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**ALTERNATIVE PROTEINS FEEDING
THE WORLD: INCLUSION OF
CRICKET POWDER IN CEREAL-
BASED PRODUCTS**

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A thesis submitted in partial fulfilment of the requirements of London South Bank University for the degree of Doctor of Philosophy

July 2022

ALTERNATIVE PROTEINS FEEDING THE WORLD: INCLUSION OF CRICKET POWDER IN CEREAL- BASED PRODUCTS

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AUTHORS DECLARATION

I declare that the work in this doctorate thesis was carried out by following the requirements of the University's Regulations. Therefore, ensuring that the Code of Practice for Research Degree Programmes was adhered and that the research has not been submitted for any other academic award. Where indicated by specific reference in the text, the work is the candidate's research. Research done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Signature:

ABSTRACT

With the growing population worldwide and subsequently, the increasing demand for protein from livestock (poultry, fish, pigs and cattle) causes concern and future challenges. Entomophagy (the practice of consuming insects) can be promoted as an alternative and sustainable food source. Although there are around 1900 edible insect species globally, these are mainly consumed in developing countries due to their nutritional composition and ease of access. For instance, crickets are high in protein, fibre and low in carbohydrate, making them suitable to feed the world as an alternative food. The objective of this research was to understand the implications when using cricket powder fortificants within baked products. Three different sample replacement levels, wheat flour and cricket powder – 30% (WW+CP), wheat flour, cricket powder, quinoa- and Khorasan flour – 30:20:20% (WW+CP+Q+KH) and wheat flour, cricket powder, quinoa- Khorasan flour and mixed seeds – 30:20:20:25% (WW+CP+Q+KH+MS), were tested against a control sample (wheat flour – WW). Dough and bread samples were subjected to rheological, technological, chemical and sensory analysis to determine the individual analysis parameters. A negative linear correlation was observed between the number of inclusions within samples. Thus, impacting the rheological dough parameters, particularly a statistical significance ($p < .05$) for secondary parameters (protein weakening, starch gelatinisation and enzyme degradation). Crumb brightness and slice volume parameters decreased through the C-Cell illumination system as the replacement levels increased for all treatments. WW+CP+Q+KH+MS samples showed a decrease in the area occupied by air cells, the average air cell diameter, and cell wall thickness. However, the number of gas air cells increased for WW+CP+Q+KH and WW+CP+Q+KH+MS, indicating a good fermentation process within the bread samples. Texture profile analysis (TPA) was monitored at 1, 3, 5 and 7 days, showing a positive correlation between the higher number of flour inclusions and a reduced hardness within samples. Likewise, resilience decreased as the replacement levels increased for all treatments. Bread samples were analysed for nutritional composition and revealed an increase in crude- protein, fat and fibre as the replacement levels increased. This correlated with a positive linear increase between the increase in replacement levels and the macronutrients. Furthermore, this directly impacted the texture for sample WW+CP+Q+KH+MS, as

it maintained the softest crumb reducing the staling rate. Finally, bread samples made with the combination of WW+CP+Q+KH+MS, showed a liking by 145 untrained panellists (appearance – 57%, taste – 74%, texture – 64%). Data collected highlighted a practical use of cricket powder, ancient grains and mixed seeds to produce enriched bread products. Meanwhile, a JISC survey found that consumers worldwide prefer insects as agricultural feed rather than a direct food source ($r = .6$). However, this changed when participants heard about crickets' potential health benefits, and a shift to accepting crickets as a direct food source was noticed ($r = .89$). Furthermore, the food neophobia levels showed a decrease compared to previous studies, suggesting more acceptance of this alternative protein

Keywords: entomophagy, fortified bread products, insects for human consumption, optimum nutritional composition, dough rheology, crumb microstructure (C-Cell), texture profile analysis (TPA), consumer perception

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DEDICATION

To my family members, especially those who have supported, helped and tolerated me throughout my studies.

My Grandfather and Aunt, Andrew Cameron Petrie and Dessree Anne Addison, passed away whilst completing this thesis.

The people of Southern Africa

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Abbreviations

$^2\text{H}_2\text{O}$ – Heavy Hydrogen or Heavy Weight Water
AACCI – American Association of Cereals and Chemists International
AGI – Artificial General Intelligence
AI – Artificial Intelligence
CBP – Chorleywood Bread Process
CCP – Critical Control Point
CFV – Compression Force Value
 CO_2 – Carbon Dioxide Emissions
DALY – Disability-adjusted life years
 D_2O – Deuterium Oxide
DNA – Deoxyribonucleic Acid
EPA – Eicosapentaenoic Acid
ESEM – Environmental Scanning Electron Microscope
EU – European Union
FAO – Food and Agriculture Organisation
FAOSTAT – Food and Agriculture Organisation Statistics
FBF – Fortified Blended Foods
FSA – Food Standards Agency
GHG – Greenhouse Gas Emissions
GHI – Global Hunger Index
GI – Glycaemic Index
GM – Genetically Modified
GR – Glycaemic Response
 H_2O – Water
HCl – Hydrochloric Acid
HMW – High Molecular Weight
IFRPI – International Food Research Policy Institution
IFST – International Food Science and Technology
ISO – International Standards Agency
LMW – Low Molecular Weight
MUFA – Monosaturated Fatty Acids
NPD – New Product Development
PBC – Perceived Behaviour Control
PFSG – Professional Food Sensory Group
PPGR – Postprandial Glycaemia Response
PUFA – Polysaturated Fatty Acids
SFA – Saturated Fatty Acids
SME – Small to Medium Enterprises
T2DM – Type Two Diabetes Mellitus

TPA – Texture Profile Analysis

TPB – Theory of Planned Behaviour

UNICEF – United Nations International Children’s Emergency Fund

UNU – United Nations University

USD – United States Dollar

WHO – World Health Organisation

Ethics Declaration

Ethical approval for this thesis was submitted to London South Bank University, School of Applied Sciences Ethics committee, and obtained in July 2018 (reference: SAS1804). The professional code of conduct for the sensory analysis was observed through the Professional Food Sensory Group (PFSG) of the Institution of Food Science and Technology (IFST) in the United Kingdom. This ensured that participant safety was adhered and discrimination against the products being tested during the sensory analysis was kept to a minimum. The application also authorised a worldwide survey to engage with participants on all continents to understand their willingness to eat insects (either as agriculture feed or a direct food source). The survey also identified the consumers' food neophobia levels across all continents, which determined cross-culture differences in accepting insects as a direct food source.

Chapter 1 – Introduction: Understanding ‘Horizon 2020’ Targets

Clearly, the challenge of feeding the world will depend increasingly on meeting the demand for meat. The challenge for producers of agricultural commodities will be to keep up with the demand for animal feed. As demand for meat increases, there is no doubt that more and more questions will arise about how much meat the world can afford to eat. The world food situation will depend on how much meat people want to eat.
(Pelletier, 2012)

According to the Food and Agriculture Organisation (FAO) of the World Health Organisation (WHO) report, the world’s population will reach nine billion people by 2050 (van Huis and van Itterbeeck, 2013). This is evident (Perez-Fajardo, 2016; McClements et al., 2021) as the world population has been increasing and is expected to continue increasing yearly. As the human population grows exponentially, according to the authors’ opinion, the world is currently experiencing a global food crisis and famine (De Foliar, 1992; van Huis, 2003). Furthermore, Henchion et al., (2017) reported that the increase in population also means increased incomes and urbanisation, which will impact food consumption patterns. Inevitably, this raises the question of long-term food sustainability and food security.

Research on alternative proteins, especially insects as food substitutions, has been growing across different disciplines and countries (Mancini et al., 2019; Ruby and Rozin, 2019; Motoki et al., 2020; Barbera et al., 2021). The broad interest in insects as alternative proteins is rooted in the basic idea that direct entomophagy (the consumption of insects) and indirect entomophagy (the eating of animals fed with insects) may contribute significantly to sustainable food consumption and global food security (Van Huis, 2015; Barbera et al., 2021). Recent data suggests that ~800 million people are undernourished, of which 780 million reside in low-to-middle income countries, especially in Sub-Saharan Africa and South Asia (Webb et al., 2018). In 2015, inadequate food intake and poor dietary quality were responsible directly or indirectly for causing ill-health, with six of the top 11 global risk factors being associated with dietary imbalances (Webb et al., 2018). In 2017, this caused

11 million deaths, and 255 million disability-adjusted life years (DALYs) were attributable to dietary risk factors (Afshin et al., 2019).

The IFRPI published their 2018 Global Hunger Index (GHI) report (Global Food Policy Report, 2018), which provides a comprehensive measurement of hunger at both global- and country- level. These GHI scores reflect the multidimensional nature of hunger by combining four standard indicators into one index that falls within the range from 0 to 100. These are:

1. Percentage of the population that is undernourished
2. Percentage of children under five who suffer from wasting (low weight for height)
3. Percentage of children under five suffering from stunting (low height for age)
4. Percentage of children who die before the age of five (child mortality)

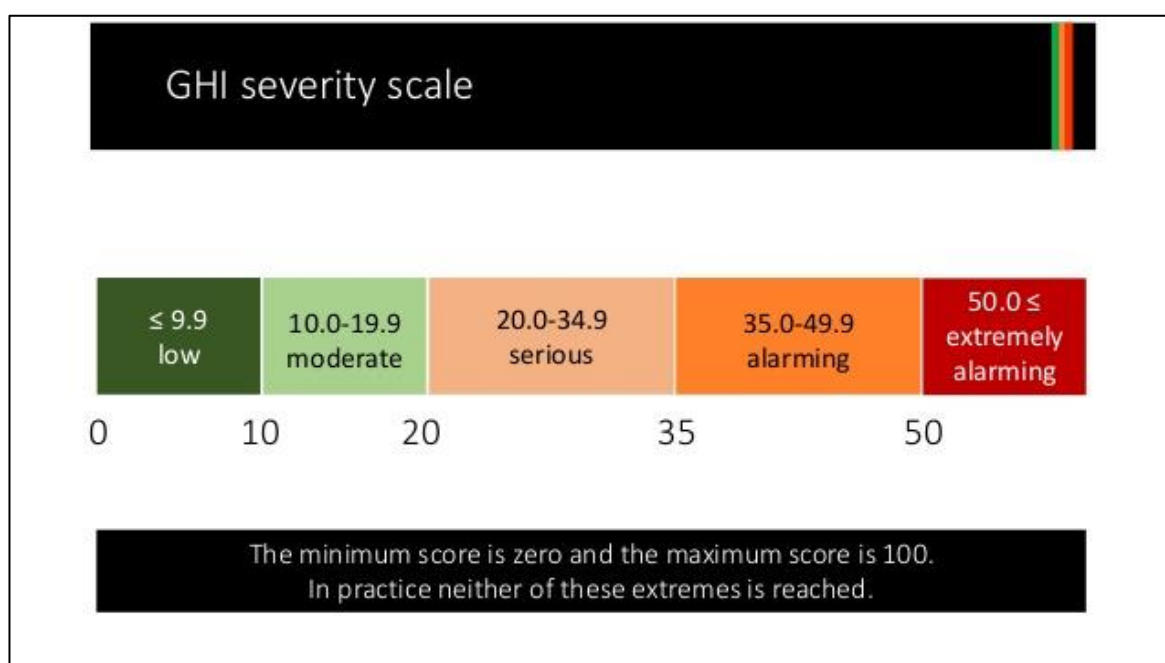


Figure 1:1: GHI Severity Scale According to the GHI Score

Source: Global Food Policy Report, 2018

A higher score indicates greater hunger and malnutrition, whereas lower indicators suggest that the country has sufficient food to feed the population. As seen in Figure 1:1, expresses the different ranges from 0 to 100, and the terminology for each different indicator/bracket. This method was designed to raise awareness and understand regional and individual country differences in the struggle against

hunger, poverty, and malnutrition. Children under the age of 5 years are highly vulnerable to malnutrition, with estimates suggesting that in 2019, globally, 144 million children under the age of five were stunted (short for his/her age), 47 million wasted (thin for his/her height) and 38 million overweight (abnormal or excess bodyweight) (UNICEF WHO, 2020). In adults, obesity is becoming more prevalent worldwide, with ~38.9% of the adult population being overweight or obese (Development Initiatives, 2018).

Furthermore, the demand for food is set to continually increase (Springmann et al., 2016; Hartmann and Siegrist, 2017; Magrini et al., 2018; Hedin et al., 2019), thus endorsing the reason to find alternative ways to feed the world. Notably, the demand for animal-based protein has been estimated to double by 2050 (Henchion et al., 2017). This increase in demand has caused concerns for sustainability and food security and has led researchers to find alternative ways to meet the protein requirements needed as the population increases. Additionally, many consumers cannot afford to purchase meat due to its high cost or in developing countries due to its limited supply (Fokou and Domnanagang, 1989). Therefore, further pressure is created to ensure that consumers in developed and developing countries have access to alternative protein sources. Thus, providing a better quality of life while minimising natural resources, which can jeopardise future generations' needs (Oslo Roundtable on Sustainable Production and Consumption, 1994).

1.1 Driving Factors for Sustainability and Environmental Change

The supply of protein for human consumption is critical, and the integration of a variety of new or alternative protein sources from both terrestrial and aquatic origin into new or existing processes/products, need to be explored. The necessity for ensuring sufficient nutritious, safe and affordable food to a fast-growing world population with changing dietary habits is becoming increasingly challenging. Brundtland's (1987) report 'Our Common Future' describes sustainability as "*development that meets the needs of the present without compromising the ability of future generations to meet their own needs*" (Brundtland, 1987, page 37) Whereas food security, as defined by the Food and Agriculture Organisation (FAO) of the United Nations Committee "*that all people, at all times, have physical, social,*

and economic access to sufficient, safe, and nutritious food that meets their food preferences and dietary needs for an active and healthy life” (FAO, 2009, webpage). Consequently, methods need to be reviewed to avoid sacrificing the environment for food security and nutrition by focusing on sustainable intensification (FAO, 2011; Mekonnen and Hoekstra, 2012). Increasing food production goes hand in hand with more efficient use of natural resources and reducing environmental impact. The research of Janssen (2018) and Malek et al., (2019) highlighted that consumers are focused on health-related concerns, which is a particular driver for pro-environmental and sustainable foods. While there are many different sustainable intensification strategies, this research looked at the use of cricket powder in cereal-based products. Particularly the emphasis on health benefits through the promotion of nutritional baked products. The current trends and trajectory for sustainable foods are worrisome. Firstly, the increasing number of people consuming more calories than they need for a healthy, active life. Secondly, rising numbers of people consuming more protein than required, and thirdly, shifting people’s consumption toward animal-based proteins.

1.2 Cricket Powder: Motivation and Innovation

In February 2022, the European Union (EU) recognised three insects as a novel food, particularly House cricket (*Acheta domestica*), mealworm (*Tenebrio molitor*) and buffalo worm (*Alphitobius diaperinus*) (European Commission, 2022). Insects are food for the future and are already part of the diet of 2.5 billion people worldwide. The FAO considers crickets healthy, nutritious, efficient, and sustainable food (Van Huis et al., 2011; Van Huis and OOninex, 2017). They are considered a source of protein and contribute to fat, minerals, vitamins, and fibre (Van Huis et al., 2011), while providing a resource for the food chain, especially for applications within human food. Consequently, incorporating crickets in the form of powders within functional food products has made a recent appearance in the Western diet as people are looking for a more sustainable food source (Global Market Insights Inc, 2015; Shockley and Dossey, 2014). While there is research regarding the inclusion of such protein powders within meat and sweet baked products (Skotnicka et al., 2021; Garcia-Segovia et al., 2020; Osimani et al., 2018; Nissen et al., 2020; Biró et

al., 2020), there is limited research regarding the development of new bread products ready for the consumer market.

Gahukar (2011) research discusses that crickets are increasingly promoted as the future meat, based primarily on their arguments of sustainability. Vermeir et al., (2020) identified consumers seek pro-environmental and sustainable benefits through their food choices. Moreover, consumers who demonstrate a pro-environmental choice experience a “*higher enjoyment of the accompanying consumption experience*” (Tezer and Boudur, 2020). This ‘green consumption effect’ is driven by increased perceived social worth, which endorses the ‘feel good factor’ adding value to the motivation. Furthermore, sensory appeal and taste are the influential drivers for the acceptance of novel products, such as insects (Renko et al., 2011; Lammers et al., 2019). This approach to marketing novel food products highlights how the consumers use sensory factors in selecting food choice. Moreover, Berger et al., (2018) research highlights one aspect to overcome this would be to consider the promotion of the health and environmental aspects to the consumer. Whilst hunger and poor health in developing countries exist alongside overconsumption and obesity in developed countries, this all underlines the importance of a global dietary rebalance. This would free up considerable resources, eradicate hunger and increase health, and ensure an adequate, sustainable food supply for future generations. Hence, an alternative nutritional food source must complement both the developing countries where food is scarce and developed countries where food is in abundance. Therefore, the driving factors identified in this research and reasons for change can provide solutions for developing countries and broader areas worldwide.

1.3 The Bread Market

Bread is one of the staple foods most widely used and consumed worldwide and is one of the major constituents of the human diet since ancient times. The Statista Research Department (2019) published that bread consumption patterns within the European Union had an average consumption of 50kg of bread per *capita* per year; between 2013 and 2018. In comparison, in Southern Africa, Zimbabwe consumes only around 18kg of bread per *capita* per year. However, it is reported, this is on a steady rise, on average, three percent growth per year. Although consumption of

bread in Zimbabwe is considerably less than the EU, this is due to the availability of the second staple carbohydrate – maize meal produced from milling dried maize.

Nevertheless, Zimbabwe was deemed the leading supplier of wheat to Southern Africa, especially between 1996 to 2003, as shown in figure 1:2. During this period the country produced around 320,000 tonnes of wheat and created demand for wheat derivative products. From 2004 onwards, farms were being overtaken by government officials during the political uprising, thereby decreasing wheat production in the country. However, the consumption of wheat products did not diminish, which created a drive to begin developing products using various grains.

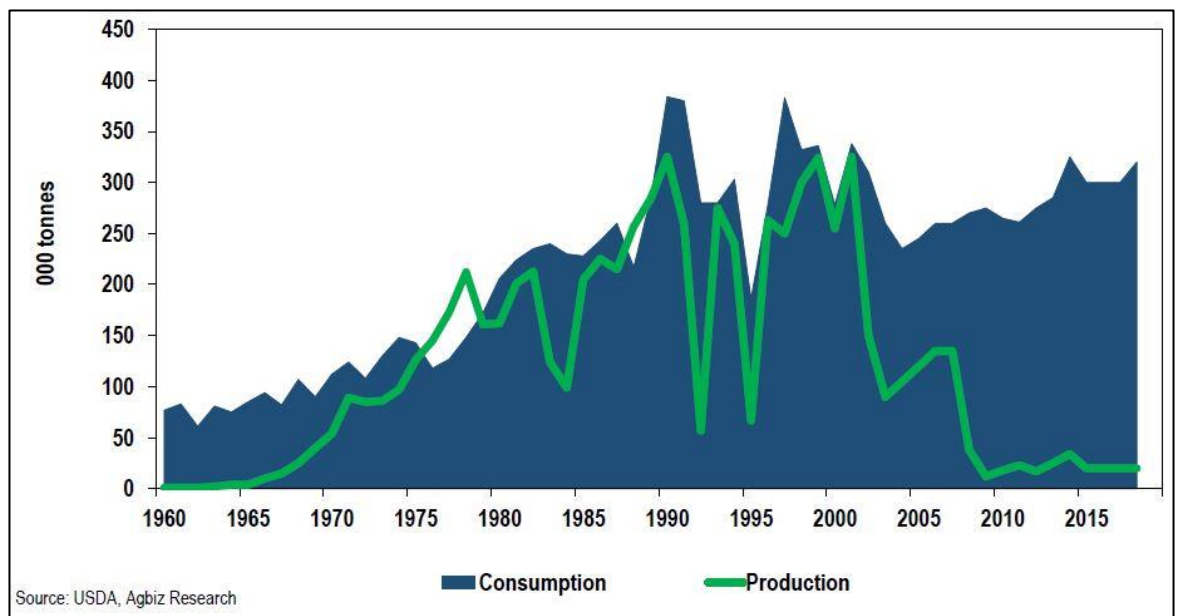


Figure 1:2: Zimbabwe's Wheat Production and Consumption

Source: Agbiz Agribusiness Research, 2018

1.4 Key Attributes of Bread Products

Several quality aspects need to be determined, to ensure bread products are viable on the market (dough rheology, aeration, gas cell structure, texture and sensory analysis). For instance, the most idealistic characteristic of bread products are the number of even and open distributed gas cells. These gas cells are originated during the mixing, in which gas pockets are formed, and manipulated throughout the breadmaking process to achieve these desirable characteristics. As bread is sold via weight, a product that has a fine crumb structure is deemed to be a more desirable commodity. Consequently, a larger volume loaf suggests it is lighter with

sufficient aeration, and less force is required to chew the product. Another critical attribute for bread products is the softness of the overall product associated with its freshness in the consumer's mind. This softness is dependent on the gas cell structure present in the product: an open crumb structure will produce a lighter texture; whereas a close crumb structure will produce a heavier, denser product.

Although bread products have evolved to take many forms, each is based on different characteristics depending on the country (baguettes in France, flatbread of the Middle East, steamed buns in Asia and sliced bread in Commonwealth countries). Through innovation and accumulated knowledge, bakers have adapted local bread varieties depending on the available ingredients to achieve the consumer's desired bread quality. Although there is a slight decline in the consumption of the preferred white sliced bread, consumers' are looking for a change, increasing their intake of alternative and energy-dense foods that are often enriched in carbohydrates and fat (Statista Research Department, 2019). Nevertheless, in recent years no matter one's nationality, there has been an increase in the awareness of the relationship between food and health due to the growing demand for healthy, natural and innovative foods.

Although the use of wheat flour (*Triticum aestivum*) for human consumption is rooted in tradition and is the dominant crop for breadmaking, there are additional minor 'small-grains' in Southern Africa that are gaining interest in the market. In particular, Khorasan- and quinoa- flours are derived from highly nutritious grains and are more suitable for healthy food production, especially for special dietary requirements. These small-grain cereals are a rich source of micronutrients (minerals) because the seed coatings are not removed before baking. Each cereal crop has specific growing advantages, particularly Khorasan and quinoa, which are heat- and drought-tolerant. For instance; fonio, a variety of quinoa, can produce grain within six weeks, thus providing food at the critical mid-season interval of harvesting. Meanwhile, wheat and Khorasan grains are high yielding under optimal conditions, and they are more adaptable and reliable in extreme weather temperatures or poor soil; hence they contribute to farmer resiliency.

1.5 Research Aim, Objectives and Hypotheses

This thesis research set out to explore and develop the viability of baked products fortified with cricket powder, which can be used as part of a balanced diet. By developing and promoting a prototype product fortified with cricket powder, ancient grains and mixed seeds, this can be marketed as a nutritional bakery bread with potential benefits. The formulation of ingredients would provide a high protein, -fibre and low in carbohydrate value product. This would entice consumers' as products may potentially be branded as either 'high protein', 'high fibre' and 'low carbohydrate' content (Millward et al., 2008; Rosenbloom, 2015). Products branded as 'high protein', 'high fibre' and 'low carbohydrate' content can attract consumers through the personae of low glycaemic index and substantial nutritional composition. The intake of ancient grains and cricket powder within a bread would provide more health benefits than refined wheat flour bread, commonly associated with coronary heart disease and Type Two Diabetes Mellitus (T2DM) (Blandino et al., 2003). Furthermore, incorporating cricket powder into a familiar baked bread product may reduce the sensitivity to 'food neophobia' and help consumers realise the potential of the nutritional value of edible insects.

1.5.1 Research Objectives and Hypotheses

This study aimed to investigate alternative ways to feed the world through the fortification of baked products using cricket powder, ancient grains, and seeds. With the movement to meet the Horizon 2020 societal challenge, this thesis looked at two main elements; hunger and health; which could combat the GHI of developing countries and dietary rebalance of Western countries. The schematic outline of the research hypotheses is summarised in table 1:1, which highlights the areas of concentration and the connecting hypotheses to each area. However, the specific objectives of this study are addressed as follows:

- To assess the doughs' rheological and enzymatic behaviour (water absorption, gluten development, viscosity, starch hydrolysis and retrogradation) when incorporating cricket powder within baked bread products. The desirable flour characteristics (protein and starch) would be identified through this analysis of rheological properties.

- To investigate the nutritional composition of baked bread products fortified with cricket powder which can lead to an increase in consumption of protein, fibre and fat within the consumers' diet.
- To evaluate the crumb structure and texture of baked bread products fortified with cricket powder. This determined the quality factors of the products' texture and staling rate, especially the association between fortification and its impact within the crumb structure.
- To investigate the overall acceptability of baked products fortified with cricket powder. Using recognised sensory analysis techniques, a preferential taste test determined the acceptability of baked bread products fortified with cricket powder.
- To evaluate the consumers' perception of, and behaviour towards, sustainable foods; for instance, cricket powder when incorporated into baked bread products. The worldwide survey was used to determine the acceptability and viability of new proteins within diets and the associated food neophobia levels. This looked at the corresponding results between continents to determine the drive for new foods fortified with insects.

	Null Hypotheses	Alternative Hypotheses	Source	Statistical Test Method
Theory One				
1	H₀: The additions of cricket powder and ancient grains within a baked product, would not affect the doughs' rheology behaviour (water absorption, gluten development, viscosity, starch amylase and retrogradation).	H₁: The additions of cricket powder and ancient grains within a baked product, would affect the doughs' rheology behaviour (water absorption, gluten development, viscosity, starch amylase and retrogradation).	Dubat (2013), AACCI 54-10:01 (AACCI 2010)	Univariate One-Way ANOVA
Theory Two				
2	H₀: The addition of cricket powder and ancient grains in baked products does not affect the macro- nutritional composition (crude fat, crude protein, ash, fibre and carbohydrate).	H₁: The addition of cricket powder and ancient grains in baked products does affect the macro- nutritional composition (crude fat, crude protein, ash, fibre and carbohydrate).	AOAC 2001:11 (AOAC, 2001) AOAC 2003.05 (AOACI, 2003) ISO 6884:2008 (ISO, 2008) ISO 712:2009 (ISO, 2009) AOAC 991.43 (AOAC, 1985)	Univariate One-Way ANOVA
3	H₀: Incorporating cricket powder and ancient grains within a bread product would not affect the glycaemic index.	H₁: Incorporating cricket powder and ancient grains within a bread product would affect the glycaemic index.		Univariate One-Way ANOVA

Theory Three				
4	H₀: The addition of cricket powder and ancient grains in baked products does not influence the crumb structure (brightness, crumb cell, volume and shape).	H₁: The addition of cricket powder and ancient grains in baked products does influence the crumb structure (brightness, crumb cell, volume and shape).	AACC 10-18.01	Univariate One-Way ANOVA
5	H₀: The inclusion of cricket powder and ancient grains in baked products does not affect the textural properties and the staling rate.	H₁: The Inclusion of cricket powder and ancient grains in baked products does affect the textural properties and the staling rate.	AACCI 74-09:01 (AACC, 1999)	Univariate One-Way ANOVA
Theory Four				
6	H₀: Participants' perception of sensory attributes (appearance, aroma and texture) of cricket powder and ancient grains within baked bread products is perceived negatively compared to a standard baked bread product.	H₁: Participants' perception of sensory attributes (appearance, aroma and texture) of cricket powder and ancient grains within baked bread products is perceived positively compared to a standard baked bread product.	Campden BRI (2018a), BS ISO 6658:2017 (BS ISO, 2017)	Crosstabulation and Chi Squared (χ^2)
7	H₀: Consumer willingness and food neophobia levels are not affected when insects are a direct food source for human diets.	H₁: Consumer willingness and food neophobia levels are affected when insects are a direct food source for human diets.	Menozzi et al., (2017) Tan et al., (2015), Caparros Megido et al., (2016), Rumpold and Schluter (2013)	Multivariate MANOVA Cronbach α , Chi Square 'goodness of fit' and Pearson correlation

Table 1:1: Hypotheses Developed Based on the Research Objectives

1.6 Thesis Requirements and Scope

Searches through scientific databases investigated research on baked bread products fortified with cricket powder. This revealed the overall absence of research using the novel ingredient within baked products, significantly the nutritional composition, dough rheology, crumb structure and sensory analysis. There are around two thousand different types of edible insects worldwide, which could help address the Horizon 2020 societal challenge. A preliminary study conducted in 2016 looking at different percentages that could be included in bread baked products and saw that 10-12% inclusion gave a good quality and open crumb structure (Petrie, 2016). However, as much as 25-30% could be incorporated into baked bread products; but at this level, the loaf volume, crumb structure and overall taste could be affected. Nevertheless, to achieve the high protein, fat and fibre required for diets

in Southern Africa, a higher percentage (around 30%) will reach the essential levels of micro- and macro-nutrients.

There was a similar issue with quinoa flour: 8-10% was required for good quality and loaf volume, but increasing levels beyond 30% reveals a bitter aftertaste. However, when blended with other flours, levels between 20-25% improves the grains' flavour. Furthermore, at this percentage the protein, fat and fibre are at their optimum levels ranging from 13-40% for protein, 4-9.7% for fat (at least twice as high in most cereals) and fibre ranging from 21-69%. Looking at fortified bread with quinoa- and Khorasan flour in the UK, most of these bread products either had 20-25% inclusion of the flours. The closest product currently found on the market was a Kamut® Khorasan wheat bread with quinoa grains, made from a high percentage of wheat flour for the sourdough. Subsequently, the inclusion of Khorasan- and quinoa- grains were incorporated for the flavour and nutritional values. Understanding how these ingredients (Khorasan-, quinoa- flour and cricket powder) contribute to creating aerated structures within baked products can help create a variety of products for all consumers while retaining the desirable characteristics of standard baked bread products.

As dough aeration and rheology are interdependent (Campbell and Martin, 2012; Chin et al., 2009), small changes in cell concentration can affect the final baked product (Lorenz and Coulter, 1991). Rheology is the study of how material flows and was used to characterise and predict dough behaviour at different stages of the breadmaking process (Dobraszczyk et al., 2001; Campden and Martin, 2012). Studying dough rheology is also crucial as sensory detection of texture depends on its rheological properties (Daubert and Foegeding, 2003) which changes throughout the breadmaking process (Dobraszczyk et al., 2001).

Dough aeration is measured in terms of gas cell distribution within the dough (Campbell, 1991). Gas cells in bread products are fundamental as they make up a large proportion of the products' volume, which affects the bread crumb and eating capabilities. Understanding the link between the cell size, distribution and properties of the baked products can improve existing products and develop new products fortified with cricket powder. In this thesis, the Calibre C-Cell (see Chapter Five, for

photos and operational use) was used to understand and compare cell volume, gas cell structure and slice area when incorporating cricket powder, Khorasan-, quinoa-flours in baked products. The C-Cell produces 48 measurements per analysis, and these are broken down into sub-groups to determine product quality in baked products. These categories are colour, dimensions, shape, cell size, elongation, inclusions, external features, and bread score. This thesis looked at the main element – ‘the cell’ size, which provided detailed information regarding cell- number, size and distribution throughout the crumb structure. The C-Cell software had a resolution limit near to 0.1mm^2 , therefore by using this method; individual holes, wall thickness, cell areas and volume were quantifiable.

This thesis’s scope looked at the following chapters; Chapter Two offers a background of the thesis. In this literature review section, it looked at three key areas, firstly the need for food security through bread products and entomophagy. This identified the global eating culture of entomophagy, including the innovation of products and culture shift promotion. The second key area looked at the breadmaking process, including individual functionality of the ingredients and their impact within the dough development. The final section discussed the ingredients impact, especially on the consumer GI response. This chapter also included the legal framework and food safety aspect of using novel ingredients within baked bread products.

Chapter Three provided an understanding of the flours used throughout the thesis to determine the doughs’ rheological and enzymatic behaviour. The Mixolab measured flour quality by exposing the sample to predetermined heating and cooling cycles while placing the sample under a strain field (Dubat, 2013). This analysis of both primary parameters (water absorption, mixing capabilities, gluten matrix, viscosity, amylase and retrogradation) and secondary parameters (protein weakening, starch gelatinisation and enzyme gradation) enabled the understanding of the effects of the hybrid flours including their properties and capabilities. The data was then collected as a set of stress-strain plots, which were then analysed via an algorithm for multigraph data structure analysis. Included in this section was an outline of the doughs’ rheological behaviour, which overall determined the dough’s elasticity and extensibility.

Chapter Four reviewed a comprehensive analysis of the nutritional composition aspects of the baked bread products. It covered characteristics such as crude-protein, fat, ash- and moisture- content which allowed the calculation of the total carbohydrates available in the baked bread product. While there is no obligation to declare dietary fibre content; this analysis allowed the results to be reported to enhance the nutritional composition data. The chapter assessed the general theory of the available nutritional composition in baked bread products fortified with cricket powder, ancient grains and seeds.

Chapter Five looked at the crumb structure, texture and staling of the baked bread products. The C-Cell analysed five main parameters: slice colour, gas cell size, cell volume, crumb structure, and elongation. Through these individual parameters, the overall characteristics of the baked bread slices were determined. The illumination system was designed to accentuate the cell structure on the surface of the bread slice. Included within this chapter, the moisture migration (cause of staling) of the baked products, was investigated through a texture profile analyser over seven days. This experiment looked at the staling rate, expressed as firmness and resilience, which is commonly used to determine shelf-life and elasticity of the baked products.

Chapter Six provided a comprehensive review regarding the results and discussion on the sensory analysis. Consumers were presented with two samples, in a preferential test, to determine which sample is most acceptable for appearance, texture, taste and overall preferred sample. Finally, a worldwide survey was conducted to determine the push and pull mechanisms of consuming insects as either agriculture feed or as a direct food source. Within this chapter, the food neophobia levels were analysed to determine the consumers eating habits' and their limit of consuming insects within foods.

Chapter Seven gave a general discussion drawing on all aspects of this research to ensuring that, holistically, all theories and hypotheses were resolved. These topics covered: the consumers' food choice, a drive in the decrease in animal-based products to meet the consumers' requirements and overall consumers' behaviour

and motivation. This chapter also explored the sustainability impact and how it could help countries globally, especially as some food supply chains are not resilient.

Chapter Eight concluded with recommendations for how research in this area can be taken forward and, more importantly, how this research would impact societal groups like developing countries. Chapter Nine provides all references and appendices regarding this thesis.

Chapter 2 – Literature Review: Viability of Cricket powder as a Fortificant in Baked Bread Products

In terms of sustainability and what we eat and what its footprint is on the environment and the consequences of eating one thing versus another, obviously, it makes a lot of sense to be eating insects. They're incredibly plentiful. They've got a very short turnover rate.
(Roach, 2010)

2.1 Introduction

The introduction of this thesis outlined in Chapter One, summarised the purpose of the research, to report findings of the novel ingredient 'cricket powder' as a fortificant in baked products. This included the nutritional composition of the ingredients and the interrelationship between aeration and dough rheology, which impacts the physicochemical properties of the baked product. Finally, consumer behaviourism and willingness to consume products fortified with cricket powder was identified through the sensory analysis and survey. This chapter provided an in-depth review of the current literature appertaining to this thesis and its scope. The literature review chapter covered food security, sustainability aspects, technological breadmaking methods, UK food laws, legislation and safety aspects concerning products containing cricket powder.

2.2 Theory of Planned Behaviour and Perceived Behaviour Control

There have been different models of consumer behaviour developed over the past three decades to understand what encourages and motivates individuals to engage in pro-environmental behaviours or make sustainable consumption choices generally. These models are primarily based on the Theory of Planned Behaviour (TPB) and Perceived Behaviour Control (PBC), which proposes that people are generally familiar with their food choice and tend to have some element of planning in their behaviour choice (Ajzen, 1991). The TPB works via the individual consumer's attitude towards the subject, which does not determine behaviour but influences behavioural intentions, thereby shaping the consumer's actions. However, actions are also influenced by attitudes, social 'normative' pressures, perceived behavioural control, barriers and self-efficacy (Ajzen, 1991). Figure 2:1

shows how one of the most significant barriers to promoting sustainable food consumption is the so-called 'gap' between attitude - intention and behaviour (Vermeir and Verbeke, 2006).

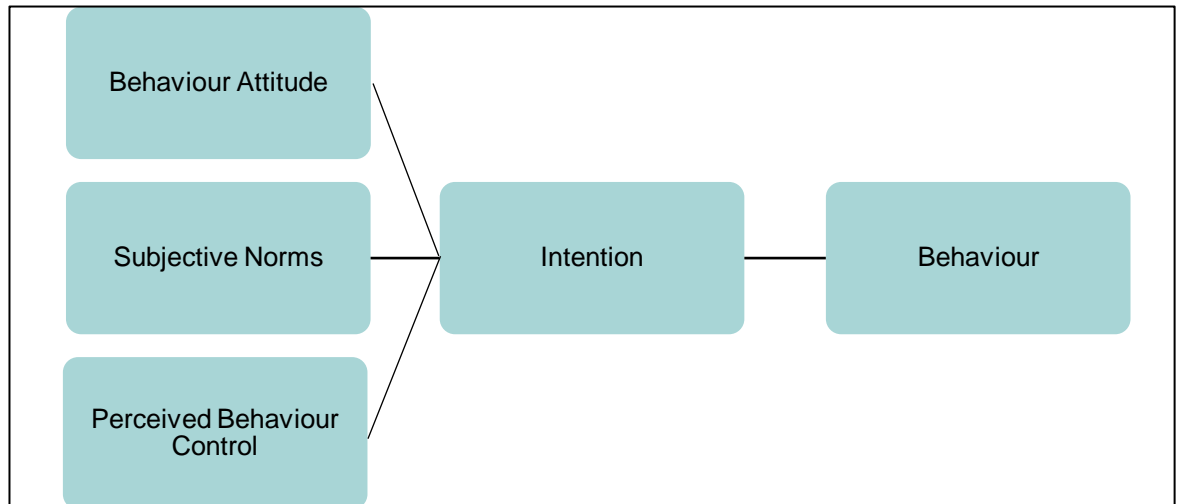


Figure 2:1: Adapted from the Theory of Planned Behaviour

Source: Ajzen, 1991

In the past, this theory has been applied to food categories to predict and explain the relationship between food-related behaviours and food consumption, more specifically relating to food choice. Ajzen (1991) and Menozzi et al., (2017) research outlined the necessity to achieve all three constructs (behaviour attitude, subjective norms, PBC) to attain the intention and overall behaviour choice. However, Ajzen's theory outlines that if one of the constructs is rejected by the consumer, the intention of the product and overall behaviour becomes null and void. The psychosocial determinants of this approach/theory are only relevant to the individual's beliefs, which can be analysed to help define targeted interventions. The individual consumer's intention captures the motivational factors that influence the overall behaviour and the PBC, which accounts for the variance in the actual behaviour. The TPB has been widely applied to predict intentions and behaviour in many fields throughout the food industry, especially in the New Product Development arena.

Conversely, within Ajzen's theory, the three primary constructs and the intention towards the planned behaviour can generate an overall 'gap'. This associated gap can integrate different disciplinary perspectives that can elucidate the complexity of consumer acceptance. One limitation with the TPB theory is the variables that factor

into behaviour intention, such as past experience, fear, threat, or mood (LaMorte, 2019). Depending on the consumers' circumstances or cultural up-bringing; the individual's experience, cultural exposure or willingness to change can be affected by other broad intersecting external factors; for example, education, socio-culture and contextual factors.

Some consumers are not aware of the environmental impact of meat production around the world. As consumers mainly purchase meat through supermarkets, the focal point has shifted to the belief that 'supermarkets' are the food producers. Likewise, the willingness to change meat consumption behaviour through reducing or substituting meat in consumer diets is low (Hartmann and Siegrist, 2017). This is due to the ideology that consumers shop in supermarkets where food is always in abundance. Therefore, the psychological factors of food -knowledge, -provisioning and eating behaviour is impacted by availability and practicality (Tan and House, 2018). Some studies interpret sustainable foods as an individual's intention, based on personal values, moral norms and internal ethics, which overall determines the actual behaviour (Arvola et al., 2008). From a cultural point of view, food protein is essential for the human body, and the source can vary from country to country. For instance, Western countries do not eat insects, even though they are a good protein source. Due to the consumer perception and the 'disgust' factor, the ideology that insects are either found on or feed on decaying matter negativity deters the consumer.

Furthermore, Western consumers' food habits and eating model indicates meals are generally not eaten because consumers are hungry, but due to the time of the day. Thus, leading to overconsumption of food commodities which leads to obesity and health problems. Conversely, consumers in developing countries are often restricted in their food purchasing and therefore consume food as an essential commodity and may sometimes be limited to only eating once a day. Given the novelty of cricket protein flour and the unpractised behaviour in developed countries, the food behaviour and the eating model effect was incorporated within Ajzen's TPB. The demand from side factors (changing consumers perceptions) and supply-side factors (visual perception and accessibility of the products) is essential for gaining consumer acceptance (Tan and House, 2018). This model's new approach is based

on three additional constructs; these include contextual-, social- and practical-factors. Whereas Ajzen's TPB model requires all three constructs (behavioural attitude, subjective norms and PBC) to allude to the consumers' intention and overall behaviour.

Based on the three-additional social norms constructs, the younger generation is predicted to increase 'willingness' to consume new foods. This is more predominant with younger males accepting newer food ideas, for instance, insects within familiar food products. Petrie (2016), study correlates these findings where the negative attitude or perception towards foods containing insects was higher in the age range 35-55 years old. On the other hand, Hartmann et al., (2016) provided evidence that the Western culture opinions were strongly dependent on the visibility of the insects themselves. This correlates with Gmuer et al., research (2016), where 109 participants were asked to rate the likelihood of consuming three types of processed insects in tortilla chips. The first sample was a combination of both ground crickets and maize; the second sample was made with larger grainy ground cricket granules and maize; the third sample was made with plain maize tortilla chips mixed with whole crickets. The consumers preferred the first sample where the crickets were ground into a powder and incorporated as an ingredient. Sixty three percent of the consumers showed a positive attitude towards the first sample, demonstrating that consumers are more open to insect products if the ingredient is processed and less visible.

Though, in both Hartmann and Gmuer research, this only looked at the attitude towards consuming the novel ingredients. With a probability of 'one in three' and the only choice being one of the three samples, consumers would be more inclined to choose the sample where the insects were disguised within a food product. Menozzi et al., (2017) outlined that the TPB consumers' attitude was reduced when presented with food choice and intention. The attitude construct in correlation to the intention mainly reduced due to food choice, which has a similar taste to the known product. This was considered the most critical predictor by the consumer when considering 'dietary intention'; the PCB construct intends to be the significant predictor in 'dietary behaviour'.

Additionally, higher predictive power in the intention was identified when consumers were educated through contextual-, social- and practical- factors when consuming insects as a novel ingredient. Therefore, this correlation between 'belief and intention' intersecting with the three additional constructs can identify cricket powder potential within baked products (see Chapter Six). This new way of introducing novel ingredients within Western dietary patterns can foster compatibility with consumer food culture and enable adopting novel foods into diets.

2.3 Food Security: Benefits of Bread and Entomophagy

2.3.1 Cereal and Grain Production Worldwide

An estimated 925 million people are currently deprived of food worldwide, and a further one billion who are not hungry suffer from 'hidden hunger' from not obtaining enough vitamins and minerals in their diets (IFPRI, 2016). Addressing the current levels of food insecurity worldwide, the population will rise to an estimated nine billion people by 2050, there is undoubtedly a need for new sustainable food resources. Since the Second World War, the UK and Europe's food policy has focused on developing the capacity to increase food output and ensure adequate food distribution. This was to address malnutrition and hunger while providing a constant supply of affordable food (East Midlands Rural Affairs, 2003).

However, even though cereals and grains are vitally important and are consumed worldwide, they are an affordable commodity that forms part of everyone's diet. The Department of Health recommends a third of the calorific intake should be starchy carbohydrates (bread, pasta, potatoes or rice) and cereals which are a good source of nourishment. As previously mentioned in Chapter One, the country where one resides determines what type of carbohydrates are readily available (steamed buns in China, flatbread in the Middle East, sliced bread in Commonwealth countries). Nevertheless, bread products are becoming popular, and with the growth in sales for healthy bread, options are on the rise in Europe and Southern Africa (Statista Research Department, 2019; Agbiz Agribusiness Research, 2018). This increase in the consumption of bread products can be seen by the growth in production/yield of cereals and grains. Figure 2:2 shows the worldwide yield of grains, cereals and

pulses. Although rice has the highest grain yield worldwide with over 45,000 hectograms per hectare, wheat followed by over 35,000 hectograms per hectare.

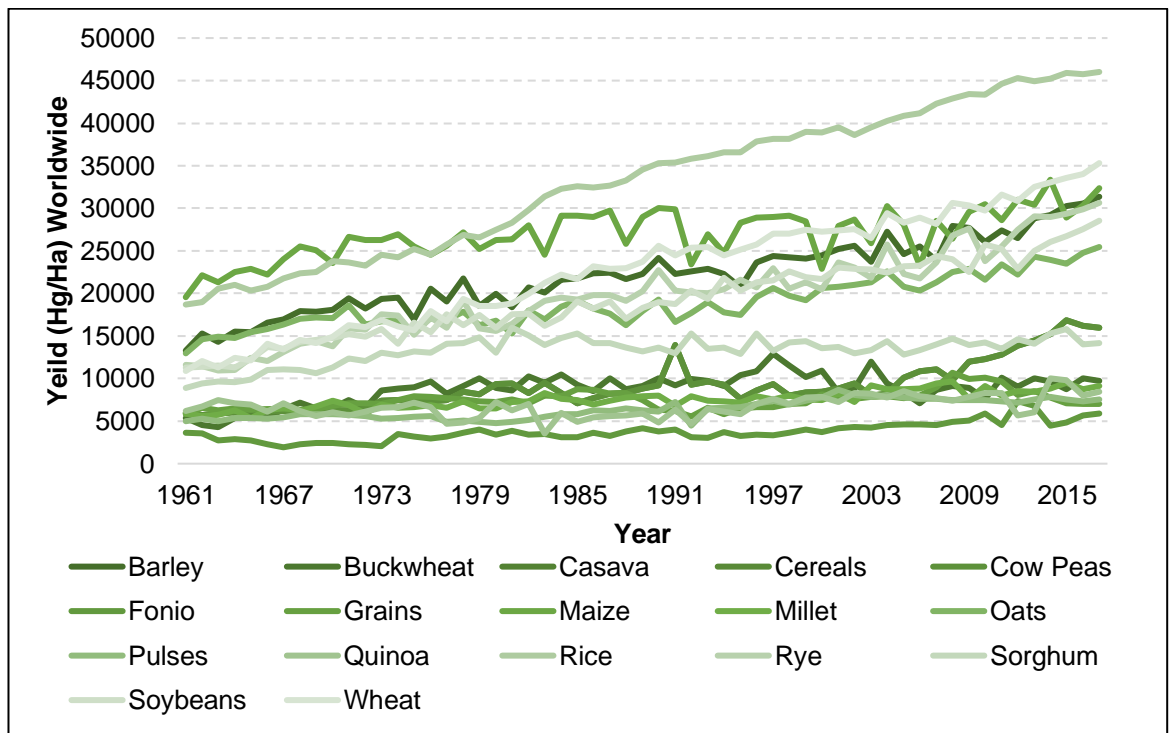


Figure 2:2: Worldwide Production of Grains, Cereals and Pulses

Source: FAOSTAT, 2019a

Analysing this further, looking at the production of grains and cereals in Africa, it can be determined that wheat is the primary source of carbohydrate produced due to its viability and multifunctional use. Figure 2:3 shows the yield of grains and cereals produced in Africa. With wheat dominating the primary grain production, this equates to nearly 75% of the entire world's wheat production (over 26,000 hectograms per hectare). While maize, general cereals and fonio (quinoa variety) respectively produced just over 20,000; 15,000 and 7,000 hectograms per hectare.

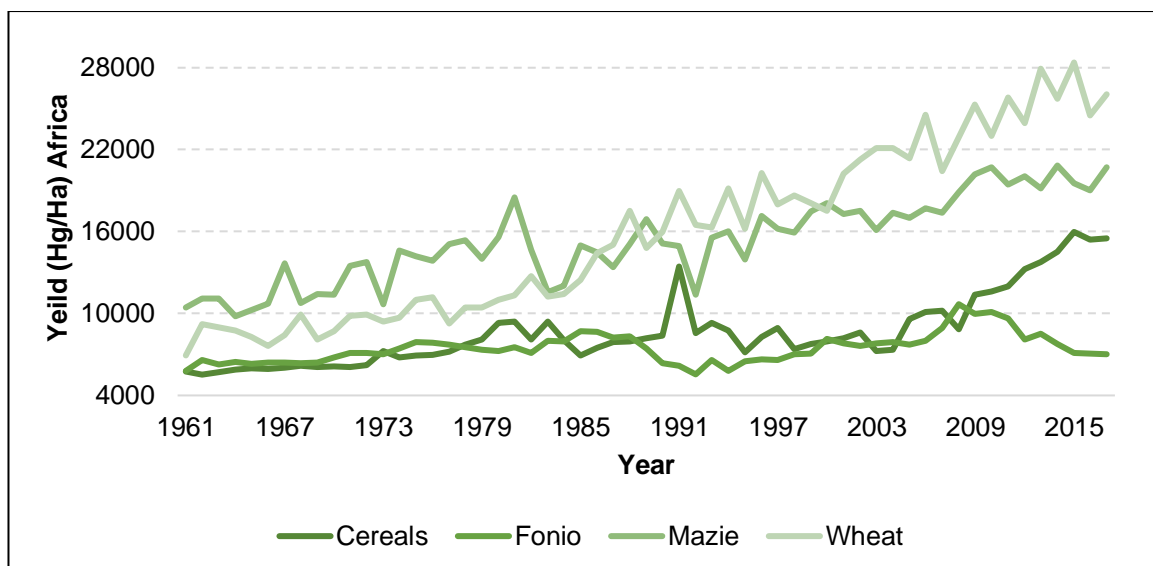


Figure 2:3: Africa Production of Grains and Cereals

Source: FAOSTAT, 2019b

Edible insects form part of the traditional diets of at least two billion people. More than 1900 species have been reported to be used as food throughout the world; with the majority located in South America, Africa and Asia. However, insects are more than just a food source; they deliver a host of ecological services that are fundamental to humankind's survival. They are a good source of protein, fibre, fats, vitamins and minerals; and provide a variety of valuable products and functions for humans such as honey, silk, medical procedures¹ and red colouring from cochineal beetles. Insects as food and feed are emerging as the pioneering method to feed the nation, especially within the twenty-first-century due to environmental pressures. With an increase in protein costs, population growth, food insecurity in natural disaster areas and developing countries, the consumption of insects or entomophagy contributes positively to the environment, health and livelihoods around the world. This research explored using grain and cereal resources located

¹ The chemical structure of insects has inspired a wealth of life-changing medicine procedures and an alternative to modern therapy. Honey is valued as an antioxidant and antimicrobial agent, suitable for consumers whom are battling heart disease and skin disorders. Venom from bees and wasps, have also shown to be an alternative medicine for inflammation and cancer therapy patients. Crickets located in Africa are used for natural treatment against scabies, asthma, eczema and urine retention. In some cases' the desert locust has found to be rich in sterols and therefore used as cardiovascular protection. These same species have also been found to contain anti-inflammatory and anticancer agents (pancratistatin, narciclasine and ungeremine) which can be used to treat lymphocytic leukaemia cells and certain brain tumour cells. More commonly in the UK, patients who have had plastic or reconstructive surgery are prescribed leech therapy to ensure that the new skin has sufficient blood circulation. As leeches remove any blood clots and ensure that blood flows within the small vessels to prevent the tissue from dying.

in Southern Africa, by incorporating natural raw ingredients (insects). Thereby clarifying the potential use of insects could offer improved food security worldwide, thus, adhering two of the Horizon 2020 societal challenge objectives.

2.3.2 Promoting Insects as a Sustainable Food Source

The environmental impact of food production is increasingly being brought to the forefront of sustainability, particularly concerning reducing carbon dioxide emissions (CO₂) and methane gases (Dobermann et al., 2017). The consensus is that the most significant contributing factor to global climate change is from the greenhouse gas emissions (GHGs), which are predominantly CO₂, nitrous oxide and methane. The agriculture sector contributes the most to GHG emissions with livestock accounting for 18% of the CO₂ (van Huis and van Itterbeeck, 2013; Sachs, 2015). On the other hand, insects are promoted as food which emits considerably fewer GHGs than most livestock. Figure 2:4 shows the carbon dioxide, energy and land use for the production of rearing livestock, sub-derivatives (milk) and insects.

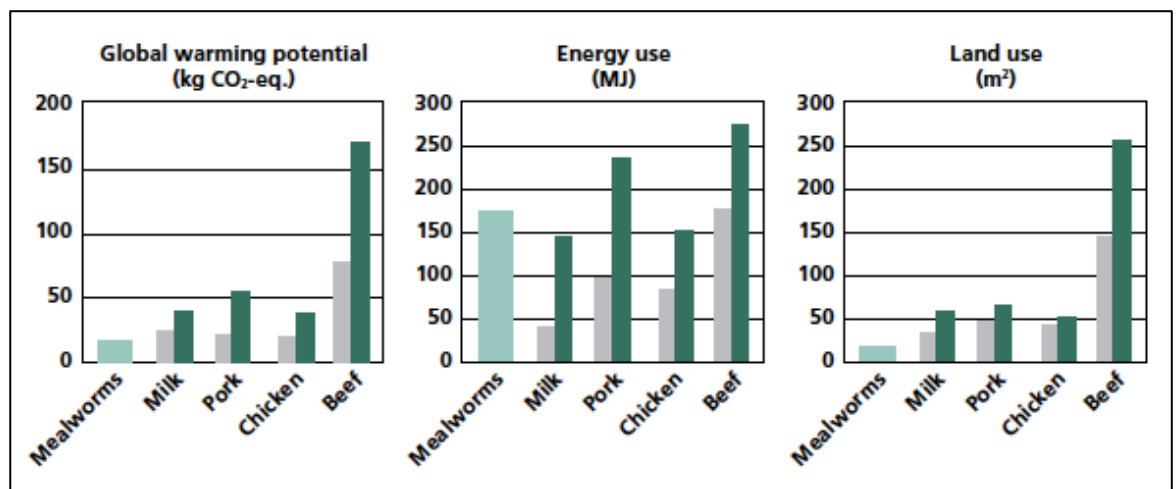


Figure 2:4: Production of Carbon Dioxide Equivalents during the Rearing of Commercial Livestock, Sub-derivatives and Insects

Source: Oonincx and de Boer, 2012

Nevertheless, other critical environmental factors are often ignored, for instance; water and land use (Dobermann et al., 2017). It is predicted that by 2025, at least 1.8 million people will be living in regions with inadequate freshwater supplies, and two-thirds of the global population will be in areas under pressure from dwindling water resources (FAO, 2012). Freshwater is a finite resource, of which an estimated

70% is used by livestock and agriculture industries (Doreau et al., 2012). Agriculture uses water directly to grow crops and indirectly to grow fodder for the production of livestock.

Land availability is an issue that frequently arises in the discussion of sustainable agriculture. As the demand for meat grows, the producers are increasing pressure to farm more livestock, which requires more land. The increase in livestock requires more feed, which leads to farmers increasing the amount of land being cultivated, which usually involves deforestation and an increase in fertiliser. Figure 2:5 shows the maximum documented levels of land, feed and water use for the three main groups of commercial livestock (cattle, pigs, chickens) and the two insect species (crickets and mealworms).

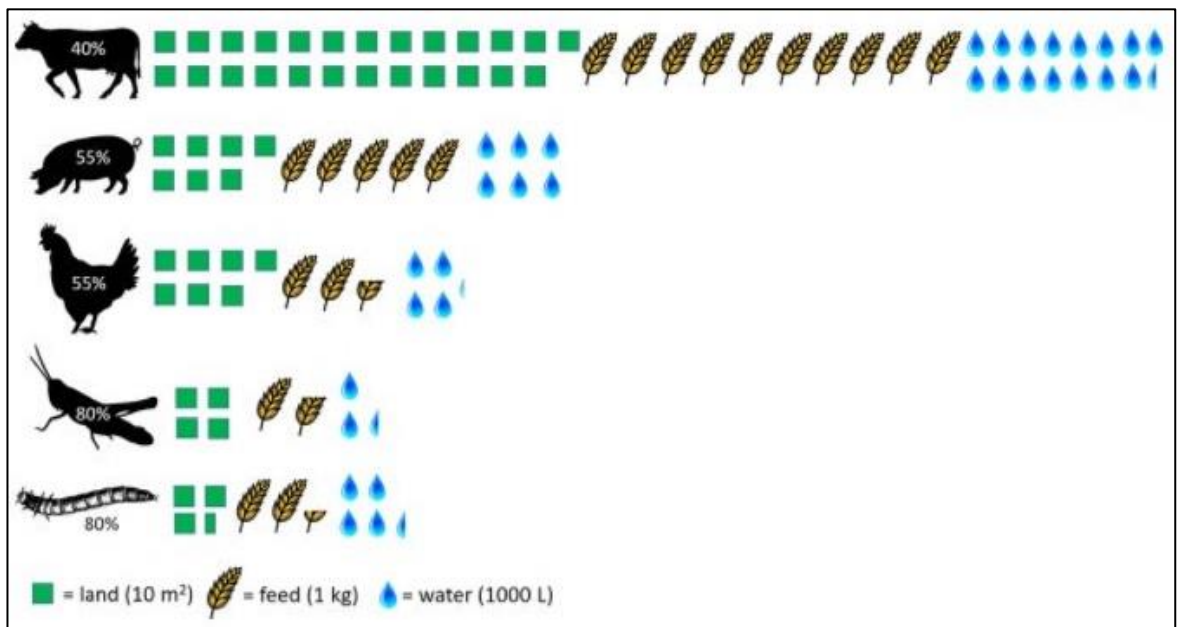


Figure 2:5: Amount of Land, Feed and Water Required to Produce 1kg of Live Animal Weight

Source: Dobermann et al., 2017

In addition to the environmental and nutritional benefits of feeding insects to humans, there are also links to consumer livelihood, especially the economic benefits. South-East Asia is the only continent that has capitalised on the consumable insect trade, where there are established farms and trade routes mainly from Thailand. The import and export of insects for food play a vital economic role throughout South-East Asia: in Thailand, this alone is valued at 3 million USD/year (FAO Thailand, 2013). Figure 2:6 shows the market values for various other staple

food sources. Given that the market value for insects often exceeds that of other standard protein sources, insect farming can provide a stable income for established farmers. The estimated value of insects as food and feed for the combined market for Europe, South-East Asia and South America is predicted to grow to 523 million USD/year by 2023. This growth is predicted to be primarily driven by increased consumer awareness and acceptance of insects as food (Global Market Insights Inc., 2015). In South Korea, the current market was estimated at 143 million USD/year for 2017 alone. This is set to quadruple to 457 million USD/year due to the increased acceptance from the global community that insects play a vital role in global food security (Han et al., 2017). This presents an excellent opportunity for new products to be developed to enhance the consumer market with growth and predictions.

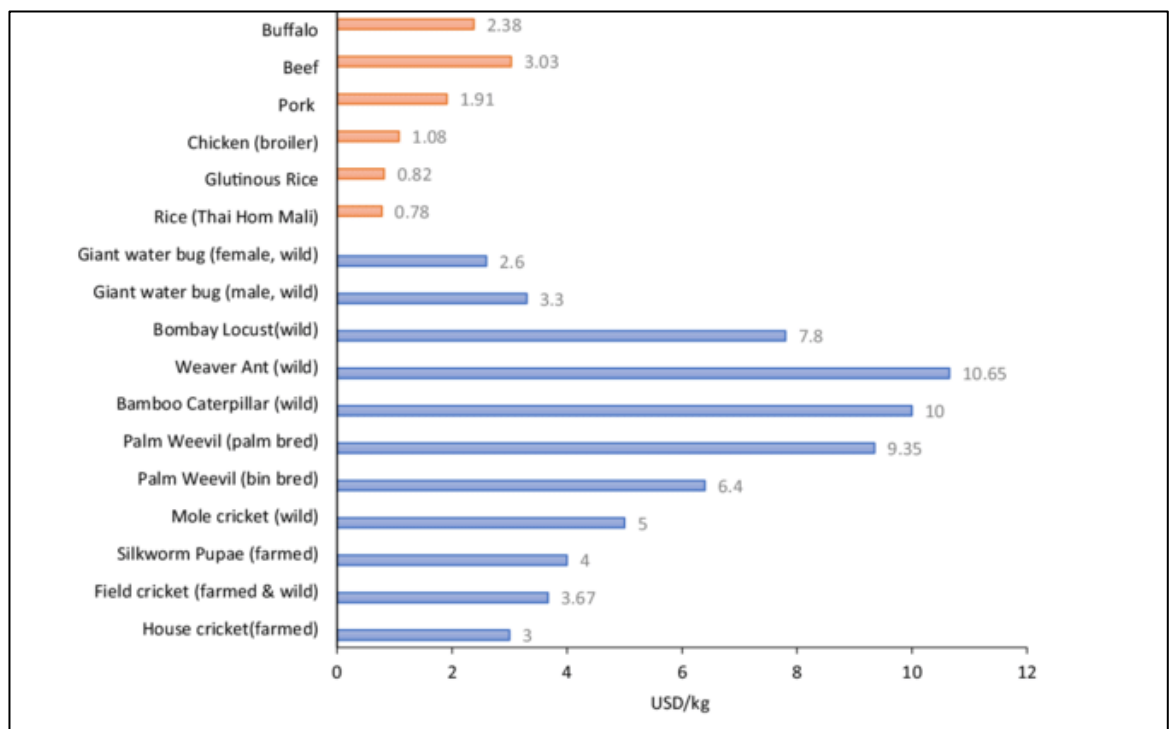


Figure 2:6: Market Value of Insects and Staple Foods in Thailand

Source: FAO Thailand, 2013

2.3.3 Entomophagy Global Eating Pattern

A report published by the United Nations Food and Agriculture Organisation titled *Edible insects: Future Prospects for Food and Feed Security* (2013), discusses the need for entomophagy around the world. Although this is already practiced within

developing countries (Africa, South America and Asia), some European countries are starting to follow this trend through the modernisation of entomophagy. Insects are promoted as a nutritious and sustainable food source, and as such researchers and companies, are encouraged to consider them as a primary protein source. Anthropologist Harris (1998), argues his theory on optimal edible insect foraging and why these smaller food sources are passed-up as they are relatively small and widely dispersed. He continues with his theory, commenting that edible insects will only be consumed in regions rich in insect fauna. In this case, insects should be widely consumed in Europe, North America and Russia where the fauna is vibrant and offers habitat for various insect species.

Jongema (2017) compiled an inventory of all edible insect species only using their Latin names provided by the number of literature researches available. However, several difficulties were encountered: humanity would be inclined to describe an insect by its *Linnaean Nomenclature* name, making official figures and estimates difficult. This becomes slightly more complicated when many different cultures use variations of the same insect species' vernacular names. Figure 2:7 shows the number of edible insects per country.

The overall outcome of the research found that over 1900 edible species are listed worldwide as of April 2012. Regional figures have been published as follows:

- 250 edible species in Africa, (van Huis, 2005)
- 549 edible species in Mexico, (Ramos-Elorduy, 2005)
- 170 edible species in China, (Chen et al., 2009)
- 164 edible species in Lao, Myanmar, Thailand and Vietnam, (Young-Aree and Viwatpanic, 2005)
- 428 edible species in the Amazon, (Paoletti and Dufour, 2005)

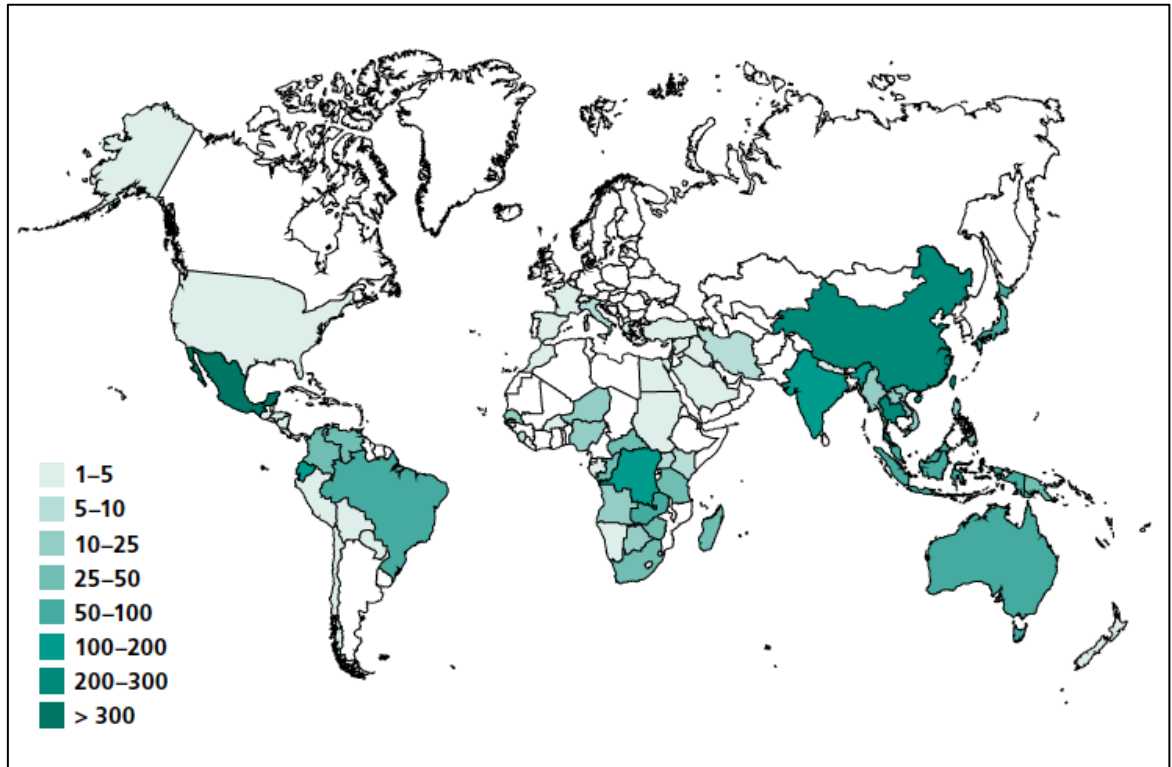


Figure 2:7: Recorded Number of Edible Insects per Country

Source: Jongema, 2017

With many edible species around the world, there are six common commercial edible insect species at present; crickets' (*Acheta domesticus* and *Grylodes sigillatus*), honey bee (*Apis mellifera*), domesticated silkworm (*Bombyxmori*), mopane caterpillar (*Imbrasia belina*), African palm weevil (*Rhynchoporus phoenicis*) and the yellow mealworm (*Tenebrio molitor*). Some types of insects are typically consumed in larva form due to the fat content (*I. belina*, *R. phoenicis* and *T. molitor*), which can provide additional flavour (Tang et al., 2019). Compared with other commercial species, crickets are particularly easy to rear and harvest with a typical turnaround rate of 43 days from egg to the di-pulse phase. Consuming insects is becoming a healthy and trendy solution to eliminate food poverty and hunger. They play an essential role within various food systems, and they are a vital source of essential nutrients for developing countries and protein-energy malnourished regions.

2.3.4 Promoting Cultural Shift Towards Entomophagy

Contemporary food production is becoming global and industrialised in which products are standardised no matter what time of the year. Due to genetically modified (GM) foods and technology (AI and AGI), the advancements in food production have increased the efficiency rate of plant productivity for human consumption. Consumers are impelling supermarkets to stock seasonal foods throughout the year, and therefore no longer benefit from seasonal products. However, consumers knowledge of seasonality and regional supply has withered over the years due to supermarkets stocking all-year-round foods. There is also an increase in farms selling their output directly to complex supermarket supply chains, which has caused a shift in the market where consumer trends have changed both culturally and environmentally from locally produced to pre-packed foods from other countries. Consumers have become increasingly estranged from food production, where consumers were previously focused on slow grown organic produce. Even more disconcerting, it has been noticed in the European food market that bifurcation is taking place between health variety and consumer value products. The health variety is marked at a notably higher price, whereas the consumer value products are relatively cheaper, and often processed with high fat and sugar (Eswaran et al., 1999).

Nowadays, sustainable food styles need to fit into people's lifestyles, especially being 'feasible', 'available', 'affordable' and 'accessible'. Defining sustainable food, which is safe, healthy and nutritious is essential and needs careful planning to meet the demands of consumers at a country level and push towards a global scale. This includes environmental welfare limits in production and processing for affordable food in all sectors of society. Therefore, sustainable food can provide a viable livelihood for the food industry hierarchy such as farmers, processors, retailers and consumers. With an increase in drought and worries about food waste management, this has an adverse effect within the food and drink industry, mainly in supply and production. From a recent publication by Mintel (2016), it was reported that sustainability and new product development needs to be considered for the common good. Several companies have launched conservation initiatives; from finding a different alternative use of oil, to new sources of protein and food waste

management - in which food that would have been thrown away is now used to create new foods or prepared for the less fortunate.

There is also an increase in new food trends depending on the social, economic and demographic areas, and through consumerism, there is a continual paradigm shift within the food industry. Food habits are also shaped by evolution, cultural tradition, fashion and physiological needs where personal experiences and exposure to availability or accessibility impact social consumerism. Baumgartner and Steenkamp (1996), suggested that culture has a significant impact on consumerism behaviour towards food products in any given market. The research continues to outline that consumers who share similar social status, education, religion and occupation are bound to have similar beliefs, norms, values and behaviours that are uniform. Therefore, the Baumgartner and Steenkamp research advocates the clustering of consumers into groups representing different 'nutritional or food styles' so that they can be targeted by social marketing messages. However, Sogari et al., (2017) advised that a consumer's perceived control over the ability to eat a product containing insects is a weak determinant of intentions. The constraint is mostly influenced by the incompatibility with the local food culture, therefore, lacking these products on the Western culture market.

Tan and House (2018), found three aspects relating to consumer acceptance, especially in novel foods such as insects. It was reported that food consumption practices of consumers should be designed around conventional vegetarian products. This allows for limited knowledge on preparing and cooking novel foods, for instance, if they are already in the shape of burger patties. Consequently, consumers do not require new culinary skills or reconfiguration of cooking practices. In contrast, motivation and repeat consumption of novel foods can be addressed through the contextual- and social factors. House (2016), has shown that nutritional and environmental value are motivational factors. However, this did not mean that the consumers were ready to consume the product and expressed that social factors needed to be considered (taste, appearance, availability). This is particularly important when assessing the willingness and repeatability of the consumers' purchase.

Finally, consumer perception and psychological impact factor would determine the experience of the consumer. Knowledge and repeatability of the product are essential, and these two aspects go hand in hand with the consumer's experience. Positive and negative experiences influence the interpretation and evaluation of insect as food, where positive experiences can lead to repeat consumption (House, 2016). Thus, the accumulation of encouraging experiences towards novel ingredients creates learning associations within the cultural context and generates a new way of preparing and presenting food.

2.3.5 Consumer Acceptability of Entomophagy

A recent study by Caparros Megido et al., (2016) looked at the consumer acceptance of insect-based alternative meat products in Western countries. The study highlighted that meat substitutes are relatively low, principally due to food neophobia and poor sensory qualities compared to meat products. To reduce insect food neophobia, it was suggested that food samples containing insects as a meat alternative should be invisible, and more importantly, associated with known flavours. Lensvelt and Steenbekkers (2014) research suggested to reduce insect neophobia; the first possible solution is to educate consumers on culture, nutritional and ecological issues associated with entomophagy (Mignon, 2002; Verbeke, 2015). A second solution is to increase edible insect exposure and experimental tasting (Caparros Megido et al., 2014). People who have already eaten insects have significantly more positive attitudes toward entomophagy and are more willing to eat insects in the future. (Lensvelt and Steenbekkers, 2014; Caparros Megido et al., 2014).

However, during the hedonic evaluation of the products in Caparros Megido's research study, it was noted that taste is an essential factor in the acceptance of novel food products. Effectively, the willingness to try a new food product with limited taste experience are more likely to depend on the participants level of interest and their neophobia levels, than on their expectations about the sensory experience. Therefore, it is vital to develop products familiar to consumers' sensory aspects (sight, smell and taste), consequently increasing the participants' engagement for sustainable protein sources. Furthermore, Caparros Megido's research provided

details of consumers' habits, including information vital to this research. The 2016 research provided data stating that out of the 159 participants who took part in the study, ~70% of the participants were convinced that the population, especially Westerners, were ready to include insects (principally minced or powder form) into their diets. This is in contrast with the studies undertaken by Vanhonacker et al., (2013), de Boer et al., (2013) and Verbeke (2015), who suggest that the potential for the introduction of insects as food is currently is non-existent.

2.4 Processing Cricket powder for Human Consumption

As previously mentioned in 2.3.3, crickets are one of the most common commercial edible insect species available to consumers due to its versatility. The addition of insects in powder form makes it possible to introduce insects into the diet without altering habits. The powder also meets the needs of both the consumer and the food industry by enabling the production of various products without majorly affecting their texture or functional properties. In food applications, functionality refers to the properties of ingredients that contribute to the flavour, texture, stability, and appearance of the final food (Kristinsson and Rasco, 2000); resulting from their solubility, emulsification, foaming, and gelling characteristics.

Figure 2:8 shows a flow chart detailing the processing method for cricket powder (*Grylloides sigillatus*) and mealworms (*Tenebrio molitor*), see Appendix A for CCP flow chart for processing *Grylloides sigillatus* and *Tenebrio molitor*. The most efficient method to create insect flour is through drying, in which the crickets are first washed and boiled in water to remove any pathogens and bacteria present on the species. These were then dried at 110 degrees Celsius for 120 minutes. The drying method stage reduces both the moisture present and water activity within the product, from a microbiological point of view, this guarantees a longer shelf-life. The crickets are assessed for quality purposes and then progressed onwards to the milling plant, where they are ground down into flour before being packaged and shipped. By milling the whole cricket ensures that the flour retains all their essential components, i.e. protein, fat, vitamins, minerals and chitin.

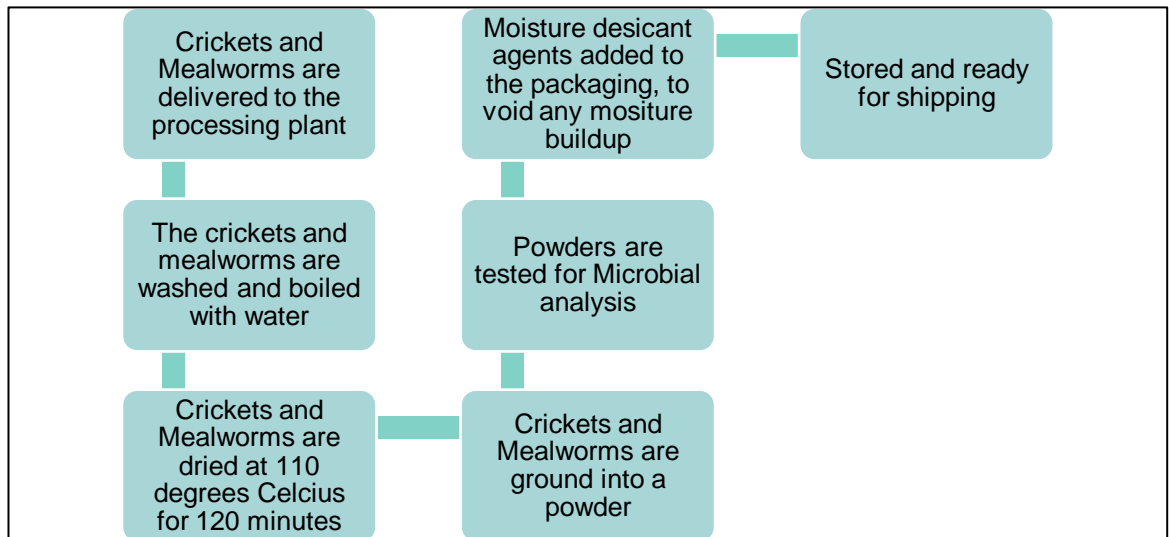


Figure 2:8: Processing Method for Crickets (*Grylloides sigillatus*) and Mealworms (*Tenebrio molitor*) as a Food Source

Source: Goldin (Entomo Farm), 2018

Chitin content in insects can widely vary between different insect sources (Jones, 2020). However, as this is ingested with other micro-and macronutrients available within the insect powders, it is promoted as a health benefit to consumers. The high chitin content found within all dried insects (around 10%) can be a good source of dietary fibre in the human diet (Imathiu, 2020). Additionally, chitin can impact the gut microbiota, which has been reported to improve gastrointestinal health due to its prebiotic potential (Selenius, Korpela and Gallego, 2018). When consumed in foods, chitin and its derivative (chitosan) are the functional dietary fibre that can reduce LDL-cholesterol levels in the blood (Choi et al., 2012; Megido et al., 2018). Consumption of chitin has been shown to improve glucose intolerance, increase insulin secretion, relieve dyslipidaemia, and protect intestinal integrity and the gut microbiota (Zheng et al., 2018). Chitin and Chitosan also appear to have antiviral and antifungal activity and antimicrobial properties. Thus, the inclusion of insects within food products has the potential health benefits for the consumer and is a suitable addition to baked products to decrease mould activity.

2.5 Methods of Breadmaking

All of the breadmaking processes (bulk fermentation, Chorleywood bread process and sour starters) have evolved for production on a large scale and have a single common aim: to convert flour into an aerated baked product. Several common steps

across all three processes are required to achieve this conversion, outlined in this subsection. The three main differences of the breadmaking stage are the different fermentation processes (long, short, bulk, bacterial – yeast vs sourdough). However, the dough processing is usually the same across the board: mixing, kneading, air incorporation and the creation/development of the gluten structure. Cauvain (2015) describes the dough development as a term that covers several complex changes in bread ingredients when the individual ingredients are incorporated together (mixing). These changes are associated first with the formation and development of the gluten network, through the hydration of the flour's proteins and the energy transferred through the kneading process. Through this process, the necessary changes to the physical properties of gluten are made, especially its ability to retain carbon dioxide gas which will be later generated during fermentation by yeast or sourdough bacterial cultures.

During the breadmaking process, there are four physical properties of the dough which need to be fundamentally understood; resistance to deformation, extensibility, elasticity and stickiness. The first three physical properties relate to the doughs' formation, especially during the kneading stage of the breadmaking process as the dough is stretched and developed over a short period. The molecular structure of the gluten-forming proteins contains glutenin and gliadin which, when wetted and developed form the gluten network, and is termed as a visco-elastic material. Although the dough is not usually measured during this kneading process, its rheological properties are used as guidance, influencing the final baked product structure (Cauvain, 2012). For instance, dough that is processed immediately post-kneading is commonly less extensible and more elastic than dough that was sufficiently rested between processing stages: this dough becomes more extensible and less resistant to deformation. This can enhance the finished loaf structure and texture, giving a larger and more open crumb structure once it has finished baking. The final physical property (stickiness) can cause processing problems due to the dough sticking to surfaces it encounters. Over a period of time, and especially in larger highly automated bakeries, the sticky dough tends to smear onto the surfaces of processing equipment and can impede the dough's progress through the machinery. Thus, the most desirable dough should have the ability to retain gas bubbles and permit the uniform expansion of the dough piece, under the influence

of carbon dioxide gas expansion during the yeast fermentation, during proofing, and in the early stages of baking.

2.5.1 Bulk Fermentation Doughs

This method of dough development is the most common and traditional/natural method of breadmaking. Before bulk fermentation, there is usually a low-speed kneading step which can be carried out either with hands or low-speed mixing machines. Throughout the process, this method is the most homogenous of the three groups, and essentially the variations are confined to the fermentation and controlling the yeast temperature or level (Cauvain, 2015). However, there are only a few essential features of the bulk fermentation process which can be summed up as follows:

- Mixing the ingredients to form a homogeneous dough.
- Rest the dough for a period of time, this is known as bulk fermentation. The yeast level, temperature, flour quality, and variety of bread being produced will determine fermentation time.
- Partway through the bulk fermentation, the dough is 'knocked back' and folded left to right, right to the left and top to bottom. Through the knock backstage, the dough is degassed of any carbon dioxide gas formed during the fermentation. Stretching the gluten-forming proteins through the folding method encourages the disulphide bonds within the dough to cross-link between the high molecular weight subunits and polypeptide chains.

2.5.2 Chorleywood Bread Process

The Chorleywood Bread Processes (CBP) basic principle involves mixing and developing the dough in a single operation lasting between two and five minutes at a fixed energy input. This optimised mechanical dough development with the addition of oxidising agents and pressure/vacuum technology gives a uniform cell size, which helps with the bread volume. The mechanical processes of the CBP are yet to be fully understood. However, researchers Chamberlain and Collins (1985) advise that the high energy input mechanically breaks the disulphide bonds in the gluten network and increases the molecular structure's oxidation (Cauvain, 2015).

The CBP process was designed in 1961 (post World War Two), due to the limited strong bread flour available and the consumer looking for a better-looking bread compared with the national wartime loaf. The main difference between the CBP and bulk fermentation process lies in the dough's rapid development in the mixer rather than through a prolonged resting period (Cauvain, 2015). Therefore, through this process, the dough's protein structure improves and can stretch (extensibility and elasticity) and retain gas from the yeast fermentation. However, arguably, a growing population would prefer cleaner labels (no oxidising agents) when producing bread products. The other concern with such processes is that the machines are expensive to purchase and operate. These are not available to all countries, therefore standard mixers and bulk fermentation processes are more suited.

2.5.3 Sour Starters and Levains

Sour starters and levains have a long history and are based on spontaneous fermentation through the symbiotic relationship between bacteria and wild yeasts (Cauvain, 2015). The microflora, fermentation conditions and ratio of the raw materials used in the recipe will determine the differences in the sour's functionality and the overall flavour within the baked product. Baked products with sour starters and levains incorporated in them have a distinctive flavour due to the ratio of acetic to lactic acid produced by the available *Lactobacilli* within the sour starter, and this acidity will also have an impact on the rheological behaviour of the dough. For instance, this can influence the flavour profile, creating a specific crust and crumb characteristic (Autton, 1982). However, the sour starter's metabolic vigour can impact the bread structure; and these types of bread usually are denser compared with those produced by bulk fermentation.

Most common sour starters are formed from water and flour, and usually at a ratio of 1:1. However, this can be amended depending on the type of flour and bread product. For instance, rye or wholemeal flour will need a higher ratio water percentage to ensure that the bran, glutenins and gliadins present in the flour are fully hydrated. Depending on the location in which the sour starters and levains are produced, can determine which bacteria are present within the culture. Furthermore, the fermentation conditions can be adapted to encourage the dominance of homo-

fermentative and/or hetero-fermentative bacteria in the culture. Thus, the available bacteria within the culture can confer different characteristics to the sourdough and therefore, the finished product.

Country	Product	<i>Lactobacillus</i> Homo-fermentative	<i>Lactobacillus</i> Hetero-fermentative
France	Wheat Levain	<i>Lb plantarum</i> <i>Lb casei</i> <i>Lb paraalimentarius</i>	<i>Lb brevis</i> <i>Lb hammesii</i> <i>Lb spicheri</i>
Germany	Rye Levain	<i>Lb acidophilus</i> <i>Lb farciminis</i> <i>Lb casei</i>	<i>Lb fermentum</i> <i>Lb fructivorans</i>
Italy	Panettone	<i>Lb plantarum</i>	<i>Lb fermentum</i> <i>Lb sanfranciscensis</i>
Denmark	Rye Levain	<i>Lb amplivarus</i>	<i>Lb reuteri</i> <i>Lb panis</i>
USA	Wheat Levain		<i>Lb sanfranciscensis</i>

Table 2:1 Examples Common *Lactobacillus* Strains by Country

Source: Craft Bakers Association. Autton, 1982

Recently different *lactobacilli* strains are spreading to different countries, for instance, the popular *Lb sanfranciscensis* culture strain was recently detected in Spain. Some researchers posit that climate change may be responsible for this cross over in individual countries' strains (Autton, 1982; Cauvain, 2015). Each microorganism present in the microflora mix has an optimal set of conditions in which it works best; including temperature, hydration, evolving pH during fermentation, length of fermentation time and the temperature at which the fermenting sourdough is stored. Moreover, the basic principles of the sour culture rely on symbiotic fermentation between bacteria and yeast; where there is also a competitive relationship. If one organism's storage conditions are favoured more than the other, this can induce multiplication of the favourable organism and limit the potential of others.

2.6 Types of Flours for the Breadmaking Process

2.6.1 Wheat Flours

As previously discussed, protein is a vital component of the wheat grain and determines the wheat quality and nutritional content of the wheat cereal, which considerably influences the breadmaking process (Mills et al., 2003; Amieur et al., 2002). The gluten-forming proteins (gliadins and glutenins) found within the molecular structure are an essential component of the breadmaking process. The

gluten-forming proteins' level and quality depend heavily on the wheat variety, agriculture practices, and environmental effects (Cornell, 2012). As a result, a higher level of gluten-forming proteins present in the flour will result in a larger loaf. This correlates with the gluten network's capability to retain and trap carbon dioxide gas during the breadmaking process, including the proving stage. Efficient hydration and kneading of the dough during mixing will result in more gas cells formed within the bread, and through this formation, create an open crumb structure while providing a lighter, less chewy texture.

Subsequent further studies by Shewry and Jones (2012) have shown that gluten-forming proteins can be separated into two fractions. These fractions are vital as they have functional significance, with the glutenin being mainly responsible for gluten and dough viscosity and elasticity; the gliadins for plasticity and extensibility. Gliadins are soluble in alcohol-water mixtures (for instance 60–70% ethanol), whereas the glutenin is insoluble and remains present. However, the two fractions of these gluten-forming proteins are structurally related. The differences in solubility resulting from their presence as monomers that interact by non-covalent forces (gliadins), or as high molecular mass polymers stabilised by inter-chain disulphide bonds. When present as reduced subunits, the glutenin proteins are also soluble in alcohol-water mixtures; therefore, by defined together with the gliadins as prolamins. The ratio of gliadin to glutenin proteins in dough and gluten is generally about 1:1. Although this ratio may vary with the wheat's genotype and growth conditions, it will result in different effects on dough strengths (Doekes and Wennekes, 1982; Graybosch et al., 1995; Vereijken et al., 2000; Johansson et al., 2001).

2.6.2 Ancient Grains

2.6.2.1 Khorasan Flour

Interest in KAMUT® Khorasan flour has increased among the minor cereals; and is derived from the ancient hybrid wheat *Triticum -durum* and *-polonicum*. In the early 1900s, Khorasan wheat was cultivated on a small scale, and only in 1977, when two farmers in the USA decided to cultivate this ancient grain on a larger scale, its popularity started to increase. In 1990, the grain was registered as a protected

cultivated variety – KAMUT® Khorasan flour. However, in Africa and Asia where the wheat can be found naturally, this is cultivated on a small scale and is preferred by low household incomes due to its higher nutritional content, minerals, vitamins, amino acids, lipids and dietary fibre (Balestra et al., 2015).

Nevertheless, Khorasan wheat is becoming popular as it was perceived as a more natural, tasty and healthy ingredient within breadmaking industry. One possibility that the ingredient was adopted within the breadmaking industry is its organoleptic characteristics, and as it is considered a highly nutritional grain. Thus, the addition of this ingredient within the bread can have a considerable positive effect, especially on the texture, flavour and nutritional value (Clarke et al., 2004). Balestra et al., (2015) found that Khorasan bread showed good sensory properties and loaf volume, resembling bread created with hard wheat. It also indicated that Khorasan bread had acceptable sensory features (appearance, consistency and flavour), and resulted in the highest total phenolic, flavonoid and crude fibre content (Sumczynski et al., 2015).

2.6.2.2 Quinoa Flour

Quinoa (*Chenopodium quinoa*) grains and other cereals that do not contain gluten-forming proteins are known as pseudocereals. Pseudocereals are not part of the Poaceae family but are nutritionally similar and are comparable to wheat cereals (Lutz and Bascuñán-Godoy, 2017). The use of such cereals and grains within breadmaking presents significant challenges as these flours do not produce a viscoelastic dough that can retain gas and expand (Duodu and Taylor, 2012). Quinoa is an endemic crop originally from the Andean region which has been recognised as a nutritious grain as it contains sufficient quality and quantity of proteins, and essential fatty acids (Stikic et al., 2012; Wolter et al., 2013). According to the National Academy of Sciences, the minerals present in quinoa meet all ages' daily requirements for iron, copper, magnesium, and manganese (James, 2009). Some of these minerals' bioavailability is questionable due to anti-nutrients, such as phytic acid and saponins (Nascimento et al., 2014). In general, it is possible to classify quinoa as a source of vitamin E, riboflavin, folic acid and thiamine (Taylor and Parker, 2002). Furthermore, the high biological value means that a high

percentage of the proteins (15% protein with a broad amino acid profile) presented in the seed can be absorbed and utilised by the body.

Recently, through gene DNA sequencing, a similar variety has been discovered in Africa known as fonio. This grain has been growing for over 5000 years and is possibly the oldest cultivated cereal in Africa (Idowu, 2017). Fonio cereal can currently be grown and maintained in temperatures ranging from -8 to 38 degrees Celsius and is not impacted by low moisture (Sousa and Raizada, 2020). Quinoa and fonio cereals are drought tolerant and desirable for poor soils, and the growth cycle/turnaround rate makes these grains an ideal crop for many of the countries in Africa.

The fortification of pseudocereals within breadmaking can additionally improve overall bread nutritional profiles. Rizzello et al., (2016) researched the incorporation of quinoa within baked goods. It was reported that baked products fortified with 25% quinoa flour resulted in a soft crumb and overall good consumer acceptability. Rizzello suggested that a more considerable percentage increase in quinoa will result in a bitter taste and increased staling rate and lower consumer acceptability. Further studies by Stikic et al., (2012) identified that quinoa's addition at 25% improves the rheological behaviour. This suggests that the optimum amount of quinoa fortification is 25%, which can enhance the nutritional profile of the baked product and improve the dough rheological behaviour as previously mentioned in the above sub-section 2.6.1.

2.7 GI Response and Index within Baked Products

Glycaemic response (GR) is the change in the blood glucose concentration induced by ingested food. Meanwhile, the glycaemic index (GI) is the difference in the glycaemic response of a food product in relation to the response generated by pure glucose (100%). It is typically measured as the area under the curve of glucose concentration in blood following food ingestion. There are three classes which products fall into; low GI (>55%), medium GI (55-70%) and high GI (<70%) (De Angelis et al., 2009). Low GI and rich fibre foods are linked to lower heart disease, diabetes, obesity, colorectal cancer and breast cancer. As starch is a polymer of

glucose and, together with the disaccharide sucrose (consisting of glucose and fructose) and lactose (glucose and galactose), makes up most of the carbohydrate consumed in the human diet. The patterns of postprandial glucose and insulin responses elicited by sucrose and fructose differ substantially from those following the digestion of starches. The GI and insulin index (II) values for glucose are significantly greater than those of bread, whereas those for fructose are less than bread. Jenkins et al., (1988) found that starch in bread is rapidly digested and absorbed, producing a high glycaemic response. However, the incorporation of whole- and ancient- grains, especially Khorasan wheat, within the breadmaking procedure can reduce the glycaemic response due to high content of phenolic and carotenoids which have a positive effect on the body function in humans (Kitts and Weiler, 2003). Furthermore, sourdough fermentation has been reported to improve starch's nutritional properties related to the glycaemic response (Iiljeberg et al., 1995). The beneficial effect of sourdough fermentation on glycaemia has already been proposed by Bjorck et al., (1994) and Jenkins et al., (1986), but in both studies, it was impossible to discern between the effect of sourdough fermentation and other factors also able to potentially influence glucose response such as dietary fibre and starch accessibility.

However, new research has come to light contradicting the evidence of whole grains and sourdough with reducing GI in human health (Korem et al., 2017; Biesiekierski et al., 2019). The main argument linking whole grains and sourdough fermentation with reducing GI is poorly documented as there is a large variability in bread types that stems from different bread ingredients, including additives. Moreover, in sourdough production, the strains of the sour cultures are not identified and may vary between countries, let alone bakeries. Matthan et al., (2016) and Zeevi et al., (2015) recently published papers regarding the high interpersonal variability in the postprandial glycaemia response (PPGR) and therefore disregards different studies and the effects of bread consumption on human physiology. However, a significant factor associated with the variability in PPGR's is the host gut microbiome which could contribute to human health and risk to obesity, glucose intolerance, insulin resistance and metabolic complications (Korem et al., 2017; Sonnenburg and Backhed, 2016). Korem's research showed that the participants PPGR response to both bread samples (white loaf bread and sourdough bread) were identical during

the GI testing. This indicated that no matter what type of bread product was consumed, there was no difference in the GI results. Furthermore, when analysing the GI results and gut microbiome data, a correlation allowed the research to predict the GI response through the gut microbiome. While exact mechanisms for the gut microbiome and GI control effect are yet to be elucidated, predicting and analysing microbiome can bear clinical significance.

2.8 Legal Framework Using Novel Ingredients

On 31st December 2015, a new regulation came into force, covering novel foods for consumption within the EU. This regulation aimed to improve the controls that both businesses and manufacturing organisations need to comply with when they process and innovate novel foods to introduce into the EU market. In the previous regulation, whole insects did not fall within the Novel Foods Regulation scope, whilst the consumption of parts of the insect did. This was due to the wording of the regulation which captured products isolated from insects. The whole insect could be ground or milled into flour, which would not be classed as a novel ingredient. However, this changed with the wording in the newly agreed EU Regulation. The new regulation also clarified that the whole insect, if not consumed to a significant degree before 15th May 1997, will fall within the definition of 'novel foods'.

Moreover, Member States across the EU have different interpretations of the existing Novel Foods legislation; in Finland, Luxembourg, and France the importation, selling, marketing or rearing of whole or processed insects for use as food is, at present, forbidden. Whereas Belgium tolerates a list of ten insects and the Netherlands tolerates three (FSA, 2016). The UK currently allows the transition of insect products and commodities onto the new system via application and authorisation of the novel product. However, the supply of 'small quantities' of primary products can be sold directly to consumers or local retail establishments. This falls under the exemption legislation and registration of supplying consumers, but the company must ensure that the insects supplied are food grade and safe to eat (FSA, 2016). Such as insect farms, establishments have to be registered with the authorities to ensure that the company is following the correct procedures and are not illegally rearing insects. Firstly, this is to ensure that the product does not

pose a safety risk. Secondly, consumer information is not misleading, especially not replacing another food product of differing nutritional value. Thirdly, the insect cannot differ from a portion of food that may be intended to replace, such that consumers are nutritionally disadvantaged. Fourth, insect farmers need to ensure that the country's natural biological diversity is not put at risk, from the potential escape of a non-native species of insects into the environment.

2.9 Food Safety Aspect

There are concerns of practising entomophagy from the general public, but this can be said for most other kinds of foods consumed and available within this country. Conventional commercially viable products can have adverse health effects on consumers. Constant articles and reports on existing and new food sources express concern regarding health and eating habits, which negatively affect consumer behaviourism and acceptance. It is unclear regarding the extent of the insect species that are toxic or can cause potential problems. Conversely, wild-harvested insects could be exposed to vast amounts of pesticides and other toxins in the local areas. Whereas insects reared and bred on trusted conventional farms and given clean food and water are less likely to be contaminated with pesticides, chemicals or even fungi spores.

Klunder et al., (2012) identified that high-temperature treatments, for instance, boiling and drying, could reduce the number of viral pathogens found within insects. However, subjecting the insects to such high temperatures can negatively impact and decrease the quality of the vitamins, minerals and nutrients. The other control of the pathogenic activity is through the chitin (exoskeleton mass of the insect), which was found to have potential antimicrobial activity within food (fish, clinical and food-borne pathogens) (Bulet et al., 1999). The chitin available within the cricket powder could extend the shelf-life of baked products and therefore tested within Chapter Five (shelf-life and texture analysis).

2.10 Summary

There is an increasing interest in the consumption of insects as an alternative protein source around the world. A constant drive to encourage consumers to accept and retain insects as a nutritional form of protein is an ongoing drive. While most

Western consumers react to novel foods with disgust and rejection, the literature on consumer acceptance outlines positive impact methods used within society. Resistance to entomophagy is mainly based on the consumers' attitude, subjective norms and perceived behavioural control. Consequently, three integrations of different disciplinary perspectives elucidate the complexity of consumer acceptance, which predicts their willingness to eat novel foods.

For this reason, the additional constructs are added to Ajzen's model due to the demanding factors and changes in consumers perception. The intersection of the social-cultural, practical and contextual factors indicated a positive impact on the consumer and reduced their food neophobia levels. Thus, enabling companies to determine the consumers' initial motivations and repeated willingness to eat insects, can help predict the consumers' intentions. Additionally, the food system around the world is a significant driver of human and animal welfare. The social aspects of foods, especially sustainable novel foods such as cricket powder, offer potential solutions to end hunger and improve health following the Horizon 2020 goals.

Reviewing the individual flours incorporated within the breadmaking process determined the characteristics of the overall product. The microstructure of baked products plays an essential role in consumer choice and behaviour, where bread products are purchased for their different qualities. The cell size and distribution impacts on the shelf-life and texture qualities of the product. Large open crumb structures found in ciabatta can stale faster than a standard loaf tin bread due to the alveoli's increased surface area from which moisture evaporates. Faster moisture migration and evaporation would increase more rapid starch retrogradation. The challenge presented for the baking industry is the allowable use of cricket powder, ancient grains and seeds to be incorporated within bread products in such a way as to be acceptable to a consumer. This cost-effective ingredient can provide a staple commodity with a high nutritional value for human consumption worldwide. Moreover, a bread product fortified with cricket powder can be presented as a sustainable food ingredient. Therefore, proceeding with this thesis, individual chapters looked at the unique properties of bread products developed with cricket powder in terms of the rheological behaviour, nutritional composition, shelf-life, texture, sensory and worldwide survey.

2.10.1 Thesis Phases Developed Based on Theoretical Concept

Experimental Phases	Description	Source
Part 1 – Dough Rheological Behaviour and Enzymatic Analysis		
Rheology and Enzymatic Analysis (Mixolab2)	The Mixolab is a standard procedure for research and development that allows academics and scientists to measure the dough's rheological behaviour that is subject to both kneading and temperature. This application allows the complete characterisation of flour blend (water absorption, protein, starch gelatinisation, stability, starch retrogradation).	Chopin (2017), Collar et al., (2014), Novotni (2009), Mohamed et al., (2006)
Part 2 – Nutritional Composition		
Kjeldahl Protein analysis	Nitrogen-based method for detection of crude protein determinations	Kjeldahl Method – AACC 30-25.01 (AACCI, 2001)
Soxtec Crude Fat Analysis	Soxhlet extraction is one of the most commonly used methods for determining total lipids in dried foods. This is because it is relatively simple to use and is the officially recognised method for a wide range of fat content determinations.	Soxtec Extraction for Crude Fat – ISO 20483:2013 (BSI ISO, 2013)
Ash Content Analysis	This allows the determination of ash present in foods which applies to all human and animal products.	ISO 6884:2008 (BSI ISO, 2008)
Moisture content Analysis	This method allows for the determination of the moisture content of cereals and cereal products.	ISO 712:2009 (BSI ISO, 2009)
Crude Fibre Analysis	There is no obligation to declare dietary fibre content within food; however, to determine the products' energy and carbohydrate content, this needs to be established. Therefore, the baked product's total dietary fibre will be determined to establish the carbohydrate content and identify how much fibre is available in the product, which can affect the crumb structure.	AOAC 991.43 (AACCI, 1985)
Overall Carbohydrate analysis	This protocol looks at the overall carbohydrates found in the product. It summarises the other nutritional composition (protein, fat, fibre, ash and moisture) deduced from a base of 100. This gives an overall percentage of carbohydrates available in the product, and therefore the calorie content can be determined.	
Part 3 - Crumb Structure, Texture and Staling Analysis		
Crumb Structure: Calibre C-Cell	Crumb structure characteristics of bread and other baked products are traditionally measured subjectively. In this method, the C-Cell imaging system uses image analysis software to measure baked products' crumb structure characteristics. The monochrome system takes a side-lit image in 256 grayscale and applies software algorithms to determine the internal crumb structure parameters.	AACCI 10-18.01
Stable Micro System TA.Tx	Texture Profile Analysis (TPA) is a widely used test based on double compression of a material between two flat plates. For bread it is widely used because it mimics the biting and	AACCI 74-09:01

Plus Texture Analyser	chewing action of the jaw by compressing the sample, allowing it to recover briefly and then compressing again	
Part 4 - Sensory Analysis and Consumer Perception		
Acceptance of insects as food	Edible insects have attracted Westerns interest in recent years due to their nutritional and environmental advantages. However, consumers remain aversive towards non-traditional classification food (insects). Therefore, the exploration into consumer perception and individual experience contributes to the acceptance and rejection of novel foods within Western Society.	Tan et al., (2015), Caparros Megido et al., (2016), Rumpold and Schluter (2013)
Food Neophobia Determination	This aims to measure the intention and behaviour of eating novel food products containing cricket powder. In this instance, the promotion of consuming cricket powder products provides consumers with essential nutrients (proteins) with a low environmental impact.	Menozzi et al., (2017)
Sensory evaluation	Given the lead time associated with product development, robust but rapid and cost-effective product evaluation approaches are required. Most customers approach to use perceived visual differences. Therefore, a 'paired Comparison Test' will allow the consumer to compare and detect the samples' apparent sensory difference or similarity based on the defined criteria.	Campden BRI (2018), BS ISO 6658:2017 (BS ISO, 2017)

Table 2:2 Experimental Phases Developed Based on Hypotheses and Theoretical Concept

2.10.2 Flowchart Showing the Experimental Phases of the PhD Study

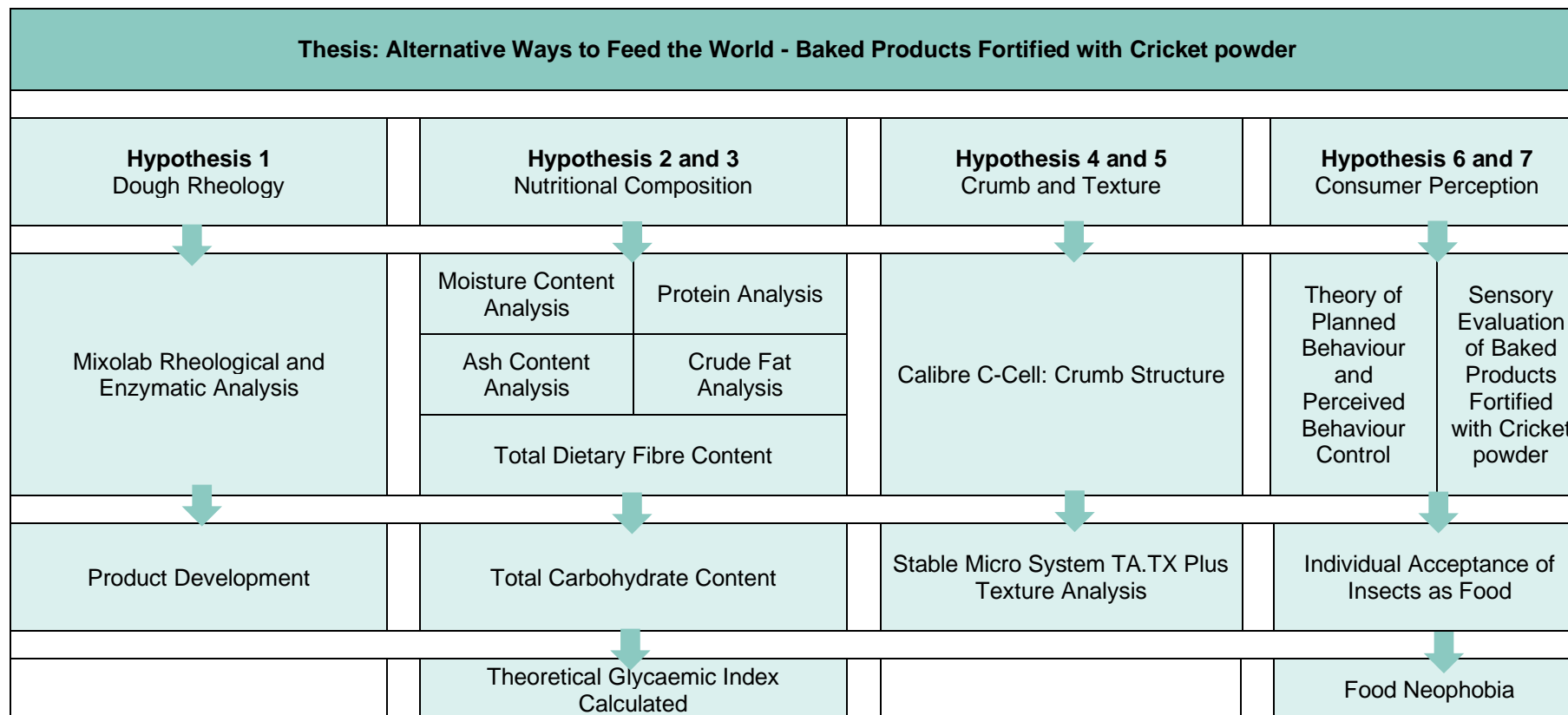


Figure 2:9: Flowchart Outlining the Experimental Phases of the PhD Study

Chapter 3 – Dough Rheological and Enzymatic Behaviour

From black pudding to pickled jellyfish, beauty lies in the eye of the beholder. What we see and taste as beautiful depends largely on what our family and friends approve of; with just a little room for personal preference.

(Mound, 1988)

3.1 – Introduction

The rheological and enzymatic behaviour of flours depends on the structure and ingredient formation (gluten network), fundamental for rheological and gas retention. It is vital to understand and observe the behaviour of doughs' made from wheat, pseudocereals and cricket powder, by examining their rheology after water absorption. Bread doughs exhibit a viscoelastic behaviour which is a combination of both the viscous fluid and elastic state. Gluten and starch have an impact on the breadmaking process and determine the final product characteristics. However, the baked product's quality depends on the flours' molecular structure, which impacts the nutritional content, volume, crumb quality and texture of the bread (later discussed in Chapter Four and Five). This chapter looked at the fundamentals, including the mechanical and molecular properties to test the null hypothesis (**H₀**): the additions of cricket powder and ancient grains within a baked product, would not affect the doughs' rheology behaviour (water absorption, gluten development, viscosity, starch amylase and retrogradation). Through these observations, the significance of the doughs' rheological and microstructure interactions helped the development of new products containing cricket powder.

3.2 Literature Review: Rheological and Enzymatic Behaviour

The most concerning cereal enzymes in bread making are the amylase, particularly the α -amylase, as it is mainly responsible for converting starch into sugars. Without this critical process occurring in the dough, fermentation would not occur as yeast requires simple sugars to produce carbon dioxide. Therefore, an appropriate balance of naturally occurring amylase in wheat flour is desirable to produce bread that is fermented with a richly coloured crust and well-developed flavour.

Enzymatic treatment is a useful method for improving the functional properties of wheat flour. These functional properties are dependent on the gluten proteins available within the flour. Moreover, the gluten quality will depend on the wheat variety and area of the world in which it is cultivated. The use of enzymes has enabled an increase in the mechanisation of the breadmaking industry, as they are a catalyst for the biochemical reaction, turning the wheat's glucose chains within the starches into simple sugars (maltose). The Incorporation of enzymes within the dough can provide high tolerance to handling, mixing, fermentation and stability. However, as Wiggins (1998) stated, insufficient amylase activity can restrict loaf volume, as the starch structure becomes rigid too soon. In contrast, too much activity caused by wet harvests can affect the dough structure and become fluid, thus collapsing the loaf.

There are four basic starch converting enzyme groups, (i) endoamylases, (ii) exoamylases, (iii) debranching enzymes, and (iv) transferases. Endoamylases can split α ,1-4 glycosidic bonds present in the inner part (endo-) of the amylose or amylopectin chain. Van Oort (2010) described α -amylase as an 'endo acting enzyme' that randomly hydrolyses the α -1,4 and α -1,6 linkages in polysaccharides, resulting in short-chain dextrans. The amylases convert starch into smaller units, such as glucose, maltose, and maltotriose units (Rajagopalan and Krishnan, 2008; Gupta et al., 2003; Kandra, 2003), therefore aiding to the dough's rheological properties. Exoamylases, are an amylase that hydrolyses glycosidic bonds near the end of the polysaccharide. This is either exclusively cleave α ,1-4 glycosidic bonds such as β -amylase or splits both α ,1-4 and α ,1-6 glycosidic bonds like amyloglucosidase/ glucoamylase and α -glucosidase (van der Maarel et al., 2002). This shortens the external side chains of amylopectin by cleaving maltose or glucose molecules, respectively. Wursch and Gumy (1994) research suggested that both enzymes present can delay bread staling by reducing the tendency of the amylopectin compound in bakery products to retrograde.

Debranching enzymes exclusively hydrolyse α ,1-6 glycosidic bonds into isoamylase and pullanase type I. The significant difference between pullulanases and isoamylase is the ability to hydrolyse pullulan, a polysaccharide with a repeating unit of maltotriose that is α ,1-6 link (Bender et al., 1959; Israilides et al., 1999).

Pullulanases hydrolyse the $\alpha,1-6$ glycosidic bond in pullulan and amylopectin, while isoamylase can only hydrolyse the $\alpha,1-6$ bond in amylopectin. These enzymes exclusively degrade amylopectin, thus leaving long linear polysaccharides. Debranching enzymes are claimed to decrease the problems associated with the use of α -amylases as anti-staling agents in baking. The thermostable pullulanase, and an α -amylase are used together. The pullulanase rapidly hydrolyses the branched maltodextrins of DP20-100 produced by the α -amylase, while they have little effect on the amylopectin itself (Carroll et al., 1987). Pullulanase thus specifically removes the compound responsible for the gumminess associated with the α -amylase treated bakery products. Moreover, transferases that cleave an $\alpha,1-4$ glycosidic bond of the donor molecule and transfer part of the donor to a glycosidic acceptor to form a new glycosidic bond. Enzymes such as amyloamylase and cyclodextrin glycosyltransferase form a new $\alpha,1-4$ glycosidic bond, while branching enzyme forms a new $\alpha,1-6$ glycosidic bond.

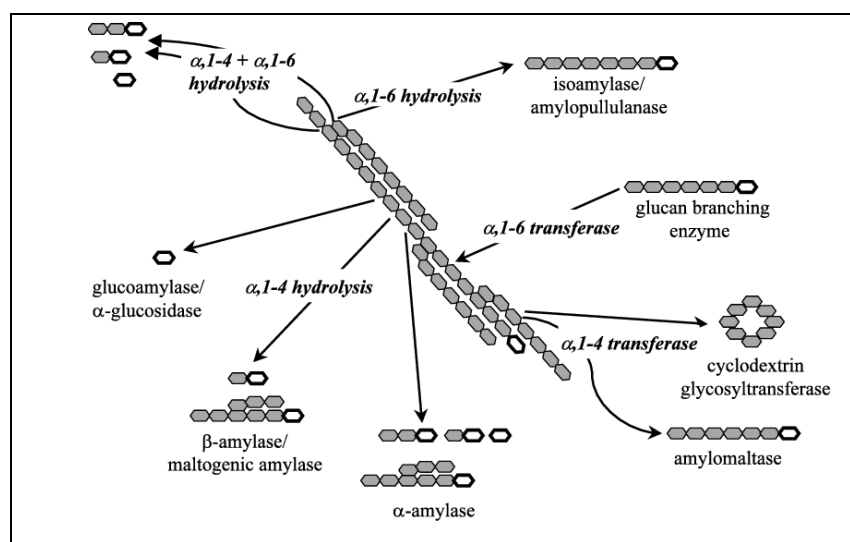


Figure 3:1 Different enzymes involved in the degradation of starch
The open ring structure symbolises the reducing end of a polyglucose molecule
Source: van der Maarel et al., (2002)

The use of enzymes with biochemical additives can induce synergistic effects on dough behaviour, thereby improving overall product quality. Therefore, allowing yeast to work continuously during dough fermentation, proofing, and the early baking stage improves bread volume and crumb texture. In addition, the small oligosaccharides and sugars such as glucose and maltose produced by amylases enhance the reactions for the browning of the crust and baked flavour. However, If

the amylase content is low, this leads to low dextrin production and poor gas production. This in turn, results in inferior quality bread with reduced size and poor crust colour (Baking Quality Analyser, n.d).

Enzymatic treatments of wheat flour assist the doughs' structure and consequently improve the functional properties. Nowadays, the baking industry is continuously looking for alternatives to chemical enzymes and additives due to previous hazards to consumers. This has increased the drive for 'clean labelled' products that either uses the natural level of enzymes present within flours or enhanced by manufacturing sprouting grains. Although enzymes added for this purpose may be manufactured, they are still natural. For example, diastatic malt is obtained from sprouting grains contributes to the increased enzyme activity within doughs. As these enzymes are natural and most of them are deactivated during the baking process, they do not appear on the label as processing aids.

Enzymes are required to promote the formation of covalent bonds between the polypeptide chains within the gluten-forming proteins, thus improving the dough's rheological behaviour during the breadmaking process. The functional property of doughs dramatically depends on the available proteins within the flour, especially the gluten-forming proteins. Enzymes allow the strengthening of different proteins (glutenins, gliadins, albumins or globulins) depending on the particular action mechanism. Therefore, this thesis explored the use of additional wheat flours, pseudocereals, and seeds as sources of naturally occurring enzymes, to help with the doughs' functionality. This allowed the association of different natural modifying enzymes, particular α -amylase, β -amylase, and proteases, as an option to improve the overall quality of the baked product. Thereby strengthening the gluten network and increasing the loaf volume, while playing an essential role in textural and crumb grain properties.

3.2.1 Dough Rheological Properties: High and Low Molecular Weight Subunits

Water is a fundamental ingredient within the breadmaking process to plasticise the dough as a physical change and create the molecular structure of the dough. Belton et al., (1994) provided evidence that water-to-proteins ratio is very sensitive for both

high and low molecular weighted subunits (HMW and LMW) of gluten². There is extensive debate on the role of molecular subunits, especially in the formation of gluten development and dough rheology. Large networks of glutenins (polymeric, including HMW and LMW) and gliadins (monomeric) exist within dough and evidence from rheological research shows their direct involvement (Belton, 2012). Glutenin is deemed as the primary protein within wheat flour, making up 47% of the total protein content available within the flour. The glutenin proteins are aggregates for high molecular weight subunits within wheat flour (Wrigley et al., 2006; Belton, 2012). This provides the strength and elasticity within dough development, which has the most significant impact on breadmaking, whereas gliadin is generally perceived as contributing to the dough's viscous nature (flow properties) and provides up to 30% of the total protein in wheat flour. Moreover, the combination of monomeric-, polymeric- proteins and HWM to LWM glutenin subunits give the overall elasticity and extensibility of the doughs' characteristics (Oliver and Allen, 1992; Bushuk, 1998; Shewry et al., 2000; Bushuk and Bekes, 2002; Wrigley et al., 2006).

The complexity of these network structures in the formation of bread doughs is multifaceted as many components interlink within the structure. For example, the proteins within flour in its dry state are mainly disordered with no interaction or structure, but with the addition of water or other hydrating substances, the HWM or LWM starts to hydrate and form a mobile material. The mobility of the material varies depending on the hydration content, for instance, HMW subunits within the flour proteins become mobile within a liquid (Belton et al., 1994; Belton et al., 1995). However, as the hydration levels continue to increase the number of beta-sheets (secondary structure in proteins containing the polypeptide chain) within the HMW subunits subsequently increases and reaches a maximum point. If any further hydration is incorporated within the subunit, the beta-sheets start to decline and

² This can be seen in the breadmaking making process, where the minimal variant levels of hydration can have a large effect on the behaviour of the proteins. Tkachuk and Hlynka (1968) research identified this, where standard water (H₂O) was substituted for deuterium oxide (D₂O or otherwise known as ²H₂O – heavy hydrogen or heavy weight water) and the results indicated the effect of dough strengthening. The results indicated that hydrogen bonds are formed within the dough, moreover, particularly when using D₂O due to the increase in hydrogen molecules found within the liquid. It is this research that contributed to the understanding of dough strengthening and necessity of hydrogen bonding that helps with the doughs rheological state.

replaced with a beta-turn. This was demonstrated through Belton (1999) and Shewry et al., (2003) model outlining the interaction of hydration of HMW subunits.

Belton and Shewry outline in their model that glutamine residues are present within the HWM subunits. These can readily form inter-chain hydrogen bonds with neighbouring chains and therefore make a continuous chain throughout the protein structure. As water levels increase even further, the molecular motion allows the formation of inter-chain beta-sheets. While the water levels continue to increase further, there is a competition between the water and glutamine for hydrogen bonds formation. Resulting in the replacement of the interchain hydrogen bonds by water and therefore, replacement of interchain-beta sheets (Belton, 2012). Thus, forming the extensive network of proteins and hydrated gluten necessary in dough development. Consequently, the combination of flours used throughout this thesis questions the capabilities of monomeric-, polymeric- proteins and HWM to LWM glutenin subunits present in each flour. The use of the Mixolab observed these effects (Shewry et al., 2002), and gave a greater understanding of the individual flours' capabilities.

3.2.2 Dough Rheological and Mechanical Properties: Cricket Powder

The substitution of wheat flours for insects, such as cricket powder, can generate poor dough rheological behaviour. Cappelli et al., (2020) research showed that the substitution of 15% cricket powder affects the dough's rheological properties (stability, water absorption, degree of softening, tenacity, extensibility, index of swelling, deformation energy and curve configuration ratio). Rumpold and Schluter (2013) and Cappelli et al. (2020) identified that the water absorption increased when using cricket powder due to the flour and fibre composition required to hydrate. This was supported by Osimani et al., (2018) research, which found that the water absorption increased as the percentage of cricket powder increased from 10% to 30%. One possible factor for this is the production of cricket powder (roasting and grinding of insects), making it hydrophobic instead of hydrophilic (Bassett, 2018; Kowalczewski et al., 2019). Their results suggest that the inclusion of cricket powder leads to greater availability of water for the biopolymers in the dough. This influences

the viscoelastic properties of the dough – a network formed by starch and hydrocolloids (Kowalczewski et al., 2019).

Furthermore Kowalczewski et al., (2019) research, identified that the bulk water fraction in relation to the bound water fraction decreases with the increasing percentage of cricket powder. Yet the baking process resulted in the removal of free water, meaning that the water available from the biopolymers and hydrocolloids was primarily retained in the structure (Kowalczewski et al., 2019). While the dough stability of such flour's substitutions increases, this can be attributed to the different protein compositions (Rumpold and Schluter, 2013; Gonzalez et al., 2019; Cappelli et al., 2020). This was linked to the degree of softening as highlighted by Kohajdovaa et al., (2013) and Osimani et al., (2018), who emphasised the interaction between wheat flour- and insect- proteins delays hydration and development of the gluten network, thus leading to the increase in dough development and stability. Moreover, the inclusion of cricket powder within doughs can have an impact on their textural properties. While emulsifiers are used in baking technology to reduce crumb hardness, the softening effect of cricket powder could be connected to the emulsifying properties of cricket proteins (Kowalczewski et al., 2019; Giannou and Tzia, 2007; Gray and Bemiller, 2003; Conte et al., 2018; Alvarez-Jubete et al., 2010; Denirkesen et al., 2010). Therefore, the incorporation of cricket powder can significantly increase the ability of the crumb to return to its original state after compression (Kowalczewski et al., 2019; Onyango et al., 2011).

Osimani et al., (2018) study showed that the dough tenacity (the ability of the dough to retain gas, Rosell et al., 2001) increased when replacing wheat flour with cricket powder. Thus, the correlation as the substitution increases, consistent with the protein rise, the tenacity would increase (Cappelli et al., 2018; Cappelli et al., 2019). Moreover, the research also highlighted that the dough extensibility and index of swelling decreased as the percentage of substitution of the cricket powder increases (Cappelli et al., 2018; Cappelli et al., 2019). This is due to the different composition of flours, particularly the increase in total protein content, which reduces starch and gluten content. Therefore, despite the high protein content in cricket powder, the starch content is reduced, influencing the gluten network formation by lowering their strength, which is consistent with the reduction in dough extensibility and index of

swelling (Cappelli et al., 2019; Gonzalez et al., 2019; Osimani et al., 2018; Roncolini et al., 2019; Pasqualone et al., 2017). According to Garcia-Segovia et al., (2020) research, when using mealworms and beetles, these do not modify the extensibility and index of swelling, indicating that the flour characteristics can impact the rheological behaviours. While the deformation energy increases when using cricket powder as a flour substitution, this is due to the changes in the area under the alveograph curve.

3.2.3 Water Absorption and Hydration Capacity

In 2018, Gonzalez et al., identified that the hydration levels increase when using a blend of cricket powder and wheat flour. This characteristic is due to the fibre's hydration present within the cricket powder, giving dough stability. The presence of the cricket powder within bread doughs can inhibit the availability of water for starch-pasting properties, disrupt the viscoelastic properties and result in a weaker gluten matrix (Rosell et al., 2010). Generally, the amount and type of amino acids present within the flour will determine the hydration and overall doughs' stability. For instance, insect flours are known to have high amounts of hydrophilic proteins (cysteine amino acids), contributing to the hydration, water absorption and dough stability. During the hydration period the cysteine amino acid in wheat flour forms together as two residues connecting with different parts of the α -helix structure. The α -helix forms disulphide (S-S) bridges which contribute to the stability of the dough (Gonzalez, et al., 2018).

Understanding the doughs' rheological behaviour helped assess the impact of cricket powder within bread doughs. Due to the natural presence of fibre found within cricket powder, the breadmaking process was adjusted. Katina et al., (2006) recommended using a 'wet hydration' when incorporating high fibre flours to improve the overall bread products' texture, volume, and quality. This technique allows the hydration of gluten-forming proteins first to ensure the gluten network is sufficiently hydrated.

3.2.4 Dough Formation

Insect flours do not contain starch and gluten-forming proteins, and in Gonzalez et al., (2018) research, despite their high protein content, water adsorption was reduced in fermented bread products. Rumpold and Schlüter (2013) research identified that the amino acid composition of the proteins was responsible for lowering the water absorption. Therefore, water absorption is only required for the hydration of the fibre present in insect flours. The addition of fibre within doughs tends to increase the final fermentation/proving time, which can be counteracted by increasing yeast levels (around 1% in a straight dough process). Although the poor baking volume may arise from the fibre enrichment, this can be compensated by adding dry gluten into the flour (Seibel and Brummer, 1991) or by using higher protein flour, for instance, Canadian or Khorasan Flour. These flours have a protein percentage between 14%-16%, which is best for the production of baked goods that require high strength and structure. It is suggested that if the amount of fibre is over 10%, the gluten-forming protein content of flour should be around 16%. Furthermore, the amount of water used should be sufficient to hydrate both the fibre and the additional gluten components, see previous subsection 3.2.2 for details (Stear, 1990).

Enzymes can also be used to improve the dough's development and the overall bread qualities (texture, volume and shelf-life). Collar et al., (2007) reported that the inclusion of high fibre ingredients could lead to a shorter shelf-life due to the diluted gluten network, shorter dough extension and lower resistance to the extension of doughs. While cricket powder has high fibre content, there is concern that this can create a 'hardening effect' in baked products. However, cricket powder contains chitin and can naturally extend baked products' shelf-life due to the antimicrobial properties. This was apparent in Gonzalez et al., (2018) research which showed that with a 5% inclusion of cricket powder, the texture parameters improved. Although the previous research only incorporated 5%, this research looks at larger quantities incorporated within the dough to accommodate the potential nutritional composition of the crickets (expressed in subsection 4:2:1)

3.3 Mixolab Materials and Methodology

Mixolab is a mechanical instrument that allows the dough formation, rheological behaviour, and determination of the dough's gluten matrix, as it is subjected to both kneading and temperature changes. It measures, in real-time the torque (N.m) produced by the dough between the blades and the temperature either subjected to the dough or generated during kneading. The ingredients are initially mixed to ensure that they are hydrated to form a dough mass and obtain a target consistency during the first phase. During this initial first phase, the dough is kept at 30 degrees Celsius for 8 minutes. While the dough was kneaded, the Mixolab looked at the deformation and weakening of the gluten available in the dough. It was at this point in the first phase of the dough behaviour data is measured. During the second phase, the temperature increased to 60 degrees Celsius to denature the protein structure, thus contributing to the protein weakening measurement. As the temperature increases further, a reduction in the torque can be observed due to the swelling of the starch granules. This drop corresponds to the weakening of the proteins when the temperature increases, indicating protein quality. Phase three is known as the gelatinisation of the dough. The dough is heated as the temperature increases 90 degrees Celsius, where the dough's absorption levels and gelatinisation measurements were observed. These conditions being similar to those used during commercial dough processing, mainly during the baking process.

The values presented in phase three depend on the starch characteristics and the amylase activity of the sample. During the fourth phase, known as the stability baking phase analysis, the heat source is held at 90 degrees Celsius. Throughout this period, the differences between phase three and four are monitored and indicated the starch gel's stability when heated. The drop during this phase may be more pronounced depending on the amylase activity levels within the flour. For instance, a higher amylase level will create a more significant dip on the graph indicating that the bread crumb will be sticky and produce a low bread volume. Phase five is known as the retrogradation stage, where the temperature reduces from 90 to 50 degrees Celsius. The torque increase between phase four and phase five indicates how the starch retrogrades when the temperature is decreased. This retrogradation can be correlated with the staling process in the sample.

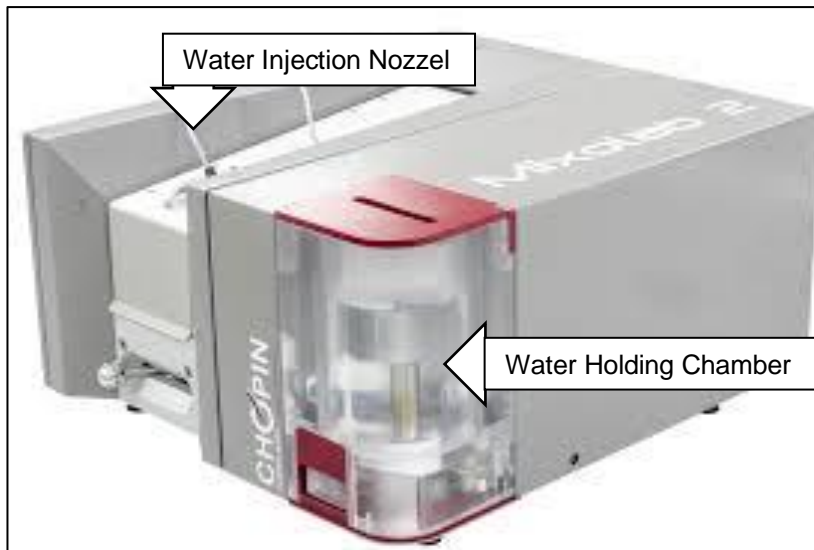


Figure 3:2 Mixolab2 – Dough Rheological Behaviour and Enzymatic Analysis Machine

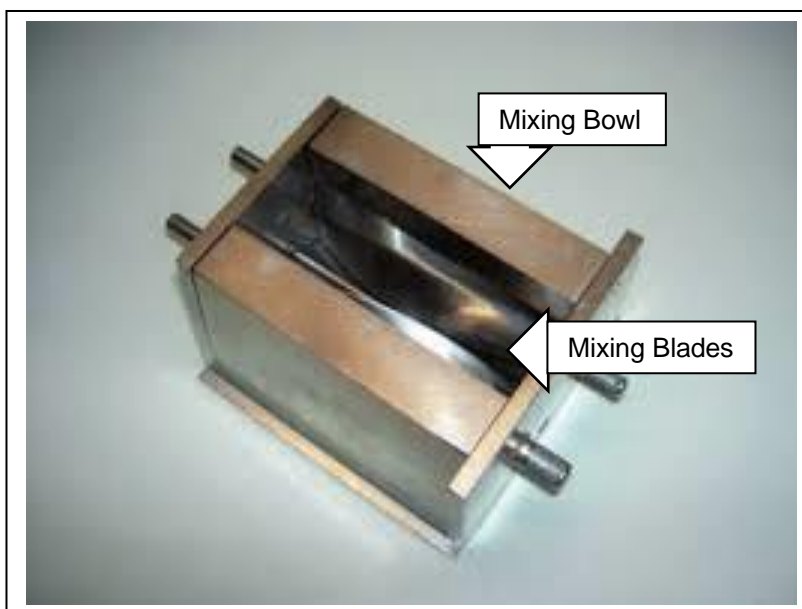


Figure 3:3 Mixolab2 – Dough Rheological Behaviour and Enzymatic Analysis Mixing Bowl

3.3.1 Mixolab Procedure – Method

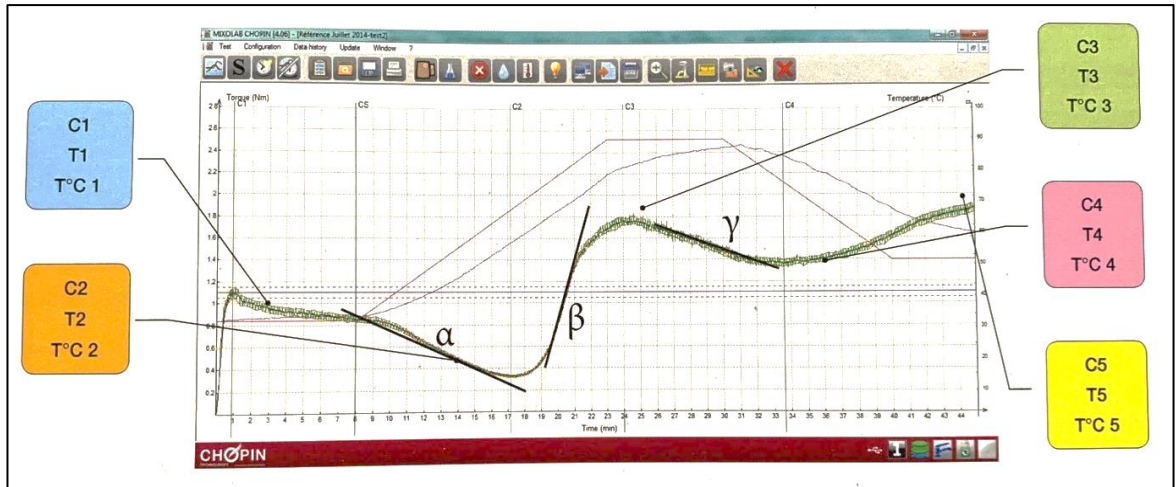
The Mixolab test was based on preparing a constant weight dough sample hydrated to obtain a target consistency during the first test phase. In the Chopin+ protocol, the optimal dough weight is 75 grams, and the target torque consistency being 1.1 ± 0.05 N.m. By using the standard method outlined by AACCI 54-10.01, the test procedure was adhered to, ensuring the test's repeatability. There are several different testing options available when using the Mixolab, but the standard Chopin+ protocol was used for this thesis. The Chopin+ protocol's operational method allows the software to calculate the sample weight according to the particular criteria

(sample moisture and hydration base). The software calculates the flour weight and water required for injection during the mixing process through these two criteria. The flour was then funnelled into the mixing bowl, and the water injection nozzle was positioned over the bowl. If the consistency of phase one was outside the tolerances, the test was stopped, and the bowl was cleaned to restart the test. However, using the integrated calculation tool to predict the actual hydration of the sample, allows accounting for the previous testing results (hydration used, phase one torque obtained and moisture present). Table 3:1 outlines the standard Chopin+ Protocol parameters to which the dough is subjected during the 45-minute test.

Chopin+ Protocol	
Mixing Speed	80rpm
Constant/ Adapted Hydration	b14 / 1.1±0.05 N.m
Dough Weight	75g
Tank Temperature	30 degree Celsius
Temperature 1st Step	30 degree Celsius
Duration 1st Step	8 minutes
Temperature 2nd Step	90 degree Celsius
1st Temperature Gradient	4 degree Celsius / minutes
Duration 2nd Step	7 minutes
2nd Temperature Gradient	-4 degree Celsius / minutes
Temperature 3rd Step	50 degree Celsius
Duration 3rd Step	5 minutes
Total Analysis Time	45 minutes

Table 3:1 Overview of Chopin+ Protocols for Testing Flour Samples

3.3.2 Mixolab Procedure – Calculation and Expression of the Results



Point	Explanation	Associated Parameters
C1	Used to determine water adsorption	T-Degree Celsius 1 and T1
C2	Measures protein weakening as a function of mechanical work and temperature	T-Degree Celsius 2 and T2
C3	Measures starch gelatinisation	T-Degree Celsius 3 and T3
C4	Measures hot gel stability	T-Degree Celsius 4 and T4
C5	Measures starch retrogradation in the cooling phase	T-Degree Celsius 5 and T5

Parameter	Calculation Method	Explanation
Water absorption (%)	Amount of water required to obtain C1 = 1.1±0.05Nm	Amount of water that flour can absorb to obtain a given consistency during the phase at constant temperature
Time for C1 (minutes)	Time required to obtain C1	Dough development time, the stronger the flour, the longer the development time
Stability (minutes)	Time during which the upper frame is > C1 – 11% (phase at constant temperature)	Mixing resistance of dough, the longer this time is, the more the flour is said to be 'strong'
Amplitude (Nm)	Width of the curve to C1	Dough elasticity, the higher the value the greater the elasticity of the flour

Parameter	Calculation Method	Explanation
Slope α	Slope of the curve between end of period at 30-degree Celsius and C2	Protein weakening speed under the effect of heat
Slope β	Slope of the curve between C2 and C3	Starch gelatinisation speed
Slope γ	Slope of the curve between C3 and C4	Enzyme degradation speed

Table 3:2 Mixolab Parameters and Description of each Parameter Tested

3.4 Overall Dough Rheological and Enzymatic Flour Results

Petrie (2016) outlined the individual flours (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) parameters; which can be seen in table 3.3. This identifies the individual points calculated through the Mixolab, which affects the associated dough rheological and enzymatic parameters phase one to five; including the doughs' development and stability time. The results provided by the Mixolab created a fundamental data matrix which allowed the determination of optimum flour combinations in terms of water absorption, mixing properties, gluten quality, viscosity, amylase and retrogradation.

The individual flours tested throughout this thesis are as follows:

1. Wheat Flour: 100% T55 la tradition Française wheat flour (Viron flour). The T55 indicates the level of ash present within the flour, which can assist with the fermentation.
2. Cricket powder: *Grylloides sigillatus* (tropical house cricket) sourced from Entomo farm in Canada. 100% organic and gluten-free.
3. Quinoa Flour (*Chenopodium quinoa*): 100% white quinoa seeds, these were then milled into flour at the National Bakery School.
4. Khorasan Flour: Sourced from Moffett Milling and Malting in South Africa which was endorsed to produce Khorasan Flour in Southern Africa.

Sample	Dough Rheology and Enzymatic Behaviours							Secondary Parameters		
	WA (%b14)	Primary Parameters					Dev. Time (min)	Stability (min)	C3-C2 (N.m)	C3-C4 (N.m)
		C2 (N.m)	C3 (N.m)	C4 (N.m)	C5 (N.m)					
Cricket Powder 100%	45	0.31	0.45	0.32	0.69	10.16	6.2	0.16	0.13	0.37
Quinoa Flour 100%	55	0.43	2.17	1.67	2.22	0.58	0.3	1.74	0.5	0.55
Khorasan Flour 100%	53.3	0.82	2.21	2.30	3.83	1.58	5.16	1.39	-0.09	1.53
Cricket Powder 10%	58.3	0.42	1.59	1.34	2.87	6.58	9.37	1.17	0.25	1.53
Cricket Powder 20%	58.3	0.37	1.46	1.18	2.33	7.94	7.32	1.09	0.28	1.15
Quinoa 10%	53.4	0.45	2.28	2.04	2.90	0.45	0.45	1.83	0.24	0.86
Quinoa 20%	53.4	0.43	2.25	1.83	2.49	0.42	8.4	1.82	0.42	0.66
Quinoa 50%	56	0.24	2.08	1.58	1.91	0.40	1.28	1.84	0.50	0.33
Khorasan Flour 5%	53.3	0.46	2.21	3.50	1.25	9.47	1.81	0.06	1.29	0.46
Khorasan Flour 10%	53.3	0.47	2.23	3.55	1.07	9.56	1.82	0.06	1.32	0.47
Khorasan Flour 20%	53.3	0.51	2.18	3.51	2.53	9.57	1.77	0.10	1.33	0.51
Khorasan Flour 50%	54	0.61	2.19	3.71	1.18	9.45	1.62	0.04	1.52	0.61

Table 3:3 Overall Preliminary of Flours and their Dough Rheology and Enzymatic Behaviours

Source: Petrie, 2016

Table 3.3 shows the various flours combined at different percentages which were selected to develop the baked products. These hybrid combinations were then analysed through the Mixolab to ascertain the characteristics of the final product. The rheological and enzymatic behaviours of these hybrid flours are presented in table 3:4, which summarises the primary (C1, CS, C2, C3, C4 and C5); and secondary parameters (alpha, beta and gamma slopes). The results were then processed through IBM SPSS (1x4) general linear model (Univariate analysis of variance: ANOVA) to determine which hybrid flour combination had a statistical significance ($p < .05$) compared with the control wheat flour sample. The letters in the table denote which samples are significantly different across all samples.

Sample	Dough Rheology and Enzymatic Behaviours							Secondary Parameters		
	WA (%b14)	C1 (N.m)	CS (N.m)	C2 (N.m)	C3 (N.m)	C4 (N.m)	C5 (N.m)	Alpha (N.m)	Beta (N.m)	Gamma (N.m)
T55 Wheat Flour – Control Sample (a)	63.7	1.12± 0.01	1.11± 0 b,c,d	0.56± 0.01 c,d	1.92± 0.01 b,c,d	1.85± 0 b,c,d	3.01± 0.07 b,c,d	- 0.11± 0.01 c,d	0.43± 0.01 d	-0.04± 0.04
T55 +Cricket Powder (b)	70.4	1.16± 0.01	1.14± 0.01 a,c,d	0.4± 0 a,c,d	1.47± 0.01 a,c,d	1.3± 0.03 a,c,d	2.26± 0.01 a,c,d	-0.1± 0 d	0.27± 0.01	-0.05± 0.02
T55 + Cricket Powder + Quinoa + Khorasan Flour (c)	75	1.11± 0.3	1± 0.02 b,c,d	0.34± 0.2 a,c,b	1.52± 0.01 a,d	0.94± 0.12 a,b,d	1.29± 0 a,b,d	- 0.09± 0 a	0.26± 0.07	-0.1± 0.02 d
T55 + Cricket Powder + Quinoa + Khorasan Flour + Mixed Seeds (d)	77.54	1.1± 0.04	0.8± 0.01 a,b,c	0.31± 0.01 a,c,b	1.08± 0.01 a,b,c	0.88± 0.01 a,b,c	1.45± 0.02 a,b,c	- 0.06± 0.01 a,b,c	0.17± 0.06 a	-0.01± 0 c

Table 3:4 Overall of the Control and Hybrid Flour Samples and their Dough Rheology and Enzymatic Behaviours

Results are displayed in rows. Letters in superscript denote the samples compared to which the value is statistically different ($p < .05$). The symbol of the sample is given in the bracket. All values are means obtained from three replications, $n = 3$.

Table 3.4 displays the water absorption, stability and mechanical force required at each parameter. The combination of flours blended and hydrated led to developing a three-dimensional viscoelastic structure due to the gluten-forming proteins available in the wheat- and Khorasan- flour. The amount of water required was calculated based on the flour samples containing a 14% moisture base. The optimum kneading torque of 1.1 ± 0.05 N.m was used, representing the doughs' consistency of 500 Brabender units (BU).

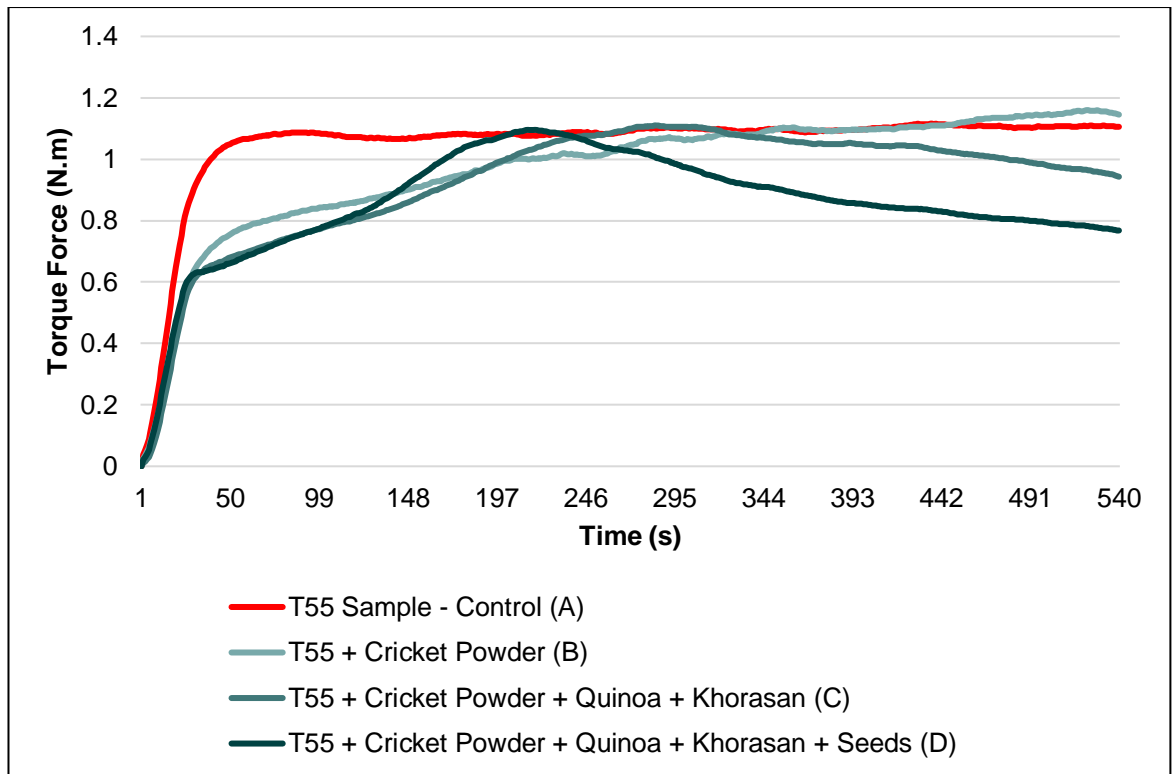


Figure 3:4 Initial Torque Required for the Beginning of the Mixing Stage for all Flour Samples

Total torque required at the initial mixing stage of breads samples (wheat flour control and wheat flour samples fortified with cricket powder and ancient grains); all values are means from three replications $n = 3$.

During this mixing period at C1, the torque represented in figure 3.3 shows the dough development stage. The mean of the control sample for C1 parameter was $M = 1.11$ ($SD = 0.01$); and for the three other samples, T55 + cricket powder (B), T55 + cricket powder + quinoa and Khorasan flour (C) and T55 + cricket powder + quinoa- + Khorasan- flour and mixed seeds (D); the means were $M = 1.16$ ($SD = 0.01$), $M = 1.11$ ($SD = 0.03$), $M = 1.10$ ($SD = 0.04$) respectively. This showed no statistical significance: $F(1,3) = 1.1$; $p > .05$, $\eta^2 = 0.28$, as both the control flour and the fortified samples achieved sufficient hydration, ensuring that the dough development stage could be achieved.

Throughout the initial mixing phase (C1 and CS), two processes occur; firstly, the hydration of the flours proteins and secondly the application of energy during the kneading/mixing stage. When the individual flours are hydrated with water and kneaded, this allows the proteins' intermolecular interactions, resulting in the changes to the disulphide and hydrogen bonds, creating a three-dimensional gluten

network. The control wheat flour sample (A) took less than 50 seconds to reach the optimum torque of 1.1 ± 0.05 N.m, while the rest of the samples B, C and D gradually reached the required torque of 1.1 ± 0.05 N.m at around 197 seconds. The doughs' stability stage (CS), shortly measured after the water absorption stage is normally recorded for 8 minutes from the experiment's start. The mean of the control sample at the CS parameter was $M = 1.11$ ($SD = 0$), and for the three other samples: B, C and D the means were $M = 1.14$ ($SD = 0.01$), $M = 1$ ($SD = 0.02$), $M = 0.80$ ($SD = 0.01$) respectively. The results showed a statistical significance beyond the .05 level: $F(1,3) = 272.84$; $p < .05$, $\eta^2 = 0.99$. With the results showing a significant between the samples, a Tukey HSD test was performed for post-hoc comparison means among the individual CS parameters. There was a statistical significance among the post-hoc comparison mean results, consisting of three groups ranging from lowest to highest; D, C and A&B respectively, in the homogeneous subsets.

This indicated that while most of the samples lasted for the 8 minutes in the region of 1.1 ± 0.05 N.m, only sample D dropped slightly to 0.8 N.m; i.e all samples except for sample D had stronger resistance to the mixing stage. Although the final sample mixing resistance declined over the 8-minute period, this could be due to the additional seeds included from the start of the mixing period weakening the gluten structure. This means that sample D could not form the gluten proteins as fast compared with the other samples, due to the additional seeds. Conversely, in a bakery setting, this would be incorporated at the end of the mixing period to ensure that the gluten-forming proteins are sufficiently hydrated and kneaded. Whereas the Mixolab test, all the ingredients need to be added initially as the procedure cannot be paused or stopped once the experiment has started.

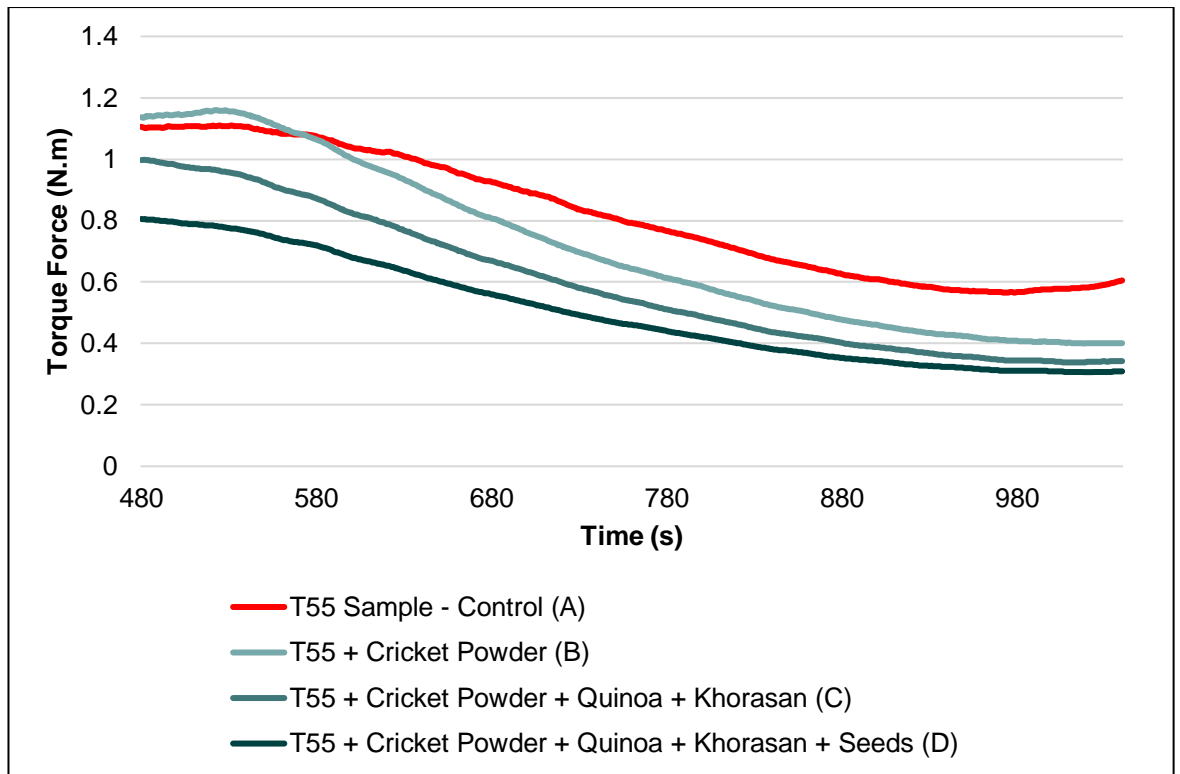


Figure 3:5 Torque and Protein Weakening: Alpha Slope of the Samples

Total torque required to weaken the available proteins under the effect of heat, across the content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder and ancient grains); all values are means from three replications $n = 3$.

Figures 3:4 and 3:5, shows the protein weakening under mechanical shearing and increase in the mixing bowl's temperature. The proteins available in the flours are fully hydrated at this stage, and the starch granules start to swell. The interaction between the protein and starch leads to the doughs' development of a viscoelastic starch gel. Consequently, the kinetics of the doughs' network formation and breakage increase as the temperature increases. Therefore, the viscosity of the dough decreases, and less kinetic energy required. This decrease can be seen on the slope of the α curve in figure 3:4.

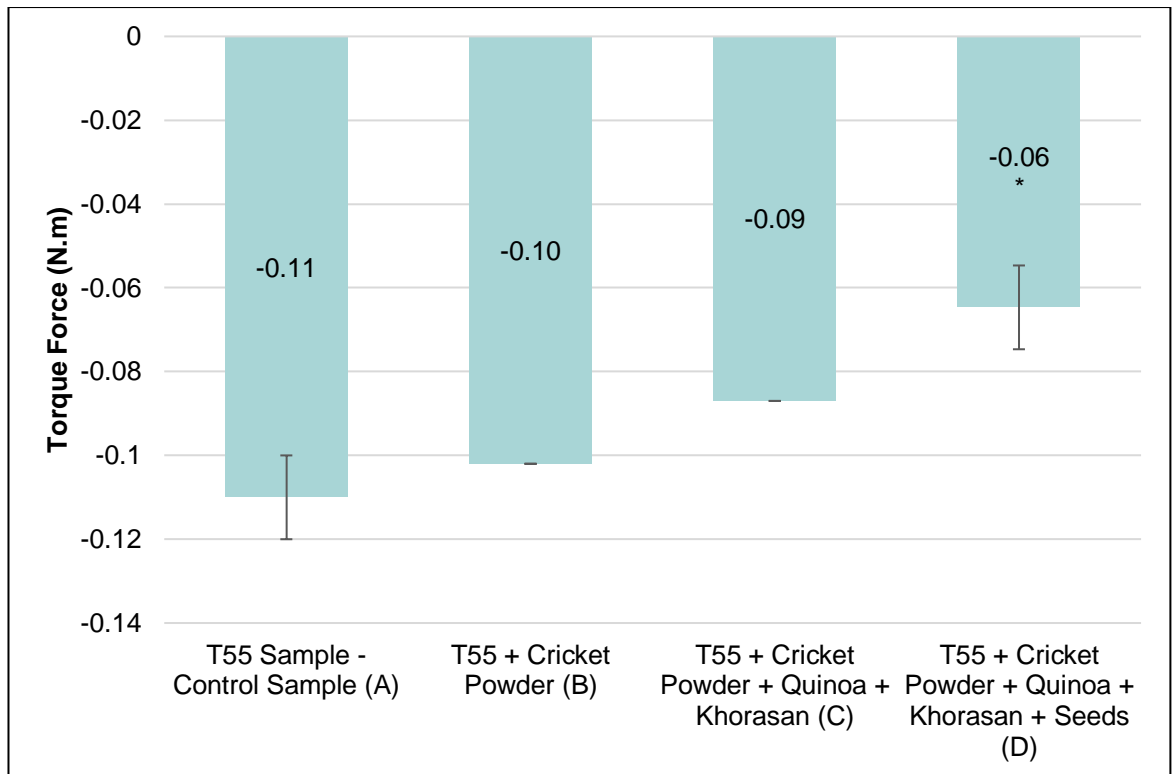


Figure 3:6 Torque and Protein Weakening: Alpha Slope of the Samples Showing Statistical Significance

Total torque required to weaken the available proteins under the effect of heat, across the content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds); all values are means from three replications $n = 3$.

*- $p < .05$ in comparison to the control wheat flour sample bread

The mean of the control sample (A) at the α parameter was $M = -0.11$ ($SD = 0.01$), and for the three other samples: B, C and D the means were $M = -0.1$ ($SD = 0.01$), $M = -0.09$ ($SD = 0$), $M = -0.06$ ($SD = 0.01$) respectively. This showed F to have a statistical significance beyond the .05 level: $F(1,3) = 15.21$; $p < .05$, $\eta^2 = 0.81$. With the results showing a significance between the samples, a Tukey HSD test was performed for post-hoc comparison means among the individual α parameters. As there was a statistical significance among the mean results, this consisted of three groups ranging from lowest to highest; A&B, B&C and D respectively, in the homogeneous subsets. The protein in the flour sample weakened faster within sample D as this had less gluten-forming proteins due to the combination of flours. Furthermore, the added grounded seeds had an impact as they interrupt the gluten-forming network. Sample C is identical in flour combination but does not include ground seeds. These results indicate that under the block's rising heat conditions and the kinetics of mechanical shearing, the protein network present in sample D

starts to degrade fast compared to other samples. Thus, less kinetic mechanical force (torque) is required as the dough proteins and starches start to coagulate from the sufficient water hydration during C1, and increase temperature leading to C2 parameter.

During the C2 parameter stage, the torque was at its minimum due to the protein weakening stage. It was within this boundary that the starch granules began to swell (leading to gelatinisation), therefore distinguishing different torque variations. The mean of the control sample (A) was $M = 0.56$ ($SD = 0.01$), and for the three other samples; B, C and D the means were $M = 0.4$ ($SD = 0$), $M = 0.34$ ($SD = 0.02$), $M = 0.31$ ($SD = 0.01$) respectively. The results showed a statistically significant difference: $F(1,3) = 77.06$; $p < .05$, $\eta^2 = 0.96$. Due to the significance between the test samples, a Tukey HSD test was performed for post-hoc comparison among the individual C2 parameters. This showed a statistically significant difference among the post-hoc comparison mean results consisting of three groups ranging from lowest to highest; D&C, B and A, respectively, in the homogeneous subsets. As the mixing bowl's temperature increased to 90 degrees Celsius (the optimum temperature), the dough's viscosity increased as the starch began to gelatinise and the proteins polymerise. This indicates the products' potential volume and crumb structure. Thus, a higher curve would typically present a baked product with sufficient volume and open crumb structure, while a lower curve would indicate a tight crumb and lower volume baked product. Figure 3:6 shows the beta slope where the torque is measured while the starch goes through the full gelatinisation stage. This is generally through two stages; the first stage is when the dough reaches a temperature of around 60-70 degrees Celsius where the starch gelatinisation linkages present in the dough begin to weaken or break. The second stage occurs when the temperature reaches 80-90 degrees Celsius, and the moisture content decreases, resulting in fewer linkages due to the branched amylopectin structures. Thus, the crumb structure develops due to the starch gelatinisation and protein coagulation as they compete for water.

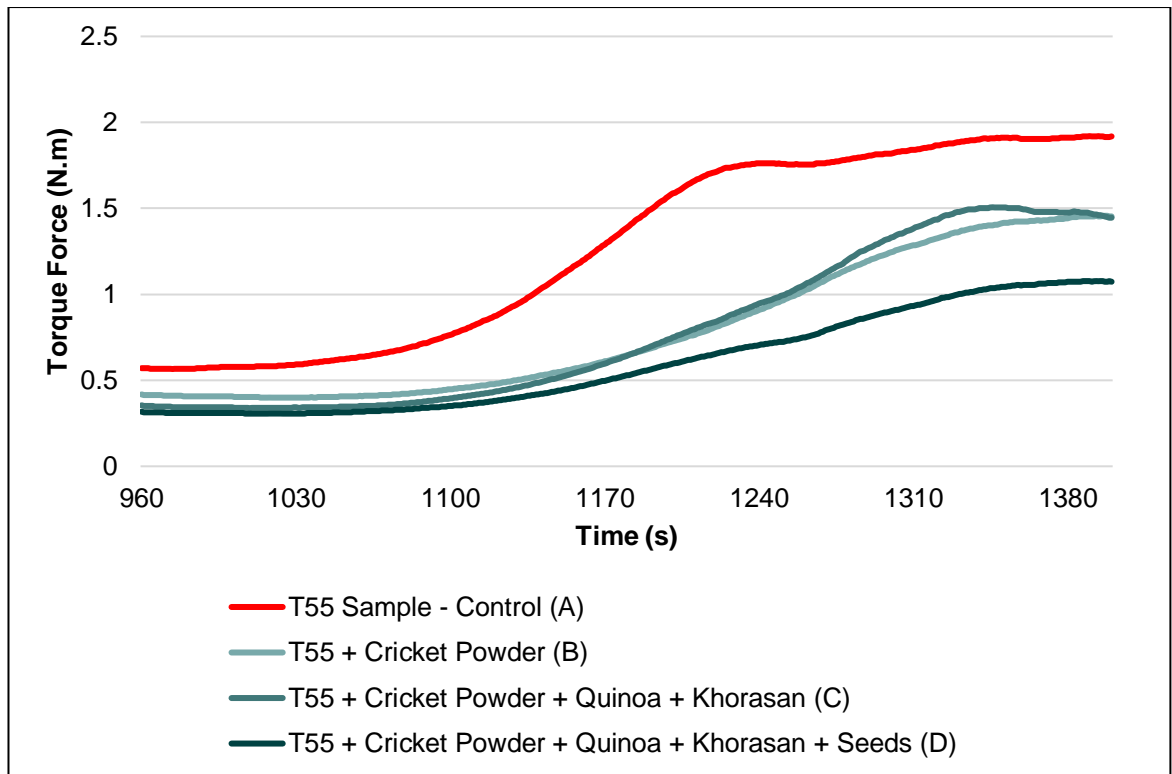


Figure 3:7 Torque and Starch Gelatinisation: Beta Slope of all Samples

Torque required to breakdown the starch gelatinisation, across the content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds) all values are means from three replications $n = 3$.

Figures 3:6 and 3:7 display the Mixolab β curve for all four samples. The mean of the control sample (A) was $M = 0.43$ ($SD = 0.01$) for the control sample (A), and for the three other samples; B, C and D the means were $M = 0.27$ ($SD = 0.02$), $M = 0.26$ ($SD = 0.09$), $M = 0.17$ ($SD = 0.07$) respectively. results showed a statistical significance: $F(1,3) = 6.11$; $p < .05$, $\eta^2 = 0.59$. As there was a significance with the statistics between the samples, a Tukey HSD test was performed for post-hoc comparison means among the individual β parameters. The outcome showed a statistical significance among the mean results, consisting of two groups ranging from lowest to highest; D,C&B and C,B&A respectively, in the homogeneous subsets. The results indicated that samples A,B&C did not have a statistical significance ($p > .05$). However, sample D had a statistical significance ($p < .05$) due to the insufficient starch gelatinisation and protein coagulation. This could be due to the seeds present in the dough as the identical dough without seeds had a sufficient viscosification stage (behaviour of starch and amylase); as sample C is identical in hybrid flours but without the addition of seeds.

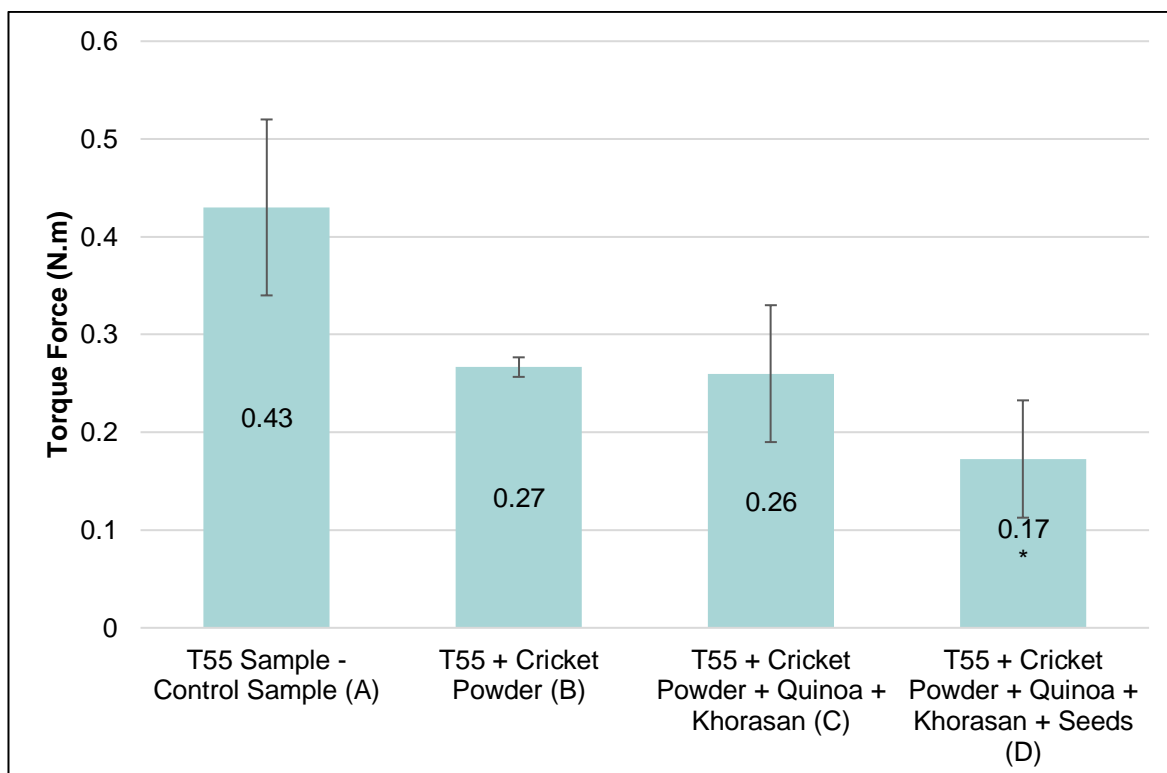


Figure 3:8 Torque and Starch Gelatinisation: Beta Slope of all Sample and the Statistical Significance

Starch gelatinisation under the total torque of the Mixolab, across the content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds); all values are means from three replications $n = 3$.

*- $p < .05$ in comparison to the control wheat flour sample bread

At the point where C3 parameter is recorded, the starch granules swell sufficiently to bursting point. This causes an affect with the amylose chains, as they start to branch out and cause an increase in viscosity, which leads to an increase in torque. The mean of the control sample (A) at C3 Parameter was $M = 1.92$ ($SD = 0.01$), and for the three other samples; B, C and D the means were $M = 1.47$ ($SD = 0.01$), $M = 1.52$ ($SD = 0.01$), $M = 1.08$ ($SD = 0.01$) respectively. The results showed a statistical significance beyond the .05 level: $F(1,3) = 591.60$; $p < .05$, $\eta^2 = 0.99$. From this a Tukey HSD test was performed for post-hoc comparison means among the individual C3 parameters. The outcome showed a statistically significant difference among the mean results, consisting of three groups ranging from lowest to highest; D, B&C and A respectively, in the homogeneous subsets. The reduction in peak viscosity was due to the limited available water within the dough and the water holding capacity at its maximum. In addition, the lack of adequate starch available

in the cricket powder had an impact on the available amylose to branch out on a molecular scale. However, it is possible that sample D reduced the torque values due to the additional seeds, which had impacted the gel formation. This can be seen by comparing samples C and D, where the only difference was the additional seeds in sample D which appear to have reduced, the torque by 0.13N.m.

The final part of the secondary results from the Mixolab was the gamma slope, which was an indicator of the starch gel's enzyme degradation. During this process, as the starch gelatinises, the viscosity increases, but the peak reduces with the α -amylase. As the Mixolab exhibits heat during the process, the viscosity can be monitored and measured similarly to inside baked products during the baking process. This is normally measured through the Hagberg Falling Number, which is used to determine whether the flour composition has sufficient enzymes available for the baker's need during the breadmaking process. The two main amylases present in the wheat and Khorasan flour are α - and β amylases. The chemical reaction of the α -amylase hydrolyses of starch breaks down into glucose subunits which are used for yeast fermentation. Figure 3:8 shows the gamma slope determining the enzyme degradation present in all flour samples. Comparing the results of the Gamma parameter across all samples, the control sample (A) was $M = -0.04$ ($SD = 0.04$), and for the three other samples; B, C and D the means were $M = -0.05$ ($SD = 0.02$), $M = -0.1$ ($SD = 0.02$), $M = -0.01$ ($SD = 0$) respectively. The results showed a statistically significant difference beyond the .05 level: $F(1,3) = 27.84$; $p < .05$, $\eta^2 = 0.91$.

Due to the significant difference between the means samples, a Tukey HSD test was performed for post-hoc comparison means among the individual gamma parameters. The outcome showed a statistical significance among the mean results, consisting of three groups ranging from lowest to highest; D, C,B&A and B&A, respectively, in the homogeneous subsets. The results indicate the natural presence of enzymes within the hybrid flour combinations. These enzymes help with the anti-firming effect through the weakening of the starch structure and networks. This reduces the re-crystallisation rate providing a longer shelf-life of the baked product. As seen in the results, the control sample is predicted to have an accelerated shelf-life, while on the opposite end of the scale the fortified product with ancient grains

and seeds has a high level of natural enzymes and therefore will increase the shelf-life.

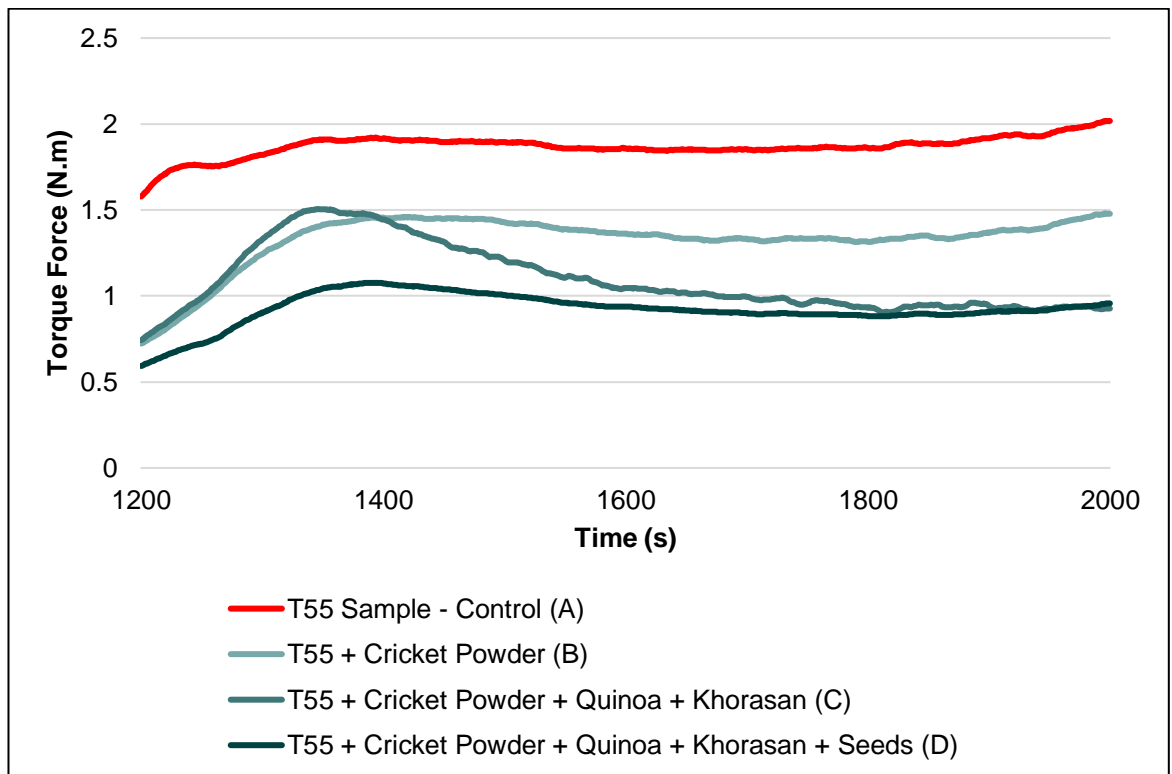


Figure 3:9 Torque and Enzyme Degradation: Gamma Slope of all Samples

Total torque required to weaken the enzyme degradation, across the content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds); all values are means from three replications $n = 3$.

Figure 3:9 shows the enzyme degradation for samples where sample D showed a statistical significance difference ($p < .05$). The only sample that had a similar enzyme degradation to the control is sample C. However, the inclusion of the seeds at the start of the mixing stage impacted the sample D dough's enzyme activity. As previously discussed, in a bakery, inclusions like seeds would be added towards the end of the mixing to avoid impact on the structure and enzymes present in the flour.

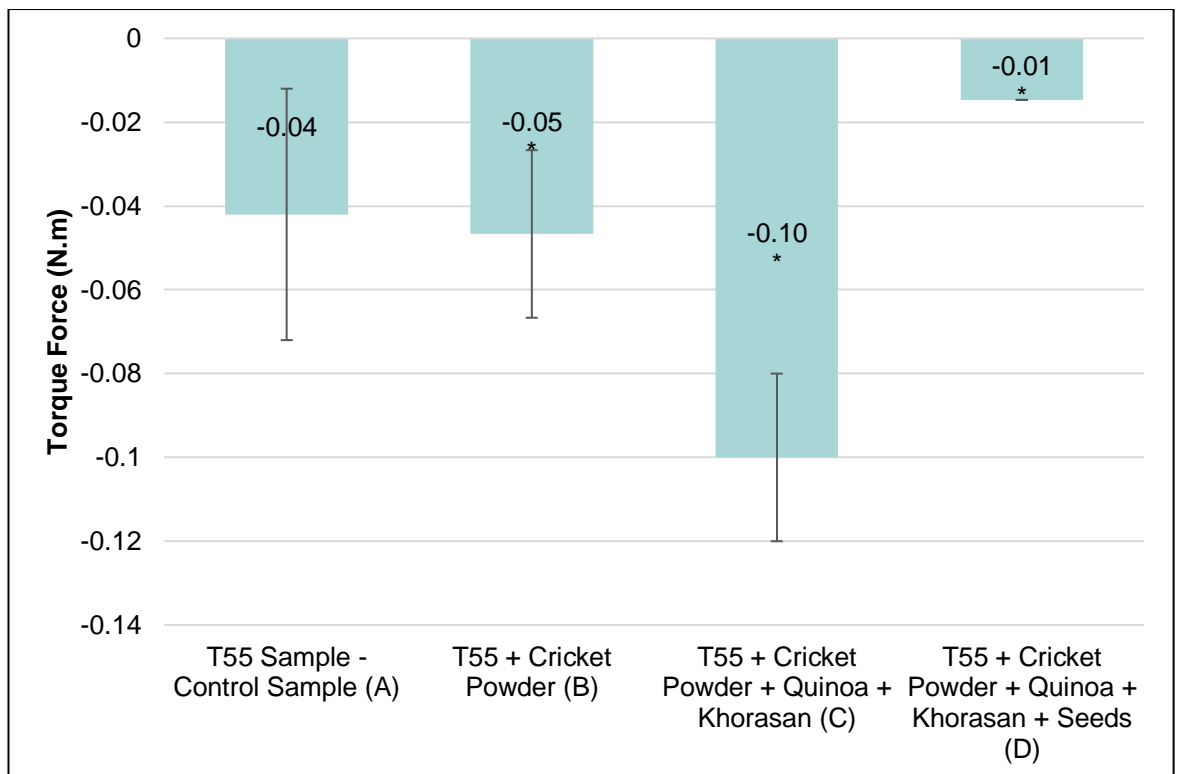


Figure 3:10 Torque and Enzyme Degradation: Gamma Slope of all Samples and the Statistical Significance

Total torque required to weaken the enzyme degradation, across the content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds) all values are means from three replications $n = 3$.

*- $p < .05$ in comparison to the control wheat flour sample bread

During the C4 parameter, the stability of the flour was recorded. This was after the heating process where the mechanical shearing physically breaks down the starch gel causing a decreased viscosity. The mean of the control sample (A) at the C4 was $M = 1.85$ ($SD = 0.01$), and for the three other samples, and for the three other samples; B, C and D the means were $M = 1.3$ ($SD = 0.03$), $M = 0.94$ ($SD = 0.12$), $M = 0.88$ ($SD = 0.01$) respectively. The results showed a statistically significant difference beyond the .05 level: $F(1,3) = 179.5$; $p < .05$, $\eta^2 = .98$. A Tukey HSD test was performed for post-hoc comparison means among the individual C4 parameters. The outcome showed a statistically significant difference among the mean results, consisting of three groups ranging from lowest to highest, D&C, B and A respectively, in the homogeneous subsets. This decrease in torque recorded, could be due to the lower peak viscosities present within the dough. This was apparent from the beginning of the sample testing, where the shear force has always

been lower. Therefore, the shearing force created during the mixing/kneading began to physically breaking down the starch within the dough.

The overall final stage of the Mixolab was the C5 parameter, which looked at the dough's final consistency after cooling. This is commonly known as recrystallisation of the amylose chains, more formally known as retrogradation. Consumers perceive this phenomenon as the firming of the crumb structure they associate with staling and a poor quality characteristic of baked products. Therefore, as a sign of freshness and a quality product, the crumb should be soft. The mean of the control sample (A) was $M = 3.01$ ($SD = .07$), and for the three other samples; B, C and D the means were $M = 2.26$ ($SD = .01$), $M = 1.29$ ($SD = .02$), $M = 1.45$ ($SD = .02$) respectively. The results showed a statistically significant difference beyond the .05 level: $F(1,3) = 1023.56$; $p < .05$, $\eta^2 = 1$. Due to the statistically significant differences across all samples, a Tukey HSD test was performed for post-hoc comparison between the individual C5 parameters. As there was a statistically significant difference among the mean results consisting of four groups ranging from lowest to highest, C, D, B and A respectively, in the homogeneous subsets. This shows that sample B tends to recrystallise faster than any other fortified sample, whereas the samples which had the inclusions of quinoa-, Khorasan- flour and seeds had a slower recrystallisation rate; particularly sample D with the seeds where the torque is notably less than the other samples. This indicated that cricket powder incorporated within the samples can shorten the shelf-life of the baked products. However, when incorporated with ancient grain and mixed seeds, the results indicated that the shelf-life could be increased naturally without added enzymes and preservatives.

3.5 Dough Rheological and Enzymatic Discussion

Water hydration is an important parameter within the breadmaking process, especially when producing the optimal development of the dough's viscoelastic properties, as this gives the best possible finished product quality in terms of volume and crumb structure. An adequate amount of water is necessary during the hydration and conjunction with the mixing stage to ensure the dough is formed into the right consistency, which is essential for optimised breadmaking. The three

processing parameters needed to be achieved, to reach optimum dough development are: firstly, the dough needs to be mixed at an optimal intensity to develop the doughs' viscosity. Secondly, the dough's energy input was crucial as this will affect the volume and bake characteristics (crumb, texture, cell size and shape) of the products. Thirdly, the flour particles' complete hydration and creation of a continuous gluten network. These three parameters are necessary to produce the final bread product, in particular the quality. With the continuous gluten network essential as this will give the dough extensibility required during the breadmaking process. This allows the dough to inflate and resist collapse during the proving and baking steps of the process. This was especially important to understand when including bran or fibre equivalent ingredients into bread products, which can disrupt the gluten network's continuity. Preliminary assessment of the flours identified a higher water absorption level than the standard wheat flour. Therefore, requiring more water to hydrate the flour proteins to achieve optimal development of the dough. At the constant hydration rate (53%), the individual flours' (cricket powder, quinoa- and Khorasan- flour) dough development was affected, identifying that the hydration levels were not achieved. The substitution of wheat flour with cricket powder, quinoa-, Khorasan- flour and seeds changed the water absorption capacity requiring a higher hydration level to achieve optimum hydration and, therefore, optimal dough development. This is in lined with with Cappelli et al., (2018), Osimani et al., (2018) Cappelli et al., (2019) research, which showed that the water absorption was affected as the cricket flour increased from 10% to 30%. Consequently, when using cricket powder as fortification within bread products, it is desirable to increase the hydration for two reasons. Firstly, allowing sufficient water absorption leads to better development of the dough, thus positively affecting the baked product's crumb structure and texture. Secondly, increased water levels directly increase the dough's weight, thereby increasing bread yield (Hammed et al., 2015).

During the Mixolab C1 parameter, all samples were required to reach 1.1 ± 0.05 N.m torque. This indicated that the mixing and pasting behaviours of the dough had reached its optimally developed stage. Samples fortified with either cricket powder, ancient grains or seeds took an additional period of time to reach the optimum torque. This conforms with the research of Kohajdova, Karovicova and Magala

(2013), who identified that the slow dough development time appears to be linked to interaction between wheat proteins, legume proteins and seeds, which delays gluten hydration and development (Mohammed et al., 2012). The increase in development time was due to the additional fibre, and bran found within the additional flours and seeds. These flours and seeds affected the water holding capacity and therefore the strength of the gluten matrix, which overall created a lengthier dough development time required at C1. The network of the disulphide bonds (SS) formed by the oxidation of the sulphhydryl groups (SH) and the high molecular weight (HMW) subunits within the control flour, allowed the dough's strength to reach the C1 torque parameter under the optimal water absorption. The strength of the SS and SH bonds between the HWM subunits gave the dough its resistance, allowing the dough to peak faster due to mechanical and shearing force produced by the Mixolab. Cricket powder notably had low molecular weight polymers and diluted the SS and SH bonds within the wheat flour, affecting the doughs' development, which peaked later within the test.

Additionally, the doughs' water holding capacity and protein structure impacted the stability, which is coherent with Zhou et al's (2018) research. This verifies that the interaction of hydrogen bonding and the gluten forming protein increases dough's stability. The use of Khorasan flour, which has an increased number of gluten-forming proteins increased the water absorption in the fortified doughs, thus increasing the dough's hydrogen bonds. Perez-Fajardo (2020) observed that additional fibre and proteins act as a physical interference to the hydration ratios. This affects the wheat proteins by altering the ionic and hydrophobic interactions, and covalent and hydrogen bonds (Perez-Fajardo, 2020). The fortification of cricket powder showed a similar interaction within the doughs, delaying the gluten-forming proteins' hydration during mixing stage. Therefore, it is necessary to fully hydrate the cricket powder separately and add it later within the mixing/development stage. This allows the gluten-forming proteins present in the ancient grains and wheat flour to sufficiently hydrate and develop. Caution was required as prolonged mixing action would have affected the overall dough structure and texture. However, it is critically necessary to unravel the gluten-forming proteins present in all the flours at the right time. At point C1 and CS, the water acts as both a lubricant and inert filler, imparting high mobility levels to the HMW subunits and facilitating hydrogen bonding between

these two groups, which were responsible for providing dough elasticity (Perez-Fajardo, 2020). In addition, the shearing force applied during the mixing stage of C1 and CS helps to develop the gluten network by stretching these HMW glutenin subunits into more extended conformations (Perez-Fajardo, 2020). C1 and CS allowed energy and mechanical shearing to unravel the HMW glutenin molecules during this period. However, insufficient hydration could create a discontinuous protein network, decreasing the mobility and bonding of hydrogen molecules, creating a close crumb structure, leading to a dense baked bread product (Belton, 1999).

Furthermore, reduced hydration within the dough would impact the rheological characteristics (extensibility, elasticity, and gas retention), resulting in lower quality doughs and final bread products. The longer development times observed in dough samples containing cricket powder are similar to Rumpold and Schluter (2013), Perez-Fajardo (2016), Osimani et al., (2018) and Gonzalez et al., (2019) research on fortification of cricket powder within gluten-free and baked products. The research showed that at C1 parameter, the time increased as the fortification levels increased. Rumpold and Schluter (2013), Perez-Fajardo (2016), Osimani et al., (2018) and Gonzalez et al., (2019) research outlined that one reason for this increase in time for dough stability is attributed to the different protein composition. Moreover, during the CS parameter, sample D's stability time decreased the most, compared to the control due to the fortification of flours and seeds' addition as there was not much gluten to develop. The decrease in C2 torque was recorded for the fortified samples due to the change in polymers' molecular weight distribution. The addition of cricket powder produced a weaker dough, which led to a decreased curve from the torque at the C2 parameter. Another possibility for the weakening at C2 could be the disulphide interference and the sulfhydryl interchange reaction, which inhibits the development of the gluten network. The interference with the gluten network formation resulted in a weaker dough as the cricket powder, quinoa and seeds may have hindered the development of disulphide bonds, especially among the glutenin subunits in the hybrid flours blend.

With the increase in temperature, the proteins began to denature, and starch granules started to swell. Onset gelatinisation was not delayed in the control sample

as the starch granules began to absorb any available water and swell. These starch granules swelled to bursting point, which resulted in the amylose chains leaching out. This effect causes an increase in viscosity, impacting the peak torque (C3 torque). The branching of amylose within the dough accounts for the increase in peak viscosity as the starch and protein available in the flours' competitively interact when water was introduced to the flour. In general, doughs containing fortification ingredients like cricket powder and pseudocereals, show more of a decrease in torque, especially during the C3 parameter. Doughs' fortified with cricket powder competed for water as the starches within wheat flours need this hydration to swell and burst to form the gelatinisation in the baked products. Therefore, due to this water competition during the C3 parameter, the amylose within the flours' cannot branch out. Consequently, a result was the decreased peak viscosity which can be seen on the graph, indicating a lengthier time required to reach peak viscosity (C3 parameter). The decrease in C3 torque across all fortified cricket samples could be due to cricket proteins' inadequate having the capacity to gel and form a structure with a bread product. This is consistent with Cappelli et al., (2018) and Cappelli et al., (2020), which found that both dough extensibility and index of swelling decrease as the percentage of substitution ingredients increase. Malunga et al., (2014) outlines that this is due to the different composition of flours in particular the increase in protein content which reduces starch and gluten content, consistent with dough extensibility and index of swelling. Additionally, the interruption of the disulphide bonds previously discussed could have reduced the binding of available wheat proteins to starch due to the dilution of these proteins from cricket powder and pseudocereals' fortifications. This would decrease hydrogen bonds available within the dough, especially between the starch and gluten, as this primary function is to promote gelatinisation.

The lowering of the C5 torque values indicated that the samples had less free water within the dough. Allowing an increased water holding capacity within doughs', especially when using fortifications such as cricket powder, inhibits the protein binding capabilities and the amylose mobility. The use of the cricket powder within doughs' requires more water, as seen through the Mixolab results, thus allowing the flour proteins to hydrate fully. This water limitation can create several problems, of which one of them being the limitation of binding the starch to the proteins.

Therefore, doughs containing cricket powder could have had inhibited amylose chain mobility. The lower torque observed during the C5 parameter, especially with the doughs fortified with cricket powder, identifies the effect of the water holding capacity. This correlates with the reduced retrogradation in bread due to the water remaining bound within the starch, which will delay subsequent retrogradation and recrystallisation, thereby retarding the staling rate and improving the shelf-life of the bread.

3.6 Dough Rheological Conclusion

The C1 torque measurement indicated the optimal torque required during the mixing/kneading stage, which is ultimately affected by both hydration of the gluten and energy required. Water/hydration is a crucial component within the breadmaking process; it acts as both a lubricant and inert filler. Therefore, affecting the flours glutenin subunits and more importantly, the mobility of these subunits. As the samples fortified with cricket powder and pseudocereals required a high-water percentage to hydrate the proteins and fibre fully, the amount of free water available decreased. Thus, also affecting the gluten network, which is fundamental in the structure of the baked product.

Another factor to consider was the energy input required to develop the dough, the control sample (wheat flour) took longer to reach stability stage, which indicates that this has high amounts of HMW proteins. Since wheat flour contains more HMW proteins levels than cricket powder and pseudocereals, more energy was required to develop the dough. Compared with doughs fortified with cricket powder and quinoa flour, which contain LMW proteins, required less dough development time. This can be seen from the longer stability time required during the C1 parameter, across all samples containing cricket powder due to the presence of LMW proteins within the doughs. These LMW proteins with cricket powder and quinoa caused a delay in reaching the optimal peak during the C1 parameter. Meanwhile, the stability time subsequently increased for all dough samples' containing cricket powder, ancient grains and seeds. This is due to the greater LMW proteins levels found within cricket powder, pseudocereals and seeds, decreasing the dough samples strength.

Overall, samples with additional flours (cricket powder, quinoa- and Khorasan- flour) and seeds displayed a decreased torque within the C2 parameter. As the torque reduces during the C2 parameter (protein weakening stage), this provides evidence that cricket powder within doughs produces lower stability and takes a longer time to reach peak torque. The presence of cricket powder and pseudocereals within the samples resulted in a lower torque during the C3 phase. The graph line showed a more significant drop when the additional inclusions of ancient grains and seeds were added to the cricket powder sample. Due to the competition of water between starch and proteins, delayed the peak viscosity time. Therefore, the dough samples took a lengthier period to undergo gelatinisation. The increased water holding capacity of wheat flour starches, cricket powder, ancient grains, and seeds increased competition for water. Thus, reducing the starches within the flour mixture to swell and reach bursting point. This also reduced the amylase and amylopectin from leaching out, creating a weaker gel, which is evidenced by the low peak viscosity torque. It is during this period, in which the leaching of amylose is responsible for the peak viscosity. However, due to water competition between the wheat flour starches and the cricket powder, ancient grains and seeds prevent the starch from swelling quickly.

During the C4 parameter, the torque decreased for all samples containing cricket powder, significantly more for samples containing ancient grains and seeds, as the hybrid flour percentage increased in value and the wheat flour percentage decreased. The mixer's shear force thinned the hybrid flour proteins (cricket powder and ancient grains) giving lower C4 torques values. This identifies that the inclusions of cricket powder and ancient grains can increase the enzymatic behaviours within the doughs. Therefore, this can be used to extend the shelf life of the baked products naturally. Finally, during the C5 parameter, the torque was significantly higher in the control sample, indicating a shorter shelf life. Meanwhile, doughs containing cricket powder, ancient grains, and seeds showed a lower value, indicating a longer shelf life. Two reasons can explain this; firstly the lipid content with the ingredients naturally coat the gas cells with a film, thus slowing down the water migration. Secondly, the high water percentage and water holding capacity found within sample D is causing a delay in the water migration.

3.6.1 Key Summary Conclusion Points

- A higher hydration level was required when using cricket powder, ancient grains and mixed seeds, due to their high-water holding capacity.
- Moving forward with NPD in Chapter Four, it is necessary to fully hydrate the cricket powder separately and add it later within the mixing/development stage. This would allow the gluten-forming proteins present in the ancient grains and wheat flour to sufficiently hydrate and develop. Caution would be required, as prolonged mixing action can overall affect the dough structure and texture.
- Samples fortified with either cricket powder, ancient grains or seeds took a lengthier time to obtain optimal torque. This development time increase could be linked to the additional fibre, and bran found within the additional flours and seeds.
- This longer development time correlates with doughs that are gluten-free or contain pseudocereals, where the C1 parameter time increases as the fortification levels increase.
- A decrease in C3 torque was noticed in all doughs' in direct comparison with the control dough. During the gelatinisation stage, little water is available due to the competition between the starch high-water holding flours. Therefore, as a result, the amylose chains cannot branch out, thus decreasing the peak viscosity.
- The doughs' containing ancient grains and seeds reduced retrogradation in bread due to the water within the starch. The reduced retrogradation and recrystallisation corresponds with an improvement in the shelf-life of the bread.

Chapter 4 – Nutritional Composition of Baked Bread Products Fortified with Cricket powder

Insects have a very good conversion rate from feed to meat. They make up part of the diet of two billion people and are commonly eaten in many parts of the world. Eating insects is good for the environment and balanced diets.

(Annan, 2015)

4.1 – Introduction

Insects form part of the human diet in many countries around the world (De Folliart, 2012). In parts of Africa alone, 50% of all dietary protein comes from insects of which the market value is considerably higher than alternative sources of commercially reared animal protein (Paoletti and Dreon, 2005; Rothman et al., 2011). The United Nations has recommended the practice of entomophagy as a potential solution to the world food shortage, and this chapter looked at the nutritional composition of baked products fortified with cricket powder. Included within this section, was a review of the minimal requirements levels for the amino acids as recommended by WHO/FAO/UNU (2007); and this is directly compared with cricket powder, wheat-, quinoa- and Khorasan- flour. Whether the argument for adopting entomophagy focuses on reducing environmental pressures or making food accessible to both developing- and developed- countries; the need to reduce malnutrition is a topic of extensive debate. Therefore, the understanding of dough rheological and enzyme behaviour from the previous chapter; led to the development bread products fortified with cricket powder, quinoa- and Khorasan flour. These were then tested for their nutritional composition to determine which product was best suited for human consumption and the marketplace. The following null hypotheses were tested: firstly, (**H₀**): The addition of cricket powder and ancient grains in baked products does not affect negatively the macro- nutritional composition (crude fat, crude protein, ash, fibre and carbohydrate). Secondly, (**H₀**): Incorporating cricket powder and ancient grains within a bread product would not affect the glycaemic index.

4.2 Literature Review: Nutritional Value of Edible Insects

The nutritional composition of edible insects is under great discussion between researchers (Rothman et al., 2014; Payne et al., 2016; Xiaoming et al., 2010; Nowak

et al., 2016; Jonas-Levi, 2017) and numerous studies provide the nutritional values comprising of macro- and micro- nutritional composition. The inconsistencies and ongoing discussions are due to the multitude of insects that are deemed edible and several external factors. For instance; the origin of the insect, life stage/cycle, diet and environmental factors (temperature and humidity) will affect its amino acid profile (Finke and Oonincx, 2014), mineral content and vitamins. Ramos-Elorduy et al., (2012) documented that 78 different edible insects have an estimated energy value range from 293-762Kcal/100g. However, Rumpold and Schluter (2013) found that 79.65% of all edible insects are characterised by energy content above 400Kcal/100g and 40.94% above 500Kcal/100g. This difference in energy values is from the individual insect specie and feed given before the di-pulse state (at the point where the insect is killed).

Nevertheless, researchers agree that insects contain large quantities of the adequate substitutional protein of acceptable quality biological value (Zielinska et al., 2018). According to recent data published by WHO, FAO and UNU (2007), in terms of protein content; insects are superior to other protein sources available or reared throughout the world due to their protein-to-weight ratio (Finke and Oonincx, 2017). The structure and function of all proteins are related to their amino acid composition, the number and order of linkages, folding, interchain linkages and interaction with other groups to induce chemical change (Jackson and Truswell, 2012). Through the structure of the individual amino acids and patterns of their linkages gives unique properties to an individual protein. Essential and non-essential amino acids' qualities must comply with human consumption needs, especially the essential amino acids. These are the building blocks and biosynthesis of all protein structures through human metabolism to ensure proper growth, development and the necessary maintenance of human muscles.

4.2.1 Essential Amino Acids Profile

Utilising insects to develop new food products can help supplement human diets due to their protein, mono- and poly-saturated fat, fibre and micro-nutrient content as consumers push for healthier, novel foods and new protein sources as a primary dietary target. Optimal protein intake can be beneficial in preventing chronic

diseases (heart disease, obesity, high cholesterol and T2MD) and helpful in weight loss/management and meeting the minimal requirements of calorie intake for consumers (Lonnie et al., 2018). Table 4:1 shows the standard essential amino acid reference levels according to FAO/WHO/UNU (2007). The essential amino acids are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine.

Essential Amino Acids	WHO/FAO/UNU 2007 Recommendations
Isoleucine	20
Leucine	39
Lysine	30
Methionine	10
Phenylalanine	25
Threonine	15
Tryptophan	4
Valine	26
Histidine	10

Table 4:1 Essential Amino Acids Content (mg/kg body mass of an adult) Standards According to WHO/ FAO/UNU

Source: Millward, 2008

Table 4:2, shows the amino acid profile for cricket species *Gryllodes sigillatus*, wheat-, Khorasan-, and quinoa- flour. A significant consideration regarding insects' inclusion within baked products is the quality of the dietary protein and fibre. As previously mentioned, protein quality depends on the type of amino acids present in their sequence and protein digestibility. To determine the quality of the proteins (animal and plant), it is fundamental to assess their essential amino acids. As cereals constitute a significant part of the human diet and consumed every day in many ways, it is important to identify their amino acids and compare these with the FAO/WHO/UNU recommendations as seen in table 4:2. Comparing the wheat-, Khorasan- and quinoa- flour proteins with the FAO/WHO/UNU recommendations, shows that these are considerably lower. Therefore, these need to be augmented with additional protein to achieve the recommended levels of essential amino acids. Incorporating cricket powder (*Gryllodes sigillatus*) within baked products can increase the essential amino acid to satisfy the WHO/FAO/UNU recommendations.

Essential Amino Acid	<i>Grylloides sigillatus</i> ⁽¹⁾	Wheat Flour ⁽²⁾	Khorasan Flour ⁽³⁾	Quinoa Flour ⁽²⁾
Isoleucine	3.64	3.9	2.5	3.6
Leucine	6.62	7	7.1	5.9
Lysine	5.11	1.9	2.3	5.4
Methionine	1.46	1.6	1.5	1.5
Phenylalanine	3.17	4.8	4.5	6.1
Threonine	3.1	2.7	2.9	3.0
Tryptophan	2.53	1.1	1.2	1.2
Valine	4.84	4.4	4.2	4.2
Histidine	2.27	2.1	2.2	2.9

Table 4:2 Essential Amino Acid Reference Levels for *Grylloides Sigillatus*, Wheat-, Khorasan-, and Quinoa- Flour (g/100g)

Source: Zielinska et al., 2018⁽¹⁾; Abdel-Aal and Hucl, 2002⁽²⁾; Dakhili, 2020⁽³⁾

Table 4:3 indicates the conventional food macro-nutrient content and the *Grylloides sigillatus* cricket species, used around the world as human nutrition food (Zielinska et al., 2018). Cricket powder is deemed to have a higher protein content (ranging from 55% to 73%) than conventional protein sources (soya bean 16.6%, chicken 39.3%, eggs 41.1% and beef 43%), but it does not have a high protein content when compared with fish (van Huis and Itterbeeck, 2013; Zielinska et al., 2018 & Magara et al., 2021). However, most edible insects' protein content depends on many factors as previously mentioned: life cycle, feeding mixtures, water content, methods of processing and determination for protein (Dumas technique or Kjeldahl method). The key difference between the Dumas and Kjeldahl methods the technique, wherein the former procedure, the sample is combusted at high temperatures in an oxygen atmosphere. Whereas the latter method, the sample is subjected to digestion through concentrated sulfuric acid and a catalyst. Both methods have similar precision, but Kjeldahl is still the most recognised and highly effective when run in batches (Muller, 2017). Therefore, many scientists and academics use this method to determine the protein content of products. This procedure evaluates the total concentration of nitrogen within the food, which is then multiplied by a nitrogen-to-protein conversion factor. Depending on the commodity being tested will determine the nitrogen to protein conversion factor, ranging from 5.7 for common wheat to 6.25 for meat products (see Table 4.8 for all commodities and their conversion factor).

Insect Specie	Protein (%)	Fat (%)	Fibre (%)	Carbohydrate (%)
<i>Grylloides sigillatus</i> (₁)	62	24	6	8
Soya Bean(₂)	16.6	9	6	9.9
Chicken(₂)	39.3	3.6	0	0
Egg(₂)	41.1	36.6	0	1.3
Beef(₂)	43	0.5	0	0.8
Fish(₂)	81	5.5	0	0

Table 4:3 Macro-Nutritional Content of *Grylloides Sigillatus* and Conventional Foods. Results Expressed on a Dry Weight Basis and Based on 100g Samples.

Source: Zielinska et al., 2018 (₁); van Huis and Itterbeeck, 2013

Based on Dry Matter	<i>Grylloides Sigillatus</i> (₁)	White Wheat Flour (₂)	Khorasan Flour (₂)	Quinoa Flour (₂)
Nutrient Composition (%)				
Protein	64.38-73.6	10.7	14.5	14.1
Fat	2.41-22.8	2	2.1	6.1
Fibre	3.65-22.08	12.7	11.1	7
Carbohydrates	2.60-5.8	75	71	64.2

Table 4:4 Approximate Nutrition Composition of Grains, Pseudocereals and *Grylloides Sigillatus*

Source: Rumpold and Schluter, 2013(₁); Boukid et al., 2017(₂)

Table 4:4, shows the nutrient composition (crude protein, crude fat, fibre and carbohydrates) of *Grylloides sigillatus*, wheat-, Khorasan- and quinoa- flour. Fat is one of the macronutrients essential for human health, not only for energy but also to maintain the structure and integrity of all cell membranes. Many studies have found that replacing saturated fatty acids (SFA) with monosaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in diets reduces the risk of cardiovascular diseases. The recommended ratio of fatty acids recommended for human nutrition is SFA/MUFA/PUFA 1.25-1.5-1 (Zielinska et al., 2015); however, *Grylloides sigillatus* contains an estimated ratio of 1-1-1 (32.80-33.50-33.90) (Tang et al., 2019). With SFA usually higher in edible insects than that of MUFA due to the growth stage, *Grylloides sigillatus* tends to have a good ratio of SFA/MUFA/PUFA. Tang et al., (2019) further suggests that it is essential to obtain MUFA in the human diet as this contains oleic acid, which reduces blood pressure and can prevent/cure inflammatory diseases and help boost the immune system. PUFA in insects, especially in *Grylloides sigillatus* species, is a good source of linoleic acid, they are considered anti-inflammatory acne reductive, while the α -linolenic acid present also helps to protect the brain from strokes (Blondeau et al., 2015).

Edible insects' crude fibre content differs depending on the species and can range from 2% for silkworms to 22.08% for crickets. The large difference in the fibre levels is due to the composition of the insect. For instance, insects that have an exoskeleton will contain large quantities of chitin linked to fibre content. Jayanegara et al., (2017) research demonstrated that removing the exoskeleton decreased the protein and chitin content of the cricket powder. However, the chemical extraction of the chitin within the cricket powder increased the protein. While crickets are relatively highly digestible, Jayanegara et al., (2017) research indicated that exoskeleton removal and chemical extraction did not further improve the digestibility of the cricket powder.

4.2.2 Mineral Content Profile

Table 4:5 shows the mineral compositions (calcium, potassium, magnesium, phosphorous, sodium, iron) of *Grylloides sigillatus*, wheat-, Khorasan- and quinoa-flour. Humans must obtain the necessary minerals for growth and development to stimulate the metabolic process and enhance the immune system (Zielinska et al., 2018). Edible insects can supply essential minerals to humans, especially those with either iron or zinc deficiencies (mainly in developing countries). Rumpold and Schluter (2012) and Boukid et al., (2017) suggested that most edible insects have a mineral content either equal to or higher than beef; however, they can be lacking in calcium and magnesium. Compared to grains and pseudocereals, the *Grylloides sigillatus* specie has a higher concentration of all mineral components except magnesium, where Khorasan- and quinoa- flour offers a higher level of this mineral (Rumpold and Schluter, 2012; Boukid et al., 2017).

Based on Dry Matter	<i>Grylloides Sigillatus</i> ⁽¹⁾	White Wheat Flour ⁽²⁾	Khorasan Flour ⁽²⁾	Quinoa Flour ⁽²⁾
Minerals (mg/100g)				
Calcium	132.14-210	34	22	47
Potassium	1,126.62	435	403	563
Magnesium	80-109.42	90	130	197
Phosphorous	780-957.79	402	364	457
Sodium	435.06	2	5	5
Iron	6.27-11.23	5.4	3.8	4.57
Zinc	18.64-21.79	3.5	3.7	3.1

Table 4:5 Mineral Composition of Grains, Pseudocereals and *Grylloides sigillatus*

Source: Rumpold and Schluter, 2012⁽¹⁾; Boukid et al., 2017⁽²⁾

It has been suggested that there could be an influence on the mineral content of insect species, especially the *Gryllodes sigillatus*, by human intervention and a recent study (Gyrlloco, 2016) showed that farmers could increase either the mineral and/or the vitamin content according to the consumers' requirement. This increase can be achieved by adjusting the cricket's diet or additional supplement intake up to 3 days before the di-pulse state (see Appendix C for the cricket life cycle). Therefore, the essential mineral or vitamin is in the cricket's metabolic system and then processed accordingly. This can potentially impact the nutritional value of the product and the benefits to the final consumer. The additional mineral and vitamin contents incorporated in baked products are tailored to consumers' needs and demographics (i.e. gender, age, different ethnic groups).

4.3 Nutritional Composition Methodology

4.3.1 Bread Production Recipes

The best combination of the four different flours used during product development has been explained in Chapter Three. The preliminary results obtained in Chapter Three analysed various flours at incremental percentages (Table 3.3). Their rheological behaviour outlined the hybrid combinations that formed the shape of this research, where the four flours were used in this study: wheat, cricket powder, quinoa and Khorasan. These hybrid flours become the focus of this research where the individual nutritional aspects (Chapter Four), crumb structure including texture (Chapter Five) and overall acceptability product (Chapter Six) were observed. There is a mixture of different classifications of flours (weak, strong and gluten-free), and the four flours were chosen for their potential to increase one or several aspects of the overall baked bread product. The flour combinations are as follows;

- Reference bread sample A contains 100% T55 la tradition Française wheat flour (Viron flour).
- Reference bread sample B contains 70% T55 la tradition Française wheat flour (Viron flour) and 30% cricket powder.

- Reference bread sample C contains 30% T55 la tradition Française wheat flour (Viron flour), 30% cricket powder, 20% quinoa- and 20% Khorasan-flour.
- Reference bread sample D contains 30% T55 la tradition Française wheat flour (Viron flour), 30% cricket powder, 20% quinoa-, 20% Khorasan- flour and 25% multi-seed mixture.

These reference bread samples are described in tables 4:6 and 4:7. This shows the ingredients required in the individual recipes and the baker's percentage³ to ensure consistency. Within each formulation, the baker's percentage for the ingredients changed depending on the bread sample. The second change was the quantity of water, adjusted according to the proportion of cricket powder, ancient grains and seeds incorporated into the recipes. This hydration percentage was taken from the results obtained in Chapter Three (dough rheology and enzyme behaviour). Maintaining the water (hydration) levels at the required level, ensuring that the gluten and cricket powder remained sufficiently hydrated.

Consequently, the high fibre flour (cricket powder) was hydrated separately and added later during dough development (see Chapter Three, subsection 3.2.3 dough formation). These adjustments were implemented to ensure that the doughs' rheological behaviour and the ingredients' functionality improved the bread quality. Therefore, creating a relatively soft dough which is important for successful breadmaking. (See Chapter Three for an in-depth discussion and details of the flours, where the dough rheology including hydration is also outlined).

³ Bakers percentage is the cornerstone within the baking sector. This allows for all ingredients to be weighed for accuracy where their individual weight is in relation to the total flour within the recipe. A positive when using bakers' percentages is an immediate understanding of the bread and the ingredients represented within the recipe. It also allows for a quick scale up and down of the recipes or when the need to remove ingredients without affecting the entire recipe.

Ingredients	T55 Wheat Flour – Control Sample ^(a)	T55 + Cricket powder ^(b)	T55 + Cricket powder + Quinoa + Khorasan Flour ^(c)	T55 + Cricket powder + Quinoa + Khorasan Flour + Mixed Seeds ^(d)
T55 Flour	100	70	30	30
Cricket powder	0	30	30	30
Quinoa Flour	0	0	20	20
Khorasan Flour	0	0	20	20
Yeast	3.3	3.3	3.3	3.3
Salt	1.8	1.8	1.8	1.8
Water	55.6	70.1	75	77.54
5 Seed Mix	0	0	0	25

Table 4:6 Bread Reference Sample Recipes, Expressed in ‘Bakers’ Percentages’.

Ingredients	T55 Wheat Flour – Control Sample ^(a)	T55 + Cricket powder ^(b)	T55 + Cricket powder + Quinoa + Khorasan Flour ^(c)	T55 + Cricket powder + Quinoa + Khorasan Flour + Mixed Seeds ^(d)
T55 Flour	296	204	85	83
Cricket powder	0	88	85	83
Quinoa Flour	0	0	57	55
Khorasan Flour	0	0	57	55
Yeast	10	9	9	9
Salt	5	5	5	5
Water	193	204	213	221
5 Seed Mix	0	0	0	127.5

Table 4:7 Bread Reference Sample Recipes, Indicating Ingredient Weight (Grams)

There was a concern regarding the high ratio of water levels required when producing the bread, increasing dough stickiness and cause processing difficulties. However, as previously discussed in Chapter Three; the increased water levels were necessary to hydrate the wheat flours, ancient grains, cricket powders, and seeds. This ensured that the gluten-forming proteins were fully hydrated; so that the full functionality of the ingredients could be exploited when incorporated into the dough mixture. Figure 4:1 provides an overview of the breadmaking process used for all of the bread samples produced for this thesis, including the control sample.

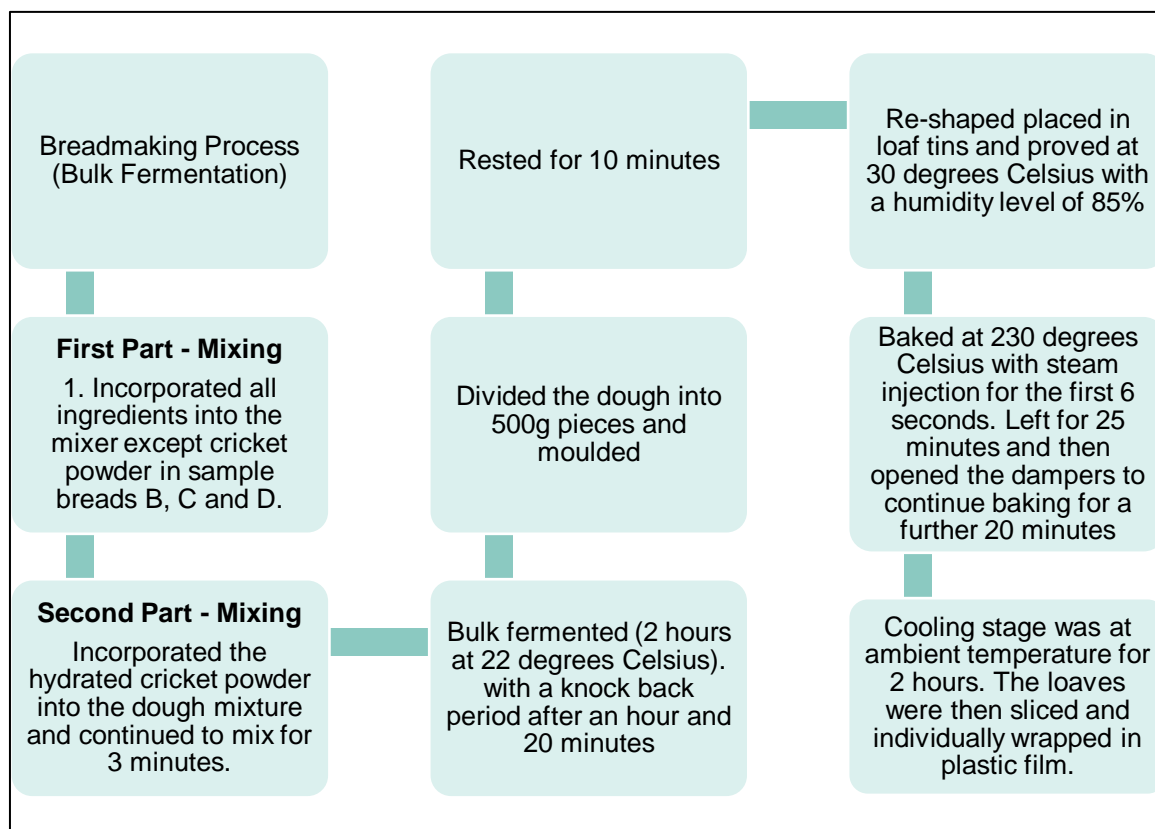


Figure 4:1 Breadmaking Stages used Throughout this Thesis

4.3.2 Preparation of Nutritional Composition Samples

The specific standard operating procedure (ISO – International standard operation, AOAC- Association of Official Agricultural Chemists and AACCI- American Association of Cereals and Chemists International), were used to determine the nutritional composition of products. Before the nutritional composition analysis for fat, protein, ash and fibre content, all reference bread samples were dried out to be completely void of any moisture content (ISO 712:2009). For example, drying the baked samples at a temperature between 130 to 133 degrees Celsius for 120 minutes. However, processing methods commonly used, including drying, can impact nutritional composition. In this case, the proteins denaturing due to the drying method's temperature. After drying, the samples were then ground down to ensure that the macro-nutrients' full extraction was achieved. These samples are then used to determine the rest of the nutritional composition analysis.

4.4 Foss Soxtec ST243 - Crude Fat Analysis

Crude fat is generally defined as the substance that can be extracted by an appropriate organic solvent such as Diethyl Ether. Traditionally, organic solvent fat extractions for quantitative fat determinations are carried out through Soxhlet or other related extraction apparatus. For this thesis, the AACCI method 30-25.01 was used to determine the total crude fat present within the samples. During the extraction process, the fat was dissolved in the solvent which was later evaporated into the condenser leaving the remaining fat in the extraction cup.

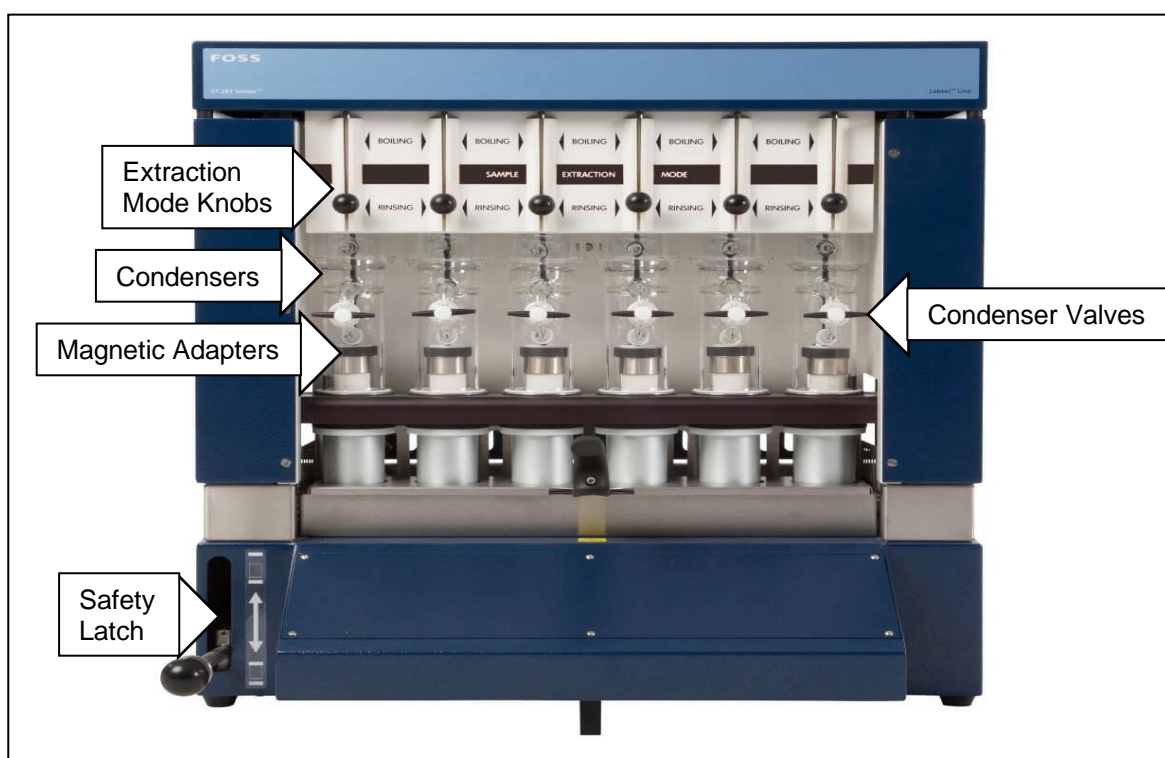


Figure 4:2 Foss Soxtec ST2043 – Crude Fat Analysis Machine

4.4.1 Crude Fat Analysis Procedure – Method

When operating the Foss Soxtec ST2043 machine, the first step was to attach the thimbles to the magnetic adapters. This allows the samples to be suspended within the extraction cup during the fat analysis process. Approximately two grams of the sample was placed in the thimble (the sample result is mathematically calculated based on weight percentage) and covered with de-fatted cotton wool (to ensure that no cross-contamination can invalidate the data). The thimbles were then individually inserted into the Soxtec machine and lifted into the condensers. It is important to note that the sample extraction mode knobs should be raised to rinsing position at

the start of the process. Each extraction cup was filled with 50ml of Diethyl Ether. These were then placed under each individual condenser, ensuring the safety latch engaged.

The extraction process (AACCI 30-25.01, 2019) was a four-stage process (boiling, extraction, condensing and drying). The first step, 'boiling stage' was when the thimbles are immersed in the solvent solution for 35 minutes. During this period, the samples are boiled to remove any crude fat present. The second step 'extraction' was where the samples were rinsed (through the heating and cooling of the Diethyl Ether within the machine), as the thimbles hung above the solvent surface and any fat residues were flushed out into the extraction cups. To ensure that the solvent thoroughly flushed the crude fat out of the samples, this process lasted 30 minutes. Once the samples had been completely flushed of any fat residue, the extraction cup went through the third stage, known as 'condensing'. The condensing valves are closed, allowing the extraction cups to evaporate any solvent into the individual condensers; this took around 20 minutes. Any solvent that remained in the individual extraction cups with the fat is given an additional 10 minutes 'drying' time to ensure the solvent's full evaporation is achieved. The extraction cups and thimbles were then removed from the machine, the samples were disposed accordingly, and the extraction cup was weighed for the results.

4.4.2 Crude Fat Analysis Procedure – Expression of the Results

The crude fat content, W_F results are expressed as a mass fraction of dry product and a percentage (%) is obtained using the following formula:

$$W_F(\%) = \left(\frac{M_3 - M_2}{M_1} \right) \times 100$$

Where:

M_1 – is the mass in grams of the test portion sample

M_2 – is the mass in grams of the extraction cup before processing

M_3 – is the mass in grams of the extraction cup and the dried crude fat extract residue obtained

4.5 Foss Kjeldahl 2100 – Protein Analysis

This method's objective was to test the total nitrogen content of cereals and pulses, which this measurement was then used to calculate the crude protein content. The Kjeldahl method is a standardised test following the ISO 20483:2013 protocol. Details of this protocol are described below: the main principle of the method was to digest the sample in sulfuric acid in the presence of a catalyst. The product's reaction was made into an alkaline solution, which was then distilled in boric acid, followed by titration with hydrochloric acid.

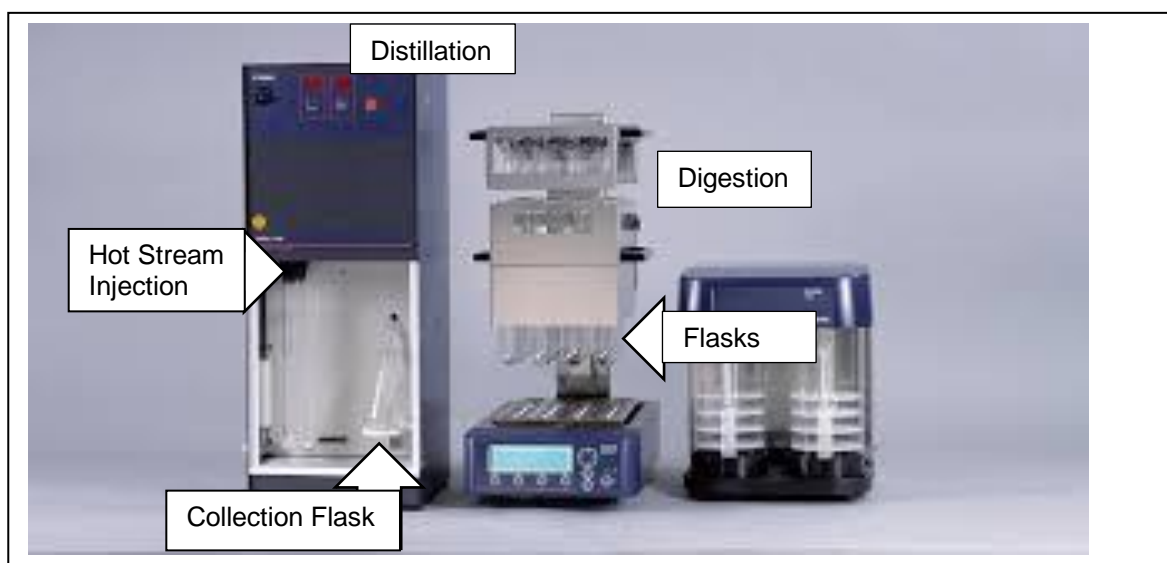


Figure 4:3 Foss Kjeldahl 2100 – Crude Protein Analysis Machines (Digestion and Distillation)

4.5.1 Protein Analysis Procedure – Digestion Method

The samples' digestion was conducted in a Foss Digester apparatus where the sample (approximately between two to three grams, as the results are mathematically calculated based on weight percentage) was transferred onto a general-purpose filter paper. The filter paper was folded to ensure the sample was fully encapsulated and placed into a flask with a catalyst tablet. The catalyst tablet used was a Copper (II) Sulphate Pentahydrate ($\text{CuSO}_{4.5}\text{H}_2\text{O}$) at 2.8% for this test. Then 15ml of sulfuric acid (H_2SO_4) 18Mol/ litre was decanted into the flask and placed in the digester, then heated to 420 degrees Celsius. Two blank samples were also generated to calculate the results, where only plain filter paper (no samples) were placed in the flask. The digestion process was conducted under a well ventilated, sulfuric acid-resistant hood to ensure that no gases escaped. The

samples were left to fully digest for two hours, measured from the time the unit temperature reached 420 degrees Celsius. After completion, the flasks were left to cool for 30 minutes before continuing with the process.

4.5.2 Protein Analysis Procedure – Distillation Method

The distillation of the samples was conducted in a Foss Kjeldahl 2100 apparatus. Once the flasks had completely cooled, 50ml distilled water was added to each flask. Each flask was then placed into the Foss Kjeldahl 2100 apparatus, including a corresponding individual collecting flask with 20ml of boric acid (H_3BO_3) 40g/litre. The Foss Kjeldahl machine automatically transferred 60ml of sodium hydroxide (NaOH) solution to the flask with the cereal sample, catalyst and sulfuric acid. This solution turns blue when the sodium hydroxide is added due to the chemical reaction between both mixtures. Through this process, the blue solution in the flask indicates the presence of nitrogen within the samples. The Foss Kjeldahl 2100 apparatus then heated the solutions by injecting hot steam into each flask, continuously for four minutes to generate the nitrogen gas. This gas was then condensed and filtered into the individual boric acid collecting flasks. Once the distillation was completed, the flask containing the cereal sample, catalyst, sulfuric acid and sodium hydroxide was removed and disposed of accordingly. The collection flask containing the boric acid and distilled nitrogen gas was removed for the final titration stage. Like all the samples, the blank sample was processed as a standard to provide a baseline measurement to determine the results.

4.5.3 Protein Analysis Procedure – Titration Method

Once the distillation method was completed, the flasks containing the boric acid and distilled nitrogen gas were titrated back to their original colour (reddish/ violet colour). The completed titration was determined by visual colourimetry of the solution and pH level of 5.1. A 50ml burette containing hydrochloric acid (HCl) 4g/ litre was used to drip the HCl acid into the distilled sample solution until the desired colour and pH level was reached. The amount of the HCl acid required for the titration was recorded and used to calculate the sample's nitrogen. The blank sample was processed as standard to provide a baseline measurement to determine the results.

4.5.4 Protein Analysis Procedure – Expression of the Results (Nitrogen Content)

The nitrogen content, W_N results are expressed as a mass fraction of dry product and a percentage (%) is obtained using the following formula:

$$W_N(\%) = \frac{(V_1 - V_0) \times T \times 0.014 \times 100}{M}$$

Where:

V_0 – is the volume in millilitres of the hydrochloric acid (HCl) required for the blank test.

V_1 – is the volume in millilitres of the hydrochloric acid (HCl) required for the sample test.

0.014 – is the value in grams of the quantity of nitrogen equivalent when using 1ml of the 0.5Mol/ litre sulfuric acid solution.

T – is the normality of the hydrochloric acid used for the titration.

M – is the mass in grams of the test sample.

4.5.5 Protein Analysis Procedure – Expression of the Results (Crude Protein Content)

Calculation of the crude protein of the dry product was achieved by multiplying the value obtained when determining the nitrogen content (table 4.8) by a conversion factor (outlined by ISO 20483:2013) and adapted to the types of cereal or pulse. Results were expressed to a two-decimal place. Some of the nitrogen to protein conversion factors used for cereals and pulses are given below. The protein content is then calculated by multiplying the nitrogen content with the nitrogen-to-protein conversion factor. This factor originated from the work of Mulder (1839), in which the research presents several proteins in a highly purified state from natural substances. This led to a standard elemental composition of $C_{40}H_{62}N_{10}O_{12}$ for protein. Since the nitrogen content of the formula is 16%, its reciprocal, 6.25, was used as nitrogen to protein factor. Most frequently, factors published by Jones' 'research (1941) are used, which are proposed as a factor for animal proteins and as default factor for unknown proteins, being the value of 6.25. Therefore, any

samples containing cricket powder would be calculated using 6.25. However, for the control sample using just common wheat, a conversion factor of 5.7 was used.

Commodity	Nitrogen to Protein Conversion Factor
Common Wheat	5.7
Durum Wheat	5.7
Wheat Milling Products	5.7 or 6.25
Wheat for Feed	6.25
Barley	6.25
Oats	5.7 or 6.25
Rye	5.7
Corn	6.25
Pulses	6.25
Other types of Wheats	6.25

Table 4:8 Nitrogen to Protein Conversion Factor

Source: ISO Standard 20483:2013

4.6 Ash Analysis

4.6.1 Ash Analysis Procedure – Method

The ash crucibles were washed thoroughly and dried in the oven to ensure there was no moisture present. Approximately two grams of the sample were weighed into the crucibles (the sample results are mathematically calculated based on weight percentage) and burnt using a mantle for around 20 to 30 minutes to remove any organic matter. The samples in the crucibles were then transferred to a muffle furnace and heated to 550 degrees Celsius for 12 hours. A white residue was left within the crucible, which is the final ash content. The crucibles were then cooled and weighed to determine the results.

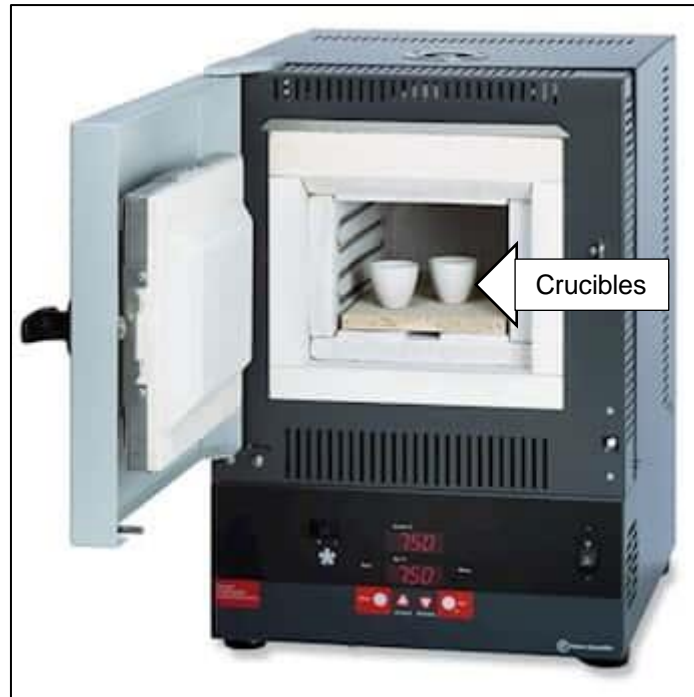


Figure 4:4 Muffle-Furnace – Ash Content Analysis Machine

4.6.2 Ash Analysis Procedure – Expression of the Results

The ash yield, W_A results are expressed as a percentage of the mass fraction. The result was achieved using the formula below:

$$W_A(\%) = \frac{(M_2 - M_1)}{M_0} \times 100$$

Where:

M_0 – is the mass in grams of the test portion on the dry basis

M_1 – is the mass in grams of the empty crucible

M_2 – is the mass in grams of the crucible and ash together

4.7 Moisture Analysis

4.7.1 Moisture Analysis Procedure – Drying Method

All samples were placed into individual open dishes and placed into the oven (preheated between 130 to 133 degrees Celsius) and left for 120 minutes. It is important to note that the oven remained closed during this time to prevent the atmospheric moisture from rehydrating the samples during the drying process. Once the drying was complete, the samples were quickly removed from the oven and

placed into a desiccator and covered. This ensured that no atmospheric moisture was reintroduced to the sample during the cooling stage. Once the dish had cooled to laboratory temperature (around 30-45 minutes), the samples were weighed.

4.7.2 Moisture Analysis Procedure – Expression of the Results without Preconditioning

The moisture content w_{H_2O} results are expressed in grams per 100g of the product as indicated using the formula below;

$$WH_2O = \left(1 - \frac{M_1}{M_2}\right) \times \frac{100}{1}$$

Where:

M_1 – is the mass in grams of the test portion after drying

M_2 – is the mass in grams of the test portion before drying

4.8 Carbohydrate Analysis

Many analytical techniques have been developed to measure the total concentration and type of carbohydrates present in food products. In 1997, FAO/WHO designed the carbohydrate analysis presenting a detailed description of the various types of carbohydrates and the method. This thesis used the standardised carbohydrate formula to determine the baked products' carbohydrate content (Bender, 2009). The carbohydrate content (dextrans, starches and sugars) was determined by calculating the percentage of the individual nutritional components and subtracting them from 100.

4.8.1 Carbohydrate Analysis Procedure – Method and Expression of Results

For many years, the total available carbohydrate content of foods has been calculated by difference, which defines carbohydrate as the difference between 100 and the sum of the weight percentages for protein, fat, water and ash (Lyons-Wall, 2012). Under this method, the other elements in the food (protein, fat, water and ash) were determined separately, added together, and then deducted from the food's total weight. Inaccuracies can arise when using this approach because of the summation errors in estimating these four constituents. However, carbohydrate' by

difference' was used for comparison informational on nutritional purposes, consistent with other researchers (Cappelli et al., 2020; Cappelli et al., 2019; Gonzalez et al., 2019) who looked at similar ingredient substitutions. This method calculated the available carbohydrates within each baked product, by using the following formula:

$$\text{Total carbohydrate \%} = 100 - (\text{weight in grams } [W_f + W_p + W_A + W_M])$$

Where:

W_C – is the total weight of the crude fat content in grams of the test portion

W_P – is the total weight of the crude protein content in grams of the test portion

W_A – is the total weight of the ash content in grams of the test portion

W_M – is the total weight of the moisture content in grams of the test portion

4.9 Gerhardt – Total Dietary Fibre Analysis

The principle of this procedure was to subject the sample to a multi-stage enzymatic process. All components, other than total dietary fibre, was dissolved and flushed out of the sample. The precipitate left from the sample was processed to determine the remaining nitrogen/proteins and ash content. A difference calculation resulted in the total dietary fibre within the samples. This method was conducted according to the AOAC 991.43 process: total, soluble and insoluble dietary fibre in foods.

4.9.1 Fibre Analysis Procedure – Preparation of the Sample

All samples were degreased with petroleum spirit several times, to ensure that all fat content was removed before testing. The resulting samples were then allowed to completely dry (around 15 minutes) before continuing with the fibre analysis.

4.9.2 Fibre Analysis Procedure – Enzymatic Decomposition Method

Approximately two grams (the sample results are mathematically calculated based on weight percentage) of the dried degreased samples was transferred to individual 400 ml glass beakers. Forty milliliters of the MES/TRIS buffer solution and small magnetic stirring staffs were added to each individual beaker. This was allowed to stir for 5 minutes to homogenise the mixture thoroughly. Using a pipette, 50µl of the

α -amylase suspension was then added to the individual beakers, stirred and then covered with aluminium foil. These beakers were then placed in a water bath at 99 degrees Celsius for 30 minutes and rotated slowly every few minutes. The beakers were removed from the water bath and cooled at room temperature until 60 degrees Celsius was reached. Once this temperature had been achieved, 100 μ l protease solution was pipetted into each beaker and briefly stirred with a magnetic stirrer. The beakers were then transferred back to the water bath at 60 degrees Celsius for 30 minutes and slightly rotated every 10 minutes. The samples were then removed from the water bath, and 5ml HCl (hydrochloric acid solution - 0.56Mol/litre) was added to each individual beaker. Using a pH meter, the level was then adjusted between 4.0 to 4.7 (using pH buffer solutions). Once the optimum pH was reached, 200 μ l amyloglucosidase suspension was pipetted to the solutions, and the beakers were transferred back to the water bath for 30 minutes. These were held at 60 degree Celsius, and the beakers were rotated every 10 minutes. Two blank samples were processed as standard to provide the baseline measurement for the results.

4.9.3 Fibre Analysis Procedure – Precipitation Method

The sample mixtures were removed from the water bath, and 220ml 95% ethanol was added to each beaker. The mixture was then left for 60 minutes at room temperature to allow the particles in the solutions to settle. Where sample solutions had high levels of particles suspended, these were left overnight to provide better results.

4.9.4 Fibre Analysis Procedure – Filtration Method

Once the sample solutions had settled, the mixtures were filtered through FibreBags®. After transferring the sample mixture through the FibreBag®, any remaining precipitate left in the beakers was removed by rinsing individually, first with 10ml of 78% ethanol and then with an additional 20ml of 78% ethanol. Each rinsing was passed through the corresponding Fibrebag®, to catch all the precipitate. Subsequently, all FibreBags® were left overnight at 18 degrees Celsius to dry for nitrogen/protein and ash incineration analysis. Once the samples were dried, these were sub-divided into two samples; one of which was used to determine the total crude protein using the Kjeldahl method (see Chapter Four, subsection

4.5), and the other was used to determine the ash content (see Chapter Four, subsection 4.6).

4.9.5 Fibre Analysis Procedure – Expression of the Results

The fibre yield, F results are expressed as a percentage of the mass fraction. The result was determined using the formula below:

$$F\% = \frac{\frac{M_{R1} - M_{R2}}{2} - M_{Pr} - M_A - M_{BI}}{\frac{M_1 - M_2}{2}} \times 100$$

To ascertain M_{BI} , the following formula is required:

$$M_{BI} = \frac{M_{R1BI} - M_{R2BI}}{2} - M_{PrBI} - M_{ABI}$$

Where:

M_{R1} – is the mass of the residue of sample

M_{R2} – is the mass of the residue of sample

M_{Pr} – is the mass of the proteins

M_A – is the mass of the mineral compounds (ash)

M_{BI} – is the mass of blind value

M_1 – is the mass of the sample for protein determination

M_2 – is the mass of the sample for ash determination

4.10 Overall Nutritional Compositional Results

The nutritional composition analysis results are presented in the summary table (see Appendix D), which gives the breakdown of the fat, protein, ash, moisture, and the fibre content of each baked product samples. These results of the individual nutritional composition factors were analysed through a 1x3 statistical analysis: 1. control - T55 wheat flour vs cricket powder, 2. control - T55 wheat flour vs T55 wheat flour and cricket powder, 3. control - T55 wheat flour vs T55 wheat flour, cricket powder, quinoa- and Khorasan- flour, 4. control - T55 wheat flour vs T55 wheat flour, cricket powder, quinoa-, Khorasan- flour and mixed seeds).

4.10.1 Results: Crude Fat Analysis

Figure 4:5 shows the mean values for the crude fat content of all the bread samples analysed in each of the four experimental conditions: the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan- flour and mixed seeds). The crude fat mean values for the samples were as follows, sample A $M = 0.08$ (SD = 0.01), sample B $M = 2.31$ (SD = 0.12), sample C $M = 1.56$ (SD = 0.02) and sample D $M = 10.08$ (SD = 0.04). Analysing the total crude fat results, samples B, C and D showed an increase in the fat content: 2.31g, 1.56g, 10.08g per 100g, respectively.

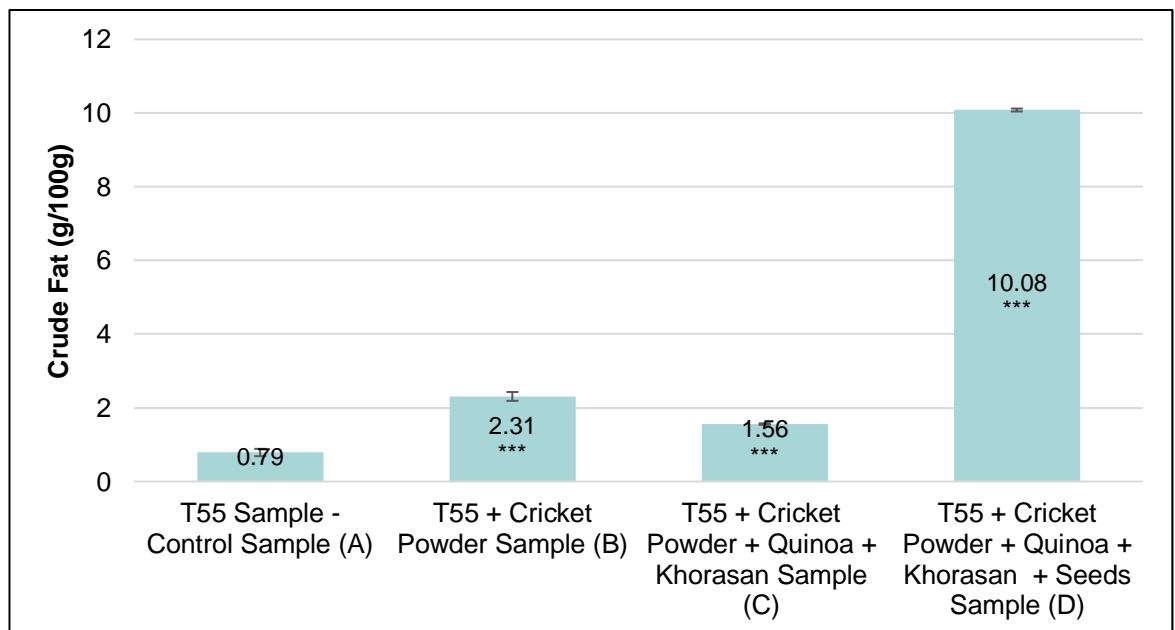


Figure 4:5 Total Crude Fat Content for all Samples

Total mean value of the crude fat content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds)

All values are means from three replications $n = 3$

***- $p < .001$ in comparison to the control wheat flour sample bread

The one-way ANOVA showed F to be statistically significant, $F(1,3) = 14454.98$, $p < .001$, partial $\eta^2 = 1$. All bread samples showed a statistical significance $p < .001$ to indicate a difference in the crude fat content compared with the control sample (T55 wheat flour). Due to the statistical difference, a Tukey HSD test was performed for post-hoc comparison among the individual samples. There was a statistically

significant difference among the mean results (individual crude fat parameter samples), consisting of four groups ranging from lowest to highest: A, C, B and D respectively, in the homogeneous subsets. The results show that samples B and D displayed a higher crude fat content due to the natural occurrence of lipid content in seeds and cricket powder as they are both rich in fatty acids.

The fat content in sample B, the inclusion of 30% cricket powder, contributed to the increase in fat content within the baked product, indicating that 100g of sample B would contribute 20.79kcal of energy. However, compared with sample C, the crude fat decreased due to incorporating additional flours (quinoa- and Khorasan- flour), which proportionately decreased the total cricket powder quantity within the baked product. The crude fat for sample D increased vastly due to the inclusion of the 25% seeds within the recipe, increasing the fat content by 8.5 grams compared with sample C.

4.10.2 Results: Crude Protein Analysis

The crude protein mean values of all bread samples are displayed in figure 4:6. These were analysed in each of the four experimental conditions: the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan- flour and mixed seeds). The crude protein mean values for the samples were as follows, sample A $M = 12.2$ (SD = 0.25) sample B $M = 30.63$ (SD = 0.24), sample C $M = 31$ (SD = 0.18) and sample D $M = 41.74$ (SD = 2.14). Analysing the total crude protein results, the inclusion of cricket powder, (and) ancient grains, (and) mixed seeds) increased the total protein content for samples B, C and D: 30.6g, 31g, 41.7g per 100g, respectively.

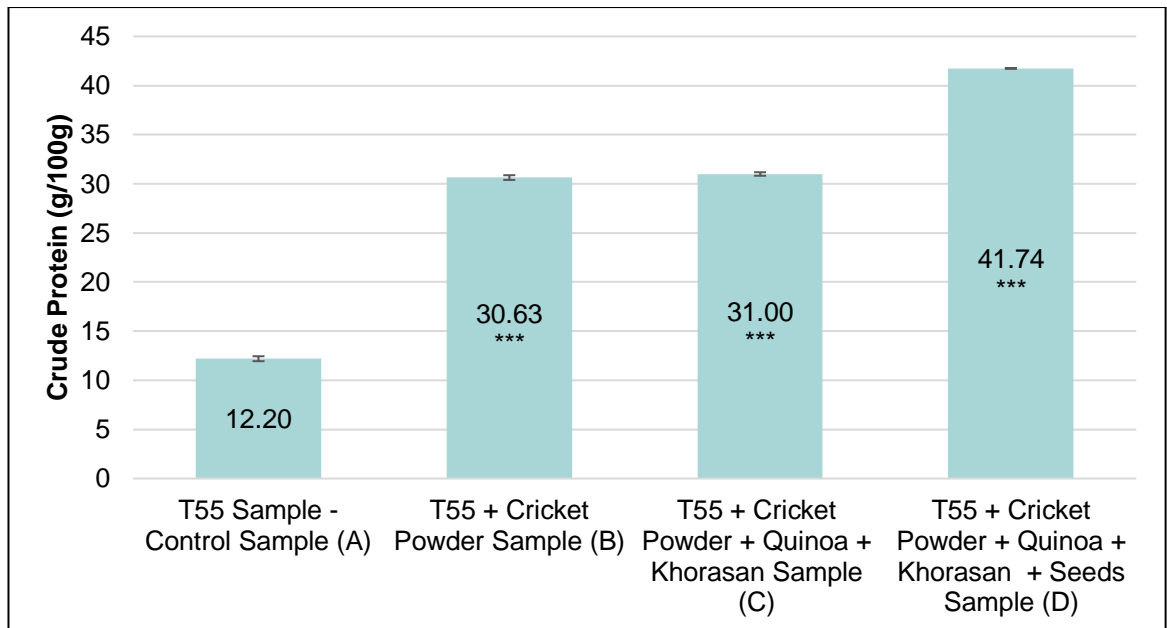


Figure 4:6 Total Crude Protein Content for all Samples

Total mean value of the crude protein content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds)

All values are means from three replications $n = 3$

***- $p < .001$ in comparison with the control wheat flour sample bread

The one-way ANOVA showed F to be statistically significant, $F(1,3) = 378.86$; $p < .001$, partial $\eta^2 = .99$. All bread samples showed a statistical significance $p < .001$ to indicate a difference in the crude protein content compared to the control sample. As there was a significance through the statistical analysis, a Tukey HSD test was performed for post-hoc comparison means among the individual crude protein parameters. The results indicate that there was a statistically significant difference among the mean results (individual crude protein parameter samples), consisting of three groups ranging from lowest to highest: A, B&C and D respectively, in the homogeneous subsets. Sample D displayed a higher crude protein content due to the combination of seeds and cricket powder. The high concentration of the crude protein content within the bread samples B, C and D was from the cricket powder fortification, as this crude protein level increased by 18.43g between sample A and B. Furthermore, a combination of cricket powder and mixed seeds can increase the protein content exponentially. This can be seen with the protein increase by 29.54g between sample A and D, and an increase by 10.74g between sample C and D.

4.10.3 Results: Ash Analysis

Figure 4:7 shows the mean values for the ash fat content of all bread samples analysed in each of the four experimental conditions: the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan- flour and mixed seeds). The ash content mean values for the samples were as follows, sample A $M = 2.61$ (SD = 0), sample B $M = 3.5$ (SD = 0), sample C $M = 3.58$ (SD = 0.3) and sample D $M = 3.89$ (SD = 0). Analysing the total ash results, samples B, C and D showed an increase in the fat content: 3.5g, 3.58g, 3.89g per 100g respectively.

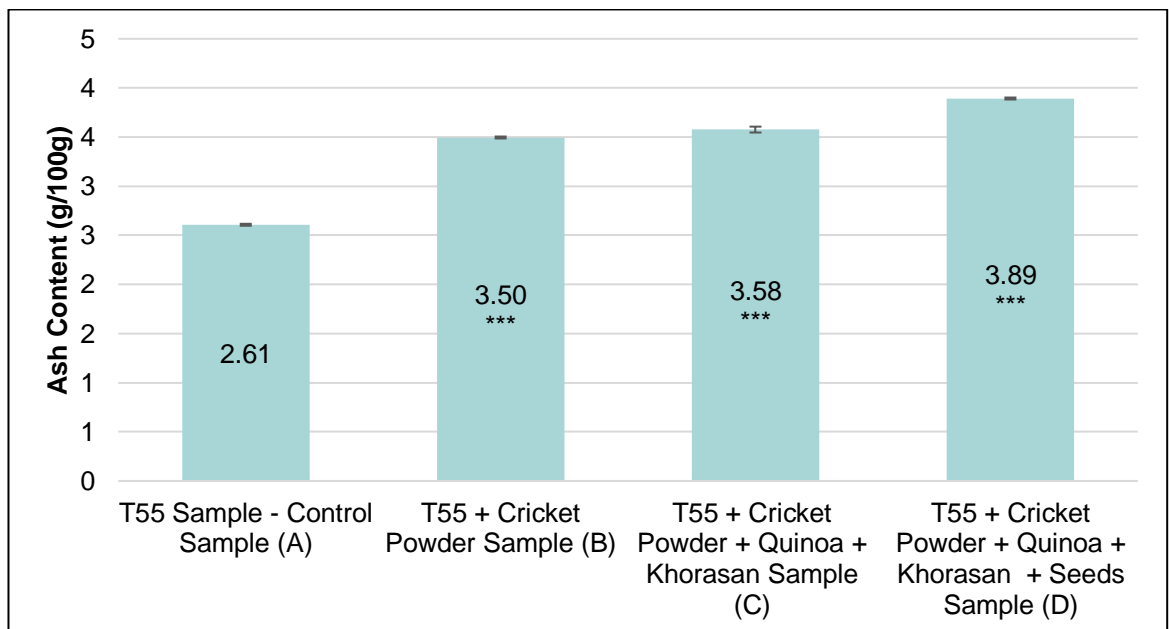


Figure 4:7 Total Ash Content for all Samples

Total mean value of the ash content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds)

All values are means from three replications $n = 3$

***- $p < .001$ in comparison with the control wheat flour sample bread

The one-way ANOVA showed F to be statistically significant, $F(1,3) = 3595.77$, $p < .001$, partial $\eta^2 = 1$. All samples showed a statistical significance $p < .001$, which indicated a difference in the ash content than the control sample A. Following the ash content results, a Tukey HSD test was performed for post-hoc comparison means among the individual ash content parameters. Therefore, there was a statistically significant difference among the mean results (individual ash content

parameter samples), consisting of three groups ranging from lowest to highest: A, B&C and D respectively, in the homogeneous subsets. The ash content for samples B, C and D, had a substantial increase due to the cricket powder, ancient grains and seeds. This indicates that the higher ash content within the bread products could have increased the fermentation activity. Consequently, a reduction in yeast should be used to avoid a fast fermentation and consequent impact on baking performance, especially the aeration and gas cell structure

4.10.4 Results: Moisture Content

The moisture content mean values of all bread samples are displayed in figure 4:8. These were analysed in each of the four experimental conditions the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan-flour and mixed seeds). The moisture content mean values for the samples were as follows, sample A $M = 16.46$ (SD = 0.71) sample B $M = 18.5$ (SD = 2.12), sample C $M = 18.93$ (SD = 1.89) and sample D $M = 20.83$ (SD = 2.41). Analysing the total moisture content results, the inclusion of cricket powder, (and) ancient grains, (and) mixed seeds) increased the moisture content for samples B, C and D: 2.04g, 2.47g, 4.37g per 100g respectively.

The one-way ANOVA showed no statistical significance, $F(1,3) = 2.69$; $p > .05$, partial $\eta^2 = 0.32$. As there was no statistical significance $p > .05$ between the samples (A B, C and D), this indicates that all samples had similar moisture content. This result was expected as all bread products had a similar moisture content. Although there was a slight increase in moisture content for sample B, C and D, this is due to the additional hydration of the cricket powder and the seeds in the recipe.

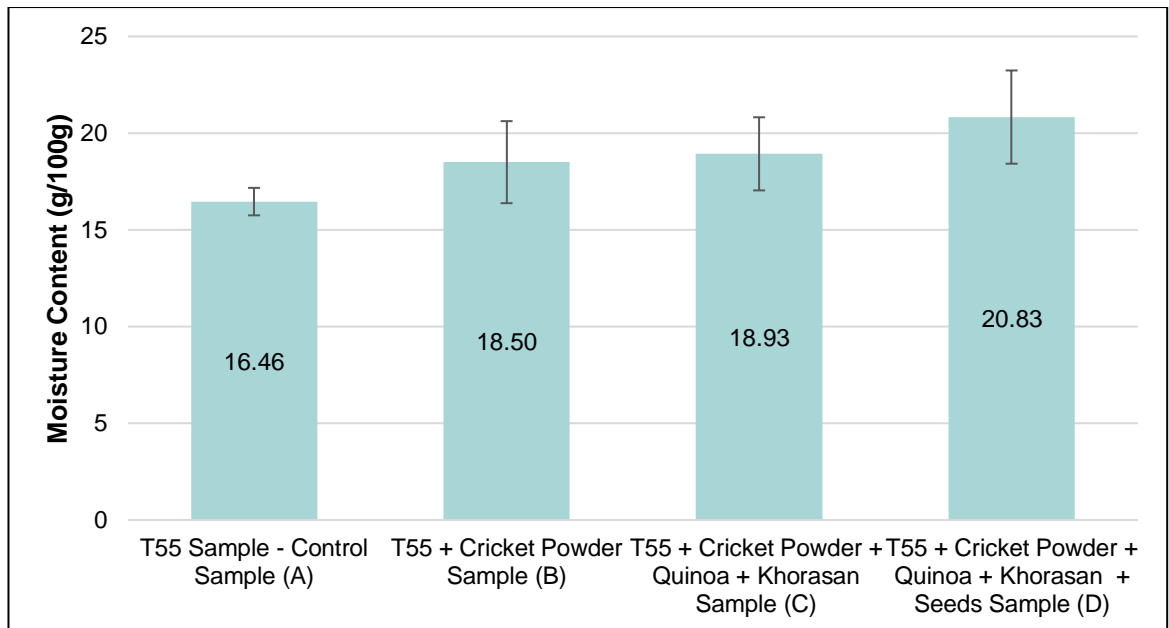


Figure 4:8 Total Moisture Content for all Samples

Total moisture content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds)

All values are means from three replications $n = 3$

4.10.5 Results: Total Dietary Fibre Analysis

Figure 4:9 shows the mean values for the total dietary fibre content of all bread samples analysed in each of the four experimental conditions: the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan- flour and mixed seeds). The total dietary fibre mean values for the samples were as follows, sample A $M = 9.38$ (SD = 1.34), sample B $M = 13.09$ (SD = 0.6), sample C $M = 10.55$ (SD = 1.75), and sample D $M = 11.79$ (SD = 1.07). Analysing the total dietary fibre results, samples B, C and D showed an increase in the fibre content: 3.71g, 1.17g, 2.41g per 100g respectively.

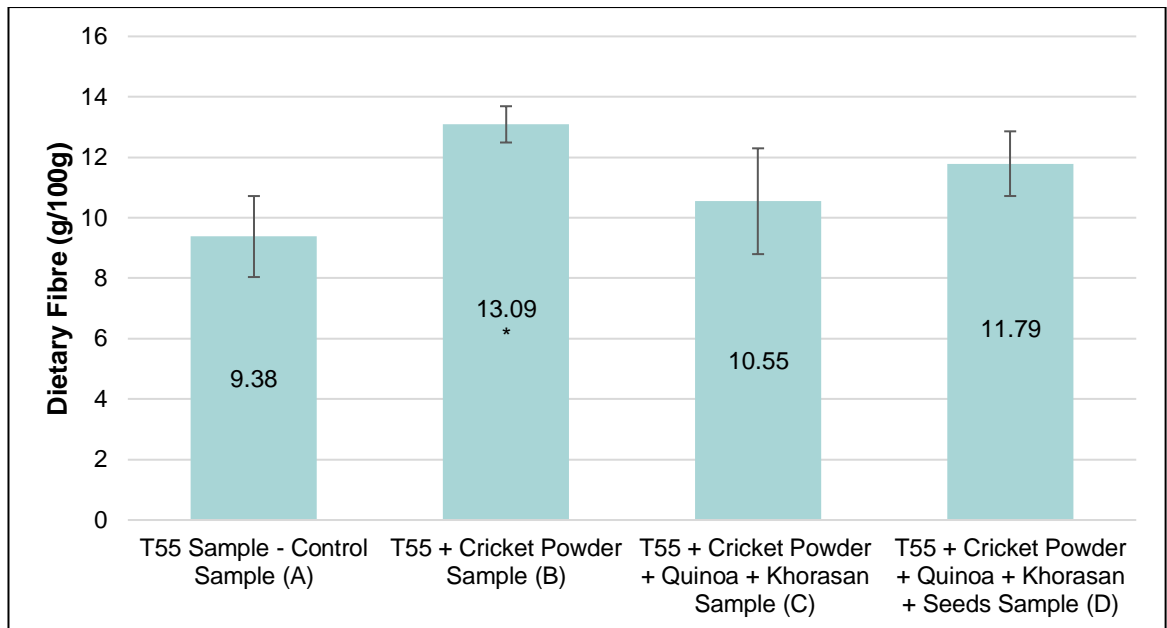


Figure 4:9 Total Dietary Fibre Content for all Samples

Total mean value of the dietary fibre content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds)

All values are means from three replications $n = 3$

*- $p < .05$ in comparison with the control wheat flour sample bread

The one-way ANOVA showed F to be statistically significant, $F(1,3) = 4.81$; $p < .05$, partial $\eta^2 = 0.64$. There was a statistical significance ($p < .05$) between samples A and B due to the concentrated cricket powder present in the sample. Due to the statistical difference, a Tukey HSD test was performed for post-hoc comparison means among the individual total dietary fibre parameters. Therefore, there was a statistically significant difference among the mean results (individual total dietary fibre parameter samples), consisting of two groups ranging from lowest to highest: A, C&D and C,D&B respectively, in the homogeneous subsets.

Sample D increased the fibre content of the bread products specifically due to the percentage of cricket powder. Analysing sample B, the total dietary fibre, increased due to the amount of cricket powder present in the sample (30%). However, in samples C and D, the cricket powder is diluted due to the additions of further flours (quinoa- and Khorasan- flour). Likewise, sample D's fibre level increased by a small change compared with sample C due to the fibre content of the added mixed seeds. Although this was only a small change, it shows that the addition of seeds can impact a baked product's overall fibre content.

4.10.6 Results: Carbohydrate Analysis

Figure 4:10 shows the mean values for the total dietary fibre content of all bread samples analysed in each of the four experimental conditions: the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan- flour and mixed seeds). The carbohydrate content mean values for the samples were as follows, sample A $M = 67.95$ ($SD = 0.41$), sample B $M = 45.06$ ($SD = 1.77$), sample C $M = 44.94$ ($SD = 1.57$), and sample D $M = 23.46$ ($SD = 1.09$). Analysing the total carbohydrate results, samples B, C and D showed a decrease in the carbohydrate content: 22.89g, 22.95g, 44.49g per 100g respectively.

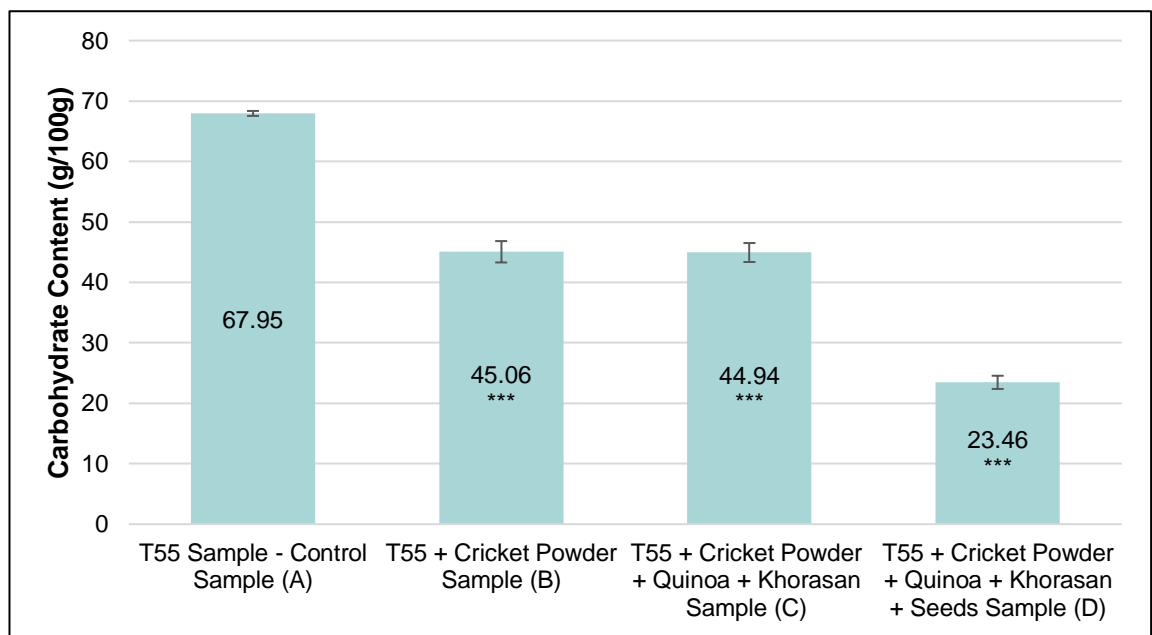


Figure 4:10 Total Carbohydrate Content for all Samples

Total mean value of the carbohydrate content of breads samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds)

All values are means from three replications $n = 3$

***- $p < .001$ in comparison with the control wheat flour sample bread

The one-way ANOVA showed F to be statistically significant, $F(1,3) = 497.03$; $p < .001$, partial $\eta^2 = 0.99$. All bread samples showed a statistical significance $p < .001$ to indicate a difference in the carbohydrate content. Following the carbohydrate content results, a Tukey HSD test was performed for post-hoc comparison means

among the individual overall carbohydrate parameters. Therefore, there was a statistically significant difference among the mean results (individual overall carbohydrate parameter samples), consisting of three groups ranging from lowest to highest: D, B&C and A respectively, in the homogeneous subsets. Sample D containing cricket powder, ancient grain and seeds, displayed the lowest carbohydrate content. Nevertheless, the individual values of the contributing ingredients (cricket powder, quinoa-, Khorasan- flour and seeds) had an overall impact on the baked products. Moreover, in sample D the major reduction in carbohydrate content was due to the mixed seeds, which increased the fat and protein content, decreasing the carbohydrate calculation.

4.11 Nutritional Compositional Discussion

The baked products' nutritional values increased based on the fortification level of the cricket powder, ancient grains, and seeds in the recipe. Like most foods, the preparation and processing methods would influence the final nutritional composition. However, the baked product's internal temperature achieved through baking (to gelatinise the starches and gluten-forming proteins), does not entirely affect the nutritional composition; unlike frying and boiling which would have subjected the nutrient components to higher heat, thus, reducing the individual compounds. The amalgamation of flours (cricket powder and ancient grains) within a baked product, successfully generated a nutritional product suitable for human consumption. This provides evidence that hybrid flour fortification, affects the nutritional values by increasing the individual macro-nutrient levels. Furthermore, the baked products provided high nutritional values, especially in fat, protein, and fibre, essential for human health. Over the past several years, WHO, FAO, and UNU have endorsed insect consumption, which has become a trendy solution to poverty struck and developing countries. Edible insects play a significant role in sustainable food diets as they contain vital micro- and macro- nutrients. These specific components are vital in developing countries like Zimbabwe, where local flours and ingredients can be successfully used to combat malnutrition. Through this knowledge and evidence of blending flours with cricket powder, an optimised product can be produced which exploits the full nutritional potential of local ingredients to feed the population. From a practical point of view, it could be considered easier to adjust traditional food products than encourage entomophagy.

Nevertheless, through this research, an unfamiliar food such as cricket powder can be usefully and successfully incorporated into baked products.

The baked products' protein content's final results ranged from 12.2grams to 41.74grams, with the highest protein content in the final sample (cricket powder, ancient grains and seeds). This is in line with Montowska et al., (2019), González et al., (2019), Khuenpet (2020), and Wu et al., (2020) research which showed that cricket powder is a rich source of protein. Despite the widespread recognition of insects as a good source of protein (FAO, 2013), a formulated recipe with a combination of ingredients shows that an increase in protein can be achieved. Thus, incorporating these local flours and ingredients from Southern Africa into the dough mixture can increase the finished product's protein content. The Pearson correlation indicated that as the fortificant levels increased the protein content improved $r = 0.88$. High values of protein within baked products can replace human consumption of animal protein, especially within developing countries where animal protein is limited or absent in many consumers diets due to cost or lack of availability. In developed countries, individuals consume larger amounts of protein *per capita* than developing nations: usually, around 96grams per person/day, of which 65% is obtained through meat consumption. In developing countries, an estimated 56grams of protein per person/day is consumed, of which 25% is obtained through meat consumption (Yen, 2010).

Crickets are a source of energy for human consumption, due to the fat found within the insects. The fat content of the bread samples analysed ranged from 0.08g to 10.08g per 100g. However, the bread samples with only cricket powder showed that average fat content increased to 2.31g per 100g. The two samples, which included ancient grains, only increased the fat content to 1.56g per 100g compared with the control sample. As a general trend, baked products containing cricket powder were considerably highest in fibre, protein and fat. This was similar to research conducted by de Oliveira et al., (2017), González et al., (2019), Wu et al., (2020) and Osimani et al., (2018).

Nevertheless, the major increase in the fat content is sample D which contained seeds. This indicated that most of the fat content in bread sample D was due to the

natural oils present in the mixed seeds. However, the fat content in the insects can be influenced by their feed whilst they are still being reared. Bukkens' (1997) research was evident, which concluded that the essential fatty acid content could change according to the insect species' feeding. Although this thesis's main intention was not to individually study the fatty acid compositions; Yang et al., (2006) provided evidence that crickets contain fatty acids, especially long-chain omega-3 fatty acids α -linoleic, linoleic and eicosapentaenoic- acids (EPA). Moreover, the unsaturated fatty acid profile of crickets was similar to white fish and poultry. However, crickets contain higher concentrations of polyunsaturated fatty acids than poultry and red meat. The polyunsaturated fatty acid (PUFA) and docosahexaenoic acid are important for brain and eye development for humans. The natural occurrence of fatty acids within crickets makes these products nutritionally important for children and infants' development. This is a further endorsement for cricket powder consumption as a direct food source for humans.

Insects containing significant amounts of fibre are commonly found to have increased chitin levels due to the presence of the insect's exoskeleton. The importance of consuming dietary fibre within the human body allows this to be fermented in the colon where it is converted to short-chain fatty acids which can be absorbed from the large bowel and oxidized for energy. The fibre level present within the bread samples influences consumers' satiety as it reduces the rate of gastric emptying and gastrointestinal transit which is in line with Gasco et al., (2018) research. Carbohydrate- containing foods that are high in fat and protein have a low Glycaemic Index because of the effects of these components on the gastric emptying: higher-in-satiety value products (containing high levels of protein, fat and fibre) will remain in the stomach for a longer period, thus, limiting the food intake after reaching satiety value, making the consumer feel full for a longer period and reducing obesity problems. Moreover, the fibre found within the baked products can have a physiological effect on the body, reducing the prevalence of chronic diseases such as diabetes, heart disease and cancer (Osimani et al., 2018; Lopez Santamarina et al., 2020; Tu et al., 2016). The mechanisms behind these findings are still unclear to scientists and clinicians; however, one possibility is attributed to fibre, where an increase in consumption can lead to several beneficial effects which is in line with Roca-Saavedra (2018) research. These include increasing bile acid

excretion, reduced caloric consumption, increased short-chain fatty acid production, carcinogen binding effects, increased antioxidants, and increased absorption of vitamins and minerals (Lattimer and Haub, 2010).

4.12 Nutritional Compositional Conclusion

Overall, the addition of cricket powder affects the nutritional composition of baked products. This can be seen from the results and discussion which demonstrates the necessity of using insects as a direct food source. Legumes and pseudocereals can be used to fortify baked products for nutritionally enriched bread. However, results obtained in the thesis show that crickets provide an alternative method of acquiring sufficient protein, fat and fibre within baked bread products. There are limited studies indicating the effect and process of these ingredients within baked products. Nevertheless, the formation of bread dough is similarly achieved worldwide, and therefore inclusions of such flours can be adapted within each country.

As previously mentioned in Chapter One, this thesis's purpose was to identify the nutritional composition of locally sourced ingredients in Southern Africa which can benefit the Country's population. There is great potential for such ingredients to support meet the millennium development goals as an intervention for malnutrition, helping with two key areas – Health and Food. With bread products delivering essential micro- and macro nutrients, fortification with cricket powder can help developing countries with vital nutrition. Through the use of these sustainable ingredients, global diseases can decrease, and life expectancy increase not only in developing countries but also, possibly, in developed countries. Therefore, the positive impact of such products may be seen not only on the environment where GHG emissions can be significantly reduced but also by reducing the land, water and carbon footprint to produce adequate edible protein, fibre and fat for human consumption.

4.12.1 Key Summary Conclusion Points

- When comparing the nutritional composition of the bread products, cricket powder, ancient grains and seeds, showed to increase the protein, fat and fibre content.

- Zimbabwe can use crickets for protein, fibre and fat fortification as an intervention to combat malnutrition. Through this fortification, consumers may receive high concentrations of micro- and macro-nutrients required for their diet.
- In addition to the fortification of baked products with cricket powder, ancient grains and mixed seeds can act as a functional hybrid product. This can help with the following:
 - Provide a functional product that offers health benefits, such as maintaining a healthy, balanced diet (protein, fat, fibre and low carbohydrates).
 - Producing a commodity that can be made in the local country with local resources. Therefore, reducing undesired or unhealthy ingredients within the consumers' diet, for example, high salt, sugar and saturated fat ingredients.
 - As a functional ingredient to enhance the food as a vehicle for superior nutritional value.

Chapter 5 – Crumb Structure and Texture Analysis

‘We don’t give a damn to the insects on our Earth, but if we could find even a single insect on Mars, the whole world would cherish it like crazy’.

(Ildan, no date)

5.1 Introduction

The quality of baked products, especially bread, depends on the control of staling and microbiology. There are only a few ingredients involved in the breadmaking process and, therefore, there is a particular reliance on the quality and functionality of the ingredients, dough formation and baking environment to develop a perfect loaf of bread. The internal bread structure is set by the formation and gelatinisation of the protein and starch network available within the dough, provided by cereal flours. This network creates a multitude of air bubbles within the dough that are filled with carbon dioxide generated through the fermentation process. The gas-filled matrix expands under the influence of heat during baking and is stabilised by the starch gelatinisation and denaturing of the proteins during the baking process (Sozer et al., 2011). The basic perceptions (size, shape, texture, appearance and colour) of baked bread products can influence consumers and subsequently impact their behaviour towards the product. For instance, large and soft loaves give a visual appearance that consumers deem value for money.

Meanwhile, an open, porous crumb structure appeals to the consumer as potentially meeting their organoleptic senses. However, the increase in the number of air bubbles in the crumb texture can accelerate the staling rate (Megahey et al., 2005). Thus, the slowing of the bread product's staling rate can be highly dependent on the formulation of the recipe and the breadmaking process used to make it. The null hypotheses tested in this chapter are as follows, firstly (**H₀**): The addition of cricket powder and ancient grains in baked products did not influence the crumb structure (brightness, crumb cell, volume and shape). Secondly, (**H₀**): The inclusion of cricket powder and ancient grains in baked products did not affect the textural properties and the staling rate. Therefore, this chapter investigated whether the hybrid flour blends (cricket powder, quinoa-, Khorasan- flour and mixed seeds) impacted the

baked products' texture and crumb structure without any additional additives or preservatives.

5.2 Literature Review: Crumb Structure and Impact on the Texture

Dough aeration and rheology are the fundamental interactions throughout the breadmaking process, creating the distinctive and appealing structure of bread (Campbell and Martin, 2012). Gluten development is generated through the mixing and kneading of the dough, which is then subjected to fermentation activity of the yeast. It is through this dough development and inclusion of yeast which provides carbon dioxide. This fermentation activity allows the cells to be filled with carbon dioxide, which inflates the cell under the influence of heat, during the proving and baking process. As the gas expands when heated up, this creates the doughs' aeration within the bread product. Allowing the gas cells to be created and inflate, surrounded by the developed gluten creates the baked products' volume. The protein network was then set by the baking process creating the required crumb (Campbell and Martin, 2012).

Cereal flours (wheat, rye, spelt, barley) are ultimately the best ingredients to form an elastic dough as it contains by far the greatest level of gluten-forming proteins that have a unique property. The cereals' gluten-forming proteins form a visco-elastic dough when hydrated and kneaded (Bushuk, 1985). For this study, dough made from 100% wheat flour was compared with doughs made from combinations of cricket powder, quinoa- and Khorasan- flour. Particular observations were made by the physical changes in soluble and insoluble proteins and their impact on the dough's visco-elasticity. The desirable outcome of the dough formation and aeration truly depends on two factors when producing bread products:

- The ability to retain carbon dioxide gas which is generated during the fermentation process.
- A balance of a viscous flow and elastic strength so that the loaf can expand adequately during proving and retain its shape (Cauvain, 2012).

Throughout the breadmaking process, the flour and dough components are classified into seven groups (Cauvain, 2012):

1. Starch
2. Gluten-forming proteins
3. Non-starch polysaccharides (pentosans)
4. Fats and lipids
5. Water-soluble proteins
6. Inorganic compounds
7. Celluloses associated with the bran

These individual groups are actively involved with dough formation during the mixing and subsequent processing. For instance, the starch present in wheat flour makes up about 65%-75% of its composition, consisting of around 25% amylose and 75% amylopectin (Nakamura et al., 1995). These two types of starch granule chain polymers influence the doughs' elasticity once hydrated with water and depending on the damaged starch, thus easier to form a gel. It is the 'damaged' starch granules that can absorb up to four times as much as water than intact granules. The gluten-forming proteins and non-starch polysaccharides (water-soluble pentosans) are influenced depending on the level of water, which would affect the rheological behaviour and this function of hydration and development of the gluten network that affects the visco-elastic behaviour. The catalysed reaction between pentosans (both water-soluble and insoluble), for instance the use of an oxidising agent can generate free radicals and promote cross-links between polymer chains and during the heating stage, gelatinisation of both water-soluble and insoluble pentosans.

Fats and lipids are naturally present in flour, and these are necessary during the dough development stage. Additional fats and lipids incorporated within the dough have the most influence on the bread's baking performance, especially relating to oven spring (volume) and the final crumb quality. The inclusion of lipids during the breadmaking process improves the dough handling, gas retention, crumb structure and flavour (Melis and Declour, 2020). This facilitates air incorporation during the dough mixing stage by plasticising and lubricating the dough, which the fat crystals are incorporated within the gluten network and stabilise. During the baking process, the fat crystals melt into an oil layer that surrounds the expanding gas cell, lubricating the crumb and creating a 'tenderising' effect (Melis and Declour, 2020). Bran and ash are found naturally in flour, and the increasing levels by fortification

can impact the volume and crumb structure, including the dough's gas retention ability. The additional bran reduces the dough extensibility and resistance to extension, which decreases the specific volume of bread and crumb. Although bran is required in the human diet, this can affect the dough formation and stability, particularly the bran's particle size. Therefore, it was important to manage both fat and bran's qualities within the dough during development to ensure they do not negatively impact the volume and crumb structure.

Figure 5:1, shows the interaction between aeration and dough rheology, especially regarding the dough formation, by either mixer design and mixer operation throughout the mixing, proving and baking process. Understanding dough rheology and aeration is essential in developing new designs and concepts as the dough interaction during different stages determines the final baked loaf volume and crumb structure.

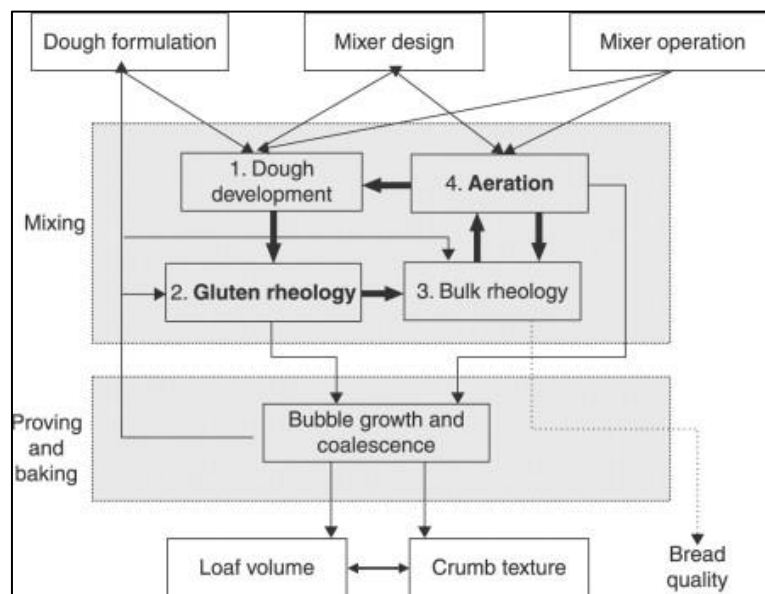


Figure 5:1: Interactions Between Aeration and Rheology as Influenced by Dough Formation, Mixer Design and Mixer Operation, during Mixing, Proving and Baking

Source: Campden and Martin, 2012

5.2.1 Challenges in Developing Cricket Powder Baked Products

The addition of cricket powder within the dough influences the finished products' volume and crumb structure. As previously discussed, the inclusion of bran within baked products impacts the volume of the product and the elasticity of the crumb.

Cricket powder acts similarly to bran, as this dilutes the non-starch and gluten-forming proteins within wheat flour, which would affect the structure and texture of the baked product. In their research on improving the quality of high-fibre bread, Hartikainen and Katina (2012) discussed obtaining sufficient aeration in high fibre bread. They concluded that obtaining an acceptable loaf volume with sufficient aeration in high fibre bread is difficult to accomplish. Salmenkallio-Marttila et al., (2001) research, reported that 20% bran inclusion within a baked product reduces the final volume by 19%. The same research confirmed that fibre-enriched bread firmness could increase as much as 41% compared with bread without fibre-enrichment. It is suggested that the deleterious effects of the fibre incorporated in the dough are due to the dilution of the gluten network structure, thus impairing the gas retention properties of the dough, rather than any effect on gas production (Shetlar and Lyman, 1944). This was confirmed in 1977 by researcher Pomeranz which detected differences in the crumb structure when compared with a control white wheat bread product through a microscope. The wheat bread's crumb structure comprises thin sheets and filaments, which are practically non-existent in bran enriched bread. This explains the reduced volume and dense crumb structure in bran bread due to the dilution of the gluten-forming proteins (Galliard and Gallagher, 1988).

5.2.2 Cricket Powder Fibre and The Gluten Matrix

The inclusion of high bran or fibre ingredients within doughs can disrupt the starch-gluten matrix. It can also restrict and force the gas cells to expand in a particular dimension (Gan et al., 1992). The presence of fibre prevents the gluten protein's aggregation, distorting the gas cell structure and contributing to the resultant crumb morphology, thus resulting in the dense crumb texture. The inclusion of cricket powder causes differences in volume, which reduces due to the addition of the fibre present in the cricket powder. This attribute reduces extensibility and weakens the gluten network; due to dilution, reduced hydration, and interaction with non-starch carbohydrates and non-gluten proteins, it also reduces the gas-retention capability. This correlates with Gonzalez et al., (2019) research investigating the inclusion of *Acheta Domestica* (another cricket specie) within baked products at 5% of the flour weight; and found that the rheological properties were affected by the fibre present.

Using cricket powder required changes to the processing method for production to make baked goods that are acceptable for the consumer. The cricket powder ingredient's particle size is important for the fibre distribution, which affects the texture of the baked product. For instance, the inclusion of fine particle cricket powder (500 microns) within the baked product produces a denser crumb compared to coarse cricket powder (1500 microns). However, as the loaf texture and physiological effect influences consumer acceptability, cricket powder also differs between fine and coarse cricket powder inclusion. Overall, there is a limit to the potential enrichment of baked products with cricket powder, as it has a detrimental effect on the organoleptic characteristics, including appearance, mouthfeel, flavour and textures when specific concentrations are exceeded.

5.3 Materials and Methodology

5.4 Materials and Method Calibre C-Cell

C-Cell illumination system was designed to accentuate the cell structure of the surface of the slices. The images are processed to measure the data and displays 48 parameters for each slice analysed. The results provide a general description of the product characteristics compared to a similar product type. It is important to standardise the method used to slice the bread to ensure a flat, clean-cut surface to ensure consistency of the results achieved. All samples were frozen for 24 hours and then cut to 25mm thickness using a universal electric slicer to ensure even thickness. This standard is in accordance with the AACCI standard method 10-18.01.

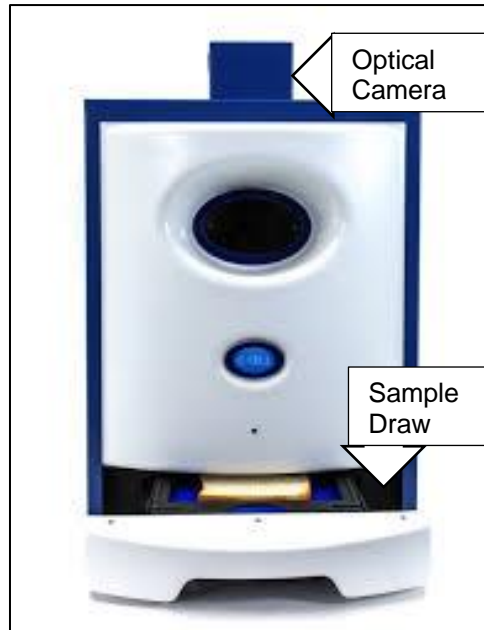


Figure 5:2 Calibre C-Cell – Crumb Structure Analysis Machine

5.4.1 Crumb Analysis Procedure – Method

The bread slice (25mm thickness) was placed into the sample draw, ensuring the sample orientation and the software orientation are both aligned. Selecting ‘analyse image’, allows the processed image window to be displayed along with the crumb data. This enables the general description of the product characteristics to be compared against each sample readings.

5.4.2 Crumb Analysis Procedure – Expression of Results

Crumb Data Calculation and Expression Results	
Slice Colour	Brightness: The mean grey level is expressed between 0-255 of the pixels within the slice. The value is lower for products with a darker crumb, for example, wholemeal and rye breads. Products that have larger or deeper cells that contribute to greater shadows can affect the brightness scale.
Gas Cell Size	<p>Number of Cells: The number of discrete cells detected within the slice. A high value may be due to finer structure or a larger total slice area. The cells are shown in the C-Cell Image, and thus when interpreting this image, cells only touching diagonally are considered.</p> <p>Area of Cells: The total area of cells as a percentage of the slice area. Large values indicate a more open texture. The value typically underestimates the actual volume fraction of air in a product. Even though there is limited sensitivity with which microscopic cells and thin</p>

cell walls can be represented in the image, the value provides a measurement of the relative visual appearance of slices.

Cell Diameter: The average diameter of the cells (pixels) is presented based on the average cell area measurements. This is a good general-purpose indicator of the coarseness of the texture but does not take the depth of the cells into account. Cell volume provides an alternative measurement that also includes consideration of the cell depth.

Cell Volume

Cell Wall thickness: the average thickness of the gas cell walls produced during the proving and baking stage.

Non-Uniformity: A measurement of the lack of uniformity between fine and coarse texture (including holes) across the slice. High values indicate less uniformity of texture. The value is useful for comparing slices of similar types of products.

Cell Volume: A measurement of the average volume of cells. This provides a good general-purpose indicator of the coarseness of the texture. Unlike Cell Diameter, this measurement takes the depth of the cell into account.

Crumb Structure Shape

Slice Area: The total area of the product slice.

Max Slice Height: The height of a product slice from its base to its highest point, indicated by the bounding box's height in the shape image. This is typically more appropriate for the height for measurements of products with a domed top surface, such as unlidged bread.

Table 5:1 Crumb Data Calculation and Expression Results

5.5 Materials and Methods: Stable Micro System TA.TX Plus Texture

Analyser

This method's objective was to quantitatively determine the force required to compress a baked product by the distance between the sample and the overhead. The firmness was taken as a measure of freshness and quality. For example, a freshly baked product will be soft and therefore, less force is required for compression; unlike a stale product which would be harder and required more force. The AACC 74-09 method applied to this research and the quality control evaluation of pan/tin bread. The Stable Micro System TA.TX was set with a compression load cell of 250Newton force. An aluminium plunger (36mm diameter) with the edges blunted to remove sharpness and prevent cutting the baked product, was connected to the machine and used for all texture testing.

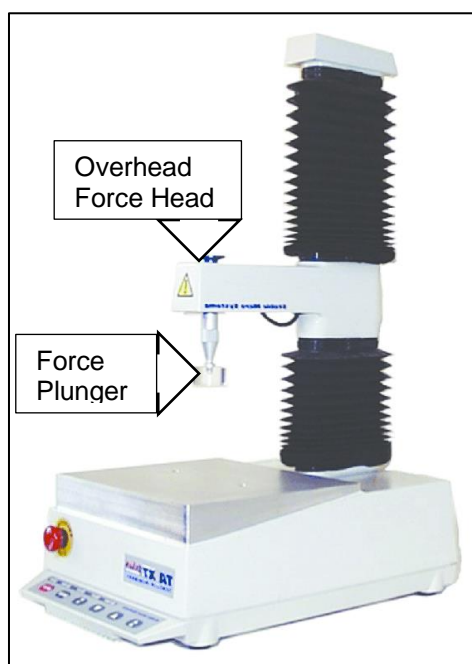


Figure 5:3 Stable Micro System TA.XT Plus – Texture Analyser and Staling Detection Machine

5.5.1 Texture Analysis Procedure – Preparation of the Sample

The thickness of the sample slices was 25mm, and the loaves were mechanically sliced to ensure consistency in all of the tests. Slices were only used once for the test sample, and no heel/end slices of the loaf were used as this may have compromised the data. To ensure accurate identification of the curve characteristics, three slices per loaf were used to evaluate the texture.

5.5.2 Texture Analysis Procedure – Method

The correct load cell should be fitted to the upper crosshead for the application undertaken; in this instance, the 250Newton force loading cell was used to ensure no damage to the machine or the crosshead. The upper crosshead limit position was set, so the compression plunger was 1mm above the sample's centre surface. The crosshead limit was set at 40% (10mm compression depth) to allow sufficient compression of the sample, at which the crosshead speed (rate of compression) was set at 100mm/minute. The speed was set at 500mm/minute (5:1 ratio of the chart to crosshead extension). To ensure the products achieved a reasonable compression rate. The sample was placed centre under the compression plunger and avoiding any irregular or non-representative areas of the crumb.

5.5.3 Texture Analysis Procedure – Calculation and Expression of Results

Figure 5:2 shows the identification of the curve characteristics. Compression force readings were taken at the point on the curve where the sample had been compressed by 25%. The chart's ratio to crosshead speed was set to 5:1 (chart-to-crosshead); therefore, for every 1mm of crosshead extension, the chart recorded 5mm. Consequently, if a 25% compression was exerted on a 25mm thickness sample, this was indicated by the crosshead's extension, which would be 6.2mm. Thus, a 6.2mm crosshead extension x 5:1 ratio equated to 31mm chart extension, resulting in a 25% compression from 31mm from the beginning of the curve. For the compression force value (CFV) measurement, a line is drawn through the first half of the curve (first slope) to baseline. CFV is taken 31mm from the start of this interpolated intersect of the curve with baseline. Meaning the CFV reading of the sample can be read in grams of force directly off the chart.

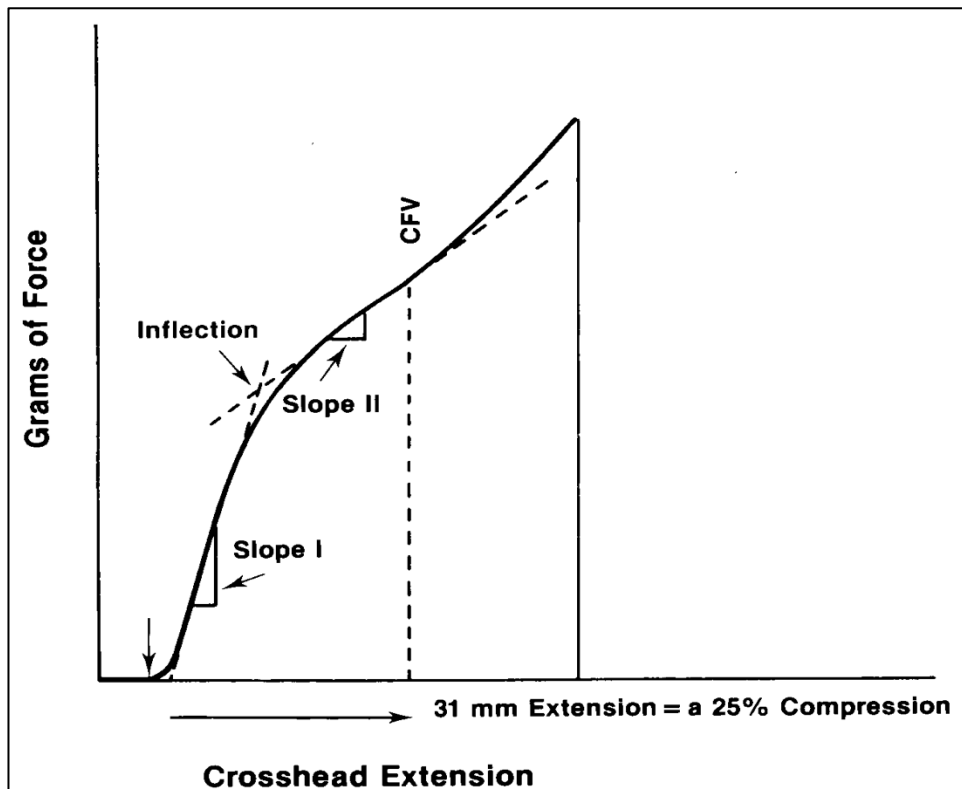


Figure 5:4 Firmness Curve. CFV = Compression Force Value

Source: AACC International, AACC method 74-09

5.6 Overall Crumb Structure and Texture Results

5.6.1 Results: Calibre C-Cell – Crumb Structure

Table 5:2 below outlines the results of the crumb structure from the C-Cell. Only a few parameters were selected out of the number of results obtained through the machine. For this thesis, the main three main parameters analysed were slice brightness, crumb cells and dimension. Each main parameter had one or more means to determine the outcome; for instance, the slice brightness looked at one measurement than the crumb cell size, which had six measurements. All three parameters helped to discern the overall slices' structure and the fortifications' effect within the samples.

C-Cell Crumb Measurements					
Parameter	Measurement	T55 Wheat Flour – Control Sample ^(a)	T55 + Cricket Powder ^(b)	T55 + Cricket Powder + Quinoa + Khorasan Flour ^(c)	T55 + Cricket Powder + Quinoa + Khorasan Flour + Mixed Seeds ^(d)
Crumb Brightness	Brightness**	121.14±	62.88±	57.13±	53.37±
		0.32 b,c,d	1.78 a,d	4.34 a	2.48 a,b
Slice Crumb Cell Size	Number of Cells (per slice)	6981.33±	5981±	6808.33±	5903.67±
		146.58 b,d	114.66 a,c	163.35 b,d	55.1 a,c
	Area of Cells (% of slice area)	55.3±	55.3±	53.8±	55.2±
		0.1 c	0.21 c	0.21 a,b,d	0.31 c
	Cell Diameter (pixel)	17.1±	17.5±	14.0±	17.1±
		0.13 c	0.7 c	0.09 a,b,d	0.5 c
	Cell Volume (% of the slice)	8.71±	11.33±	8.7±	11.21±
		0.06 b,d	0.13 a,c	0.23 b,d	0.34 a,c
Wall Thickness (pixels)	3.15±	3.22±	2.98±	3.13±	
	0.02 c	0.06	0.05 a	0.03	
Non-Uniformity	4.8±	2.5±	1.8±	2.23±	
	1.01 c	0.53	1.62 a	1.12	
Slice Dimensions	Bread Slice Area (pixels)	572027.3±	517617±	480935±	489457.3±
		4680.78 b,c,d	4920.65 a,c,d	2598.22 a,b	5437.29 a,b
	Height (pixels)	1008.37±	925.1±	853.52±	891.99±
		5.39 b,c,d	11.13 a,c,d	8.01 a,b,d	5.56 a,b,c

Table 5:2 Calibre C-Cell – Crumb Structure and Statistical Significance across all Samples

Results are displayed in columns. Letters in superscript denote the samples in comparison to which the value is statistically different ($p < .05$). The symbol of the sample is given in the bracket. All values are means obtained from three replications, $n = 3$.

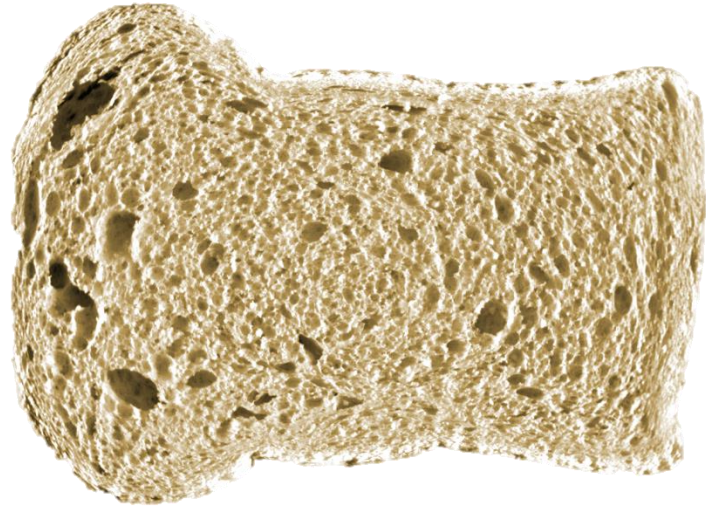
The first main parameter of the C-Cell looked at the brightness of the crumb. This measurement indicated light reflectance by the product slice. The outcome of the results can range from 0 to 255, depending on how dark the crumb is; therefore, indicating two aspects. Firstly, the quality control to ensure that the product is within the tolerances of the product specification. Secondly, the slice colour can visually impact consumers' perception as a darker bread crumb is sometimes perceived as healthier than white bread. Table 5:2 shows the mean values for the brightness of the crumb of all bread samples analysed in each of the four experimental conditions: the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan- flour and mixed seeds). The brightness mean values for the samples were as follows, sample A $M = 121.14$ ($SD = 0.32$) sample B $M = 62.88$ ($SD = 1.78$), sample C $M = 57.13$ ($SD = 4.34$) and sample D $M = 53.37$ ($SD = 2.48$). Analysing the crumb brightness, samples B, C and D showed decreased values (58.26, 64.01, 67.77 respectively).

The one-way ANOVA showed F to be statistically significant, $F(1,3) = 432.51$; $p < .05$, partial $\eta^2 = 0.99$. All samples showed a statistical significance $p < .05$, which indicated a difference in crumb brightness than the control sample A. Following the results, a Tukey HSD test was performed for post-hoc comparison among the individual slice brightness parameters. Therefore, there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest; D&C and C,B&A, respectively, in the homogeneous subsets. The crumb brightness for samples B, C and D had decreased due to cricket powder, ancient grains and seeds. This indicated a difference in the crumb colour for all samples, which can be seen by the photos in Figure 5:5. The photos show that the fortificants (cricket powder, ancient grains, seeds) decrease the slice's brightness, indicating a darker crumb from the additional flours and seeds included within the baked product. However, fortifying with seeds can skew the data as the seeds can

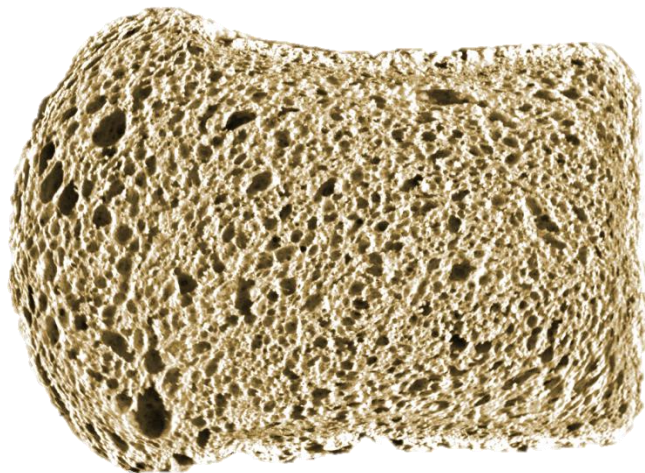
sometimes be recorded as deeper cells or create shadows, affecting the brightness scale.

Crumb Structure Brightness

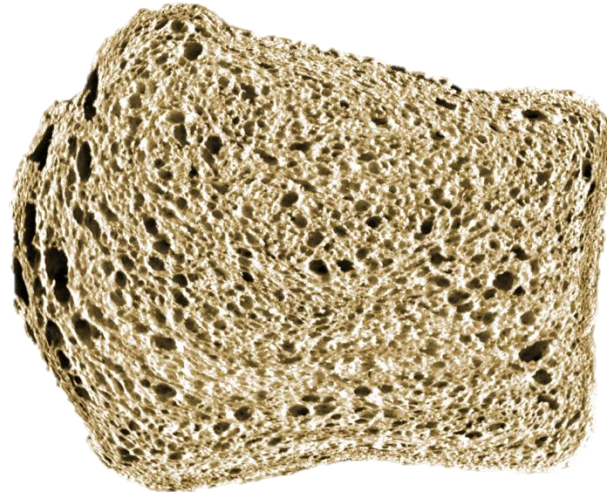
T55 Wheat Flour
– Control Sample



T55 + Cricket
powder



T55 + Cricket
powder + Quinoa
+ Khorasan Flour



T55 + Cricket
powder + Quinoa
+ Khorasan Flour
+ Mixed Seeds

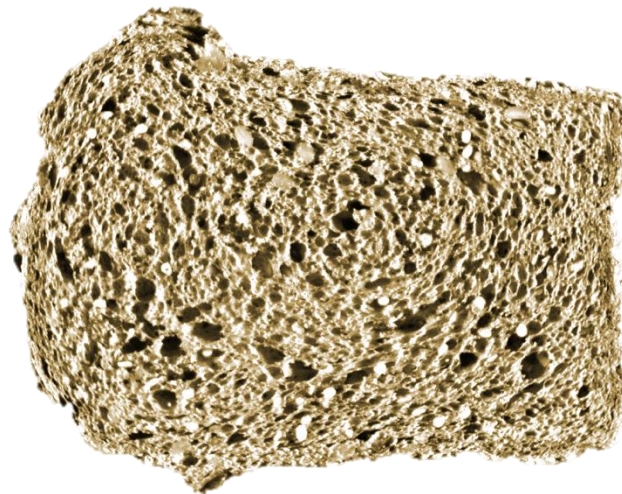


Figure 5:5 Images of the Crumb Structure Brightness

The second parameter measured the slice crumb cell size relating to the baked products' internal structure. This parameter measured the crumbs subunits of the cell and volume, which gave an internal measurement for the structure of the samples; specifically looking at the gas cells in which the gluten-forming matrix is greatly associated in breadmaking. The first, subunit 'cell' involved the measurements: number of cells, area of the slice and cell diameter; and Table 5:3 shows the mean values of these measurements. Each bread reference sample was analysed in the four experimental conditions: the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan- flour and mixed seeds). The number of cells mean values for the samples were as follows, sample A $M = 6981.33$

(SD = 146.58), sample B $M = 5981$ (SD = 114.66), sample C $M = 6808.33$ (SD = 163.35), and sample D $M = 5903.67$ (SD = 55.1). Compared to the area of the slice, the mean values were as follows: sample A $M = 55.33$ (SD = 0.97), sample B $M = 55.39$ (SD = 0.21), sample C $M = 53.87$ (SD = 0.21) and sample D $M = 55.26$ (SD = 0.31). Finally, cell diameter the mean values were as follows: sample A $M = 17.11$ (SD = 0.13), sample B $M = 17.5$ (SD = 0.7), sample C $M = 14$ (SD = 0.9) and sample D $M = 17.11$ (SD = 0.5).

The number of cells one-way ANOVA showed F to be statistically significant, $F(1,3) = 57.51$; $p < .05$, partial $\eta^2 = 0.96$. Samples B and D showed a statistical significance $p < .05$, which indicated a difference in the number of cells compared to the control sample A. Following the results, a Tukey HSD test was performed for post-hoc comparison among the particular area of cells parameters. As there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest; D&B and C&A, respectively, in the homogeneous subsets. The number of cells for samples B and D had a substantial decrease due to the inclusion of cricket powder, ancient grains, seeds to the samples. This indicated that samples A and C had a finer cell structure, compared to sample D and B, which had a coarser crumb. The conclusion is that this coarse crumb is specifically linked to an open crumb structure which is created during the fermentation process.

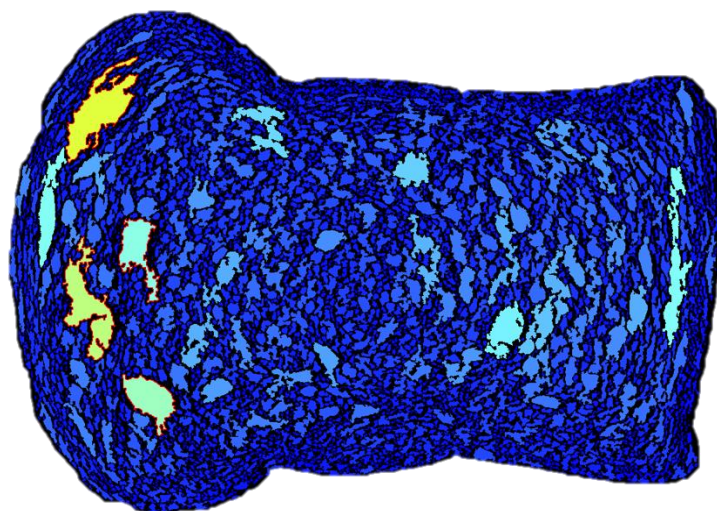
The area of cells one-way ANOVA showed F to be statistically significant, $F(1,3) = 32.41$; $p < .05$, partial $\eta^2 = 0.92$. Sample C showed a statistical significance $p < .05$ which indicated a difference in the area of cells compared to the sample A, B and D. Following the results, a Tukey HSD test was performed for post-hoc comparison means among the individual area of cells parameters. Therefore, there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest; C and D, A&B, respectively, in the homogeneous subsets. The area of cells decreased as the structure was dense with a closed crumb, compared to samples A, B and D. The total area of cells presented through this analysis were expressed as a percentage of the slice area. Larger values expressed through the results (sample A, B and D) indicated that the cell structure was more open and affected the overall texture. From the results, the control sample

had a larger percentage indicating a more open cell structure, as it did not have any additional fortification with flours and seeds. Moreover, samples B and D had similar outcomes compared with the control sample and, although this would be expected of sample B (with only cricket powder fortification); sample D should have a further reduced percentage due to the addition of quinoa-, Khorasan- flour and seeds.

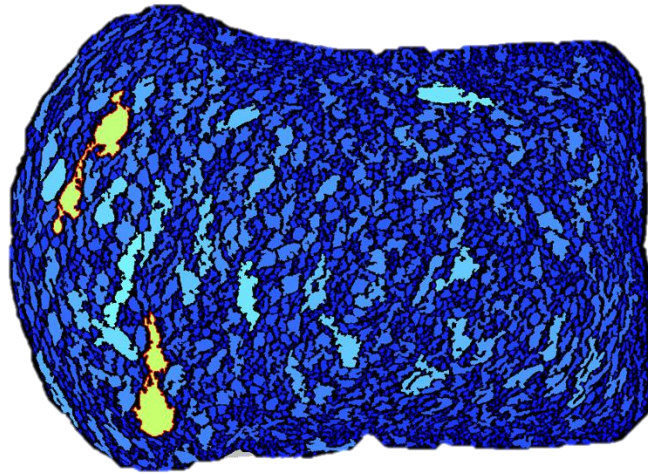
The cell diameter one-way ANOVA showed F to be statistically significant, $F(1,3) = 40.67$; $p < .05$, partial $\eta^2 = 0.94$. Sample C showed a statistical significance $p < .05$ which indicated a difference in the cell diameter compared to samples A, B and D. Following the results, a Tukey HSD test was performed for post-hoc comparison against the individual cell diameter parameters. There was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest; A and D, B&C, respectively, in the homogeneous subsets. Likewise, this correlates with the t-test where the cell diameter results show a decrease in cell structure as this was close and dense, not an open cell structure compared with samples A, B and D. These results gave a good indicator regarding the cell structure, from the number of the cells present in a slice of bread, the area of cells and the correlating cell diameters. The results correlated similarly between the area of cells and cell diameters, where samples A, B and D are similar in cell structure, compared with sample C, which had the lowest value due to the dough's poor fermentation.

Crumb Structure Gas Cell

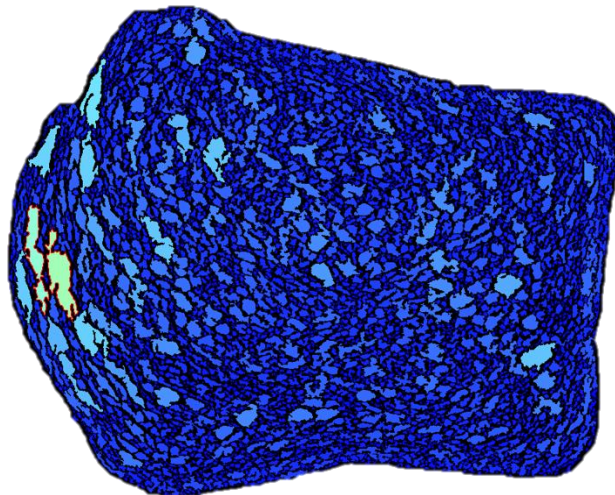
T55 Wheat Flour
– Control
Sample



T55 + Cricket
powder



T55 + Cricket
powder +
Quinoa +
Khorasan Flour



T55 + Cricket
powder +
Quinoa +
Khorasan Flour
+ Mixed Seeds

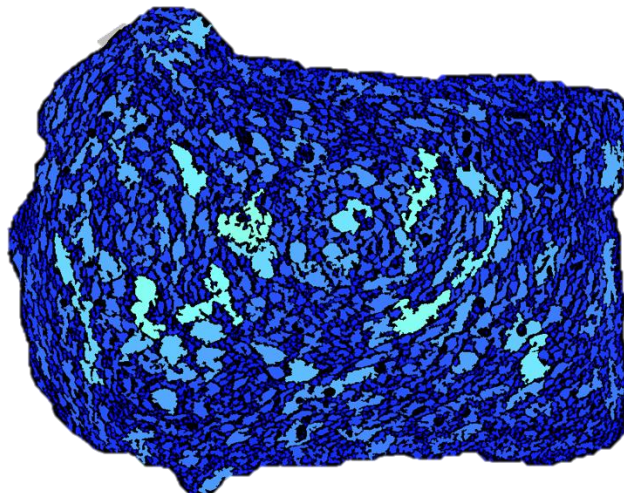


Figure 5:6 Images of the Crumb Structure Gas Cells

Within the slice crumb cell parameter, the volume crumb subunit (cell volume, cell wall thickness, cell non-uniformity) measurements were analysed. Table 5:3 shows the mean values for the individual measurements: cell volume, cell wall thickness, cell non-uniformity. The bread reference samples analysed in the four experimental conditions were: the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan- flour and mixed seeds). The cell volume mean values for the samples were as follows, sample A $M = 8.01$ (SD = 0.06), sample B $M = 11.33$ (SD = 0.13), sample C $M = 8.7$ (SD = 0.23), and sample D $M = 11.21$ (SD = 0.34). Compared to the cell wall thickness, the mean values were as follows: sample A $M = 3.15$ (SD = 0.02), sample B $M = 3.22$ (SD = 0.06), sample C $M = 2.98$ (SD = 0.05) and sample D $M = 3.13$ (SD = 0.03). Finally, cell non-uniformity the mean values were as follows: sample A $M = 4.89$ (SD = 1.01), sample B $M = 2.53$ (SD = 0.5), sample C $M = 1.85$ (SD = 1.62) and sample D $M = 2.23$ (SD = 1.12).

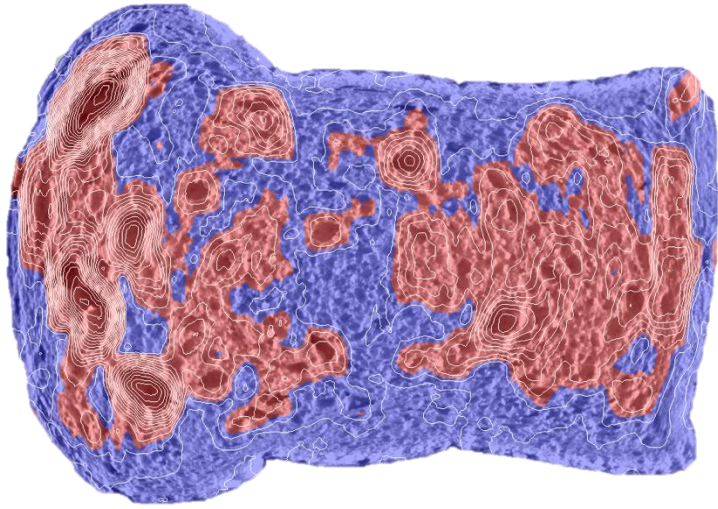
The cell volume one-way ANOVA showed F to be statistically significant, $F(1,3) = 141.67$; $p < .05$, $\eta^2 = 0.98$. Sample B and D showed a statistical significance $p < .05$, which indicated a difference in the cell volume compared with the control sample A and C. The cell volume for sample A and C decreased, which showed that the cell structure was fine and close, whereas sample B and D had an open, coarse crumb. Following the results, a Tukey HSD test was performed for post-hoc comparison among the individual non-uniformity parameters. Therefore, there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest; C&A and D&B, respectively, in the homogeneous subsets. In the first subunit of slice crumb size, the cell diameter was reported with sample C having a finer crumb. This provided a general-purpose indication of the coarseness of the texture. Unlike cell diameter, this measurement took the depth of the cell into account. Therefore, the outcome showed that sample B and D had a coarse cell crumb texture compared with A and C samples where they had a finer cell crumb texture.

The cell wall thickness one-way ANOVA showed F to be statistically significant, $F(1,3) = 19.83$; $p < .05$, $\eta^2 = 0.86$. Sample C showed a statistical significance $p < .05$ which indicated a difference in the cell wall thickness compared to the control sample A, B and D. Following the results, a Tukey HSD test was performed for post-hoc comparison means among the individual cell wall thickness parameters. Therefore, there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest: C and D,A&B, respectively, in the homogeneous subsets. This indicated that sample C cell wall thickness was affected the most during the dough development and processing stage due to ancient grains' inclusion. This correlates with the parameter measurements: cell diameter and cell area, which showed a decrease in values due to the additional ancient grains. Whereas sample B had the best cell wall thickness as only cricket powder was added to the flour mixture.

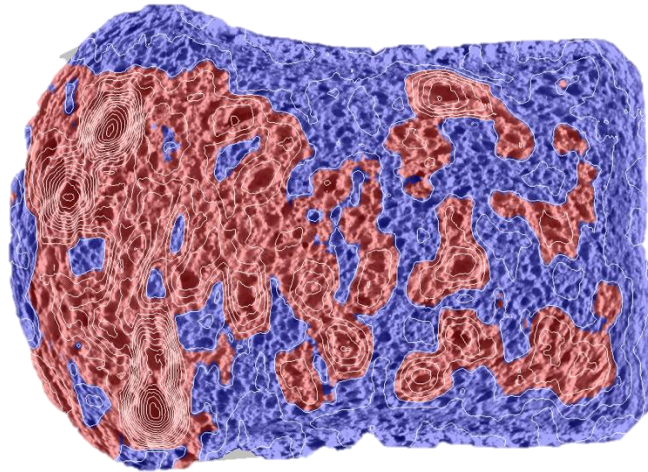
The cell non-uniformity one-way ANOVA showed F to be statistically significant, $F(1,3) = 4.38$; $p < .05$, $\eta^2 = 0.62$. Sample C showed a statistical significance $p < .05$ which indicated that there was a difference in the cell non-uniformity compared to the control sample A, B and D. The cell non-uniformity for sample A, B and D had an increase in values, indicating that these samples had similarity in textures (fine and coarse). In comparison, sample C results showed that there was a lack of uniformity. Following the results, a Tukey HSD test was performed for post-hoc comparison among the individual non-uniformity parameters. Therefore, there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest; C, D&B and D, B&A respectively, in the homogeneous subsets. The means of the values represented within this parameter showed the non-uniformity between fine and coarse texture (including holes) across the slice. The high values indicate less non-uniformity of texture which can be seen mainly in the control sample A. This can be seen in the figure (5:7), where the clustered red cells are present at the top of the slice. Compared to sample B and D showed a similar outcome, but more evenly distributed cells throughout the slice. However, sample C had the lowest value representing a more uniform texture across the slice.

Crumb Structure Cell Volume

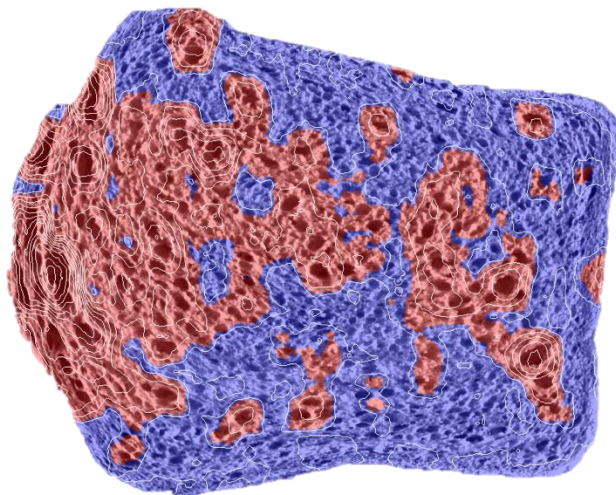
T55 Wheat Flour
– Control
Sample



T55 + Cricket
powder



T55 + Cricket
powder +
Quinoa +
Khorasan Flour



T55 + Cricket
powder +
Quinoa +
Khorasan Flour
+ Mixed Seeds

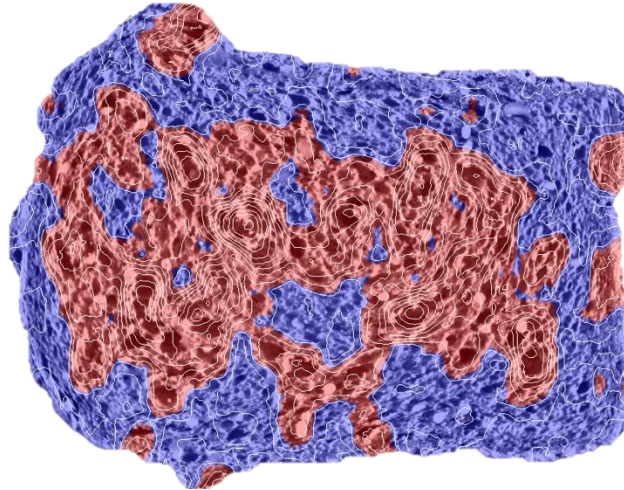


Figure 5:7 Images of the Crumb Structure Cell Volume

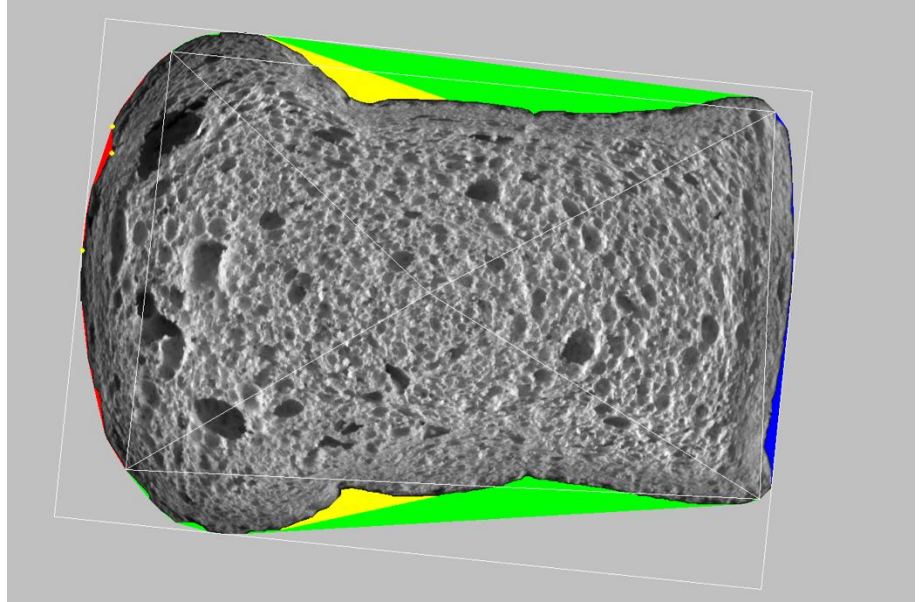
The final parameter explored the height of the sample and the overall area of the bread slice. The results help determine the height of the product, contributing to the overall volume of the product, and the area of the slice. Table 5:3 shows the mean values for the individual measurements: bread slice area and slice height. However, the values are reported via pixels, and therefore conversion is required to give a true indication for slice height. The bread reference samples analysed in the four experimental conditions were: the control product – sample A (T55 wheat flour), sample B (T55 wheat flour and cricket powder), sample C (T55 wheat flour, cricket powder, quinoa- and Khorasan- flour) and sample D (T55 wheat flour, cricket powder vs cricket powder, quinoa-, Khorasan- flour and mixed seeds). The bread slice area values for the samples were as follows, sample A $M = 572,027.33$ (SD = 4,680.78), sample B $M = 517,617$ (SD = 4,920.65), sample C $M = 480,935$ (SD = 2,598.22), and sample D $M = 489,457.33$ (SD = 5,437.29). Finally, the slice height mean values were as follows: sample A $M = 1008.37$ (SD = 5.39), sample B $M = 925.1$ (SD = 11.13), sample C $M = 853.52$ (SD = 8.01) and sample D $M = 891.99$ (SD = 5.56). However, as previously mentioned to convert the sample height to mm a conversion factor of 0.14 needed to be multiplied against the sample. Therefore, the sample heights in cm, are as follows: sample A $M = 14.11\text{cm}$ (SD = 0.79cm), sample B $M = 12.95\text{cm}$ (SD = 1.56cm), sample C $M = 11.95\text{cm}$ (SD = 1.12cm) and sample D $M = 12.49\text{cm}$ (SD = 0.78cm).

The bread slice area one-way ANOVA showed F to be statistically significant: $F(1,3) = 246.09$; $p < .05$, partial $\eta^2 = 0.99$. All samples showed a statistical significance $p < .05$, which indicated a difference in crumb brightness compared with the control sample A. Following the results, a Tukey HSD test was performed for post-hoc comparison among the individual slice area parameters. Therefore, there was a statistically significant difference among the mean, consisting of three groups ranging from lowest to highest; C&D, B and A respectively, in the homogeneous subsets. The results indicated that the bread slice areas reduce in area with cricket powder additions, ancient grains, and seeds. The slice area for samples B, C and D had decreased due to cricket powder's inclusions, ancient grains and seeds.

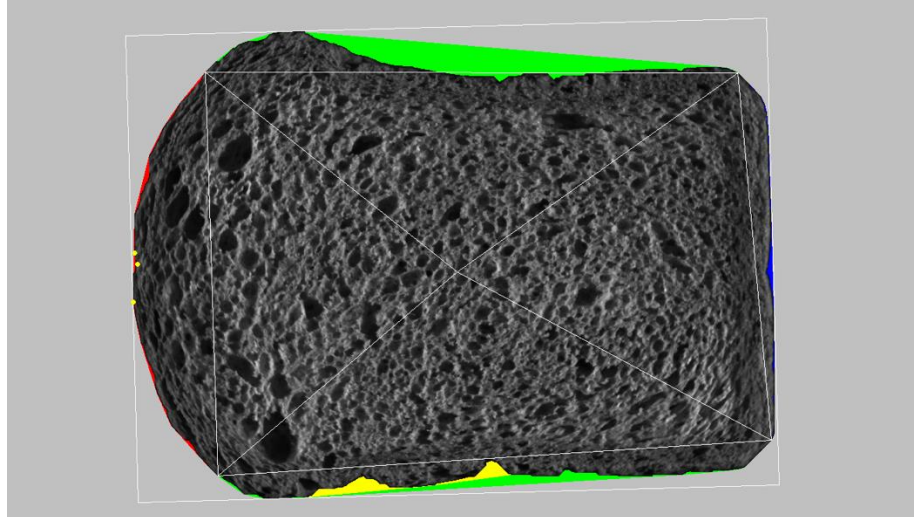
The bread slice height one-way ANOVA showed F to be statistically significant beyond the .05 level: $F(1,3) = 210.28$; $p < .05$, partial $\eta^2 = 0.99$. All samples showed a statistical significance $p < .05$, which indicated a difference in crumb brightness compared with the control sample A. The slice height for samples B, C and D had decreased due to cricket powder's inclusions, (and) ancient grains, (and) seeds. Following the results, a Tukey HSD test was performed for post-hoc comparison among the individual slice area parameters. Therefore, there was a statistically significant difference among the mean results, consisting of four groups ranging from lowest to highest; C, D, B and A respectively, in the homogeneous subsets. These results can be seen in figure 5.8, which shows the height of a product slice from its base to its highest point. Moreover, the inclusion of cricket powder, ancient grains, and seeds, reduced the loaf volume, therefore impacting the slice height and area.

Crumb Structure Shape

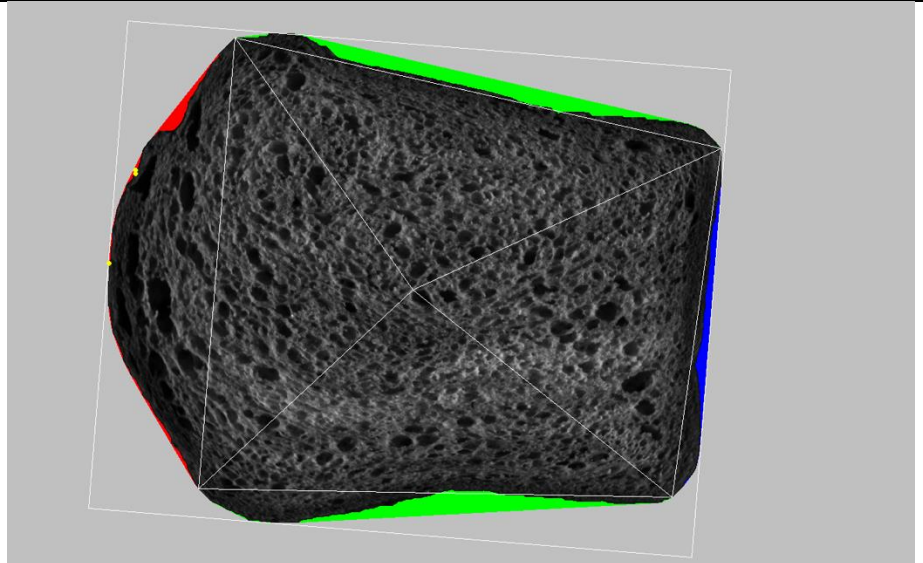
T55 Wheat Flour
– Control
Sample



T55 + Cricket
powder



T55 + Cricket
powder +
Quinoa +
Khorasan Flour



T55 + Cricket
powder +
Quinoa +
Khorasan Flour
+ Mixed Seeds

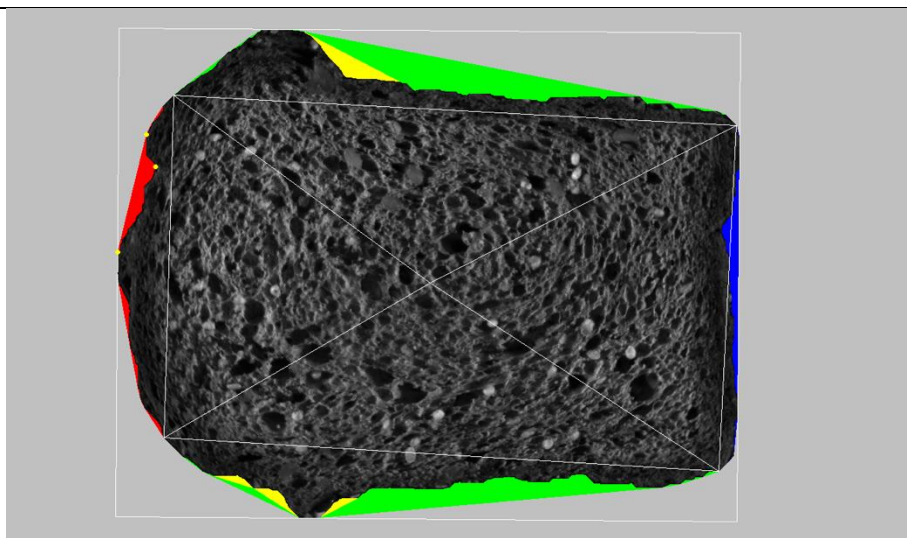


Figure 5:8 Images of the Crumb Structure Shape

5.6.2 Results: Stable Micro System TA.TX Texture Analyser

The texture analyses of bread products are important parameters in determining the product quality and staling effects. With current advances in science, consumers expect longer shelf-life products that help with sustainability and reduce food waste. The inclusion of cricket powder, ancient grains and seeds as a functional ingredient to naturally extend the shelf-life of baked products can be useful for developing countries. Although several baked products were tested for the texture and staling effect, only the middle of the bread samples was reported. The full data results and analysis can be located in Appendix E.

Day	Middle Crumb Firmness			
	T55 Wheat Flour – Control Sample ^(a)	T55 + Cricket Powder ^(b)	T55 + Cricket Powder + Quinoa + Khorasan Flour ^(c)	T55 + Cricket Powder + Quinoa + Khorasan Flour + Mixed Seeds ^(d)
Day 1	964.23±183.91 d	1078.99±49.7 d	1293.94±116.76 d	901.53±74.62 a,b,c
Day 3	1539.56±349.15 d	1340.21±180.99 d	1798.29±153.06 d	1131.91±118.63 a,b,c
Day 5	2568.28±410.66 c,d	2402.29±194.79 c,d	1409.51±305.17 a,b	1622.41±110.58 a,b
Day 7	3799.13±225.16 b,c,d	1851.29±125.08 a	2580.04±592.78 a	1759.25±206.25 a

Table 5:3 Crumb Firmness: Middle of the Slice of Bread (Day 1 to 7) of the Bread Samples

All values are means (grams force) from three replications $n = 3$

* - $p < .05$ in comparison with the control wheat flour sample bread

Table 5:3 indicates the compression force changes over the seven days; Appendix E provides the mean values of the compression and resilience force over the seven days, including the bottom and top of the bread samples. Between day one and seven, sample D (a combination of flours and seeds) had shown to be the most effective in slowing retrogradation which can delay onset staling. This was followed by sample B, which was initially similar to the control product between day one and day five. However, between day five and seven, the staling rate reduced. The mean of the crumb firmness for sample D, over the seven-day period, was 901.53 (SD = 74.62), 1131.91 (SD = 118.63), 1622.41 (SD = 110.58), 1759.25 (SD = 206.25) respectively. Compared with the control sample (A), the compression force value steadily increased over the seven days. This indicated that the water in the product was naturally beginning to migrate from the centre of the product over this period; indicated by the mean values 964.23 (SD = 183.91), 1539.56 (SD = 349.15), 2568.28 (SD = 410.66), 3799.13 (SD = 225.16) respectively.

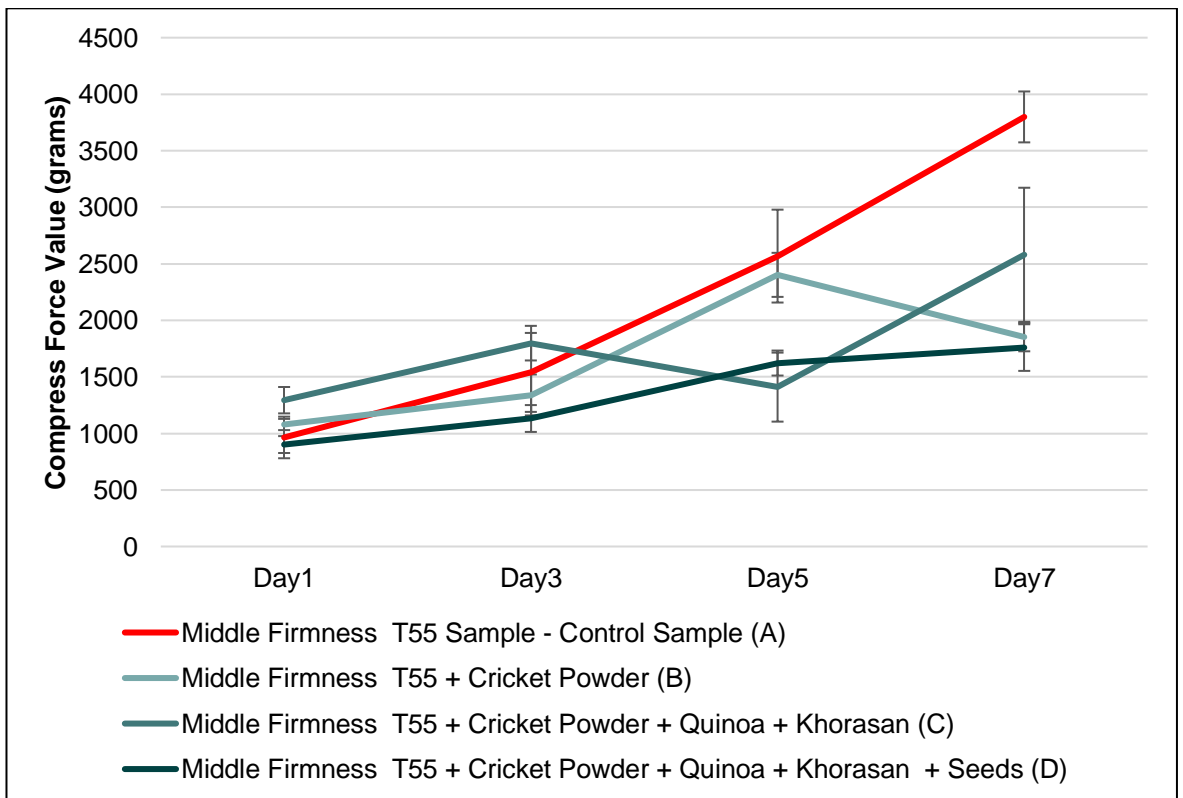


Figure 5:9 Crumb Firmness: Middle of the Slice (Day 1 to 7) of Bread Samples (wheat flour control and wheat flour samples fortified with cricket powder, ancient grains and seeds)

All values are means from three replications $n = 3$

The general linear model (univariate analysis of variance: ANOVA) was used to determine whether there was a significant difference between test samples. Day one showed F to be statistically significant, $F(1,3) = 6.44$; $p < .05$, $\eta^2 = 0.71$. A Tukey HSD test was performed for post-hoc comparison means among the individual slice area parameters. Therefore, there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest: D, A&B and B&C, respectively, in the homogeneous subsets. The results indicated that sample D had the softest crumb structure compared with all of the other samples. This is due to the combination of the lipids within seeds and the additional hydration required for the cricket powder, ancient grains and seeds.

By day three, sample A crumb firmness increased by 59.67%, compared with sample D, which increased by 25.55%. Analysing the results through ANOVA, the F value showed there to be a statistical significance, $F(1,3) = 5.05$; $p < .05$, $\eta^2 = 0.65$. A Tukey HSD test was performed for post-hoc comparison means among the individual slice area parameters. Therefore, there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest; D, B&A and A&C, respectively, in the homogeneous subsets. This means that sample D still had the softest crumb compared to the rest of the samples, due to the additional mixed seeds. Moreover, sample C had the highest incidence of staling despite containing similar ingredients to sample D, indicating that the additional seeds positively impacted the shelf-life.

On day five, sample A crumb firmness increased by 60.11%, compared with samples C and D, which increased by 8.93% and 79.96% respectively. The data was analysed through ANOVA which indicated the F value to be statistically significant, $F(1,3) = 12.52$; $p < .05$, $\eta^2 = 0.82$. A Tukey HSD test was performed for post-hoc comparison means among the individual slice area parameters. Therefore, there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest: C&D and B&A, respectively, in the homogeneous subsets. By day five, it can be seen that the combination of cricket powder, ancient grains and mixed seeds have had a positive impact on the starch retrogradation.

By Day seven staling of all products was demonstrated by increased the compression force value across all samples. Sample A crumb firmness increased by 294%, compared with sample B, C and D, which increased by 71.58%, 99.39% and 95.14% respectively. The results indicate that there was a statistical significance, $F(1,3) = 23.15$; $p < .05$, $\eta^2 = 0.9$. A Tukey HSD test was performed for post-hoc comparison means among the individual slice area parameters. Therefore, there was a statistically significant difference among the mean results, consisting of two groups ranging from lowest to highest: D, B&C and A respectively, in the homogeneous subsets. This means that samples C, B and D had the slowest staling rate by day seven, of which sample D was still the least firm across all seven days. Although cricket powder impacted the texture and shelf-life, the lipids within the seeds gave the softest crumb.

5.7 Crumb Structure and Texture Discussion

Bread is the most popular household commodity that meets the consumers' standard carbohydrate intake. In any discussion about the fortification of products with both micro- and macro- nutrients to improve the nutritional profile, the final product characteristics must meet the consumers' standards for quality; i.e. loaf volume, gas cell structure, crumb structure and overall texture; along with a good shelf-life. Incorporating fibre/bran ingredients such as cricket powder in baked products will affect the final product characteristics. More importantly, the consumers' visual expectations may not be met due to these additional ingredients. Through the NPD process, the product characteristics were optimised not to drastically disappoint the consumers' expectations.

The bread sample slices' crumb brightness decreased in value due to the cricket powder (from 121.14 to 62.88). Additions of ancient grains and seeds within sample C and D also decreased the brightness due to the inclusions (57.13 and 53.37 respectively). In addition, the cricket powder is naturally dark brown, and when incorporated with water, this oxidises and intensifies the colouring. Thus, when cricket powder was incorporated within the doughs' the crumb colour value decreased, and there was an impact on the bread's appearance. There was a correlation ($r = 0.99$) between the crumb colour and high protein content across all

samples: as the crumb colour value decreased, the corresponding protein value increased.

The number of cells detected within the bread slice samples showed a decrease in value as the samples progressed from A to D. All samples were processed simultaneously: placed in identical loaf tins, proved at the same temperature and humidity. This ensured that the slice area, volume and crumb was subjected to identical parameters. Thus, the difference in the value for the gas cell structure, more specifically, the number of cells present in the slice, would result from the bread height. The area occupied by the air cells decreased with cricket powder, ancient grains and seeds. Sample C increased in value compared with samples B and D, which may be attributed to the addition of Khorasan flour which contains an abundance of gluten-forming proteins. This counteracted the detrimental effect of the cricket powder's addition, giving volume to the loaf. However, when additional seeds were added to the dough, the volume decreased due to the seeds' interference with the gluten matrix.

The area of cells across all samples was significant, with sample C showing the least value percentage (53.87%) compared with samples D, A and B (55.26%, 55.33% and 55.39%) respectively. With a larger value present with sample D, A and B, this suggests a more open crumb texture. However, unlike the number of cells, this value typically underestimates the actual volume fraction of air in a product. The seeds in sample D could have been interpreted by the apparatus as cells, as the value is a measurement of the slice's relative visual appearance. Moreover, the cell diameter presented is an average diameter of the cells based on the average cell area measurements. This is a general-purpose indicator of the coarseness of the texture. However, it does not account for the depth of the cells. Thus, the results correlate with the area of cells where sample C showed a coarser product compared with sample A, D and B (17.11, 17.11 and 17.5) respectively. Although the coarse cell clustering needs to be examined further to determine if the seeds in sample D, are interpreted as 'gas cells' rather than 'volume'. As the overall number of gas cells within sample D indicated that the seeds' additions impacted the volume.

The cell volume is a combination of several measurements, not only just the cell volume but a combination of several measurements mainly: clustering of cells and non-uniformity. Firstly, the cell clustering analysis can be determined by the extent to which areas of the slice have fine and coarse clustering as indicated by the blue and red areas seen in figure 5:5. Fine cells are signified by the blue areas, whereas the red indicates the coarse cells. There was no statistical significance in the clustering, and all values were relatively low (under 0.06) would indicate that all sample slices were similar and had a mixture of fine and coarse texture areas. There were a reduced number of coarse cells located around the outside of the slice, especially the sides and bottom. The coarse cells accumulate mainly towards the middle and top of the slice, as this is a typical pattern of expanding gas cells. This is where the loaf expands upwards when the bread is being proved and baked within a pan. Thus, the expansion of the cells at the dough's outer surface is naturally inhibited by the restriction of the bread pan. Therefore, the crumb texture in the slice area where the dough has been pressed against the pan is generally close, fine and even; with smaller sized cells.

The samples were then analysed for non-uniformity, as the coarse and fine clustering could detract from the product's texture. Therefore misrepresenting the data, and the overall quality of the bread product. There was a statistical significance across the samples as the control sample indicated less uniformity in texture. Samples B, C and D, showed lower values, which indicated that the mixture of both coarse and fine clustering of cells did not impact the texture. Sample C showed to be the most adequate in terms of visual appearance and ratio of coarse and fine clustering, suggesting that this would appear to have the most acceptable eating qualities. This was followed by sample D, which indicated that the mixture of cricket powder, ancient grains and seeds displayed the best results for overall cell volume despite the fortification with these ingredients.

The volume and height of the bread were impacted by the water-binding capacity of the final bread. The cricket powder has a high water absorption capacity so that samples containing it needed increased water levels in their recipes to optimise the dough's hydration and development. However, as the cricket powder has an increased water holding capacity, this meant that more water was retained during

the baking process. This resulted in a reduction of water available for evaporation, thus, providing the bread product with greater moisture retention and in turn impacting the crumb softness. The same phenomenon was apparent with the additions of seeds and ancient grains where the increase in water absorption levels affected the bread products properties, in particular the volume. Despite the low specific volume, the crumb for sample D was the softest across all seven days during the texture analysis. Another factor that might have led to the decreased volume could have been the gelatinisation and viscosity of the dough (previously discussed in Chapter Three). This correlates with Zhou et al., (2018) research, which outlined the relationship between the baked product volume to the gelatinisation and viscosity. The research showed that when there was an increase in viscosity, this would provide structure for the gas cells to expand and offer support for the gas retention.

Furthermore, from Chapter Three, the torque from the C3 parameter showed this delay during the gelatinisation stage when cricket powder and seeds were incorporated within the dough. This would affect the starch gelatinisation, thereby creating a decreased viscosity which indicates a weaker gelatinisation stage. The weakening in gelatinisation would lead to low gas holding capabilities, resulting in a bread product with poor volume. The results from both the Mixolab and the C-Cell, supports the correlation between peak viscosity, the loaf height and slice area.

The final factor to be considered was the interference with the gluten network, which would have impacted the gas retention, leading to lower height and volume. Cricket powder contains a larger number of insoluble proteins and chitin and with its inclusion in the recipe led to a reduced extensible dough. Such ingredients reduce the volume and increase the close crumb structure as there is no gas expansion. Fibre is one of the main factors interfering with the gluten forming proteins and inevitably the network, thus limiting the expansion of the gas cells. Therefore, the gas cells would continue to decrease as the continual addition of non-gluten forming flours are added within the dough (cricket powder, pseudocereals and seeds). There was a similar correlation when looking at the cell diameter, as this declined as additional non-gluten flours and seeds were added to the dough. Subsequently there are two ways to stabilise the gas bubbles produced during the fermentation

stage through the three-dimensional network, this is through the gluten-starch matrix and liquid lamella films. However, as previously conversed, the fibre inhibits the gluten matrix; secondly, the level of LMW polymeric proteins cause a decrease in the dough's extensibility. In this case, both forms of interference led to reduced gas retention and extensibility, which affected the dough's gas cell expansion. The decrease in the area occupied by gas cells could be explained by the chitin fibres present, which interfered with the gluten matrix, causing a reduction in gas holding capabilities. This decrease was noticeable in the amount and areas occupied by the gas cells, and since there was a decreased extensibility, the dough expansion was lower. Therefore, affecting the gas holding capabilities, thus creating a close crumb bread.

During the texture analysis, the control sample showed the most accelerated hardness/firmness levels across all seven days compared with all of the other samples. The sample containing cricket powder, ancient grains and seeds had the lowest compression force value on day one, indicating this was the softest of all samples. In contrast to sample C, which had the same ingredients except for the inclusion of the seeds. Sample D was the only sample with the addition of seeds within the product, and this increased the fat content by 8.3g compared to the control sample. The additional fat content within the seeds also impacted the amylopectin's molecular associations and the gelatinised starch. By day three, there was an increase in firmness across samples A, B, and C. This increase in compression force recognises two stages having taken place, firstly, the changes in the molecular organisation of the starch polymers. Secondly, the starch molecules which re-crystallise (starch retrogradation) follows from the migration of water drawn away from the middle of the slice towards the crust. The change in water mobility and distribution causes changes in diffusion properties in the crumb and crust, which gave the perception of the crumb becoming too stiff and dry (Lodi and Vodovotz, 2008). The general loss of texture and freshness characteristics over this short period of time is due to the starch retrogradation/recrystallisation, especially the short amylopectin side chains. This, in turn, led to a firmer and less resilient crumb, and a softer crust due to the moisture migration outwards.

By Day five there is a drastic change in the compression force required for samples A and B. These two samples required significant compression force, compared with samples C and D. Sample C became softer between day three and day five. Slade and Levine, (1991) explained that non-starch polysaccharides, gluten proteins and lipids can naturally extend the shelf-life. This is due to the interaction between the non-starch polysaccharides, gluten proteins and lipids that impact the starch and gluten networks. By day seven, the compression force required increased for all samples. Sample A had the highest value indicating that the control flour had the fastest and highest staling rate than all other samples.

Meanwhile, the fortified samples, especially the sample with additional seeds, showed the softest crumb. Again, this is due to the lipid content of the seeds, which impacted the crumb texture. This decreased water migration rate, which allowed bread samples fortified with cricket powder to retain water within their structure. Thus, the cricket powder water holding capacity reduced the amount of water available and increased water retention. This is also confirmed by the Mixolab results (C5 parameter), which demonstrated that the retrogradation slowed down due to cricket powder's addition within the baked products.

5.8 Crumb Structure and Texture Conclusion

The area occupied by gas cells showed a reduction across all hybrid flour samples, but more particularly in sample D. These results correlate with the decrease in loaf volume, as the fortification levels of cricket powder, ancient grains and seeds increased. This was due to the reduced gas holding capabilities and increased resistance to dough expansion caused by interference with the gluten network, similarly in Perez-Fajardo (2020) research. The inclusion of cricket powder in doughs produced a denser bread due to the amount of LMW proteins that inhibited the dough's extensibility and, therefore, decreased the gas retention. Another possibility could be the presence of crude fat within the seeds and the cricket powder. In Perez-Fajardo (2020) study, this showed that the presence of linoleic and linolenic acids within ingredients could act as foam breakers due to the expansion of the monolayers. As cricket powder contains linoleic and linolenic acids,

this could explain one reason why there is a destabilisation of the elasticity of the gluten, therefore inhibiting gas retention.

In general, the control sample was the firmest across all seven days with the significance of staling increasing day by day: especially by day seven. There was no significant difference between day one and three for sample B, which increased in staling by day five. Sample B had a similar staling pattern as the control sample, and therefore the lower molecular weight subunits present within the dough did not act as a dough softener. However, by day seven, the crumb becomes softer and reduced in firmness drastically compared with any other sample. Comparing samples C and D, these two samples showed a soft crumb over the seven days. However, sample D showed one of the softest crumbs and consistent in softness over the seven days.

This could not be attributed to the added cricket powder otherwise the correlation would be significant with sample B and D. Likewise, although there was a reduction in staling between samples C and D, the ancient grains only slow down the staling effect. It was the additional seeds that have a major impact on the products' texture and staling rate. This can be seen in the correlation ($r = 0.99$) between additional crude fat and staling rate within baked products. As previously discussed, water holding capabilities, gas retention and loaf volume are functions of the crumb's hardness. Bread hardness decreases due to the greater compression of the gas cells in volume bread where the crumb texture is lubricated with lipids. Therefore, another explanation for the slower staling rate in sample D is the cell volume as seen in sub-section 5.5.1, where the decreased gas retention and reduced dough expansion were accountable for the low volume C-Cell results. The dough extensibility was impacted by the lack of gluten-forming proteins which had a detrimental effect on the viscoelastic properties, consequently averting the crumb from springing back. Therefore, it can be determined that the inclusion of cricket powder pseudocereals and seeds in bread impacts the springiness of the crumb over time.

5.8.1 Key Summary Conclusion Points

- The decrease in crumb brightness corresponded with increased protein level, and therefore, the darkening of the crumb could give a perception of a healthier loaf.
- Results for Sample D indicated that the mixture of cricket powder, ancient grains and seeds displayed the best results for overall cell volume despite the fortification with these ingredients.
- A decrease in extensibility led to a reduced volume and increased density due to gas cell expansion prevention. This decrease in the area occupied by gas cells could be explained by the chitin fibres present, which interferes with the gluten forming proteins, thus decreased gas retention.
- The increase in the water holding capacity allows the cricket powder, pseudocereals and seeds to hold onto more water, resulting in less available water for evaporation. This ability to retain moisture enhanced the crumb softness as Sample D had the lowest firmness value across all seven days.
- Fortified samples, especially with sample D, where the additional seeds have shown to provide the softest crumb. Again, this is due to the lipid content available in the seeds, which improved the crumb texture.

Chapter 6 – Consumer Acceptance and Worldwide Survey

'It is the duty of scientists to use science to relieve hunger and poverty. If more of us did that, it would help overturn the political mismanagement and corruption that is the biggest cause of human misery'.

(Sato, 1998)

6.1 Introduction

Around two billion people regularly consume insects worldwide, and the majority of these people are found in developing countries. This is due to the accessibility of insects within natural environments. People in Western society view the ideology of consuming insects or insect products negatively, possibly due to insects' perception as pests and/or disease carriers and their association with decay (van Huis et al., 2013). The research presented within this chapter revolved around consumer perception and acceptability of baked bread products fortified with cricket powder, ancient grains and seeds. The results suggested that the inclusion of these ingredients impacts the doughs behaviour, nutritional composition, texture and shelf-life; and that the inclusion of cricket powder can be beneficial within the food system. From the previous chapter results the following null hypotheses were formulated, firstly (**H₀**): Participants' perception of sensory attributes (appearance, aroma and texture) of cricket powder and ancient grains within baked bread products are perceived negatively compared to a standard baked bread product. Secondly, (**H₀**): Consumer willingness and food neophobia levels are not affected when insects are a direct food source for human diets. This chapter was divided into two sections, the first section, explored the acceptance of baked bread products fortified with cricket powder, ancient grains and seeds among consumers around the world. The second section looked at the worldwide survey, where two aspects of consumer acceptance were investigated. This included consumerism, which was explored to determine the individual continent's response to insects' consumption as a direct food source or as an agriculture feed for livestock and aquaculture. This was to evaluate consumers' willingness around the world to accept insects as part of human diets. Within this section, research data helped identify whether communication and marketing can affect consumers' readiness to eat insects as an alternative food source. Finally, food neophobia levels were explored to assess

consumers' willingness to eat insects across all continents. These results evaluate the cross-cultural differences in accepting insects as a food source.

6.2 Literature Review: Consideration of Insects as a Food Source

Edible insects are a valuable, viable and sustainable resource, with over 1900 different species suitable for human consumption (Jongema, 2012). In some parts of the world entomophagy is well established (the tropics), but in areas where food security is fragile insects need to be promoted as key sustainable foods. Recently the COVID-19 pandemic has exposed the fragility of 'just-in-time' food supply chains within the UK (Jackson and Cameron, 2020). From this crisis, it has been made clear that current food systems are unsustainable and need real-world solutions. This sustainable product aims to ensure that everyone has enough healthy food to eat, produced in ways that will restore, rather than destroy, the natural world (Jackson and Cameron, 2020). Recognising this as both a socio-cultural and systematic challenge, there is a need for an innovative approach. The adoption of insects as a nutritional food source in many countries is currently negatively perceived. Harvesting and consuming insects has been associated with our ancestors as a primitive food acquisition method (van Huis et al., 2013). Prominently in Western culture, where the willingness to adopt insects as a direct food source is the least accepted (van Huis and van Itterbeek, 2013; Siegrist et al., 2013; and Looy et al., 2014) and argued that insects are continuously rejected because they are a non-food commodity. They continue to argue that insects are perceived as unclean because they consume food scraps or degrade food and pose a health risk to consumers (van Huis and van Itterbeek, 2013; Siegrist et al., 2013; and Looy et al., 2014). In contrast, in the tropics where most developing countries are situated, the population views entomophagy as a normal part of their daily lifestyle. Moreover, the UN has repeatedly urged that insects need to be viewed as a secure food source globally, rather than just as pests, which means that consumers need to perceive insects as sustainable food rather than vermin (van Huis, 2013).

6.2.1 Implications: Insects as a Food Source

Pliner and Salvy (2006) identified that when a new protein product is introduced into society, it can sometimes generate fear due to its uncertainty. This can lead to the

final refusal to consume the product, known as food neophobia (Pliner and Salvy, 2006). Caparros Megido et al., (2014) and Verbeke, (2015) argued that the likelihood of incorporating insects within human diets would increase the consumers' food neophobia levels due to the unfamiliarity with the ingredient. Laureati et al., (2016) explained that there are two ways consumers perceive the association between entomophagy and food neophobia. The first is the rejection of insects as a direct food source, which hinges on the consumers' knowledge of rearing, origin and habitat. The second is rejection which can be based on negative post-ingestion consequences; allergens and natural ecosystems. These factors have shown to be most effective in establishing behavioural changes regarding food exposure.

Consequently, the question needs to be asked, 'how do you encourage more people to eat insects?'. This is a recurring debated topic that involves experts in all sectors (food, social-, life- sciences, engineering) to provide holistic and innovative solutions. One method is through food exposure which can increase positive associations with, and familiarity for, the ingredient. This can reduce stimulus error (individual perception) of the ingredient and increase the food product's uptake (Laureati et al., 2014). Another mechanism when introducing this as a new food source is to gradually increase the new ingredient in familiar products, thus reducing the food neophobia reactions. An example of this mechanism was the response to a publication from Moss (2013): *Salt, Sugar and Fat*, in which the food industry was blamed for causing diabetes, heart disease, hypertension and certain types of cancers. The American food company General Mills responded to this publication by gradually decreasing the levels of salt, sugar and fat; while increasing whole grains, fibre and nutrients; within their baked products. Therefore, a similar approach could also be used to encourage the incorporation of insects within baked products. As consumers become more familiar with these products and the idea of insect fortification generally, the ingredient percentage can increase.

6.2.2 Promoting Entomophagy: Psychological and Socio-Culture Perspectives

Consumer acceptance is frequently identified as a significant barrier to the adoption of insects as a food source (Payne et al., 2016). This is common in populations

which do not have a history of insect consumption (Sidali et al., 2019). Hence one of the reasons why entomophagy is not common within Western societies. A few studies have considered the behavioural aspect of consumer perception particularly willingness to consume insects meaning that the consumers' attitude, culture or demographic variable will determine the acceptance of insects as food. A study by Sogari et al., (2017) considered individual behaviour attributes and perceived behaviour control when consuming insects in Italy. The outcome of the participants' willingness to consume insects was based on their knowledge regarding the impact on the environment and potential health benefits (Fasogbon, 2020). However, the individual product attributes' and contextual factors could have impacted the participants' acceptance as outlined in Tan et al., (2016) research. This shows that a combination of consumer traits, for instance: culture exposure, social- and practical factors can motivate the consumer in the context of food provisioning and the consumption of products containing insects. Moreover, these traits can increase the acceptability of products containing insects (Tan et al., 2016).

In non-insect-eating countries, the culture exposure of insects as food can be harnessed through media influence. For instance, with the modern movement towards veganism, gluten-free diets and free-from products, the media could portray insects as a novel, healthy and ethically preferable alternatives to meat. Verneau et al., (2015) research correlated the idea that communication overrides other factors influencing consumer attributes. Thus, marketing and communication of entomophagy's benefits are likely to be a more successful strategy than relying on a swell of changing opinion from the younger generation. Therefore, giving all generations, gender and nationalities a robust knowledge of the impact of consuming insects as food can increase acceptability. Practical factors to consider are applying insects within the consumers' food system and encouraging repeat purchases; i.e. the product characteristics will determine consumer willingness to 'try and buy'. Applications, such as ground insects, which do not radically change the products familiar characteristics, can be gradually incorporated and increased over time. The second method is through the endorsements of organic certification, high fibre and protein branding, and the incorporation of ancient grains to entice consumers to accept the product. As has been clearly shown, there is a link between

the psychology of accepting insects as food through the intrinsic means of communication and social and practical contextual factors.

This research looked at four consumer traits: firstly, to determine the consumer acceptance of a product which was fortified with cricket powder, ancient grains and seeds. The participant had to identify which attribute they preferred than a control sample (identical in the ingredients, but no cricket powder). Secondly, the willingness to consume insects (either as agriculture feed or direct food source food) without prior knowledge of the benefits. The aim of this was to evaluate the willingness of consumers around the world to adopt insects as part of either agriculture feed or a direct food source within human diets. Thirdly, the willingness to consume insects after being provided with knowledge about health and sustainable aspects. This provided evidence to determine if communication and marketing can affect the readiness to adopt insects as an alternative food source. Fourth, this research was used to assess the consumers' food neophobia levels across all continents. The results evaluated the cross-culture differences in accepting insects as food.

6.3 Introduction: Sensory Analysis

The world's current population estimation is to reach over 9 billion people by 2050 is undeniable. The task of producing sufficient and sustainable protein to meet the dietary demand for this number of people is never-ending from traditionally reared livestock, commercially farmed aquaculture to alternative protein forms (Quorn, tofu and majority plant-based proteins). Although there is interest in insects as a sustainable source of protein, there are multiple determinants that influence insects' acceptance as food (Motoki et al., 2020). Insects are a highly sustainable and nutritious source of protein, which are environmentally friendly to rear. On this basis alone, insect consumption could address significant worldwide challenges such as global warming, deforestation and diminishing water supplies (Looy et al., 2014; Rumpold and Schluter, 2013). Cavallo and Materia (2018) identified product characteristics as a crucial factor in shaping acceptance, and by incorporating insect flour within a product can increase its acceptance rate. The acceptance of insects as food is intrinsically linked to communication, which can change perceived behaviour. Furthermore, innovations in food design potentially increase the

development of this new food commodity. Thus, insects combined with a familiar food can theoretically offer an avenue within the food market: a sustainable food product that can deliver vital nutrients, minerals, and vitamins.

6.3.1 Sensory Analysis – Method

In the present study, the assumption is that Westerners would not accept a baked bread product fortified with cricket powder, ancient grains and seeds. This argument's basis is the negative perception of eating insects in Western Society (Shelomi, 2015; van Huis et al., 2013; Hartmann et al., 2015). The lack of insects' availability for consumers to buy, coupled with a combination of social norms, culture exposure, and perceived behaviour control, are considered the main barriers to entomophagy. As previously mentioned, Cavallo and Materia (2015) study identified the acceptance of insects by incorporation with flour in a familiar product; the association of the familiar food product with an unfamiliar ingredient can influence the liking and willingness to accept. Even though the consumer might not have tasted the product before, the learned associations and positive experience can repeat consumption (Tan et al., 2015; Tan and House, 2018). The sensory analysis objective for this thesis was to determine which baked bread sample (similar samples; just one sample did not include cricket powder) had the best specific desired attributes. Attribute-specific testing directs the consumer to focus on one specific characteristic of the baked product.

A combination of staff and students (145 participants) at London South Bank University participated in this study. The participants were recruited through announcements, emails, and word-of-mouth to partake with the product development sensory analysis, looking at alternative proteins within baked bread products. Information sheets were handed out previously to the participants, and they were fully briefed on the sensory analysis procedure before-hand. This ensured that everyone participating had some knowledge about the project and were aware that the products to be consumed were not suitable for vegans and/or vegetarians or people with allergies/intolerance to wheat and shellfish. No other information was presented to the participants who could influence the data collection. During the sensory analysis, participants were evenly spaced within the room and advised not

to communicate with anyone, thus keeping distractions to a minimum. Every participant was assigned a randomised six-digit number (participant number) and asked to complete the consent form. (See Appendix F for a sample of the paired comparison test sheet.) The research assistants uniformly distributed the samples, using a three-digit number chosen at random for each test. Each pair of samples comprised two identical samples in shape and size, just different in one ingredient (cricket powder). All scoring sheets were collected at the end of the sensory analysis session, which the participants were debriefed and allowed to leave the room. Participants were only allowed to exit the room once the sensory analysis was completed, and everyone had handed in their sensory evaluation forms. Due to the uncertainty of the proposed hypothesis, a two-tailed test was used to observe both the distribution's negative and positive tails. The test allowed the possibility for the negative and positive differences, unlike a one-sided tailed test, would only observe if there was a difference between the groups. Thus, to observe both sides of the distribution, α and β risk were calculated accordingly, with α at .05 and β risk set at 30%. This indicated that 145 participants were sufficient to test the hypothesis. Therefore, from the randomised group of participants, the minimum number needed to agree with the hypothesis/objective was 77. This was calculated through the following equation:

$$x = \frac{n + 1}{2} + z\sqrt{0.25 n}$$

Where:

n – is the number of assessors calculated via β risk of a two-side paired test.

z – is the function of the significance level, in this case with α set at .05 the z will equate to 1.96.

6.3.2 Sensory Analysis – Results

The sensory analysis results can be seen in figure 6:1, where the individual attributes (appearance, taste and texture) for the baked products can be summarised in percentages. Based on 145 participants, with the significance level set at .05 for each attribute, the minimum number of identical responses required to determine that one sample's preference exists at 5% significance level ($p = .05$) was

77. Figure 6.1, shows the results in a percentage and the overall threshold to determine which sample attribute was preferred equates to 53%. In general, the majority of the participants who undertook the sensory analysis test preferred the alternative baked product fortified with cricket powder, quinoa-, Khorasan flour and mixed seeds compared with the control sample. The results indicated that the fortified product was above the 53% threshold for each attribute: appearance, taste and texture; scoring 57%, 74% and 64%, respectively. When the participants were asked which of the two samples they preferred, the responses identified that 71% preferred the fortified sample.

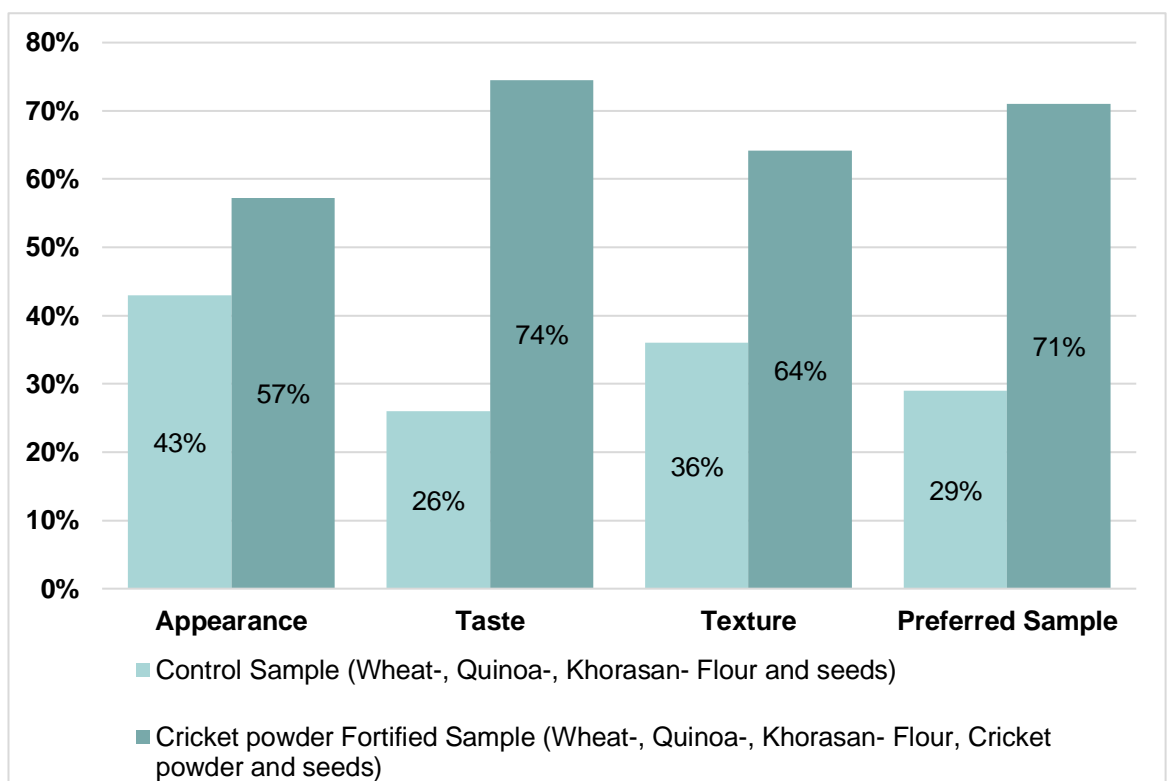


Figure 6:1 Sensory Analysis to Determine which Bread Sample Attribute was Most Preferred.

Analysing the data through Crosstabulation allowed the relationship between the multiple data variables to be examined. This gave a greater understanding of group variables and their correlation. Meanwhile, the use of Chi-squared measured the difference between the observed and expected frequencies of the variables' outcome. Therefore, comparing the total percentages of participants through Crosstabulation and Chi-square, a 2x2 mixed design (Appearance x Preferred

Sample, Taste x Preferred Sample and Texture x Preferred Sample) showed a significant interaction between each attribute and the preferred sample.

Samples		Preferred Samples	
		Control Sample	Cricket powder Fortified Sample
Appearance of Samples	Control Sample (Wheat-, Quinoa-, Khorasan- Flour and seeds)	28	14
	Cricket powder Fortified Sample (Wheat-, Quinoa-, Khorasan- Flour, Cricket powder and seeds)	34	69

Table 6:1 Crosstabulation of Preferred Samples vs Appearance of the Samples

The first 2x2 Crosstabulation (Appearance x Preferred sample) identified that 47.59% ($n = 69$) of the participants favoured the appearance, and overall preferred, the fortified product. However, 9.64% ($n = 14$) of the participants liked the fortified sample's appearance, but overall preferred the control sample. Participants who preferred the control sample for the appearance 23.4% ($n = 34$), changed their preference when asked which 'overall preferred sample' they favoured. While 19.3% of the participants ($n = 28$), kept their original choice of the control sample for both appearance and overall preferred sample. Through the Chi-squared analysis this showed that there was a statistical significance beyond the .05 level: $\chi^2(2, n = 145) = 13.81$; $p < .05$. A Pearson product-moment correlation coefficient was computed to assess the relationship between the samples' appearance and, overall, which sampled was most preferred. There was a moderate correlation between the two variables, $r = 0.31$, $n = 145$, $p = .001$. This indicated that although initially, some consumers liked the control product, they chose the cricket powder bread in the end for the appearance of the sample.

	Samples	Preferred Samples	
		Control Sample	Cricket powder Fortified Sample
Taste of Samples	Control Sample (Wheat-, Quinoa-, Khorasan- Flour and seeds)	34	3
	Cricket powder Fortified Sample (Wheat-, Quinoa-, Khorasan- Flour, Cricket powder and seeds)	8	100

Table 6:2 Crosstabulation of Preferred Samples vs Taste of the Samples

The 2x2 Crosstabulation (Taste x Preferred sample), showed a significant interaction between the two attributes. The majority of the participants (68.9%, $n = 100$) favoured the fortified product for taste and overall preferred the sample, compared to 5.5% ($n = 8$) of the participants who liked the fortified product, but overall preferred the control sample in the end. Even with this slight movement, this showed that most participants liked the taste of the fortified product and would choose this over the control sample. Comparing this with the participants who favoured the control sample in taste, only 2.1% ($n = 3$) overall preferred the fortified product. Meanwhile, 23.45% ($n = 34$) liked the control sample taste and selected the control sample at the end of the experiment. The Chi-squared analysis showed that there was a statistical significance beyond the .05 level: $\chi^2(2, n = 145) = 95.61$; $p < .05$. A Pearson product-moment correlation coefficient was computed to assess the relationship between the samples' taste and, overall, which sampled was most preferred. There was a strong correlation between the two variables, $r = 0.81$, $n = 145$, $p = .001$. This means that most participants preferred the taste of the cricket powder fortified bread product from the onset. Therefore, very few changed their minds and remained with their original choice (cricket powder bread) as their preferred product.

	Samples	Preferred Samples	
		Control Sample	Cricket powder Fortified Sample
Texture of Samples	Control Sample (Wheat-, Quinoa-, Khorasan- Flour and seeds)	23	29
	Cricket powder Fortified Sample (Wheat-, Quinoa-, Khorasan- Flour, Cricket powder and seeds)	19	74

Table 6:3 Crosstabulation of Preferred Samples vs Texture of the Samples

The final 2x2 Crosstabulation, looking at Texture x Preferred sample, showed a significant interaction between the two attributes. While 51% (n = 74) of the participants favoured the fortified product in both texture and overall preferred sample; 13.1% (n = 19) preferred the fortified products texture but selected the control sample at the end for the most preferred sample. In comparison, 20% (n = 29) participants who favoured the control sample for texture, chose the fortified bread as their overall preferred sample in the end. However, the remainder 15.9% (n = 23) of the participants selected the control sample for both texture and overall preferred sample. The Chi-squared analysis showed a statistical significance beyond the .05 level: $\chi^2(2, n = 145) = 9.18$. This indicated that most participants preferred the texture of the cricket powder fortified bread product from the onset. Therefore, very few participants changed their minds and remained with their original choice (cricket powder fortified bread) as their preferred product.

6.3.3 Sensory Analysis – Discussion

Although sensory booths are usually used for sensory analysis, these at London South Bank University have been decommissioned. Hence, a lecture theatre was used for sensory analysis. One of the aims is around the idea of producing a final product for the Southern African market/consumer, the sensory analysis was conducted within the UK. Therefore, the results and responses were based on staff and students within LSBU and did not consider the Southern African consumer. Although the sample population was made up from many different ethnicities, these data points were not collected and so cannot be spoken about. Causse et al., (2010)

highlighted that consumers from different countries with different cultures and food practices have similar preferences when analysing sensory descriptors. However, their research showed that diversification of taste, texture and overall preference of worldwide consumers differ by region and country. Consequently, a limitation to this project and sensory analysis is that it should have recruited either Southern African consumers or expanded the project to Southern Africa to validate the results.

The sensory analysis results appeared that the consumers preferred the baked bread product fortified with cricket powder, ancient grains, and seeds. Consumers showed positive attitude behaviour and more receptivity to the theme of insects as food. This shift in attitude behaviour and acceptance of such products fundamentally plays a major role in accepting new foods. Even with insect-based ingredients, reducing the insects' visibility by processing them into flour and incorporating this into a familiar and well-liked general product, improved the consumers' willingness to eat an insect-based food. Familiarity can help with the consumers' uptake of a product, as the baked product's visual aspect resembled similarly as sliced bread suggests that people were more receptive to the product. Furthermore, ancient grains and seeds incorporated within the baked products allowed to disguise the cricket powder's appearance within the bread samples. By incorporating these ingredients together to create a baked product, provides a commodity that the consumers are willing to purchase due to the visual appearance, taste and texture. The visual acceptance of the fortified product correlated with a previous study by Schosler et al., (2012), where insect flour was used within baked products. The correlation identified the acceptability and success of insect inclusion in food products requires them to be disguised or milled into a fine powder to avoid visible parts. These findings correlate with Laureati et al., 2016 research that found that insects within a traditionally well-liked and known food, is an effective strategy to enhance overall liking.

Included within the sensory analysis within this study, respondents were able to comment on the products. The full list of comments can be found in Appendix F; however, a few are given below. Many participants enjoyed the taste of the inclusion of the cricket powder with one respondent commenting:

'More depth of taste with the [cricket sample], the crumb structure was open and light.'*

However, a couple of consumers found that the cricket powder impacted the samples' quality, especially the texture and aftertaste. The comment below suggested that the cricket powder influenced the texture of the baked product. This could have been due to the quinoa flour as this was freshly milled before production of the bread samples.

'Both taste similar, however, the sample with [cricket flour] had a gritty aftertaste.'*

Finally, the majority of the comments concerned the perception of the baked product. Most of the consumers found the appearance of the fortified bread was better than the control sample. The similarity of comments on the seeds and colour of the crumb confirms that cricket powder's inclusion within this type of baked product could be acceptable to consumers in Western society.

'Looked just like wholemeal bread, which makes it look like a healthier option. Good inclusion of seeds'

'The [cricket bread] had a rich flavour and with the additional seeds perceived healthier.'*

'The [cricket product] was delicious and looked great. I loved the seeds on the outside of the crust, which made this healthier.'*

These comments identified that the cricket powder prototype bread had a good ratio of seeds, a richer flavour and a darker crumb colour than the control sample. Overall, the prototype gave the consumers a great impression of the product being 'a healthy natural bread'.

**author changed the sample number to denote the key ingredient*

6.4 Introduction: Worldwide Consumer Survey

Using insects as food is a promising way to overcome the global food challenges (Piha et al., 2018) that persist around the world (fragile food systems or as a sustainable food source). Whether they could be used as feed for livestock and aquaculture, or food for consumers as a direct source, insect husbandry is more efficient than regular meat production (see Chapter Two, subsection 2.3.2).

Consumer willingness and food neophobia is regarded as one of the main reasons for the absence of insects within Western diets (Piha et al., 2018). Food choices are a combination of social, culture, demographic and cognitive factors, and evidence shows that consumer knowledge and experience can increase willingness to adopt entomophagy within societies. Willingness to consume insects can be culturally relative due to endemic food choices and attitude towards insects, which can vary not only according to culture but also between countries and continents.

Many studies have investigated consumer willingness and food neophobia levels that are linked to psychological factors. For instance, consumer knowledge falls into three areas: product-related experience, objective- and subjective- knowledge. Although product experience is linked to the consumers' attitude and perceived behaviour, this can be related to the number of product-related experiences that have been accumulated by the consumer (Rose et al., 2011). This included the knowledge and experience of the food item attained through friends and family members, advertisement and visual involvement. Subjective knowledge is based on the individuals' experience and engagement with the product, thus, representing the individuals' thought about the product category. Objective knowledge is actual information regarding the product, and this is normally measured with a tailored test (Piha et al., 2018). Overall, several factors need to be considered within the consumers' willingness and food neophobia behaviour experience: mainly behavioural attitude, product-related experience, cognitive function (subjective- and objective- knowledge) and personal beliefs. Therefore, the purpose of the survey was to capture individual customer knowledge (subjective and objective) in their willingness to consume insects, and their levels of food neophobia to such ingredients. The aim of this research was to investigate three aspects of entomophagy around the world. First, the willingness to consume insects as feed for livestock and aquaculture, and as a sustainable food source without prior knowledge of the benefits. Secondly, the willingness to consume insects after being provided with knowledge about the health and sustainable aspects of insects. Thirdly, to assess the consumers' food neophobia levels across all continents.

6.4 Worldwide Consumer Survey

6.4.1 Worldwide Consumer Survey – Method

The online consumer survey was created through Jisc Online Survey, formally known as Bristol Online Surveys. Jisc Online Survey is GDPR compliant, and ISO 27001 accredited, ensuring all data collected could not be linked back to the participant. The survey was distributed through social media (LinkedIn, Instagram, Facebook and Twitter) and email, which was then dispersed further by colleagues and participants. There were no financial incentives to complete the survey, and while 2521 respondents viewed and started the survey, a total of 1392 completed all three sections of the survey. The socio-demographic profiles of the subsets are presented in table 6.1.

Demographic Features of Participants and Subsets							
Demographic Features	Africa	Asia	Australasia	Europe	North America	South America	UK
Number of Participants	35	36	100	281	208	17	715
Male	11.4%	16.7%	4%	11.4%	26.4%	35.3%	7.3%
Female	88.6%	80.6%	96%	88.6%	72.2%	64.7%	92.3%
Other	-	2.8%	-	-	1.4%	-	0.4%
18-24 years	28.6%	51.4%	69%	60%	47.1%	50%	72.5%
25-34 years	37.1%	29.7%	21%	20%	35.6%	43.8%	15.4%
35-44 years	11.4%	5.4%	3%	12.9%	13%	6.3%	5.6%
45-54 years	8.6%	10.8%	6%	5.7%	3.4%	-	3.4%
55-64 years	8.6%	2.7%	1%	1.1%	1%	-	2%
65-74 years	2.9%	-	-	0.4%	-	-	1%
75+ years	2.9%	-	-	-	-	-	0.3%

Table 6:4 Demographic Features of Participants and Subsets

- indicates no data presented

Figure 6:2 shows the frequency of the responses, allowing an overview analysis of the countries and continents who responded to the survey. The number of participants and the overall percentage for each continent are as follows, Africa $n = 35$ (2.5%), Asia $n = 36$ (2.6%), Australasia $n = 100$ (7.2%), Europe $n = 281$ (20.1%), North America $n = 208$ (14.9%), South America $n = 17$ (1.3%) and UK $n = 715$ (51.4%).

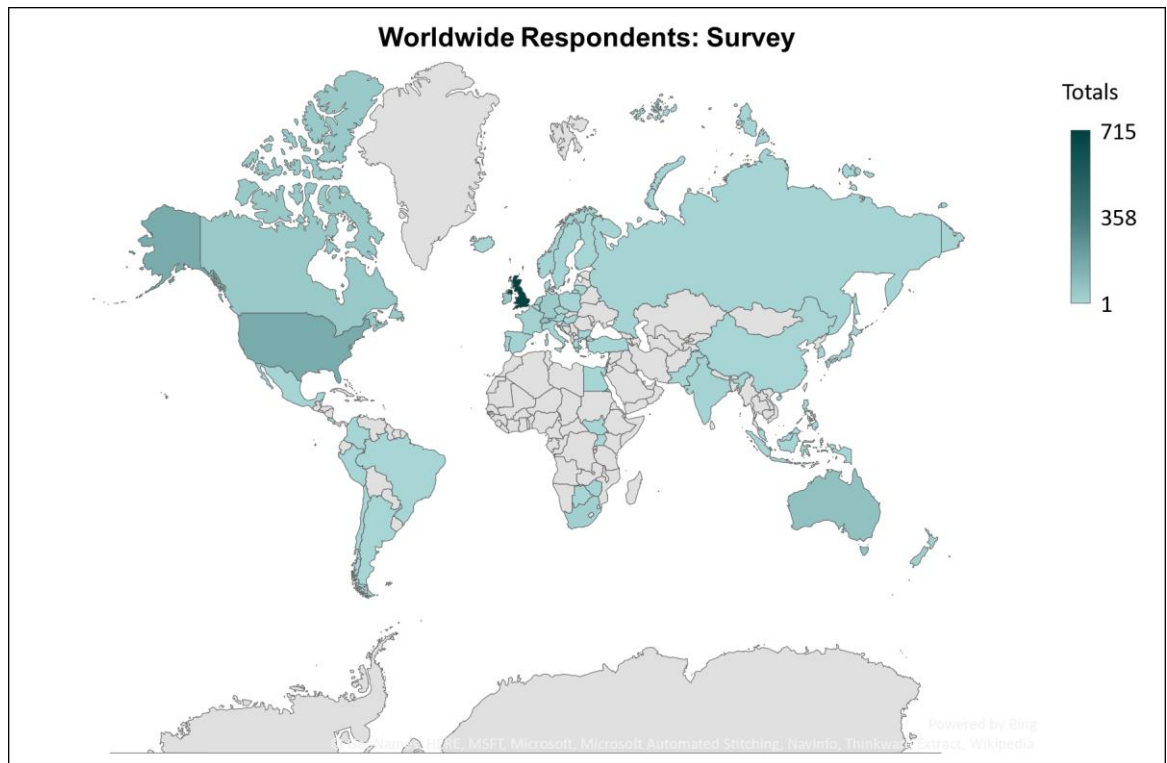


Figure 6:2 Worldwide Map showing which Continents and Countries Participated in the Survey. Light to darker colour indicates the number of responses, darker areas signify a higher response rate. Countries areas in grey, denote no response from the country.

6.4.2 Worldwide Consumer Survey – Measures

The survey entailed 15 questions divided into two sections, willingness to consume insects as food or feed (five questions) and overall food neophobia levels (ten questions). The first section observed the consumers attitude towards insects: either as livestock and aquaculture feed or as a food ingredient. The attitude towards the behaviour was monitored using a 5-point hedonic scale from very unlikely to very likely. Lastly an intervention was included within this section, consumers were advised about the possible health benefits, sustainable aspects and nutritional values of insect consumption. The consumer was then assessed after the intervention for their willingness to consume insects as food and their reaction to the objective knowledge. The second section of the survey was used to assess the social norms of the participants, based on their food neophobia level. This food-neophobia scale was developed by Pliner and Hobden (1992) which allowed the belief strength and outcome to be monitored. This part of the survey assessed the participants ethical morals on novel foods such as insects and eating at novel food restaurants which use insects as food. For each statement, the participants had to

respond using a 5-point hedonic scale from strongly disagree to strongly agree. (Appendix G shows the full list of survey questions that were generated and posted online through the survey platform).

6.4.3 Worldwide Consumer Survey – Results

A cross-regional comparison allowed the analysis of individual continents rather than individual countries. The comparison of continents allows geographical cross-regional evaluations. Although the UK is part of Europe, this was kept separate to analyse the UK consumer market. Table 6.2 showed that consumers had a positive attitude (behaviour) towards insects as feed for livestock and aquaculture (mean score: Africa 3.94, Asia 2.95, Australasia 3.65, Europe 3.35, North America 3.74, South America 3.81, UK 3.31). A moderately negative attitude (behaviour) towards insects as food was recorded (mean score: Africa 3.1, Asia 2.53, Australasia 2.95, Europe 2.88, North America 2.96, South America 2.96, UK 2.62). After the intervention participants showed a moderately positive attitude (behaviour) in the willingness to consume insects as food (mean score: Africa 3.31, Asia 2.81, Australasia 3.21, Europe 3.07, North America 3.11, South America 3.75, UK 2.82).

Overall, participants reported having a medium neophobia tolerance according to the Pliner and Hobden (1992) scale (low 0-23, medium 24-44 or high 45-100). The overall mean scores for the food neophobia levels were as follows: Africa 35.23, Asia 35.68, Australasia 38.52, Europe 37.83, North America 37.91, South America 38.94, UK 37.52). Cronbach's α was generated to determine if the internal consistency results were satisfactory for the following: (a) insects as food, (b) intervention communicating the health benefits and nutritional content of insects and (c) food neophobia scale. The outcome showed that the results were significantly acceptable with Cronbach's α : 0.91 (attitude towards insects as food), 0.95 (attitude towards insects as food after the intervention) and 0.82 (perceived behaviour control towards food neophobia levels). The results suggested these are homogenous, meaning that the participants all had similar viewpoints towards the questions.

Comparison of Gender vs Continent for the individual Constructs										
Construct	Gender			Africa	Asia	Australasia	Europe	North America	South America	UK
	Male	Female	Other							
Willingness as Feed	4.13±1.26	3.33±1.56	3.23±1.8	3.94±1.26	2.95±1.37	3.65±1.54	3.35±1.54	3.74±1.48	3.81±1.6	3.31±1.58
Willingness as Food	3.34±1.36	2.7±1.33	2.76±1.77	3.1±1.13	2.53±1.31	2.95±1.35	2.88±1.34	2.96±1.4	3.52±1.51	2.62±1.33
Willingness as Food (Intervention)	3.43±1.37	2.9±1.5	2.86±2.04	3.31±1.45	2.81±1.63	3.21±1.47	3.07±1.47	3.11±1.54	3.75±1.57	2.82±1.48
Food Neophobia Levels	38.85±6.36	37.49±6.56	33±11.69	35.23±6.07	35.68±7.9	38.52±5.46	37.83±5.95	37.91±6.8	38.94±5.74	37.52±6.84

Table 6:5 Comparison of Gender vs Continent for the Individual Constructs

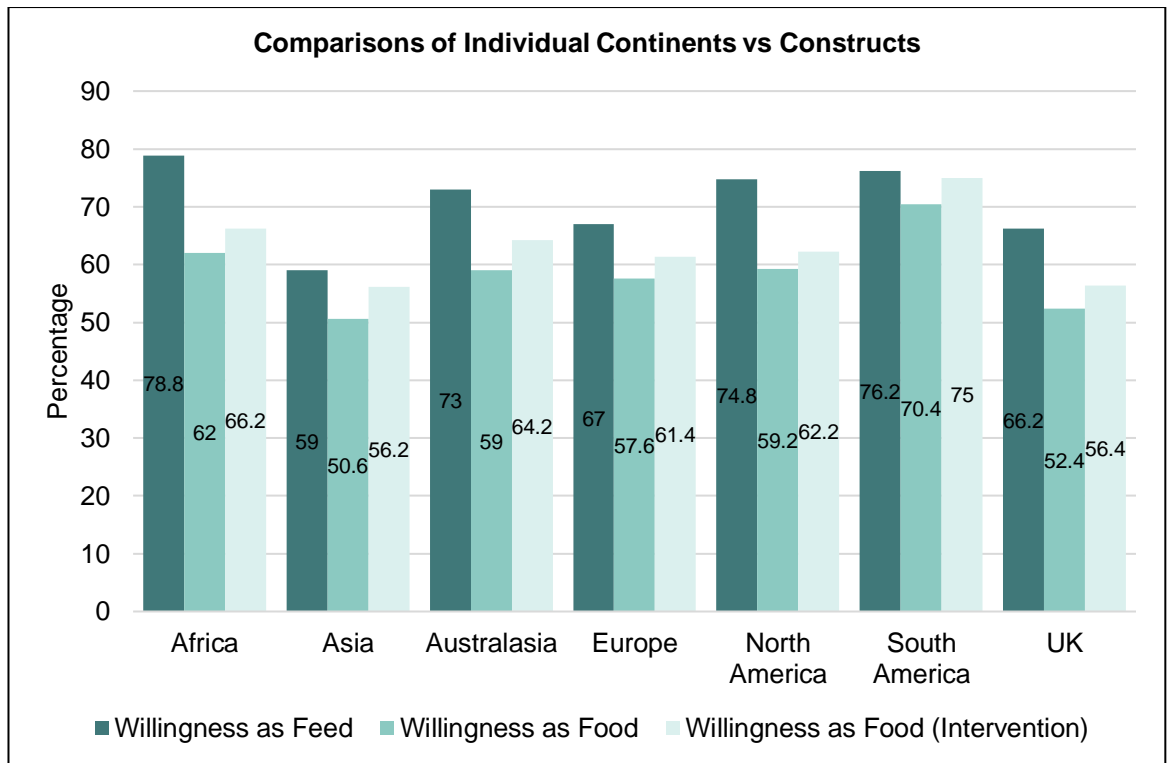


Figure 6:3 Comparisons of Individual Continents vs Constructs Before and After Intervention

Comparing the constructs in table 6.2, Chi-square (χ^2) was used to assess model 'goodness-of-fit' test, with the results showing a significance at $p < .05$. The results showed that a proportion of participants preferred insects as feed for livestock and aquaculture rather than as a direct food source, $\chi^2(4, n = 1392) = 199.72$; $p < .001$. A Pearson product-moment correlation coefficient was computed to assess the relationship between the constructs (insects as feed for livestock and aquaculture rather than as a direct food source). There was a large correlation between the two variables, $r = 0.6$, $n = 1392$, $p < .01$. This indicated that participants preferred the idea of insects as feed within agriculture (livestock and aquaculture) rather than for direct human consumption. The participants' results were then analysed before the intervention to determine if communication regarding insects could promote a shift in the results. The outcome showed that participants preferred insects as feed for livestock and aquaculture even before the intervention, $\chi^2(4, n = 1392) = 126.27$; $p < .001$. Pearson product-moment correlation coefficient was computed to assess the intervention. There was a large correlation between the two variables, $r = 0.59$, $n = 1392$, $p < .01$.

As previously mentioned, the intervention was assessed against the willingness to consume insects as either feed for agriculture or as a direct food source. The results showed that a large proportion of participants changed their preference for insects as a direct food source after the intervention, $\chi^2(4, n = 1392) = 12.95; p < .01$. A Pearson product-moment correlation coefficient was computed to assess the relationship between the constructs (insects as a direct food source before and after the intervention). There was a large correlation between the two variables, $r = 0.89, n = 1392, p < .01$. This indicated that participants were more receptive to the idea of insects as feed within agriculture (livestock and aquaculture) rather than for direct human consumption. However, after the intervention, the participants attitude behaviour changed and became more open to the ideology of insects as a direct food source. Communication regarding insects is important as indicated by the change in consumers attitude once they had heard about insects, particularly about cricket powder being high in protein, low in carbohydrate, gluten-free and generally highly nutritional. Analysing the food neophobia levels through MANOVA, allowed comparison between continents. This showed no statistical significance; $F(1,6) = 1.85; p > .05$ with $\eta^2 = 0$, meaning that all consumers around the world had similar food neophobia levels (medium level) with no cross-regional variance.

6.4.4 Worldwide Consumer Survey – Discussion

There were a number of variables investigated to determine the impact of the participants willingness to accept insects as either agricultural feed or direct food source, and the individual food neophobia levels. It should be noted that both subsets (gender and age) were biased towards females and a younger cohort of people. The spread of gender and age skewed the data, especially the overall effect/balance of the results. These kinds of biases are common in surveys and online questionnaire methodologies (Piha et al., 2018; Duffy et al., 2005). It was found in previous studies (van Huis et al., 2013; Vanhonacker et al., 2013; Verbeke, 2015; Hartmann et al., 2015, Menozzi et al., 2017) that the male population were more positive towards eating insects. The reduced number of participants for some continents was due to limiting factors; access to social media platforms, survey in English, and internet access. For example, the online survey was promoted through social media platforms which can be a hindrance for some demographic groups.

Another limiting factor was that some media platforms are barred in certain countries; for example, Asian countries due to political situations in China, Russia and North Korea. Although a few respondents obtained a link to the survey, there is a possibility that some participants would not understand the questions as these were not translated into the local languages. Especially considering developing countries is the access to the internet, many of these countries do not have the digital infrastructure to enable access to the survey. Therefore, caution was required when making generalisations about the overall population (Henrich et al., 2010). The final limitation was the use of certain questions from Pliner and Hobden Food Neophobia Scale (1992) to assess willingness of unfamiliar foods. The Food Neophobia Scale is a simple assessment consisting of 10 descriptors, where the terminology could be mis-understood. This means that consideration of such terminology descriptors, for instance 'exotic foods' and 'ethnic foods', are applicable to individual context. While the research was conducted with the original Food Neophobia Scale, if the survey was to be redesigned and distributed again this would be adapted to reflect this. For example, asking if a food that would be considered 'exotic in your own culture' would be adapted accordingly.

The results of the study indicated that consumers are clearly ready (positive attitude behaviour) for the incorporation of insects into feed supplies for agriculture (livestock and aquaculture). Majority of the participants within the survey declared that they would consider supplementing their diets by consuming livestock and aquaculture that had been reared on insects. This outcome is understandable since insects are already a natural food source for some livestock, e.g. fish and birds. Therefore, this singularity makes the consumer accept insects as agriculture feed more positively. Oonincx and de Boer (2012) advised that feeding livestock and aquaculture with insects can improve the welfare of the animals as they are encouraged to forage (free-range behaviour) for their own food. This has shown to decrease the animals' stress and increase their body weight when offered this method of feeding. More recently in the Netherlands, a company (Albert Heijn) have started to sell this 'nostalgia' of natural feeding of poultry to consumers, for example, promoting eggs that are organic and free range laid by birds whose intended soy feed had been replaced with live insects.

Introducing insects as a direct food source within society was perceived moderately negative in this study. However, after the intervention this was marginally increased to a positive outcome suggesting that consumers would consider incorporating insects into their diets. The percentages of participants willing to introduce insects into their diets increased after the intervention (see figure 6.3), and these are higher compared with those observed in other studies conducted within individual countries (Vanhonacker et al, 2013; Verbeke, 2015; de Boer et al., 2013; Laureati et al., 2016). It should be noted that these previous studies range from 5-7 years ago and the level of communications regarding the value of insects as a food source has increased over the past few years. Moreover, the acceptance of insects as a direct food source in Africa and South America were comparatively more positive than any other continent. The education and upbringing of children in these areas (social, culture and demographic factors) mean that consumers have different eating habits compared to those in Western countries. This demonstrates that a combination of contextual factors (social, culture, demographic) and consumer- knowledge and experience can increase willingness for entomophagy within societies. Communication to, and education of, young children is vital in encouraging the acceptance of insects as a direct food source. This would allow consumers to be more receptive to the topic, idea and acceptance of novel foods made with insects.

In contrast to all other continents, Asia had the highest negative response to both insects used in agriculture and as a direct food source. Even though insects are in abundance and found throughout Asia, this negative response can be attributed to one of two theories. The first theory being the age range of respondents who participated within the survey. With the majority of these participants aged between 18 and 35 years, and due to the repression of the individual countries' culture and current affairs in developing countries. This was particularly evident with the introduction of Western culture in Asia, resulting in the movement away from local cuisines and cultural standards. Through the introduction of fast food restaurants (MacDonald, KFC and Burger King) and Western lifestyle commodities within Asia (Starbucks coffee, fashion luxury wear) young consumers are moving away from their traditional cuisines for Western food. It is estimated that, by 2025, China are forecasted to account for 46% of all luxury goods purchased worldwide as consumers wish to move towards a Western culture and be seen to be 'fitting in'

rather than keeping to their local cuisines and culture (Bain and Company, 2018). The second theory is based on the perception of insects as food. Although the overall value for insects as agriculture feed is low, this could be due to the fact that insects are already perceived as food. Therefore, if insects were to be classed as feed, there is an increase in energy, time and cost for double processing (conversion for feed, then into food). However, based on the results from Asia, the willingness to adopt insects as a direct food source is perceivably low due to the former theory that the respondents age and drive for Western culture would diminish the openness to consume insects as food.

In comparison assessing the food neophobia levels and gender, males showed to be more receptive to novel foods. In this research, males seemed to have a marginal higher food neophobia level compared to females. However, analysing the results on willingness to consume insects as agriculture- or as a direct- food, showed them to have the highest response rate. Caparros Megido et al., (2014), Verbeke (2015) and Laureati et al., (2016) research made the assertion that males would have a lower food neophobia, due to their acceptance of insects as a direct food source. These previous studies were solely based in Belgium and Italy and not based on other countries around the world. Therefore, this worldwide survey analysing the acceptance of insects as agriculture feed, direct food source and the food neophobia levels gives a greater understanding of the consumers' perception. Previous comparisons were analysed through individual countries' research and assumed the constructs of similar areas. The outcome from these individual studies between 2010-2015, had a negative response with regard to the acceptance of insects as a direct food source. However, over the intervening period of time there has been an increasing shift in subjective-, objective- knowledge and perceived behaviour control compared with the earlier studies (van Huis et al., 2011; Vanhonacker et al., 2013; Verbeke, 2015; Hartmann et al., 2015, Menozzi et al., 2017).

Through this worldwide survey, there is an indication that the consumers' perception shift of insects over the past ten-year period is changing. There is a willingness to accept insects as a direct food source and food neophobia levels have reduced; although, the subset contains a large range of participants from a wide range of countries/continents and backgrounds. Gender was found to be the most influential

factor for the willingness to accept insects as a direct food source. Males were more likely to accept insects as a direct food source than females which is consistent with the findings of Schosler et al., (2012) and Verbeke (2015). While there is a contrast with the age range reported in the Scholser research, this could be due to the number of participants in the category 'between 18 to 24-years-old and female'. This would have skewed the data reducing the overall mean score. Nevertheless, the willingness increases as the age range reached over 25+, which allows for the higher levels of openness and curiosity of young adults. However, strategies can be used to continue this advocacy and uptake of entomophagy. Through the education and assessment of consumer acceptance of the new food products, this can increase the demand of such products and overall acceptance. The impact of the food neophobia levels would continue to decrease, as people who are already familiar with the product would have positive view to share with others.

6.5 Consumer Acceptance and Worldwide Survey Conclusion

In summary, the results of this study revealed that consumers are willing to accept baked bread products fortified complementary ingredients cricket powder, ancient grains and seeds. Furthermore, consumers preferred the appearance, taste and texture of the bread fortified with cricket powder, ancient grains and seeds, over the control product. Reducing the visibility of the insects by grinding them into a flour and incorporating this into a familiar and well-liked general product, improved the consumers' willingness to eat an insect-based food.

The worldwide survey revealed that there is more potential for the use of insects in agriculture feed (livestock and agriculture) than as a direct food source in human diets although, there was an increase in uptake and drive for change in the participants' diets following the intervention. The increase in consumer acceptability ratings for insect products grew substantially after they had received positive information about insects – high protein, sustainable, low fat, low carbohydrate, etc. This shows that communication and marketing are key when encouraging people to change their existing food choice. Changing consumers food choices is not an easy task and requires more than an increase in the familiarity of insects as ingredients in food. To fully ensure that novel foods like insects (cricket powder) to increase in consumption, there is a need for people's internal beliefs and values to be changed

through knowledge and intervention. As the worldwide study validates, basic psychological processes, such as cognitive structures of food choice and attitude behaviour, vary differently through the cross-regional comparisons. Meaning that one strategic outcome in North America would not necessarily be the same in Australasia. However, continual enhancement and communication regarding the positive aspects of adopting insects as food can slowly further reduce food neophobias and increase uptake.

6.5.1 Key Summary Conclusion Points

- Consumers are willing to accept baked bread products fortified with cricket powder, ancient grains and seeds, as long as the crickets are processed into a fine powder before use.
- Reducing the visibility of the insects by grinding them into flour and incorporating this into a familiar and well-liked general product, improved the consumers' willingness to eat an insect-based food.
- Participants prefer the idea of insects as an agricultural feed rather than as a direct food source in their own diet.
- Food neophobia levels are decreasing, which can be seen in previous studies (earlier than 2010). This decrease in neophobia levels can increase the uptake of insects as food.
- Communication and marketing are key when promoting insects as food. An increase in consumer acceptability ratings for insect products grew substantially after they had received positive information about insects – high protein, sustainable, low fat, low carbohydrate etc.

Chapter 7 – General Discussion, Conclusion and Future Research

Food security is a primary concern worldwide, whether in developed- or developing-countries, food is required for the human diet. The ideal diet consists of healthy food, sufficient quality and quantity, affordable, safe, and culturally acceptable for human health status (Franzo et al., 2020). There is a delicate balance to be struck when it comes to an ideal diet as each country is affected by different forms of malnutrition. This can manifest as poor nutrition or micronutrient deficiency compared to obesity due to gluttony and excessive calories consumed on the other end of the scale. All forms present significant problems in achieving sustainable development, impacting human health, the environment and human capabilities (Franzo, 2019). There are many underlying determinants for malnutrition, the predominant factor being poor nutrition. No matter what the diet might consist of, they stem from the food systems in place, and these food systems must adapt and deliver nutritious diets without damaging the environment.

This thesis targeted one potential way of addressing hunger, malnutrition, and improving food sustainability within developing- and developed countries. The research aimed to explore the viability of baked bread products fortified with cricket powder, which can be used as part of a balanced diet. The results indicated that cricket powder is beneficial for human diets when incorporated within baked products. The findings do not contradict but extend existing research by providing further evidence and creating awareness of other possible food security methods in developing and developed countries. Finding economically viable foods to feed an ever-increasing population, especially in nations with severe poverty, has become more urgent than ever before. Human impact on the environment has never been more telling than over the last ten years. Drought, flooding and wildfires are all sure signs that the world is quickly changing. The impact of these global problems raises the importance of food- sustainability and production. Meeting these two driving factors in the race for human survival, while maintaining the consumers' acceptance of the final product is vital. As consumer acceptance and perception are a major driving force for product integration within a culture.

7.1 Cricket powder as a Functional Ingredient

One of this project's relative influences was determining the doughs' rheological and enzyme behaviour after the cricket powder was incorporated as a functional ingredient. This was analysed before developing the prototype product to consider factors between the flours, including water absorption, gluten development, viscosity, starch amylase, and retrogradation. It was necessary to understand these factors because they ensure a good quality bread structure. For instance, pseudocereals (quinoa) and cricket powder impacted the doughs' crumb structure as they do not contain gluten. A continuous gluten network gives dough extensibility, allowing it to inflate and resist collapse during the process's proving and baking steps. Adding bran or fibre equivalent ingredients (such as cricket powder) into bread products can disrupt the continuity of the gluten network. Understanding the causes and effects of working with high bran/fibre ingredients, allows the recipe to be altered with wheat- and Khorasan flour to help with the gluten structure.

Further considerations were also given to the theoretical and applied implications of the findings presented within Chapter Three. Forming the extensive network of proteins and hydrated gluten necessary in dough development. Consequently, the combination of flours used throughout this thesis questions the capabilities of monomeric-, polymeric- proteins and HWM to LWM glutenin subunits present in each flour. The use of the Mixolab observed these effects (Shewry et al., 2002), and gave a greater understanding of the individual flours' capabilities. Moreover, the cricket powder dough had an inadequate gelling capacity, consequently decreasing the binding of wheat proteins to starch. The diminished hydrogen bond between starch and gluten was promoted during gelatinisation, leading to a lower viscosity. Therefore, the results provided clarity when incorporating ingredients, such as cricket powder, ancient grains and seeds within doughs.

The addition of cricket powder, quinoa flour and seeds impacted the water hydration percentage, which significantly increased the time required to develop the dough due to the gluten matrix's dilution. This interference with the gluten network formed a weaker dough as the fibre present within the dough would have hindered forming the disulphide bonds (Belton, 2012). Ensuring sufficient hydration of the gluten-forming proteins within the wheat- and Khorasan flours ensured that the

development of the dough's integrity was preserved. Thus, the length of time required for kneading was examined for dough stability, to ensure that the development of the gluten. Caution was required because prolonged mixing action would have affected the doughs' structure and texture.

Due to the natural presence of fibre found within cricket powder, the breadmaking process was adjusted. As an insufficient hydration, would impact the dough's rheological characteristics such as extensibility, elasticity, and gas retention, thereby lowering the quality of the doughs. Katina et al., (2006) recommended using a 'wet hydration' when incorporating high fibre flours to improve the overall bread products' texture, volume, and quality. This technique allowed the hydration of gluten-forming proteins first to ensure the gluten network is sufficiently hydrated. However, as previously discussed in Chapter Three; the increased water levels were necessary to optimally hydrate the wheat flours, ancient grains and cricket powders and seeds. This ensured that the gluten-forming proteins were fully hydrated; so that the full functionality of the ingredients could be exploited when incorporated into the dough mixture. Otherwise, a continuous mixing action can overdevelop the gluten, creating a solid barrier thus trapping the gasses (carbon dioxide), created by the yeast, from being expelled and therefore not pushing the dough upward. As a result, the overall interior crumb would become very dry, dense and crumbly, which would not be accepted by consumers. Finally, it was also noted that through the fortification of dough with cricket powder, ancient grains and seeds, the retrogradation within the bread products was slowed and therefore the shelf-life of the bread product was naturally extended. This is due to the natural occurrence of lipids found in the flours and the seeds as this creates a liquid fat film which coats the gluten forming proteins.

The second study showed a significant increase in the nutritional composition profile when a combination of ingredients is incorporated, such as cricket powder and/or ancient grains and/or seeds into bread products. Protein is essential for human existence, especially for mental and physical development (Foresight: The Future of Food and Farming, 2011). The potential to increase the protein, fat and fibre content within a staple and familiar product that can be consumed worldwide is an achievement. A bread that can deliver essential micro- and macro- nutrients within

developing- and developed- countries, a staple food source for the growing population (ending malnutrition – either protein or micronutrients). Nutritional deficiencies, especially during pregnancy and at the early stages of child growth, can have detrimental health impacts, some with lifelong effects (Mousa et al., 2019). Insects are highly nutritious and a healthy food source of fat, protein, vitamins, fibre and mineral content. The utilisation of these sustainable ingredients can decrease global diseases and extend life expectancy in developing countries. Moreover, within developing countries, this product could be auspicious in tackling micronutrient deficiency. The study provides guidance and outlines possibilities for the use of cricket powder within baked products

Therefore, cricket powder's inclusion increases the nutritional composition and the organoleptic properties (Chapter Six). The darkening of the crumb colour also gave the perception of a healthier loaf, similar to a rye bread which consumers consider to be a healthy trendy product (see Chapter Six – Consumer Perception and Sensory Analysis). This would not be misleading the consumers, as the product not only looks acceptable, but findings from Chapter Four (Nutritional Composition) regarding the protein, fat, fibre, and carbohydrate content supported this theory of perception. Therefore, the product is acceptable and supported by health claims that can benefit human societal groups. For the poor, obtaining an essential number of calories becomes a central focal point for survival activity, regardless of the quality of those calories. The inclusion of cricket powder, ancient grains and seeds (in baked products) increased protein, fibre, and fat levels within the baked products. The increase in fat content had a beneficial impact, significantly affecting the softness of the baked crumb, which can be seen in Chapter Five (sub-section 5.6.2).

These fortified baked products could be used to manage malnutrition within developing countries, although not suited for severe protein-energy malnutrition. This is because nutritional rehabilitation protein and fat need to be around 12% and 53% retrospectively, baked products with fortified cricket powder, ancient grains and seeds increase protein and fat by 41.74% 10.08%, respectively. Therefore, by consuming a high protein, fat, and fibre baked product, the nutrition required to prevent Kwashiorkor and Marasmus's future cases within developing countries could be attained (see Chapter Four). Moreover, the dried ingredients could be

blended and stored at an ambient temperature, making it ideal for developing countries. This could be marketed as a 'ready mix', in which water just needs to be added and produced into a product known to the local community. Thus, where infrastructure to support food storage and distribution at low temperatures can be limiting, the storage of dried hybrid ingredient mix would be more suitable, than a ready-made product. The use of such products can prevent future starvation and save lives around the world, especially within Red Cross camps, disaster-stricken areas or malnourished societies.

The third aspect of this research was to determine cricket powder's functionality within baked products, specifically looking at the crumb structure and texture to see if the staling rate accelerated. Cricket powder does not have any viscosity and elasticity capabilities which is vital when producing bread. This was represented by the C3 torque value (Chapter Three – Dough Rheological Behaviour), which showed that cricket powder's inclusion resulted in no viscosity capabilities. Therefore, as the viscosity and elasticity would be affected, the baked products' volume and crumb structure become dense and did not have the same open crumb structure as the control bread. However, a combination of 30% of cricket powder, 40% of ancient grains and seeds gave the best results for any fortified product, for the overall cell volume. Although the fortification with cricket powder, ancient grains and seeds showed to have a decrease in gas retention and lower dough expansion, caused by the fibre interference of the gluten network. It was this prototype bread sample that retained the softest texture over the 7-day period.

7.2 Consumer Perception of Cricket Protein Cereal-Based Products

Consumer food choice has a considerable impact on the environment and more importantly, climate change. Animal-based protein production is resource-intensive and has a significant impact on the environment (Bazoche and Poret, 2020). Seventy-one percent of the world is considered habitable, of which 50% of that proportion of land is used for farming. Furthermore, 77% of the 50% is solely used for livestock rearing or as graze/feed for commercially reared animals (Our World in Data, 2018). Consumers exert choices and preferences which poses the challenge of accepting insect-based foods, more so, within Western society. Due to emotion, disgust is the primary motivational barrier towards the acceptance of insects as food.

As shown in Chapter Six, individuals reject new and unfamiliar foods as a typical survival reaction to prevent ingestion of potentially poisonous substances. However, in this thesis (current research), untrained participants were presented with a familiar product (baked bread) fortified with cricket powder in a blind tasting experiment using a similar product, but without cricket powder, as the control sample. Everyone participating had some knowledge about the project and were aware that the products to be consumed were not suitable for vegans and/or vegetarians or people with allergies/intolerance to wheat and shellfish. The sensory analysis results found that consumers preferred the appearance, taste, and texture of the prototype bread product fortified with cricket powder, ancient grains, and seeds when compared with the control product. This was a resounding success as consumers were willing to ingest insects when incorporated within a familiar baked product. This suggests that the consumers' emotions were not affected as they were aware one of the products contained (cricket powder) and therefore, the emotion disgust responsiveness was low. Moreover, it is hypothesised that this was only acceptable due to the crickets being ground into a fine powder, thus reducing the food neophobia levels. The survey demonstrated that consumers were willing to accept insects within their diet after the education intervention, consequently demonstrating an increase in acceptability ratings as they were educated about insects' benefits (high protein, low fat, low carbohydrate). This suggests that communication and marketing are vital when consumers are encouraged to change their current food choices. The results indicated that the consumers' internal beliefs and values changed through knowledge and intervention while partaking in the study, suggesting that information marketing is an important factor in encouraging adoption.

7.3 Research Impact

Given the current failings of the food system and the considerable challenges consumers face, especially regarding health and food, each person needs to have nutritional food access. Much can be achieved immediately with current knowledge and technology aspects (given sufficient investment) but coping with radical changes to the food system to provide new solutions to world problems is the challenge everyone faces. The impact of this research is more than just academia. It is about helping humanity, especially in the less fortunate countries without a

nutritional staple diet. This research generated a clear connection between the food science realm and benefits to the consumer. Although potential change may come later, there is usually no measurement to capture this value. It is vital that this research's impact is continued and the only way to proceed with this study is to achieve and demonstrate the overall influence insects can have within our diets. By providing this pioneering product to society, this philosophical research helped change the world for the better. The research created a positive outcome with the consumers, especially within the Western Society, who preferred bread products fortified with cricket powder.

Diets play a pivotal role in humans, mostly as this is associated with health. A recommendation to ensure that consumers sufficiently obtain their necessary calories, including the essential micro- and macro- nutrients, is well known and acknowledged by the public. Conversely, the vast majority of the population occasionally do not meet this recommendation. Tackling this issue is a challenge given that preferences and socio-economic circumstances drive food choice. Using foods familiar with consumers can be vehicles for delivering vital dietary nutrients, improving public health. Overall, cricket powder can offer both short and long term health benefits, mainly providing essential nutrition to poor or under-resourced areas. This research has shown that cricket powder could offer a convenient way to increase consumption without significant lifestyle changes. Bread is widely consumed as a staple product, making this an ideal vehicle for delivering cricket powder as a nutritious and functional ingredient in human diets. Public engagement activities have shown that bread products fortified with cricket powder are well accepted. Moreover, consumers liked the taste, texture and appearance of the bread and the associated concept and health benefits. Alongside the survey, this research indicates that consumers are ready for speciality bread containing cricket powder. Furthermore, bread products fortified with cricket powder provided better nutritional profiles. The novel bread products developed in this research could increase health benefits.

The pathway to sustainability is not paved with gold as many researchers hope it to be, but more peaks and troughs. Introducing an alternative protein within the society

based on sustainability and nutritional claims falls on the consumers and the new product development and existing product development departments. Food manufacturing companies must make critical decision points that will deliver sustainable food across the world, generating new knowledge and innovation, impacting consumers' dietary choice. However, there is still a requirement to understand the environmental impact beyond product development, such as packaging and transportation. In many circumstances, the impact of new product development will consider the environmental impact with the consumer in mind to use the product to full optimal usage and minimal wastage. For instance, cricket powder supplied through supermarkets could increase this novel future food commodity uptake. Consumers can purchase these ingredients and incorporate within either gluten-free baking or non-gluten-free baking, cooking or pastry. This can be achieved for specific protein foods, that strategic protein choices for human consumption are based on pressures associated with supply, environmental impact and consumer demand. These are influenced by different issues including availability of alternative protein- sources, production, requirements and consumer attitudes to dietary choice.

Inventing ground-breaking new products for human consumption that can enhance the quality of life and health is not difficult; it is more about the cost and overall impact on the world. For instance, some diets can either be environmentally sustainable and not healthy (as they impact nature) or be healthy and not environmentally sustainable. The use of insects merged with a combination of sustainable ancient grains such as quinoa and Khorasan flour, which can be planted and harvested twice a year, making this bread product within the sustainable food scope. A blend of protein flours can provide an array of benefits for both humans and environmental health. However, ensuring these benefits are combined with health and sustainable food that reflects the complexity of the relationships between food systems, health and environment.

7.4 Recommendations and Future Research

- The measurement of the GI in humans and their gut microbiome of bread products fortified with cricket powder would be an essential factor to perform.

This will explain the mechanisms through which GI response is reduced, particularly how the gut microbiome has an impact on the blood sugar levels.

- An environmental scanning electron microscope (ESEM), will help clarify the impact of cricket powder within bread products. This machine uses electrons rather than light to create the 3D image, achieving magnifications up to 30,000 μ m. This will magnify the starch cells, gluten film surrounding the gas cells and more importantly, the impact of fibre from the cricket powder within the cell structure. Although some preliminary work was started on this, due to COVID-19, this was removed from the research. However, this project will continue to identify the fibres' impact from cricket powder, which would generally result in a low volume fortified bread product.
- The upscale of insect ingredients and production of fortificant bread products would provide understanding in relation to appropriate efficiency and costs to breeding, production and transportation. Nowadays most industrial production are based on high efficiency drying or freeze-drying processes, which considerably increase production costs. If sustainable environmental development is to be a key feature, mass production of insects for human consumption is necessary to be conducted. These findings would have potential within broader applications within the food and baking sector, as small production experiments conducted within this thesis would not fully impact large scale manufacturing.
- Further studies involving the worldwide survey by translating the questions into the local languages will provide greater accuracy. This would provide a more realistic result when surveying insects as a direct food source, particularly looking at consumer perception, eating habits, willingness to consume and food neophobia levels. The results would provide a better understanding of the consumers' food behaviour, looking mainly at developing countries and how insects' direct consumption is incorporated within their day-to-day food choice. Through this investigation and over a wider time frame than in the present study, facilitating insects' inclusion within diets and monitoring lifestyles factors such as habitual diets will provide greater knowledge in how consumers engage with insects as a direct food source.

There is no easy solution to a sustainable diet that facilitates both developing and developed countries, especially one that will prevent the collapse of whole societies and ecosystems. By taking an ordinary, small insect, such as a cricket and realising the potential that something so small can have on humankind, creating new innovative products ready for the consumer market, has been one of this research project's highlights. New radical rethinking of consumer values and priorities are required for the future generation. This research was always intended to help and benefit consumers around the world through entomophagy. These products are vital for our future, especially for generations to come. As the continual fight for a nutritional and sustainable food source which can both serve developing- and developed countries continue to plague us. It is crucial that consumers consider sustainable environmental solutions to end world hunger and increase global health can only emerge as people accept alternative protein sources, fibre and fat. Food choices that can eliminate world hunger are those that take the least toll on the environment and contribute most to long-term health and safest to consume. The use of cricket powder is one innovative solution to create a more sustainable environment and necessary to ensure future food security. The humble cricket is one way to help solve the current problems concerning sustainable food and malnutrition, especially in poor or disaster-stricken areas.

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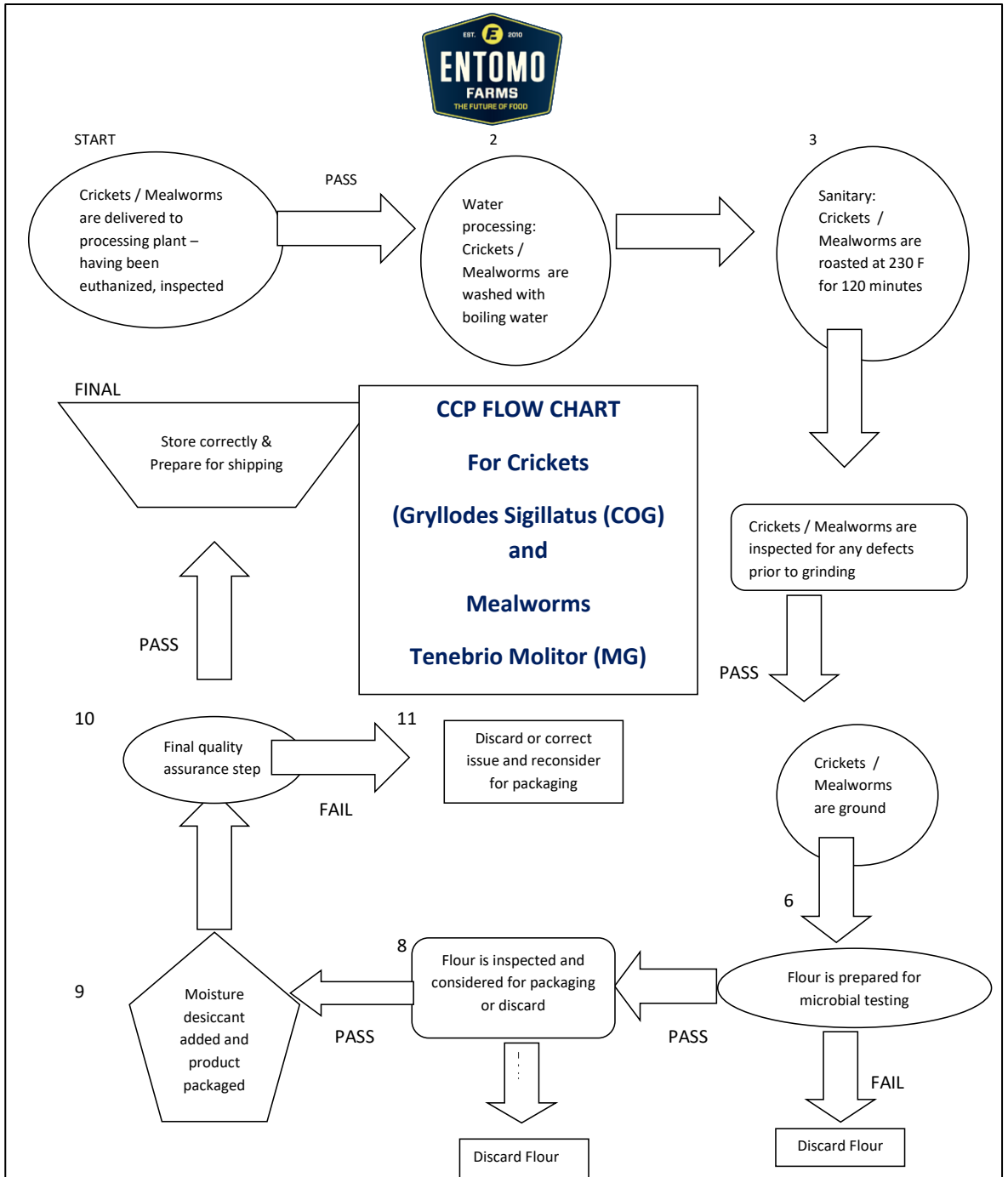
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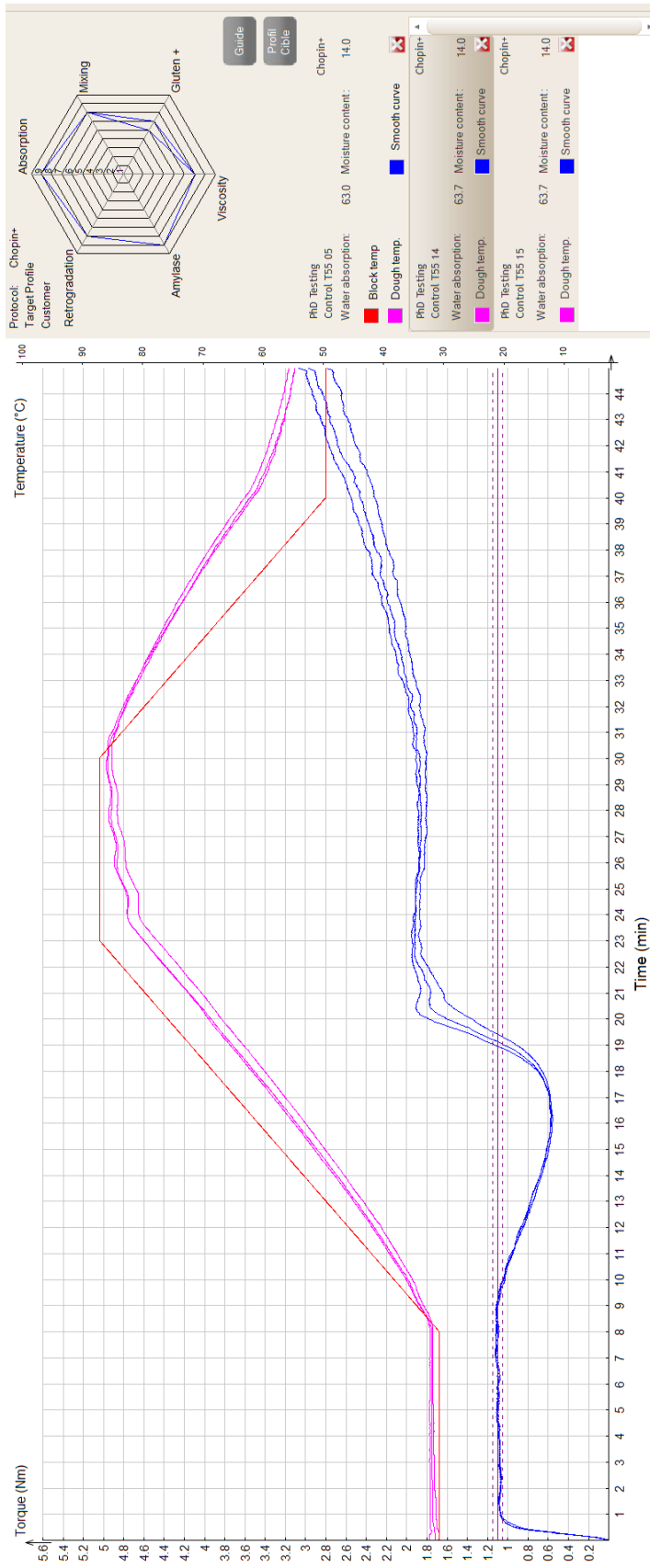
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Appendix A: CCP Flow Chart for *Gryllobes Sigillatus* (tropical house cricket) and *Tenebrio Molitor* (Mealworm)

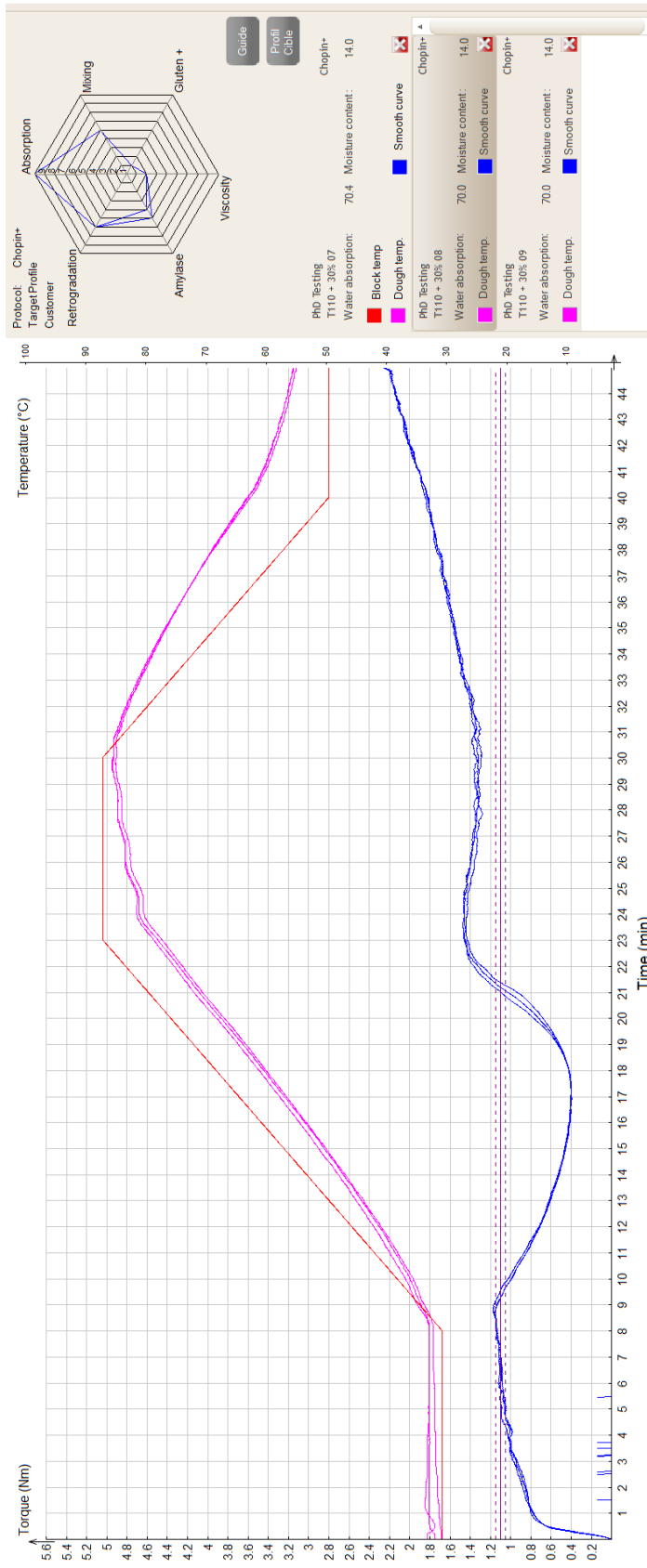


Appendix B: Mixolab Graphs

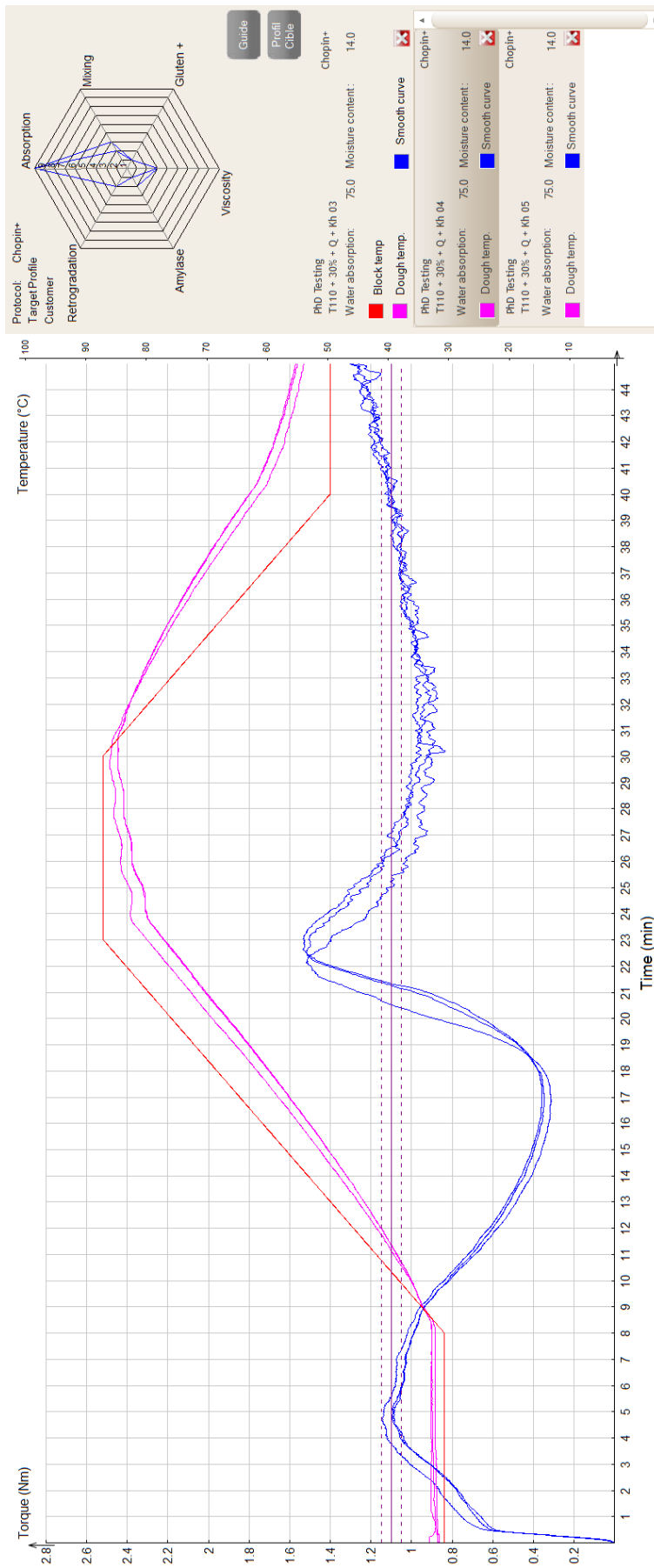
(i) T55 Wheat Flour Sample



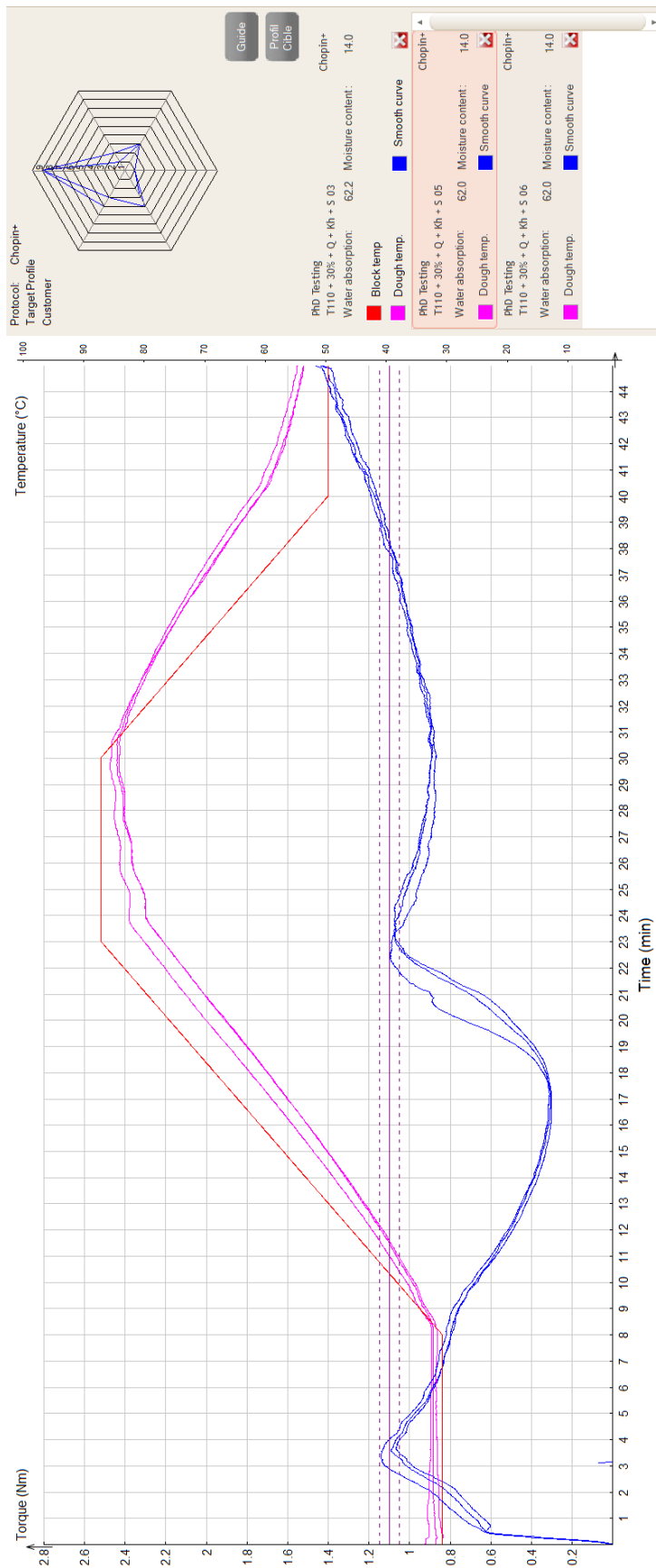
(ii) T55 Wheat Flour and Cricket Powder



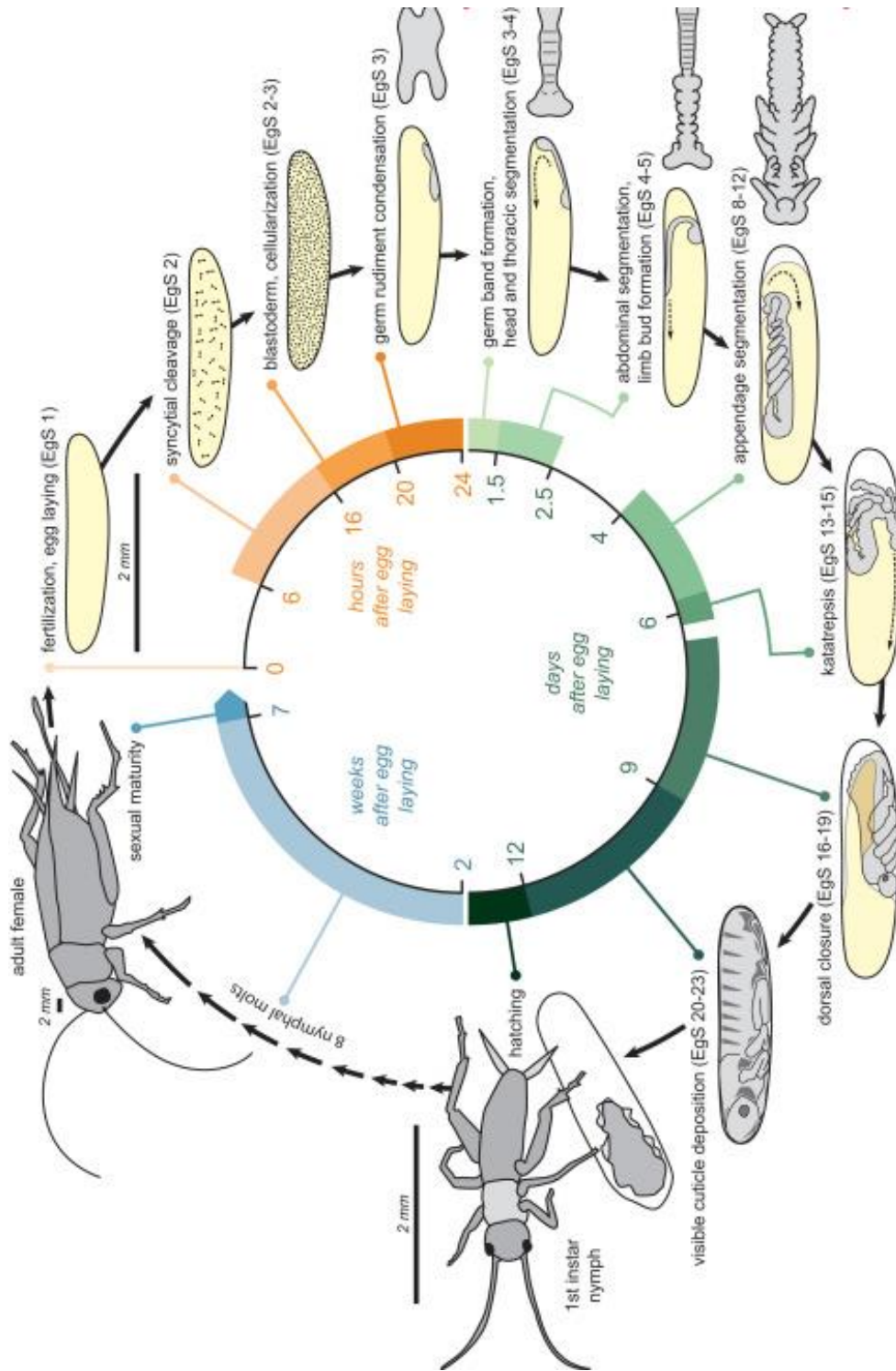
(iii) T55 Wheat Flour, Cricket Powder, Quinoa- and Khorasan- Flour



(iv) T55 Wheat Flour, Cricket Powder, Quinoa- and Khorasan- Flour and Seeds



Appendix C: Life Cycle of the Cricket



Appendix D: Nutritional Composition Data

Sample	Nutritional Composition			Moisture	Fibre
	Fat	Protein	Ash		
T55 Wheat Flour – Control Sample ^(a)	0.79±0.1 b,c,d	12.2±0.25 b,c,d	2.61±0.01 b,c,d	16.46±0.71	9.38±1.34 b,d
T55 +Cricket Powder ^(b)	2.31±0.12 a,c,d	30.63±0.24 a,d	3.5±0.01 a,c,d	18.5±4.33	13.09±0.6 a,c
T55 + Cricket Powder + Quinoa + Khorasan Flour ^(c)	1.56±0.02 a,b,d	31±0.18 a,b,d	3.58±0.03 a,b,d	18.93±1.89	10.55±1.75 c,d
T55 + Cricket Powder + Quinoa + Khorasan Flour + Mixed Seeds ^(d)	10.08±0.04 a,b,c	41.74±0.04 a,b,c	3.89±0.01 a,b,c	20.83±3.56	11.79±1.07 a,c

Nutritional composition analysis of baked products fortified with cricket powder, ancient grains and seeds

Results are displayed in rows. Letters in superscript denote the samples in comparison to which the value is statistically different ($p < .001$). The symbol of the sample is given in the bracket. All values are means obtained from three replications, $n = 3$.

Sample	Total Carbohydrate Content	
	Carbohydrate	
T55 Wheat Flour – Control Sample ^(a)	67.95±0.5 b,c,d	
T55 +Cricket Powder ^(b)	45.06±1.77 a,d	
T55 + Cricket Powder + Quinoa + Khorasan Flour ^(c)	44.94±1.57 a,d	
T55 + Cricket Powder + Quinoa + Khorasan Flour + Mixed Seeds ^(d)	23.46±1.09 a,b,c	

Total carbohydrate and Kcal value of the breads samples (wheat flour control and wheat flour samples fortified with cricket powder and ancient grains)

Results are displayed in rows. Letters in superscript denote the samples in comparison to which the value is statistically different ($p < .001$). The symbol of the sample is given in the bracket. All values are means obtained from three replications, $n = 3$.

Appendix E: Stable Micro Texture Analyser Data

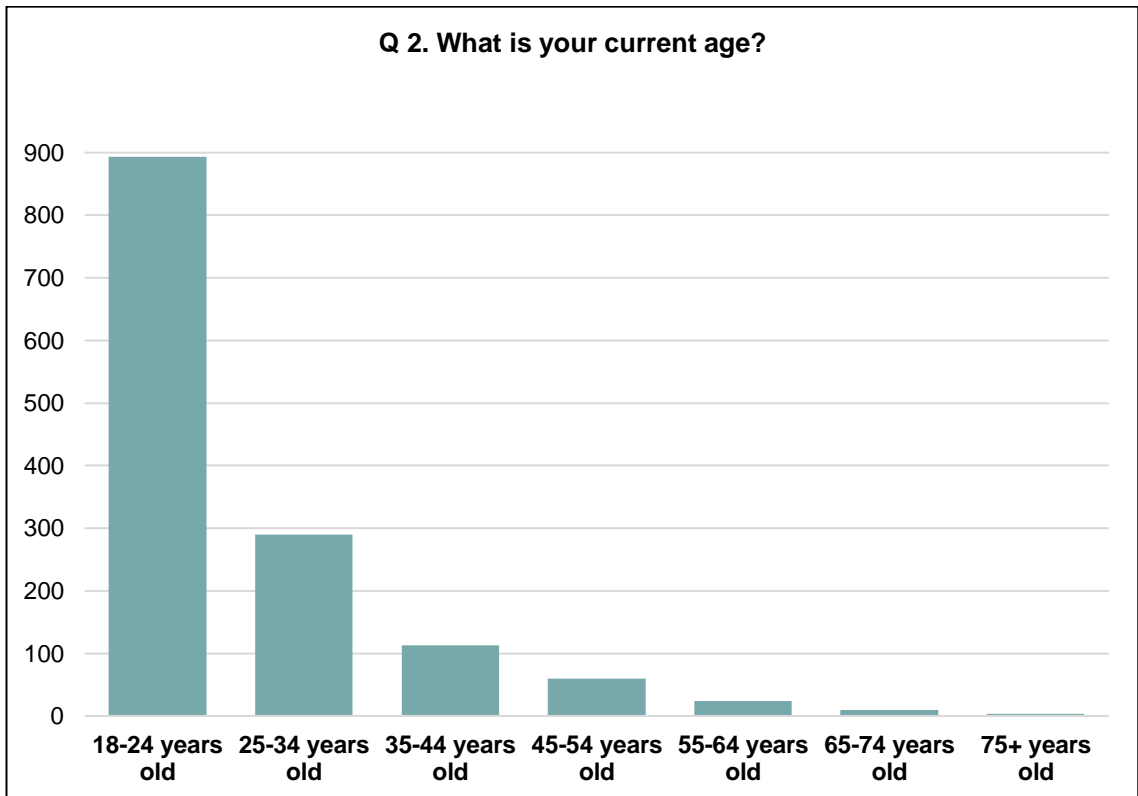
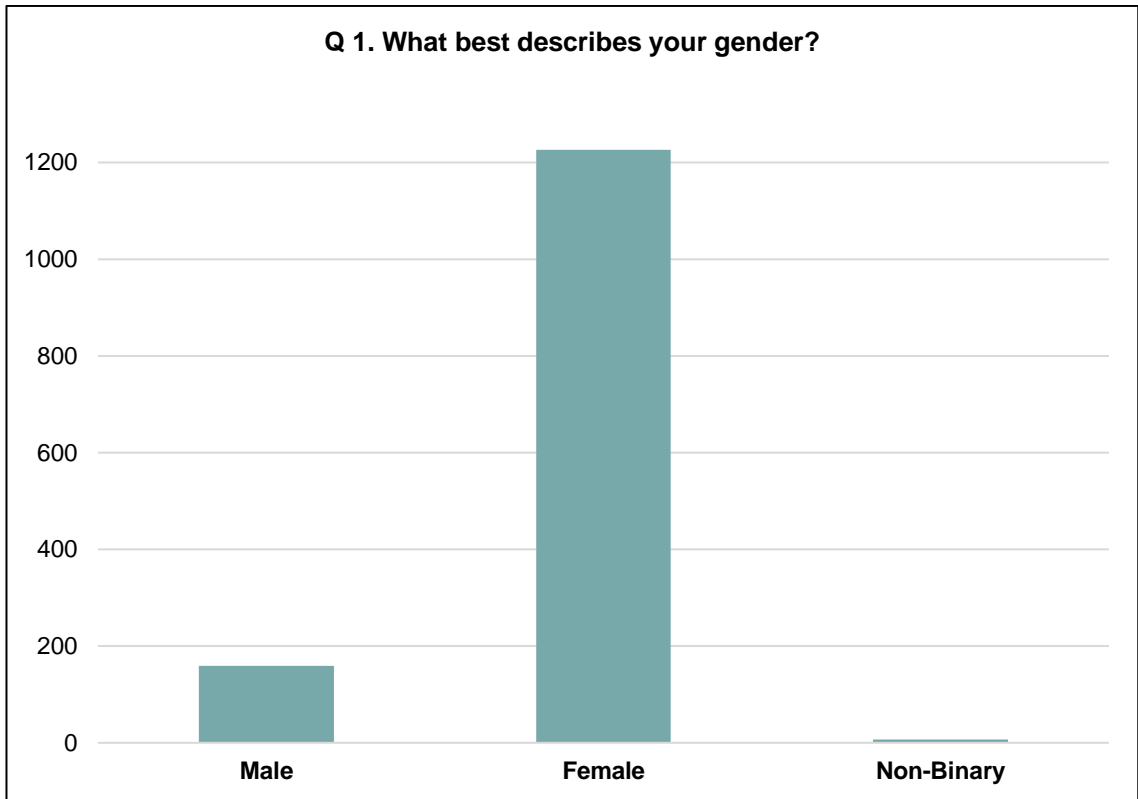
Sample	Crumb Texture: Middle of the Loaf																
	Day 1				Day 3				Day 5				Day 7				
	Crumb Firmness – Middle	Mean	SE	Crumb Resilience – Middle	Mean	SE	Crumb Firmness – Middle	Mean	SE	Crumb Resilience – Middle	Mean	SE	Crumb Firmness – Middle	Mean	SE	Crumb Resilience – Middle	Mean
T55 Wheat Flour – Control Sample ^(a)	964.23 ±183.91 _d	106.18	0.01	0.79 ±0.01	1539.56 ±343.15 _d	201.58	0.01	0.78 ±0.01	2568.28 ±410.66 _{c,d}	237.10	0.01	0.70 ±0.02 _d	3799.13 ±225.16 _{b,c,d}	130.00	0.02	0.78 ±0.04	0.02
T110 Wheat Flour Sample ^(b)	818.70 ±83.18 _d	48.02	0.03	0.85 ±0.06	2501.47 ±83.96 _{b,c,d,e}	48.47	0.01	0.84 ±0.03	2776.52 ±17.67 _{c,d}	10.20	0.01	0.81 ±0.11	3508.84 ±184.68 _{b,c,d}	106.63	0.02	0.85 ±0.04	0.02
T55 + Cricket Powder ^(b)	1078.99 ±49.70	28.69	0.03	0.85 ±0.06	1340.2 ±180.99 _d	104.50	0.02	0.86 ±0.04	2402.28 ±194.79 _{c,d}	112.46	0.01	0.68 ±0.01 _{c,d}	1851.29 ±125.08	72.22	0.03	0.76 ±0.05	0.03
T55 + Cricket Powder + Quinoa + Khorasan Flour ^(c)	1293.93 ±116.76 _d	67.41	0.02	0.87 ±0.03	1798.2 ±153.06 _d	86.37	0.03	0.84 ±0.06	1409.51 ±305.17 _{c,d}	176.19	0.01	0.89 ±0.02 _{a,b}	2680.04 ±592.78 _a	342.24	0.03	0.81 ±0.04	0.03
T55 + Cricket Powder + Quinoa + Khorasan Flour + Mixed Seeds ^(c)	901.52 ±74.62 _{a,b,c}	43.08	0.21	1 ±0.36	1131.91 ±118.63 _{a,b,c}	68.49	0.01	0.84 ±0	1622.41 ±110.58 _{a,b,c}	63.84	0.00	0.81 ±0 _b	1759.25 ±206.25 _a	119.08	0.01	0.81 ±0.02	0.01

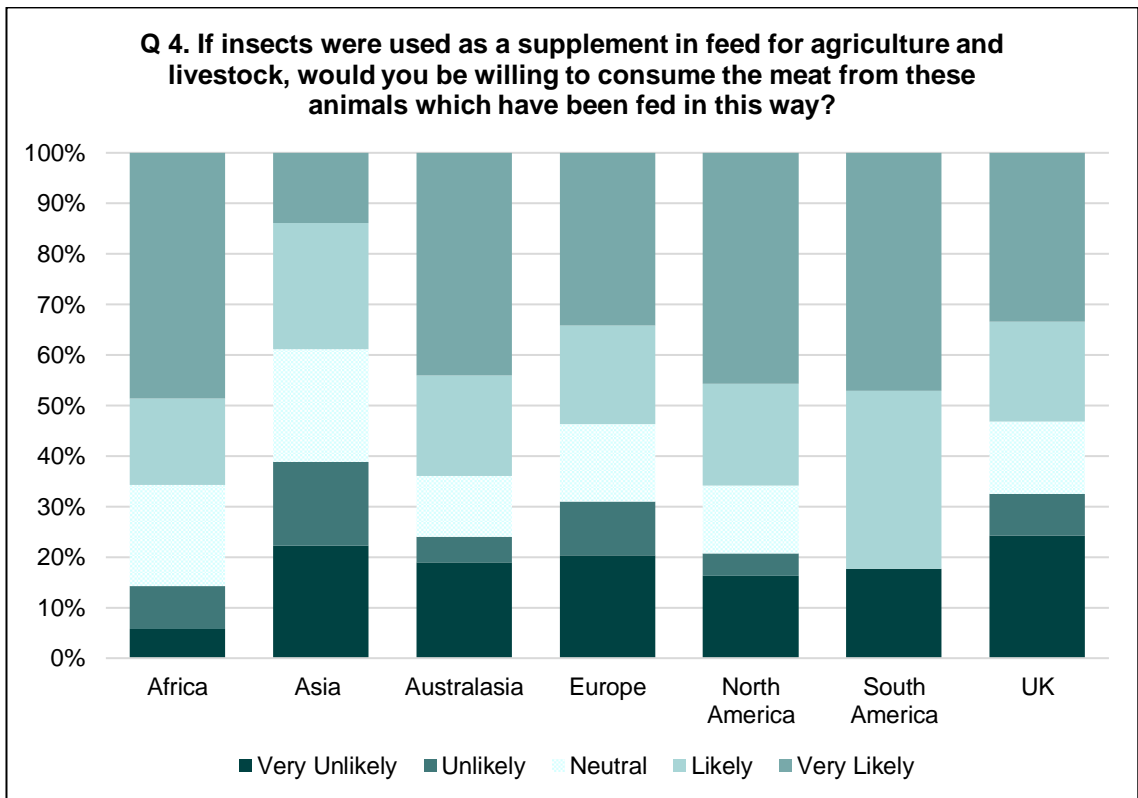
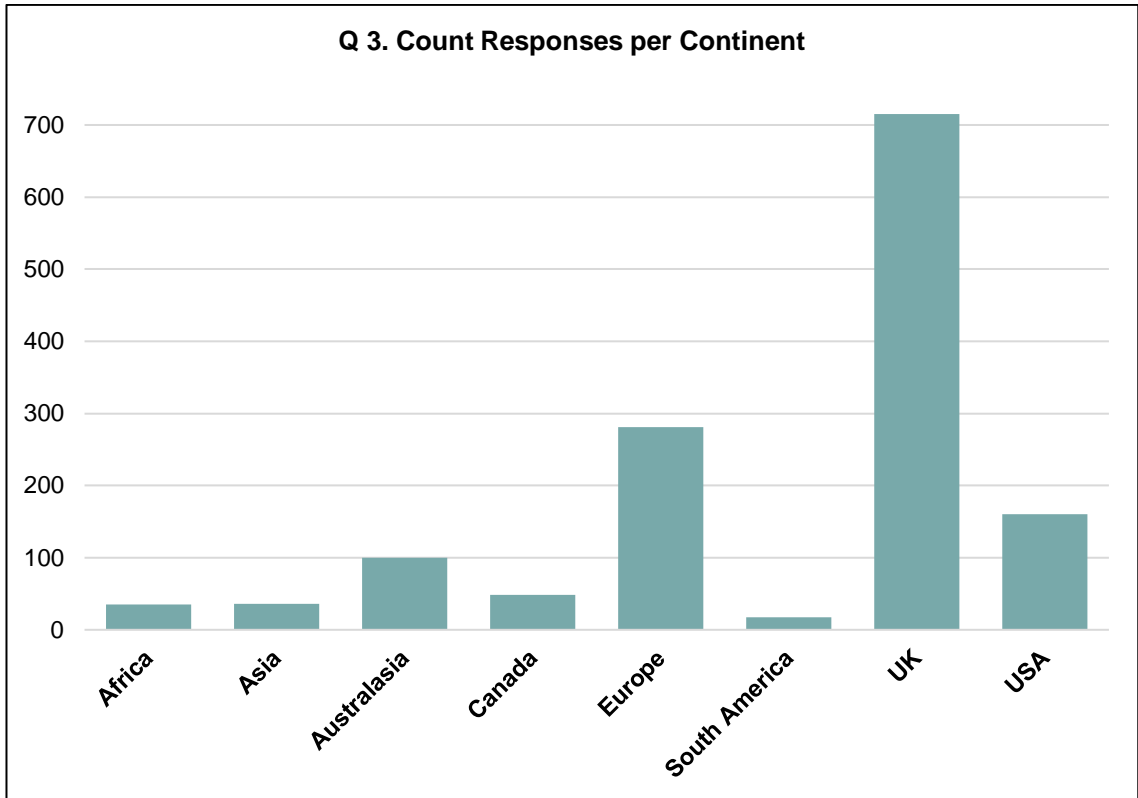
Appendix F: Sensory Analysis

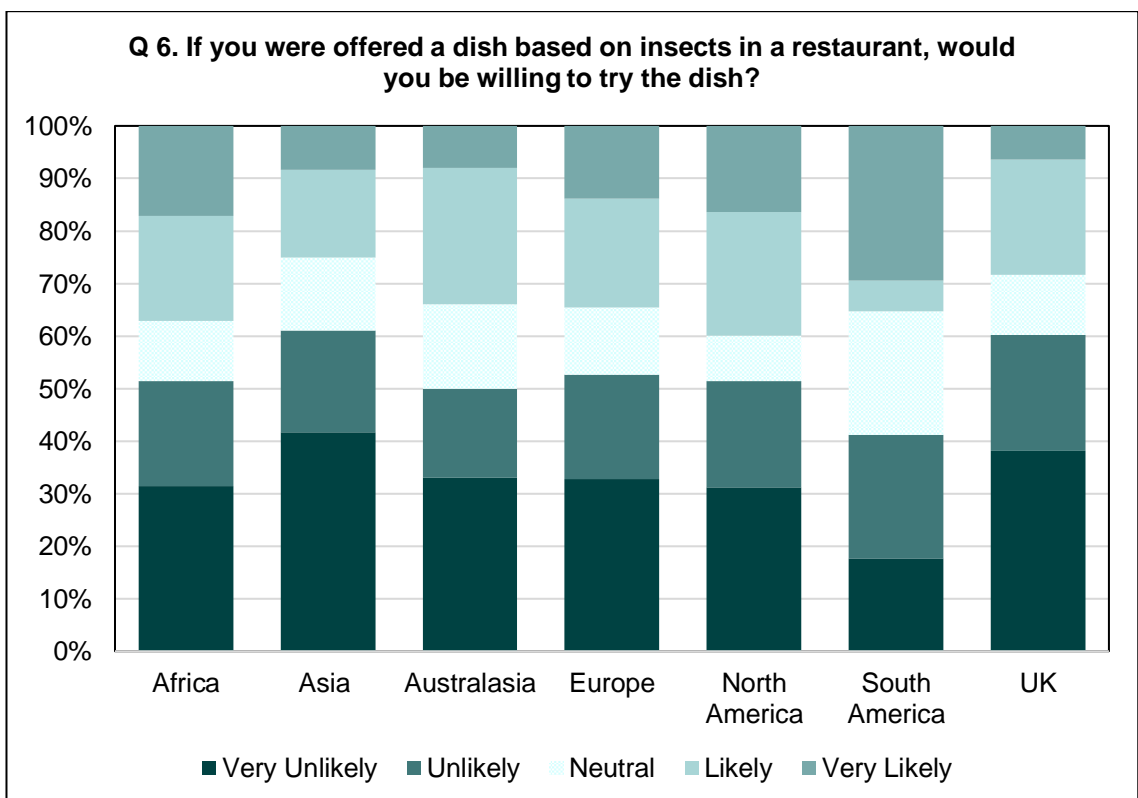
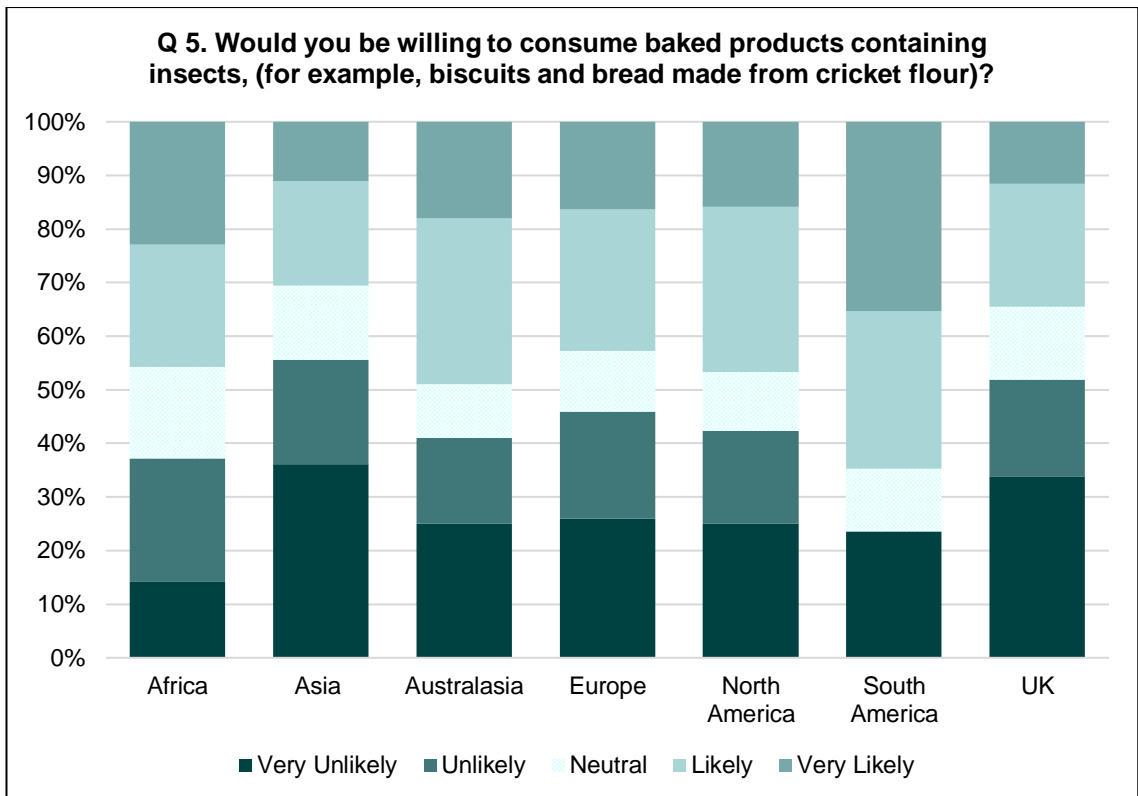
- *At first, I thought this was brown bread, but after tasting the sample it had better flavour and easier to chew*
- *The cricket bread looks healthier and gives this perception that it is better for you compared to the control sample*
- *Dark Colour of the cricket sample, perceived a healthier option*
- *The crust of the cricket sample tasted amazing, nice crusty and roasted flavour. However, was slightly too dry*
- *Loved the textures and appearance of cricket sample, however, preferred the flavour of the control sample*
- *Both samples tasted similar, however, the one with cricket powder tasted better*
- *The seeds incorporated within and around the product made it more appealing*
- *Taste of the cricket sample was preferred, however, the control sample was better in appearance*
- *In appearance and texture, both samples were similar*

Appendix G: Worldwide Survey

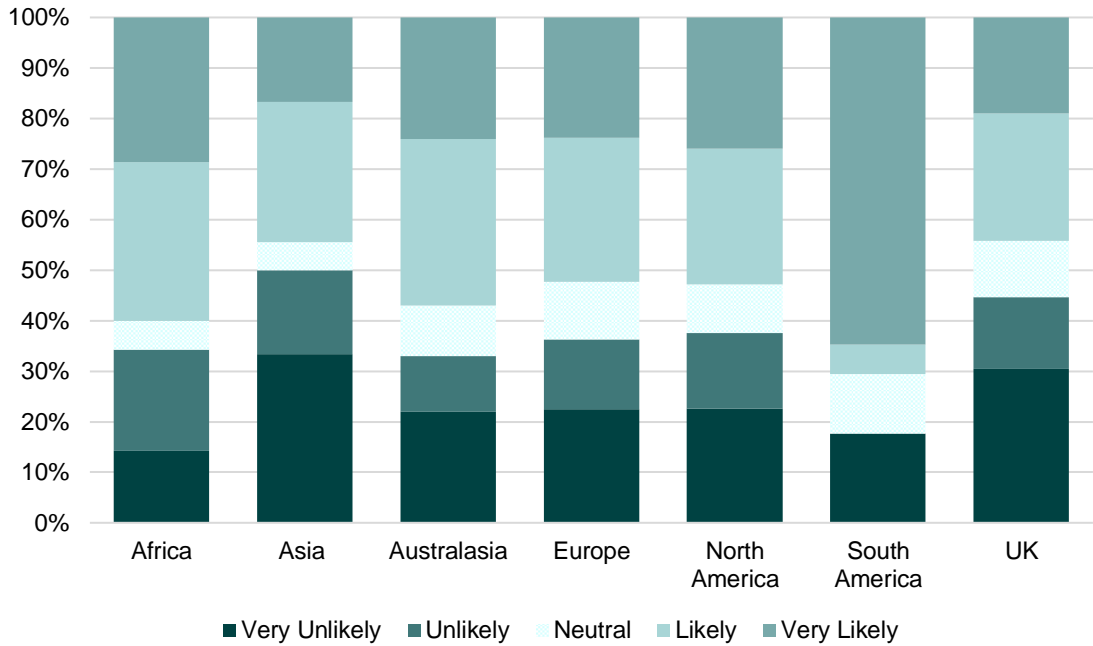
Results Insects as Feed Vs Insects as a Direct Food Source







7. Would you be willing to consume a product that contained ground crickets (these crickets are ground to a fine powder and are not visible to the human eye)?



8. Ground crickets offer great opportunities for the baking industry and the consumer, for instance they are gluten free, contain high protein and very little carbohydrates. What is the likelihood you would buy a baked product fortified with ground crickets

