464  0,18464  0,18464  60,00  0,000
Error        14  0,04308  0,04308  0,00308
Total        15  0,22772


S = 0,0554731   R-Sq = 81,08%   R-Sq(adj) = 79,73%


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
M       8   2,0  A
C       8   1,8    B

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 oil binding capacity (OBC) of mealworm powder*

General Linear Model: Mealworm OBC versus Pressure; Temperature

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


Analysis of Variance for Mealworm OBC, using Adjusted SS for Tests

Source                DF    Seq SS    Adj SS    Adj MS       F      P
Pressure               1  0,100845  0,100845  0,100845  615,99  0,000
Temperature            1  0,002798  0,002798  0,002798   17,09  0,014
Pressure*Temperature   1  0,000054  0,000054  0,000054    0,33  0,596
Error                  4  0,000655  0,000655  0,000164
Total                  7  0,104352


S = 0,0127950   R-Sq = 99,37%   R-Sq(adj) = 98,90%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   1,8  A
1         4   1,6    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   1,7  A
40           4   1,6    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
0         30           2   1,8  A
0         40           2   1,8  A
1         30           2   1,6    B
1         40           2   1,5    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 oil binding capacity (OBC) of cricket powder.*

General Linear Model: Cricket OBC versus Pressure; Temperature

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


Analysis of Variance for Cricket OBC, using Adjusted SS for Tests

Source                DF    Seq SS    Adj SS    Adj MS      F      P
Pressure               1  0,038851  0,038851  0,038851  15,38  0,017
Temperature            1  0,011758  0,011758  0,011758   4,66  0,097
Pressure*Temperature   1  0,002077  0,002077  0,002077   0,82  0,416
Error                  4  0,010103  0,010103  0,002526
Total                  7  0,062789


S = 0,0502580   R-Sq = 83,91%   R-Sq(adj) = 71,84%


Grouping Information Using Tukey Method and 95,0% Confidence

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

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 total phenolic content of mealworm powder.*

General Linear Model: Mealworm (mg GAE versus Pressure; Temperature

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


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

Source                DF   Seq SS   Adj SS   Adj MS      F      P
Pressure               1  0,55125  0,55125  0,55125  35,85  0,004
Temperature            1  0,10125  0,10125  0,10125   6,58  0,062
Pressure*Temperature   1  0,17314  0,17314  0,17314  11,26  0,028
Error                  4  0,06151  0,06151  0,01538
Total                  7  0,88715


S = 0,124005   R-Sq = 93,07%   R-Sq(adj) = 87,87%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   6,2  A
0         4   5,7    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   6,1  A
30           4   5,9  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
1         30           2   6,3  A
1         40           2   6,2  A
0         40           2   6,0  A
0         30           2   5,4    B

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 total phenolic content of cricket powder.*

General Linear Model: Cricket (mg GAE/ versus Pressure; Temperature

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


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

Source                DF   Seq SS   Adj SS   Adj MS       F      P
Pressure               1  0,40500  0,40500  0,40500  144,86  0,000
Temperature            1  0,16642  0,16642  0,16642   59,52  0,002
Pressure*Temperature   1  0,27266  0,27266  0,27266   97,52  0,001
Error                  4  0,01118  0,01118  0,00280
Total                  7  0,85527


S = 0,0528759   R-Sq = 98,69%   R-Sq(adj) = 97,71%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4  10,6  A
0         4  10,2    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  10,6  A
30           4  10,3    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
1         40           2  11,0  A
1         30           2  10,3    B
0         30           2  10,2    B
0         40           2  10,2    B

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 antioxidant activity of mealworm powder with DPPH Assay.*

General Linear Model: Mealworm (mg tro 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  0,0001206  0,0001206  0,0001206  0,55  0,501
Temperature            1  0,0010855  0,0010855  0,0010855  4,91  0,091
Pressure*Temperature   1  0,0019298  0,0019298  0,0019298  8,73  0,042
Error                  4  0,0008845  0,0008845  0,0002211
Total                  7  0,0040203


S = 0,0148701   R-Sq = 78,00%   R-Sq(adj) = 61,50%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
0         4   2,6  A
1         4   2,6  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   2,6  A
40           4   2,6  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
1         30           2   2,6  A
0         40           2   2,6  A
0         30           2   2,6  A
1         40           2   2,5  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 antioxidant activity of cricket powder with DPPH Assay.*

General Linear Model: Cricket (mg trol 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  0,0000302  0,0000302  0,0000302  0,18  0,696
Temperature            1  0,0000302  0,0000302  0,0000302  0,18  0,696
Pressure*Temperature   1  0,0014775  0,0014775  0,0014775  8,65  0,042
Error                  4  0,0006835  0,0006835  0,0001709
Total                  7  0,0022212


S = 0,0130715   R-Sq = 69,23%   R-Sq(adj) = 46,15%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4   2,6  A
0         4   2,6  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
30           4   2,6  A
40           4   2,6  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
1         30           2   2,6  A
0         40           2   2,6  A
1         40           2   2,6  A
0         30           2   2,6  A

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

Table C. 18. *Three way ANOVA and Tukey's Comparison Test with 95% confidence level for determination of the difference between cricket and mealworm antioxidants with DPPH Method.*

General Linear Model: Antioxidant Acti versus Pressure; Temperature; ...

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


Analysis of Variance for Antioxidant Activity (DPPH), using Adjusted SS
for
     Tests

Source                             DF     Seq SS     Adj SS     Adj MS
F
Pressure                            1  0,0001929  0,0001929  0,0001929
3,19
Temperature                         1  0,0000103  0,0000103  0,0000103
0,17
Insect Type                         1  0,0032065  0,0032065  0,0032065
53,00
Pressure*Temperature                1  0,0000925  0,0000925  0,0000925
1,53
Pressure*Insect Type                1  0,0003299  0,0003299  0,0003299
5,45
Temperature*Insect Type             1  0,0000011  0,0000011  0,0000011
0,02
Pressure*Temperature*Insect Type    1  0,0005034  0,0005034  0,0005034
8,32
Error                               8  0,0004840  0,0004840  0,0000605
Total                              15  0,0048205

Source                                 P
Pressure                           0,112
Temperature                        0,691
Insect Type                        0,000
Pressure*Temperature               0,251
Pressure*Insect Type               0,048
Temperature*Insect Type            0,894
Pressure*Temperature*Insect Type   0,020
Error
Total


S = 0,00777813   R-Sq = 89,96%   R-Sq(adj) = 81,17%


Unusual Observations for Antioxidant Activity (DPPH)

     Antioxidant
        Activity
Obs       (DPPH)      Fit   SE Fit  Residual  St Resid
  1      1,01755  1,03037  0,00550  -0,01282     -2,33 R
  2      1,04319  1,03037  0,00550   0,01282      2,33 R

R denotes an observation with a large standardized residual.


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

Pressure	N	Mean	Grouping
1	8	1,0	A
0	8	1,0	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	8	1,0	A
40	8	1,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Insect Type	N	Mean	Grouping
M	8	1,0	A
C	8	1,0	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	4	1,0	A
1	40	4	1,0	A
0	40	4	1,0	A
0	30	4	1,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Insect Type	N	Mean	Grouping
0	M	4	1,0	A
1	M	4	1,0	A
1	C	4	1,0	B
0	C	4	1,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Insect Type	N	Mean	Grouping
30	M	4	1,0	A
40	M	4	1,0	A
30	C	4	1,0	B
40	C	4	1,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Insect Type	N	Mean	Grouping
0	30	M	2	1,0	A
1	40	M	2	1,0	A B
1	30	M	2	1,0	A B
0	40	M	2	1,0	A B
1	30	C	2	1,0	A B
1	40	C	2	1,0	B C
0	40	C	2	1,0	B C
0	30	C	2	1,0	C

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 antioxidant activity of mealworm powder with Cuprac Assay.*

General Linear Model: Mealworm (mg tro 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   9,7607  9,7607  9,7607  163,35  0,000
Temperature            1   0,6045  0,6045  0,6045   10,12  0,034
Pressure*Temperature   1   0,3040  0,3040  0,3040    5,09  0,087
Error                  4   0,2390  0,2390  0,0598
Total                  7  10,9082


S = 0,244446   R-Sq = 97,81%   R-Sq(adj) = 96,17%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4  10,2  A
0         4   8,0    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   9,4  A
40           4   8,8    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  10,7  A
1         40           2   9,7  A
0         30           2   8,1    B
0         40           2   7,9    B

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 antioxidant activity of cricket powder with Cuprac Assay.*

General Linear Model: Cricket (mg trol 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   0,6267  0,6267  0,6267  0,80  0,423
Temperature            1   0,2046  0,2046  0,2046  0,26  0,637
Pressure*Temperature   1   6,1904  6,1904  6,1904  7,87  0,049
Error                  4   3,1464  3,1464  0,7866
Total                  7  10,1682


S = 0,886901   R-Sq = 69,06%   R-Sq(adj) = 45,85%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N  Mean  Grouping
1         4  24,7  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  24,6  A
30           4  24,3  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  25,8  A
0         30           2  24,9  A
1         30           2  23,7  A
0         40           2  23,4  A

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

Table C. 21. *One way ANOVA to examine the effect of concentration on T2 results of mealworm.*

General Linear Model: Mealworm T2 versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  5; 10; 15; 20; 25


Analysis of Variance for Mealworm T2, using Adjusted SS for Tests

Source          DF  Seq SS  Adj SS  Adj MS     F      P
Concentration    4   93615   93615   23404  5,14  0,001
Error          115  523243  523243    4550
Total          119  616858


S = 67,4532   R-Sq = 15,18%   R-Sq(adj) = 12,23%


Unusual Observations for Mealworm T2

Obs  Mealworm T2      Fit  SE Fit  Residual  St Resid
 70      300,672  165,003  13,769   135,668      2,05 R
 71      298,954  165,003  13,769   133,951      2,03 R
 72      299,626  165,003  13,769   134,622      2,04 R

R denotes an observation with a large standardized residual.
```

Table C. 22. *One way ANOVA to examine the effect of pressure on T2 results of mealworm*

General Linear Model: Mealworm T2 versus Pressure

```
Factor    Type   Levels  Values
Pressure  fixed       2  0; 1


Analysis of Variance for Mealworm T2, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
Pressure    1    5481    5481    5481  1,06  0,306
Error     118  611377  611377    5181
Total     119  616858


S = 71,9803   R-Sq = 0,89%   R-Sq(adj) = 0,05%

Unusual Observations for Mealworm T2

Obs  Mealworm T2      Fit  SE Fit  Residual  St Resid
106      341,113  182,412   9,293   158,702      2,22 R
107      344,046  182,412   9,293   161,634      2,26 R
108      344,184  182,412   9,293   161,773      2,27 R

R denotes an observation with a large standardized residual.
```

Table C. 23. *One way ANOVA to examine the effect of temperature on T_2 results of mealworm*

General Linear Model: Mealworm T2 versus Temperature

```
Factor       Type   Levels  Values
Temperature  fixed       2  30; 40


Analysis of Variance for Mealworm T2, using Adjusted SS for Tests

Source        DF  Seq SS  Adj SS  Adj MS     F      P
Temperature    1   12492   12492   12492  2,44  0,121
Error        118  604366  604366    5122
Total        119  616858


S = 71,5664   R-Sq = 2,03%   R-Sq(adj) = 1,19%


Unusual Observations for Mealworm T2

Obs  Mealworm T2      Fit  SE Fit  Residual  St Resid
107      344,046  199,373   9,239   144,673      2,04 R
108      344,184  199,373   9,239   144,811      2,04 R

R denotes an observation with a large standardized residual.
```

Table C. 24. *One way ANOVA to examine the effect of heat on T2 results of mealworm.*

General Linear Model: Mealworm T2 versus Gelling Process

```
Factor           Type   Levels  Values
Gelling Process  fixed       2  H; N


Analysis of Variance for Mealworm T2, using Adjusted SS for Tests

Source            DF  Seq SS  Adj SS  Adj MS       F      P
Gelling Process    1  404965  404965  404965  225,52  0,000
Error            118  211893  211893    1796
Total            119  616858


S = 42,3757   R-Sq = 65,65%   R-Sq(adj) = 65,36%


Unusual Observations for Mealworm T2

Obs  Mealworm T2      Fit  SE Fit  Residual  St Resid
100      157,752  247,262   5,471   -89,510     -2,13 R
101      155,537  247,262   5,471   -91,725     -2,18 R
102      155,022  247,262   5,471   -92,241     -2,20 R
106      341,113  247,262   5,471    93,851      2,23 R
107      344,046  247,262   5,471    96,783      2,30 R
108      344,184  247,262   5,471    96,922      2,31 R

R denotes an observation with a large standardized residual.
```

Table C. 25. *One way ANOVA to examine the effect of concentration on T_2 results of cricket*

General Linear Model: Cricket T2 versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  5; 10; 15; 20; 25


Analysis of Variance for Cricket T2, using Adjusted SS for Tests

Source          DF  Seq SS  Adj SS  Adj MS       F      P
Concentration    4   94177   94177   23544  402,87  0,000
Error          115    6721    6721      58
Total          119  100898


S = 7,64465   R-Sq = 93,34%   R-Sq(adj) = 93,11%
```

Table C. 26. *One way ANOVA to examine the effect of pressure on T_2 results of cricket*

General Linear Model: Cricket T2 versus Pressure

```
Factor    Type   Levels  Values
Pressure  fixed       2  0; 1


Analysis of Variance for Cricket T2, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS  Adj MS     F      P
Pressure    1     589,3     589,3   589,3  0,69  0,407
Error     118  100308,4  100308,4   850,1
Total     119  100897,7


S = 29,1560   R-Sq = 0,58%   R-Sq(adj) = 0,00%


Unusual Observations for Cricket T2

Obs  Cricket T2      Fit  SE Fit  Residual  St Resid
 76     195,815  135,100   3,764    60,715      2,10 R
 77     196,017  135,100   3,764    60,917      2,11 R
 78     193,568  135,100   3,764    58,468      2,02 R

R denotes an observation with a large standardized residual.
```

Table C. 27. *One way ANOVA to examine the effect of temperature on T2 results of cricket*

General Linear Model: Cricket T2 versus Temperature

```
Factor       Type   Levels  Values
Temperature  fixed       2  30; 40


Analysis of Variance for Cricket T2, using Adjusted SS for Tests

Source        DF    Seq SS    Adj SS  Adj MS     F      P
Temperature    1      83,2      83,2    83,2  0,10  0,755
Error        118  100814,4  100814,4   854,4
Total        119  100897,7


S = 29,2294   R-Sq = 0,08%   R-Sq(adj) = 0,00%


Unusual Observations for Cricket T2

Obs  Cricket T2      Fit  SE Fit  Residual  St Resid
 76     195,815  136,483   3,774    59,332      2,05 R
 77     196,017  136,483   3,774    59,534      2,05 R

R denotes an observation with a large standardized residual.
```

Table C. 28. *One way ANOVA to examine the effect of heat on T2 results of cricket*

General Linear Model: Cricket T2 versus Gelling Process

```
Factor           Type   Levels  Values
Gelling Process  fixed       2  H; N


Analysis of Variance for Cricket T2, using Adjusted SS for Tests

Source            DF    Seq SS   Adj SS  Adj MS     F      P
Gelling Process    1    5044,6   5044,6  5044,6  6,21  0,014
Error            118   95853,1  95853,1   812,3
Total            119  100897,7


S = 28,5011   R-Sq = 5,00%   R-Sq(adj) = 4,19%
```

Table C. 29. *One way ANOVA to examine the effect of concentration on T1 results of mealworm*

General Linear Model: Mealworm T1 versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  5; 10; 15; 20; 25


Analysis of Variance for Mealworm T1, using Adjusted SS for Tests

Source         DF   Seq SS  Adj SS  Adj MS      F      P
Concentration   4   428043  428043  107011  10,19  0,000
Error          75   787822  787822   10504
Total          79  1215865


S = 102,490   R-Sq = 35,20%   R-Sq(adj) = 31,75%


Unusual Observations for Mealworm T1

Obs  Mealworm T1      Fit  SE Fit  Residual  St Resid
 71      700,085  488,741  25,623   211,344      2,13 R
 72      708,684  488,741  25,623   219,944      2,22 R

R denotes an observation with a large standardized residual.
```

Table C. 30. *One way ANOVA to examine the effect of pressure on T1 results of mealworm*

General Linear Model: Mealworm T1 versus Pressure

```
Factor    Type   Levels  Values
Pressure  fixed       2  0; 1

Analysis of Variance for Mealworm T1, using Adjusted SS for Tests

Source    DF   Seq SS   Adj SS  Adj MS     F      P
Pressure   1    28014    28014   28014  1,84  0,179
Error     78  1187851  1187851   15229
Total     79  1215865


S = 123,405   R-Sq = 2,30%   R-Sq(adj) = 1,05%


Unusual Observations for Mealworm T1

Obs  Mealworm T1      Fit  SE Fit  Residual  St Resid
 41      683,107  376,639  19,512   306,469      2,52 R
 42      686,053  376,639  19,512   309,415      2,54 R
 71      700,085  339,212  19,512   360,872      2,96 R
 72      708,684  339,212  19,512   369,472      3,03 R

R denotes an observation with a large standardized residual.
```

Table C. 31. *One way ANOVA to examine the effect of temperature on T_1 results of mealworm*

General Linear Model: Mealworm T1 versus Temperature

```
Factor       Type   Levels  Values
Temperature  fixed       2  30; 40


Analysis of Variance for Mealworm T1, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS  Adj MS     F      P
Temperature   1    24029    24029   24029  1,57  0,214
Error        78  1191836  1191836   15280
Total        79  1215865


S = 123,612   R-Sq = 1,98%   R-Sq(adj) = 0,72%


Unusual Observations for Mealworm T1

Obs  Mealworm T1      Fit  SE Fit  Residual  St Resid
 41      683,107  340,594  19,545   342,513      2,81 R
 42      686,053  340,594  19,545   345,459      2,83 R
 71      700,085  375,257  19,545   324,828      2,66 R
 72      708,684  375,257  19,545   333,428      2,73 R

R denotes an observation with a large standardized residual.
```

Table C. 32. *One way ANOVA to examine the effect of heat on T1 results of mealworm*

General Linear Model: Mealworm T1 versus Gelling Process

```
Factor           Type   Levels  Values
Gelling Process  fixed       2  H; N

Analysis of Variance for Mealworm T1, using Adjusted SS for Tests

Source           DF   Seq SS  Adj SS  Adj MS      F      P
Gelling Process   1   523898  523898  523898  59,05  0,000
Error            78   691968  691968    8871
Total            79  1215865

S = 94,1880   R-Sq = 43,09%   R-Sq(adj) = 42,36%


Unusual Observations for Mealworm T1

Obs  Mealworm T1      Fit  SE Fit  Residual  St Resid
 41      683,107  438,850  14,892   244,258      2,63 R
 42      686,053  438,850  14,892   247,204      2,66 R
 71      700,085  438,850  14,892   261,235      2,81 R
 72      708,684  438,850  14,892   269,835      2,90 R

R denotes an observation with a large standardized residual.
```

Table C. 33. *One way ANOVA to examine the effect of concentration on T1 results of cricket*

General Linear Model: Cricket T1 versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  5; 10; 15; 20; 25


Analysis of Variance for Cricket T1, using Adjusted SS for Tests

Source         DF  Seq SS  Adj SS  Adj MS       F      P
Concentration   4  150902  150902   37726  214,77  0,000
Error          75   13174   13174     176
Total          79  164076


S = 13,2534   R-Sq = 91,97%   R-Sq(adj) = 91,54%


Unusual Observations for Cricket T1

Obs  Cricket T1      Fit  SE Fit  Residual  St Resid
  1     322,699  351,184   3,313   -28,485     -2,22 R
  2     324,766  351,184   3,313   -26,418     -2,06 R
 11     322,344  351,184   3,313   -28,840     -2,25 R
 12     322,570  351,184   3,313   -28,615     -2,23 R

R denotes an observation with a large standardized residual.
```

Table C. 34. *One way ANOVA to examine the effect of pressure on T1 results of cricket*

General Linear Model: Cricket T1 versus Pressure

```
Factor    Type   Levels  Values
Pressure  fixed       2  0; 1

Analysis of Variance for Cricket T1, using Adjusted SS for Tests

Source    DF  Seq SS  Adj SS  Adj MS     F      P
Pressure   1    1445    1445    1445  0,69  0,408
Error     78  162631  162631    2085
Total     79  164076


S = 45,6619   R-Sq = 0,88%   R-Sq(adj) = 0,00%


Unusual Observations for Cricket T1

Obs  Cricket T1      Fit  SE Fit  Residual  St Resid
 51     373,974  275,042   7,220    98,933      2,19 R
 52     374,034  275,042   7,220    98,992      2,20 R


R denotes an observation with a large standardized residual.
```

Table C. 35. *One way ANOVA to examine the effect of temperature on T_1 results of cricket*

General Linear Model: Cricket T1 versus Temperature

```
Factor       Type   Levels  Values
Temperature  fixed       2  30; 40


Analysis of Variance for Cricket T1, using Adjusted SS for Tests

Source       DF  Seq SS  Adj SS  Adj MS     F      P
Temperature   1       9       9       9  0,00  0,947
Error        78  164067  164067    2103
Total        79  164076


S = 45,8631   R-Sq = 0,01%   R-Sq(adj) = 0,00%


Unusual Observations for Cricket T1

Obs  Cricket T1      Fit  SE Fit  Residual  St Resid
 51     373,974  279,631   7,252    94,343      2,08 R
 52     374,034  279,631   7,252    94,403      2,08 R
 71     370,932  279,631   7,252    91,301      2,02 R
 72     370,890  279,631   7,252    91,259      2,02 R

R denotes an observation with a large standardized residual.
```

Table C. 36. *One way ANOVA to examine the effect of heat on T_1 results of cricket*

General Linear Model: Cricket T1 versus Gelling Process

```
Factor           Type   Levels  Values
Gelling Process  fixed       2  H; N


Analysis of Variance for Cricket T1, using Adjusted SS for Tests

Source           DF  Seq SS  Adj SS  Adj MS     F      P
Gelling Process   1    9433    9433    9433  4,76  0,032
Error            78  154643  154643    1983
Total            79  164076


S = 44,5265   R-Sq = 5,75%   R-Sq(adj) = 4,54%
```

Table C. 37. *Three way ANOVA and Tukey's Comparison Test with 95% confidence level to observe the effect of different concentrations, salt addition and heat on T2 results of mealworm*

(General Linear Model: Mealworm T2 versus Concentration; Salt; ...

```
Factor           Type   Levels  Values
Concentration    fixed       5  5; 10; 15; 20; 25
Salt             fixed       3  CaCl2; MnCl2; None
Gelling Process  fixed       2  H; N


Analysis of Variance for Mealworm T2, using Adjusted SS for Tests

Source                               DF    Seq SS    Adj SS   Adj MS
F
Concentration                         4   4239588   4239588  1059897
22460,66
Salt                                  2  11671219  11671219  5835609
123664,49
Gelling Process                       1      6970      6970     6970
147,71
Concentration*Salt                    8   2470951   2470951   308869
6545,35
Concentration*Gelling Process         4      6687      6687     1672
35,42
Salt*Gelling Process                  2    346883    346883   173441
3675,46
Concentration*Salt*Gelling Process    8      9308      9308     1164
24,66
Error                                60      2831      2831       47
Total                                89  18754437

Source                                   P
Concentration                        0,000
Salt                                 0,000
Gelling Process                      0,000
Concentration*Salt                   0,000
Concentration*Gelling Process        0,000
Salt*Gelling Process                 0,000
Concentration*Salt*Gelling Process   0,000
Error
Total


S = 6,86943   R-Sq = 99,98%   R-Sq(adj) = 99,98%


Unusual Observations for Mealworm T2

Obs  Mealworm T2      Fit  SE Fit  Residual  St Resid
 46       508,13   492,68    3,97     15,45      2,75 R
 58      1406,57  1418,24    3,97    -11,67     -2,08 R
 67       941,46   954,70    3,97    -13,24     -2,36 R
 81       683,39   696,95    3,97    -13,56     -2,42 R
 87      1182,42  1168,38    3,97     14,04      2,50 R
 90      1499,33  1487,84    3,97     11,50      2,05 R

R denotes an observation with a large standardized residual.

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

Concentration	N	Mean	Grouping
5	18	1060,8	A
10	18	811,7	B
15	18	654,9	C
20	18	521,6	D
25	18	457,0	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Salt	N	Mean	Grouping
CaCl2	30	994,6	A
None	30	915,0	B
MnCl2	30	194,0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Gelling Process	N	Mean	Grouping
N	45	710,0	A
H	45	692,4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Salt	N	Mean	Grouping
5	CaCl2	6	1524,4	A
5	None	6	1495,7	B
10	CaCl2	6	1185,8	C
10	None	6	1048,1	D
15	CaCl2	6	935,3	E
15	None	6	831,9	F
20	CaCl2	6	733,6	G
20	None	6	652,3	H
25	CaCl2	6	594,1	I
25	None	6	546,7	J
25	MnCl2	6	230,2	K
10	MnCl2	6	201,1	L
15	MnCl2	6	197,6	L
20	MnCl2	6	178,9	M
5	MnCl2	6	162,2	N

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Gelling Process	N	Mean	Grouping
5	N	9	1074,6	A
5	H	9	1046,9	B
10	N	9	814,2	C
10	H	9	809,2	C
15	N	9	660,3	D
15	H	9	649,6	E
20	N	9	544,7	F
20	H	9	498,5	G
25	H	9	457,7	H
25	N	9	456,2	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Salt	Gelling Process	N	Mean	Grouping
CaCl2	N	15	1020,0	A
None	N	15	990,1	B
CaCl2	H	15	969,3	C
None	H	15	839,8	D
MnCl2	H	15	268,1	E
MnCl2	N	15	119,9	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Salt	Gelling Process	N	Mean
5	None	N	3	1573,2
5	CaCl2	N	3	1560,9
5	CaCl2	H	3	1487,8
5	None	H	3	1418,2
10	CaCl2	N	3	1203,3
10	CaCl2	H	3	1168,4
10	None	N	3	1137,0
10	None	H	3	959,3
15	CaCl2	N	3	954,7
15	CaCl2	H	3	915,9
15	None	N	3	911,0
20	CaCl2	N	3	770,3
15	None	H	3	752,8
20	None	N	3	728,9
20	CaCl2	H	3	697,0
25	CaCl2	N	3	610,8
25	None	N	3	600,7
25	CaCl2	H	3	577,3
20	None	H	3	575,8
25	None	H	3	492,7
25	MnCl2	H	3	303,3
10	MnCl2	H	3	299,8
15	MnCl2	H	3	280,1
5	MnCl2	H	3	234,7
20	MnCl2	H	3	222,9
25	MnCl2	N	3	157,1
20	MnCl2	N	3	134,9
15	MnCl2	N	3	115,0
10	MnCl2	N	3	102,4
5	MnCl2	N	3	89,7

Concentration	Salt	Gelling Process	Grouping
5	None	N	A
5	CaCl2	N	A
5	CaCl2	H	B
5	None	H	C
10	CaCl2	N	D
10	CaCl2	H	E
10	None	N	F
10	None	H	G
15	CaCl2	N	G
15	CaCl2	H	H
15	None	N	H
20	CaCl2	N	I
15	None	H	I
20	None	N	J
20	CaCl2	H	K
25	CaCl2	N	L
25	None	N	L
25	CaCl2	H	M
20	None	H	M
25	None	H	N
25	MnCl2	H	O
10	MnCl2	H	O P
15	MnCl2	H	P
5	MnCl2	H	Q
20	MnCl2	H	Q
25	MnCl2	N	R
20	MnCl2	N	S
15	MnCl2	N	S T
10	MnCl2	N	T U
5	MnCl2	N	U

Means that do not share a letter are significantly different.

Table C. 38. *Three way ANOVA and Tukey's Comparison Test with 95% confidence level to observe the effect of different concentrations, salt addition and heat on T2 results of cricket*

General Linear Model: Cricket T2 versus Concentratio; Salt; Gelling Proc

```
Factor           Type   Levels  Values
Concentration    fixed       5  5; 10; 15; 20; 25
Salt             fixed       3  CaCl2; MnCl2; None
Gelling Process  fixed       2  H; N


Analysis of Variance for Cricket T2, using Adjusted SS for Tests

Source                               DF   Seq SS   Adj SS   Adj MS
F
Concentration                         4  2427204  2427204   606801
17225,98
Salt                                  2  4220855  4220855  2110428
59911,22
Gelling Process                       1     2642     2642     2642
75,02
Concentration*Salt                    8  1606722  1606722   200840
5701,49
Concentration*Gelling Process         4     1675     1675      419
11,89
Salt*Gelling Process                  2    22269    22269    11134
316,08
Concentration*Salt*Gelling Process    8     1580     1580      198
5,61
Error                                60     2114     2114       35
Total                                89  8285061

Source                                   P
Concentration                         0,000
Salt                                  0,000
Gelling Process                       0,000
Concentration*Salt                    0,000
Concentration*Gelling Process         0,000
Salt*Gelling Process                  0,000
Concentration*Salt*Gelling Process    0,000
Error
Total


S = 5,93514   R-Sq = 99,97%   R-Sq(adj) = 99,96%


Unusual Observations for Cricket T2

Obs  Cricket T2      Fit  SE Fit  Residual  St Resid
 55      691,13   707,69    3,43    -16,56     -3,42 R
 57      718,66   707,69    3,43     10,97      2,26 R
 58     1107,78  1085,44    3,43     22,34      4,61 R
 60     1070,64  1085,44    3,43    -14,80     -3,05 R
 85      605,45   585,79    3,43     19,66      4,06 R
 86      574,53   585,79    3,43    -11,26     -2,32 R

R denotes an observation with a large standardized residual.


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

Concentration	N	Mean	Grouping
5	18	737,5	A
10	18	481,3	B
15	18	377,1	C
20	18	315,8	D
25	18	281,0	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Salt	N	Mean	Grouping
None	30	626,0	A
CaCl2	30	554,6	B
MnCl2	30	135,1	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Gelling Process	N	Mean	Grouping
N	45	444,0	A
H	45	433,1	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Salt	N	Mean	Grouping
5	None	6	1081,4	A
5	CaCl2	6	1031,1	B
10	None	6	710,6	C
10	CaCl2	6	617,7	D
15	None	6	541,9	E
15	CaCl2	6	459,5	F
20	None	6	432,2	G
25	None	6	364,0	H
20	CaCl2	6	362,2	H
25	CaCl2	6	302,2	I
25	MnCl2	6	176,9	J
20	MnCl2	6	153,1	K
15	MnCl2	6	129,9	L
10	MnCl2	6	115,6	M
5	MnCl2	6	100,0	N

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Gelling Process	N	Mean	Grouping
5	N	9	749,5	A
5	H	9	725,5	B
10	N	9	490,1	C
10	H	9	472,5	D
15	N	9	380,4	E
15	H	9	373,7	E
20	N	9	318,3	F
20	H	9	313,4	F
25	N	9	281,5	G
25	H	9	280,6	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Salt	Gelling Process	N	Mean	Grouping
None	H	15	629,7	A
None	N	15	622,3	B
CaCl2	N	15	582,1	C
CaCl2	H	15	527,0	D
MnCl2	H	15	142,7	E
MnCl2	N	15	127,5	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Salt	Gelling Process	N	Mean	Grouping
5	None	H	3	1085,4	A
5	None	N	3	1077,3	A
5	CaCl2	N	3	1075,3	A
5	CaCl2	H	3	987,0	B
10	None	N	3	713,6	C
10	None	H	3	707,7	C
10	CaCl2	N	3	649,6	D
10	CaCl2	H	3	585,8	E
15	None	H	3	546,3	F
15	None	N	3	537,5	F
15	CaCl2	N	3	480,8	G
15	CaCl2	H	3	438,2	H
20	None	H	3	435,5	H
20	None	N	3	428,8	H
20	CaCl2	N	3	382,9	I
25	None	H	3	373,6	I
25	None	N	3	354,3	J
20	CaCl2	H	3	341,6	J
25	CaCl2	N	3	322,0	K
25	CaCl2	H	3	282,4	L
25	MnCl2	H	3	185,7	M
25	MnCl2	N	3	168,0	M N
20	MnCl2	H	3	163,0	N
20	MnCl2	N	3	143,2	O
15	MnCl2	H	3	136,7	O P
10	MnCl2	H	3	124,0	P
Q					
15	MnCl2	N	3	123,0	P
Q R					
10	MnCl2	N	3	107,2	
Q R S					
5	MnCl2	H	3	104,0	
R S					
5	MnCl2	N	3	96,0	
S					

Means that do not share a letter are significantly different.

Table C. 39. *Three way ANOVA and Tukey's Comparison Test with 95% confidence level to observe the effect of different concentrations, salt addition and heat on T1 results of mealworm*

General Linear Model: Mealworm T1 versus Concentration; Salt; ...

```
Factor           Type   Levels  Values
Concentration    fixed       5  5; 10; 15; 20; 25
Salt             fixed       3  CaCl2; MnCl2; None
Gelling Process  fixed       2  H; N


Analysis of Variance for Mealworm T1, using Adjusted SS for Tests

Source                               DF    Seq SS    Adj SS   Adj MS
F
Concentration                         4   3025309   3025309   756327
31313,05
Salt                                  2  19861008  19861008  9930504
411137,26
Gelling Process                       1     52171     52171    52171
2159,94
Concentration*Salt                    8   2330942   2330942   291368
12063,05
Concentration*Gelling Process         4     17738     17738     4434
183,59
Salt*Gelling Process                  2    437566    437566   218783
9057,93
Concentration*Salt*Gelling Process    8     55519     55519     6940
287,32
Error                                30       725       725       24
Total                                59  25780977

Source                                   P
Concentration                        0,000
Salt                                 0,000
Gelling Process                      0,000
Concentration*Salt                   0,000
Concentration*Gelling Process        0,000
Salt*Gelling Process                 0,000
Concentration*Salt*Gelling Process   0,000
Error
Total

S = 4,91465   R-Sq = 100,00%   R-Sq(adj) =  99,99%


Unusual Observations for Mealworm T1

Obs  Mealworm T1      Fit  SE Fit  Residual  St Resid
 53      1492,21  1500,27    3,48     -8,07     -2,32 R
 54      1508,34  1500,27    3,48      8,07      2,32 R

R denotes an observation with a large standardized residual.
```

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	N	Mean	Grouping
5	12	1543,7	A
10	12	1288,6	B
15	12	1123,3	C
20	12	983,1	D
25	12	922,5	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Salt	N	Mean	Grouping
CaCl2	20	1721,6	A
None	20	1417,3	B
MnCl2	20	377,8	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Gelling Process	N	Mean	Grouping
H	30	1201,7	A
N	30	1142,7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Salt	N	Mean	Grouping
5	CaCl2	4	2208,6	A
5	None	4	2089,0	B
10	CaCl2	4	1925,0	C
15	CaCl2	4	1689,3	D
10	None	4	1616,9	E
20	CaCl2	4	1476,0	F
15	None	4	1316,7	G
25	CaCl2	4	1309,2	G
20	None	4	1105,6	H
25	None	4	958,2	I
25	MnCl2	4	499,9	J
20	MnCl2	4	367,5	K
15	MnCl2	4	363,9	K
5	MnCl2	4	333,5	L
10	MnCl2	4	323,9	L

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Gelling Process	N	Mean	Grouping
5	H	6	1552,4	A
5	N	6	1535,0	B
10	H	6	1307,0	C
10	N	6	1270,1	D
15	H	6	1156,3	E
15	N	6	1090,3	F
20	H	6	1010,8	G
25	H	6	982,1	H
20	N	6	955,3	I
25	N	6	862,8	J

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Salt	Gelling Process	N	Mean	Grouping
CaCl2	H	10	1758,2	A
CaCl2	N	10	1685,1	B
None	N	10	1495,7	C
None	H	10	1338,8	D
MnCl2	H	10	508,1	E
MnCl2	N	10	247,4	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Salt	Gelling Process	N	Mean
5	CaCl2	H	2	2252,8
5	None	N	2	2242,7
5	CaCl2	N	2	2164,3
10	CaCl2	H	2	1983,7
5	None	H	2	1935,3
10	CaCl2	N	2	1866,3
15	CaCl2	H	2	1733,3
10	None	N	2	1731,1
15	CaCl2	N	2	1645,3
10	None	H	2	1502,7
20	CaCl2	H	2	1500,3
20	CaCl2	N	2	1451,7
15	None	N	2	1385,9
25	CaCl2	H	2	1320,8
25	CaCl2	N	2	1297,6
15	None	H	2	1247,4
20	None	N	2	1143,5
20	None	H	2	1067,8
25	None	N	2	975,6
25	None	H	2	940,9
25	MnCl2	H	2	684,6
15	MnCl2	H	2	488,1
5	MnCl2	H	2	469,1
20	MnCl2	H	2	464,2
10	MnCl2	H	2	434,8
25	MnCl2	N	2	315,3
20	MnCl2	N	2	270,9

15	MnCl2	N	2	239,8
10	MnCl2	N	2	213,0
5	MnCl2	N	2	198,0

Concentration	Salt	Gelling Process	Grouping
5	CaCl2	H	A
5	None	N	A
5	CaCl2	N	B
10	CaCl2	H	C
5	None	H	D
10	CaCl2	N	E
15	CaCl2	H	F
10	None	N	F
15	CaCl2	N	G
10	None	H	H
20	CaCl2	H	H
20	CaCl2	N	I
15	None	N	J
25	CaCl2	H	K
25	CaCl2	N	L
15	None	H	M
20	None	N	N
20	None	H	O
25	None	N	P
25	None	H	Q
25	MnCl2	H	R
15	MnCl2	H	S
5	MnCl2	H	S T
20	MnCl2	H	T
10	MnCl2	H	U
25	MnCl2	N	
V			
20	MnCl2	N	
W			
15	MnCl2	N	
X			
10	MnCl2	N	
Y			
5	MnCl2	N	
Y			

Means that do not share a letter are significantly different.

Table C. 40. *Three way ANOVA and Tukey's Comparison Test with 95% confidence level to observe the effect of different concentrations, salt addition and heat on T_1 results of cricket*

General Linear Model: Cricket T1 versus Concentratio; Salt; Gelling Proc

```
Factor           Type   Levels  Values
Concentration    fixed       5  5; 10; 15; 20; 25
Salt             fixed       3  CaCl2; MnCl2; None
Gelling Process  fixed       2  H; N


Analysis of Variance for Cricket T1, using Adjusted SS for Tests

Source                               DF    Seq SS    Adj SS   Adj MS
F
Concentration                         4   3360983   3360983   840246
105734,39
Salt                                  2  10246220  10246220  5123110
644679,08
Gelling Process                       1     11113     11113    11113
1398,37
Concentration*Salt                    8   2280387   2280387   285048
35869,75
Concentration*Gelling Process         4      6554      6554     1638
206,17
Salt*Gelling Process                  2     10550     10550     5275
663,82
Concentration*Salt*Gelling Process    8      8624      8624     1078
135,65
Error                                30       238       238        8
Total                                59  15924669

Source                                   P
Concentration                        0,000
Salt                                 0,000
Gelling Process                      0,000
Concentration*Salt                   0,000
Concentration*Gelling Process        0,000
Salt*Gelling Process                 0,000
Concentration*Salt*Gelling Process   0,000
Error
Total


S = 2,81900   R-Sq = 100,00%   R-Sq(adj) = 100,00%


Unusual Observations for Cricket T1

Obs  Cricket T1      Fit  SE Fit  Residual  St Resid
 29     1573,41  1568,56    1,99      4,84      2,43 R
 30     1563,72  1568,56    1,99     -4,84     -2,43 R
 39     1653,59  1649,32    1,99      4,27      2,14 R
 40     1645,06  1649,32    1,99     -4,27     -2,14 R
 53      902,73   907,85    1,99     -5,12     -2,57 R
 54      912,96   907,85    1,99      5,12      2,57 R

R denotes an observation with a large standardized residual.
```

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	N	Mean	Grouping
5	12	1245,6	A
10	12	960,7	B
15	12	762,2	C
20	12	656,8	D
25	12	592,1	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Salt	N	Mean	Grouping
CaCl2	20	1243,5	A
None	20	1012,5	B
MnCl2	20	274,5	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Gelling Process	N	Mean	Grouping
H	30	857,1	A
N	30	829,9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Salt	N	Mean	Grouping
5	CaCl2	4	1899,8	A
5	None	4	1608,9	B
10	CaCl2	4	1473,8	C
10	None	4	1166,8	D
15	CaCl2	4	1116,2	E
20	CaCl2	4	929,4	F
15	None	4	907,8	G
25	CaCl2	4	798,3	H
20	None	4	742,7	I
25	None	4	636,1	J
25	MnCl2	4	342,0	K
20	MnCl2	4	298,2	L
15	MnCl2	4	262,7	M
10	MnCl2	4	241,5	N
5	MnCl2	4	228,0	O

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Gelling Process	N	Mean	Grouping
5	H	6	1269,2	A
5	N	6	1222,0	B
10	H	6	983,2	C
10	N	6	938,2	D
15	N	6	765,8	E
15	H	6	758,7	F
20	H	6	663,5	G
20	N	6	650,0	H
25	H	6	610,8	I
25	N	6	573,4	J

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Salt	Gelling Process	N	Mean	Grouping
CaCl2	N	10	1245,1	A
CaCl2	H	10	1241,9	A
None	H	10	1043,2	B
None	N	10	981,7	C
MnCl2	H	10	286,2	D
MnCl2	N	10	262,8	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Salt	Gelling Process	N	Mean
5	CaCl2	H	2	1923,1
5	CaCl2	N	2	1876,5
5	None	H	2	1649,3
5	None	N	2	1568,6
10	CaCl2	H	2	1503,3
10	CaCl2	N	2	1444,3
10	None	H	2	1195,3
15	CaCl2	N	2	1156,1
10	None	N	2	1138,4
15	CaCl2	H	2	1076,3
20	CaCl2	N	2	951,0
15	None	H	2	928,2
20	CaCl2	H	2	907,8
15	None	N	2	887,5
25	CaCl2	H	2	799,1
25	CaCl2	N	2	797,5
20	None	H	2	770,2
20	None	N	2	715,2
25	None	H	2	673,1
25	None	N	2	599,1
25	MnCl2	H	2	360,3
25	MnCl2	N	2	323,7
20	MnCl2	H	2	312,5
20	MnCl2	N	2	283,9
15	MnCl2	H	2	271,7
15	MnCl2	N	2	253,8
10	MnCl2	H	2	251,1
5	MnCl2	H	2	235,3
10	MnCl2	N	2	232,0
5	MnCl2	N	2	220,8

Concentration	Salt	Gelling Process	Grouping
5	CaCl2	H	A
5	CaCl2	N	B
5	None	H	C
5	None	N	D
10	CaCl2	H	E
10	CaCl2	N	F
10	None	H	G
15	CaCl2	N	H
10	None	N	I
15	CaCl2	H	J
20	CaCl2	N	K
15	None	H	L
20	CaCl2	H	M
15	None	N	N
25	CaCl2	H	O
25	CaCl2	N	O
20	None	H	P
20	None	N	Q
25	None	H	R
25	None	N	S
25	MnCl2	H	T
25	MnCl2	N	U
20	MnCl2	H	U
20	MnCl2	N	
V			
15	MnCl2	H	
W			
15	MnCl2	N	
X			
10	MnCl2	H	
X			
5	MnCl2	H	
Y			
10	MnCl2	N	
Y Z			
5	MnCl2	N	
Z			

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 absorbance peak observed at the frequency of 1026 cm^{-1} in mealworm powders*

General Linear Model: 1026 versus Pressure; Temperature

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


Analysis of Variance for 1026, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0000401  0,0000401  0,0000401   0,37  0,577
Temperature            1  0,0004852  0,0004852  0,0004852   4,46  0,102
Pressure*Temperature   1  0,0014231  0,0014231  0,0014231  13,09  0,022
Error                  4  0,0004347  0,0004347  0,0001087
Total                  7  0,0023831


S = 0,0104253   R-Sq = 81,76%   R-Sq(adj) = 68,07%


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
0         40           2   0,2  A
1         30           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. 42. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1234 cm^{-1} in mealworm powders*

General Linear Model: 1234 versus Pressure; Temperature

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


Analysis of Variance for 1234, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0000525  0,0000525  0,0000525   4,95  0,090
Temperature            1  0,0000263  0,0000263  0,0000263   2,48  0,191
Pressure*Temperature   1  0,0007163  0,0007163  0,0007163  67,55  0,001
Error                  4  0,0000424  0,0000424  0,0000106
Total                  7  0,0008375


S = 0,00325634   R-Sq = 94,94%   R-Sq(adj) = 91,14%


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    B
0         30           2   0,1    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 absorbance peak observed at the frequency of 1392 cm^{-1} in mealworm powders*

General Linear Model: 1392 versus Pressure; Temperature

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


Analysis of Variance for 1392, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0000611  0,0000611  0,0000611   3,02  0,157
Temperature            1  0,0000690  0,0000690  0,0000690   3,42  0,138
Pressure*Temperature   1  0,0007782  0,0007782  0,0007782  38,53  0,003
Error                  4  0,0000808  0,0000808  0,0000202
Total                  7  0,0009890


S = 0,00449375   R-Sq = 91,83%   R-Sq(adj) = 85,71%


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
0         40           2   0,1  A
1         30           2   0,1  A
1         40           2   0,1  A B
0         30           2   0,1    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 absorbance peak observed at the frequency of 1516 cm^{-1} in mealworm powders*

General Linear Model: 1516 versus Pressure; Temperature

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


Analysis of Variance for 1516, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0001767  0,0001767  0,0001767   3,89  0,120
Temperature            1  0,0000952  0,0000952  0,0000952   2,09  0,221
Pressure*Temperature   1  0,0014634  0,0014634  0,0014634  32,19  0,005
Error                  4  0,0001818  0,0001818  0,0000455
Total                  7  0,0019172


S = 0,00674222   R-Sq = 90,52%   R-Sq(adj) = 83,40%


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 B
0         30           2   0,1    B

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 absorbance peak observed at the frequency of 1625 cm^{-1} in mealworm powders*

General Linear Model: 1625 versus Pressure; Temperature

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


Analysis of Variance for 1625, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0000128  0,0000128  0,0000128   0,22  0,666
Temperature            1  0,0001256  0,0001256  0,0001256   2,13  0,218
Pressure*Temperature   1  0,0022412  0,0022412  0,0022412  38,00  0,004
Error                  4  0,0002359  0,0002359  0,0000590
Total                  7  0,0026154


S = 0,00767993   R-Sq = 90,98%   R-Sq(adj) = 84,21%


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
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
0         40           2   0,2  A
1         30           2   0,2  A
1         40           2   0,1  A B
0         30           2   0,1    B

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 absorbance peak observed at the frequency of 2358 cm^{-1} in mealworm powders*

General Linear Model: 2358 versus Pressure; Temperature

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


Analysis of Variance for 2358, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0004234  0,0004234  0,0004234  6,67  0,061
Temperature            1  0,0001824  0,0001824  0,0001824  2,87  0,165
Pressure*Temperature   1  0,0000120  0,0000120  0,0000120  0,19  0,686
Error                  4  0,0002540  0,0002540  0,0000635
Total                  7  0,0008718


S = 0,00796900   R-Sq = 70,86%   R-Sq(adj) = 49,01%


Grouping Information Using Tukey Method and 95,0% Confidence

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

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

Table C. 47. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 2924 cm^{-1} in mealworm powders*

General Linear Model: 2924 versus Pressure; Temperature

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


Analysis of Variance for 2924, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0000788  0,0000788  0,0000788   3,24  0,146
Temperature            1  0,0000447  0,0000447  0,0000447   1,84  0,247
Pressure*Temperature   1  0,0008841  0,0008841  0,0008841  36,34  0,004
Error                  4  0,0000973  0,0000973  0,0000243
Total                  7  0,0011048


S = 0,00493242   R-Sq = 91,19%   R-Sq(adj) = 84,59%


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 B
0         30           2   0,1    B

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

Table C. 48. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 3267 cm^{-1} in mealworm powders*

General Linear Model: 3267 versus Pressure; Temperature

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


Analysis of Variance for 3267, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0000202  0,0000202  0,0000202   0,70  0,450
Temperature            1  0,0002050  0,0002050  0,0002050   7,12  0,056
Pressure*Temperature   1  0,0009924  0,0009924  0,0009924  34,45  0,004
Error                  4  0,0001152  0,0001152  0,0000288
Total                  7  0,0013328


S = 0,00536715   R-Sq = 91,35%   R-Sq(adj) = 84,87%


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
0         40           2   0,1  A
1         30           2   0,1  A
1         40           2   0,1  A B
0         30           2   0,1    B

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

Table C. 49. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1064 cm^{-1} in cricket powders*

General Linear Model: 1064 versus Pressure; Temperature

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


Analysis of Variance for 1064, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0000320  0,0000320  0,0000320  0,91  0,393
Temperature            1  0,0002020  0,0002020  0,0002020  5,76  0,074
Pressure*Temperature   1  0,0000180  0,0000180  0,0000180  0,51  0,513
Error                  4  0,0001402  0,0001402  0,0000351
Total                  7  0,0003922


S = 0,00592052   R-Sq = 64,25%   R-Sq(adj) = 37,44%


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
0         40           2   0,1  A
1         30           2   0,1  A
0         30           2   0,1  A

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

Table C. 50. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1230 cm^{-1} in cricket powders*

General Linear Model: 1230 versus Pressure; Temperature

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


Analysis of Variance for 1230, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0000252  0,0000252  0,0000252  1,00  0,373
Temperature            1  0,0001411  0,0001411  0,0001411  5,62  0,077
Pressure*Temperature   1  0,0000106  0,0000106  0,0000106  0,42  0,552
Error                  4  0,0001005  0,0001005  0,0000251
Total                  7  0,0002774


S = 0,00501174   R-Sq = 63,78%   R-Sq(adj) = 36,61%


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
0         40           2   0,1  A
1         30           2   0,1  A
0         30           2   0,1  A

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

Table C. 51. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1396 cm^{-1} in cricket powders*

General Linear Model: 1396 versus Pressure; Temperature

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


Analysis of Variance for 1396, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0002311  0,0002311  0,0002311   7,57  0,051
Temperature            1  0,0003226  0,0003226  0,0003226  10,57  0,031
Pressure*Temperature   1  0,0000245  0,0000245  0,0000245   0,80  0,421
Error                  4  0,0001221  0,0001221  0,0000305
Total                  7  0,0007003


S = 0,00552426   R-Sq = 82,57%   R-Sq(adj) = 69,49%


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    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   0,1  A
0         40           2   0,1  A B
1         30           2   0,1  A B
0         30           2   0,1    B

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

Table C. 52. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1508 cm^{-1} in cricket powders*

General Linear Model: 1508 versus Pressure; Temperature

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


Analysis of Variance for 1508, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS      F      P
Pressure               1  0,0010534  0,0010534  0,0010534  13,89  0,020
Temperature            1  0,0007880  0,0007880  0,0007880  10,39  0,032
Pressure*Temperature   1  0,0001264  0,0001264  0,0001264   1,67  0,266
Error                  4  0,0003033  0,0003033  0,0000758
Total                  7  0,0022711


S = 0,00870718   R-Sq = 86,65%   R-Sq(adj) = 76,63%


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
1         30           2   0,2  A B
0         40           2   0,2  A B
0         30           2   0,1    B

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

Table C. 53. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1624 cm^{-1} in cricket powders*

General Linear Model: 1624 versus Pressure; Temperature

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


Analysis of Variance for 1624, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0002634  0,0002634  0,0002634  2,93  0,162
Temperature            1  0,0007742  0,0007742  0,0007742  8,62  0,043
Pressure*Temperature   1  0,0000278  0,0000278  0,0000278  0,31  0,608
Error                  4  0,0003591  0,0003591  0,0000898
Total                  7  0,0014244


S = 0,00947543   R-Sq = 74,79%   R-Sq(adj) = 55,88%


Grouping Information Using Tukey Method and 95,0% Confidence

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

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

Table C. 54. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 2358 cm^{-1} in cricket powders*

General Linear Model: 2358 versus Pressure; Temperature

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


Analysis of Variance for 2358, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0001531  0,0001531  0,0001531  7,35  0,053
Temperature            1  0,0001786  0,0001786  0,0001786  8,57  0,043
Pressure*Temperature   1  0,0000238  0,0000238  0,0000238  1,14  0,345
Error                  4  0,0000833  0,0000833  0,0000208
Total                  7  0,0004389


S = 0,00456454   R-Sq = 81,01%   R-Sq(adj) = 66,77%


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
30           4   0,1  A
40           4   0,1    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         30           2   0,1  A
1         30           2   0,1  A
0         40           2   0,1  A
1         40           2   0,1  A

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

Table C. 55. *Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 2924 cm^{-1} in cricket powders*

General Linear Model: 2924 versus Pressure; Temperature

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


Analysis of Variance for 2924, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0000168  0,0000168  0,0000168  1,83  0,247
Temperature            1  0,0000490  0,0000490  0,0000490  5,33  0,082
Pressure*Temperature   1  0,0000002  0,0000002  0,0000002  0,02  0,895
Error                  4  0,0000367  0,0000367  0,0000092
Total                  7  0,0001028


S = 0,00303109   R-Sq = 64,24%   R-Sq(adj) = 37,41%


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
0         40           2   0,1  A
1         30           2   0,1  A
0         30           2   0,1  A

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

Table C. 56. Two way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 3271 cm^{-1} in cricket powders

General Linear Model: 3271 versus Pressure; Temperature

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


Analysis of Variance for 3271, using Adjusted SS for Tests

Source                DF     Seq SS     Adj SS     Adj MS     F      P
Pressure               1  0,0000557  0,0000557  0,0000557  4,37  0,105
Temperature            1  0,0000932  0,0000932  0,0000932  7,31  0,054
Pressure*Temperature   1  0,0000023  0,0000023  0,0000023  0,18  0,692
Error                  4  0,0000510  0,0000510  0,0000127
Total                  7  0,0002021


S = 0,00357019   R-Sq = 74,77%   R-Sq(adj) = 55,85%


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
0         40           2   0,1  A
1         30           2   0,1  A
0         30           2   0,1  A

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

Table C. 57. *One way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1026 and 1064 cm^{-1} considering insect type as sole factor*

General Linear Model: 1026-1064 versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for 1026-1064, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS     F      P
Insect Type   1  0,0007358  0,0007358  0,0007358  3,71  0,075
Error        14  0,0027753  0,0027753  0,0001982
Total        15  0,0035110


S = 0,0140796   R-Sq = 20,96%   R-Sq(adj) = 15,31%


Unusual Observations for 1026-1064

Obs  1026-1064       Fit    SE Fit  Residual  St Resid
  4   0,165700  0,127988  0,004978  0,037712      2,86 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
M       8   0,1  A
C       8   0,1  A

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

Table C. 58. *One way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1230 and 1234 cm^{-1} considering insect type as sole factor*

General Linear Model: 1230-1234 versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for 1230-1234, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS      F      P
Insect Type   1  0,0009471  0,0009471  0,0009471  11,89  0,004
Error        14  0,0011149  0,0011149  0,0000796
Total        15  0,0020620


S = 0,00892394   R-Sq = 45,93%   R-Sq(adj) = 42,07%


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
C       8   0,1  A
M       8   0,1    B

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

Table C. 59. One way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1392 and 1396 cm^{-1} considering insect type as sole factor

General Linear Model: 1392-1396 versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for 1392-1396, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS      F      P
Insect Type   1  0,0032747  0,0032747  0,0032747  27,14  0,000
Error        14  0,0016893  0,0016893  0,0001207
Total        15  0,0049640


S = 0,0109847   R-Sq = 65,97%   R-Sq(adj) = 63,54%


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
C       8   0,1  A
M       8   0,1    B

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

Table C. 60. One way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1508 and 1516 cm^{-1} considering insect type as sole factor

General Linear Model: 1508-1516 versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for 1508-1516, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS      F      P
Insect Type   1  0,0099600  0,0099600  0,0099600  33,29  0,000
Error        14  0,0041883  0,0041883  0,0002992
Total        15  0,0141483


S = 0,0172963   R-Sq = 70,40%   R-Sq(adj) = 68,28%


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
C       8   0,2  A
M       8   0,1    B

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

Table C. 61. *One way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 1624 and 1625 cm^{-1} considering insect type as sole factor*

General Linear Model: 1624-1625 versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for 1624-1625, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS      F      P
Insect Type   1  0,0051912  0,0051912  0,0051912  17,99  0,001
Error        14  0,0040399  0,0040399  0,0002886
Total        15  0,0092311


S = 0,0169872   R-Sq = 56,24%   R-Sq(adj) = 53,11%


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
C       8   0,2  A
M       8   0,1    B

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

Table C. 62. *One way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 2358 cm^{-1} considering insect type as sole factor*

General Linear Model: 2358 versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for 2358, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS      F      P
Insect Type   1  0,0012320  0,0012320  0,0012320  13,16  0,003
Error        14  0,0013107  0,0013107  0,0000936
Total        15  0,0025427


S = 0,00967585   R-Sq = 48,45%   R-Sq(adj) = 44,77%


Unusual Observations for 2358

Obs      2358       Fit    SE Fit   Residual  St Resid
  3  0,060300  0,041825  0,003421   0,018475      2,04 R
  6  0,022600  0,041825  0,003421  -0,019225     -2,12 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
C       8   0,1  A
M       8   0,0    B

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

Table C. 63. *One way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 2924 cm^{-1} considering insect type as sole factor*

General Linear Model: 2924 versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for 2924, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS     F      P
Insect Type   1  0,0000452  0,0000452  0,0000452  0,52  0,481
Error        14  0,0012076  0,0012076  0,0000863
Total        15  0,0012528


S = 0,00928737   R-Sq = 3,61%   R-Sq(adj) = 0,00%


Unusual Observations for 2924

Obs      2924       Fit    SE Fit   Residual  St Resid
  2  0,051700  0,071462  0,003284  -0,019762     -2,27 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
C       8   0,1  A
M       8   0,1  A

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

Table C. 64. *One way ANOVA and Tukey's Comparison Test with 95% confidence level for absorbance peak observed at the frequency of 3267 and 3271 cm^{-1} considering insect type as sole factor*

General Linear Model: 3267-3271 versus Insect Type

```
Factor       Type   Levels  Values
Insect Type  fixed       2  C; M


Analysis of Variance for 3267-3271, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS     F      P
Insect Type   1  0,0000010  0,0000010  0,0000010  0,01  0,925
Error        14  0,0015349  0,0015349  0,0001096
Total        15  0,0015359


S = 0,0104706   R-Sq = 0,07%   R-Sq(adj) = 0,00%


Unusual Observations for 3267-3271

Obs  3267-3271       Fit    SE Fit   Residual  St Resid
  2   0,056100  0,077887  0,003702  -0,021788     -2,22 R
  4   0,098500  0,077887  0,003702   0,020613      2,10 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Insect
Type    N  Mean  Grouping
C       8   0,1  A
M       8   0,1  A

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