IN-VITRO PROTEIN DIGESTIBILITY AND TEXTURAL PROPERTIES OF HIGH MOISTURE EXTRUDED CRICKET-SOY MEAT ANALOGUES

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In vitro Protein Digestibility and Textural Properties of High Moisture Extruded Cricket-Soy Meat Analogues

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

To my adored parents Esther and Moses Kiiru and treasured brothers- Benson, Michael and Eric. Thank you for the support, prayers and believing in me. Be blessed abundantly!

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ABBREVIATIONS AND ACRONYMS

CF	Cricket Flour
FCF	Full-Fat Cricket Flour
LCF	Low-Fat Cricket Flour
SPI	Soy Protein Isolate
SPC	Soy Protein Concentrate
DSF	defatted soy flour
TI	Trypsin Inhibitor
CP	Crude Protein
IVPD	In vitro Protein Digestibility
DH	Degree of Hydrolysis
OPA	O-Phthaldialdehyde
DM	Dry Matter
CHOCDF	Calculated Carbohydrate by Difference
SSF	Simulated Salivary Fluid
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
TNBS	Trinitro-benzene-Sulfonic Acid
HMEC	High Moisture Extrusion Cooking
WFR	Water Flow Rate
RPM	Rotation Per Minute
TS	Temperature Section
DS	Die Section
W*H*L	Width*Height*Length
Kg/h	Kilogram per hour
ml/min	Millilitres per minute
AI	Anisotropic Index
N	Newton
SEM	Scanning Electron Microscopy
TIFF	Tagged Image File Format
°C	Degree Celsius
T	Temperature
AOAC	Association of Analytical Chemists
ANOVA	Analysis of Variance
FA	Fatty Acids
PUFA	Poly-unsaturated Fatty Acids
MUFA	Mono-unsaturated Fatty Acids
SFA	Saturated Fatty Acids
NDF	Neutral detergent fiber
UNCTAD	United Nations Conference on Trade and Development

FAO	Food and Agriculture Organisation
IFAD	International Fund for Agricultural Development
WFP	World Food Programme
EFSA	European Food Safety Authority
WHO	World Health Organisation
UNU	United Nations University
ADF-N	Acid detergent fiber nitrogen
FAOSTAT	Food and Agriculture Organisation Corporate Statistical Database
USDA	United States Department of Agriculture
USA	United States of America
UK	United Kingdom

ABSTRACT

The rapid increase in global population and the unsustainable conventional meat production have created demand for alternative animal-derived protein. Partial replacement of meat with soy protein-based meat analogues have been traditionally done through high moisture extrusion cooking and presently, edible insects such as house crickets which are a sustainable alternative source of proteins has been suggested. However, the composition and amount of insect content is a challenge to effective texturisation as well as protein digestibility of the meat analogues. This research was aimed at developing meat analogues from a mixture of soy protein isolate (SPI) substituted with full or low- fat cricket flour at 0, 15, 30 and 45%. A laboratory co-rotating twin-screw extruder with a throughput of 1 kg/h and 150 rpm screw speed was used while, the cooking temperature was varied at 120, 140 and 160 °C and water flow rate (WFR) set to 9 or 10 ml/min. The impact of cricket flour (CF) inclusion on in vitro protein digestibility (IVPD), firmness, parallel (L) and perpendicular (V) tensile stress and anisotropy index (AI) was evaluated. IVPD analysis was conducted on 15 and 45% CFs blends at 120 and 160°C while, texture profile analysis was analysed on all treatments. The results showed that, full and low-fat CFs addition had a significant ($P<0.05$) increase in IVPD ($r=0.93$ and 0.88) at 120°C and a decrease ($r=$ -0.36 and -0.49) at 160°C. The highest IVPD (50.21%) was obtained from 45% full-fat CF inclusion at 120°C. Textural properties significantly correlated and were affected ($P<0.01$) by all treatments. At WFR of 10 and 9ml/min, the samples firmness increased ($r= 0.56, 0.57$) with process temperatures but reduced ($r\approx -0.47, -0.72$) with CF inclusions, respectively. Correspondingly, defatting of CF negatively influenced firmness ($r= -0.62$ and -0.5921). Comparatively, all blends exhibited stronger tensile stress L than V at all treatments. At WFR of 10 and 9 ml/min, defatting CF, inclusion of full and low- fat reduced ($r= -0.50, -0.24, -0.54$) and ($r= -0.48, -0.47, -0.57$) tensile L, and ($r= -0.49, -0.16, -0.56$) and ($r= - 0.53, - 0.47, -0.55$) tensile V. On the same however, process temperature increased ($r= 0.69, 0.60$) tensile stress L and ($r= 0.65$, 0.57) tensile V. Increasing WFR reduced ($r= -0.17$) tensile stress L and (-0.20) tensile V. From SEM and AI analysis, CFs inclusion up to 30% CF and high water flow rate and process temperatures improved fibrousness. Inclusion of 30% low-fat CF and

extruded at 160°C and WFR of 10ml/min had most pronounceable anisotropic structure with highest AI of 2.8. These findings showed that, CF can be utilized to structure meat-like products with superior textural and protein digestibility thus a valuable ingredient for manufacturing animal-derived meat analogues.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Human population is a function of food availability; with the world's population projected to reach 9 billion by 2050, about 70% more food will be required to meet this demand (Aiking, 2011; Guoyao, Wu., Bazer, Fuller., Cross, & Russell, 2014). These factors have not only mount pressure to agricultural sector but it has also put mankind at risk of food insecurity and deficiencies such as protein energy malnutrition; which are one of the greatest human challenges in imminent decades (FAO, 2009a; FAO, IFAD, & WFP., 2015). For instance, there has been an increasing pressure on the livestock sector to meet the growing demand for high-value animal protein (Guoyao, Wu., Bazer, Fuller., Cross, W., Russell, 2014). In particular meat, which is an important component in many human diets across the world. However, meat production and consumption has become a major contributor to environmental pressure such as fresh water depletion, land use and global warming (Steinfeld et al., 2006). It has also been in focus on ethical and health concerns (Mayfield et al., 2007). These factors have promoted new research for more environmentally friendly sources to partially replace meat protein. Some of the promising sources to reduce the impact of meat production and consumption include plant proteins such as soy (Kumar et al., 2017) and the unconventional animal proteins such as edible insects (Pal & Roy, 2014).

With over 2000 edible insects, insects have been shown to be a highly valuable, tasty and nutritious food to over 2 billion people, particularly in Asia, Africa and South America (Jongema, 2015). Insect rearing and consumption has been accrued to their high feed to protein conversion rate (Megido et al., 2016), high concentrations of carbohydrates, fat and/or proteins (Raubenheimer, D., Rothman, 2013), dense mineral content, low environmental impact and low land use (Rumpold & Schlüter, 2013; Van Huis et al., 2013). In specific, house cricket (Acheta domesticus) is a valuable source of protein, up to 66.6%, which is significantly higher than the conventional protein

sources. It is also reported to have high amounts of unsaturated fatty acid and dietary fibre and dense vitamin and mineral content (Von Hackewitz, 2018).

On top its farming has been shown to be easy and economical and currently in Kenya it is promoted for large-scale rearing for human consumption through value added products (J.N. Kinyuru et al., 2015; John N Kinyuru, Mogendi, Riwa, & Ndungu, 2015). Nevertheless, the consumption of edible insects and products as so called 'Novel Foods', have been met with cultural, psychological, safety regulation and nutritional quality barriers (Pambo et al., 2016; EFSA, 2015). Research has shown that, presenting insects in invisibly forms by creating appealing products is a good strategy to vehicle insects as foods and to increase consumer acceptance (Goff, G., Delarue, 2016; John N. Kinyuru, Silvenus O. Konyole, Nanna Roos, Christine A. Onyango, Victor O. Owino, Bethwell O. Owuor, Benson B. Estambale, Henrik Friis, Jens Aagaard-Hansen, 2013). One of the promising way of incorporating insects as an acceptable food in human diet is through extruded protein products that mimic the texture and appearance of meat.

Traditionally, use of low moisture extrusion cooking (< 35%) has been employed to produce meat analogues from starches and proteins as extrusion feed. However, these extruded products have a spongy-like texture and need rehydration before consumption (Guy, 2001). Currently, the use of a twin extruder at high moisture conditions of above 40%, is one of the suitable technology and efficient process for production of meat analogues using starches and proteins as raw materials (Lin, Huff, & Hsieh, 2002; Osen & Schweiggert-Weisz, 2016). This process also allows transformation (via molecular and chemical reactions) of unconventional food sources into meat-like substitutes by combination of thermo-mechanical shear treatment. By varying the process conditions such as barrel temperature, moisture content, shear and pressure, the protein- water mixture is plasticized and textured in a cooling die into a desired anisotropic fibrous structure; which resembles muscle meat (Noguchi, 1990; Osen, Toelstede, Eisner, & Schweiggert-Weisz, 2015).

Some of the key features in development of high protein meat analogues from high moisture cooking (HMEC) is their fibrous structure and protein digestibility. Several

methods for investigating the aforementioned features have been used, however the common methods include textural profile analysis and in vitro protein analysis, respectively. In particular, determination of tensile force and deformation at rupture upon stretching of extrudates (parallel and perpendicular to extrusion flow direction) have been reported to relate to fibre strength/ tenderness (de Huidobro, Miguel, Blázquez, Onega, 2005) whereas compression force have been shown to relate little to fibre formation (Lin, Huff, & Hsieh, 2000b).

Use of standardized static in vitro in evaluation of protein digestion is one of the suitable methods in monitoring protein hydrolysis in the human gastro-intestinal physiological condition (Minekus et al., 2014). The textural properties and protein digestibility outcome of extrudates can be influenced by the composition of the ingredients and extrusion conditions. Process and system parameters inside the extruder and cooling die such as cooking temperature, screw speed and mass flow were reported to influence the textural properties such as fibre length, thickness and orientation of the meat analogues (Chen, Wei, & Zhang, 2011; Noguchi, 1989). On the other hand in vitro protein digestibility (IVPD) of the high moisture products was found to be significantly affected by process temperature, lipid content (Akdogan, 1999) and fibre content (Smetana et al., 2018). Ideally, changes in the HMEC conditions cause macromolecular transformations such as starch gelatinization, Maillard reactions, protein denaturation, plasticization and texturisation (Guy, 2001) in the proteinaceous matrix in the extruder barrel and cooling die, altering the rheological and chemical properties/ characteristics of the resulting extrudate (Chen, Zhang & Ojokoh., 2010). Consequently, the product characteristics help to monitor and compare changes in apparent changes in the HMEC conditions (material and process parameters) or experimental settings.

In this study, a twin-screw extruder was used to extrude different formulations of full and low-fat cricket flours and SPI under different temperatures settings and moisture contents. The aim was therefore to investigate high moisture extrusion of the different insect flours inclusion and extruder parameters with regard to the textural properties and in vitro protein digestibility of the cricket-soy protein meat analogues. For this purpose, various formulations were extruded in which the SPI powder was replaced at

0, 15, 30 and 45% with full or low-fat cricket flour at cooking temperature of 120, 140 and 160 °C and water flow rate (WFR) of 9 and 10 ml/min respectively.

1.2 Problem statement

The rapidly growing population and a privation of traditional protein sources has mounted a great deal of pressure on the livestock sector to meet the increasing demand for animal protein. This meat sector however, have proven to be highly unsustainable due to its disproportionate contribution to environmental impact in the food sector (Steinfeld et al., 2006).

Over the years, HMEC have focused on plant protein products from soybean- as known as 'poor man's meat'- in partial replacement of meat protein in processing of meat analogues. However, in recent years the cultivation of soy in large scale all over the world has subsequently been faced with serious social, economic and ecological problems such as scarcity for agricultural land and environmental pollutions (UNCTAD, 2016). On top, it is associated with presence allergenic factors and considerable high amounts of anti-nutritional factors (Friedman & Brandon, 2001). Amongst the widely used form of soy is the soy protein isolate; a very important functional ingredient, in development of meat analogues due to its superior quality and high amounts of proteins (Banaszkiewicz, 2011; Friedman & Brandon, 2001). It has been reported that, extrusion of pure SPI presents a homogenous structure that is very hard in texture and a have bland flavour (Cheftel et al.,1992). Compared to animal proteins, the soy protein isolate extrudates has been shown to have lower protein quality and digestibility (Sadler, 2004).

Acheta domesticus biomass- an animal derived protein- have been underutilized in spite of the multiple potential benefits. A few studies have mainly focused on use of A. domesticus and other insects as extruded feeds/ pellets. Findings showed that, some of the key variables during insect inclusion in the extrusion feed formulation is the lipid and chitin content (Ottoboni et al., 2018); high lipid content limits extrusion efficiency, desired texture and IVPD (Akdogan, 1999) whereas, high chitin/ fibre content reduce IVPD as well (Marono et al., 2015a). These components (22% lipids,19% chitin) are significantly high in house cricket (Rumpold & Schluter, 2013)

and thus modifications (defatting, blending among others) of raw material used as extrusion feed is required. These shortcomings, alongside the aforementioned extrusion conditions possess a challenge to the inclusion of house cricket in development of cricket-soy based meat analogues.

1.3 Justification

There is undoubtedly need to come up with sustainable production/ foods especially for the protein rich foods. Earlier work on HMEC of soy protein isolate and other plant based flour ingredients showed that it was possible to produce a meat analogues with better anisotropic structure (Cheftel et al., 1992; Lin et al., 2002). As an alternative to plant based, animal protein-from edible insects- is of special interest due to the nutritional characteristics and low potential of environmental impact. One promising edible insects is Acheta domesticus, it is among the most widely cultivated insect species and in many regions of the world- including East Africa where it is already part of human diet.

On top, the functionality of proteins from Acheta domesticus has been found to very much applicable in the food industry (Ndiritu, Kinyuru, Kenji, & Gichuhi, 2017). According to (Hoek, Elzerman, Hageman, Kok, & Luning, 2013; Hoek et al., 2011), there is a room for development of more acceptable insect based products, through scientific and technological approach, that would imitate the physical properties such as texture and appearance of meat in its full complexity (Smetana, Pernutz, Toepfl, Heinz, & Van Campenhout, 2018). However, only limited studies have been undertaken using edible insects for texturisation under HMEC, and to best of our knowledge no work has been published on HMEC with house crickets and, in detail, about full and low-fat flours. For instance, mixtures of SPC and insect biomass from lesser mealworm larvae (Alphitobius diaperinus) (Smetana et al., 2018) and yellow mealworm Tenebrio molitor (Smetana et al., 2018) have been found to be suitable in development of fibrous meat analogues. Findings from these studies highlighted that process conditions, soy-insect ratio and compositions (lipids, fibre/ chitin content among others) have an effect on the outcome of the texturized extrudates. Indeed, lipid content is a key variable during insect inclusion in extrusion (Ottoboni et al., 2018) as

well as moisture content (Lin, Huff & Hsieh, 2000; Lin et al., 2002). Therefore, modification of raw materials through defatting is bound to have an effect- concentration of other components such as proteins, sugars and fibres- that play a role/ affect extrusion behaviour and outcome of extrudates (Osen & Schweiggert-Weisz, 2016).

The objective of this study was to investigate the outcome- protein digestibility and textural properties- of HMEC of defatted and whole cricket flour blended with SPI at different process conditions. This could lead to improved understanding of ways to tailor cricket- soy based meat analogues. Nevertheless, the blending of plant and animal-based proteins has been suggested to serve as a means of improving protein quality in food products (Boland et al., 2013). On top, the inclusion of insect biomass could provide an opportunity to increase the utilisation of A. domesticus. One of the approach shown to improve willingness to consume edible insects is when they are transformed into known conventional food items (Balzan et al., 2016; Hartmann & Siegrist, 2017).

1.4 Objectives

1.4.1 General objective

To evaluate the influence of A. domesticus on the textural properties and protein digestibility of extruded soy protein isolate- based meat analogues

1.4.2 Specific objectives

1. To evaluate the composition of A. domesticus flours and soy blends and extrusion parameters suitable for effective extrusion and texturisation of meat analogues.
2. To determine the effect of A. domesticus flours on in vitro protein digestibility of the meat analogues.
3. To determine the effect of A. domesticus flours on firmness, tensile properties or anisotropy indices of the meat analogues.

4. To evaluate the effect of A. domesticus flours on the microstructure/ appearance of the meat analogues.

1.5 Hypothesis

NULL: Inclusion of A. domesticus flours and different extrusion conditions have no significant effect on the in vitro protein digestibility, firmness, tensile properties and microstructure of the cricket-soy based meat analogues.

CHAPTER TWO

LITERATURE REVIEW

2.1 Food Security and Entomophagy

Food security is one of the greatest challenge for mankind in the coming decades (FAO et al., 2015). Some of the pillars of food security include food safety, nutritious to meet dietary needs, food availability and access (physical and economic access) (FAO, 2006). When people can afford to buy more and change their consumption pattern towards the western standard, it is expected that an increase in demand for a higher share of animal protein, thus increasing scarcity (Alexandratos & Bruinsma, 2012). The meat production and consumption is known to contribute to numerous health diseases (Smil, 2002) and a significant share of greenhouse gases (GHG) emissions (Guoyao, Wu., Bazer, Fuller., Cross, & Russell, 2014). In light of food scarcity, climate change and health concerns, it is therefore imperative to seek alternatives to meat that are sustainable, healthy and nutritious (van Huis & Oonincx, 2017).

According to Jongema, (2015) more than 2000 species of insects are considered safe for consumption as food in a practice known as entomophagy or human-insect eating habits. Globally, about 2 billion of people- approximately 30% of the global population- readily taking them as food (Gjerris, Gamborg, & Röcklinsberg, 2016; Van Huis, 2013). Commonly consumed insect Orders include Ortboptera (locusts and grasshoppers), Lepidoptera (moths and butterflies), Hemiptera (true bugs), Coleoptera (beetles), Hymenoptera (bees and ants) and lsoptera (termites) (Durst & Johnson, 2010). Much interest on edible insects as food, gradually rose when Meyer-rochow (1975) suggested that they could play a key role in future global food security. This was primarily anchored on fact that, there is need for sustainable food source to meet the future demands (Van Huis et al., 2013). In addition, edible insects are ‘perfect foods’ for alleviation of undernutrition particularly, in developing countries (Nadeau, Nadeau, Franklin, & Dunkel, 2015). Insects have a higher feed conversion ratio, require a low space for production than livestock and in addition to their high fecundity they can be multivoltine (Rumpold & Schlüter, 2013). They can also be reared on bio-waste streams and have a low environmental footprint over their whole life cycle. In

addition, edible insects have been reported to emit fewer greenhouse gases and low risk of spreading zoonotic infections to human (Makkar, Tran, Heuzé, & Ankers, 2014). Altogether, these shows a great potential of edible insects in reducing cases of malnutrition particularly in the developing and/ or rural regions (Kinyuru et al., 2013; Obopile & Seeletso, 2013).

Edible insects are rich in essential nutrients however, the nutritional values vary with insect species, season, distinct life stages (Van Huis et al., 2013) and cooking preparation (Kinyuru, Kenji & Njoroge, 2009). Nutritional profile study on five edible insect species used as food and feed in Korea: Allomyrina dichotoma (Rhinoceros beetle), Protaetia brevitarsis (White spotted flower chafer),, Tenebrio molitor (Mealworm), Teleogryllus emma (Emma field cricket) and Gryllus bimaculatus (Black cricket), were reported to have high protein content of 44.2 to 58.3% , fat of 11.9 to 34.5 %, a total of 17 amino acids, 8 essential, 1 conditional essential and 8 non-essential, and 26 fatty acids which include 12 saturated fatty acids, 6 mono saturated fatty acids (MUFA) and 8 poly unsaturated fatty acids (PUFA) (Ghosh, Jung, & Meyer-Rochow, 2016). In comparison to conventional animal sources, the quality of the protein- amino acid composition- the edible insects had comparable the essential amino acid content (Ghosh et al., 2016). Mineral content - iron and zinc contents-in of edible insects have also been reported to be higher than that of animal meats and chicken eggs with phosphorus being the most abundant (Chakravorty et al., 2016). In terms of affordability, edible insects have been shown to be more affordable compared to animal proteins.

Despite these benefits, consumer acceptance is a barrier to adoption of insects as foodstuff or protein source but willingness to consume seemed to improve after making insects invisible in the foodstuffs such as processed insect proteins as ingredient (Schösler, Boer, & Boersema, 2012). Nevertheless, the use of insects as substitute in animal meat industry should alleviate the excessive burden on livestock as conventional source and sequentially the adverse environmental effects.

2.1.1 House cricket (Acheta domesticus)

House cricket is classified under order Orthoptera, suborders Ensifera (Legendre et al., 2010). It originated from Southwestern Asia. Its lifecycle takes 2-3 months to be complete when grown within temperature conditions of 26-32 ^{o}C. An adult cricket ranges within 16-21mm long, a body colour of yellowish brown and wings covering the abdomen (Walker, 2014). The domestication of cricket has been done for feeds or food, owing to their superior nutritional composition and feed conversion efficiency compared to the conventional livestock (Van Huis et al., 2013). However, there is a need for further processing of these insects to more acceptable and invisible forms (Yi et al., 2013). Currently in Kenya, A.domesticus, is now being promoted for large-scale rearing for human consumption and is utilised in value added products such as fortified foods (Tenagashaw et al., 2017).

2.1.1.1 Nutritional value of house cricket

The species of Orthoptera, including the A. domesticus have high amounts of proteins and are globally represented as an alternative protein source (Bu, Rumpold, Jander, & Rawel, 2016; Ndiritu et al., 2017) Table 2.1. Its protein content was reported to be 66.6 percent and met all the recommended essential amino acids levels as per (FAO/WHO/UNU, 2001). Moreover, proteins from A. domesticus have been reported to be of higher quality to that of soy proteins in terms of maximum nitrogen retention and weight gain (Finke, DeFoliart, & Benevenga, 1989). Additionally, according to Ghosh, Lee, Jung, & Meyer-Rochow, (2017), crickets showed higher amounts of zinc and magnesium and possessed fats of lower SFAs but more PUFAs (with the exception of A. dichotoma).

Table 2.1: Proximate nutrient composition of Acheta domesticus species

% Dry Matter						
Stages of growth	Water	Crude fat	Total nitrogen	ADF-N	NDF	Ash
Adult	73.2 ± 1.9	22.8 ± 1.5	10.3 ± 0.4	0.7 ± 0.1	19.1 ± 2.5	5.1 ± 1.4
Juvenile	66.8 ± 9.8	9.8 ± 1.4	8.8 ± 0.5	0.6 ± 0.1	16.4 ± 5.6	9.1 ± 6.7

Values are means ± standard deviation; ADF-N= Acid Detergent Fiber Nitrogen; NDF=Neutral Detergent Fiber.

Source: Barker, Fitzpatrick, and Dierenfeld, (1998)

2.2 Soy and forms

2.2.1 Soybean (Glycine max)

Soybean is a leguminous crop belonging to the family leguminoceae. It originated in china and has been cultivated in China and other Asian countries for more than 5000 years (Allaire & Taylor, 2007). According to (FAOSTAT, 2014), it has been ranked the seventh most important food product after milk, rice, different types of meat, and wheat. The current producer is USA with 7 x 10^7 tons; Brazil, 5.8 x 10^7 tons; Argentine, 5.8 x 10^7 tons; India, 1.0 x 10^7 tons (Kitamura, 2010). Today, the leading cultivation areas include, the United States, Brazil and Argentina (USDA, 2016). Amongst the crop plants, soybean is the most important for the production of grain and it contains 40- 50% protein and 20% oil as shown in Table 2.2.

Table 2.2: Composition of soybean grain (SBG), defatted soy flour (DSF), soy protein concentrate (SPC) and soy protein isolate (SPI)

Component	SBG (%)	DSF (%)	SPC (%)	SPI (%)
Protein	35	45.5	58.1	80.7
Crude fibre	3	5.8	3.8	0.3
Water	13	6.9	5.8	5.0
Fat	17	2.4	0.5	3.4
Carbohydrates	31	40.1	31.2	7.4

Source: Perkins, (1995) and Liu, (1997)

This is higher than other crop plants in the world (Lokuruka, 2010). Furthermore, soybean also contains some significantly high amounts of phospholipids, isoflavones, edible fiber and oligosaccharides as well as some high levels of anti-nutrients including saponins, trypsin inhibitors, lectins and goitrogens (Burssens et al., 2011). However, heat processing of soybean has been one of the key steps in soybean consumption due to its effectiveness in deactivation of trypsin inhibitor, hemagglutinin and other harmful substances. Heating also modifies the soybean protein by softening the tissues of the bean and importantly, is lessens the beany flavour that is inherent in raw soybeans (Nishinari, Fang, Guo, & Phillips, 2014).

2.2.2 Soy protein concentrates (SPC)

Soy protein concentrate is one of many products obtained during the processing of mature soybeans. It is prepared from high quality, dehulled soybean seeds by removal of most part of water soluble non-protein constituents such as soluble sugars from the defatted flour (US5097017A, 1992). The final product is usually in form of granules or spray dried. It is lower in the levels of glycinin, B-conglycinin, saponins, lectins, trypsin inhibitors and oligosaccharides that are considered to be anti-nutritional factors than raw soybean. In addition, the SPC is required to have at least 55% protein on a moisture-free basis as shown in Table 2.2. Usually, most of SPC is used to produce textured SPC through extrusion technology. This extruded, texturized SPC is very common and suitable product for aquaculture feeds.

2.2.3 Soy protein isolate (SPI)

This is the most refined form of soy protein with over 80% protein content, obtained from dehulled soybeans by solubilizing and separating proteins out of the soy flakes and finally precipitating in the isoelectric point at pH 4 to 5 (Jiang, Jie, & Xiong, 2009). SPI have nearly all of the fat, fiber and soluble carbohydrates removed. It is characterized by significantly reduced levels of anti-nutritional factors due to the extraction process. Thus, it is of high nutritive value as shown in Table 2.2. Generally, SPI is a superior ingredient and is commonly used as a component of various meat or meatless products (Kumar et al., 2017).

2.3 Soy protein

Soy protein refers to the protein found in soy beans, it is characterized by a well balance of amino acids- contains all the essential amino acid. Thus, it is used to replace animal proteins in diets especially in vegetarian and vegan foods. Moreover, soy protein has been shown to be physiologically beneficial in mitigating certain cholesterol related diseases such as cardiovascular and of hyperlipidemia (Nishinari et al., 2014). In addition, soy proteins have excellent functional properties such as emulsifying, gelling ability and water- and oil- holding capacity (Jiang et al., 2009).

2.3.1 Soy protein digestibility

The most significant chemical constituent to oy consumption is the storage proteins trypsin inhibitors (TI) and lectins as well as β-conglycinin and glycinin (Campos-Vega, Loarca-Piña, & Oomah, 2010). The most important TI are the Kunitz trypsin inhibitor and the Bowman-Birk inhibitor when it comes to human consumption (Dipietro & Liener, 1989). These proteins are responsible for the decreased protein digestibility. They are also associated with causing pancreatic hypertrophy mainly after consumption of raw or inadequately cooked beans (Campos-Vega et al., 2010). Heat treatment has been shown to inactivate these proteins; but, a 100% inactivation of trypsin inhibitor may cause overheating, which damages soy proteins by destroying lysine, tryptophan, and cysteine, as reported in soy milk (Yuan, Chang, Liu, & Xu, 2008).

2.4 Meat and meat production

Meat is the edible carcass (include flesh and organs) of an animal or fowl that has been reared and slaughtered. The flesh of cattle, sheep, goat and pigs is referred as red meat while the flesh from poultry is termed as white meat (Bender, 1992). Meat is an important part of diet due to its high protein content, minerals (such as Fe, Zn, Cu, Se), vitamins (vitamin B_6, B_{12}, B_1 and A, niacin, pantothenic acid, riboflavin) and essential fatty acids (FA) (Williams, 2007). Meat is the single largest source of animal proteins worldwide, and is regarded as a high status in most countries (Mann, 2000). Meat is highly desirable due to its unique set of properties/ qualities: color, appearance, flavor, juiciness/ water holding, odor and texture. In particular, meat texture is dictated by three categories of meat proteins namely stromal (connective tissue such as collagen, elastin, reticulin), sarcoplasmic and myofibrillar. The latter- myofibrillar (majorly myosin and actin)- constitute up to 50-60% of total proteins, and is largely responsible for structural and textural properties of meat (Nair & Zhai, 2019).

With the rising population and with the increased in incomes, the global production of meat will go from 580 to 1043 million tons (Steinfeld et al., 2006). The livestock production is being faced with issues of animal welfare, human health and ecological sustainability with respect to its carbon and water footprint (Mancini & Antonioli, 2018; Steinfeld et al., 2006). In particular, bovine meat production greatly impact on environment than other meat sources. Its food production efficiency- converting vegetable protein and water as well as other limited natural resources such as land, energy and labor into meat is very low (Raney et al., 2009; Steinfeld et al., 2006). In the common agricultural systems, livestock production is shown to be responsible for the emission of the main greenhouse gases: about 9%, carbon dioxide, 39% methane and 65% nitrous oxide. In addition it requires about 40% of total grains and 75% of total soya for animal feeding and on top, a 70% of the total arable land is required for rearing (Aiking, 2011). Thus, these activities have led to a huge impact on ecological and socioeconomic issues such as deforestation, food security, pollution and have created need for sustainable solutions (Aiking, Boer, & Vereijken, 2006). Due to this challenges, there is growing need for the development of meat analogues to meet the

market demand and efforts to promote environment-friendly production with little adverse effects to the environment (Tijhuis et al., 2012).

2.5 Meat analogues

Meat analogues or alternatives are protein-rich foods made from non-animal protein and their aesthetic qualities and/ chemical characteristics are similar to meat (Sadler, 2004). According to the US legislation, they can also be termed as 'imitations' if they have low nutritional value or termed as a substitute if their nutritional value is equal to that of meat. Suitable alternatives should be produced in a more cheap and sustainable way than meat, and should be developed in a way that simulate the meat like products in terms of color, flavor, texture and nutritive value including better digestibility (Kumar et al., 2017). In fact, most of meat analogues are based on plant materials such as soy, pulses, cereals and vegetables followed by the animal proteins including milk, insects and lastly the mycoproteins (Smetana, Mathys, Knoch, & Heinz, 2015).

These foods are cheaper to the processing industry and most have a longer shelf life and easier storage (Smetana, et al., 2015). Indeed, the growing interest in meat analogues has been attributed to the health and nutritional benefits, sustainability concerns, cultural and religious beliefs and animal rights concerns (Aiking, 2011; Smetana et al., 2015). Examples of technologies used to make these meat analogues include use of extrusion shear and heat (Noguchi, 1989). Soybean in form of soy protein isolate and concentrates have been the widely utilized vegetable protein to make such products that resemble the meat tissues such as textured products, sausages and frankfurters (Dahl & Villota, 1991).

2.6 Extrusion and type of extruders

The extrusion process is a thermo-mechanical processing operation. It involves mixing of feeds, application of shear and use of rotating screws to convey, heat and as shown in Figure 2.1 (Alvarez-Martinez, Kondury, & Harper, 1988). In food extrusion, the feed is subjected to controlled high temperature, pressure and shear which ultimately lead to a variety of physiochemical changes that change the properties of the extrudates (Alvarez-Martinez et al., 1988; Cheftel et al., 1992). The extrusion processing is very

versatile- capable of utilizing dry or wet, liquid or solids feeds and produces a wide variety of product types- as well as retain most of nutrients (Guy, 2001). Furthermore, the combination of unit operations in a simultaneous and continuous way, makes it efficient in labor, floor space, energy saving whilst increasing the productivity (Guy, 2001; Moscicki, L., & van Zuilichem, 2011).

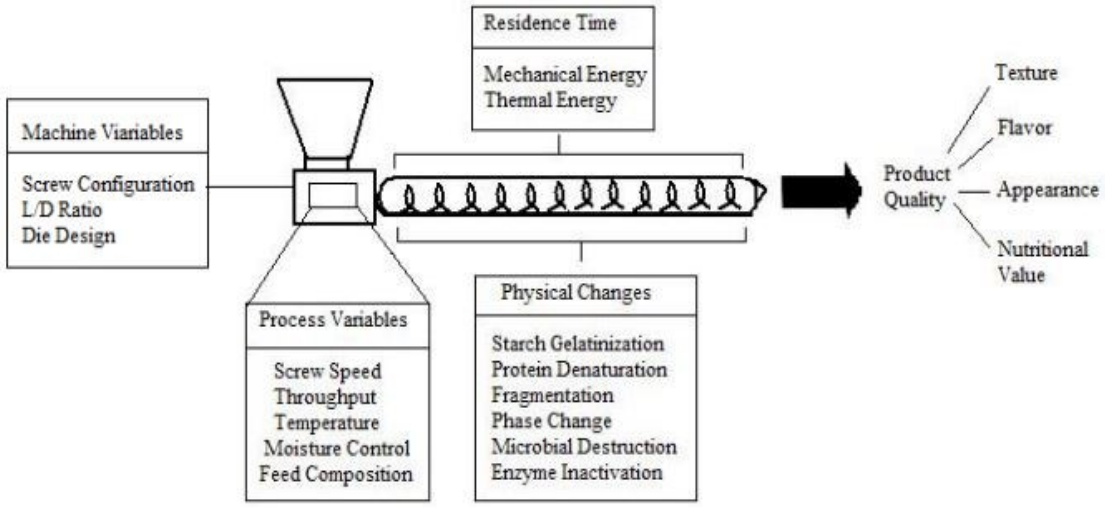


Figure 2.1: Schematic of extrusion processing.

Source: Choudhury and Gogoi, (1996)

In the food industry, single and the twin-screw extruders are commonly used. The single- screw extruders were first used in the 1940s to make puffed snacks from cereal flour and grits, then in 1950s it was further developed to manufacture dry expanded 'ready to eat foods- pet foods and textured vegetable proteins (Akdogan, 1999; Guy, 2001). Due to use of a single screw, this extruder has a relatively poor mixing ability, it is preferable to use premixes and pre-conditioned materials as feed materials. The operation of the single-screw extrusion mainly depends on several circumstances such as the pressure requirements of the die and the degree the screw is filled, this can be altered by feed rate, screw speed, melt characteristics and viscosity. In addition, the slip of the barrel wall, which is controlled by the barrel wall pressure or wall groove or both. This combination of variables make this type of extrusion limited to the range and its flexibility. (Dobraszczyk, Ainsworth, Ibanoglu, & Bouchon, 2006).

The twin-screw extruders Figure 2.2, are extruders with two screws of equal length and run together in same barrel (Guy, 2001). They were developed in the late 1970s and are now extensively and widely applied in food processing due to their better process control, versatility and flexible design that can accommodate complex formulations.

Figure 2.2: Typical twin-screw extruders

Source: Guy, (2001)

They are categorized according to the arrangement of the twin screws, as either intermeshing (in which the screw threads engage one another) or non-intermeshing (where screws threads do not engage one another), thus allowing one screw to turn just as in the case of single-screw extruders (Guy, 2001).

The twin-screw extruders can also have co-rotating or counter-rotating screws Figure 2.3. However, these types differ in their transport characteristics and thus differing in their technological applications. In counter-rotating extruders, the screws rotate in the opposed direction whereas, in the co-rotating, they rotate in the same direction. But, these type of screws can further differentiated in the position of the screw with respect to each other (Vlachopoulos & Strutt, 2003) as illustrated in Figure 2.3. According to Guy, (2001), the twin screw extruder has superior material transportation capabilities, product is transferred in bulk from one screw to the other, compared to the single screw extruders, thus the diverse applications.

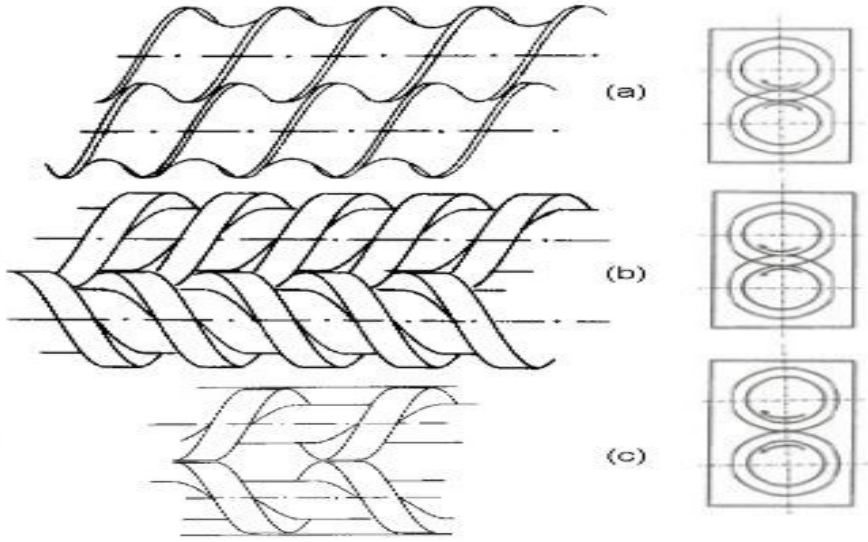


Figure 2.3: Types of twin-screw extruders; (a) Intermeshing co-rotating; (b) Intermeshing counter-rotating; (c) Non- intermeshing counter-rotating.

Source: Vlachopoulos & Strutt, (2003)

2.6.1 Extrusion of meat analogues

Extrusion cooking is used to produce meat analogues using common protein rich and starchy raw materials. Usually, meat analogues are produced by a low moisture (<35%) extrusion and are mostly used as meat extenders or ground meat substitutes (Cheftel et al., 1992; Guy, 2001). However, these products do not mimic the appearance and texture of fibrous whole-muscle meat, have a spongy texture and have to be rehydrated for them to be consumed (Guy, 2001). Currently, use of high moisture extrusion cooking (HMEC) is the most suitable technology for obtaining fibrous meat-like structures from protein rich materials (Osen & Schweiggert-Weisz, 2016) which is also known as 'wet extrusion' (Guy, 2001).

2.6.1.1 High moisture extrusion cooking (HMEC)

High moisture extrusion cooking is characterized with a moisture content of between 40- 70%; thus it is also referred to as 'wet extrusion' (Noguchi, 1989). When combined with twin screw extruder and a long cooling die it is very effective in tailoring of meat analogues. It is also very versatile and allows manipulation of feed material and

process conditions such as varying moisture, temperature, pressure and shear materials (Osen & Schweiggert-Weisz, 2016). During high moisture extrusion, the feed and water are introduced separately to the extruder barrel and 'cooked' to temperatures between 130°C to 170 °C, and it is at this cooking zone that the texturisation mechanism sets in (Guy, 2001).

2.6.1.1.1 Texturisation process

The process of structure creation occurs under set conditions: protein concentration is above 40%, water levels of 35-40%, temperatures of above 150 °C and under high shear and pressure (Fellows, 2009). During this process, protein solubility and cross-linking reactions occur as well as formation of some new covalent bonds. The cause of protein mass to plasticize is postulated to the weakening of protein-protein interactions under high shear from the screws and the pressure as the hot mass is conveyed towards the cooling die. (Chen et al., 2011). A cooling die is a vital component in the extruder apparatus; its function is to prevent expansion due to increased viscosity by dissipating energy from the food matrix to below 100 °C. In addition, solidification of the viscous mass occurs here at the cooling die, this facilitates the formation of the fibrous structure. Texturisation occurs between the molecules as they flow streamlined to each other by some form of laminar cross bonding (Lin, Huff, & Hsieh, 2000a; Lin et al., 2002). In addition, the cooling die allows protein mass to develop longitudinally, similar to the layered meat texture characteristics (Osen & Schweiggert-Weisz, 2016).

2.6.2.1.2 Factors influencing extrusion texturisation

Fibrous structure or texture is one of the key characteristics of meat analogues. This is controlled by several factors, among them being the process conditions and the material properties seen in Figure 2.4. These factors indirectly affect the extrudates properties; they influence the system parameters which includes residence time, mass temperature, viscosity, and shear and pressure the extruder response inside the barrel. Therefore, proper control of this process variables will ensure that the die temperature, die pressure, flow rate through the die and screw mechanical energy will be maintained at desired values hence a consistent product properties (Wolz & Kulozik, 2017). Thus,

maintaining a good degree of texturisation and fibrous structure of meat analogues will need proper control of the variables.

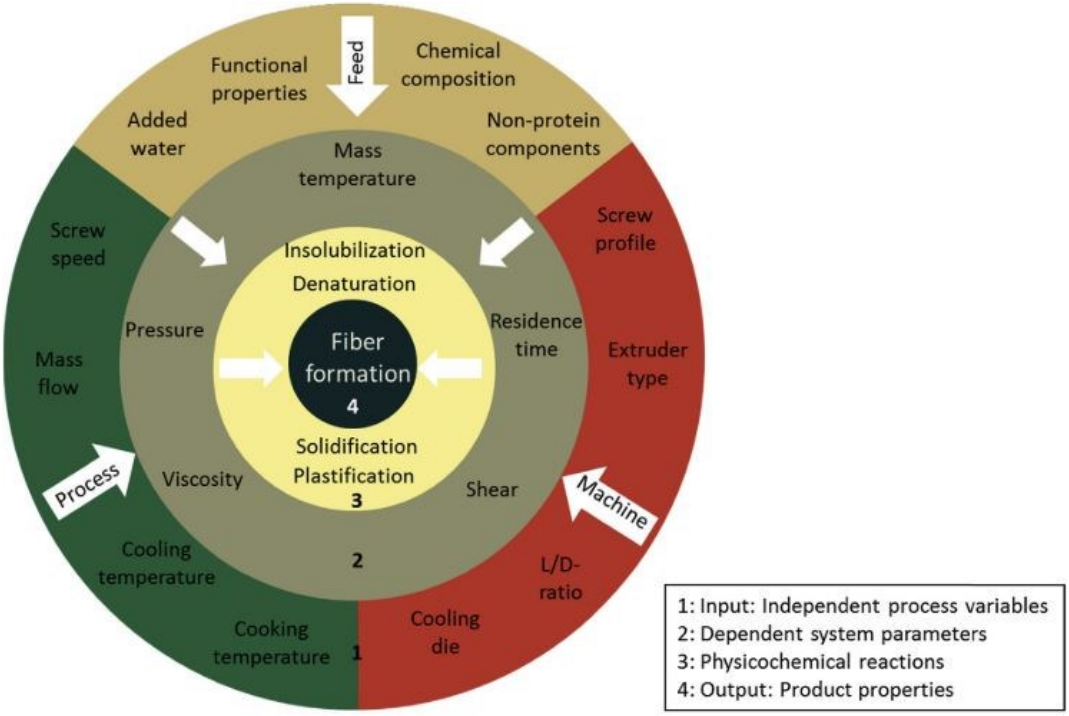


Figure 2.4: Input variables and dependent parameters during high-moisture extrusion cooking.

Source: Osen and Schweiggert-Weisz, 2016)

a. Process parameters

(i) Barrel temperatures

This is the most important parameter in texturisation; it affects the products temperature which in turn alters the degree of cook and melt viscosity (Moscicki, L., & van Zuilichem, 2011). This melt temperature is critical to protein cross-linking reactions. At temperatures above 90 °C, the expansion and layer formation is hindered whereas at 140-180 °C , a significant decrease in disulfide bonds occurs (Cheftel et al.,1992). The barrel temperatures have been reported to affect the tensile strength - both parallel (L) and perpendicular (V) to die directions- of extrudates, with parallel

tensile strengths being affected more (Akdogan, 1999). Usually, a greater ratio of L to V suggests presence of an aligned structure (Krintiras, Göbel, Van Der Goot, & Stefanidis, 2015).

(ii) Screw speed

According to Thiebaud, Dumay, & Cheftel, (1996), screw speed affect the texturisation by varying the mean residence time in the barrel, the amount of shear and friction generated as well as the degree of barrel fill.

(iii) Water injection/ flow rate

The flow rate of injected water determines the barrel moisture content which in turn affects the friction and thus the melt viscosity. Moisture content have been reported to cause a lubrication effect, which reduces the shear and friction in the screw section. This is similar to effects produced by the presence of lipids as well (Fellows, 2009; Guy, 2001).

(iv) Feed rate

This extrusion parameter affect the extrusion outcome similarly to the screw speed. They control the frictional energy, residence time and barrel fill. It also controls degree of texturisation by determining the linear speed of the food mix section (Guy, 2001).

b. Material parameters

(i) **Water/ Moisture content**

In HMEC, water can be added directly to the feed or injected from a pump into the barrel or added as steam. However, in food extrusion, the moisture content of the feed is regulated by flow rate and the temperature. Water is responsible for the product density, rehydration and solvation of starch (gelatinization) and protein polymers (Akdogan, 1999). At water levels of above 10%, the polymers begin to move and interact with each other and start to acquire a glassy physical state that progresses to a viscous one. But, at low water level the screw shearing tend to expend a large amount

of energy and causes the mass to heat up, and in turn more polymer degradation follows. The reverse happens as the water level increases, the viscosity falls and fluidity of the mass increases giving a low mechanical energy. (Fellows, 2009; Guy, 2001; Moscicki, L., & van Zuilichem, 2011).

(ii) Proteins

Proteins are formed from chains of amino acids which form structures of different physical sizes and forms. In an extrusion mix, different types of proteins form dispersed phases- the water soluble will get denatured and coagulated into a soft mass while the globular proteins will hydrate and form larger structures of viscoelastic dough by agglomeration with water (Guy, 2001). These high viscous complexes form crude films and retain some of the expanding vapor. Some type of proteins such as the prolamin and glutenin, forms viscoelastic dough that is macerated to small particles, while the meat proteins form resistant particles under shear forces of extruder and retain the sizes (Fellows, 2009; Guy, 2001).

(iii) Carbohydrates/ Sugars

Starch granules are easily gelatinized and dispersed upon extrusion resulting in the formation of a continuous phase of the melt. In addition, the average molecular weight is decreased which allows optimum air cell formation and stability at the die exit. It is also reported that, the amylose and amylopectin give the best expansion characteristics (Bemiller & Lafayette, 1997; Huber & Bemiller, 2001). In fact pure starch fluids are very elastic and they deform once they are in the die while still storing their elastic energy in their molecular structures, this energy is later released as it leaves the die causing a swelling effect normal to the direction of flow (Guy, 2001).

Sugars are commonly used in extruded breakfast cereals. They contribute to binding, flavor and browning/ Maillard's reaction characteristics which are key to texture and overall mouthfeel (Kokkinidou, Peterson, Bloch, & Bronston, 2018). In addition, more significant effects of sugar are attributed to competition of moisture, inhibition of gelatinization and plasticization of starch –based systems. Other key effects are the amylose-lipid complexing has also been noted (Dobraszczyk et al., 2006).

(iv) **Lipids**

Lipids influence the quality of the product (textural properties, strength, size and weight) and this is primarily attributed to the complexing ability of lipids with other biopolymers such as protein, sugars, starch, fibers or other lipids (Ilo, Schoenlechner, & Berghofe, 2000; Riaz, 2000). During extrusion, the added heat, water and biopolymers can react by cleavage, binding, recombination or losing the native form or degrading to form unreactive complexes. Therefore, most processors will aim at defatting materials to prevent these deleterious effects (Riaz, 2000).

During extrusion, lipids can create a lubrication effect when starch, fiber and proteins exist, by melting at 40 °C, which decreases shear/ mechanical energy and the melt /cooking phase which forms the final properties of extrudates (Ilo et al., 2000). At levels of 2-3%, oils and fats produce large effects in processing of starch, but higher levels may reduce the breakdown of starch polymer in that no expansion is obtained (Guy, 2001; G. Zhang & Hamaker, 1998). Notably, most of these lipid related reactions are temperature controlled. In essence, most lipid reactions and interactions include lipid oxidation (Bjorck & Asps, 1983) and lipid-derived aldehydes that create substituted pyrazines that are primary to the Maillard reaction (Bruechert & Huang, 1988).

(v) **Fibers**

Apart from their nutritive importance in the diet, fiber materials such as bran can be part of the dispersed phase of extrudates which is in the continuous phase (Guy, 2001). Interestingly, fiber is chemically unchanged by extrusion process, but it influences the expansion of the products. This is because the fragments in the fibers disrupts the air cell walls thus reducing their formation and the swelling consequently altering the air cell size (Huber & Bemiller, 2001).

2.6.2 Quality analysis of meat analogues

In order to describe the quality features of meat analogues, several analytical methods are applied. Actually, most of these methods focus on the textural characteristics,

structure/ microstructure features of the fibrous meat analogues as well as the in vitro protein digestibility. The most common are based on microscopy (Lin et al., 2002; Ranasinghesagara, Hsieh, & Yao, 2005) and textural profile analysis (Lin et al., 2000a; Nishinari et al., 2014). It is worth to note that, these quantification methods cannot replace the sensory evaluation since they do not give enough data that give a collective information of all texture attributes (Osen & Schweiggert-Weisz, 2016).

2.6.2.1 Textural profile analysis

Food texture have been a major factor in sensory evaluation of food quality, during food grading and marketing of food as well (Lu, 2013). Evidently, there is a close relationship between the texture and structure or microstructure (Aussanasuwannakul, Slider, Salem, Yao, & Kenney, 2012). Bourne (2002), stated that, the textural properties of a food are that group of physical characteristics that arise from the structural element of the food. These elements are sensed primarily by the touch, are related to the deformation, fragmentation, and flow of the food under a force, and are measured quantitatively by functions of mass, time, and distance. Mainly, the objective of measurement of the textural properties of food is to ensure food standards are met. Usually, the force/deformation methods are widely used to measure the mechanical properties (force or damage) since they mimic the sensory evaluation by humans in the hand or the mouth (Lu, 2013).

These measurement methods/tests are classified into fundamental, empirical and or imitative (Bourne, 2002). The fundamental methods measure well-defined mechanical properties, usually done to understand the theory and practice of materials of construction and may not be very useful in measuring the actual sense in mouth during mastication (Lu, 2013). Meanwhile, the empirical methods measure those mechanical properties that are not directly defined but correlate with sensory evaluation of food. These tests are easy to perform, rapid and use relatively inexpensive equipment, and they are widely used in food industry (Lu, 2013).

The imitative tests imitate the conditions to which the food material is under and a good example is the use of Farinograph in dough testing to imitate the handling and working of bread dough (Bourne, 2002). There are two basic approaches for

force/deformation of food texture: destructive and non-destructive. The former measurement relate closely to sensory evaluation but cannot be used in sorting products due to their destructive nature (Bourne, 2002; Lu, 2013). Force measuring instruments are the most common in texture measurement. Ideally, force is a product of mass x length x time^{-2}, and the standard unit is (N). These tests include tensile test, puncture test, cutting/shear, compression and compression-extrusion among others (Lu, 2013).

2.6.2.1.1 Puncture test

These test measures the maximum force required to push a multiple/ single probe into a food sample up to a pre-specified depth. This test involves both compression and shearing, and as an empirical test it also imitates the biting of the food in mouth (Bourne, 2002). The probe can be moved a food on a constant speed as seen in testing machines such as Texture Analyzer model TA.XT2. During measurement, the probe draws out and touches the sample surface causing a deformation under the load but with no puncturing yet. Then immediately the punch begins at yield point, a sudden slope is seen (Lu, 2013). This yield point marks the moment the penetration begins and it is the point of great interest. Thereafter, the direction of force changes after yield point and the force can continue to increase/remain constant or decrease (Bourne, 2002).

The following empirical equation was proposed by (Bourne, 1966) and it relates the puncture yield force to the sample area and perimeter of the probe:

$$Fs = KcA + KsP + C \quad \text{.....Eq. 1}$$

where Fs is the force in N acting on the probe; Kc is the compression coefficient in N/mm^2 and Ks is the shear coefficient in N/mm; A and P are the cross-sectional area (mm^2) and the perimeter (mm) of the probe, respectively; and C is a constant in N.

2.6.2.1.2 Tensile test

Tensile tests involve measurement of the force required in stretching a food specimen or an intact food sample apart. They explain the mechanical properties and structural failure characteristics of a food (Lu, 2013). A common application of tensile test is in the understanding the structural changes in muscle foods during processing (Lu, 2013) and fracture processed foods (Katagiri, Masuda, Tani, & Kitabatake, 2011). A recent study conducted by Krintiras et al. (2015), demonstrated the tensile stress and strain anisotropy indices on structured meat analogues made from soy protein isolate and wheat gluten dispersion by a Couette Cell device. The study used the Zwick Roell Z005 universal testing machine (Zwick Roell AG., Ulm, Germany) to determine the different mechanical properties parallel and perpendicular to the direction of the fiber, the results were correlated to the fibrousness. Basically depending on the treatment, samples developed fibrous structures of different mechanical properties parallel and perpendicular to the direction of the fibers. The parallel direction is that of outflow from the extruder, while the direction perpendicular to the fibers is that along the height of the cooling die.

Thereafter, a tensile anisotropy index (AI) on both directions; parallel to perpendicular is calculated. The anisotropy index indicates material anisotropic structures and degree of fibrousness. Moreover, AI can quantify the textural and sensorial characteristics of the meat substitutes which are key to a product's market acceptance (Manski, Der, & Boom, 2007). However, tensile tests are not common in food texture measurements because the process of mastication is mainly a compression of foods. These tests however, are more difficult to perform as they involve gripping or holding a food sample (Lu, 2013).

2.6.2.2 Scanning electron microscopy (Cryo-SEM) and SEM observations

According to Lin et al. (2002), microscopy is an important method in observing and describing the microstructure characteristics of the fibrous meat analogues. In fact, cryo-SEM has emerged as a powerful tool for observation of biological specimens at below ambient temperature (between -100^{o}C to -175^{o}C). The first cryo-SEM was performed in the 1960's, and by 1980's the first commercial system was made available. This method is very rapid and allows in-situ investigation of a specimen; provides life-like appearance of the sample as it allows a sample to remain hydrated and chemically unmodified (Watt, 1997). Undeniably, it has found a great purpose in visualization of delicate or labile structures such as plant xylem cells (McCully et al., 2000). First, the sample is cryo-fixed by putting it into liquid nitrogen (-210^{o}C), if fracturing is necessary the sample is transferred to the cryo-preparation chamber where fracturing is performed if necessary.

Besides, the fracturing is done to remove ice contamination on the surface of the specimen structure which would result into blurred cloudy images. Thereafter, a sputter coating with metal (gold or platinum) can be done so as to reduce 'charging problems' the samples. Finally, the specimen is moved to SEM chamber where is remains frozen during imaging using a field emission scanning electron microscope (FEG) under high vacuum. An appropriate detector is also chosen for a particular imaging (Echlin, 2013).

2.6.2.3 Digital imaging

In this study digital imaging was done by use of digital camera to acquire digital images of the physical color and appearance of the samples. It was done for the purpose of qualitative analysis which involves visual inspection and comparison. The visual examination is however subjective and does not provide an objective numerical index. Usually, the digital imaging is relatively cheaper and uses simpler experimental set-ups than most commercial microscopy imaging systems. In addition, the use of digital imaging is suitable in analyzing the color and structures of larger objects such extrudates of meat analogues (Ranasinghesagara et al., 2005).

A digital camera records an image on an electric light sensor made up of pixels, usually a camera with a high-resolution (2.1 mega-pixel or above is recommended). The resolution and the file compression are key to image quality. The former is related to number of pixels, the higher the resolution the better while the latter reduces the amount of memory taken up by the image and non-compressed file (TIFF format) is preferred. In addition, it is necessary the camera to have a macro and zoom feature. The sample should be placed under a consistent and uniform lighting. This proper lighting can be done in a calibrated photo box that is illuminated with a standardized C, D_{65} or D light source (Lawless & Heymann, 1998), is very important since the food color of the food sample depends on the part of spectrum reflected from it. Meanwhile, the angle between light source and the camera lens axis should be set at 45° so as to diffuse the reflection.

2.6.2.2 In vitro protein digestibility

2.6.2.2.1 Effect of high moisture meat analogues on protein digestibility

Proteins are biopolymers that are important nutritional components for all mammals (Reeds, 2000). According to Phillips (1989), the protein quality vary depending on the composition, particularly in terms of essential amino acids, degree of denaturation of the protein and reduction in amino acid availability by cross linking, degradation and complexion. In particular, thermal treatment have been shown to modify both the physical properties and chemical composition of proteins and thus affect protein digestibility (Phillips, 1989). Like any other food processing and/ or extrusion have both beneficial and undesirable effects on the nutritional value of proteins. The combination of the process conditions and presence of other materials play a role of the interaction and changes of these proteins. The proteins/ polypeptides may undergo unfolding and or aggregation/ denaturation of the protein to random configurations that improve the nutrition quality by increasing accessibility to proteases thus becomes high digestibility (K. S. Liu & Hsieh, 2007). In some cases, these proteins are enzyme inhibitors and this denaturation helps to reduce their effects on digestibility (Bjorck & Asps, 1983; Phillips, 1989).

Conversely, during the protein hydrolysis, some iso-peptides can be produced which cross-link in a manner that interfere with protease action thus reducing the digestibility. Maillard reaction may also occur while extruding protein foods that contain reducing sugars (Martins, Jongen, & Boekel, 2000). These reaction involves a free amino acid mainly lysine and reducing sugars, at high temperatures (>180 °C) and shear (>100 rpm) to form complexes that are non-digestible and have a brown coloring (melanoidins). Therefore, this decreases the protein quality by lowering the digestibility and loss of amino acids while forming un-utilized products (Camire, Camire, & Krumhar, 1990).

2.6.2.2.2 In vitro protein digestion and degree of hydrolysis (DH)

A key determinant of quality of protein quality is digestibility, amounts of indispensable amino acids and bioavailability of amino acids (FAO/WHO, 1991) and many in vitro methods have been offered (Kerese, 1976). One of the recent method the static in vitro digestion method which monitors the degree of protein hydrolysis after in vitro digestion (Minekus et al., 2014). This method mimics the mastication, gastric and small intestinal processes and parameters of digestion. The end products are measured, which mainly include the amino acids are monitored using methods such as osmometry, trinitro-benzene-sulfonic acid (TNBS) (Adler-Nissen, 1986) and soluble nitrogen content method (Margot, Flaschelb, & Renkena, 1994). However, a suitable method which is based on the reaction of primary amino acids with o-phthaldialdehyde (OPA) in presence of beta-mercaptoethanol is recommended (Church, Swaisgood, Porter, & Catignani, 1947).

The available data has shown that, edible house cricket biomass is a valuable and a sustainable animal-derived protein that can provide an opportunity to be utilized in making of meat analogues. Secondly, it is possible to develop meat analogues using high moisture extrusion cooking however, suitable formulations are needed for effective texturisation. There are available tools to quantify and or evaluate the key meat characteristics; textural properties/ structure fibrousness as well as protein digestibility. These tools include the commonly used texture profile analysis and the latest in vitro protein digestion protocols.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Raw materials

Soy protein isolate (Glycine max) (SUPRO EX 33 IP) was provided by Fraunhofer Institute for Process Engineering and Packaging IVV, (Freising, Germany). Cricket flour (CF) as whole / full-fat CF was purchased from EIF, (Chiang Mai, Thailand) while the low- fat CF was obtained by defatting the whole CF using ethanol as the solvent.

3.1.1 Preparation of the low-fat cricket flour

The flour was defatted according to the method described by L'Hocine et al. (2006) with modifications on ratio of solvent and ingredients defatted. In brief, 100g of full-fat CF was mixed with analytical grade ethanol in ratio of 1:2 and stirred for 30 minutes using magnetic stirrers. The solvent layer was then filtered out using a vacuum pump (Vacuubrand, Inc., USA) and the extraction step repeated two more times to obtain maximum defatting. A final rinsing with fresh absolute ethanol was done. Finally, the flour was dried overnight in a fume hood (Frontier® Acid Digestion™, Singapore) at room temperature, while the recovered filtrate was re-distilled using a rotary evaporator (labTech, Italy) for the next fat extraction. Thereafter, the residual fat content of the dried defatted flours was determined using Soxhlet method.

3.2 Formulation and preparation of extrusion blends

According other extrusion trials, a lipid content greater than 10% in the extrusion feed caused a decrease on the extruder efficiency and less fibre formation. Thus, based on the raw flours' proximate composition, the following percentages of full or low fat CF; 0%: 15%, 30% and 45%, were selected to substitute the SPI to make the blended formulas. Before combining the flours, the cricket flours were ground in a thermomix (TM31) (Vorwerk, Wuppertal, Germany) which is shown in Figure 3.1 a. Grinding was done (without a mixing whisk installed) for 30 sec at 6000 rpm and then passed through 900 μm stainless sieve (Gilson Inc., USA). Later on, each blend was mixed

for 30 seconds and at 2000 rpm, in the thermomix fitted with the mixing whisk as shown in Figure 3.1 b. Before extrusion, pre-moistening was done on the blends to improve the feeder outflow; a calculated amount of distilled water was sprayed until a moisture content of about 21% was achieved. The moisture content was monitored using an instant/ infrared moisture analyser from Sartorius (MA 160); which digitally calculates the moisture by weight loss as shown in Figure 3.1 c.

a.  **b.**  c. 

Figure 3.1: (a) Thermomix, (b) mixing whisk installed in Thermomix, (c) Infrared moisture analyzer

3.3 High moisture extrusion cooking

3.3.1 Experimental set up

The extrusion cooking were conducted at Fraunhofer Institute for Process Engineering and Packaging IVV, (Freising, Germany) using a laboratory, co-rotating, intermeshing twin screw Haake Rheocord extruder (Thermo Fisher Scientific, Massachusetts, USA). During the extrusion, process parameters such as water flow rate (WFR) and cooking temperature were controlled, while the screw speed and mass flow were kept constant. The configuration of the extrusion system consisting of the extruder, dry mix and water inlet as well texturisation process is shown schematically in Figure 3.2.

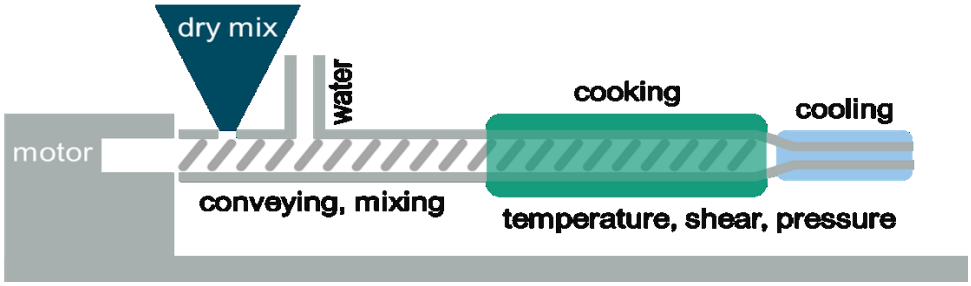


Figure 3.2: Extruder design and texturisation of extrusion feed under high moisture conditions using a cooling die.

Source: Osen and Schweiggert-Weisz, 2016).

3.3.1.1 Feeder and water dosage

A twin screw gravimetric feeder type KCM (K-tron, Niederlenz, Switzerland) Figure 3.3 a with a control monitor Figure 3.3 b was used to feed the dry ingredients into the extruder. In addition, the extruder was equipped with a water pump (Alpha 50 Plus, ECOM, Prague, Czech Rep.), the flow rate would be changed to achieve different water content of the extrudates. On the side, a computer installed with PolyLab Software was connected and used to set/control process parameters such as screw speed and temperature while simultaneously, it recorded the system parameters such as product temperature, pressure and torque.

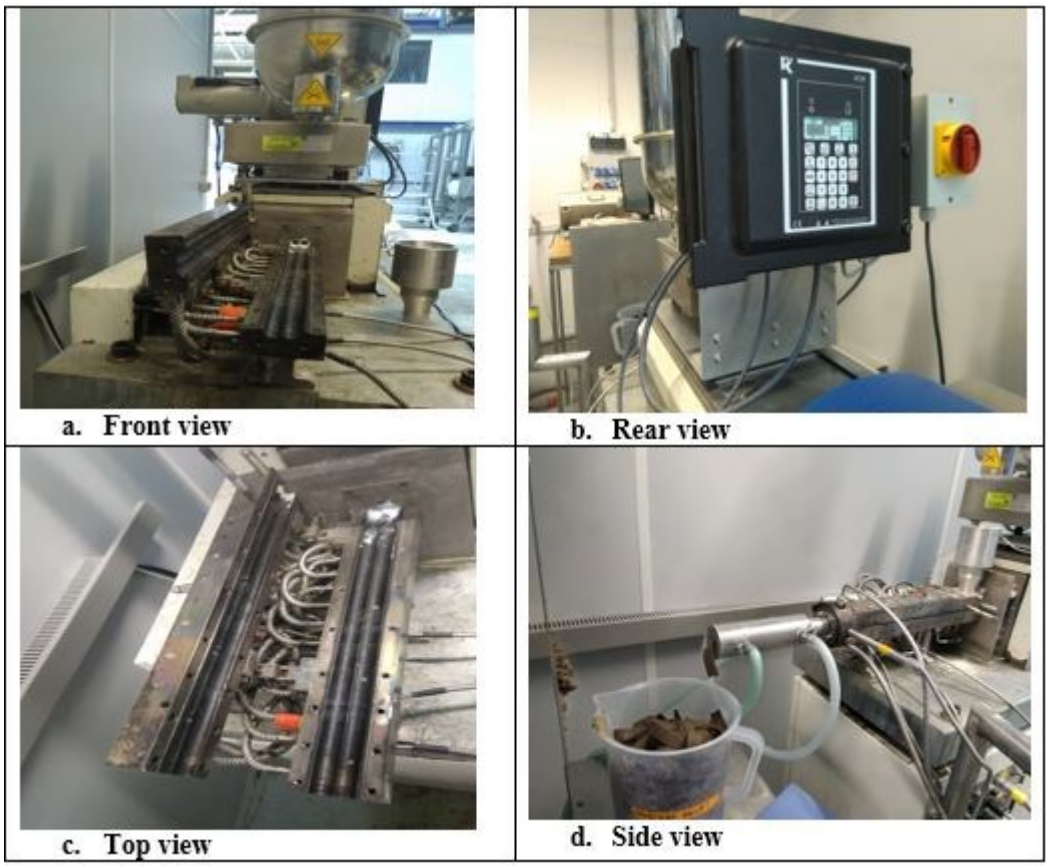


Figure 3.3: An overview of Haake Rheocord extruder. Front view: (a) Overview of extruder and feeder. Rear view: (b) showing the feeder control panel. Top view: (c) Smooth barrel, sensors connected to different zones. Side view: (d) Cooling die with product exiting on its nozzle.

3.3.1.2 Screw configuration

The extruder used twin screws that were co-rotating and intermeshing in the forward direction from the feeding zone. The extruder had screws of 16 mm diameter, a smooth barrel and a length ratio of 25:1. The screw profile could be assembled in hexagon-shape shafts and comprised of (from feed to exit): 192 mm, twin lead feed screw; 8 mm, 45° forwarding paddles; 16 mm, twin lead feed screw; 16 mm, 45° forwarding paddles; 16 mm, twin lead feed screw; 8 mm, 45° forwarding paddles; 32 mm, twin lead feed screw; 4 mm, 45° for- warding paddles; 8 mm, twin lead feed screw; 8 mm, 45° forwarding paddles; 64 mm, twin lead feed screw; and 24 mm, single lead screw as shown in Figure 3.4.

As a whole the screw configuration is made up of right-handed transport (A) and kneading elements (B), as well as left-handed transport elements (C) together.

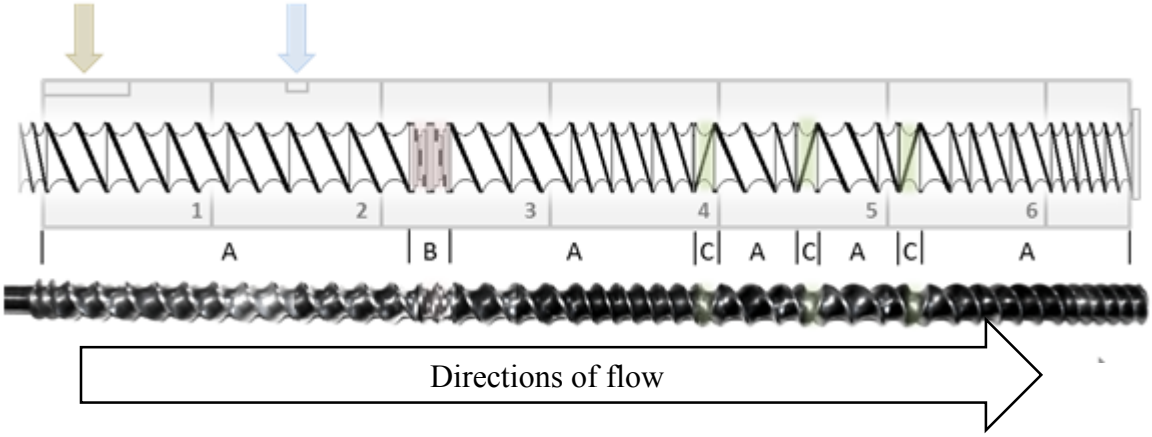


Figure 3.4: Screw configuration

3.3.1.3 Temperature- controlled zones

The extruder barrel was segmented into 5 temperature-controlled zones**,** which independently heated by an electric cartridge heating system and cooled with water; from TS 1, TS 2, TS 3, TS 4 and D1 respectively as shown in Figure 3.5. Each of the TS segments had a temperature sensor, while the end section TS4 additionally harboured a pressure sensor at the nozzle exit.

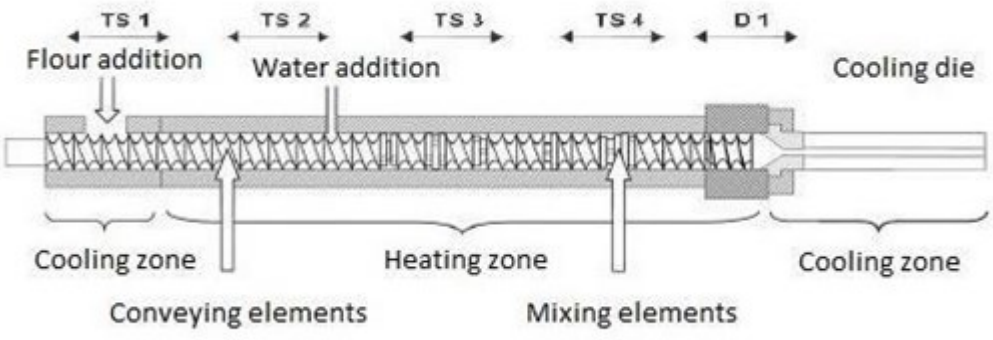


Figure 3.5: Schematic representation of the extruder barrel and cooling die.

Source: Choudhury & Gogoi, (1996)

3.3.1.4 Cooling die

At the end of the cooking barrel, a cooling die with dimensions of 19 x 2 x 210 mm (W x H x L) was attached to the end of the extruder, with water as a cooling medium, flowing at 3.4 l/min as shown in Figure 3.6.

Figure 3.6: A cooling die

3.3.2 Experimental procedure

3.3.2.1 Preparation of feed and extruder

Each formulation was extruded per experiment. Before the test, a total of 0.5 kg of feed were prepared and introduced into the feeder and feeder mass flow was calibrated. Before extrusion the screws ends were greased using a food grade grease, then assembled correctly in a rotating-intermeshing manner. Thereafter, they were fitted to the rotating shaft and encased tightly in the barrel. The extrusion was initiated by starting the process with PolyLab Software and setting the required parameters.

3.3.2.2 Extrusion

During extrusion, seven different formulations (100% SPI, 15%, 30%, 45% full or low- fat CF) were used, the extrusion temperature and water flow were varied to study the impact on product quality. Table 3.1 show the temperature profile used is shown. First, the barrel temperatures were increased stepwise from 40 °C with a temperature

profile of 40, 60, 80 and 100 °C from the first feeding zone to the fourth zone while the last zone (fifth) was set at the desired cooking temperature of (T) 120 or 140 or 160 °C, respectively.

Table 3.1: Temperature profile

Heating zone	1	2	3	4	5
Temperature (° C)	40	60	80	100	T

The cooling die was set at constant temperature of 80 °C throughout the experiment. Once the set temperature was reached in the process chamber, other process parameters were varied. The water flow rate (WFR) was varied by adjusting the HPLC pump to a flow rate of 9 or 10 ml/min. Besides, the screw speed was run at constant 150 rpm and the feeder run at feed rate of 0.4kg/h as shown in Table 3.2. Following adjustment of the WFR and attainment of a continuous product outflow out of the cooling die, samples were collected.

Table 3.2: Extrusion parameters

Parameter	**Conditions**
Temperature (° C)	120, 140, 160
Water mass flow (ml/min)	9, 10
Feed mass flow (kg/h)	0.40
Screw speed (rpm)	150

3.3.2.3 Sampling

Samples were collected when the extruder attained steady state conditions at the required temperature range. At least three samples were collected in an interval of 10 seconds. Scissors were used to cut the extrudates at about 15cm length, then packed in aluminum weighing pan lids in a vacuum plastic bags and finally stored at -20 °C.

3.4 Analysis

3.4.1 Proximate analysis of raw ingredients

The proximate composition (protein, fat, moisture and ash) for full- fat CF, low- fat CF and soy protein isolate, were determined according to (AOAC, 2005)**.** Total carbohydrates was calculated by difference as described by (WHO/FAO, 2002). All the analyses were performed in triplicate.

3.4.1.1 Proximate composition of formulations

The composition of blends was calculated based on raw flour ratios used as presented in Eq. 2.

$$Xf = AXs + BXc \quad \text{..... Eq. 2}$$

Where; Xf= % composition of formulation, for example protein

A=5%, 30% or 45%

Xs= % proximate composition of soy for example protein

B= (100-A) %

Xc= % proximate composition of cricket flour for example protein

3.4.1.2 Determination of crude protein content by Kjeldahl method

A sample weighing 1.002 g was placed into a digestion flask after heating to 38°C. Thereafter, 10g of K_2SO_4, 0.7g $CuSO_4$ and 10ml of concentrated H_2SO_4 were added and mixed thoroughly. The digesting flask was tilted gently and closed with stopper for digestion. The digester was heated gently (200 ^oC) until frothing subsided and then boiled to 380°C for 30 min until the solution became clear. The solution was cooled, and 90 ml of distilled water was added. Thereafter, 25ml of sulphide solution was added. The flask was then tilted and 80ml of NaOH was added to form two layers. A condenser unit was connected and flask was heated to collect the ammonia distillate

in a 50 ml boric acid solution. Later, the distillate was titrated against standard acid solution of HCl. Similar steps were done for blank sample. Calculation was carried out as presented in equation 3.

$$\text{Nitrogen (\%)} = 1.4007 \times (Vs - Vb) \times M / W \quad \text{.....Eq. 3.}$$

Where; Vs and Vb = ml HCl used for test portion and blank respectively;

M =molarity of HCl solution

W = test portion weight in gram.

Crude protein % = nitrogen x protein factor of 6.25.

3.4.1.3 Determination of lipid content by Soxhlet method using ethanol as solvent

About 25g of sample was weighed onto Whatman® thimble (33mmx 94 mm), then a cotton wool was used to cover the sample and total weight recorded. Then, a round bottom flask with 3 boiling stones placed inside and cork were weighed and then cramped and immersed into a water bath at 90°C. Thereafter, the Soxhlet apparatus was cramped and about 180 ml of absolute ethanol was added over the thimble into the flask. A condenser was connected with running water at 10 °C and the extraction was run for 12 hours. The boiling flask and its content was taken for ethanol distillation. Finally the flask was reweighed, and the difference was used to calculate the lipid content as presented below.

$$\text{Lipid content \%} = \left[\frac{A-B}{C}\right] \text{ x } 100 \quad \text{..... Eq. 4}$$

Where; A= Weight of flask before extraction (g)

B= Weight of flask after extraction in (g)

C= Weigh of the sample

3.4.1.4 Determination of ash content by incineration

Crucibles were dried on a Bunsen burner flame for 2-3 minutes, then cooled in a desiccator, model DSGL210 (United Scientific supplies, Illinois, USA) at the

weighing room for 2h. Afterwards, each crucible was weighed and then each crucible with about 1g of samples were weighed and recorded using an analytical balance - model Sartorius ENTRIS64-1S (Sartorius lab instruments, Gottingen, Germany). The crucibles were then heated on the Bunsen burner until no smoke was observed. Finally, ashing was done in a muffle furnace- model HTC 01/15 (Nabertherm, Lilienthal, Germany) at 550 ^{o}C for 24 hours as presented below.

$$\text{Ash content \%} = [100 - (\frac{A-B}{C}) \text{ x } 100] \quad \text{..... Eq. 5}$$

Where; A= Weight of crucible + sample before ashing (g)

B= Weight of crucible + sample after ashing (g)

C= Weigh of the sample (g)

3.4.1.5 Determination of moisture content by oven drying

Glass crucibles were dried in an oven at 103 ^{o}C for 48 hours: The crucibles were then put in desiccator and cooled in a weighing room for 1 hour, then about 1.5g of samples were weighed into the crucibles using an analytical balance. Finally, the crucibles were dried in an oven (Memmert, Schwabach, Germany) at 103 ^{o}C for 24 hours and then weighed. Drying was repeated until no weight change was observed, calculation was done as presented below.

$$\text{Moisture content \%} = (\frac{A-B}{C}) \text{ x } 100 \text{.....Eq. 6}$$

Where; A= Weight of crucible + sample before drying (g)

B= Weight of crucible + sample after drying (g)

C= Weigh of the sample (g)

3.4.1.6 Determination of total carbohydrate content

Total carbohydrates (CHOCDF/ fibre) were determined by calculation as shown below.

$$\text{CHOCDF/ fibre} = (100 - X) \quad \text{.....Eq. 7}$$

Where X= total weight in grams of protein, fat, water and ash in 100g of food

3.5 Analyses of extrudates

3.5.1 In vitro protein digestibility

3.5.1.2 Static in vitro digestion method

A standardised static in vitro digestion protocol developed within the COST FA1005 INFODIGEST Network was used to evaluate the protein digestibility of the extruded meat analogues (Minekus et al. 2014) as shown in Figure 3.7. Prior to digestion, samples were freeze dried and milled using a laboratory mill model A 11, (IKA, Staufen, Germany) for 10 seconds and sieved through stainless steel sieves to obtain a particle size between 0.5 to 1 mm. Preparation of stock solutions; simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) was done according to Minekus et al., (2014). All chemicals for in vitro digestion were purchased from Sigma- Aldrich, Germany (Merck KGaA, Darmstadt, Germany). An amount of 0.5 g milled sample was weighed in 50 ml lid glass and 4.5 ml deionized water was added. Thereafter, 4 ml SSF stock solution and 25 µl of 0.3 M $CaCl_2$ were added then mixed under magnetic stirrer to reproduce the oral phase. In simulating gastric phase, 7.5 ml SGF stock solution was added followed by 1.6 ml SGF containing pepsin (P6887-5G, CAS n. 9001-75-6) and 5 µl of 0.3 M $CaCl_2$. The pH was adjusted to 3.0 by addition of 1 M HCl in the final mixture and/ or necessary amount of purified water to reach a total volume of 895 µl to be added. Air was flushed out using N_2 and the lid was closed, and the samples were incubated in a water bath for 2 h set at 37 °C under agitation. Hourly, the pH was checked and if necessary corrected using 1 M NaOH.

The chyme from the gastric phase was mixed with 11 ml of SIF stock solution, followed by 5 ml SIF supplemented with pancreatin (P7545-100G, CAS n. 8049-47-6) and 2.5 ml SIF containing bile extract from porcine (B8631-100G, CAS n. 8006-63-7). Then 40 µl of 0.3 M $CaCl_2$ were added to the mixture and pH was adjusted to 7.0 by addition of 1 M NaOH and/ or purified water to reach 1.46 ml. Again, air was flushed out using N_2 followed by incubation, for 2 h at 37 °C with a (if necessary), correction of the pH using 1 M HCl. Thereafter, the supernatant was collected into 2 ml Eppendorf tubes, snap-freezed in liquid nitrogen and stored at −20 °C until analysis. The digestion experiments were performed in triplicates.

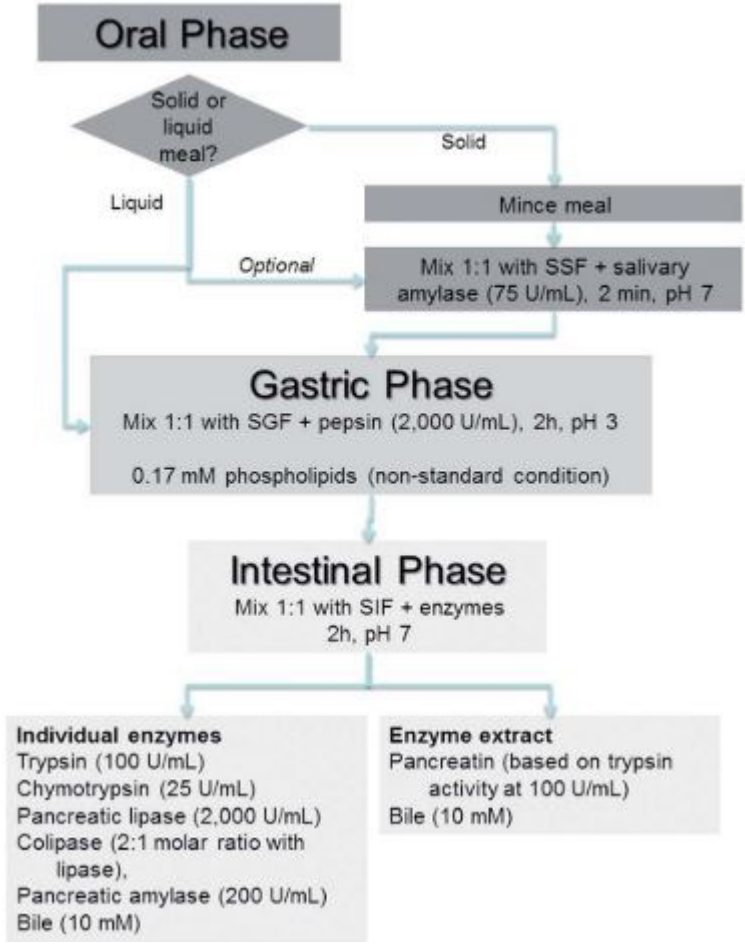


Figure 3.7: Simulated in vitro digestion method. SSF, SGF and SIF are Simulated Salivary Fluid, Simulated Gastric Fluid and Simulated Intestinal Fluid, respectively.

Source: Minekus et al., (2014)

3.5.1.3 Determination of the degree of hydrolysis (DH)

After in vitro digestion, the degree of protein hydrolysis (DH) which in this case is an indicator of protein digestibility, was determined based on the reaction of primary amino groups (NH_2) in the digestate supernatant with o-phthaldialdehyde (OPA) (Nielsen, Petersen, & Dambmann, 2001). Beforehand, the supernatants were thawed and centrifuged at 7000 rpm for 10 minutes. The OPA reagent was freshly prepared for use within 2 h. The OPA reagent (20 ml for 21 measurements) was prepared as follows: 0.762 g di-sodium tetra borate decahydrate and 0.02 g sodium dodecyl sulfate (SDS) were dissolved in 15 ml purified water in a 20 ml volumetric flask which was mixed under ultra-sound for 2 h. Meanwhile, 0.016 g o-phthaldialdehyde (P1378-5G, CAS n.643-79-8) was dissolved in 400 µL ethanol and completely transferred to the SDS solution. Then, 17.3 g of 99% dithiothreitol (0281-5G), was added to the mix by rinsing with purified water. Finally, the solution was filled to 20 ml mark with purified water. A reference solution of L-serine (Art.7769 Merck, Darmstadt, Germany) was prepared by diluting 25 mg of the amino acid in 250 ml purified water. The final solutions were prepared by mixing 120 µl sample with 900 µl OPA reagent.

Degree of hydrolysis was quantified using a spectrophotometer (Varian Inc, California, USA) and readings were taken at 340 nm using. Purified water was used a as the control and L-serine as the standard to make an equation curve that was later used to compute values of sample digestibility as presented in equations 8, 9 and 10.

The assay and measuring sequence was as illustrated in (Nielsen et al., 2001).

$$DH = h/h_{tot} * 100\% \quad \text{.....Eq. 8.}$$

Where h is the number of hydrolyzed bonds and is determined:

$$\text{Serine-NH2} = OD_{sample} - OD_{blank}/OD_{standard} - OD_{blank} * 0.9516 \text{ meqv/L} * 0.1 * 100/ X * P \quad \text{.....Eq. 9}$$

Where serine-NH_2 = meqv serine NH_2/g protein; X = g sample; P = protein % in sample; 0.1 is the sample volume in liter (L). h is then:

$$h = (\text{serine-}NH_2 - \beta) / \alpha \text{ meqv/g protein} \quad \text{.....Eq. 10}$$

Where α and β for soy protein isolate was 0.970 and 0.342 while for the A. domesticus flour and its formulations (not been examined) are estimated to be 1.00 and 0.40, respectively, according to Adler-Nissen, (1986).

Where h_{tot} is the total number of peptide bonds per protein equivalent and is given as 7.8 for soy protein isolate and 7.6 as the estimate for the A. domesticus flour and the resulting formulations (also not examined), respectively, according to Adler-Nissen, (1986).

3.5.2 Textural properties

3.5.2.1 Firmness by puncture test

For evaluation of the textural properties, the firmness of the extrudates was done as described in Bourne, (2002). First, samples were defrosted to room temperature and analysed immediately. For each treatment three samples were cut; 10 cm long and had a 1.85 mm thickness then, placed on the platform where puncture test was done using a TA-XT2 texture analyser (Stable Micro Systems Ltd., Godalming, UK) fitted with a 2 mm cylindrical probe as shown in Figure 3.8. The probe penetrated at a test speed of 1 mm/sec to a distance of 2 mm. Seven measurements were done on random positions of the sample. The maximum force in Newton (N) was recorded and used as an indicator of product texture (hardness/ shear force).

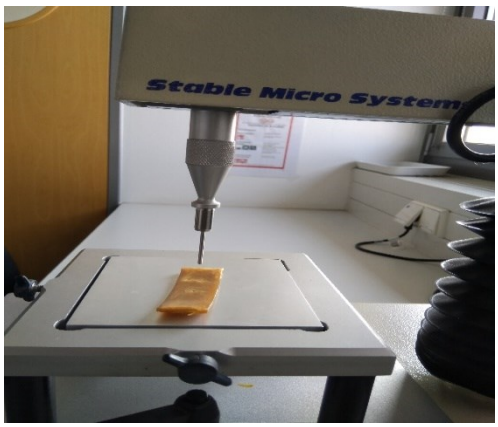


Figure 3.8: Puncture test using a texture analyser (model TA-XT2)

3.5.2.2 Tensile properties

The tensile stress of the extrudates were evaluated using a Zwick Roell Z005 universal testing machine (Zwick Roell AG., Ulm, Germany) according to the modified procedure of Krintiras et al. (2015), in order to calculate the degree of stress anisotropy. Prior to analysis, samples were defrosted to room temperature.

For each treatment, three samples were taken and ten measurements were done on random specimens. Each specimen was cut (19 x 19 mm) and a thickness of 1.85 mm. These calculated cross-sectional (3.515 x 10^{-5} m^2) was key to calculating the normal stress. The tensile tests were conducted with a constant deformation rate of 0.5 mm/s at room temperature and the distance between the points of application of roller clamps was 10 mm as shown in Figure 3.9 a. Each of the above experiment was done for tensile stress parallel in the direction parallel to the formed fibers and tensile stress perpendicular in the direction of formed fibers as shown in (Figure 3.9 b). The average was used to calculate the relevant tensile anisotropy indices (AI) through Eq. 11.

$$AI\delta = \frac{\delta\parallel}{\delta\perp} \quad \text{.....Eq.11}$$

Where AIδ is the stress anisotropy index, δ ‖ is the normal stress for specimens cut parallel to the fibres and δ ‖ is the normal stress for specimens cut perpendicular to the fibre.

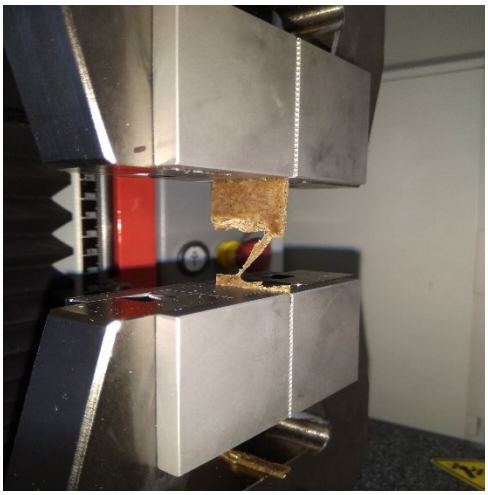
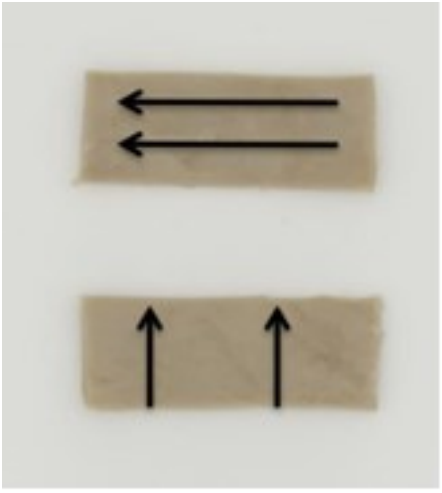
(a)  (b) 

Figure 3.9: (a) Zwick Roell Z005 universal testing machine conducting tensile properties (b) Tensile stress parallel at top and perpendicular at bottom, in the direction of formed fibers

3.5.3 Scanning electron microscopy (cryo-SEM) analysis

Cryo-SEM was performed for characterization and to provide visual confirmation of samples microstructure in-situ according to Krintiras et al. (2016) with some modification. Samples were sliced horizontally (direction of parallel to the fibers) with a scalpel and immediately fixed on a sample transfer shuttle fitted with a conductive mounting medium (1:1 mix of Tissue-Tek® O.C.T™ compound and colloidal graphite) (Agar Scientific Ltd., Stansted, United Kingdom).

Thereafter, they were plunged in liquid nitrogen slush (ca. -210 °C) and promptly transferred to the cryo chamber, model PP2000 T (Quorum Technologies Ltd., Laughton, United Kingdom) which was precooled to -135 °C. Whilst in the cryo chamber, the sample was sublimated at – 90 °C for 15 min in order to get rid of residual surface ice contamination. In addition, a sputter of platinum in Argon atmosphere (60 s coating at ca. 5-10 mA current) was sputtered on the sample. Finally, the sample was transferred to the cryo stage in the SEM chamber (T = -135 °C) for imaging using a Quanta 250 FEG field emission scanning electron microscope (FEI, Brno, Czech Republic) under high vacuum (~$3 \cdot 10^{-7}$ mbar). In this case, an Everhart-Thornley detector at a working distance of 5 mm and an accelerating voltage of 10 kV was used.

Images with a magnification of 100x/ 500x/ 1000x were taken on three different places on each sample for comparison.

3.5.4 Digital imaging

Digital imaging was done according to Yam & Papadakis, (2004). In brief, two extrudates were randomly selected and placed on a well illuminated photo box. One of the extrudate was hand-peeled by hand to reveal fibrous structures. A Canon digital camera, model EOS 80D (Canon, Vienna, Austria) was connected onto a computer and anchored above the samples in the photo box. Camera settings were as follows: aperture at f18, 1/8, no flash and remote control on. The raw images of the top surface of the extrudates were taken and saved as CR2 files.

3.6 Statistical analysis

The quantitative data was analyzed using Stata SE version 12 (Stata Corp LP, Texas USA). One-way ANOVA was performed to compare the effect of cricket flours inclusions and means were separated using Bonferroni's test adjusted at 95% confidence level. Thereafter, Pearson's correlation coefficient at $P<0.01$ and at $P<0.05$ was used to check the linear relation between the treatments or extrusion parameters with response variables.

CHAPTER FOUR

RESULTS AND DISCUSSION

Analysed data and summaries was presented in figures and tables. Trends were identified and correlations done between sample blends and process conditions. Significant observations on in vitro protein digestibility and textural properties were highlighted, discussed and theories postulated. Finally, several conclusions were drawn from observations depending on the significant effects observed.

4.1 Proximate composition of raw materials and formulation

Chemical composition of raw ingredients are presented in Table 4.1. Soy proteins had the highest protein content of 82%, these results were found comparable to those reported by Van Eys, Offner and Bach, (2005). Whole/ full fat cricket flour composition was found to be similar to findings by Rumpold and Schlüter, (2013a). Upon defatting the full-fat CF, the fat content was reduced by approximately 51%, which indicated that defatting at the set conditions described in the protocol was successful. However, a study by James and Nwabueze, (2013) reported a higher defatting efficiency of about 80% on soy bean with ethanol but on different conditions (3 hour soaking in ethanol and centrifuging at 4000 rpm).

Table 4.1: Chemical composition of raw materials in dry weight basis (g/100g)

Ingredients	Protein	Fat	Moisture	Ash	CHOCDF/ fibre
100% SPI	81.64± 0.03	0.99± 0.16	9.83 ± 0.48	3.90 ± 0.25	3.64 ± 0.13
Full-fat CF	61.39± 0.33	24.80±0.67	3.70 ± 0.17	5.09 ± 0.01	5.02 ± 1.22
Low-fat CF	68.48± 0.16	12.12±0.65	5.33 ± 0.34	4.12 ± 0.92	9.95 ± 0.59

[1]SPI= Soy protein isolate; CF= Cricket flour, CHOCDF= Carbohydrate calculated by difference; [2]Values are means ± standard error.

The full-fat CF had a crude protein (CP) content of approximately 61%, and this increased by 7.48% upon defatting to a CP content of 68.48%. This high protein content is likely to have come from the increased dry matter which composed primarily

of chitin-a polysaccharide of glucosamine and N-acetylglucosamine, both containing N atoms (Van Huis et al.,2013). However, the CP in this case was expected to be overestimated using Kjeldahl analysis and conversion factor * 6.25 (Finke, 2002). In comparison to other animal protein sources, the obtained protein content of the cricket flours were higher.

The CP content of beef is 17.37%, chicken is 17.44%, pork is 15.41%, salmon is 19.84% and milk is 3.28% (Von Hackewitz, 2018). From Table 4.1, the total carbohydrates (CHOCDF/ fibre) of CF was found to double: this was perhaps due to concentration of the insoluble matter which according to Irungu et al. (2018) is made up of 54% crude fibre. Defatting using alcohol has been reported to extract the ethanol-soluble carbohydrates primarily the simple sugars (Theander & Westerlund, 1987) and some proteins (Pace et al., 2004). These sugars have been reported to be very key to chemical changes during thermal processing such as Maillards reaction (Bjorck & Asps, 1983).

According to Table 4.2, the full-fat CF containing blends had a higher fat content compared to the low-fat blends, with the highest being 11.70% obtained at 45% full-fat CF inclusion. This lipid content was in the range for an efficient extrusion/ texturisation. Notably, the low-fat CF containing blends had higher protein, ash and fibre contents as compared to the full-fat blends. These blend compositions were assumed to also reflect the composition in the final extrudates.

Table 4.2: Chemical composition of formulations prepared for extrusion (g/100g)

Formulations	Protein	Fat	Moisture	Ash	CHOCDF/ Fibre
0% CF/Control	81.64 ± 0.03	0.99 ± 0.14	9.83 ± 0.40	3.90 ± 0.20	3.64 ± 0.30
15% Full fat CF	78.60 ± 0.07	4.56 ± 0.06	8.91 ± 0.33	4.07 ± 0.26	3.84 ± 0.14
15% Low fat CF	79.66 ± 0.02	2.65 ± 0.04	9.15 ± 0.36	3.93 ± 0.17	4.58 ± 0.21
30% Full fat CF	75.56 ± 0.10	8.13 ± 0.10	7.99 ± 0.26	4.26 ± 0.34	4.03 ± 0.34
30% Low fat CF	77.69 ± 0.03	4.30 ± 0.06	8.48 ± 0.32	3.97 ± 0.14	5.54 ± 0.13
45% Full fat CF	72.52 ± 0.14	11.70±0.19	7.07 ± 0.20	4.43 ± 0.42	4.26 ± 0.61
45% Low fat CF	75.71 ± 0.05	5.99 ± 0.15	7.80 ± 0.29	3.99 ± 0.11	6.47 ± 0.09

[1]SPI = Soy protein isolate, CF = Cricket flour, CHOCDF= Carbohydrate calculated by difference; [2]Entries are means + standard error

4.2 In vitro protein digestibility (IVPD)

4.2.1 In vitro protein digestibility of raw/ unextruded flours

The IVPD of SPI was 39.81% and this higher compared to that of cricket flours; 28.73% for full-fat and 29.95% for low-fat CF as shown in Table 4.3. Usually, a higher DH would indicate a better digestibility and hence a high availability of amino acid to the body (Finke et al., 1989). The high IVPD in SPI was attributed to high and free digestible amino acids and low amounts of anti-nutritional factors as a result of manufacturing process (Jiang et al., 2009); Stein, 2008). Conversely, the raw cricket flour have been found to contain about 19% of indigestible chitin (a polysaccharide of glucosamine and N-acetylglucosamine) which is reportedly difficult to digest more so in humans gut due to lack of enzyme chitinase (Belluco et al., 2013; Finke, 2007). In addition, there was no significant difference (P=0.901) observed in IVPD between the cricket flours, indicating that defatting had no measurable effect on the IVPD.

Table 4.3: Degree of hydrolysis of raw flours

Ingredients	Degree of hydrolysis (%)
SPI	39.81 ± 0.34^{a}
Full- fat CF	28.73 ± 0.68^{b}
Low- fat CF	29.95 ± 0.67^{b}

[1]SPI= Soy protein isolate, CF= Cricket flour; [2]Values are means ± standard error; [2]Values with different superscripts indicate significant difference (P<0.05).

4.2.2 In vitro protein digestibility of extruded blends

The results for in vitro protein digestibility for 120°C and 160°C cricket- soy extruded samples are shown in Table 4.4. Generally, the IVPD values for the extrudates were higher than those of the raw flours. This could be attributed to the effect of extrusion cooking. The combined thermal-mechanical process (shearing action of the rotating screws and the heating in the extruder barrel) have been reported to have a significant and positive effect on protein digestibility (Chen et al., 2011; Guy, 2001). This is through the modification of native protein structure leading to aggregation and

denaturation resulting to a larger surface area for proteolysis (Chen et al., 2011; Liu & Hsieh, 2007; Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014). For instance, there was a 2.63% increase in IVPD of the 100% SPI flour upon extrusion at 120°C. This observation could be explained by possible deactivation of the heat labile inhibitors: Ordinarily SPI may contain as high as 40% of trypsin inhibitors depending on the method of preparation (Baker & Rackis, 1986).

Table 4.4: Degree of hydrolysis (%) of extrudates at selected temperatures

Extrudates	120°C	160°C
0% CF/ 100% SPI	42.44 ± 0.20^{d}	48.79 ± 0.35^{a}
15% Full fat CF	47.37 ± 0.26^{b}	37.87 ± 0.81^{b}
15% Low fat CF	44.95 ± 0.47^{c}	38.96 ± 1.73^{b}
45% Full fat CF	50.21 ± 0.43^{a}	42.88 ± 0.75^{b}
45% Low fat CF	46.18 ± 0.15^{bc}	41.85 ± 0.18^{b}

[1]SPI= Soy protein isolate, CF= Cricket flour; [2]Values are means values ± standard error; [3]Different superscripts ($^{a, b, c}$) on the same column and different subscripts ($_{A, B}$) on the same row indicate means are significantly different ($P<0.05$).

At 120°C, the inclusion of CFs showed a significant difference ($P< 0.05$) on the IVPD. There was no significant difference ($P= 0.632$ and 0.574) on the 15% full fat CF and 45% low fat CF blends, and 15% and 45% low fat CF blends respectively. The IVPD was highest at the 45% full-fat CF inclusion with a DH value of 50%. The inclusion of the low and full fat CFs showed a significant ($P<0.05$) and strong positive correlation ($r= 0.8597, 0.9276$) with IVPD respectively as shown in Table 4.5. This increase could be attributed protein modification by heat treatment and high quality animal-derived protein of the A. domesticus, which according to Finke, DeFoliart, and Benevenga, (1989), demonstrated a better protein digestibility than the plant-based soy protein. A similar study by Azzollini et al. (2017) recorded an improvement of IVPD upon addition of grinded mealworm to wheat-based extruded blends. Notably, the partial defatting of CF gave a significant ($P< 0.05$) and strong negative correlation ($r= -0.7898$) with IVPD as shown in Table 4.5. This could be observed from the lower IVPDs values of low- fat CF blends as compared with the full-fat CF. This observation was attributed to the higher CHOCDF/fibre than in full-fat CF (See Table 4.2); these

CHOCDF/fibre portion is reported to mainly compose of indigestible chitin (Chapman, 2013; Finke, 2002). These results agree with Marono et al., (2015) who reported a lower IVPD in insect meals with higher levels of chitin.

Table 4.5: Pearson correlation coefficient test results for the relationship between material and extrusion parameters with in vitro protein digestibility at water flow rate (WFRs) of 10 ml/min.

Parameter		In vitro protein digestibility	
		r	Sig. value (p)
	Temperature (°C)		
Defatting CF	120	-0.7898**	0.0002
	160	0.0046	0.3674
Inclusion level			
Full- fat CF	120	0.9276**	0.0003
	160	-0.3597	0.3417
Low- fat CF	120	0.8759**	0.0090
	160	-0.4932	0.1773
Temperature	-	-0.5131**	0.0037

[1]CF= cricket flour, r= correlation coefficient, sig= significant, obs=observations
[2]**Correlation is significant at P<0.01, level. * Correlation is significant at P< 0.05, level.

At 160°C, the inclusion of CFs on blends resulted to significant difference (P< 0.05) on the IVPD. From correlation results, increasing the extrusion temperature from 120 to 160°C showed a significant (P< 0.05) and a strong negative relationship (r= -0.5131) with IVPDs of the extruded blends. Overall, the IVPD of all the extruded blends were lower than those at 120°C, except for the control (100% SPI). This exception which had an improvement (+ 6%) was ascribed to possible further deactivation of the heat labile inhibitors at higher temperatures. Conversely, the decrease on IVPD for rest of blends was attributed to the deleterious effects of physiochemical reactions such as Maillard's reaction and heat induced protein-lipid reactions among others (Guy, 2001).

According to Bjorck and Asps (1983), the HMEC conditions (moisture of 35% and temperature of 140-160°C) induces these adverse interactions that impede IVPD. It is anticipated that during HMEC at lower temperatures such as below 120°C, fewer adverse and slower reactions occur.

Meanwhile, at 160°C their reactivity increases causing deleterious and sometimes irreversible changes (Bhattacharya et al., 1988). For instance, the Maillard's reaction which is a reaction between reducing sugars and amino acids is reported to reduce the degree of protein hydrolysis (Smith & Friedman, 1984). This is through formation of indigestible complexes and the consequential loss of amino acids and in particular lysine- which is the most reactive protein-bound amino acid due to its free ε-amino group (Camire et al., 1990). In this study, the extruded blends were presumed to contain high amounts of lysine from the unsubstituted soy protein (Stein, Roth, Sotak, & Rojas, 2013) and about 7% sugars from A. domesticus flour (Irungu et al., 2018).

At 160°C there was no significant effect (P= 0.292) from partial defatting of CF, inclusion level of low and full-fat CF. However, defatting had a weak positive correlation (r=0.0046) with IVPD (at 160°C) while low- fat CF inclusion had a relatively stronger negative correlation (r= -0.4932) than the full-fat CF (r= -0.3597). This decrease in IVPD with low and full- fat CF inclusion levels was attributed to the gradual addition of reactants- sugars and indigestible chitin from CFs, coupled with the rapid reactivity at higher process temperatures. The use of INFOGEST in vitro digestion procedure to assess the digestibility is considered most reliable, but due to its newness it proved a difficulty in comparing data from other literature (Azzollini et al., 2017).

4.3 Textural properties

4.3.1 Penetration force (N) of extruded blends at the selected temperatures and water flow rates

Figure 4.1 show the effect of the cricket flours inclusion on the penetration force/ firmness at selected temperatures and at extrusion WFR of 10 and Figure 4.2 at WFR of 9 ml/min respectively. The correlation between the different extrusions conditions

with firmness are shown in Table 4.6. The firmness was significantly affected (P< 0.05) by full/ low-fat CFs inclusions in the three process temperatures for both extrusion WFRs of 10 and 9 ml/min. At WFR of 10ml/min, the firmness values were lowest at extrusion temperature 120° ranging from 0.36 to 3.85N, then it gradually increased at 140°C (1.00 to 12.83N) and 160°C (2.61 to 17.04N) as presented in Figure 4.1. Likewise at WFR of 9ml/min, the firmness of extruded blends increased with temperature but at higher rate as compared to those at WFR of 10ml/min. At 120°C, the firmness ranged from 0.59 to 6.09N and 140°C it ranged from 1.92 to 18.98N then at 160°C it was from 5.37 to 22.40N as shown in Figure 4.2. According to literature, high extrusion temperature is the most important parameter in promoting the texturisation and structure formation process (Guy, 2001; Cheftel et al.,1992).

Table 4.6: Pearson correlation coefficient test results for the relationship between material and extrusion parameters with penetration stress/ firmness (N) at water flow rates (WFRs) of 10 and 9 ml/min.

Parameter		Penetration stress	
		r	Sig. value (p)
	WFR		
Defatting CF	10	-0.6255**	<0.0001
	9	-0.5921**	<0.0001
Inclusion level			<0.0001
Full- fat CF	10	-0.3074**	<0.0001
	9	-0.4660**	<0.0001
Low-fat CF	10	-0.6452**	<0.0001
	9	-0.5204**	<0.0001
Temperature	10	0.5582**	<0.0001
	9	0.5728**	<0.0001
Water flow rate	-	-0.2178**	<0.0001

[1]CF= cricket flour, r= correlation coefficient, sig= significant, obs= observations;

[2]**Correlation significant at p<0.01, level. *Correlation significant at p< 0.05, level

Pearson correlation results in Table 4.6, show that the process temperature had a significant ($P< 0.05$) and strong positive relationship ($r= 0.5582, 0.5728$) with the penetration force of extruded blends at WFRs of 10 and 9 ml/ min respectively. The reason for lower values at WFR of 10 ml/ min however, was accrued to the lubricating effect of water content. The variation of WFR had a significant ($P< 0.05$) but weak negative relationship with the firmness ($r= -0.2178$). According to Ilo et al. (2000), high levels of moisture causes a poor texture by making the 'melting' incomplete due to dilution and this dilution in turn leads to a low friction and less shear which can impede texturisation/ structure formations.

Notably, the full- fat CF blends yielded relatively higher firmness values compared to the low- fat CF counterparts at both WFRs in exception of 45% low- fat CF blend which had comparable firmness with 30 and 45% full- fat CF extruded blends at 160°C and WFR of 9ml/min. Further results from Pearson correlation showed that partial defatting of CF had a significant ($P<0.01$) and strong negative relationship ($r= -0.6255$ and -0.5921) with firmness at extrusion WFR of 10 and 9 ml/min respectively. This observation was attributed to higher lipid content in the full- fat CF, which according to literature extrusion cooking of samples with a lipid content of above 5% can result to formation of rigid surface structure through surface moisture evaporation and subsequent cooling (Dobraszczyk et al., 2006).

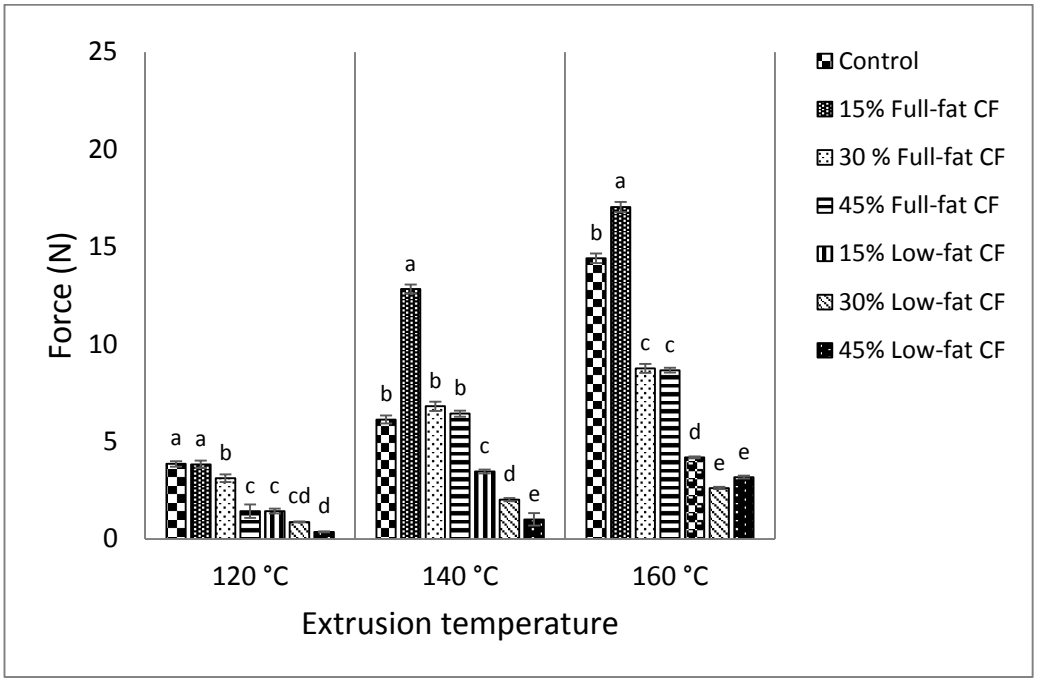


Figure 4.1: Penetration force (N) of extrudates at the selected temperatures and at water flow rate of 10 ml/min.

[1]CF= Cricket flour; [2]Values are means values ± standard error; [3]; [4]Different letters ([a, b, c]) on the same grouped bars indicate means are significantly different (P<0.05).

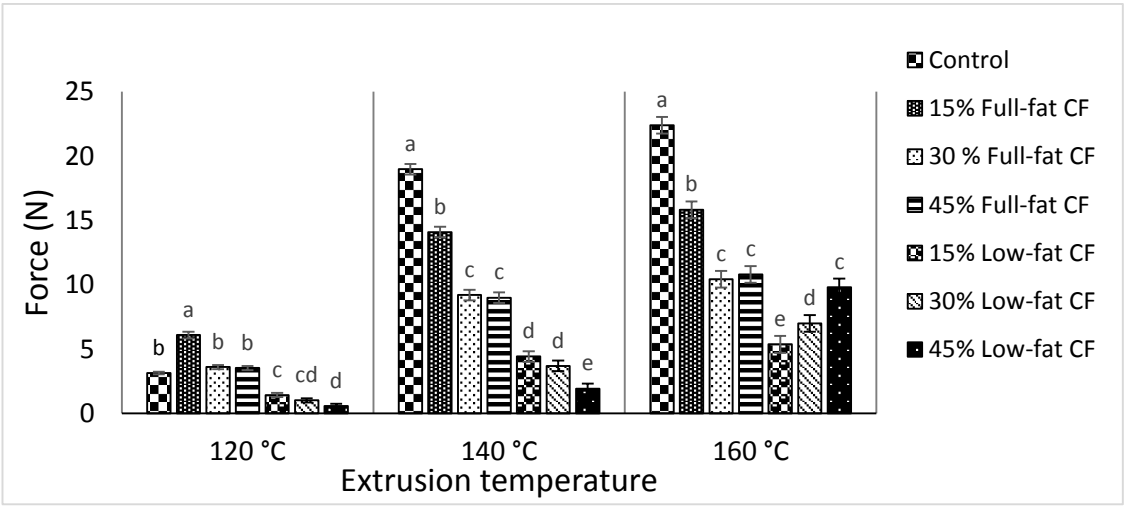


Figure 4.2: Penetration force (N) of extrudates at the selected temperatures and at water flow rate of 9 ml/min.

[1]CF= Cricket flour; [2]Values are means values ± standard error; [3]; [4]Different letters ([a, b, c]) on the same grouped bars indicate means are significantly different (P<0.05).

During firmness measurement, these full- fat samples produced two distinctive peaks as shown in Figure 4.3 (b) whereas low- fat samples measurement plotted a single smooth peak during probe penetration as seen in Figure 4.3 (c). This observation confirmed the surface hardening (top and bottom layer) on full-fat samples.

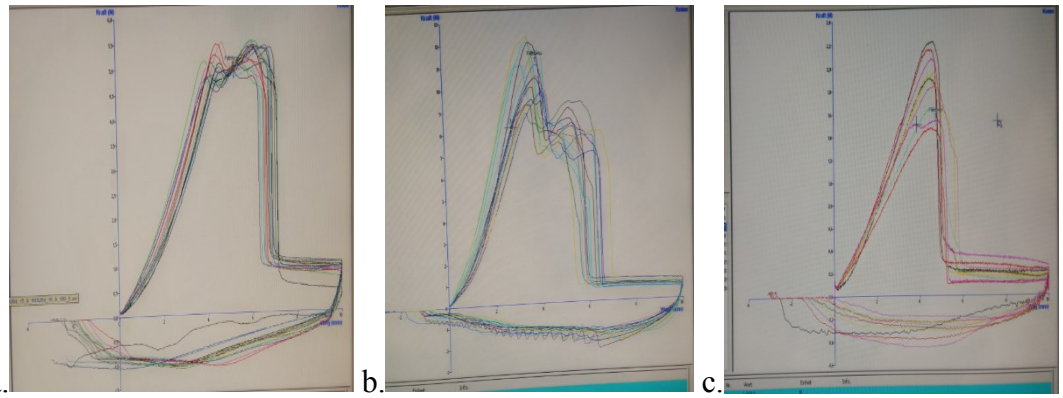


Figure 4.3: Two peaks observed when probe penetrated through the (a) soy protein isolate, (b) full-fat CF blend extrudates and (c) low-fat CF blend extrudates.

Higher amounts of cricket flour inclusion had an overall decrease in the firmness of extruded blends at both WFRs. In particular, inclusion of full and low-fat CFs had a significant ($P<0.01$) and strong negative relationship ($r= -0.3074, -0.6452$) at WFR of 10ml/ min and ($r= -0.4660, -0.5204$) at WFR of 9ml/ min respectively. Ideally, pure SPI had the highest values on firmness, followed by blends substituted with 15, 30 and 45% of full/ low- fat CF. Previous extrusion studies involving pure SPI at high temperatures have demonstrated that it forms a plasticized rigid structure of layered fibres (Osen & Schweiggert-Weisz, 2016) and tough homogenous structures (Akdogan, 1999). Meanwhile, the reduction in firmness with CF inclusion was postulated to be as a result of a shift from formation of from 100% soy protein stiffening, to a softer texturized/ fibrous texture. However, there was an exception on 15% full- fat CF blend at 140 and 160 °C which had higher firmness values than the control at WFR of 10ml/ min also, the 15% full- fat CF blend at 120°C had a higher firmness compared to the control. These observations could possibly be due to the combined positive effect of stiffening of the high soy content (formation of rigid multi-layers of fibre) as well as the surface hardening owing to the lipid content (5%).

The reduction in firmness values is attributed to the influence of ingredients from the CFs which probably transform the structure from rigid to softer fibrous texture. According to Smetana et al. (2018), ingredients such as fiber of 5 and 10% were reported to improve the structure formation to a more directional and finer meat-like structure. In this study, the following blends obtained at WFR of 10ml/ min, reached a firmness similar to that of chicken breast meat (~5.00 to 14.00 N) (Owens, Cavitt, and Meullenet, 2004); inclusion of 15, 30 and 45% full- fat CF (at 140°C) and inclusion of 30 and 45% low- fat CF (at 160°C). Equally, at WFR of 9 ml/ min, blends from inclusion of 15, 30 and 45% low- fat CFs (at 140 °C) and inclusion of 30 and 45% full or low- fat CFs also reached the firmness of chicken breast. However, inclusion of 45% CF exhibited the lowest firmness results. These results agree with those from Smetana et al. (2018) who reported a significant decrease in the cutting force of textured high moisture intermediates upon substitution of soy concentrates with high amounts of defatted concentrates of Tenebrio molitor (Mealworm) and Alphitobius diaperinus (Lesser mealworm larvae).

4.3.2 Tensile stress (N) of extruded blends

4.3.2.1 Parallel tensile stress (L)

Results in Figure 4.4 show the parallel tensile stress (L) for the cricket- soy extruded samples at selected temperatures (120, 140 and 160°C) at WFRs of 10 and Figure 4.5 at 9ml/ min.

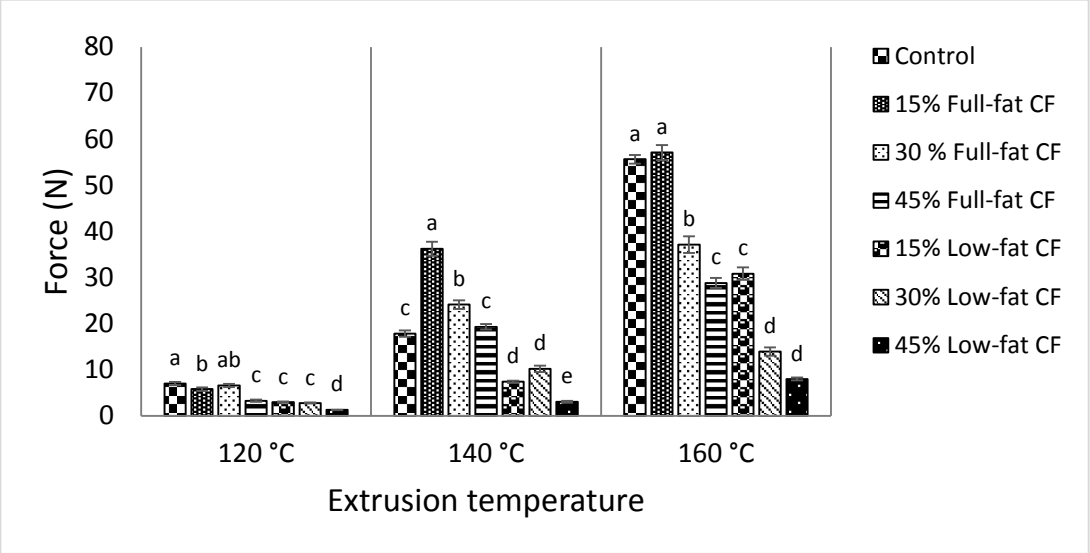


Figure 4.4: Parallel tensile stress (N) of extruded blends at the selected temperatures and water flow rate of 10 ml/min.

[1]CF= Cricket flour; [2]Values are means values ± standard error; [3]; [4]Different letters ([a, b, c]) on the same grouped bars indicate means are significantly different by Bonferroni's test (P<0.05).

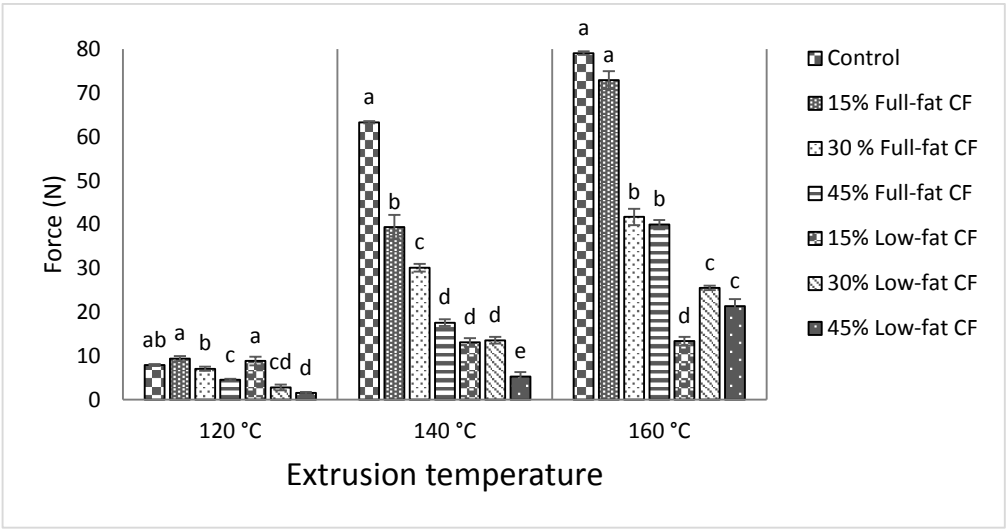


Figure 4.5: Parallel tensile stress (N) of extruded blends at the selected temperatures and water flow rate of 9 ml/min

[1]CF= Cricket flour; [2]Values are means values ± standard error; [3]; [4]Different letters ([a, b, c]) on the same grouped bars indicate means are significantly different by Bonferroni's test (P<0.05).

Pearson correlation analysis results of the material and extrusion parameters on tensile stress L are shown in Table 4.7. At extrusion WFR of 10ml/ min, the tensile stress L were weakest at 120°C which ranged from 1.28 to 7.02N, at temperatures of 140°C and 160°C the tensile stress L of the samples were stronger (3.02 to 36.24N) and (7.99 to 57.10N) respectively. Correspondingly, at WFR of 9ml/ min, similar trends on tensile stress L values were recorded however, the values were higher to their counterparts at WFR of 10ml/ min; at 120°C (1.51 to 9.36N), at 140°C (5.27 to 77.17N) and for 160°C (13.35 to 80.34N). These results suggest that, high process temperature as well as lower moisture level can induce changes in the material that result to increased tensile strengths and a possibility of positive effect on fibre formation. Pearson correlation results showed that the process temperature had a significant (P< 0.01) and a strong positive correlation (r= 0.7002) with tensile stress L at extrusion WFR of 10 and (r= 0.6755) at 9 ml/min respectively.

Table 4.7: Pearson correlation coefficient results for the relationship between material and extrusion parameters with tensile stress (parallel and perpendicular) (N) at water flow rates (WFRs) of 10 and 9 ml/min.

Parameter		Parallel tensile stress (L)		Perpendicular tensile stress (V)	
		r	Sig. value (p)	r	Sig. value (p)
	WFR				
Defatting CF	10	-0.4964**	<0.0001	-0.4849**	<0.0001
	9	-0.4804**	<0.0001	-0.5278**	<0.0001
Inclusion level					
Full- fat CF	10	-0.2444*	0.0251	-0.1639	0.1364
	9	-0.4795**	<0.0001	-0.4747**	<0.0001
Low-fat CF	10	-0.5435**	<0.0001	-0.5604**	<0.0001
	9	-0.5748**	<0.0001	-0.5511**	<0.0001
Temperature	10	0.7002**	<0.0001	0.6491**	<0.0001
	9	0.6755**	<0.0001	0.6366**	<0.0001
Water flow rate	-	-0.1727**	0.0030	-0.2088**	0.0003

[1]CF= cricket flour, r= correlation coefficient, sig= significant, obs=observations

[2]**Correlation is significant at p<0.01, level. * Correlation is significant at p< 0.05, level

A study by Osen and Schweiggert-Weisz, (2016) on high moisture extruded pea protein isolates found that, samples had minimal texturisation between 100 and 120°C with optimum at 140°C while at high temperatures of 160°C they were rigid and dense/ multilayered structures. Subsequently, variation of the water flow rate had a significant ($P< 0.01$) and negative relationship ($r= -0.1727$) with the tensile L. This weakening in tensile stress L with higher water content was attributed to the lubrication effect, fall of viscosity and increase of mass fluidity that give a low mechanical/ shearing energy hence low texturisation/ structure formation.

Meanwhile, at both WFRs, the inclusion of full- fat CFs produced stronger tensile stress L than the low- fat CF counterparts. In specific, defatting of the CF had a significant ($P< 0.01$) and strong negative relationship ($r= -0.4964$) with tensile stress L at extrusion WFR of 10 and ($r= -0.4804$) at 9 ml/min. The higher tensile stress observed on full- fat CF extruded blends was explained by the aforementioned tough and surface layers formed, these samples were more elastic and would typically have a longer deformation/ strain path compared to low- fat blends as seen in Figure 4.3 (b) and (c) respectively. The short deformation in low- fat blends was characterised by tearing at different areas and this was presumed to be from the fibres/ fibrousness.

Generally, inclusion amounts of CFs decreased the tensile stress L except for 15 and 30% full- fat CF. The correlation results, the inclusion of full- fat CF to the blends had a significant ($P< 0.01$) and negative relationship ($r= -0.2444$ and -0.4795) with tensile stress L at extrusion WFR of 10 and 9 ml/min respectively. Similarly, addition of low-fat CF had a significant ($P< 0.01$) and negative relationship ($r= -0.5435$ and -0.5748) with tensile stress L at extrusion WFR of 10 and 9 ml/min respectively. This negative correlation of tensile stress L with addition insect biomass (fiber, carbohydrates) affirms the reduction in over-texturisation of soy proteins isolate; from rigid, dense and layered structures to a fibrous structure and sometimes no structures like in the case of high amount of CFs inclusion. These observations suggest that there is need for a balance on composition of ingredients added such as protein, carbohydrates, lipid and waters among others, that can help in aggregation and cross linking during fiber formation (Akdogan, 1999). In particular, polysaccharides help to form a separate phase which enhances protein aggregation which promote fiber formation more so in

parallel directions (Akdogan, 1999)., At WFR of 10ml/min, the inclusion of 30% low-fat CF had a tensile stress L of 13.54N (at 140°C) and 13.94N (at 160°C), the tensile stress values were comparable to that of cooked beef of 12..4N (Lu, Chen, Solomon & Berry, 1998).

4.3.2.2 Perpendicular tensile stress (L)

The results for tensile stress V for the cricket- soy extruded samples at the selected temperatures (120, 140 and 160°C) and at WFRs of 10ml/ min are shown in Figure 4.6 and those at WFR of 9ml/ min in Figure 4.7.

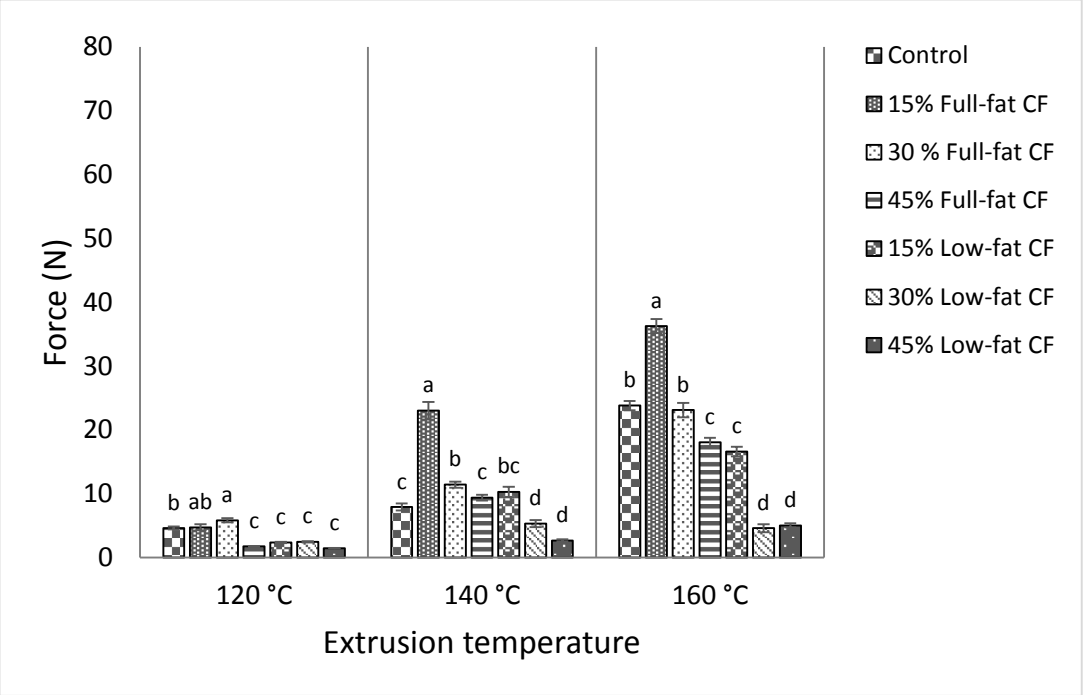


Figure 4.6: Perpendicular tensile stress (N) of extruded blends at the selected temperatures and water flow rate of 10 ml/min.

[1]CF= Cricket flour; [2]Values are means values ± standard error; [3]; [4]Different letters ([a, b, c]) on the same grouped bars indicate means are significantly different by Bonferroni's test ($P<0.05$).

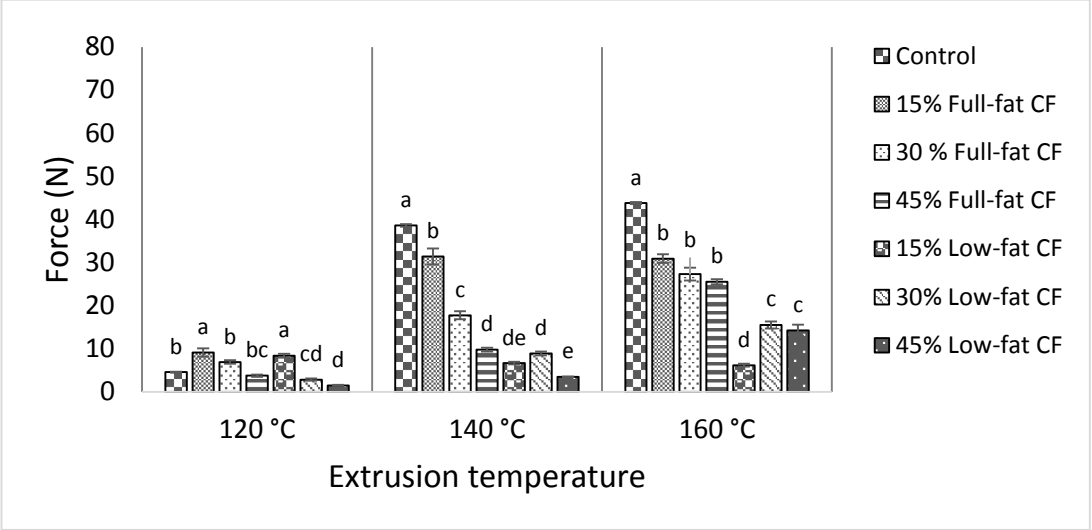


Figure 4.7: Perpendicular tensile stress (N) of extruded blends at the selected temperatures and water flow rate of 9 ml/min.

[1]CF= Cricket flour; [2]Values are means values ± standard error; [3]; [4]Different letters ([a, b, c]) on the same grouped bars indicate means are significantly different by Bonferroni's test ($P<0.05$).

The values for Pearson correlation coefficient of defatting of CF, CF inclusion levels, process temperature and moisture content with tensile stress V are presented in Table 4.7. It was found that tensile stress V significantly ($P< 0.01$) increased with process temperatures ($r= 0.6491$) at WFR of 10 and ($r= 0.6366$) at 9 ml/min. On the other hand, tensile V significantly ($P< 0.01$) decreased upon variation of water flow rate from 9 to 10ml/min ($r= -0.2088$). For instance at WFR of 9ml/ min the tensile stress V values of samples were 1.48 to 9.10N at 120°C, 3.46 to 43.87N at 140°C and 6.18 to 48.77N at 160°C however, after an increase in WFR to 10ml/ min the tensile V ranged from 1.50 to 4.75N at 120°C, 2.70 to 23.02N at 140°C and 5.05 to 36.25N at 160°C. It was noted that, the tensile stress V were lower as compared to those of tensile stress L. According to literature fibre formation predominantly occurs parallel to the cooling die (Noguchi, 1989) and that the parallel tensile stress (L) are more affected by factors such as barrel temperatures (Akdogan, 1999).

The CFs defatting had a significant ($P< 0.01$) and strong negative relationship ($r= - 0.4849$ and -0.5278) with tensile stress V at extrusion WFR of 10 and 9 ml/min

respectively. The inclusion of full- fat CF showed a negative and not significant correlation (r= -0.1639) with tensile stress V at extrusion WFR of 10 ml/min. However at WFR of 9 ml/min, the same CF inclusions revealed a strong and significant negative correlation with tensile V. This could be explained by the positive synergistic effects of high shearing energy at the low water flow rate and surface hardening due to lipid content with tensile stress V. On the other hand, inclusions of low- fat CF had a significant (P< 0.01) and strong negative relationship (r= -0.5604 and -0.5511) with the tensile stress V at extrusion WFR of 10 and 9 ml/min. These material and extrusion factors affecting the tensile stress V were presumed to act in the same mechanistic at tensile stress L. These results show that both CFs can influence tensile properties on both parallel and perpendicular directions giving anisotropic structures.

4.3.3 Tensile stress anisotropy indices (AI) of extruded blends

Figure 4.8 shows the values for anisotropy indices (AI) for the extruded blends at selected temperatures and water flow rate of 10 and 9 ml/min.

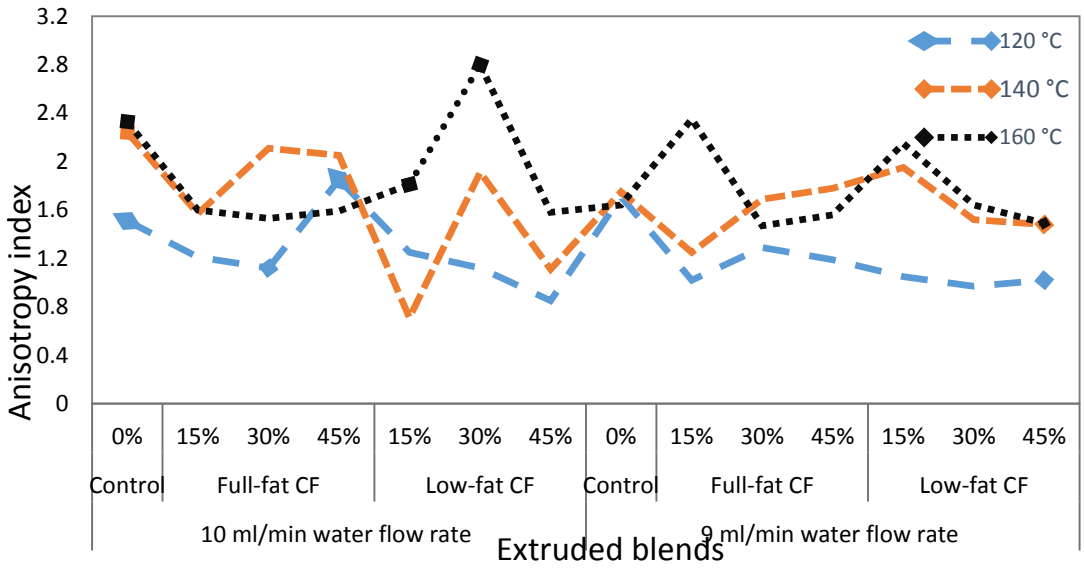


Figure 4.8: Anisotropy indices of extruded blends at selected temperatures and water flow rate of 10 and 9 ml/min.

[1]SPI- Soy protein isolate, CF= cricket flour; [2]Values are ratio between parallel tensile stress (L) and perpendicular tensile stress (V) in the direction to the formed fibres/ extrusion flow.

Anisotropic index is a good indicator of samples degree of fibre formation as well as samples inhomogeneity in structural orientation. In this study, the degree of fibrousness was based on the ratio of the yield stresses measured parallel and perpendicular to the fiber direction. From literature, a greater anisotropic index (parallel to perpendicular stress) suggests presence of an aligned structure/ significant differences in tensile stress values between parallel and perpendicular directions (Krintiras et al., 2015).

Table 4.8 shows process temperature correlated positively, r = .6512 and 0.3458 with stress AI at WFR of 9 and 10 ml/min, respectively. AI varied between 0.85-2.80 and 1.05-1.72 at WFR of 10 and 9 ml/min respectively, depending on the process temperature and composition. It was found that the AI was highest at 160°C and ranged from 1.49–2.35 and 1.53–2.80 from the extrusion WFR of 9 and 10 ml/ min, respectively. This suggests that formation of anisotropic structures can be related to an increase in thermal treatment irrespective of the water flow rate used.

Table 4.8: Pearson correlation coefficient results for the relationship between selected cricket flour inclusion, process temperature and water flow rate (WFR) with stress anisotropic index

Parameter		Stress anisotropic index	
		r	Sig. value (p)
	WFR		
CF inclusion	10	0.0279**	<0.0001
	9	-0.2971**	<0.0001
Temperature	10	0.3458**	<0.0001
	9	0.6512**	<0.0001
Water flow rate	-	-0.1705**	<0.0001

The stress AI correlated positively (r = .1705) with variation of WFRs from 9 to 10 ml/min (Table 4). Illustratively, at 160°C, samples extruded at WFR of 10 ml/min had higher stress AI as compared to their counterparts extruded at 9 ml/min except for the 15% CF blends. This suggests that increasing WFR, the structures of the samples

would become less layered and exhibit anisotropic. According to Emin, Quevedo, Wilhelm, and Karbstein, (2017), increasing water content would lead to significant increase in the reaction rates of protein and the disulphide bonds, hydrogen bonds, and hydrophobic interactions would promote high degree of fibrous structure formation (Hong et al., 2016).

The inclusion of CFs at WFR of 9 ml/min resulted in a reduction (r = −.2971) in the stress AI. For instance, at the highest texturisation temperature (160°C), the inclusion of CF gave a lower stress AI than the control, except for 15% full-/low-fat CF inclusions with a stress AI of 2.35 and 2.15, respectively. On the other hand, inclusion of CFs at WFR of 10 ml/min showed an increase (r = .0279) in stress AI. This processing WFR produced the highest stress AI of 2.80 and was obtained from 30% low-fat CF inclusion processed at 160°C. According to literature, at this high temperatures, increasing moisture content would increase protein reactions (Emin et al., 2017; Osen et al., 2015b), enhance quality of texturization, promote alignment of protein (Akdogan, 1999), cause less formation of lipid complexes (Zhang, C., Wei, Y. M., Zhang, B., and Kang, 2007), and thus exhibit high anisotropy.

At both WFRs, the blends containing 30 and 15% full/low fat and processed at 140°C and 160°C achieved comparable stress AI values to those of raw beef ∼2 (Krintiras, Göbel, Bouwman, & Goot, 2014). These AI results show that it is possible to effectively tailor a cricket–soy meat analogue using 15 and 30% CFs by controlling the process temperature and water flow rates during HMEC.

4.3.4 Scanning electron microscopy (Cryo-SEM) and digital image analysis of extruded blends

The cryo-SEM analysis allowed for identification of structure formation within the extrudates at 160 °C as shown in Figure 4.9a and b. According to literature, minimum texturisation temperature of most high moisture extruded samples has been observed at 140°C and the structure formation improves at 160°C (Osen & Schweiggert-Weisz, 2015; Yuan et al., 2008). The Cryo-SEM analysis on the control blends (pure SPI) displayed distinct multi-layers of parallel fibres Figure 4.9a (1a and 1b). Similar results were reported by Osen & Schweiggert-Weisz, (2016) during extrusion of pure pea

protein isolates (PPI) at 140°C and 160°C. Similarly, the 15% full- fat CF extruded blend displayed multi-layers of fibres as shown in Figure 4.9a (2a and 2b). These observations validates the high firmness and tensile stress recorded on these samples as a result of the over-texturisation and or stiffening of layers respectively.

The 30% low- fat CF blend exhibited very distinct and dense fibrous structures with connected fibres Figure 4.9b (5a and 5b). This was the most pronounced anisotropic structure and corresponded with highest AI of 2.8. This fibrousness was suggested to be as a result of a balance of ingredients such as proteins, CHOCDF/fiber and <5% lipid as reported in Table 4.2. These set conditions can promote protein aggregation and fiber formation by forming a separate phase and, on top, increase screw mechanical energy (SME) for fiber alignment (Akdogan, 1999; Zhang et al., 2020).

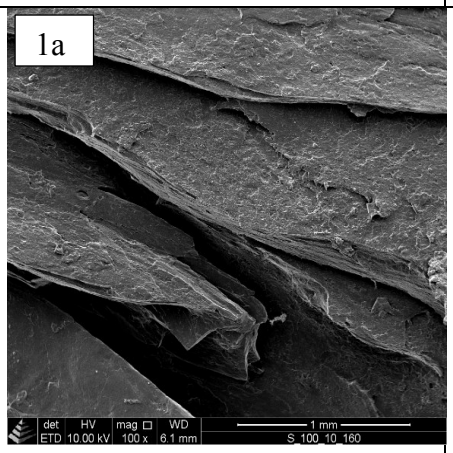
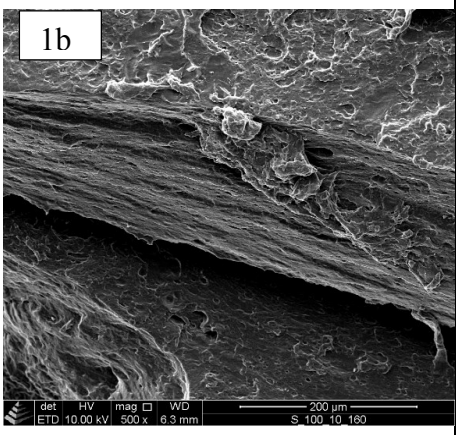
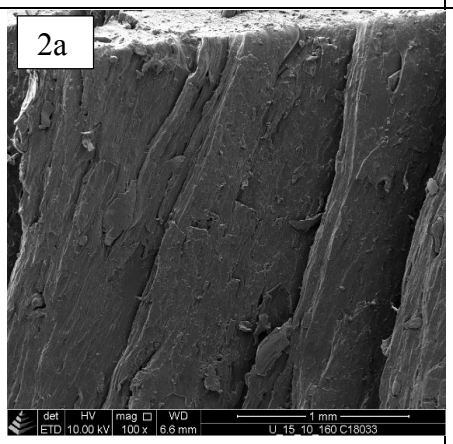
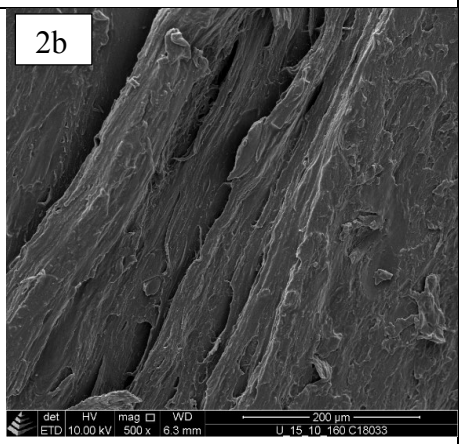
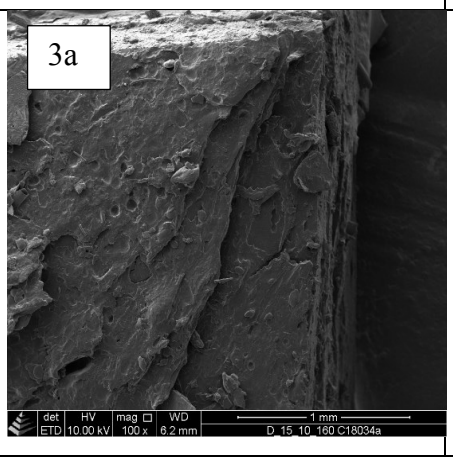
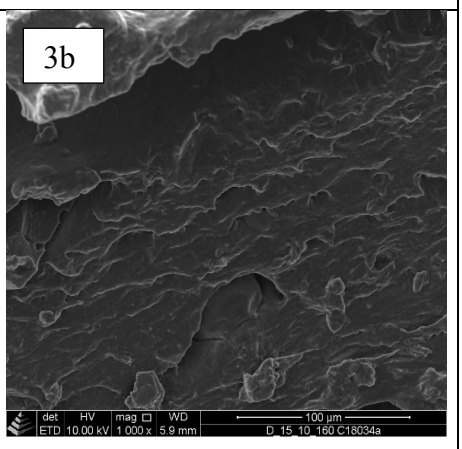
Extrudates		Magnification	
		X100	X500
100%	SPI		
15%	FFC		
	LFC		

Figure 4.9a: Scanning electron micrographs of extrudates at different magnifications.

[1]SPI= Soy protein isolate, LCF= low-fat cricket flour, FCF= full-fat cricket flour

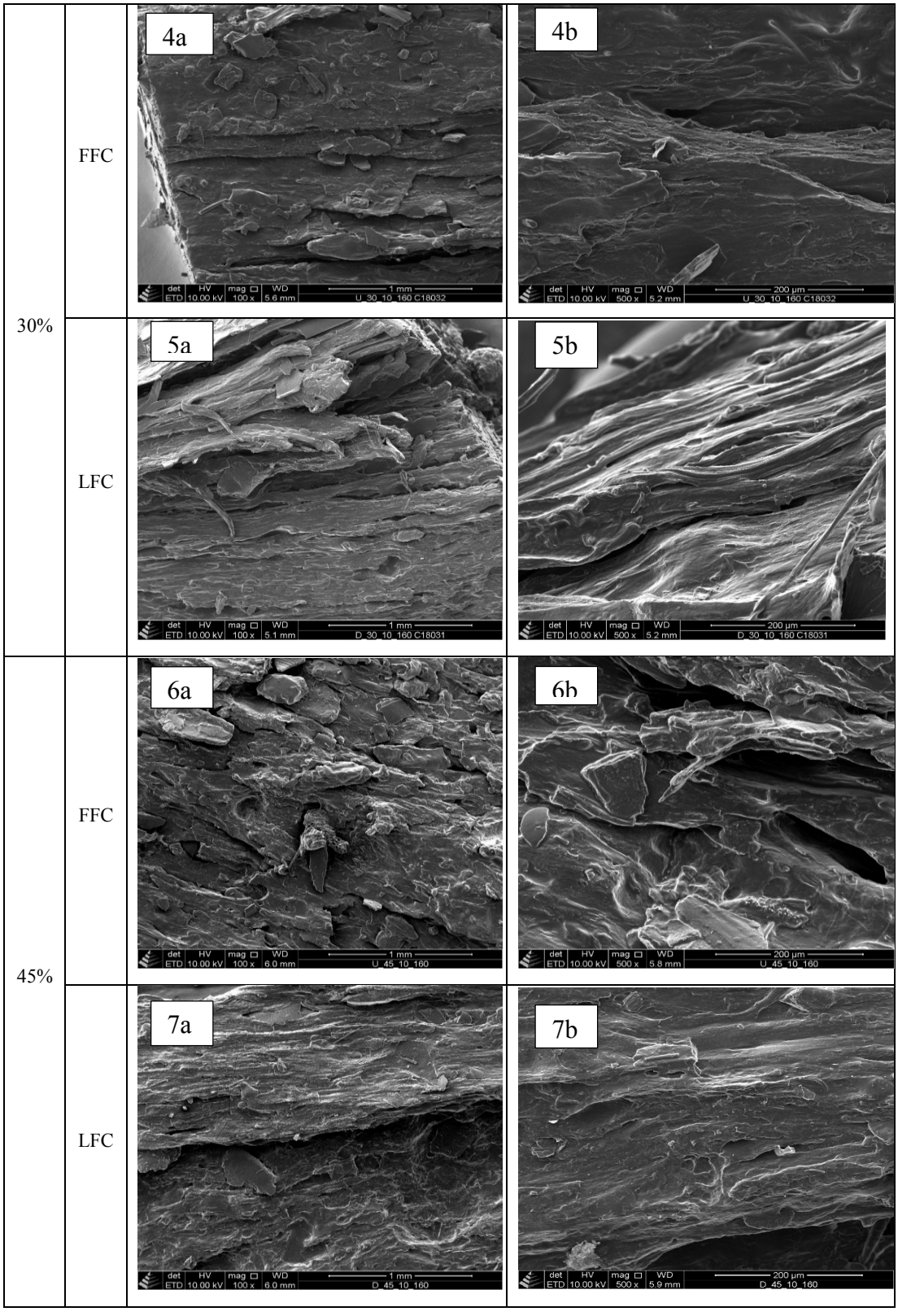


Figure 4.9b: Scanning electron micrographs of extrudates

[1]SPI= Soy protein isolate, LCF= low-fat cricket flour, FCF= full-fat cricket flour

Comparison between the 30% CFs blends, the full-fat had less visible fibrous structures and we speculate the effect of higher lipid content. This observation also validated that high tensile stress in full-fat blends did not necessarily translate to better structure/fiber formation. We expect that during extrusion, complexes of lipids and other macromolecules formed and got distributed on surface of protein aggregates preventing the aggregation of protein molecules and stabilization of the fibrous structure (Zhang et al., 2020). Finally, at 45% CF inclusion, there were no distinct structures observed. This affirms that high ≥45% insect biomass deteriorates structure or fibre formations.

Digital images were taken for qualitative analysis by visual inspection, of the color and overall surface appearance/ texture of extruded blends changes both WFRs (Figure 4.10 and 4.11). An increase in process temperature and CF inclusion had observable changes on color and surface appearance however, WFR variation had no observable changes on the extruded blends. Samples obtained at 160°C were characterized with a darker color compared to those extruded at 120 °C and 140 °C. Similarly, samples with 30 and 45% CF inclusion were relatively darker than the control samples and those containing 15% CF. This color change was associate with the melanoidin formation during the sugar-amino browning/ Maillard's reactions (Brands, 2002); and the dark colour intensifies at high temperatures and in presence of reactants (Martins et al., 2000). In this case, reactants would include carbohydrates/sugars from cricket flour, and consequently, these same samples at 160°C reported a lower protein digestibility.

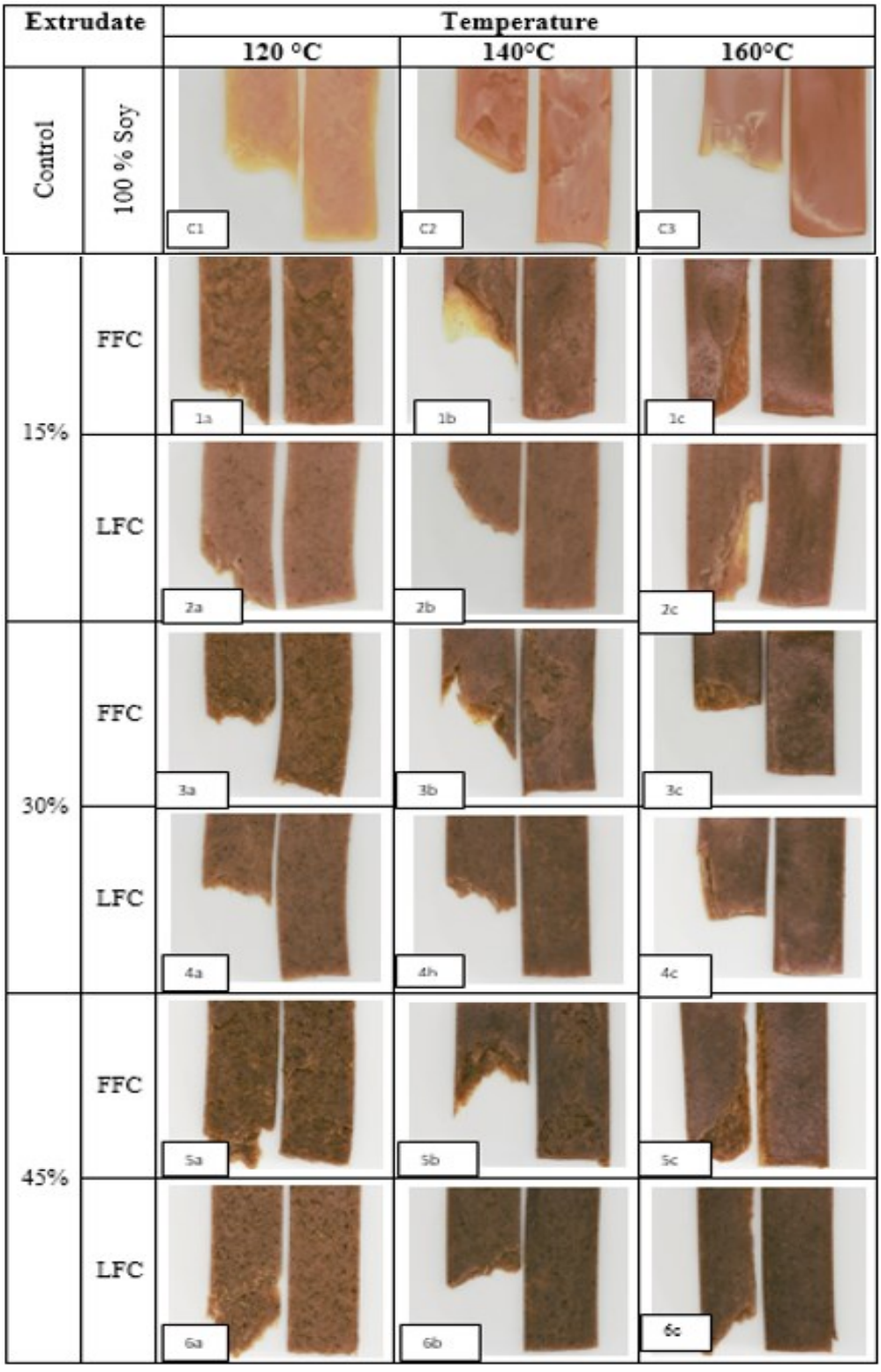


Figure 4.10: Digital images of extrudates at water flow rate of 10 ml/min.

[1]SPI= Soy protein isolate, FCF= full-fat cricket flour, LCF= low-fat cricket flour

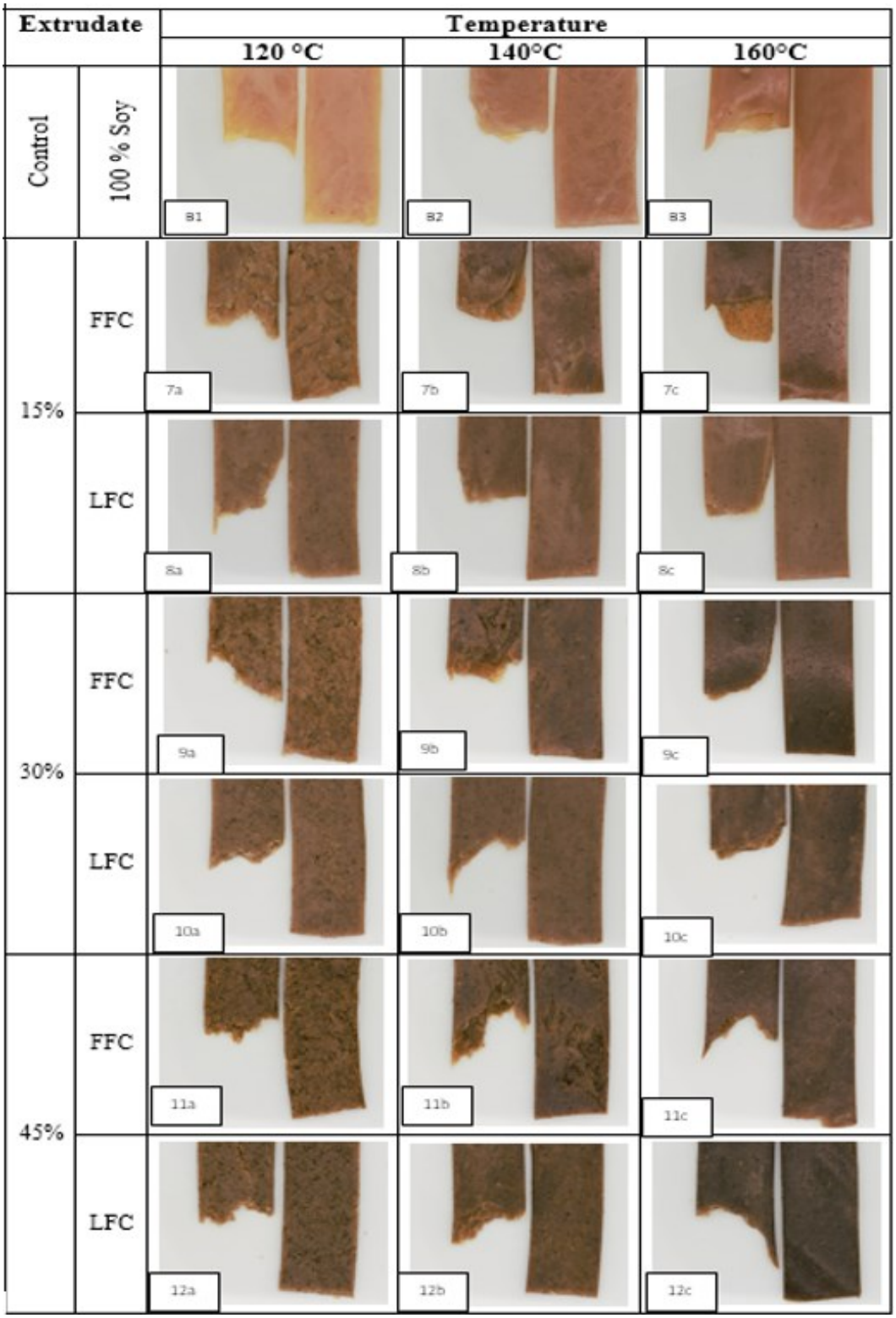


Figure 4.11: Digital images of extrudates at water flow rate of 9 ml/min.

[1]SPI= Soy protein isolate, FCF= full-fat cricket flour, LCF= low-fat cricket flour

The texture and surfaces of samples at lower temperatures of 120°C appeared non-uniform and cracked whereas, at higher temperatures they were uniform and had smoother surfaces. This could be due to the high shearing at high energy and friction as temperature increased. Moreover, the control and full- fat CF samples exhibited a more reflective surface- 'plastic and shiny' compared to the low- fat CF counterparts especially at 160°C. These reflective appearance was linked to stiffening (100% SPI) at high temperature and shear as well as a thin lipid coating on the extruded product (full- fat CF samples) as they exited the die and subsequent cooling.

The blending and/ or defatting of Acheta domesticus helped to achieve a suitable formulation that could be suitably extruded. Data obtained from the in vitro protein digestibility helped to highlight the effect of formulation and the process conditions have on protein digestibility. Overall, the textural profile and microstructure analyses revealed possible mimicking of meat structural properties.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1.1 Conclusion

This research shows potential for utilization of edible house cricket (Acheta domesticus) in development of meat analogues using high moisture extrusion cooking. It confirmed that the compositions and inclusion levels of A. domesticus flour affect the properties of the meat analogues at different process temperatures and water flow rates. The house cricket-soy meat analogues were found to have a higher in vitro protein digestibility (especially those containing full- fat CF flour) at process temperature of 120°C. This suggest that A. domesticus flour can be utilized without defatting to develop meat analogues with better protein digestibility.

The textural properties- firmness and tensile stress (parallel and perpendicular) of the cricket-soy meat analogues were found to be significantly reduced with the inclusion of full or low A. domesticus flour. However, the textural properties would increase at higher process temperatures and at lower water flow rates. Firmness and tensile stress' comparable to chicken breast and beef were attained respectively at process temperatures of 140 and 160°C and water flow rates of 9 and 10ml/ min. Overall, the meat analogues containing low- fat CF were found to have lower values for firmness and tensile stress' as compared to full-fat CF counterparts. The cricket-soy meat analogue containing 30% low- fat CF had the highest anisotropic index of 2.8. It had the most pronounceable fibrous structures. However, high amounts of CFs inclusion (45% full or low fat CF) was characterized with no observable fibrous structures.

The relationship between textural properties, microstructure and digestibility should be considered to design high quality meat analogues. Our findings from this research pave way for development of fibrous meat analogues containing A. domesticus with tailored protein digestibility characteristics. This provides a good opportunity to reduce dependence of meat production and consumption for animal-derived protein.

5.1.2 Recommendations

In light of the findings of the study, full and low-fat cricket flours can therefore be used to partially replace SPI in soy-based meat analogues using HMEC. In production of cricket-soy meat analogoues, up to 45% full-fat CF and lower extrusion temperatures of 120°C are recommended for improved protein digestibility. High process temperatures of 140 to 160 °C will be suitable for structuring anisotropic structure/ fibrous cricket-soy meat analogues. Similarly, partially defatting CF and using about 30% inclusion level is necessary in obtaining better anisotropic structure. However, other ways to improve protein digestibility should be considered if high temperatures and low-CF are used. Finally, constant water injection into extrusion at flow rate of 10 ml/min can be used for better anisotropic structures.

Further research should be considered to experiment on fully defatted cricket flours. Subsequently, other food grade and improved defatting protocols that can reduce fat content to lower levels need to be explored. This would improve the CF biomass utilisation and substitution of SPI in development of high moisture meat analogoues. In addition, the lipid extracted should be considered for further characterization and utilisation. Finally, sensory and shelf life analysis of the developed meat analogoues need to be researched to further the successful utilisation of cricket-soy meat analogues.

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