CAPE PENINSULA UNIVERSITY OF TECHNOLOGY

Determination and characterisation of the function of black soldier fly larva protein before and after conjugation by Maillard reaction

Bongisiwe Zozo

Thesis submitted in fulfilment of the requirements for the degree Master of Applied Science in Chemistry in the Faculty of Applied Science

Supervisor

Prof Merrill Wicht (CPUT, Department of Chemistry)

Co-Supervisor

Prof Jessy van Wyk (CPUT, Department of Food Science and Technology)

August 2020

CPUT copyright information

DECLARATION

I, Bongisiwe Zozo, declare that the content of this thesis represents my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Bongisiwe Zozo
Signed

31 August 2020
Dated

ABSTRACT

The increasing global population and consumer demand for protein will render the provision of protein a serious future challenge, thus placing substantial pressure on the food industry to provide for the human population. The lower environmental impact of insect farming makes the consumption of insects such as Black soldier fly larvae (BSFL) an appealing solution, although consumers in developed countries often respond to the idea of eating insects with aversion. One approach to adapt consumers to insects as part of their diet is through application of making insect-based products in an unrecognised form. Nutritional value and structural properties of the BSFL flours (full fat and defatted) were assessed. The BSFL flours were obtained by freeze drying and by the removal of fat using hexane and isopropanol solvent (at ratio 3:2 v/v). The nutritional analysis (protein, fat, fiber, ash and moisture) and structural analysis (Universal Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (UATR-FTIR), Thermal gravimetry (TG) and Differential scanning calorimetry (DSC) were carried out using standard methods proposed by Association of Official Analytical Chemists (AOAC). This part of the study has shown that the defatted BSFL flour holds good nutritional value and enhanced structural properties, therefore can be incorporated into food products. Thereafter, the defatted flour was subjected to an alkaline protein extraction procedure. The protein functional properties were improved by inducing the Maillard reaction in protein-carbohydrate mixtures. An aqueous solution of the protein alone and protein:glucose (2:1 w/w, pH 9) mixtures were heated at 50, 70 and 90 °C for 30, 60, 90, and 120 min. The products obtained were then characterised and compared. The pH and zeta potential change were monitored. The protein extract displayed a good essential amino acid (EAA) profile. UATR-FTIR was able to discriminate between glycated and non-glycated proteins, when the data were analysed by the multivariate statistical method, principal component analysis (PCA). FTIR, TGA, DSC and Scanning electron microscopy (SEM) analysis indicated the formation of Maillard reaction products (MRP). The thermal stability results suggested that the Maillard reaction with the incorporation of glucose can be a promising way to improve the properties of the BSFL protein.

ACKNOWLEDGEMENTS

I wish to thank:

Professor M. Wicht, my supervisor, Senior Lecturer at the Department of Chemistry, Cape Peninsula University of Technology for her support and mentoring, which has helped me tremendously in my research study. Without her input and encouragement throughout this study, I would have failed without a shadow of doubt;

Professor J. van Wyk, my co supervisor and the Head of Department at Food Science and Technology, Cape Peninsula University of Technology for her guidance, support and assistance during the course of my study;

Mr V. Mshayisa, Lecturer at the Department of Food Science and Technology, Cape Peninsula University of Technology, I am forever grateful to him for always believing in me and his assistance, guidance, patience and his willingness to assist at all times in the laboratory.

Mr N. Mshicileli, Senior Technologist at Agri Food Technology Station for his expertise and assistance with analysis and interpretation of results.

The financial assistance of the National Research Foundation (NRF) and Mauerberger towards this research is acknowledged.

At last, I would like to thank God, my parents, family and friends for their endless love, support and encouragement.

DEDICATION

To my late grandmother, Nokruxeshe Zozo

LIST OF OUTPUTS

The following outputs reflect the contributions by the candidate to scientific literacy and progress during her Masters degree (2018-2020)

The following conference presentations were delivered on research studies related to this thesis.

"The nutritional quality and techno functional properties of Black soldier fly larva before and after defatting", Poster presentation at the First African Conference on Edible Insects, Harare, Zimbabwe (14 - 16 August 2019). **Bongisiwe Zozo**, Merrill Wicht, Vusi Mshayisa and Jessy Van Wyk.

Bongisiwe Zozo received an award for the best poster at this conference.

"Nutritional and structural characterisation of Black soldier fly larva before and after freeze drying", Poster presentation at SAAFoST's 23rd Biennial International Congress and Exhibition, Johannesburg, South Africa (1 – 4 September 2019). **Bongisiwe Zozo**, Merrill Wicht, Vusi Mshayisa and Jessy Van Wyk.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

1.1. Background

The world population is increasing rapidly and by the year 2050, it is expected to reach 9 billion (DESA, 2009). The increasing human population is causing high demand for food thus placing extensive pressure on the food industry to provide (Cribb, 2010). End to end with this increase, is the global disposable income (Alexandratos & Bruinsma, 2012). Consumers with higher incomes continue to eat large quantities of animal products and consumers currently eating at lower rates will increase their consumption as their income increases, causing a subsequent elevation of the animal protein demand. The animal production industry experiences elevated nutritional costs which is a reflection of this increase due to the disagreement developing for the limited plant protein source distribution between human and animal consumption (FAO, 2009).

Cattle are one of the primary sources of protein for human consumption, providing fundamental source of essential amino acids, hence prominence is also directed on the demand for cattle meat (Hoffman, 2012). This then forms an unkind circle in which involvement is required through global adaptation. These concerns are further enlarged by the effect of global climate change, which might lead to decrease of crop yield over the next 50 years (Dar, 2013). The challenges of freeing the world population from hunger may only be addressed by a global agricultural production increase of 70 to 100% by the year 2050 (Bruinsma, 2009). This global production increase requires improvements in the efficiency and cost-effectiveness of food production systems having a minimal environmental effect (Berg et al., 2013). The utilization of the limited resources, such as water and lands, are improved by agricultural investments as a requirement. To counteract the adverse climate change effects, extensive research should be conducted into production systems that are sustainable, so that global food security may be sustained (Dar, 2013). Climate change affects food availability and accessibility and the stability of the food system directly through changes in productivity, quality of yield, crop failures, loss of livestock, farming costs and the effects of changing weather conditions on agricultural practices.

Therefore, to feed the ever-increasing human population, production would have to be increased by the agricultural industry to an output of approximately 200 million tonnes of meat

from livestock per annum (Bruinsma, 2009). Thus, the demand will increase continually, increasing prices for these meat proteins (Hoffman, 2012). Despite the aversion that many humans have towards the consumption of insects, one of the options is to serve insect meals to achieve effective sustainable production of protein (Newton et al., 2005).

Therefore, this research aims to explore the use of a cheaper, available source of protein, namely Black soldier fly larvae (BSFL). Black soldier fly (BSF) protein (also known as mag-meal) is a derived product obtained from insects fed on organic waste. After removal of the chitinous integument (the natural covering of an organism or an organ, such as its skin), followed by defatting of the larvae, the product that remains is the mag-meal. Other than protein from some conventional sources, mag-meal is a high-quality protein source with a guaranteed sustainability by which great quantities of the product have been produced in South Africa.

In this study, the extracted protein obtained from BSFL was characterised and then it was conjugated with a suitable sugar by the Maillard reaction. The Maillard reaction is one of the most important processes that takes place in food processing and storage. In 1912, Louis Camille Maillard discovered chemical changes during food processing, describing the reaction between the protein and sugars at the elevated temperatures typical of food processing (Delgado-Andrade et al., 2010). The conjugation of protein with glucose is done to modify protein using internal changes in protein structure by physical and enzymatic means. This will alter their functional properties in order to increase food applications. Enzymatic protein modification may entail partial proteolysis, incorporation of crosslinks within the protein conformation, or attachment of specific functional groups to the protein. Physical techniques to change protein functionality include thermal treatment, biopolymer complexing, and texturisation (Kester, 1984).

In regards to consumption of insects, it is interesting that there are a number of countries (South Asia and Sub-Saharan Africa countries) where they form a staple food (Tao & Li, 2018). Africa has a long history of eating many edible insects (Van Huis, 2013) such as caterpillars, termites, crickets and palm weevils. As a food source, BSF larvae are nutritious and rich in protein and they provide minerals and vitamins. For consumer acceptance, protein extracts from BSFL for further use in food products (conjugation or as a meal ingredient on its own) may be relevant for countries that have no history of consuming insects (Del Valle, 1982). Compared to other insects, BSFL protein has already been considered to be the animal grade alternative to other animal feeds due to its high protein and lipid content even when fed from plant-based streams (Kroeckel, 2012).

BSF larvae have also been used as the partial replacement for maize or soy-based feeds, mainly because the species naturally colonizes and breaks down poultry manure resulting in populations being kept by poultry farms for the purpose of waste management and pollution reduction (Bradley, 1984). BSF larvae are also known to reduce the nutrient content and mass of swine manure with efficiencies similar to that for poultry manure (Zhou, 2012) and with benefits for improved farm hygiene and reduced house fly populations.

1.2. Research Design

The research process used to carry out this study involved sampling, sample pre-treatment, sample treatment and sample analysis.
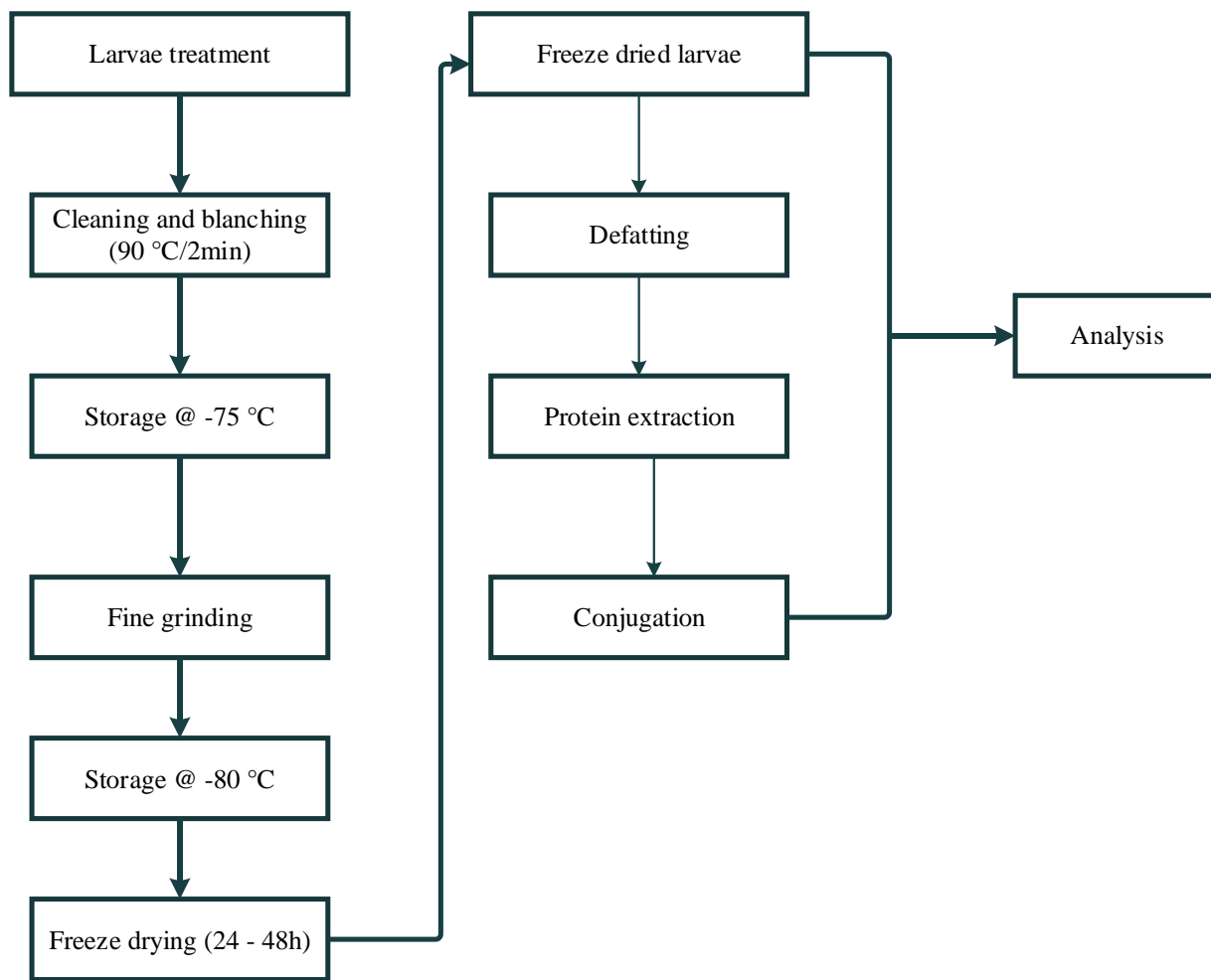
Figure 1.0 Brief design of sampling, sample pretreatment, sample preparation and sample analysis.

1.3. Research problem

Although protein is available and affordable in the form of fish, meat, poultry and legumes, its per capita availability is decreasing due to population growth. Therefore, to secure the future supply of proteins for food and feed, new sources of protein are needed. Although high-protein products such as nuts, soy and beans are readily available, protein from algae, duckweed and insects are also accessible on a smaller scale. To increase the consumption scale of the native protein, this study conducted research on BSF larvae to be used as a source of protein which was conjugated with glucose to form a functional source of human or animal nutrients.

1.4. Broad objective of the study

The aim of this study was to extract and characterise the native and conjugated protein from BSF larvae. Conjugation produced a modified protein with new functional properties which will eventually be used as an ingredient in food products. The ingredient thus has two properties i.e. high value in terms of nutrition as well as a functional property due to conjugation.

1.4.1. Specific objectives

- The first specific objective was to extract oil (by-product) from insect larvae (BSF) using hexane:isopropanol solvent mixture and characterise the flours (full fat and defatted).
- The second specific objective was to use the defatted BSF larvae sample and extract protein by alkaline precipitation method.
- The third specific objective was to determine and characterise the extracted native protein.
- The fourth specific objective was to conjugate the extracted native protein with a sugar, namely glucose.
- The fifth specific objective was to subject the protein and glycated conjugates to determination and characterisation by analytical instrumentation.

1.5. Hypothesis

The Food and Agricultural Organization (FAO) dictate that animal and human food contains a range of nutrients including protein, lipids and carbohydrates. In this study, the characterisation of native and conjugated larval protein were investigated. It was hypothesized that conjugated BSF larvae protein will have improved functionality such as thermal stability.

1.6. Assumptions

Insect protein can be used for animal and human food if it is properly prepared and tested. The extraction of protein from BSF larvae to be developed for animal and human food is currently assumed to be an option for increasing availability of protein.

1.7. Delineation of the Research

All studies were carried out on BSFL, no studies were conducted on other insects. The hexane and isopropanol mixture was used for the defatting stage. The protein extraction was performed using the alkaline precipitation method. The Maillard reaction products were obtained using a single sugar protein conjugate by utilizing predetermined reaction temperatures (60, 70 and 90 °C), and time intervals (30, 60, 90 and 120 min) at a fixed pH (pH 9). All instrumentation studies were limited to High Performance Liquid Chromatography (HPLC), Leco protein analyzer, Universal Attenuated Total Reflectance Fourier-Transform Infrared spectroscopy (UATR-FTIR), Differential Scanning Calorimetry (DSC), Thermal Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and Zeta Potential.

1.8. Significance of Research

The importance of this research is to reach a situation where the diminishing levels of protein sources for the growing population has been considered and a means of alternative protein implementation is developed. The reduction of environmental waste thus lowering waste pollution is an important benefit of this research. The formation of conjugated protein from the insects will produce an outcome suitable for human consumption.

1.9. Expected outcomes, results and contributions of the research

Research study of Maillard reaction conditions between glucose and protein will be conducted.

Identification and characterisation methods of the native and conjugated protein using the analytical instruments will be established.

A potential solution to problems of protein shortage will be reached.

Development of an acceptable human food source or ingredient(s) is an outcome of the research.

Publication of research paper/s.

Masters graduation.

1.10. Ethical considerations

Due to the animal (insect) use in the study, an approval to conduct the research was obtained from Cape Peninsula University of Technology Ethics Committee before commencing data collection.

The Committee requires that research participants are assured of protection from any potential negative consequences that may arise as a result of participating in the research.

1.11. Overview of the study

Figure 1.2 shows the structure of the thesis. The study was conducted and reported as follows:

Chapter One - Orientation and background of the study

This chapter identified the research topic and an introduction was given about the study. The study problem and aim of the research was defined and the objectives stated. The researcher defined the delineation of the study, which research methods were used and why, and discusses ethical considerations as well the significance of the research.

Chapter Two – Literature overview

Chapter two reviewed background relating to the BSFL, the growing stages and its acceptance by the continent of Africa. A discussion with regard to the ways to change people's perspective regarding the reason or the need behind the consumption of BSFL. Furthermore, the Maillard reaction was briefly reviewed.

Chapter Three – First research chapter

This chapter detailed the evaluation of full-fat and defatted BSF flour, their nutritional value, structural and thermal stability.

Chapter Four –Second research chapter

In this section, the detailing of protein extraction from the defatted flour was given. Conjugation of the extracted protein with glucose was detailed. FTIR, PCA, TGA, DSC, SEM, Zeta potential and HPLC (for amino acids) instrumentation used for analysis before and after conjugation were discussed.

Chapter Five – General summary and conclusion

Chapter five contains the summary of the research, giving general conclusion and recommendations of the study.
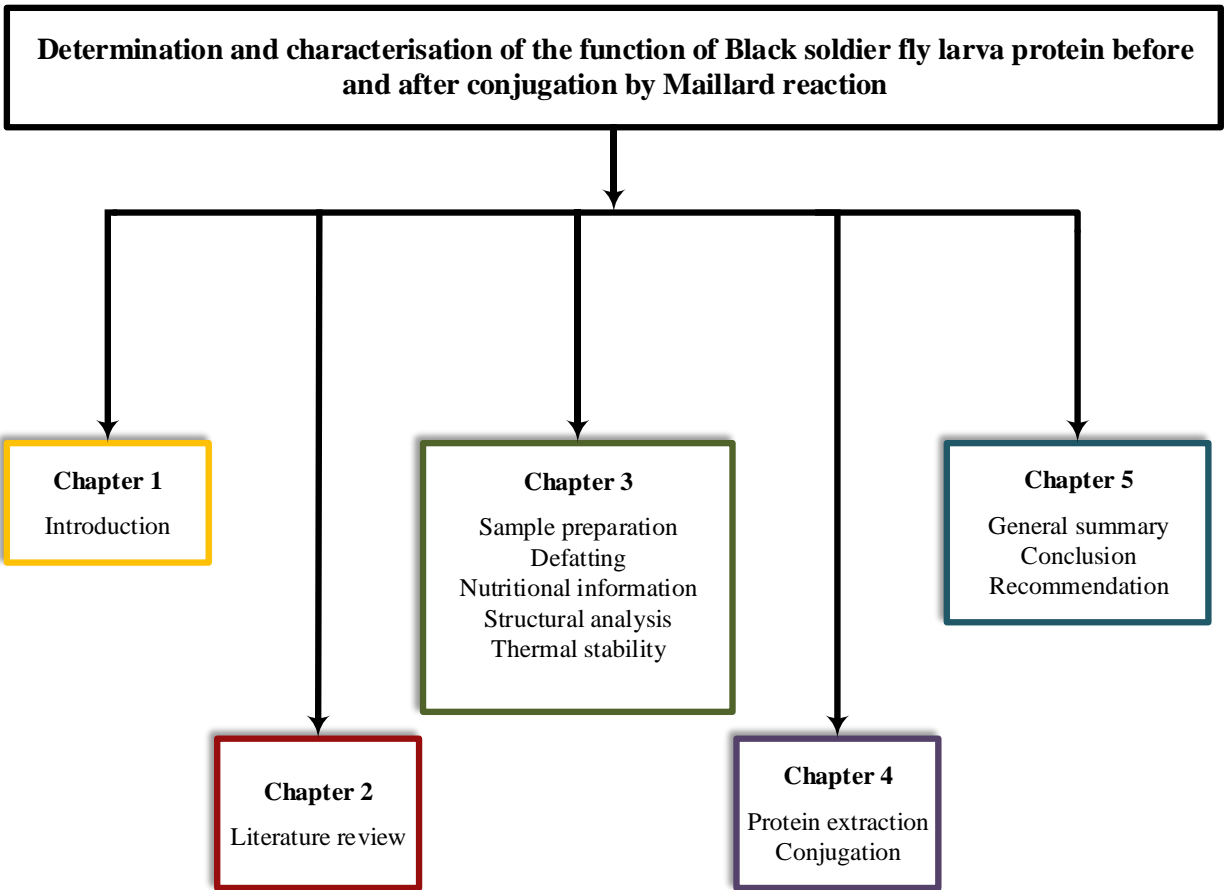
Figure 1.2 The structure of the thesis and the study that was conducted.

References

Alexandratos, N. & Bruinsma, J. 2012. World agriculture: Towards 2010. *Food Policy*, 22(1): 101–103.

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.

Berg, A., de Noblet-Ducoudré, N., Sultan, B., Lengaigne, M. & Guimberteau, M. 2013. Projections of climate change impacts on potential C4 crop productivity over tropical regions. *Agricultural and Forest Meteorology*, 170: 89–102.

Bradley, S.W., Booth, D.C. & Sheppard, D.C. 1984. Parasitism of the Black Soldier Fly by Trichopria sp. (Hymenoptera: Diapriidae) in Poultry Houses. *Environmental Entomology*, 13(2): 451–454.

Bruinsma, J. 2009. The resource outlook to 2050: by how much do land, water and crop yields need to increase by 2050? *How to feed the World in 2050. Proceedings of a technical meeting of experts, Rome, Italy, 24-26 June 2009*: 1–33.

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.

Cribb, J. 2010. The coming famine : the global food crisis and what we can do to avoid it. *University of California Press. Clinical Biochemist Reviews*, 80(3): 120–103.

Dar, W.D. & Gowda, C.L.L. 2013. *Combating Climate Change*. M. S. Kang & S. S. Banga, eds. CRC Press, 40(3) : 7–12.

Food and Agriculture Organization of the United Nations. 2009. The state of food and agriculture, 1974. *Lancet (London, England)*, 2(2): 313–4.

Gahukar, R.T. 2011. Entomophagy and human food security. *International Journal of Tropical Insect Science*, 31(03): 129–144.

Gu, F.L., Kim, J.M., Abbas, S., Zhang, X.M., Xia, S.Q. & Chen, Z.X. 2010. Structure and antioxidant activity of high molecular weight Maillard reaction products from casein-glucose. *Food Chemistry*, 120(2): 505–511.

Hoffman, L. 2012. Consumer preferences in meat. *Proceeding of the 2nd International Seminar on Animal Industry*, 88:9-22.

Kester, J.J. & Richardson, T. 1984. Modification of Whey Proteins to Improve Functionality. *Journal of Dairy Science*, 67(11): 2757–2774.

Maillard, J.C. 1912. Formation de matieres humiques par action de polypeptides sur sucres. *Comptes rendus de l'Académie des Sciences*, 156:148–149.

Newton, G.L., Sheppard, D.C., Watson, D.W., Burtle, G.J., Dove, C.R., Tomberlin, J.K. & Thelen, E.E. 2004. The Black Soldier Fly, Hermetia Illucens, as a Manure Management / Resource Recovery Tool. *Manure management system utilizes wild fly populations*, 16(2): 0–5.

Purschke, B., Meinlschmidt, P., Horn, C., Rieder, Oskar & Jäger, Henry. 2018. Improvement of techno-functional properties of edible insect protein from migratory locust by enzymatic hydrolysis. *European Food Research and Technology*, 244: 999–1013.

Purschke, B., Meinlschmidt, P., Horn, C., Rieder, O. & Jäger, H. 2017. Improvement of techno-functional properties of edible insect protein from migratory locust by enzymatic hydrolysis.

European Food Research and Technology, 22(3): 1–15.

Valle, F.R., Mena, M.H. & Bourges, H. 1982. An investigation into insect protein. *Journal of Food Processing and Preservation*, 6(2): 99–110.

Van Huis, A. 2013. Edible insects. Future prospects for food and feed security. *Annual Review of Entomology*, 171(2): 1–201.

Van Huis, A. 2003. Insects as Food in sub-Saharan Africa. *International Journal of Tropical Insect Science*, 23(03): 163–185.

Wang, Y.-S. & Shelomi, M. 2017. Review of Black Soldier Fly (Hermetia illucens) as Animal Feed and Human Food. *Foods*, 6(10): 91.

Zhou, F., Tomberlin, J.K., Zheng, L., Yu, Z. & Zhang, J. 2013. Developmental and Waste Reduction Plasticity of Three Black Soldier Fly Strains (Diptera: Stratiomyidae) Raised on Different Livestock Manures. *Journal of Medical Entomology*, 50(6): 1224–1230.

CHAPTER 2

Literature review

2.1 Introduction

Globally, there is a rapid increase in the human population, leading to an immense demand on food supply, which has placed extreme pressure on the agriculture sector in ensuring food security (Dar, 2013). The global animal production industry is suffering from a shortage in the supply of feed ingredients for animal use, due to the increase in human population, which has resulted in suboptimal animal protein quantities being produced for human consumption (Capper, 2013). This vicious cycle has placed emphasis on the key term "sustainable", which may be defined as "meeting the needs of the present without compromising the ability of future generations to meet their own needs" (Burton, 1987). Thus, it has become increasingly important to find good-quality, renewable protein sources that are of at least equal in quality to already existing ones, which are able to substitute the traditional sources used in animal nutrition (Newton et al., 2005). An opportunity has presented itself, to explore the broad spectrum of other possible protein sources for human nutrition as these sources can be obtained from a variety of organisms, both flora and fauna (Newton et al., 1984; Pieterse, 2014).

Entomophagy, meaning consumption of insects, has been practiced by humans on every inhabited continent including the African and American continents, not only historically (Ramos-Elorduy et al., 1997), but also until the present day in African countries (Yen et al., 2010; Johnson, 2010; Meyer-Rochow & Chakravorty, 2013). Insects are taken as taboo or at least an unappetizing meal in the developed countries. Over the past years, however, starting perhaps with Holt's essay in 1885, "Why Not Eat Insects?", the discussion of the role insects should play in modern, urban, or Western food has increased. Consumers where food is not conditioned by religion, tradition, fashion or in a word by culture are more accepting to the idea of insect consumption and believe that they have benefits over other foods. These consumers are showing greater interest in producing insect dishes (Vane-Wright, 1991; Lensvelt & Steenbekkers, 2014; Caparros Megido et al., 2014); this may be out of personal curiosity, ecological concerns (Tucker, 2014) or any other possible reason (Verbeke, 2015).

The interest in entomophagy has reached a point where suppliers of insects cannot keep the pace with the demand, and some edible species (freshwater and marine species) are already under threat of overexploitation (Johnson, 2010). Networking organisations like international conferences have been held (Huis, 2013; Halloran et al., 2015) and cookbooks about insect recipes have been printed (Gahukar, 2011; Nadeau et al., 2015; Shelomi, 2016; Huis, 2013). Significant attention has been paid to the potential of edible insects as a solution to present or future food crises (Gahukar, 2011), in particular fears of global food insecurity due to climate change and rising populations (Shelomi, 2016).

Besides the fact that they taste good when prepared properly, insects are high in protein, good fats, and certain trace elements. They have a minimal environmental impact; this is their advantage over animal meats, which underlies their frequent championing as saviours in a food-insecure world. One presentable problem with industrializing edible insects today, is the knowledge of insect choice relative to its health benefits and ease of extraction and preparation. While thousands of species are consumed worldwide (Nadeau et al., 2015) all but a dozen or so are caught in the wild by more traditional societies and cannot at this time be farmed, with consequences for regular supply and for conservation (Defoliart, 1995). The species usually sold and consumed in the West, such as house crickets and mealworms, are thus not necessarily the most sustainable species nor those with the most desirable organoleptic properties such as taste and texture. Therefore, authors of different fields have noted that, if done improperly, entomophagy can be environmentally damaging rather than helpful (Gahukar, 2011).

Furthermore, the agricultural sector yields a high tonnage of waste that is not utilised but has the potential to be used and recovered in a different sector (Cordell et al., 2009). There are many methods provided by nature to manage animal origin waste products. These may include bacteria, protozoa, fungi and insects (Bondari & Sheppard, 1987). Therefore, an effective, eco-friendly means of solving the problem of waste management has been presented by nature and insects have been given the opportunity to be a useful, sustainable protein source (Bondari, 1987).

There are studies that have proven the insect larvae meals consumed by pigs to be a renewable protein source (Newton et al., 1977). In addition, they are used in poultry as supplements (Awoniyi et al., 2004) and fish (Bondari, 1987) nutrition. The production of these animals, their good growth and development is as a result of larvae flours they utilised (El Boushy, 1991) and proved beneficial in certain environmental (El Boushy, 1991), health and economical

(Newton et al., 2005) aspects. Thus, results achieved have led to insects being utilised as sustainable organic waste decomposers (waste management potential) and as a recovery tool for useful nutrients. However, the protein obtained from these insects has not yet been looked at as to be presented for human consumption. One model would be to conjugate the extracted protein with sugars to make it suitable for human consumption.

Due to the lack of studies carried out in conjugation of insect protein, there is very little documentation with similar results. This chapter therefore, aims to give a review on the Black soldier fly larvae (BSFL), the nutritional quality of the insects, the conjugation of the protein and potential analysis conducted thereafter.

2.2 Insect species suitable for nutrient reintroduction

In the nutrient system of recirculation, there are various organisms that are suitable for consumption, specifically there are three orders to take note of, namely *Diptera*, *Coleoptera* and *Haplotaxida* (Bondari & Sheppard, 1987). Insects that are commonly known as true flies or two-winged flies are included in the order of *Diptera.* Fruit flies, Black soldier flies (BSF), house flies, midges and mosquitoes are insects that are familiar in this group (Resh & Cardé, 2003). *Diptera* insects are described as abundant, because they have the ability to colonize any habit on earth (Scholtz & Holm, 2003). BSF are included in the *Stratiomyidae* family, near the larval habitats is where the adults are found, mostly in damp places, in soil, in wetlands, under bark, in animal manure and in decaying organic matter, they are therefore found in a wide array of locations (Rozkošný, 1982). Only insects from the *Stratiomyidae* family will be discussed further, for the purpose of this research.

2.2.1 *Stratiomyidae* family

*Hermetia illucens***,** the BSF, is a widespread and common fly without any functioning mouthparts, hence it is non-biting flies (Bondari & Sheppard, 1987). These flies are ubiquitous and their tendency is to gather around suitable breeding sites, which include garbage heaps, faeces (manure) and decaying matter. The larvae of these flies are also common *detrivores* in compost heaps and are also found in association with carrion, where they have a significant potential for their use in forensic entomology (Lord et al., 1994). The BSF has potential for great nutrition as a source of protein in animal nutrition according to researchers, where it has already proved itself in studies undertaken in pigs (Newton et al., 1977), poultry (Hale et al., 1973; Pieterse et al., 2014) and fish

(St-Hilaire et al., 2007) nutrition. BSF is already being used for animal nutrition but not for human consumption.
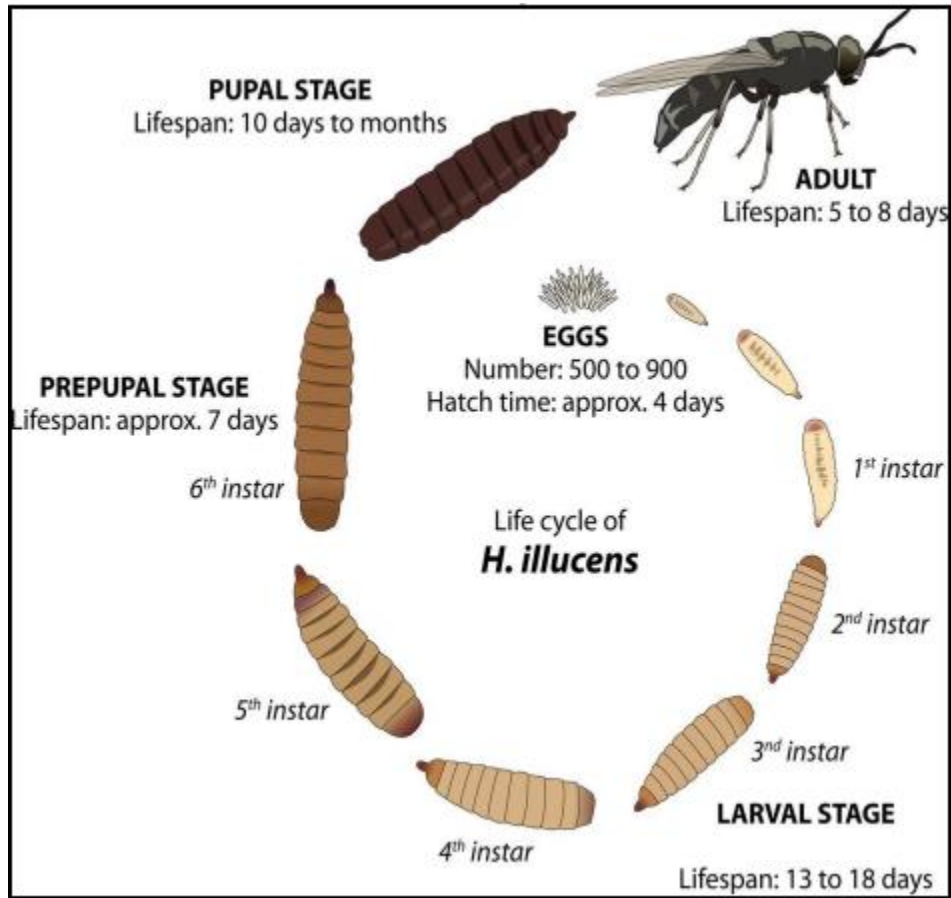
Figure 2.1 Description and life cycle of Black Soldier Fly, *Hermetia illucens* (Adopted from St-Hilaire et al., 2007).

Figure 2.1 provides a visual of the life cycle of BSF, which is approximately 13 to 18 days (St-Hilaire et al., 2007). The number of eggs produced by the female range from 350 to 700, producing in the short life of 13 to 18 days. By difference, the house fly, *Musca domestica*, lives up to almost thirty days and during those days they must eat, and by doing so, they are engaged actively in the spread of diseases between animals (Sheppard et al., 2002). The fact that BSFs do not consume their food in their short life span as an adult is the reason behind them not being vectors of human pathogens (Bondari & Sheppard, 1987). On the surface, the BSF eggs are usually deposited in close proximity to decaying matter, such as manure and take about four days to hatch (Sheppard, 1992). The larvae that are light cream colour, crawl onto the waste that is the site where they are able to utilize the feed at a very high speed. Under normal conditions, it takes the larvae two weeks

to reach maturity, thus, if the temperature conditions are not normal and if there is not enough food, then the two-week period may increase up to several months (Sheppard et al., 2002). BSF pass through four stages, namely the egg, larvae (six instars), pupae and adult stage (Hall & Gerhardt, 2002). The intervals between moults are called instars. When they are matured, they are about 6 mm in diameter and 25 mm in length and weigh approximately 0.2 g. The larvae and pupae can survive under conditions of high oxygen deprivation, they are very tough and robust.

The Black soldier fly larvae (BSFL) is a mimic of organ pipe mud dauber wasp (*Trypoxylon politum*), they are close in size, colour and appearance (Sheppard et al., 2002). This particular kind of wasp mimicry is so enhanced that the fly's antennae are long and wasp-like, its hind tarsi are pale and the fly has two transparent 'windows' in the basal abdominal segments that make the fly appear to have a narrow wasp-like waist. This mimicry acts as a predator repellent, providing the fly with time that is enough to breed in its short life span (Sheppard et al., 2002).

2.2.2   Benefits of the black soldier fly

BSF are essential and they have proven themselves to have high nutritional value and in a number of biological processes they are also beneficial. These processes have been established to aid in the production of animal systems. They aid by controlling insect pests, being part of the food chain and can also act as pollinators (second, right behind bees, as the main pollinators of plants), recyclers and scavengers (Sheppard et al., 2002). The BSF larvae and fully-grown flies are considered to be useful in the following ways:

They avert blow flies and houseflies from laying eggs in the inhabited BSFL material. They are called detrivores and the female bearing an egg is close to manure and decomposing waste, however their attraction is not to human habitation or foods (Sheppard et al., 2002). BSF, contrary to other fly species, are not considered pests, because they do not fly around as compared to other house flies and they do not bite and are sanitary. Their number is reduced easily by killing the larvae in a grub bin, just before they grow into flies (Bondari & Sheppard, 1987).

BSF, unlike any other fly species, is not a disease transporter and the larvae have resulted in magnificent reductions of *Escherichia coli* and *Salmonella enterica* in manure by enhancing the intestinal digestion (Bondari & Sheppard, 1987). They decrease the volume and weight of would-be waste quickly, where the larvae break down its food and then converts it to a valuable nutritional source, carbon dioxide respired by the grubs and symbiotic microorganisms (Sheppard et al., 2002).

2.3 Chemical composition of Black soldier fly larvae: Factors affecting the chemical composition

Data on the physico-chemical composition of insect larvae meal (dried flour) and its suitability as a protein source varies throughout the available publications. These variances are related to the differences in the species, age at harvest (Calvert & Martin, 1969; Inaoka et al., 1999; Aniebo et al., 2008), method of drying (Fasakin et al., 2003) and larval feed substrate (Newton et al., 1977; Pieterse, 2014; Fasakin et al., 2003). Moreover, also the method of processing the species would have an impact on the chemical composition of the larvae meal (Table 2.1), where authors attributed this to the dilution effect of either the water or the fat on the remaining nutrients. However, the raw material (feed formulation) processing is essential as it allows for processors to adjust the chemical composition of the materials in order to make them more suitable for consumption by different species and different production stages of livestock. Table 2.1 illustrates the different methods of processing the fly larvae meal for fat or lipids and moisture extraction consequently resulted in variations in protein content between 43.30% and 45.75% on a dry matter basis. The crude protein content of the defatted larvae meal is high with a decrease in the crude fat content of the meal. Generally, when oil is removed from the larvae meal, the percentage protein content relative to the fat content increases (Shiau et al., 1990).

Table 0.1 Influence of processing method on chemical composition of BSF larvae meal. Values represent mean ± standard deviation (Fasakin et al., 2003).

Larvae processing method	Moisture (%)	Crude Protein (%)	Crude fat (%)	Ash (%)
Full fat oven-dried	8.25 ± 0.02	43.45 ± 0.03	14.30 ± 0.03	14.35 ± 0.02
Hydrolysed oven-dried	8.06 ± 0.05	45.60 ± 0.02	13.28 ± 0.03	13.20 ± 0.02
Defatted oven-dried	9.20 ± 0.01	45.75 ± 0.03	7.00 ± 0.02	13.35 ± 0.02
Full fat sun-dried	8.55 ± 0.04	43.30 ± 0.01	14.35 ± 0.03	14.65 ± 0.01
Hydrolysed sun-dried	8.40 ± 0.01	44.30 ± 0.06	13.65 ± 0.01	13.25 ± 0.01
Defatted sun-dried	9.65 ± 0.04	45.10 ± 0.05	7.40 ± 0.01	13.45 ± 0.02

Additionally, Aniebo and Owen (2010) published that the nutritional value of the fly larvae meal is also affected by, not only the processing method in which water is removed, but also the size, stage of growth and age at which they are harvested (Table 2.2); as they grow the percentage protein content of the larvae decreases ($P<0.05$). These authors reported that the protein content decreased, from 59.6 to 50.8% dry matter (DM), and an increase in the fat content, from 22.4 to 27.3% on a dry matter basis, when water was removed from the larvae at two, three and four days

of age, respectively, as shown in Table 2.2. The causes for this decrease are related to the fact that as the larvae approach the pupae phase in metamorphosis they begin to store more energy in the form of lipids (Pearincott, 1960). Secondly, the larvae utilize the proteins in enzymatic reactions in the formation of the chitin layer (Kramer & Koga, 1986). Aniebo and Owen (2010) further illustrated that, compared with oven drying, sun-drying resulted in larvae with lower protein and higher fat content.

Table 0.2 Average (± standard error) crude protein and fat content (% DM basis) of BSF larvae as affected by age and method of drying (Aniebo & Owen, 2010).

	Day 2 Harvested	Day 3 Harvested	Day 4 Harvested
Oven-dried			
Crude protein	59.6[a] ± 0.05	54.2[b] ± 0.03	50.8[b] ± 0.04
Crude fat	22.4[a] ± 0.14	23.9[b] ± 0.14	27.3[c] ± 0.35
Sun-dried			
Crude protein	55.3[a] ± 0.14	51.3[b] ± 0.04	45.5[c] ± 0.74
Crude fat	25.2[a] ± 0.14	28.0[b] ± 0.14	32.0[a] ± 0.35

[a-c] Means within a row, different superscript differ ($P<0.05$)

The effects are often not linear, an experiment done to use food waste mixes with known protein/fat compositions found that using high protein resulted in high proteinaceous BSFL, but the fat percentage in the substrate did not correlate with the larval fat percentages (Oonincx et al., 2015). There are a lot of sources that cause variation, even in the experimental methods. The experimental methods used to extract proteins from BSFL meals, whether studies are being used specifically pre-pupae or all larvae (Liu et al., 2017) and whether they performed chitin correction, can all influence the results given (Makkar et al., 2014) with chitin correction reducing the reported percent protein content by 2–5% (Spranghers et al., 2017). The method to perform chitin correction consists of the development of a set of equations based on the stoichiometric contents of nitrogen of chitin and protein whereby the amounts of each component can be estimated from the value of the total nitrogen content, provided the rest of the proximate composition of the sample is accurately known. Chitin correction was not performed for this study.

The differences between the individuals and batches can be important: commercially available BSFL, had percentages ranging from 31.7% to 47.6% crude protein and 11.8–34.3% fat in different studies (Kroeckel et al., 2012; Bußler et al., 2016; De Marco et al., 2015). Various

sources from around the world also shows the same substrates differently, at least in terms of development time and the ability of one genotype to produce more than one phenotype when exposed to different environments (Zhou et al., 2013).

Regardless of these differences, all values consistently point to BSFL as a good source of proteins and lipids, averaging $40.8 \pm 3.8\%$ protein and $28.6 \pm 8.6\%$ fat (Table 2.3), both for reared and wild specimens (Nyakeri et al., 2017). The literature places BSFL's position among other insects as a possible alternative protein source in the scenarios envisioned by entomophagy advocates, such as climate change and overpopulation induced food insecurity. In every case, nutrients were concentrated from the substrate, which always had less fat and protein than the untreated insects (Spranghers et al., 2017). When defatted, BSFL meal can have crude protein levels over 60% (Spranghers et al., 2017) which is comparable to other insect meals (Makkar et al., 2014), and will have lower lipid percentages, assuaging concerns over the saturated fat content.

Table 0.3 Mean % crude protein (not chitin-corrected) and % fat (ether extract) per dry weight of BSFL meal, using mean values from the studies listed.

Diet or Source	%Protein	%Fat	Source
Poultry manure	37.90	18.73	Arango Gutiérrez et al. 2004
Municipal organic vegetable waste	39.80	30.10	Mutafela 2015
Horse manure	40.90	12.90	Mutafela 2015
Fresh fruit waste	37.80	41.70	Mutafela 2015
Swine manure	43.20	27.00	Newton et al. 2005
Poultry manure	42.10	34.80	Newton et al. 2005
Food manufacturing by-product mixes	38-46	21-35	Oonincx et al. 2015
TOTAL 77 Chicken feed	41.20	33.60	Spranghers et al. 2017b
Biogas digestate	42.20	21.80	Spranghers et al. 2017b
Restaurant waste (vegan)	43.10	38.60	Spranghers et al. 2017b

2.4 Protein: General introduction

Amino acids are building blocks for proteins in the human body. Furthermore, proteins contribute to the flavour of food and colour formation in processing and food storage. Next to this, proteins are important for the physical properties of food regarding texture, emulsification, foam and gel formation. Using crude protein extracts has its limitations for food applications. These limitations include undesirable colour or taste, and weak gel forming ability (Belitz et al, 2004). Therefore,

further purification of proteins from crude protein extract could be used to enhance the physical and sensory properties of foods. A variety of protein extraction and separation techniques is used in food production. These separation techniques are widely applied and make use of the biochemical properties of food proteins, e.g. protein solubility, size, charge, or adsorption characteristics (Smith & Nielsen, 2010).

2.4.1   Protein quality of Black soldier fly larvae

According to Jones, Cooper, and Harding (1972); Martin, Rivers, and Cowgill (1976); Pennino, Dierenfeld, and Behler (1991); Barker, Fitzpatrick, and Dierenfeld (1998); Finke (2002); Ghaly and Alkoaik (2009), the proximate crude protein content of BSFL ranges from 48 to 66% on a dry basis (about 19 - 26% on a fresh basis), while other components include lipids, carbohydrates, fibre and ash. The crude protein content of BSFL is comparable, or even higher than that of conventional meat sources that have a protein content of about 15 to 22% on a fresh basis. The chemical composition or nutritional value of intact edible insects has been reported in many articles (Finke, 2002; Ramos-Elorduy et al., 2002; Finke, 2002), often from an entomological and zoo-biology science point of view; still very little information from a food chemistry point of view is available. In order to evaluate insect protein quality, the amino acid composition of BSFL was determined by various authors and their results are compared in Table 2.4. The amino acid profiles reported differs greatly between the various authors (Table 2.4) which, despite the variety in feed substrate, days at harvest and drying methods, is also attributed to the techniques undertaken in different laboratories by the respective authors. When amino acid profile of the fly larvae was analysed by Newton et al. (2005) and Pieterse (2014), both hydrolysed their samples before analysis.

Newton et al. (2005) utilized a Durrum Model D-500 amino acid analyser and Pieterse (2014) determined the specific amino acid content through pre-column derivatisation, which separated them using high performance liquid chromatography. The fluorescence detector was used to complete the procedure of amino acid detection. St-Hilaire et al. (2007) conducted the amino acid analysis utilizing the Association of Official Analytical Chemists (A.O.A.C) approved amino acid method. It is noted from the literature that Newton et al. (2005) were the only authors that tested for and reported on traces of tryptophan in their analysis, where they utilized the procedure of Amaya-F et al. (1976) which has a high recovery rate for this specific amino acid. The methods undertaken by other authors are presented in Table 2.4. These studies did not allow

for the detection of tryptophan as the cost associated with the test is very high. The nutritional value of a food protein is evaluated not only in terms of amino acid composition, but also in protein digestibility. Few studies gave an indication that some insect species might give poor digestibility, such as, termites, locusts, and grasshoppers due to their hard exoskeletons containing chitin (consisting of nitrogen). Most of these studies measured protein by using nitrogen determination. This could result in an inaccurate estimation of protein content determination because of the presence of non-protein nitrogen (Bukkens, 1997).

Table 0.4 Amino acid profile of Black soldier fly larvae and pre-pupae (g/100g DM) receiving different feed substrates.

Amino acids (g/100g DM)	FAO, 2015	Newton et al., 2005	St-Hilaire et al., 2007	Pieterse, 2014
Feed substrate		Beef manure	Pig manure	Kitchen waste
Stage of harvest		Prepupae	Prepupae	Larvae
Processing method		Oven dried at 70 °C	Oven dried at 80 °C	Oven dried at 60 °C
Alanine	3.24	3.69	2.45	2.05
Arginine	2.36	2.24	1.78	2.38
Aspartic acid	4.63	4.56	4.09	3.19
Cystine	0.04	0.06	-	-
Glutamic acid	4.59	3.81	4.42	3.74
Glycine	2.40	2.88	1.72	1.99
Histidine	1.26	1.91	0.76	1.23
Isoleucine	2.15	1.96	1.83	1.62
Leucine	3.33	3.53	2.66	2.48
Lysine	2.78	3.37	2.05	2.41
Methionine	0.88	0.86	0.77	0.69
Phenylalanine	2.19	2.20	1.83	1.52
Proline	2.78	3.26	-	1.91
Serine	1.31	0.12	1.37	1.62
Threonine	1.56	0.55	1.58	0.51
Tyrosine	2.90	2.51	2.22	2.27
Valine	3.45	3.41	2.99	2.03

2.5 Maillard Reaction

Due to its promising results (products), the Maillard reaction (MR) is one particular area that has been focused on. The MR is named after Louis-Camille Maillard who first described it in 1912 in a session of the Academy of Sciences in Paris, upon heating sugars and amino acids in water, a yellow-brown colour developed (Martins et al., 2001). In food systems, the MR is precisely demonstrated, but when it occurs in living organisms the process is called glycation. The effect of MR has been observed and analysed as the reaction is vital in both medicine, chemistry and food science. The reaction is the series of non- enzymatic reactions, including the reaction between the carbonyl groups of the reducing sugar with the amino group of the amino acids, polypeptides, proteins, nucleic acids or phospholipids, forming Schiff-bases (Figure 2.2), and followed by subsequent rearrangement (Chawla et al., 2007; Hwang et al., 2011). The MR develops into a complex sets of reactions that generates numerous products, including early volatile compounds, intermediate products, and large molecular weight polymers (Jing & Kitts, 2002; Zeng et al., 2011). The formed compounds contribute specifically to aroma and characteristics of colour and they are all referred to as Maillard reaction products (MRPs). Advanced Glycation End Products (AGEs) is the term used in biological emems for the end products. During thermal processing and home-cooking, the MRPs are derived via the interaction of amino-carbonyl compounds, to modify important food properties such as colour, flavour and stability during processing, storage and distribution (Maillard et al., 2007; Yu et al., 2013).
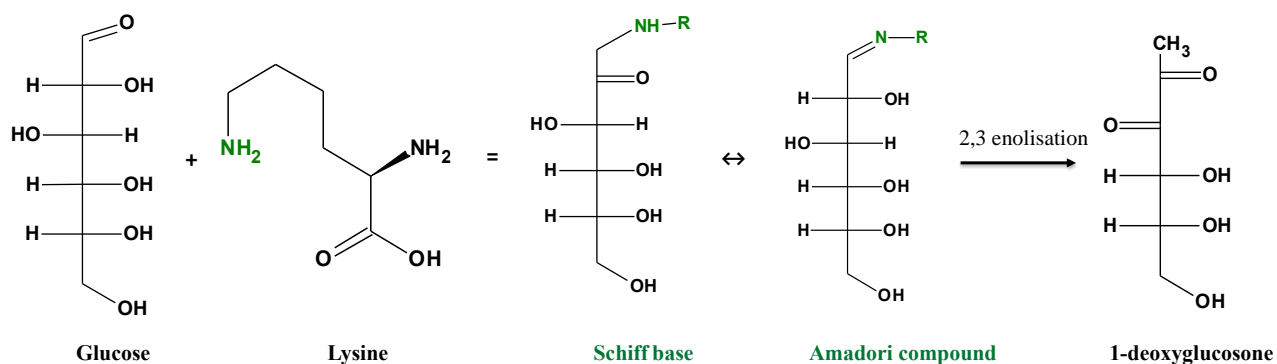
Figure 0.2 Mechanism of a Schiff base, Amadori compound and 1-deoxyglucosone formation from glucose/lysine (Adopted from Yaylayan and Haffenden, 2003).

Although the complete pathways for all the MRPs have not been fully elucidated (Figure 2.2), it is well recognised that three distinct stages of MR development occur as proposed by Hodge in

1953 (Sun & Zhuang, 2011). The mechanism occur in seven steps (A–G) of the reaction with three main steps as first, middle, and last stage:

I. First stage (colourless, no absorption in the near-UV range)

    A. Sugar-amine condensation

    B. Amadori rearrangement

II. Intermediate stage (colourless, or yellow with strong absorption in the near-UV range)

    C. Sugar dehydration

    D. Sugar fragmentation

    E. Amino acid degradation

III. Final stage (highly coloured)

    F. Aldol condensation

    G. Aldehyde-amine polymerization; formation of heterocyclic nitrogen compounds (melanoidins).

The initial stage of the Maillard reaction is a condensation reaction that occurs between the carbonyl group of an aldose or ketose and the free amino group of amino acid, followed by the elimination of water to give an N-substituted aldosylamine or ketosylamine. This reaction, which is initiated by an attack of the nucleophilic amino compound on the carbonyl carbon, is reversible and requires an acidic catalyst (Martins et al., 2001).

In the next step, the Amadori rearrangement is important for the Maillard reaction to proceed. This is an acid-base catalysed conversion of the N-substituted aldosylamine to an N-substituted 1-amino-1-deoxy-2-ketose. The Amadori reaction is catalysed by weak acids, where the protonation of the Schiff base and the subsequent proton moves to constitute the critical steps; Their own acid catalysts is served by amino acids, so the reaction is fast even in the absence of added acid (Manzocco et al., 2001). If a ketose can react with the amino compound, a ketosylamine is formed. This leads to a 2-amino-2-deoxyaldose through Heyn's rearrangement, which is similar to the Amadori rearrangement. Both rearrangements result in the conversion of an alphahydroxyamino compound to an alpha-aminocarbonyl compound (Van Boekel, 2006; Gogus et al., 2009). The formation of the Amadori rearrangement products (ARPs) proceed slowly at room temperature because a tautomeric shift to the open chain form of the reducing sugar is required for the initial reaction to occur. In the reaction of aldoses with the amino acids blocking

the Amadori rearrangement step the formation of brown pigment is stopped completely (Gogus et al., 2009). Amadori rearrangement products are more stable when formed than the glycosylamines in environments that are acidic, although they rely on heat to change. Once heated, the Amadori rearrangement products undergo the process of fission and colourless reductones and fluorescent substances are formed. Amadori compounds have been isolated in various heated and stored food products including soy sauce, malt, roasted cocoa and dehydrated vegetables.

In the intermediate stage, three degradation pathways exist; sugar dehydration, sugar fragmentation, and Strecker degradation (Manzocco et al., 2001; Van Boekel, 2006; Gogus et al., 2009).

The process of sugar dehydration is divided into two types; they both are pH dependent. In acidic conditions, at pH 7 or below, it undergoes mainly 1,2-enolization with the formation of furfural (when pentoses are involved) or hydroxy methyl furfural (HMF) (when hexoses are involved). In basic conditions, at pH higher than 7, the Amadori compound degradation is thought to involve mainly 2,3-enolization, where reductones, such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one, and a variety of fission products (Figure 2.3), including acetol, pyruvaldehyde, and diacetyl are formed (Manzocco et al., 2001; Gogus et al., 2009). The next reaction is sugar fragmentation; the accepted mechanism is dealdolization, the reverse of an aldol condensation. The products are aldols, amino-free polymers, and free amino compounds (Gogus et al., 2009).
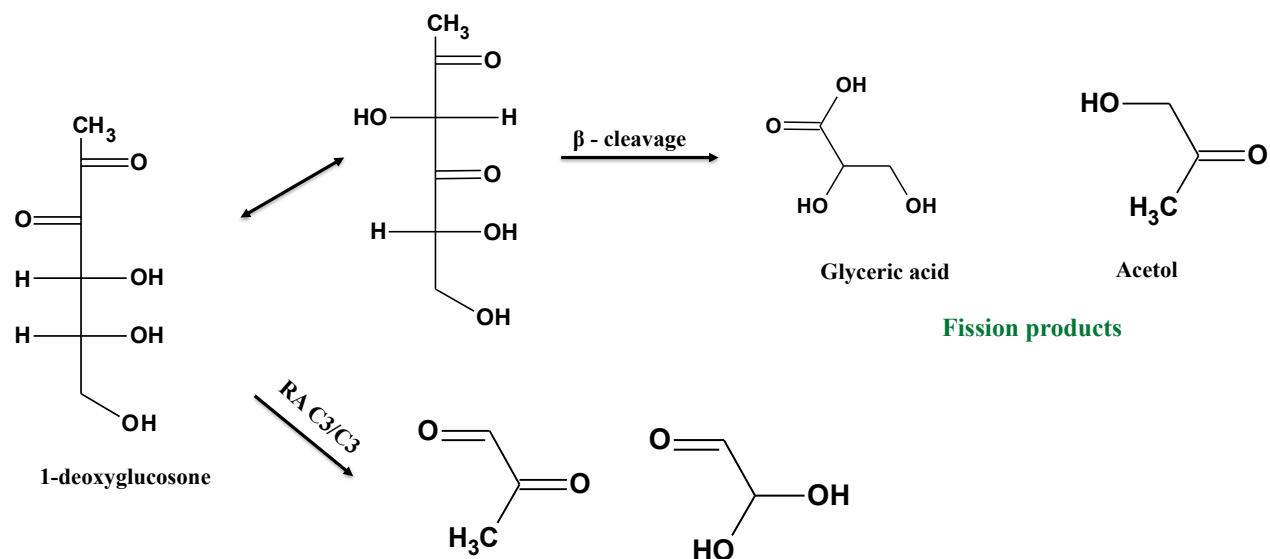
Figure 0.3 Mechanism of fission products formation from 1 deoxyglucosone (Adopted from Yaylayan and Haffenden, 2003).

In the Strecker degradation reaction, the fission products are highly complex, and they are involved in further reactions. Carbonyl groups can condense with free amino groups, which may result in the incorporation of nitrogen into the reaction products. This is caused by the reaction of a reductone and an α-amino acid. At this stage, the C=O is free to form an aldehyde with one less carbon than that of the original amino acid. The formed aldehyde may be important as auxiliary flavour; these may further condense with themselves, with sugar fragments, with furfurals, and with the other degradation products to form brown pigments (Gogus et al., 2009; Jaeger et al., 2010).

The reactions taking place in the final stage are aldol condensation, aldehyde-amine polymerization, and formation of heterocyclic nitrogen compounds (pyrroles, imidazoles, pyridines, and pyrazines). The polymerization of products from the second step and co-polymerization with amino compounds yield the coloured products. Finally, both water-soluble and water-insoluble pigments, called melanoidins, are produced, and their structures are amino acid, sugar and sugar: amino acid ratio dependent. Melanoidins differ highly in molecular mass and contain different discrete chromophores (Matmoroh et al., 2006). On the basis of their molecular weight and solubility, this is used to distinguish between types of melanoidins.

Dialysis of sugar-amino acid reaction mixtures may be used to prepare melanoidins with a molecular weight 12 000 Da, as the browning reactions proceed the melanoidins eventually become insoluble in water and precipitate to form unknown compounds (Manzocco et al., 2001; Jaeger et al., 2010).

+ Amino compounds

Aldose Sugar → N-substituted glycosylamine　　+ H_2O

↓

Amadori Rearrangement Products (ARP)
1-Amino-1-deoxy-2-ketose

$-2H_2O$　>pH 7　　　　>pH 7　　　　$-3H_2O$　　≤ pH 7

Reductones　　**Fission products**　　**Schiff's base of hydroxymethylfurfural (HMF) or furfural**
　　　　　　　(Acetol, diacetyl, pyruvaldehyde)

$-2H$　　$+2H$

Dehydroreductones

$-CO_2$
+ amino acids

Strecker degradation　　　　+ Amino compounds　　- Amino compounds

Aldehydes　　　　　**HMF or furfural**

Aldose and N-free polymes

+ Amino compounds　　　　　　　　　　+ Amino compounds

Aldimenes and ketimes

Melanoidins (brown nitrogenous polymers)

Scheme 1.1 General pathway for the Maillard reaction (adopted from Martins et al., 2001).

2.5.1 Factors affecting the Maillard reaction

It has been shown that the MR pathways and subsequent products can be modulated by many factors. These can be distinguished as those relating to the reactant e.g. type of amino acid, sugar

type, sugar: amino acid ratio, and external factors such as moisture level, temperature and pH (Morales & Jimenez-Perez, 2001, Jing & Kitts, 2002; Matmoroh et al., 2006). In order to reduce the complexity, sugar-amino acid model systems have commonly been used to study the phenomena and mechanism of the MR. The following subsections explain these factors in detail.

2.5.1.1 Amino Acid type

The amino acid type which is present in the solution is significant in the MR. Some amino acids have two reactive groups therefore they react fast with the sugars to produce brown pigments (melanoidins). Liu et al. (2008) classified the amino acids according to their reactivity with the sugars at pH 9.0 with lysine, glycine, tryptophan, and tyrosine, classified as the most reactive amino acids. They have measured the colour intensity of 60 model systems heated for 3 h at pH 6.5 and 100 °C and found that the colour intensity of the MRPs was caused by the high lysine presence (Gogus et al., 2009).

2.5.1.2 Type of Sugar

Reactants that are essential in these reactions are the reducing sugars, mainly the interaction of the carbonyl group with the free amino groups of amino acids from the proteins. Most reactive compounds are the ones with low molecular weight compared with the high molecular weight compounds, this is due to greater steric hindrance in the latter. Consequently, aldopentoses are normally more reactive than aldohexoses and monosaccharides are more reactive than di- or oligosaccharides. Aldose sugars are more reactive ketoses, due to greater steric hindrance of the carbonyl group of ketoses. Jing & Kitts (2002) demonstrated that the pentose xylose, showed much higher reactivity than hexoses glucose and fructose (Guofeng et al., 2012).

2.5.1.3 Effect of pH

Both the initial pH of the reactants and the buffering capacity of the system influence the rate and direction of the Maillard reaction. Kim and Lee (2008) systematically investigated the influence of pH on the extent of interaction of glucose and free amino-nitrogen. They made quantitative observations on the interaction of glucose with glycine, and of glucose with various enzymatic digests of protein material. Determinations of pH and Van Slyke amino nitrogen were made with time; in each case the initial pH values were set using phosphate buffers and solutions consisted of 1% glycine and 13.2% glucose. An appreciable loss of amino-nitrogen occurred in 48 h and the loss increased with increasing pH. It was shown that the optimum pH for the whole reaction is in the range pH 6 – 9. With regards to the influence of pH on the MR, studies by Lee et al. (2006)

showed that the products increases with increasing pH, up to a pH 10, with little browning occurring below pH 6. The pH-dependence of the MR can qualitatively be described by the effect of protonation of the amino acid. The amount of unprotonated amino group, the reactive species, increases with increasing pH (Kim & Lee, 2008).

2.5.1.4 Temperature

The kinetics of the MR appear to be influenced by temperature, with an increase resulting in a rapidly increasing rate of browning. The temperature does not only determine the rate of browning but also the character of the reaction. It has been proven that the rate of browning increases 2 – 3 times for each 10 °C rise in temperature (Bersuder et al., 2007). With the exception for foods containing fructose, the increase may be 5 – 10 times for each 10 °C rise (Xiaohong et al., 2010). The composition of the MRPs formed as a result of temperature increase results in an increase in colour intensity. This is due to an increase in the carbon content of the pigment (Bersuder et al., 2007; Guofeng et al., 2012). Ajandouz et al. (2008), when working with equimolecular amounts of D-xylose and glycine in aqueous solution at a range of temperatures, reported that an increase in temperature leads to an increase in aromatic character in both high and low molecular weight products. The structure of the melanoidins synthesised at room temperature differs considerably from those synthesised at higher temperatures in that they have different types of aliphatic carbons and fewer unsaturated carbons (Guofeng et al., 2012).

2.5.1.5 Water activity

Heating, dehydrating, or concentrating food constituents may cause non-enzymatic browning reactions. Several authors have investigated the role of bound and unbound water in browning reactions. Almost all of the findings showed that a maximum browning rate occurs at water activity between 0.4 and 0.6, however this is dependent on the type of food substance. Reaction rate decreases at low water activities as a result of the increasing diffusion resistance due to high viscosity (Tazi et al., 2009). The dilution of the reactants slows down the reaction rate at higher water activities (Yilmaz & Toledo, 2005). Additionally, water behaves as a reaction product at higher water activities and prevents the formation of reaction intermediates that are produced together with water (Yilmaz & Toledo, 2005; Tazi et al., 2009).

2.6 The role of Maillard reaction products in food acceptability

The Maillard reaction is the well-known reaction which results from food processing. The products of the reaction (MRPs) have significant influence on food quality attributes such as aroma, flavour,

texture and colour. Although the Maillard reaction may cause food quality deterioration, this reaction can be useful in designing foods that present sensory attributes demanded by the consumer (Ames, 1990; Yu & Zang, 2010).

2.6.1 Colour

The Maillard reaction is primarily characterised by the formation of colour. In the last decade, studies have been taken to determine Maillard reaction kinetics and the coloured compounds formation ratio, mainly with the use of model systems. The development of the brown colour during storage and processing is desirable for many products such as baked foods, coffee, cookies while in some kinds of food products such as orange juice, white chocolate, milk and powder egg this colour development is undesired. Particularly significant is the control and prediction of food colour development which companies require to satisfy consumer preference. Melanoidins produced by the Maillard reaction are strongly dependent on technological conditions of the reaction (Wang et al., 2011). Sugar caramelisation can form melanoidins without the amino groups reacting. For example, high molecular weight pigments, roasted foods such as coffee, cocoa, bread and malt. Well documented in literature is the positive correlation between melanoidins content in food and antioxidant activity.

2.6.2 Flavour and aroma

The development of flavour and aroma due to the Maillard reaction is dependent on the reaction temperature, time, pH, water content and the type of sugars and amino acids involved (Yu & Zhang, 2010; Van Boekel, 2006). In general, the kinetic parameters are influenced by the reaction temperature, while the type of flavour compounds formed is determined by time, the second factor. The Maillard reaction intermediate and final stages such as Strecker degradation step are the most important to flavour development. The Strecker degradation reaction, where amino acids are degraded by dicarbonyls leads to deamination and decarboxylation of the amino acids (Ames, 1990; Rizzi, 2008).

The Maillard reaction products that are volatile can be labelled according to the sugar dehydration/fragmentation products as furans, pyrones, cyclopentenes, carbonyls and acids; the aldehydes and sulfur compounds are the amino acid degradation products; and pyrroles, pyridines, imidazoles, pyrazines, oxazoles, thiazoles, and others are volatile products that are produced by further interactions. Pyrazines and alkylpyrazines are linked with the flavour and aroma of roasted and nutty. Alkylpyridines confer to food flavours and aromas of green, bitter, astringent and burnt

with furans, furanones and pyranones relating to sweet, burnt, pungent and caramel-like flavours/aromas.

Compounds that are generally present at trace levels are essential to the characteristic flavour and aroma of food products. The oxygen-containing aroma compounds 2,3-butanedione, 2,3-pentanedione, methylpropanal, 3-methylbutanal, phenylacetaldehyde, 3-hydroxy-4,5-dimethyl-2(3H) furanone and 2,5-dimethyl- 4-hydroxy-3(2H)-furanone occur in concentrations ranging from 1 μg/kg up to 100 mg/kg. The nitrogen-containing aroma compounds 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine and 2-acetyl-1-pyrroline are present in food in an order of magnitude of 0.001–10 mg/kg (Stanimirova et al., 2011). Overall, the most powerful aroma compounds are sulfur containing Maillard odorants and often play, although at low levels, a dominant role in the flavour of cooked meats. The flavour and aroma of stewed beef juice, boiled trout, french fries, bread crust, cooked chicken, roasted chicken, boiled beef, cocoa powder, peanuts, pilsner, roasted beef, popcorn and coffee are as the result of volatile compounds (Cerny, 2008).

The sulfur containing compounds are meat-related flavour compounds derived from cysteine and ribose while typical bread, rice and popcorn flavours are attributable to amino acid proline. The intense meat-like and sulfur smell are given off by cysteine-containing mixtures. The highly intense smell of potatoes is generated by the sulfur containing amino acid methionine and this is used in the food industry to enhance the soft flavour of potatoes. Mixtures containing amino acids other than cysteine or methionine in combination with reducing sugars are characterised mostly by a caramel and jam smell (Stanimirova et al., 2011; Van Boekel, 2006).

The food industry invests in reconstituting combinations of these compounds to create synthetic flavours and aromas. The subtleties of flavour perception are many and varied. This is used in the process of creating synthetic flavours and although these compounds may be detected by Electronic Noses, human sensory perception is considered essential to validate instrumental data (Gerrard, 2002a; Schaller et al., 1998).

2.6.3   Texture

The definition of texture is complex. According to Szczesniak (2002), "texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics''. The texture of food is influenced by the Maillard reaction via protein cross-linking. The functional properties of food can be

modified by manipulating the extent and nature of protein cross-linking during food processing. The effect and control of protein cross-linking to maximize food quality is not yet known (Gerrard, 2002b). The resultant Maillard reaction by protein cross-linking will affect the texture, and the protein digestibility. Although the well-understood MR effects on food colour, flavour and aroma are used by the food industry, attention is required for the effects on food texture development.

2.7 Analyses of Maillard reaction products

Many authors highlighted the health benefits of MRPs and risks present in the diet. For that reason, in common foods it is important that MRPs are characterised and quantified, to get the best balance between advantages (benefits) and disadvantages (potential risks), and to establish guidelines for food health (Delgado-Andrade et al., 2009). Sophisticated analytical technique developments have made it possible to isolate, characterise, and quantify several specific non-browning reaction compounds formed *in vitro* and *in vivo*, both at the early and advanced stages of Maillard reaction. The most common are: Amadori compounds (indirectly analysed as furosine), Nε-(carboxymethyl)lysine (CML) and some intermediate derivatives of the reaction, such as hydroxymethylfurfural. Measurement of fluorescent compounds formed during the reaction is also a reliable tool to evaluate the value of nutritional loss due to thermal processing of foods (Delgado-Andrade et al., 2009; Friedman, 1996).

2.7.1   Fluorescent compounds

Conventionally, the progress of Maillard reaction in food processing and food products was monitored based on the spectrophotometric evaluation of colour development at 420 nm (Rufiàn-Henares et al., 2002). However, recently, the evaluation of fluorescent compounds generated by the Amadori rearrangement product undergoing dehydration and fission, has become usual. The fluorescence measurement is used to assess Maillard reaction under physiological conditions, meaning, advanced glycation end products (AGEs) generation, and also to access AGE's correlated pathologies development (Delgado-Andrade et al., 2006).

Fluorescent compounds (FC) are regarded as precursors to the brown pigments formed in the MR. Evaluating the extent of the reaction in food products (Morales & Van Boekel, 1998; Rufiàn-Henares & Delgado-Andrade, 2009). Formation of MRPs was first evaluated using fluorescence in milk and now it is used to monitor the processing of cereals, cookies, soybeans, infant formula, cooked salmon and bakery products (Morales & Van Boekel, 1998).

Fluorescent compounds may be free in the matrix or linked to the protein fraction. Total FC (free + linked to protein) determination demands a previous enzymatic hydrolysis, during which the use of a nonspecific protease (pronase) is required (Delgado-Andrade et al., 2008). In foods such as milk, breakfast cereals, cooked salmon, roasted soy and enteral-free baby formula and total FC has been tested (Delgado-Andrade et al., 2008; Rufiàn-Henares et al., 2002).

A well-established method called Fluorescence of Advanced Maillard products and Soluble Tryptophan (FAST) has been applied to evaluate nutritional and lysine loss. FAST is based on the quantification of protein denaturation using fluorescence: (1) fluorescence of the advanced Maillard products (FAMP), such as pyrrole and imidazole derivatives, at excitation/emission 330/420 nm; and (2) tryptophan fluorescence (FTrp) at excitation/emission 290/340 nm at pH 4.6 (Friedman, 1996).

2.7.2 Hydroxymethyfurfural (HMF)

HMF is an intermediate compound formed during the Maillard reaction by the degradation of hexoses at high temperatures under acid conditions (Arribas-Lorenzo & Morales, 2010).
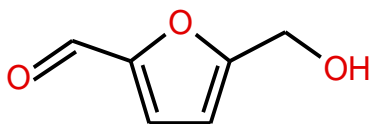
Figure 0.4 Hydroxymethyfurfural structure.

The most known methods for HMF (Figure 2.4) determination in food are spectrophotometric (colorimetric) methods, they are of limited accuracy since other chromophores in food may absorb in the same wavelength region, causing inconsistency in the results. Moreover, colorimetric methods have low sensitivity, while on the other hand chromatographic methods (such as liquid or gas chromatography) are more accurate and sensitive, and the individual determination of HMF and furfural is the major advantage of the use of chromatographic methods (Erbersdobler & Somoza, 2007; Morales et al., 1997; Rufiàn-Henares et al., 2001). Heat intensity applied to food results in direct HMF formation, and in products containing high carbohydrate concentrations, it is considered a thermal damage marker. Additionally, it can be useful monitoring the thermal process applied to several food products namely: baked goods, breakfast cereals, caramel, honey and certain meats (Rufiàn-Henares & Delgado-Andrade, 2009; Rufiàn-Henares et al., 2006).

2.7.3 Furosine

Furosine (ε-N-2-furoylmethyl-L-lysine) products are measured as Amadori compounds (Figure 2.5). The amount of furosine present in foods is dependent on the kind of heat treatment and the storage time. After prolonged storage or after overheating, the levels of furosine tend to decrease giving rise to other compounds such as CML (Delgado-Andrade et al., 2005; Friedman, 1996; Rufiàn-Henares et al., 2009).
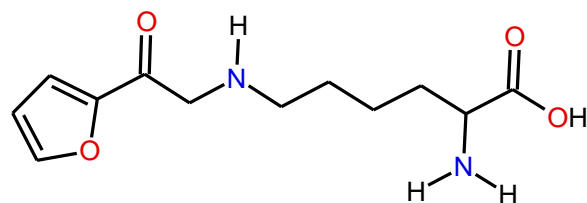
Figure 0.5 Furosine chemical structure.

The most specific and important sign of the initial phase of the Maillard reaction is the presence of furosine. Lysine is the limiting amino acid of this product, thus the presence of furosine is an important marker of protein value loss therefore it is widely used in the analysis of cereal products. To tailor the processing conditions one must monitor furosine formation and content in order to guarantee the preservation of the nutritional value of food products (Rufiàn-Henares et al., 2006; Resmini et al., 1990). Concerning analytical techniques, in 1992, an ion pairing HPLC based methodology was proposed and successfully applied in a series of studies (Alexandratos & Bruinsma, 2012). In 1996, when furosine became commercially available, Reversed Phase-HPLC became the method of choice for furosine detection in foods. As CML transforms into furosine during heating it is therefore necessary for the acid hydrolysis to be performed in an inert atmosphere, which impairs furosine degradation (Erbersdobler & Somoza, 2007).

2.7.4 Nε- (carboxymethyl)lysine

A stable, low reactivity advanced Maillard product is called Nε-(carboxymethyl)lysine (CML) (Figure 2.6). Amadori compound degradation produces CML products, such as Nε-(fructosyl)lysine (FL). FL undergoes oxidation to form, Nε-(carboxymethyl)lysine (CML). R-dicarbonyls such as glyoxal (GO), formed during the oxidation of the sugar or the Amadori rearrangement products are immediate precursors of CML. Another route to CML is through the lipid peroxidation, and GO has been suggested as an intermediate. CML is referred as one of the

most important markers of bioactive Maillard products and its content is usually correlated to the health risk of ingestion of heat-treated foods (Charissou et al., 2007).
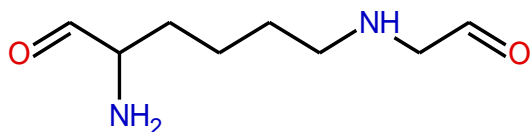
Figure 0.6 Chemical structure of Nε-(carboxymethyl)lysine.

There are three main methodologies proposed to evaluate CML in foods: a) Reversed Phase High Performance Liquid Chromatography (RP-HPLC), b) Gas Chromatography Mass Spectrometry (GC/MS) following methylation of the carboxylic group and acylation of the amine group and c) enzyme-linked immunosorbent assay based on a monoclonal anti-CML antibody (ELISA) (Charissou et al., 2007). Presently, CML analyses in foods are performed by specific immunosorbent assay (AGE - ELISA). This test is suitable for quantitative CML analysis both in biological samples and food (Goldberg, 2004). Among the Maillard reaction products, the best characterised end product is CML. It is applied as an advanced glycation end product or advanced lipoxidation end product (AGEs/ALEs) marker in research (Goldberg, 2004).
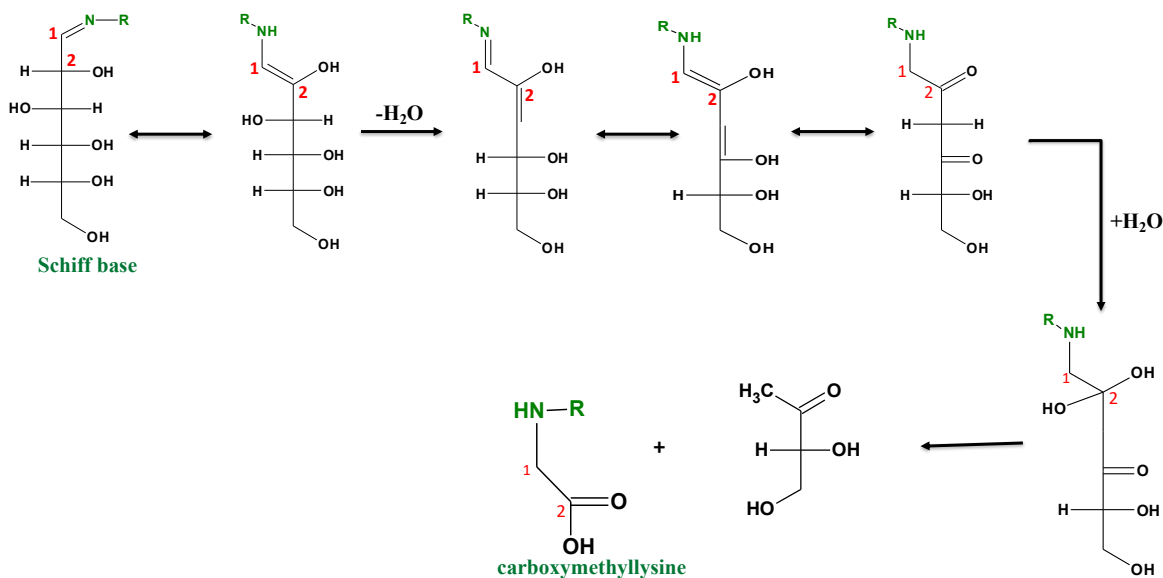
Figure 0.7 Mechanism of Nε-(carboxymethyl)lysine formation from a Schiff base (Adopted from Kasper and Schieberle, 2005).

2.8 Current study analysis methods

High performance liquid chromatography, differential scanning calorimetry, thermogravimetric analysis, Fourier-transform infrared spectroscopy and scanning electron microscopy, were employed to determine, characterise and analyse the products before and after conjugation.

2.8.1 High performance liquid chromatography (HPLC)

HPLC is a widely applied analytical technique as evidenced by the number of instruments installed, practitioners, analyses performed, and research papers published annually. In HPLC, sample constituents are separated as they migrate through a column at different velocities and elute from the system at different times. The analytes take part in a three-mode interaction between the stationary phase, the sample, and the mobile phase. Analytes that are strongly retained on stationary phase elute late, while analytes that do not interact strongly migrate faster and elute sooner through the system. HPLC utilises a variety of column formats, dimensions, and chemistries, while mobile phases include a wide range of organic solvent and buffer mixtures. Detection of eluted analytes can be achieved by a single detector or multiple detectors connected in series. This study used HPLC for the separation of amino acids from the protein before and after conjugation using a UV detector.

2.8.2 Universal Attenuated total reflectance Fourier-transform infrared spectroscopy (UATR-FTIR)

Infrared spectroscopy is the study of the interaction of infrared light with matter, which can be used to identify unknown materials, examine the quality of a sample or determine the number of components in a mixture. The middle infrared region refers to electromagnetic radiation with wavenumber ranging from $13000 - 10$ cm^{-1} (corresponding wavelength from $0.78 - 1000$ μm). Infrared region is further divided into three sub-regions: near-infrared ($13000 - 4000$ cm^{-1} or $0.78 - 2.5$ μm), mid-infrared ($4000 - 400$ cm^{-1} or $2.5 - 25$ μm) and far-infrared ($400 - 10$ cm^{-1} or $25 - 1000$ μm). The most commonly used is the mid-infrared region, since molecules can absorb radiation in this region to induce the vibrational excitation of functional groups. Recently, applications of near infrared spectroscopy have also been developed, especially for use in the food and agricultural industries (Pavia et al., 2009).

By passing infrared light through a sample and measuring the absorption or transmittance of light at each frequency, an infrared spectrum is obtained, with peaks corresponding to the frequency of absorbed radiation. Since all groups have their characteristic vibrational frequencies,

information regarding molecular structure can be gained from the spectrum. Infrared spectroscopy is capable of analysing samples in almost any phase (liquid, solid, or gas), and can be used alone or in combination with other instruments following different sampling procedures. Besides fundamental vibrational modes, other factors such as overtone and combination bands, Fermi resonance, coupling and vibration-rotational bands also appear in the spectrum. Due to the high information content on the spectrum, infrared spectroscopy has been a very common and useful tool for structure elucidation and substance identification (Holler et al., 2007).

2.8.3 Thermal stability

Thermal analysis refers to the variety of techniques developed and used in which any physical property of a given system is continuously measured as a function of temperature, though temperature and time may be related by the term called the heating rate namely dT/dt. The temperature profile can be a prefixed schedule like temperature programming or temperature jump or flash heating. Thermal analysis techniques are employed in virtually every area of modern science and technology (Menczel et al., 2008; Wagner, 2007). The basic information that the variety of techniques can provide includes crystallinity, specific heat, expansion and information on a variety of physical and chemical transformations that can take place in the sample under inspection. The thermal techniques used in this study are the thermal gravimetric analysis and differential scanning calorimetry.

2.8.3.1 Thermal gravimetric analysis (TGA)

TGA is a technique in which the mass of a substance is monitored as a function of temperature or time, as the sample is subjected to controlled temperature program and atmospheric environments. This technique can characterise materials that exhibit weight loss or gain due to desolvation, decomposition or oxidation. The thermogravimetric instrument consists of a furnace, a temperature programmer, a microgram balance, a sample holder and a thermobalance where the curves are recorded. The sample under study is placed in a crucible, which is attached to the weighing arm of the microgram balance; also, the thermobalance has a clamp which is used to hold the microbalance arm. As the sample is heated, the change in weight of the sample is measured by the microbalance while the temperature is checked by a thermobalance (Wagner, 2017).

2.8.3.2 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is an experimental technique to measure the heat energy uptake that takes place in a sample in a temperature controlled environment. There are two main

types of DSCs; power compensated DSC and heat-flux DSC. In our laboratory we use the heat-flux DSC, hence only that will be discussed. Heat-flux DSC: Thermocouples are attached to the base of the sample and reference material vessels (a single heat source is employed to heat both the reference material and the sample). The second set of thermocouples measures the temperature of the furnace and of the heat-sensitive plate. The temperature difference between the sample and reference material is measured as a function of time or temperature. This temperature difference is proportional to the change in the heat flux (energy input per unit time). As phase change occurs heat is absorbed or emitted by the sample, changing the heat flux through the heat sensitive plate. The variation in heat flux causes an increase in temperature difference to be measured between the heat-sensitive plate and the furnace (Menczel et al., 2008).

2.8.4 Inductively Coupled Plasma (ICP) Spectroscopy

ICP is one method of optical emission spectrometry. When plasma energy is given to an analysis sample from outside, the component elements (atoms) are excited. When the excited atoms return to low energy state, emission rays (spectrum rays) are released, and these are based on their measured wavelength. The element type is based on the wavelength and the content of each element is determined based on the rays' intensity. To generate plasma, first, argon gas is supplied to the torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature and this energy is used in the excitation of the sample. Solution samples are introduced into the plasma in an atomized state through the capillary tube in the centre of the torch tube (Wilschefski & Baxter, 2019).

2.8.5 Scanning Electron Microscope (SEM)

Scanning Electron Microscopy (SEM) is an analytical technique used to determine the morphology and crystalline structure of the sample. The Scanning Electron Microscope (hereinafter "SEM") enables a clear observation of very small surface structures, which is not possible with an optical microscope (hereinafter "OM"). Moreover, as it can provide images with deeper focal depth, it enables observations of 3-dimensional images, with a similar sense as when we look at a substance with the naked-eye, by enlarging the specimen surface which has a rough structure (Rochow & Rochow, 1978).

References

Ajandouz, E., Desseaux, V., Tazi, S. & Puigserve, A. 2008. Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems. *Food Chemistry*, 107(3): 1244–1252.

Amaya-F. J., Young, C. & Chichester, C. 1976. Automated determination of Tryptophan in legumes and cereals. *Agricultural and Food Chemistry*, 25:139-143.

Ames, J.M. 1990. Control of the Maillard reaction in food systems. *Trends in Food Science & Technology*, 1: 150–154.

Ames, J.M., Guy, R.C.E. & Kipping, G.J. 2001. Effect of pH, Temperature, and Moisture on the Formation of Volatile Compounds in Glycine/Glucose Model Systems. *Journal of Agricultural and Food Chemistry*, 49(9): 4315–4323.

Aniebo, A.O., Erondu, E.S. & Owen, O.J. 2008. Proximate composition of housefly larvae (Musca domestica) meal generated from mixture of cattle blood and wheat bran. *Livestock Research for Rural Development*, 20(12): 2–9.

Aniebo, A.O. & Owen, O.J. 2010. Effects of age and method of drying on the proximate composition of housefly larvae (Musca domestica Linnaeus) meal (HFLM). *Pak. J. Nutr*, 9(5): 485-487.

Ansorena, D., Gimeno, O., Astiasarán, I. & Bello, J. 2001. Analysis of volatile compounds by GC–MS of a dry fermented sausage: chorizo de Pamplona. *Food Research International*, 34(1): 67–75.

Arango Gutierrez, G.P., Vergara Ruiz, R.A. & Mejia Velez, H. 2004. Compositional, microbiological and protein digestibility analysis of larval meal of *Hermetia illucens* (*Diptera:Stratiomyidae*). Revista Facultad Nacional de Agronomía, Medellín, 57 (2): 2491-2499.

Awoniyi, T.A.M., Adetuyi, F.C. & Akinyosoye, F.A. 2004. Microbiological investigation of maggot meal, stored for use as livestock feed component. *Journal of Food Agriculture & Environment*, 50(3): 3-4.

Barker, D., Fitzpatrick, M.P. & Dierenfeld, E.S. 1998. Nutrient composition of selected whole invertebrates. *Zoo Biology*, 17(2): 123–134.

Belitz, H.D., Grosch, W. & Schieberle, P. 2009. Food Chemistry. Berlin, Heidelberg: Springer

Berlin Heidelberg. *Food Chemistry Journal*, 20(4): 1-1070.

Bensmira, M., Jiang, B., Nsabimana, C. & Jian, T. 2007. Effect of Lavender and Thyme incorporation in sunflower seed oil on its resistance to frying temperatures. *Food Research International*, 40(3): 341–346.

Bondari, K. & Sheppard, D.C. 1987. Soldier fly, Hermetia illucens L., larvae as feed for channel catfish, Ictalurus punctatus (Rafinesque), and blue tilapia, Oreochromis aureus (Steindachner). *Aquaculture Research*, 18(3): 209–220.

Burton, I. 1987. Report on reports: Our common future: The world commission on environment and development. *Environment: Sci. and Policy Sust. Dev*, 29(5): 25-29.

Bukkens, S.G.F. 1997. The nutritional value of edible insects. *Ecology of Food and Nutrition*, 36(2–4): 287–319.

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(12).

Calvert, C.C. & Martin, R.D.A. 1969. Housefly pupae as food for poultry. Journal of *Economic Entomology*, 62: 938-939.

Caparros Megido, R., Sablon, L., Geuens, M., Brostaux, Y., Alabi, T., Blecker, C., Drugmand, D., Haubruge, É. & Francis, F. 2014. Edible Insects Acceptance by Belgian Consumers: Promising Attitude for Entomophagy Development. *Journal of Sensory Studies*, 29(1): 14–20.

Capper, J. 2013. Should we reject animal source foods to save the planet? A review of the sustainability of global livestock production. *South African Journal of Animal Science*, 43(3): 233.

Cerny, C. 2008. The Aroma Side of the Maillard Reaction. *Annals of the New York Academy of Sciences*, 1126(1): 66–71.

Charissou, A., Ait-Ameur, L. & Birlouez-Aragon, I. 2007. Evaluation of a gas chromatography/mass spectrometry method for the quantification of carboxymethyllysine in food samples. *Journal of Chromatography A*, 1140(1–2): 189–194.

Chawla, S.P., Chander, R. & Sharma, A. 2007. Antioxidant formation by γ-irradiation of glucose–amino acid model systems. *Food Chemistry*, 103(4): 1297–1304.

Cordell, D., Drangert, J.O. & White, S. 2009. The story of phosphorus: Global food security and

food for thought. *Global Environmental Change*, 19(2): 292–305.

Dar, W.D. & Gowda, C.L.L. 2013. *Combating Climate Change*. M. S. Kang & S. S. Banga, eds. CRC Press.

Defoliart, G.R. 1995. Edible insects as minilivestock. *Biodiversity and Conservation*, 4(3): 306–321.

Delgado-Andrade, C., Rufián-Henares, J.A. & Morales, F.J. 2006. Study on fluorescence of Maillard reaction compounds in breakfast cereals. *Molecular Nutrition & Food Research*, 50(9): 799–804.

De Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero, D., Katz, H., Dabbou, S., Kovitvadhi, A., Zoccarato, I., Gasco, L. & Schiavone, A. 2015. Nutritional value of two insect larval meals (Tenebrio molitor and Hermetia illucens) for broiler chickens: Apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. *Animal Feed Science and Technology*, 209: 211–218.

El Boushy, A.R. 1991. House-fly pupae as poultry manure converters for animal feed: A review. *Bioresource Technology*, 38(1): 45–49.

Erbersdobler, H.F. & Somoza, V. 2007. Forty years of furosine – Forty years of using Maillard reaction products as indicators of the nutritional quality of foods. *Molecular Nutrition & Food Research*, 51(4): 423–430.

Food and Agriculture Organization (FAO). 2013. Edible insects: Future prospects for food and feed security: 1–201.

Fasakin, E.A., Balogun, A.M. & Ajayi, O.O. 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish Clarias gariepinus fingerlings. *Aquaculture Research*, 34(9): 733–738.

Finke, M.D. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biology*, 21(3): 269–285.

Finke, M.D. 2015. Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biology*, 34(6): 554–564.

Friedman, M. 1996. Food Browning and Its Prevention: An Overview [†]. *Journal of Agricultural and Food Chemistry*, 44(3): 631–653.

Gahukar, R.T. 2011. Entomophagy and human food security. *International Journal of Tropical Insect Science*, 31(03): 129–144.

Gerrard, J.A. 2002a. New Aspects of an AGEing Chemistry — Recent Developments Concerning the Maillard Reaction. *Australian Journal of Chemistry*, 55(5): 299.

Gerrard, J.A. 2002b. Protein–protein crosslinking in food: methods, consequences, applications. *Trends in Food Science & Technology*, 13(12): 391–399.

Ghaly, A.E. & Alkoaik, F.N. 2009. The Yellow Mealworm as a Novel Source of Protein. *American Journal of Agricultural and Biological Sciences*, 4(4): 319–331.

Goldberg, T., Cai, W., Peppa, M., Dardaine, V., Baliga, B.S., Uribarri, J. & Vlassara, H. 2004. Advanced glycoxidation end products in commonly consumed foods. *Journal of the American Dietetic Association*, 104(8): 1287–1291.

Hale, O.M. 1973. Dried Hermetia illucens larvae (Diptera: Stratiomyidae) as a feed additive for poultry. *Journal of the Georgia Entomological Society*, 8(2): 16–20.

Hall, J.E., Crook, E.D., Jones, D.W., Wofford, M.R. & Dubbert, P.M. 2002. Mechanisms of Obesity-Associated Cardiovascular and Renal Disease. *The American Journal of the Medical Sciences*, 324(3): 127–137.

Halloran, A., Flore, R. & Mercier, C. 2015. Notes from the 'Insects in a gastronomic context' workshop in Bangkok, Thailand. *Journal of Insects as Food and Feed*, 1(3): 241–243.

Hodge, J.E. 1953. Dehydrated Foods, Chemistry of Browning Reactions in Model Systems. *Journal of Agricultural and Food Chemistry*, 1(15): 928–943.

Holler, J., Skoog, D. & Crouch, S. 2007. Quantum-mechanical properties of radiation. In *Principles of Instrumental Analysis*. 144–156.

Hwang, I.G., Kim, H.Y., Woo, K.S., Lee, J. & Jeong, H.S. 2011. Biological activities of Maillard reaction products (MRPs) in a sugar–amino acid model system. *Food Chemistry*, 126(1): 221–227.

Inaoka, T., Okubo, G., Yokota, M. & Takemasa, M. 1999. Nutritive Value of House Fly Larvae and Pupae Fed on Chicken Feces as Food Source for Poultry. *Japanese poultry science*, 36(3): 174–180.

Jaeger, H., Janositz, A. & Knorr, D. 2010. The Maillard reaction and its control during food processing. The potential of emerging technologies. *Pathologie Biologie*, 58(3): 207–213.

Jin, G., He, L., Zhang, J., Yu, X., Wang, J. & Huang, F. 2012. Effects of temperature and NaCl percentage on lipid oxidation in pork muscle and exploration of the controlling method using response surface methodology (RSM). *Food Chemistry*, 131(3): 817–825.

Jing, H. & Kitts, D.. 2002. Chemical and biochemical properties of casein–sugar Maillard reaction products. *Food and Chemical Toxicology*, 40(7): 1007–1015.

Johnson, D. V. 2010. The contribution of edible forest insects to human nutrition and to forest management. In *Forest insects as food: humans bite back. Proceedings of a workshop on Asia-Pacific resources and their potential for development,* 50(8): 15–20.

Jones, L.D., Cooper, R.W. & Harding, R.S. 1972. Composition of Mealworm Tenebrio molitor Larvae. *The Journal of Zoo Animal Medicine*, 3(4): 34.

Jusino, M.G., Ho, C.T. & Tong, C.H. 1997. Formation Kinetics of 2,5-Dimethylpyrazine and 2-Methylpyrazine in a Solid Model System Consisting of Amioca Starch, Lysine, and Glucose. *Journal of Agricultural and Food Chemistry*, 45(8): 3164–3170.

Kasper, M. & Schieberle, P. 2005. Labeling studies on the formation pathway of Nε-carboxymethyllysine in Maillard-type reactions. In *Annals of the New York Academy of Sciences*. New York Academy of Sciences: 59–62.

Keeble, B.R. 1988. The Brundtland report: 'Our common future'. *Medicine and War*, 4(1): 17–25.

Kim, J.S. & Lee, Y.S. 2009. Enolization and racemization reactions of glucose and fructose on heating with amino-acid enantiomers and the formation of melanoidins as a result of the Maillard reaction. *Amino Acids*, 36(3): 465–474.

Koehler, P.E. & Odell, G.V. 1970. Factors affecting the formation of pyrazine compounds in sugar-amine reactions. *Journal of Agricultural and Food Chemistry*, 18(5): 895–898.

Kramer, K.J. & Koga, D. 1986. Insect chitin: Physical state, synthesis, degradation and metabolic regulation. *Insect Biochemistry*, 16(6): 851–877.

Kroeckel, S., Harjes, A., Roth, I., Katz, H., Wuertz, S., Susenbeth, A. & Schulz, C. 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (Hermetia illucens) as fish meal substitute — Growth performance and chitin degradation in juvenile turbot (Psetta maxima). *Aquaculture,* 352(2): 345-352.

Lan, X., Liu, P., Xia, S., Jia, C., Mukunzi, D., Zhang, X., Xia, W., Tian, H. & Xiao, Z. 2010. Temperature effect on the non-volatile compounds of Maillard reaction products derived from xylose–soybean peptide system: Further insights into thermal degradation and cross-linking. *Food Chemistry*, 120(4): 967–972.

Larroche, C., Besson, I. & Gros, J.-B. 1999. High pyrazine production by Bacillus subtilis in solid substrate fermentation on ground soybeans. *Process Biochemistry*, 34(6–7): 667–674.

Lasekan, O.., Feijao Teixeira, J.. & Salva, T.J.. 2001. Volatile flavour compounds of cooked acha (Digitaria exilis Stapf). *Food Chemistry*, 75(3): 333–337.

Lee, Y.S., Kim, Jae-Hun, K.J., Lee, J.W., Kim, M.R., Yook, H.S. & Byun, M.W. 2006. Effect of pH on non-enzymatic browning reaction during γ-irradiation processing using sugar and sugar–glycine solutions. *Food Chemistry*, 94(3): 420–427.

Lei, C.Y., Zhou, D.Z., Zhao, R., Deng, Q.H., Yu, A.N. & Yang, X.H. 2011. Effect of Metal Ions (Cu^{2+}/Fe^{2+}) on the Antioxidant Activity of L-Ascorbic Acid-Glycine Model System. *Advanced Materials Research*, 396–398: 28–31.

Lenaerts, S., Van Der Borght, M., Callens, A. & Van Campenhout, L. 2018. Suitability of microwave drying for mealworms (Tenebrio molitor) as alternative to freeze drying: Impact on nutritional quality and colour. *Food Chemistry*, 254: 129–136.

Lensvelt, E.J.S. & Steenbekkers, L.P.A. 2014. Exploring Consumer Acceptance of Entomophagy: A Survey and Experiment in Australia and the Netherlands. *Ecology of Food and Nutrition*, 53(5): 543–561.

Liu, S.C., Yang, D.J., Jin, S.Y., Hsu, C.H. & Chen, S.L. 2008. Kinetics of color development, pH decreasing, and anti-oxidative activity reduction of Maillard reaction in galactose/glycine model systems. *Food Chemistry*, 108(2): 533–541.

Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., Cai, M., Li, Q., Mazza, L., Zhang, J., Yu, Z. & Zheng, L. 2017. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly P. Falabella, ed. *PLOS ONE*, 12(8).

Lord, W.D., Goff, M.L., Adkins, T.R. & Haskell, N.H. 1994. The Black Soldier Fly *Hermetia illucens* (Diptera: Stratiomyidae) As a Potential Measure of Human Postmortem Interval: Observations and Case Histories. *Journal of Forensic Sciences*, 39(1).

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.

Maillard, J.C. 1912. Formation de matieres humiques par action de polypeptides sur sucres. *Comptes rendus de l'Académie des Sciences*, 156:148–149.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M.C. & Lerici, C.R. 2001. Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science & Technology*, 11(9–10): 340–346.

Martin, R.D., Rivers, J.P.W. & Cowgill, U.M. 1976. Culturing mealworms as food for animals in

captivity. *International Zoo Yearbook*, 16(1): 63–70.

Martins, S.I.F., Jongen, W.M. & van Boekel, M.A.J. 2001. A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology*, 11(9–10): 364–373.

Matroh, K., Benjakul, S. & Tanaka, M. 2006. Effect of reactant concentrations on the Maillard reaction in a fructose–glycine model system and the inhibition of black tiger shrimp polyphenoloxidase. *Food Chemistry*, 98(1): 1–8.

Menczel, J.D., Judovits, L., Prime, R.B., Bair, H.E., Reading, M. & Swier, S. 2008. Differential Scanning Calorimetry (DSC). In *Thermal Analysis of Polymers*. Hoboken, NJ, USA: John Wiley & Sons, Inc.: 7–239.

Meyer-Rochow, V.B. & Chakravorty, J. 2013. Notes on entomophagy and entomotherapy generally and information on the situation in India in particular. *Applied Entomology and Zoology*, 48(2): 105–112.

Morales, F.J. 2005. Assessing the non-specific hydroxyl radical scavenging properties of melanoidins in a Fenton-type reaction system. *Analytica Chimica Acta*, 534(1): 171–176.

Mshayisa, V. 2016. Antioxidant effects of Maillard reaction products (MRPs) derived from glucose-casein model systems. Master's thesis. Cape Peninsula University of Technology, South Africa.

Mutafela, R.N. 2015. High value organic waste treatment via black soldier fly bioconversion. Msc thesis. KTH Royal Institute of Technology, Sweden.

Nadeau, L., Nadeau, I., Franklin, F. & Dunkel, F. 2015. The Potential for Entomophagy to Address Undernutrition. *Ecology of Food and Nutrition*, 54(3): 200–208.

Newton, G., Sheppard, D., Watson, D., Burtle, G., Dove, C. and Tomberlin, J., 2005. The black soldier fly, Hermetia illucens , as a manure management /resource recovery tool. *Symposium on the state of the science of animal manure and waste management*.

Newton, G.L., Booram, C. V., Barker, R.W. & Hale, O.M. 1977. Dried Hermetia Illucens Larvae Meal as a Supplement for Swine. *Journal of Animal Science*, 44(3): 395–400.

Newton, G.L., Sheppard, D.C. Watson, D.W. Burtle, G.J. Dove, C.R. Tomberlin, J. K. & Thelen, E. E. 1984. The Black Soldier Fly, Hermetia illucens , as a manure management/resource recovery tool. *Symposium on the state of the science of Animal Manure and Waste Management*. 5-7.

Nyakeri, E. Ogola, H. Ayieko, M. & Amimo, F. An open system for farming black soldier fly larvae as a source of proteins for smallscale poultry and fish production. *Journal of Insects as Food and Feed*, 3(1): 51-56.

Oonincx, D.G.A.B., van Broekhoven, S., van Huis, A. & van Loon, J.J.A. 2015. Feed Conversion, Survival and Development, and Composition of Four Insect Species on Diets Composed of Food By-Products N. T. Papadopoulos, ed. *PLOS ONE*, 10(12).

Pavia, L.D., Lampman, M.G., Kriz, S.G. & Vyvyan, R.J. 2009. Introduction to Spectroscopy. *Brooks/Cole, Cengage Learning*, 55(3):200-600.

Pearincott, J.V. 1960. Changes in the lipid content during growth and metamorphosis of the house fly, Musca domestica linnaeus. *Journal of Cellular and Comparative Physiology*, 55(2): 167–174.

Peng, F. & Yang, X.H. 2013. Effect of Cu^{2+} on Ascorbic Acid - Methionine Pattern is Formed by the Reaction of Aroma Compounds. *Advanced Materials Research*, 803: 103–107.

Pennino, M., Dierenfeld, E.S. & Behler, J.L. 1991. Retinol, α-tocopherol and proximate nutrient composition of invertebrates used as feed. *International Zoo Yearbook*, 50(2): 10–20.

Pieterse, E., Pretorius, Q., Hoffman, L.C. & Drew, D.W. 2014. The carcass quality, meat quality and sensory characteristics of broilers raised on diets containing either Musca domestica larvae meal, fish meal or soya bean meal as the main protein source. *Animal Production Science*, 54(5): 622.

Purschke, B., Meinlschmidt, P., Horn, C., Rieder, O. & Jäger, H. 2017. Improvement of techno-functional properties of edible insect protein from migratory locust by enzymatic hydrolysis. *European Food Research and Technology*, 0(0): 1–15.

Ramos-elorduy, J. 2009. Anthropo-entomophagy: Cultures, evolution and sustainability. *Entomological Research*, 39(5): 271–288.

Ramos-Elorduy, J., Moreno, J.M.P., Prado, E.E., Perez, M.A., Otero, J.L. & De Guevara, O.L. 1997. Nutritional value of edible insects from the state of Oaxaca, Mexico. *Journal of Food Composition and Analysis*, 10(2): 142–157.

Ramos-Elorduy, J., Gonzalez, E.A., Hernandez, A.R. & Pino, J.M. 2002. Use of *Tenebrio molitor* (coleoptera: *Tenebrionidae*) to recycle organic wastes and as feed for broiler chickens. *Journal* of *Economic Entomology*, 95(1): 214–220.

Ramos-Elorduy, J., Moreno, J.M.P., Prado, E.E., Perez, M.A., Otero, J.L. & De Guevara, O.L. 1997. Nutritional value of edible insects from the state of Oaxaca, Mexico. *Journal of Food Composition and Analysis*, 10(2): 142–157.

Ramos-Elorduy, J., Neto, E.M.C., dos Santos, J.F., Moreno, J.M. & Torres, I.L., 2006. Estudio comparativo del valor nutritivo de varios coleoptera comestibles de méxico y pachymerus nucleorum de brasil. Interciencia: Revista De Ciencia y Tecnología De América. 31(7): 512–516.

Resh, V.H., & Cardé, R.T. 2003. Encyclopedia of insects. Academic Press San Diego, CA.

Resmini, P., Pellegrino, L. & Batelli, G. 1990. Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Journal of Food Science*, 3:173.

Rizzi, G.P. 2008. The Strecker Degradation of Amino Acids: Newer Avenues for Flavor Formation. *Food Reviews International*, 24(4): 416–435.

Rufián-Henares, J.A. & Delgado-Andrade, C. 2009. Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals. *Food Research International*, 42(3): 394–400.

Schaller, E., Bosset, J.O. & Escher, F. 1998. 'Electronic Noses' and Their Application to Food. *LWT - Food Science and Technology*, 31(4): 305–316.

Schiavone, A., De Marco, M., Martínez, S., Dabbou, S., Renna, M., Madrid, J., Hernandez, F., Rotolo, L., Costa, P., Gai, F. & Gasco, L. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (Hermetia illucens L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology*, 8(1): 51.

Scholtz, C.H. & Holm, E. 1985. Insects of Southern Africa. Butterworths Durban.

Shelomi, M. 2016. The meat of affliction: Insects and the future of food as seen in Expo 2015. *Trends in Food Science & Technology*, 56: 175–179.

Sheppard, D.C., Tomberlin, J.K., Joyce, J.A., Kiser, B.C. & Sumner, S.M. 2002. Rearing Methods for the Black Soldier Fly (Diptera: Stratiomyidae): Table 1. *Journal of Medical Entomology*, 39(4): 695–698.

Sheppard, D.C., 1992. Large-scale feed production from animal manures with a non-pest native fly . *Food Insects Newsletter,* 5(2): 1-7.

Shiau, S.Y., Lin, S.F., Yu, S.L., Lin, A.L. & Kwok, C.C. 1990. Defatted and full-fat soybean meal as partial replacements for fishmeal in tilapia (Oreochromis niloticus×O. aureus) diets at low

protein level. *Aquaculture*, 86(4): 401–407.

Smith, D.M. 2017. Protein Separation and Characterization Procedures. In Springer, 50: 431–453.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. & De Smet, S. 2017. Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*, 97(8): 2594–2600.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W. & Sealey, W. 2007. Fly Prepupae as a Feedstuff for Rainbow Trout, Oncorhynchus mykiss. *Journal of the World Aquaculture Society*, 38(1): 59–67.

Stanimirova, I., Boucon, C. & Walczak, B. 2011. Relating gas chromatographic profiles to sensory measurements describing the end products of the Maillard reaction. *Talanta*, 83(4): 1239–1246.

Szczesniak, A.S. 2002. Texture is a sensory property. *Food Quality and Preference*, 13(4): 215–225.

Tazi, S., Plantevin, F., Falco, C.D., Puigserver, A. & Ajandouz, E.H. 2009. Effects of light, temperature and water activity on the kinetics of lipoxidation in almond-based products. *Food Chemistry*, 115(3): 958–964.

Tucker, C.A. 2014. The significance of sensory appeal for reduced meat consumption. *Appetite*, 81: 168–179.

Van Boekel, M.A.J.S. 2006. Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances*, 24(2): 230–233.

Vane-Wright, R.I. 1991. Why Not Eat Insects? *Bulletin of Entomological Research*, 81(1): 1–4.

Van Huis, A. 2013. Edible insects. Future prospects for food and feed security. *Annual Review of Entomology*, 171(2): 1–201.

Van Huis, A. 2013. Potential of Insects as Food and Feed in Assuring Food Security. *Annual Review of Entomology*, 58(1): 563–583.

Vhangani, L.N. & Van Wyk, J. 2013. Antioxidant activity of Maillard reaction products (MRPs) derived from fructose-lysine and ribose-lysine model systems. *Food Chemistry*, 137(1–4): 92–98.

Vázquez-Araújo, L., Verdú, A., Navarro, P., Martínez-Sánchez, F. & Carbonell-Barrachina, Á.A. 2009. Changes in volatile compounds and sensory quality during toasting of Spanish

almonds. *International Journal of Food Science & Technology*, 44(11): 2225–2233.

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.

Wagner, M. & Wagner, M. 2017. Thermal Analysis in Practice. In *Thermal Analysis in Practice*. Carl Hanser Verlag GmbH & Co. KG:1–9.

Wang, H.Y., Qian, H. & Yao, W.R. 2011. Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chemistry*, 128: 573–584.

Wang, Y.-S. & Shelomi, M. 2017. Review of Black Soldier Fly (Hermetia illucens) as Animal Feed and Human Food. *Foods*, 6(10): 91.

Wilschefski, S. & Baxter, M. 2019. Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects. Clinical Biochemist Reviews, 40(3): 115–133.

Yaylayan, V.A. & Haffenden, L.J.W. 2003. Mechanism of pyrazole formation in [13C-2] labeled glycine model systems: N-N bond formation during Maillard reaction. *Food Research International*, 36(6): 571–577.

Yen, A.L., Durst, P.B., Johnson, D. V, Leslie, R.N. & Shono, K. 2010. Edible insects and other invertebrates in Australia: future prospects. In *Biology*. 65–84.

Yilmaz, Y. & Toledo, R. 2005. Antioxidant activity of water-soluble Maillard reaction products. *Food Chemistry*, 93(2): 273–278.

Yu, A.N. & Zhang, A.D. 2010. The effect of pH on the formation of aroma compounds produced by heating a model system containing l-ascorbic acid with l-threonine/l-serine. *Food Chemistry*, 119(1): 214–219.

Yu, H., Chin, M., West, J.J., Atherton, C.S., Bellouin, N., Bergmann, D., Bey, I., Bian, H., Diehl T., Forberth, G., Hess, P., Schulz, M. & Shindell, T. 2013. A multimodel assessment of the influence of regional anthropogenic emission reductions on aerosol direct radiative forcing and the role of intercontinental transport. *Journal of Geophysical Research: Atmospheres*, 118(2):700-720.

Zeng, Y., Zhang, X., Guan, Y. & Sun, Y. 2011. Characteristics and Antioxidant Activity of Maillard Reaction Products from Psicose-Lysine and Fructose-Lysine Model Systems. *Journal of Food Science*, 76(3): C398–C403.

Zhou, F., Tomberlin, J.K., Zheng, L., Yu, Z. & Zhang, J. 2013. Developmental and Waste Reduction Plasticity of Three Black Soldier Fly Strains (Diptera: Stratiomyidae) Raised on

Different Livestock Manures. *Journal of Medical Entomology*, 50(6): 1224–1230.

Zhang, X., Shen, Y., Prinyawiwatkol, W. & Xu, Z. 2012. Volatile Compounds in Fresh-Cut Pineapple Heated at Different Temperatures. *Journal of Food Processing and Preservation*, 36(6): 567–573.

CHAPTER 3

The nutritional quality and structural analysis of Black soldier fly larvae flour before and after defatting

3.1 Abstract

This study aimed to assess the nutritional information and structural overview of the BSFL flours (full fat and defatted). The BSFL flours were obtained by freeze drying the larvae and the removal of fat using hexane and isopropanol mixture ratio of 3:2 (v/v). Nutritional and structural analyses were conducted using standard methods. All results were statistically analysed. The full-fat and defatted flours had high protein (43.70% and 54.38% respectively). Defatting significantly increases the protein content by approximately 10%, while the fat content decreased from 8% in full fat larvae to 1.8% in defatted larvae. The compositional data was qualitatively confirmed with Universal Attenuated Total Reflectance - Fourier Transform Infrared spectroscopy (UATR-FTIR) mainly in the amide I and II regions. Thermal gravimetry (TG) and differential scanning calorimeter (DSC) analysis, showed the conformational physical changes produced due to removal of fat which affected protein denaturation. DSC analysis displayed curves of both endothermic and exothermic reaction. Both samples exhibited the wide endothermic heating peaks from 42 °C to 112 °C during the first heating program, which could be attributed to the evaporation of water content in the samples. TGA curves showed that the first stage of the decomposition process was relevant with loss of free and loosely bound water up to 150 °C. The second stage of decomposition was from 150 to 400 °C, during which stage protein and carbohydrates volatilized. And the third stage from 450 to 550 °C may be associated with polypeptide decomposition. FTIR revealed that the defatting process induced structural modifications on the amide I (1650 cm^{-1}) and amide II (1540 cm^{-1}) regions. In the functional group region at $\pm$ 2930 cm^{-1} referred to as sp^2, sp^3 C-H bonds, which are as the result of the fat bond's absorption, less intense peaks for the defatted flour were observed. Defatting has significant effect on the functional properties and nutritional value of the BSFL. Defatted as well as full-fat flour both show good nutritional and structural characteristics for use in many food applications, however the improved proximate composition of the defatted BSFL can be applied to food products using BSFL flour as an alternative ingredient.

3.2 Introduction

Insects have been a part of the human diet for centuries and are currently consumed by humans in many parts of Africa (Bukkens, 1997). They are considered to supplement diets of approximately 2 billion people (FAO, 2009). Due to the current food insecurity situation prevailing in many developing countries and future challenges of feeding over 9 billion people in 2050, insects have received global attention as a potential alternate major source of proteins (FAO, 2009). As a result of increasing incomes, urbanization, environment and nutritional concerns and other anthropogenic pressures, the global food system is undergoing a profound change. Due to these reasons there has been a major shift to diets which include the consumption of insect products, and this change is likely to continue in the coming decades.

The evaluation of alternative ingredients that are affordable and locally available as substitutes for conventional protein meals is therefore required. In this context, insects have captured the interest as a complementary source of protein, amino acids (AA), fat, carbohydrates, vitamins and trace elements (Chen et al., 2009). Removal of fat from an insect is the technique used to separate the high insect protein for use in animal feed, and insect oil which may be used in feed and biofuel (Makkar et al., 2014). This has become a frequent practice to process the insect into defatted protein-rich meal and oil fractions to minimise the variations in biochemical composition and attenuate the risk of lipid oxidation (Dumas et al., 2018).

As stated before, the nutrient composition varies, i.e. crude protein and crude lipid can vary from 40 to 54% and 15 to 49%, respectively, depending on the substrates fed to larvae and processing methods the larvae have undergone (Lock et al., 2016; Makkar et al., 2014). The insect larvae can therefore contain such essential nutrients from sources not directly suitable for human or animal nutrition, an ability that can be used to tailor the composition of the insect larvae towards desired nutrient profiles to be used as feed ingredients.

Even with the great research interest in insects such as Black soldier fly larvae (BSFL), data on nutrition information studies of defatted BSFL meal remain hard to find. Currently, only few studies have been conducted to evaluate effects of defatted BSFL meal on several aquatic animals (Belghit et al., 2018; Renna et al., 2017; Dumas et al., 2018). Results observed among these studies were inconsistent. This is probably due to larvae reared on various feeds or the defatting methods used (i.e. mechanical press without solvents, Soxhlet method, solvent extraction), which can be challenging for obtaining consistent quality of insects' meal and

formulating diets with consistent nutrient composition. For this study, solvent extraction was chosen and a choice of solvents was made based on several additional factors: volatility (for ready removal later), freedom from toxic or reactive impurities (to avoid reaction with fats), ability to form a two-phase system with water (to remove nonlipids), and price. In this study a hexane: isopropanol blend was chosen. According to Schaumburg and Spencer (1978), hexane in high concentration is a known neurotoxin, through metabolism to the 2,5-diketo compounds, but it is relatively non-toxic in laboratory usage and is not a hazard after sewage processing. While many solvents have been recommended (Hara & Radin, 1978; Nelson et al., 1986), the most popular is the chloroform:methanol system of Folch et al. (1957) and its various modifications. In this regard, chloroform can produce tumours in animals and methanol is well known for its damage to the visual system.

Recently, research on different substrates affecting the composition of the BSFL have been well documented (Tschirner & Simon, 2015; Liland et al., 2017; Meneguz et al., 2018). However, the nutrient quality and structure overview of both whole (full fat) and defatted BSFL has not been studied as a suitable alternative protein source. The objective of this study was to evaluate the effect of defatting on the BSFL nutrient quality, mineral composition and structural analysis. In addition, DSC and TGA were carried out to investigate the compositions and structural variation of the insect product to understand the mechanistic speculation of enhanced protein yield of this insect species.

3.3 Material and Methods
3.3.1 Chemicals
All chemicals used in the study were of analytical grade unless stated otherwise. Hexane (Analytical reagent grade), isopropanol (88%), petroleum ether (Analytical reagent grade), HNO_3 (55%), Ethylenediaminetetraacetic acid (EDTA, Certified Reference Material, 41% Carbon, 5.5% Hydrogen, 9.56% Nitrogen) and potassium hydroxide were obtained from Sigma-Aldrich (South Africa). Stock standard solution (1000 ppm) of the elements were also obtained from Sigma-Aldrich (South Africa). Prepared solutions and reagents were stored under conditions that prevented deterioration or contamination. All solutions were prepared using deionized water (Milli-Q system, Millipore, Gradient model) with a resistivity of 18.2 MΩ.cm.

3.3.2 Sample processing

Black soldier flies in the larval stage were supplied by AgriProtein, Phillipi, Cape Town. They were immediately cleaned by the blanching method to prevent browning and were stored at −80 °C until further processing. The insect was ground in a blender and then freeze-dried (VirTis SP Scientific, wizard 2.0) for 3 days at 500 mTorr; freeze-drying was used to obtain a more stable and easier product to use.

Some of the freeze-dried samples were subjected to a defatting process. The insect was defatted using hexane and isopropanol at a ratio of 3:2 (v/v). One part of the whole BSFL sample (g) and five parts of the solvent mixture (mL) were stirred on a magnetic stirrer (Labotec, Cape Town, South Africa) for 2 h. Following sedimentation of the solids, the solvent-fat-mixture was decanted. The procedure was repeated twice. Residual solvent was removed by evaporation in the fume hood overnight. Subsequent fine grinding of the defatted samples using mortar and pestle, and sieving through a stainless steel filter sieve with pore size of 500 μm to remove the integument, produced defatted Black soldier fly flour.

3.3.3 Nutritional analysis

Proximate analysis, including protein content (method 920.53), moisture content (method 934.01), ash content (method 923.03) and crude fat was performed according to the standard methods proposed by Association of Official Analytical Chemists (AOAC). Carbohydrates were calculated by difference: carbohydrates = 100 - (% proteins + % lipids + % ash) according to the method described by Pearson's Chemical Analysis of Foods.

3.3.3.1 Moisture content

Whole and defatted insects were separately pulverized using a mortar and pestle before analysis. The moisture content was determined by drying the samples in a drying oven (Scientific series 900) at 100 °C. A crucible was taken from an oven (100 °C) and placed in a desiccator to cool for 30 min. The crucible was weighed accurately using an analytical balance (Chem-Lab Supplies, South Africa). The scale was tared and 5 g of sample was accurately weighed into the crucible (61 x 37mm porcelain, Chem-Lab Supplies, South Africa). The crucible and sample were placed in the oven (set at 100 °C to prevent denaturation of protein) for 12 h. After drying, the crucibles were taken out of the oven and placed into a desiccator to cool for 30 min. The moisture free samples were weighed. All the masses were recorded for calculation purposes using Equation 3.1.

Calculation:

$$\text{Moisture (\%)} = \frac{(A + B - C)}{B} \text{x100}$$

<div align="right">**Equation 3.1**</div>

- A- crucible weight
- B-sample weight
- C-weight of the crucible and sample after completion of the oven-drying procedure

3.3.3.2 Ash content

Whole and defatted insects were grounded separately using a mortar and pestle or a blender (Philips, Eindhoven, The Netherlands) until fine powder was obtained before analysis. The ash content was determined after ashing the samples in a muffle furnace (Carbolite Sheffield LMF 4) at 500 °C for 12 h. The sample was first dried according to the method of determining the moisture content as previously described. Thereafter, the sample was transferred to the furnace. Afterwards, the furnace was switched off and the sample left to cool for at least 2 h before transferring it to a desiccator for 30 min followed by accurate weighing. Masses were recorded for further calculations using Equation 3.2.

Calculation:

$$\text{Ash (\%)} = \frac{D - A}{\text{sample weight}} \text{ x 100}$$

<div align="right">**Equation 3.2**</div>

- A – weight of the clean crucible
- D – weight of the crucible and ash

3.3.3.3 Protein content

Crude protein content was determined using Dumas (TruSpec[TM] Leco Carbon/Hydrogen/Nitrogen Series) which was calibrated with EDTA (AOAC, 2000), using a protein-to-nitrogen conversion factor of 6.25. A small piece of tin foil cup (Leco Corparation 502167) was placed on the scale. The scale was tared and 0.1 g of the sample was placed in the foil piece. The sample-filled foil was removed from the scale and sealed to form a teardrop. The teardrop shaped foil was placed in the carousel of the TruSpec[TM] Leco. From here the sample was analysed and returned a nitrogen (N) value on the computer screen. The N value was recorded for further calculation using Equation 3.3.

Calculation:

$$\text{Crude protein } (\%) = \text{Nitrogen value } \% \text{ x } 6.25 \qquad \textbf{Equation 3.3}$$

To give a chemical breakdown of this method, a sample of known mass is combusted in a high temperature range of 800 - 900 °C chamber in the presence of oxygen. This leads to the release of carbon dioxide, water and nitrogen. The gases are then passed over special columns, namely potassium hydroxide aqueous solution, which absorb the carbon dioxide and water. A column containing a thermal conductivity detector at the end is then used to separate the nitrogen from any residual carbon dioxide and water and the remaining nitrogen content is measured.

3.3.3.4 Fat content

Fat content was analysed using the Soxhlet method as described by the Association of German Agricultural Investigation and Research Institutions (VDLUFA, 1976), with some modifications. Homogenized samples (3 g) were transferred into extraction thimbles (26x60, Bio-Smart Scientific, South Africa). The thimbles were placed into the Soxhlet compartment (ST 243 Soxhlet 1040 extraction unit). The extraction was implemented using 50 mL petroleum ether (bp. 40 – 60 °C) into the extraction cups during 30 min at 60 °C in the Soxhlet apparatus. After extraction, the petroleum ether was evaporated using a heating block at 50 °C under fume hood using extraction cups. The fat residue was cooled in a desiccator for 30 min and weighed. Masses were recorded for further calculations using Equation 3.4.

Calculation:

$$\text{Fat } (\%) = \frac{(\text{crucible} + \text{oil}) - \text{empty crucible}}{\text{sample weight}} \text{ x } 100 \qquad \textbf{Equation 3.4}$$

3.3.3.5 Element composition

The mineral analysis was done at the Cape Peninsula University of Technology, Chemistry Department at Bellville Campus. Mineral content was determined on 5 g of dried and finely ground samples. Each sample was ashed at 500 °C for 5 h and left to cool to ambient temperature. Afterwards, 5 mL of 1M HNO_3 was added. Subsequently this was filtered into100 mL volumetric flask, which was made up to volume with 1 M HNO_3. Elements were measured on an iCAP 6000 Series Inductively Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. Elemental concentrations were calculated using iTEVA Analyst software. Argon

gas flow rate was maintained at 2-5 mL/min and instrument settings were as follows: a camera temp -27 °C, generator temp 24 °C, optics temp 38 °C, RF power 1150 W, pump rate 50 rpm, aux gas flow 0.5 L/min, nebulizer 0.7 L/min, coolant gas 12 L/min and normal purge gas flow. Wavelengths for the elements were as follows: Ca 396.847 nm, Fe 259.941 nm, K 766.491 nm, Mg 279.553 nm, Mn 257.611 nm, Na 588.995 nm, and Zn 213.859 nm. Working standards of 0.1, 0.2, 0.4, 1.0, 2.0 and 3.0 ppm were prepared in 0.5M HNO_3.

3.3.4 Structural analysis
3.3.4.1 Universal Attenuated total reflectance Fourier-transform infrared spectroscopy (UATR-FTIR)

All samples were analysed using a Perkin Elmer FTIR equipped with a UATR polarization accessory (Thermo Electron, USA). All spectra were collected by co-addition of 32 scans at a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1}. Prior to data collection of each sample, a background spectrum was collected. The UATR crystal was cleaned with acetone to remove any residual contribution of the previous samples. Sample powders obtained by grinding in a mortar and pestle were placed directly covering the surface of the ATR crystal for the FTIR analysis.

3.3.5 Thermal Analysis
3.3.5.1 Thermal Gravimetric Analysis (TGA)

The thermal degradation properties were evaluated using a thermogravimetric analyser (Perkin Elmer, TGA 7, USA). Approximately 10 mg from each sample was weighed and placed on the heating pan. The tests were conducted in the ramp mode with a temperature range from 30 °C to 600 °C at constant heating rate of 10 °C min^{-1} under nitrogen atmosphere (20 mL min^{-1}) to avoid thermo-oxidative degradation.

3.3.5.2 Differential Scanning Calorimetry (DSC)

The thermal test was done using a differential scanning calorimeter (DSC822e, Mettler Toledo, Greifensee, Switzerland) in accordance to ASTM D3418 standard. Approximately 10 mg of the samples were heated in a semi hermetic pan from room temperature to 200 °C with a heating rate of 10 °C min^{-1} under a nitrogen atmosphere. After 5 min of holding time for the elimination of the thermal history, the samples were cooled to −30 °C, held for 5 min, and heated to 400 °C at a heating rate of 10 °C min^{-1}.

3.3.6 Statistical analysis

All experiments were analysed in triplicate and the results were presented as means ± standard deviation. No statistical analysis was done on the results of this chapter. Results were only observed and compared according to the numerical value.

3.4 Results and discussion

The composition of the BSF larvae meal is summarised in Table 3.1. in terms of results achieved by proximate analysis. The comparisons in this chapter's discussion and conclusion sections are from numerical comparison and were not statistically analysed, however all analyses were done in triplicate (n = 3). These results and those published were compared, and it is noted that the values for the crude protein content of the whole BSFL ranged between 30.6% (Pieterse, 2014) and 43.6% (St-Hilaire et al., 2007) in literature.

Table 0.1 Proximate analyses (dry-matter base) of the whole BSF and defatted (DF) BSF larvae meal. Values represent mean ± standard deviation (n = 3).

Treatment	Moisture %	Protein %	Fat %	Ash %	CHO %
Whole BSFL	4.14 ± 0.05	45.82 ± 0.14	25.78 ± 1.67	6.85 ± 0.34	17.41 ± 0.03
DF BSFL	6.46 ± 0.02	56.11 ± 0.53	4.86 ± 0.06	8.08 ± 0.14	24.49 ± 0.56

The crude protein content obtained in this specific study (45.8%) was comparable to that reported by St-Hilaire et al. (2007), this may be linked with the fact that the larvae were reared on the same feed substrate, harvested at the same stage and processed by the same method (freeze-drying). The slight difference may be explained by a difference in the day of harvest within the larval stage, for example, Aniebo and Owen (2010) reported that there was a decrease in crude protein content from 59.6% to 54.2% to 50.8% in oven dried BSF larvae harvested at day two, three and four respectively. The crude protein content achieved in the current study, when compared to results achieved by Pieterse (2014) of 30.6%, was significantly higher. These high crude protein values reported by the respective authors could be related to the difference in the larval growth medium, stage at harvest and the method of processing. This difference can also be explained by the fact that the prepupae are covered with a chitin layer that consists of nitrogen-hydrogen bonds (Kramer & Koga, 1986). This higher nitrogen content of the prepupae caused an increase in the calculated

protein content, due to the method of analyses utilized; Association of Official Analytical Chemists International (2000), Official Method 4.2.07.

The moisture in BSF from the literature (Newton et al., 1977; St-Hilaire et al., 2007) revealed levels in the region of 0.73% lower than the results observed from the whole BSFL in this study. The defatted BSFL showed a higher moisture content than the whole BSFL, it would therefore indicate that there was moisture absorption during the removing of the fat. As the fat content of the whole larvae decreased, there was an increase in protein content. Defatted BSFL is equal to the whole BSFL minus the fat removed (100 – 25.78 = 74.22g). Therefore, after defatting the proximate analyses values should theoretically be similar to the experimental values. Despite the fact that the theoretical proximate values are based on the initial experimental values of the whole BSFL, the assayed values of the defatted BSFL are well correlated. The values (experimental (theoretical)) are as follows: moisture 6.46 (5.59); protein 56.11 (61.74); ash 8.08 (9.23); CHO 24.49 (23.45).

The mineral content of the samples are shown below in Table 3.2. It is evident from the results that the BSF larvae are a good source of manganese (Mn) as compared to the meat results (660 mg/kg) reported by Demirezen and Uruc, 2006. The iron (Fe) levels in both larvae meals were close to the results of chicken (120 mg/kg), lamb (200 mg/kg) and beef (160 mg/kg) reported by Gerber et al. (2009). The results in Table 3.2 suggest that the content of some minerals increased with decreased fat content (Fe and Mn) but for other minerals its content decreased even though the ash content increased slightly. It would have been expected that the absolute amount would increase due to defatting which results in a relative increase in the amount of these minerals. The processing of food can affect the mineral binding and availability (Fairweather-Tait & Hurrell, 1996). For each essential mineral, the human body has a specific amount required, for example, calcium which is for building strong bones, these results fall within the recommended dietary allowance (Council, 1989). BSFL has 630.34 mg/kg more calcium than a fish meal (Makkar et al., 2014), providing a considerable advantage to BSFL over other food sources, nutritionally.

Table 0.2 Mineral composition of whole BSF, defatted (DF) BSF larvae meal and recommended dietary allowance (Council, 1989).

mg/kg	BSFL		Recommended dietary intake (mg per day)
	Whole	**Defatted**	
Ca	634.77	634.18	1980
Fe	142.62	156.37	96.40
Mg	1901.14	1886.01	2090
Mn	71766.20	79488.50	40.10
K	9457.26	9212.04	4290
Na	1041.68	785.44	185.00
Zn	155.90	89.69	24.70

TGA curves of the whole and defatted BSFL are shown in Figure 3.1. It showed that the first stage of the decomposition process was relevant with loss of free and loosely bound water up to 150 °C. The second stage of decomposition was from 150 to 420 °C, protein and carbohydrates volatilized in this stage. And the third stage from 420 to 550 °C may be associated with polypeptide decomposition. The whole and defatted BSFL samples remained with weight of 25% and 27%, respectively, this is due to polymers which decompose at very high temperatures. Further denaturation and unfolding of the BSFL protein structure occurred at higher temperatures for both samples, therefore, they are thermally stable.
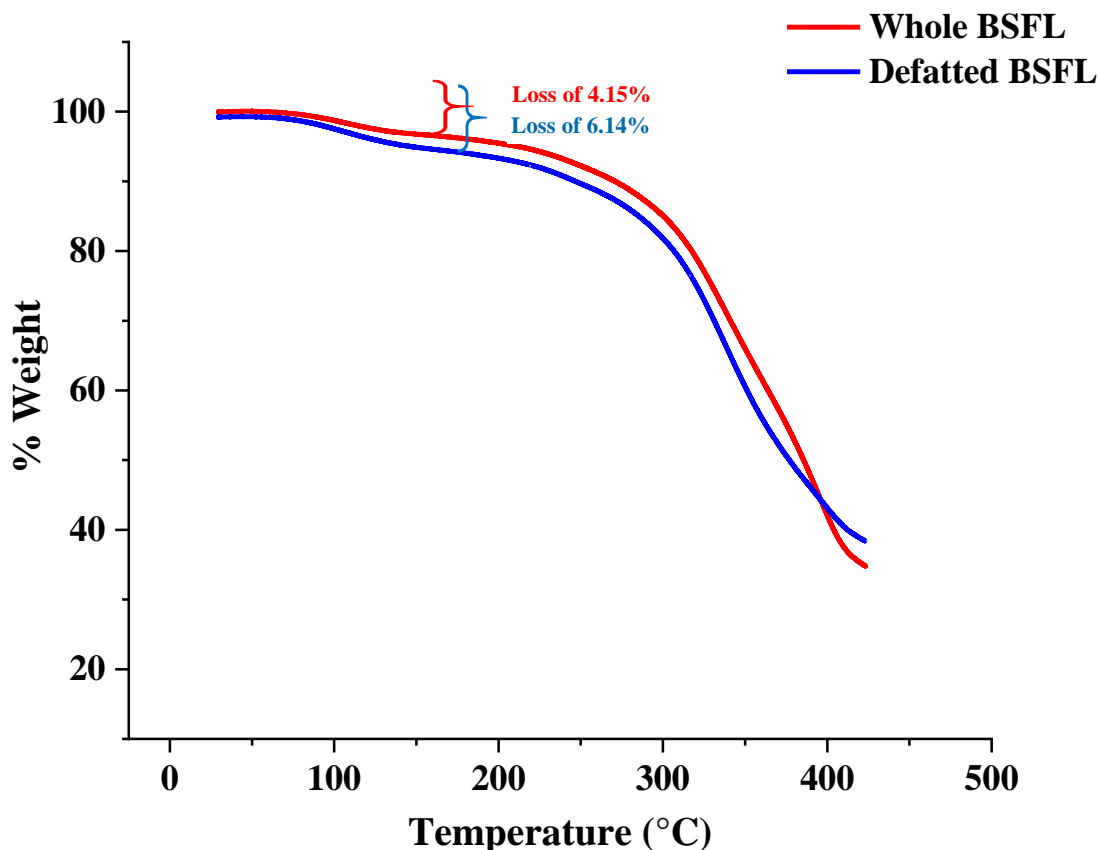
Figure 3.1 The TGA curves of the whole and defatted BSFL.

The DSC profile of BSF larvae whole and BSF larvae defatted are shown in Figure 3.2. It was observed that the two samples showed endothermic peaks and the highest enthalpy changes occurred at temperature 80 to 110 °C. Table 3.3 displays the thermal profile of the whole and defatted (DF) samples.

Table 0.3 Thermal profile of the whole and defatted (DF) samples from DSC analysis (T_O, onset temperature; T_P, peak temperature and ΔH, peak enthalpy).

	T_0 (°C)	T_P (°C)	ΔH (Jg^{-1})
Whole BSFL	40.76	86.68	197.29
DF BSFL	40.76	81.74	153.98

This is observed because the whole sample is deemed to be more energy dense than defatted samples. Thus, the whole sample needs more energy to promote polypeptide decomposition

(Larrosa et al., 2018). This phenomenon is in accord with TGA analysis. A higher temperature was required for denaturation (TGA) and more energy required for decomposition (DSC).
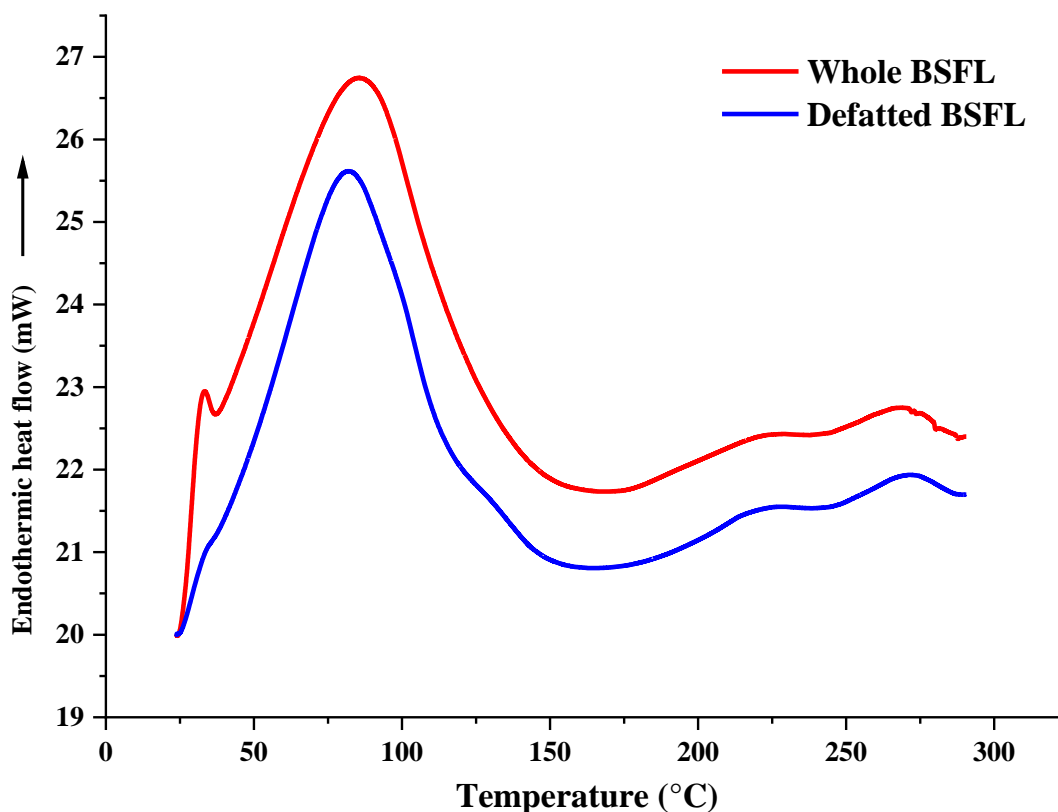
Figure 3.2 The DSC profile of BSF larvae whole and BSF larvae defatted (DF).

Figure 3.3 showed the FTIR spectra of the whole and defatted BSFL samples under the optimum extraction condition. Diagrams revealed that the regions of 1650 cm^{-1} and 1540 cm^{-1} which referred to C=O and C–N stretching from amide I and II were modified by the defatting, because the intensity of the whole BSFL decreased compared to DF BSFL sample. Functional group region at 2930 cm^{-1} referred to as sp^2, sp^3 C-H as the results of fat showed less intense peaks for the DF BSFL. As shown in Figure 3.3, the strong absorbance between 2853.43 and 2923.30 cm^{-1} corresponded to the aliphatic C-H stretching vibration, revealing a large amount of methyl and methylene groups. The absorption peak near 1742.86 cm^{-1} represented the CO group stretching vibration in ketones or carboxylic acids, which was consistent with the high content of ketones in the lipid.

The peaks corresponding to the presence of alkenes (-C=C- stretch) were found at 1574.38 cm^{-1}. These peaks confirmed the presence of triglyceride functional groups in the insect flours. The presence of aromatic amine (C-N stretch) was confirmed by the absorption band at 1165.75 cm^{-1}. The ester group (C-O stretch or C-H bend) was identified at 1095.45 cm^{-1}. Similar results were previously reported for the extraction of lipid from yellow mealworm beetle (Yan et al., 2016; Reshad et al., 2015; Dutta et al., 2014).
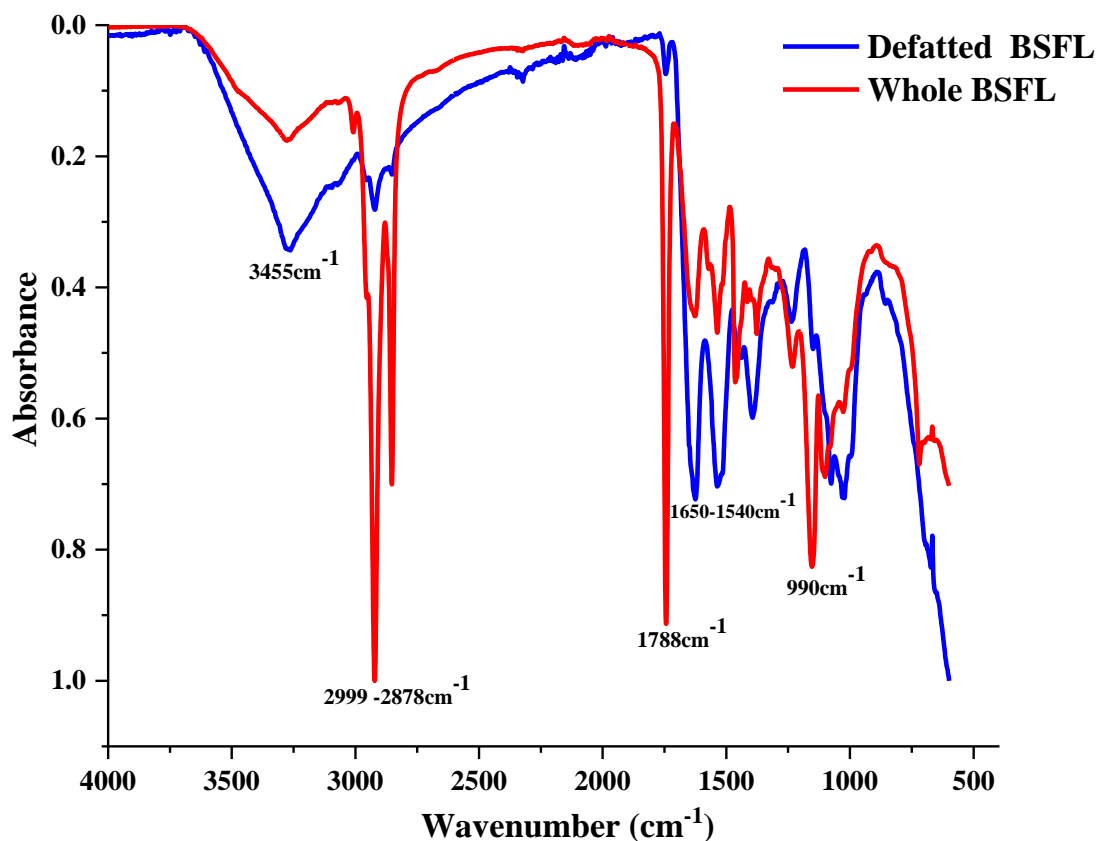
Figure 3.3 The FTIR spectra of the whole and defatted BSF larvae.

3.5 Conclusion

It was concluded that both the whole and defatted larvae are high in crude protein which can be considered for further investigation as a protein rich concentrate or feed ingredients in ruminant nutrition. Further trials should be conducted on different methods to isolate or drain the oil completely from the samples. More importantly, from the current study, it could be inferred that Black soldier fly meal (BSFLM) can be successfully utilised as an alternative protein source to partially replace other protein sources in the ability to sustain normal diets. However, it is

recommended that, in addition to the removal of fat and oil, the residual flour should be processed for protein extraction and characterisation thereof. Additionally, both BSFL meals are high in trace minerals. Finally, it was observed that the defatting treatments produced higher crude protein values than the full-fat treatment. The effect of defatting has the advantage of being able to make use of the fat for other sustainable purpose such as biofuels and yields a higher-level protein for further development for consumption by animals and humans.

References

AOAC, 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, USA.

Aniebo, A.O., Erondu, E.S. & Owen, O.J. 2008. Proximate composition of housefly larvae (Musca domestica) meal generated from mixture of cattle blood and wheat bran. *Livestock Research for Rural Development*, 20(12).

Aniebo, A.O. & Owen, O.J. 2010. Effects of age and method of drying on the proximate composition of housefly larvae (Musca domestica Linnaeus) meal (HFLM). *Pakistan Journal of Nutrition*, 9(5): 485-487.

Aniebo, A.O., Odukwe C.A, Ebenebe, C.I., Ajuogu, P.K., Owen, O.J. & Onu, P.N. 2011. Effect of Housefly Larvae (Musca domestica) Meal on the Carcass and Sensory Qualities of the Mud Catfish, (Clarias gariepinus). *Advances in Food and Energy Security*, 1: 24–28.

Belghit, I., Liland, N.S., Waagbø, R., Biancarosa, I., Pelusio, N., Li, Y., Krogdahl, Å. & Lock, E.J. 2018. Potential of insect-based diets for Atlantic salmon (Salmo salar). *Aquaculture*, 491: 72–81.

Bukkens, S.G.F. 1997. The nutritional value of edible insects. *Ecology of Food and Nutrition*, 36(2–4): 287–319.

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.

Chen, X., Feng, Y. & Chen, Z. 2009. Common edible insects and their utilization in China. *Entomological Research*, 39(5): 299–303.

Council, N.R. 1989. *Recommended Dietary Allowances*. National Academics Press.

De Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero,

D., Katz, H., Dabbou, S., Kovitvadhi, A., Zoccarato, I., Gasco, L. & Schiavone, A. 2015. Nutritional value of two insect larval meals (Tenebrio molitor and Hermetia illucens) for broiler chickens: Apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. *Animal Feed Science and Technology*, 209(2): 211–218.

Demirezen, D. & Uruç, K. 2006. Comparative study of trace elements in certain fish, meat and meat products. *Meat Science*, 74(2): 255–260.

Dumas, A., Raggi, T., Barkhouse, J., Lewis, E. & Weltzien, E. 2018. The oil fraction and partially defatted meal of black soldier fly larvae (Hermetia illucens) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (Oncorhynchus mykiss). *Aquaculture*, 492: 24–34.

Dutta, R., Sarkar, U. & Mukherjee, A. 2014. Extraction of oil from Crotalaria Juncea seeds in a modified Soxhlet apparatus: Physical and chemical characterization of a prospective bio-fuel. *Fuel*, 116: 794–802.

Fairweather-Tait, S. & Hurrell, R.F. 1996. Bioavailability of Minerals and Trace Elements. *Nutrition Research Reviews*, 9(1): 295–324.

Folch, J., Lees, M. & Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. The *Journal of Biological Chemistry*, 226(1): 497–509.

Gerber, N., Brogioli, R., Hattendorf, B., Scheeder, M.R.L., Wenk, C. & Günther, D. 2009. Variability of selected trace elements of different meat cuts determined by ICP-MS and DRC-ICPMS. *Animal*, 3(1): 166–172.Hara, A. & Radin, N.S. 1978. Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry*, 90(1): 420–426.

Huang, C., Feng, W., Xiong, J., Wang, T., Wang, W., Wang, C. & Yang, F. 2019. Impact of drying method on the nutritional value of the edible insect protein from black soldier fly (Hermetia illucens L.) larvae: amino acid composition, nutritional value evaluation, in vitro digestibility, and thermal properties. *European Food Research and Technology*, 245(1): 11–21.

Inaoka, T., Okubo, G., Yokota, M. & Takemasa, M. 1999. Nutritive Value of House Fly Larvae and Pupae Fed on Chicken Feces as Food Source for Poultry. *Japanese Poultry Science*, 36(3): 174–180.

Kramer, K.J. & Koga, D. 1986. Insect chitin: Physical state, synthesis, degradation and metabolic regulation. *Insect Biochemistry*, 16(6): 851–877.

Larrosa, A.P.Q., Camara, Á.S., Pohndorf, R.S., da Rocha, S.F. & Pinto, L.A. de A. 2018.

Physicochemical, biochemical, and thermal properties of Arthrospira (Spirulina) biomass dried in spouted bed at different conditions. *Journal of Applied Phycology*, 30(2): 1019–1029.

Leni, G., Caligiani, A. & Sforza, S. 2019. Killing method a ff ects 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(May 2018): 116–125.

Liland, Nina S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C.G., Waagbø, R., Torstensen, B.E. & Lock, E.-J. 2017. Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae by feeding seaweed-enriched media C. Wegener, ed. *PLOS ONE*, 12(8): 20-30.

Liland, N.S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C.G., Waagbø, R., Torstensen, B.E. & Lock, E.-J. 2017. Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae by feeding seaweed-enriched media C. Wegener, ed. *PLOS ONE*, 12(8): 1–30.

Lock, E.R., Arsiwalla, T. & Waagbø, R. 2016. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. *Aquaculture Nutrition*, 22(6): 1202–1213.

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.

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.

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M. & Gasco, L. 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. *Journal of the Science of Food and Agriculture*, 98(15): 5776–5784.

Nelson, G.J., Kelley, D.S. & Hunt, J.E. 1986. Effect of nutritional status on the fatty acid composition of rat liver and cultured hepatocytes. *Lipids*, 21(7): 454–459.

Newton, G.L., Booram, C.V., Barker, R.W. and Hale, O.M., 1977. Dried Hermetia illucens larvae meal as a supplement for swine. *J. Anim. Sci*, 44(3): 395-400.

Pieterse, E., Pretorius, Q., Hoffman, L.C. & Drew, D.W. 2014. The carcass quality, meat quality and sensory characteristics of broilers raised on diets containing either Musca domestica larvae meal, fish meal or soya bean meal as the main protein source. *Animal Production*

Science, 54(5): 622.

Ramos-Elorduy, J., Moreno, J.M.P., Prado, E.E., Perez, M.A., Otero, J.L. & De Guevara, O.L. 1997. Nutritional value of edible insects from the state of Oaxaca, Mexico. *Journal of Food Composition and Analysis*, 10(2): 142–157.

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M.T., Biasato, I., Biasibetti, E., De Marco, M., Brugiapaglia, A., Zoccarato, I. & Gasco, L. 2017a. Evaluation of the suitability of a partially defatted black soldier fly (Hermetia illucens L.) larvae meal as ingredient for rainbow trout (Oncorhynchus mykiss Walbaum) diets. *Journal of Animal Science and Biotechnology*, 8(1): 57.

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M.T., Biasato, I., Biasibetti, E., De Marco, M., Brugiapaglia, A., Zoccarato, I. & Gasco, L. 2017b. Evaluation of the suitability of a partially defatted black soldier fly (Hermetia illucens L.) larvae meal as ingredient for rainbow trout (Oncorhynchus mykiss Walbaum) diets. *Journal of Animal Science and Biotechnology*, 8(1): 57.

Reshad, A.S., Tiwari, P. & Goud, V. V. 2015. Extraction of oil from rubber seeds for biodiesel application: Optimization of parameters. *Fuel*, 150: 636–644.

Schaumburg, H. & Spencer, P. 1978. Environmental hydrocarbons produce degeneration in cat hypothalamus and optic tract. *Science*, 199(4325): 199–200.

Schiavone, A., De Marco, M., Martínez, S., Dabbou, S., Renna, M., Madrid, J., Hernandez, F., Rotolo, L., Costa, P., Gai, F. & Gasco, L. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (Hermetia illucens L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology*, 8(1): 51.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. & De Smet, S. 2017c. Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*, 97(8): 2594–2600.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W. & Sealey, W. 2007. Fly Prepupae as a Feedstuff for Rainbow Trout, Oncorhynchus mykiss. *Journal of the World Aquaculture Society*, 38(1): 59–67.

Tschirner, M. & Simon, A. 2015. Influence of different growing substrates and processing on the

nutrient composition of black soldier fly larvae destined for animal feed. *Journal of Insects as Food and Feed*, 1(4): 249–259.

Wang, G., Peng, K., Hu, J., Yi, C., Chen, X., Wu, H. & Huang, Y. 2019. Evaluation of defatted black soldier fly (Hermetia illucens L.) larvae meal as an alternative protein ingredient for juvenile Japanese seabass (Lateolabrax japonicus) diets. *Aquaculture*, 507: 144–154.

Yan, W.H., Duan, P.G., Wang, F. & Xu, Y.P. 2016. Composition of the bio-oil from the hydrothermal liquefaction of duckweed and the influence of the extraction solvents. *Fuel*, 185: 229–235.

CHAPTER 4

Characterisation of Black soldier fly larvae protein before and after conjugation by the Maillard reaction

4.1. Abstract

Black soldier fly has been proposed as an alternative protein source sustainable for both food and feed for its nutritional composition. The functional properties of this protein can be improved by inducing the Maillard reaction (MR) in protein-carbohydrate mixtures. This study focused on the extraction, conjugation and characterisation of Black soldier fly larvae (BSFL) protein. The defatted BSFL larvae were subjected to protein extraction at an alkaline pH. The protein extract was then conjugated to glucose. The protein extract and glucose were mixed (in ratio 2:1 w/w, at pH 9), incubated at 50, 70, and 90 °C for 30, 60, 90 and 120 min. The products obtained were then characterised and compared. Change in pH and stabilisation properties of the conjugates were analysed by zeta potential measurement. The changes were confirmed by Universal Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (UATR-FTIR), Scanning Electron Microscopy (SEM), Thermal Gravimetric Analysis (TGA) and Differential Scanning Analysis (DSC). UATR-FTIR combined with Principal Component Analysis (PCA) monitored the protein-sugar conjugates, to show the structural difference among heated proteins (control) and Maillard Reaction Products (MRPs). The protein extract from the larvae was rich in protein content (75%), and displayed good essential amino acids (EAA) in sufficient quantities to meet the dietary requirements for humans. The EAA quantities of the Maillard reaction products (MRPs) decreased due to the MR treatment. FTIR indicated that the amide I and II bands of the protein were altered by the MR. The increased T_{max} (the temperature at which decomposition is completed) demonstrated that the thermal stability of protein was remarkably improved by its conjugation with glucose. These results suggested that MR with glucose can be a promising way to improve the functional properties of BSFL protein.

4.2. Introduction

The rapid increase of the population in the world has placed pressure on conventional food sources (Wang et al., 2017). To be specific, the protein demand that is growing cannot be met by the current protein sources namely, livestock animals, poultry animals and plants. Therefore, for this reason, insects are proposed as an alternative protein source for both food and feed because of their nutritional composition (Janssen et al., 2017; Osimani et al., 2017; Purschke et al., 2018). Black soldier fly (BSF) is one of the promising insects for food production in the world (Muller at al., 2017). Because of its high protein content, it has been used to feed chickens (Schiavone et al., 2017), fish (Renna et al., 2017; Henry e al., 2015; Belforti et al., 2015) and swine (Makkar et al., 2014). Additionally, it has also been exploited for biodiesel production because of the high content of fat (Feng et al., 2018; Wang et al., 2017). Despite people's perception against insects as food, it was discovered that consumers are willing to have insects in their dishes if they are in an unrecognised form, such as a modified product (Schosler., 2012; Balzan et al., 2016). Therefore, the BSF larvae protein has the potential of being exploited for human consumption in the future. For this reason, its protein quality information is extremely necessary to be studied further.

Proteins are important food ingredients because they can change the taste, texture and the appearance of the foods. However, proteins are very unstable in certain conditions of temperature, pH or the presence of organic solvents. Thus, researchers have enhanced protein stability under these unfavourable conditions by using saccharides (Akhtar et al., 2017; Dickinson, 2015; Evans et al., 2013). Saccharides are high molecular weight polymers with very high stability (de Oliveira et al., 2016). Therefore, by combining the properties of these two through conjugation, it is possible to obtain a product with higher stability (Akhtar et al., 2017; Dickinson, 2015; Evans et al., 2013).

The better approach to obtain these conjugates is by the Maillard reaction (MR) (Akhtar et al., 2017; de Oliveira et al., 2016; Liu et al., 2016). MR is the ideal and safest method in protein modification (Feng et al., 2018; Ifeduba et al, 2016; Leiva et al., 2017). At present, there are two main types of MR: dry and wet methods. In this study, the wet method, was used. Wet heating largely shortens the reaction time at high temperature for the Maillard reaction to provide better browning products. Until now, different proteins, such as soy-bean (Qi et al., 2009), whey protein (Jimenez-Castano et al., 2007) and rice protein (Li et al., 2009) have been conjugated with different saccharides to improve functional properties. Therefore, well-controlled MR can be a good method for the BSF larvae protein modification.

In this study glucose was used to conjugate with BSFL protein. Many studies have shown that Maillard Reaction Products (MRPs) prepared by protein and glucose could significantly improve protein functionality (Leiva et al., 2017). BSFL flour is reported to be rich in amino acids, especially the essential amino acids such as lysine, methionine and threonine (Huang et al., 2019). However, there is no published information about the amino acid profile of BSFL protein or the improvement of the functional properties of the protein using MR.

Therefore, the aim of this study was to extract protein from the BSFL, characterise the protein, conjugate the protein with glucose and determine the amino acid profile before and after conjugation by the Maillard reaction. In order to elucidate conformational changes such as in the chemical structure, protein degradation was monitored in terms of zeta potential. Furthermore, in this investigation, Scanning Electron Microscopy (SEM) and Thermal analyses were studied to obtain information that supply details on the molecular structure and to detail conformational modifications that occur during glycation. Universal Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (UATR-FTIR) technique along with Principal Component Analysis (PCA) were used to probe changes in the protein structure and PCA was also used as an exploratory technique to provide evidence of structural differences in the data, cluster or detect outliers and to improve the classification. PCA will help to obtain valuable information from the entire set of data from the FTIR technique using multivariate data analysis.

4.3. Materials and methods
4.3.1. Chemicals

Defatted BSFL sample was prepared according to the method in the previous chapter. The glucose used in this study was obtained from Sigma-Aldrich (South Africa). Acetonitrile (LC grade), methanol (LC grade), borate buffer, ethylenediaminetetraacetic acid (EDTA), Tris-HCl, o-phthaldialdehyde (OPA), fluorenyl-methyl chloroformate (FMOC) reagents and standard solutions mixture of 18 amino acids were also obtained from Sigma-Aldrich (South Africa). Amber vials, caps were purchased from Merck (Modderfontein, South Africa). All chemicals and reagents were of analytical grade unless stated otherwise. Chemicals used for protein extraction were NaOH and HCl purchased from Merck (South Africa) and they were also used to adjust the pH of the solutions. Prepared solutions and reagents were stored under conditions that prevented deterioration or contamination. All solutions were prepared using deionized water (Milli-Q system, Millipore, Gradient model) with a resistivity of 18.2 MΩ.cm.

4.3.2. Protein extraction

Protein extraction was conducted according to the method of Azagoh et al. (2016) with slight modifications. Protein was extracted from the defatted BSF larvae meal using solubilisation into water at an alkaline pH. The 5 g of the defatted sample was weighed accurately into separate 500 mL standard beakers, and 200 mL of distilled water was added. The pH of the mixture was adjusted immediately to pH 10 (pH meter; Metrohm, Switzerland) using 1 N NaOH solution. The mixture was stirred (magnetic stirrer) on a hot plate at a rate designed to prevent the formation of a vortex, for 1 h at 45 °C. The pH was monitored intermittently and maintained at 10 throughout the stirring period. After stirring, distilled water at pH 10 adjusted by NaOH, was added to reach 250 mL of solution. The mixture was centrifuged at 10,000 g for 30 min. After centrifuging, the pellets were freeze-dried (VirTis Sentry 5SL, United States) before use for the subsequent experiments. The protein content was analysed using the Dumas method with a protein-to-nitrogen conversion factor of 6.25 (AOAC, 2000).

4.3.3. Preparation of protein-glucose conjugates

Maillard reaction products (MRPs) were prepared in accordance with the method of Mshayisa (2016) with slight modifications. BSFL protein (2.0 g) and glucose (1.0 g) were dissolved in 100 mL of 0.1 M Tris-HCl buffer at pH 9. The samples were transferred into 250 mL Schott bottles and heated for 30, 60, 90 and 120 min at 50, 70 and 90 °C in a water bath, respectively. Samples of protein without glucose were subjected to the same treatment and were prepared as the control. After the heating period had elapsed, the resulting MRPs were immediately cooled in an ice bath. MRP solutions were subject to pH measurement (pH meter; Metrohm, Switzerland). The solutions were subsequently freeze-dried to obtain the products in powder form. The powders were stored in air-tight screw-capped glass bottles at -80 °C until analysis.

4.3.4. Characterisation

4.3.4.1. Protein content

Crude protein content was determined using Dumas (TruSpec[TM] Leco Carbon/Hydrogen/Nitrogen Series) which was calibrated with EDTA (AOAC, 2000), using a protein-to-nitrogen conversion factor of 6.25. The Dumas method consists of combusting a sample of known mass in a high temperature range of 800-900°C chamber in the presence of oxygen to release CO_2, H_2O and N_2.

4.3.4.2. Amino acids

Amino acids were prepared according to the method of Jajić et al. (2013) with slight modifications.

Preparation of protein hydrolysates: All samples were ground into a fine powder. First, 0.5 g was weighed (equivalent to 10 mg nitrogen content) into a screw-capped test tube and 7 mL of 6M HCl was added. The tubes were capped, and the samples were hydrolysed for 6 h at 110 °C. After the hydrolysis, the samples were cooled to room temperature and evaporated to dryness using a Reacti-Therm^{TM} heating/stirring module, at 70 °C under a stream of nitrogen. The residues were quantitatively transferred into 100 mL volumetric flasks using 0.1M HCl. The solutions were filtered using 0.22 μm pore size cellulose membrane syringe filters (Sigma-Aldrich, South Africa) into High Performance Liquid Chromatography (HPLC) vials.

HPLC Determination: The chromatographic conditions employed were in accordance with the Agilent method (Henderson et al., 2000). Mobile phase A was acetonitrile:methanol:water (45:45:10, vol. %). The mobile phase B, consisted of 5.5 g of Na_2HPO_4 per 1 L of distilled water, adjusted to the pH 7.8 using 10 M NaOH solution. Briefly, the hydrolysed samples and solutions of the standard amino acid mixture were automatically derivatised with OPA and FMOC by programming the autosampler to mix the solution following the steps below:

1. draw 2.5 µl from vial 1 (borate buffer)

2. draw 0.5 µl from the sample (position X)

3. mix 3 µl in air, max. speed, 2×

4. wait 0.5 min

5. draw 0 µl from vial 2 (water, uncapped vial)

6. draw 0.5 µl from vial 3 (OPA)

7. mix 3.5 µl in air, max speed, 6×

8. draw 0 µl from vial 2 (water, uncapped vial)

9. draw 0.5 µl from vial 4 (FMOC)

10. mix 4 µl in air, max speed, 6×

11. draw 32 µl from vial 5 (water),

12. mix 18 µl (4 µl of the sample + 14 µl of water) in air, max speed, 2× and

13. inject 0.5 µl of each sample into a Zorbax Eclipse-AAA column

at 40 °C, with detection at $\lambda 1 = 338$ nm and $\lambda 2 = 262$ nm. The separation was performed at a flow rate of 1 ml min^{-1} employing a solvent gradient (vol. %,) as follows:

Table 4.1 Gradient elution program used for chromatographic separation of the amino acids.

Time (min)	% Mobile phase (A)	% Mobile phase (B)
0	0	100
1.9	0	100
18.1	60	40
18.6	100	0
22.3	100	0
23.2	0	100
30	0	100

(A): ACN:MeOH:H$_2$O; (B): Na$_2$HPO$_4$ (40 mM, pH 7.8)

Amino acid standards of 25, 50, 100, 250 and 1000 nmol/L were prepared in 0.1M HCl. This method was validated. Appendix A displays the specificity (Table A.1), linearity (Table A.2), limit of detection and limit of quantification (Table A.3) data.

4.3.4.3. Thermal Gravimetric Analysis (TGA)

Thermal stability behaviour of the extracted protein, proteins heated alone and MRPs was analysed using TGA system (Perkin Elmer, TGA 7, USA). To ensure that the reaction was free from interparticle, heat and mass transfer resistances, the mass of the samples were retained within 10 ± 0.2 mg from each sample and were carefully placed on the heating pan. The tests were conducted in the ramp mode of temperature range from 30 °C to 700 °C at a constant heating rate of 10 °C/min. Nitrogen was used as carrier gas (flow rate of 20 mL/min) to avoid thermo-oxidative degradation.

4.3.4.4. Differential Scanning Calorimetry (DSC)

The DSC profile of the samples was evaluated using a Perkin-Elmer DSC thermal analysis system. The samples (3–5 mg) were placed in hermetically sealed aluminum pans, heated from 30 to 300 °C at a constant rate of 10 °C/min with a constant purging of nitrogen at a rate of 20 mL/min. A

sealed empty aluminum pan was used as a reference. Thermal transitions of samples were measured for the denaturation temperature. The transition temperature (T_{max}) and total transition enthalpy changes (ΔH) were recorded.

4.3.4.5. Multivariate analysis: Principal Component Analysis (PCA)

PCA is one of the most used chemometric techniques, and is useful for infra-red or near infra-red spectra due to the large amount of data. The original amount of data is consolidated or reduced using classification aims. Measured values or variables are filtered out and bundled into groups depending on the content. With the determination of relationships and structures in the data, information is received to find characteristics or properties in samples. Data evaluation draws conclusions on the sample composition using a sensor signal or pattern of sensor signals. These factors are called Principal Components (PC) (Petrozzi, 2013). This projection method uses only the most important principal components of the PCA. PCA calculation is based on a singular value decomposition of the data array of the fingerprint region of the FTIR spectrum. The first two scores of the PCA results were used to make a projection plot that provided a visual determination of the similarity among the fingerprints. The samples are in the wavelength space known as the variance, which is represented by the drawing of an ellipsoid around the sample. Unscrambler 9.7 for Windows® was used for the PCA in this work.

4.3.4.6. Universal Attenuated total reflectance Fourier-transform infrared spectroscopy (UATR-FTIR)

FTIR can be utilized to identify some of the functional groups present in the solids. In the present study, all samples were analysed using a Perkin Elmer FTIR equipped with an (UATR) polarization accessory. A total of 32 scans were conducted at a resolution of 4 cm^{-1} in the range of 4000–600 cm^{-1} and saved in JCAMP-DX format, which is one of many adapted formats for further statistical treatment by various software. Prior to data collection of each sample, the background air spectrum, water vapour and CO_2 interferences were subtracted from these spectra. The UATR crystal was cleaned with acetone to remove any residual contribution of the previous samples. Then, sample powders obtained by grinding in a mortar and pestle were placed directly covering the surface of the ATR crystal. After baseline correction and smoothing was performed using the Savitzky-Golay function on OriginPro 8.6 software for Windows®, the spectrum data were imported to Unscrambler 9.7 software for Windows®, to standardise the normal variations.

4.3.4.7. Scanning Electron Microscopy (SEM)

The SEM (Electron Microscopy Sciences, Hatfield, PA) was employed to observe microstructure and morphologies of all samples. Before the examination, samples were mounted directly on aluminum specimen stubs with a conductive double-sided adhesive carbon tabs (EM-Tec CT12, Innovative Microscopy Supplier, South Africa) prior coating. Specimens were coated with gold using a Polaron SC 515 Sputter Coater. Digital images of topographical features of the samples were collected using a Quanta 200 FEG environmental SEM operated in the high vacuum/secondary electron imaging mode at an accelerating voltage of 10 kV. The magnification of the images ranged from 150x to 2000x. The images were used to find the difference in the structure of the samples.

4.3.4.8. Measurements of zeta potential

Electrostatic interactions may be involved in protein adsorption phenomena as well as van der Waals, hydrophobic, and steric interactions (Salgın et al., 2013). The Zeta potential was measured in order to evaluate the charge accumulated at the surface of soluble proteins, using a Zetasizer Nano system (Malvern Instruments, South Africa). This provides information on the electrostatic repulsion of charged particles which modifies the distribution of ions in their environment (Malhotra & Coupland, 2004). The zeta potential of the samples was measured as a solution of 1% w/v of the samples prepared using Milli-Q water, followed by filtration prior to taking measurements. Zeta potential measurements were performed at 25 °C in folded capillary cells without the presence of air. Each measurement was the average of six determinations and the entire experiment was performed in triplicate.

4.4. Results and discussion

4.4.1. Amino acids

The BSFL protein extracted from the defatted larvae according to Azagoh's method was analysed for the protein content using the Dumas method. The value obtained (75%) had increased by 19% from the initial Dumas figure of 56% (Table 3.1). The amino acid profile of this protein extract, analysed by HPLC, is shown in Table 4.2. The protein extract was used in the conjugation process with glucose using MR.

Table 4.2 Amino acid profile of protein extract from defatted Black soldier fly larvae (current study), casein and soybean protein (Young & Pellet, 1991) and the recommended dietary allowance for human nutrition from Food Agricultural Organization (FAO), 2013.

Amino acid (mg/g protein)	BSFL protein (current study)	Casein	Soybean	FAO[a] (2013) mg/g protein
Essential amino acids (EAA)				
Histidine	47 ± 0.88	32	25	16
Isoleucine	26 ± 0.52	54	47	30
Leucine	83 ± 0.41	95	85	61
Lysine	46 ± 1.44	85	63	48
Methionine	80 ± 0.85	35	24	23
Phenylalanine	33 ± 0.94	111	97	41
Threonine	46 ± 0.42	42	38	25
Tryptophan	-	-	-	7
Valine	123 ± 1.36	63	49	40
Sum of EAA	486 ± 6.83	517	428	291
Non-essential amino acids				
Alanine	72 ± 0.62			
Arginine	55 ± 0.52			
Aspartic acid	119 ± 0.56			
Glutamic acid	152 ± 0.99			
Glycine	105 ± 0.82			
Proline	34 ± 0.41			
Serine	59 ± 0.38			
Tyrosine	64 ± 0.14			
Sum of total AA	1107 ± 4.42			

[a] The dietary allowance as reported by FAO, 2013; for adult maintenance in mg/g protein
Mean values ± standard deviation (n = 3)

The content for tryptophan, cysteine and proline were not included in the analysis due to their correlation coefficient being lower than the standard value, 0.9999. Appendix A displays the calibration curves and chromatograms of the protein and standard mixture.

The crude protein determination gives the approximation of the protein content in a sample, but amino acid compositions show the quality of the protein. BSFL protein (Table 4.2) displays an amino acid profile comparable to casein and soy-bean, with presence of both essential and non-essential amino acids, and a similar amino acid profile was reported by Azagoh et al. (2016) on

mealworm proteins. The BSFL protein contained all the essential amino acids in quantities generally higher than those necessary for adult human maintenance (FAO, 2013). The sum of the total essential amino acids (EAA) was comparable to that of soy bean protein, but slightly lower than that of casein, as reported by Young and Pellet (1991).

Most importantly, the concentration of the nine essential amino acids were higher in BSFL than the recommended daily intake. This included lysine, methionine and valine which are limiting amino acids in human diet. Only isoleucine and phenylalanine were 5 g/kg lower than the FAO recommended value. Limiting amino acids are not synthesised by mammals and are therefore essential in the human diet. As their name stipulates, they are indispensable nutrients. These nutrients are found in plant food sources and gelatine. With BSFL protein showing a high concentration of these amino acids, it is therefore an ideal protein source.

The amino acid composition of the protein extract, heated protein and MRPs are summarised in Table 4.2, 4.3 and 4.4, respectively. To our knowledge, no literature is reported on the amino acid profile of the BSFL protein after conjugation by Maillard reaction (MR). Compared to the protein amino acid composition, MR caused a decrease in the amino acid content in MRPs (Figure 4.2b, 4.2c and 4.2d). Similar results were also observed by Gu et al. (2010) and Liu et al. (2016) on casein-glucose and whey protein isolate-glucose, respectively. The reduction of the available amino acid group is attributed to the binding of protein and sugar during glycation. Overall, heat treatment results in the decrease of amino acids, in both heated protein (Table 4.3) and MRPs (Table 4.4), especially MRPs heated for 120 min at 90 °C (Figure 4.2d). This means that heating at higher temperature for a longer period induces interactions of free amino acids. When the protein is heated alone, it is clear that heating leads to the alteration of the amino acid levels with notable decrease of lysine content. It is important to highlight lysine because it is the most reactive amino acid for the Maillard reaction.

Table 4.3 Amino acid profile of the heated Black soldier fly larvae proteins for 30, 60, 90 and 120 min at 50, 70 and 90 °C.

Amino acid, unit (g/kg protein)	50 °C				70 °C				90 °C			
	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
Essential amino acids (EAA)												
Histidine	16.6 ± 0.22	10.3 ± 0.06	8.6 ± 0.83	3.5 ± 0.25	5.3 ± 0.12	3.6 ± 0.25	3.60± 0.80	2.10 ± 0.95	6.2 ± 0.42	3.2 ± 0.32	1.2 ± 0.40	2.8 ± 0.60
Isoleucine	9.6 ± 0.17	4.5 ± 0.40	4.3 ± 0.36	3.2 ± 0.74	5.4 ± 0.07	5.0 ± 0.25	4.2 ± 0.38	2.2 ± 1.18	5.6 ± 0.04	2.2 ±1. 82	1.3 ± 1.61	4.4 ± 0.34
Leucine	72.1 ± 0.39	61.2 ± 0.14	26.2 ± 0.74	13.9 ± 0.12	26.5 ± 0.25	24.7 ± 0.57	23.6 ± 1.39	24.7 ± 1.02	25.2 ± 0.90	23.8 ± 1.08	23.6 ± 1.39	20.2 ± 0.73
Lysine	32.4 ± 0.12	17.3 ± 0.81	14.2 ± 0.24	1.0 ± 0.43	4.8 ± 0.25	3.8 ± 0.32	3.2 ± 0.85	2.7 ± 0.39	6.2 ± 0.75	4.7 ± 0.70	2.1 ± 1.37	3.7 ± 0.85
Methionine	9.5 ± 0.08	4.8 ± 0.20	4.1 ± 0.22	3.0 ± 0.73	3.6 ± 0.16	3.0 ± 1.06	2.6 ± 0.92	2.8 ± 0.84	2.3 ± 0.77	3.0 ± 1.06	2.6 ± 0.92	2.8 ± 0.84
Phenylalanine	21.4 ± 0.16	14.5 ± 0.35	13.8 ± 0.05	4.4 ± 0.63	5.0 ± 0.33	4.9 ± 0.32	4.2 ± 0.90	5.4 ± 0.68	7.2 ± 0.75	4.1 ± 0.71	4.2 ± 0.90	4.5 ± 0.62
Threonine	21.6 ± 0.17	13.6 ± 0.21	6.1 ± 0.78	2.4 ± 0.22	20.2 ± 0.25	17.6 ± 0.91	15.6 ± 1.07	11.9 ± 0.50	8.8 ± 0.85	7.9 ± 1.19	5.6 ± 0.84	7.6 ± 1.30
Valine	20.6 ± 0.08	10.0 ± 0.10	8.7 ± 0.68	3.2 ± 0.26	2.9 ± 0.86	2.4 ± 1.01	2.3 ± 1.11	3.4 ± 0.22	1.1 ± 1.64	0.4 ± 0.81	0.6 ± 1.90	0.3 ± 1.53
Sum of EAA	183 ± 1.39	126 ± 2.28	77 ± 3.89	31 ± 3.39	71 ± 2.29	63 ± 4.60	57 ± 7.42	52 ± 5.77	62 ± 6.12	49 ± 7.69	41 ± 9.33	46 ± 6.81
Non-essential amino acids												
Alanine	21.6 ± 0.26	9.6 ± 0.15	8.0 ± 0.74	1.8 ± 0.39	2.8 ± 0.34	2.7 ± 0.70	2.0 ± 0.82	1.9 ± 0.40	9.2 ± 1.72	6.3 ± 0.69	2.0 ± 0.82	0.9 ± 0.48
Arginine	24.5 ± 0.08	12.2 ± 0.25	10.1 ± 0.82	2.3 ± 0.29	4.5 ± 0.62	3.2 ± 0.61	2.8 ± 0.52	2.2 ± 0.33	2.2 ± 1.61	3.2 ± 0.61	2.8 ± 0.52	2.2 ± 0.33
Aspartic acid	28.7 ± 0.21	11.0 ± 0.25	8.7 ± 0.83	1.3 ± 0.21	5.9 ± 0.31	5.7 ± 1.15	4.5 ± 0.71	4.5 ± 1.68	5.8 ± 0.33	3.3 ± 0.67	2.2 ± 1.79	1.2 ± 0.26
Glutamic acid	35.7 ± 0.39	15.5 ± 036	14.4 ± 0.53	1.9 ± 0.05	2.6 ± 0.24	4.4 ± 1.36	3.3 ± 0.70	4.6 ± 1.25	5.5 ± 1.58	4.4 ± 1.36	4.0 ± 0.47	5.3 ± 1.59
Glycine	35.4 ± 0.28	18.8 ± 0.10	10.2 ± 0.43	0.7 ± 0.11	7.0 ± 0.50	5.3 ± 0.46	4.7 ±0.56	4.3 ± 1.68	10.2 ± 1.34	12.5 ± 1.26	11.0 ± 2.06	5.2 ± 1.72
Serine	17.0 ± 0.22	7.6 ± 0.31	1.3 ± 0.21	0.2 ± 0.54	2.3 ± 0.56	2.2 ± 0.36	1.6 ± 0.26	1.8 ± 0.33	4.4 ± 1.00	4.1 ± 1.39	1.8 ± 0.33	1.6 ± 0.26
Tyrosine	20.9 ± 0.05	10.6 ± 0.25	8.6 ± 0.79	4.1 ± 0.33	4.0 ± 0.33	3.2 ± 0.72	2.9 ±0.58	2.9 ± 0.85	1.8 ± 1.55	1.6 ± 1.46	2.2 ± 0.28	1.6 ± 1.11
Sum of total AA	368 ± 1.49	211 ± 1.67	138 ± 4.34	43 ± 1.91	100 ± 2.90	90 ± 5.37	79 ± 4.14	74.0 ± 6.52	101 ± 8.90	84 ± 7.43	67 ± 6.28	64 ± 5.98

Mean values ± standard deviation (n = 3)

Table 4.4 Amino acid profile of the Maillard reaction products heated for 30, 60, 90 and 120 min at 50, 70 and 90 °C.

| Amino acid, unit (g/kg protein)	50 °C				70 °C				90 °C			
	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
Essential amino acids (EAA)												
Histidine	4.80 ± 0.52	4.4 ± 1.37	4.8 ± 1.63	4.5 ± 0.35	2.0 ± 0.15	2.7 ± 0.25	2.7 ± 1.13	1.9 ± 1.34	2.2 ± 0.51	1.1 ± 0.32	1.1 ± 0.57	0.8 ± 0.85
Isoleucine	6.20 ± 0.97	2.9 ± 0.82	2.3 ± 1.39	2.1 ± 1.22	2.9 ± 0.09	3.6 ± 0.25	2.7 ± 0.46	3.1 ± 1.44	2.7 ± 0.05	2.5 ± 1.82	2.2 ± 1.98	1.6 ± 0.42
Leucine	29.2 ± 1.77	32.0 ± 1.25	30.7 ± 2.80	30.9 ± 1.29	30.6 ± 0.36	18.0 ± 0.57	17.2 ± 1.70	14.9 ± 1.25	28.3 ± 1.10	15.1 ± 1.08	10.1 ± 1.70	10.2 ± 0.89
Lysine	6.0 ± 0.82	6.1 ± 0.66	5.3 ± 1.37	5.2 ± 1.56	5.2 ± 0.31	3.3 ± 0.32	3.1 ± 1.04	2.8 ± 0.47	6.2 ± 0.92	4.2 ± 0.70	3.2 ± 1.67	1.1 ± 1.04
Methionine	2.0 ± 1.25	1.8 ± 1.52	1.8 ± 1.59	1.8 ± 1.30	2.3 ± 0.20	2.6 ± 1.06	1.7 ± 1.13	1.6 ± 1.03	2.2 ± 0.94	1.2 ± 1.06	1.2 ± 1.13	1.0 ± 1.03
Phenylalanine	8.1 ± 1.20	3.9 ± 5.01	6.9 ± 1.57	7.0 ± 1.22	3.8 ± 0.40	3.5 ± 0.32	3.4 ± 1.10	1.6 ± 0.84	6.2 ± 0.92	3.4 ± 0.71	2.3 ± 1.10	2.4 ± 0.76
Threonine	4.1 ± 0.82	3.8 ± 0.29	4.9 ± 1.58	5.8 ± 1.39	7.7 ± 0.31	3.6 ± 0.91	2.7 ± 1.31	2.7 ± 0.61	4.2 ± 1.04	3.1 ± 1.19	1.1 ± 1.03	2.1 ± 1.59
Valine	4.0 ± 0.82	3.8 ± 0.90	3.5 ± 1.32	3.6 ± 0.21	2.5 ± 1.05	1.8 ± 1.01	1.3 ± 1.37	1.4 ± 0.26	5.4 ± 2.01	3.8 ± 0.81	2.6 ± 2.33	1.5 ± 1.87
Sum of EAA	61 ± 8.16	62 ± 11.72	60 ± 12.24	61 ± 8.54	54 ± 2.86	39 ± 4.69	36 ± 9.24	32 ± 7.25	57 ± 7.50	34 ± 7.69	24 ± 11.50	21 ± 8.45
Non-essential amino acids												
Alanine	4.7 ± 0.88	4.3 ± 1.97	2.9 ± 1.54	4.3 ± 1.65	2.4 ± 0.42	2.1 ± 0.70	1.3 ± 1.00	1.0 ± 0.49	2.0 ± 2.10	2.2 ± 0.69	1.4 ± 1.00	1.5 ± 0.58
Arginine	5.4 ± 0.66	5.3 ± 0.42	5.1 ± 1.05	5.4 ± 1.27	2.5 ± 0.76	3.1 ± 0.61	2.9 ± 0.61	2.9 ± 0.40	4.1 ± 1.97	2.3 ± 0.61	2.0 ± 0.64	1.2 ± 0.40
Aspartic acid	6.5 ± 1.87	8.2 ± 1.49	6.8 ± 1.62	7.2 ± 1.18	4.7 ± 0.38	2.8 ± 1.15	4.0 ± 0.87	3.0 ± 2.06	5.9 ± 0.40	4.8 ± 0.67	3.2 ± 2.19	2.8 ± 0.20
Glutamic acid	6.3 ± 1.14	6.8 ± 0.52	6.3 ± 1.50	6.8 ± 0.48	2.6 ± 0.30	3.6 ± 1.36	3.3 ± 0.85	2.4 ± 1.53	1.9 ± 1.94	2.0 ± 1.36	1.6 ± 0.57	1.1 ± 1.95
Glycine	8.2 ± 0.74	8.7 ± 0.94	8.1 ± 1.47	8.5 ± 1.55	5.0 ± 0.61	4.4 ± 0.46	4.0 ± 0.68	3.2 ± 2.06	7.6 ± 1.64	7.6 ± 1.26	2.0 ± 2.53	1.1 ± 2.10
Serine	3.2 ± 0.49	3.4 ± 1.95	3.1 ± 1.10	3.4 ± 1.03	5.2 ± 0.68	4.5 ± 0.36	1.8 ± 0.32	1.1 ± 0.40	6.2 ± 1.22	5.5 ± 1.39	2.8 ± 0.40	2.1 ± 0.32
Tyrosine	4.4 ± 0.62	4.2 ± 1.16	4.0 ± 1.53	4.4 ± 0.25	12.9 ± 0.40	10.2 ± 0.72	2.6 ± 0.71	1.4 ± 1.04	13.9 ± 1.63	10.2 ± 1.46	3.6 ± 0.34	1.8 ± 1.77
Sum of total AA	100 ± 6.40	103 ± 8.45	96 ± 9.81	101 ± 7.39	89 ± 3.55	70 ± 5.37	56 ± 5.07	47 ± 7.99	96 ± 10.90	69 ± 7.43	41 ± 7.69	33 ± 7.33

Mean values ± standard deviation (n = 3)
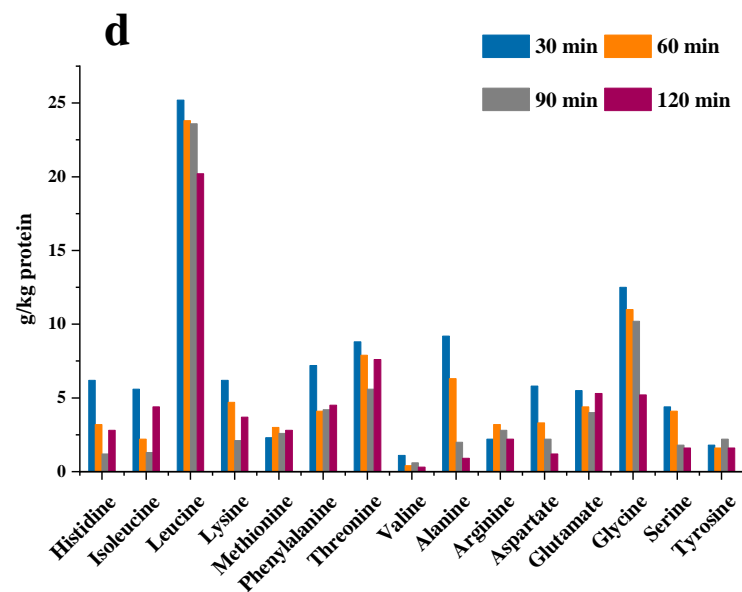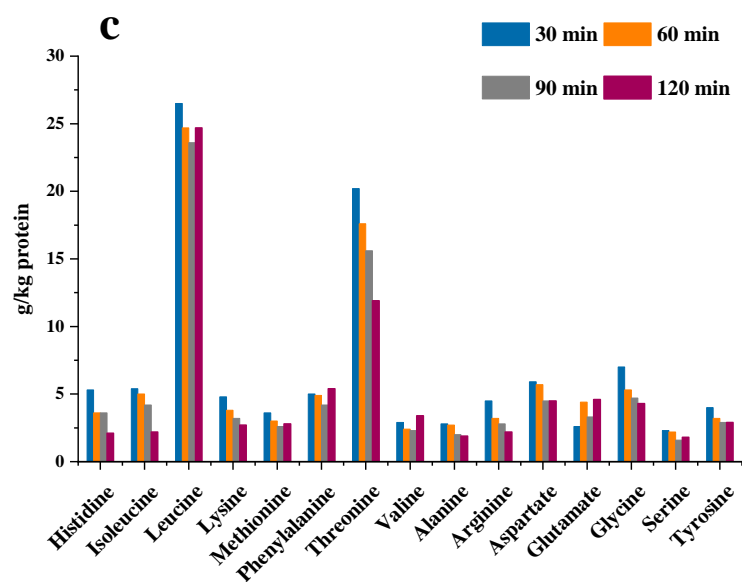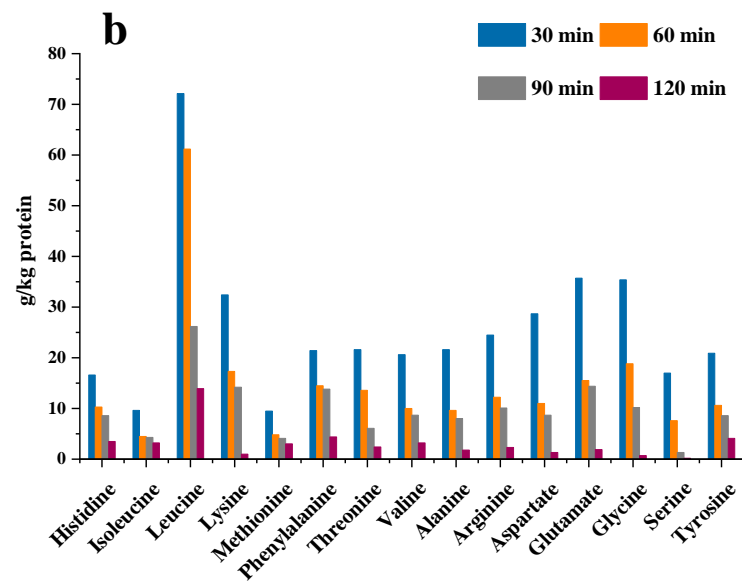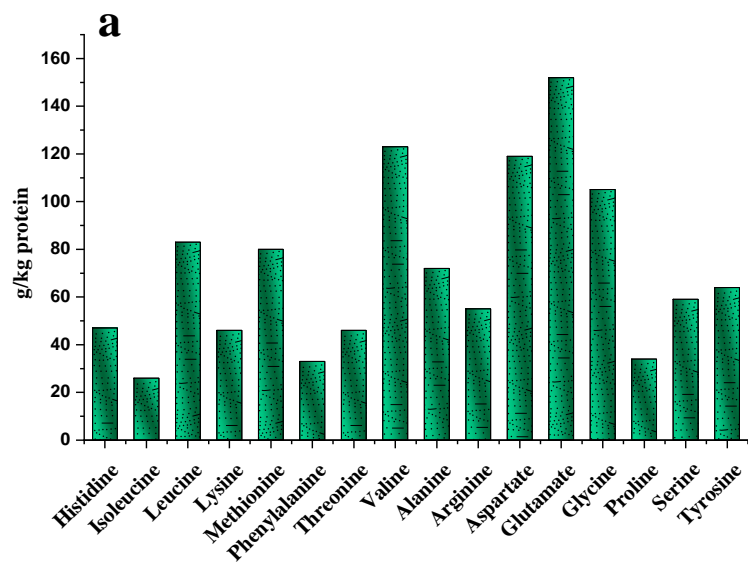
Figure 4.1 Bar graph showing protein extract (a), heated proteins at (b) 50 °C, (c) 70 °C, (d) 90 °C for 30, 60, 90 and 120 min.
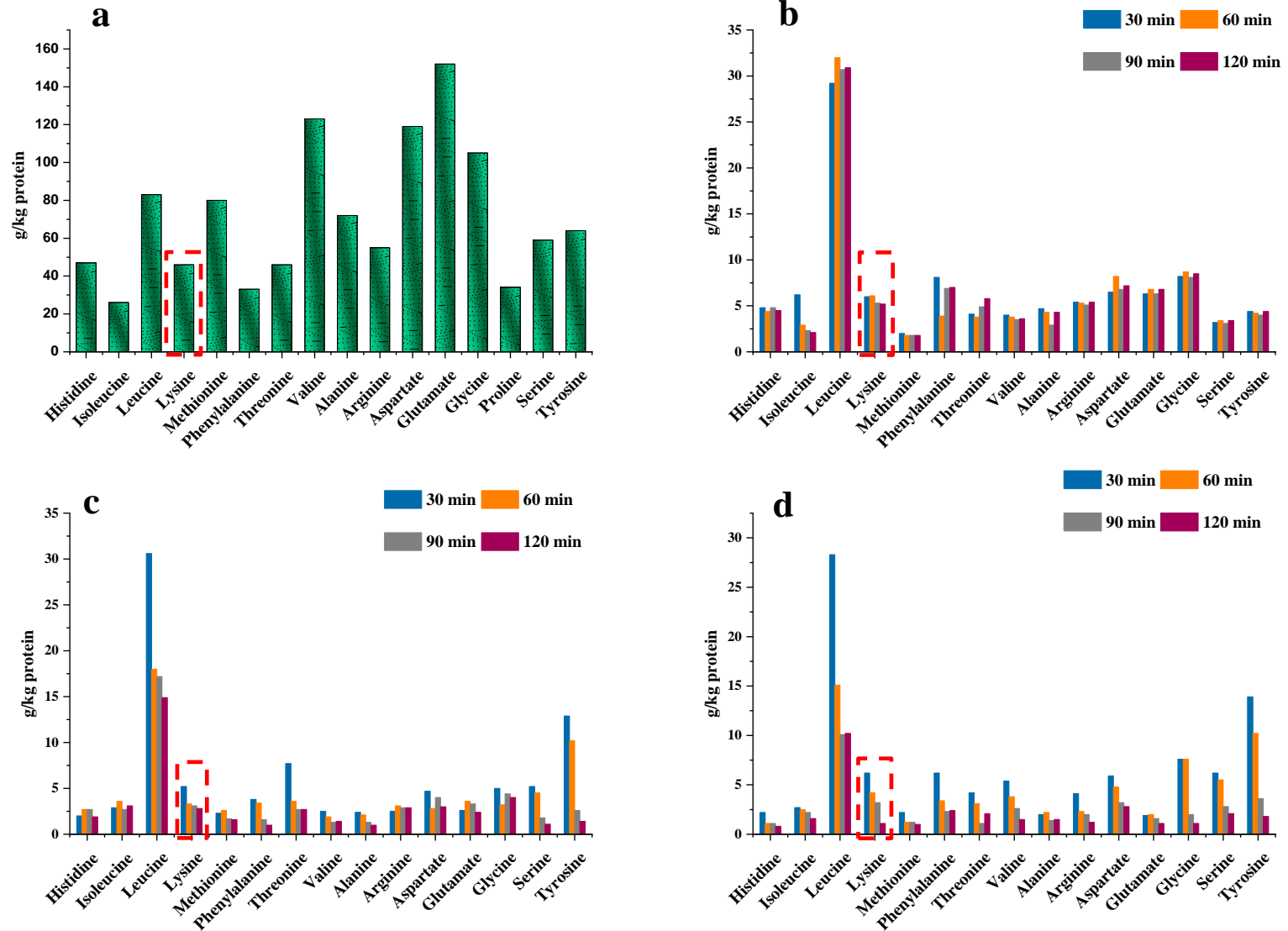
Figure 4.2 Bar graph showing protein extract (a), Maillard reaction products at (b) 50 °C, (c) 70 °C, (d) 90 °C for 30, 60, 90 and 120 min.

Also other amino acids displayed noticeable decreases, namely, phenylalanine, valine, alanine and tyrosine (Table 4.3). Similar results were reported by Zhang et al. (2012) from β-conglycinin and dextran conjugation.

After the first temperature (50 °C) treatment, the amino acids declined for both proteins and MRPs but not significantly for the MRPs (Figure 4.2b). It was suggested that although these amino groups participate in the initial stage of MR to react with carbonyl groups to form a Schiff base, longer heating period and higher temperature results in the degradation and Amadori rearrangement groups formation (Sun et al., 2006). Meanwhile from the results, it was clear that the reactivity of the amino groups at 50 °C (Figure 4.2b) was less than at 90 °C (Figure 4.2d). Similar results were reported previously, where a decrease in amino acid content of the conjugates resulted due to the MRPs formation (Jiménez-Castaño et al., 2007) during the glycation of whey protein and dextran.

Figure 4.2d focused on identifying the impact of temperature in the MRPs. As can be seen, during heat treatment at 50 °C (Figure 4.2b), no significant change is observed. As the temperature increased, the content of the essential amino acids decreased. Again, this is expected as the amino acids are used up during the MR. It can be concluded that as the temperature and heating time are increased, the essential amino acids are reduced.

The EAA profile of the BSFL protein (Table 4.2) revealed that the levels of EAAs were comparable with those of FAO amino acid reference (FAO, 2013) recommended for human maintenance nutrition. Amino acid composition is an important chemical property of proteins, as it determines their nutritional value. Therefore, from these results it can be concluded that BSFL protein could be an alternative source of essential amino acids for human nutrition.

4.4.2. Thermal Gravimetric Analysis (TGA)

The weight loss curve obtained by TGA for heated proteins and MRPs at three different temperatures, which are 50 °C (a), 70 °C (b) and 90 °C (c) for 30 min, 60 min, 90 min and 120 min, are presented in Figure 4.3 and 4.4, respectively. The TGA curves show the remaining weight from 50 to 700 °C. Additionally, Appendix B (Figure B.1 and B.2) displays the TG (thermal gravimetric) and Differential Thermogravimetric (DTG) curves of the non-heated protein and the MRP at time zero, correspondingly. All the samples showed the initial mass degradation step at ~50 °C to 150 °C, which indicates the evaporation of moisture in the samples. For the heated

proteins (Figure 4.3), the mass loss occurring in the range of ~250 - 550 °C may be associated with polypeptide decomposition. These TGA curves are similar to the results reported by Huang et al. (2019) for the BSFL dried flours.
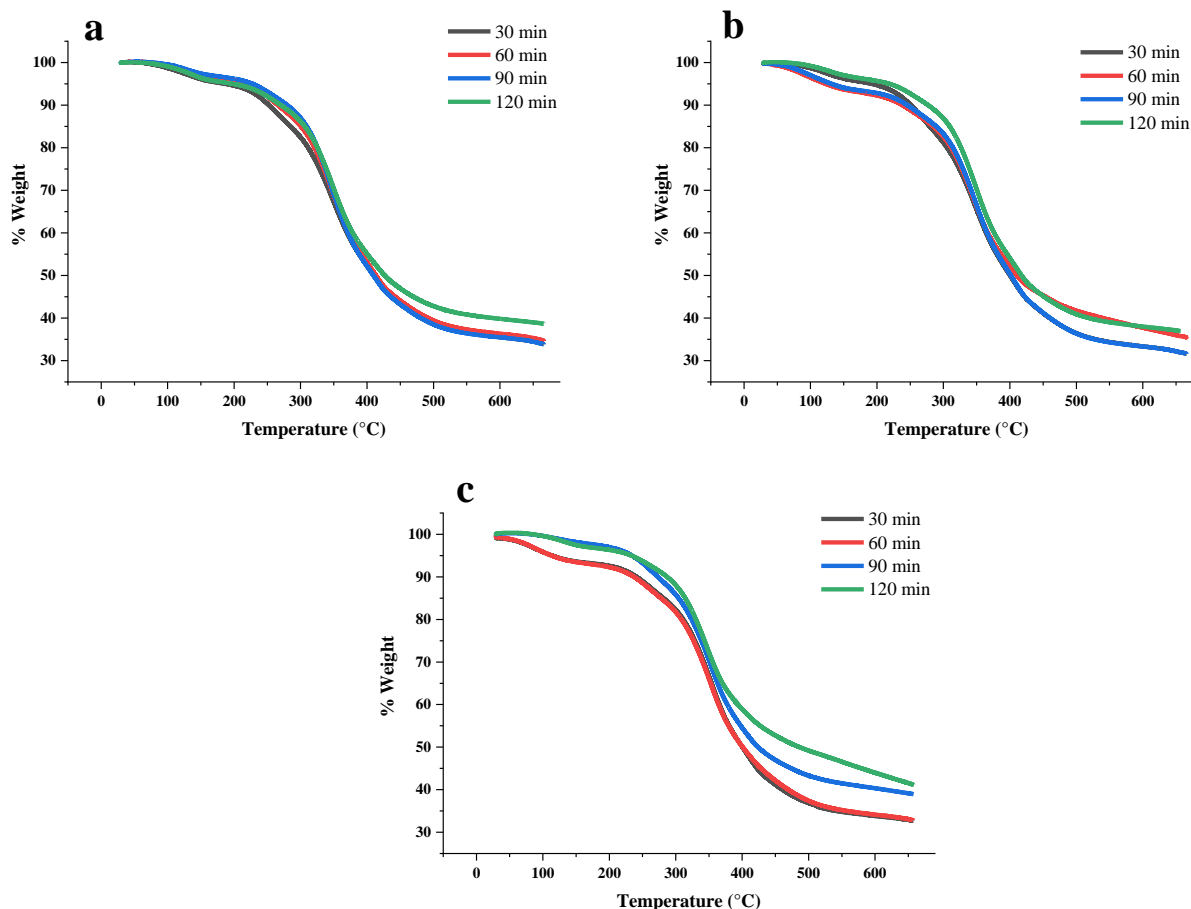

Figure 4.3 Thermal gravimetric analysis of the heated protein for 30, 60, 90 and 120 min at (a) 50 °C, (b) 70 °C and (c) 90 °C.

In the mass losses of the MRPs (Figure 4.4), two stages of decomposition were observed, when compared to the heated proteins. Again, the first stage is the loss of the bound water and some volatile substances in the glycosylated products between ~50 and 200 °C, causing the decline in the weight. The drop in mass took longer than in the case of the proteins, possibly indicating slightly stronger bonding between the volatiles and conjugate products. The loss is also higher than in the case of the proteins. Approximately 15% loss occurs in the conjugates, but only 5 to 8% in the proteins. The second stage of the decomposition took place between ~250 to 550 °C heating temperature, the percentage weight decreased due to the decomposition of the glycation products and Maillard reaction intermediates, the pyrolysis of some other macromolecules; the remaining

products after 550 °C could be ash and other residues; the residue was 35% of the total. The weight loss percentage of the protein heated alone is lower than that of the MRPs, this observation is clearly shown in Appendix B (Figure B.2).
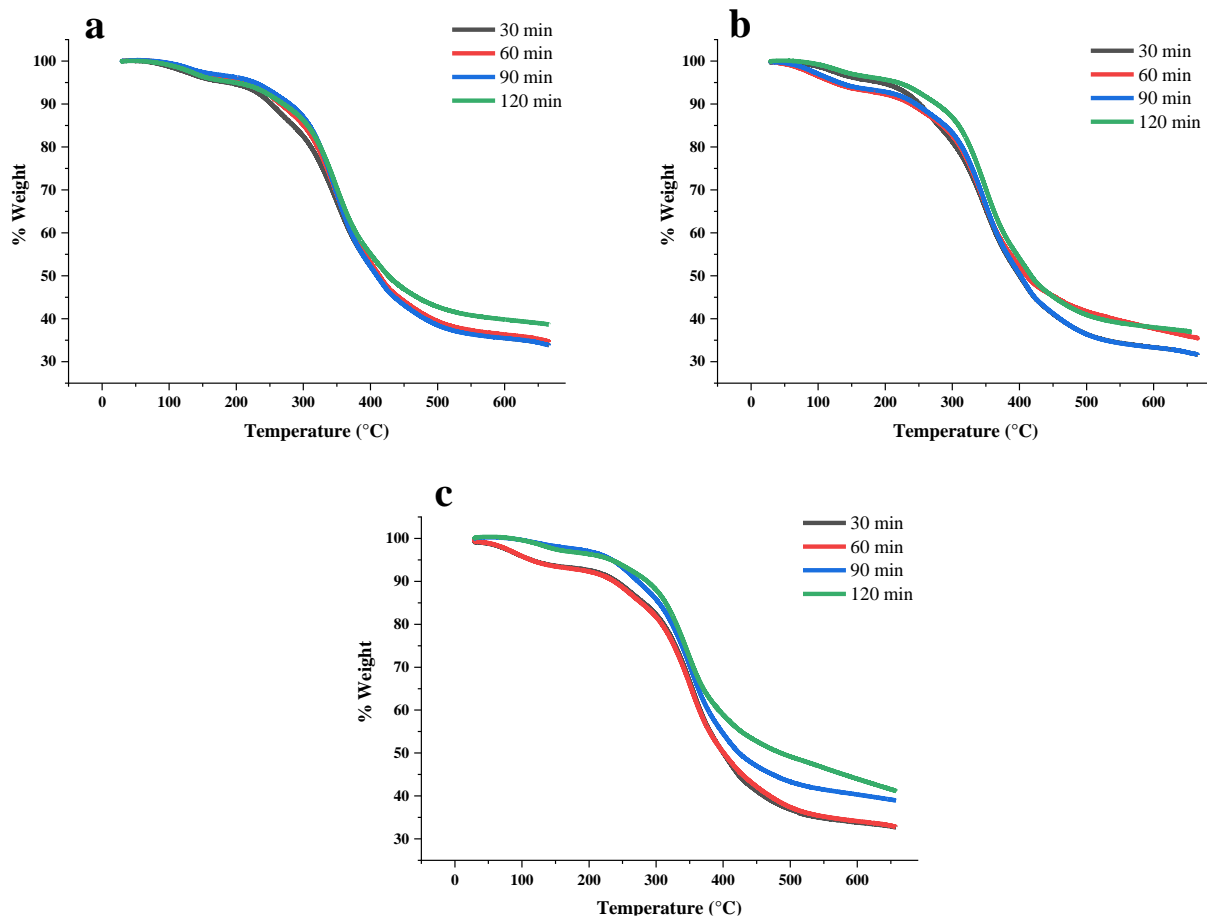

Figure 4.0 Thermal gravimetric analysis of the protein-glucose conjugates heated for 30, 60, 90 and 120 min at (a) 50 °C, (b) 70 °C and (c) 90 °C.

These results are in close agreement with those reported by the other researchers (Liou, 2004; Liu, 2014) who studied the thermal decomposition of rice husk and whey protein isolate conjugated with glucose, respectively. By comparing Figure 4.3 and 4.4, it can be observed that it took higher temperature to decompose the bound water on the MRP samples, compared to heated proteins. Comparing the heating times and temperatures within Figure 4.3, there was no significant difference between protein heated alone for 4.3a and 4.3b. Figure 4.3c showed a significant difference between 30, 60 and 90, 120 min. The longer heating times remained with more than 40% residues (Figure 4.3c). It is important to note that the second decompositions on Figure 4.4 occurred at a higher temperature as compared to Figure 4.3. This observation deviates from the

results reported from other insects, where other researchers have reported to have more than two steps of mass loss, but they were subjected to different drying methods; conventional drying and microwave drying (Wasko et al., 2016; Huang et al., 2019).

Figure 4.4a and 4.4b show no differences in heating times. Meanwhile Figure 4.4c shows that during heating for 120 min at 90 °C, the products formed during MR have higher decomposition temperature and they continued to decompose above the set temperature. The weight loss was similar for both Figure 4.4a and 4.4b in the first and second decomposition.

Comparing all the results shown, the decomposition temperature position depends markedly on the sample, in this case on how long and at what temperature they were heated. Increasing the heating time and the temperature is accompanied by an increase in the decomposition temperature, meaning the non-heated protein will have lower decomposition temperature than the MRPs.

The best preparation conditions of BSFL protein:glucose glycation, was the reaction temperature at 90 °C for 120 min, in view of the fact that ~40% residue remained, which demonstrated that the sample had larger molecules as compared to the original sample, therefore, better heat stability.

4.4.3. Differential Scanning Calorimetry (DSC)

The DSC profiles of heated proteins and MRPs are shown in Figure 4.5 and 4.6, respectively. Figure 4.5 presents the changes in peak temperature for heated proteins for 30 – 120 min at 50 °C(a), 70 °C(b) and 90 °C(c). Endothermic peaks at 90 °C, 240 °C, 320 °C and 400 °C were observed for the heated proteins. The observed sharp endothermic peaks above 90 °C in the heated proteins might be due to melting peptides. The heated proteins showed a higher endothermic transition when compared with non-heated protein, Appendix C (Figure C.1). Therefore, the heating treatment resulted in an increase in enthalpy; this phenomenon is indirectly proportional for the increase in heating time. These findings prove that, after higher heating time, the proteins are more stable.
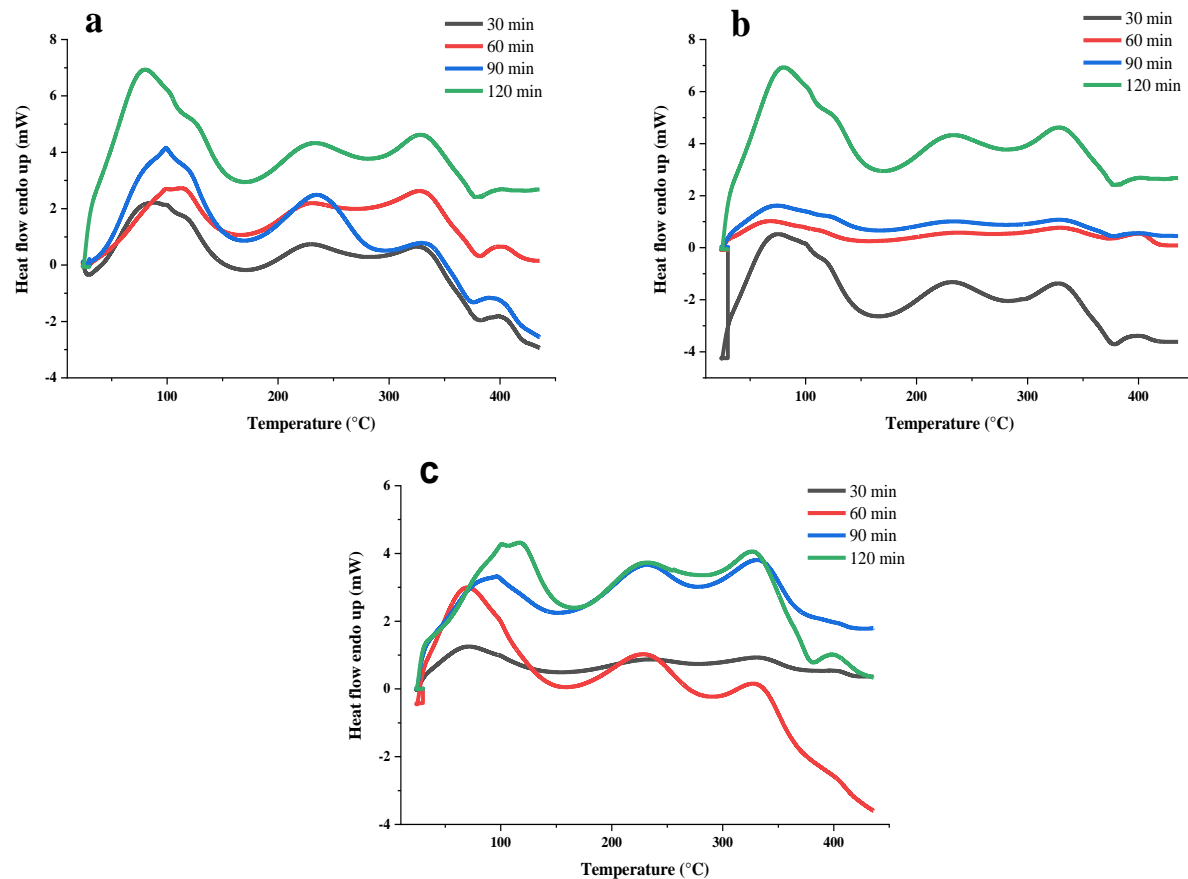
Figure 4.5 Differential scanning calorimetry thermograms of the heated protein for 30, 60, 90 and 120 min at (a) 50 °C, (b) 70 °C and (c) 90 °C.

Therefore, more energy will be required to breakdown the polypeptides. This phenomenon is in accord with TGA analysis. The shape of the obtained calorimetric curve of the heated proteins are in agreement with results reported by Fitzsimons et al. (2008), who studied endothermic peaks in whey protein. The heating time increased thermal stability of the MRPs, while increasing temperature decreased the thermal stability, as shown by Figure 4.6 (a, b and c). Furthermore, the enthalpy of MRPs gradually decreased with increasing temperature of the treatments (Figure 4.6a and 4.6c). These findings are in agreement with the results reported by Garcia et al. (2012). These authors studied cassava starch and whey protein glycation. During the heating, it is possible that the initially ordered structure of the BSFL protein is gradually destroyed.
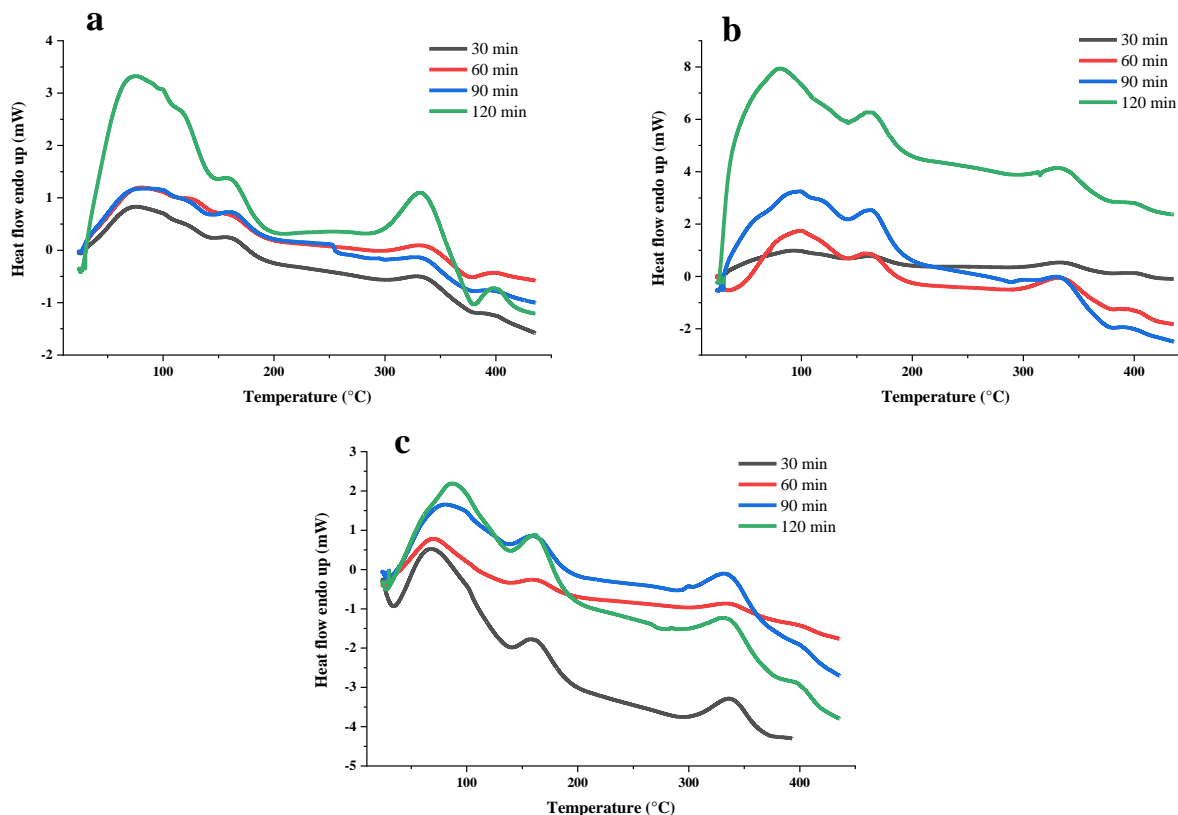
Figure 4.6 Differential scanning calorimetry of the protein-glucose conjugates heated for 30, 60, 90 and 120 min at (a) 50 °C, (b) 70 °C and (c) 90 °C.

Nuthong et al. (2009) confirmed that the cross-linking of molecules induced by the Maillard reaction might contribute to the increase in denaturation temperature. The reduced magnitude of the enthalpy after the conjugation reaction may be attributed to the disruption of the intramolecular forces of the BSFL samples when it is covalently bound to carbohydrate molecules. In this study, the glucose molecules may also reduce the steric spacers between the protein molecules, which would promote aggregation by increasing the interactions of the hydrophobic binding sites on the surface.

Also, Ibanoglu (2005) noted that a lower enthalpy energy value of the conjugates suggests that less energy is required for their denaturation, which may be attributed to the partial denaturation of the protein prior to the heat treatment due to the high charge density of the biopolymers or the aggregation of proteins as a result of phase separation.

4.4.4. Universal Attenuated Total Reflectance Fourier-transform infrared spectroscopy (UATR-FTIR)

Protein molecules are complex due to their molecular vibrations arising from numerous amino acid atoms, therefore spectroscopic analysis is used to deduce that information. Namely, FTIR spectroscopy is used for such analysis, in this study it is particularly useful for the study of chemical bonds of the protein-carbohydrates. FTIR is used in order to confirm the change or formation of glycated samples, when protein molecules are covalently bonded to the sugar, the typical bond intensity will increase or decrease. Therefore, the formation of new bonds between protein and carbonyl group of the reducing sugar can be reflected by FTIR (Liu et al., 2014).

The dominant peaks of the spectra (Figure 4.7 & 4.8) are in the region of 1180 - 953 cm^{-1}, which represent vibrational modes such as the stretching of C-C and C-O and the bending mode of C-H bonds. In addition, the above-mentioned absorptions are weak in the spectra of most proteins (Oliver et al., 2009). The characteristic absorption bands in the ranges of 3480 - 3440, 3260 - 3270, and 2960 - 2878 cm^{-1} represent the -OH, -COO, and the -CH stretching regions, respectively (Su et al., 2009). The most distinctive peaks for proteins located approximately between 1650 cm^{-1} and 1540 cm^{-1} result from the strong amide I and II bands, respectively (Oliver et al., 2009).

The FTIR bands were shown in Figure 4.7 and 4.8 for 50 °C, 70 °C and 90 °C by four different heating times (30, 60, 90 and 120 min). As shown in Figure 4.7a, no significant changes in the FTIR spectroscopy amide region of the protein-heated alone were observed on heating at different times at 50 °C temperature. This result is in accord with the DSC, TGA and SEM analysis, which were used to monitor the denaturation and conformation changes in structures. As incubation with glucose progressed, the absorptions in the region of amide showed an increase in intensity with time, indicating that glucose molecules may have attached to the BSFL protein, forming a Schiff base compound with amine groups (Figure 4.8a). Compared with the native protein, conjugates rendered low intensity on the amide regions, the two most important vibrational modes of amides: the amide 1 vibrations (1600 – 1700 cm^{-1}), which is primarily caused as the result of C=O bonds, and the amide II vibrations (1510 – 1580 cm^{-1}), which is caused by N-H bonds due to its deformation and stretching of the C-N bonds. It might be expected that the –OH group in glucose and the amino groups in BSFL protein are consumed during the heating process.
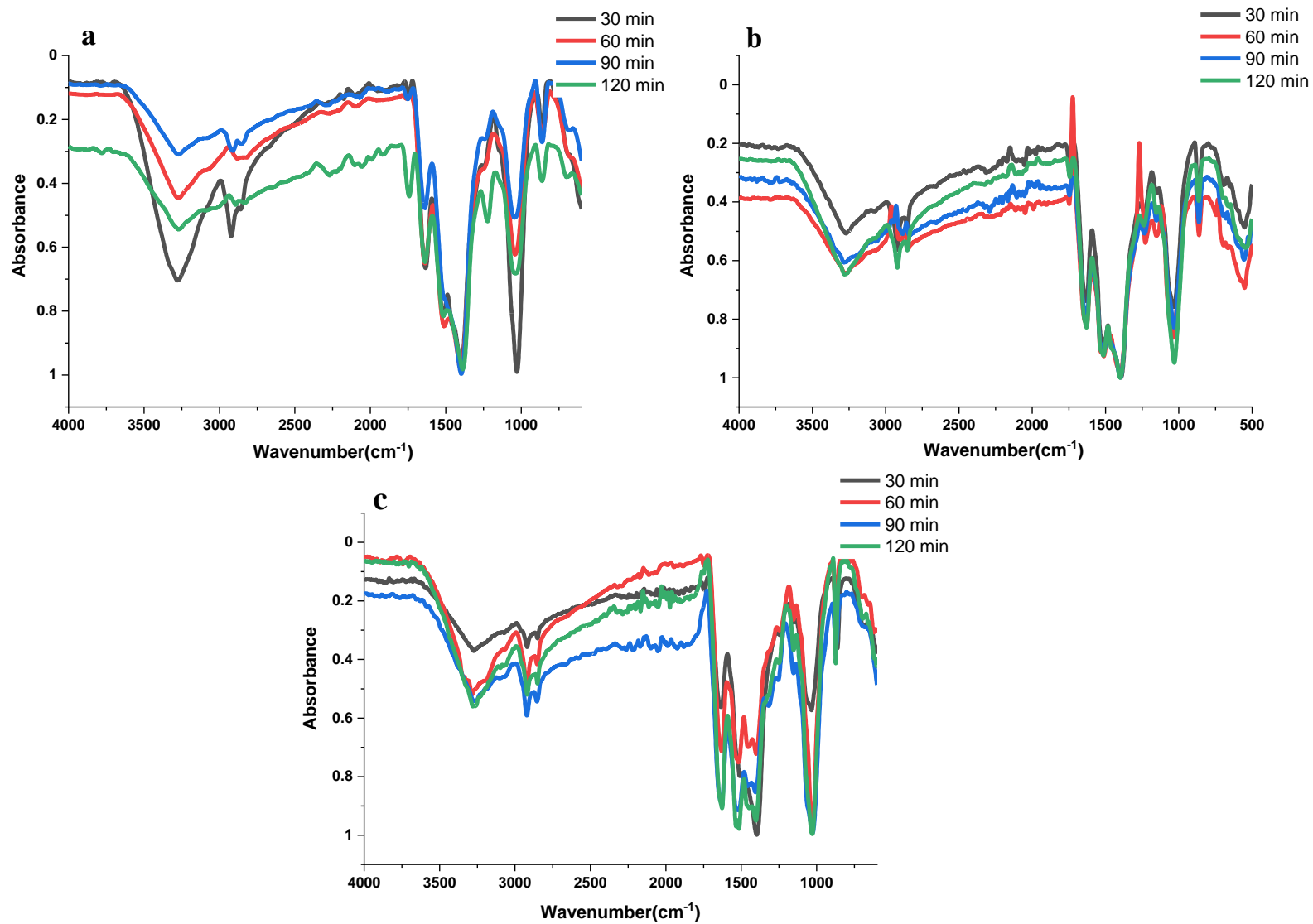
Figure 4.7 Fourier-transform infrared spectroscopy of heated proteins at 50 °C (a), 70 °C (b) and 90 °C (c) for 30, 60, 90 and 120 min.
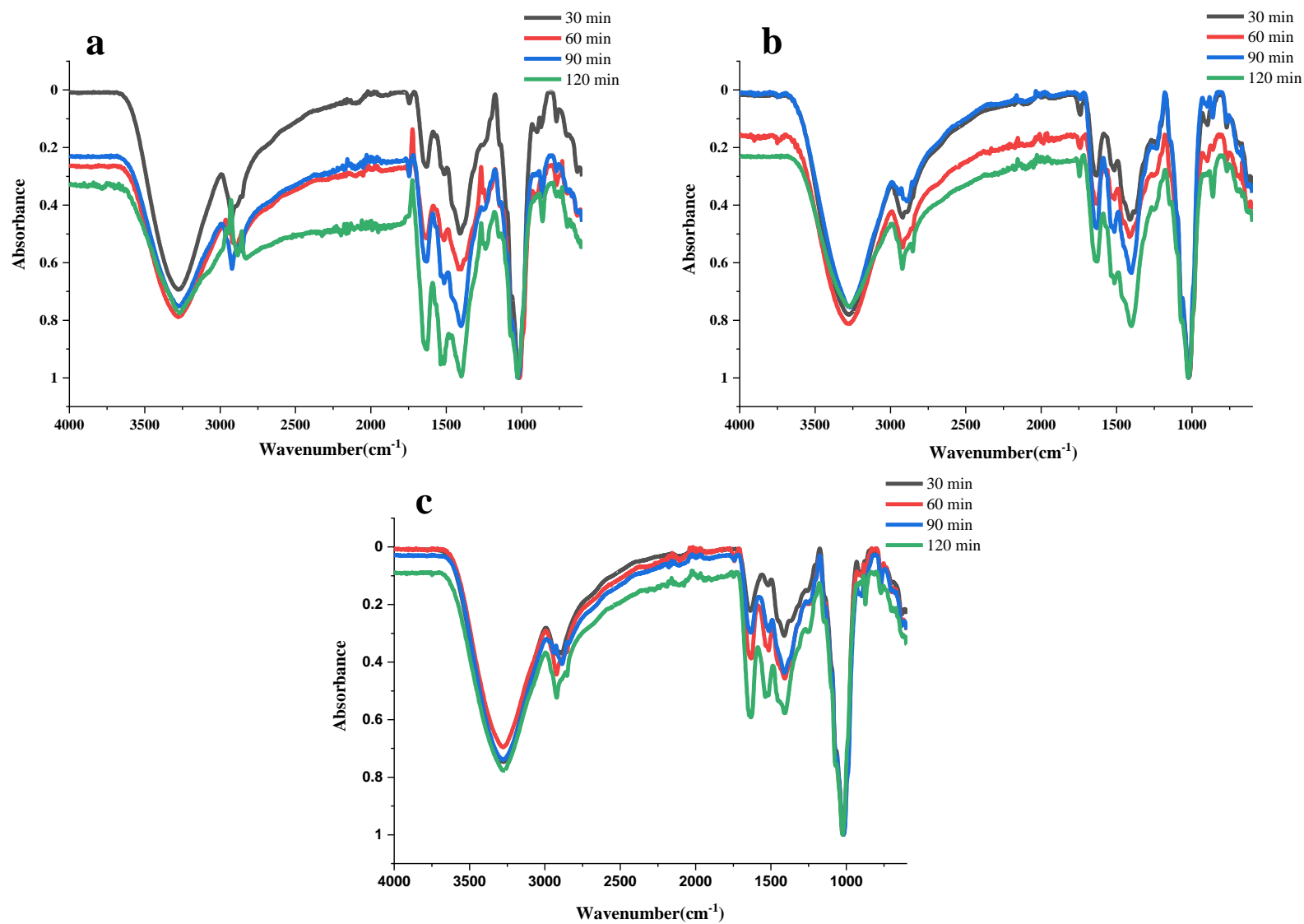
Figure 4.8 Fourier-transform infrared spectroscopy of Maillard reaction products at 50 °C (a), 70 °C(b) and 90 °C(c) for 30, 60, 90 and 120 min.

Su et al. (2009) found a gradual decrease in the intensity of the bands at 1600–1400 cm^{-1} during the Maillard reaction between carboxyl methyl cellulose (CMC) and soy protein isolate (SPI). Gu et al. (2010) also noted that functional groups, including NH_2, especially those in lysine, may be decreased, whereas the amount of Maillard products, such as the Amadori compound (C=O), Schiff bases (C=N), and pyrazines (C-N), may be increased. This result might be supported by the lower amino acid content (such as arginine, lysine and histidine) of the MRP conjugates formed during the Maillard reaction (Table 4.4).

In addition, Figure 4.8c, illustrates no change in the 1033 – 601 cm^{-1} region. Chang and Tanaka (2002) speculated that this could correspond to Maillard reaction completion, which, although these bands cannot be classified as a specific functional group, they are also important in indicating an alteration in the protein structure, compared with proteins heated alone, this region increased in the glycated samples.

Consequently, it can be concluded that complicated cross-linking or aggregation may have occurred between the glycated samples. Furthermore, the free amino groups of the conjugates decreased rapidly upon increased reaction time and temperature (Table 4.4). The FTIR results implied that the glucose had attached to the BSFL protein.

4.4.5. Principal Component Analysis (PCA)

As it can be observed, the changes to spectral bands were too small to be easily discerned especially when differentiating between heating times and temperatures. Therefore, the FTIR analysis required a multivariate statistical approach to extract the spectral changes, that is, a statistical method that is used to discriminate samples, shows the structure and systematic separation or outliers. Principal Component Analysis (PCA) of the entire data set spectra was performed on the heated proteins and glycated samples (heated at 50 °C, 70 °C and 90 °C for 30, 60, 90 and 120 min), using the spectral region where the major hydroxyl, amide and carbohydrate bands appear (600 - 4000cm^{-1}). PCA essentially breaks the dataset down into orthogonal components that describe the variance in the dataset and allows one to examine only the spectral differences (Esbensen & Swarbrick, 2018). The PCA scores plots showed that the spectra from the different samples clustered individually and the loadings plots show the coefficient of the linear combinations associated with each Principal Component (PC). This shows how the actual spectral

profile correlates to the positive and negative PC direction, and also describes the greater source of variance which corresponds to the first principal axis of the ellipsoid. The central axis is called the first principal component (PC1). PC1 not only lies along the direction of maximum variance in the data set, but it is also the direction of maximum variance in any data set. Then the second principal component (PC2), per definition, lies along a direction orthogonal to the first PC and along the direction of the second largest data set variance and the second main axis of the ellipsoid.
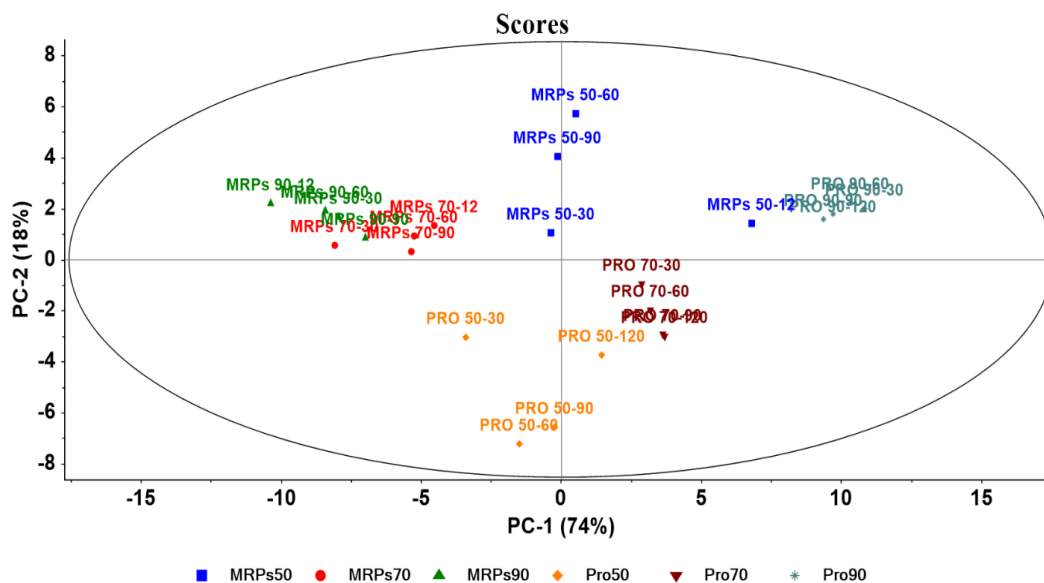

Figure 4.9 Principal Component Analysis scores of the full Fourier-transform infrared spectroscopy spectrum. Principal Component 1 and Principal Component 2 are responsible for 74% and 18% variance respectively, of the heated proteins and Maillard reaction products. Maillard reaction products (MRPs) at 50, 70, 90 °C; Heated protein (Pro) at 50, 70, 90 °C.

The PCA model was performed with full cross validation. The score plot for the first two PCs from the FTIR normalised data of MRPs and heated proteins is shown in Figure 4.9. Through PCA, it was observed that 92% of data variances were explained by the analysis of the two principal components, 74% explained by the first and 18% in the second PC. The ellipse (known as Hotelling's T^2 ellipse) represents a 100-confidence interval around the model mean and is one criterion for detecting outliers. As can be seen, there are no outliers in the model space when PC1 and PC2 are jointly assessed. An outlying sample will appear in the score plot as separate from the rest of the samples, to a larger or smaller degree. They are characterised by excessively high scores (with either sign) as compared to all other samples. Based on the position of the samples in the

score plot, the first component corresponds to the north-south axis, whereas the second component describes the complementary east-west axis. With reference to Figure 4.9, it can be seen that the MRPs at 90 and 70 °C, are in the far left of the plot. They have the most negative score for PC1, approximately -10 and -5, respectively and positive score for PC2, approximately 2.0 for both temperatures. It can be observed that the MRP samples move asymmetrically down the PC2, while the heated protein moves symmetrically from left to right along the PC1, from lower to higher temperature.
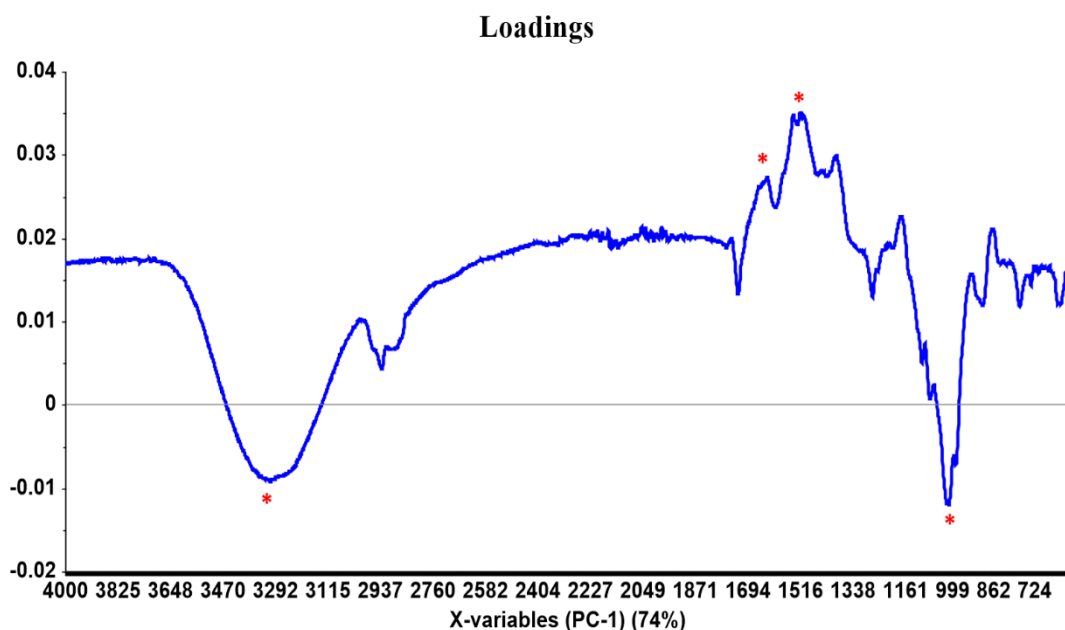

Figure 4.10 Loading plot Principal Component 1, responsible for 74% variance. The most dominant peaks are red starred and are responsible for the variance.

In other words, the PC1 positive axis is associated with the heated protein samples, while the positive axis of the PC2 is associated with MRPs. The contribution of the heated protein is strongly to PC1 but not much to PC2, since its scores on the PC2 axis are very close to zero. Additionally, MRPs also contribute to PC1, but at the opposite end of the plot axis. For example, MRPs at 90 °C are approximately at -10 of the PC1 while the heated proteins at 90 °C are far out +10 of the same axis. Therefore, samples that lie at approximately 180° from each other along the PC direction from each other typically represent opposite features along the PC1.

This is most likely due to the state of glycation varying through the course of the heat treatment. The clustering of the sample for protein heated alone is significantly different from the

MRP samples showing that the observed differences were due to both glycation and heating. Therefore, the more extensive is the heat treatment (i.e. the extent of glycation), the greater the dissimilarity in the amide I and amide II structural region of the samples (loading plots). Especially, the clustering distance for the samples heated for 120 min was considered greater than that of the physical blend or for those samples exposed to shorter heating times and lower temperatures.

Additionally, PC1 separates the heated protein samples, with positive values, from the MRP samples, in negative values. On the other hand, PC2, in turn, manages to separate both the MRPs heated at 90 and 50 °C and protein heated at 90 °C, in positive values, from the protein heated at 50 and 70 °C, in negative values of the scores chart. It is observed that heating at 50 °C has no influence on the separation of the 74% variance of the spectrum. It is important to note that the samples (proteins and MRPs) heated at 50 °C are located at zero position of the PC1 score plot, but they are not clustering together, when they are observed through PC2 axis. This is justified by FTIR data shown on Figure 4.7a and Figure 4.8a.
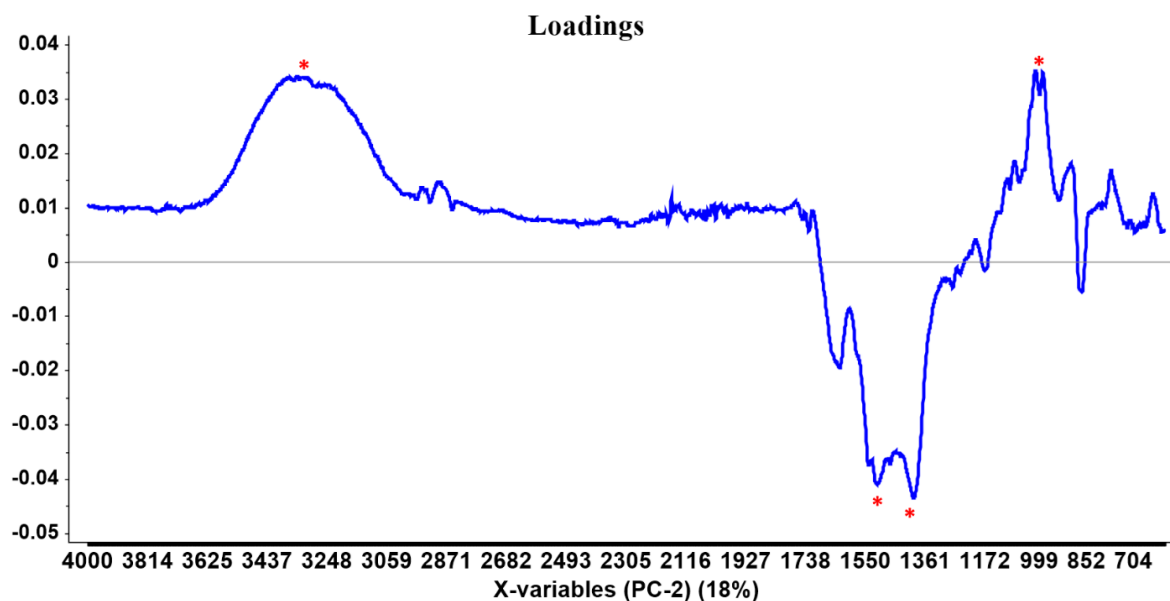
Figure 4.11 Loading plot Principal Component 2, responsible for 18% variance. The most dominant peaks are red starred and are responsible for the variance.

Figure 4.10 and 4.11 illustrate the interpretation of the loadings plot, which provides further interpretation of the variable responsible for the separation. It is important to note that variables of large loadings lie away from the origin and variables of little importance lie near the origin.

However, when assessing importance, it is mandatory also to consider the proportions of the total explained variance along each component, in this case, PC1 explains 74% and PC2 only 18%, then variables with large loadings in PC1 are much more important than those with large loadings in PC2.

The variable that is most dominant in PC1 is from a region of 1441 – 1609 cm^{-1}. In particular, the major source of variability can be interpreted as when the band around 1514 cm^{-1} increases or decreases. The first PC (Figure 4.10) also separates according to the 3292, 1694 and 999 cm^{-1} regions (C=O importance variable). The second PC (Figure 4.11) is responsible for the separation according to the 3248, 1550, 1361 and 999 cm^{-1} regions. Order of relevance in the separation of these principal components is listed in Table 4.5.

Table 4.5 Original variables (wavenumbers) from PCA with more impact on the first two principal components and the vibrational modes associated with each component (Esbensen & Swarbrick, 2018).

| Principal Component | Order of relevance | Wavenumber (cm^{-1}) | | Associated to |
		band (from - to)	max band	
PC1	1	1441 -1609	1514	C-N stretching, C-O stretching and C-C Stretching (Amide II)
	2	1609 - 1694	1692	C=O stretching and N-H bending (Amide I)
	3	926 - 1044	999	Stretching of the carbohydrate carbonyl groups
PC2	1	886 - 1001	999	Stretching of the carbohydrate carbonyl groups
	2	3069 - 3620	3248	O-H stretching
	3	1500 - 1610	1550	C-N stretching, C-O stretching and C-C stretching (Amide II)

From these results, it can be concluded that the UATR-FTIR managed to show structural changes of the protein heated alone and MRPs. It was also evident from the PCA (scores and loadings) that the major differences in the FTIR spectrum were caused by the protein region (amide I & II region). These results compliment those displayed by TGA and DSC.

4.4.6. Scanning Electron Microscopy (SEM)

It is well known that food is made up of molecule structures which are too small to be viewed by the human eye. Therefore, SEM is a useful technique for visual perception of food assessment. SEM provides detailed information on the outer and cutting surface of a product. It was important to reveal the similarities and the differences between the starting material, heated protein and Maillard reaction products (MRPs). The photomicrographs were taken at 150x to 2000x magnification for all the samples.
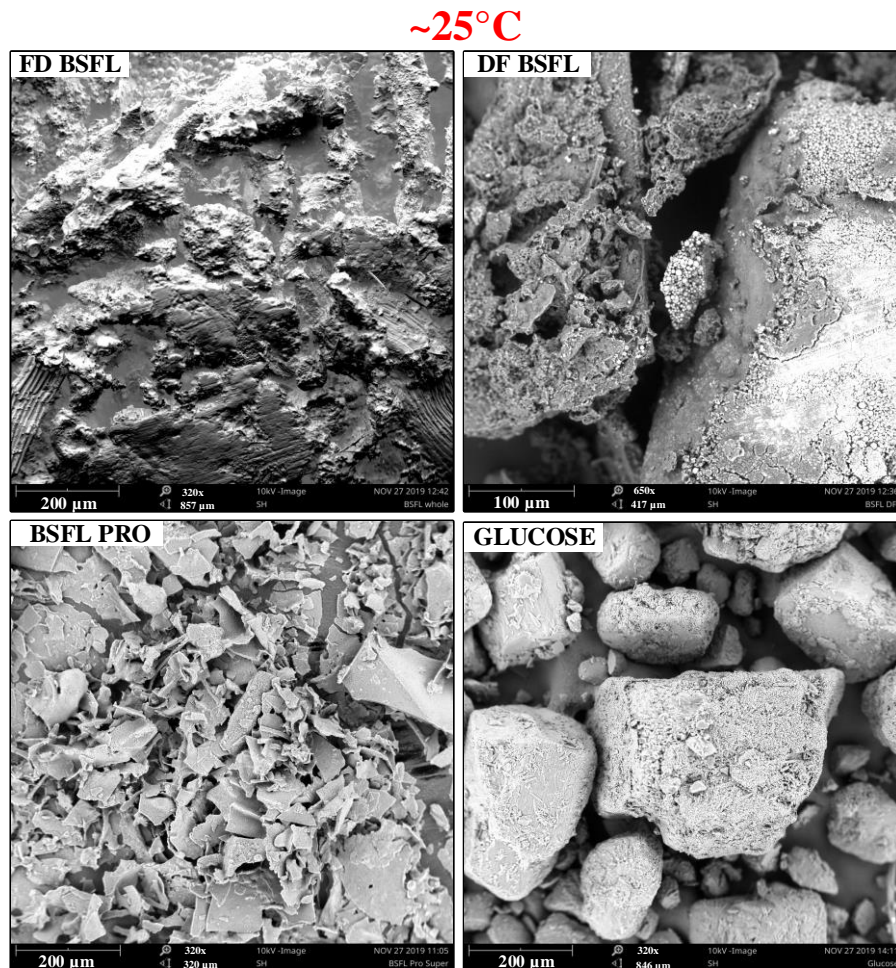

~25°C

Figure 4.12 Scanning electron microscope analysis of FD BSFL, DF BSFL, BSFL extracted protein and glucose 320 to 650x magnification.

Figure 4.12. displays the surface morphology of the non-heated BSFL samples (freeze dried (FD) and defatted (DF) flours, the extracted protein and glucose. The surface of the FD BSFL microstructure displays particles that are uneven, rigid and compact. A closer view of an integument could be observed on the FD sample. After the treatment of defatting, it can be

observed that the surface is more porous, it is apparent that defatting caused significant changes on the BSFL flour structure. The photomicrography of the protein extract is significantly different from the starting materials. These results are in agreement with other reports on the surface morphology of the wheat germ protein (Niu et al., 2011), silver carp (Liu et al., 2016) and chitin isolate extract from *H. illucens* by Wasko et al. (2016).
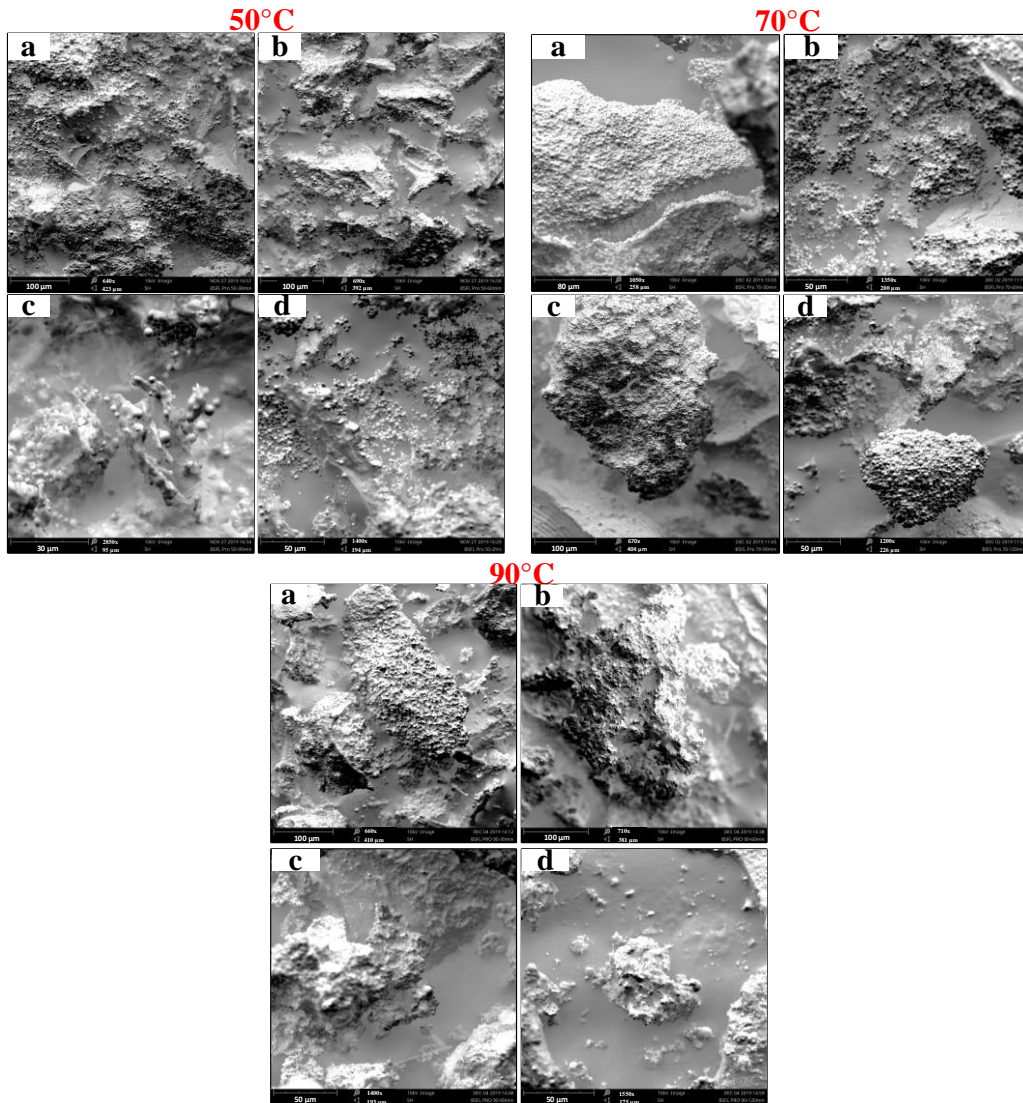

Figure 4.13 Scanning electron microscope analysis of heated protein at 50 °C, 70 °C and 90 °C for (a) 30 min, (b) 60 min, (c) 90 min and (d) 120 min at 640 to 2850x magnification.

Photomicrographs of heated proteins are shown in Figure 4.13a - d. The proteins were heated at three different temperatures (50 °C, 70 °C and 90 °C) for 30 min (Figures a), 60 min (Figures b) 90 min (Figures c) and 120 min (Figures d). It is observed that the increase in temperature produces a reduction of space between the sample molecules. This suggests the formation of a compact matrix because of the increase in protein-protein interactions. It can be observed that as you increase the temperature to 70 °C, the particles were clustered in separate aggregates, they are uneven and irregular. As the temperature is increased further to 90 °C, the protein molecules are
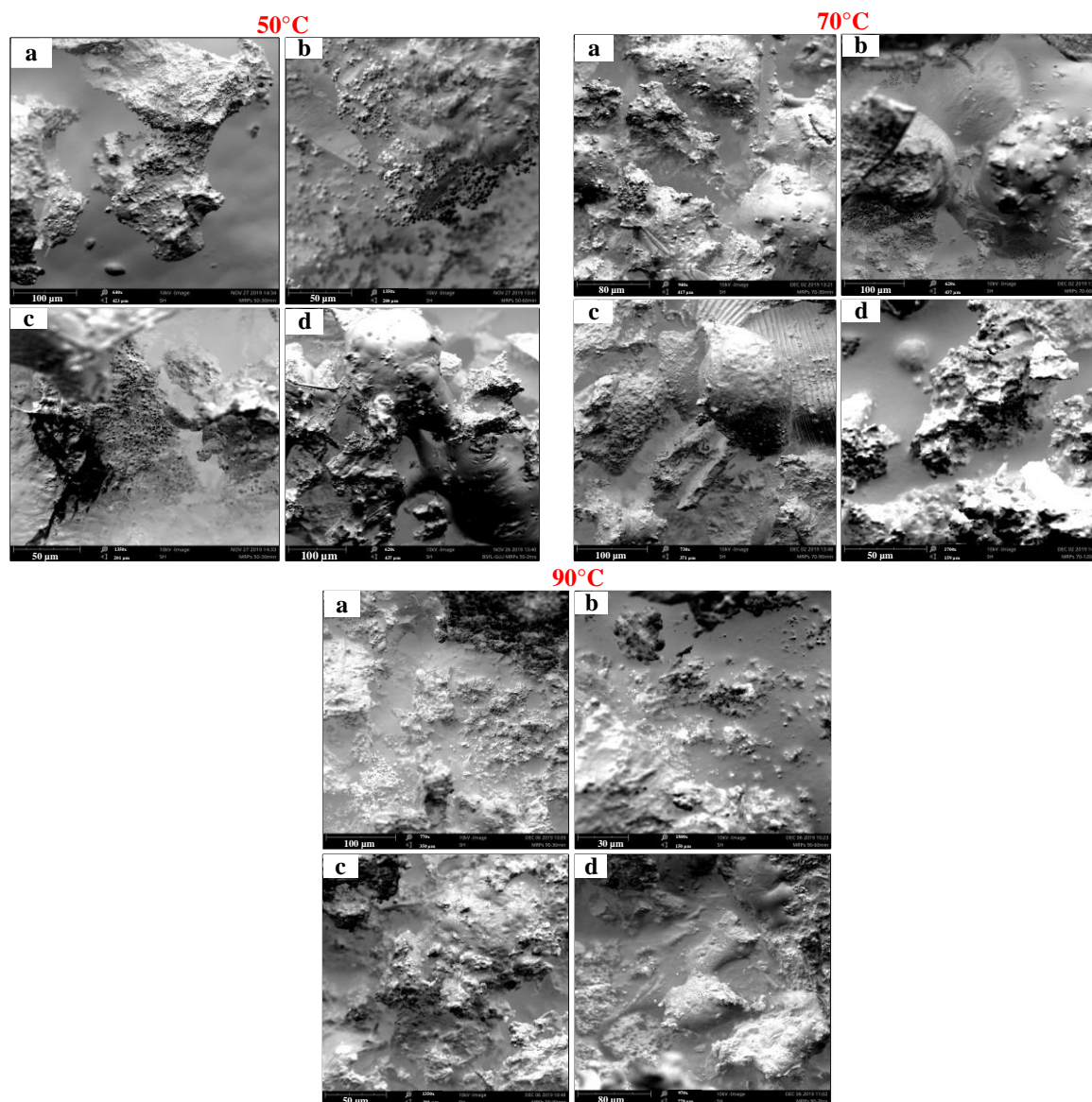

Figure 4.14 Scanning electron microscope of Maillard reaction products at 50 °C, 70 °C and 90 °C for (a) 30 min, (b) 60 min, (c) 90 min and (d) 120 min at 620 to 1800x magnification.

broken down, shown in Figure 4.13d. This formation is probably due to the filamentous nature of the BSFL protein. Huang et al. (2019) reported that heating the proteins for long period polymerizes the particles and prepares them to be ready for interaction because they have unfolded.

Therefore, it can be concluded that treatment at temperatures close to or higher than the denaturation temperature, 70 °C and 90 °C, resulted in the protein molecules unfolding. Research on insect studies have shown two different surface morphologies that have been described until now, they are (1) conventional dried BSFL flour, and (2) microwave dried BSFL (Huang et al., 2019). In the present study, we researched further and observed the surface morphology of the freeze dried BSFL protein extracts.

After conjugation, the large particles irregular in shape with rough surface texture were observed in Figure 4.14, which originated from both proteins and sugar molecules (Figure 4.12). At 90 °C the sugar and protein aggregates spread out more, which agrees with Niu's report (Niu et al., 2011) in the study of wheat germ protein. The particles of the MRPs were now porous, in a thinner sheet. This is also because the glucose can break the non-covalent bond of the protein during Maillard reaction (MR), which makes the protein bonds and molecules unfold and reduces the protein molecule aggregation. On heating at 70 °C the MRPs exhibited some reduction of the molecule space (Figure 4.14b), which disappeared at 90 °C (Figure 4.14c). The collapse of the cuticle layer was noticed on the material subjected to higher temperature. When the temperatures were kept constant, an increase in heating time also resulted in an increase in matrix order. Similar results of surface morphology were observed in MRP products of wheat germ protein (Niu et al., 2011). The morphology showed different structures depending on the method of protein extraction.

4.4.7. Measurements of zeta potential

Zeta potential is a physical property that is displayed by a particle in suspension or material surface. Zeta potential can be used to optimise the formulations of protein solutions and emulsions. And the knowledge of the zeta potential is important because it can reduce the time needed to produce trial formulations. It also aids in predicting long term stability. The liquid layer surrounding the particle exists as two parts; an inner region (Stern layer) where the ions are strongly bound and an outer (diffuse) region where they are less firmly associated. Within the diffuse layer there is a notional boundary inside which the ions and particles form a stable entity. When a particle moves

(e.g., due to gravity), ions within the boundary cause its mobility. Those ions beyond the boundary remain with the bulk dispersant. The potential at this boundary (surface of hydrodynamic shear) is the zeta potential. The general dividing line between stable and unstable suspensions is generally taken at either +30 or -30 mV. Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable. The curve of zeta potential versus pH will be positive at low pH and lower or negative at high pH. The point where the plot passes through zero zeta potential is called the isoelectric point and is very important from a practical consideration.
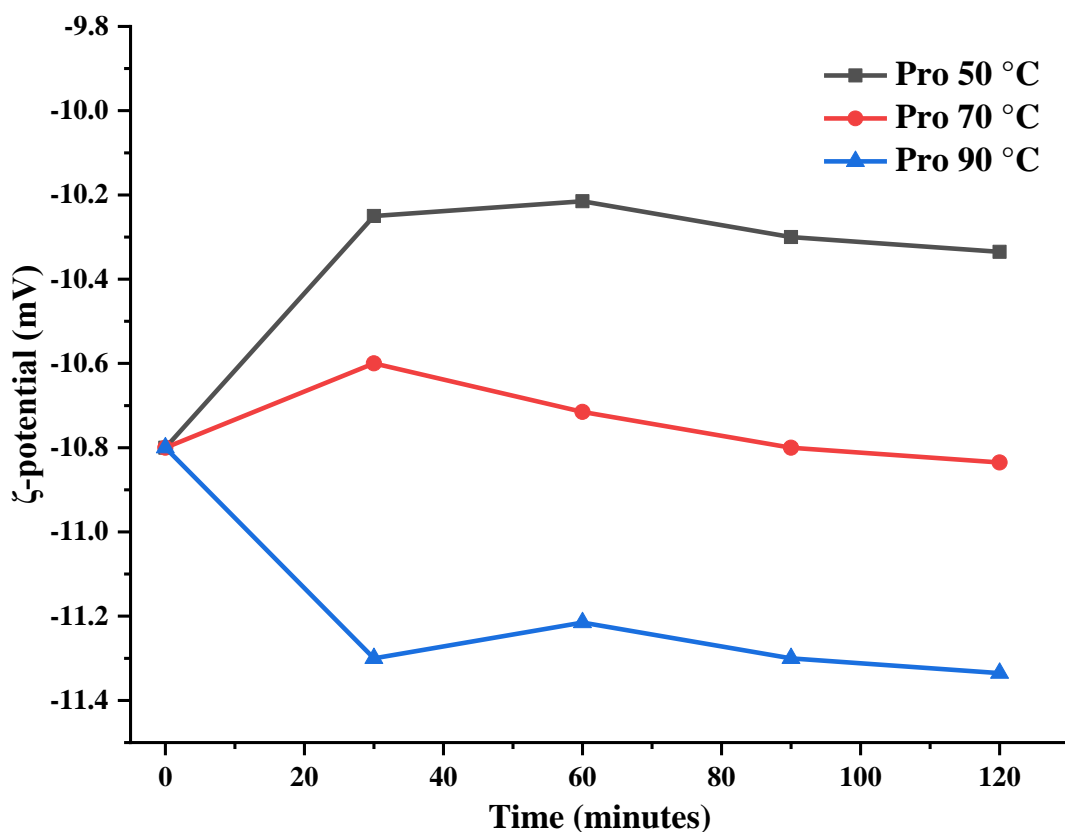

Figure 4.15 Zeta potential profile of proteins heated alone at 50 °C, 70 °C and 90 °C for 0, 30, 60, 90 and 120 min at their different isoelectric points.

Measurements of the zeta potential of the proteins heated alone and Maillard reaction products (MRPs) are displayed in Figure 4.15 and Figure 4.16, respectively. No significant change is observed in Figure 4.15, for the proteins heated alone. Malhotra et al. (2004) also found similar results in the study on soy proteins. After heating treatment, the MRP (Figure 4.16) pH decreased from 9 (Appendix F), meaning the solution was less basic as compared to the more alkaline starting

material. Zeta potential increased when the pH decreased, with positive values at more acid pH and negative values at more alkaline pH. The trends for Figure 4.16 curves could be explained by protonation at an acidic pH and deprotonation at an alkaline pH, of the amino acids in the protein groups (Bouzid et al., 2008).

At pH 9 (control at 0 min), the proteins heated alone and MRPs had the different zeta potential of -10.8 mV and -45mV, respectively. As the reaction progressed, the pH decreases, zeta potential for MRPs at 90 °C (-43,5 mV) at 70 °C (-30,2 mV) and at 50 °C (-22,4 mV) at 60 min. MRPs at 70 °C were close to or equal to the threshold absolute value of (-30 mV and +30 mV) described as the stability boundary (Carneiro-Da-Cunha et al., 2011).
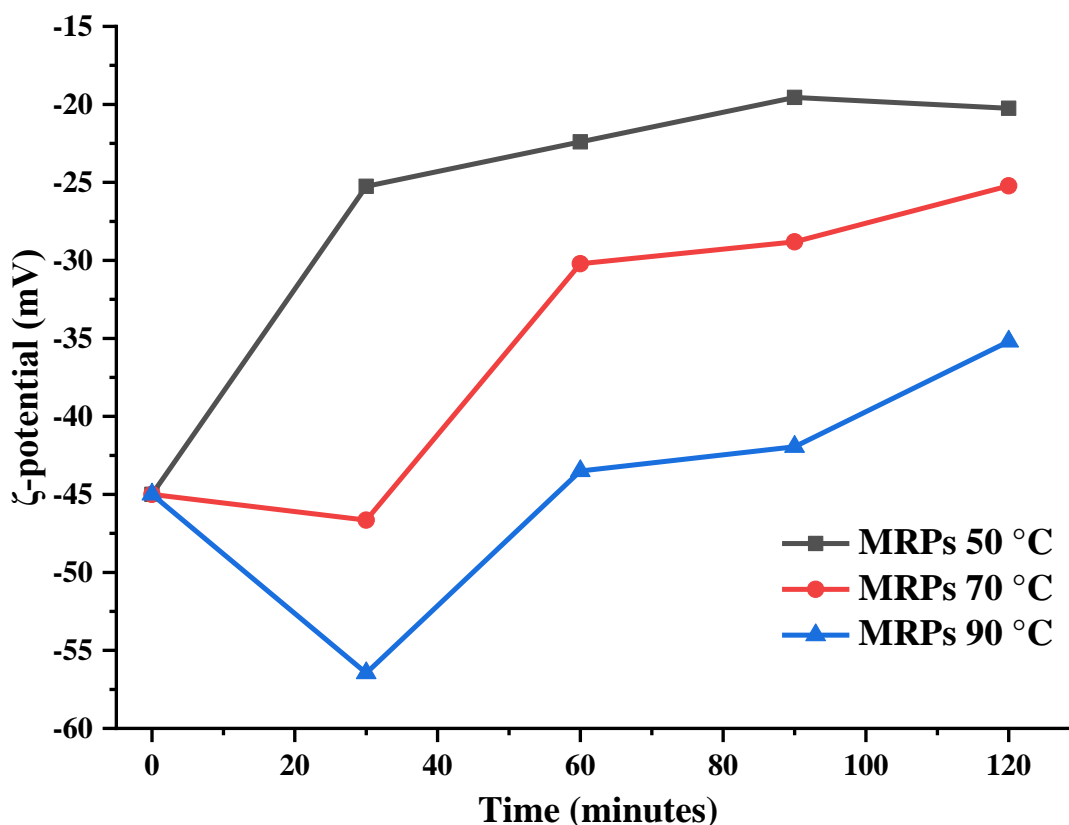
Figure 4.16 Zeta potential profile of the Maillard reaction products at 50°C, 70°C and 90°C for 0, 30, 60, 90 and 120 min at their different isoelectric points.

In general, as the reaction progressed, the pH decreased and zeta potential increased. These results reveal that MRPs were less sensitive to aggregation (more stable, more negative than -30mV) when compared to proteins heated alone (less stable, less negative than -30mV). The zeta-potential stability of the heated protein is decreased slightly on further heating to 120 min. These results are in agreement with the DSC analysis results.

4.5. Conclusion

In conclusion, it was possible to obtain MRPs by MR between protein extracted from the BSFL flour and glucose. Under the conditions used in this study, a modification was observed due to MR. The amino acids level of the protein was found to be comparable with those of FAO/WHO. The difference in the microstructure between the protein and MRPs was also determined by SEM. FTIR confirmed that an increase in the degree of glycation leads to an increase in the structural change of the protein. At the same time, the spectroscopic analysis of FTIR indicated that amide I and II were modified by MR. Upon comparing the samples in the FTIR through the PCA, it becomes evident that the protein has different properties on the PCs compared with the MRP samples. The shifts of amide bands in the FTIR analysis and the DSC peak shifts of the MRPs further testifies to the formation of the conjugates. In the case of TGA, longer reaction times do improve the properties and there was evidence that at this stage other products may have been formed. Compared with proteins heated alone, the conjugates exhibited similar thermal stability as demonstrated by the denaturation temperature of ~250 - 550 °C. As shown in the DSC analysis, the results of this study also demonstrate that the thermal stability of the protein was effectively improved by conjugation with glucose. Therefore, the present results suggest that protein conjugated with glucose under Maillard reaction conditions can be a promising way to improve the properties of the BSFL protein.

References

AOAC, 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, USA.

Akhtar, M. & Ding, R. 2017. Covalently cross-linked proteins & polysaccharides: Formation, characterisation and potential applications. *Current Opinion in Colloid and Interface Science*, 28: 31–36.

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.

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(2): 318–326.

Belforti, M., Gai, F., Lussiana, C., Renna, M., Malfatto, V., Rotolo, L., De Marco, M., Dabbou,

S., Schiavone, A., Zoccarato, I. & Gasco, L. 2015. Tenebrio molitor meal in rainbow trout (Oncorhynchus mykiss) diets: Effects on animal performance, nutrient digestibility and chemical composition of fillets. *Italian Journal of Animal Science*, 14(4): 670–676.

Bollen, J., Van de Sompel, H., Hagberg, A. & Chute, R. 2009. A Principal Component Analysis of 39 Scientific Impact Measures T. Mailund, ed. *PLoS ONE*, 4(6): 1–11.

Bouzid, H., Rabiller-Baudry, M., Paugam, L., Rousseau, F., Derriche, Z. & Bettahar, N.E. 2008. Impact of zeta potential and size of caseins as precursors of fouling deposit on limiting and critical fluxes in spiral ultrafiltration of modified skim milks. *Journal of Membrane Science*, 314(1–2): 67–75.

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.

Cardoso, J.C., Albuquerque, R.L.C., Padilha, F.F., Bittencourt, F.O., De Freitas, O., Nunes, P.S., Pereira, N.L., Fonseca, M.J.V. & Araújo, A.A.S. 2011. Effect of the Maillard reaction on properties of casein and casein films. In *Journal of Thermal Analysis and Calorimetry*. Kluwer Academic Publishers: 249–254.

Carneiro-Da-Cunha, M.G., Cerqueira, M.A., Souza, B.W.S., Teixeira, J.A. & Vicente, A.A. 2011. Influence of concentration, ionic strength and pH on zeta potential and mean hydrodynamic diameter of edible polysaccharide solutions envisaged for multinanolayered films production. *Carbohydrate Polymers*, 85(3): 522–528.

Chang, M.C. & Tanaka, J. 2002. FT-IR study for hydroxyapatite/collagen nanocomposite cross-linked by glutaraldehyde. *Biomaterials*, 23(24): 4811–4818.

De Oliveira, F.C., Coimbra, J.S. dos R., de Oliveira, E.B., Zuñiga, A.D.G. & Rojas, E.E.G. 2016. Food Protein-polysaccharide Conjugates Obtained via the Maillard Reaction: A Review. *Critical Reviews in Food Science and Nutrition*, 56(7): 1108–1125.

Destefanis, G., Barge, M.T., Brugiapaglia, A. & Tassone, S. 2000. The use of principal component analysis (PCA) to characterize beef. *Meat Science*, 56(3): 255–259.

Dias, E.C., Borém, F.M., Pereira, R.G.F.A. & Guerreiro, M.C. 2012. Amino acid profiles in unripe Arabica coffee fruits processed using wet and dry methods. *European Food Research and Technology*, 234(1): 25–32.

Dickinson, E. 2015. Colloids in Food: Ingredients, Structure, and Stability. *Annual Review of Food*

Science and Technology, 6(1): 211–233.

Esbensen, K.H. & Swarbrick, B. 2018. Multivariate Data Analysis 6th Edition An introduction to Multivariate Analysis. *Process Analytical Technology and Quality by Design*, 50(2): 69–83.

Evans, M., Ratcliffe, I. & Williams, P.A. 2013. Emulsion stabilisation using polysaccharide-protein complexes. *Current Opinion in Colloid and Interface Science*, 18(4): 272–282.

FAO/WHO. (2013). Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. Food and nutrition paper no. 92. Food and Agriculture Organization and the World Health Organization, Rome.

Feng, W., Qian, L., Wang, W., Wang, T., Deng, Z., Yang, F., Xiong, J. & Wang, C. 2018. Exploring the potential of lipids from black soldier fly: New paradigm for biodiesel production (II)—Extraction kinetics and thermodynamic. *Renewable Energy*, 119: 12–18.

Fitzsimons, S. Mulvihill, D. & Morris, E. 2008. Large enhancements in thermogelation of whey protein isolate by incorporation of very low concentrations of guar gum. *Food hydrocolloids,* 22 (4): 576-586.

García, L., Cova, A., Sandoval, A.J., Müller, A.J. & Carrasquel, L.M. 2012. Glass transition temperatures of cassava starch-whey protein concentrate systems at low and intermediate water content. *Carbohydrate Polymers*, 87(2): 1375–1382.

Gu, F.L., Kim, J.M., Abbas, S., Zhang, X.M., Xia, S.Q. & Chen, Z.X. 2010. Structure and antioxidant activity of high molecular weight Maillard reaction products from casein-glucose. *Food Chemistry*, 120(2): 505–511.

Guerrero, P., Kerry, J.P. & de la Caba, K. 2014. FTIR characterization of protein–polysaccharide interactions in extruded blends. *Carbohydrate Polymers*, 111: 598–605.

Hashim, D.M., Man, Y.B.C., Norakasha, R., Shuhaimi, M., Salmah, Y. & Syahariza, Z.A. 2010. Potential use of Fourier transform infrared spectroscopy for differentiation of bovine and porcine gelatins. *Food Chemistry*, 118(3): 856–860.

Henderson, J.W., Ricker, R.D., Bidlingmeyer, B.A. & Woodward, C. 2000. Rapid, Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids Amino Acid Analysis Using Zorbax Eclipse-AAA Columns and the Agilent 1100 HPLC. *Agilent*, 20(4): 1–10.

Henry, M., Gasco, L., Piccolo, G. & Fountoulaki, E. 2015. Review on the use of insects in the diet of farmed fish: Past and future. *Animal Feed Science and Technology*, 203(1): 1–22.

Huang, C., Feng, W., Xiong, J., Wang, T., Wang, W., Wang, C. & Yang, F. 2019. Impact of drying

method on the nutritional value of the edible insect protein from black soldier fly (Hermetia illucens L.) larvae: amino acid composition, nutritional value evaluation, in vitro digestibility, and thermal properties. *European Food Research and Technology*, 245(1): 11–21.

Ibanoglu, E. 2005. Effect of hydrocolloids on the thermal denaturation of proteins. *Food Chemistry*, 90(4): 621–626.

Ifeduba, E.A. & Akoh, C.C. 2016. Microencapsulation of stearidonic acid soybean oil in Maillard reaction-modified complex coacervates. *Food Chemistry*, 199: 524–532.

Jajić, I., Krstović, S., Glamočić, D., Jakšić, S. & Abramović, B. 2013. Validation of an HPLC method for the determination of amino acids in feed. *J. Serb. Chem. Soc*, 78(6): 839–850.

Janssen, R.H., Vincken, J.P., Van Den Broek, L.A.M., Fogliano, V. & Lakemond, C.M.M. 2017. Nitrogen-to-Protein Conversion Factors for Three Edible Insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. *Journal of Agricultural and Food Chemistry*, 65(11): 2275–2278.

Jiménez-Castaño, L., Villamiel, M. & López-Fandiño, R. 2007. Glycosylation of individual whey proteins by Maillard reaction using dextran of different molecular mass. *Food Hydrocolloids*, 21(3): 433–443.

Kaya, M., Mujtaba, M., Bulut, E., Akyuz, B., Zelencova, L. & Sofi, K. 2015. Fluctuation in physicochemical properties of chitins extracted from different body parts of honeybee. *Carbohydrate Polymers*, 132: 9–16.

Kim, H.E. & Brad, S. 2018. Multivariate Data Analysis. CAMO Software AS. 67-100

L'Hocine, L., Boye, J.I. & Arcand, Y. 2006. Composition and functional properties of soy protein isolates prepared using alternative defatting and extraction procedures. *Journal of Food Science*, 71(3): C137–C145.

Leiva, G.E., Naranjo, G.B. & Malec, L.S. 2017. A study of different indicators of Maillard reaction with whey proteins and different carbohydrates under adverse storage conditions. *Food Chemistry*, 215: 410–416.

Li, Y., Lu, F., Luo, C., Chen, Z., Mao, J., Shoemaker, C. & Zhong, F. 2009. Functional properties of the Maillard reaction products of rice protein with sugar. *Food Chemistry*, 117(1): 69–74.

Li, Y.Q., Kong, D.X. & Wu, H. 2013. Analysis and evaluation of essential oil components of cinnamon barks using GC-MS and FTIR spectroscopy. *Industrial Crops and Products*, 41(1): 269–278.

Liang, C., Yuan, F., Liu, F., Wang, Y. & Gao, Y. 2014. Structure and antimicrobial mechanism of ε-polylysine–chitosan conjugates through Maillard reaction. *International Journal of Biological Macromolecules*, 70: 427–434.

Liou, T.H. 2004. Evolution of chemistry and morphology during the carbonization and combustion of rice husk. *Carbon*, 42(4): 785–794.

Liu, F., Wang, D., Sun, C., McClements, D.J. & Gao, Y. 2016. Utilization of interfacial engineering to improve physicochemical stability of β-carotene emulsions: Multilayer coatings formed using protein and protein-polyphenol conjugates. *Food Chemistry*, 205: 129–139.

Liu, J., Xu, Q., Zhang, J., Zhao, P. & Ding, Y. 2016. Characterization of silver carp (Hypophthalmichthys molitrix) myosin protein glycated with konjac oligo-glucomannan. *Food Hydrocolloids*, 57: 114–121.

Liu, Q., Kong, B., Han, J., Sun, C. & Li, P. 2014. Structure and antioxidant activity of whey protein isolate conjugated with glucose via the Maillard reaction under dry-heating conditions. *Food Structure*, 1(2): 145–154.

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.

Malhotra, A. & Coupland, J.N. 2004. The effect of surfactants on the solubility, zeta potential, and viscosity of soy protein isolates. *Food Hydrocolloids*, 18(1): 101–108.

Marei, N.H., El-Samie, E.A., Salah, T., Saad, G.R. & Elwahy, A.H.M. 2016. Isolation and characterization of chitosan from different local insects in Egypt. *International Journal of Biological Macromolecules*, 82: 871–877.

Mellado-Carretero, J., Kaade, W., Ferrando, M., Güell, C. & de Lamo-Castellví, S. 2019. Attenuated Total Reflectance Fourier Transform Midinfrared Spectroscopy Combined with Multivariate Analysis, a Novel Approach to Monitor Maillard Reaction. *Journal of Food Science*, 84(10): 2777–2784.

Mshayisa, V. 2016. Antioxidant effects of Maillard reaction products (MRPs) derived from glucose-casein model systems. Master's thesis. Cape Peninsula University of Technology.

Muehlethaler, C., Massonnet, G. & Esseiva, P. 2011. The application of chemometrics on Infrared and Raman spectra as a tool for the forensic analysis of paints. *Forensic Science International*, 209(1–3): 173–182.

Mueller, D., Ferrão, M., Marder, L., da Costa, A. & de Cássia de Souza Schneider, R. 2013. Fourier Transform Infrared Spectroscopy (FTIR) and Multivariate Analysis for Identification of Different Vegetable Oils Used in Biodiesel Production. *Sensors*, 13(4): 4258–4271.

Mularczyk-Oliwa, M., Bombalska, A., Kaliszewski, M., Włodarski, M., Kopczyński, K., Kwaśny, M., Szpakowska, M. & Trafny, E.A. 2012. Comparison of fluorescence spectroscopy and FTIR in differentiation of plant pollens. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 97: 246–254.

Müller, A., Wolf, D. & Gutzeit, H.O. 2017. The black soldier fly, Hermetia illucens - A promising source for sustainable production of proteins, lipids and bioactive substances. *Zeitschrift fur Naturforschung - Section C Journal of Biosciences*, 72(9–10): 351–363.

Niu, L.Y., Jiang, S.T., Pan, L.J. & Zhai, Y.S. 2011. Characteristics and functional properties of wheat germ protein glycated with saccharides through Maillard reaction. *International Journal of Food Science and Technology*, 46(10): 2197–2203.

Nuthong, P., Benjakul, S. & Prodpran, T. 2009. Characterization of porcine plasma protein-based films as affected by pretreatment and cross-linking agents. *International Journal of Biological Macromolecules*, 44: 143–148.

Oliver, C.M., Kher, A., McNaughton, D. & Augustin, M.A. 2009. Use of FTIR and mass spectrometry for characterization of glycated caseins. *Journal of Dairy Research*, 76(1): 105–110.

Osimani, A., Garofalo, C., Milanović, V., Taccari, M., Cardinali, F., Aquilanti, L., Pasquini, M., Mozzon, M., Raffaelli, N., Ruschioni, S., Riolo, P., Isidoro, N. & Clementi, F. 2017. Insight into the proximate composition and microbial diversity of edible insects marketed in the European Union. *European Food Research and Technology*, 243(7): 1157–1171.

Petrozzi, S. 2013. Practical Instrumental Analysis; Methods, Quality Assurance and Laboratory Management. Wiley-VCH Verlag & Co.KGaA. Germany, 274-278.

Purschke, B., Meinlschmidt, P., Horn, C., Rieder, · Oskar & Jäger, · Henry. 2018. Improvement of techno-functional properties of edible insect protein from migratory locust by enzymatic hydrolysis. *European Food Research and Technology*, 244(1): 999–1013.

Qi, J.R., Yang, X.Q. & Liao, J.S. 2009. Improvement of functional properties of acid-precipitated soy protein by the attachment of dextran through maillard reaction. *International Journal of Food Science and Technology*, 44(11): 2296–2302.

Qiu, J., Zheng, Q., Fang, L., Wang, Y., Min, M., Shen, C., Tong, Z. & Xiong, C. 2018. Preparation and characterization of casein-carrageenan conjugates and self-assembled microcapsules for encapsulation of red pigment from paprika. *Carbohydrate Polymers*, 196: 322–331.

Rai, S., Kureel, A.K., Dutta, P.K. & Mehrotra, G.K. 2018. Phenolic compounds based conjugates from dextran aldehyde and BSA: Preparation, characterization and evaluation of their anti-cancer efficacy for therapeutic applications. *International Journal of Biological Macromolecules*, 110: 425–436.

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M.T., Biasato, I., Biasibetti, E., De Marco, M., Brugiapaglia, A., Zoccarato, I. & Gasco, L. 2017. Evaluation of the suitability of a partially defatted black soldier fly (Hermetia illucens L.) larvae meal as ingredient for rainbow trout (Oncorhynchus mykiss Walbaum) diets. *Journal of Animal Science and Biotechnology*, 8(1): 1–13.

Rhim, J.-W., Mohanty, K.A., Singh, S.P. & Ng, P.K.W. 2006. Preparation and Properties of Biodegradable Multilayer Films Based on Soy Protein Isolate and Poly(lactide). *Industrial & Engineering Chemistry*, 2(1): 3059–3066.

Salgın, S., Salgın, U. & Bahadır, S. 2013. Zeta Potentials and Isoelectric Points of Biomolecules: The Effects of Ion Types and Ionic Strengths. *Tetrahedron Letters*, 26(33): 3999–4002.

Schösler, H., Boer, J. de & Boersema, J.J. 2012. Can we cut out the meat of the dish? Constructing consumer-oriented pathways towards meat substitution. *Appetite*, 58(1): 39–47.

Schiavone, A., De Marco, M., Martínez, S., Dabbou, S., Renna, M., Madrid, J., Hernandez, F., Rotolo, L., Costa, P., Gai, F. & Gasco, L. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (Hermetia illucens L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology*, 8(1): 51.

Soon, C.Y., Tee, Y.B., Tan, C.H., Rosnita, A.T. & Khalina, A. 2018. Extraction and physicochemical characterization of chitin and chitosan from Zophobas morio larvae in varying sodium hydroxide concentration. *International Journal of Biological Macromolecules*, 108(1): 135–142.

Su, J.F., Huang, Z., Yuan, X.Y., Wang, X.Y. & Li, M. 2009. Structure and properties of carboxymethyl cellulose/soy protein isolate blend edible films crosslinked by Maillard reactions. *Carbohydrate Polymers*, 79: 145–153.

Sun, Y., Hayakawa, S., Puangmanee, S. & Izumori, K. 2006. Chemical properties and antioxidative activity of glycated α-lactalbumin with a rare sugar, D-allose, by Maillard reaction. *Food Chemistry*, 95(3): 509–517.

Vhangani, L.N. & Van Wyk, J. 2013. Antioxidant activity of Maillard reaction products (MRPs) derived from fructose-lysine and ribose-lysine model systems. *Food Chemistry*, 137(1–4): 92–98.

Wang, C., Qian, L., Wang, W., Wang, T., Deng, Z., Yang, F., Xiong, J. & Feng, W. 2017. Exploring the potential of lipids from black soldier fly: New paradigm for biodiesel production (I). *Renewable Energy*, 111: 749–756.

Wang, C., Qian, L., Wang, W., Wang, T., Deng, Z., Yang, F., Xiong, J. & Feng, W. 2017a. Exploring the potential of lipids from black soldier fly: New paradigm for biodiesel production (I). *Renewable Energy*, 111: 749–756.

Waśko, A., Bulak, P., Polak-Berecka, M., Nowak, K., Polakowski, C. & Bieganowski, A. 2016. The first report of the physicochemical structure of chitin isolated from Hermetia illucens. *International Journal of Biological Macromolecules*, 92: 316–320.

Yaman, N. & Durakli Velioglu, S. 2019. Use of Attenuated Total Reflectance—Fourier Transform Infrared (ATR-FTIR) Spectroscopy in Combination with Multivariate Methods for the Rapid Determination of the Adulteration of Grape, Carob and Mulberry Pekmez. *Foods*, 8(7): 1–12.

Yang, F., Huang, X., Zhang, C., Zhang, M., Huang, C. & Yang, H. 2018. Amino acid composition and nutritional value evaluation of Chinese chestnut (*Castanea mollissima* Blume) and its protein subunit. *RSC Advances*, 8(5): 2653–2659.

Young, V. R. & Pellet, P. L. 1991. Protein evaluation, amino acid scoring and the food and drug administration's proposed food labelling regulations. *Journal of Nutrition*, 121(1): 145- 150.

Zhang, B., Chi, Y.J. & Li, B. 2014. Effect of ultrasound treatment on the wet heating Maillard reaction between β-conglycinin and maltodextrin and on the emulsifying properties of conjugates. *European Food Research and Technology*, 238(1): 129–138.

Zhang, J.B., Wu, N.N., Yang, X.Q., He, X.T. & Wang, L.J. 2012. Improvement of emulsifying properties of Maillard reaction products from β-conglycinin and dextran using controlled enzymatic hydrolysis. *Food Hydrocolloids*, 28(2): 301–312.

CHAPTER 5

Conclusion and recommendations

5.1. Conclusion

Prior work has documented the importance of insect consumption, insects such as Black soldier fly (BSF), to meet the pressure of imminent food scarcity. As an alternative protein source, insect meals have been reported to have various beneficial effects in both production and health of animals, for example in the animal nutrition industry it has become a necessity to seek sustainable and alternative protein sources for animal production. However, these studies have either been short-term studies or have not focused on the exploitation of the BSF insect for human consumption. In this study we defatted freeze dried BSF larvae, extracted the protein and glycated the product with glucose under temperature and time conditions. The characterisation and analytical tests were conducted before and after conjugation. In Chapter 1, a general introduction to the study is given followed by a more complete literature overview in Chapter 2.

The first research experimental part of this study was the extraction of the oil (by-product) from BSFL using hexane: isopropanol mixture. This solvent mixture successfully removed most of the fat only leaving behind 4.86%. The hypothesis that the defatted BSFL will be used for protein extraction, was accepted. The effect of removing fat from the BSF larvae was also evaluated. Nutritional information was determined, and further evaluation was completed by structural and thermal instrumentation. It was discovered that the whole larvae and defatted larvae both have high nutritional value, with 45.82 and 56.11% protein content, respectively. The thermal stability of the defatted larvae was improved on removal of the lipid fraction.

The second phase of this study aimed to investigate the extracted BSFL protein properties before and after conjugation by Maillard reaction (MR) with glucose. The protein was extracted using the alkaline extraction method. The amino acid level of the protein was found to be comparable with the amino acid level recommended by FAO/WHO for dietary allowance for human nutrition. It was possible to obtain Maillard reaction products (MRPs) by the reaction between BSFL protein and glucose for a range of diverse temperature and time spans. Under the conditions used in this study, the modification was observed due to MR. Differences in the

microstructure between the protein and MRPs were observed using measurements by SEM, FTIR, TGA and DSC. It was concluded that the increase in the degree of glycation leads to an increase in structural change of the protein. This was in accord with the modification of amide I and II due to MR. Upon comparing the samples using Principal Component Analysis, it became evident that the protein (proteins heated at 90 °C) has different properties when compared to products (MRPs heated at 90 °C) of the Maillard reaction. Compared with proteins heated alone, the conjugates exhibited a better thermal stability, therefore effective improvement of the protein was obtained by conjugation with glucose in the Maillard reaction.

Although human and/or animal food products still need to be produced, the fact that similar amino acid results were achieved between soybean, casein and BSFL protein leads to the conclusion that BSF could be regarded as a protein source that can be utilised to partially replace other traditional sources in its ability to sustain food security.

Although Black Soldier Fly cannot be classified as the "perfect insect" in the quest for an environmentally sustainable and alternative protein source, this dissertation has established that it is a favourable option for the development of foodstuffs with acceptable protein make-up for animal and human subjects.

5.2. Recommendations

The following recommendations are suggested for further research.

- Further studies should include more trials on defatting methods in order to determine the most effective one. These methods should also be applied to different insect species to study the effect they may have on a variety of edible insect species.

- Another beneficial area of research is the analysis of nutrient components such as vitamins and fatty acids in order to facilitate nutritional information prior to the use of the larvae for human food development.

- It is recommended to investigate the BSFL protein and MRPs further using other different analysis instrumentation, such as Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), Circular Dichroism (CD) and Liquid Chromatography with tandem mass spectrometry (LC-MS-MS).

- Scrutiny using food analysis techniques to determine the credibility of the MRPs to be used as an ingredient in human foodstuffs such as technofunctional and physicochemical properties as well as sensory evaluation.

APPENDIX A

Essential amino acids

a

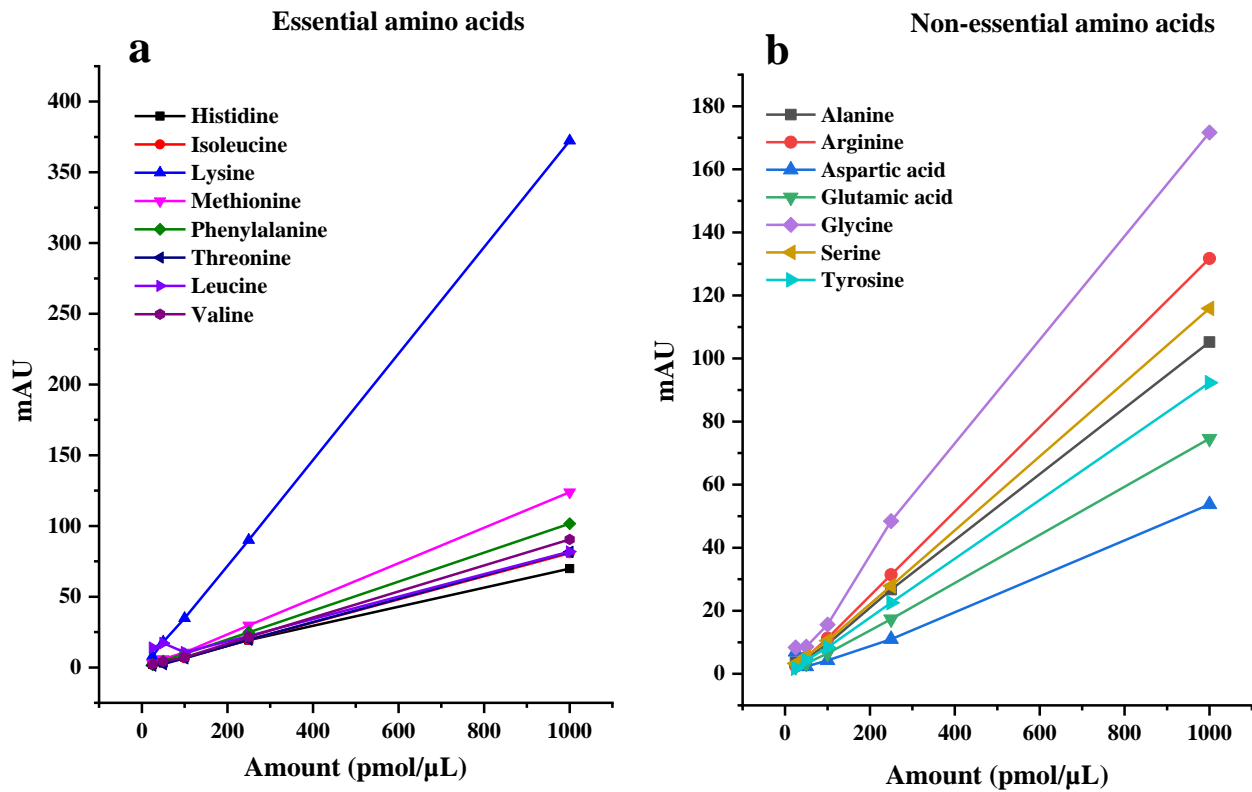**Non-essential amino acids**

b

Figure A.1 Calibration curves of the amino acid standards (25, 50, 250 and 1000 pmol/μL) for the essential amino acids (a) and non-essential amino acids (b), drawn using High Performance Liquid Chromatography software called Open Lab.
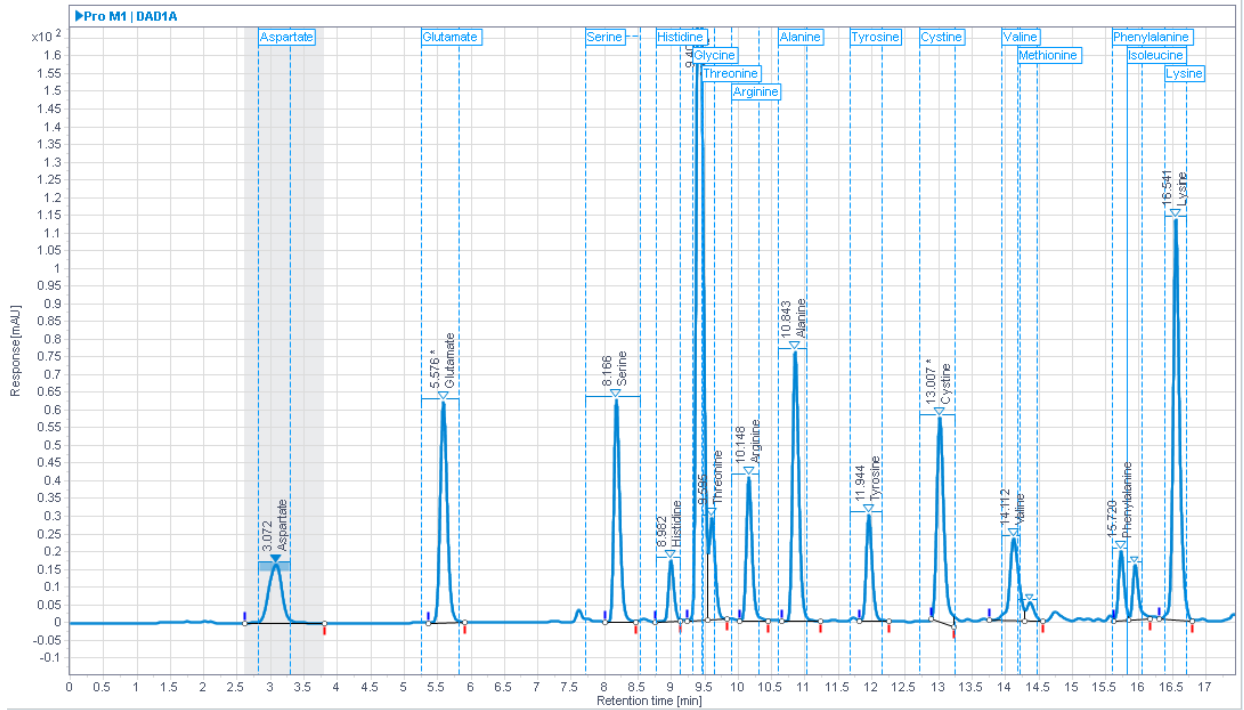
Figure A.2 High Performance Liquid Chromatogram of Black soldier fly larvae protein, eluting from 3 to 17 min.
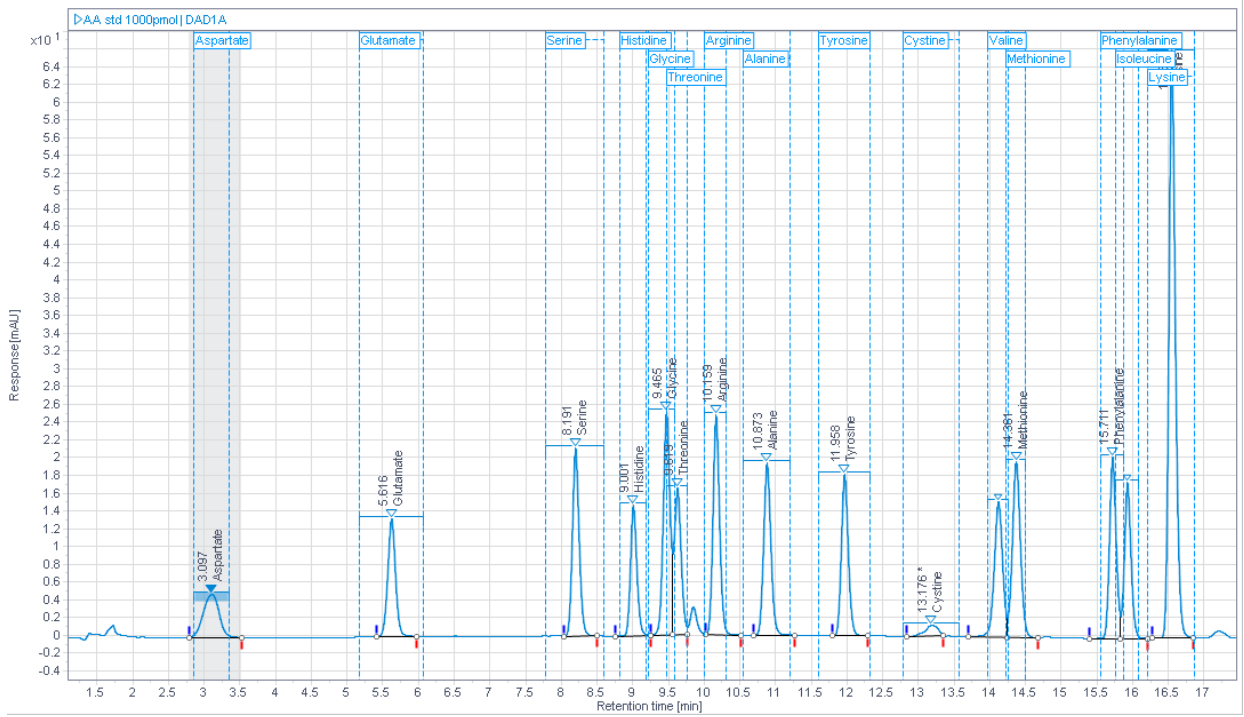

Figure A.3 High Performance Liquid Chromatogram of the standard mixture (1000 nmol/L), eluting from 3 to 17 min.

Table 01 Specificity evaluation comparing retention times; retention times of the standard amino acid mixture (n = 3).

Amino acid	Concentration, nmol/L					Average	RSD%
	25	50	100	250	1000		
Aspartic acid	3.07	3.08	3.08	3.07	3.07	3.07	0.08
Glutamic acid	5.58	5.58	5.58	5.57	5.57	5.58	0.05
Serine	8.17	8.17	8.17	8.16	8.16	8.17	0.03
Histidine	8.98	8.99	8.98	8.99	8.98	8.98	0.04
Glycine	9.40	9.40	9.40	9.41	9.44	9.41	0.16
Threonine	9.60	9.59	9.60	9.59	9.59	9.59	0.03
Arginine	10.15	10.15	10.15	10.15	10.15	10.15	0.02
Alanine	10.84	10.84	10.85	10.84	10.85	10.85	0.03
Tyrosine	11.94	11.94	11.94	11.94	11.94	11.94	0.01
Valine	14.11	14.12	14.12	14.11	14.11	14.11	0.02
Methionine	14.60	14.61	14.60	14.60	14.61	14.60	0.02
Phenylalanine	15.72	15.72	15.73	15.73	15.72	15.72	0.01
Isoleucine	15.93	15.93	15.92	15.93	15.93	15.93	0.02
Lysine	16.54	16.55	16.54	16.55	16.54	16.54	0.02
Leucine	16.55	16.55	16.55	16.56	16.56	16.55	0.02
Proline	20.64	20.65	20.64	20.65	20.64	20.64	0.01

Table A.2 Linearity data for the standard amino acids mixtures.

Amino acid	Slope	Intercept	R^2
Aspartic acid	0.054	-0.808	0.9999
Glutamic acid	0.075	-0.420	0.9999
Serine	0.116	-0.375	0.9999
Histidine	0.070	0.349	0.9999
Glycine	0.171	1.537	0.9999
Threonine	0.083	-1.018	0.9999
Arginine	0.133	-1.163	0.9999
Alanine	0.105	0.015	0.9999
Tyrosine	0.093	-0.464	0.9997
Valine	0.091	-0.628	0.9999
Methionine	0.124	-0.674	0.9999
Phenylalanine	0.102	-0.432	0.9999
Isoleucine	0.081	-0.549	0.9999
Lysine	0.372	-1.359	0.9999
Leucine	0.004	-0.464	0.9999
Proline	0.014	-1.018	0.9998

Table 03 Limit of detection (LOD) and limit of quantification (LOQ) values.

Amino acid	LOD µmol/L	LOQ µmol/L
Aspartic acid	0.277	0.838
Glutamic acid	0.044	0.134
Serine	0.027	0.082
Histidine	0.059	0.177
Glycine	0.083	0.250
Threonine	0.026	0.079
Arginine	0.015	0.046
Alanine	0.036	0.108
Tyrosine	0.011	0.033
Valine	0.043	0.132
Methionine	0.029	0.089
Phenylalanine	0.006	0.017
Isoleucine	0.032	0.097
Lysine	0.015	0.044
Leucine	0.031	0.033
Proline	0.020	0.045

APPENDIX B
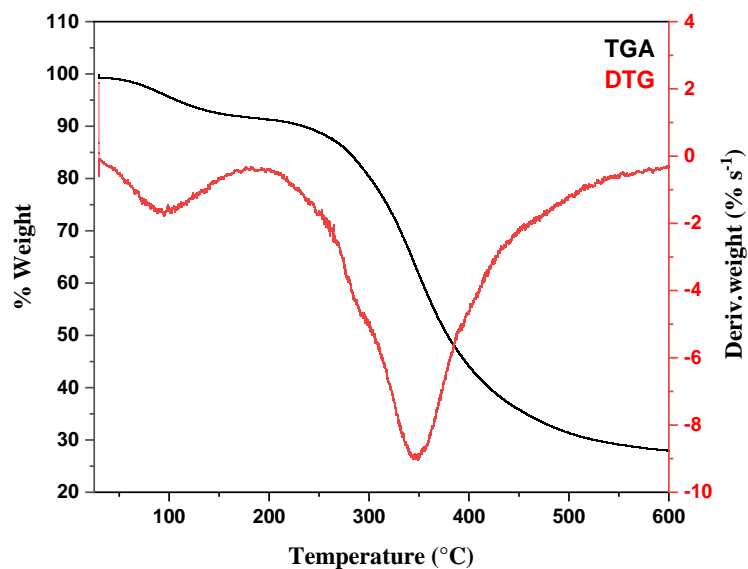
Figure B.1 Thermal Gravimetric Analysis and Differential Thermogravimetric of the non-heated Black soldier fly larvae protein.
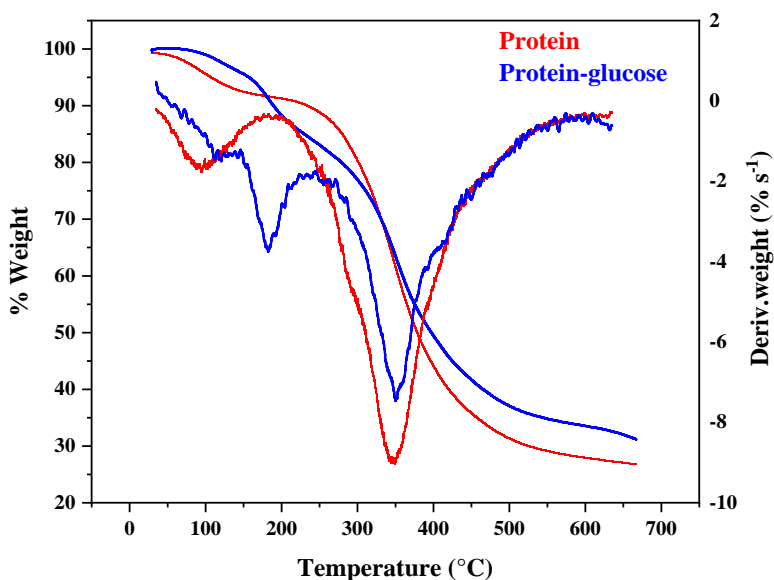

Figure B.2 Thermal Gravimetric Analysis and Differential Thermogravimetric of the heated Black soldier fly larvae protein(red) and Maillard reaction product (blue) both heated for 30 min at 50 °C.
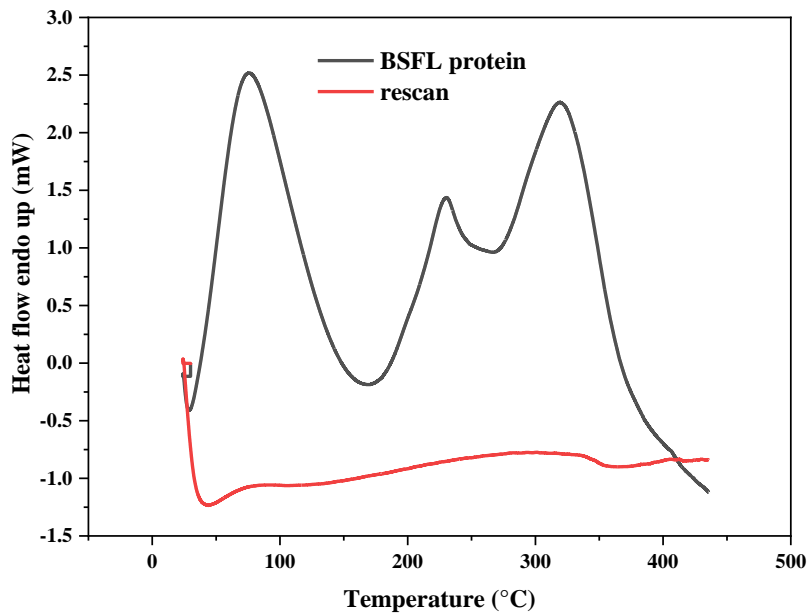
APPENDIX C

Figure C.1 Differential Scanning Calorimetry of Black soldier fly larvae protein and rescan of the non-heated BSFL protein.
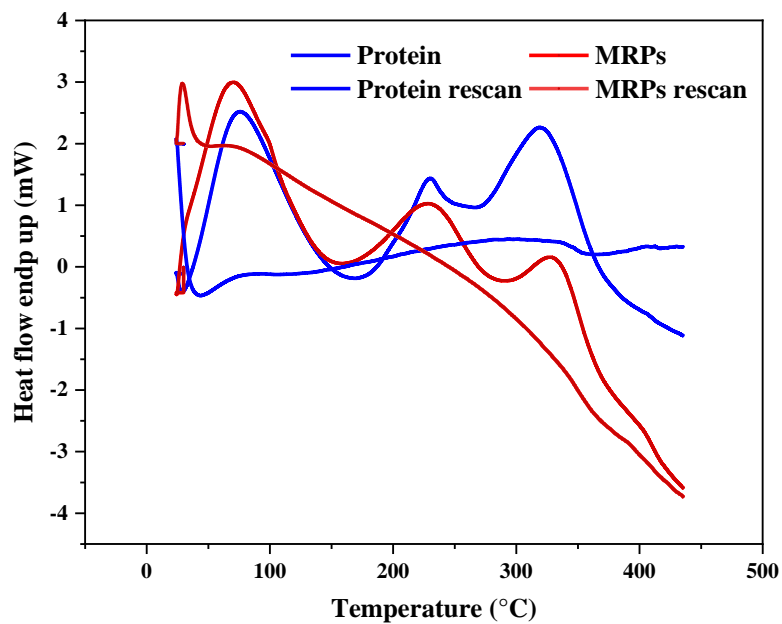

Figure C.2 Differential Scanning Calorimetry and rescans of the Black soldier fly larvae protein (red) and Maillard reaction product (blue) both heated for 30 min at 50 °C.
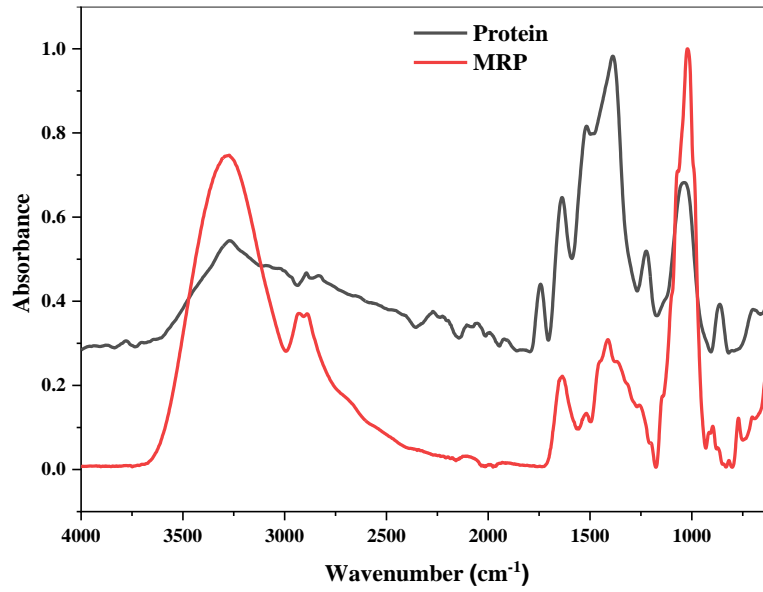
APPENDIX D

Figure D.1 Infrared spectrum of the Black soldier fly larvae protein (black) and Maillard reaction product (red) both heated for 30 min at 50 °C.
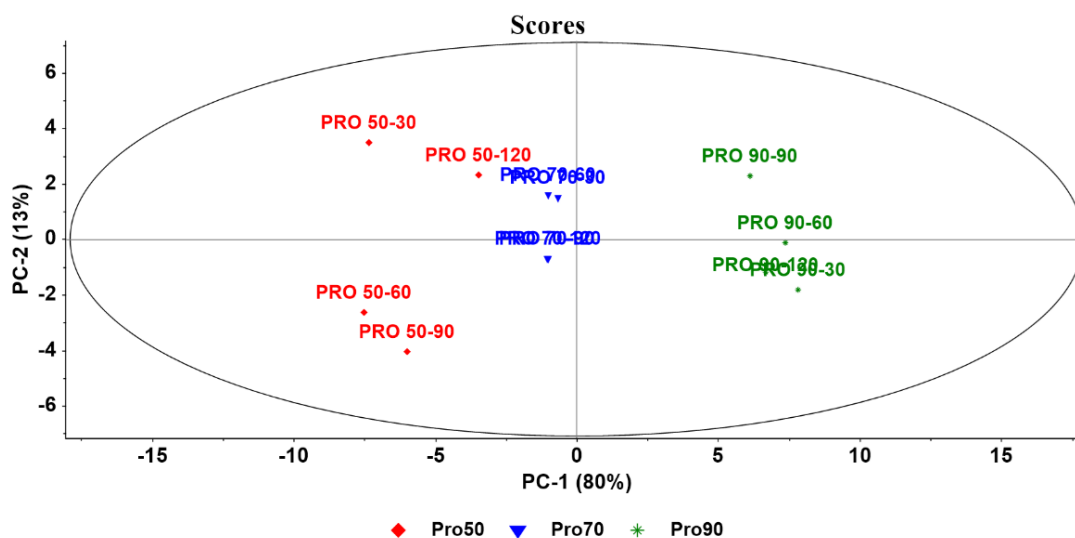
APPENDIX E

Figure E.1 Principal Component Analysis scores of the full Fourier-transform infrared spectrum. Principal Component 1 and Principal Component 2 are responsible for 80% and 13% respectively, of the heated proteins. Heated protein (Pro) at 50, 70, 90 °C.
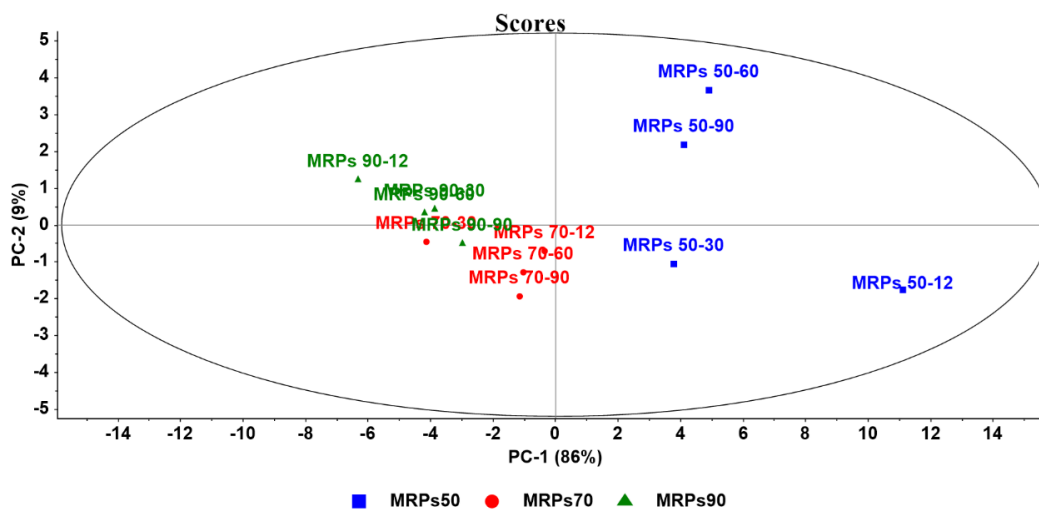

Figure E.2 Principal Component Analysis scores of the full Fourier-transform infrared spectrum. Principal Component 1 and Principal Component 2 are responsible for 86% and 9% respectively, of the Maillard reaction products. Maillard reaction products (MRPs) at 50, 70, 90 °C.

APPENDIX F

Table F.1 Measurements of the initial pH and after heat treatment for Black soldier fly larvae protein and Maillard reaction products.

Temperature (°C)	Time (minutes)	pH measurements	
		Black soldier fly larvae protein	Maillard reaction products
50	0	9.00	9.00
	30	8.81	8.61
	60	8.82	8.67
	90	8.66	8.55
	120	8.60	8.68
70	0	9.00	9.00
	30	8.71	8.66
	60	8.74	8.68
	90	8.57	8.58
	120	8.60	8.73
90	0	9.00	9.00
	30	8.72	8.67
	60	8.76	8.62
	90	8.56	8.64
	120	8.53	8.53