Study of consumer attitudes towards insect-based foods utilising Q method & the exploration of insect protein gels formulation

Submitted in partial fulfilment of the conditions for the award of the degree of Master by Research in Food science and Engineering

Viktoria Iliopoulou

4341998

Supervised by

Dr. Joanne Gould (Food Nutrition & Dietetics)

Dr. Anastasios Pagiaslis (Nottingham University Business School)

School of Biosciences

University of Nottingham

I hereby declare that this dissertation is all my own work, expect as indicated in the text:

Signature:_____

Date ____ /____ /____

September 2019

ABSTRACT

The food industry will have to take advantage of novel sustainable food ingredients to meet the needs of the growing world population whilst attempting to reduce malnutrition, and the environmental impact of food production. Of particular concern is the need to meet the demand for protein due to the environmental cost of livestock production, changing diets and in many countries an ageing population. One of the novel sources of sustainable proteins is insects, due to their high protein content and low environmental impact, although there is a lack of acceptance by consumers in developed countries.

This study aimed to identify and describe the viewpoints held about insect-based foods by consumers at the University of Nottingham, via Q methodology. Forty consumers were asked to rank-order forty-seven statements in a table. The factor analysis revealed that consumers' viewpoints clustered around four different point of views: the Informed and positive, the Informed and negative, the Informed with low sensory expectation and the Informed and satisfied but price-worried. The characteristics of each factor are presented and descriptors including socio-demographic, food neophobia, personal involvement, willingness to consume insect-based foods, awareness and preference of insect-based food product. These findings regarding consumers' viewpoints on insect-based foods will be helpful in the development of marketing strategies and food product design with the overall aim of increasing insect consumption in developed countries.

The second part of this research aimed to investigate how insects could be incorporated into food products in an invisible manner, which has been shown to be more appealing to consumers. As an exemplar the protein from mealworm beetle (*T. molitor*) was extracted, characterised and gelled to assess whether these insects could

not only be incorporated but add a functional property to a food product. Mealworm beetles were subjected to four protein extraction processes: an alkaline solubilisation and isoelectric precipitation (nAEIP); a salt extraction in phosphate buffer (SE); a sequential extraction (SEq); and an Osborne fractionation (OF). The protein extracts were compared in terms of extraction yield and molecular weight distribution using SDS-PAGE. It was found that OF and SEq had the highest extraction yield 19.3 %, followed by nAEIP and SE (15.4 and 5.9 %, respectively). While the molecular weight of all fractions ranged from 130 kDa to ≤ 14 kDa, the OF fraction gave more and better separated bands compared with the other methods. Moreover, AEIP and SE proteins had 3 bands with similar Mw (55-40, 18 and ≤ 14 kDa) while AEIP had one extra band (35-25 kDa).

Following characterisation, the gelation ability of two of the extracts (AEIP and SE) was assessed using two processing methods (heat and salt). The non-purified (nAEIP) protein extract was the only extract capable of forming gel, while purified AEIP (pAEIP) and SE (pSE) proteins did not form gel. For gelation of the nAEIP extract to occur a protein concentration above 12 % was required for thermal gelation, however with the addition of 100mM $CaCl_2$ the protein concentration requirement decreased to 8%.

This research has highlighted the different viewpoints of consumers to insect-based foods and identified potential routes to market and designed food products to increase insect consumption. In addition, the gelation of protein extracted from mealworm beetles has been investigated and provides processing steps to incorporate insects into food products invisibly, which could also have the potential to increase consumers' willingness to eat insects.

Table of Contents

ABSTRACT ii

List of Tables vii

List of Figures ix

Acknowledgements xi

Abbreviations xii

Chapter 1. General Introduction 1

1.1 Introduction to the Thesis 3

Chapter 2. Study of consumer acceptance of insect-based foods containing 'invisible' insect ingredients via an exploratory study utilising Q Method 5

2.1 Literature Review 5

2.1.2 Food Neophobia 7

2.1.3 Awareness of the benefits of insect consumption 8

2.1.4 Consumers' perception of insect consumption 8

2.1.5 Impact of product description and prior experience 10

2.1.6 Q methodology 12

2.2 Methodology 16

2.2.1 Study area and participants 16

2.2.2 Data collection and measures 17

2.2.3 Questionnaire 17

2.2.4 Application of Q methodology 19

2.3Analysis and Results 22

2.3.1 Descriptive Statistic 22

2.3.2 Q-methodology Results 23
2.4 Discussion 38
2.4.2 Strengths and limitations 42
2.5 Conclusions 42
Chapter 3. Formulation of insect protein gels 44
3.1 Introduction 44
3.1.1 Insect protein 45
3.1.2 Insect protein functionality 46
3.1.3 Protein extraction 47
3.1.2 Gelation 51
3.2 MATERIAL AND METHODS 58
3.2.1. Insect 58
3.2.2 Chemicals and references samples 58
3.2.3 Whole insect samples preparation 58
3.2.4 Water content 59
3.2.5 Fat Extraction 59
3.2.6 Protein Extraction 60
3.2.7 Freeze Drying 65
3.2.8 Protein Analysis 66
3.2.9. Thermal Analysis 71
3.2.10 Fourier Transform Infrared (FT-IR) Spectroscopy 72
3.2.11 Gelation preparation and analysis 73
3.3RESULTS AND DISCUSSION 76

3.3.1 Mealworm beetles (T. molitor) protein extraction yield 76

3.3.2 Characterisation of the protein extracts 82

3.3.3 Formation of a protein gel 91

3.4 CONCLUSIONS AND FUTURE WORK 97

Chapter 4 General Conclusions 99

4.1 Future work 101

Appendices 103

Appendix 1: Questionnaire 103

Appendix 2: STATEMENTS 110

Appendix 3: Q methodology tables 112

Appendix 4: Ideal Q sorts for each factor 118

Bibliography 122

List of Tables

Table 1. Participants' socio-demographic characteristics. 22

Table 2. Socio-demographic characteristics, food neophobia, awareness, personal involvement inventory levels, willingness to consume insect-based food of representatives of factor 1..... 26

Table 3. Most preferable insect-based food product rank of distinguished Q sorters of factor 1..... 28

Table 4. Socio-demographic characteristics, food neophobia, awareness, personal involvement inventory levels and willingness to consume insect-based food of representatives of factor 2..... 29

Table 5. Most preferable insect-based food product rank of distinguished Q sorters of factor 2..... 31

Table 6. Socio demographic characteristics, food neophobia, awareness, personal involvement inventory levels and willingness to consume insect-based food of representatives of factor 3..... 32

Table 7. Most preferable insect-based food product rank of distiguished Q sorters of factor 3..... 34

Table 8. Socio-demographic characteristics, food neophobia, awareness, personal involvement inventory levels and willingness to consume insect-based food of representatives of factor 4..... 35

Table 9. Most preferable insect-based food product rank of distinguished Q sorters of factor 4..... 37

Table 10. Crude protein content on dry mass of different insect species 45

Table 11. The different types of protein that have been found in *T. molitor* as reported by Yi et al., (2016). 49

Table 12. Examples of standard dilutions for preparation of a calibration curve. 67

Table 13. Total protein extraction yields (%) of mealworm beetles, by four extraction methods: Alkali solubilisation and isoelectric precipitation, protein extraction using NaCl in phosphate buffer, sequential extraction and Osborne fractionation and the yield of the extracted fraction[a]. 80

Table 14. G el formation of protein extracted with AEIP and SE methods after heating. (X: no gelation, A: aggregates and G: Gelation) 92

Table 15. Gel formation of protein extracted with AEIP and SE methods after pre-heating and salt addition. (X: no gelation, V: viscous fluid G: Gelation) 93

Table 16. Correlation matrix of Q sorters. 112

Table 17. The rotated factor loadings matrix with shaded the Q sorts that define a particular factor. 113

Table 18. N ormalized factor scores (z socres) and the rank order for all factors..... 114

Table 19. Factor score correlations. 115

Table 20. Factor Characteristics. 116

Table 21. Factor arrays for the four study factors. 116

List of Figures

Figure 1. Illustration of the table used by the participants to sort the statements 13

Figure 2. Centroid Factor Analysis scree plot of factors.**Error! Bookmark not defined.**

Figure 3. Freeze dried whole insects (L) and insect powder (R) after grinding 58

Figure 4. Example of a calibration curve produced by absorbance measurement of the standard samples. 68

Figure 5. Visual Observation of vials; (A) no gel formed (pSE), (B) Formed gel (nAEIP) 74

Figure 6. Schematic representation of the four extraction methods (AEIP, SE, SEq and OF) of *T. molitor* adult accompanied with the percentage of extraction yield and extracts' protein content (%).....**Error! Bookmark not defined.**

Figure 7. Molecular weight distribution of *T. molitor* extracted fractions, non-purified nAEIP, Alkali-soluble glutelins by Osborne fractionation (gtOF), water-soluble albumins by Sequential Extraction (aSEq), water-soluble albumins by Osborne Fractionation (OFa). 83

Figure 8. DSC Analysis of the aSEq (1), nAEIP (2), pSE (3), and gbSEq (4). The arrows A and B, C and D, E and F, and G and H show the first and second endothermic peaks heating. 86

Figure 9. FT-IR spectra of insect powder (1), chitin of shrimps (2), and defatted materials (3). 87

Figure 10. FT-IR spectra of insect powder (1), nAEIP (2) and pSE (3) 89

Figure 11. A zoomed in FT-IR spectra of insect powder (1), nAEIP (2) and pSE (3) between 1700 cm^{-1} and 1470 cm^{-1}..... 90

Figure 12. Aggregated particles, forming unhomogeneous solution 30% (w/v) pSE after 30 min heating and salt addition 94

Figure 13. Microscopic observation of the nAEIP during the gelation process. A &B protein solution before heating, C. stained aggregates with Rhodamine b after heating and salt addition and D. formed gel of nAEIP after heating Scale bar= 100..... **Error! Bookmark not defined.**

Figure 14. Microscopic observation of the pSE during the gelation process. Aggregates formed after heating (A). pSE protein solution before heating (B), stained with Rhodamine b (C) and not stained aggregates (D) after heating and salt addition. Scale bar= 100 .....**Error! Bookmark not defined.**

Figure 15. Factor array for Factor 1 exemplifying Q sort always conforms to the same distribution used in the original data collection and it is constructed by reference to the size and ultimately the rank order of the z scores. 118

Figure 16. Factor array for Factor 2 exemplifying Q sort always conforms to the same distribution used in the original data collection and it is constructed by reference to the size and ultimately the rank order of the z scores. 119

Figure 17. Factor array for Factor 3 exemplifying Q sort always conforms to the same distribution used in the original data collection and it is constructed by reference to the size and ultimately the rank order of the z scores. 120

Figure 18. Factor array for Factor 4 exemplifying Q sort always conforms to the same distribution used in the original data collection and it is constructed by reference to the size and ultimately the rank order of the z scores. 121

Acknowledgements

This thesis would not have been possible without the help and support of a large number of individuals. First and foremost I would like to thank my family, especially my beloved parents, who have endured my absence during my research, supported me, encouraged me and helped me tremendously in all possible ways. Without your continued help, support and guidance, this would never have been possible. Additionally, I devote my dissertation to my precious sisters who give me emotional strength and always stand by my side. Thank you.

I am deeply indebted to my supervisors Assistant Professor of University of Nottingham, Dr. Joanne Gould and Dr. Anastasios Pagiaslis who entrusted me with that position and provided me stimulating advice, guidance and encouragement to me every step of my way. Writing updates, monthly reports, always pushed me, by forcing me to quantify my progress and obtain critical feedback on a periodic basis. I would like to thank Panagiota, Alexandra, Khat, Darrell, Val, Bill, Dan for helping me whenever I need it.

Abbreviations

AEIP: Alkali Extraction and Isoelectric Precipitation Method

aSEq: albumins extracted with Sequential Extraction method (aqueous soluble)

BAPs: Bioactive peptides

BCA: Bicinchoninic acid assay

BSA: Bovine serum albumin

CP: Crude Protein

gSEq: globulins extracted with Sequential method (salt soluble)

nAEIP: non purified Alkali and Isoelectric precipitation extracts

NPN: Non-protein nitrogen.

nSE: non purified Salt Extracted

OF: Osborne Fractionation

OFa: albumins extracted through Osborne Fractionation

OFgb: globulins extracted through Osborne Fractionation

OFgt: glutelins extracted through Osborne Fractionation

pAEIP: purified Alkali and Isoelectric precipitation extracts

PII: Personal Involvement Inventory

pSE: purified Salt Extracted

SE: Salt Extraction Method

SEq: Sequential Extraction

SDS-PAGE: Sodium dodecyl sulphate - polyacrylamide gel electrophoresis

SSP: Salt Soluble Proteins

TP: True protein

Chapter 1. General Introduction

A number of global organizations warn that humanity has negatively affected the global ecosystem in more than one way, including overpopulation, greenhouse gas emissions, melting of ice caps, desertification of the land and rise of the life expectancy (IPCC, 2014; WFF, 2018). Food Security, ensuring the provision of food for the growing global population, which is expected to reach 9.8 billion in 2050 (UN DESA, 2017), is an important challenge. The growth in population will be accompanied by a 70% increased demand for human food, feed for livestock used for feeding humans and a need for land, as well as cropping intensity for plant production (FAO, 2012).

Of particular concern is the need to meet the demand for protein due to the environmental cost of livestock production, increasingly popularity for high protein foods and in many countries an ageing population. Proteins have a major impact on the body, being fundamental for the body's growth, replenishment of amino acids and proper function of the metabolism (British Nutrition Foundation, 2012). The improvement in production efficiency of current protein sources as well as, the discovery and study of novel protein sources is therefore necessary to ensure we can provide essential sufficient protein to the population. Insects are considered to be one of the promising alternative and sustainable sources of nutrients for food and feed and hence the FAO has actively promoted the consumption of insects (van Huis, 2013, New, 2011). Insects are also one of the most abundant and diverse groups of organisms on our planet, the number of species estimated to exist ranges from 4 to 30 (Dossey, 2010).

There are two key reasons for the active promotion of insect consumption; nutrition and the lower environmental impact of insect farming. Nutritionally, insects contain a substantial quantity of fat, minerals and vitamins (Bovera et al., 2015, Rumpold and

Schlüter, 2013, Kouřimská and Adámková, 2016), and have a high protein content ranging from 30 to 90 % depending on the species and the developmental stage of the insect (Aguilar-Miranda et al., 2002, Kulma et al., 2016). Mealworm larvae (*T. molitor)* has been shown to have a high protein content of around 60 % (Zielińska et al., 2015, Bußler et al., 2016, Azagoh et al., 2016).

Insects have the ability to transform an extensive range of organic matter into body mass efficiently (e.g. a high feed conversion ratio) (van Huis, 2013, Oonincx et al., 2010). The feed conversion ratio is much higher than most vertebrate livestock, being two times more efficient than pigs and chickens, and four times more efficient than sheep (Gahukar, 2011, Capinera, 2004). Insects themselves are prolific with shorter life spans, with some insects such as house crickets laying around 1200- 1500 eggs in less than a month (Gahukar, 2011). Moreover, the water requirement and the emission of greenhouse gases (Oonincx et al., 2010) are lower than livestock. Insects can be bred on plants or agricultural by-products that are not connected with the human food supply.

The unfamiliarity with insect-based foods of consumers in developed countries is a limiting factor for insect consumption, which could be overcome by processing insects or fractions of the insect alongside other ingredients enabling the insects to be invisible in a food product (Hartmann et al., 2015). It has been shown that the presence of invisible insects in processed products resulted in higher willingness to consume insects than when the insects were visible (Balzan et al., 2016, Piha et al., 2016, Hartmann and Siegrist, 2016). Additionally, many studies have shown that the acceptance and the willingness to consume whole or processed insects in the developed countries is relatively low due to neophobia, limited prior experience or information and risk perception (Hartmann and Siegrist, 2016; Baker, Shin and Kim, 2016; Caparros Megido et al., 2016; Piha et al., 2016; Fischer and Steenbekkers, 2018; Hartmann et al., 2018; Van Thielen et al., 2019, Sidali et al, 2019). However,

based on study by Sidali et al., (2019) persuasion strategies such as covering with cocoa, hiding or modification of insects' appearance, marketing strategies, which will influence the acceptance and tasting experience, could increase the regularity of insect consumption. In the food industry, the use of naturally derived food hydrocolloids, such as proteins and polysaccharides is common to perform a variety of functions including thickening, gelling, aqueous dispersions, stabilising foams, emulsions and dispersions (Banerjee and Bhattacharya, 2012). The ability of proteins extracted from some insect species such as honey bee brood (*Apis mellifera)* (Mishyna et al., 2019b) or *Acheta domesticus, Tenebrio molitor, Alphitobius diaperinus, Zophobas morio* and *Blaptica dubia* (Yi et al., 2013) to form gels has previously been investigated. A more extensive understanding of the gelation mechanism of insect proteins is needed to enable the control of the characteristics of gel to be applied in food products. Knowledge about the interactions under changing environmental conditions that induce gelation or the rheological and textural properties of the formed gels, could help researchers to predict the behaviour of the molecules during food processing (Matuszek, 2001).

Therefore, there is a need for further investigation of willingness, acceptance and risk perception of insects as food among consumers as well as, food product design to improve the introduction of insects to the markets and diet of population.

1.1 Introduction to the Thesis

The overall aim of the research presented in this thesis was to develop a greater understanding of the perception of insect-based foods by UK consumers and investigate one method of incorporating insects into food products. The research conducted in this thesis has arisen from a joint collaboration between the Division of Marketing (Business School) and the Division of Food, Nutrition and Dietetics (School of Bioscience) at the University of Nottingham to enable a greater insight into the viability of insect consumption in the UK.

The present study examines the different point of views held by United Kingdom consumers about the insect-based foods. Awareness, food neophobia, personal involvement, socio-demographic characteristics and a list of consumer preferred insect-based products were studied to provide an insight into how to incorporate insect-based foods to future consumers. As the UK has a wide shopping audience, it is a host-country for a number of students from all over the world and it is part of the EU, where Belgium and Netherlands are already accepting insects as food, it makes studying UK consumers an interesting sample. Then, protein extraction from mealworm beetles using four different methods and the extracted protein fractions of two methods were studied for their ability to form gels after heating and after addition of salt.

The objectives of this MRes were therefore:

1. To characterise the viewpoints held by UK consumers about insect-based foods using the Q methodology;
2. To find out insect-based food products that could be potential acceptable;
3. To extract protein from mealworm beetles produced using different extraction protocols – evaluating the purity and yields of the obtained extracts;
4. To characterise the extracted proteins molecular weight and thermal properties;
5. To assess the gelling ability of two of the protein extracts.

This thesis is structured into four chapters. Chapter 1 includes a general introduction into the thesis. The Chapter 2 presents the results of the Q method study of 40 UK consumers (Objectives 1-2). Chapter 3 deals with Objectives 3-5, as it contains research on the extraction, characterisation and gelation of insect protein. Each chapter contains a review of relevant literature and specific methods. General conclusions with recommendations for further research are made in Chapter 4.

Chapter 2. Study of consumer acceptance of insect-based foods containing ‘invisible’ insect ingredients via an exploratory study utilising Q Method

2.1 Literature Review

It is widely acknowledged that there is a need for protein with a specific amino acid profile in the human body and there is a need to find novel sources of protein to ensure provision of food for the growing population. Insects are one example of a novel protein source being suggested as a solution as they have high protein content and they present the required amino acid profile that the human body need (Yi et al., 2013). However, a novel source of protein must be acceptable and consumed by the consumers to offer a benefit.

Entomophagy, the consumption of insects, is not a new practice globally, but it is more popular outside of Europe and US, in areas such as Africa and Asia. In the EU, New Zealand and America entomophagy is a quite unfamiliar practice. However, Evans (2015) reported historic references of insect consumption such as nymph of cicadas’ as a delicacy in ancient Greece and the consumption of ‘cossus' larvae on flour and wine by Romans 2000 years ago. In the following years, insect consumption was not a common practice and there were not or were extremely limited reports referred in entomophagy in many European countries. However, in other countries, the kind of edible insects (Coleopteran, Lepidoptera, Hymenoptera, Orthopteran, Hemipteran, Isopteran, Odonatan, Dipteran, Dictyopteran, Megalopteran and others) (van Huis, 2013) differs among countries or even among the different areas of a country, similarly with consumption of different types of meat across the world. Based on van Huis (2003), different species of insects in different developmental stage (eggs, larvae, pupae or adult) are consumed. There are cases where the larvae or

nymph may be consumed, while the other developmental stages to be morally forbidden such as Pygmies the larvae and nymph stage of Goliath beetle is eaten while the consumption of adult is not permitted for religious purposes (Bergier, 1941). The consumption of insect or insect-based foods is much more popular in some countries like Thailand, Kenya, China and Mexico. However, the developed countries such as UK and USA until recently have not been familiar with insect consumption finding insects disgusting, contaminants and not joyful.

Interestingly, there may be people who already consume insect-based foods without knowing it, a typical example is the red colorant carmine (E120), extracted from cochineal insects (*Dactylopius coccus Costa // Coccus cacti L*). E120 is used in many products such as cakes and yoghurts as well as in cosmetics.

In addition, for EU countries the Novel food legislation will influence insect consumption. A novel food defined by the Novel Food Regulation (EC) No 258/97, refers to any food or food ingredient, which had not been used for human consumption to a significant degree before 15 May 1997. As the consumption of insects was not a common habit before 1997 in Europe, whole insects and/or products of insects are now subjected to the Novel Food Regulation. Due to this legislation change, all products containing insects will not be able to be sold after January 2020, unless Novel Food authorisation has been granted.

Regardless of the history of consumption, in the last thirty years, the number of published articles has increased and researchers seem to be interested about insect consumption. The researchers, and indeed food manufacturers will face the challenge of persuading and inducing the individuals of developed countries to consume insects. Prior research has shown that willingness, food neophobia, perception of insects, sensory experience and product description can contribute to consumers' attitudes towards insect-based foods.

2.1.2 Food Neophobia

One of the most important factors capable of affecting the human behaviour, attitude and acceptance or rejection towards insect-based foods seems to be food neophobia (Pliner and Hobden, 1992, Caparros Megido et al., 2016). The word neophobia is a Greek compound word consist of the word 'neos' which means new, novel and the word phobia which means fear. Therefore, the negative attitude, rejection and the irrational fear for unpleasant tasting experience towards any unfamiliar and unknown food is defined as neophobia. Food neophobia is a necessary defence mechanism of the body targeted to safeguard from potentially harmful foods (Pliner and Hobden, 1992). An individual tends to have an expectation and perception before they taste something, predicting the possible pleasant or unpleasant experience that they will have, after taking into account that arbitrary hypothesis will act by having the willingness to eat the food. Food neophobia has a negative impact on the willingness to buy or to eat insects and plays a noteworthy role (Piha et al., 2016, Baker et al., 2016, Hartmann et al., 2015, Hartmann and Siegrist, 2016). Pliner and Hobden, (1992) developed a Food Neophobia Scale (FNS), which is a questionnaire with 10 statements, concerning novel foods and eating habits measured on a 7-point bipolar scale (1= strongly disagree to 7= strongly agree). The level of neophobia is different among the individuals and it is affected by cultural norms, gender, age, educational level, income and social peer group (Flight et al., 2003, Tuorila et al., 2001). Consequently, some persuasion strategies are selected and aimed to decrease the level of neophobia, which will trigger less aversion towards insects and more acceptance of insect-based foods. The most competent mechanism to achieve a decrease in the level of neophobia is the familiarisation of consumers with the insects as a food through processed insect powder, increasing awareness of the nutritional value and the general positive impact of insect-based foods on the environment and the incorporation of insects into familiar foods. A study conducted in the Italian Alps reported that the

food neophobia decreased after using persuasion strategies including the combination of insect with familiar foods such as chocolate; or the promotion of products with processed insects which are invisible (Sidali et al., 2019).

2.1.3 Awareness of the benefits of insect consumption

Willingness to consume insects is significantly affected by the knowledge held by consumers about the nutritional value and the contribution to the environmental protection. Consumers in many developed countries are not familiar with insect-based foods. Awareness is quite low among the residents of the developed countries of EU and US, which could be explained by the fact that this habit is not rooted in traditional diet (Hartmann et al., 2015) as a part of their conventional food chain but as a part of a delicacy menu in alternative restaurants (Verkerk et al, 2017). There is a lack of information about the nutritional benefits of insects such as protein content, calcium, iron, zinc and fats (Baker et al., 2016). Moreover, many consumers are not aware of the contribution of environmental protection and preservation; causing lower environmental deterioration and requiring a smaller use of space. Insect food rejection is driven by the feeling of disgust, considering them as contaminated and unhealthy, with little awareness that insects food are conformed with restricted regulation for cleanliness and health. However, recently with the insertion of the insects into the European market it could be hypothesised that consumers have started to get more informed about the benefits of insect-based foods consumption and awareness of the existence of insect-based foods globally both of which could enhance willingness to eat and acceptance of consuming insects.

2.1.4 Consumers' perception of insect consumption

The mistaken perception that insects are dirty and living contaminants, are part of the list of various factors that affect the acceptance of insect-based foods creating the

feeling of disgust (Poortvliet et al., 2019, Myers and Pettigrew, 2018). This perception is rooted in the connection of insects with a more unsanitary way of living, which makes it easy conceive that insects could be carriers of impurities and diseases. Myers and Pettigrew (2018) report that insects are considered as starvation food, which are consumed due to desperation. Additionally, the consideration of insects as unhealthy and contaminants of food is more common, instead of perceiving their nutritive benefits (Hartmann and Siegrist, 2016). According to Hartmann *et al* (2015), the impression that people coming from lower economic status' are typical insect consumers leads to the rejection of insects as a source of food and therefore the rejection of insect-based foods as an alternative source of protein. Insect consumption has been a challenge for food scientists with many barriers to be overcome, with first the expected distaste (Hartmann et al., 2015). The real challenge is to make consumers recognize how delicious and tasteful insects could be by consuming them as a whole natural or processed ingredient. As the population of consumers have various attitudes, it would be more effective for the researchers to develop different strategies to target different groups of the population. People open to explore new tastes and foods, need different attraction techniques like extravagant decorations of whole insects, while the part of population which tend to reject the insects need proposals that combine insects with high gastronomical cuisines or the embodiment of insects as part of common dishes like salads or bread (Hartmann and Siegrist, 2016).

However, European legislation declares insects to be a novel food (Food Safety - European Commission, 2019). This legislation guarantees that the ingredients have met the food safety requirementsbefore the products are placed on the market. As long as the Food Safety European Commission gives the permission of a product's release, this certifies its safety and the belief that insect-based foods are dirty and living contaminants is not scientifically valid. Nevertheless, Poortvliet *et al* (2019) conducted a study which found that Dutch consumers preferred to eat a product

containing meat that they were not aware of the derived-source rather than eat an insect-bovine meat product. Moreover, the outcomes showed that the meat-insect foods were perceived as both healthier and more disgusting compared with bovine-meat products. Additionally, the consideration of insects as unhealthy and contaminants of food is more common, instead of perceiving their nutritive benefits (Hartmann and Siegrist, 2016).

2.1.5 Impact of product description and prior experience

Product description

The impact of sensory properties of insect-based foods on consumers' willingness and acceptance has been investigated (Hartmann *et al.*, 2015). The expectation of an unpleasant experience, prejudices the consumers, preventing them from accepting or eating insects. The acceptance of insects has been demonstrated to be affected by the degree of processing, as the consumers' exposure to processed insects such as incorporating powdered or minced insects in familiar foods. Processing of insects could change the negative attitude and make consumers more open to finally making insect-based foods part of their dietary behaviour (Hartmann and Siegrist, 2016, Caparros Megido et al., 2016, Sidali et al., 2019, Van Thielen et al., 2018, Schösler et al., 2012). For instance, Schösler *et al* (2012) reported that a pizza containing invisible insects was more preferable than the chocolate glazed whole insect. The product description and the image can contribute to the intention of consumer to purchase. When the description is explicit and there is an image on the package meaning consumers were totally aware of the insects' presence, the result was an increase in the risk perception, which becomes a barrier to acceptance (Baker et al., 2016).

Prior experience

A key aim of research studies dealing with novel food is how the general population can become more open to insect consumption. House (2016) concluded that future research should be targeted on enhancement of the positive perception, experience and convenience of insect-based foods, as once consumers have tasted insects they have a high intention to do it again, which will stabilise a permanent marketing audience. Research to date has shown that the majority of the consumers with one or more prior experience of insect-based food is quite low. A few studies have evaluated prior experience and found that only 11% of Belgian consumers (Van Thielen et al., 2018), 33% of the sample recruited at the Paul Lambin Institute in Belgium (Caparros Megido et al., 2016), 20 % and 38% of the control and experimental group in Zurich (Hartmann and Siegrist, 2016) have tried insect-based food. Based on these studies, the aim of the food manufacturers / governments / FAO should be the conversion of the once-experience to a repeated habit. Improving the taste, the appearance and the availability of insect-based products will aid this conversion.

In addition to the affective, emotional and psychological factors, which affect food selection, there are additional factors such as price, taste, availability of product and degree of fit with the traditional dietary behaviours that are product related and substantially relevant to the food choice (House, 2016, Pambo et al., 2016, Alemu et al., 2017).

Given that insect-based foods have started to come in the markets progressively and that United Kingdom is inhabited by people from all over the world who are or are not familiar with insects as food makes the UK a good field for research of the factors that could affect the willingness and acceptance of insect-based foods. It seems that numerous persuasion and marketing strategies should be developed for changing the attitude of the consumers in developed countries. In order for this to be achieved, researchers should be aware of what affects people's willingness, perception and

intention to consume meat. To the best of the author's knowledge, this has not been investigated in UK. The main contribution of this study was to investigate the different viewpoints of the participants, concerning insect-based foods using Q methodology.

2.1.6 Q methodology

Q methodology is a technique developed by William Stephenson (1953) and is used in a variety of fields such psychology, political sciences, communications, marketing and health sciences. Q methodology combines the advantages of qualitative and quantitative research and it has a major role on the analysis of individuals' subjectivity which means the study of an individual's perceptions, opinions, beliefs, attitudes and preferences towards groups, situations or objectives. The researcher does not impose the categories to the respondent but the method itself determines operant categories. To this extent, the participants are self-categorized on the basis of the Q sort they produce. The usefulness of this method is the exploration of beliefs, preferences, viewpoints, motives and goals of individual, which affect the behaviour of participant but remains unexplored with the other methods. At the end of the analyses, we may come to understand an individual's terms of their association with a particular group or factor.

The Q method can be separated to 4 stages which are (1) Research Design, (2) Data Collection, (3) Analysis and (4) Interpretation. The research design includes the building of the concourse, which is defined as the full range of items/statements representing all possible opinions referred to the topic to be examined. Then among the various statements there are some distinguishing statements, which are short 'stand-alone' sentences, easy to be read and understandable and are used for the sorting (Q-set). The participants are the P-sample and can be selected randomly or not. Data collection is conducted through the ranking of the statements by the P-sample

where the cards should be ranked on a table with graduated columns of different values from strongly disagree to strongly agree. The ranking should be based on the extent to which the individual is represented by those statements. The quasi-normal distribution with the 11 categories (-5 to +5) (Figure1) used in this study is the house standard distribution and is prearranged (forced). The benefits of forced method are explained by the fact that the shape of distribution is coherent to a distribution fitting the normal curve of error within same way as many person measurements for a single trait tend to be normally distributed.

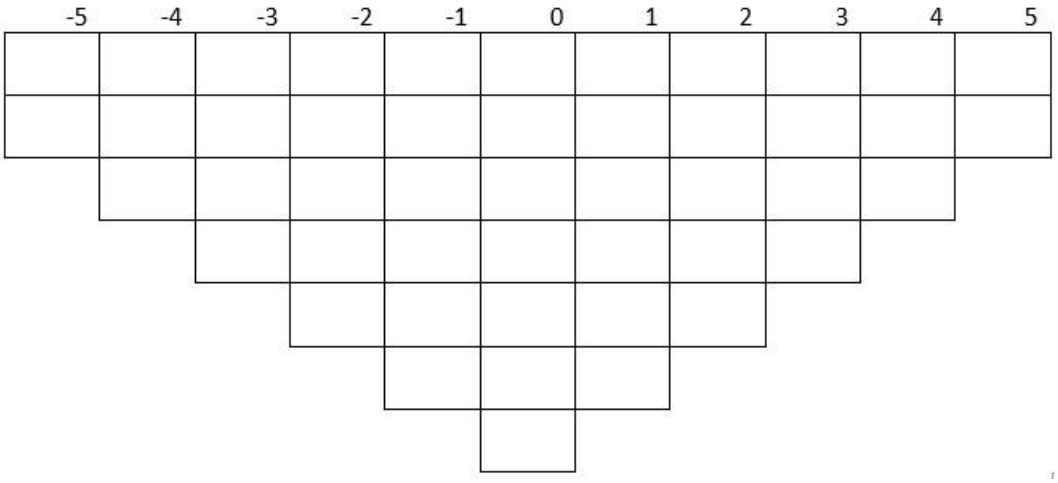


Figure 1. Illustration of the table used by the participants to sort the statements.

Evidently, a large number of items are shaped towards the midpoint of the distribution and permits fewer at the verges. The only values which are to be referred to are the column numbers as the rows are insignificant (Webler et al., 2007).

After the design of the research, the participants are invited to face-to-face interviews or through online platforms, where the participants should rank the statements based on their opinion. The participants after being informed about the way that they should sort the statements, following some standard rules, will take the Q set in the form of a pack of randomly numbered cards, each card containing one of the statements for the Q set and the score sheet on which they should place the cards. Initially the statements

should be separated into three categories agree, disagree and neutral. Over the period of the Q sorting any participants' comments are recorded as this information is helpful for the interpretation of factors.

This is then followed by data analysis, which is carried out using different software packages relative to the method. The analysis starts with the calculation of the intercorrelation matrix of all Q sorts, which shows how similar or dissimilar the viewpoints of the Q sorters are (participants). Then, in order to explain as much as possible about the relationships that hold between the many Q sorts, the identification of shared meanings, which are called factors in the present data, then requires a factor analysis. Factor analysis is a reduction technique, which aims to create fewer factors than there are Q sorts in the group. The analysis tries to identify patterns of similarity in the Q sorts structures and therefore to show evidently how many different point of views participants expressed. The first step of the factor analysis is the decision of the number of factors that the researcher will keep. The decision is based on the variance of the Q sorts. The relation between each respondent and factor is displayed by the factor loadings. The sum of the squared loadings of all the Q sorts on a factor is resulted in the eigenvalue (EVs). The eigenvalues (EVs) and scree plot consists extra criteria for making the decision easier. The eigenvalue is a measure of how much of the variance of the observed variables a factor explains. Any value with an EVs ≥1explains more than a single observed variable and contributes to summarising all individual responses into a few representative responses (Watts, 2012). The scree plot presents the line of the eigenvalues of the factors and the point where the slope of the curve is gradually tapering off indicates the number of factors that should be generated. Based on Brown (1980) 'Experience has indicated that 'the magic number 7' is generally suitable' however it is recommended to extract more factors than those that will be kept after the next step which is rotation. Therefore, the result of factor extraction is the factor loadings which shows how much associated or correlated each

Q sort is with a factor. Rotating the factors, which is the next step is relative to changing the point of view where results are observed in order to obtain a clearer result, which will be easier to explain. The rotation could be either objective, based on some statistical principle (like varimax), or theoretical / judgemental, or based on theoretical concerns and presupposes prior knowledge or preassumed appraisal of the researcher. The latter type of rotation in essence tries to confirm an idea or a theory and the researcher is responsible for the judgement about the acceptability of this solution. After the rotation the amount of variance doesn't change but there will be changes in factor loadings and factor EVs due to changing perspectives. Next, the most representative responses (Q-sorts) of a given factor are flagged and they will be used for subsequent calculations. The aim of flagging is to distinguish perspectives and that can occur automatically or manually (by an experienced researcher). The condition for choosing a Q sort as representative for a factor is to have significantly high factor loading >0.38 (at the $p< 0.01$ level) (Watts, 2012). The next step before interpretation is the calculation of the z-scores, which indicate the relationship between statements and factors. The z-score is the weighted average of the scores that similar respondents (flagged Q-sorts) gave to the statements. Then the factor scores are calculating by rounding the z-scores towards the array of discrete values in the grid. The outcomes from that is the composition of an idealised Q sort for each factor, indicating how a representative of a factor would rank the items.

What follows is the calculation of some characteristics in order to compare the factors. The correlations between factor scores and the standard errors for differences in factor z-scores are provided by the software and contribute to the factors comparison. After the comparison of the z-scores of a statements, the researcher is able to identify the consensus statements –if there are any-, which are statements that have similar z scores across all factors and the distinguished statement(s) which is/are the statements that compared factors by pairs have significant differences between their z-scores of a

statement. The statement with distinguished z-score is more engaged with that factor than the others.

Interpretation

The interpretation of factors is based on the ideal Q sort, which is constructed for each factor from the factor scores, the distinctiveness of the statements, the qualitative data and the understanding of the researcher. The description of a factor is based on the composition of the Q sort, paying initial attention to the extreme ends of the composite sort, which are the characterising statements, and build the base for the interpretation. Next, the different viewpoints and the interpretation of the differences among factors is indicated by the distinguishing statements. Based on the characteristics of the factor, the factor is usually given a meaningful label in order to give readers a first idea about the factor, but it is not essential. Finally, the combination of extra information that the researcher has collected and could affect the interpretation such as demographics or comments during the interview can contribute to the interpretation of each factor.

2.2 Methodology

2.2.1 Study area and participants

The study was conducted at the School of Biosciences at the Sutton Bonington Campus of University of Nottingham (UK). The study area was purposively selected for its high student density and multicultural population, which means that there were consumers who were or were not familiar with the insect-based foods. Therefore, using an explanatory research design, the study area would provide insights into aspects of belief, acceptability and perception of insect-based foods. The recruitment of the participants was achieved via the University of Nottingham student's database, distribution and posting of a leaflet, and the individuals became aware of the study.

The sample consisted of 40 residents / students (30 females) ranging between 20-50 years old. Participants were rewarded with a voucher and a consent form was shared with them to be signed to show that they agreed that they have been informed about the content of the research.

2.2.2 Data collection and measures

Data for this survey was collected from participants in two parts. The first part included the use of an online structured questionnaire and the second part involved a face-to-face interview. The first part of the research method, an online questionnaire was shared in order to gain general information for assessing the awareness and general attitudes towards novel foods, food neophobia, product related preferences, individual's attitudes, prior experience and demographics. The second part consisted of some statements, which the interviewees were asked to rank on a table following the Q- method in order to compare the viewpoints of the participants and the Ken-Q Analysis 1.1.0 program was used for the analysis of the results. Ken-Q helped to collect, organise, analyse, interpret and present the results. The data collected from the questionnaires were processed to remove the non-completed questionnaires and entered in Genstat for analysis.

2.2.3 Questionnaire

The questionnaire was created using Qualtrics, an online survey platform which included 13 questions or group of questions which covered various topics such as awareness, neophobia, beliefs and attitudes toward insect-based foods, prior tasting experience, intention to taste it, preference of the desirable products incorporated with insects, physical- and socio- demographics, which were asked to the participants. The online survey offered several advantages by providing higher flexibility in its design and saving time for the participant and the researcher (Selm and Jankowski, 2006).

The data quality improves as the programming filter of the platform provides different technological settings which could control the order of the question, tailor the questionnaire to different respondents and checking the completeness of the questionnaire (Evans and Mathur, 2005). The duration of the entire response to the questionnaire was targeted to be between 10-15 minutes to avoid respondent's tiredness and the vocabulary using was familiar to regular English speakers as among the participants were English speakers from all over the world.

The questionnaire began with a group of four dichotomous types of question (response yes or no) borrowed from Barsics et al., (2017*)*, and related to the awareness and the general attitude of the participants. The questions were; 'Have you ever heard that edible insects are eaten in some parts of the world?', Have you ever heard that there are insect-based products available on the market', 'Have you ever heard that edible insects contain essential nutrients for humans?' and 'Have you ever tasted edible insects or a meal that contains edible insects?'. The second group of questions referred to food neophobia and was assessed used the Food Neophobia Scale (a=81) (Pliner and Hobden, 1992). The scale consisted of 10 items (questions); 'I am constantly sampling new and different foods', 'I don't trust new foods', If I don't know what is in a food', 'I like foods too weird to eat', 'at dinner parties, I will try new food', 'I am afraid to eat things, I have never had before', 'I am very particular about the foods I will eat', 'I will eat almost everything', 'I like to try new ethnic restaurants'. Responses ranged from 'strongly agree' to 'strongly disagree' on a 7-point Likert scale from '1=very strongly disagree' to '7=very strongly agree'. A low score characterises a person as neophilic while a high score characterises a person being neophobic. The next group of items was called Personal Involvement Inventory (PII) (Zaichkowsky, 1994)which tested the personal involvement of the participant and contains terms with 14 adjectives classically associated with involvement and others associated with attitude rated on a 10-point Likert scale from '1=strongly

agree' and '10=strongly disagree'. Participants were asked to reply to 'Have you ever tried insects based foods before' question, responded with 'yes' or 'no' and those who replied 'no', were then asked 'Have you ever consider trying insects based foods' on a 10-point Likert scale from '0=definitely no' and '10=definitely yes'. The next question borrowed from the 'Consumer acceptance of foods containing edible insects in Belgium two years after their introduction to the market' (Van Thielen et al., 2018) focused on the insects' incorporation in food. The participants should rank 18 product types (burger, snack, energy shake, energy shake, energy bar, protein bars, casserole, ready-to-eat meal, spreads, fried snack, pasta sauce, soup, pasta, cookie/cake, candy, yoghurt, desert, chocolate and ice cream) suitable for insect incorporation based on their personal preference. Finally, the last part of questionnaire were six questions which asked demographic details such as sex, nationality, age, highest level of education, current job and eating habits. The full questionnaire can be found in Appendix 1.

2.2.4 Application of Q methodology

Data Collection

The Q method begun with the identification of all comments made about insect-based foods. The concourse was assembled form prior research. From the large concourse, 47 statements (Appendix 2) were finally selected to be used for the survey. The meaning of the sentences was checked by giving them to two native speakers and a non-native speaker, to be assured that the statements were clear and understandable and then two copies of the statements were printed in small cards. Participants were informed about what they should do and how they should distribute the cards on the table / chart (Figure 1**)** which was placed in front of them. Initially, the participants were encouraged to read and divide the statements into three general categories – a 'most likely I disagree' pile, a 'I am not really sure if I agree or disagree' pile and

'most likely I agree' pile before they started to distribute them on the table. The participants were asked if there were any queries. After completing the questionnaire, the participants had the opportunity to make any changes if they disagreed with the ranking, including comments about having more agreements or disagreements or other comments concerning their attitude or belief towards insect-based foods. This additional information contributes to the interpretation of the results during the analysis. Once participants were sure that they had finished, the sorting was recorded.

Data Analysis

The Ken-Q Analysis 1.1.0 (Banasick, 2019) was used for the data analysis. The input of the statements and results was followed by the calculation of the correlation matrix of all Q-sorts.

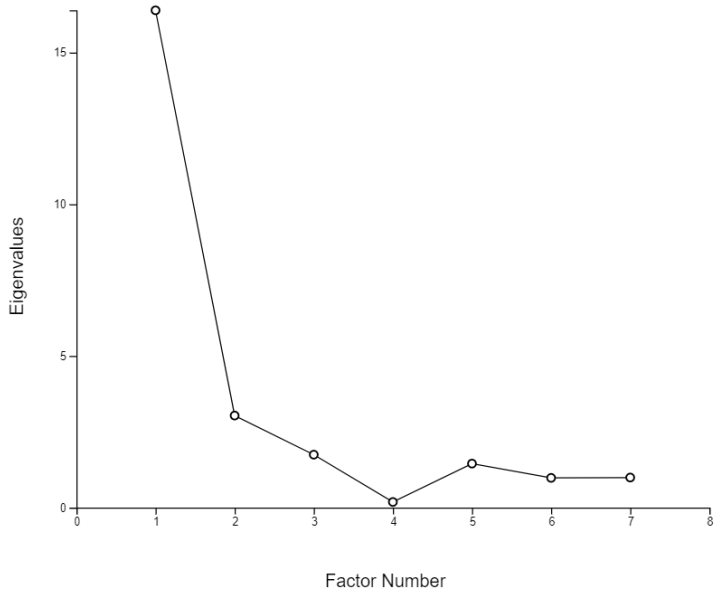


Figure 2. Centroid Factor Analysis scree plot of factors.

The correlation represents the similarities of the differences in the viewpoints between the individual Q sorts. Then, the centroid factor analysis as proposed by Watts and Stenner (2012), will group the individual viewpoints, based on the correlation matrix,

exploring practically, how many different Q sorts exist. The number of the factors depends on the variability of the Q sorts. Eigenvalue and their scree plot deal with factor extractions, pointing out how many factors the researcher should want to use in the analysis (Webler et al., 2007, Watts, 2012). The scree plot of Figure 2 presents the line of the eigenvalues of the seven extracted factors and the point where the slope of the curve is gradually tapering off, which indicates the number of factors that should be generated. Any factor with eigenvalue higher than 1.0 explains more variance than a single variable and it is used in the analysis. The seven unrotated factors accounted for 61% variance across participants' Q sorts and had eigenvalues of 16.39, 3.03, 1.74, 0.18, 1.45, 0.97 and 0.99, which means that the Factors 1, 2, 3 and 5 will be used. The next step is the rotation of factors using varimax rotation, as recommended by Watts and Stenner (2012). The rotation minimizes the complexity of the factor loadings to make the structure simpler to interpret, without affecting the consistency or the differences between the individual Q sorts. New factor loadings come off after rotation, the KADE application gives the choice of automatic flagging identified Q-sorts with higher factor loadings, which explains each factor with the best way by loading highly on only one factor. Factor loadings of ± 0.4 or above were significant at the $p < 0.05$ level. Upon rotating the factors, the factor loadings change and the particular Q sorts may change how closely the approximate a factor's viewpoint. Table 17 (Appendix 3) presents the rotated factor loadings with grey colour the ones showing significant values and the factor's eigenvalues, which are 8.9, 3.69, 4.94 and 3.32. Among the 40 Q sorts, 23 of them were flagged and the factor loadings ranged from -0.60 to 0.79, averaged at 0.56. Afterwards, the software conducted the calculation of z-scores and the rank order for all factors (Table 18, Appendix 3); correlations between factor scores (Table 19, Appendix 3); and factor characteristics (Table 20, Appendix 3). Next, the factor arrays were constructed (Table 21, Appendix 3) and the ideal Q-sorts (Figure 17, 18, 19 and 20, Appendix 4) were presented. Interpretation of the results followed.

2.3Analysis and Results

This section is divided into two sub-sections. The first sub-section describes the characteristics of the population obtained by the questionnaire. In the second sub-section, the results of Q methods are presented.

2.3.1 Descriptive Statistic

This sub-section describes the general characteristics of the sample used for the Q method. Table 1 provides an overview of the 40 participants self-reported demographic characteristics. The age of participants ranged from 21 to 50 years (*M= 29, SD-7*) and the majority of the participants were female (75%); bias towards females is common in surveys. The level of educational status was high with 37.5% of participants having completed undergraduate studies and 35% pursuing post graduate studies, such as MA, MSc, MRes and 15% had completed a PhD. Half of the participants were in full time education and around one quarter were working full time (30 hours per week), while 22.5% of the participants that had a part time job were searching for a job or did not work at all. The participants were mostly British (40%), French and Greek (10% each), Nigerian and Chinese (5% each) and the rest (30%) were Bulgarian, Canadian, German, Irish, Iranian, Italian, Mexico, Nepalese, Polish, Portuguese, Thai and Tunisian. Finally, almost all the participants were meat eaters (40%) or flexitarian (52.5%).

Table 1. Participants' socio-demographic characteristics.

Gender	**N**	**(%)**
Female	30	75
Male	10	25
Education	**N**	**(%)**
Secondary education / High school	5	12.5
Tertiary education / university / vocational	15	37.5
post graduate education / MSc / MA / MBA / MRes	14	35
Doctoral education / PhD	6	15
Work	**N**	**(%)**
Working full-time (30 hours per week plus)	11	27.5

working part-time (under 30 hours per week)	4	10
Looking for a job	2	5
In full-time education	20	50
Not working	3	7.5
Eating Habits	**N**	**(%)**
A meat-eater (consuming meat (almost) every day of the week)	16	40
A flexitarian (consuming meat only a few days per week)	21	52.5
A vegetarian (not consume any meat at all)	2	5
A vegan (not using or eating animal products)	1	2.5
Nationality	**N**	**(%)**
Europeans	28	70
British	16	40
French	4	10
Greek	4	10
Bulgarian	1	2.5
German	1	2.5
Irish	1	2.5
Italian	1	2.5
International	12	30
Mexico	1	2.5
Nepalese	1	2.5
Polish	1	2.5
Portuguese	1	2.5
Nigeria	2	5
Chinese	2	5
Canadian	1	2.5
Iranian	1	2.5
Thai	1	2.5
Tunisian	1	2.5
Age	Mean	St.Dev
21-50	29	7

2.3.2 Q-methodology Results

In this section the results of Q methodology are presented in combination with the self-reported characteristics of the participants. The data analysis was completed using the Ken-Q Analysis application (Banasick, 2019). The ratio of participants to sortable statements was 40:47. The total of 40 Q sorters were intercorrelated, whose correlation matrix is shown in Table 16 (Appendix 3), which presents the nature and the extent of the relationship of each Q sort with every other sort in the study. When

the intercorrelation is below ± 0.45 shows a lack of connection, with a few or non similarities between the two Q sorts. The factor analysis was carried out and seven factors were extracted (Centroid Factor Analysis), which together explained 61% of the study variance. That means that the set of factors are highly correlated and the predictions are valid. The next step was the rotation that ensures that each factor offers the most meaningful vantage point from which to view our subject matter. With other words, the rotation aims to achieve to increase the factor loadings of Q sorts on a factor, while working to decrease their loadings on all the other factors. Table 17 (Appendix 3) presents the rotated factor loadings matrix of the four factors that were kept and their factor's eigenvalues which were 8.9, 3.6, 4.94 and 3.32 and explained 51% variance The factor loadings ranged from -0.57 (negative) to 0.79 (positive), indicating that among the Q sorts there were many that were represented by the extracted factors. The shaded factor loadings of the Table 17 (Appendix 3) are the Q sorts loaded significantly on one of these four factors and show how much or less the factor represents the individuals' Q sorts. Table 18 presents the z-scores of the factors statements, which makes the interpretation easier as the factor array and the ideal Q sort of every factor is based on the weighted average of the scores that similar respondents (flagged Q-sorts) gave to the statements (z-scores). Table 21 (Appendix 3) contains all the factor arrays, demonstrating what an ideal Q sort for each factor would look like and served as a primary tool for interpretation, providing the rank order for the entire Q-set. Identification of the most and least distinguished statements among the factor arrays contributes to an interpretation and to naming of each factor the analysis based on its unique themes**.** The extent to which each factor array intercorrelated with other factor arrays are presented in Table 19 (Appendix 3), and reveal that factor 1 is correlated with factor 3 and 4 (0.54 and 0.59, respectively), while factor 3 is correlated with factor 2 (0.46) and factor 3 is correlated with factor 4 (0.48). That means that the intercorrelated factors have common characteristics. Each correlation above 0.39 is considered as statistically significant. Therefore, the

correlation between the factors could be taken as evidence for the similar patterns that the distinguished participants presented in ranking part of the survey. Finally, the Table 20 (Appendix 3) presents the factor characteristics, which contains the reliability and error measures for each of the factor arrays. The following interpretation of each factor was based on the information provided these tables.

Factor 1: Informed and positive

Factor 1 was named '*Informed and positive*'. Starting with the socio-demographic characteristics presented in Table 2. Factor 1 has an eigenvalue of 8.90 and explains 22% of the study variance (Table 17, Appendix 3). Eleven participants were significantly associated with this factor, and 82 % were females with an average age of 27 years. The majority of the participants were Europeans (72.7%) and they have pursued Tertiary education (36.4%), Post Graduate Education (36.4%), and Doctoral education (18.2%). While 45.5% of the individuals were in full time education or in employment full-time (36.4%). All the distinguished participants of factor 1 are meat eaters, with 54.5% eating meat almost every day (meat-eaters) and 45.5% only a few days per week (flexitarians).

The results from the food neophobia score revealed that the participants in this factor were neophobic with mean score equal to 3.6 *(M = 3.6; SD = 2.1)* which is considered high, as overall scores under 2.0 are perceived as low (neophilic individual) while score above 3.0 are perceived as high (Arvola et al., 1999). However, the standard deviation (2.1) showed that the data for food neophobia was moderately spread out over a wide range of value. All of the participants were aware of insect-based foods benefits. A percentage of 45% *(n = 5)* had a prior insect-based food consumption while the rest 55% *(n = 6)* that have never eaten insects but showed

Table 2. Socio-demographic characteristics, food neophobia, awareness, personal involvement inventory levels, willingness to consume insect-based food of representatives of factor 1

	Range or n	Mean or %	SD
Age (years)	23-32	26.9	3.4
Gender (Female)	8	72.7%	
Nationality			
Europeans	8	72.7%	
Internationals	3	27.3%	
Education			
Secondary Education / High school	1	9.1%	
Tertiary Education / University	4	36.4%	
Post Graduate Education / MSc/MA/MBA/MRes	4	36.4%	
Doctoral education /PhD	2	18.2%	
Working			
Working full-time (30 hours per week plus)	4	36.4%	
In full-time education	5	45.5%	
Not working	1	9.1%	
Looking for a job	1	9.1%	
Eating Habits			
A meat-eater (consuming meat (almost) every day of the week)	6	54.5%	
A flexiterian (consuming meat only a few days per week)	5	45.5%	
Food Neophobia	1-7	3.6	2.1
Awareness		100%	
Personal involvement Inventory (PII)	14-140	79	9.3
Prior insect consumption (No)		54.45%	
Willingness to consume	1-10	8.5	1.8

high mean of willingness *(M = 8.5; SD = 1.8)*. The mean score of PII was 79 *(M =79; SD = 1.9)* which means that the participants are moderately involved.

From the outcomes of the analysis, it has been clear that factor 1 is highly associated with a positive attitude towards insect-based foods. The distinguished statements of that factor 1 presented at Table 21 (Appendix 3), concern the sensory appeal, considering insect-based foods as clean (number of statement: position of sorting) (26: +2) and fresh (27: +1) with pleasant texture. In terms of healthiness and nutritional value, the most correlated participants strongly agreed that insect-based foods are rich in protein (11: +5), in vitamins & minerals (12: +4) and contribute to individuals' well-being (14: +3). While it seems that the participants in this factor, were not of the opinion that the insect-based foods could be rich in fibre or could be dangerous to their health (28: -3). Insect-based foods seemed not to be considered risky from that group of participants as they disagreed with the belief of causing physical risks (32: -4), leading in sickness (35: -4) and damaging the body (33: -5), while the quality of insect-based foods considered trustworthy (40: +1). The idea of insect-based food consumption seems to offer positive emotions such as enjoyment (3: +3), satisfaction (22: +2) and not arouse the feeling of repulsion (37: -3), disgust (36: -2) and scary (38: -3), which explains why respondents did not find the consumption of insect-based food a personal challenge (39:-2). Furthermore, the other people and society seem not to influence the participants, as they responded negatively to the statement referring to insect consumption after someone's abetment (46: -4) and on the statement if they would feel ashamed to consume insects (45: -5). Trust in sustainability of insect proteins as an alternative source of protein (20: +4) and good source of protein (2: +5) was high not only in that factor but to others as well.

The participants who belong to factor 1 seemed to be high potential consumers and their preferences would be helpful for the food industries that are planning to launch insect-based foods. Table 3 presents the ranking of the most preferred products with

insect-based foods and it shows that Burger (1), Snack (2) and Protein Bars (3) were the most preferred while the less preferred are Ice-cream (16), Yoghurt (17) and Candy (18). That a burger is considered to be suitable for inclusion of insects does not come as a surprise because burger is a familiar and well-liked product. A familiar preparation in combination with invisible incorporation of the insects resulted in an increased willingness to taste (Caparros Megido et al., 2014). Moreover, the consideration of the insect proteins as a good source and healthy comes in line with the high preference of insect-based protein inclusion in protein bars, energy bars and energy shakes.

Table 3. Most preferable insect-based food product rank of distinguished Q sorters of factor 1.

PRODUCTS	Rank	PRODUCTS	Rank
Burger	1	Cookies/Cake	10
Snack	2	Casserole	11
Protein Bars	3	Desert	12
Fried snack	4	Pasta sauce	13
Energy bar	5	Soup	14
Energy shake	6	Spreads	15
Pasta	7	Ice-cream	16
Chocolate	8	Yoghurt	17
Ready-to-eat meal	9	Candy	18

Factor 2 Informed and negative

Factor 2 was named '*informed and negative*'. Factor 2 had an eigenvalue of 3.69 and explained 9% of the study variance (Table 17 Appendix 3). Four participants were significantly associated with this factor and all of them are female with an average age of 30.5 years. As shown in Table 4, the majority of the participants were international (75%) and the half of them pursued tertiary education, the one fourth followed post graduate studies such as MSc/MA/MRes (25%), and one quarter pursued doctoral education. 50% of the individuals were in full time education and the rest was equally

working full time (25%) or didn't work (25%). Finally, the eating habits ranged from meat eaters (50%) to flexitarian (25%) and vegetarian (25%).

The results from the food neophobia score 3.6 (M = 3.6; SD = 1.5) revealed that the participants in this factor are neophobic like people of factor 1. The level of awareness was high as the awareness mean of the participants is 92%. None of the participants had consumed insect-based food before, and they had low willingness to eat (M = 2.0; S D= 0). The mean of personal involvement inventory score was 91 (M = 91; SD = 1).

Table 4. Socio-demographic characteristics, food neophobia, awareness, personal involvement inventory levels and willingness to consume insect-based food of representatives of factor 2.

	Range or n	**Mean or %**	**SD**
Age (years)	26-39	30.5	3.4
Gender (Female)	4	100%	5.1
Nationality			
Europeans	1	25%	
Internationals	3	75%	
Education			
Tertiary Education / University	2	50%	
Post Graduate Education / MSc/MA/MBA/MRes	1	25%	
Doctoral education /PhD	1	25%	
Working			
Working full-time (30 hours per week plus)	1	25%	
In full-time education	2	50%	
Not working	1	25%	
Eating Habits			
A meat-eater (consuming meat (almost) every day of the week)	2	50%	

A flexiterian (consuming meat only a few days per week)	1	25%	
A vegetarian (consuming any meat at all)	1	25%	
Food Neophobia	1-7	3.6	1.5
Awareness		92%	
Personal involvement Inventory	14-140	91	1
Prior insect consumption (No)		100%	
Willingness to consume	1-10	2	0

Factor 2 was characterized by the recognition of the nutritional value of insect-based foods, ranking statements concerning health like 'I believe insect-based foods are rich in protein' (11: +5), 'I believe insect-based foods are rich in vitamins and minerals' (12: +4), 'I believe insect-based foods can keep me healthy' (14: +3) and 'I believe insect-based foods are good for my skin/teeth/hair/nails etc' (9: +1). Taking into account the consideration of simplicity of insect-based foods for cooking or preparation, the recognition as a good source of protein and distinguishing insect-based foods as a good value for money (5: +4) could explain the willingness to taste these kind of foods after someone's abetment (46: +1).

However, the respondents tended to find insect-based foods repulsive (37: +2), disgusting (36: +1), scary (38: +3), unpleasant (21: -2), not exciting (8: -3), not satisfying (22: -2) and not to have a positive influence in what other people think about them (1: -3). The lack of feeling successful in case of insect-based foods consumption (45: -4), came close with the inability to feel proud of it (44: -5), lack of excitement (8: -3), the feeling of guilty and satisfaction (22: -2).

The negative attitude of participants of factor 2 towards insect-based foods makes them possibly non potential consumers. Table 5 presents the ranking of the most preferred products for insect-based foods and it shows that Protein Bars (1), Energy

bars (2) and Snacks (3) were the most preferred while the less preferred were Pasta sauce (16), Yoghurt (17) and Ice-cream (18). The participants' preference for inclusive of proteins into protein bars, energy bars and snacks are in line with their acceptance of the nutritional value of insect-based foods. That could be very useful for the food industry. The promotion of insect-based foods as healthy and sustainable could increase the acceptance and the willingness of the non potential consumers to start eating insect-based foods. The less preferable options are similar with that of the participants of factor 1.

Table 5. Most preferable insect-based food product rank of distinguished Q sorters of factor 2.

PRODUCTS	F2	PRODUCTS	F2
Protein Bars	1	Ready-to-eat meal	10
Energy bar	2	Casserole	11
Snack	3	Desert	12
Fried snack	4	Spreads	13
Cookies/Cake	5	Soup	14
Burger	6	Pasta	15
Chocolate	7	Pasta sauce	16
Candy	8	Yoghurt	17
Energy shake	9	Ice-cream	18

Factor 3: Informed with low sensory expectations

Factor 3 was named '*Informed with low sensory expectation*'. Factor 3 had an eigenvalue of 4.94 and explains 12% of the study variance (Table 17, Appendix 3). Five participants were significantly associated with this factor and Table 6 presents their characteristics. More than a half of the distinguished participants were female (60%) with an average age of 24.8 years. The majority of the participants were Europeans (80%) and 60% of them pursued tertiary education, 20% followed post graduate studies such as MSc/MA/MRes (20%), and the rest of them pursued doctoral education (20%). Equally, 40% of the individuals were in full time education while

around 20% were working full time. All the distinguished participants of factor 3 were eating meat, differing on the frequency that they did it with 54.5% of them to eat meat almost every day (meat-eaters) and 45.5% to consume eat only a few days per week (flexitarians).

The mean of food neophobia score was 3.6 *(M = 3.6; SD = 1.8)* revealing that the participants in this factor were neophobic, like the participants of factor 1 and 2. The level of awareness was high with a mean of 80 %. The participants (60%), who did not have a prior insect-based food consumption experience, showed a low willingness to eat with mean of willingness score 4.0 *(M = 4.0; SD = 4.3).* Participants' personal involvement inventory score mean score is 91 (*M = 90; SD = 8.7)* which means that the participants were somewhat involved. The standard deviation of willingness and PII declare that data of awareness are moderately spread out over a wide range of values.

Table 6. Socio demographic characteristics, food neophobia, awareness, personal involvement inventory levels and willingness to consume insect-based food of representatives of factor 3.

	Range or n	**Mean or %**	**SD**
Age (years)	19-30	24.8	3.6
Gender (Female)	3	60 %	
Nationality			
Europeans	4	80 %	
Internationals	1	20 %	
Education			
Tertiary Education / University	3	60 %	
Post Graduate Education / MSc/MA/MBA/MRes	1	20 %	
Doctoral education /PhD	1	20 %	
Working			

Working full-time (30 hours per week plus)	2	40 %	
In full-time education	2	40 %	
Working part-time (under 30 hours per week)	1	20 %	
Eating Habits			
A meat-eater (consuming meat (almost) every day of the week)	3	60 %	
A flexiterian (consuming meat only a few days per week)	2	40 %	
Food Neophobia	1-7	3.6	1.8
Awareness		80 %	
Personal involvement Inventory	14-140	91	8.7
Prior insect consumption (No)		60 %	
Willingness to consume	1-10	4	4.3

Q set statements most associated with factor 3 when compared to other factors included statements about the way the participants would feel after insect-based food consumption. Respondents who fit better with this factor would feel proud (44: +4) and good about themselves (42: +2) after eating insect-based foods, which was considered a trendy habit (30: +2) not being a passing fad (17: +5). Finding the insect-based foods, a personal challenge (39: +3), which made them feel uncomfortable (47: -4) and not feel ashamed (4: -5), could explain why the insect-based foods boost their confidence and affected them positively. The informed with low sensory expectation could be an explanation as well. This factor was highly associated with statements concerning senses such as the texture (16: -4), appearance (18: -3), freshness (27: -3) and taste (15: -2) which were expected not to be good. Some of the higher associated statements with factor 3 had environmental content, which was about the contribution to global carbon emissions reduction (13: +5) and the sustainability of insect proteins (7: +5), which both were ranking on the strongly agree spectrum.

Table 7 presents the ranking of the most preferred products with insect-based foods and it shows that Burger (1), Snacks (2) and Pasta (3) were the most preferred while the less preferred were Yoghurt (16), Ice-cream (17) and Spreads (18). That burger, snacks and pasta are considered to be suitable for inclusion of insects from a consumer point of view does not come as a surprise, because all of them are familiar and well-liked products. It seemed important that the product fits in with the existing food consumption pattern of a specific group.

Table 7. Most preferable insect-based food product rank of distiguished Q sorters of factor 3.

PRODUCTS	Rank	PRODUCTS	Rank
Burger	1	Soup	10
Snack	2	Candy	11
Pasta	3	Energy shake	12
Fried snack	4	Ready-to-eat meal	13
Pasta sauce	5	Casserole	14
Protein Bars	6	Desert	15
Cookies/Cake	7	Yoghurt	16
Chocolate	8	Ice-cream	17
Energy bar	9	Spreads	18

Factor 4 Informed and satisfied but price-worried

Factor 4 was named '*Informed and satisfied but price-worried*'. Factor 4 had an eigenvalue of 3.32 and explains 8% of the study variance (Table 17, Appendix 3). Three participants were significantly associated with this factor. Table 8 presents the characteristics of the participants. All the most associated Q sorts belonging to this factor were females with an average age of 23.3. The 66.6% of the participants were Europeans, have pursued tertiary education (66.6%), and doctoral education / PhD (33.3%). A 66.6% of the individuals were studying in full time or was searching for a job (36.4%). All the distinguished participants of factor 4 were eating meat, differing

on the frequency that they did it with 66.6 % of them to eat meat only a few days per week (flexitarians) and 33.3% to consume meat almost every day (meat-eaters).

The results from the food neophobia revealed that the participants in that factor were more neophilic (2.8) than all the other factors. The mean of food neophobia scores 2.8 *(M = 2.8; SD = 1.4)* was somewhat low which makes the participants of factor 4 less neophobic than all the others. The mean of awareness score was 100, which means that all the participants are completely aware of the insect-based foods benefits. A percentage of 33.3% had a prior insect-based food consumption while the rest 66.6% that had never eaten insect showed a high willingness to eat with mean of willingness score 7.5 (M = 7.5; SD = 1.5). The personal involvement inventory mean score was 77 (M = 77; SD = 5.1) which is lower among the factors. The standard deviation of PII declares that data of awareness are moderately spread out over a wide range of values.

Table 8. Socio-demographic characteristics, food neophobia, awareness, personal involvement inventory levels and willingness to consume insect-based food of representatives of factor 4.

	Range or n	**Mean or %**	**SD**
Age (years)	20-28	23.3	3.4
Gender (Female)	3	100 %	
Nationality			
Europeans	2	66.6 %	
Internationals	1	33.3 %	
Education			
Tertiary Education / University	1	33.3 %	
Post Graduate Education / MSc/MA/MBA/MRes	1	33.3 %	
Doctoral education /PhD	1	33.3 %	
Working			

In full-time education	2	66.6 %	
Looking for a job	1	33.3 %	
Eating Habits			
A meat-eater (consuming meat (almost) every day of the week)	1	33.3 %	
A flexitarian (consuming meat only a few days per week)	2	66.6 %	
Food Neophobia	1-7	2.87	1.4
Awareness		100 %	
Personal involvement Inventory	14-140	77	5.1
Prior insect consumption (No)		66.6%	
Willingness to consume	1-10	7.5	1.5

Factor 4 contained highlighted statements, which concerned the price (6: +5) of insect-based food and the food taste (15: +4), which both were the most associated statements with factor 4. The results revealed that the participants who represented this factor better, had positive viewpoints for insect-based foods, finding them funny (4: +4), well-appeared (18: +3), pleasurable (21: +3) and exciting (8: +2). In terms of disgust, they do not find them neither disgusting (36:-2) nor repulsive (37: -3) However the nutritional (12: -1) and healthy value (14: -2) were not trusted even when the sensory appeal is high, considering insect-based foods well-appeared (18: +3), nice smell, (19:+1) and good taste (15:+4). They are not of the opinion that insect-based food consumption could affect positively their public image (1: -3) but they would definitely not feel guilty (43: -5) or ashamed (41: -5) in case of insect-based food consumption. The contribution of insect-based foods to controlling weight (34: -4) or the way that they think about themselves (42: -2) was low, based on the Q sorts of the most correlated participants of the factor.

Table 9 presents the ranking of the most preferred products to be incorporated with insect showed that ready-to-eat meals (1), Burger (2) and Snacks (3) were the most

preferred while the less preferred were Candy (16), Ice-cream (17) and Yoghurt (18). At general, ready to eat meals, burgers and snacks are cheap and well-liked options for the people nowadays. Therefore, the willingness to eat insect-based food and the worries of participants about the price could balance through the incorporation of the insect proteins into foods such as ready-to-eat meals, burger, snacks and protein bars.

Table 9. Most preferable insect-based food product rank of distinguished Q sorters of factor 4

PRODUCTS	Rank	PRODUCTS	Rank
Ready-to-eat meal	1	Pasta	10
Burger	2	Soup	11
Snack	3	Pasta sauce	12
Fried snack	4	Cookies/Cake	13
Protein Bars	5	Desert	14
Spreads	6	Chocolate	15
Energy shake	7	Candy	16
Energy bar	8	Ice-cream	17
Casserole	9	Yoghurt	18

Consensus statements

Among the statements associated with factors, five consensus statements (pink cells of Figures 15, 16, 17, and 18) were determined. These indicated that the consumers disagreed that the insect-based foods could have a positive influence in what the other people think about them (1) and yet strongly agree that insect-based foods could be an alternative source of protein to meat/fish/dairy (2), nutritious (10), low in fat (25) and slightly agreed on the quality (40).

2.4 Discussion

The aim of this study was to spherically explore major viewpoints consumers hold about insect-based food at the Sutton Bonington Campus, as assessed through Q methodology, a method that combines qualitative and quantitative elements. To date, there is a lack of prior research studies on insect-based foods using Q method, to the best of our knowledge, ours is the first study to apply the Q-methodology to insect-based foods in UK. Consumers studies of insect-based foods have been conducted in other countries such as German, Switzerland, Italy and Netherlands (Hartmann and Siegrist, 2016, House, 2016, Hartmann et al., 2015, Mancini et al., 2019). Using Q-methodology, different viewpoints about insect-based foods of the participants are collated. It was clear that population as a whole does not share the same viewpoint but the viewpoints could be split up into different factors with individuals belonging to the same factor sharing similar opinions about insect-based foods.

Four main factors were identified, each demonstrating how, in general, persons from this specific factor think about the insect-based foods. Factor 1: '*informed and positive'* demonstrated the positive attitude some consumers hold towards insect-based food by having a high willingness to taste insect-based foods, being aware of the high nutritional value of insects and not agreeing with risk perception. Focusing on the negative feeling activated by the high feeling of disgust and repulsion, supporters of factor 2 were termed the '*informed and negative*', where the nutritional value had no impact on their attitude. Factor 3: '*informed with low sensory expectation'* demonstrated a repulsion towards insect-based foods, as representatives of this factor have low expectations of insect-based foods in terms of taste, while it is noteworthy that they would feel confident and proud of themselves and as if they have made an achievement if they ever were to eat an insect-based food. Lastly, factor 4:

'*Informed and satisfied but price-worried*' advocates that the supporters find insect-based-foods pleasurable, fun and exciting but they were concerned about the price.

The profiles of the distinguished participants of all the factors were on agreement about the most preferable insect-based food products having burger, snacks, energy bars, energy bars and ready-to-eat products on the top while they did not have differences in terms of educational and employment status or eating habits.

The findings extend previous research aimed at uncovering the perceptions and the viewpoints of consumers towards insect-based foods. The awareness of consumers in general is one of the main determinants consumption of insect-based foods in combination with food neophobia level, prior experience and insect contamination disgust (Hartmann and Siegrist, 2016, Mancini et al., 2019). Awareness and prior experience have a positive effect on acceptance while high food neophobia and insect contamination disgust affect the acceptance negatively. Therefore, making the consumers aware of insect-based foods using different manners, will contribute to the acceptance and willingness for insect-based foods. Each factor found in this study may agree with the results of previously published studies. For example, the representatives of factor 1: *informed and positive* appeared to hold a moderate food neophobia level which could had effect on insect-based foods rejection. However, the high level of awareness in combination with the prior insect-based food experience had the result of not only reducing the disgust towards insect-based foods but also a high willingness of the participants to consume insect-based foods. Similarly, it has been reported that the participants of their survey showed a decreased disgust towards insect-based foods after attending a seminar informing the consumers about insect consumption (Mancini et al., 2019, Caparros Megido et al., 2014). Another study demonstrated that the awareness of consumers through education and promotion strategies in Northern Europe could explain their more positive attitude, compared

with people from Central Europe where entomophagy is less common (Piha et al., 2016). Moreover, prior experience of insect-based usually affects acceptance and willingness to eat insect-based foods positively, as demonstrated in previous studies, which indicated that previous entomophagy experience is capable of making the people more open to novel foods (Caparros Megido et al., 2016, Hartmann et al., 2015). Additionally, the insect-based food product preference of the potential consumer was similar with what Van Thielen. L et al., (2017) revealed in their research about the consumer acceptance of foods containing edible insects in Belgium. Both agreed that burgers, protein bars and snacks were at the top of the consumers preference, giving a guideline to food industry, while ice cream, yoghurt, candy and spreads are at the bottom of the consumers preferences.

However, as factor 2 '*informed and negative*' reveals, awareness is not always enough to affect the consumers' opinion positively about insect-based-food. In addition to awareness, the age and the gender of the participants could affect their opinion. The participants represented in factor 2 were older than all the other factors. It has been shown that well-established viewpoints of older people are more difficult to change to 'new' habits, which could be described as incompatible with their current knowledge and therefore the introduction of insect-based foods is often accompanied by disgust (Myers and Pettigrew, 2018, Verbeke, 2015). In factor 2, the distinguished participants were all females with zero prior insect-based foods experience and with a very low intention to taste insect-based food. Gender has also previously been shown to influence the acceptance of insect-based foods, with women shown to be more neophobic compared to men (Verbeke, 2015, Caparros Megido et al., 2016). Therefore, the participants even they recognised the nutritional value, they had negative attitude towards insect-based foods, which makes them possibly less potential consumers. However, the recognition of the high nutritional value could be useful for the food industry. Launching foods such protein bars, protein snacks and

energy bars, which include insect-based proteins and based on the participants ranking are at the top of the list, and promoting them as healthy and nutritional the acceptance and the willingness could be increased.

The factor 3: '*informed with low sensory expectations'* was characterised by participants that are aware of the nutritional value of the insect-based foods but they have low expectations in terms of taste which explains their low willingness to taste insect-based foods. Similarly, an Italian study explored the attitude of Italian consumers towards insect-based foods and revealed that the idea that insect-based foods might be disgusting, discourages the consumers from tasting insect-based foods (Cicatiello et al., 2016). Therefore, due to low sensory expectations the participants would prefer to eat already well-liked and familiar foods, which will include insect proteins such as burger, snack and pasta. The incorporation of insects into foods that generally are acceptable and well liked, could boost the participants to try them.

The factor 4 '*Informed and satisfied but price-worried*' distinguished participants regarded insect-based-foods as an exciting, pleasurable and with high sensory appeal eating habit. Participants in this factor showed a low food neophobia level – the lowest level amongst all the factors - in combination with the awareness indicated a positive attitude towards foods with highlighted concern about the perception of a high price. A survey conducted in Kenya has also shown that price, availability and convenience are among the nutritional and sensorial determinants of insect-based foods acceptance (Pambo et al., 2016). Similarly, consumer acceptance of insect-based foods in the Netherlands revealed that the price, availability and taste are subordinate factors that affect the insect-foods in terms of repetitive habit (House, 2016). The ranking of the most preferable possibly insect-based foods lead in the preference of foods that generally have a low price and are well-liked. Ready-to-eat meals, burgers and snacks are foods that are considered cheap and well-tasted.

2.4.2 Strengths and limitations

Results yielded with Q methodology relate to the identification of major viewpoints on a certain topic in society. It is important to note that this study included a small amount of consumers (n=40) over a short period of time (less than 3 months). In addition, the study took place on Sutton Bonington Campus, which is home to the School of Biosciences and School of Veterinary Medicine and Science and this location and speciality in terms of education could affect the results due to an increased level of awareness of food security and agriculture. With these limitations considered the general is ability of findings should be considered carefully. However, the study strictly adhered to the pre-described ratio between sample size and number of items (Watts, 2012) and therefore does provide a nuanced insight into the main viewpoints held about insect-based foods among the participants. Future studies should look to investigate how common these viewpoints are in the population and how robust the theories may be across different cultures. It would be important to include a sample set more representative of the population in terms of education level and working environment, as the scientific situation of the location could have influenced willingness and acceptance of consumers towards insect-based foods. Generally, viewpoints on eating habits can be influenced by social discourse as well as by official and public informational sources (Robinson and Higgs, 2012).

2.5 Conclusions

The study aimed to explore the different viewpoints held by consumers about insect-based foods in the UK, employing Q methodology for the first time. Four factors (groups of participants) emerged: (1) *informed and positive,* (2) *informed and negative,* (3) *informed with low sensory expectation* and (4) *Informed and satisfied but price-worried*. While there is some research that investigates the factors that affect acceptance of insect-based food, very little if any, segregates the viewpoints into

categories and identifies different profiles of potential consumers. The study highlighted that the majority of the participants are aware of the nutritional value of insect-based food and develop different attitudes towards insect-based foods based on the prior experience, food neophobia levels, gender, age, sensory expectations and subordinate determinants such as price and convenience. Factor 2 and especially factor 4 would be possible consumers for insect-based foods. The low food neophobia of participants represented the factor 4 could be the key to contributing to the enhancement of insect consumption. The product types in which incorporation of insects is accepted are similar among the factors, with –obviously- substantially differentiation for some product type in each factor. Burgers, snacks, energy bars, protein bars and ready-eat-meal appeared to be the most preferable insect-based food products. Best arguments to be used for promotion and increase of the potential consumers' willingness, are the fact that the insects are healthy, sustainable food source, cheap and the incorporation of the insects into familiar and well-liked foods.

Chapter 3. Formulation of insect protein gels

3.1 Introduction

Entomophagy it is not a common practice in Europe, therefore as discussed in the previous chapter in order to meet the growing demands of the global population, there is a challenge to increase people's willingness to eat and accept insects as a whole or as an ingredient. The Q method study demonstrated that the majority of the participants were aware of the nutritional value of insect-based food and developed different attitudes towards insect-based foods based on the prior experience, food neophobia levels, gender, age, sensory expectations and subordinate determinants such as price and convenience. One route to increase consumption would be to enhance prior experience as well as reduce food neophobia would be the introduction of food products containing invisible insects to consumers in developed countries. A method to formulate these insect-based foods would be through the use of insect protein extracts, insect lipid fraction or chitin from insects into foods before the direct consumption of an insect as a whole.

Researchers should take into account the consumers' need to mask the insect's appearance through different processing methods. Insects have been shown to be able to be incorporated into some manufactured food products such as salty and sweet spreads, fruit and nuts bars, candy, biscuits and crackers, chocolates and macarons, burgers, nuggets and schnitzel, often the insects are added as a flour and offer no functional properties to the product (Mariod and Fadul, 2015, MailOnline, 2019). Adding insects for nutrition and functionality is an exciting area of research. In order for the successful incorporation of insect ingredients, such as proteins, into foods further study of their physicochemical and functional properties including solubility, amino acid profile, water and oil binding, the ability to form gels, foams and emulsions is needed. A limited number of studies have been published about the functional properties of insect properties focusing on gelation, emulsions and

foaming; in particular, the mealworm larvae's ability to form emulsions (Gould and Wolf, 2017), foaming and gelation (Mishyna et al., 2019b). However, there is a lack of studies investigating the protein content, extraction and the gelation properties of proteins from the adult *Tenebrio molitor*.

The aim of this study was to characterise the four different protein extracts and study the ability of the extracted fractions to form a gel after heating and salt addition.

3.1.1 Insect protein

Insects, apart from being rich in fat, minerals and vitamins (Bovera et al., 2015, Rumpold and Schlüter, 2013, Kouřimská and Adámková, 2016), have a high protein content ranging from 30 to 90 % depending on the species and the developmental stage of the insect (Aguilar-Miranda et al., 2002, Kulma et al., 2016). The reported protein composition of some species is shown in Table 10.

Table 10. Crude protein content on dry mass of different insect species

	Protein (%) Dry Mass	**Reference**
Tenebrio molitor larvae	60 ± 5.2	(Zielińska et al., 2015)*
Schistocera gregaria larvae	76	(Zielińska et al., 2015)
Schistocera gregaria adult	48.4	(Mishyna et al., 2019a)
Gryllodes sigillatus	70	(Zielińska et al., 2015)
Alphitobius diaperinus	58.03	(Yi et al., 2013)
Acheta domesticus	70.8	(Yi et al., 2013)
Z morio	59.9	(Yi et al., 2013)
Blaptica dubia	67.4	(Yi et al., 2013)
Hermetica Illucens	32	(Bußler et al., 2016)**
Apis mellifera larvae	75.7	(Mishyna et al., 2019a)

*average protein content of *Tenebrio molitor* larvae on dry mass(n=5)(Bußler et al., 2016), (Bovera et al., 2015), (Aguilar-Miranda et al., 2002), (Azagoh et al., 2016)
**average protein content of *H. illucens* larvae on dry mass (n=2) (Caligiani et al., 2018)

Insect protein contains not only all the essential amino acids for human body (Azagoh et al., 2016, Makkar et al., 2014) but also their digestibility is high (Marono et al., 2015).

In addition to the provision of amino acids, there is the potential for insects to provide insect protein peptides, and their associated biological effect, as they are released during fermentation or digestion of food proteins by proteolytic enzymes. These types of bioactive peptides have been found in milk. Antihypertensive, mineral-binding, anti-oxidative, antimicrobial, immune- and cyto-modulating biological activities are some of the biological activities realised by the peptides (Walther, et al., 2011). Enzymatic hydrolysis procedures applied to insect proteins may lead to exploration and application of novel bioactive peptides (BAPs) as sustainable protein/peptide sources for human nutrition (Nongonierma and Fitzgerald, 2017).

3.1.2 Insect protein functionality

In the literature, there are a limited number of publications referring to the protein properties. Protein charge and the surface hydrophobicity of proteins were studied for all fractions obtained by alkaline extraction or sonication assisted extraction of proteins of grasshopper (*Schistocera gregaria)* and honey bee (*Apis mellifera)*. This study indicated that the defatting step did not affect the protein charge. However, the extraction methods had an impact on the protein content and the hydrophobicity of grasshopper (*S. gregaria),* but not honey bee (*A. Mellifera,)* with the alkali extraction products for both species honey bee brood and grasshopper to led to lower protein content but a higher hydrophobicity (Mishyna et al., 2019a). Moreover, interfacial and emulsifying properties of protein extracted from mealworm larvae (*Tenebrio molitor)* were studied and compared with that of whey protein, resulting in the conclusion that mealworm larvae proteins are a promising food emulsifier and seemed to be a medium for the incorporation of invisible insects into products in foods (Gould and Wolf, 2017). While hexane and aqueous extracted protein fractions of edible crickets

(*Acheta domesticus)* possess emulsion capacity and foam stability, their ability depended on the extraction method (Ndiritu et al., 2017).

3.1.3 Protein extraction

In order to extract protein from insects, the insects are often starved and then killed. The method the insects are killed can affect the physicochemical properties of proteins. A study about the effect of two killing methods of *H. mellifera* on the nutritional quality of the protein indicated that the freezing killing method elicited different enzymatic pathways, which affected the nutritional quality of proteins; whilst blanching inhibited them. Moreover, blanching led to a more extractable fraction compared with that of freezing (Leni et al., 2019).

Following the kill step, protein extraction should be rapid and effective. Enzymes such as endogenous phenoloxidases take part in an enzymatic browning from which O-quinones are produced. Brown melanin pigments are created from the polymerisation of the O-quinones or cross-linked structures are formed by the reaction of O-quinones with assorted groups of proteins or amino acids, which could be detrimental for the quality of protein, affecting protein solubility and digestibility (Janssen et al., 2019). Moreover, it has been reported that endogenous phenoloxidases could be responsible for browning during the insect grinding (Janssen et al., 2017) and affect the soluble protein yield and the digestibility of *T. molitor, A. diaperinus and H. Illucens*. The endogenous proteases of processed and non-processed sample show biological activity at pH 8, which means that the pH of extraction protocols should be controlled, as they may affect the production of the insect-based food (Janssen et al., 2019).

The conditions of extraction should be moderate in order to obtain non-denatured proteins. The denaturation of proteins causes changes to the physicochemical properties such as solubility or conformation. Heating, the use of chemicals and extreme pH values are capable of causing denaturation by breaking polypeptide

bonds, which leads to unfolding of polypeptide chains, and exposure of the hydrophobic domains of the protein molecule. Therefore, the interaction of the protein molecules with the solvent is affected. High water solubility is one of the main characteristics of protein, which is desired to allow use in foods and beverages. The use of proteins in foams, emulsions and gels depends on the protein solubility (Zayas, 1997). The need for investigating the techno-functional properties is essential for fulfilling the targeted exchange of conventional proteins with novel proteins.

The optimisation of solubility of some insect proteins have been investigated under various condition such as pH, ionic strength or the extraction temperature of the solvent (Bußler et al., 2016, Zhao et al., 2016, Yi et al., 2013, Broekman et al., 2015). As it has been described by Broekman et al., (2015) thermal processing is responsible for reducing the solubility of insect proteins, while when the pH is close to pI the solubility is also low (Zhao et al., 2016, Bußler et al., 2016).

The purpose of the extraction protocol is to obtain protein in the purest form, excluding the presence other materials for an accurate study of the techno-physicochemical properties of the extracted proteins. Some materials such as phenolic compounds, which exhibit anti-nutritional activities or others with nutritional activity such as chitin, can interact with the proteins and function as ‘co-passengers’ in the final protein extraction. Insects should undergo fractionation under controlled conditions to prepare protein extracts with as few as possible of these materials, as is common for plants derived proteins targeted to replace meat proteins (Boland et al., 2012).

In general, the most common protein extraction methods are aqueous (Yi et al., 2013, Ndiritu et al., 2017), alkali and isoelectric precipitation (Mishyna et al., 2019a, Azagoh et al., 2016) and a sequential fractionation often termed the Osborne fractionation (Aguilar-Miranda et al., 2002, Caligiani et al., 2018). The functional properties of extracted proteins differ, depending on extraction method or different

types of proteins in the extracts, for example the presence of cuticular, haemolymph and/or muscle proteins. Table 11 presents the different types of proteins that have been found in *Mealworm beetles* as reported by Yi et al., (2016).

Table 11. The different types of protein that have been found in *T. molitor* as reported by Yi et al., (2016).

Type of protein	***Size (kDa)***	***Solubility***	*Proteins identified & MW*
Haemolymph	<14 ~ 80	Water soluble	Haemolymph protein (13 kDa), putative serine proteinase (~28 kDa) alpha-amylase (~50 kDa), vitellogenins, ovalbumin-like (43.2 kDa)*
Cuticular	14-30	Insoluble	chymotrypsin-like proteinase (24kDa)**,
Muscle proteins	18-220	Salt-soluble or insoluble	Myosin heavy (262.3 kDa) light chain (16.8 kDa), tropomyosin 2 (32.5 kDa), troponins (18.3- 47.3 kDa) a-actinin (107 kDa),

*(Elpidina et al., 2005, Tsybina et al., 2005)
** (Yi et al., 2013)

In the interior of the arthropod body the haemolymph flows which is analogous to the blood in vertebrates and remains in direct contact with the animal's tissues. Insect haemolymph has lack of erythrocytes and the main component is water. The latter acts as a solvent for many different molecules of haemolymph and while the insects are in the larval stage has a larger volume than adults (Capinera, 2004). Therefore the proteins of insect haemolymph, which are water soluble, vary in quantity during the

course of development and they are classified based on their functions in chromo proteins, protease inhibitors, storage proteins, lipid transport, enzymes, the vitellogenins and those involved in the immune systems of arthropods (Chapman, 1998). Based on Yi et al., (2016), *T.molitor* larva haemolymph includes storage proteins like alpha-amylase (~50 kDa), lipid transport protein such as vitellogenin and antifreeze proteins (< 14 kDa) (Graham et al., 2000, Graham et al., 1997) and others among them are haemolymph protein (13 kDa), putative serine proteinase (~28 kDa), ovalbumin-like (43.2 kDa), arginine kinase (40.1 kDa) and tubulin a α – 1 chain (50.6 kDa) (Yi et al., 2016). The cuticle, which is a complex matrix consisting of proteins and chitin filaments, contributes to part of an insect's exoskeleton. The cuticular proteins have molecular weight mainly between 14 to 35 kDa (Andersen et al., 1995) such as chymotrypsin (32 kDa) (Yi et al., 2013). Finally, the other group of proteins, which are abundant in insects, are the muscle proteins, which are salt soluble and are present with various molecular weights. Among the muscle proteins of *T. molitor* were actin, a α-actinin-4 (107 kDa), myosin heavy chain (262 kDa), myosin-2 essential light chain (16.8 kDa), tropomyosin 2 (32.5 kDa), troponin I (23.8 kDa), troponin T (47.3 kDa), and putative troponin C (18.3 kDa) (Yi et al., 2016). Aside from *T. molitor*, in the literature there are other species, which have been studied for their molecular weight distribution. Among these are *S. gregaria* proteins whose low molecular weight have been reported to potentially represent a protein which bind with fatty acids (15 kDa) (Haunerl and Chisholm, 1990). While the proteins with molecular weight ranging from 14 to 94 kDa are typical of proteins existing in the brain, corpus cardiacum, subesophageal ganglion and haemolymph of *S. gregaria* (Annadurai et al., 1992). In similar analysis, *L. migratoria* has been reported to contain various proteins with more characteristic bands those ones that corresponds to muscle proteins tropomyosin and tubulin (Purschke et al., 2018) while bands with molecular weights 16, 26, 29, 70 and 82 kDa are correlated with heat shock proteins of *Apis mellifera L.* bee (Alqarni et al., 2019).

3.1.2 Gelation

The term gel is defined as 'a form of matter which is an intermediate between solid and liquid having both elastic (solid) and viscous (liquid) properties (Aguilera and Rademacher, 2004). Gels possess the ability to be deformed under stress and revert to the initial shape when the stress is removed, as deformation affects the bonds between protein particles. When a transition from solution (liquid) to gel (solid) occurs, it results in gel formation. The high moisture three-dimensional network formed by the interaction of biopolymers and water exhibits viscoelastic properties. Gels have the ability to be distorted under pressure like solids but to retain the initial shape as liquids do, when the applied stress is removed (Nazir et al., 2016, Banerjee and Bhattacharya, 2012). Gels can be formed from polysaccharides, proteins or a mixture of both biopolymers. Based on Aguilera and Rademacher (2004), gels have three microstructural characteristics, which differentiate them from other biomaterials, including (1) the formation of constant network of connected molecules or aggregates that spans the solution; (2) avoidance of flowing due to the existence of some specific points in the formed network that keep the molecules interconnected; and (3) a big part of the liquid phase swells the polymeric network (Aguilar-Miranda et al., 2002). Gelling is a capacity possessed by biopolymers such as proteins and polysaccharides that provide semi-solid characteristics in a range of food products. The gelling agents utilised in many goods are food hydrocolloids extracted from natural sources (Banerjee and Bhattacharya, 2012).

Some food proteins include, casein; whey proteins; egg proteins; muscle proteins and legume proteins that possess an important function to form gels, making them extremely useful in food manufacturing. There are a number of food products on the market either in a form of gel or in different forms containing a gelling ingredient. Some of these products are jams, jellies, milk gels, bread doughs, confectionary, cream cheese, margarine, desserts, yoghurt and cake fillings (Ward and Courts, 1977, Aguilera and Rademacher, 2004, Bhattacharya and Jena, 2007). Gels find multiple

functions in foods such as thickening, gelling, stabilising foams and emulsions, inhibit or decrease syneresis, to name but a few. Moreover, food proteins usually are evaluated based on their gelling capacity which determines the quality properties of many foods such as textural and rheological characteristics (Jayas, 1997). The real application of gels is the development of new gel-type food products with unique textural, sensorial and nutritional characteristics. Therefore, it is a challenge for researchers to study the conditions under which protein gels are formed and their properties, aimed to (1) replace one protein for another - maybe novel protein such as insect protein - in food systems and (2) regulate the gelation process, managing to be able to express the textural and sensory outcome characteristics of the final product in advance. The physicochemical properties of the proteins such as molecular weight, hydrophobicity and thermal properties or experimental treatments such as heating/cooling, pressure/shear stress application, ionic strength, pH and water activity can affect the forming of the three dimensional network. Accordingly, the study of the effect of all these different conditions and the interaction with other molecules in a solution demand research on a multidisciplinary level (Nazir et al., 2016). There are many ways for the classification of food gels, based on gelation mechanism, interactions among the protein and other biopolymers and, structural and morphological characteristics. However, in this study the attention is on the formation of protein gels.

A deep understanding of the structure of the protein molecules is needed prior to describing protein gels. Globular proteins and fibrous proteins are commonly used in preparation of food gels. The tertiary structure of globular proteins is folded that the non-polar hydrophobic parts are arranged on the inside of the molecule while the polar hydrophilic parts are exposed on the outside of the molecule. This folded structure has a key role in solubility of the globular proteins. On the other hand, the fibrous proteins form long shapes where the polypeptides are placed antiparallel to each other. This arrangement makes fibrous proteins more stable and strong but water insoluble. To

achieve the complex process of gelation the protein structure should have reached a certain degree of unfolding (denaturation or destabilisation) in order to increase the interaction of the molecules. The gelation of proteins necessitates a force that will leads to unfolding of the native proteins and exposes the hydrophobic parts of the proteins, which will result in a range of intermolecular interactions. Then the interconnected protein molecules start aggregating which leads in a three-dimensional network of aggregates of molecules cross-linked by non-covalent bonds or less frequently by covalent bonds (Totosaus et al., 2002). The covalent interactions are usually irreversible and involve sulfhydryl (-SH)/ disulfide (S-S) interchange reactions, while the non-covalent interactions are possibly reversible, including hydrogen bonds, hydrophobic interactions, and electrostatic interactions (Wijayanti et al., 2014). The involvement of all these interactions in the gelation mechanism could explain why the understanding of gelation mechanisms is a quite challenging case.

The gelation mechanisms can be categorised based on the different methods of gelation such as, physically induced (heat, pressure) and chemically induced (acid, ionic, enzymatic) (Aguilera and Rademacher, 2004).

Heat-Induced Gelation

The most important and common way to form gels is heat-induced gelation, since the preparation of many foods involves heat treatment (Banerjee and Bhattacharya, 2012). Generally, heat induced gelation is a two-step process. The first step, which may be reversible, is the unfolding or dissociation of the protein molecule due to the input of energy as a result of heating. The second step, which is usually an irreversible reaction is when the exposed parts of protein molecules are associated and aggregated, forming complexes of higher molecular weight. The formation of the three dimensional network that spans the entire container volume can occur if the protein concentration is higher than a particular value, if an adequate degree of protein unfolding (partial or total denaturation) has occurred and if the environmental conditions are appropriate

(Aguilera and Rademacher, 2004). In general, the particle size and porosity of the aggregates can be determined by both steps, unfolding and aggregation relying on the ratio of the reaction rates of each step in specific temperature range. Moreover, the heating rate of the solution is capable of affecting the particle size of aggregates since the slower rate of the reaction the larger the aggregates formed. However, protein solutions usually contain a mixture of proteins instead of pure fractions, which means that different protein types have different behaviour under heat treatment. The nature of the bonds that participate in gelation varies including hydrophobic interactions and disulphide (-S-S-) bridges.

Pressure-Induced Gelation

High pressure can be applied as a single process or in combination with other factors and more specifically with changing of temperature, which is a positive advantage in studying the modifications of the functional properties of proteins. Generally, high pressure promotes reactions, which lead to the decrease of the volume of both suspended particles and solvent contained in the system. In the case of solvent, the pressure dissociates the water and the pH becomes more acidic. Moreover, the negative reaction volume created under pressure promotes the hydrophobic bonds although the hydrophobic interactions are becoming weaker. In terms of proteins, the conformation that a protein adapts caused by the high pressure, depends if is stabilised by covalent disulfide bonds or by non-covalent if the protein concentration is above to critical value. High pressure denaturation and aggregation show time dependent behavior and are to some extent reversible (Dumay et al., 1994). High pressure induced gelation is affected by various operating parameters such as operating pressure, duration time and repeated compressions, pH and type of solvent, although heat induced and high pressure induced gels present differences in appearance and in rheological properties (Van Camp and Huyghebaert, 1995).

Ionic Strength- Induced Gelation

The increase of the ionic strength in a protein solution is capable of reducing the electrostatic charges at the surface of the molecules or aggregates, which lead to the reduction of the electrostatic repulsion of the molecules, favouring the gelation. The addition of salts such as calcium or sodium chloride in a solution which contains pre-denatured proteins aims to induce gelation called cold-gelation (Bryant and McClements, 1998). In order to form a gel, the electrostatic forces should be balanced. The unfolding of the proteins after heating causes changes in protein hydrophobicity. The salt attracts the water molecules and decreases the water molecules available to interact with the charged part of the protein and the hydrophobic effect will drive the proteins to aggregation.

pH-Induced Gelation

The alteration of pH after the addition of acid or microbial fermentation causes alterations to the net charge of the protein molecules, which as a result changes the attractive and repulsive forces between the protein molecules and the interactions between solvent and protein. The net charges of proteins close to their isoelectric point is zero, which means that the hydrophobic interaction and aggregation of the molecules is promoted due to the low repulsive forces. When the pH of the solution is not close to pI gelation is prevented. However, changes to the pH do not only affect the proteins but also may affect the salt in the solution. It has been reported for acid induced coagulation of casein micelles in milk, the addition of acid increases the solubility of colloidal calcium phosphate. Acid-induced gelation based on the assumption that spherical particles with known radius can move by Brownian motion and can aggregate when they encounter each other. Next, the aggregates are interacting with other aggregates, creating fractal clusters which may be the building block of the gel (Lucey and Singh, 1997).

Enzyme-Induced Gelation

Enzyme-induced gelation is based on the introduction of artificial covalent cross-links into food proteins. Examples of suitable catalysts for cross-linking of proteins that will lead to gel formation are transglutaminase, peroxidase and polyphenol oxidase (Aguilera and Rademacher, 2004).

3.1.2.1 Insect Protein Gels

Studies of insect protein have shown their ability to form gels. Yi et al., (2013) studied the functional properties of five insect species (*Tenebrio molitor, Zophobas morio, Alphitobius diaperinus, Acheta domesticus* and *Blaptica dubia)* focusing on gelation and foaming. The results revealed that the water soluble supernatant fractions of all the species were capable of gel formation at 30% (w/v) protein concentration when the pH values were 7 and 10, but not at pH 3 and 5 which are close to the isoelectric point of the proteins. However, the only fraction which formed gel at a lower concentration was that of house crickets (*A. domesticus)* at the concentration of 3% (w/v) at pH 7 (Yi et al., 2013). Another study on the rheological properties of alkali extracted and isoelectric precipitated protein fraction of yellow mealworms (*T. molitor)* showed that protein concentration, pH, temperature, addition of NaCl and transglutaminase affected the elastic modulus (Zhao et al., 2016). Honey bee brood (*A. mellifera)* aggregation and gelation studies revealed that aggregation of soluble proteins was dependent on temperature and pH value and the highest coagulation was reached at 85 °C at pH 5 and 7. While alteration in protein surface hydrophobicity, net charged and amount of available and buried sulfhydryl groups demonstrated the contribution of either covalent intermolecular interaction resulted in aggregation at various pH. However, the critical concentration value was 5 and 11 % on raw powder basis at pH 7 and 3, respectively (Mishyna et al., 2019b). Nevertheless, a study of heat coagulation of raw (ground powder), deffated and extracted protein powder with alkali and isoelectric precipitation revealed raw powder and defatted had the higher protein coagulation (73.3 and 61.6 %) at pH 7 (Mishyna et al., 2019a).

Moreover, studies related to the ability of insect proteins to form gels are limited. It has been reported that gelatin isolated by two edible Sudanese insects, melon bug (*Coridius viduatus)* and sorghum bug (*Agonoscelis versicoloratus versicoloratus)* can replace commercial gelatin in ice cream production. Therefore, the study investigated the replacement of commercial gelatin with insect gelatin and the effect that it had on taste and texture. In terms of functionality as a gelling agent to absorb free water in ice-cream mix and help prevent the formation of large crystals in the ice cream, insect gelatin fulfilled the conditions that Sudanese Standard and Metrology have set for ice cream and was categorised as safe, but in terms of texture and taste there were significant differences (Mariod and Fadul, 2014).

Therefore, insect proteins extracted from different species have shown their rich nutritional value and their ability to form gel are comparable to conventional protein sources indicating the potential as an ingredient for insect-based gel product. Based on this finding, protein from mealworm beetles was extracted using four methods, characterised and two extracts were tested for gel forming properties, for which the results are presented in the next sections.

3.2 MATERIAL AND METHODS

3.2.1. Insect

Freeze dried mealworm beetles (*Tenebrio Molitor*) in the adult stage were bought from Norfolk Pet Supplies (Kings Lynn, UK). *Tenebrio molitor* is the latin name given by Linnaeus (1758) and express the genus *(Tenebrio)* and the species (*molitor*).

3.2.2 Chemicals and references samples

Ethanol, calcium chloride, sodium chloride, sodium phosphate dibasic, sodium phosphate monobasic, hydrochloric acid (2M), sodium hydroxide, Coomassie blue R250 and Biodesign™ Dialysis Tube (8 kDa) by Honeywell Fluka and the Pierce™ BCA protein assay kit was purchased from Thermo Fisher Scientific (Fisher Scientific, Loughborough, UK). Chitin from shrimp shells was purchased from Sigma Aldrich (Gillingham, UK). The SDS – PAGE kit and all the reagents were purchased from BioRad (BioRad, Watford, UK).

3.2.3 Whole insect samples preparation

30g of whole insects were ground to a fine powder as shown in Figure 4 using a coffee grinder for 45 secs (De'Longhi KG49, Havant, UK).



Figure 3. Freeze dried whole insects (L) and insect powder (R) after grinding

3.2.4 Water content

Theory

Water content is defined as the mass loss of a sample that occurs when the material is heated due to water vaporisation. The water content has a key role in the conformational and chemical state of the protein and the determination is essential in resolving stability issues (Towns, 1995). The determination was carried out based on the method written in *Official Methods of Analysis*(AOAC, 2000).

Experimental Procedure

An empty dish was dried in the oven Memmert U110 (CamLab, Cambridge, UK) at 105 °C for 3 h, transferred to a desiccator to be cooled and weighed. Afterwards, the sample (3 g ± 0.03) was added to the disk spreading it until uniformly distributed across the dish. The dish was then placed in the oven 105 °C. The samples were dried for 3 h at 105 °C (Memmert U110, CamLab, Cambridge, UK), transferred to the desiccator to cool. The dish was reweighed with the dried sample and equation 1 was used to calculate the moisture content.

Equation 1.

$$\text{Moisture}(\%) = \frac{(W1 - W2)}{W1} * 100$$

Where: W1 = weight (g) of samples before drying

W2 = weight (g) of sample after drying

3.2.5 Fat Extraction

Theory

Fat extraction is based on the ability of the fat to be solubilised in organic solvents such as ethanol or hexane. Insects typically contain between 6 to 35%, which can

interfer with protein extraction, therefore fat extraction is necessary to enhance the efficiency of protein extraction (Choi et al., 2017, Zhao et al., 2016). Moreover, it has been reported that fat extraction contributes to the enhancement of particular functional properties of the proteins (L'Hocine et al., 2006, Lee et al., 2016, Choi et al., 2017). The use of 99.5 vol % ethanol is quite advantageous as it is an efficient lipid solvent which removes adequately the fats as well as being food grade. As the purpose of this project is the study of insect proteins' ability to form gels, the maximum purity of isolated proteins is desirable, minimising any interactions with other components such as fats.

Experimental procedure

Based on the procedure of a previously published study by Zhao et al., (2016) 30 g of sample was dispersed in 200 mL ethanol and incubated (Covey Laboratory Engineering Co Ltd, Guildford, United Kingdom) at 40 °C for 60 min. The solids were filtered using Qualitative Whatman filter paper circle grade 1 (Whatman, Little Chalfont, UK), re-suspended in 200 mL and extracted as described again. The defatted solids were dried in a vacuum oven (Fistreem International Ltd, Loughborough, United Kingdom) at 40°C overnight and were stored in ambient conditions until use. The amount of extracted fat was determined gravimetrically and the yield was expressed on a wet weight basis.

3.2.6 Protein Extraction

Theory

The process of extraction is based on the ability of organic compounds to be separated from a mixture of compounds using an appropriate solvent in which the target compound, in this research the proteins, is selectively solubilised while the other components are insoluble. Aqueous, saline and alkaline solvents were used to extract protein. In this research four methods of protein extraction were utilised. The one

method based on the protein extraction after solubilising proteins in a solution while the other method demanded the initial solubilisation of the protein in a solution and then precipitation by adjusting the pH close to the isoelectric point of the proteins. The former method was used for salt extraction and sequential extraction while the latter method used for modified Osborne fractionation and alkali extraction and isoelectric precipitation. For alkaline solubilisation and isoelectric precipitation (AEIP), the proteins were solubilised in alkali solution (pH 13) and then the pH was adjusted to the isoelectric point of proteins in order to precipitate the proteins. Proteins are not soluble at their isoelectric point because at that pH the proteins have no net charge causing protein molecules to aggregate which leads to protein precipitation. Osborne fractionation (Osborne, 1908) is a sequential extraction of proteins using different solvents. Water, salt and alkaline solutions were used to separate different protein fractions and then the pH of each isolated fraction was adjusted to pI of the proteins.

3.2.6.1. Alkali solubilisation and Isoelectric Point Precipitation (AEIP)

Experimental procedure

The defatted material of mealworm beetles was used for extraction of soluble proteins in alkali solvent followed by the pH based precipitation (Zhao et al., 2016). Initially, the defatted materials were dispersed in 400 g of 0.25 M sodium hydroxide, and incubated (Covey Laboratory Engineering Co Ltd, Guildford, United Kingdom) for 1 h in 40 °Cfollowed by centrifugation (JA-10 Beckman, Beckman Coulter, California, USA) at 4 °C for 20 min at 3,500 g. The supernatant, which contains the alkali soluble proteins were collected and the pellet re-suspended in alkali solution, and a second protein extraction was carried out. Both of the supernatants were combined and the pH was adjusted to 4.3-4.5 (Mettler- Toledo Ltd, Leicester, UK) by adding 2 M HCl (Zhao et al., 2016). The dispersion was centrifuged (JA-10 Beckman, Beckman Coulter, California, USA) at 4 °C for 15 min at 2,500 g. Following that, the pellet was washed by adding a small amount of water and centrifuged (JA-10 Beckman,

Beckman Coulter, California, USA) at 4 ℃ for 10 min at 2,500 g twice. The pellet containing the proteins was re-suspended in 200 mL water and the pH (Mettler-Toledo Ltd, Leicester, UK) was adjusted to 7. This pH value was selected based on a previously published study (Azagoh et al., 2016) which shows that the alkali soluble proteins extracted from mealworm larvae have highest solubility at pH 7. The re-suspended protein slurry was agitated overnight in order re-solubilise the alkali soluble proteins followed by centrifugation (JA-10 Beckman, Beckman Coulter, California, USA) at 4 ℃ for 10 min at 2,500 g to attain the pH 7 soluble protein fraction. The supernatant was retained and stored at –80 ℃ (Denley, Massachusetts, United States) until freeze drying using a Modulyo Freeze Dryer (Edwards, Burgess Hill, UK). The freeze dried sample was called nAEIP.

For obtaining a purified AEIP fraction, 3g of nAEIP was solubilised in distilled water adjusted pH 7 (by using 0.5 M NaCl or 1 M HCl) and then using BiodesignInc dialysis tube with a molecular weight cut-off 6-8 kDa (Fisher Scientific, Loughborough, UK) for 48 h against 2 changes of deionised water per day at 4 ℃. The sample was then freeze dried using a Modulyo Freeze Dryer (Edwards, Burgess Hill, UK) and the dried samples was called pAEIP.

3.2.6.2 Protein extraction using NaCl in phosphate buffer (SE)

Experimental procedure

The SE protein extraction was conducted following the procedure of Liu et al (2008) with slight modifications. The defatted sample 20 g was dispersed in 200 mL of 0.5 M sodium chloride solution in 0.1 M phosphate buffer, adjusting the pH to 7 by using 0.5M NaOH or 1M HCl; by stirring at ambient temperature for 1 h. The solution was centrifuged (JA-10 Beckman, Beckman Coulter, California, USA) at 10,000 g for 20 min, the supernatant collectedand the pellet was re-suspended following the same conditions as the previous extraction. Supernatants from both extractions were

dialysed using BiodesignInc dialysis tube with a molecular weight cut-off 8 kDa (Fisher Scientific, Loughborough, UK) for 48 h against 2 changes of deionised water per day at 4 ℃ (Polysec Coldrooms Ltd, Worcester, United Kingdom). A small aliquot of deionised water was kept before the water change and the protein concentration measured with BCA Assay in order to determine if protein was lost during purification. The content of dialysis box was stored at -80 ℃ (Denley, Massachusetts, United States) and freeze dried using a Modulyo Freeze Dryer (Edwards, Burgess Hill, UK).

3.2.6.3 Sequential protein extraction (SEq)

Experimental procedure

A modified method was developed based on Osborne fractionation (Osborne, 1908) and method of de la Rosa et al., (1992) with some modifications.

Isolation of water soluble fractionation – albumins

Initially the defatted material (20g) was dispersed in distilled water in a ratio 1:10, and stirred for 1h at 4 ℃ (Polysec Coldrooms Ltd, Worcester, United Kingdom) followed by centrifugation (JA-10 Beckman, Beckman Coulter, California, USA) at 9,000 g for 20min at 4 ℃. The supernatant was kept in freezer (-80 ℃) (Denley, Massachusetts, United States) until freeze drying using a Modulyo Freeze Dryer (Edwards, Burgess Hill, UK).

Salt soluble fraction – globulins

After albumin extraction, the salt soluble protein fraction was extracted from the remaining residue by suspending in 200 mL of 0.5 M sodium chloride, and the pH was adjusted to pH 7, by adding 1 M HCl. After 1 hour stirring at 4 ℃ (Polysec Coldrooms Ltd, Worcester, United Kingdom) the solution was centrifuged (JA-10 Beckman, Beckman Coulter, California, USA) at 9,000 g for 20 min and the supernatant was collected and dialysed using BiodesignInc dialysis tube with a

molecular weight cut-off 8 kDa (Fisher Scientific, Loughborough, UK) against distilled water for 2 days at 4 ℃ (Polysec Coldrooms Ltd, Worcester, United Kingdom). The water was refreshed 2 times per day. After dialysis, the tube's contents were centrifuged (JA-10 Beckman, Beckman Coulter, California, USA) at 4 ℃ for 10 min at 9,000 g and the supernatant retained and stored at -80 ℃ (Denley, Massachusetts, United States) until freeze drying using a Modulyo Freeze Dryer (Edwards, Burgess Hill, UK) to attain the salt soluble proteins.

3.2.6.4 Osborne fractionation (OF)

Experimental procedure

The procedure of Osborne (1908) was used in order to be extracted albumins, globulins and glutenis and the step of prolamin extraction was skipped. 20g of defatted materials were dispersed in 100 mL water and agitated for 2 h at 4 ℃ (Polysec Coldrooms Ltd, Worcester, United Kingdom). Consequently, the solution was centrifuged (JA-10 Beckman, Beckman Coulter, California, USA) for 30 min at 4,800 g at 4 ℃. The supernatant, which contained the water soluble albumins, was kept and the pellet was re-suspended in 100 mL of 1M sodium chloride again agitated for 2 h at 4 ℃ (Polysec Coldrooms Ltd, Worcester, United Kingdom), centrifuged (JA-10 Beckman, Beckman Coulter, California, USA) for 30 min at 4,800 g at 4 ℃. The salt soluble globulins contained in the supernatant were collected and the pellet washed twice with distilled water and centrifuged (JA-10 Beckman, Beckman Coulter, California, USA) for 10 min at 4.800 g at 4℃ in order to remove as much sodium chloride residues as possible. Afterwards, the pellet was suspended in 0.1 M sodium hydroxide, stirred for 2 h at 4 ℃ (Polysec Coldrooms Ltd, Worcester, United Kingdom) and then centrifuged (JA-10 Beckman, Beckman Coulter, California, USA) for 30 min at 4,800 g at 4 ℃ and the alkali soluble glutelins were collected. The pH of the collected supernatants was adjusted to the isoelectric point of each protein fraction (Mettler- Toledo Ltd, Leicester, UK). Hence, the pH was adjusted to 3.6 for water

soluble albumins, to 4.8 for the salt soluble globulins and to 2.6 for alkali soluble glutelins. After pH adjustment, the samples were centrifuged for 15 min at 4.800 g at 4 °C (JA-10 Beckman, Beckman Coulter, California, USA). Consequently, the pellets were frozen overnight at -80 °C (Denley, Massachusetts, United States) and freeze dried the next day using a Modulyo Freeze Dryer (Edwards, Burgess Hill, UK).

3.2.6.5 Feasibility of protein extraction in industrial level

Protein extraction could be expensive with respect to the cost of the chemicals, energy, materials and manpower particularly in a fluctuating market. A less expensive method of protein utilization would involve a minimum of prior treatment of the insect powder. However, the proteins needed for studying their ability to form gels needs requires more complicated processes with some additional stages, which are not economically worth including (Stafford et al., 1979). Therefore, apart from the chemicals, which are used for the protein solubilisation and separation of the protein phase the cost increased by the additional stages like purification or freeze drying which are both costly processes.

Therefore, the sequential protein extraction and salt extraction methods, which include the purification are the most costly method among others. Dialysis tubes are expensive and increase the budget needed. Then, Osborne fractionation is more expensive than alkali extraction and isoelectric precipitation method as it requires more chemicals. The need to have protein concentrated requires a drying stage. For the purpose of our experiment freeze drying is the most expensive and time-consuming and the same time most appropriate method. The condition in the freeze dryer do not affect the protein structure comparing with other water-removal treatments (dehydrated or use of microwaves) that the temperature is high and it is capable of affecting the structure of the proteins.

3.2.7 Freeze Drying

Theory

Freeze drying or lyophilisation is a process in which the water passes directly from the solid state (ice) to the vapour state without passing through the liquid state. The sample is frozen and placed in a cabinet under low heat and a sublimation process occurs wherethe frozen water crystals (ice) evaporate. The basic advantage of this method is the avoidance of the evaporation process using heat that could induce protein denaturation. Denaturation would cause changes to the secondary structure of the protein such as the exposure of the hydrophobic amino acids of the protein affecting protein functionality.

Experimental procedure

Frozen samples from sections 2.3.6.1, 2.3.6.2, 2.3.6.3, and 2.3.6.4 containing the isolated protein fractions were placed in the Modulyo Freeze Dryer (Edwards, Burgess Hill, UK), covered with pierced aluminum foil for the avoiding dispersion and contamination of the equipment. The samples were freeze dried for between 10 to 12 days, until dry.

3.2.8 Protein Analysis

3.2.8.1 BCA Assay

Theory

The protein concentration was measured, using the BCA assay. The principle of this method is that proteins can reduce the Cu^{2+} to Cu^{1+} in an alkaline solution and result in a purple color formation by the chelation of bicinchoninic acid with Cu^{1+}. Bicinchoninic acid interacts with Cu^{2+} forming a purple coloured product that absorbs at 562 nm. The Cu^{2+} ions are reduced by the peptide bonds of proteins, which means, that the amount of protein is proportional to the amount of Cu^{2+} reduced (Smith et al., 1985).

Experimental procedure

The BCA assay was carried out using the Pierce BCA Protein Assay kit (Fisher Scientific, Loughborough, UK). Following Table 12, protein concentration standards solutions were prepared using bovine serum albumen (BSA).

Table 12. Examples of standard dilutions for preparation of a calibration curve.

Vial	**Volume of Diluent (µL)**	**Volume and source of BSA (µL)**	**Final BSA concentration (µg/mL)**
A	0	300 of stock	2000
B	125	375 of stock	1500
C	325	325 of stock	1000
D	175	175 of vial B dilution	500
E	325	325 of vial C dilution	750
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0=blank

The working reagent (WR) which contains reagent A and reagent B in a ratio of 50:1, was prepared. Samples for analysis were prepared by combining 100 µl of standards or samples with 2 mL of working reagent. The tubes were incubated in a water bath (Covey Laboratory Engineering Co Ltd, Guildford, United Kingdom) at 37°C for 30 min. The absorbance of the standards and samples was read at a wavelength of 562 nm (Agilent, Santa Clara, United States); the machine was zeroed with water before measurement and all the measurements undertaken within 10 min of incubation period ending. The average absorbance of blank sample was subtracted from all the standards and samples. A calibration curve was produced based on the standard samples absorbance against their protein concentration, an example of which is shown in Figure 4. The protein content of the samples was calculated from the equation produced by linear regression.

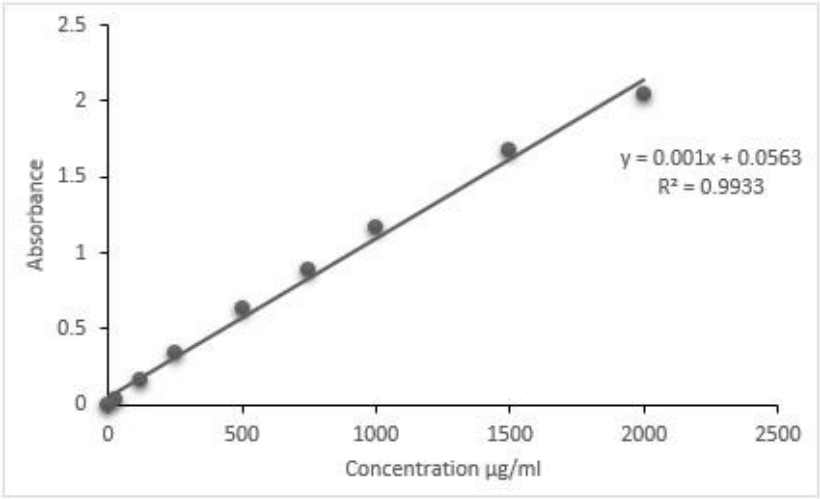


Figure 4. Example of a calibration curve produced by absorbance measurement of the standard samples.

3.2.8.2. Nitrogen analysis

Theory

Nitrogen content of a sample is calculated from the combustion of a sample of known mass at high temperature (more than 1000 °C) in the presence of oxygen, where the carbon is converted to carbon hydroxide; hydrogen to water; nitrogen to oxides of nitrogen and sulphur to sulphur dioxide. The carbon dioxide, water and nitrogen released are then passed through a column, that absorbs the carbon dioxide and water and transform the oxides of nitrogen to nitrogen gas. A column containing a thermal conductivity detector at the end is then used to separate the nitrogen from any residual carbon dioxide, water, and the remaining nitrogen content is measured. The measured signal from the thermal conductivity detector for the unknown sample is converted based on calibration samples into a nitrogen content. The protein concentration can be calculated by using the nitrogen-to-protein conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) which is commonly used for food materials. It is worth referring to a recent publication by Janssen et al., (2017) that reported a 6.25 conversion factor results in an overestimation of the protein content due to the presence of non-protein nitrogen in insects, while the most appropriate conversion

factor for *Alphitobius diaperinus*, and *Hermetica illucens*, based on their study was 5.60. For this study the conversion factor of 6.25 was used. The detected nitrogen content is multiplied with the conversion factor and the crude protein (CP) is determined. The CP is the total nitrogen which includes protein nitrogen and non-protein nitrogen.

Experimental procedure

For the experimental procedure a Nitrogen Analyser CHNS-O Analyser (CE Instruments Ltd, Wigan, UK) was used. The standard (D-Methionine), the defatted powder and the samples of extracted proteins were weighed directly in the tin capsule in the range of 2- 3 mg for the standard and 5 – 6 mg for the defatted and protein samples and then submitted to the equipment for analysis.

3.2.8.3 Calculation of the extraction yield

In order to calculate the protein extraction yield, the calculation of dry mass of each powder (ground insect powder, defatted materials, and freeze dried powder) was measured utilising methods described in section 3.2.4. The results from the calculation of water content in the ground powder using the official method of analysis (AOAC, 2000) and the results from the calculation of moisture content using the Thermogravimetric analysis (TGA) used for the calculation of the dry mass. Following, the equation 2, the extraction yield was calculated water.

Equation 2

$$\%\ extraction\ yield = \left(\frac{dry\ mass\ extract\ * \%\ crude\ protein\ (CP) in\ extract}{dry\ mass\ ground\ insect * \%\ crude\ protein\ (CP)\ in\ ground\ insect}\right) * 100$$

3.2.8.4. Sodium dodecyl sulphate- polyacrylamide electrophoresis (SDS-PAGE)

Theory

SDS-PAGE separates proteins in a gel matrix by molecular weight. The sodium dodecyl sulphate (SDS) is a detergent with a strong protein denaturing effect, binds to the protein backbone causing protein unfolding and coating the protein chain with negatively charged surfactant. In reducing conditions, the combination of SDS and a reducing agent, for example mercaptoethanol, which reduces the intramolecular and intermolecular disulphide, bonds causing complete denaturation of protein into linear chains, which may provide a more accurate molecular weight characterisation. In both conditions, the protein molecules will obtain a uniformly negative charge proportional to the polypeptide chain length. When an electric current is applied across the gel, the proteins are therefore separated by molecular weight. The lower a molecular weight a protein has the faster the protein will move through the netting-like gel matrix therefore being found at the bottom of the gel (Laemmli, 1970).

Experimental procedure

The extracted proteins were analysed using SDS-PAGE to determine the molecular weight (M_W) of the sample. In this research the mini protean tetra electrophoresis cell system (Biorad, United States) and 4-15% polyacrylamide gel was used. The preparation of 1ml Laemmli buffer was based on the developed protocol (He, 2011) for reducing (950 µl Laemmli buffer and 50 µl β- mercaptoethanol) and for non-reducing conditions (1000 µl Laemmli buffer). The protein samples were solubilised in the buffer at a 1:1 ratio (20 µl sample: 20 µl buffer) and were heated in a test tube heater (Stuart Scientific, Stone, UK) for 5 min at 90-100 °C. The samples were then placed in ice for 5 min and then centrifuged for 2 min at 10,000 g (Thermo Fisher Scientific, Langenselbold, Germany). Consequently, 15 µl of each sample was loaded in the wells of the gel and the proteins were subjected to 150 V for 30-40 min. Afterwards the gel was washed three times with water and then stained for 1 hour with Coomassie Brilliant Blue stain solution (50% methanol, 10% acetic acid, 39.5% H_2O, 0.5% Coomassie). The gel was then de-stained in an orbital incubator (Covey

Laboratory Engineering Co Ltd, Guildford, United Kingdom) for 10 hours, with 2-3 changes of destaining solution (5% methanol, 7.5% acetic acid, 87.5% H_2O) at ambient temperature in order to increase the intensity of bands and finally, the gel was imaged with the Gel Doc System (BioRad, California, US).

3.2.9. Thermal Analysis

3.2.9.1. Thermogravimetric Analysis (TGA)

Theory

Thermogravimetric analysis is used for the determination of the thermal stability and water content of a sample. The mass of a sample is monitored as a function of time or temperature when the sample is subjected to a controlled temperature programme in a controlled atmosphere. As the material is heated, the mass changes due to decomposition, reduction or evaporation.

Experimental procedure

Measurement of water content in the freeze dried samples was calculated using the Thermogravimetric analysis (TGA) carried out on a Mettler Toledo Star TGA/DSC1 unit (Mettler Toledo Corp., Zurich, Switzerland). Freeze dried protein samples (5-9 mg) were placed in oxide aluminium pans and heated from 0-500 °C at a heating rate 1 °C/sec. The degradation temperature and weight loss were recorded and analysed with the STAR°SW (DEMO version).

3.2.9.2. Differential Scanning Calorimetry (DSC)

Theory

DSC measures the temperature and the heat flows associated with transitions in materials as a function of time and temperature in a controlled atmosphere. More precisely, when a sample is heated or cooled or held at a constant temperature, the amount of energy (heat) absorbed or released is measured and the transitions are recorded and compared with those of a reference. For this research, the heating of

protein samples can cause unfolding of polypeptide chains which is an endothermic and cooperative process which is observable with DSC. DSC will provide information about the temperature of protein denaturation. Determining the denaturation temperature of the proteins, aids the gelation method development as protein unfolding and denaturation is a necessary step for the protein gelation.

Experimental procedure

Freeze dried samples were analysed using Mettler Toledo Star TGA/DSC1 unit (Mettler Toledo Corp., Zurich, Switzerland). The freeze dried samples (780-800 mg) and deionised water were placed in aluminium pan and hermetically sealed. The temperature protocol used was designed in steps, initially the temperature was set from 25 ℃ to 20 ℃ at a heating rate 50 K/min, stabilising at 20 ℃ for 2 min. The samplewas then heated to 140 ℃ with a heating rate of 10 K/min and cooled to 20 ℃ at a rate 20 K/min. Afterwards it was held at 20 ℃ for 2 min, heating again to 140 ℃with a heating rate of 10 K/min and cooled back to 20℃ with a cooling rate of 50 K/min. The analysis of the data was carried out with the use of STARe software demo version (Mettler Toledo, Leicester, UK).

3.2.10 Fourier Transform Infrared (FT-IR) Spectroscopy

Theory

FT-IR is a technique, which is used to study the molecular composition of the samples and the secondary structure of proteins. The chemical bonds and the bond angles of the molecules have the ability to be vibrated and stretched under the effect of radiation. The high energy of infrared radiation is able to cause vibration to all the molecules of a sample and the different bonds in the sample will absorb this energy and will elicit higher energy band with different frequency. Each compound has its own infrared spectrum in which has characteristic absorption bands. For example, polypeptides have nine characteristic absorption bands in the infrared spectra which are associated with the amide bonds that link the amino acids. The amide I and II are

the most vibrational bands of the backbone (Kong and Yu, 2007). The absorption which results in stretching vibrations of the C=O bond of the amide, coincides with the Amide I band, while the absorption resulted in stretching vibrations of the N-H bond is associated with the Amide II. However, both of these bonds are part of hydrogen bonding that occurs among the different elements of secondary structure, which means that the both amides are sensitive to the secondary structure content of a protein. The systematic correlation of the shape of Amide I and II to secondary structure of proteins with known structure have been reported (Casal et al., 1988).

Experimental procedure

A FT-IR spectrometer (Tensor 27, Bruker, Coventry, UK) with an attenuated total reflectance (ATR) accessory equipped with a single reflection diamond crystal in a horizontal configuration, was used to analyse ground insects powder, defatted insect powder and extracted insect protein powder. A small amount of powder was placed on the ATR surface and the experiment was carried out at ambient temperature. The different samples were scanned 124 times from 500 to 4000 $cm^{-1}$with a resolution of 4 cm^{-1}. Three replicate samples were collected and the recorded spectra were analysed using OPUS Spectroscopy Software (Bruker, Coventry, UK).

3.2.11 Gelation preparation and analysis

3.2.11.1 Gel preparation

Theory

Gels are formed under controlled conditions where a solution (liquid) is converted to a gel (solid). The formation of three-dimensional network between proteins and water by having protein-protein and protein-water interaction can be induced by different conditions. The gelation of proteins impacted by temperature, protein concentration, ionic strength, enzymes, pressure and pH as discussed in section 3.1.2.

Experimental procedure

The nAEIP protein solutions were prepared by solubilising the freeze dried material in deionised water at different concentrations 4, 6, 12, and 14%, the pSE protein solutions were prepared by solubilising 4,6, 12, 14 and 30% protein concentration. The dispersions were stirred for30 min at ambient temperature followed by pH adjustment to 7 using 0.1 M NaOH. The protein dispersions were then placed in glass vials with a final volume 1.5 mL. The vials were heated for 30 min and 90 min at 85 - 90 ℃ in closed glass vials. After which, the vials were cooled at room temperature (24 ±1 ℃) for 2 hours and then visual observations made in order to determine gelation. If the liquid was not moving upon turning tube, it was considered a gel (Figure 5).

In order to see if the presence of 100 mM $CaCl_2$could induce gelation at a lower protein concentration, or causes gelation in samples that did not gel after heating, using the method described by Barbut and Foedging (1993) with slight modifications, the preheated samples that did not form gel were combined with 100 mM of $CaCl_2$, stored overnight at room temperature and observed the 24 hours later to assess gel formation. pAEIP purified to only contain proteins > 8 kDa were dispersed in water at 15% protein concentration and stirred for 30 min.

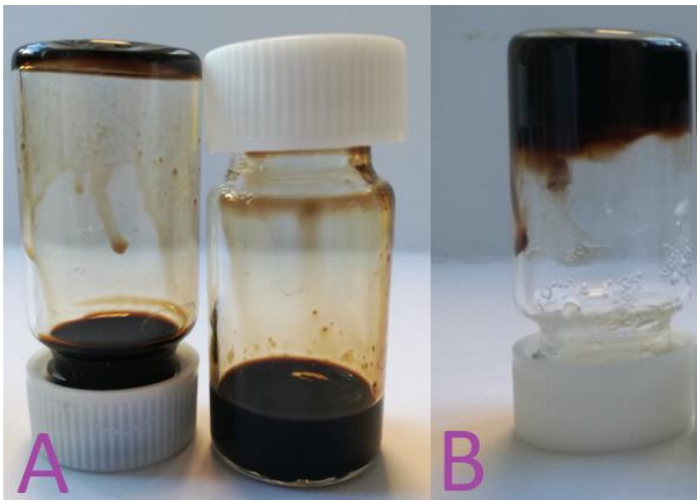


Figure 5. Visual observation of sample vials;(A) no gel formed (pSE), and (B)gel formed (nAEIP)

The pH was adjusted to 7 and the samples were heated for 30 min at 85 - 90 ℃ in closed glass vials. Three replicates were tested.

3.2.11.2 Microscopic Analysis of Gel

Theory

The fluorescence microscope uses light cubes of specific wavelength to illuminate the sample. The light excites fluorescent species in the sample, which then emit light of a longer wavelength. The image produced is based on the second light source or the emission wavelength of the fluorescent species rather than from the light originally used to illuminate and excite the sample. The microscopy observation of the samples provides an initial assessment of the gel structure formed. The use of fluorescent stain Rhodamine b selectively binds with proteins and will fluoresce upon illumination at a 560 nm wavelength.

Experimental procedure

The microscopy observations were carried out using an EVOS F1 microscope (AMG, Washington, DC, USA) and the samples were observed before and after staining with Rhodamine b (diluted 5 times). A drop from the sample was placed on the objective glass slide and if the sample was too thick, it was diluted with one drop of water and the coverslip was placed above the sample.

3.3 RESULTS AND DISCUSSION

Utilising the methods described in 3.2 proteins were extracted from mealworm beetles using 4 protocols, following extraction the protein content and molecular weight of the extracts were measured. Extraction using alkaline solution or aqueous solutions has been used for protein extraction from insects, including *T.molitor* larvae (Azagoh et al., 2016, Zhao et al., 2016) but not for *T. molitor* adult. Moreover, to date, Osborne fractionation or salt extraction has not been used for protein extraction of adult mealworm beetles. The results of the extraction of protein from mealworm beetle will be presented first, followed by exploratory gelation experiments utilising two of the extracts.

3.3.1 Mealworm beetles (T. molitor) protein extraction yield

Soluble proteins of mealworm beetles were extracted using four different extraction methods, including alkali solubilisation and isoelectric precipitation (AEIP), salt extraction (SE), sequential extraction (SEq) and Osborne fractionation (OF). The schematic representation of processing and fractionation of *T. molitor* adult, accompanied with the percentage of protein extracted and extracts' protein content (CP %) is given in Figure 6, as well as the composition presented in Table 14. The results showed that the extraction yield decreases through the steps of extraction. While the crude protein (CP) of the fractions ranged from 16 to 87 %, depending on the protocol.

Ground insect powder had 28.7 % fat content. Azagoh et al., (2016) reported a 33.1% fat content for mealworm larva whilst Yi et al., (2013) found 9.9% fat content. The protein content of ground insect powder (CP) was 74 % on dry matter, which is higher than the dry matter content reported for mealworm larvae ranging from 38.5 to 41.9 % (Ghaly and Alkoaik, 2009), 54 % (Bußler et al., 2016) and almost 50 % higher than what Yi et al., (2013) reported. Similarly, *S. gregaria* and *A. mellifera* had protein content 38.1% and 22.1 % , respectively (Mishyna et al., 2019a).

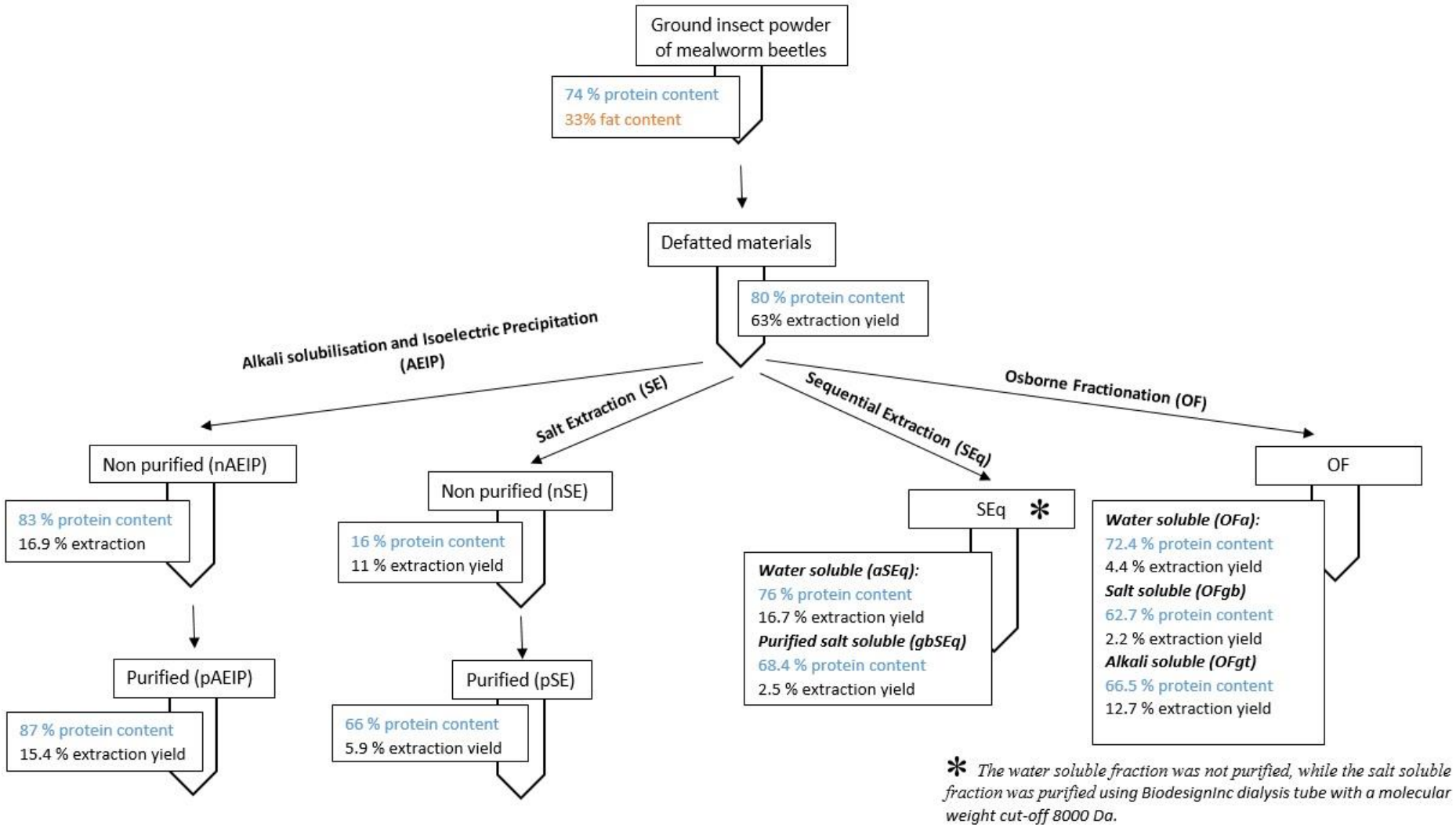


Figure 6. Schematic representation of the four extraction methods (AEIP, SE, SEq and OF) of mealworm beetles accompanied with the percentage of extraction yield and extracts' protein content (%).

While, other insects such as honey-ants (94.5%), and black bees (81.7%) (Aguilar-Miranda et al., 2002) were also found to have high protein content as well. Therefore, this study revealed differences between the mealworm larva and beetles crude protein content which occurs due to different developmental stage.

As described by van Huis (2003) the nutritional value and the protein content of insects could be different among different species, subspecies or even among different developmental stages of the same species. The most studied metamorphic stage of *T. molitor* is the larvae stage (mealworm larva); the study of adult stage (mealworm beetles) is limited. Therefore, the results of this study revealed that the mealworm beetles have higher protein content than the mealworm larvae. Similarly, it has been reported that the larvae and pupa of *A. mellifera* have lower protein contents than the adult, containing 35.3, 45.9 and 51.0 % protein (Ghosh et al., 2016). Ghosh et al., (2016) also reported that *A. mellifera* larva, pupa and adult have different fat contents, reducing with age, causing an increase in overall protein content.

In addition, differences may occur due to conversion factor used in nitrogen analysis for protein content measurement. Janssen *et al.* (2017) reported that the conversion factor 6.25 used for measurement of nitrogen content of the sample could overestimate the content due to presence of non-protein nitrogen (NPN), for example from chitin which is present in mealworms. In literature, there are reports that agree with Janssen et al., (2017) such as Mishyna et al., (2019) reported that used as conversion factor 4.5 for adult grasshopper (*A. domesticus)*; 4.9 and 5.6 for pupae and larvae of honeybee (*A. mellifera)* respectively, demonstrating the conversion factor can also change with age. Caligiani *et al* (2018) reported that for obtaining an accurate proximate composition of *H. illucens* a nitrogen conversion factor of 5.71 specific should be used which is lower than the most standard conversion factor used for proteins. The nitrogen conversion factor for mealworm beetle has not been published,

to the best of the author's knowledge, and as many publications still use 6.25, it was decided to use 6.25 as the conversion factor in this study.

Subsequently, the crude protein content increased by 6% from 74 to 80% after defatting of the sample, being comparable with the reports of Bußler et al., (2016), where the protein content of mealworm larva (*T. molitor)* and black solder fly (*H. illucens)* increased after defatting from 60 to 68% and from 36 to 47%, respectively.

The crude protein content of extracts of the different extraction methods varied depending on the level of processing. For AEIP, the extract had a protein content of 83%, which increased after dialysis to 87% (pAEIP), as expected. For SE, the extract had a much lower protein content compared to AEIP initially (16%), indicating the extraction protocol was not selective for protein. The protein content of SE increased after dialysis to 66% (pSE), which although higher did not compare to AEIP. The lower protein content of pSE is due to the presence of residues of the body in the powder as it was confirmed by microscopy observations (section 3.3.3.1, Figure 14B). The increase of the CP of pAEIP and pSE, however showed that the purification protocols removed non protein molecules such salt.

Extracts from OF and SEq were of a substantial protein content between 60-70%. It is important to note that SEq (CP) was calculated only after purification and for OF the salt soluble protein precipitated so the salt remained solubilised in the solution and therefore in the precipitated and freeze-dried fraction the salt presence was low.

The total protein extraction yields (g/100g) of mealworm beetles using the four extraction methods; Alkali solubilisation and isoelectric precipitation (AEIP), protein extraction using NaCl in phosphate buffer (SE), sequential extraction (SEq) and Osborne fractionation (OF) and a breakdown of the quantity of each fraction (if appropriate) are presented in Table 13. The Osborne fractionation (OF) and sequential extraction (SE) had the highest total extraction yields (19.3 %).

It has been reported that the protein extraction method affects the protein yields, the protein content and the type of proteins that will be extracted. The pH, temperature and extraction time have been reported to affect the extraction yield as well (Wang et al., 2011).

Table 13. Total protein extraction yields (%) of mealworm beetles, by four extraction methods: Alkali solubilisation and isoelectric precipitation, protein extraction using NaCl in phosphate buffer, sequential extraction and Osborne fractionation and the yield of the extracted fraction[a].

Extraction Method	**Yield of total extracted protein (g/100g)**	**Yield of extracted fraction (g/100g)**		
		albumins	**globulins**	**glutelins**
Alkali solubilisation and Isoelectric precipitation				
nAEIP	16.9 ± 2.3	-	-	16.9 ± 2.3
pAEIP	15.4 ± 0.6	-	-	15.4 ± 0.6
Salt extraction				
nSE	11 ± 0.5	-	11 ± 0.5	-
pSE	5.9 ± 2.2	-	5.9 ± 2.2	-
Sequential Extraction	19.3 ± 0.3	16.6 ± 0.7	2.5 ± 0.9	-
Osborne Fractionation	19.3 ± 0.3	4.4 ±0.1	2.2 ±0.1	12.7 ± 0.1

[a] the values are means of three or more replicates for some samples (AEIP & SE) ± standard deviation

Zhao et al., (2016) in an effort to optimise the protein extraction conditions for mealworm larvae studied the effect of NaOH concentration, pH, temperature and time on extraction yield and reported that there were significant differences. In general, each extraction medium solubilises a specific type of protein. In Osborne fractionation, and sequential extraction, the proteins are solubilised in two and/or three different solutions, respectively, which means that different type of protein extracted in each fraction vary, giving a greater overall protein content. Whereas proteins extracted using salt or alkali, and for isoelectric precipitation, are solubilised in one

solution, which will restrict the type of proteins extracted therefore potentially reducing the extraction yield.

During the protein extraction, all the different methods ended up to decreased extraction yield as shown in Table 13 and Figure 6. The nAEIP protein extraction yield was decreased from 63 % to 16.9 %. The decrease could be due to the presence of a high amount of different type of proteins in the defatted material, which are not soluble in the alkali solution. Another reason could be that after the resolubilisation of the sample at a pH 7 changed the amount of protein which remained soluble at that pH which was confirmed by the SDS-PAGE electrophoresis (section 3.3.2.1, figure 7). Moreover, the extraction yield is calculated using the Equation 2 (section 3.2.8.3) where the ground insect contain chitin which is removed throughout the extraction as it is confirmed by the FT-IR results (section 3.3.2.3, Figure 9). The chitin contains non-protein nitrogen, which is detected by the Nitrogen Analyser and it leads in the overestimation of the protein content. Finally, purification negatively affected the protein extraction yield. In this study, the dialysis tube used was impermeable to molecules $\geq$ 8 kDa, and therefore proteins of a molecular weight $\leq$ 8 kDa would be lost during dialysis, explaining the decrease in protein extraction yield. The calculation of protein loss was conducted by measurement of the protein concentration of the distilled water in which the dialysis bag had been placed with BCA assay. The results revealed that was a loss of around 0.7 g of proteins with molecular weight lower than 8 kDa. Similarly, these could be the reasons why the extraction yield decreased during all the processes.

In addition to protein content and yield information, the different extraction protocols allow the categorisation of the types of protein extracted. It can be assumed that the alkali soluble fraction of AEIP and OF were glutelins, the water soluble fraction of OF, and SEq were albumins and the salt soluble fraction of SE, OF and SEq were globulins. Based on these assumptions, the alkaline soluble glutelins are the major

protein fraction of the mealworm beetles, as evidenced by AEIP and OF results. Similarly, Aguilar-Miranda et al., (2002) reported that the main protein fraction of mealworm larva corresponded to glutelins. Other insects such as *H. Illucens* also have been shown to have a dominant protein fraction made up of glutelin (37%, alkaline soluble), albumins (27%, aqueous soluble) and globulins (16%, salt soluble) (Caligiani et al., 2018). However, the application of those methods in peas, faba bean, chickpeas and fenugreek revealed opposite results. The extraction yield of the SE method was higher than AEIP (Stone et al., 2015, Abdel-Aal et al., 1986).

3.3.2 Characterisation of the protein extracts

3.3.2.1 Molecular weight distribution of proteins

Soluble proteins from mealworm beetles were separated by SDS-PAGE and were classified into different groups according to their molecular weight. Figure 7 presents the molecular distribution of mealworm beetle protein extracts from the four different extraction methods.

The water soluble fraction of sequential extraction protocol (aSEq) contained only one band at 18 kD a while the water soluble fraction of Osborne fractionation (OFa) contained five different groups with Mw~120 kDa, 55-40 kDa, 35-25 kDa, 18 kDa and ≤ 14 kDa. The salt soluble fraction of sequential method (gbSEq) and the purified salt extraction (pSE) gave the same Mw distribution containing three bands, one faded at 40 kDa, one intense at 18 kDa and one intense ≤ 14 kDa, while the salt soluble fraction of Osborne fractionation (OFgb) contained six bands with Mw ranging from 100 kDa to 18 kDa. There are slight differences in the two methods, for example, aSEq is hydrated in water for 1 hr whereas OFa is hydrated for 2 hrs, which may have caused the differing results as the hydration of proteins increases the solubility of the proteins in the water (Bagchi, 2013).

Protein present in the nAEIP extract contained in three bands between 55 kDa and 18 kDa. While the alkali soluble fraction of Osborne fractionation (OFgt) had three additional bands at 130 kDa, 110 kDa and at 70 kDa.

The distribution of molecular weights (Mw) of all fractions from the Osborne method were more distinctive than any other extraction method. As presented in Figure 7 the bands in nAEIP (4 bands), aSEq (1 band), gbSEq (3 bands) and pSE (3 bands) are less than OFa, OFgb and OFgt. The presence of proteins with a molecular weight higher ≥ 55 kDa have reduced which could be due the pH adjustment at 7 during the processing.

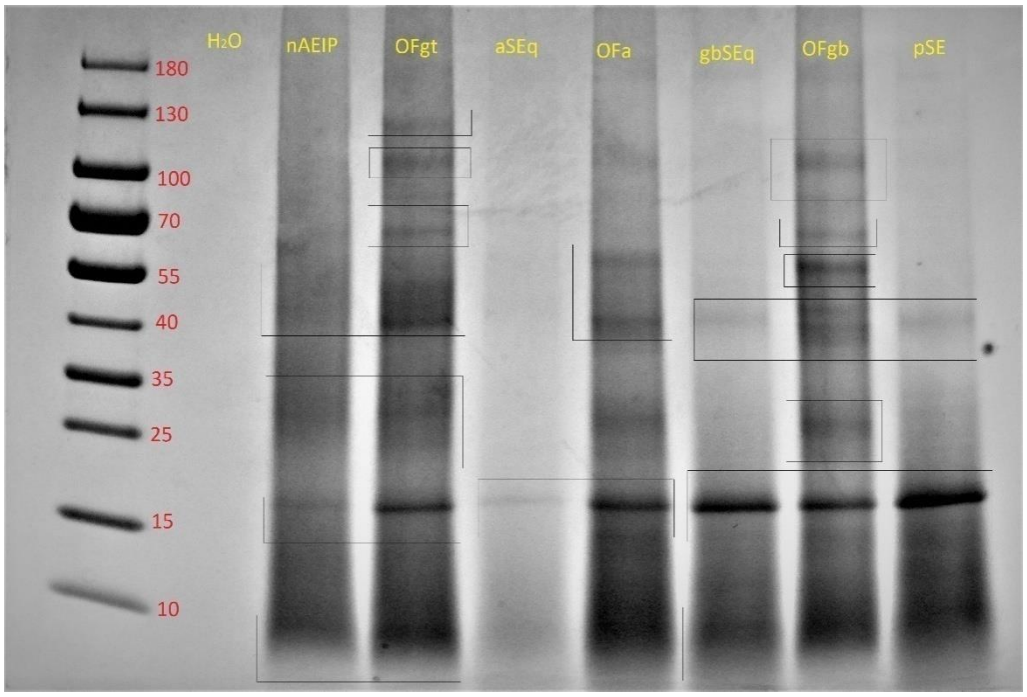


Figure 7. Molecular weight distribution of protein extracted from mealworm beetles:non-purified nAEIP, Alkali-soluble glutelins by Osborne fractionation (OFgt), water-soluble albumins by Sequential Extraction (aSEq), water-soluble albumins by Osborne Fractionation (OFa).

The protein fractions extracted with OF (OFa, OFgb and OFgt) after precipitation were not resolubilised in pH 7, instead they were pH adjusted to their pI (3.6, 2.6 and 4.8) for maximum extraction. Bußler et al.,et al., (2016) reported that the pH affected the molecular distribution, by changing the proportion of high molecular weight

proteins (67 to 250 kDa) and low molecular weight proteins (≤ 67 kDa). It was reported that protein fractions extracted at lower pH (pH 2 and 3), had proteins with higher Mw (67-250 kDa) while at higher pH values from 5 to 8 the proteins with molecular weight from 48 to 65 kDa and 13 to 18 kDa are increased. In this study the results were similar, as the OF fraction with pHs ranging from 2.6 to 4.8 had bands with Mw > 55 kDa in contrast with the fractions that the pH had been adjusted to pH 7.

Yi et al., (2016) observed bands from 55 to 25 kDa corresponding to trypsin (26.5), arginine kinase (40.1 kDa), tubulin a – 1 chain (50.6 kDa), α-amylase (51.7 kDa) and ovalbumin-like (43.2 kDa), which could be seen in several extracts Lee et al., (2000) also reported that bands >95 kDa, also found in OFgt , gbSEq and OFgb of this study, could possibly correspond to a vitellogenin-like protein with a molecular weight of 160 kDa and α-actinin-4 (107 kDa) (Yi et al., 2016). Moreover, the insect haemolymph includes storage proteins like alpha-amylase (~50 kDa), lipid transport protein such as vitellogenin and anti-freeze proteins (< 14 kDa) (Graham et al., 2000, Graham et al., 1997) and chymotrypsin-like proteinase (24kDa) (Elpidina et al., 2005, Tsybina et al., 2005). Bußler *et al.,* (2016) described the bands ranging from 15 to 40 kDa to originate from *T.molitor* cuticle proteins with molecular weight ranging from 14 to 32 kDa (Andersen *et al.,*1995). The group of proteins with molecular weight ranging from 95 to 40 kDa could also possibly originate from enzymes and other proteins, such as the muscle protein actin- 7E (42 kDa), melanization-inhibitng protein (43kDa), β-glycosidase (59 kDa), trypsin-like proteinases (59 kDa) and melanisation-engaging types of protein (85 kDa) or muscle proteins such as troponin T (47.3 kDa) tropomyosin 1 (75.2 kDa) (Cho *et al.,* 1999; Ferreira *et al.* ,2001; Zhao et al., 2005; Prabhakar et al., 2007; (Yi et al., 2016, Yi et al., 2013, Leni et al., 2019).

Muscle proteins like tropomyosin 2 (32.5 kDa), troponin I (23.8 kDa), troponin C (18.3 kDa) and actin (32 kDa) could possible correspond to the bands of 35-25 kDa

(Leni et al., 2019). The 18 kDa band observed in all the extracts could coincide with muscle proteins such as the putative troponin C (18.3 kDa) or calponin (20.3 kDa) (Yi et al., 2016). The majority of muscle proteins are salt soluble which explains the more intense bands of pSE and gbSEq at 18 kDa whereas those with a molecular weight ≤ 14 kDa possibly originate from anti-freeze type of protein ranging from 8.5 to 13 kDa (Yi et al., 2013, Graham et al., 1997, Rothemund et al., 1997).

Therefore, the SDS PAGE revealed that the fractions of Osborne fractionation (OF) had more bands, which could be a result of the pH of solvent used during extraction. Moreover, the mealworm beetle contains different types of proteins, whose presence and/or quantity depending on the extraction method. The bands 55-40 kDa, 35-25 kDa, 18 kDa and ≤ 14 kDa are present in all methods apart from aSEq, which contained only one band at 18 kDa. The presence of proteins with a low molecular weight may be attributed to the hydrolysis of proteins, which has previously been shown to occur when exposed to alkaline solutions (Caligiani et al., 2018, Wang et al., 2011).

3.3.2.2 Differential Scanning Calorimetry (DSC)

DSC thermograms of extracted proteins produced by the different extraction processes are shown in Figure 8. There were two small endothermic peaks. Seen upon heating, which attributed to the denaturation of the proteins (Wright and Boulter, 1980). The endothermic peaks of nAEIP were observed, the first (C) at 50.65 °C (ΔH = 0.72 J/g) and the second (D) at 80.62 °C (ΔH = 0.09 J/g). The endothermic peaks of the pSE proteins were observed, the first (E) at 52.3 °C (ΔH = 0.81 J/g) and the second (F) at 80.68 °C (ΔH = 0.075 J/g).

The globulins extracted through the SEq method showed two endothermic peaks as well, the first (G) at 56.18 °C (ΔH = 1.13 J/g) and the second (H) at 80.49 °C (ΔH = 0.075 J/g).

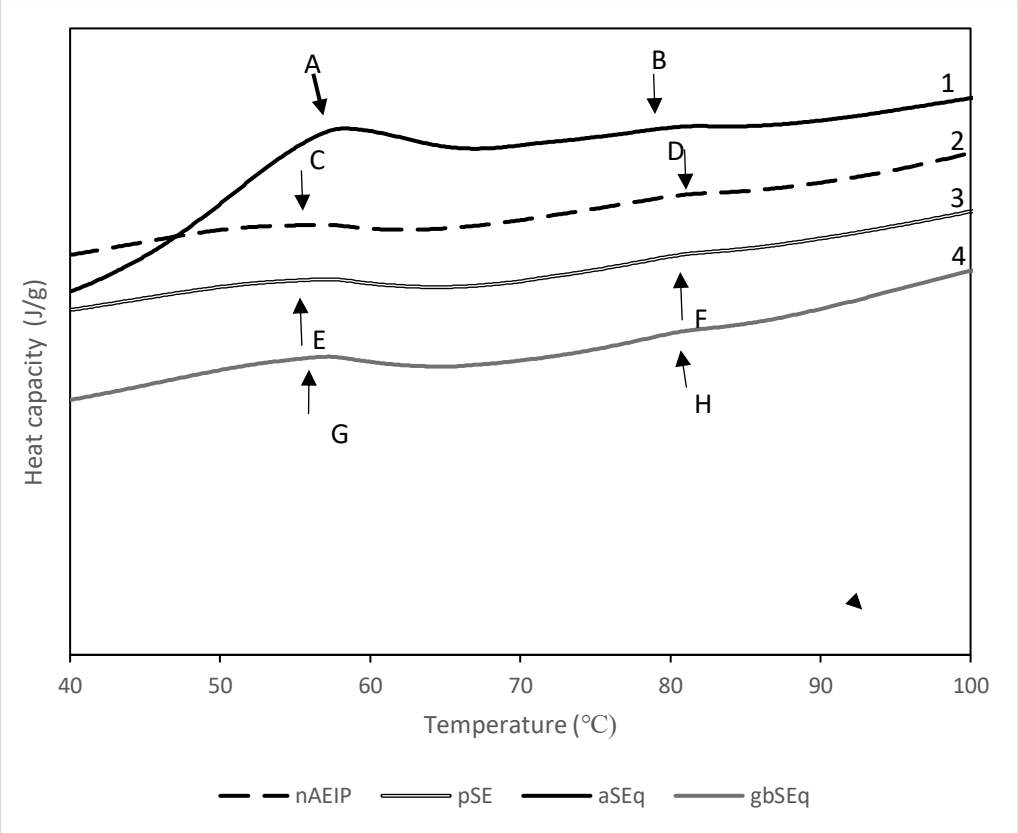


Figure 8. DSC Analysis of the aSEq (1), nAEIP (2), pSE (3), and gbSEq (4). The arrows A and B, C and D, E and F, and G and H show the first and second upon heating.

However, a key difference seen in the aSEq was the presence of a glass transition, which is an important property of amorphous and semi-crystalline materials, e.g. polymers, pharmaceutical and food ingredients. The glass transition occurs in a temperature range where the polymers change form a hard and rigid glassy state to a more flexible rabbery state. The aSEq presented two peaks one attributed to a glass transition starting at 43 °C until 51 °C (ΔCp = 0.72 J/g) and the second peak was at 108 °C (H = 3.22 J/g). The results showed that the aSEq solution was contaminated with other polymers such as chitin which transition from the glassy state and this sample also showed an increase in denaturation temperature increased.

The small protein transitions of all samples (all less than 1.2 J/g) show the low order of structure of the proteins in these samples, which was likely to be caused during the processing. During the extraction of lipids, the samples were heated for 60 min at 40 °C, which may have caused the partial unfolding of the proteins. The thermogram

results for all the extracts (Figure 8) indicated that protein denaturation temperature was between 50-80 °C. Moreover, the proteins extracted with AEIP method have been treated with a high alkali solution (pH 13) which has also been reported to affect the protein structure.

3.3.2.3 Fourier Transform Infrared Spectroscopy (FT-IR)

Infrared spectroscopy was used to determine the presence of lipid and chitin and any differences in protein structure of the extracts. Figure 9 presents the absorption peaks of samples from ground and freeze dried insects, defatted insect material and commercially available shrimp chitin at a spectrum ranging from 4000 cm^{-1} to 600 cm^{-1}.

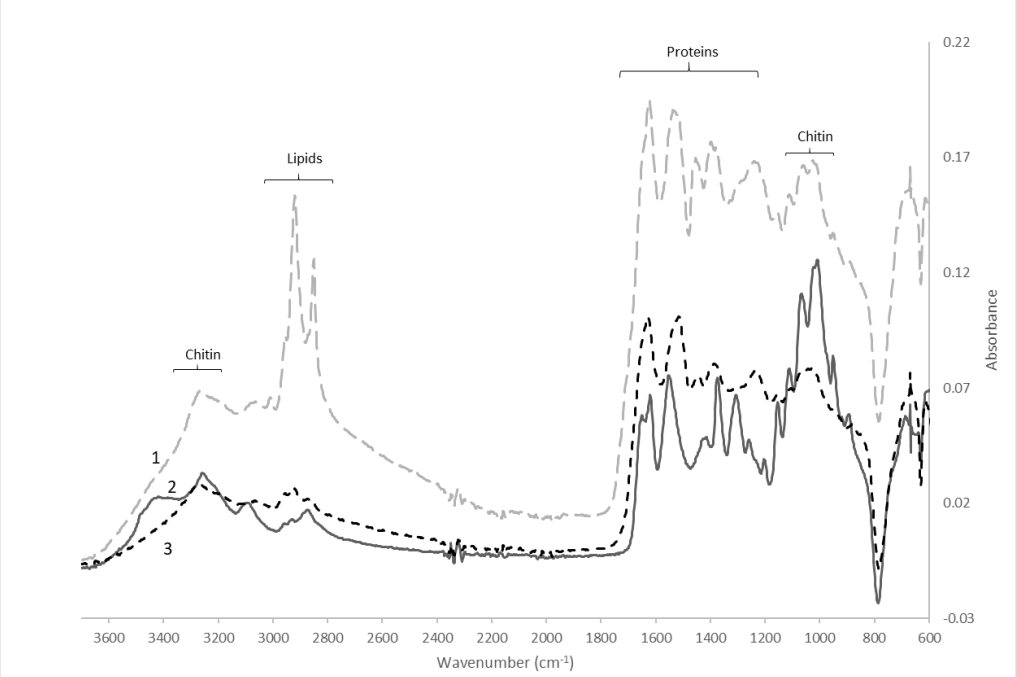


Figure 9. FT-IR spectra of insect powder (1), chitin of shrimps (2), and defatted materials (3).

The ground insect powder sample had absorption peaks at 3300 and 1100 cm^{-1}which are due to the chitin-residue of the insect exoskeleton. Furthermore, the ground insect powder had two peaks at 3000 and 2800 cm^{-1}; an earlier change in the line at 1780 $cm^{-1}$where compared with the other two samples (shrimp chitin and defatted sample); and

a peak at 1240 cm^{-1}which corresponded to the characteristic phospholipid infrared bands. It has been reported that the phospholipid infrared bands in the spectrum, are dividing into three regions which corresponded to the hydrophobic region of C-H; the interfacial region (C=O); and to the polar region ($P-O_2$)(Arrondo and Goñi, 1998).

Proteins give rise to IR absorption bands, namely amide, with amide I, II and III to be the most prominent vibrational bands of the protein backbone (Kong and Yu, 2007). Therefore, the three peaks present in the ground insect powder at 1600 cm^{-1},1550 $cm^{-1}$and at 14000 cm^{-1}, coincide with Amide I, Amide II and Amide III, respectively.

The defatted insect material showed the peaks in lipids spectrum decreased substantially as expected as the ethanol would have removed lipid and at the same time protein absorbance decreased, indicating that some of the protein could have been extracted into the ethanol. There were peaks are still present 1650 cm^{-1}, 1550 cm^{-1} and1350 to 1200 cm^{-1} which coincide with Amide I, Amide II and Amide III, respectively. While the decrease in the absorbance of the proteins could also occur due to the changes of the protein structure after the fat extraction. The heating of the samples at 40 °C during fat extraction and after fat extraction for drying the sample caused changes to protein structure that decreased the absorbance.

Finally, the commercial chitin derived from shrimps had two peaks as well, at 3250 cm^{-1} and 1100 cm^{-1}, which are characteristic of a chitin, previously found by (Chen et al., 2013). The two peaks at 1650 cm^{-1} and 1550 cm^{-1}, which correspond to amide I and amide II, respectively could correspond to the proteins, which are bound with chitin.

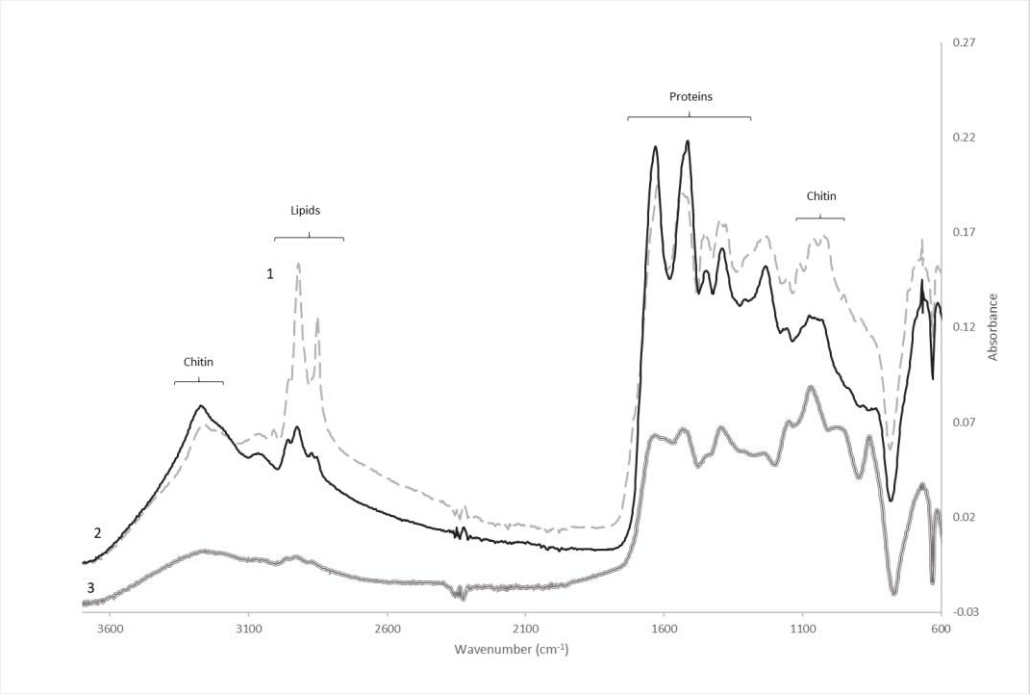


Figure 10. FT-IR spectra of ground powder of mealworm beetles (1), nAEIP (2) and pSE(3).

FT-IR used to determine if there were changes to the absorption spectra, which could provide an insight into the efficiency of extraction of the nAEIP and pSE extracts compared to the ground powder. The results presented in Figure 10 revealed that the chitin absorption reduced in the nAEIP sample. For the protein absorption spectrum there were two peaks denoting Amide I and II, which had almost the same absorption as the ground insect powder. In the ground insects the absorption at 1550 $cm^{-1}$was lower than the absorption at 1650 cm^{-1} while the nAEIP had almost same absorption at these to wavenumbers, indicated that the protein structure of the proteins has been affected by the extraction. The pSE samples presented a peak in an α-chitin absorption spectrum (1200 cm^{-1}), while the absorption by the protein molecules is lower than nAEIP and insect powder, giving two small peaks in the carbonyl spectrum at 1650cm^{-1} and 1525 cm^{-1} reflecting the amide I and II. The presence of other non protein materials is confirmed by the low crude protein (Figure 6 and Figure 14B) as well, the nAEIP sample had an 83% protein content on dry mass while the pSE have 66%.

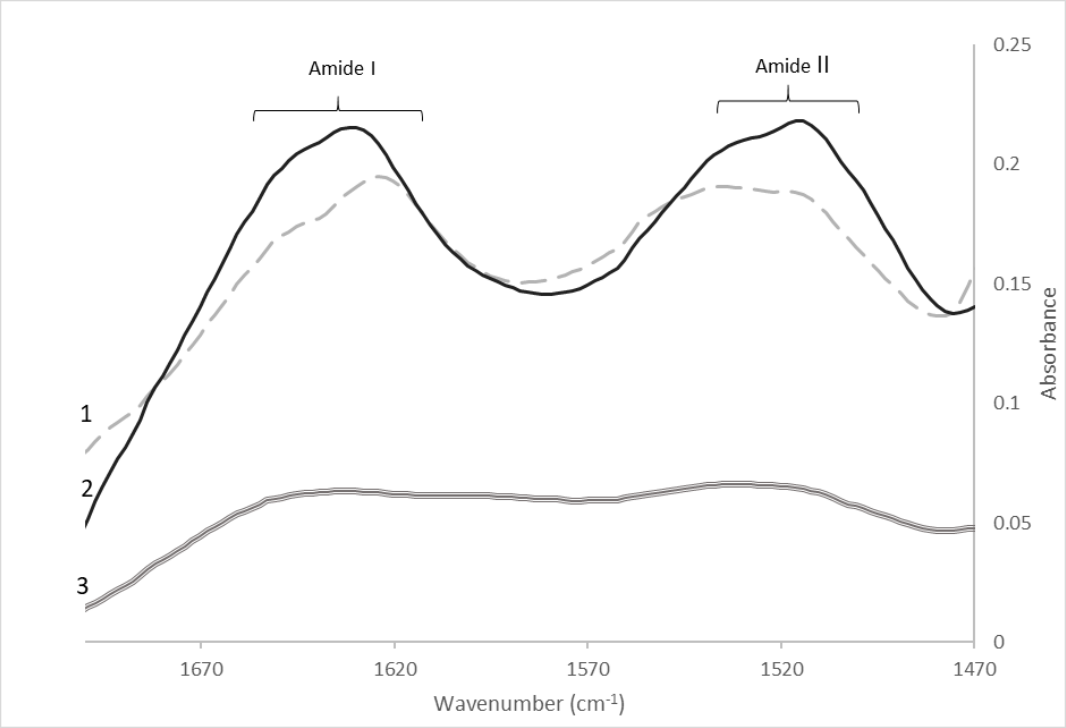


Figure 11. A zoomed in FT-IR spectra of mealworm beetles ground powder (1), nAEIP (2) and pSE (3) between 1700 cm^{-1} and 1470 cm^{-1}.

The Amide I and II regions of typical infrared absorption wavelength 1700 cm^{-1} to 1470 cm^{-1} was inspected further as shown in Figure 11.The absorption associated with Amide I leads to stretching vibrations of C=O while the Amide II band leads to bending vibrations of the N-H bond. Both of these bonds are involving in the hydrogen bonding which contribute to the secondary structure of the protein. Therefore, changes at the secondary structure of the proteins which could occur after heating, will lead to changes of the absorption spectrum. The nAEIP sample shows the Amide I and II bands centred at 1650 cm^{-1} and 1525 cm^{-1}, respectively, had almost equal absorption. After protein denaturation, changes occur in the protein secondary structure as the hydrogen bonds break. In comparison the absorption of pSE of the Amide I and II of pSE is four times less than that of nAEIP. Moreover, the pSE presents a broadening causing differentiation of the two different peaks of Amide I and Amide II. The disappearance of the bands at that spectrum are attributed to the bonding processed, which means that the protein structure has been affected. In

general, the FT-IR spectra changes observed in the intensity of the ~1650 cm^{-1} (Amide I) and ~ 1525 cm^{-1}(Amide II) spectra are attributed to changes in an α-helical, antipararell β-sheet and β-sheet structures of the protein. For example, β–lactoglobulin absorption spectra changes depending on the physicochemical factors, namely but a few, pH, heating and ionic strength (Boye et al., 1995). However, the protein extracts used for analysis are a mixture of isolated insect proteins and therefore not a single purified type of protein.

Therefore, we concluded that the FT-IR contributed to confirm the effectiveness of the defatting. Moreover, the AEIP method excluded chitin more effectively than the pSE extract, but the protein structure was affected by the process.

3.3.3 Formation of a protein gel

The SE and AEIP extracts were selected for studying of the gelation abilities as these two methods are used often as the protein extraction method used when studying the gelation properties of legumes and meat protein (Stone et al., 2015, Soetrisno and Holmes, 1992, Rosa et al., 1992, McCord et al., 1998). Protein concentration ranged from 4 to 14% (w/v) for nAEIP and from 4 to 30% for pSE after heating for 30 and 90 min in a water bath at 85- 90 ℃, cooled for 2 h and inspected for gel formation and then100 mM $CaCl_2$ were added in the vials that had not formed a gel.

There was no difference in gel formation between the different heating times for both extracted fractions, as DSC indicated denaturation was between 75 ℃ and 85 ℃ and the heating temperature used was 85-90 ℃, which indicates that the protein because unfolded during the extraction process. Yi et al., (2013) reported similarly that heating time did not affect the gelation. However, the results revealed that the extraction methods, the protein concentration, heating and the ionic strength affected the gel properties.

Table 14. G el formation of protein extracted with AEIP and SE methods after heating. (X: no gelation, A: aggregates and G: Gelation)

	Protein Concentration (w/v)				
	4%	**8%**	**12%**	**14%**	**30%**
30min					
nAEIP	X	X	G*	G	-
pSE	X	X	X	X	X
90min					
nAEIP	X	X	G	G	-
pSE	X	X	X	X	X

*at that concentration sometimes the gel was more ductile.

Table 14 shows the impact of protein concentration on gelation. Proteins extracted after AEIP (nAEIP) formed a gel at a protein concentration above 12% (w/v) (DM) and pH 7 after both heating durations. However, the gel at 12% concentration and 30 min heating sometimes was more ductile. Similarly, the chickpea and pea extracted with alkali solubilisation and isoelectric precipitated proteins demonstrated a heating duration-dependency (Kiosseoglou and Paraskevopoulou, 2011). Water soluble fraction of *T. molitor, Z. morio, A. diaperinus, A. domesticus* and *B. dubia* were found to form a gel only in much higher concentrations than that of nAEIP, a 30% (w/v) protein concentration at pH 7 and 10 was needed,and only house crickets (*A. domesticus)* formed a gel at 3 % (w/v) concentration at pH 7 (Yi et al., 2013). Similarly, protein concentrate derived from migratory locust (*Locust migratoria)* formed a brittle gel at a concentration of 20% at pH 7.9 (Purschke et al., 2018), honey bee brood (*A. mellifera)*(Mishyna et al., 2019b), *cirina forda* (Omotoso, 2006), silkworm *(Bombyx mori)* (Omotoso, 2015) whey protein concentrates and egg white (Tang, 1993), which were found to form a gel at the minimum concentration of 5, 6, 6, 6 and 1.8% respectively.

Table 15. Gel formation of protein extracted with AEIP and SE methods after pre-heating and salt addition. (X: no gelation, V: viscous fluid G: Gelation)

	Protein Concentration (w/v)				
	4%	**8%**	**12%**	**14%**	**30%**
30min + 100mM Ca_2Cl					
nAEIP	X	G	-	-	-
pSE	X	X	A	A	A
90min + 100mM Ca_2Cl					
nAEIP	X	G	-	-	-
pSE	X	X	A	A	A

The effect of $CaCl_2$ addition was also studied, the results of which are shown in Table 15. Into the preheated samples that did not form a gel (Table 14), 100mM $CaCl_2$ was directly added to the solution. The vials were left overnight and the next day were visually assessed for gelation. The calcium chloride induced gelation occurred by forming a network by effective charge screening, favouring the protein-protein interactions. For nAEIP the critical concentration changed and the solutions at 8% (w/v) concentration formed gel at pH 7 under both heating durations. Azagoh et al., (2016) showed that the presence of NaCl in the solution , changed the protein charge and the solubility of the protein. The AEIP proteins had a lower solubility in a solution which contained NaCl compared with that in aqueous solution. Although the solubility is lower in the presence of salt, the addition of $CaCl_2$ affected the electrostatic charges of the proteins and resulted in gelation at lower protein concentration, as seen for β-lactoglobulin (Boye et al., 1995, Renard and Lefebvre, 1992).

However, pSE proteins did not form gels under heat treatment no matter the protein concentration, the heating duration or the addiction of $CaCl_2$, although at both heating durations above 14% (w/v) concentration the formation of large aggregates was evident, as shown in Figure 12.

A possible explanation for this is that the $CaCl_2$ in combination with heating induced aggregation but the high amount of other materials (CP on dry mass was 66% Figure 6) prevented the creation of a homogeneous gel. Therefore, the non-protein materials of pSE fraction need to be removed in order to test the gelation ability.



Figure 12. Aggregated particles, forming an inhomogeneous solution of 30% (w/v) pSE after 30 min heating and salt addition

The high solubility and high hydrophobicity can positively contribute to gelation. Stone et al., (2015), demonstrated that pea proteins extracted with AEIP had higher hydrophobicity at pH 7 than the proteins extracted with SE method but the solubility at pH 7 was reversed. Other studies support that the solubility of AEIP extracted proteins was higher than that of SE (Karaca et al., 2011, Sumner et al., 1981). The lower solubility of pSE in water at pH 7 could lead to precipitation of proteins and lower hydrophobicity, which both prevent the gelation process, and could therefore explain why the pSE did not gel.

To test this hypothesis, that the proteins with Mw ≤ 8 kDa contribute to gelation, pAEIP samples at a 15% protein concentration were heated for 30 min at 85-90°C. Figure 6 presents the decrease in extraction yield of pAEIP (from 16.9 to 15.4) which means that the 1.5% of extracted proteins had Mw ≤ 8 kDa. The results revealed that pAEIP sample did not form gel. These results could indicate that the proteins with lower molecular weight do contribute to crosslinking during gelation, as nAEIP does form a gel. The contribution of proteins with such low Mw could also be the cause for the inability of pSE samples to form gel.

3.3.3.1 Microscopic Analysis of Gel

Banerjee and Bhattacharya (2012) recommend that as well as the visual observation of the vials, the gels should be assessed using different types of measurements. The microscopic observation before and after gelation treatment were carried out using rhodamine b stain, which indicates the location of the protein.

The nAEIP protein extract, shown in Figure 13 (A & B), appears to show a small degree of aggregation. Similarly, Zhao et al., (2016) described that the mealworm larvae showed a small particle size dispersion after the adjustment of pH at 7, as with this method.

After heating and addition of $CaCl_2$ the solution contained larger aggregates as it can be seen in Figure 13C, however the low concentration of protein inhibited gel formation. Whereas at a higher concentration of protein, as shown in Figure 13D, a gel formed, to a degree that prevented staining. Dilutions of the gel were not possible as the gel was quite hard. As a general trend, observing the vials with concentration at 4%and 8% after heat treatment, the lower the protein concentration in the solution the smaller the size of aggregates formed after heating.

In contrast, pSE extract had a lower protein content, see Figure 6, therefore to compare at the same protein concentration more dry mass was addedand therefore insect body residues may be present, as evidenced in Figure 14A.

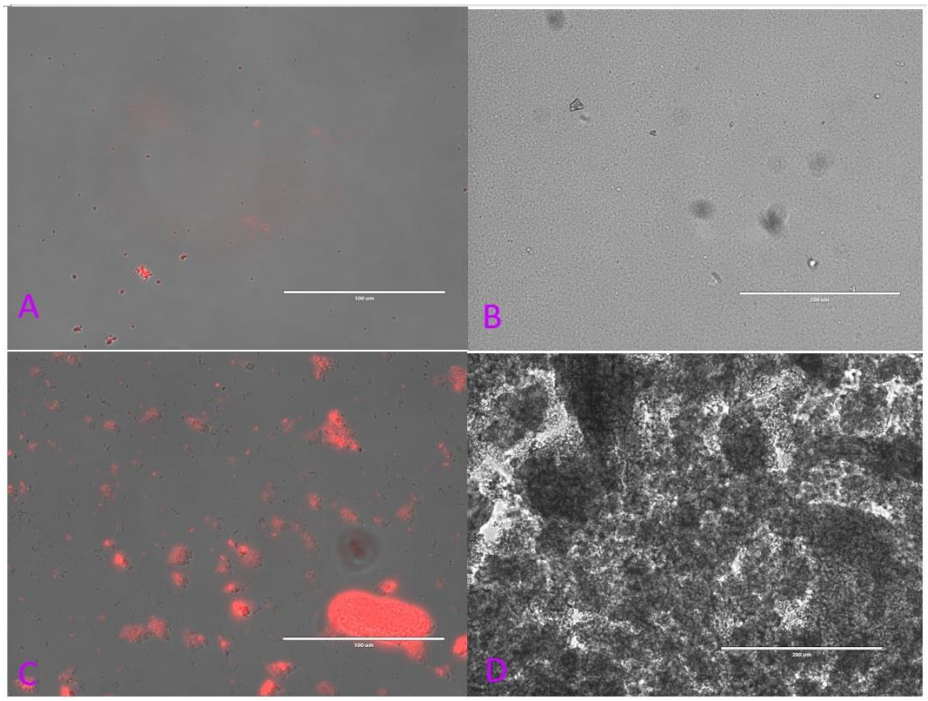


Figure 13. Typical examples of micrographs of the nAEIP during the gelation process; protein solution before heating with rhodamine b (A) and without rhodamine b (B), protein aggregation seen after heating and salt addition at a low concentration of protein (C) and protein gelation seen after heating and salt addition due to the presence of a high concentration (D). Scale bar =100 µm.

After heating, the proteins formed small aggregates as shown in Figure 14B, significantly larger aggregates were formed after addition of $CaCl_2$ (Figure 14 C&D), however no self-supporting gel was formed with pSE.

The micrographs are in agreement the visual observations of the gels. Staining with Rhodamine b has confirmed the changes in protein structure leading to gel formation.

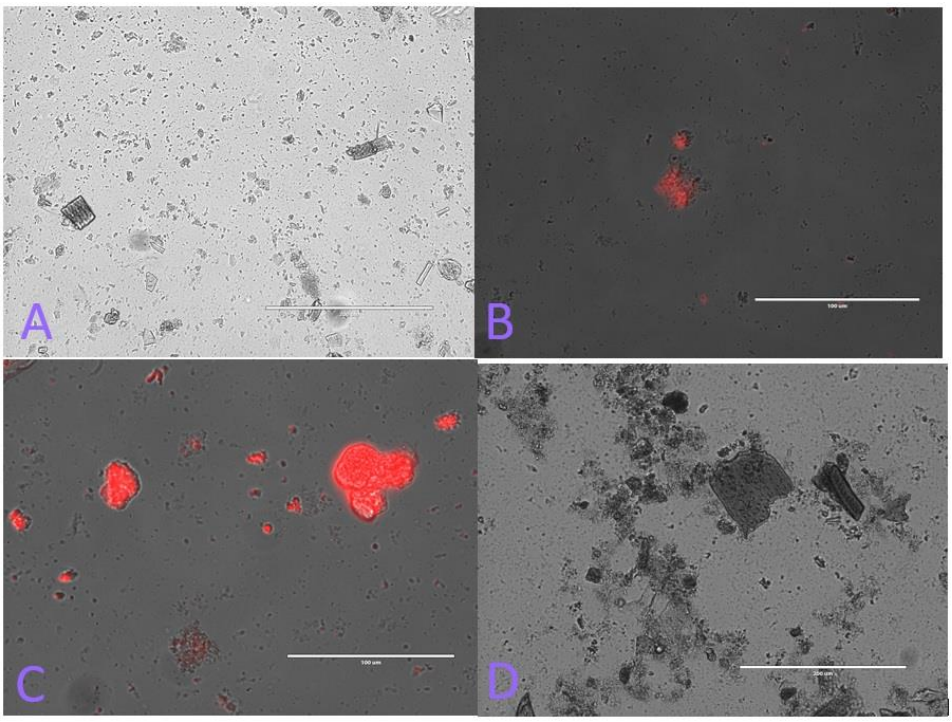


Figure 14. Typical examples of micrographs of the pSE during the gelation process; protein dispersion (A), protein aggregates formed after heating (B), and after thermal processing and salt addition with Rhodamine b (C) and without (D). Scale bar =100 µm.

3.4 CONCLUSIONS AND FUTURE WORK

The present study investigated the gelation ability of mealworm beetle (*T. molitor)* at the adult stage. Soluble proteins from adult mealworm beetle using four different extraction methods were characterized for their physicochemical properties. The salt extraction (SE) and alkali solubilisation and isoelectric precipitation (AEIP) were selected for studying the gelation ability of the extracted protein, as these two methods are used often for extraction of legumes and meats proteins for studying the gelation properties. The determination of the crude protein contents (CP) of mealworm beetles and the extraction yields showed that had a high protein content ranging from 62 to 87% and low extraction yield ranging from 5.9 to 19.2 % were affected by the extraction methods. Alkali extraction and isoelectric precipitation (AEIP) was a more

effective extraction method compared with the salt extraction (SE). While the molecular weight range for nAEIP protein was similar to that of pSE samples ranging from 45 to ≤ 14 kDa. The purification of SE protein in the dialysis tubes was not an efficient way of purification as there was a loss of protein and the removal of the chitin residues was not efficient as shown by the FT-IR results.

The protein gelation was demonstrated to be affected by the extraction method, protein concentration and the presence of salt. Proteins extracted with alkaline solution and isoelectric precipitation were capable of gel formation after heating (85-90 °C), at pH 7, for 30 min and 90 min at a protein concentration above 12% (w/v), while the addition of salt in the preheated induced gelation in lower protein concentration that of 8%, while the salt extracted proteins were incapable of forming gel.

Chapter 4 General Conclusions

The objective of this research was to describe and categorise different viewpoints held about consumption of insect-based foods by a sample of UK consumers and investigate the potential of using insect protein as a gelling agent in food formulations.

Q methodology and a questionnaire were used to explore consumer acceptance of insect-based foods. Forty individuals completed a questionnaire, which included questions about their socio-demographics, food neophobia, awareness of insect consumption, personal involvement inventory and preference insect-based product following completion of the questionnaire, participants took part in Q-method. The predominant feature of this Q method study was the exploration of different viewpoints held by consumers about insect-based foods. To the best of the author's knowledge, this the first time the Q methodology has been employed for studying insect consumption. The age of participants ranged from 21 to 50 years and the majority of the participants were female (75%). The participants were mostly British (40%), other nationalities present included French and Greek (10% each), Nigerian and Chinese (5% each) and the remainder were Bulgarian, Canadian, German, Irish, Iranian, Italian, Mexico, Nepalese, Polish, Portuguese, Thai and Tunisian. Finally, almost all the participants were meat eaters (40%) or flexitarian (53%) participants.

From the Q methodology four groups (factors) emerged: (1) *informed and positive*, (2) *informed andnegative*, (3) *informed with low sensory expectation* and (4) *Informed and satisfied but price-worried*. While there is some research that investigates the factors affecting the acceptance of insect-based foods, very little if any, categorises the viewpoints into factors allowing the identification of different profiles of potential consumers. The study highlighted that the majority of the participants are aware of the nutritional value of insect-based food. Participant attitudes towards insect-based foods

were found to develop based on their prior experience, food neophobia levels, gender, age, sensory expectations and subordinate determinants such as price and convenience. The results of Q-method revealed that the participants of factor 1 and factor 4 could be potential consumers of an insect-based food product, in comparison participants of factors 2 and 3 showed a very low willingness to try insects. Therefore, the preference of the participants who represented the factor 1 and 4 could determine which insect-based food would have higher acceptance by the public. Burger and snacks were the most preferred food products for insect inclusion, while candy, ice cream and yoghurt least preferred. Therefore, insect-based burgers and snack could meet acceptance. Ensuring the price point of the insect-based food deemed affordable is an important aspect for participants in factor 4.

Grinding insects into a flour is a typical method of incorporating insects into food products. The presence of insects can influence the flavour, colour, nutrition, sustainability of a food product. Insects, in particular insect protein, can provide a functional role in providing structure and texture to food products such as stabilising emulsion and foams, fibre formation or gel structures. For this study the potential to formulate an insect protein gel using mealworm beetles was explored, providing a potential route for incorporation of insects in food products.

The research presented in Chapter 3 evaluated the production of a water soluble protein fraction from mealworm beetles. Four different extraction methods (AEIP, SE, OF, SEq) were used in this study. The crude protein, the extraction yield, the purity and the molecular weight distribution of the protein extracts were evaluated. The determination of crude protein content of extractsand the extraction yield of the methods showed the extraction yield of all the extracts was low, ranging from 5.9 to 19.3 while the protein content of all the extracts, some requiring dialysis, presented high protein content from 63% to 83%, except the SE extract which before the

purification had 16% protein content. The ability of two protein extracts, from AEIP and SE, to form gel were studied after thermal processing as well as salt addition. AEIP and SE were selected as these two extraction methods are often used for to extract of protein from legumes and meat prior to gelation. Alkali extraction and isoelectric precipitation was a more effective extraction method compared with the salt extraction method. While the molecular weight range for nAEIP protein was similar to that of pSE samples ranging from 45 to ≤ 14 kDa. The purification of SE protein using dialysis tubes caused a loss of protein and the removal of the chitin residues was not complete as shown in the FT-IR spectra.

AEIP protein extract was able to form a gel after thermal processing (12% protein concentration) and at a lower protein concentration was required (8%) when salt was added after thermal processing. In contrast, the purified SE was not able to form gel. This exploratory study in the gelation properties of protein extracted from mealworm beetles has shown that the extraction conditions, protein concentration and ionic strength (salt addition) all affected the ability to form a gel.

4.1 Future work

There are still aspects left to explore to increase our understanding of how to successfully incorporate insects into food products, as well as ensure consumers purchase the products. Further investigation should include:

- The Q method developed for insect-based foods in this research needs to be applied to a larger study population, ensuring a broader range of participants in terms of social status, to reflect the general population.
- For the individuals who presented positive attitudes (Factor 1 and 4) towards the insect-based foods more behavioural exploratory tests should be carried out in order to confirm what the intention of the individuals to eat insects, would really is.

- In order to allow for incorporation of insects in food products the optimisation of protein extraction is required, as in this study, the protein yields could have been higher.
- Further characterisation of the insect protein extracts in terms of hydrophobicity, solubility, particle charge and particle size would indicate other application of the ingredient.
- This study has shown that insect protein gels from mealworm beetles are possible under certain extraction and environmental conditions, the rheological and textural properties of these gels now needs to be studied.

Appendices

Appendix 1: Questionnaire

Q1

NOVEL FOODS IN UK

Information Sheet and Consent Form

You are invited to participate in a survey over the coming weeks for a research project conducted by Viktoria Iliopoulou for her MRes Thesis and supervised by Dr Anastasios Pagiaslis, Marketing Division, Nottingham University Business School, the University of Nottingham.

The purpose of this study is to study peoples' attitudes and beliefs about novel foods.

Your participation in this study will consist of attending one interview and replying to an online questionnaire. The first part of the participation is the completion of a questionnaire (~10min) and then the interview is a short (~30min.) face to face interview whose purpose is to sort a set of statements in a specific task. The interview will take place after the questionnaire completion. Initially, (1) you will be asked to answer to some socio-demographic, and general attitudinal questions. (2) Afterwards, in the interview you will be asked to sort a set of general attitudinal statements.

The researcher assures you that your participation in this study is ***entirely voluntary***. *You are free to withdraw from the experiment at any time*. Should you choose to withdraw the data that you have provided will be destroyed wherever possible and will not be used for any analysis.

You will be paid a participation reward of £10 (in the form of an Amazon voucher) for fully taking part in these study. The funding for this study has been approved and allocated from the Nottingham University Business School.

Your anonymity is this project will be protected. This Information Sheet and Consent Form will be kept in a separate box from the rest of the data collected during the study. The researcher assures you that the information that you provide will be kept strictly confidential. The data collected from this study will be stored in a locked cabinet in the researchers' office for a period of 5 years. Only the researchers will have access to the data whether it is the original interviews or the data files on the computer. The results obtained from this project are for research purposes only and publication of the results in scientific journals will be limited to aggregated group responses rather than individual ones.

The present study is conducted according to ethical standards of the American Psychological Association (APA) and has been granted ethical approval from Nottingham University's Research Ethics Committee.

If you have any questions about the study, or if you would like to receive a report of the results when they come available, contact the principal investigator Viktoria Iliopoulou at: **stxvi2@nottingham.ac.uk.**

❍*I agree to participate in the study entitled* ***—Novel foods in UK****- conducted by Viktoria Iliopoulou, Mres student and Dr Anastasios Pagiaslis, Marketing Division, Nottingham University Business School. I understand that by accepting to participate in this study, I am in no way waiving my right to withdraw from the study.*

Q2: The following questions are looking into awareness and general attitudes you may have towards novel foods. Please answer each question. There are no right or wrong answers just answer each question as quickly as possible.

	Yes	No
Have you heard that edible insects are eaten in some parts of the word?	❍	❍
Have you heard that there are insect-based products available on the market?	❍	❍
Have you heard that edible insects contain essential nutrients for humans?	❍	❍
Have you ever tasted edible insects or a meal that contains edible insects?	❍	❍

Q3: This question is looking at your opinions regarding new/novel foods. New/ Novel foods are foods that may be unknown or unfamiliar to you.

	Strongly Agree	Agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Disagree	Strongly disagree
I am constantly sampling new and different foods	❍	❍	❍	❍	❍	❍	❍
I don't trust new foods	❍	❍	❍	❍	❍	❍	❍
If I don't know what is in a food, I won't try it	❍	❍	❍	❍	❍	❍	❍
I like foods from different countries	❍	❍	❍	❍	❍	❍	❍
Ethnic food looks too weird to eat	❍	❍	❍	❍	❍	❍	❍
At dinner parties, I will try new food	❍	❍	❍	❍	❍	❍	❍
I am afraid to eat things, I have never had before	❍	❍	❍	❍	❍	❍	❍
I am very particular about The foods I will eat	❍	❍	❍	❍	❍	❍	❍
I will eat almost everything	❍	❍	❍	❍	❍	❍	❍
I like to try new ethnic restaurants	❍	❍	❍	❍	❍	❍	❍

Q4: To me insect-based food is:

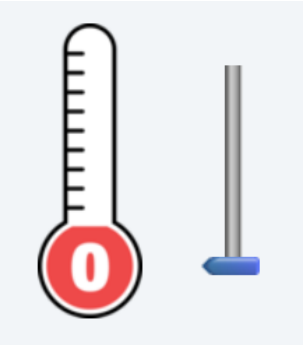
Important	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Unimportant
Of no concern to me	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Of concern to me
Irrelevant	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Relevant
Means a lot to me	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Means nothing to me
Doesn't matter to me	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Matters to me
No Risk	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Risky
Easy to go wrong	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Hard to go wrong
Hard to pick	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Easy to choose
Appealing	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Unappealing
Boring	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Interesting
Unexciting	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Right
Fun	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Not Fun
Says something about me	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Says nothing about me
Shows something about me	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	Show nothing about person

Q5: Have you ever tried insect-based food before?

- ○ Yes
- ○ No

(Only participants that responded negatively to the precious question had to answer next question (Q6)

Q6: Would you ever consider trying insect-based foods?



Q7: Below you will see a list of insect-based foods that are currently available in the market.
Please rank them, based on **your current preferences if you have** tasted insect-based foods before or your **potential preferences haven't** tasted insect-based foods before. Please sort the foods according to your liking. The current order is random. *(If*

you are using a mobile you will need to scroll very quickly in order to be able to move to the next question.)

Burger	1	Pasta	10
Snack	2	Soup	11
Energy shake	3	Pasta	12
Energy bar	4	Cookies/Cake	13
Protein Bars	5	Candy	14
Casserole	6	Yoghurt	15
Ready-to-go meal	7	Desert	16
Spreads	8	Chocolate	17
Fried snacks	9	Ice- cream	18

Q8: Finally, we would like to ask you a couple of questions about yourself.

Q9: what is your sex?

- Female
- Male
- Other, do not wish to say

Q10: What is your age? (Please enter a number) _____

Q11: What is your nationality? _____

Q12: What is the highest level of education you completed?

a) Primary Education
b) Secondary Education / Highschool
c) Tertiary Education / University / Vocational Education
d) Post Graduate Education / MSc/MA/MBA/MRes
e) Doctoral education /PhD

Q13: Are you currently working?

a) Working full-time (30 hours per week plus)
b) Working part-time (under 30 hours per week)
c) Looking for a job
d) In full-time education
e) Retired
f) Not working

Q14: Regarding your eating habits, are you?

a) A meat-eater (consuming meat (almost) every day of the week)
b) A flexiterian (consuming meat only a few days per week)
c) A vegeterian (not consume any meat at all)
d) A vegan (not using or eating animal products)

Appendix 2: STATEMENTS

1. I believe (consuming) insect-based foods has (may have) a positive influence in what other people think about me.
2. I believe insect-based foods are an alternative source of protein to meat / fish / dairy.
3. I believe insect-based foods are (can be) enjoyable.
4. I believe insect-based foods are (can be) fun.
5. I believe insect-based foods are (could be) good value for money.
6. I believe insect-based foods are (could be) too expensive.
7. I believe insect-based foods are a more sustainable source of protein compared to the equivalent meat / dairy / fish products.
8. I believe insect-based foods are exciting.
9. I believe insect-based foods are good for my skin/teeth/hair/nails etc.
10. I believe insect-based foods are nutritious.
11. I believe insect-based foods are rich in protein.
12. I believe insect-based foods are rich in vitamins and minerals.
13. I believe insect-based foods can help in reducing global carbon emissions.
14. I believe insect-based foods can keep me healthy.
15. I believe insect-based foods have (would have) a good taste.
16. I believe insect-based foods have (would have) a pleasant texture.
17. I believe insect-based foods is a passing fad.
18. I believe insect-based foods look (would look) nice.
19. I believe insect-based foods smell (would smell) nice.
20. I believe insect-based foods are (can be) an alternative source of protein for my lifestyle.
21. I believe insect-based foods are (can be) pleasurable.
22. I believe insect-based foods are (could be) satisfying.
23. I believe insect-based foods are (would be) easy to prepare.
24. I believe insect-based foods are (would be) low in calories.
25. I believe insect-based foods are (would be) low in fat.
26. I believe insect-based foods are clean.
27. I believe insect-based foods are fresh.
28. I believe insect-based foods are not safe to eat.
29. I believe insect-based foods are rich in fibre and roughage.
30. I believe insect-based foods are trendy.
31. I believe insect-based foods can (could be) cooked very simply.
32. I believe insect-based foods can (could) cause physical risks.
33. I believe insect-based foods can (could) damage your body in the long term.
34. I believe insect-based foods can (could) help me control my weight.
35. I believe insect-based foods can (could) make you sick.
36. I find insect-based foods to be disgusting.
37. I find insect-based foods to be repulsive.
38. I find insect-based foods to be scary.
39. I see (would see) insect-based foods as a personal challenge.
40. I trust (would trust) the quality of insect-based foods.
41. I would feel ashamed to consume insect-based foods.
42. I would feel good about myself if I consumed insect-based foods.
43. I would feel guilty to consume insect-based foods.
44. I would feel proud of myself if I ate insect-based foods.
45. I would feel successful if I consumed insect-based foods.

46. I would only consume insect-based foods if someone else told me to.
47. Insect-based foods make me feel comfortable.

Appendix 3: Q methodology tables

Table 16. Correlation matrix of Q sorters.

Partici	SK1	ZL2	ND3	YC4	OC5	OP6	CM7	IO8	RP9	NK10	LLT11	FB12	JM13	OB14	JS15	JW16	MT17	KH18	SK19	AH20	AS21	IS22	PM23	DC24	WW25	AD26	MS27	SL28	HF29	CA30	MP31	CM32	CA33	KA34	MK35	YX36	JI37	AT38	AK39	SM40
SK1	100	57	-57	43	28	27	51	46	39	45	35	52	33	53	31	48	20	45	28	18	25	62	58	65	27	39	45	46	50	63	35	47	44	51	16	52	41	39	41	25
ZL2	57	100	-49	40	47	49	54	26	57	47	38	53	20	59	42	50	29	60	20	43	29	65	48	62	50	45	54	63	53	57	32	50	47	67	34	52	44	56	56	23
ND3	-57	-49	100	-48	-31	-28	-27	-29	-33	-43	-36	-51	-56	-39	-18	-44	-41	-43	-24	-21	-10	-69	-53	-60	-21	-39	-62	-41	-53	-67	-49	-35	-45	-52	-3	-58	-40	-38	-41	-47
YC4	43	40	-48	100	25	21	22	9	24	-2	18	35	48	3	0	11	20	19	29	7	3	23	23	43	4	28	47	34	46	52	18	8	26	37	6	33	-3	5	19	45
OC5	28	47	-31	25	100	56	37	21	38	42	30	51	19	52	53	46	40	31	13	33	55	33	49	42	66	43	33	45	51	40	20	37	44	37	29	46	48	46	65	17
OP6	27	49	-28	21	56	100	33	19	55	38	40	43	24	48	29	58	32	36	3	40	33	33	33	27	37	44	35	38	57	41	24	33	28	50	32	40	28	45	63	13
CM7	51	54	-27	22	37	33	100	26	49	31	23	45	9	56	37	44	21	39	27	40	17	37	46	36	43	27	22	34	39	37	23	40	55	35	32	33	35	42	38	9
IO8	46	26	-29	9	21	19	26	100	30	58	38	52	21	59	52	43	15	53	23	34	7	53	64	42	36	3	30	48	20	27	17	59	48	42	33	48	31	53	43	18
RP9	39	57	-33	24	38	55	49	30	100	18	37	49	20	48	40	38	32	50	50	24	18	52	35	46	39	48	43	33	45	38	33	38	46	52	40	44	25	53	55	19
NK10	45	47	-43	-2	42	38	31	58	18	100	40	66	20	70	63	67	21	52	3	48	33	62	75	51	52	23	51	58	40	35	30	59	53	50	31	57	61	69	58	16
LLT11	35	38	-36	18	30	40	23	38	37	40	100	36	40	43	37	50	33	28	19	29	13	55	48	37	29	49	42	24	57	29	25	38	29	25	2	29	37	46	58	41
FB12	52	53	-51	35	51	43	45	52	49	66	36	100	30	63	64	54	17	57	27	47	44	59	73	54	53	39	61	74	56	51	42	54	67	63	34	70	47	73	69	18
JM13	33	20	-56	48	19	24	9	21	20	20	40	30	100	4	-8	16	39	23	13	36	12	34	25	36	2	21	45	12	36	38	33	4	19	27	-3	18	3	13	10	40
OB14	53	59	-39	3	52	48	56	59	48	70	43	63	4	100	72	68	31	53	10	38	43	69	73	48	64	43	35	58	49	31	29	75	72	46	43	57	55	73	65	9
JS15	31	42	-18	0	53	29	37	52	40	63	37	64	-8	72	100	53	18	53	5	30	36	51	65	39	69	19	34	53	39	29	20	61	64	40	44	60	58	69	55	-10
JW16	48	50	-44	11	46	58	44	43	38	67	50	54	16	68	53	100	29	43	12	50	27	57	69	42	59	41	36	50	58	48	40	60	47	45	29	58	51	67	63	28
MT17	20	29	-41	20	40	32	21	15	32	21	33	17	39	31	18	29	100	29	28	26	5	31	35	25	34	49	32	-1	52	18	34	11	25	18	6	26	27	13	30	30
KH18	45	60	-43	19	31	36	39	53	50	52	28	57	23	53	53	43	29	100	16	40	24	62	51	54	57	24	45	58	40	51	22	50	38	72	48	64	34	56	50	6
SK19	28	20	-24	29	13	3	27	23	50	3	19	27	13	10	5	12	28	16	100	14	-8	30	14	43	8	23	34	10	23	11	25	2	25	8	-8	11	21	19	25	37
AH20	18	43	-21	7	33	40	40	34	24	48	29	47	36	38	30	50	26	40	14	100	6	37	36	22	39	17	33	44	31	18	36	26	35	32	12	25	28	47	29	8
AS21	25	29	-10	3	55	33	17	7	18	33	13	44	12	43	36	27	5	24	-8	6	100	19	38	22	45	27	24	23	32	11	-7	41	19	33	19	27	34	36	43	-16
IS22	62	65	-69	23	33	33	37	53	52	62	55	59	34	69	51	57	31	62	30	37	19	100	59	63	45	42	55	53	49	47	47	52	63	60	24	52	58	67	53	35
PM23	58	48	-53	23	49	33	46	64	35	75	48	73	25	73	65	69	35	51	14	36	38	59	100	59	66	42	55	56	59	47	44	69	63	50	38	71	55	70	63	33
DC24	65	62	-60	43	42	27	36	42	46	51	37	54	36	48	39	42	25	54	43	22	22	63	59	100	33	37	67	55	49	67	34	36	41	52	26	48	30	46	49	36
WW25	27	50	-21	4	66	37	43	36	39	52	29	53	2	64	69	59	34	57	8	39	45	45	66	33	100	31	21	40	49	32	32	60	54	34	49	63	48	65	58	14
AD26	39	45	-39	28	43	44	27	3	48	23	49	39	21	43	19	41	49	24	23	17	27	42	42	37	31	100	43	21	71	25	45	22	30	29	24	30	31	37	60	48
MS27	45	54	-62	47	33	35	22	30	43	51	42	61	45	35	34	36	32	45	34	33	24	55	55	67	21	43	100	51	50	53	33	30	40	63	16	52	45	52	50	33
SL28	46	63	-41	34	45	38	34	48	33	58	24	74	12	58	53	50	-1	58	10	44	23	53	56	55	40	21	51	100	32	54	29	48	55	64	34	61	37	68	56	5
HF29	50	53	-53	46	51	57	39	20	45	40	57	56	36	49	39	58	52	40	23	31	32	49	59	49	49	71	50	32	100	48	48	44	45	45	27	50	45	55	64	43
CA30	63	57	-67	52	40	41	37	27	38	35	29	51	38	31	29	48	18	51	11	18	11	47	47	67	32	25	53	54	48	100	37	38	30	63	29	63	23	40	53	30
MP31	35	32	-49	18	20	24	23	17	33	30	25	42	33	29	20	40	34	22	25	36	-7	47	44	34	32	45	33	29	48	37	100	14	38	20	17	33	40	42	31	45
CM32	47	50	-35	8	37	33	40	59	38	59	38	54	4	75	61	60	11	50	2	26	41	52	69	36	60	22	30	48	44	38	14	100	63	44	42	64	52	71	60	8
CA33	44	47	-45	26	44	28	55	48	46	53	29	67	19	72	64	47	25	38	25	35	19	63	63	41	54	30	40	55	45	30	38	63	100	36	29	54	58	71	46	10
KA34	51	67	-52	37	37	50	35	42	52	50	25	63	27	46	40	45	18	72	8	32	33	60	50	52	34	29	63	64	45	63	20	44	36	100	51	63	36	53	57	1
MK35	16	34	-3	6	29	32	32	33	40	31	2	34	-3	43	44	29	6	48	-8	12	19	24	38	26	49	24	16	34	27	29	17	42	29	51	100	36	4	43	35	2
YX36	52	52	-58	33	46	40	33	48	44	57	29	70	18	57	60	58	26	64	11	25	27	52	71	48	63	30	52	61	50	63	33	64	54	63	36	100	47	56	61	18
JI37	41	44	-40	-3	48	28	35	31	25	61	37	47	3	55	58	51	27	34	21	28	34	58	55	30	48	31	45	37	45	23	40	52	58	36	4	47	100	62	53	10
AT38	39	56	-38	5	46	45	42	53	53	69	46	73	13	73	69	67	13	56	19	47	36	67	70	46	65	37	52	68	55	40	42	71	71	53	43	56	62	100	70	13
AK39	41	56	-41	19	65	63	38	43	55	58	58	69	10	65	55	63	30	50	25	29	43	53	63	49	58	60	50	56	64	53	31	60	46	57	35	61	53	70	100	35
SM40	25	23	-47	45	17	13	9	18	19	16	41	18	40	9	-10	28	30	6	37	8	-16	35	33	36	14	48	33	5	43	30	45	8	10	1	2	18	10	13	35	100

Table 17. The rotated factor loadings matrix with shaded the Q sorts that define a particular factor.

	Participant	Factor 1	Factor 2	Factor 3	Factor 4
1	SK1	0.40	0.26	0.53	0.04
2	ZL2	0.34	0.12	0.47	0.33
3	ND3	-0.27	-0.55	-0.57	0.01
4	YC4	-0.15	0.34	0.61	0.15
5	OC5	0.32	0.19	0.14	0.68
6	OP6	0.16	0.23	0.19	0.55
7	CM7	0.37	0.11	0.16	0.16
8	IO8	0.67	0.10	0.23	-0.16
9	RP9	0.24	0.10	0.25	0.24
10	NK10	0.79	0.15	0.22	0.12
11	LLT11	0.35	0.44	0.10	0.20
12	FB12	0.60	0.14	0.42	0.33
13	JM13	-0.03	0.62	0.35	-0.01
14	OB14	0.79	0.06	0.13	0.25
15	JS15	0.75	-0.09	0.12	0.31
16	JW16	0.62	0.37	0.11	0.30
17	MT17	0.09	0.55	0.01	0.24
18	KH18	0.41	0.03	0.51	0.11
19	SK19	0.04	0.25	0.13	0.01
20	AH20	0.33	0.25	0.06	0.00
21	AS21	0.29	-0.17	0.20	0.51
22	IS22	0.61	0.33	0.34	-0.05
23	PM23	0.75	0.32	0.29	0.25
24	DC24	0.34	0.28	0.63	0.07
25	WW25	0.61	0.11	0.02	0.47
26	AD26	0.13	0.44	0.11	0.49
27	MS27	0.30	0.32	0.58	0.17
28	SL28	0.54	-0.03	0.52	0.16
29	HF29	0.30	0.52	0.25	0.52
30	CA30	0.24	0.28	0.69	0.16
31	MP31	0.24	0.48	0.14	0.06
32	CM32	0.75	0.01	0.18	0.22
33	CA33	0.64	0.13	0.19	0.13
34	KA34	0.27	-0.06	0.76	0.25
35	MK35	0.29	-0.14	0.19	0.25
36	YX36	0.56	0.16	0.49	0.27
37	JI37	0.63	0.18	0.02	0.22
38	AT38	0.75	0.07	0.18	0.27
39	AK39	0.53	0.25	0.24	0.56
40	SM40	0.02	0.72	0.18	-0.03
Eigenvalues		8.90	3.69	4.94	3.32

% Explained Variance	22	9	12	8

Table 18. N ormalized factor scores (z socres) and the rank order for all factors.

Statement Number	factor 1		factor 2		factor 3		factor 5	
	Z-score	Rank	Z-score	Rank	Z-score	Rank	Z-score	Rank
S1	-0.69	34	-1.12	39	-1.17	41	-1.37	42
S2	1.77	2	2.28	1	1.49	5	1.94	1
S3	1.02	7	-1.03	38	0.3	16	0.26	22
S4	0.57	13	-1.41	46	-0.2	30	1.27	3
S5	0.53	15	1.37	5	0.16	20	0.51	18
S6	-0.41	32	-0.85	34	0.1	24	1.72	2
S7	1.54	3	1.54	4	2.15	1	1.06	7
S8	0.18	24	-1.16	41	-0.16	27	0.93	10
S9	0.26	21	0.18	20	0.18	19	-0.6	36
S10	1.15	6	0.73	12	1.06	9	0.91	11
S11	2.07	1	1.75	2	1.68	3	1.19	4
S12	1.16	5	1.57	3	0.24	17	-0.11	26
S13	0.99	9	1.33	6	2.12	2	0.53	17
S14	1.01	8	0.91	9	0.22	18	-0.47	33
S15	0.55	14	-0.46	30	-0.61	34	1.18	5
S16	0.22	22	-1.18	42	-1.25	43	-0.52	35
S17	-0.8	36	-0.16	25	-1.82	46	-0.95	39
S18	0.17	25	0.14	22	-1.04	39	1.08	6
S19	-0.11	31	-0.23	27	-1.52	45	0.3	20
S20	1.43	4	-1.12	40	1.11	7	0.11	25
S21	0.38	18	-0.89	35	0.11	23	0.96	8
S22	0.61	12	-0.97	36	-0.19	29	0.13	23
S23	0.11	28	0.83	11	-0.58	33	0.32	19
S24	0.44	16	1.26	7	0.49	14	0.9	12
S25	0.71	10	0.64	16	1.07	8	0.95	9
S26	0.64	11	-0.97	37	0	26	-0.25	27
S27	0.42	17	-0.82	33	-0.98	37	-0.35	31

S28	-1.32	39	-0.48	31	-0.9	36	-0.25	28
S29	-0.69	35	0.57	17	0.56	12	-0.28	29
S30	0.12	27	-0.2	26	0.7	11	-1.15	41
S31	0.29	20	0.66	13	-0.4	32	0.77	13
S32	-1.62	45	-0.31	29	-1.21	42	0.12	24
S33	-1.69	46	-1.29	43	-1.02	38	-1.93	45
S34	0.09	29	0.42	18	0.08	25	-1.49	44
S35	-1.55	44	0.17	21	-0.38	31	-0.4	32
S36	-1.32	38	0.64	15	0.12	21	-0.5	34
S37	-1.46	42	0.84	10	0.12	22	-1.07	40
S38	-1.41	41	1.08	8	0.48	15	-0.32	30
S39	-0.99	37	0.65	14	1.18	6	0.72	14
S40	0.35	19	0.03	23	-0.17	28	0.62	15
S41	-1.69	47	-1.33	44	-1.84	47	-2.3	47
S42	0.13	26	-0.3	28	0.8	10	-0.72	37
S43	-1.33	40	-0.09	24	-1.11	40	-1.97	46
S44	-0.02	30	-1.58	47	1.54	4	0.29	21
S45	-0.55	33	-1.4	45	0.56	13	0.55	16
S46	-1.46	43	0.37	19	-0.65	35	-1.48	43
S47	0.2	23	-0.61	32	-1.41	44	-0.87	38

Table 19. Factor score correlations.

	F1	**F2**	**F3**	**F4**
F1	1			
F2	0.2855	1		
F3	0.5433	0.4641	1	
F4	0.5928	0.2559	0.4871	1

Table 20. Factor Characteristics.

	F1	F2	F3	F4
No. of Defining Variables	11	4	5	3
Avg. Rel. Coef.	0.8	0.8	0.8	0.8
Composite Reliability	0.978	0.941	0.952	0.923
S.E. of Factor Z-scores	0.148	0.243	0.219	0.277

Table 21. Factor arrays for the four study factors.

	Statement	F1	F2	F3	F4
1	I believe (consuming) insect-based foods has (may have) a positive influence in what other people think about me.	-2	-3	-3	-3
2	I believe insect-based foods are (can be) an alternative source of protein to meat / fish / dairy.	5	5	4	5
3	I believe insect-based foods are (can be) enjoyable.	3	-2	1	0
4	I believe insect-based foods are (can be) fun.	2	-5	-1	4
5	I believe insect-based foods are (could be) good value for money.	1	4	1	1
6	I believe insect-based foods are (could be) too expensive.	-1	-2	0	5
7	I believe insect-based foods are a more sustainable source of protein compared to the equivalent meat / dairy / fish products.	4	4	5	3
8	I believe insect-based foods are exciting.	0	-3	0	2
9	I believe insect-based foods are good for my skin/teeth/hair/nails etc.	0	1	1	-2
10	I believe insect-based foods are nutritious.	3	2	3	2
11	I believe insect-based foods are rich in protein	5	5	4	4
12	I believe insect-based foods are rich in vitamins and minerals.	4	4	1	0
13	I believe insect-based foods can help in reducing global carbon emissions	3	3	5	1
14	I believe insect-based foods can keep me healthy.	3	3	1	-1
15	I believe insect-based foods have (would have) a good taste.	2	-1	-2	4
16	I believe insect-based foods have (would have) a pleasant texture.	0	-3	-4	-2
17	I believe insect-based foods is a passing fad.	-2	0	-5	-3
18	I believe insect-based foods look (would look) nice.	0	0	-3	3
19	I believe insect-based foods smell (would smell) nice.	-1	0	-4	1
20	I believe insect-based foods are (can be) an alternative	4	-3	3	0

	source of protein for my lifestyle.				
21	I believe insect-based foods are (can be) pleasurable.	1	-2	0	3
22	I believe insect-based foods are (could be) satisfying.	2	-2	-1	0
23	I believe insect-based foods are (would be) easy to prepare.	-1	2	-1	1
24	I believe insect-based foods are (would be) low in calories.	1	3	2	2
25	I believe insect-based foods are (would be) low in fat.	2	1	3	3
26	I believe insect-based foods are clean.	2	-2	0	0
27	I believe insect-based foods are fresh.	1	-1	-2	-1
28	I believe insect-based foods are not safe to eat.	-3	-1	-2	-1
29	I believe insect-based foods are rich in fibre and roughage.	-2	1	2	-1
30	I believe insect-based foods are trendy.	0	0	2	-3
31	I believe insect-based foods can (could be) cooked very simply.	1	2	-1	2
32	I believe insect-based foods can (could) cause physical risks.	-4	-1	-3	0
33	I believe insect-based foods can (could) damage your body in the long term.	-5	-4	-2	-4
34	I believe insect-based foods can (could) help me control my weight.	-1	1	0	-4
35	I believe insect-based foods can (could) make you sick.	-4	0	-1	-1
36	I find insect-based foods to be disgusting.	-2	1	0	-2
37	I find insect-based foods to be repulsive.	-3	2	0	-3
38	I find insect-based foods to be scary.	-3	3	1	-1
39	I see (would see) insect-based foods as a personal challenge.	-2	2	3	2
40	I trust (would trust) the quality of insect-based foods.	1	0	-1	1
41	I would feel ashamed to consume insect-based foods.	-5	-4	-5	-5
42	I would feel good about myself if I consumed insect-based foods.	0	-1	2	-2
43	I would feel guilty to consume insect-based foods.	-3	0	-3	-5
44	I would feel proud of myself if I ate insect-based foods.	-1	-5	4	0
45	I would feel successful if I consumed insect-based foods.	-1	-4	2	1
46	I would only consume insect-based foods if someone else told me to.	-4	1	-2	-4
47	Insect-based foods make me feel comfortable.	0	-1	-4	-2

Appendix 4: Ideal Q sorts for each factor

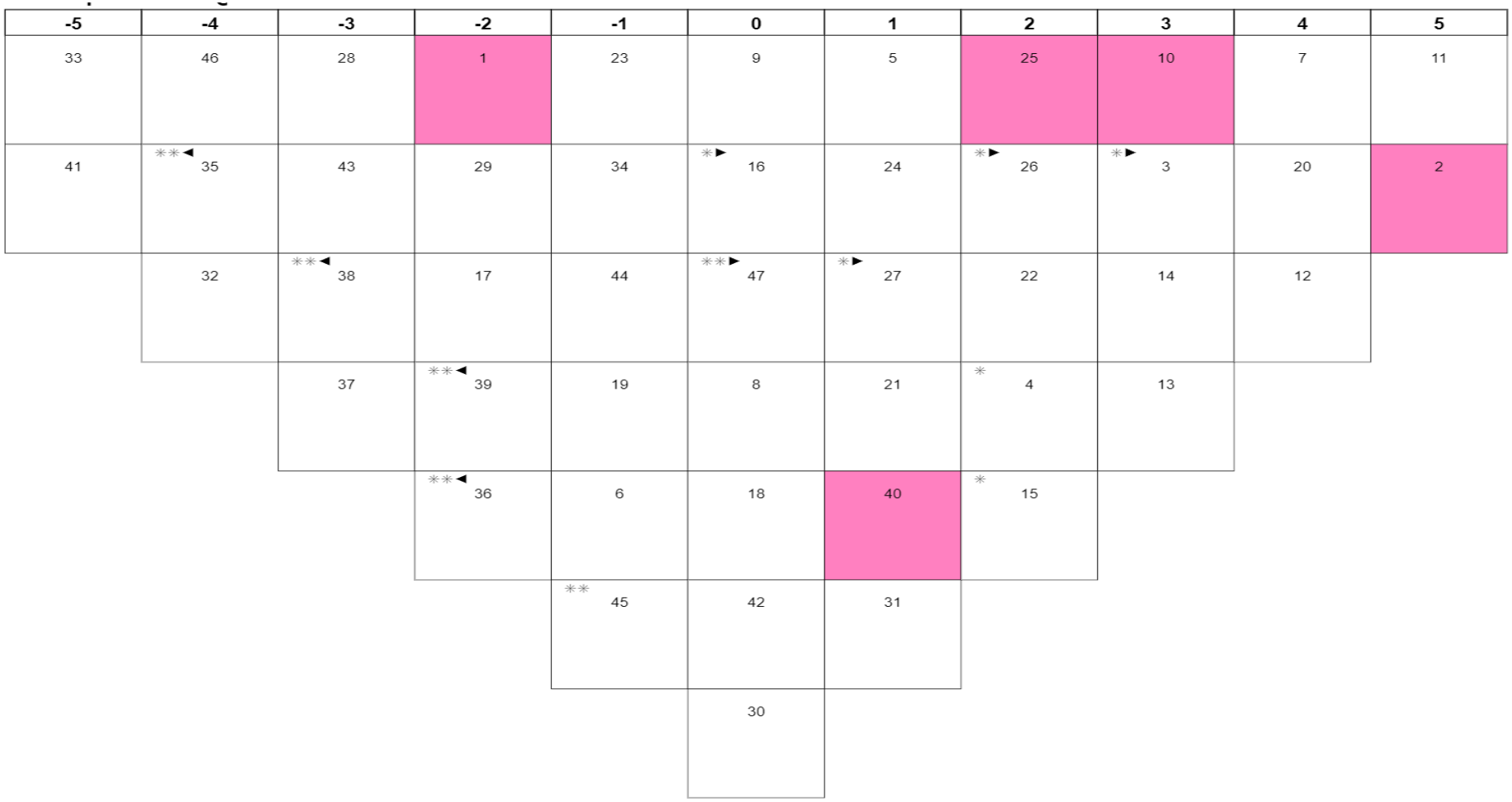


Figure 15. Factor array for Factor 1 exemplifying Q sort always conforms to the same distribution used in the original data collection and it is constructed by reference to the size and ultimately the rank order of the z scores.

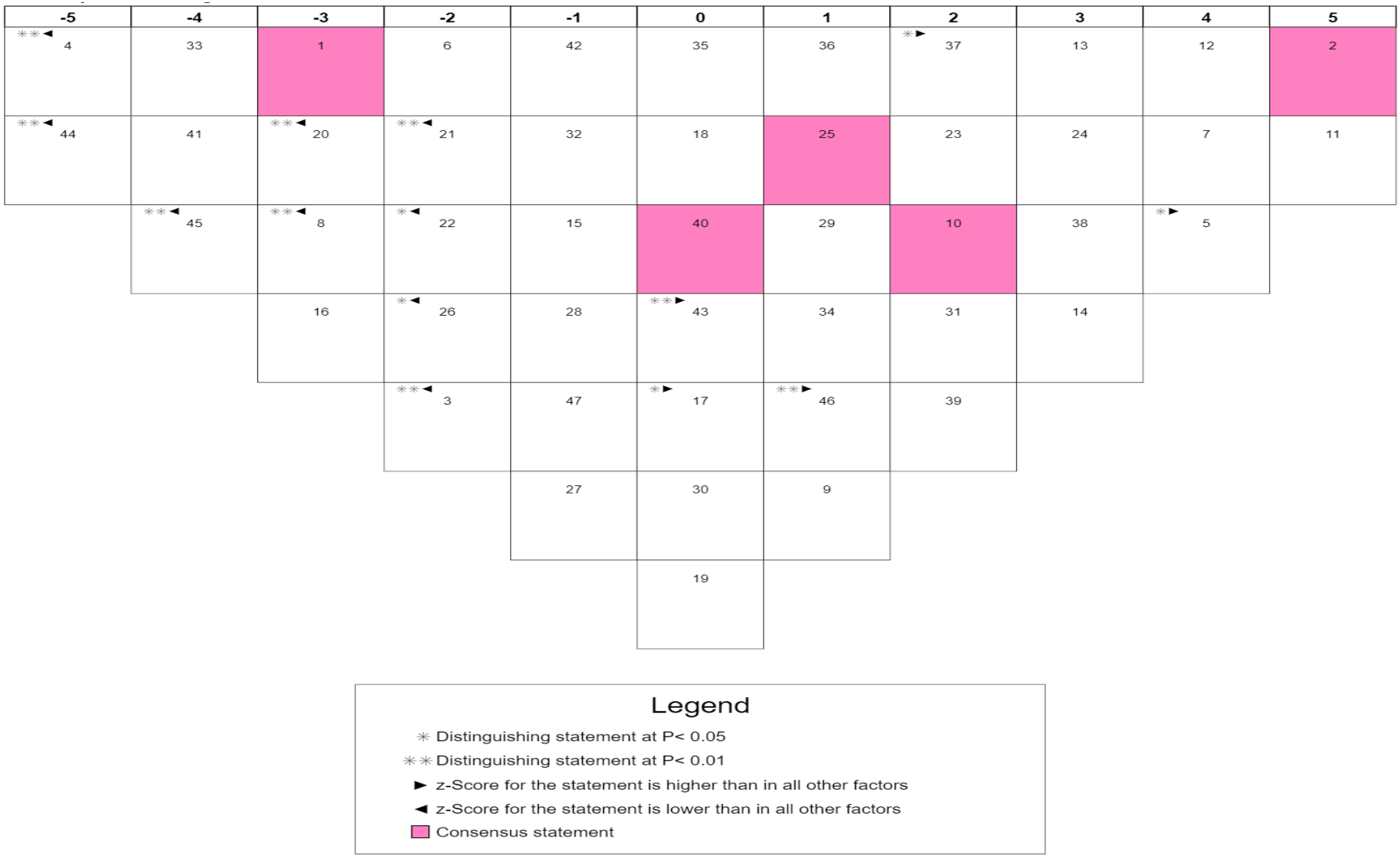


Figure 16. Factor array for Factor 2 exemplifying Q sort always conforms to the same distribution used in the original data collection and it is constructed by reference to the size and ultimately the rank order of the z scores.

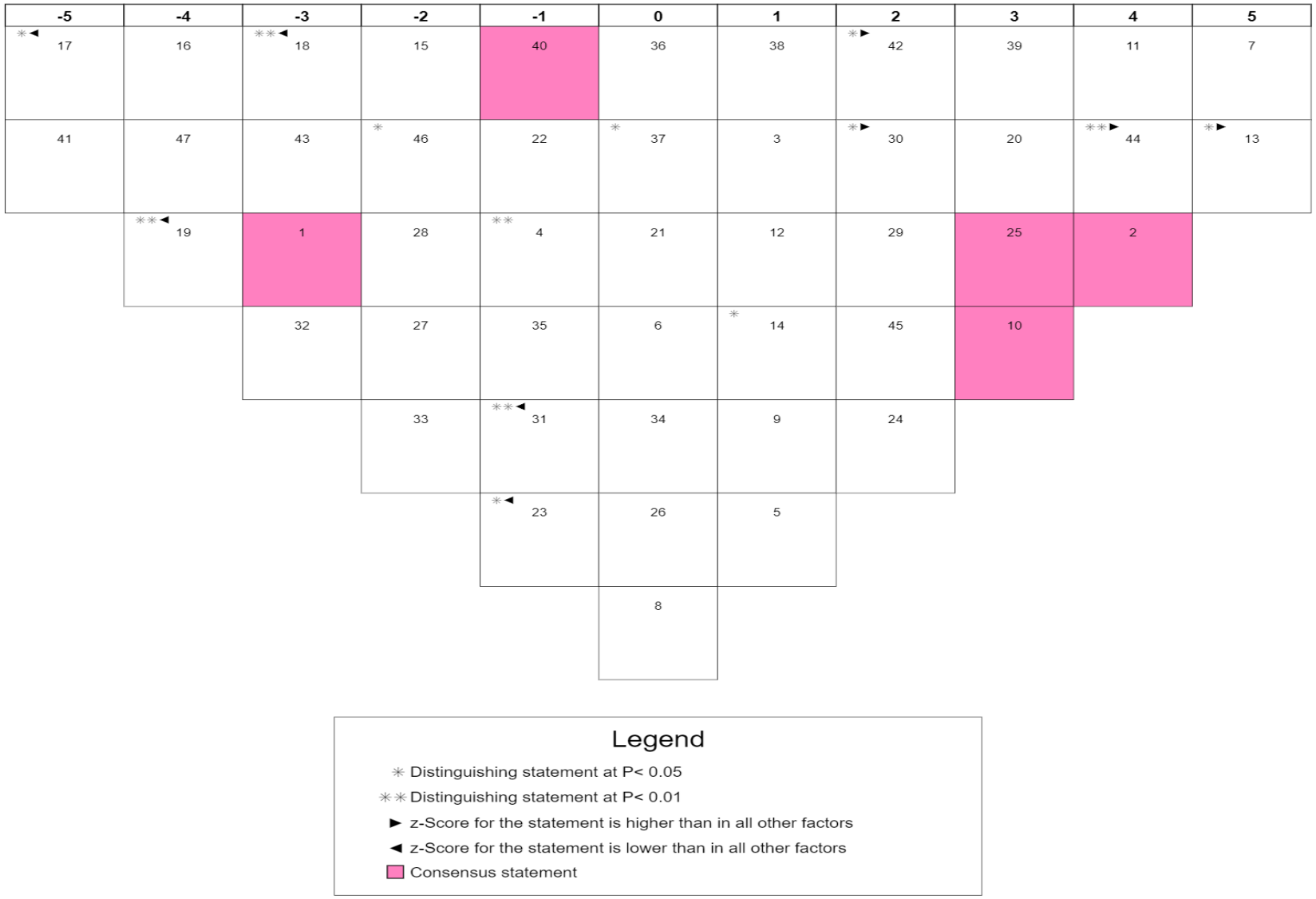


Figure 17. Factor array for Factor 3 exemplifying Q sort always conforms to the same distribution used in the original data collection and it is constructed by reference to the size and ultimately the rank order of the z scores.

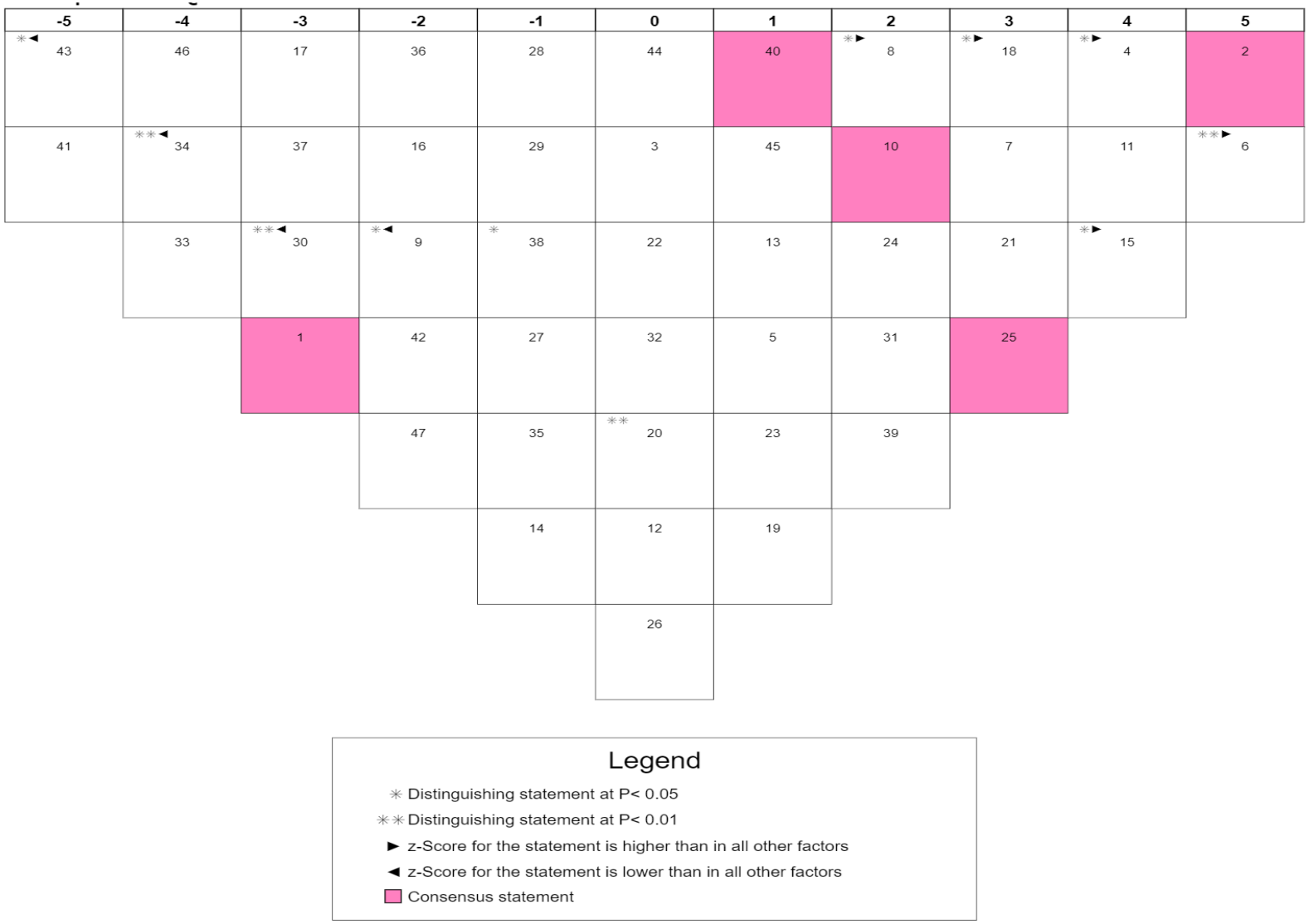


Figure 18. Factor array for Factor 4 exemplifying Q sort always conforms to the same distribution used in the original data collection and it is constructed by reference to the size and ultimately the rank order of the z scores.

Bibliography

Bagchi, 2013. Hydration of proteins. *In:* BAGCHI, B. (ed.) *Water in Biological and Chemical Processes: From Structure and Dynamics to Function.* Cambridge: Cambridge University Press.

ABDEL-AAL, E. S. M., SHEHATA, A. A., EL-MAHDY, A. R. & YOUSSEF, M. M. 1986. Extractability and functional properties of some legume proteins isolated by three different methods. *Journal of the Science of Food and Agriculture,* 37**,** 553-559.

AGUILAR-MIRANDA, E. D., LÓPEZ, M. G., ESCAMILLA-SANTANA, C. & BARBA DE LA ROSA, A. P. 2002. Characteristics of maize flour tortilla supplemented with ground Tenebrio molitor larvae. *Journal of agricultural and food chemistry,* 50**,** 192.

AGUILERA, J. M. & RADEMACHER, B. 2004. *Protein gels,* Woodhead Publishing Ltd.

ALEMU, M. H., OLSEN, S. B., VEDEL, S. E., PAMBO, K. O. & OWINO, V. O. 2017. Combining product attributes with recommendation and shopping location attributes to assess consumer preferences for insect-based food products. *Food Quality and Preference,* 55**,** 45-57.

ALQARNI, A. S., ALI, H., IQBAL, J., OWAYSS, A. A. & SMITH, B. H. 2019. Expression of heat shock proteins in adult honey bee (Apis mellifera L.) workers under hot-arid subtropical ecosystems. *Saudi Journal of Biological Sciences*.

ANDERSEN, S. O., HOJRUP, P. & ROEPSTORFF, P. 1995. Insect cuticular proteins. *Insect Biochemistry and Molecular Biology,* 25**,** 153-176.

ANNADURAI, R. S., OBERTHÜR, W. & REMBOLDM, H. 1992. High resolution two-dimensional mapping of tissue-specific polypeptides in the desert locust, *Schistocerca gregaria*. *Electrophoresis,* 13**,** 456-458.

AOAC 2000. *Approved methods of the American Association of Cereal Chemists / compiled by the Approved Method Committee,* St. Paul, Minn., St. Paul, Minn. : AACC.

ARRONDO, J. L. R. & GOÑI, F. M. 1998. Infrared studies of protein-induced perturbation of lipids in lipoproteins and membranes. *Chemistry and Physics of Lipids,* 96**,** 53-68.

AZAGOH, C., DUCEPT, F., GARCIA, R., RAKOTOZAFY, L., CUVELIER, M. E., KELLER, S., LEWANDOWSKI, R. & MEZDOUR, S. 2016. Extraction and physicochemical characterization of Tenebrio molitor proteins. *Food Research International,* 88**,** 24-31.

BAKER, M. A., SHIN, J. T. & KIM, Y. W. 2016. An Exploration and Investigation of Edible Insect Consumption: The Impacts of Image and Description on Risk Perceptions and Purchase Intent. *Psychology & Marketing,* 33**,** 94-112.

BALZAN, S., FASOLATO, L., MANIERO, S. & NOVELLI, E. 2016. Edible insects and young adults in a north-east Italian city an exploratory study. *British Food Journal,* 118**,** 318-326.

BANERJEE, S. & BHATTACHARYA, S. 2012. Food Gels: Gelling Process and New Applications. *Critical Reviews in Food Science and Nutrition,* 52**,** 334-46.

BERGIER, E. 1941. peuples entomophages et insects comestibles: etude sure les moeurs de l'homme et de l'insecte. *Imprimerie Rulliere Feres***,** 229.

BHATTACHARYA, S. & JENA, R. 2007. Gelling behavior of defatted soybean flour dispersions due to microwave treatment: Textural, oscillatory, microstructural and sensory properties. *Journal of Food Engineering,* 78**,** 1305-1314.

BOLAND, M. J., RAE, A. N., VEREIJKEN, J. M., MEUWISSEN, M. P. M., FISCHER, A. R. H., VAN BOEKEL, M. A. J. S., RUTHERFURD, S. M., GRUPPEN, H., MOUGHAN, P. J. & HENDRIKS, W. H. 2012. The future supply of animal-derived protein for human consumption. *Trends in Food Science & Technology,* 29.

BOVERA, F., PICCOLO, G., GASCO, L., MARONO, S., LOPONTE, R., VASSALOTTI, G., MASTELLONE, V., LOMBARDI, P., ATTIA, Y. A. & NIZZA, A. 2015. Yellow mealworm larvae (Tenebrio molitor , L.) as possible alternative to soybean meal in broiler diets. *British Poultry Science,* 56.

BOYE, J. I., ISMAIL, A. A. & ALLI, I. 1995. Effects of physicochemical factors on the secondary structure of β-lactoglobulin. *Journal of Dairy Research,* 63**,** 97-109.

BRITISH NUTRITION FOUNDATION. 2012. *Protein, British Nutrition Foundation* [Online]. Available: https://www.nutrition.org.uk/nutritionscience/nutrients-food-and-ingredients/protein.html [Accessed 2012].

BROEKMAN, H., KNULST, A., DEN HARTOG JAGER, S., MONTELEONE, F., GASPARI, M., JONG, G., HOUBEN, G. & VERHOECKX, K. 2015. Effect of thermal processing on mealworm allergenicity. *Molecular Nutrition & Food Research,* 59**,** 1855-1864.

BRYANT, C. M. & MCCLEMENTS, D. J. 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. *Trends in Food Science & Technology*, 9**,** 143-151.

BUßLER, S., RUMPOLD, B. A., JANDER, E., RAWEL, H. M. & SCHLÜTER, O. K. 2016. Recovery and techno-functionality of flours and proteins from two edible insect species: Meal worm (Tenebrio molitor) and black soldier fly (Hermetia illucens) larvae. *Heliyon,* 2**,** e00218.

CALIGIANI, A., MARSEGLIA, A., LENI, G., BALDASSARRE, S., MAISTRELLO, L., DOSSENA, A. & SFORZA, S. 2018. Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin. *Food Research International,* 105**,** 812-820.

CAPARROS MEGIDO, R., GIERTS, C., BLECKER, C., BROSTAUX, Y., HAUBRUGE, É., ALABI, T. & FRANCIS, F. 2016. Consumer acceptance of insect-based alternative meat products in Western countries. *Food Quality and Preference,* 52**,** 237-243.

CAPARROS MEGIDO, R., SABLON, L., GEUENS, M., BROSTAUX, Y., ALABI, T., BLECKER, C., DRUGMAND, D., HAUBRUGE, É. & FRANCIS, F. 2014. Edible Insects Acceptance by B elgian Consumers: Promising Attitude for Entomophagy Development. *Journal of Sensory Studies,* 29**,** 14-20.

CAPINERA, J. L. 2004. *Encyclopedia of entomology / edited by John L. Capinera,* Dordrecht, London, Dordrecht, London : Kluwer Academic.

CASAL, H. L., KÖHLER, U. & MANTSCH, H. H. 1988. Structural and conformational changes of β-lactoglobulin B: an infrared spectroscopic study of the effect of pH and temperature. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology,* 957**,** 11-20.

CHAPMAN, R. F. 1998. *The insects : structure and function / R.F. Chapman,* Cambridge, Cambridge : Cambridge University Press.

CHEN, C., LI, D. & SHAO, X. 2013. High-performance nanocomposite films: reinforced with chitosan nanofiber extracted from prawn shells. *Journal of Materials Science,* 49**,** 1-7.

CHOI, B. D., WONG, N. A. K. & AUH, J.-H. 2017. Defatting and Sonication Enhances Protein Extraction from Edible Insects. *Korean Journal for Food Science of Animal Resources,* 37**,** 955-961.

CICATIELLO, C., DE ROSA, B., FRANCO, S. & LACETERA, N. 2016. Consumer approach to insects as food: barriers and potential for consumption in Italy. *British Food Journal,* 118**,** 2271-2286.

DOSSEY, A. T. 2010. Insects and their chemical weaponry: New potential for drug discovery. *Natural Product Reports,* 27**,** 1737-1757.

DUMAY, E. M., KALICHEVSKY, M. T. & CHEFTEL, J. C. 1994. High-Pressure Unfolding and Aggregation of .beta.-Lactoglobulin and the Baroprotective Effects of Sucrose. *Journal of Agricultural and Food Chemistry,* 9**,** 1861-1868.

ELPIDINA, E. N., TSYBINA, T. A., DUNAEVSKY, Y. E., BELOZERSKY, M. A., ZHUZHIKOV, D. P. & OPPERT, B. 2005. A chymotrypsin-like proteinase from the midgut of Tenebrio molitor larvae. *Biochimie,* 87**,** 771-779.

EVANS, J. R. & MATHUR, A. 2005. The value of online surveys. *Internet Research,* 15**,** 195-219.

FAO 2012. *Forest Insects as Food: Humans Bite Back,* Bangkok, Rome, Italy.

FLIGHT, I., LEPPARD, P. & COX, D. N. 2003. Food neophobia and associations with cultural diversity and socio-economic status amongst rural and urban Australian adolescents. *Appetite,* 41**,** 51-59.

GAHUKAR, R. T. 2011. Entomophagy and human food security. *International Journal of Tropical Insect Science,* 31**,** 129-144.

GHOSH, S., JUNG, C. & MEYER-ROCHOW, V. 2016. Nutritional value and chemical composition of larvae, pupae, and adults of worker honey bee, Apis mellifera ligustica as a sustainable food source. *Journal of Asia-Pacific Entomology,* 19.

GOULD, J. & WOLF, B. 2017. Interfacial and emulsifying properties of mealworm protein at the oil/water interface.

GRAHAM, L., LIOU, Y.-C., WALKER, V. & DAVIES, P. 1997. Hyperactive antifreeze protein from beetles. *Nature,* 388**,** 727-728.

GRAHAM, L., WALKER, V. & DAVIES, P. 2000. Developmental and environmental regulation of antifreeze proteins in the mealworm beetle Tenebrio molitor. *European. Journal of Biochemistry,* 267**,** 6452-6458.

HARTMANN, C., SHI, J., GIUSTO, A. & SIEGRIST, M. 2015. The psychology of eating insects: A cross-cultural comparison between Germany and China. *Food Quality and Preference,* 44**,** 148-156.

HARTMANN, C. & SIEGRIST, M. 2016. Becoming an insectivore: Results of an experiment. *Food Quality and Preference,* 51**,** 118-122.

HAUNERL, N. H. & CHISHOLM, J. M. 1990. Fatty acid binding protein in flight muscle of the locust, Schistocerca gregaria. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism,* 1047**,** 233-238.

HE, F. 2011. Laemmli-SDS-PAGE. *Bio-protocol,* 1**,** e80.

HOUSE, J. 2016. Consumer acceptance of insect-based foods in the Netherlands: Academic and commercial implications. *Appetite,* 107**,** 47-58.

JANSSEN, R. H., LAKEMOND, C. M. M., FOGLIANO, V., RENZONE, G., SCALONI, A. & VINCKEN, J.-P. 2017. Involvement of phenoloxidase in browning during grinding of Tenebrio molitor larvae. *PLoS ONE,* 12**,** e0189685.

JANSSEN, R. H., VINCKEN, J.-P., ARTS, N. J. G., FOGLIANO, V. & LAKEMOND, C. M. M. 2019. Effect of endogenous phenoloxidase on protein solubility and digestibility after processing of Tenebrio molitor, Alphitobius diaperinus and Hermetia illucens. *Food Research International,* 121**,** 684-690.

JAYAS, J. F. 1997. Gelling Properties of Proteins. *Functionality of Proteins in Food.* Berlin, Heidelberg: Springer,.

KARACA, A. C., LOW, N. & NICKERSON, M. 2011. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International,* 44**,** 2742-2750.

KIOSSEOGLOU, V. & PARASKEVOPOULOU, A. 2011. *Functional and physicochemical properties of pulse proteins-3*, Elsevier Inc.

KONG, J. & YU, S. 2007. Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta Biochimica et Biophysica Sinica*, 39**,** 549-559.

KOUŘIMSKÁ, L. & ADÁMKOVÁ, A. 2016. Nutritional and sensory quality of edible insects. *NFS Journal,* 4**,** 22-26.

KULMA, M., PLACHY, V., KOUŘIMSKÁ, L., VRABEC, V., BUBOVÁ, T., ADÁMKOVÁ, A. & HUCKO, B. 2016. Nutritional value of three Blattodea species used as feed for animals. *Journal of Animal and Feed Sciences,* 25.

L'HOCINE, L., BOYE, J. I. & ARCAND, Y. 2006. Composition and Functional Properties of Soy Protein Isolates Prepared Using Alternative Deffating and Extraction Procedures. *Journal of Food Science,* 71**,** C137-C145.

LAEMMLI, U. K. 1970. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature,* 227**,** 680.

LEE, H., YILDIZ, G., DOS SANTOS, L. C., JIANG, S., ANDRADE, J. E., ENGESETH, N. J. & FENG, H. 2016. Soy protein nano-aggregates with improved functional properties prepared by sequential pH treatment and ultrasonication. *Food Hydrocolloids,* 55**,** 200-209.

LENI, G., CALIGIANI, A. & SFORZA, S. 2019. Killing method affects the browning and the quality of the protein fraction of Black Soldier Fly (Hermetia illucens) prepupae: a metabolomics and proteomic insight. *Food Research International,* 115**,** 116-125.

LUCEY, J. A. & SINGH, H. 1997. Formation and physical properties of acid milk gels: a review. *Food Research International,* 30**,** 529-542.

MAILONLINE. 2019. *Scientists say your next meal could be a sausage made from maggots,* [Online]. [Accessed].

MAKKAR, H. P. S., TRAN, G., HEUZÉ, V. & ANKERS, P. 2014. State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology,* 197**,** 1-33.

MANCINI, S., SOGARI, G., MENOZZI, D., NUVOLONI, R., TORRACCA, B., MORUZZO, R. & PACI, G. 2019. Factors predicting the intention of eating an insect-based product. *Foods,* 8**,** <xocs:firstpage xmlns:xocs=""/>.

MARIOD, A. A. & FADUL, H. **2014**. Extraction and characterization of gelatin from two edible Sudanese insects and its applications in ice cream making. *Food Science and Technology International,* 2**,** 380-391.

MARIOD, A. A. & FADUL, H. 2015. Extraction and characterization of gelatin from two edible Sudanese insects and its applications in ice cream making. *Food Science and Technology International,* 21**,** 380-391.

MARONO, S., PICCOLO, G., LOPONTE, R., MEO, C. D., ATTIA, Y. A., NIZZA, A. & BOVERA, F. 2015. In vitro crude protein digestibility of tenebrio molitor and hermetia illucens insect meals and its correlation with chemical composition traits. *Italian Journal of Animal Science,* 14**,** 338-343.

MATUSZEK, T. 2001. Rheological properties of protein gels.

MCCORD, A., SMYTH, A. & O NEILL, E. 1998. Heat-induced gelation properties of salt-soluble muscle proteins as affected by non-meat proteins. *Journal of Food Science,* 63**,** 580-583.

MISHYNA, M., MARTINEZ, J.-J. I., CHEN, J. & BENJAMIN, O. 2019a. Extraction, characterization and functional properties of soluble proteins from edible grasshopper (Schistocerca gregaria) and honey bee (Apis mellifera). *Food Research International,* 116**,** 697-706.

MISHYNA, M., MARTINEZ, J.-J. I., CHEN, J., DAVIDOVICH-PINHAS, M. & BENJAMIN, O. 2019b. Heat-induced aggregation and gelation of proteins from edible honey bee brood (Apis mellifera) as a function of temperature and pH. *Food Hydrocolloids,* 91**,** 117-126.

MYERS, G. & PETTIGREW, S. 2018. A qualitative exploration of the factors underlying seniors' receptiveness to entomophagy. *Food Research International,* 103**,** 163-169.

NAZIR, A., ASGHAR, A. & ASLAM MAAN, A. 2016. *Food Gels: Gelling Process and New Applications*.

NDIRITU, A., KINYURU, J., KENJI, G. & GICHUHI, P. 2017. Extraction technique influences the physico-chemical characteristics and functional properties of edible crickets (Acheta domesticus) protein concentrate. *Journal of Food Measurement and Characterization,* 11**,** 2013-2021.

NEW, T. 2011. Patrick B. Durst, Dennis V. Johnson, Robin N. Leslie and Kenichi Shono (eds): Forest insects as food: humans bite back. *An international journal devoted to the conservation of insects and related invertebrates,* 15**,** 483-484.

NONGONIERMA, A. B. & FITZGERALD, R. J. 2017. Unlocking the biological potential of proteins from edible insects through enzymatic hydrolysis: A review. *Innovative Food Science and Emerging Technologies,* 43**,** 239-252.

OMOTOSO, O. T. 2006. Nutritional quality, functional properties and anti-nutrient compositions of the larva of *Cirina forda* (Westwood)(Lepidoptera: Saturniidae). *Journal of Zhejlang University - Science B,* 7**,** 51-55.

OMOTOSO, O. T. 2015. An Evaluation of the Nutrients and Some Anti-nutrients in Silkworm, Bombyxmori L. (Bombycidae: Lepidoptera). *Journal of Biological Sciences,* 8**,** 45-50.

OONINCX, D. G. A. B., VAN ITTERBEECK, J., HEETKAMP, M. J. W., VAN DEN BRAND, H., VAN LOON, J. J. A. & VAN HUIS, A. 2010. An Exploration on Greenhouse Gas and Ammonia Production by Insect Species Suitable for Animal or Human Consumption. *PLOS ONE,* 5**,** e14445.

OSBORNE, T. 1908. Our present knowledge of plant proteins. *American . Joural of Physiology,* 28**,** 417-428.

PAMBO, K. O., MBECHE, R. M., OKELLO, J. J., KINYURU, J. N. & MOSE, G. N. 2016. Consumers' salient beliefs regarding foods from edible insects in Kenya: a qualitative study using concepts from the Theory of Planned Behaviour. *African Journal of Food, Agriculture, Nutrition and Development,* 16**,** 11366-11385.

PIHA, S., POHJANHEIMO, T., LÄHTEENMÄKI-UUTELA, A., KŘEČKOVÁ, Z. & OTTERBRING, T. 2016. The effects of consumer knowledge on the willingness to buy insect food: An exploratory cross-regional study in Northern and Central Europe. *Food Quality and Preference,* 70.

PLINER, P. & HOBDEN, K. 1992. Development of a scale to measure the trait of food neophobia in humans. *Appetite,* 19**,** 105.

POORTVLIET, P. M., VAN DER PAS, L., MULDER, B. C. & FOGLIANO, V. 2019. Healthy, but Disgusting: An Investigation Into Consumers' Willingness to Try Insect Meat. *Journal of Economic Entomology,* 112**,** 1005-1010.

PURSCHKE, B., TANZMEISTER, H., MEINLSCHMIDT, P., BAUMGARTNER, S., LAUTER, K. & JÄGER, H. 2018. Recovery of soluble proteins from migratory locust (Locusta migratoria) and characterisation of their compositional and techno-functional properties. *Food Research International,* 106**,** 271-279.

RENARD, D. & LEFEBVRE, J. 1992. Gelation of globular proteins: effect of pH and ionic strength on the critical concentration for gel formation. A simple model and its application to β-lactoglobulin heat-induced gelation. *International Journal of Biological Macromolecules,* 14**,** 287-291.

ROBINSON, E. & HIGGS, S. 2012. Liking food less: the impact of social influence on food liking evaluations in female students. *PloS one,* 7**,** e48858-e48858.

ROSA, A. P. B., PAREDES-LÓPEZ, O., GUEGUEN, J. & VIROBEN, G. 1992. Fractionation Procedures, Electrophoretic Characterization, and

Amino Acid Composition of Amaranth Seed Proteins. *Journal of Agricultural and Food Chemistry,* 40**,** 931-936.

ROTHEMUND, S., LIOU, Y. C., DAVIES, P. L. & SÖNNICHSEN, F. D. 1997. Backbone structure and dynamics of a hemolymph protein from the mealworm beetle Tenebrio molitor. *Biochemistry,* 36**,** 13791.

RUMPOLD, B. A. & SCHLÜTER, O. K. 2013. Nutritional composition and safety aspects of edible insects.

SCHÖSLER, H., DE BOER, J. & BOERSEMA, J. J. 2012. Can we cut out the meat of the dish? Constructing consumer-oriented pathways towards meat substitution. *Appetite,* 58**,** 39.

SELM, M. & JANKOWSKI, N. 2006. Conducting Online Surveys. *International Journal of Methodology,* 40**,** 435-456.

SIDALI, K. L., PIZZO, S., GARRIDO-PÉREZ, E. I. & SCHAMEL, G. 2019. Between food delicacies and food taboos: A structural equation model to assess Western students' acceptance of Amazonian insect food. *Food Research International,* 115**,** 83-89.

SMITH, P. K., KROHN, R. I., HERMANSON, G. T., MALLIA, A. K., GARTNER, F. H., PROVENZANO, M. D., FUJIMOTO, E. K., GOEKE, N. M., OLSON, B. J. & KLENK, D. C. 1985. Measurement of protein using bicinchoninic acid. *Analytical Biochemistry,* 150**,** 76-85.

SOETRISNO, U. S. S. & HOLMES, Z. 1992. Functional Properties of Acid and Salt Extracted Proteins of Yellow Peas (Pisum sativum L. Miranda). *Journal of Agricultural and Food Chemistry,* 40**,** 975-980.

STAFFORD, D. A., WIMPENNY, J. W. T., HUGHES, D. E., KANE-MCGUIRE, L. & RASHEED, A. C. M. 1979. Protein recovery from biological treatment processes. *Conservation & Recycling,* 3**,** 35-43.

STONE, A. K., KARALASH, A., TYLER, R. T., WARKENTIN, T. D. & NICKERSON, M. T. 2015. Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food Research International,* 76**,** 31-38.

SUMNER, A. K., NIELSEN, M. A. & YOUNGS, C. G. 1981. Production and Evaluation of Pea Protein Isolate. *Journal of Food Science,* 46**,** 364-366.

TANG, Q. 1993. *Rheology of whey protein solutions and gels : thesis submitted for the degree of Doctor of Philosophy in Food Technology at Massey University, New Zealand.* Doctor of Philosophy (Ph.D.) Doctoral, Massey University.

TOTOSAUS, A., MONTEJANO, SALAZAR & GUERRERO 2002. Totosaus, A., Montejano, J. G., Salazar, J. A., Guerrero, I. (2002). A review of physical and chemical protein-gel induction. International Journal of Food Science and Technology. 37 (6), 589-601. *International Journal of Food Science and Technology,* 37**,** 589-601.

TOWNS, J. K. 1995. Moisture content in proteins: its effects and measurement.

TSYBINA, T., DUNAEVSKY, Y., BELOZERSKY, M., ZHUZHIKOV, D., OPPERT, B. & ELPIDINA, E. 2005. Digestive proteinases of yellow mealworm (Tenebrio molitor) larvae: Purification and characterization of a trypsin-like proteinase. *Biochemistry (Moscow),* 70**,** 300-305.

TUORILA, H., LÄHTEENMÄKI, L., POHJALAINEN, L. & LOTTI, L. 2001. Food neophobia among the Finns and related responses to familiar and unfamiliar foods. *Food Quality and Preference,* 12**,** 29-37.

UN DESA, U. N. D. O. E. A. S. A. 2017. *WORLD POPULATION PROJECTED TO REACH 9.8 BILLION IN 2050, AND 11.2 BILLION IN 2100* [Online]. Available: https:// https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html [Accessed 14 June 2019].

VAN CAMP, J. & HUYGHEBAERT, A. 1995. High pressure-induced gel formation of a whey protein and haemoglobin protein concentrate. *LWT - Food Science and Technology,* 28**,** 111-117.

VAN HUIS, A. 2013. *Edible insects : future prospects for food and feed security / by Arnold van Huis [and six others]*, Rome : Food and Agriculture Organization of the United Nations.

VAN THIELEN, L., VERMUYTEN, S., STORMS, B., RUMPOLD, B. & VAN CAMPENHOUT, L. 2018. Consumer acceptance of foods containing edible insects in Belgium two years after their introduction to the market. *Journal of Insects as Food and Feed*.

VERBEKE, W. 2015. Profiling consumers who are ready to adopt insects as a meat substitute in a Western society. *Food Quality and Preference,* 39**,** 147-155.

WANG, M., JIANG, L., LI, Y., LIU, Q., WANG, S. & SUI, X. 2011. Optimization of Extraction Process of Protein Isolate from Mung Bean. *Procedia Engineering,* 15**,** 5250-5258.

WARD, A. G. & COURTS, A. 1977. *The science and technology of gelatin / edited by A.G. Ward, A. Courts,* London, London : Academic Press.

WATTS, S. 2012. *Doing Q methodological research : theory, method and interpretation / Simon Watts and Paul Stenner*, London, London : SAGE.

WEBLER, T., DANIELSON, S. & TULER, S. 2007. Guidance on the use of Q method for evaluation of public involvement programs at contaminated sites. *In:* INSTITUTE, S. A. E. R. (ed.). Massachusetts, USA.

WIJAYANTI, H. B., BANSAL, N. & DEETH, H. C. 2014. Stability of Whey proteins during Thermal Processing: A Review. *Comprehesive Reviews in Food Science and Food Safety,* 13.

WRIGHT, D. J. & BOULTER, D. 1980. Differential scanning calorimetric study of meals and constituents of some food grain Legumes. *Journal of the Science of Food and Agriculture,* 31**,** 1231-1241.

YI, L., LAKEMOND, C. M. M., SAGIS, L. M. C., EISNER-SCHADLER, V., VAN HUIS, A. & VAN BOEKEL, M. A. J. S. 2013. Extraction and characterisation of protein fractions from five insect species. *Food Chemistry,* 141**,** 3341-3348.

YI, L., VAN BOEKEL, M., BOEREN, S. & LAKEMOND, C. 2016. Protein identification and in vitro digestion of fractions from Tenebrio molitor. *European Food Research and Technology = Zeitschrift für Lebensmittel-Untersuchung und -Forschung. A,* 242**,** 1285-1297.

ZAICHKOWSKY, J. L. 1994. The Personal Involvement Inventory: Reduction, Revision, and Application to Advertising. *Journal of Advertising,* 23**,** 59-70.

ZHAO, X., VÁZQUEZ-GUTIÉRREZ, J. L., JOHANSSON, D. P., LANDBERG, R. & LANGTON, M. 2016. Yellow Mealworm Protein for Food Purposes - Extraction and Functional Properties. *PLoS ONE,* 11**,** e0147791.

ZIELIŃSKA, E., BARANIAK, B., KARAŚ, M., RYBCZYŃSKA, K. & JAKUBCZYK, A. 2015. Selected species of edible insects as a source of nutrient composition. *Food Research International,* 77**,** 460-466.