Impact of cricket protein powder replacement on wheat protein composition, dough rheology and bread quality

by

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Abstract

The continuous rise in population, environmental concerns, and an increasing shift of consumers' belief towards eating sustainable foods has led researchers to look for alternate sources of protein. Insect proteins are novel protein sources that are environmentally friendly due to their lower greenhouse gas emissions when compared to beef, poultry, and pork. Farming insects requires less resources compared to raising livestock. Insects are high in protein, contain chitin which is a source of fiber, and are a good source of B vitamins. There is a wide variation in nutritional and functional quality of protein depending on the type of insect. The objective of this project was to understand how cricket protein powder affects the mixing, pasting and dough development characteristics of bread dough. Two different cricket protein powders, GrioPro (G) and Entomo Farms (E), were tested at replacement levels of 10 and 20% (of total flour weight). Protein powders were first characterized for their functional properties. Dough samples collected at peak torque development were subjected to size exclusion-HPLC analysis to quantify the change in soluble polymeric proteins (SPP) and insoluble polymeric proteins (IPP). MixoLab constant and optimized water absorption protocols were used to study the effect of cricket protein powder replacement on dough development. Dough extensibility was tested using the Kieffer Rig protocol. Breads were baked with 5, 10, or 20% replacement levels of cricket protein powder. Loaf volume, and color were measured, and bread slices underwent C-Cell analysis and texture profile analysis (TPA) at 0, 1, 3 and 7 days. In general, incorporation of powders G and E led to two opposite effects. Dough samples with powder E showed lower peak areas (9,432 and 17,346 mAu) of IPP compared to the control (23360 mAu) while the SPP dough samples showed higher peak areas (41,414 and 44,133 mAu) to the control (41,212 mAu). Use of powder G led to an increased stability, significantly higher C1 torque (20% level), and an increase water absorption. Replacement of wheat flour with powder E led to softer doughs with a decreased stability at the 20% replacement level and no significant difference in water absorption. Peak viscosities were significantly decreased for all replacement levels of both G and E powders. Extensibility was significantly decreased as the replacement level increased for all treatments. Loaf volume also decreased as the replacement level increased for all treatments. Color results showed a significant decrease in L-value and a significant increase in a, and b-values thus producing a crumb color like that of whole wheat breads. Powder G at 10 and 20% replacement levels significantly decreased the area occupied by air cells, the average air cell diameter, and cell wall thickness. Both powder

E and G led to a decreased amount of number of air cells. TPA results showed a significant increase in hardness at higher replacement levels with G being harder than E. Chewiness also increased as the replacement level increased while cohesiveness, springiness, and resilience decreased as the replacement levels increased for either G or E.

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Preface

My mother always said that the kitchen was the heart of the home and I have always taken it to heart. Food has the beautiful ability of bringing people together and I have always strived in finding ways to improve or create products that all people can enjoy. Therefore, this project began as a wish for finding a new ingredient to test in a gluten-free based product. However, the concept of insect “flour” being incorporated into human food is so novel that not a lot of research exists in this area. Therefore, the idea transformed into finding out how the cricket protein powder affects the dough rheological properties and final product characteristics of bread. My hope is that this thesis will inspire others to further pursue this research avenue and eventually incorporate the cricket protein powder into other grain products including gluten-free options.

Chapter 1 - Alternative Sources of Protein

As one of the macronutrients, protein plays a key role in maintaining a healthy diet and a healthy body. The daily Dietary Reference Intake (DRI) is 0.8 grams of protein per kilogram of body weight which amounts to 56 grams per day for the average man and 46 grams per day for the average woman (Institute of Medicine, 2005). Not only is the quantity important, but also the quality of the protein which depends on the source. There are many sources of protein available in the market, such as meat, plant-based proteins, dairy products, eggs, and seafood/shellfish. However, research is constantly being conducted to either improve existing proteins sources or turn to novel protein sources. This review will provide (1) a brief introduction to proteins, (2) a brief overview of the current protein sources available, (3) discuss why alternative protein sources are a necessity, (4) briefly cover novel protein sources and (5) do an in-depth examination of the potential of insect proteins to be used as an alternative protein source.

1.1. Proteins

Rodrigeuz et al. (2012) define proteins as an extremely complex polymer that is based on up to 20 different amino acids which are connected via amide (also referred to as peptide) bonds. The differences in the protein structures and their functionalities are caused by the sequence in which the amino acids are connected, the size and type of the amino acids that make up the protein, and the size of the peptide chain. Proteins are often classified by their solubility and, especially for cereal proteins, have been divided into four categories based on solubility: albumins, globulins, prolamins, and glutelins. Pihlanto et al. (2017) state that albumins are soluble in water and coagulate with heat, while globulins are soluble in saline solutions, but not in water. On the other hand, prolamins are only extractable in concentrated aqueous alcohol solutions and glutelins are only extractable in dilute aqueous acid or alkali solutions (Pihlanto et al., 2017). The amino acids that make up proteins also have their own classification system based on whether the human body can make them (non-essential) or if they can only be gained through the diet (essential). The essential amino acids are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, cysteine, and tyrosine (Friedman, 1996). The non-essential amino acids are: alanine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, proline, and serine (Friedman, 1996).

1.1.1. Functionality and Quality

In terms of nutrition, the protein's main role is to be digested and broken down into amino acids to be used as a nitrogen supply for the body to synthesize proteins and other biological molecules (Mercer et al., 1989). The concentration and ratio of the amino acids found within a protein are responsible for determining the protein quality. Table 1.1a, b shows a list of the essential amino acids found in different protein sources, as well as, the suggested human amino acid requirements in milligrams per gram of protein for certain age groups.

Table 1.1. (a) Essential amino acids of different protein sources and (b) FAO/WHO and young and pellet suggested human amino acid requirements

(adapted from Friedman, 1996)

(a)

Amino Acid	Casein mg/g protein	Beef mg/g protein	Egg White mg/g protein	Soy Protein mg/g protein	Wheat Flour mg/g protein
Thr	46.4	42.1	46.8	38.4	29.3
Cys + Met	34.9	32.7	66.4	68.1	38.7
Val	68.5	45.4	67.8	49.1	42.7
Ile	53.6	41.8	52.8	47.1	33.4
Leu	101.6	77.5	87.6	85.1	68.5
Tyr + Phe	125.4	70.2	90.8	96.6	77.8
His	29.7	32.0	22.5	25.4	21.9
Lys	84.4	79.4	69.8	63.4	26.6
Trp	13.1	9.9	14.6	11.4	11.2

(b)

Amino Acid	FAO/WHO data (mg/g protein)				Young & Pellet data (mg/g protein)
	1 yr.	2-5 yr.	10-12 yr.	Adult	Preschool-Adult
Thr	43	34	28	9	25
Cys + Met	42	25	22	17	25
Val	55	35	25	13	35
Ile	46	28	28	13	35
Leu	93	66	44	19	65
Tyr + Phe	72	63	22	19	65
His	26	19	19	16	--
Lys	66	58	44	16	50
Trp	17	11	9	5	10

The amount of the amino acids varies depending on the source of the protein (Table 1.1). Friedman (1996) also stated that proteins containing a higher ratio of essential amino acids have a higher protein quality. Furthermore, if the proteins are deficient in one or more amino acids, then

they are considered poor in quality (Friedman, 1996). Additionally, the suggested amino acid requirements vary with age (Table 1.1). Fuller and Wang (1990) explain that the amount of protein needed in the diet "depends on the digestibility and availability of amino acids supplied by a specific diet and the ability of the consumer to respond to the amino acid supply with deposition of body protein". Digestion plays a key role in determining the protein quality since the protein must be broken down into amino acids for the body to be able to use. Friedman (1996) expands on this concept by stating that another factor in determining protein quality is the biological utilization of the specific amino acids after digestion, absorption, and minimal obligatory rates of oxidation. Thus, factors that inhibit or alter the digestibility of a protein also affect the protein quality. Non-nutritive compounds such as protease inhibitors and tannins are one factor that has an impact on the digestibility and bioavailability of the protein, thus affecting the protein quality (Pihlanto et al., 2017).

1.1.2. Bioactivity

Pihlanto et al. (2017) define bioactive peptides "as protein fragments derived from food proteins with a positive impact on the body function or condition". Furthermore, bioactive peptides are inactive within the native protein matrix, but are activated by microbial fermentation, enzymatic digestion, or food processing (Pihlanto et al., 2017). Some examples on the benefits of bioactive peptides include the lowering of blood pressure, stimulating the immune system functions, antibacterial properties, help control body mass, improve the nutritive values of food, antioxidant properties and antitumoral properties (Dzuiba and Darewicz, 2007; Pihlanto et al., 2017). Moreover, active peptides are a constituent of "functional foods" which are foods that have been designed to obtain the desired functional and biological properties which are needed for the proper functioning of the body (Dzuiba and Darewicz, 2007). Thus, the consumption of protein is a necessity to maintain a healthy lifestyle.

1.2. Existing Protein Sources

Milk, meat, fish, poultry, eggs, cereals, legumes, and oilseeds are all sources of protein. The plant-based protein sources globally supply 57%, meat supply 18%, dairy supply 10%, fish and shellfish supply 6%, and other animal products supply the remaining 9% (Henchion et al., 2017). Traditionally, the western dietary pattern focuses on mostly animal based products to satisfy the recommended protein requirements (Pihlanto et al., 2017). Table 1.2 summarizes the major

protein sources found in the diet for both developing and developed countries. Developing countries rely more heavily on plant-based protein especially from cereals than on animal-based proteins such as meat, dairy products, fish, and eggs (Table 1.2). Other protein sources that are currently used in feed and biofuel production are rapeseed, algae, grass, and duckweed (Spiegal et al., 2013).

Table 1.2. Major sources of protein in the diet in developing and developed countries
(adapted from Friedman, 1996)

Source	Developing Countries (%)	Developed Countries (%)
Cereals	58.8	29.1
Meat	8.6	26.4
Pulses	7.4	1.7
Milk & Dairy	5.6	16.7
Fish & Seafood	4.1	7.3
Oil Crops	3.8	1.9
Vegetables	3.5	3.5
Starchy Roots	3.1	3.2
Eggs	1.6	4.3
Offals	1.2	2.2
Nuts	1.0	1.1

1.2.1. Meat

A common animal-based protein source is meat. Meat has three major proteins: actin, collagen, and myosin (Friedman, 1996). According to Henchion et al. (2017) the amount of protein in raw meat varies between 20-25% depending on the source and the fat content of the meat, which corresponds to 28-36% in cooked meat due to the loss of water during cooking. More variability is found in commercial meat products in terms of their amounts of connective tissue, myofibrillar, and non-muscle proteins (Friedman, 1996). Additionally, ruminants can digest fibrous material that humans cannot and convert it into high quality protein that has a high bioavailability and digestibility (Henchion et al., 2017). According to Friedman (1996) and Henchion et al. (2017) meat contains nutrients not found in plant proteins such as the amino acids: methylhistidine, and hydroxymetheyllysine; as well as, being a source of Vitamins A, B9, B12, D, and E in addition to the minerals zinc, iron, and selenium. Furthermore, meat has an ability known as the “meat factor” which allows it to enhance the iron availability from other sources (Friedman, 1996; Henchion et

al., 2017). However, there are some health concerns associated with the overconsumption of meat. Pihlanto et al. (2017) state that a high intake of meat (specifically red and processed meats) is associated with a higher risk of coronary heart disease, type 2 diabetes, and certain types of cancers.

1.2.2. Plant-Based Proteins

There are many different varieties of plant-based proteins. Examples include cereals, pseudo-cereals, legumes, brassica species, and others such as sunflower (Pihlanto et al., 2017). As such, there exists a variability in the protein quality due to the wide variety of sources. Not only are these sources used in human food but are also a necessity in feed applications. Spiegal et al. (2013) state that legumes, cereals, mushrooms and potatoes are currently used in both food and feed applications.

1.2.3. Cereals

According to Henchion et al. (2017), cereal proteins are responsible for supplying a major portion of the dietary protein intake around the world and play a crucial role in the diet of developing countries. When it comes to the Western diet, wheat makes up the largest group of plant-based protein sources (Krijne and Essink, 2011). In Europe, bread made from wheat is a crucial way for delivering protein to consumers where the loaves typically contain about 8 grams of protein (Henchion et al., 2017). Table 1.3 summarizes the amino acid composition of various cereal grains, as well as, the total percentage of protein found in said grains.

The amino acid composition varies depending on the plant source with wheat containing the highest protein content (14%) and rice having the lowest protein content (7.5%) (Table 1.3). Generally, the amount of protein found in cereals ranges from 10-15% where the storage proteins are responsible for the highest amount compared to the other types of proteins found within the cereal grains (Henchion et al., 2017). Examples of these storage proteins are prolamins, globulins, and germins (Cunsolo et al., 2012). The western part of Africa consumes a lot of millet, while in Southern India rice and millet are the main cereals consumed (Henchion et al., 2017). On the other hand, Ethiopia prefers the consumption of teff which according to Jansen et al. (1962) is responsible for delivering 41 grams of protein in their typical diet. Cavazos and Gonzalez de Mejia (2013) reported that cereal proteins have bioactivities such as antioxidant properties, anti-inflammatory properties, decreasing cholesterol, and anti-diabetic properties.

Table 1.3. Amino acid content of cereals (% amino acid in the protein)
(adapted from Handbook on Drying, Milling and Production of Cereal Foods, 2017)

Amino Acid	Brown rice (%)	HRS wheat (%)	Field maize (%)	Sorghum (%)	Pearl millet (%)	Barley (%)	Oats (%)	Rye (%)
Trp	1.08	1.24	0.61	1.12	2.18	1.25	1.29	1.13
Thr	3.92	2.88	3.98	3.58	4.00	3.38	3.31	3.70
Ile	4.69	4.34	4.62	5.44	5.57	4.26	5.16	4.26
Leu	8.61	6.71	12.96	16.06	15.32	6.95	7.50	6.72
Lys	3.95	2.82	2.88	2.72	3.36	3.38	3.67	4.08
Met	1.80	1.29	1.86	1.73	2.37	1.44	1.47	1.58
Cys	1.36	2.19	1.30	1.66	1.33	2.01	2.18	1.99
Phe	5.03	4.94	4.54	4.97	4.44	5.16	5.34	4.72
Tyr	4.57	3.74	6.11	2.75	--	3.64	3.69	3.22
Val	6.99	4.63	5.10	5.71	5.98	5.02	5.95	5.21
Arg	5.76	4.79	3.52	3.79	4.60	5.15	6.58	4.88
His	1.68	2.04	2.06	1.92	2.11	1.87	1.84	2.28
Ala	3.56	3.50	9.95	--	--	4.60	6.11	--
Asp	4.72	5.46	12.42	--	--	5.56	4.13	--
Glu	13.69	31.25	17.65	21.92	--	22.35	20.14	21.26
Gly	6.84	6.11	3.39	--	--	4.55	4.55	--
Pro	4.84	10.44	8.35	--	--	9.02	5.70	--
Ser	5.08	4.61	5.65	5.05	--	4.65	4.00	4.13
Total protein	7.50	14.00	10.00	11.00	11.40	12.80	14.20	12.10

1.2.4. Pulses (Legumes)

According to Henchion et al. (2017) pulses have a compositional profile of approximately 10% moisture, 21-25% crude protein, 1-1.5% lipids, 60-65% carbohydrates, and 2.5-4% ash. The exceptions are Chickpea as it has a higher lipids concentration of 4-5% and soybean and lupin which can have up to 45-50% protein (Henchion et al., 2017). Table 1.4 shows the protein content of various pulses in terms of grams per 100 grams dry weight.

There is a variation in protein content which is caused by genetic, environmental, and agronomic factors (Table 1.4). Pulses consist of the cotyledon, the embryonic axis, and the seed coat. The cotyledons make up the largest portion of the pulse, therefore they are responsible for contributing the highest amount of protein (Henchion et al., 2017). A review by Pihlanto et al. (2017) considered the pulse proteins of lupin, hemp, and quinoa and found that white lupin is consumed in the Mediterranean while Australia uses narrow-leaf lupin. Both types contain high amounts of protein and fiber but are poor in digestible carbohydrates (Pihlanto et al., 2017).

Additionally, lupin has been associated with having the potential health benefits of dyslipidaemia, hyperglycaemia, and hypertension prevention (Pihlanto et al., 2017). On the other hand, quinoa has an amino acid composition that is similar to milk while hemp has a high oil content of about 25% and contains about 25% protein that can be easily digested (Pihlanto et al., 2017). However, when it comes to using hemp as a protein source in the food industry, only the varieties of hemp containing low δ-9-tetrahydrocannabinol contents can be used (Pihlanto et al., 2017). Table 1.5 shows the health benefits associated with lupin, hemp, and quinoa.

There exist quite a few health benefits from these pulses; however, out of the essential amino acids, pulses have a deficiency in the sulphur containing amino acids (Table 1.5). Furthermore, anti-nutritional compounds like hydrolase inhibitors can be found in pulses as they are responsible for the defense mechanism of the seed. These compounds may inhibit various biological functions in the human body (Henchion et al., 2017). Thus, only consuming pulse protein sources would not be enough to supply all the amino acids necessary in the body. Supplementation would be a necessity to ensure all essential amino acid requirements are being met.

Table 1.4. Proximate composition of different pulse grains (g/100g dry weight)
(adapted from Henchion et al., 2017)

Source	Protein Content (g/100g)	Source	Protein Content (g/100g)
Kidney Bean	23.58	Lima Beans	21.46
Chickpea	19.29 - 19.30	Navy Beans	22.33
Lentils	25.80 - 26.10	Gt. Northern Bean	21.80 - 21.86
Mung Bean	23.86 - 27.50	French Beans	18.81
Mungo Bean	25.21 - 26.22	Winged Beans	29.65
Pigeon Pea	21.70	Hyacinth Beans	23.90
Peas	19.30 - 24.55	White Beans	23.36
Adzuki Bean	19.87	Horse Gram	22.50
Black Beans	21.60 - 23.60	Cowpea	23.85 - 24.10

Table 1.5. Health benefits of lupin, quinoa, and hemp

(adapted from Pihlanto et al., 2017)

Source	Compounds	Health Benefits
Blue or White Lupin	γ-Conglutin	Lipid lowering effect
	Fiber	Total & LDL cholesterol lowering effect Gut health
	Peptides liberated by enzymatic hydrolysis with trypsin or pepsin	Cholesterol modulating properties in a dual cell model: Caco-2 & human hepatocytes
	Protein hydrolysates produced by Izyme AL & Alcalase 2,4 L	Anti-inflammatory activity in THP-1-derived macrophages
	Phenolic compounds	Anti-carcinogenic, anti-inflammatory, antimicrobial, antioxidant, cardioprotective
	Isoflavonoids	Non-steroidal phytoestrogenic activity
Quinoa	Phytosterols	Growth-promoting, antidiabetic, immunomodulatory, hepatoprotective, neuroprotective, hypocholesterolemic, wound healing, antidepressive, & antioxidant activities
	Phenolic compounds	Inhibition of alpha glucosidase *in vitro*
	Peptides produced by LAB fermentation	Antioxidant activity *in vitro* & in human keratinocytes
	Protein hydrolysates produced by preparation with papain or a microbial papain-like enzyme	Antidiabetic & antioxidant activity *in vitro*
Hemp	Seed oil	Reduce atopic dermatitis symptoms in patients
	Protein hydrolysates produced by pepsin, alcalase, papin & papain + pancreatin	ACE & renin inhibition *in vitro* Hypotensive effects in SHR (200 mg per kg body weight)
	Protein hydrolysates produced by HT Proteolytic Concentrate (HT) & consecutive treatment with pepsin & pancreatin	Antioxidant activity *in vitro* & in SHR
	Protein hydrolysates produced by pepsin	Acetylcholinesterase-inhibitory properties

1.3. Need for Alternate Protein Sources

As can be seen from the previous section of the review, there are plenty of protein sources currently available in the market. Even so, researchers continue to look for alternate or novel sources that can be used as well. This gives rise to the question: Why is there a need for commercializing new protein sources? This section of the review will help in answering this question.

1.3.1. Population Growth

The Food and Agriculture Organization of the United Nations (FAO) estimate the world population to be 9 billion by 2050 (FAO, 2006). Figure 1.1 shows how the population has been growing since the 1950s.

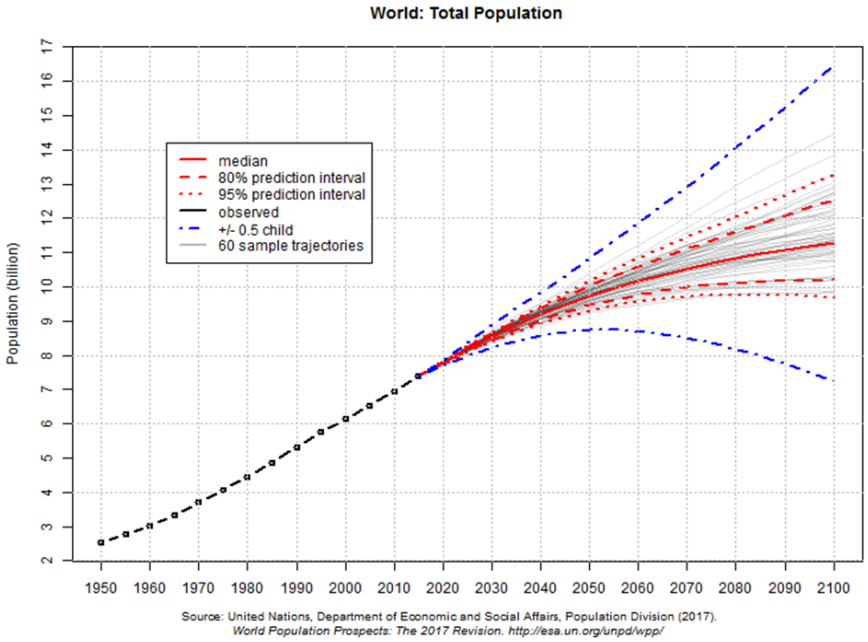

Figure 1.1. Rise of total world population (in billions) from 1950 to 2100
(United Nations Department of Economic and Social Affairs, Population Division, 2017)

The world population has been increasing and is expected to keep increasing in the future (Figure 1.1). The rise in population will increase a demand for food because there will be more mouths to feed. Henchion et al. (2017) report that the increase in population also means increased incomes and an increase in urbanization which in turn will impact food consumption patterns. Thus, the types of foods being demanded will change. Moreover, the demand for protein will increase as well since protein has been associated with contributing to healthy aging (Henchion et

al., 2017). Additionally, the demand for animal-based protein has been estimated to double by the year 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 people cannot afford to purchase meat due to its high cost, or in developing countries due to its limited supply (Fokou and Domnanagang, 1989). Thereby, adding to the need to develop alternate options for people to have easier access to protein sources.

1.3.2. Environmental Impacts

Over the years, environmental impacts have become a growing concern. In terms of animal-based proteins, the main concern comes from the fact that it relies intensely on livestock farming. Henchion et al. (2017) state that approximately 12% of greenhouse gas (GHG) emissions come from the production of livestock and that 30% of the biodiversity loss is attributed to livestock production. GHG emissions are associated with climate change (Tilman and Clark, 2014). Pihlanto el al. (2017) found that intensive livestock farming also causes a depletion of natural resources. Profetas (2008) reported that 40-%50% of the global grain harvest is currently used for feed production.

As the population continues to rise, and the demand for animal protein increases; there will be a higher need of land used for planting. This has led to there being land use concerns since the increase in demand for agricultural lands will drive an increase in deforestation and the conversion of wetlands and grasslands into areas that can be used to produce feed (Spiegal et al., 2013; Henchion et al., 2017). Changes in land use patterns can thus negatively impact the biodiversity since all of the animals living in those lands would be losing or facing reduction in habitats. Additionally, deforestation would also impact GHG emissions (Henchion et al., 2017). Speigel et al. (2017) suggested that people should eat less beef and instead eat more pork, chicken, or turn to meat substitutes to reduce land use. Not only are plant-based proteins used for feed associated with deforestation and habitat loss as stated above, but they have other environmental issues such as water use, soil degradation, and pollution (Henchion et al., 2017).

The above concerns have led researchers to come up with new crop production models such as soilless growing and indoor farming (Pihlanto et al., 2017). However, production costs and energy requirements make these options only feasible for high value protected crops and not field

grown crops (Pihlanto et al., 2017). Thus, more technological development and research is necessary to find ways of making plant and animal-based protein sources more sustainable. The alternative is to find new sources of protein to help decrease the pressure on both plant and animal-based protein supplies.

1.3.3. Health Concerns

In terms of meat, there are some health repercussions when it is overconsumed or if too much processed meat is eaten with potential problems being a higher risk of coronary heart disease, type 2 diabetes, or some cancers (Pihlanto et al., 2017). Moreover, plant-based protein sources when compared to animal-based protein sources are lower in protein quality (Pihlanto et al., 2017). Plant-based proteins especially suffer when it comes to supplying heme (Weinborn et al., 2017). Heme is obtained primarily from the consumption of myoglobin and hemoglobin found in meats and animal products and is important in preventing iron (Fe) deficiency which causes anemia (Weinborn et al., 2017). Plants contain non-heme Fe which is less bioavailable than the heme found in animal-based proteins; however, heme has been found to increase both heme and non-heme Fe while non-heme Fe has been shown to decrease the absorption of non-heme Fe (Weinborn et al., 2015). An additional concern is that plant-based proteins may contain non-nutritive compounds which are derived from secondary metabolism (Pihlanto et al., 2017). Non-nutritive compounds can impair the intake, uptake, or utilization of other foods and feed components, and can led to stress in humans and animals (Pihlanto et al., 2017). Such non-nutritive compounds can be divided into two categories including (1) proteins (such as lectins and protease inhibitors) which are sensitive to normal processing temperatures and (2) non-protein compounds which include polyphenolic compounds (condensed tannins, cyanogenic glycosides, and saponins), alkaloids, phytic acid saponins and certain oligosaccharides (Pihlanto et al., 2017). Furthermore, fortification and supplementation of these proteins can help fill in the nutritional deficiencies, or new sources can be found that have better nutritional profiles.

1.3.4. Beliefs

Some people will not consume meat for religious reasons or for moral/ethical beliefs (Fokou and Domnangang, 1989). The beliefs of consumers play an important part when it comes to demand. If they do not want to eat meat, then they will not buy it and that in turn will decrease demand. Vainio et al. (2016) reported that people's reasons for adopting a meat free diet were

associated with health concerns, weight control, animal welfare, and a sense of disgust related to meat. Consumers are beginning to change their minds on what they look for in foods. As consumer's change their eating patterns, so to must the food industry adapt and investigate new ways to meet the demands of their consumers.

1.4. Novel Protein Sources

Emerging sources of protein include algae, *in vitro* meat, and insects. Figure 1.2 shows the application of various protein sources and the time they entered into the market. Animal-based proteins have the largest application followed by plant-based proteins and both sources have been in the market for the longest time. Algae, insects, and *in vitro* meat have much smaller applications to date, but have the potential to grow (Figure 1.2). This review will briefly cover on algae and take an in depth look at the potential of insect protein for the food and feed industry.

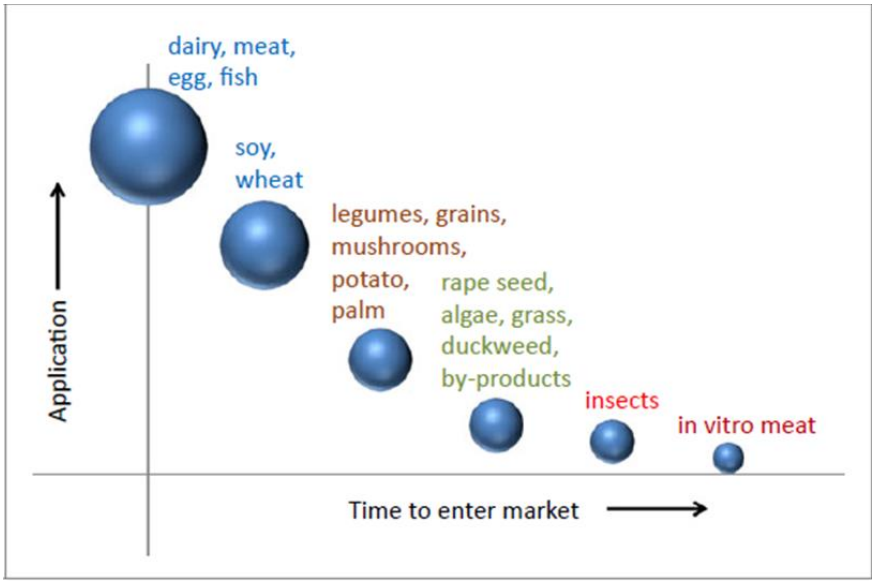

Figure 1.2. Application and time to enter market for existing and emerging protein sources (Spiegel et al., 2013)

1.4.1. Algae

Algae also use photosynthesis like terrestrial plants; however, they belong in a separate group of organisms which is large and diverse (Spiegel et al., 2013). Furthermore, algae can be differentiated as microalgae which are single celled and grow over a wide range of environmental conditions, or as seaweed which are complex multicellular and grow in saltwater (Spiegel et al., 2013). Kuhad et al. (1997) state that algae's ability to multiply with carbon dioxide as the only source of carbon is the major potential merit for using algae as a novel protein source. Currently,

24 million tonnes of algae are farmed globally with most of the production being seaweed (Henchion et al., 2017). In the EU only *Spirulina* and *Clorella* spp. are used whereas in Mexico *Spirulina platensis* is the preferred type of microalgae (Henchion et al., 2017; Kuhad et al., 1997). Japan and China also supply microalgae; however, there are issues concerning their safety (Henchion et al., 2017). Globally, about 30% of the algal production goes to the animal feed industry and the rest of the algal production is sold for human consumption or used for cosmetics (Henchion et al., 2017). Nutritionally, algae are like vegetable proteins, but contain low amounts of sulfur containing amino acids and contain a high lysine content (Henchion et al., 2017; Kuhad et al., 1997). Red and green varieties of seaweeds are high in protein (10-47% depending on species) while the brown seaweeds contain low protein amounts (3-15%) and the proteins are highly digestible (Henchion et al., 2017).

There are some safety concerns when it comes to use of algae in foods. According to Spiegel et al. (2013) about 2% of the 4000 algal varieties can contain neurotoxins and hepatoxins resulting in diseases such as paralytic shellfish poisoning, diarrheic shellfish poisoning, neurotoxic shellfish poisoning, ciguatera fish poisoning, amnesic shellfish poisoning, and microcystin. Microbial contamination from birds, insects, and rodents can also occur since *Spirulina* and *Chlorella* are cultivated in open basins (Spiegel et al., 2013). Fungi, yeast, and protozoa contamination is also common (Kuhad et al., 1997). Accumulations of heavy metals, high iodine levels, or high levels of pesticides pose other safety hazards (Henchion et al., 2017). Lastly, allergen concerns also exist since *Chlorella* has been reported to cause allergic reactions (Spiegel et al., 2013). Besides safety concerns, other cons include high production costs, difficulties in extraction/refining, and sensory/palatability issues when incorporating algae into food products (Henchion et al., 2017). All the previous factors impact the feasibility of using algae as a novel protein source in human production, and Kuhad et al. (1997) found that algae are better suited for animal feed than human foods.

1.4.2. Insects

Currently worldwide more than 2,000 species of insects are consumed, and these insects provide economic resources and nutritional intake for many societies (Belluco et al., 2013; Van Huis, 2013; Paul et al., 2016). In Africa approximately 524 insect species are consumed, 349 are consumed in Asia, 679 are consumed in Central and South America, 152 are consumed in

Australia, and only 41 are consumed in Europe (Paul et al., 2016). Furthermore, Europe not only has the lowest number of insect species consumed but is also a region of the world where insect consumption is not seen as a common practice (Belluco et al., 2013; Van Huis, 2013; Mlcek et al., 2014; Paul et al., 2016). Furthermore, Europe and North America (U.S. and Canada) treat insects as "novel" protein sources, and view insects as dirty, disgusting or dangerous pests that transmit diseases thus hindering insect consumption in these regions (Lähteenmäki-Uutela and Grmelová, 2016; Van Huis & Dunkel, 2016). According to Henchion et al. (2017), Coleoptera (beetles) are the most commonly consumed at 31%, followed by caterpillars at 18%, then Hymenoptera (bees, wasps, and ants) at 14%, then Orthoptera (grasshoppers, locusts and crickets) at 13%, then Hemiptera and Homoptera (cicadas, leafhoppers, planthoppers, scale insects, and true bugs) at 10%, then termites and dragonflies at 3%, and lastly flies at only 2%. Adult butterflies are only consumed in very few countries such as Australia where the Bogong moth (Agrotis infusa) is smoked out of rocky crevices and collected on kangaroo skins to be eaten after the scales, wings, and legs have been roasted off (Van Huis and Dunkel, 2016). More recently, organizations such as the FAO and the European commission have been promoting the use of insects as an alternative protein source in the Western world with an emphasis on crickets, the lesser mealworm, and the yellow mealworm for human food applications and the black soldier fly, the yellow mealworm, and the common housefly for applications in the feed industry (Henchion et al., 2017).

1.5. Insect Proteins as a Novel Protein Source

1.5.1. Nutrition

According to Van Huis and Dunkel (2016), generalizing nutritional information is difficult because there are many species of edible insects and their nutritional profile varies between species and within species with different diets. Additionally, factors such as gender, developmental stage, diet, environmental factors (day length, humidity, light intensity), spectral composition, and processing methods affect the nutritional composition and bioavailability (Van Huis and Dunkel, 2016).

1.5.1.1. Protein Content and Amino Acids

The average protein content of insects' ranges from 35% for termites and 61% for crickets, grasshoppers, and locusts with the latter group having species reaching protein contents up to 77% (Van Huis and Dunkel, 2016). Most insects' amino acid score ranges from 46 to 96% (Belluco et al., 2013), and have high amounts of lysine, threonine, and methionine which are the major limiting amino acids in cereal and legume-based diets (Van Huis, 2013). Furthermore, all insect orders generally meet the recommended amino acid requirements set by the WHO/FAO/ONU (Van Huis and Dunkel, 2016). However, there are some insects that are deficient in certain types of amino acids such as tryptophan or lysine (Henchion et al., 2017). The mophane worm was found to contain higher levels of threonine, valine, phenylalanine, and tryptophan than soybean or fishmeal and the mophane worm also contained lysine and methionine levels that were comparable to the amounts found in fishmeal (Madibela et al., 2007). The *Clanis bilineata* has methionine, cysteine, tyrosine and histidine contents higher than those of eggs and milk, and the ratio of essential to total amino acids was higher than that of eggs and milk (Xia et al., 2012). The insect proteins have been found to be highly digestible between 77 to 98% and the removal of the chitin not only increases the quality of the protein but makes the insect protein comparable to that of products from vertebrate animals (Belluco et al., 2013).

1.5.1.2. Fats and Fatty Acids

Insects range in fat from less than 10% to more than 30% fresh weight basis (De Foliart, 1991). Orthoptera (grasshoppers, crickets, and locusts) have an average fat content of 13%, Coleoptera (beetles, and grubs) have an average fat content of 33%, and Hemiptera (true bugs), Isoptera (termites), Blattodea (cockroaches), and some Lepidoptera (caterpillars) range between 28 to 33% fat (Van Huis and Dunkel, 2016). Furthermore, the caloric value of insects' ranges from 293 to 762 kcal per 100 grams of dry weight (Belluco et al., 2013). The degree of unsaturation of the fatty acids associated with the phospholipids is crucial for helping insects to regulate the fluidity of their membranes since they are poikilotherms (De Foliart, 1991). Triglycerides have a less important physiological role in insects; therefore, they are more variable and are influenced by diet (De Foliart, 1991). Factors such as diet and developmental stage affect the fatty acid profiles of insects especially since there is more fat accumulation during later stages of adulthood (De Foliart, 1991; Paul et al., 2016; Van Huis and Dunkel, 2016). Additionally, the sex of the insect also impacts the chemical composition since females contain more fat before ovipositioning

(Paul et al., 2016). Generally, insects tend to be high in C18 fatty acids, oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) (De Foliart, 1991). The high amounts of linoleic (18:2) and linolenic (18:3) acids are significant since these types of acids are essential meaning the human body cannot synthesize them (Van Huis and Dunkel, 2016). Locust and crickets contain arachidonic acid while mealworm is high in palmitic acid, oleic acid, and linoleic acid (De Foliart, 1991; Van Huis and Dunkel, 2016). Additionally, the saturated to unsaturated fatty acid ratio for most of the edible insects is less than 40% which compares favorably with poultry and fish; however, the amount of polyunsaturates (linoleic and linolenic) is higher in insects than in fish or poultry (De Foliart, 1991; Van Huis, 2013). Terrestrial edible insects are a better source for long chain polyunsaturated fatty acids (PUFAs) (particularly the ω-6 fatty acids) than the aquatic edible insects (Van Huis and Dunkel, 2016). PUFAs significantly impact human health since they help avert depression, increase cognitive function, reduce body weight, and reduce cholesterol (Van Huis and Dunkel, 2016). On the contrary, beef and pork have very low PUFA content and mainly consist of monounsaturated fatty acids (MUFAs) (Van Huis and Dunkel, 2016). However, the FAO recommendation for the ω-6 and ω-3 PUFAs ratio (n-6/n-3 ratio) is 10:1 and the mealworm n-6/n-3 ratio exceeded 18:1 while the cricket n-6/n-3 ratio was 16:1 (Van Huis and Dunkel, 2016). Therefore, both mealworms and crickets exceeded the FAO recommended amount.

1.5.1.3. Chitin

The second most abundant polysaccharide found in nature is chitin which is normally found in organisms such as fungi, crustaceans, and insects, but is absent in mammals (Van Huis and Dunkel, 2016). The main component of the arthropod exoskeleton in insects is chitin which accounts for approximately 10% of the whole dried insect (Belluco et al., 2013; Van Huis, 2013). For example, the amount of crude fiber found in grasshoppers ranges from 7 to 12% (Paul et al., 2016). Fiber identified in insects mainly consists of fiber but also consists of other compounds such as sclerotized proteins (Paul et al., 2016). Thus, using acid detergent fiber (ADF) to measure insect chitin tends to lead to an overestimation because amino acids account for 933% of the weight of ADF (Van Huis and Dunkel, 2016). Similarly, chitin also leads to an overestimation of protein content since chitin contain non-protein nitrogen (Bosch et al., 2014). The amount of chitin found in house crickets and yellow mealworm larvae falls between 1.6 to 2.0% while the adult yellow mealworm contains a higher amount of chitin of 7.4% (Van Huis and Dunkel, 2016). Chitin, chitosan (deacetylated chitin), and chitooligosaccharides (degraded products of chitosan or chitin)

have immunity enhancing effects and promote the growth of beneficial bacteria in the gut while inhibiting the growth and activity of the pathogenic microorganisms (Van Huis, 2013). It was generally assumed that the chitin lowered the digestibility level of insects; however, recently it has been discovered that chitin digestion by humans is possible since 2 catalytically active chitinases were discovered (AMCase and chitotriosidase) both of which belong to the 18 glycosyl hydrolases family (Belluco et al., 2013; Henchion et al., 2017). The chitinases role has been associated with defense against parasitic infections and defense to some allergic conditions (Van Huis and Dunkel, 2016). Furthermore, chitin and chitin derivatives contain nonspecific antiviral and antitumor activities and have been shown to effect innate and adaptive immune responses such as the ability to recruit and activate innate immune cells (Van Huis and Dunkel, 2016).

1.5.1.4. Vitamins and Minerals

Van Huis and Dunkel (2016) reported that 17.3% of the world's population is at risk for zinc deficiencies which has resulted in 450,000 deaths in Africa, 40% of deaths in Asia, and 2% of deaths in Latin America. Edible insects have been shown to be rich in vitamins, but in order to get the exact vitamins desired the insect species must be specifically selected (Van Huis and Dunkel, 2016). Furthermore, many edible insects contain high iron and zinc levels which could be a way to alleviate the deficiencies seen in developing countries (especially for pregnant women) and in vegetarian diets worldwide (Belluco et al., 2013). The Angolan caterpillar and *Usta Terpsichore* are rich in iron, copper, and zinc while Mophane worms are rich in calcium and phosphorous (Madibela et al., 2007; Belluco et al., 2013). Additionally, crickets and termites are also rich in iron and zinc (Van Huis and Dunkel, 2016). The ash content in grasshoppers varies from 2 to 11% depending on the developmental stage especially since some grasshopper species undergo a significant decrease in ash content during maturation from the penultimate instar to the adult stage (Paul et al., 2016). High contents B vitamins such as B1 (thiamine), B2 (riboflavin), and B3 are also found in many edible insects (Van Huis and Dunkel, 2016; Henchion et al., 2017). The insect diet has been associated with variations in the vitamin content (Van Huis and Dunkel, 2016). The *Saturnidae* species of caterpillars were found to be rich in riboflavin and niacin when smoked and dried by traditional techniques; however, the amount of thiamine and pyridoxine (B6) was low (Belluco et al., 2013). A mixture containing different species of aquatic Hemiptera called axayácatl, ahuahutle (the axayácatl eggs), and jumiles (combination of different stink bugs) consumed in Mexico contain high contents of riboflavin and niacin while axayácatl is also high in

iron (Belluco et al., 2013). The practice of gut loading has proven effective in increasing the calcium to protein ratio and vitamin A content in house crickets, yellow mealworm larvae, and silkworm larvae (Van Huis, 2013). However, it is unknown to what extent the minerals are bioavailable; therefore, more research is necessary to find the bioavailability of vitamins and minerals in insects (Van Huis and Dunkel, 2016).

1.5.2. Environmental and Sustainability

A very promising aspect of using insect proteins as an alternative protein source is that the rearing of insects has a smaller environmental impact compared to traditional animal husbandry (Stoops et al., 2016). Furthermore, Van Huis (2013) reported that 1 kg of beef had the highest impact on the environment in terms of carbon dioxide equivalents at 14.8 kg, followed by pork at 3.8 kg, and chicken at 1.1 kg. In comparison, the yellow mealworm, the house cricket, and the migratory locust compared more favorably in terms of greenhouse gas (GHG) emissions and ammonia production than conventional livestock (Van Huis, 2013; Van Huis and Dunkel, 2016). For example, commercially bred grasshoppers (Locusta migratoria) produce no methane, 2.37 g of carbon dioxide equivalents and 5.40 mg of ammonia per kg body mass per day compared to beef which produces a maximum of 0.28 g of methane, 7.08 g of carbon dioxide equivalents, and 170.00 mg of ammonia (Paul et al., 2016). The 1 kg production of two species of mealworms produced lower GHG emissions, similar amounts of energy and significantly lower amounts of land was necessary in comparison to the production of milk, chicken, pork, or beef (Van Huis and Dunkel, 2016). Moreover, rearing mealworms requires 18 m^2 to produce 1 kg of protein which is 2.5 times less than for milk, 2.6 times less than for chicken, 3.0 times less than pork, and 11 times less than cattle (Van Huis and Dunkel, 2016). Additionally, only 2.1 kg of feed is necessary to produce 1 kg of cricket protein thus making it 2.1 times more efficient than chicken, 4.3 times more efficient than pigs, and 11.9 times more efficient than cattle (Van Huis and Dunkel, 2016). In comparison, 7.5 kg of plant proteins are necessary to produce 1 kg of high-quality meat from animal sources (Paul et al., 2016). It has been assumed that the increased efficiency in the feed conversion ratio for insects is because insects are poikilothermic (cold-blooded) which allows their growth stages to not need feed to maintain a constant body temperature (Van Huis, 2013).

Another advantage to using edible insects is that many species can be grown on organic side-streams thus allowing for the conversion of low value organic byproducts into high value

protein sources (Van Huis and Dunkel, 2016). Furthermore, insects require much lower amounts of water to develop and most of them can meet their water requirement from their diet unlike cattle which require 22,000 to 43,000 L of water to produce 1 kg of beef (Paul et al., 2016). On the other hand, chicken require 4,300 L of water, pigs require 6,000 L of water, and sheep require 10,400 L of water (Van Huis and Dunkel, 2013). Additionally, cattle production 72% pastures, 23% crop ingredients, 4% fodder crops, and 1% drinking water is used while for insects no pastures or fodder crops are necessary thereby lowering the water requirements (Van Huis and Dunkel, 2016). The yellow mealworm and the lesser mealworm are drought resistant thereby also lowering the amount of water necessary for them to develop (Van Huis, 2013).

Edible insects are up to 80% edible and digestible compared to 40% for cattle and 55% for chicken and they have shorter lifespans which allows for a high yield in a shorter amount of time than traditional livestock production (Henchion et al., 2017). Lastly, Paul et al. (2016) state that rearing insects reduces the need for livestock grazing which contributes to environmental destruction and a reduction in poaching in wild game reserves has also been observed. Smetana et al. (2016) conducted a life cycle assessment of insects used for either feed or food purposes and found that insect proteins had better performance than traditional food analogues; however, the multiple insect production techniques and processing technologies made the sustainability unpredictable and challenging. Therefore, development of a standardized way of rearing insects will become necessary in order to improve the sustainability of insects.

1.5.3. Insect Rearing

The seasonality of edible insects requires them to be preserved in order to keep them available year-round when collected from the wild; therefore, a better way to rear them is as mini-livestock and feed them chicken feed, vegetables, or waste streams (Spiegel et al., 2013; Van Huis and Dunkel, 2016). The most important factors to consider when mass rearing are quality, reliability, and cost-effectiveness (Van Huis, 2013). Currently no standardized rearing method exists therefore there are many ways of rearing edible insects. One example is a residential cricket breeding system which used wild resources such as taro aerial parts, young cassava leaves and brown rice flour with or without banana slices as feed sources instead of using a broiler feed diet (Megido et al., 2015). The taro diet produced crickets with the highest percent of protein and reduced the cost of rearing (Megido et al., 2015).

On the other hand, Thailand has 20,000 farms which produce 7,500 tons per year of the house cricket and field crickets which are fed high-protein chicken feed (responsible for half the total production costs) (Van Huis and Dunkel, 2016). Feeding the mature crickets local fruits and vegetables, pumpkin, or squash not only reduced cost, but also gives the crickets a golden color once they have matured (Van Huis and Dunkel, 2016). Palm weevils are also reared in Thailand using plastic containers which are full of ground palm stems mixed with pig feed (Van Huis and Dunkel, 2016). For insect proteins to become a feasible alternative protein source, large-scale mass rearing (tons/day) needs to be developed since huge demand will be required by both the feed and food industry (Van Huis and Dunkel, 2016). Therefore, more research is necessary to create an optimized insect rearing program that can feasibly scaled up for large-scale mass rearing.

1.5.4. Safety

According to Spiegel et al. (2013) some insects are edible in some regions but not in others since they feed on certain plants or originate from polluted and pesticide treated areas of the wild; and others require special capture, preparation, storage, or transportation methods to keep them safe. As of 2010, the Codex Alimentarius Commission (CAC) has not extensively studied the food safety of edible insects since they are treated as traditional foods for indigenous people. However, the CAC has described insects as being rich in nutrients and providing a proper medium for growth of unwanted microorganisms (Spiegel et al., 2013). Viruses, bacteria, microsporidia, fungi, and nematodes carrying pathogenic bacteria are all kinds of microorganisms that can infest insects (Van Huis and Dunkel, 2016). Furthermore, changes in diet, elevated temperatures, humidity, or toxins can cause microorganisms to multiply and kill many of the insect hosts; however, the inclusion of antimicrobials in the insect diet often inhibits growth thereby impacting the developmental stages (Van Huis and Dunkel, 2016). Additionally, the processing and preservation of the edible insects can also introduce post-processing contamination (Van Huis and Dunkel, 2016). The use of waste streams as insect feed also has the potential to introduce various safety hazards such as the presence of contaminants, antinutritional factors, and allergens (Spiegel et al., 2013). Feed contaminations include mycotoxins, natural toxins, heavy metals, veterinary residues (such as antibiotics), pesticides, and pathogens (Spiegel et al., 2013). Furthermore, insects can convert or accumulate contaminants present in their diet thereby increasing the concentrations of the contaminants (Spiegel et al., 2013).

Spores can also contaminate edible insects if present in their feed (Stoops et al., 2016). Mealworm larvae and grasshoppers have been shown to contain high numbers of total aerobic plate count, Enterobacteriaceae and Lactic Acid Bacteria (LAB), as well as, a 5.3 log cfu/g level of bacterial yeast and mould counts which is a risk in food safety (Stoops et al., 2016). Additionally, both mealworm larvae and grasshoppers harbor a diverse community of bacteria mostly dominated by Proteobacteria, Firmicutes and Actinobacteria in mealworm larvae, and by Firmicutes and Proteobacteria in grasshoppers. (Stoops et al., 2016).

Due to the potential food safety issues mentioned above, a kill step during processing is necessary to ensure the safety of consuming edible insects (Stoops et al., 2016). Heat treatment such as boiling or sun drying is enough to eliminate possible neurotoxins and to eliminate E. coli, and Salmonella in insects (Spiegel et al., 2013; Van Huis, 2013). Lactic acid fermentation of composite flour to water mixtures consisting of 10 or 20% roasted mealworm powder was sufficient in controlling both enterobacteria and bacterial spores (which are not eliminating by heating) (Spiegel et al., 2013). Allergenicity concerns also exist for edible insects as insect proteins have a cross-allergenicity with shrimps and house dust mites (Spiegel et al., 2013). Symptoms can consist of drooling, difficulty swallowing, pain, and shortness of breath and some caterpillars may evoke toxic reactions (Belluco et al., 2013). Silkworm pupa has been found to contain arginine kinase which has resulted in more than 100 patients going into anaphylactic shock (Belluco et al., 2013). Crickets and yellow mealworms also contain arginine kinase and yellow mealworm also contains tropomyosin which is another well-known allergen (Van Huis and Dunkel, 2016).

1.5.5. Legislation

Currently, no specific rules or regulations exists for foods made from insects (Lähteenmäki-Uutela and Grmelová, 2016). Germany and Finland do not allow the commercial production and marketing of edible insects while the Czech Republic allows the farming and marketing of edible insects without having any specific legal regulations (Lähteenmäki-Uutela and Grmelová, 2016). On the other hand, the United Kingdom, France, Belgium, and the Netherlands allow specific types of insects to be used for food and have implemented national rules such as the mealworm beetle, the lesser mealworm beetle, and the locust (Lähteenmäki-Uutela and Grmelová, 2016). However, the EU does not have any rules on food hygiene, animal-origin food hygiene, or any microbiological criteria on food containing edible insects (Lähteenmäki-Uutela and Grmelová,

2016). Therefore, investment and innovation are forestalled due to the unclear or lack of legislation and will need to be addressed to make insect proteins more feasible and to ensure that edible insects are safe for human and animal consumption.

1.5.6. Major Barriers Faced

There exist many challenges when it comes to allowing insect proteins to be a feasible option for an alternative protein source as listed below:

1. The major hurdle is the lack of legislations and regulations when it comes to rearing and selling insects for food/feed which discourages investors and hinders the ability for the market to grow (Van Huis and Dunkel, 2016; Henchion et al., 2017).
2. The variability in processing and rearing techniques among national and international producers thereby affecting safety and quality of the edible insects produced and making sustainability challenging (Smetana et al., 2016; Van Huis and Dunkel, 2016; Henchion et al., 2017).
3. Lack of awareness among consumers/buyers about the edible insect market thus leading to a low demand for insect proteins; however, to enter the food and feed industry and increase demand it requires large scale mass rearing plans to be developed and implemented (Van Huis and Dunkel, 2016; Henchion et al., 2017).
4. People's perception of insects being inherently unsanitary and seen as pests or disease transmitters which makes the marketing of edible insects more difficult (Henchion et al., 2017). Also, the belief that eating insects is a primitive behavior which creates an embarrassment factor surrounding edible insects (Van Huis and Dunkel, 2016).
5. Westerners' belief that insect consumption is a threat to their psychological and cultural identity (Van Huis and Dunkel, 2016). Thus, meat is believed to be a healthy and necessary part of the diet in Western food cultures and is usually the main component of the meal (Vainio et al., 2016).
6. Religious practices which may encourage or inhibit people from eating edible insects. For example, the consumption of grasshoppers has been mentioned in the book of Leviticus and the Islamic religion allows for the consumption of grasshoppers (Paul et al., 2016).

A potential solution to getting consumers' acceptability in eating edible insects comes from rendering the insect unrecognizable and incorporating it as an ingredient in a familiar product (Henchion et al., 2017). Van Huis and Dunkel (2016) found that unrecognizable insects are perceived as less scary by consumers. Increased costs and risks are introduced when transforming edible insects into ingredients rather than eating them whole and requires the establishment of new value chains (Henchion et al., 2017). The value chains need to ensure a safe, reliable feedstock, large-scale mass rearing, safe processing into an insect ingredient, and a safe application of these insect ingredients in final food products (Henchion et al., 2017). Therefore, the edible insect industry needs to be furthered developed before it be seen as an economically competitive market.

1.5.7. Insects Used for Feed

Insect proteins have been shown to be acceptable replacements for fish meal in animal diets with the most promising insects being black soldier fly, the common housefly, the yellow mealworm, the lesser mealworm, the silkworm, and several species of grasshoppers (Van Huis, 2013). Additionally, the house cricket was shown to be superior to soy protein in providing amino acids for weanling rats (Belluco et al., 2013). "Magmeal" made from maggots is high in protein and rich in phosphorous, trace elements and B vitamins and was shown to be able to replace fish meal without adversely affecting the hen performance or the egg quality (Khan et al., 2016).

Silkworms are sun dried and powdered to create silkworm pupae meal and are an acceptable alternative protein in poultry rations especially since a linear increase of the silkworm pupae meal caused a corresponding decrease in the cost per unit of feed and increased the growth rate, meat yield, and profitability in broiler chicks (Khan et al., 2016). Another acceptable protein source for broiler chicks is the yellow meal worm (Khan et al., 2016). Moreover, the earthworm powder was found to be suitable as a partial replacement in fishmeal for common carp since the digestibility increased with the addition of earthworm powder and the growth rate and energy retention also increased (Ngoc et al., 2016).

Bosch et al. (2014) studied housefly pupae, adult house cricket, yellow mealworm larvae, lesser mealworm larvae, Morio worm larvae, black soldier fly larvae and pupae, six spot roach, death's head cockroach and Argentinean cockroach for their potential use in pet food formulations (for cats and dogs). It was found that all the insects had higher amounts of crude protein than that of soybean meal and were comparable to the amount found in poultry meat meal and fish meal

(Bosch et al., 2014). However, Bosch et al. (2014) concluded that feasibility of mass production, product safety, and pet owner perception would be the determining factors for if insect proteins had a future in the pet food industry.

1.5.8. Insects Used for Human Foods

There are many ways whole insects can be eaten including raw, dried, crushed, textured, pulverized, ground, heated such as cooked, roasted, boiled, fried, toasted, extruded, and canned, or they can be preserved by freeze-drying (Spiegel et al., 2013). Furthermore, insect proteins can be isolated via extraction techniques or can be converted into insect meal before being used in food products (Spiegel et al., 2013; Van Huis and Dunkel, 2016). However, their functional properties can be altered via processing such as improved protein solubility due to cooking, or a decrease in the water absorption capacity after roasting and grilling (Paul et al., 2016). Additionally, degutting a mophane caterpillar leads to an increase in crude protein and digestibility; however, cooking the caterpillar lowered both crude protein and digestibility (Van Huis, 2013). Madibela et al. (2007) showed that if processed in a controlled environment, the mophane worm quality varies only slightly depending on the type of processing and that it needed to be degutted for human consumption.

Processing methods do affect the nutrient potential of edible insects as seen in the changes in protein digestibility and vitamin content reduction in the toasted, and dried insect samples; therefore, optimal processing methods need to be developed (Kinyuru et al., 2010). On the other hand, dehydration and defatting of the *Rhynchophorus phoenicis*, *Oryctes rhinoceros*, *Imbrasia belina*, and *Macrotermes bellicosus* larvae increased the concentration of nutrients found in the proximate analysis (Ekpo, 2011). Edijala et al. (2009) reported that the type of heat treatment was the most important factor affecting the proximate composition and cholesterol concentration in the *Rhynchophorus phoenicis*, and *Oryctes rhinoceros* larvae. Tong et al. (2011) studied silkworm larvae as a potential future protein source for astronauts in the bioregenerative life support system during long-term deep space exploration missions. The silkworm larvae were fed mulberry and stem lettuce and on the third day of the fifth instar were freeze dried and ground into a silkworm powder which was found to contain high levels of vitamins A, B1, B2, and E (Tong et al., 2011). Furthermore, phosphorous and potassium levels were high, and the silkworm powder contained

12 essential amino acids, 9 minerals, and 12 fatty acids thereby making it an acceptable alternative protein source for the astronauts (Tong et al., 2011).

The functional properties of crickets include gelation concentration of 10%, a water absorption capacity of 238.47%, an oil absorption capacity of 202.1%, an emulsion capacity of 46.8%, and a foam stability of 8.5% (Adebowale et al., 2005). The lowest solubility of the cricket protein was at a pH of 4 while the highest solubility was between the pHs of 6 and 7 (Adebowale et al., 2005). Adebowale et al. (2005) concluded that the African giant cricket would be suited for foods requiring gelling and thickening, foods where water retention is desirable during cooking, and good for food products involving fat absorption such as bakery products. There does not exist much research regarding the addition or incorporation of insect proteins as ingredients into food products. Therefore, further testing is necessary to see how the insect proteins would alter the final product characteristics. However, there are products commercially available on the market which contain edible insects (Table 1.6).

1.6. Scope of this study

The high protein content and good nutritional profile of edible insects makes them promising alternative protein sources. Their necessity for less resources (water, land, feed) compared to traditional livestock and their smaller environmental foot print also makes insects a promising food. However, the use of edible insect as a protein source still requires some growth and technological development, as well as, the creation of legislations and regulations to ensure the safety and quality of the product are up to human standards. Moreover, people's negative connotations of insects also need to be addressed in order to allow the market to expand. More research is necessary to see the effect of insect protein addition into food products specifically to see if the processing not only affects the nutritional and functional properties of the insect protein itself, but also the final product characterizations as well. Since cricket protein powder is currently available on the market it was chosen for investigation in this thesis. The aim was to study 3 different areas: 1) protein characterization of the cricket proteins and their functionality, 2) how the incorporation of cricket protein powder affected wheat dough rheological and visco-elastic properties, and 3) how cricket protein powder affects the final product characterization of bread loaves. As the two cricket protein powders used in this research varied in how they were processed, the effect of processing on functional properties was also evaluated.

Table 1.6. Commercially available products that contain edible insects.

Product	Company	Description
Baked Goods	Bitty Foods	Made with cricket "flour"(3 flavors)
The Bux Burger	Bugfoundation	Burger made from lesser mealworms
Multiple Products	Bush Grub	Work with buffalo worms, crickets, mealworms, and locusts. Insect candy; crispy critters; and insect "flour"
Original Cricket Bar	Chapul	Flavored protein bars made from crickets
Burgers, Nuggets, and Schnitzel	Damhert	Insecta brand products made from buffalo worms
Multiple Products	DeliBugs	Freeze dried insects; fruit and nut bars; insect candy and lollipops. Made using worms, ants, grasshoppers, crickets, and butterflies
Chocolate and Toffees	Don Bugito	Chocolate covered crickets/superworms; toffee mealworms. Maple cricket granola; chile and lime crickets; spicy superworms
Candy and Chocolates	Ento Market	Chocolate covered ants; spicy worms; and insect lollipops
Burgers and Balls	Essento	Burgers and meatballs made from mealworms, carrots, and rice
Protein Bars	Exo Protein	Bars made from cricket "flour" (4 flavors)
Aldento-Mealworm Pasta	Goffard Sisters	Pasta made from mealworm "flour"
Ice Cream	Gourmet Grubb	Use Entomilk (dairy alternative) made from black soldier fly larvae
Salty and Sweet Spreads	Green Kow	Spreads made from mealworms: Carrot and tomato; dark chocolate; milk chocolate
Multiple Products	Grub	Sell mealworms, buffalo worms, crickets, grasshoppers, and cricket fudge.
Granola Bars	Hopper Foods	Bars made from cricket "flour" (flavors: cranberry and almond; toasted coconut; and cacao and cayenne)
Insect Candy	HotLix	Various insects inside candy and lollipops
Chocolate Covered Insects	Insectable	Made from buffalo worms, crickets, grasshoppers, and mealworms
Mealworms and Crickets	Insecto	Flavored crickets: Thai, curry and BBQ Flavored mealworms: plain curry and BBQ
Edible Insects	Jimini's	Flavored grasshoppers (fruity curry, pepper & dried tomato) and mealworms (sesame and cumin, garlic and herbs, and sweet soya)
Multiple Products	Micronutris	Work with crickets and mealworms. Insect biscuits and crackers; chocolates and macarons topped with insects
Chirps Cricket Chips	Six Foods	Baked chips made from beans and cricket powder (3 flavors)
Multiple Products	Snack Insects	Work with buffalo worms, crickets, grasshoppers, and mealworms. Chocolate covered insects; drinks made from worms

1.7. References

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Chapter 2 - Materials and Methods

2.1. Materials

For the purposes of this study, King Arthur Sir Galahad Artisan Bread Flour with a protein content of 11.7% (as is) and a moisture content of 12.7% served as the control. Two companies provided the cricket protein powders, GrioPro (All Things Bug LLC, Georgia) and Entomo Farms (Entomo Farms, Ontario). Both companies use *Acheta domesticus*, and *Gryllodes sigillatus* to create their powders; however, processing differs between the two companies. GrioPro (G) makes a slurry, pasteurizes it, and uses a spray dryer to make their powder. On the other hand, Entomo Farms (E) washes their crickets with water, roasts them in the oven, and grinds them to make their powder. Certificate of analysis (COA) data provided by Entomo Farms and GrioPro for their cricket protein powders (E and G) are summarized in Table 2.1. The aerobic plate counts (APC) performed by FSI Microbiology Testing Laboratory (Food Science Institute, Kansas State University) were found to be 6.1 log CFU/g for powder E, and less than 0.5 CFU/g for powder G.

Table 2.1. Certificate of analysis (COA) data for cricket protein powders

Composition	E	G
Moisture (%)	2.6	1.33
Ash (%)	6.5	5.6
Protein (g/100g)	61.83	67
Fiber (g/100g)	6.0	6.6
Cholesterol (mg/100g)	228	303
Calcium (mg/100g)	110	150
Iron (mg/100g)	2	6
Potassium (mg/100g)	1100	1010
Sodium (mg/100g)	310	390
B12 (µg/100g)	24	8.52

2.2. Experimental Design

This study was conducted in three main groups of analysis: (a) characterization of the cricket protein powders, (b) dough development and dough rheology, and (c) test baking and end-product quality. Full factorial designs (FFD) with two factors (insect protein powder type×replacement level) were applied as summarized below in Table 2.2.

Table 2.2. Experimental design

Tests	Replacement Levels (%)	Sample Codes
Powder Characterization Tests		
Proximate analysis	-	E, G
Water holding capacity	-	E, G @ pH 3, 5, 7 and 10
Solubility	-	E, G @ pH 3, 5, 7 and 10
SDS-PAGE	-	E, G
SEC-HPLC	0, 10, 20	E0, E10, E20, G0, G10, G20
Dough Development and Rheology		
MixoLab - Constant WA	0, 10, 20	E0, E10, E20, G0, G10, G20
MixoLab - Optimized WA	0, 10, 20	E0, E10, E20, G0, G10, G20
Dough extensibility	0, 10, 20	E0, E10, E20, G0, G10, G20
End-product Quality		
Test baking	0, 5, 10, 20	E0, E5, E10, E20, G0, G5, G10, G20
Physical properties	0, 5, 10, 20	E0, E5, E10, E20, G0, G5, G10, G20
Crumb microstructure	0, 5, 10, 20	E0, E5, E10, E20, G0, G5, G10, G20
Texture	0, 5, 10, 20	E0, E5, E10, E20, G0, G5, G10, G20

2.3. Methods

2.3.1. Powder Characterization

2.3.1.1. Moisture Content.

The moisture content for the flour was performed according to the AACCI Method 44-15.02 (AACCI, 1975) for air oven moisture. A 2 g sample was placed into a metal tin and baked uncovered in an oven (ThermoFisher Scientific, Mo. #3511FS) set to 130 $\pm$ 1°C for 60 min. Once removed from the oven, the tins were covered with lids and allowed to cool to room temperature before being weighed. The moisture content (MC) was calculated with the following equation:

$$MC(\%) = \frac{Moisture\ loss}{Original\ sample\ weight} \times 100$$

The flour samples were run in quadruplicates.

2.3.1.2. Water Holding Capacity (WHC)

The AACCI Method 56-11.02 (AACCI, 2009) for Solvent Retention Capacity was modified by using different buffers instead of distilled water to conduct the extraction. The buffers used were prepared according to Table 2.3. Fifty mL centrifuge tubes with screw caps were used. Prior to the experiment empty tube weights were recorded. A 5 g sample (of both types of cricket

protein powders) was placed into the tube and 25 g of the various buffers was added. The tube was then capped and shaken vigorously in 5 min intervals for 20 min after which the samples were centrifuged (Sorvall Biofuge Stratos) at 1000×g for 15 min at 20°C. The supernatant was removed, and the tubes were drained at a 90° angle for 10 min before being reweighed. The water holding capacity (WHC) was calculated with the following equation:

$$WHC\ (\%)\ = \frac{Gel\ weight}{Powder\ weight} \times 100$$

Table 2.3. Specifications for buffer preparations

pH	0.1 M citric acid (mL)	0.2 M Na_2HPO_4 (mL)
3	238.55	61.65
5	145.50	154.50
7	52.95	247.05
pH	**0.1 M Na_2CO_3 (mL)**	**0.1 M $NaHCO_3$ (mL)**
10	180.00	120.00

The supernatant was later used for the solubility testing. The samples were run in triplicate.

2.3.1.3. Solubility

To determine the solubility of each type of cricket protein powder, protein solubilized was determined colorimetrically using the bicinchoninic acid (BCA) protein assay (ThermoFisher Scientific, Waltham, MA). Extractions of the cricket protein powders were carried out as stated in section 2.3.1.2 with the supernatant diluted 1:100 before use. The final protein concentration value was multiplied by 100 to account for the dilution factor of the original extracts. The samples were run in triplicate.

2.3.1.4. Molecular Weight

Polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate cricket powder proteins based on their molecular weight. Both wheat and cricket protein powders were extracted using an LDS sample buffer (ThermoFisher Scientific, Waltham, MA) containing 2% (v/v) 2-mercaptoethanol and heated to 95°C for 5 min. After cooling and centrifugation at 10,000×g for 2 min, 10 μl of each sample were loaded on a12% Bis/Tris NuPAGE gel using MOPS running buffer (ThermoFisher Scientific, Waltham, MA) and run at 150 volts until the dye front reached the bottom of the gel. Molecular weight markers were run in the first lane as a reference. Gels were

stained overnight using a colloidal blue stain (ThermoFisher Colloidal Blue Staining Kit) and were destained with distilled water and scanned.

2.3.1.5. SEC-HPLC Protocol

The change in soluble polymeric (SP) and insoluble polymeric (IP) proteins during mixing were studied using size exclusion high performance liquid chromatography (SEC-HPLC) analysis. For this analysis, 50 g dough samples were prepared using the Chopin MixoLab and mixed until peak time. The dough was then removed from the Mixolab and broken down into nickel sized samples and placed into a 50 mL test tube and immediately placed in a -80°C freezer. The samples were then lyophilized and ground prior to extraction. The raw materials (wheat flour, and both types of cricket protein powders were also freeze-dried and extracted for comparison purposes.

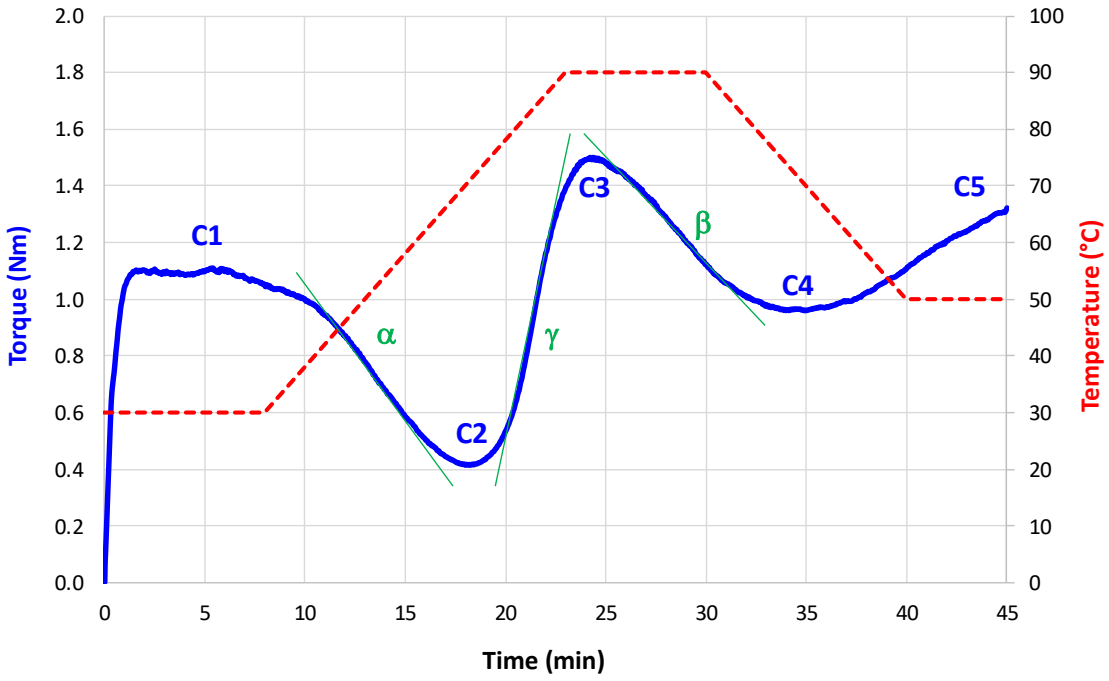
Extraction of the samples was done as described in Schober et al. (2006). One mL of pH 7 SDS buffer was added to a 100 mg sample and the samples were extracted for 5 min with continuous vortexing. After centrifuging (Sorvall Biofuge Stratos), 0.5 mL was removed into a clean centrifuge tube. The remaining supernatant was discarded. Extraction was repeated, and another 0.5 mL supernatant was added to the tube with previous extract (resulting in sample SP) This procedure was repeated one more time as a wash step were all the supernatant was discarded. For IP, the pellet from the SP extraction was used and 1 mL of SDS buffer was added and the samples sonicated for 30 s at 10W using a probe sonicator with a 0.125 in diameter probe (Sonic Dismembrator 60, Thermo Fisher Scientific, Waltham, MA). After centrifuging, the supernatant was transferred to a clean tube. After extraction both SP and IP extracts were stabilized by heating all at 80°C for 2-3 min. The samples were analyzed using an HPLC SEC-4000 column with pH 7 SDS mobile phase, at a column temp at 40°C, with a 0.5 mL/min flow rate. All samples were run in duplicate for the raw materials while the doughs were run in quadruplicate. The peak areas were calculated for each sample and for the doughs the peak areas were measured only for the polymeric region 1 resulting in samples SPI and IPI.

To determine if disulfide bonded protein complexes were present in the in the cricket protein powders, samples were reduced after extraction as described above. For the reduced samples, the reducing agent beta-mercaptoethanol (BME) was added to an aliquot of sample prior to HPLC analysis.

2.3.2. Dough Development & Dough Rheology

2.3.2.1. MixoLab - Constant Water Absorption (WA) Protocol

Dough mixing and pasting properties were studied using a MixoLab (Chopin Technologies, France) as described by AACCI Method 54-40.02 method. According to the Chopin+ protocol, flour and water were mixed in a 50-g MixoLab bowl (dough mass of 75g) for 45 min at constant speed of 80 rpm based on 14% moisture basis. Figure 2.1 is a typical MixoLab curve illustrating the changes in the dough behavior as a consequence of the mechanical shear stress and the temperature during dough development and continuous mixing.



- - - - Temperature profile:

Stage-1: Constant temperature of 30°C (8 min)
Stage-2: Heating ramp to 90°C (4°C/min)
Stage-3: Constant temperature of 90°C (7 min)
Stage-4: Cooling ramp to 50°C (4°C/min)
Stage-5: Constant temperature of 50°C (5 min)

—Critical changes:

0 to C1	Mixing and dough development, gluten network formation
C1 to C2	Over-mixing, gluten weakening, starch swelling
C2 to C3	Starch gelatinization
C3 to C4	Starch rupture, breakdown
C4 to C5	Starch re-gelling, amylose and amylopectin re-association

Figure 2.1. Typical MixoLab curve illustrating the changes in the dough behavior.

The amount of water to be added was previously determined from the Mixograph of the control dough, and then adjusted to result in a peak torque of 1.1 Nm. This water absorption (WA) level was then kept constant to study the effect of cricket protein powder on mixing and pasting behavior of the control flour. The samples tested were the wheat flour (control), 10 and 20% of

the total flour weight replacement by cricket protein. In addition, cricket protein powders were mixed with pure starch as controls at a 20% replacement level of total starch weight. The flour samples were tested using a 50 g total sample weight, while the starch was run using a sample weight of 75 g. Furthermore, the starch was run as an as is basis and not as a 14% basis. All samples were run in duplicates. The resulting MixoLab curves were analyzed for the following parameters listed in Table 2.4. A detailed explanation of these MixoLab parameters can be found in Rosell et al. (2007).

Table 2.4. Specific MixoLab parameters

(Adopted from Rosell et al. 2007)

Parameter	Description	Implication
C1 torque (Nm)	Maximum torque, C1	Dough development
C2 torque (Nm)	Minimum torque, C2	Protein reduction
C3 torque (Nm)	Maximum torque, C3	Starch gelatinization
C4 torque (Nm)	Minimum torque, C4	Amylase activity
C5 torque (Nm)	Maximum torque, C5	Starch gelling (Nm)
C1 time (min)	Time, C1	Development arriving time
C2 time (min)	Time, C2	Protein reduction time
C3 time (min)	Time, C3	Starch gelatinization time
C4 time (min)	Time, C4	Amylase activity time
C5 time (min)	Time, C5	End of the test
C1 temperature (°C)	Temperature, C1	Mixing temperature (~30°C)
C2 temperature (°C)	Temperature, C2	Initial pasting temperature; onset of gelatinization
C3 temperature (°C)	Temperature, C3	Final pasting temperature
C4 temperature (°C)	Temperature, C4	Breakdown temperature
C5 temperature (°C)	Temperature, C5	Set-back temperature
Amplitude (Nm)	Band width	Dough strength
Stability (min)	Time C1 torque drops below 1.1	Dough stability
Alpha, α (-)	Slope b/w the end of stability and C2	Protein destabilization and unfolding due to shear and temperature
Gamma, γ (-)	Slope b/w C2 and C3	Viscosity development due to starch swelling and gelatinization
Beta, β (-)	Slope b/w C3 and C4	Breakdown due to prolonged mixing at high temperature

2.3.2.2. MixoLab - Optimum Water Absorption (WA) Protocol

The same experimental procedures described in section 2.3.2.1 were applied except for pre-steps for determination of optimum water absorption (WA) for each run. The results of MixoLab constant WA test results were used as a baseline for each sample. For the samples where C1 torque values were above 1.1 Nm, optimized mixing runs (first 8 min of the test) were conducted by adding progressively more amount of water until C1 torque was adjusted back to the target value of 1.1 Nm. Similarly, for the samples where C1 torque values were below 1.1 Nm, optimized mixing runs (first 8 min of the test) were conducted by adding progressively less amount of water until C1 torque was brought to the target value of 1.1 Nm.

2.3.2.3. Extensional Rheology - Kieffer Rig Dough Extensibility Test

Uniaxial extension tests were performed using the Kieffer Dough Extensibility Rig attached to a TA-XT2 Texture Analyzer (TA-XT2 Texture Analyzer, Texture Technologist Corp. New York, NY). Each dough sample was prepared using the MixoLab with the samples mixed to peak torque as described previously. Immediately after mixing, each sample was molded using the Kieffer Rig set mold. The mold was first prepared by being brushed with oil and placing wax strips in each mold cavity. A 50 g dough piece was placed into a teflon-coated block to prepare dough strips according to the method of Kieffer at al. (1998). The excess dough that came out of the sides of the block were removed and the dough rested for 30 min before being tested. On average of 7-9 dough strips from each dough sample were tested at extension speed of 3 mm/sec, distance 75 mm, trigger force 5 g. The samples were analyzed in duplicate for a total of 14-18 strips tested. Pre- and post-test speeds were 2 mm/s and 10 mm/s, respectively. The force required to stretch the dough sample and the displacement of the hook were recorded as function of time. The peak force (resistance to extension, Rmax in g), and the distance at which this peak force occurs (extensibility, E in mm) were captured for each run.

2.3.3. Test Baking

2.3.3.1. Water Absorption

The AACCI Method 54-40.02 (AACCI 1995)for the Mixograph (National Manufacturing, NE) was followed to find the water absorption and mixing time for the control flour and 5%, 10% and 20% of the total flour weight replacement by cricket protein powder. The Mixograph was run

using a 35-g. sample. To estimate the water absorption the equation $y = 1.5x + 43.6$ was used where x is the percent flour protein content (14% moisture basis) and y is the percent water absorption. The 35 g flour sample was transferred into the mixing bowl and a spatula was used to create a well in the flour. The water was added into the well using a pipette. The bowl was placed into position and clamped down. The mixing head was lowered, and the Mixograph was started. A 10 min mixing time was used. Once optimized, each sample was run in duplicates. The average midline peak time values and the average absorption values were used in the test baking. Note that the 20% replacement with GrioPro cricket protein powder did not mix well in the pin mixer, therefore only one clear graph was attained. The data gathered from the mixographs aided in determining the optimized water absorption per sample used during the mixing part of the test baking.

2.3.3.2. Test Baking Protocol

Breads were made following AACCI Method 10-10.03 (optimized straight-dough bread-making method) (AACCI 1999). The guidelines followed were for doughs made using 100 g flour. A 5%, 10%, and 20% total flour weight replacement level with GrioPro and Entomo cricket protein powders were tested and the full formulations used can be found below in Table 2.5. The mixing time and amount of water (determined from Mixograph) are shown in Table 2.6.

Table 2.5. Formulation for 5, 10 and 20% total flour weight replacement levels

	5%		10%		20%	
Ingredient	**Amount (g)**	**Flour basis (%)**	**Amount (g)**	**Flour basis (%)**	**Amount (g)**	**Flour basis (%)**
Wheat flour	95	100	90	100	80	100
Cricket protein powder	5	5.3	10	11.1	20	25
Yeast	2	2.1	2	2.2	2	2.5
Sucrose	6	6.3	6	6.7	6	7.5
Salt	1.5	1.6	1.5	1.7	1.5	1.9
Shortening	3	3.2	3	3.3	3	3.8

After weighing the ingredients, they were transferred into a pin mixer (National Manufacturing, NE) and a well was created. 2.0 g of yeast was thoroughly mixed into the water before being transferred into the well. After mixing, the dough was rounded by hand and placed into a lightly greased plastic bucket. Plastic wrap was used to seal the top of the bucket before

placing the bucket into the fermentation cabinet (National Manufacturing, NE) set at 86±2°F. The fermentation scheduled used was a 52 min + 25 min + 13 min with the 1st punch occurring after 52 min and the 2nd punch occurring after 25 min. A sheeter set at a 3″ roll width and 3/16″ roll spacing was used for the 1st and 2nd punch. After sheeting, the dough was folded in half and in half again before being put back into the fermentation cabinet. 13 min after the 2nd punch, the dough was sheeted again using a 3″ roll width and 5/16″ roll spacing before going through the moulder (Food's Nutrition, Mo. # 345558). The moulded dough was placed into a lightly greased baking pan and allowed to proof in the fermentation cabinet for 33 min. Afterwards, the proofed dough was baked in a reel oven (Despatch, MN) for 25 min at 419°F.

Table 2.6. Mixing time and water absorption for test baking

Sample	Water Absorption (%)	Mixing Time
Control	66	6 min 18 sec
E5	65	5 min 42 sec
G5	66	6 min 18 sec
E10	64	5 min 30 sec
G10	66	6 min 30 sec
E20	62	5 min 18 sec
G20	68	6 min 30 sec

2.3.3.3. Loaf volume and Crumb Color

The loaves were cooled for an hour before being weighed. The volume was measured via rape seed displacement in accordance with AACCI method 10-05.01 (AACCI 1995). Afterwards, the loaves were sliced using a mechanical slicer (Chef's Choice Int., PA) into 0.5″ thick slices. The two slices in the middle of the loaves were used for further testing. The crumb color was measured for each slice using a WR Series colorimeter (FRU, China). Each sample was conducted in triplicate with the average value being reported.

2.3.3.4. Crumb Structure (C-Cell Imaging)

Treatment loaves were evaluated using C-Cell Imaging (Calibre Control International, Ltd, Warrington, UK) by cutting each loaf into 1.3 cm slices (±0.5 cm) with an electrical food slicer (Chef's Choice, Int., Colorado Springs, Co.). Every fifth piece from the base end with the break and shred facing upward was used for evaluation. Images were taken with the break and shred located on the left side of the slice. One slice from each loaf and three loaves from each treatment

were imaged using the system. Images were analyzed using C-Cell imaging software (C-Cell Version 2.0, Campden & Chorleywood Food Research Association Group, Gloucestershire, UK) which accompanied the equipment. Values determined for each treatment included slice area, number of cells, area of cells, area of holes, number of holes, volume of holes, cell wall thickness, and cell wall diameter.

2.3.3.5. Texture Profile Analysis (TPA) and Staling Study

The two slices in the middle were tested using the texture profile analysis (TPA) on the TA-XT2 Texture Analyzer (Texture Technology Corp., NY). A 2.5 cm cylindrical probe was used to compress the samples which consisted of two 0.5″ slices stacked on top of each other. The test parameters are listed in Table 2.7. To examine the effect of staling on the bread samples, the TPA was conducted again 1, 3, and 7 days after baking. The slices were stored inside 10×14" aluminum bags (OD PAKVF3.5M Mylar Foil bags) with a mold inhibitor (2000cc Oxygen absorber, Impak Corporation) to prevent molding before testing. Each sample was run in triplicate with the average value being reported.

Table 2.7. Texture profile analysis (TPA) test parameters

Parameters	
Sample Thickness	2.5 cm
Pre-Test Speed	1 mm/s
Test Speed	5 mm/s
Post-Test Speed	5 mm/s
Strain	75%
Holding period	5 s
Trigger Force	5 g

2.4. Statistical Analysis

The average value and standard error were reported. Levenne's test for homogeneity was conducted on the data sets. The data sets that showed a significant result in Levenne's were transformed in order to meet the assumption that all the variances were equal across the samples. The data from the experiments were then analyzed using either a one-way, two-way, or three-way analysis of variance (ANOVA), and means separations were done using the Ryan-Einot-Gabriel-Welsh and Quiot (REGWQ). All the data sets were combined, and correlations were found. SAS version 9.4 was the software used to run the analysis (SAS Institute, 2013).

2.5. References

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Chapter 3 - Results and Discussions

3.1. Cricket Protein Powder Characterization

3.1.1. Composition

The two types of cricket protein powders used were Entomo Farms and GrioPro. Both powders contain the same species of crickets, *Acheta domesticus* and *Gryllodes sigillatus*; however, processing differs between the two powders. GrioPro makes a slurry, pasteurizes it, and uses a spray dryer to make their powder. On the other hand, Entomo Farms washes their crickets with water, roasts them in the oven, and grinds them into a powder. The compositional information for both cricket protein powders can be seen in Table 2.1. The ash, fiber, calcium, potassium, and sodium levels were similar in both proteins. Both powders show high levels of protein at 61.83 and 67 g/100g, respectively for Entomo Farms and GrioPro. Both values fall within the average protein content of 61% reported for the *Orthoptera* order which includes grasshoppers, locusts, and crickets (Van Huis and Dunkel, 2016). However, Bosch et al. (2014) state that since chitin contains non-protein nitrogen and makes up 1-7% of an insect's body there can be an overestimation of the protein quantity (Bosch et al., 2014). Therefore, the values reported for both cricket protein powders could be higher than the actual amount. Additionally, both insects have a higher protein content when compared to raw meat (20-25%), cereals (10-15%), pulses (21-25%), eggs (13%), milk (3.5%), and soy (36.5%) (Henchion et al., 2017; Yi et al., 2013). The amount of cholesterol was higher in GrioPro cricket protein powder compared to Entomo Farms cricket protein powder. Insects have a saturated to unsaturated ratio of less than 40% which compares similarly to fish and poultry; however, insects have a higher ratio of the essential polyunsaturates, linoleic and linolenic (De Foliart, 1991). The polyunsaturated fatty acids can help prevent depression and increase cognitive function (Van Huis and Dunkel, 2016).

The biggest differences between the two cricket protein powders was the amount of iron, B12 and the moisture content. GrioPro cricket protein powder had a higher amount of iron at 6.00 mg/100g compared to Entomo Farms cricket protein powder at 2.00 mg/100g. Both powders' iron quantity was lower than the reported value for the large African cricket, *Gryllidae sp*, at 20.08 mg/100g (Adebowale et al., 2005). However, GrioPro cricket protein powder's iron quantity did compare favorably with the values reported for the *Cirina forda* larvae (5.34 mg/100g) and the

silkworm pupal (6.33 mg/100g) (Omotoso, 2006 and 2015). Entomo Farms cricket protein powder has a higher amount of B12 available at 24.0 μg/100g in comparison to GrioPro cricket protein powder at 8.52 μg/100g; however; it also has a much higher moisture content of 2.6% compared to the moisture content of GrioPro cricket protein powder at 1.33%. The differences seen in the nutritional factors can be due to various factors. Van Huis (2013) states that gender, developmental stage, diet, and environmental factors can alter the nutritional composition of the insects. Furthermore, processing also affects the bioavailability of nutritional compounds (Van Huis and Dunkel, 2016). Kinyuru et al. (2010), showed a general decrease in the vitamin contents from the fresh, toasted, and dried samples of termites and grasshoppers. Due to the good nutritional profile, cricket protein powder could be used as an enrichment agent in order to increase the amount of protein and improve the nutritional profile of baked goods especially gluten free products. Baked products made from gluten free flours tend to be lower in protein, lower in fiber, and lack vitamins, minerals, and antioxidant levels in comparison to their wheat-based counterparts (Missbach et al., 2015; Padalino et al., 2016; Menga et al., 2017). This lack in nutrition is caused by the gluten free flours coming from isolated starches or refined flours which are not generally enriched/fortified (Padalino et al., 2016). Therefore, enrichment of gluten free flours with cricket protein powder could help improve the nutritional profile and could potentially create products on par with their wheat based counterparts.

3.1.2. Microbial Load

The aerobic plate count (APC) indicates the level of microorganisms in a product. The Entomo Farms cricket protein powder (E) had a much higher aerobic plate count at 6.10 $\log_{10}$ CFU/g than that of GrioPro cricket protein powder at <0.50 $\log_{10}$ CFU/g. However, the certificate of analysis (COA) provided for Entomo Farms had a lower value for the aerobic plate count of 2.70 $\log_{10}$. Therefore, during storage the bacteria had time to grow since Entomo Farms has a much higher moisture content, which allowed more water to be available for microbial growth. Thus, accurate storage of the cricket protein powders is crucial to ensure their safety for consumption over time. Every microorganism has a different tolerance level in food; however, being higher than 5.00 $\log_{10}$ poses a safety concern for human consumption when it comes to shellfish (Food and Nutrition Board, 1985). Furthermore, cereals generally contain bacterial populations approximately 6.00 $\log_{10}$ which is comparable to the Entomo Farms cricket protein powder result

and do not pose a significant risk if they are used as an ingredient in a food product that undergoes further heat processing (Food and Nutrition Board, 1985). Therefore, testing of products after incorporation of cricket protein products into baked goods is also necessary to ensure consumer safety. According to Spiegel et al. (2013), the insect species, feed, environment, and processing are all factors affecting the safety hazards posed by insects. The feed can be contaminated with mycotoxins, natural toxins, heavy metals, pesticides and pathogens (Spiegel et al., 2013). Additionally, the insects themselves can lead to allergic reactions or can be carriers of pathogens (Spiegel et al., 2013). Therefore, the difference in aerobic plate count can be due to the different processing methods used (roasting and grinding *vs*. pasteurization and spray drying). Furthermore, the differences in feed and rearing practices could also be cause for Entomo Farms cricket protein powder having a higher number of aerobic bacteria present. Stoops et al. (2016) found that mealworms and grasshoppers both harbored Pseudomonads which are important spoilage organisms in meat, fish, eggs, milk, tofu and vegetables. This led them to conclude that a kill step was necessary during processing to ensure the safe consumption of insects (Stoops et al., 2016). Van Huis and Dunkel (2016) reported that a short heating step was enough to eliminate *E. coli* and Salmonella; however, spore forming bacteria introduced through soil contact cannot be fully eliminated by boiling. The silkworm pupa has been reported to contain arginine kinase as an allergenic compound (Belluco et al., 2013). Yellow mealworms were found to also contain arginine kinase and tropomyosin (Van Huis and Dunkel, 2016). *In vitro* tests have found that people with crustacean, shrimp, or house dust mite allergies have the possibility of developing allergic reactions to insects (Van Huis and Dunkel, 2016). Currently, no laws exist for the regulating of insect additions in food. Therefore, in the future should insect powders begin increasing in commercial markets then new legislations will need to be made to ensure the protein powders are food grade and safe for human consumption. Additionally, since every insect is different further studies will be necessary to create optimized rearing and processing methods that can limit the factors (feed, environment, and post-processing) which affect the contamination of the insects.

3.1.3. Water Holding Capacity

The water holding capacity (WHC) represents how much liquid the proteins can absorb. As can be seen in Figure 3.1, Entomo Farms cricket protein powder can absorb around 2.5 times

its weight while GrioPro cricket protein powder absorbed around 3 times its weight. Entomo Farms cricket protein powder showed a slight increase in WHC as the pH becomes less acidic; however, there was no significant differences between the WHC at pH 7 and pH 10 (Table 3.3). This increase of WHC in less acidic conditions was more drastic in GrioPro cricket protein powder ranging from 305.7±0.3% to 365.3±2.4% where the WHC was significantly different at every pH (Table 3.3). The values for GrioPro cricket protein powder compared favorably with *Cirina forda* larvae (300%) while the values for Entomo Farms cricket protein powder were closer to those of the giant African cricket (238.47%) (Omotoso, 2006; Adebowale et al., 2005). Both cricket protein powders had a higher value range of WHC in comparison to the silkworm larva (175%), the silkworm pupa (115%), and the *Imbrasia oyemensis* larvae (65.44 to 86.89%) (Omotoso, 2015; Akpossan et al., 2015). Overall, GrioPro cricket protein powder across all the different pHs was significantly different from Entomo Farms cricket protein powder (Table 3.2) indicating that the type of processing does influence the powder's ability to retain water. The 2-way ANOVA shows that the WHC*pH interaction was significant (Table 3.1) therefore, it can be concluded that differences in pH will influence the amount of liquid absorbed. According to Akpossan et al. (2015), the intrinsic factors that affect water binding capacities include amino acid composition, protein conformation, and surface polarity/hydrophobicity. Under acidic conditions proteins denature and unfold thereby revealing hydrophobic groups (Omotoso, 2006). Therefore, the conformation of the protein will change, and the surface polarity shifts to repel water thus affecting the water holding capacity as seen in the lower WHC values at acidic pHs. Since both proteins had high WHC values across the various pHs, it can be concluded that their structures contain hydrophilic constituents. In comparison, wheat flour has a water holding capacity of 90.7% while oat flour has a water holding capacity of 95.8% and rye flour has a much higher water holding capacity of 124.8% respectively (Mesias and Morales, 2017). Therefore, the cricket protein powders' water holding capacity is higher than cereal flours and may create a competition for water when incorporated into doughs.

3.1.4. Solubility

According to Hall et al. (2017) the first property that should be examined when developing a novel protein ingredient is solubility as it is a good indicator for the emulsifying and foaming capacities. Entomo Farms cricket protein powder, ranging from 2.44±0.39 g/ml to 4.70±0.03 g/ml, increased significantly in solubility as the pH became less acidic (Figure 3.2). In contrast, GrioPro

cricket protein powder showed no significant difference in solubility between the pHs of 3, 5, and 7 (Figure 3.2). The solubility at a pH of 10 of 1.97±0.08 g/ml was significantly higher than that of the solubility at a pH of 3 of 0.92±0.05g/ml for the GrioPro cricket protein powder (Figure 3.2). Literature states that the African giant cricket, cricket protein hydrolysates and *Cirina forda* larvae become more soluble in alkaline pH which corresponds to the results noted in Entomo Farms cricket protein powder and GrioPro cricket protein powders (Adebowale et al., 2005; Hall et al., 2017; Omotoso, 2006). However, these results differ from the solubility trends found in silkworm and *Imbrasia oyemensis* larvae where the proteins are more soluble in an acidic pH (Omotoso, 2015; Akpossan et al., 2015). Overall, Entomo Farms cricket protein powder was significantly higher across all pHs than that of GrioPro cricket protein powder; however, the solubility of Entomo Farms cricket protein powder at a pH of 3 was not significantly different than the solubility of GrioPro cricket protein powder at a pH of 10 (Table 3.3). The lower solubility seen in GrioPro cricket protein powder may have been related to molecular weight distribution (as seen in the SDS-PAGE and SE-HPLC analysis) since in general, larger molecules have lower surface areas therefore making them less soluble than smaller particles (Buckton and Beezer, 1992). Furthermore, the 2-way ANOVA showed that the solubility*pH interaction was significant (Table 3.1). Thus, the pH does influence the solubility when it comes to Entomo Farms cricket protein powder. In contrast, GrioPro cricket protein powder maintained a more consistent solubility until a drastic pH change occurs. Changes in pH cause the net charges on the proteins to change and the net charges influence protein structure and the attractive and repulsive affinities with water which affect the solubility (Akpossan et al., 2015). Furthermore, acidic pH may cause denaturation which in turn may reduce the hydration of a protein and the unfolding of the protein reveals more hydrophobic groups thus reducing the solubility of the protein (Omotoso, 2006). Lastly, below a pH of 4 carboxyl groups change to a non-ionized form which causes a reduction in the peptide's affinity to water molecules (Hall et al., 2017). This helps explain why the lowest solubility for both cricket protein powders was seen at a pH of 3. Product developers will need to consider pH when developing new products to help decide which protein powder is best to use depending on their end-product quality specifications.

3.1.5. SDS-PAGE

The total proteins (TP), the soluble proteins (SP), and the insoluble proteins (IP) were analyzed using SDS-PAGE to compare protein composition and the molecular weight of individual proteins in the samples (Figure 3.3). All three fractions for the wheat showed bands within a range of 30 to 160 kDa (Figure 3.3). According to Southan and MacRitchie (1999), 80-120 kDa correspond to high molecular weight glutenin-subunits, 30-55 kDa corresponds to low molecular weight glutenin-subunits, 20-30 kDa corresponds to gliadins, and >20 kDa corresponds to albumins and globulins. For the cricket proteins only faint bands were seen in the gels. This may have been due to poor solubility in the SDS-PAGE sample buffer and to some of the proteins only partially migrating into the gel. Only a couple of bands were visible for Entomo Farms cricket protein powder and no bands were visible for GrioPro cricket protein powder extracts. Entomo Farms cricket protein powder had bands visible at 40 kDa, at 60 kDa, and from 80 to 160 kDa (Figure 3.3). This compares to the results found by Yi et al. (2013) on 5 varieties of insects (*T. molitor*, *A. diaperinus*, *Z. morio*, *A. domesticus*, and *B. dubia*) where bands were present at 32–95 kDa and >95 kDa. Hall et al. (2017) also saw several bands ranging from 14.4 kDa to 212 kDa in unhydrolyzed cricket protein.

Extracts from both cricket powders did show dark bands at the bottom of the sample wells which suggests that there were some proteins in the extracts that did not enter the gel during analysis. As will be seen later when SE-HPLC results are discussed, large molecular weight proteins were noted in both samples, but especially in the extracts from the GrioPro cricket protein powders. High molecular weight proteins have been identified in insects previously including muscle proteins such as M-line protein present in flight and leg muscles (400 kDa), and kettin leg and flight muscle isoforms (500 kDa and 700 kDa respectively) (Yi et al., 2013). More studies need to be done to identify the range of bands corresponding to each species of insect.

3.1.6. Raw Materials SP-IP HPLC Chromatograms

For wheat, SEC- HPLC chromatograms are typically divided into four sections from larger molecular weight to smaller molecular weight proteins: large polymeric proteins (composed of both HMW-GS and LMW-GS with predominately HMW-GS between 11-13 min), smaller polymeric proteins (13-17 min), large monomeric proteins (gliadins between 17-23 min), and smaller monomeric proteins (albumins and globulins between 23-26 min) (Johansson et al., 2001;

Kuktaite et al., 2004; Suchy et al., 2003). The HPLC chromatograms for the soluble proteins (SP) and the insoluble proteins (IP) can be seen in Figure 3.4-a,b for wheat and both types of cricket protein powders. Note that the figures have been normalized by adjusting the highest peak area to 1 for visual comparison purposes. The wheat flour SP profile differs from both type of cricket protein powders and has its highest intensity peak at a retention time of 18 min (Figure 3.4-a). Both Entomo Farms and GrioPro cricket protein powders have their highest intensity peaks at retention times of 20 and 24 minutes (Figure 3.4-a). Retention time is affected by the physical size of the compound, where smaller molecules travel longer in the column. Thus, the cricket powders generally have lower molecular weight proteins in the SP fraction than wheat does and contain substantially more low molecular proteins than did the wheat flour. GrioPro cricket protein powder differs from Entomo Farms cricket protein powder by having a peak at a retention time of 11 minutes which is absent in Entomo Farms cricket protein powder (Figure 3.4-a) showing that GrioPro cricket protein powder had a small amount of high molecular weight polymeric proteins in the SP fraction compared to the Entomo Farms cricket protein powder.

The IP profiles all showed the highest intensity peak at a retention time of 11 min (Figure 3.4-b) showing that both Entomo Farms and GrioPro cricket protein powders contain large molecular weight proteins in the IP fraction. Both Entomo Farms and GrioPro cricket protein powders showed peaks at retention times 20 and 24 min not seen in the wheat flour. In wheat, the IP protein fraction tends to have only very high molecular weight polymeric proteins, thus the IP from both cricket powders had low molecular weight proteins in addition to the high molecular weight proteins seen in the chromatograms. This is especially interesting at the high molecular weight polymeric proteins found in wheat have been related to dough strength. No research has been done to characterize the insect proteins via HPLC, therefore future research is needed to identify the proteins present in the chromatograms of the cricket protein powders.

3.1.7. Reduced vs. Non-Reduced SP-IP HPLC Chromatograms

To determine if the high molecular weight proteins seen in the cricket protein powders were polymeric proteins complexes held together by disulfide bonds, samples were analyzed both under non-reducing conditions and with a reducing agent added. The addition of the reducing agent, BME, to the extracts was able to completely break down both the SP and IP high molecular weight complexes found in wheat flour as expected (Figure 3.5-a,b). Neither of the cricket proteins

showed change in their SP chromatograms (Figure 3.5-c,e) showing that none of the proteins present were disulfide bonded polymeric protein complexes. There were some changes in the chromatograms for the IP extracts, suggesting that some of the large molecular weight proteins in IP were disulfide cross-linked proteins or protein complexes, however.

Table 3.1. Two-way analysis of variance (ANOVA) for water holding capacity (WHC) and solubility

	Variable	df	Mean Square	F-value	P-value
WHC	Protein	1	21.640	5692.59	<0.0001
	pH	3	4.046	354.82	<0.0001
	Protein*pH	3	1.292	113.30	<0.0001
	Error	16	0.004		
Solubility	Protein	1	27.510	387.36	<0.0001
	pH	3	3.038	42.78	<0.0001
	Protein*pH	3	0.388	5.46	0.0089
	Error	16	0.071		

[1]WHC was transformed into a square root for analysis

Table 3.2. One-way analysis of variance (ANOVA) for water holding capacity (WHC) and solubility

Variable	df	Mean Square	F-value	P-value
WHC	7	3.854	1013.85	<0.0001
Error	16	0.004		
Solubility	7	5.398	76.01	<0.0001
Error	16	0.071		

[1]WHC was transformed into a square root for analysis

Table 3.3. Water holding capacity (WHC) and solubility of Entomo Farms (E) and GrioPro (G) cricket protein powders across a range of pHs

Powder type	pH	Water holding capacity (%)	Solubility (g/ml)
E	3	257.3 ± 0.9 g	2.44 ± 0.39 d
	5	266.7 ± 1.7 f	3.37 ± 0.14 c
	7	272.0 ± 0.6 e	4.05 ± 0.06 b
	10	273.7 ± 0.3 e	4.70 ± 0.03 a
G	3	305.7 ± 0.3 d	0.92 ± 0.05 f
	5	317.7 ± 0.3 c	1.36 ± 0.06 ef
	7	345.3 ± 1.8 b	1.75 ± 0.05 e
	10	365.3 ± 2.4 a	1.97 ± 0.08 de

[1]means with the same letter are not significant ($p > 0.05$)

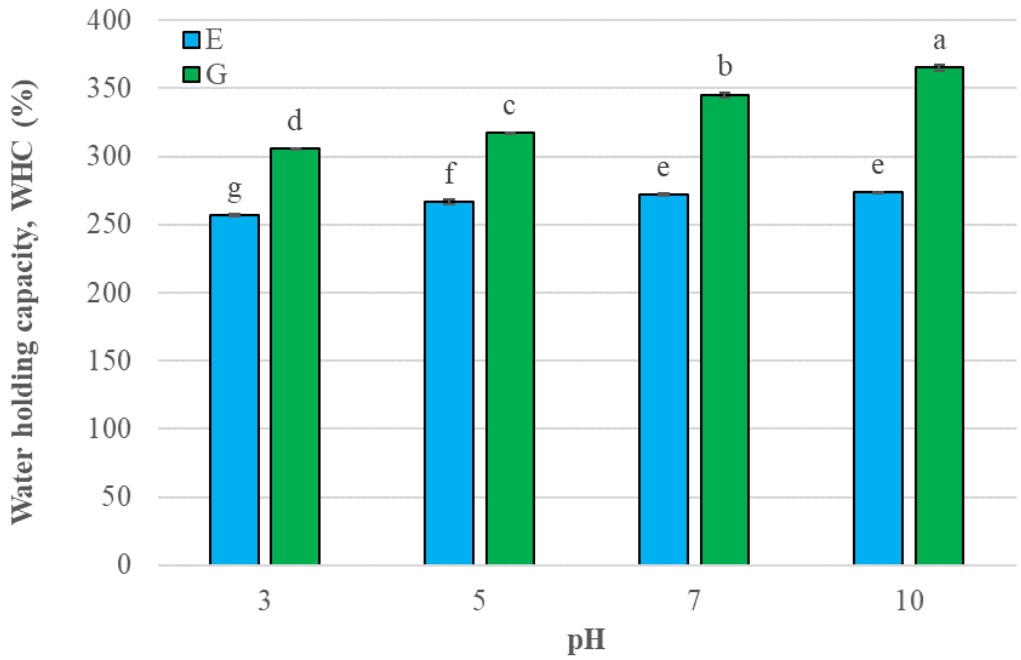


Figure 3.1. Water holding capacity of Entomo Farms (E) and GrioPro (G) cricket protein powders across a range of pHs

[1]means with the same letter are not significant ($p > 0.05$)

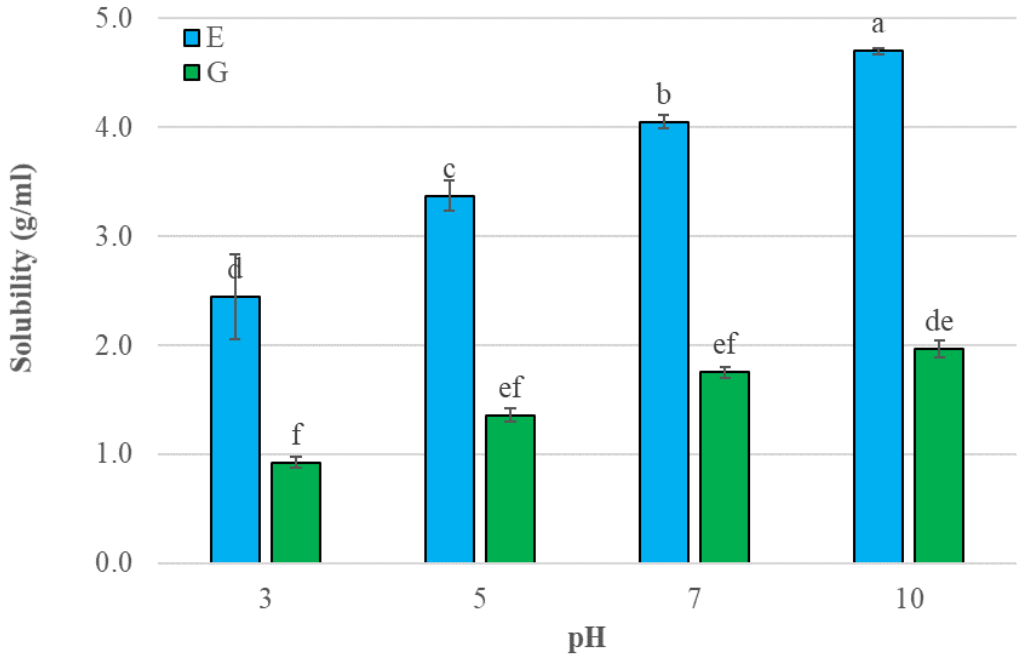


Figure 3.2. Solubility of Entomo Farms (E) and GrioPro (G) cricket protein powders across a range of pHs

[1]means with the same letter are not significant ($p > 0.05$)

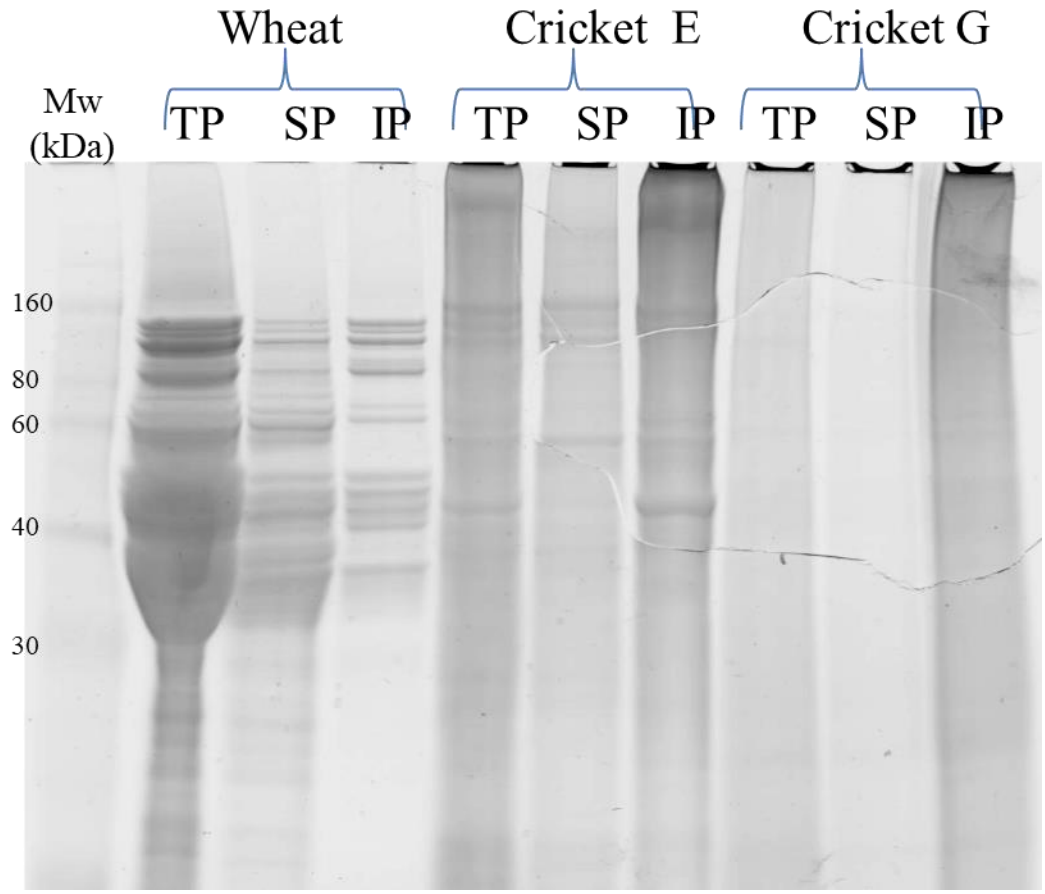
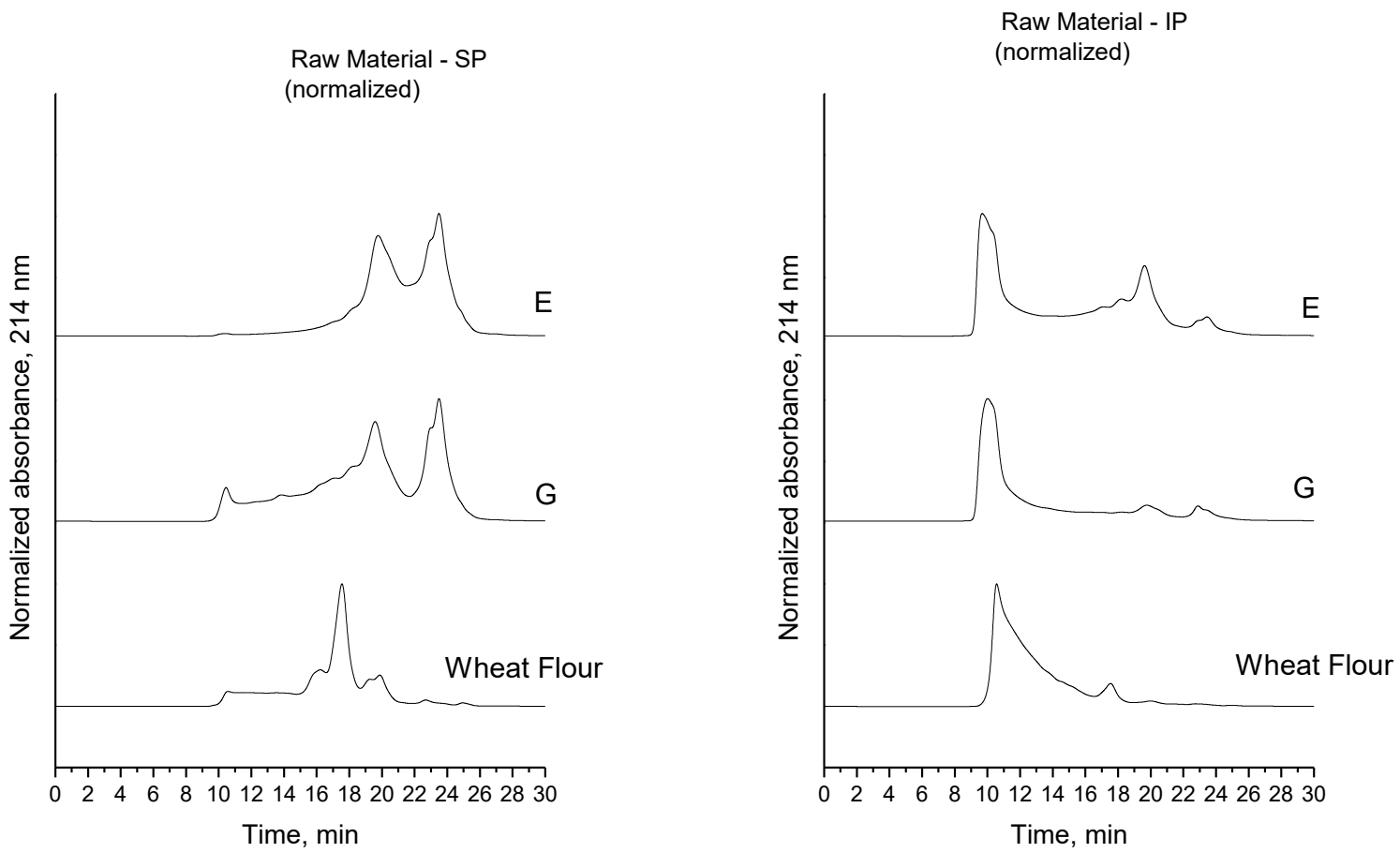


Figure 3.3. SDS-PAGE molecular weight distributions.
Total Protein (TP), Soluble Proteins (SP) and Insoluble Proteins (IP) depicted for Entomo Farms (E) and GrioPro (G) cricket protein powders
[1] Samples loaded on Bis/Tris NuPAGE gel using MOPS running buffer

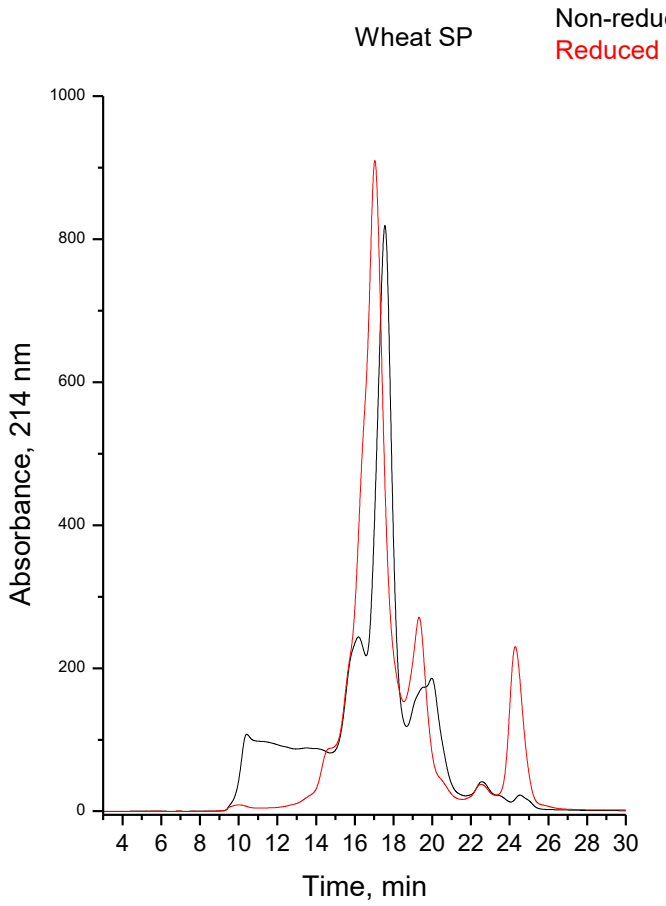


(a) (b)

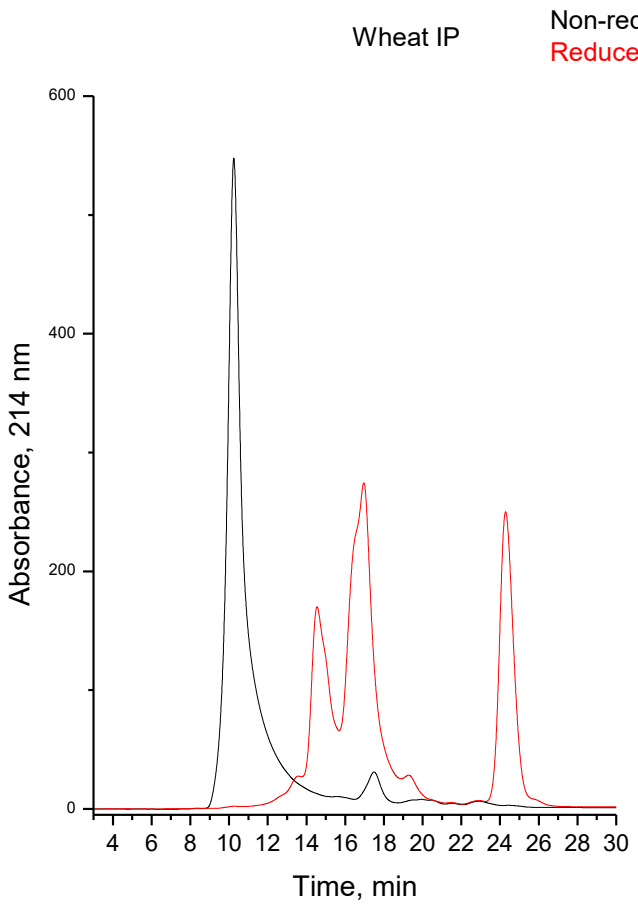
Figure 3.4. Raw materials size exclusion HPLC chromatograms[1]

(a) SP chromatogram, and (b) IP chromatogram for the wheat flour, Entomo Farms (E), and GrioPro (G) cricket protein powder.

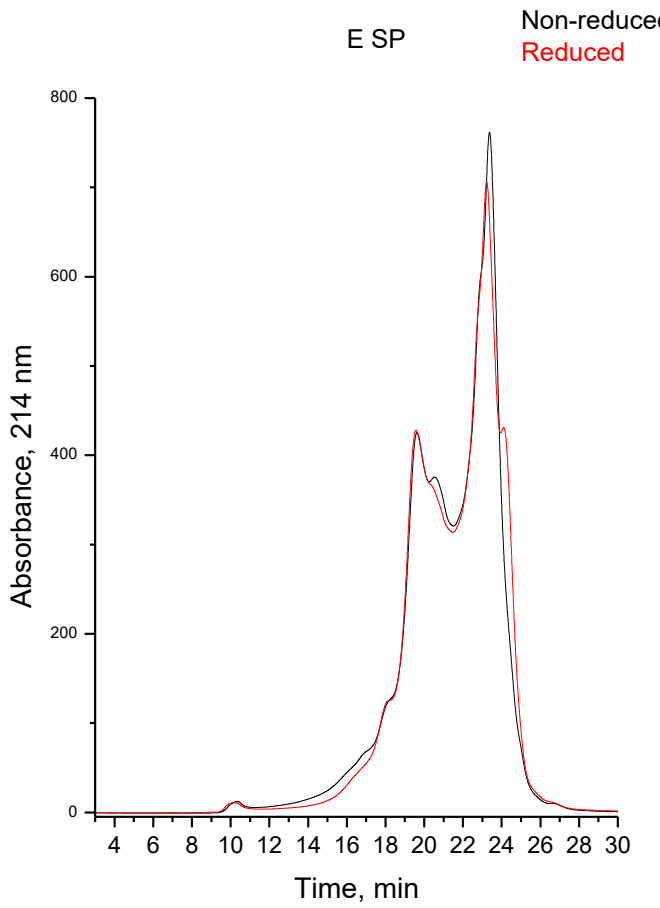
[1] Graphs have been normalized for visual comparison purposes


Wheat SP
Non-reduced
Reduced
Absorbance, 214 nm
Time, min
0
200
400
600
800
1000
4 6 8 10 12 14 16 18 20 22 24 26 28 30

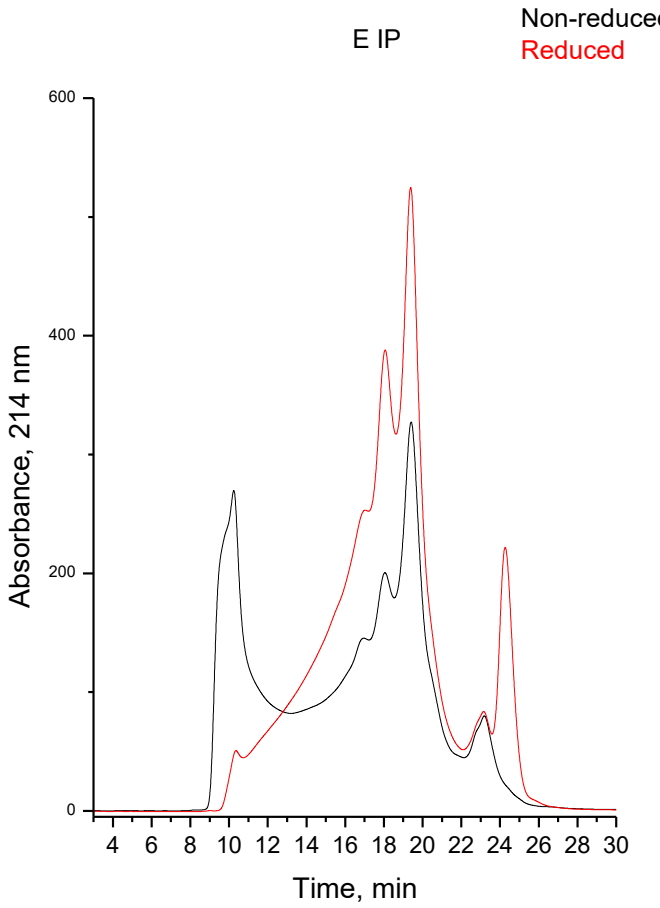
(a)


Wheat IP
Non-reduced
Reduced
Absorbance, 214 nm
Time, min
0
200
400
600
4 6 8 10 12 14 16 18 20 22 24 26 28 30

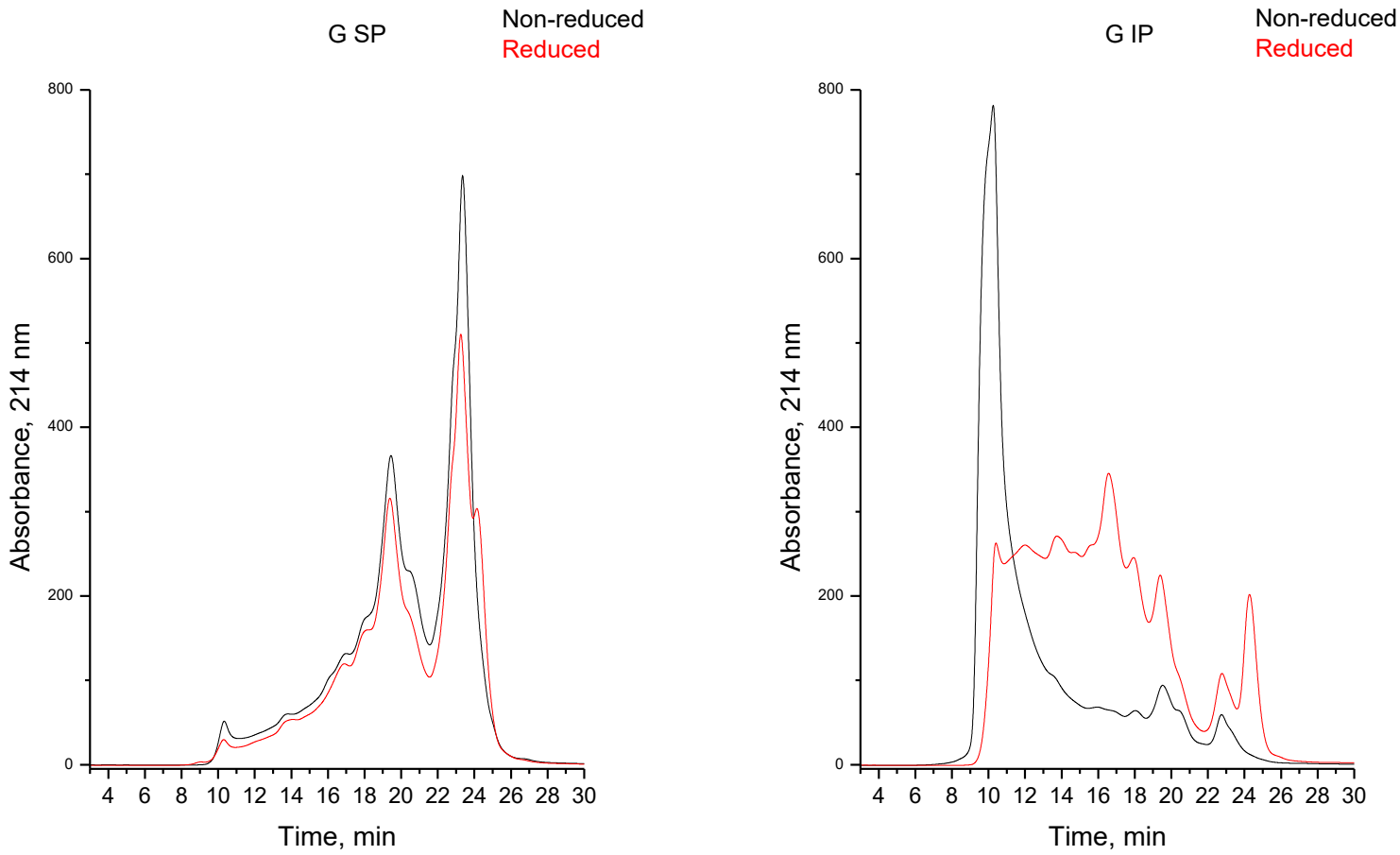
(b)


E SP
Non-reduced
Reduced
Absorbance, 214 nm
Time, min
0
200
400
600
800
4 6 8 10 12 14 16 18 20 22 24 26 28 30

(c)


E IP
Non-reduced
Reduced
Absorbance, 214 nm
Time, min
0
200
400
600
4 6 8 10 12 14 16 18 20 22 24 26 28 30

(d)



(e) (f)

Figure 3.5. Raw materials reduced vs. non-reduced size exclusion HPLC chromatograms

(a) SP, and (b) IP reduced vs. non-reduced chromatograms of the control (wheat flour)
(c) SP, and (d) IP reduced vs. non-reduced chromatograms of the Entomo Farms (E) cricket protein powder
(e) SP, and (f) IP reduced vs. non-reduced chromatograms of the GrioPro (G) cricket protein powder
[1] 0.1 BME was the reducing agent used

3.2. Dough Development

3.2.1. MixoLab at Constant Water Absorption

The three-dimensional gluten network is formed during mixing and heating causes the rheological properties of the dough to change due to protein-carbohydrate interactions between the starch granules and the gluten proteins (Singh and Singh, 2013). In order to observe the effect of the cricket protein powder on the mixing and pasting properties the MixoLab was used. The MixoLab profiles can be seen in Figure 3.6-a,b for doughs containing 0, 10, and 20% replacement levels of Entomo Farms or GrioPro cricket protein powders. The C1 torque represents the maximum torque during mixing (dough development) (Rosell et al., 2011). Only the 20% replacement level of GrioPro cricket protein powder was significantly higher in C1 torque at 1.31 Nm than the control at 1.13 Nm (Table 3.5). All other treatments showed no significant difference in C1 torque to each other or to the control (Table 3.5). Thus, the 20% replacement level of GrioPro cricket protein powder resulted in a much stronger dough than all the other treatments. These results fall in line with what has been seen in addition of mustard seed flour which caused no difference in C1 torque (Mironeasa and Codina, 2017) and the 20% GrioPro cricket protein powder replacement level corresponds with what was seen in the addition of soy protein and transglutaminase to rice flour which increased C1 torque (Rosell et al., 2011). However, a decrease in C1 torque was observed when grape seed flour was incorporated into wheat flour (Mironeasa et al., 2012).

Two distinct processes occur during mixing: hydration of the flour components and application of energy (MacRitchie, 2016). Flour and water mixed together cause the conformations and intermolecular interactions of the proteins to change and form disulphide bonds which make up the three-dimensional gluten network (Meredith and Wren, 1969; Belton, 1999).Water behaves as both a lubricant and an inert filler (Singh and Singh, 2013) therefore imparting high levels of mobility to the high molecular weight subunits (HMW) and facilitating hydrogen bonding between the HMW subunits (responsible for providing dough elasticity) (Belton, 1999). Insufficient hydration creates a discontinuous protein network (Singh and Singh, 2013) due to the decrease in mobility and decrease in hydrogen bonding thus forming a dense mass (Belton, 1999). This in turn impacts the rheological characteristics of the dough such as extensibility, elasticity, and gas retention which results in lower quality bread as explained previously (sections 3.1.6 and 3.1.7) by

SPI-IPI peak areas in doughs (Mironeasa and Codina, 2017). Therefore, the increase in C1 torque seen in the 20% replacement of GrioPro cricket protein powder could be explained by there not being enough water available to fully hydrate the wheat proteins, especially since GrioPro cricket protein powder had a higher water holding capacity than Entomo Farms cricket protein powder which could be competing for water and further preventing the flour proteins from hydrating (Figure 3.1). Such an effect was seen in rice flour + soy protein + transglutaminase mixture (gluten-free application) where the high water holding capacity of the soy protein limited the water available for the other components and caused an increase in C1 torque (Rosell et al., 2011).

In addition, the shear and tensile forces applied during mixing also help develop the continuous network by stretching the HMW glutenin subunits into more extended conformations with potentially more time and energy required to unravel larger sized glutenin molecules (Southan and MacRitchie, 1999). Thus, the increased C1 torque in the 20% GrioPro cricket protein powder replacement level could also be due to the larger amount of high molecular weight proteins found in this dough (Figure 3.9-a,b). This idea is supported by the significant correlation between the insoluble proteins found in dough with C1 torque ($r = 0.94$, P-value = 0.005).

The Entomo Farms cricket protein powder replacement levels had a significantly longer C1 time of 8.08 and 8.33 min in comparison to both the control of 1.67 min and the GrioPro cricket protein powder replacement levels of 1.80 and 0.91 min (Table 3.5). For the GrioPro cricket protein powder replacement levels, the 10% replacement was not significantly different in time from the control; however, the 20% replacement level was significantly shorter in C1 time from any other treatment (Table 3.5). The longer development time seen in doughs containing Entomo Farms cricket protein powder correspond with results reported on the addition of mustard seed flour to wheat flour or the addition of soy protein and transglutaminase on rice flour where C1 time increased as the replacement level increased (Rosell et al., 2011; Mironeasa et al., 2012). According to Rosell et al. (2011), the development time (C1 time) represents the time required for all the compounds to be hydrated. However, the opposite was seen in the C1 times when cricket protein powder replaced some wheat flour. GrioPro cricket protein powder containing doughs which contained larger amounts of high molecular weight proteins (Figure 3.9-a,b) and had a higher water holding capacity (Figure 3.1) and exhibited lower development times (C1 time). This could be explained by the fact that mixing extends the lower molecular weight proteins first before the larger molecular weight proteins (MacRitchie, 2016).

As discussed in the raw materials SP-IP HPLC chromatograms, Entomo Farms cricket protein powder contained lower molecular weight proteins not present in the GrioPro cricket protein powders in the IP fraction. Therefore, it could be possible that the increased amount of low molecular weight proteins found in Entomo Farm IP protein fraction caused a time delay in mixing doughs containing Entomo Farm cricket powder as mixing took longer to move on to the higher molecular weight proteins. The lower molecular weight proteins in the Entomo Farm cricket powder may have also served to 'dilute' larger proteins which then took longer to interact during mixing. On the other hand, the larger molecular weight proteins found in GrioPro cricket protein powder containing doughs combined with the un-hydrated wheat proteins could be producing such a dense mass that the doughs peak faster than the Entomo Farms cricket protein powder containing doughs. This is supported by the significant inverse correlation between the insoluble polymeric proteins found in the cricket protein powders with the C1 time ($r = -0.79$, P-value = 0.06, significant at $P < 0.1$).

Finally, the stability time showed a decrease in the 20% Entomo Farms cricket protein powder replacement level (6.88 min) in comparison to the control (11.04 min) (Table 3.5). A decrease in stability was also reported when soybean protein and transglutaminase was added to rice flour (Rosell et al., 2011). The 10% Entomo Farms cricket protein powder replacement level (10.59 min) was not significantly different from the control (11.04 min) in stability time (Table 3.5). On the other hand, both replacement levels of GrioPro cricket protein powder significantly increased the stability time (12.69 and 12.51 min) in comparison to the control sample (11.04 min) (Table 3.5). Additions of either mustard seed flour or grape seed flour also increased the dough stability time (Mironeasa et al., 2012; Mironeasa and Codina, 2017).

Addition of gliadins caused a decrease in dough stability (tolerance to overmixing) while addition of glutenins caused an increase in dough stability (Verbruggen et al., 2001). It could be possible that the smaller sized proteins found in Entomo Farms cricket protein powder could be mimicking the effect of adding gliadins (both represent an addition of smaller polymers) and thereby causing the stability to decrease as was seen in the 20% replacement level. Therefore, the increased stability in GrioPro cricket protein powder containing doughs could be due to the strength provided by the larger sized proteins being added through the protein addition. This increase in high molecular weight proteins may have allowed the dough to sustain itself longer during the mechanical treatment of the mixer since there are more HMW polymers to unravel and

keep up the demand for a higher mixing intensity. Again, this idea is reinforced by the significant correlation between the insoluble polymeric proteins found in the cricket protein powders and stability ($r = 0.99$, P-value < 0.0001).

C2 torque is the minimum torque found during the protein weakening stage which is detected within the range of 52-58°C wherein the starch granules begin to swell (onset of gelatinization) and become the main cause for further torque variations (Rosell et al., 2011; Mironeasa and Codina, 2017). Neither type of cricket protein addition differed in C2 torque from the control. Furthermore, the C2 torque showed no significant difference between replacement levels of either doughs containing Entomo Farms or GrioPro cricket protein powder (Table 3.5). However, GrioPro cricket protein powder containing doughs exhibited significantly higher C2 torque values than Entomo Farms cricket protein powder containing doughs (0.56 and 0.58 Nm vs. 0.44 and 0.41 Nm for 10 and 20% replacement levels, respectively) (Table 3.5). The decrease in C2 torque with the increase in replacement level was also found in addition of mustard seed flour, grape seed flour, and soy protein + transglutaminase to rice flour (Rosell et al., 2011; Mironeasa et al., 2012; Mironeasa and Codina, 2017). This difference in C2 torque could be due to the differing strength of the dough caused by the difference in molecular weight distribution of the polymers found in doughs containing Entomo Farms cricket protein powder vs. those containing GrioPro cricket protein powder. The stronger dough (containing GrioPro cricket protein powder) with increased stability could withstand the protein weakening better while the weaker dough (containing Entomo Farms) with less stability weakened the proteins to a greater extent. The significant correlations between the insoluble proteins in the cricket protein powder ($r = 0.86$, P-value $= 0.03$) and in the insoluble proteins found in doughs ($r = 0.80$, P-value $= 0.06$, significant at $P < 0.1$) with the C2 torque reinforce the previous statement. The inverse correlation between C2 torque and the soluble polymeric proteins found in dough ($r = -0.77$, P-value $= 0.07$, significant at $P < 0.1$) also reinforces the previous statement.

Neither the C2 time nor the C2 temperature (the onset of gelatinization time and temperature) were significantly different in any of the treatments (Table 3.5). Therefore, it can be said that the onset of gelatinization was not delayed, nor did it occur at differing temperatures. The onset of gelatinization occurs during the heating stage of the MixoLab where the starch granules begin absorbing water and swelling (Zhou et al., 2018). The starch granules swell to bursting point and the amylose chains leach out causing an increase in viscosity which leads to a peak torque (C3

torque) (Zhou et al., 2018). Both the 10 and 20% replacement levels of both cricket protein powders showed the same trend for the C3 torque (peak viscosity after starch gelatinization) (Rosell et al., 2011). As the replacement level increased, the C3 torque decreased (Table 3.5). The 10% replacement level of GrioPro cricket protein powder was not significantly different in C3 torque than the control (Table 3.5). The C3 torque corresponds to the dough's peak viscosity, thus it can be said that replacing wheat flour with 10% of GrioPro cricket protein powder does not change the peak viscosity. In general, the Entomo Farms cricket protein powder containing doughs had lower C3 torques (1.46 and 1.35 Nm) than the GrioPro cricket protein powder containing doughs (1.66 and 1.52 Nm) (Table 3.5). The decrease in peak viscosity as the replacement level increases was also observed in the addition of mustard seed flour; however, incorporation of grape seed flour and soy protein + transglutaminase to rice flour caused the peak viscosity to increase (Rosell et al., 2011; Mironeasa et al., 2012; Mironeasa and Codina, 2017).

For the C3 time, only the 20% GrioPro cricket protein powder replacement level was significantly later in time (25.64 min) than any other treatment including the control (24.78 min) (Table 3.5). All other treatments were not significantly different in C3 time (Table 3.5). Therefore, the limited water and higher water holding capacity in GrioPro cricket protein powder could be competing with starch for water therefore not allowing as much amylose to leach out thus decreasing the peak viscosity as seen by the longer time required for the 20% GrioPro cricket protein powder replacement level to reach peak viscosity (C3 time). Soy protein addition resulted in a higher peak viscosity due to its gel forming abilities (Rosell et al., 2011) Therefore, it could be possible that cricket proteins form weak gels as seen by the lower peak viscosity; however, further testing would be necessary to prove this.

C4 torque represents the cooking stability and is the minimum torque during the heating period where the mechanical shear stress physically breakdown the granules causing a decreased viscosity (Rosell et al., 2011; Mironeasa and Codina, 2017). The 10% replacement level of GrioPro cricket protein powder was significantly higher in C4 torque (1.47 Nm) than the control (1.24 Nm); however, the 20% GrioPro cricket protein powder replacement level showed no significant difference to both the control and the 10% GrioPro cricket protein powder replacement level (Table 3.5). GrioPro cricket protein powder containing doughs showed significantly higher C4 torque values (1.47 and 1.39 Nm) in comparison to the Entomo Farms cricket protein powder containing doughs (1.17 and 1.09 Nm) (Table 3.5). This could be because Entomo Farms cricket protein

powder containing doughs had lower peak viscosities to begin with. Therefore, when the shear force began physically breaking down the granules, the consistency was already lower than that of GrioPro cricket protein powder containing doughs and by the time the shear thinning finished the C4 torque was lower for Entomo Farms cricket protein powder containing doughs. A decrease in C4 torque was observed in addition of grape seed flour and with the addition of soy protein + transglutaminase to rice flour (Rosell et al., 2011; Mironeasa et al., 2012).

The C5 torque value is the final consistency after cooling known as setback which is caused by the recrystallization of the amylose chains (Rosell et al., 2011). For the C5 torque values, only the 10 and 20% replacement levels of GrioPro cricket protein powder were significantly different than the control (Table 3.5). All the Entomo Farms cricket protein powder containing doughs (1.54 and 1.50 Nm) were significantly lower in C5 torque values than the GrioPro cricket protein powder containing doughs (1.89 and 1.92 Nm) (Table 3.5). The results for GrioPro cricket protein powder containing doughs correspond to what was observed in the addition of Mustard Seed Flour, Grape Seed Flour, and in the addition of only soy protein to rice flour which caused an increase in C5 torque (Rosell et al., 2011; Mironeasa et al., 2012; Mironeasa and Codina, 2017). The setback is caused by the recrystallization of the amylose chains during cooling (Rosell et al., 2017). Therefore, it can be concluded that neither Entomo Farms nor GrioPro cricket protein powder prevented the setback of the amylose chains, but that GrioPro cricket protein powder allowed more recrystallization to happen as seen by the increased C5 torque. This could be since mobility of the amylose can be inhibited by foreign proteins binding with water from the dough system (Mironeasa and Codina, 2017). Therefore, the lower C5 torque found in Entomo Farms cricket protein powder containing doughs could be because these samples contained less free water in the dough system than found in the dough system of GrioPro cricket protein powder containing doughs. The higher WHC of GrioPro cricket protein powder compared to that of Entomo Farms cricket protein powder supports this idea (Figure 3.1), which requires GrioPro cricket protein powder containing doughs to need more water than the control dough to fully hydrate than what was provided under the constant water absorption protocol. This limitation in water could have limited the amount of free water found in the dough system; thereby, not allowing the cricket proteins in GrioPro cricket protein powder containing doughs to bind as much water as the cricket proteins found in Entomo Farms cricket protein powder containing doughs. Therefore, the Entomo Farms cricket protein powder containing doughs could have been able to inhibit the amylose chain

movement to a larger degree than the GrioPro cricket protein powder containing doughs. Further testing into the bulk and free water ratios would be necessary to prove this.

3.2.2. Water Absorption at Optimization

The optimization protocol for the MixoLab requires each sample's water absorption to be adapted so that the sample reaches a C1 torque value of 1.1±0.05 Nm (Schmiele et al., 2017). Optimized water absorption is an important parameter since it represents the amount of water that is necessary to mix and hydrate the flour components into a dough that is of proper consistency for breadmaking (Hammed et al., 2015). The results showed no significant change in water absorption between any of the Entomo Farms cricket protein powder replacement levels or between the 10% GrioPro cricket protein powder replacement level with the control (Figure 3.7). However, the 20% replacement level of GrioPro cricket protein powder required a significantly larger amount of water (62.7%) for the dough to reach optimum development in comparison to the control (59.8%) (Table 3.7). This corresponds with the high C1 torque found in the MixoLab curve at constant water absorption which could have been due to inadequate hydration of the flour components (Table 3.5) requiring more water to fully hydrate and reach optimum development. The 20% GrioPro cricket protein powder replacement level water absorption result corresponds to the association that high protein content in flour has higher water absorption (Hammed et al., 2015). However, both Entomo Farms and GrioPro cricket protein powders have a similar total protein content; therefore, the differences in behavior could be due to their differences in processing (dry heat vs. moist heat). Furthermore, Ohm et al. (2008) state that increasing the amount of lower molecular weight polymeric proteins were strongly associated with decreasing water absorption necessary in noodle dough. This also explains why Entomo Farms cricket protein powder containing doughs had a lower water absorption than GrioPro cricket protein powder containing doughs since there is a higher number of lower molecular weight subunits found in Entomo Farms cricket protein powder containing doughs; (Table 3.5 and Figure 3.9-a,b) this was also seen by the inverse correlation between soluble polymeric proteins found in the cricket protein powder and water absorption ($r = -0.95$, P-value = 0.004). The increase in water absorption seen in the 20% GrioPro cricket protein powder replacement level fall in line with results reported for increasing concentrations of: soy protein incorporation (Zhou et al., 2018), addition of soy protein hydrolysates (Schmiele et al., 2017), wheat bran incorporation (Xhabiri et al., 2013), and

mushroom flour incorporation (Yuan et al., 2017). However, the opposite trend (decreased water absorption at higher concentrations) was seen in addition of fructooligosaccharides (Schmiele et al., 2017), and in whey protein incorporation (Zhou et al., 2018). Water holding capacity as discussed in Mixolab at constant water absorption also affects the water absorption necessary to meet optimum development as seen in the 20% GrioPro cricket protein powder replacement level which required more hydration due to the high value of water holding capacity reported for GrioPro cricket protein powder (Figure 3.1). Therefore, product developers will be able to add more water when dealing with a larger replacement level of GrioPro cricket protein powder which economically is desirable since flour with high water absorption leads to production of more dough thus increasing bread yield (Hammed et al., 2015).

3.2.3. MixoLab at Optimum Water Absorption

Optimum dough development requires a critical mixing intensity and a critical amount of imparted energy as well as complete hydration of the flour particles to create a continuous gluten network (Singh and Singh, 2013; MacRitchie, 2016). Furthermore, optimally developed dough is a necessity to create good quality bread since a continuous gluten network has extensibility thus allowing for dough inflation and has the strength necessary to resist collapse (MacRitchie, 2016). In order to see the effect of cricket protein powder on the mixing and pasting behaviors of optimally developed dough the MixoLab was run following the optimized water absorption protocol (as reported in water absorption at optimization). The MixoLab profiles for doughs containing 0, 10, and 20% replacement levels of Entomo Farms or GrioPro cricket protein powders can be seen in Figure 3.8-a,b. Since the optimization protocol requires all samples to reach a 1.1 Nm C1 torque (Schmiele et al., 2017), it was evident that there was no significant difference found in the C1 torque (Table 3.9). There was no significant difference in C1 time for any of the GrioPro cricket protein powder containing doughs; however, the Entomo Farms cricket protein powder containing doughs took a significantly longer time to mix as the replacement level increased (from 1.60 to 6.58 to 7.94 min) (Table 3.9). An increase in development time as the concentration increased was also found for addition wheat bran (Xhabiri et al., 2013) or addition of mushroom flour (Yuan et al., 2017).

Water holding capacity and strength of the gluten matrix affect dough development time (C1 time) (Table 3.5). GrioPro cricket protein powder containing doughs, especially at the 20%

replacement levels, have an increased amount of high molecular weight subunits which were imparting strength to the dough as seen in the higher C1 torque under the constant water absorption protocol. This strength was allowing the dough to peak faster since the mixer was attempting to mix the higher molecular weight polymers (Table 3.5). On the other hand, Entomo Farms cricket protein powder containing doughs have more low molecular weight (LMW) proteins which were being mixed/unraveled first and thereby causing the dough to reach its peak later in time (MixoLab constant water absorption data presented in section 3.2.1).

Both replacement levels of GrioPro cricket protein powder extended the stability time of the dough in comparison to the control (12.73 and 12.56 vs. 11.07 min) (Table 3.9). These results correspond to what was seen in incorporation of soy protein (Zhou et al., 2018), and incorporation of wheat bran (Xhabiri et al., 2013). On the other hand, as the replacement level of Entomo Farms cricket protein powder increased the stability time decreased significantly in comparison to the control (9.37 and 7.32 vs. 11.07 min) (Table 3.9) similar to what was seen with the incorporation of soy protein hydrolysates with fructooligosaccharides (Schmiele et al., 2017), incorporation of whey protein (Zhou et al., 2018), and incorporation of mushroom flour (Yuan et al., 2017). Additionally, water holding capacity and dough structure impact dough stability (Zhou et al., 2018). Xhabiri et al. (2013) stated that an increase in dough stability was due to increased interaction of hydrogen bonding between the gluten proteins. Thus, increased water absorption for GrioPro cricket protein powder containing doughs could be allowing for increased hydrogen bonding.

Schmiele et al. (2017) observed that soy protein hydrolysates with fructooligosaccharides acted as a physical hindrance to hydration of wheat proteins by altering the ionic and hydrophobic interactions and covalent and hydrogen bonds. Therefore, it could be possible that Entomo Farms cricket protein powder was also physically hindering the hydration of the gluten proteins during mixing. Another factor is that the increased dough strength provided by the increased amount of high molecular weight proteins in GrioPro cricket protein powder containing doughs allowed the dough to withstand the mixing action longer due to the higher critical intensity necessary to unravel the larger proteins again supported by the correlation between the insoluble polymeric proteins found in the cricket protein powders and the stability ($r = 0.95$, P-value = 0.004).

As for the C2 torque, none of the GrioPro cricket protein powder replacement levels showed any significant difference in torque compared to the control (Table 3.9). However, as the

replacement level of Entomo Farms cricket protein powder increased there was a significant decrease in C2 torque (0.42 and 0.37 Nm) in comparison to the control (0.51 Nm) (Table 3.9). Increase in C2 torque was reported in mushroom flour addition (Yuan et al., 2017) and wheat bran addition (Xhabiri et al., 2013); however, whey protein also decreased C2 torque (Zhou et al., 2018) As discussed in the MixoLab at constant water absorption section, mixing after dough development leads way to a breakdown stage caused by protein weakening (MacRitchie, 2016). Therefore, the C2 torque value acts as an indication of the weakening of the gluten network (Yuan et al., 2017). As stated above, GrioPro cricket protein powder incorporation produces stronger doughs and Entomo Farms cricket protein powder produces weaker doughs. Therefore, the more dramatic decrease in C2 torque found in Entomo Farms cricket protein powder containing doughs was likely due to the weaker doughs breaking down faster due to the decreased stability. Since the increased strength coming from the larger molecular weight proteins found in GrioPro cricket protein powder were helping the dough withstand the mechanical breakdown better than Entomo Farms cricket protein powder containing doughs (MixoLab constant water absorption data presented in section 3.2.1), as seen in the correlation between C2 torque and stability ($r = 0.96$, P-value = 0.003) and between the insoluble polymeric proteins found in the cricket protein powders and C2 torque ($r = 0.90$, P-value = 0.01).

Another possibility would be that the weakening was caused by interference of the sulfhydryl/disulfide interchange reactions which would inhibit the gluten network strength (Zhou et al., 2018). Soy proteins increased disulphide linkages by undergoing sulfhydryl/disulfide interchange reactions with wheat proteins (Schmiele et al., 2017; Zhou et al., 2018). This interfered with gluten network formation and created a weaker dough since the soy protein was possibly hindering the formation of disulfide bonds between other high molecular weight glutenin subunits (Wang et al., 2017a). It could be possible that Entomo Farms cricket protein powder was chemically interfering with the gluten structure, thereby creating weaker doughs. However, further testing would be necessary to prove this. However, when comparing a theoretical IP peak value (calculated by taking 10% or 20% of the IP area for the cricket protein powder and adding 90% or 80% of the IP area of the wheat flour) and comparing it to the actual IP peak areas found in the dough (experimental value) the experimental value was lower for Entomo Farms cricket protein powder containing doughs than the calculated value (Figure 3.10-b). On the other hand, the theoretical SP values for Entomo Farms cricket protein powder containing doughs were higher

than those observed in the experimental value (Figure 3.10-a). This suggests that Entomo Farms cricket powder proteins were chemically interacting with the wheat proteins resulting in a shift in the molecular weight distribution of the proteins to lower molecular weight proteins (shift in proteins from IP to SP) which produced weaker doughs.

Conversely, GrioPro cricket protein powder addition showed no major difference between the theoretical and experimental SP value (Figure 3.10-a) while there was a major increase seen in the experimental IP value at the 10% replacement level in comparison to the theoretical IP value (Figure 3.10-b). Therefore, GrioPro cricket protein powder can increase the amount of insoluble proteins found in the dough; however, more research is necessary to understand the mechanism behind this increase and examine whether GrioPro cricket protein powder can chemically interact with the gluten network.

C2 time results showed that all the Entomo Farms cricket protein powder treatments and the 10% replacement level of GrioPro cricket protein powder had no significant difference in time compared to the control dough (Table 3.9). The 20% replacement level of GrioPro cricket protein powder was significantly later in C2 time compared to all other treatments; therefore, it can be said that there was a delay in the onset of gelatinization in the 20% GrioPro cricket protein powder replacement level. Furthermore, this is reinforced by the onset of gelatinization temperature where again only the 20% replacement level of GrioPro cricket protein powder was the only one with a significantly higher temperature of 74.9°C compared to all other treatments (Table 3.9). The delay in gelatinization caused the dough to begin gelatinizing at a higher temperature in comparison to the other treatments.

This also caused the peak viscosity to be recorded at a significantly later time for the 20% GrioPro cricket protein powder replacement level as can be seen in the C3 time column of Table 3.9. All other treatments showed no significantly difference in C3 time (Table 3.9). C3 torque results show a trend of decreasing torque as the replacement level of both cricket protein powders increases (Table 3.9). Doughs containing Entomo Farms cricket protein powder (from 1.72 to 1.59 to 1.46 Nm) showed a more drastic decrease in C3 torque compared to doughs containing GrioPro cricket protein powder (from 1.72 to 1.67 to 1.51 Nm) (Table 3.9). With the increasing temperature, the proteins begin denaturizing and the starch begins to swell (Schmiele et al., 2017) and as discussed previously (Table 3.5) the leaching amylose is responsible for the increasing peak viscosity. Starch, protein, and other flour components competitively interact with each other when

it comes to water (Wang et al., 2017b). Therefore, the increased water holding capacity found in GrioPro cricket protein powder could be preventing the starch from swelling as fast as doughs containing Entomo Farms cricket protein powder (Figure 3.1). Therefore, inhibiting the starch from escaping more readily as was seen in the delay in the onset of gelatinization temperature and the increase in onset of gelatinization temperature for the 20% replacement level of GrioPro cricket protein powder. This would also explain why the 20% replacement level had a lower peak viscosity (C3 torque) than the 10% GrioPro cricket protein powder replacement level and why it took longer for the 20% replacement level to reach peak viscosity (C3 time). The decreased peak torque seen at all the treatments containing cricket protein powder could also be due to the poor gelling capacity of the cricket proteins (Table 3.5). Lastly, the potential interruption of disulfide bond formation as discussed previously (Table 3.5) may have diminished the binding of wheat protein to starch (Wang et al., 2017b). This is turn may have diminished hydrogen bonding between the starch and gluten which was promoted during gelatinization thus leading to a lower peak viscosity (C3 torque) (Wang et al., 2017b). Further testing would be necessary to see if the cricket proteins were chemically or physically interfering with the protein-protein or protein-starch interactions. However, Figure 3.10-b shows how the experimental value of IP for GrioPro cricket protein powder containing doughs was much higher than the calculated value. Lower peak torque viscosity as the concentration increased was also reported for incorporation of mushroom flours (Yuan et al., 2017), for wheat bran addition (Xhabiri et al., 2013), and for incorporation of soy protein (Zhou et al., 2018). However, due to the gelling effect of whey protein, there was an increase in C3 torque (Zhou et al., 2018).

The 10% replacement level of Entomo Farms cricket protein powder showed no significant difference in C4 torque value compared to the control; however, the 20% replacement level was significantly lower in torque value (1.40 vs. 1.18 Nm) (Table 3.9). Additionally, the 10% replacement level of GrioPro cricket protein powder also showed no significant difference in C4 torque compared to the control and the 20% replacement level was also significantly lower in torque value (1.40 vs. 1.27 Nm) (Table 3.9). A decrease in C4 torque was also observed with incorporation of soy protein hydrolysates with Fructooligosaccharides (Schmiele et al., 2017), addition of mushroom flour (Yuan et al., 2017), and addition of bran (Xhabiri et al., 2013); however, whey protein (Zhou et al., 2018) increased the C4 torque. As discussed previously (Table 3.5) lower peak viscosities led to lower C4 torque values.

Lower C5 torque could be due to the higher water holding capacity exhibited by both types of cricket protein powder (section 3.1.3) which allowed water to be trapped within the 3-dimensional matrix (Yuan et al., 2017). As discussed in the constant MixoLab profiles, decreased water would inhibit the starch from retrogradation and thereby improve the shelf life of the bread. As stated by Yuan et al. (2017) where lower C4 and C5 torque values correlate with reduced staling in breads. However, due to an equipment error, the C5 torque was not captured therefore no data can be reported and no insight can be made on the setback. But, it could be possible that the low C4 torque values will help reduce staling in bread and increase the shelf life.

3.2.4. SPI-IPI Peak Areas Found in Dough

Perhaps one of the most important factors affecting dough development and breadmaking quality is the quantity and quality of wheat proteins (Aussenac et al., 2001; Hammed et al., 2015; Zhou et al., 2018). Studies have identified that it is not just the variation in protein content which causes differences in strain hardening, dough properties and breadmaking quality, but also due to the variation in molecular weight distributions of the flour proteins (Gupta et al., 1995; Huebner et al., 1997; Ohm et al., 2008; Zhang et al., 2008; Singh and Singh, 2013; Hammed et al., 2015; MacRitchie, 2016).

Research has identified the molecular weight distribution of the glutenin fraction to be the most important in relation to dough strength and breadmaking performances of the wheat flours (Aussenac et al., 2001; Edwards et al., 2007; Singh and Singh, 2013; MacRitchie, 2016; Wang et al., 2017a). Specifically, the high molecular weight glutenin subunits (HMW-GS) (about 15-25% of the total glutenin fraction) have been found to be more important than the low molecular weight glutenin subunits (LMW-GS) as they have been linked to the following parameters: dough strength, maximum dough resistance, peak dough mixing time, elasticity, extensibility, and loaf volume (Belton, 1999; Aussenac et al., 2001; Zhang et al., 2008; Singh and Singh, 2013; MacRitchie, 2016). Additionally, studies have further indicated that the unextractable fraction of the glutenin polymers causes variations in dough strength and breadmaking quality compared to the total amount of polymeric protein (Bean et al., 1998; Aussenac et al., 2001; Zhang et al., 2008; Singh and Singh, 2013).

Since high molecular polymeric proteins play such a crucial role in dough development the focus in this experiment was to see how the addition of the cricket protein powder changed the

amount of unextractable polymeric proteins (IPI) and extractable polymeric proteins (SPI) during mixing to peak torque. Doughs mixed to peak torque containing 0, 10, or 20% of Entomo Farms or GrioPro cricket protein powders showed no significant difference in their SPI peak areas (Figure 3.9-a); however, changes were seen in their IPI peak areas (Figure 3.9-b). Aussenac et al. (2001) stated that during mixing protein polymeric molecular chains are not capable of disentangling fast enough in response to the stress shear applied therefore bonds are broken. Furthermore, the center of the protein polymers experiences the highest tension resulting in polymers preferring to break at their centers; therefore, only proteins with a molecular mass higher than a critical size are broken down and the low molecular mass protein polymers are not broken down (Aussenac et al., 2001). This would explain why there was no significant difference in the SPI peak areas since solubility is inversely related to molecular weight, thus the SPI areas have a low molecular mass (MacRitchie, 2016).

At a 10% replacement level, Entomo Farms cricket protein powder significantly decreased the IPI peak area value compared to the control (0% replacement level), while the 20% replacement level showed no significant difference to the control (Figure 3.9-b). On the other hand, GrioPro cricket protein powder at a 10% replacement level showed no significant difference from the control and a significant increase in the IPI peak area value at the 20% replacement level (Figure 3.9-b). Dough mixing breaks up the protein aggregates either by physical separation or by breaking the covalent and non-covalent bonds holding the polymeric protein complexes together thus making them more extractable and causing a decrease in the IPI during mixing (Meredith and Wren, 1969; Aussenac et al., 2001). Addition of Entomo Farms cricket protein powder followed this trend by having lowered the IPI peak area as the replacement level increased; however, addition of GrioPro cricket protein powder did the opposite and increased the amount of IPI peak areas as the replacement level increased. This could be because GrioPro cricket protein powder contains higher molecular weight proteins than the Entomo Farms cricket protein powders to begin with (Figure 3.4-a,b). This means that there was a greater amount of large molecular weight proteins present in doughs containing GrioPro cricket protein powder vs. doughs containing Entomo Farms cricket protein powder.

In general, GrioPro cricket protein powder had significantly higher IPI peak area values (29518 and 65820 mAu for 10 and 20% replacement levels) than those for doughs containing Entomo Farms cricket protein powder (9432 and 17346 mAu for 10 and 20% replacement levels)

(Table 3.11). This further reinforces the scenario of GrioPro cricket protein powder increasing the amount of high molecular weight polymers present to begin with than Entomo Farms cricket protein powder which would cause a lower reduction in the amount of IPI peak area since the mixing action would not be able to break apart as many protein aggregates in the time the dough was mixed to peak development. A second possibility could be that the GrioPro cricket protein powder is more functional therefore allowing it to interact with the glutenin proteins found in the wheat flour. Wang et al. (2017a) state that dough rheological properties can be altered by Avenin-like proteins which can form sulfhydryl/disulfide crosslinks with gluten proteins. Verbruggen et al. (2001) found that the addition of unalkylated glutenin subunits could be partially incorporated into the gluten network by providing free sulphydryl groups. If GrioPro cricket protein powder can crosslink via disulfide bonds or some other type of interaction with the gluten proteins it could be affecting the structure and molecular weight of the polymers being formed (Gupta et al., 1995) and that structural difference could be contributing to the differences seen in the dough strength (Edwards et al., 2007). Such interaction seems to be highly likely since the calculated value for IPI was much lower than the experimental value (Figure 3.10-b) thereby implying that GrioPro cricket protein powder can chemically interact with the gluten matrix. Further testing would be necessary to confirm if the addition of GrioPro cricket protein powder is just simply adding more high molecular proteins to begin with, or capable of interacting chemically with the gluten network.

3.2.5. Kieffer-Extensional Dough Properties

In order to see the effect of the cricket protein on the extensibility of the dough the Kieffer Rig Dough Extensibility test was conducted. The Kieffer rig curves can be seen in Figure 3.11-a,b for doughs containing 0, 10, and 20% replacement levels of either Entomo Farms or GrioPro cricket protein powders. Results show that as the replacement level of Entomo Farms cricket protein powder increases, the force decreases drastically from 28.84 to 17.77 to 8.67 kg. (Table 3.13). GrioPro cricket protein powder showed the opposite trend therefore as the replacement level increased, the amount of force required to break the dough significantly increased from 28.84 to 33.20 to 39.70 kg (Table 3.13). Extensibility is dependent on two things: the loop-train ratio and the Van de Waals interactions between the linear proteins and the globular proteins (Belton, 1999). An increase in the Van de Waals interactions causes the viscous resistance of the globular proteins

to increase thus leading to an increase in the resistance to extension (Belton, 1999). Furthermore, an increase in the number of trains found in the loop-train ratio also increases the resistance to extension since it takes more energy to unzip the trains than to deform the loops (Belton, 1999).

Results show a differing in behavior when replacing wheat flour with either Entomo Farms or GrioPro cricket protein powders. This could be due to the different molecular weight distribution as seen in the SDS-PAGE and the Raw Materials SP-IP HPLC Chromatograms. GrioPro cricket protein powder has higher molecular weight molecules while Entomo Farms cricket protein powder has lower molecular weight polymers. Mixing extends the smaller molecules first and requires more time and a higher strain rate to extend the larger molecules (MacRitchie, 2016). Thus, the larger molecules found in GrioPro cricket protein powder could be responsible for the increase in maximum force since it takes more force to unfold the largest molecules thus leading to a higher mixing intensity. This is supported by the higher C1 torque values reported above for GrioPro cricket protein powder containing doughs (Table 3.5) where the toughest dough was the 20% GrioPro cricket protein powder replacement level (CT1 torque and Kieffer Force were highly correlated by $r = 0.90$, P-value = 0.01).

Previous research has also been shown that the addition of LMW-GS decreased max resistance while the addition of HMW-GS increased the maximum resistance (Verbruggen et al., 2001; Wang et al., 2017a). Therefore, the lower amount of molecular weight polymers found in Entomo Farms cricket protein powder could be acting the same as the LMW-GS and causing the decrease in maximum due to the decreased number of high molecular weight polymers. The maximum extensibility decreased for both cricket protein powders as the replacement level increased. However, the decrease was more dramatic in the addition of GrioPro cricket protein powder than that of Entomo Farms cricket protein powder where the distance decreased from 48.59 to 19.51 to 10.32 mm (Table 3.13). Gupta et al. (1995) stated that polymers above a specific size threshold may be detrimental to dough extensibility. Therefore, it could be possible that the extensibility was more drastically affected by GrioPro cricket protein powder due to its larger quantity of high molecular weight polymers. Zhang et al. (2008) showed that the soluble polymeric proteins were negatively correlated with the maximum resistance and that the insoluble polymeric proteins were positively correlated with maximum resistance when it comes to the wheat proteins. Correlations between the maximum force and the soluble polymeric proteins found in the cricket proteins showed a positive correlation of $r = 0.96$ (P-value = 0.002) which is the opposite trend of

what Zhang et al. (2008) found for the soluble polymeric proteins found in wheat. However, the soluble polymeric proteins found from doughs containing 10 and 20% replacement levels showed a negative correlation with the maximum force r = -0.66 (P-value = 0.15). Even though this correlation was not significant it still shows the interesting point where the soluble polymeric proteins found in the crickets behave differently from those found in wheat, but when both types of soluble polymeric proteins are combined the overall effect remains the same as seen by Zhang et al. (2008).

On the other hand, both the insoluble polymeric proteins found in the cricket protein itself (r = 0.92, P-value = 0.01) and in the doughs containing 10 and 20% replacement levels (r= 0.76, P-value = 0.08) were highly correlated with the maximum resistance on a P-value < 0.05 and a P-value <0.1 level respectively. Therefore, it could be said that the insoluble polymeric proteins found in the insects behave the same and cause the same impact on the maximum resistance as reported by Zhang et al. (2008). Lastly, Dobraszczyk and Salmanowicz (2007) suggested that the maximum force fell under the rheological parameters most likely to predict test baking volume, but that extensibility was not a good predictor. However, the opposite was observed in this case where results showed a high correlation between extensibility and loaf volume (r = 0.99, P-value = 0.0003). Thus, extensibility could be an adequate predictor on baking performance for insect proteins incorporated into bread.

Table 3.4. One-way analysis of variance (ANOVA) for MixoLab profiles (constant water)

Variable	df	Mean Square	F-value	P-value
C1 torque	4	0.031	18.16	0.0035
Error	5	0.002		
C2 torque	4	0.011	16.64	0.0043
Error	5	0.001		
C3 torque	4	0.036	35.98	0.0007
Error	5	0.001		
C4 torque	4	0.047	23.40	0.0020
Error	5	0.002		
C5 torque	4	0.078	25.13	0.0017
Error		0.003		
C1 time	4	27.544	996.16	<0.0001
Error	5	0.028		
C2 time	4	0.069	1.32	0.3758
Error	5	0.052		
C3 time	4	0.258	8.96	0.0167
Error	5	0.029		
Stability	4	10.963	174.27	<0.0001
Error	5	0.063		
Onset of gelatinization	5	1.115	1.45	0.3278
Error	6	0.767		

Table 3.5. MixoLab parameters for doughs under constant water absorption protocol

Powder type	Replacement level (%)	C1 torque (Nm)	C2 torque (Nm)	C3 torque (Nm)	C4 torque (Nm)	C5 torque (Nm)
E	0	1.13 ± 0.02 b	0.50 ± 0.01 ab	1.65 ± 0.04 a	1.24 ± 0.05 bc	1.64 ± 0.05 b
	10	1.02 ± 0.02 b	0.44 ± 0.02 b	1.46 ± 0.01 b	1.17 ± 0.03 c	1.54 ± 0.04 b
	20	1.00 ± 0.02 b	0.41 ± 0.01 b	1.35 ± 0.02 c	1.09 ± 0.02 c	1.50 ± 0.00 b
G	0	1.13 ± 0.02 b	0.50 ± 0.01 ab	1.65 ± 0.04 a	1.24 ± 0.05 bc	1.64 ± 0.05 b
	10	1.12 ± 0.01 b	0.56 ± 0.02 a	1.66 ± 0.00 a	1.47 ± 0.03 a	1.89 ± 0.02 a
	20	1.31 ± 0.06 a	0.58 ± 0.03 a	1.52 ± 0.03 b	1.39 ± 0.03 ab	1.92 ± 0.06 a

Powder type	Replacement level (%)	C1 time (min)	C2 time[1] (min)	C3 time (min)	Stability (min)	Onset of gelatinization[1] (°C)
E	0	1.67 ± 0.04 b	18.35 ± 0.15	24.78 ± 0.08 b	11.04 ± 0.02 b	71.3 ± 0.7
	10	8.08 ± 0.11 a	18.65 ± 0.08	24.91 ± 0.16 b	10.59 ± 0.07 b	72.3 ± 0.4
	20	8.33 ± 0.03 a	18.46 ± 0.18	24.77 ± 0.17 b	6.88 ± 0.25 c	71.4 ± 0.7
G	0	1.67 ± 0.04 b	18.35 ± 0.15	24.78 ± 0.08 b	11.04 ± 0.02 b	71.3 ± 0.7
	10	1.80 ± 0.08 b	18.59 ± 0.21	25.04 ± 0.02 b	12.69 ± 0.22 a	72.0 ± 0.8
	20	0.91 ± 0.23 c	18.84 ± 0.17	25.64 ± 0.12 a	12.51 ± 0.21 a	73.2 ± 0.5

MixoLab profiles reported for doughs containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels where water was held constant

[1]not significantly different by one-way ANOVA
df 4, F-value 1.32, P-value 0.3758 for C2 time
df 5, F-value 1.45, P-value 0.3278 for Onset of gelatinization

[2] means with the same letter are not significant ($p > 0.05$)

Table 3.6. One-way analysis of variance (ANOVA) for MixoLab water absorption

Variable	df	Mean Square	F-value	P-value
Water absorption	5	7.585	21.08	<0.0001
Error	11	0.360		

Table 3.7. Change in water absorption during optimization procedure

Powder type	Replacement level (%)	Water Absorption (%)
E	0	59.8 ± 0.4 bc
	10	58.3 ± 0.3 c
	20	58.3 ± 0.2 c
G	0	59.8 ± 0.4 bc
	10	60.3 ± 0.3 b
	20	62.7 ± 0.3 a

Water absorption reported for doughs containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels
[1]means with the same letter are not significant ($p > 0.05$)

Table 3.8. One-way analysis of variance (ANOVA) for MixoLab profiles (optimized water)

Variable	df	Mean Square	F-value	P-value
C1 torque	4	0.000	4.85	0.0568
Error	5	0.000		
C2 torque	4	0.010	93.32	<0.0001
Error	5	0.000		
C3 torque	4	0.023	164.75	<0.0001
Error	5	0.000		
C4 torque	4	0.027	37.14	0.0007
Error	5	0.000		
C5 torque[1]				
C1 time	4	20.517	87.79	<0.0001
Error	5	0.234		
C2 time	4	0.398	21.15	0.0025
Error	5	0.019		
C3 time	4	1.497	20.84	0.0026
Error	5	0.072		
Stability	4	10.439	260.77	<0.0001
Error	5	0.040		
Onset of gelatinization	5	5.165	16.31	0.0020
Error	6	0.317		

[1]was not captured due to equipment issue

Table 3.9. MixoLab profiles for doughs under optimized water absorption protocol

Powder type	Replacement level (%)	C1 torque[1] (Nm)	C2 torque (Nm)	C3 torque (Nm)	C4 torque (Nm)	C5 torque[2] (Nm)
E	0	1.11 ± 0.01	0.51 ± 0.01 a	1.72 ± 0.01 a	1.40 ± 0.04 ab	
	10	1.14 ± 0.01	0.42 ± 0.01 b	1.59 ± 0.01 c	1.34 ± 0.01 bc	
	20	1.12 ± 0.01	0.37 ± 0.01 c	1.46 ± 0.00 e	1.18 ± 0.00 d	
G	0	1.11 ± 0.01	0.51 ± 0.01 a	1.72 ± 0.01 a	1.40 ± 0.04 ab	
	10	1.10 ± 0.01	0.54 ± 0.01 a	1.67 ± 0.00 b	1.49 ± 0.02 a	
	20	1.11 ± 0.00	0.51 ± 0.01 a	1.51 ± 0.02 d	1.27 ± 0.00 cd	

Powder type	Replacement level (%)	C1 time (min)	C2 time (min)	C3 time (min)	Stability (min)	Onset of gelatinization (°C)
E	0	1.60 ± 0.02 b	18.35 ± 0.13 bc	24.96 ± 0.31 a	11.07 ± 0.27 b	71.1 ± 0.5 b
	10	6.58 ± 0.08 a	18.14 ± 0.09 c	25.86 ± 0.23 a	9.37 ± 0.10 c	70.3 ± 0.3 b
	20	7.94 ± 0.76 a	18.62 ± 0.00 bc	25.20 ± 0.18 a	7.32 ± 0.12 d	72.0 ± 0.1 b
G	0	1.60 ± 0.02 b	18.35 ± 0.13 bc	24.96 ± 0.31 a	11.07 ± 0.27 b	71.1 ± 0.5 b
	10	1.73 ± 0.01 b	18.68 ± 0.16 b	25.16 ± 0.03 a	12.73 ± 0.06 a	72.4 ± 0.6 b
	20	1.15 ± 0.03 b	19.32 ± 0.02 a	23.51 ± 0.04 b	12.56 ± 0.06 a	74.9 ± 0.1 a

MixoLab profiles reported for doughs containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels where water was not held constant

[1]Not significantly different by one-way ANOVA df 4, F-value 4.85, P-value 0.0568 for C1 torque

[2]was not captured due to equipment issue

[3]means with the same letter are not significant ($p > 0.05$)

Table 3.10. One-way analysis of variance (ANOVA) for SPI and IPI

Variable	df	Mean Square	F-value	P-value
SPI	4	15494682.590	5.28	0.0074
Error	15	2932263.300		
IPI	4	0.395	64.00	<0.0001
Error	15	0.006		

[1]IPI was transformed into a log base 10 for analysis

Table 3.11. Peak areas under the curve for extractable polymeric proteins (SPI) and unextractable polymeric proteins (IPI)

Base	Powder type	Replacement level (%)	Peak area (mAu)			
			SPI		IPI	
Wheat flour	E	0	41213 ± 883	ab	23360 ± 1788	bc
		10	44133 ± 854	a	9432 ± 1170	d
		20	41413 ± 920	ab	17346 ± 1921	c
	G	0	41213 ± 883	ab	23360 ± 1788	bc
		10	39157 ± 524	b	29518 ± 603	b
		20	39548 ± 1018	b	65820 ± 3038	a

Peak areas reported as milli absorbance units (mAu) for doughs containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels
[1]means with the same letter are not significant ($p > 0.05$)

Table 3.12. One-way analysis of variance (ANOVA) for Kieffer rig dough extensibility testing

Variable	df	Mean Square	F-value	P-value
Force	4	1.116	786.70	<0.0001
Error	5	0.001		
Distance	5	0.944	328.04	<0.0001
Error	87	0.003		

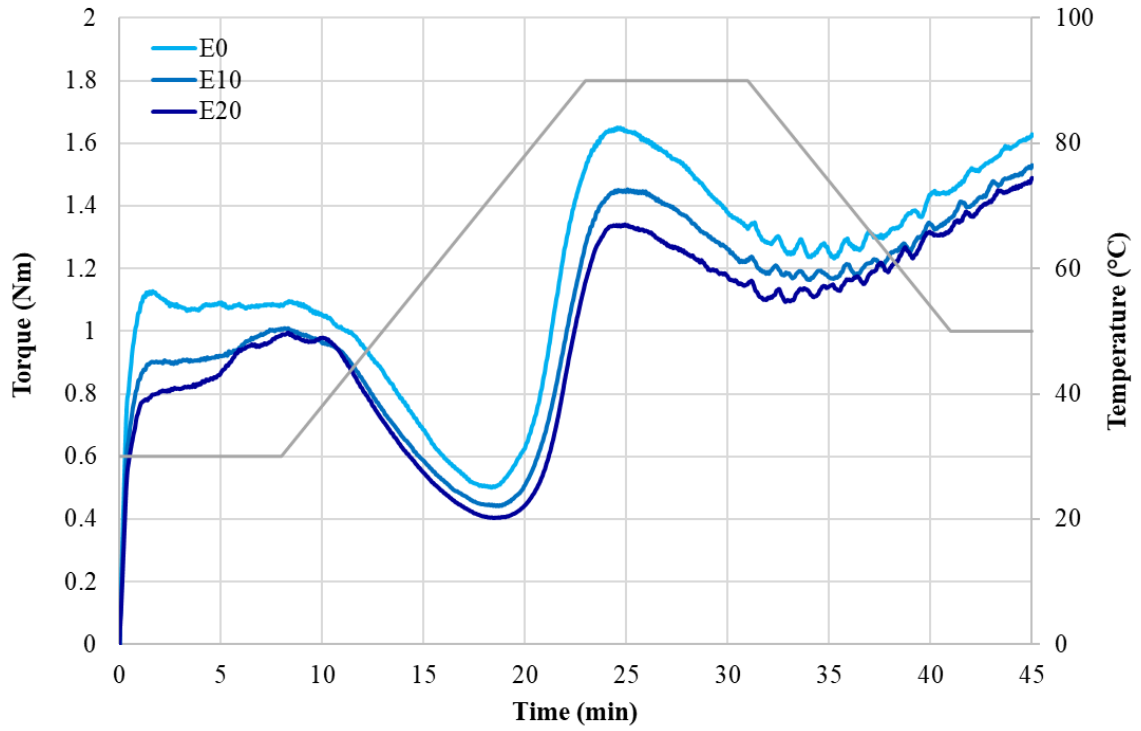
[1]Force and distance were transformed into a log base 10 for analysis

Table 3.13. Kieffer rig dough extensibility testing results

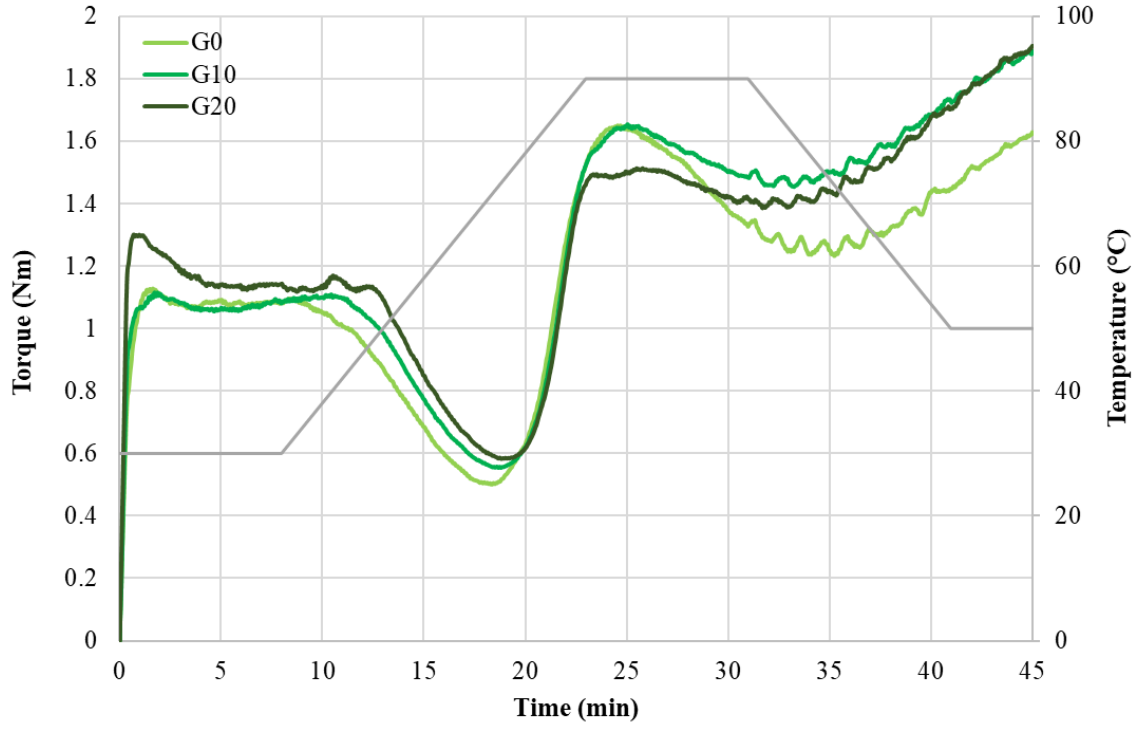
Powder type	Replacement level (%)	Force, R_{max} (kg)	Distance, Ext_{max} (mm)
E	0	28.84 ± 0.70 [c]	45.89 ± 1.67 [a]
	10	17.77 ± 0.22 [d]	35.06 ± 0.81 [b]
	20	8.67 ± 0.23 [e]	26.13 ± 0.92 [c]
G	0	28.84 ± 0.70 [c]	45.89 ± 1.67 [a]
	10	33.20 ± 0.60 [b]	19.51 ± 0.42 [d]
	20	39.70 ± 1.03 [a]	10.32 ± 0.31 [e]

Force and distance reported for doughs containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels where water absorption was held constant

[1]means with the same letter are not significant ($p > 0.05$)



(a)



(b)

Figure 3.6. MixoLab profiles under constant water absorption

For doughs containing (a) Entomo Farms (E) and (b) GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels where water absorption was held constant

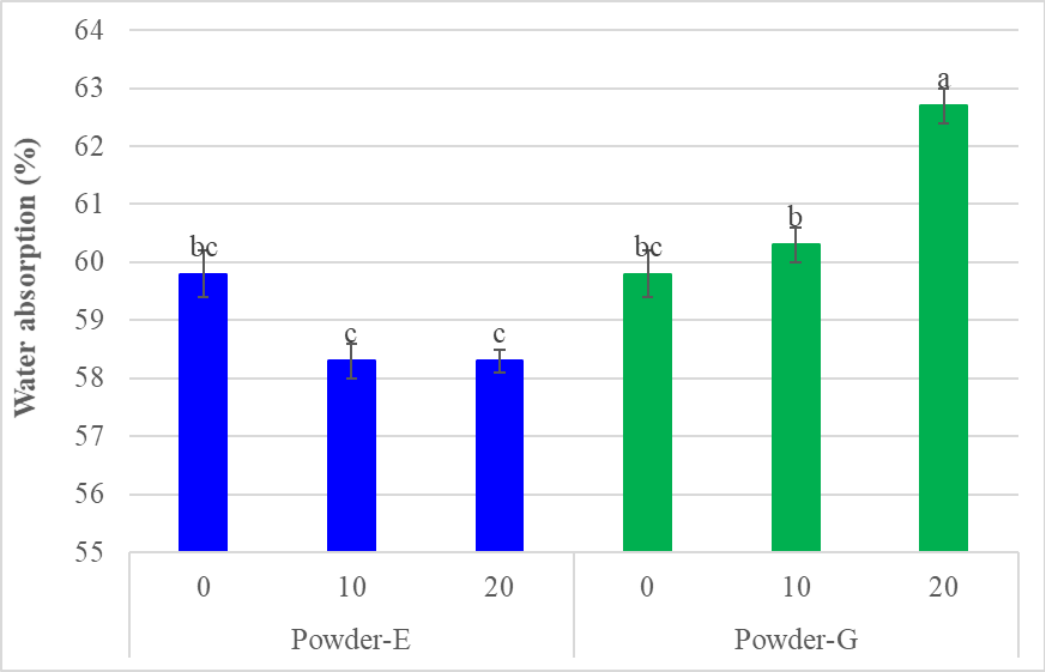
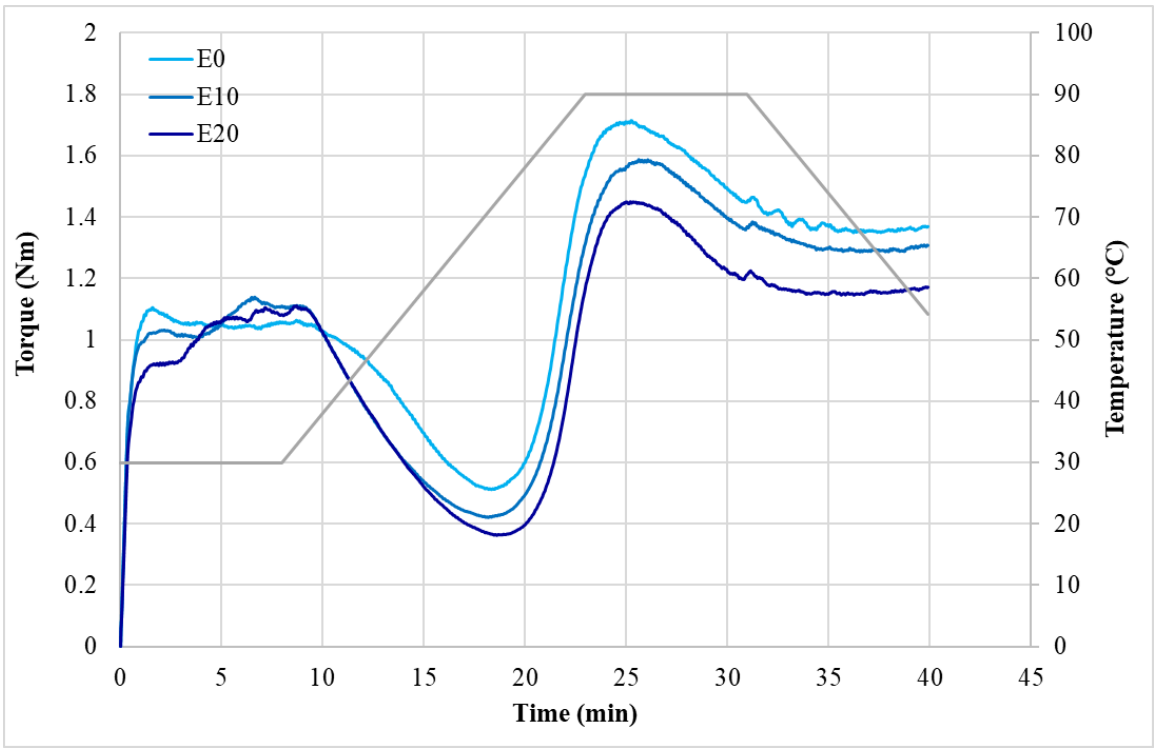


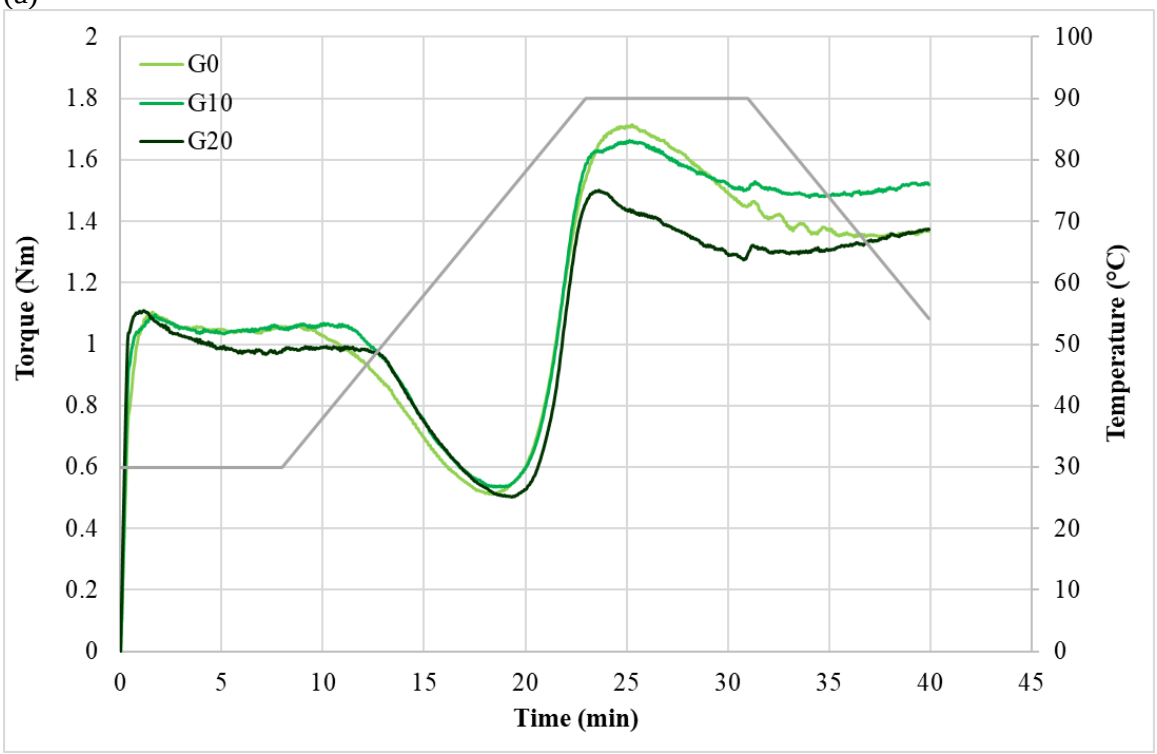
Figure 3.7. Optimized water absorption

For doughs containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels

[1]means with the same letter are not significant ($p > 0.05$)

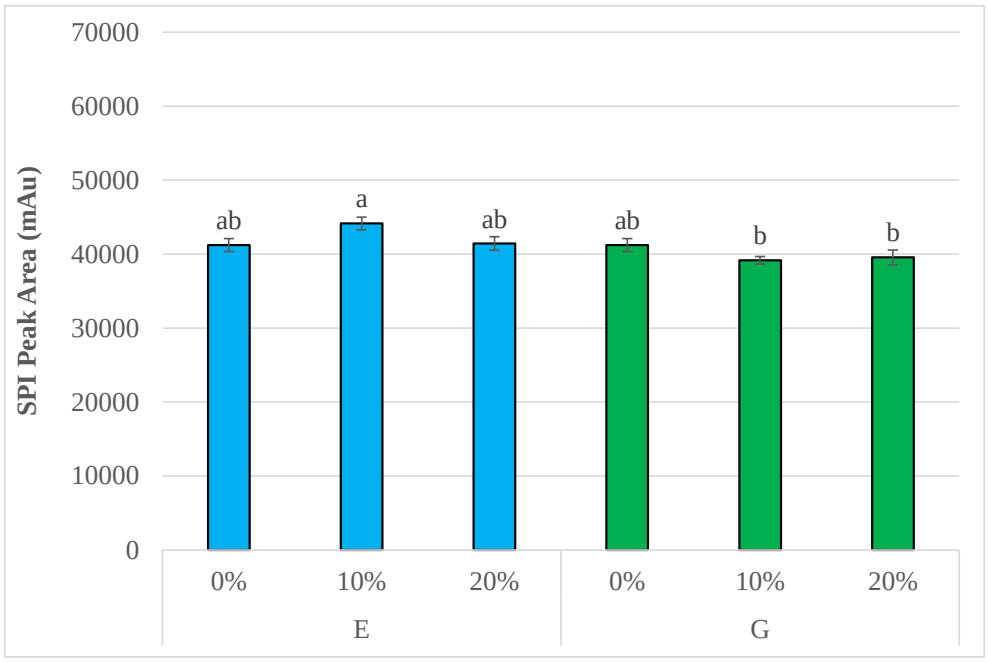


(a)

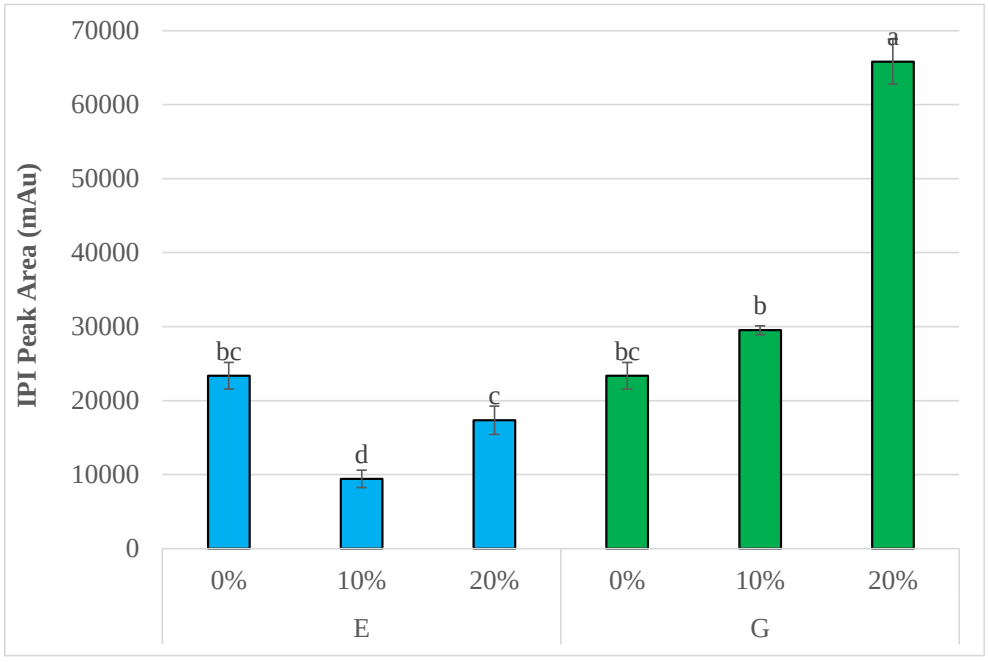


(b)

Figure 3.8. MixoLab profiles (optimized water absorption protocol)
For doughs containing (a) Entomo Farms (E) and (b) GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels where water was not held constant



(a)

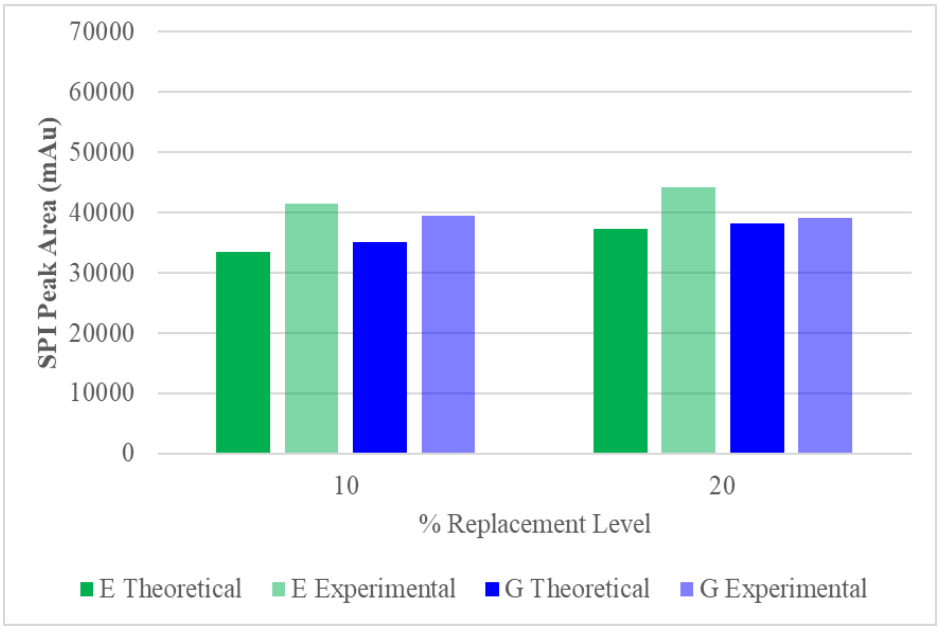


(b)

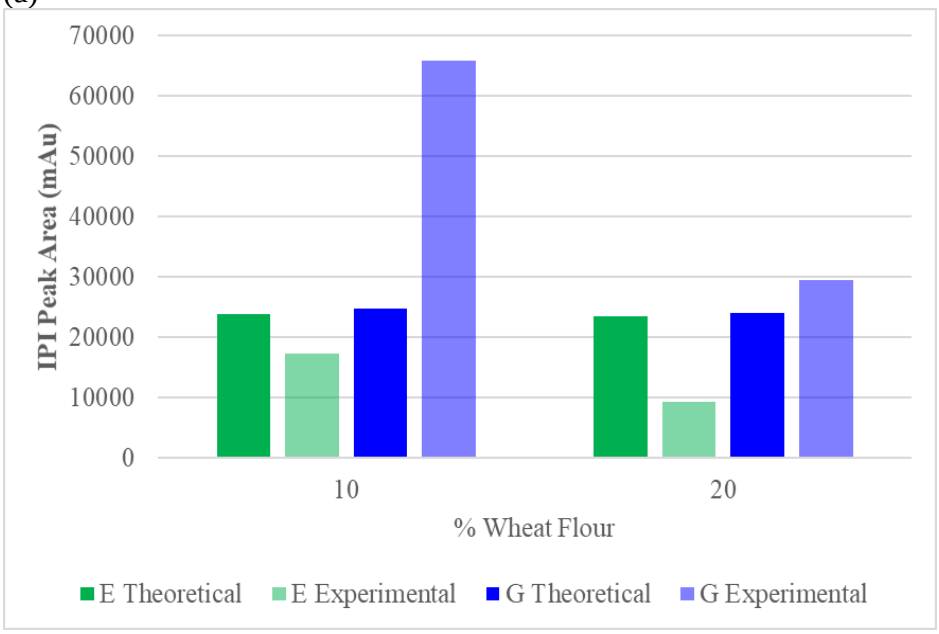
Figure 3.9. Peak areas under the curve for (a) SPI and (b) IPI

Peak areas reported as milli absorbance units (mAu) for doughs containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels

[1]means with the same letter are not significant ($p > 0.05$)



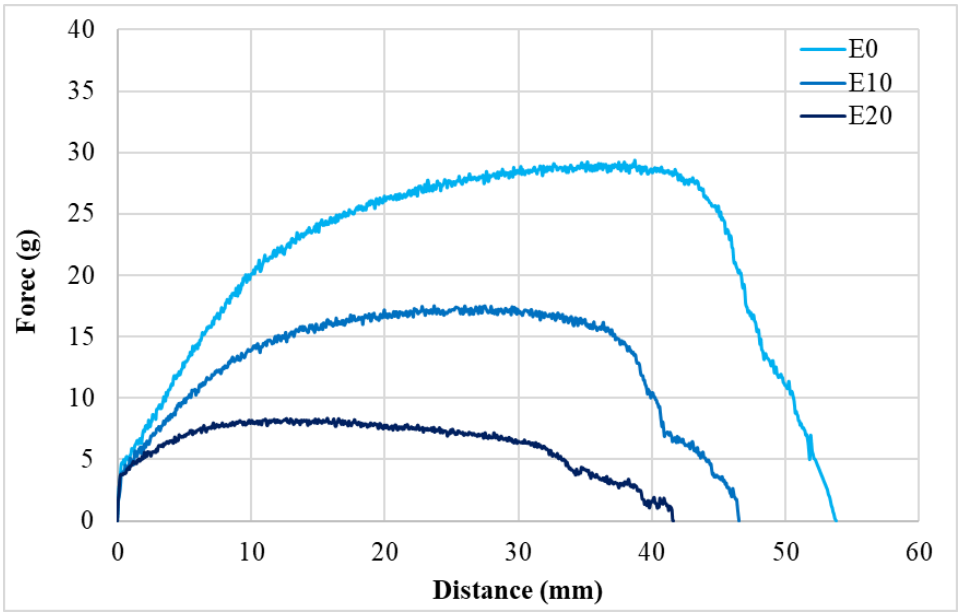
(a)



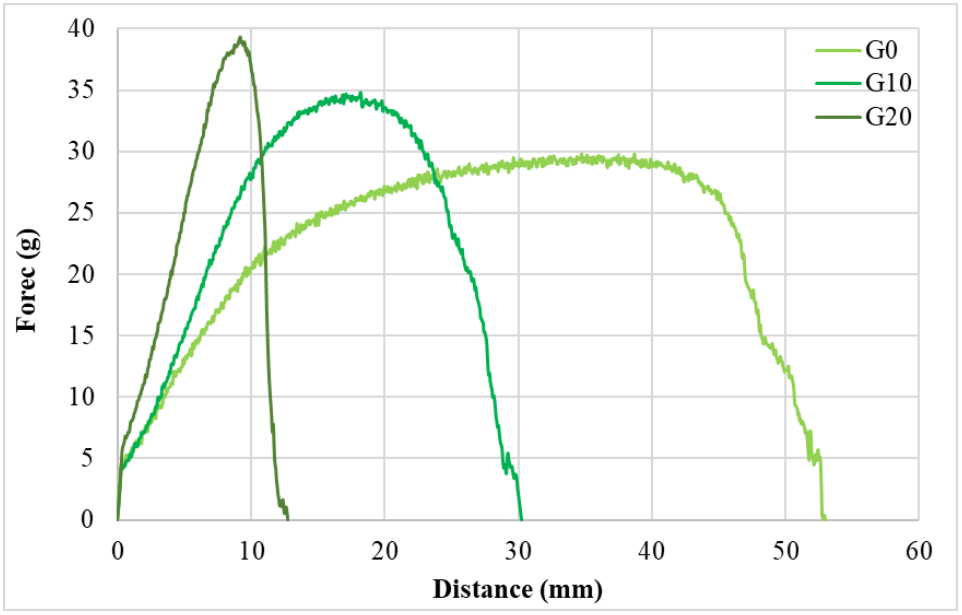
(b)

Figure 3.10. Peak areas under the curve comparing theoretical and experimental values for (a) SPI, and (b) IPI

Theoretical values calculated by using 90 or 80% of the SPI or IPI peak area value for wheat flour and adding 10 or 20% of the SPI or IPI peak area value from E or G combined with starch



(a)



(b)

Figure 3.11. Kieffer rig extensibility curves

Force and distance reported for doughs containing (a) Entomo Farms (E) and (b) GrioPro (G) cricket protein powders at 0, 10, and 20% total flour weight replacement levels where water absorption was held constant

3.3. Test Baking and End-Product Quality

3.3.1. Color/Brightness

Bread is a popular house-hold product that meets nutritional recommendations (Batista et al., 2011); however, in an endeavor to enrich the balance of essential amino acids and improve the nutritional profile the addition of different proteins and fibers have been studied (Taha et al., 1982; Batista et al., 2011; Schmiele et al., 2017). The final product characterization must be up to consumers' standards for the product to sell and when it comes to bread the quality depends on loaf volume, texture, color, flavor, and smell (Batista et al., 2011). Thus, any incorporation of new ingredients such as the cricket protein powder must be optimized in order to ensure these final product characteristics are not drastically impacted so that consumers' expectations can continue to be met.

Figure 3.12-a,b depict the images of slices from loaves containing 0, 5, 10, and 20% replacement levels of either Entomo Farms or GrioPro cricket protein powders. The crumb color of the slices was reported in terms of L (brightness), a (red-green spectrum), and b (blue-yellow spectrum) values (Menegon de Oliveira et al., 2017) which can be seen in Figures 3.13-a, b, and c. For both Entomo Farms and GrioPro cricket protein powder, as the replacement level increased, the L-value significantly decreased (Figure 3.13-a). This decrease in L-value appeared to be more drastic in Entomo Farms cricket protein powder (from 55.44 to 46.15 to 40.06) than in GrioPro cricket protein powder (from 55.74 to 49.56 to 43.77) and both types of cricket protein powders produced loaves with darker crumb colors at all replacement levels in comparison to the control (64.79) (Table 3.15). At the 5% replacement level there was no protein type (Entomo Farms vs. GrioPro) effect on the L-value (Table 3.15). Therefore, it can be concluded that Entomo Farms cricket protein powder containing doughs produced darker colored crumbs than GrioPro cricket protein powder containing doughs.

The a-value increased for both Entomo Farms and GrioPro cricket protein powder containing loaves as the replacement level increased (Figure 3.13-b). Furthermore, Entomo Farms cricket protein powder showed a more drastic increase in the a-value than GrioPro cricket protein powder (from 1.63 to 3.54 to 5.67 vs. from 0.86 to 2.96 to 3.98) (Table 3.15). The control crumb color was in the negative range (-1.27) which indicates a green reading whereas all the other treatments with their increase in positive numbers show how the cricket protein powder caused the

crumb color to shift to a redder color (Table 3.15). An increase in the b-value occurred at all replacement levels for both types of cricket protein powder in comparison to the control (Figure 3.13-c). However, the increase was more drastic in the loaves containing Entomo Farms cricket protein powder (from 15.60 to 18.26 to 21.10) (Table 3.15). For GrioPro cricket protein powder, the 10 and 20% replacement levels caused no significant increase in comparison to each other (18.48 vs. 18.33) (Table 3.15). Furthermore, the 10% replacement level of Entomo Farms cricket protein powder was not significantly different from the 10 and 20% replacement levels of GrioPro cricket protein powder (18.26 vs. 18.48 and 18.33) (Table 3.15).

Finally, there was no significant difference between the 5% replacement levels of Entomo Farms or GrioPro cricket protein powder (15.60 vs. 14.98) (Table 3.15). Positive b-values represent the yellow spectrum, therefore the addition of either protein powder caused the crumb to become more yellow in comparison to the control. While the increase remained the same regardless of the protein type for the 5 and 10% replacement levels; however, the 20% replacement level showed that at higher concentrations Entomo Farms cricket protein powder will more drastically impact the crumb color than GrioPro cricket protein powder. These results correspond with results seen by Ohm et al. (2008) where higher protein flour concentrations led to an increase in redness in noodle dough. Zhou et al. (2018) also saw a decrease in L values while the a and b values increased with the incorporation of either whey protein or soy protein. Replacement with soy protein hydrolysates and fructooligosaccharides led to darker crust color (Schmiele et al., 2017) while the additions of either cinereous cockroach flour or hard-to-cook black bean flour produced crust and crumb colors more like those found in whole wheat bread (Batista et al., 2011; Menegon de Oliveira et al., 2017). This shift to a darker color can be attributed to increased Maillard reactions due to more protein availability providing more reducing sugars/amino acid groups, as well as, an increase in enzymatic reactions and nonenzymatic reactions (caramelization) (Ohm et al., 2008; Schmiele et al., 2017; Zhou et al., 2018).

Additionally, Entomo Farms cricket protein powder is a dark brown while GrioPro cricket protein powder is a light brown thus this initial difference in color explains the color difference between the cricket protein types. The brightness decreased for all treatments as the replacement level increased (Figure 3.13-d). At 5 and 10% replacement levels, GrioPro cricket protein powder produced a more drastic decrease in brightness than Entomo Farms cricket protein powder did (103.9 and 78.4 vs. 109.2 and 86.4); however, at the 20% replacement level Entomo Farms cricket

protein powder had the lowest brightness (50.6) in comparison to all other treatments (Table 3.15). Therefore, it can be said that brightness is impacted regardless of which type of cricket protein is used. According to Yuan et al. (2017) alteration of the microstructure in bread affects the extent of light scattering which in turn would alter the brightness of the bread. Furthermore, the darker crust and crumb colors caused by the increase of protein would also lead to a decrease in brightness as seen by the correlation between the L-value and brightness ($r = 0.98$, P-value < 0.0001) and by the inverse correlations between brightness and the a ($r = -0.98$, P-value < 0.0001) and b ($r = -0.98$, P-value < 0.0001) values.

3.3.2. Loaf Weight and Volume/Slice Area

Both types of cricket protein powder caused an increase in the loaf weight in comparison to the control (144.6 g) (Figure 3.13-e). For GrioPro cricket protein powder, the loaf weight increased as the replacement level increased (from 147.8 to 152.8 to 157.3 g) (Table 3.15). However, Entomo Farms cricket protein powder showed no significant difference in loaf weight between the 5, 10, or 20% replacement levels (147.2 vs. 147.4 vs. 149.2 g) (Table 3.15). At the 5% level there was no significant difference between either type of protein powder (147.2 vs. 147.8 g) (Table 3.15). Increased loaf weight as concentration of foreign protein increased was also reported for whey protein, and mushroom flour while soy protein acted oppositely by decreasing loaf weight (Yuan et al., 2017; Zhou et al., 2018).

In contrast, the loaf volume decreased for both proteins as the replacement level increased (Figure 3.13-f). This decrease was more drastically seen in loaves containing GrioPro cricket protein powder (from 783.3 to 580.0 to 400.0 cc) in comparison to loaves containing Entomo Farms cricket protein powder (from 853.3 to 800.0 to 585.0 cc.) (Table 3.15). All treatments regardless of protein type were significantly lower in loaf volume compared to the control (905.0 cc) (Table 3.15). However, the 5% replacement of GrioPro cricket protein powder showed no difference from the 10% replacement level of Entomo Farms cricket protein powder while the 10% replacement level was not different from the 20% replacement level of Entomo Farms cricket protein powder (Table 3.15). Decrease in loaf volume or specific volume as the protein concentration increased was also seen in soy protein, cowpea flour, hard-to-cook black bean flour, mushroom flour, and cockroach flour (Batista et al., 2011; Menegon de Oliveira et al., 2017; Yuan et al., 2017; Zhou et al., 2018).

The differences in loaf height can also be seen in the c-cell images of Figure 3.12-a,b. The slice area followed the same trend of decreasing while the protein replacement level increases (Figure 3.13-g). This decrease is less pronounced in breads containing Entomo Farms cricket protein powder since the 5 and 10% replacement levels showed no significantly difference in slice area values (5014 vs. 4774 mm^2) (Table 3.15). On the other hand, GrioPro cricket protein powder containing loaves produced the lowest slice area values out of all the treatments (from 4471 to 3146 to 2172 mm^2) (Table 3.15). All replacement levels containing either of the cricket protein powders were significantly lower in slice area than the control (5974 mm^2) (Table 3.15). Since GrioPro cricket protein powder containing breads weighed the most, had the lowest volumes, and had the smallest values of slice area it can be concluded that the addition of GrioPro cricket protein powder produces denser breads than both the control and breads containing Entomo Farms cricket protein powder.

A combination of different factors could be responsible for the decrease in loaf volume and slice area and the increase in weight for GrioPro cricket protein powder containing breads. The first factor is the water absorption and water binding capacity which affect the final bread weight (Yuan et al., 2017; Zhou et al., 2018). According to Yuan et al. (2017) water evaporation also plays a significant role in the final bread weight where they suggested that mushroom flour either contained less water than wheat flour to begin with leading to a lower amount of water to evaporate during baking or that the mushroom flour allowed the breads to retain moisture more strongly. As seen in previous sections (Figure 3.1 and Figure 3.7) GrioPro cricket protein powder had both a higher water holding capacity leading to a higher water absorption necessary to optimally develop the dough. Therefore, it could be possible that the increased amount of water holding capacity allowed GrioPro cricket protein powder to retain more water and resulted in less free water available to evaporate therefore providing the bread with a higher ability to retain moisture. The same phenomenon was observed for both hard-to-cook black bean flour and cowpea flour where the increase in water absorption affected the dough properties and led to low specific volumes (Batista et al., 2011).

A second factor related to decreased specific loaf volume may have been gelation/viscosity. Zhou et al. (2018) related specific volume to gelation and viscosity since increased viscosity provide strength to the expanding gas cells therefore improving gas retention. As mentioned before (MixoLab optimum water absorption data presented in section 3.2.3) both Entomo Farms and

GrioPro cricket protein powder resulted in a decrease in peak viscosity (C3 torque value). Furthermore, a delay in gelatinization occurred only in the 20% replacement level of GrioPro cricket protein powder (MixoLab optimum water absorption data presented in section 3.2.3) which affects starch gelatinization thereby affecting the peak viscosity by creating a weaker gel. This weakening in gelation could be leading to poorer gas retention resulting in lower volume in the bread loaves. This is supported by the high correlations between the OT3 (Peak Viscosity at optimum water) value and loaf volume (r = 0.73, P-value = 0.1) or with slice area (r = 0.80, P-value = 0.06). The onset of gelatinization (OC2) also correlated significantly with loaf weight (r = 0.90, P-value = 0.02), loaf volume (r = -0.88, P-value = 0.02), and slice area (r = -0.79, P-value = 0.06). The final factor would be interference with the gluten network which impacts gas retention and leads to lower height and volume (Yuan et al., 2017). As discussed previously (Figure 3.9-a, b and Figure 3.11-a,b) GrioPro cricket protein powder contained a higher amount of insoluble proteins which led to a decrease in dough extensibility. Decreased extensibility leads to lower specific volume and increased density due to the prevention of gas expansion (Batista et al., 2011). Extensibility (r = -0.98, P-value = 0.0005) inversely correlated with loaf weight and highly correlated with both loaf volume (r = 0.99, P-value = 0.0003) and slice area (r= 0.98; P-value = 0.002). Insoluble polymeric proteins found in the doughs also correlated highly with loaf weight (r = 0.79, P-value = 0.06). Therefore, the lower volume and increased weight seen in GrioPro cricket protein powder containing breads could be due to the decreased extensibility and higher amount of insoluble polymeric proteins contained in doughs containing GrioPro cricket protein powder. As was seen in the 20% GrioPro cricket protein powder replacement level which produced the lowest volume and highest weight (densest) bread. Fiber can also interfere with the gluten network due to the interactions between the proteins (gluten or non-gluten) and the fibers which prevent the free expansion of dough and are detrimental to gas retention (Batista et al., 2011; Menegon de Oliveira et al., 2017) The chitin present in the cricket protein powders is a type of fiber therefore, it could also be decreasing the gas retention and resulting in the low volume loaves.

3.3.3. Crumb Microstructure (C-Cell)

The area occupied by air cells showed no significant difference between the control (50.8%) and the 5% replacement levels of both Entomo Farms (51.0%) and GrioPro (50.7%) cricket protein powder, or the 10% replacement level of Entomo Farms cricket protein powder

(51.6%) (Table 3.15). However, the area occupied by air cells continues to decrease significantly as the addition of GrioPro cricket protein powder increases (at the 10 and 20% replacement levels) where the values drop to 48.4 and 47.0% (Figure 3.13-h). Note that the 10% replacement level of GrioPro cricket protein powder produced no significantly difference in area occupied by air cells than in breads containing 20% of Entomo Farms cricket protein powder (48.4 vs. 49.1%) (Table 3.15). All loaves containing either type of protein powder showed a significant decrease in the number of air cells compared to the control (4134) (Figure 3.13-k, and Table 3.15). The 5% replacement of Entomo Farms cricket protein powder produced no significantly different number of air cells than that of the 10% replacement level of Entomo Farms cricket protein powder (3615 vs. 3407) (Table 3.15). The 10% replacement level of GrioPro cricket protein powder (2639) was also not significantly different than the 20% replacement level of GrioPro cricket protein powder (2514) (Table 3.15).

The average air cell diameter followed the same trends as the area occupied by air cells (Figure 3.13-i). Only the 10% and 20% replacement levels of GrioPro cricket protein powder (1.49 and 1.12 mm) and the 20% replacement level of Entomo Farms cricket protein powder (1.61 mm) were significantly lower than the control (1.81 mm) (Table 3.15). The average air cell thickness only showed a significantly lower value in the 10 and 20% replacement levels of GrioPro cricket protein powder (0.408 and 0.352 mm) in comparison to all other treatments including the control (0.428 mm) (Table 3.15).

Two mechanisms are involved in stabilizing the gas bubbles produced via fermentation: the 3-dimensional net formed by the gluten-starch matrix (primary stabilizer) and the liquid lamellae (secondary stabilizer) (Batista et al., 2011; MacRitchie, 2016). As discussed in the previous section (Table 3.16), fiber interferes with the gluten matrix and the amount of high molecular weight polymeric proteins causes a reduction in extensibility. Both types of interferences led to decreased gas retention while decreased extensibility also decreases dough expansion. Therefore, the reduction in area occupied by air cells and number of air cells could be explained by chitin (fiber) interfering with the gluten matrix and causing the decrease in gas retention. The fact that GrioPro cricket protein powder was more detrimental than that of Entomo Farms cricket protein powder could be explained by the reduced extensibility found in GrioPro cricket protein powder containing doughs due to the higher amount of high molecular weight proteins contained in these doughs (Figure 3.9-a, b and Figure 3.11-a, b) and this in turn may have

caused a more pronounced decrease in the number of air cells and area occupied by air cells since the reduced extensibility inhibited dough expansion and was detrimental to gas retention thus producing denser breads with tighter crumb structures in comparison to both the control bread and breads containing Entomo Farms cricket protein powder. This idea is reinforced by the correlations between extensibility with average area occupied by air cells ($r = 0.90$, P-value = 0.01) and with number of air cells ($r = 0.90$, P-value = 0.01). The insoluble polymeric proteins found in dough also correlated highly with average area occupied by air cells ($r = -0.83$, P-value = 0.04).

Another potential reason could be a change in the amount of lipids which could be causing destabilization in the protein-lipid stabilizing films (MacRitchie, 2016). Lipids that form expanded monolayers (linolenic acid) act as foam breakers which produce lower elastic restoring forces in dough and led to low loaf volume (MacRitchie, 2016). As described previously, insects have a higher ratio of linoleic and linolenic acids (De Foliart, 1991). Therefore, it could be possible that the increased amount of linolenic acid was acting as a foam breaker and hindering gas retention as seen in the decrease in average area occupied by air cells and the lower number of air cells.

Another factor to consider would be the poorer gel formation due to a lower peak viscosity and delay in gelatinization (observed for 20% GrioPro) hindering gas retention as discussed previously in the loaf weight and volume/slice area section. As seen in the significant correlation between OT3 (peak viscosity at optimum water absorption) and number of air cells ($r = 0.82$, P-value = 0.05) and in the significant correlations between OC2 (onset of gelatinization at optimum water absorption) with average area occupied by air cells ($r = -0.96$, P-value = 0.002), with cell diameter ($r = -0.99$, P-value = 0.0003), and with cell wall thickness ($r = -0.96$, P-value = 0.002).

Reduced extensibility and weak gel formation would also explain the reduced average air cell diameter seen in the 20% Entomo Farms cricket protein powder replacement level and the 10 and 20% GrioPro cricket protein powder replacement level since the reduced dough expansion would inhibit the air cells from growing therefore producing smaller sized air cells thus decreasing their diameters. This can be seen by the high correlations between extensibility with cell diameter ($r = 0.91$, P-value = 0.01) and between the insoluble polymeric proteins found in dough with cell diameter ($r = -0.91$, P-value = 0.01). As heat was applied in the oven, water evaporates into steam and the rate of fermentation increases releasing more carbon dioxide gas which cause the air bubbles to expand in size and pushes on cell walls causing them to stretch and become thinner (Figoni, 2011). The reduced cell wall thickness seen in the 10 and 20% GrioPro cricket protein

powder replacement levels could be due to the poor gas retention allowing for more of these gasses to escape thus causing even more pressure against the cell walls and this was supported by the correlations between extensibility with air cell wall thickness (r = 0.84, P-value = 0.04) and between the insoluble polymeric proteins found in dough with air cell wall thickness (r = -0.95, P-value = 0.003). Therefore, the cell walls would be forced to stretch more and become thinner. Thus, it can be concluded that the reduced average area occupied by air cells, lower number of air cells, reduced air cell diameter, and reduced cell wall thickness caused by a hindering of both dough expansion and gas retention correspond with the decreased loaf volume reported previously (loaf weight and volume/slice area data presented in section 3.3.2). Again, this was supported by the high correlations between loaf volume with average area occupied by air cells (r = 0.94, P-value = 0.0006), with number of air cells (r =0.92, P-value = 0.001), with cell diameter (r = 0.93, P-value = 0.0008), and with cell wall thickness (r = 0.86, P-value = 0.007). Lastly, there was no significant difference seen in any of the treatments regarding the number of holes found in the bread slices (Table 3.15). Therefore, it can be concluded that incorporation of the protein will not lead to any major cosmetic issues in the final product.

3.3.4. Texture Profile Analysis (TPA) and Staling

Texture and staling are important parameters in determining a products quality. Consumers expect the product to have a long shelf-life, therefore a staling study was conducted on the bread loaves containing 0, 5, 10, or 20% replacement levels of either cricket protein powder to see how the replacement levels impacted both the texture and the staling of the bread.

Hardness is the force required to squeeze the food between the teeth (Kowalczewski et al., 2019). Figure 3.14-a depicts the change in hardness over seven days while Table 3.18 shows the actual values. In general, the 20% addition level was the hardest sample across the seven days for both Entomo Farms and GrioPro cricket protein powder containing loaves (Figure 3.14-a). However, GrioPro cricket protein powder containing loaves were harder than Entomo Farms cricket protein powder containing ones since at every day (including day-0) the probe was overloaded when trying to take the measurement. For 20% Entomo Farms cricket protein powder replacement levels, overloading only occurred at day-3 and day-7. For Entomo Farms cricket protein powder containing loaves, there was no significance change in hardness for the 5 or 10% replacement levels in comparison to the control during day-0 and day-1 (Figure 3.14-a). At day-3

the 10% replacement level of Entomo Farms cricket protein powder shows a significant increase in hardness in comparison to the control and 5% replacement level (Figure 3.14-a). The control and 5% replacement levels showed a slight increase in hardness at day-3 in comparison to day-0 but remained not significantly different from each other at day-3 (Figure 3.14-a). By day-7 all replacement levels containing Entomo Farms cricket protein powder were significantly harder than the control; however, the 5 and 10% replacement levels showed no significant difference from each other (Figure 3.14-a).

On the other hand, breads containing GrioPro cricket protein powder showed a more drastic change in hardness over time in comparison to breads containing Entomo Farms cricket protein powder (Figure 3.14-a). At day-0, only the 5% replacement level of GrioPro cricket protein powder showed no difference in hardness compared to the control (Figure 3.14-a). By day-1 only the control showed no significant difference in hardness compared to day-0, while the 5 and 10% GrioPro cricket protein powder replacement levels showed a significant increase in hardness compared to day-0 (Figure 3.14-a). Note that at day-1 the 5% replacement level of GrioPro cricket protein powder is showing a hardness value not significantly different as what is seen at day-3 for the 5% replacement level of Entomo Farms cricket protein powder (Figure 3.14-a) further supporting that GrioPro cricket protein powder more drastically affected the hardness than Entomo Farms cricket protein powder did.

At day-3, the 10% GrioPro cricket protein powder replacement level significantly increased in hardness in comparison to day-0 and day-1, while the control and 5% replacement levels did not differ from the day-1 values (Figure 3.14-a). Finally, at day-7 the control and 5% GrioPro cricket protein powder replacement levels increased in hardness in comparison to day-0, day-1, and day-3; however, the 10% replacement level did not differ significantly from day-3 (Figure 3.14-a). Therefore, it can be concluded that GrioPro cricket protein powder containing breads will stale significantly faster than loaves containing Entomo Farms cricket protein powder and will be significantly harder at the 10 and 20% GrioPro cricket protein powder replacement levels. However, Entomo Farms cricket protein powder replacement levels of 5 and 10% will not differ from the control during the first days of staling, only showing significant increase at day-3 and beyond.

The above results coincide with results found in additions of cockroach flour which also showed no significant difference in hardness at a 5% level and beyond that an increase in hardness

with an increase in the concentration of cockroach flour (Menegon de Oliveira et al., 2017). Furthermore, additions of whey protein, soy protein, or mushroom flour also caused an increase in bread hardness at high concentrations of the foreign proteins and additions of mustard seed flour decreased bread shelf life (Mironeasa and Codina, 2017; Yuan et al., 2017; Zhou et al., 2018). However, the opposite effect (decrease in hardness) was seen in additions of grape seed flour which improved the shelf-life of the bread and in gluten free applications (starch substitution with cricket protein) which also led to a decrease in bread hardness (Mironeasa and Codina, 2017; Kowalczewski et al., 2019). Menegon de Oliveira et al. (2017) stated that bread hardness increasing is caused by a greater compression of the gas cells in breads of lower volume which increases the bread's resistance to deformation. This would explain why GrioPro cricket protein powder containing breads had higher values of hardness and worst shelf-life since the addition of GrioPro cricket protein powder produced bread with lower specific volumes (loaf weight and volume/slice area data presented in section 3.3.2). The high inverse correlation between loaf volume and hardness ($r = -0.95$, P-value = 0.0012) helps support the previous statement. Yuan et al. (2017) reported that lower carbon dioxide retention also increases bread hardness due to it causing lower volume breads. As discussed previously, a reduction in gas retention occurred with the increase in replacement level (Table 3.15) which produced low volume breads thus increasing the bread hardness. Supported by the inverse correlations between the average area occupied by the air cells ($r = -0.96$, P-value = 0.0006) and the number of air cells ($r = -0.85$, P-value = 0.01) with the bread hardness.

Kowalczewski et al. (2019) found cricket protein powder altered the bound and bulk water fractions and decreased the rate of water transport which allowed the bread to retain water in its structure. They also found that the roasting and grinding of insects made the cricket protein turn hydrophobic which was responsible for the increased water availability provided for starch gelatinization (Kowalsczewski et al., 2019). This would explain the discrepancies found between Entomo Farms and GrioPro cricket protein powders since only Entomo Farms is manufactured via roasting and grinding. Therefore, it could be possible that the pasteurization processing used to produce GrioPro allows the cricket protein powder to remain hydrophilic and provide competition with starch for water as can be seen by the delay in gelatinization in the 20% GrioPro cricket protein powder replacement level (MixoLab optimum water absorption data presented in section 3.2.3) therefore impacting the final crumb structure (onset of gelatinization, OC2, correlated highly

with hardness) (r = 0.89, P-value = 0.04). This is also supported by the increased water holding capacity seen in GrioPro cricket protein powder compared to Entomo Farms cricket protein powder (water holding capacity data presented in section 3.1.3) and by the lower amount of water needed to optimize the Entomo Farms cricket protein powder containing doughs (water absorption at optimization data presented in section 3.2.2). Therefore, it could be concluded that the increased retention of water given by Entomo Farms cricket protein powder to the breads caused the hardness at 5 and 10% to not differ from the control unlike the breads containing GrioPro cricket protein powder substitutions. This idea is supported by the significantly lower C5 torque value in the MixoLab for Entomo Farms cricket protein powder containing doughs which can be due to a decreased amount of free water due to the foreign proteins binding to the water and inhibiting the movement of the amylose chains (MixoLab constant water absorption data presented in section 3.2.1). Thereby, preventing the amylose chains from restructuring themselves causing the breads to have a longer shelf-life. As was seen in the 5 and 10% replacement levels of Entomo Farms cricket protein powder containing doughs which staled more similarly to the control during the first days than the GrioPro cricket protein powder containing doughs.

Cohesiveness is defined as the strength of the internal bonds responsible for the bread structure (Ahmed et al., 2017). The change in cohesiveness over time is depicted in Figure 3.14-b and the actual values are reported in Table 3.18. The most drastic differences between treatments can be seen at day-0 where both types of cricket protein powders significantly decreased the cohesiveness of the bread in comparison to the control as the replacement level increased (Figure 3.13-b).

However, at day-0 Entomo Farms cricket protein powder containing loaves showed no difference between the 5 and 10% replacement level with only the 20% replacement level being drastically lower than the control (Figure 3.14-b). Day-0 for GrioPro cricket protein powder showed a decrease in cohesiveness between the 5 and 10% replacement levels and was not able to measure the percent cohesiveness in the 20% replacement levels (Figure 3.14-b). At day-1 the control bread dropped in cohesiveness and showed a similar value as the 5 and 10% replacement levels containing Entomo Farms cricket protein powder and the 5% replacement level of GrioPro cricket protein powder (Figure 3.14-b). The 20% Entomo Farms cricket protein powder continued having the lowest percent of cohesiveness at day-1, but did not differ from its cohesiveness value at day-0 (Figure 3.14-b).

The same result is mirrored for the 10% GrioPro cricket protein powder replacement level with it having the lowest percent cohesiveness at day-0 compared to all other GrioPro cricket protein powder replacement levels; however, it did not differ significantly from its day-0 value or from the 20% replacement level of Entomo Farms cricket protein powder at day-1 (Figure 3.14-b). By day-3 and day-7, the control value of cohesiveness decreased to match all other replacement levels of both types of cricket protein powders (Figure 3.14-b). Therefore, it is clearly seen that replacement with either type of cricket protein powder will most drastically affect cohesiveness out of the oven, but with time the effect of both cricket protein type and replacement level lose significance. Since cohesiveness depends on the strength of internal bonds (Ahmed et al., 2017) the reduction in cohesiveness caused by the replacement levels of either type of cricket protein powder could be due to the change in the gluten network as discussed in previous sections (extensional dough properties, and loaf weight and volume/slice area data presented in sections 3.2.5 and 3.3.2, respectively). The foam destabilization occurring due to the increased amount of linolenic acid (C-cell microstructure data presented in section 3.3.3) could also be weakening the bonds holding the gluten network together and leading to lower cohesive values.

Springiness is the extent to which crumb can return to its original size after compression (Ahmed et al., 2017). Figure 3.14-c shows the change in springiness over time with the values reported in Table 3.18. Loaves containing 5 or 10% replacement levels of Entomo Farms cricket protein powder showed no significant difference in springiness from each other or the control sample across the span of 3 days (Figure 3.14-c). The 20% Entomo Farms cricket protein powder replacement level was not significantly different in springiness from the control or the other Entomo Farms cricket protein powder replacement levels at day-0, but by day-1 springiness decreased significantly for the 20% replacement level (Figure 3.14-c). At day-7 the 5% Entomo Farms cricket protein powder replacement level remained the same as the control as was seen in all previous days; however, the 10% Entomo Farms cricket protein powder replacement level showed a lower percent of springiness than the control sample (Figure 3.14-c). On the other hand, loaves containing GrioPro cricket protein powder showed a more drastic change in springiness than loaves containing Entomo Farms cricket protein powder. At day-0 only the 5% GrioPro cricket protein powder replacement level showed no difference compared to the control while the 10% GrioPro cricket protein powder replacement level was significantly lower in springiness (Figure 3.14-c). Across the span of day-1, day-3, and day-7 all treatments containing GrioPro

cricket protein powder remained consistent with the control always having the highest percent springiness followed by the 5% replacement level and the 10% replacement level showing the lowest amount of springiness (Figure 3.14-c). Therefore, it can be concluded that for breads containing Entomo Farms cricket protein powder at 5 and 10% replacement levels will not significantly impacts the breads springiness over time; however, for breads containing GrioPro cricket protein powder it is due to the replacement level and not the time which causes a significant decrease in springiness. The results reported for Entomo Farms cricket protein powder coincide with results reported for mushroom flour and cricket protein powder added to starch (non-gluten application) which caused no significant difference in springiness; however, the results reported for GrioPro cricket protein powder coincide with soy protein addition which also caused a decrease in springiness (Yuan et al., 2017; Zhou et al., 2018; Kowalczewski et al., 2019). The reduced amount of springiness seen in GrioPro cricket protein powder containing breads could be due to the detrimental effects the cricket protein powder had in dough extensibility as the concentration increased caused by the larger amount of insoluble polymeric proteins (Figure 3.11-a, b) which lowered the viscoelastic properties therefore preventing the crumb from being able to 'spring' back to its original size.

Chewiness is the energy required to chew food before swallowing (Ahmed et al., 2017). The change in chewiness over time is shown in Figure 3.14-d while the values can be seen in Table 3.18. At day-0 and day-1 loaves containing 5 and 10% replacement levels of Entomo Farms cricket protein powder showed no significant difference from each other or the control (Figure 3.14-d). However, at day-0 and day-1 the 20% Entomo Farms cricket protein powder replacement level was significantly higher than any other of the Entomo Farms cricket protein powder replacement level and the control (Figure 3.14-d). By day-3, the 5% replacement level of Entomo Farms cricket protein powder maintained the same amount of chewiness as the day-3 control; however, the 10% Entomo Farms cricket protein powder replacement level significantly increased in comparison to the day-3 control (Figure 3.14-d). At day-7, there was an increase in chewiness for the control and both the 5 and 10% replacement levels containing Entomo Farms cricket protein powder had higher amount of chewiness (Figure 3.14-d). In general, loaves containing 10% replacement level of GrioPro cricket protein powder had the highest values of chewiness over time in comparison to all other treatments (Figure 3.14-d). The 10% GrioPro cricket protein powder replacement level showed the same trend as the 20% GrioPro cricket protein powder replacement level since it also

increased in chewiness over time (Figure 3.14-d). Note that only at day-3 did the control loaf show no significant difference in chewiness as the 5% GrioPro cricket protein powder replacement level (Figure 3.14-d). Therefore, it can be said that loaves containing 5 and 10% replacement levels of Entomo Farms cricket protein powder do not significantly impact the breads chewiness for the first 3 days. Furthermore, breads containing GrioPro cricket protein powder are significantly different in chewiness across the span of time with the chewiness being more pronounced in higher replacement levels.

Chewiness is calculated by hardness×cohesiveness×springiness (Ahmed et al., 2017). Therefore, the results are closely tied to the results found in all three parameters (Table 3.18). The harder the bread, the more energy required to chew the food therefore the increased chewiness at higher concentration for Entomo Farms cricket protein powder and for any GrioPro cricket protein powder replacement level is tied to the higher hardness values. As discussed in the hardness section, water retention, gas retention and loaf volume are tied to hardness. Therefore, the increase in chewiness could be due to the denser breads produced at higher concentrations of the protein (more drastically seen in GrioPro cricket protein powder containing breads) caused by the lower gas retention (loaf weight and volume/slice area, and C-cell microstructure data presented in sections 3.3.2 and 3.3.3, respectively). Supported by the high correlations between chewiness with loaf weight ($r = 0.90$, P-value = 0.005) and with loaf volume ($r = -0.93$, P-value = 0.002).

Resilience is the ability of crumb to return to its original state (Kowalczewski et al., 2019). Figure 3.14-e shows the change in resilience over time with the actual values reported in Table 3.18. For this TPA parameter, both types of cricket protein powders showed the same trend. At day-0, the resilience decreased as the replacement level increased for loaves containing Entomo Farms or GrioPro cricket protein powder (Figure 3.14-e). However, the 5 and 10% Entomo Farms cricket protein powder replacement levels were significantly higher than the 5 and 10% GrioPro cricket protein powder replacement levels (Figure 3.14-e). All the other days (1, 3, and 7) showed no significant difference in resilience in any of the treatments including the control samples (Figure 3.14-e). Thus, the type and amount of cricket protein used is only significant out of the oven and is not as important beyond day-1. The more resilient a food is, the more capable it is to return to its original state after deformation (Kowalczewski et al., 2019). Therefore, the springier a food is, the more resilient it is as well. Therefore, the decreased springiness due to a weaker gluten matrix

(Figure 3.14-c) would lower the ability for the bread crumb's ability to return to its original state thus reducing its resilience.

Table 3.14. One-way analysis of variance (ANOVA) for physical properties of baked products

Variable	df	Mean Square	F-value	P-value
L	6	429.354	518.29	<0.0001
Error	35	0.828		
a	6	31.176	1281.42	<0.0001
Error	35	0.024		
b	6	0.058	225.87	<0.0001
Error	35	0.000		
Brightness	6	3386.382	3705.79	<0.0001
Error	14	0.914		
Loaf weight	6	54.554	55.83	<0.0001
Error	14	0.977		
Loaf volume	6	100049.603	1183.69	<0.0001
Error	14	84.5238		
Slice area	6	0.077	397.12	<0.0001
Error	14	0.000		
Area occupied by air cells	6	0.001	59.91	<0.0001
Error	14	0.000		
Avg. air cell diameter	6	0.219	62.97	<0.0001
Error	14	0.003		
Avg. air cell wall thickness	7	0.002	67.58	<0.0001
Error	16	0.000		
Number of air cell	6	0.026	125.61	<0.0001
Error	14	0.000		
Number of holes	6	0.760	1.12	0.4005
Error	14	0.680		

[1]b, slice area, area occupied by air cells, and number of air cell were transformed into a log base 10 for analysis

Table 3.15. Physical properties of baked products

Powder type	Replacement level (%)	Color						Brightness (-)	
		L		a		b			
E	0	64.8 ± 0.6	a	-1.27 ± 0.04	g	10.6 ± 0.3	d	151.0 ± 0.9	a
	5	55.4 ± 0.2	b	1.63 ± 0.10	e	15.6 ± 0.4	c	109.2 ± 0.3	b
	10	46.2 ± 0.4	d	3.54 ± 0.08	c	18.3 ± 0.1	b	86.4 ± 0.8	d
	20	40.1 ± 0.3	f	5.67 ± 0.06	a	21.1 ± 0.1	a	50.6 ± 0.1	g
G	0	64.8 ± 0.6	a	-1.27 ± 0.04	g	10.6 ± 0.3	d	151.0 ± 0.9	a
	5	55.7 ± 0.3	b	0.86 ± 0.05	f	15.0 ± 0.2	c	103.9 ± 0.6	c
	10	49.6 ± 0.4	c	2.96 ± 0.05	d	18.5 ± 0.1	b	78.4 ± 0.3	e
	20	43.8 ± 0.3	e	3.98 ± 0.04	b	18.3 ± 0.1	b	61.5 ± 0.1	f

Powder type	Replacement level (%)	Loaf weight (g)		Loaf volume (cc)		Slice area (mm^2)		Area occupied by air cells (%)	
E	0	144.6 ± 1.0	d	905.0 ± 7.6	a	5974 ± 196	a	50.8 ± 0.03	a
	5	147.2 ± 0.1	c	853.3 ± 6.7	b	5014 ± 71	b	51.0 ± 0.15	a
	10	147.4 ± 0.1	c	800.0 ± 5.8	c	4774 ± 59	bc	51.6 ± 0.12	a
	20	149.2 ± 0.4	c	585.0 ± 2.9	d	2747 ± 6	e	49.1 ± 0.50	b
G	0	144.6 ± 1.0	d	905.0 ± 7.6	a	5974 ± 196	a	50.8 ± 0.03	a
	5	147.8 ± 0.5	c	783.3 ± 4.4	c	4471 ± 116	c	50.7 ± 0.17	a
	10	152.8 ± 0.8	b	580.0 ± 5.8	d	3146 ± 23	d	48.4 ± 0.07	b
	20	157.3 ± 0.5	a	400.0 ± 0.0	e	2172 ± 30	f	47.0 ± 0.09	c

Powder type	Replacement level (%)	Ave. air cell diameter (mm)		Ave. air cell wall thickness (mm)		Number of air cell		Number of Holes[1]
E	0	1.81 ± 0.02	a	0.428 ± 0.003	a	4134 ± 153	a	1.59 ± 0.66
	5	1.83 ± 0.05	a	0.426 ± 0.005	a	3615 ± 61	b	1.12 ± 0.69
	10	1.85 ± 0.01	a	0.428 ± 0.002	a	3407 ± 28	bc	0.37 ± 0.32
	20	1.61 ± 0.05	b	0.418 ± 0.004	ab	2291 ± 58	e	0.65 ± 0.29
G	0	1.81 ± 0.02	a	0.428 ± 0.003	a	4134 ± 153	a	1.59 ± 0.66
	5	1.82 ± 0.04	a	0.428 ± 0.004	a	3278 ± 34	c	1.10 ± 0.58
	10	1.49 ± 0.02	b	0.408 ± 0.001	b	2639 ± 17	d	0.12 ± 0.07
	20	1.12 ± 0.01	c	0.352 ± 0.002	c	2514 ± 13	d	1.03 ± 0.40

Values reported for breads containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 5, 10, and 20% total flour weight replacement levels

[1] not significantly different by one-way ANOVA df 6, F-value 1.12, P-value 0.4005 for Number of Holes

[2] means with the same letter are not significant ($p > 0.05$)

Table 3.16. Three-way analysis of variance (ANOVA) for physical properties of baked products

Variable	df	Mean Square	F-value	P-value
Protein	1	952022895.000	568.56	< 0.0001
Day	3	542883695.000	324.22	< 0.0001
Level	2	1268926236.000	757.82	< 0.0001
Protein*day	3	5052716.000	3.02	0.0377
Day*level	6	27499966.000	16.42	< 0.0001
Protein*level	2	600087313.000	358.38	< 0.0001
Error	54	1674438.000		
Protein	1	20.465	1.29	0.2617
Day	3	1793.982	112.83	< 0.0001
Level	2	1013.084	63.72	< 0.0001
Protein*day	3	101.562	6.39	0.0009
Day*level	6	95.636	6.01	< 0.0001
Protein*level	2	330.071	20.76	< 0.0001
Error	53	15.900		
Protein	1	0.077	26.10	< 0.0001
Day	3	0.016	5.36	0.0027
Level	2	0.108	36.50	< 0.0001
Protein*day	3	0.004	1.26	0.2964
Day*level	6	0.002	0.77	0.5966
Protein*level	2	0.037	12.55	< 0.0001
Error	53	0.003		
Protein	1	21615175.760	49.45	< 0.0001
Day	3	17266736.990	39.50	< 0.0001
Level	2	34957769.800	79.98	< 0.0001
Protein*day	3	2080971.880	4.76	0.0052
Day*level	6	1192225.390	2.73	0.0220
Protein*level	2	15174243.420	34.72	< 0.0001
Error	53	437092.4000		
Protein	1	0.001	11.20	0.0015
Day	3	0.017	211.14	< 0.0001
Level	2	0.005	66.50	< 0.0001
Protein*day	3	0.000	6.00	0.0013
Day*level	6	0.003	36.16	< 0.0001
Protein*level	2	0.000	5.39	0.0073
Error	54	0.000		

[1]Cohesiveness was transformed into a log base 10 for analysis

[2]Hardness, Cohesiveness, Springiness, Chewiness, & Resilience ANOVA values reported from top to bottom

Table 3.17. One-way analysis of variance (ANOVA) for physical properties of baked products

Variable	DF	Mean Square	F-value	P-value
Hardness	23	283399001.000	191.23	<0.0001
Error	48	1481953.000		
Cohesiveness	23	0.035	121.92	<0.0001
Error	47	0.000		
Springiness	23	196.633	6.34	<0.0001
Error	47	30.993		
Chewiness	23	8381930.700	22.58	<0.0001
Error	47	371190.100		
Resilience	23	35.203	50.55	<0.0001
Error	48	843.098		

[1]Cohesiveness was transformed into a log base 10 for analysis

Table 3.18. Change in texture profile analysis (TPA) parameters over time

Powder type	Storage (day)	Replacement level (%)	Hardness (g)	Cohesiveness (%)	Springiness (%)	Chewiness (g)	Resilience (%)
E	0	0	2289 ± 62 i	68.7 ± 2.1 a	86.8 ± 0.6 ab	1366 ± 70 ij	22.4 ± 1.1 a
		5	2619 ± 24 i	54.9 ± 1.2 b	84.6 ± 0.6 ab	1216 ± 20 j	16.4 ± 0.5 b
		10	3501 ± 215 hi	54.3 ± 0.7 b	82.3 ± 0.9 abc	1564 ± 93 hij	15.6 ± 0.5 b
		20	16663 ± 950 e	29.7 ± 0.6 h	88.1 ± 4.5 a	4361 ± 99 c	9.3 ± 0.7 e
	1	0	4846 ± 124 hi	45.0 ± 0.6 c	84.4 ± 1.5 ab	1844 ± 89 ghi	11.7 ± 0.2 cde
		5	4832 ± 182 hi	43.7 ± 0.8 c	78.4 ± 1.4 abcde	1655 ± 81 hij	11.1 ± 0.4 cde
		10	5250 ± 268 hi	43.1 ± 0.6 c	75.9 ± 2.6 bcde	1714 ± 78 hij	10.4 ± 0.2 de
		20	15709 ± 637 e	32.1 ± 0.6 gh	55.2 ± 3.2 i	2774 ± 102 e	9.5 ± 0.1 e
	3	0	6874 ± 277 gh	41.8 ± 1.3 cd	82.8 ± 0.6 abc	2372 ± 69 efg	11.5 ± 0.7 cde
		5	8858 ± 38 fg	37.7 ± 0.8 def	77.4 ± 0.7 abcde	2587 ± 53 ef	10.2 ± 0.4 de
		10	16961 ± 465 e	34.1 ± 0.6 efgh	84.4 ± 4.1 ab	4866 ± 218 c	10.5 ± 0.2 de
		20					10.6 ± 0.3 de
	7	0	12100 ± 375 f	36.4 ± 1.2 efg	83.0 ± 3.4 abc	3646 ± 138 d	11.1 ± 0.3 cde
		5	16215 ± 142 e	35.8 ± 0.3 efg	76.3 ± 0.3 abcde	4432 ± 48 c	11.7 ± 0.2 cde
		10	18553 ± 1138 de	32.9 ± 0.8 fgh	70.9 ± 1.6 defg	4316 ± 150 c	11.1 ± 0.5 cde
		20					11.6 ± 0.5 cde

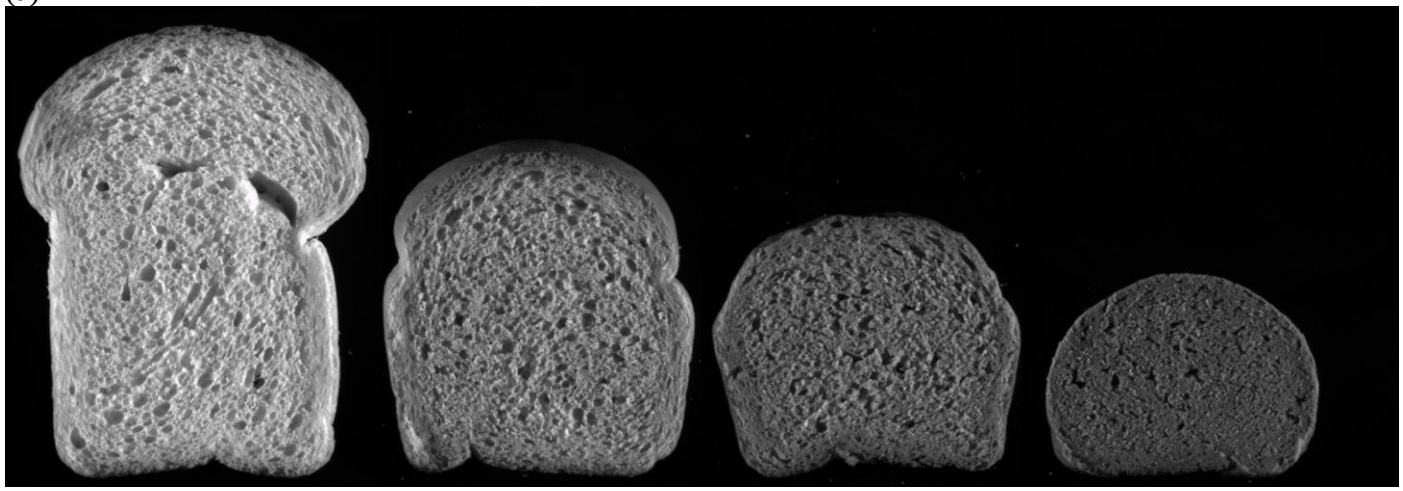
Table 3.18- cont'd. Change in texture profile analysis (TPA) parameters over time

Powder type	Storage (day)	Replacement level (%)	Hardness (g)	Cohesiveness (%)	Springiness (%)	Chewiness (g)	Resilience (%)
G	0	0	2289 ± 62 i	68.7 ± 2.1 a	86.8 ± 0.6 ab	1366 ± 70 ij	22.4 ± 1.1 a
		5	4919 ± 205 hi	50.8 ± 0.8 b	81.7 ± 0.5 abcd	2044 ± 115 fgh	13.3 ± 0.2 c
		10	23328 ± 1781 c	38.0 ± 0.5 de	55.5 ± 0.0 i	6185 ± 294 c	12.1 ± 0.5 cd
		20					10.4 ± 0.8 de
	1	0	4846 ± 124 hi	45.0 ± 0.5 c	84.4 ± 1.5 ab	1844 ± 89 ghi	11.7 ± 0.2 cde
		5	9372 ± 291 fg	42.1 ± 0.7 cd	72.3 ± 1.5 cdef	2852 ± 133 e	9.9 ± 0.3 de
		10	26819 ± 396 b	33.5 ± 0.2 efgh	61.1 ± 1.2 ghi	5485 ± 132 b	11.0 ± 0.1 cde
		20					12.3 ± 0.2 cd
	3	0	6874 ± 277 gh	41.8 ± 1.3 cd	82.8 ± 0.6 abc	2372 ± 69 efg	11.5 ± 0.7 cde
		5	10680 ± 372 f	34.5 ± 0.5 efgh	69.8 ± 0.9 efgh	2565 ± 27 ef	9.5 ± 0.1 e
		10	33873 ± 1298 a	29.5 ± 0.5 h	59.9 ± 0.8 hi	5975 ± 225 b	11.1 ± 0.2 cde
		20					12.2 ± 0.1 cd
	7	0	12100 ± 375 f	36.4 ± 1.2 efg	83.0 ± 3.4 abc	3646 ± 138 d	11.1 ± 0.4 cde
		5	20115 ± 1731 d	32.3 ± 0.5 gh	71.8 ± 4.4 cdef	4623 ± 174 c	10.2 ± 0.4 de
		10	33774 ± 317 a	32.8 ± 1.6 fgh	62.7 ± 3.3 fghi	6929 ± 92 a	11.6 ± 0.4 cde
		20					11.3 ± 0.1 cde

Values reported for breads containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 5, 10, and 20% total flour weight replacement levels over a period of 0, 1, 3, and 7 days

[1]means with the same letter are not significant ($p > 0.05$)

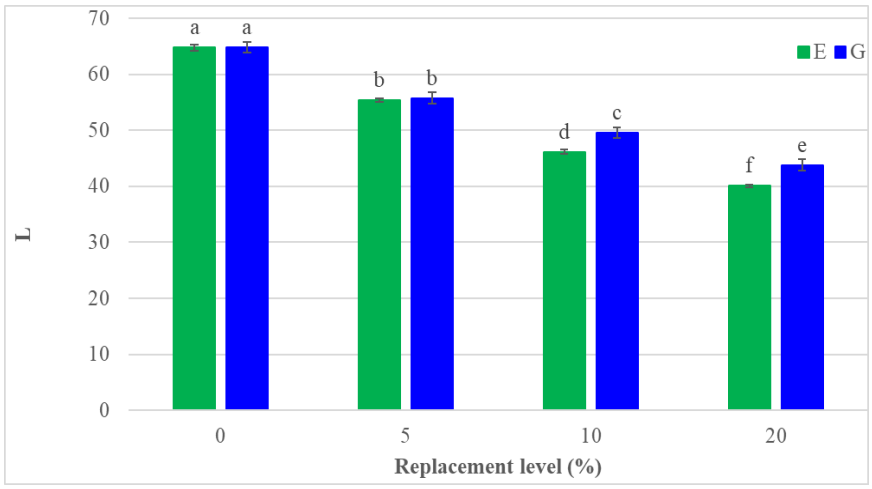
(a)



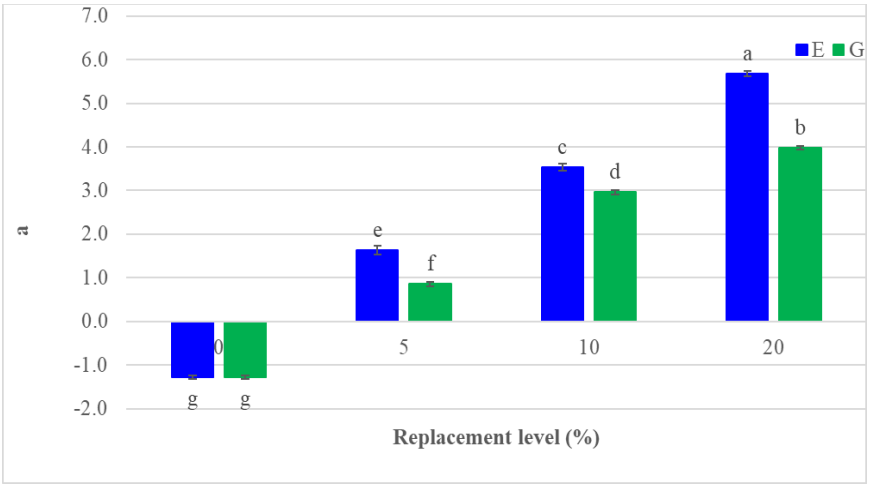
(b)

Figure 3.12. C-Cell images (a) for Entomo Farms, and (b) for GrioPro cricket protein powder containing breads
Breads containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 5, 10, and 20% (from left to right) total flour weight replacement levels

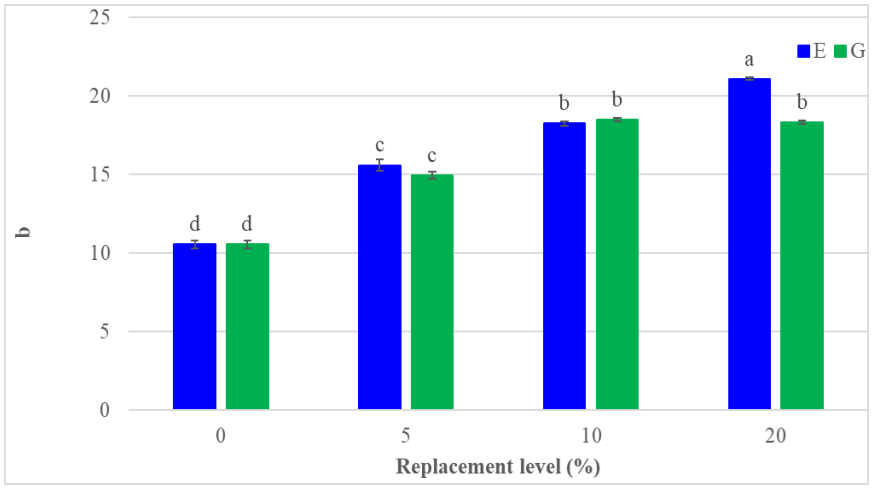
(a)


70
60
50
40
30
20
10
0
L
a a
b b
d c
f e
E G
0
5
10
20
Replacement level (%)

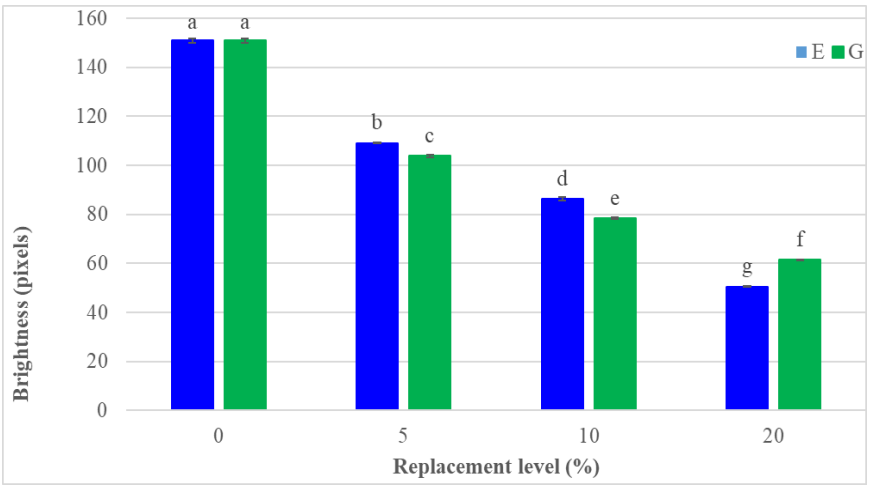
(b)


7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
a
g g
e f
c d
a b
E G
0
5
10
20
Replacement level (%)

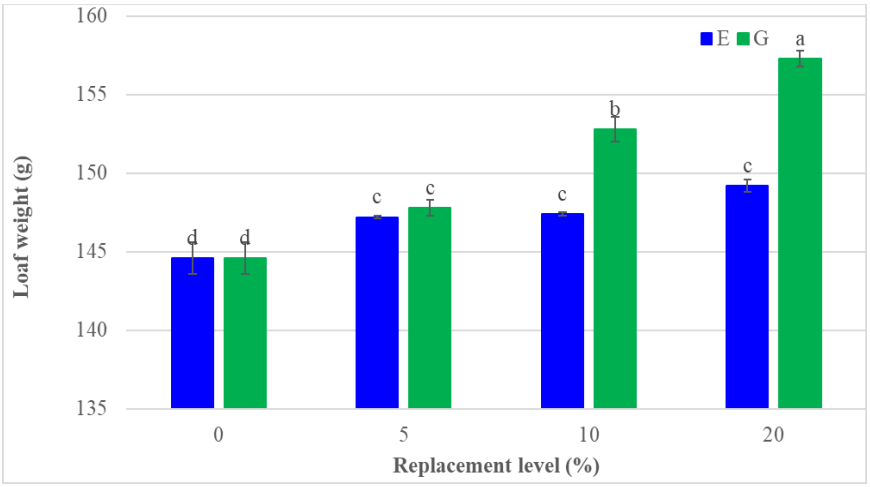
(c)


25
20
15
10
5
0
b
d d
c c
b b
a b
E G
0
5
10
20
Replacement level (%)

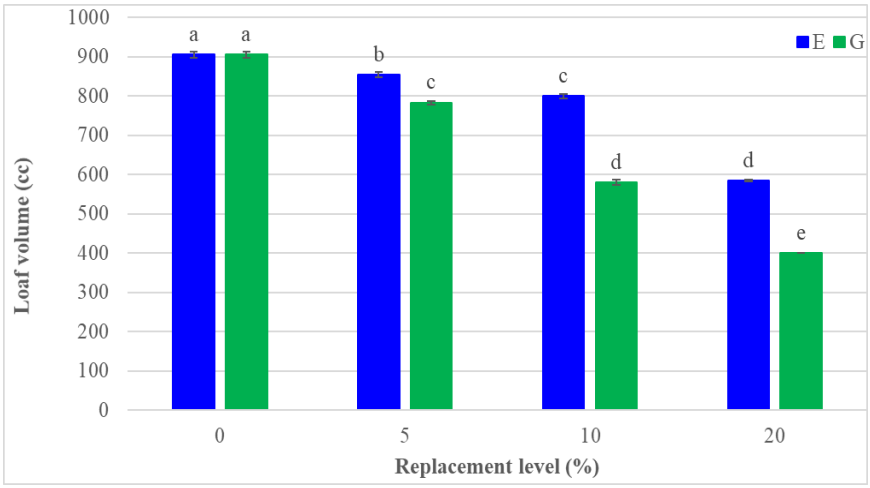
(d)


Brightness (pixels)
160
140
120
100
80
60
40
20
0
a a
b c
d e
g f
E G
0
5
10
20
Replacement level (%)

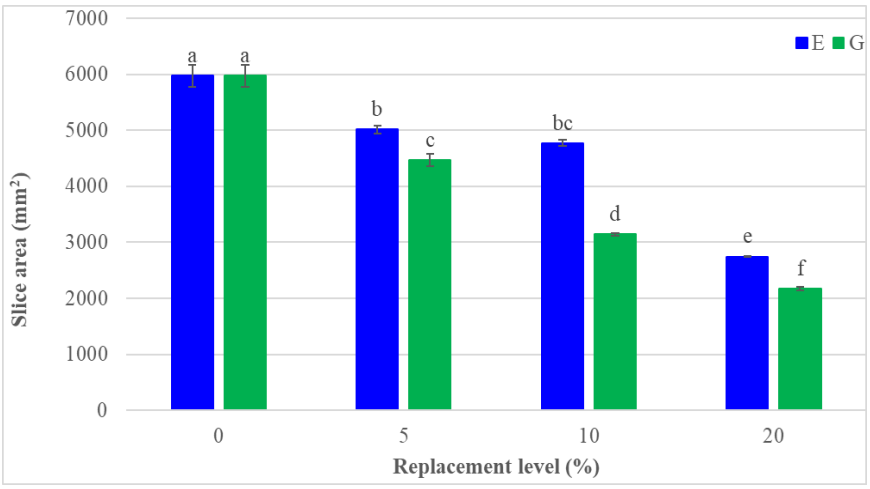
(e)


Loaf weight (g)
160
155
150
145
140
135
d d
c c
c b
c a
E G
0
5
10
20
Replacement level (%)

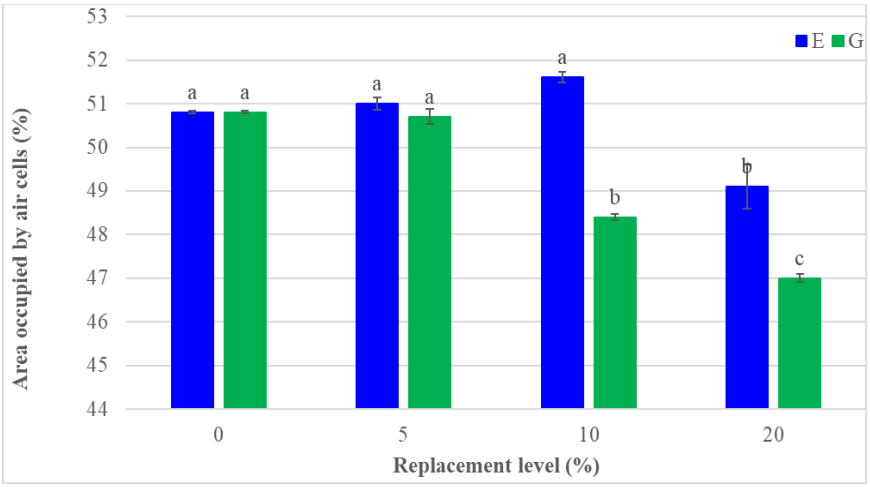
(f)


Loaf volume (cc)
1000
900
800
700
600
500
400
300
200
100
0
a a
b c
c d
d e
E G
0
5
10
20
Replacement level (%)

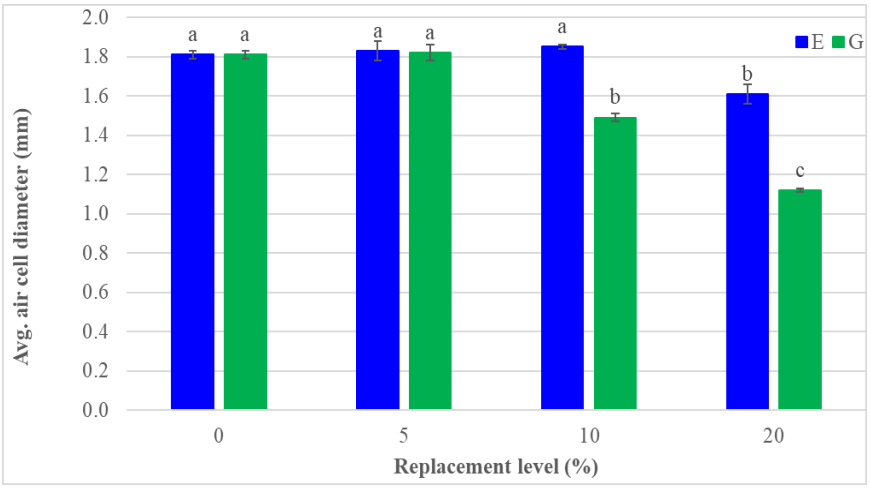
(g)


Slice area (mm²)
7000
6000
5000
4000
3000
2000
1000
0
E G
a a
b c
bc d
e f
0 5 10 20
Replacement level (%)

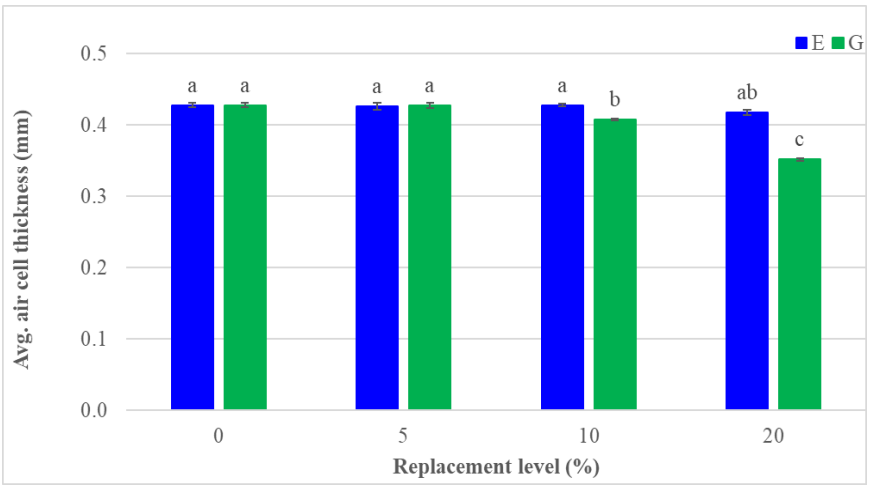
(h)


Area occupied by air cells (%)
53
52
51
50
49
48
47
46
45
44
E G
a a
a a
a b
b c
0 5 10 20
Replacement level (%)

(i)


Avg. air cell diameter (mm)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
E G
a a
a a
a b
b c
0 5 10 20
Replacement level (%)

(j)



(k)

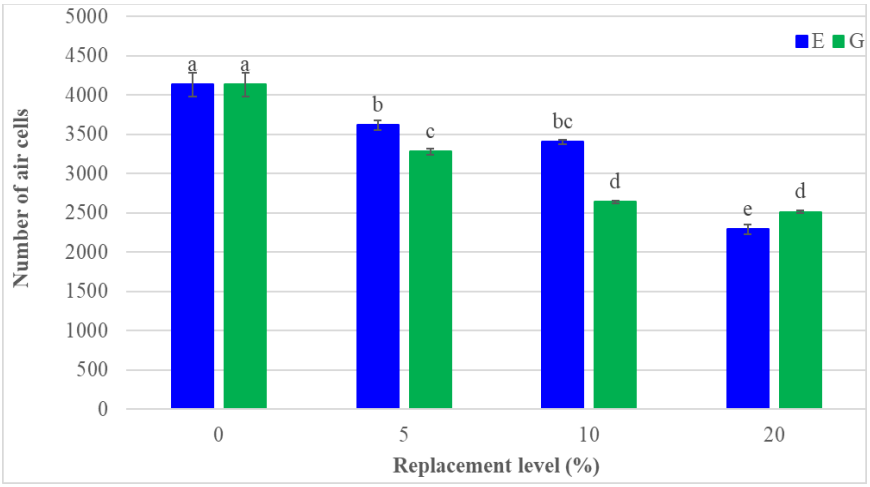
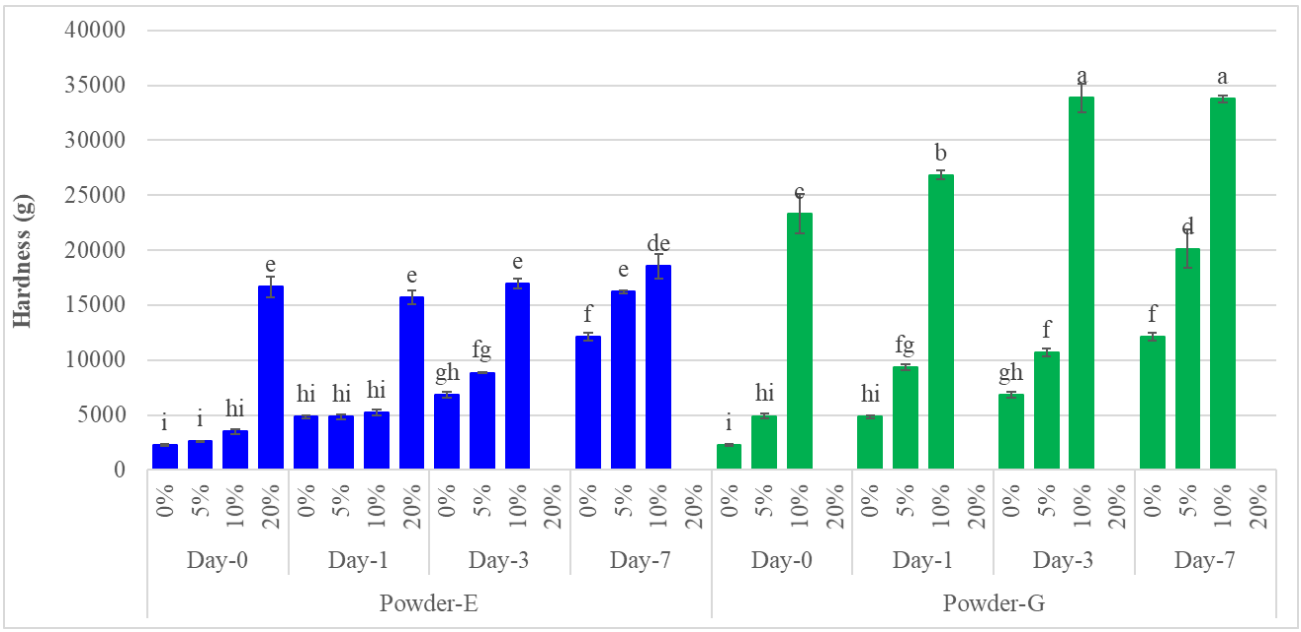


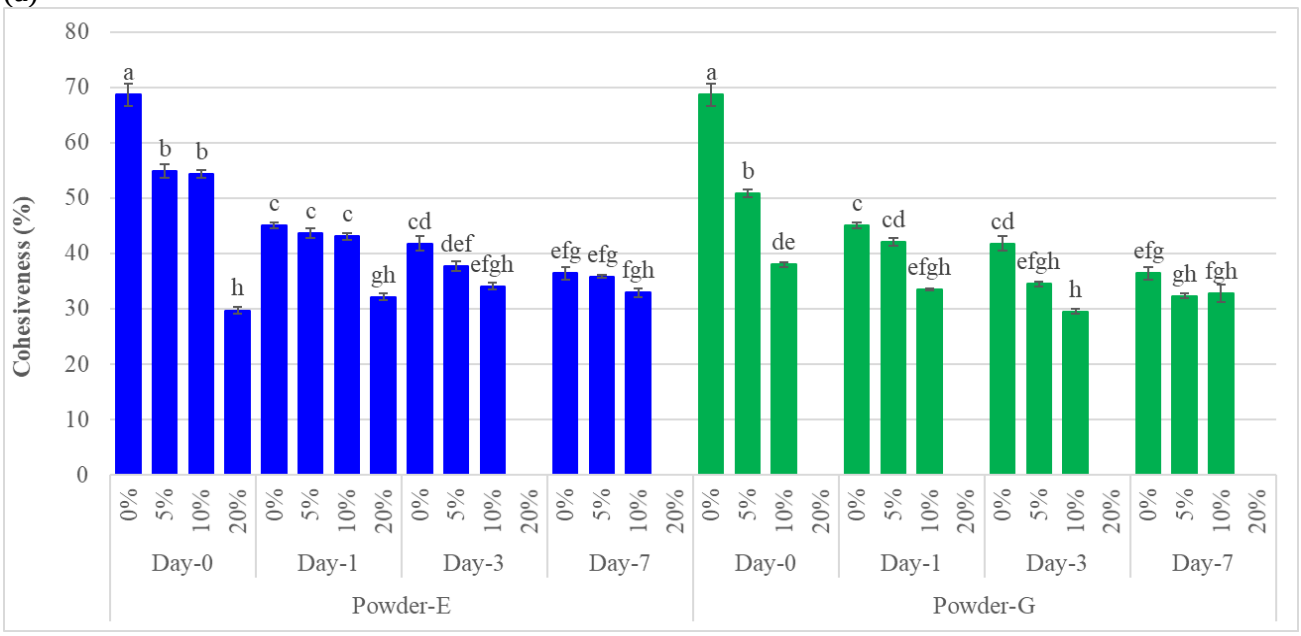
Figure 3.13. Physical properties of baked products

C-cell values reported in (a) through (k) for breads containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 5, 10, and 20% total flour weight replacement levels

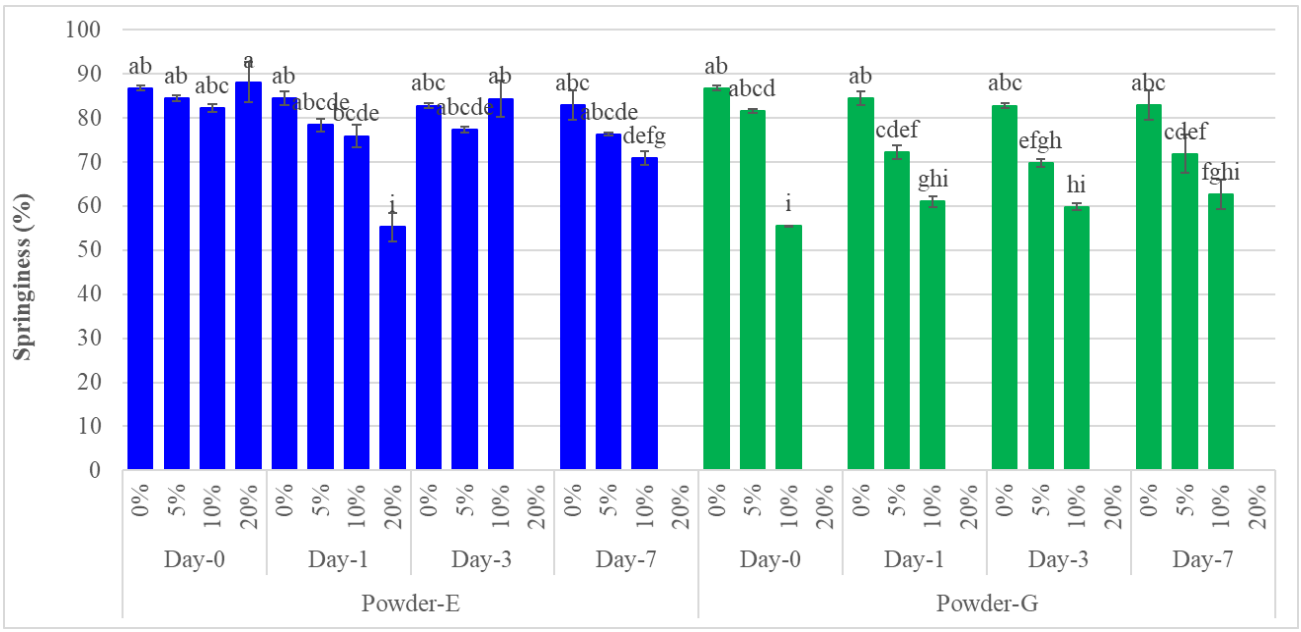
[1]means with the same letter are not significant ($p > 0.05$)


Hardness (g)
40000
35000
30000
25000
20000
15000
10000
5000
0
0%
5%
10%
20%
Day-0
Day-1
Day-3
Day-7
Powder-E
Powder-G

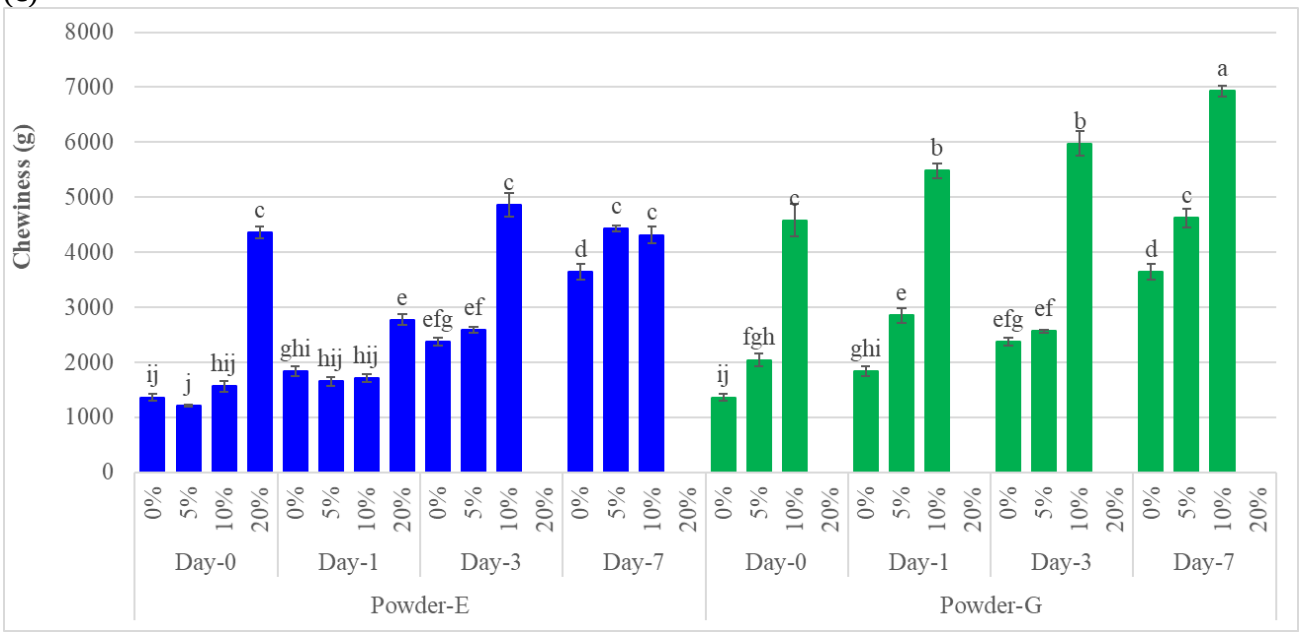
(a)


Cohesiveness (%)
80
70
60
50
40
30
20
10
0
0%
5%
10%
20%
Day-0
Day-1
Day-3
Day-7
Powder-E
Powder-G

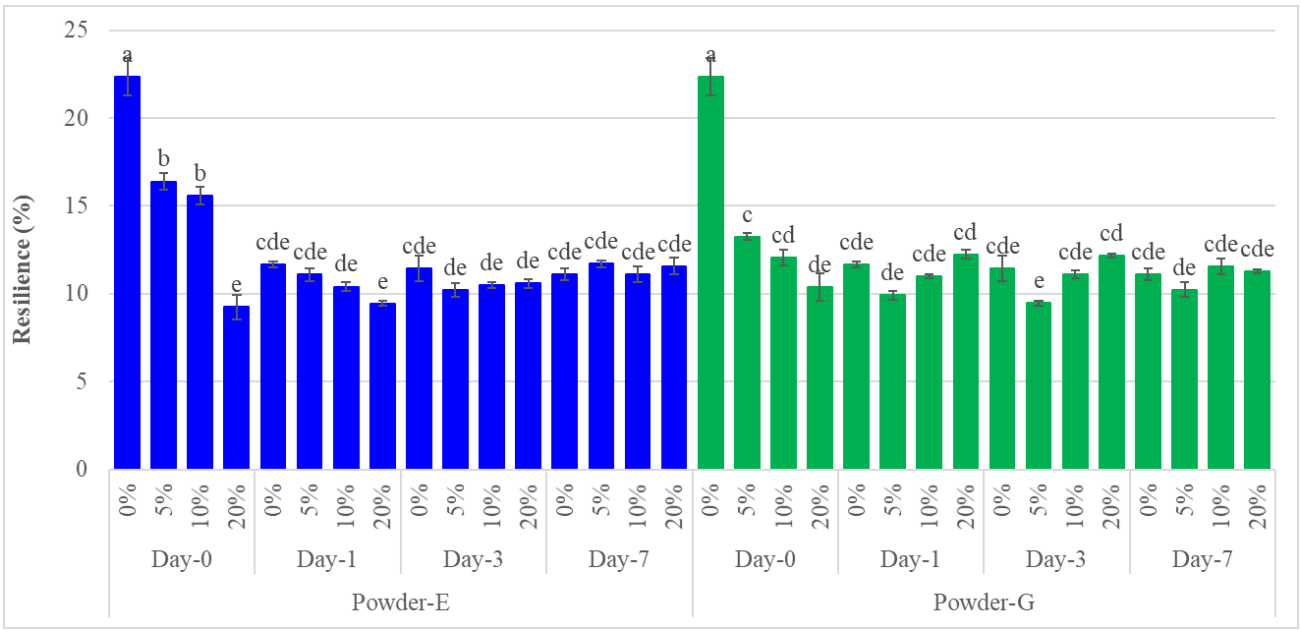
(b)


Springiness (%)
100
90
80
70
60
50
40
30
20
10
0
ab ab abc a ab abcde bcde i abc abcde ab abc abcde defg ab abcd i ab cdef ghi abc efgh hi abc cdef fghi
0% 5% 10% 20%
Day-0 Day-1 Day-3 Day-7
Powder-E
Powder-G

(c)


Chewiness (g)
8000
7000
6000
5000
4000
3000
2000
1000
0
ij j hij c ghi hij hij e efg ef c d c c ij fgh c ghi e b efg ef b d c a
0% 5% 10% 20%
Day-0 Day-1 Day-3 Day-7
Powder-E
Powder-G

(d)



(e)

Figure 3.14. Change in texture profile analysis (TPA) parameters over time

TPA values reported in (a) through (e) for breads containing Entomo Farms (E) and GrioPro (G) cricket protein powders at 0, 5, 10, and 20% total flour weight replacement levels over a period of 0, 1, 3, and 7 days

[1]means with the same letter are not significant ($p > 0.05$)

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Chapter 4 - Conclusions

4.1. Powder Characterization

4.1.1. Composition

Entomo Farms and GrioPro cricket protein powders contain high levels of protein (62% vs. 67%) which are higher than the amount found in raw meat (20-25%), cereals (10-15%), pulses (21-25%), eggs (13%), milk (3.5%), and soy (36.5%). Insect proteins have a higher ratio of essential polyunsaturates (linoleic and linolenic) and contain a good nutritional profile. The differences in moisture, iron, B12, and cholesterol between the two different types of cricket protein powders could be due to various factors such as: gender, developmental stage, diet, and environmental factors. All these factors alter the nutritional profile of the insect and the type of processing affects the bioavailability of the nutritional profile.

4.1.2. Microbial Load

Entomo Farms cricket protein powder had a higher CFU than GrioPro cricket protein powder thereby showing that pasteurization (G) worked more effectively than roasting (E) in controlling the aerobic bacteria. Contaminants found in insects may include mycotoxins, natural toxins, heavy metals, pesticides, and pathogens. Furthermore, insects can cause allergic reactions since they contain arginine kinase. Processing, diet, and rearing practices are all sources of contamination to the insects themselves while post-processing contamination can also occur after the insects have been converted into the cricket protein powders. Mealworms and grasshoppers have been shown to contain the spoilage organism, *Psuedomonads*. Furthermore, insects act as carriers for pathogens thus making a kill step during processing crucial in order to ensure the powders are safe for human consumption. Acidification is the only way for the spore forming bacteria to be eliminated during processing. However, currently no legislation exists to regulate the rearing and processing of insects used in food.

4.1.3. Water Holding Capacity

Entomo Farms cricket protein powder can hold approximately 2.5 times its weight in water while GrioPro cricket protein powder can hold approximately 3 times its weight in water. Thus, GrioPro cricket protein powder has a greater water holding capacity than Entomo Farms cricket

protein powder. For Entomo Farms cricket protein powder, as the pH became more alkaline there was a slight increase in the water holding capacity. However, there was no significant difference observed between the pH of 7 and 10. For GrioPro cricket protein powder, as the pH became more alkaline there was a drastic increase in water holding capacity. The differences seen between the two types of cricket protein powders could be due to the differences in processing. Entomo Farms cricket protein powder is roasted while GrioPro cricket protein powder is pasteurized which can alter the proteins functionality. Other factors influencing the water holding capacities include the amino acid composition, the protein conformation, and the surface polarity/hydrophobicity of the compounds found within the cricket protein powders.

4.1.4. Solubility

In general, both Entomo Farms and GrioPro cricket protein powders were the most soluble during alkaline conditions. For Entomo Farms cricket protein powder, there was a significant increase in solubility as the pH became more alkaline. For GrioPro cricket protein powder, there was no difference observed at pH 3, 5, or 7, but there was a significant increase in solubility at the pH of 10. Therefore, Entomo Farms cricket protein powder is more soluble than GrioPro cricket protein powder. Differences in the molecular weight distribution (as seen in the SDS-PAGE) affects solubility since the bigger molecules tend to be less soluble due to a decreased surface area. GrioPro cricket protein powder contains larger molecules thereby explaining its lower solubility. pH changes the net charges on the proteins and these charges influence the attractive and repulsive affinities with water especially at acidic conditions where protein denaturation reveals hydrophobic groups thus reducing solubility. Furthermore, below a pH of 4, the carboxyl groups become un-ionized thus reducing a peptide's affinity to water which also causes a reduction in solubility.

4.1.5. SDS-PAGE

For the wheat flour, bands ranged within 30-160 kDa where the bands correspond with high molecular weight glutelin subunits at a range from 80-120 kDA, low molecular glutelin subunits at a range from 30-55 kDa, gliadins at a range from 20-30 kDa, and albumins and globulins falling under 20 kDa. For both types of cricket protein powders, the MOPS buffer was unable to fully solubilize the cricket proteins therefore only a few visible bands appeared for Entomo Farms cricket protein powder and no bands were visible in GrioPro cricket protein

powder. The results for Entomo Farms cricket protein powder were comparable to a study done on unhydrolyzed cricket protein which resulted in bands ranging from 14.4 to 212 kDa. For GrioPro cricket protein powder, proteins that were solubilized in the sample buffer appeared to be too large to migrate into the gel thus explaining why no bands were visible. Therefore, it can be concluded that GrioPro cricket protein powder contains larger molecules than Entomo Farms cricket protein powder which was also supported by SEC-HPLC results discussed below.

4.1.6. Raw Materials SP-IP HPLC Chromatograms

For the wheat flour, the SP chromatogram differed from both Entomo Farms and GrioPro cricket protein powders since the highest intensity peak was at 18 minutes. On the other hand, both Entomo Farms and GrioPro cricket protein powders had their highest intensity peaks at 20 and 24 minutes thereby containing smaller molecular weight compounds not seen in wheat flour. The wheat flour, and both types of cricket protein powders all had the highest intensity peak at 11 minutes which in wheat corresponds to the high molecular weight proteins. Therefore, both proteins contain large sized molecular compounds similar in size to high molecular weight glutelin subunits. As was seen in the SDS-PAGE where the GrioPro cricket protein powder had no visible bands due to the largeness in size not allowing the compounds to migrate through the gel.

4.1.7. Reduced vs. Non-reduced SP-IP HPLC Chromatograms

The reducing agent, BME, fully reduced the disulfide bonds found in the SP and IP profiles of wheat flour. For Entomo Farms and GrioPro cricket protein powders, no change was observed in the SP profiles while an incomplete reduction was observed in the IP profiles. Thus, it can be concluded that other interactions other than disulfide bonds are holding together the SP and IP molecules for both Entomo Farms and GrioPro cricket protein powders.

4.2. Dough Development

4.2.1. MixoLab at Constant Water Absorption

Only the 20% replacement level of GrioPro cricket protein powder was significantly higher in C1 torque value than the control. The C1 torque represents the maximum torque during mixing and is affected by both hydration and energy application. Water acts as a lubricant and inert filler thus affecting the mobility of the high molecular weight glutenin subunits. Due to the higher water

holding capacity of GrioPro cricket protein powder, the amount of free water would have been limited thus decreasing the mobility and creating a discontinuous gluten network.

The other factor is the energy application since it takes longer to fully develop dough with greater amounts of high molecular weight proteins, and it requires more energy. Since GrioPro cricket protein powder contains greater amounts of larger molecular weight proteins than Entomo Farms cricket protein powder does, it would require more energy to develop.

On the other hand, the lower molecular weight protein are unraveled first before the larger molecular weight ones. Therefore, the significantly longer C1 time seen in Entomo Farms cricket protein powder could be due to the larger amount of lower molecular weight proteins found in Entomo Farms cricket protein powder as seen in both the SDS-PAGE and in the SP and IP chromatograms. The lower molecular weight proteins in Entomo Farms cricket protein powder were causing a time delay to reach the maximum peak torque during mixing. The stability time decreased significantly for the 20% replacement level of Entomo Farms cricket protein powder; however, both the 10 and 20% replacement levels of GrioPro cricket protein powder significantly increased in stability time. This could be due to the larger amount of higher molecular weight proteins found in GrioPro cricket protein powder which added strength to the dough by sustaining the demand for larger molecules to withstand the mechanical shear and tensile forces applied by the mixer.

The minimum torque during mixing (C2 torque) was significantly higher in GrioPro cricket protein powder replacement levels than in the Entomo Farms cricket protein powder replacement levels. Overall, both cricket protein powders displayed the same behavior of decreasing in C2 torque as the replacement level increased. The C2 torque represents the protein weakening stage, therefore it can be concluded that GrioPro cricket protein powder containing doughs were stronger as was seen by the increased stability and increased peak torque. On the other hand, Entomo Farm containing doughs were softer and broke down faster. There was no significant difference between any of the treatments in the C2 time and temperature (the onset of gelatinization time and temperature) therefore it can be concluded that there was no delay in gelatinization for any of the treatments.

The C3 torque (peak viscosity) showed the same trend for both types of cricket protein powders whereas the replacement level increased there was a decrease in peak viscosity. This trend was more drastic in the doughs containing Entomo Farms cricket protein powder. The C3 time

was only significantly delayed in the 20% GrioPro cricket protein powder replacement level. A limited water supply delays the peak viscosity time since there is a competition between the starch and other ingredients for water therefore taking a longer time for the dough to undergo gelatinization. The increased water holding capacity for Entomo Farms and GrioPro cricket protein powder would increase the amount of competition for water thereby allowing less starch to swell to bursting point. This would lower the amount of amylase and amylopectin leaching out and create a weaker gel as was seen by the lower peak viscosity values (C3 torque).

The C4 torque decreased for Entomo Farms cricket protein powder containing doughs as the replacement level increased. For GrioPro cricket protein powder containing doughs, the C4 torque increased as the replacement level increased. Entomo Farms cricket protein powder containing doughs had lower C4 torques since they had lower C3 torques than GrioPro cricket protein powder containing doughs. Therefore, the shear force applied by the mixer thinned the Entomo Farms cricket protein powder containing doughs more than the GrioPro cricket protein powder containing doughs. Finally, the C5 torque was significantly higher in doughs containing GrioPro cricket protein powder, while doughs containing Entomo Farms cricket protein powder showed no difference to the control. This could be due to the higher water holding capacity found in GrioPro cricket protein powder reducing the amount of free water in the dough system thereby not allowing the cricket proteins in GrioPro from binding to as much water as the cricket proteins in Entomo Farms. Thus, the Entomo Farms cricket protein powder containing doughs could have been able to more fully inhibit the amylose movement.

4.2.2. Water Absorption at Optimization

Optimized water is crucial in dough development since it represents the amount of water necessary to fully hydrate the flour components into a dough. Only the 20% replacement level of GrioPro cricket protein powder required a significantly higher percentage of water for the dough to reach optimum development. This corresponds with the C1 torque (under constant water) for the 20% GrioPro cricket protein powder replacement level which was significantly higher than all the other treatments. A larger amount of lower molecular weight compounds were strongly associated with a lower water absorption which is the case in Entomo Farms cricket protein powder containing doughs since they contain smaller sized molecules (SDS-PAGE). Water holding capacity also impacts water absorption and GrioPro cricket protein powder has a higher water

holding capacity than Entomo Farms cricket protein powder thereby also explaining why the 20% replacement level of GrioPro cricket protein powder needed more water to fully hydrate all of the ingredients in the dough.

4.2.3. MixoLab at Optimum Water Absorption

Since the optimization protocol requires all samples to reach 1.1 Nm C1 torque, it was expected for there not to be any significant difference between any of the treatments. The C1 time mirrored what was seen in the MixoLab profiles at constant water absorption. Only the Entomo Farms cricket protein powder containing doughs took a significantly longer time to reach the maximum torque (C1 torque). GrioPro cricket protein powder containing doughs have a larger amount of high molecular weight subunits which impart toughness to the dough as was seen in the MixoLab curve under constant water absorption where the 20% replacement level was significantly higher in C1 torque. The larger amount of lower molecular weight proteins found in Entomo Farms cricket protein powder containing doughs were acting like a dough softener since the smaller molecules are unraveled first thereby delaying when the dough reaches its maximum torque during mixing (C1 torque). Furthermore, this increased amount of lower molecular weight compounds is also responsible for the decreased stability as seen in the Entomo Farm containing doughs since there was not enough large sized molecules to resist the shear forces from the mixer.

GrioPro cricket protein powder replacement levels increased dough stability time since the amount of high molecular weight proteins was increased with the addition of the cricket protein powder. Water holding capacity also affected the dough stability since an increase interaction between hydrogen bonding between the gluten proteins adds strength. Therefore, the increased water absorption required for the GrioPro cricket protein powder containing doughs could be allowing there to be more free water available to form more hydrogen bonds.

No significant difference was observed in the C2 torque for any of the doughs containing GrioPro cricket protein powder; however, as the replacement level increased there was a significant decrease in the C2 torque of Entomo Farms cricket protein powder containing doughs. The C2 torque values acts as an indicator for the weakening of the gluten network. Since GrioPro cricket protein powder replacement produces stronger doughs they can withstand the weakening better than the Entomo Farm doughs which break down faster. The linkage of GrioPro cricket protein powder to the high molecular weight wheat polymeric proteins would impart strength to

the dough and allow for the GrioPro cricket protein powder containing doughs to withstand the protein weakening stage better.

Only the 20% replacement level of GrioPro cricket protein powder had a significantly later C2 time therefore it can be concluded that there was a delay in gelatinization. This corresponds to the significantly higher C2 temperature in the 20% GrioPro cricket protein powder replacement level since the gelatinization delay caused the dough to begin gelatinizing at a higher temperature. The delay also caused a shift in the peak viscosity for the 20% GrioPro cricket protein powder replacement level as was seen in the delayed C3 time. No other treatments had a significant change in C3 time. However, both types of cricket protein powders resulted in a decrease in C3 torque as the replacement level increased. The leaching amylose is responsible for the peak viscosity (C3 torque) therefore the competition between the cricket protein powders and the starch for water (due to the cricket protein powders high water holding capacity) prevents the starch from swelling as fast as when there is no cricket protein present. This competition for water would be greater in doughs containing GrioPro cricket protein powder since it has a higher water holding capacity than Entomo Farms cricket protein powder. As was seen in the 20% replacement level being the only treatment where gelatinization was delayed and resulting in a lower peak viscosity.

. The strong possibility of GrioPro cricket protein powder chemically interacting with the wheat starch would inhibit the binding of the wheat proteins to the starch and reduce the peak viscosity. Both the 20% replacement levels of Entomo Farms and GrioPro cricket protein powders showed a decrease in C4 torque which is due to a lower peak viscosity. No C5 values were reported due to equipment error.

4.2.4. SPI-IPI Peak Areas Found in Dough

No significant difference was seen in the SPI peak areas for either Entomo Farms or GrioPro cricket protein powder containing doughs. However, the IPI peak area value was significantly lower at the 10% replacement level of Entomo Farms cricket protein powder and significantly higher at the 20% replacement level of GrioPro cricket protein powder. Mixing breaks up the protein aggregates by physical separation and by breaking the covalent and non-covalent bonds holding the high molecular weight wheat polymeric proteins together thereby making them more extractable and decreasing the IPI peak areas. Therefore, the lower IPI peak areas in Entomo Farms cricket protein powder could be due to the larger molecules breaking down

while the increased amount of IPI peak area in GrioPro cricket protein powder could be due to there being a larger amount of high molecular weight proteins to begin with thereby withstanding the mixer better. Furthermore, GrioPro cricket protein powder's ability to chemically interact with the gluten matrix would also increase the dough strength and create more high molecular weight subunits.

4.2.5. Kieffer-Extensional Dough Properties

As the replacement level increased, the force significantly decreased for Entomo Farms cricket protein powder containing doughs. On the other hand, as the replacement level increased the force significantly increased for GrioPro cricket protein powder containing doughs. This corresponds with GrioPro cricket protein powder being the stronger dough and Entomo Farms cricket protein powder being a weaker dough due to their differences in molecular weight distributions as seen in the SDS-PAGE and the SPI-IPI dough peak areas. However, the larger proteins found in GrioPro cricket protein powder containing doughs was detrimental to the dough's extensibility since they caused an increased resistance to extension. Thus, it can be concluded that GrioPro cricket protein powder will produce lower volume loaf breads than Entomo Farms cricket protein powder due to the increased resistance to extension seen in the GrioPro cricket protein powder containing doughs. Since Entomo Farms cricket protein powder containing doughs also decreased the extensibility it is highly likely that loaf volume will also be impacted when compared to the control.

4.3 Test Baking and End-Product Quality

4.3.1. Color/Brightness

For both Entomo Farms and GrioPro cricket protein powder containing doughs the L-value decreased as the replacement level increased with the decrease being more drastic in Entomo Farm containing doughs. The a-value became a positive value for all treatments containing cricket protein powder and an increase in the b-value was also seen. Therefore, it can be concluded that adding either type of cricket protein powder changes the bread color to be more like whole wheat bread. The shift in darker color is attributed to an increase in Maillard reactions due to more reducing sugars/amino acid groups being available, as well as, an increase in enzymatic browning reactions and in caramelization reactions (non-enzymatic). Brightness decreased for all treatments

containing cricket protein powder with the 20% Entomo Farms cricket protein powder replacement level having the lowest value. Alteration of the microstructure from the replacement with either type of cricket protein powder affects the extent of light scattering which in turn altered the brightness of the bread slice.

4.3.2. Loaf Weight and Volume/Slice Area

Replacement with either Entomo Farms or GrioPro cricket protein powder led to an increased weight for the bread loaves in comparison to the control; however, there was no significant difference between any of the replacement levels in Entomo Farms cricket protein powder containing breads. In contrast, the loaf volume decreased for all breads containing either type of cricket protein powders as the replacement levels increased. The decreased loaf volume was more drastic in the GrioPro cricket protein powder containing breads. The slice area also decreased for either type of cricket protein powders as the replacement level increased. Since GrioPro cricket protein powder containing breads weighed the most, had the lowest volumes, and the smallest slice area it can be concluded that GrioPro cricket protein powder replacement produces denser breads.

Both water absorption and water binding capacity affect the final bread weight since water evaporation is what influences the bread weight. More free water available means there is more water free to evaporate during the baking process. Furthermore, more bound water allows the bread to retain moisture more strongly. GrioPro cricket protein powder had a higher water holding capacity and a higher water absorption than Entomo Farms cricket protein powder thereby allowing the bread to bind water and lose less to evaporation thus leading to an increased weight volume. The volume decrease was due to the weak peak viscosities as seen in the MixoLab which were not strong enough to provide strength to the expanding gas cells thereby inhibiting gas retention. Interference from the cricket proteins to the gluten matrix also impacts gas retention by increasing the doughs resistance to extensibility as seen for GrioPro cricket protein powder containing doughs in the Kieffer section. This prevents dough expansion thereby producing loaves that were lower in volume.

4.3.3. Crumb Microstructure (C-Cell)

The area occupied by air cells showed a significant decrease in both 20% replacement levels and in the 5 and 10% replacement levels of GrioPro cricket protein powder. All loaves

containing cricket protein powder showed a decreased amount in the number of air cells. Only the 10 and 20% replacement levels of GrioPro cricket protein powder and the 20% replacement level of Entomo Farms cricket protein powder had significantly lower average air cell diameter. The average cell thickness was only significantly lower in the 10 and 20% replacement levels of GrioPro cricket protein powder. These results correspond with the lower loaf volume seen as the replacement levels increased due to the decreased gas retention and increased resistance to dough expansion caused by interference with the gluten network. GrioPro cricket protein powder produced the densest breads since the higher amount of high molecular weight proteins inhibited the dough extensibility and was detrimental in the gas retention as seen by the denser breads produced. Another possibility could be that the linoleic and linolenic acids present in the cricket protein powders formed expanded monolayers which acted as foam breakers and destabilized the elastic restoring forces thus inhibiting gas retention.

4.3.4. Texture Profile Analysis (TPA) and Staling

The force required to squeeze the food between the teeth is the force. In general, the 20% replacement levels of either cricket protein powder was the hardest across a span of seven days. However, GrioPro cricket protein powder containing breads were harder than Entomo Farms cricket protein powder containing breads. No significant difference occurred between the 5 and 10% Entomo Farms cricket protein powder replacement levels with the control during day-0 and day-1. On the other hand, GrioPro cricket protein powder containing breads did not stale similarly to the control. Therefore, it can be concluded that GrioPro cricket protein powder containing breads stale faster than both the control bread and Entomo Farms cricket protein powder containing breads. Bread hardness increasing is caused by a greater compression of the gas cells in lower volume breads therefore explaining why GrioPro cricket protein powder had drastically higher values since it produced the lowest volume breads. Lower gas retention and decreased dough expansion were responsible for the lower specific volume as seen in the C-Cell results.

At day-0 the cohesiveness for all treatments containing cricket protein powder was significantly lower than the control. Beyond day-3, the control cohesiveness is no longer significantly different than any of the treatments containing cricket protein powder. Since cohesiveness depends on the strength of the internal bonds, the reduction in cohesiveness caused by the replacement levels of either type of cricket protein powder could be due to the change in

the gluten network as mentioned in the extensibility, and loaf weight/volume. Loaves containing 5 or 10% replacement levels of Entomo Farms cricket protein powder showed no significant difference in springiness from each other or the control sample across the span of 3 days.

On the other hand, loaves containing GrioPro cricket protein powder showed a more drastic change in springiness than loaves containing Entomo Farms cricket protein powder. At day-0 only the 5% GrioPro cricket protein powder replacement level showed no difference compared to the control while the 10% GrioPro cricket protein powder replacement level was significantly lower in springiness. Across the span of day-1, day-3, and day-7 all treatments containing GrioPro cricket protein powder remained consistent with the control always having the highest percent springiness followed by the 5% replacement level and the 10% replacement level showing the lowest amount of springiness. Therefore, it can be concluded that for breads containing Entomo Farms cricket protein powder at 5 and 10% replacement levels will not significantly impacts the breads springiness over time; however, for breads containing GrioPro cricket protein powder it is due to the replacement level and not the time which causes a significant decrease in springiness. The detriment caused in the dough extensibility impacted the viscoelastic properties thus preventing the crumbs from springing back to its original size as easily as the control bread.

The energy required to chew food before swallowing is known as chewiness and it is calculated by hardness×cohesiveness×springiness. In general, loaves containing 10% replacement level of GrioPro cricket protein powder had the highest values of chewiness over time in comparison to all other treatments. The 10 and 20% GrioPro cricket protein powder replacement levels showed the same trend since both resulted in an increase in chewiness over time. At day-0 and day-1 loaves containing 5 and 10% replacement levels of Entomo Farms cricket protein powder showed no significant difference from each other or the control. At day-7, there was an increase in chewiness for the control and both the 5 and 10% replacement levels containing Entomo Farms cricket protein powder had higher amount of chewiness. Therefore, it can be said that Entomo Farms cricket protein powder containing loaves containing 5 and 10% replacement levels do not significantly impact the breads chewiness for the first 3 days. Furthermore, breads containing GrioPro cricket protein powder were significantly different in chewiness across the span of time with the chewiness being more pronounced in higher replacement levels. Since hardness, cohesiveness, and springiness are used to calculate chewiness, the results were influenced by the previous values. As discussed in the hardness section, water retention, gas

retention and loaf volume are tied to hardness. Therefore, the increase in chewiness due to the denser breads produced at higher concentrations of the protein (more drastically seen in GrioPro cricket protein powder containing breads) caused by the lower gas retention (as seen in the loaf weight and volume/slice area and C-Cell sections).

The ability of the crumb to return to its original state is known as resilience. both types of cricket protein powders showed the same trend. At day-0, the resilience decreased as the replacement level increased for loaves containing Entomo Farms or GrioPro cricket protein powder. However, the 5 and 10% Entomo Farms cricket protein powder replacement levels were significantly higher than the 5 and 10% GrioPro cricket protein powder replacement levels. All the other days (1, 3, and 7) showed no significant difference in resilience in any of the treatments including the control samples. Thus, the type and amount of cricket protein used is only significant out of the oven and is not as important beyond day-1. The springier a food is, the more resilient it is as well. Therefore, the decreased springiness due to a weaker gluten matrix (as seen in springiness section) would lower the ability for the bread crumb's ability to return to its original state thus reducing its resilience.

4.4. Overall Conclusions

This study showed that incorporating cricket protein powder into bread is feasible if added at lower quantities (5 or 10% replacement level for Entomo Farms cricket protein powder and 5% level for GrioPro cricket protein powder). At higher replacement levels the detriment to the gluten network causes the loaf volume to significantly lower and not be on par with consumers standards. In GrioPro cricket protein powder this is due to the larger amount of high molecular weight proteins which led to stronger doughs as seen in the increased MixoLab C1 torque and the increased Kieffer Rig resistance. Additionally, the higher molecular weight proteins led to GrioPro cricket protein powder having a lower solubility than that of Entomo Farms cricket protein powder.

Both proteins produced a high-water holding capacity which affected the gelatinization at the 20% GrioPro cricket protein powder replacement level. Dough stability time increased with the addition of GrioPro cricket protein powder; however, the addition of Entomo Farms cricket protein powder caused the stability to decrease. Extensibility was highly impacted by addition of either type of cricket protein powder where a significant decrease occurred as the replacement level increased. GrioPro cricket protein powder led to a more drastic decrease in extensibility. All

these changes led to poor dough expansion and poor gas retention as was seen in the significantly lower loaf volumes and the C-Cell results. The area occupied by cells, the average cell diameter, and the cell wall thickness decreased at the 10 and 20% GrioPro cricket protein powder replacement levels. Furthermore, all replacement levels showed a decrease in the number of air cells. Texture profile analyses showed an increase in hardness at high replacement levels which was more pronounced in GrioPro cricket protein powder. The cohesiveness, springiness, and resilience decreased for all replacement levels. The chewiness increased for all replacement levels. The staling revealed that Entomo Farms cricket protein powder replacement levels remained more like the control at low replacement levels longer than the GrioPro cricket protein powder replacement levels. Therefore, GrioPro cricket protein powder incorporations lead the breads to stale faster. Thus, further studies are needed to optimize the bread formulations to ensure the final product characteristics are acceptable to the consumers.

Furthermore, the differences in molecular weight distribution caused Entomo Farms cricket protein powder to behave similar to a dough weakener and GrioPro cricket protein powder to behave like a dough strengthener. Therefore, there is a potential for either type of protein powder to be used as a Genetically Modified Organism (GMO) free additive (weakener or strengthener) to flour in order to achieve the desired flour specifications before baking. However, further studies are necessary to study the effects of GrioPro cricket protein powder on soft wheat flours and the effects of Entomo Farms cricket protein powder on hard wheat flours in order to confirm if they can be used as a strengthener or weakener.

Chapter 5 - Future Work

5.1. Powder Characterization

Optimizing a rearing method for the crickets to find the best diet, gender, developmental stage, and environmental factors which would produce insects with the best nutritional profile and bioavailability of the nutrients during processing. To do so different diets would be tested, as well as, different environmental factors and the crickets would be harvested during various times in their development cycle to see which produces the insect with the greatest nutritional profile. Future work in the SDS-PAGE would be identifying what the molecules are in the cricket protein bands and what they correspond to in the insect. This could be done along with more HPLC work to identify the peaks seen in the chromatograms. Furthermore, trying different enzymes such chitanase and then analyze proteins again by SDS-PAGE and SEC-HPLC to see if chitin binding was responsible for holding together the molecules seen in both the raw materials and the dough HPLC chromatograms. Further testing on characterizing the proteins could be done by testing the emulsion capacities of the cricket protein powders to see if they act as foam stabilizers or destabilizers. Digestibility of the cricket protein powders could be done using In Vitro or In Vivo tests. Conducting a lipid analysis on both cricket protein powders to quantify which lipids are found in the protein powders. Finally, conducting a particle size analysis to examine how processing is affecting the particle size distribution in either type of cricket protein powder.

5.2 Dough Development and Test Baking

FTIR spectroscopy could be used to see how the secondary structure of the proteins in the wheat doughs changed with the addition of the cricket protein powders. This would give more detail on if the conformations of the gluten proteins changed which affects the viscoelastic properties of the dough.

Recreating the processing with live crickets in order to fractionate the cricket into pure protein, chitin, and lipids to see if lipid removal or chitin removal would change the results seen in the dough development and the final bread qualities when the whole insect was added. Treating the cricket protein powders with chitinase to break up the chitin before incorporating these modified powders into dough and bread to see the role chitin may have on the bread baking. Another control option would be to add commercially pure chitin to wheat flour to see the effect

of chitin on the dough and bread systems. ^{1}H Nuclear Magnetic Resonance (NMR) to examine the difference in water activity with the addition of either type of cricket protein powder. Thus, studying how the bulk and free water ratios are changed with the addition of the cricket protein powders to confirm that Entomo Farms cricket protein powder produces a hydrophobic powder and see if GrioPro cricket protein powder retains its hydrophilic nature during pasteurization. Use of advanced imaging techniques such as Confocal laser scanning microscopy (CSLM) or Scanning Electron Microscopy (SEM) on the doughs to explore how the replacement levels affected the microstructure of the dough. Thus, gaining information on how the crickets affected the gluten matrix and dough development. Use of fundamental rheology such as stress relaxation, creep & creep recovery to study the effect of cricket protein powders on the gluten network on a molecular level to study if any chemical interaction between the cricket proteins or chitin and the wheat proteins or wheat starch occurred. Using the dough inflation system on the TA-XT2 texture analyzer to see how the cricket protein powders inhibit dough expansion.

Adding in dough improvers such as hydrocolloids and/or emulsifiers to examine if the final product qualities such as loaf volume can be improved by optimizing the bread formulation. Trying different bread baking methods such as delaying the addition of the cricket protein powder to examine if the same results are seen when addition was not delayed. Instead of replacing the wheat flour, the cricket protein powders could be tested as various addition levels to examine if that would help strengthen the Entomo Farms cricket protein powder containing dough which lacked some higher molecular weight molecules. Conducting a sensory panel on the cricket protein breads to explore how they are ranked and if they are acceptable to consumers' standards. Conducting proximate analysis on the bread samples to examine how the nutritional profile is improved with the replacement of the cricket protein powders. Recreating the project with a more acidic product such as sourdough and a more basic product such as a pound cake to create a baseline behavior for the cricket protein powders across the pH spectrum since water holding capacity and solubility are affected by pH. Exploring how the cricket protein powders behave in an extruded product such as a chip, cereal, pasta, or as an extruded meat substitute. Adding cricket protein powders to cookies or tortillas to explore how the color, flavor, and spreadability are impacted and examine how the cricket protein powders behave when the gluten network is not being developed. To explore the addition of cricket protein powders in gluten-free applications to see if GrioPro cricket protein powder can add strength to the gluten free products and retain moisture in the doughs, as well as,

improve the nutritional profile of a gluten free product. Exploring the addition of Entomo Farms or GrioPro cricket protein powder to a variety of soft and hard wheat flours to quantify their potentials as a dough weakener/strengthener respectively.

Using different types of insects to create other types of insect protein powders and re-doing the experiments to examine how they behave in the dough and bread systems, as well as, in the nutritional profile of the final product. Conducting a cost analysis on the incorporation of insect protein powders in baked goods to further quantify the feasibility of these novel protein sources.