PHYSICAL AND FUNCTIONAL PROPERTIES OF CRICKETS FOR THEIR USE AS INGREDIENTS IN FOOD

by

CATHERINE MICALI

(Under the Direction of WILLIAM L. KERR)

ABSTRACT

Two species of crickets, *Acheta domesticus* and *Gryllodes sigillatus*, were evaluated for their use as an ingredient in food products. Raw crickets were freeze-dried and evaluated for color, flowability, hygroscopicity, moisture isotherm, protein content, fat content, rheological properties, emulsification capacity, stability, foamability, and solubility. Raw cricket paste was also incorporated into an extruded puffed snack product and evaluated for its consumer acceptability as well as color, density, and expansion ratio. The cricket species *A. domesticus* was the lightest colored powder with the best flowability and lowest hygroscopicity, while there was no significant difference between the rheological properties, emulsification capacity, stability, foamability, or solubility of either species. Extruded samples with 5 g/100 g *A. domesticus* paste had the highest likeability rating from consumers, indicating that an acceptable product that incorporates cricket paste can be produced and accepted by consumers.

INDEX WORDS: Freeze-drying, Entomophagy, Cricket, Extrusion, Food powder, Rheology

PHYSICAL AND FUNCTIONAL PROPERTIES OF CRICKETS FOR THEIR USE AS INGREDIENTS IN FOOD

by

CATHERINE MICALI

B.S.A. The University of Georgia, 2017

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2017

PHYSICAL AND FUNCTIONAL PROPERTIES OF CRICKETS FOR THEIR USE AS INGREDIENTS IN FOOD

by

CATHERINE MICALI

Major Professor:	William L. Kerr
Committee:	Mark A. Harrison
	Marianne Shockley

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
May 2017

ACKNOWLEDGEMENTS

I would like to acknowledge and thank Dr. Harrison for helping me do this strange and perhaps misguided program of combined Bachelor/Master's degrees. I first went into your office as a small caterpillar of an undergrad and I have finally emerged as a Masterful butterfly. Thank you to Dr. Kerr for letting me hangout in the FPL for the past 4ish years and agreeing to take me on without really knowing what we were getting into. You were always willing to help and answer any question I had. Enormous thanks to Dr. Shockley whose enthusiasm was endless and procured all the crickets I needed to make this happen. This project would not have happened without Carl Ruiz, his knowledge of all the equipment everywhere, and his never-ending stream of advice. Thank you to all the past FPL and otherwise graduate students- Audrey Varner, Meg Liu, Kat Kitchen, Emily Wagener, Daniel Parish, Lee Carella, and Suejee Park - who let me tag alone and teach me so many things. I would be remiss if I did not mention the amazing people and friends I have made here who provided mental support and wine and cheese parties when that did not cut it- Natalie Oswell, Luke Wallace, Victoria Ramirez, Erica Kenney, Martina Buchholz, Lauren Hudson, Chase Golden, Luna Lin, Carlyn Oswald, Caroline Taylor, Ana Jaworski, Alex Maxwell, Po-An Chi, and all of you beautiful people. Shoutout to Kate Klein and Real Food UGA who originally put the idea of doing entomophagy in my head and told me about Dr. Shockley. Thank you to Karen Simmons, Lisa Cash, Doris Lord, and Lisa Porterfield for being so good at what you do and pleasant to work with. Thank you to my parents for putting up with so much school for so long and supporting me. Much love and thanks to Nick, Landon, and CB for your unwavering support and ability to make a bad day a good day.

Lastly, special thanks to Gwen Hirsch. You helped make Athens and UGA Food Science my home, and I am not sure I would have survived without you, Martin, Nathan, Buglet, Ben, Tucker, Dillion, and Sam.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

The human consumption of insects, known as entomophagy, is nothing new. Many cultures around the globe consume insects regularly, both as a seasonal staple and as a delicacy. In a few areas of the world, such as Europe and North America, most insects are thought to be nothing but a pest and nuisance and not a food source. Several movements over the years have attempted to push people to overcome their impractical fear and acknowledge insects for their nutritious qualities and potential to be a sustainable food source, and one way to do this is to incorporate insects as a powder into common food products (Shockley & Dossey, 2014).

Insects have become popular due to claims of sustainable farming and nutritional benefits. Most species are high in protein with little to no carbohydrates, and a fat composition of mostly unsaturated fatty acids. Additionally, some are high in important micronutrients such as iron (Bukkens, 1997; Huis et al., 2013a; Shockley & Dossey, 2014).

Currently, few processed foods incorporate insects and little is known about the properties of insects as an ingredient in food. If the consumption of insects is to be increased and brought into the mainstream of countries that do not regularly consume insects, it would help to understand and utilize the functional properties of the protein and fat within insects. Most studies around entomophagy have focused on the relationship that consumers have with insects in their diet, either from a consumer acceptance standpoint or from a nutritional standpoint. There are a small number of studies that have looked at the functional properties of insects (Cilia et al., 2009;

Mariod & Adam, 2013; Xu, Zhang, Song, & Sun; Yi et al., 2013; Zhao, Vázquez-Gutiérrez, Johansson, Landberg, & Langton, 2016), yet these mostly deal with protein that has been extracted through multi-step processing. While these studies are useful, utilizing the whole insect in products will decrease processing costs and increase accessibility for small food producers.

This study involves two species of crickets, *Acheta domesticus* and *Gryllodes sigillatus*, in minimally processed powder and paste forms. These were tested for their rheological, flow and other functional properties. In addition, consumer acceptance of an extruded snack made with cricket paste was examined. More specifically, the major objectives were:

1. To evaluate the physical properties of two species of crickets in dried powder form for characteristics such as color, flowability, dynamic hygroscopicity, moisture isotherms, protein content, and fat content.
2. To evaluate the rheological properties and protein functionality of two species of cricket paste over different temperatures and pH.
3. To evaluate an extruded snack product made from different amounts (4 levels) of cricket paste for consumer acceptability characteristics such as texture, color, flavor, expansion ratio, and protein content

CHAPTER 2

LITERATURE REVIEW

Insects have played a role in the diets of humans around the world for millennia. They are consumed as a regular part of many people's diet, both as a seasonal staple and a delicacy. Most people of western culture would not consume insects, as they consider them to be disgusting and carriers of disease, but there have been several movements over the years to push people to overcome their impractical fear and acknowledge insects for their nutritious qualities and potential to be a sustainable food source (Kellert, 1993).

Insects are highly nutritious, generally being a good source of protein and fat, although the exact nutritional content depends upon the species being consumed. Recently there has been a rising interest in using farmed insects as a food ingredient in processed food. There are claims that insects are more sustainable and have a better nutritional profile than livestock such as cattle and pigs (Shockley & Dossey, 2014). This review encompasses the general nutritional properties and composition of edible insects, the physical properties of the dried material, the properties of the wet material, and the food products that can be created from them.

Nutritional profile of edible insects

The term "entomophagy" is used to describe the consumption of insects in general, but in this paper it refers specifically to the consumption of insects by humans. Wageningen University's Laboratory of Entomology listed 2,163 species reported in the literature as being consumed by humans. The three most common orders of insects consumed by humans are

Lepidoptera (butterflies and moths, mostly in the form of caterpillars), Hemiptera ("true bugs"), and Coleoptera (beetles and grubs) (Shockley & Dossey, 2014).

Nutritional values vary widely between species and within species themselves. The metamorphic stage of an insect (caterpillar vs butterfly), the diet of the insect, and the processing that the insect has undergone influences the nutritional composition. There are some generalizations that can be made thanks to the thorough study of hundreds of insects at different life stages over many years. In general, edible insects are high in protein and fat with little to no digestible carbohydrates. Many are nutritionally comparable to other animal meat such as beef and pork. Their amino acid profile is equivalent or superior to soy protein, and studies have shown that the digestibility of insect powder is as high as 91% (Bukkens, 1997; Huis et al., 2013a; Shockley & Dossey, 2014).

Edible insects have around 60% crude protein by dry weight. Several feeding trials have found the house cricket, *Acheta domesticus*, to be equal or superior to soy when fed to weanling rats and poultry (Finke, DeFoliart, & Benevenga, 1989). Their amino acid makeup tends to be low in methionine/cysteine but high in lysine and threonine, which are the most deficient amino acids in plant based diets (DeFoliart, 1992). In addition to their high protein content, insects have what is considered a "healthy" fatty acid profile. Fat content is widely variable and highly dependent on species and diet, but Isoptera (termites) and Lepidoptera (caterpillars) have the highest fat content overall. The fatty acid profile for insects is similar to that of poultry and fish in the degree of unsaturation. They are high in monounsaturated and polyunsaturated fatty acids, especially the essential fatty acids linoleic acid (18:2) and linolenic acid (18:3) (Huis et al., 2013a). Finke (2002) found that the three primary fatty acids in house crickets (*A. domesticus*) are oleic, palmitic, and linoleic.

A study of cholesterol in insects found none in some species and similar levels to that found in other animal flesh, that is ~1mg sterol/g tissue. Again, this was very dependent upon the species and diet of the insect (DeFoliart, 1992).

Although rich in some micronutrients, insects are largely lacking in others. Some insects have been shown to be very high in iron which can be vital in the fight toward combatting widespread iron deficiency. Locusts (*Locusta migratoria*), for example, can have between 8 and 20 mg per 100 g dry weight, compared to an iron content of 6 mg per 100 g dry weight for beef (Huis et al., 2013a). Phosphorus, copper, zinc, calcium, and several other trace minerals were found in varying quantities. The presence of certain vitamins is very species specific. Most B vitamins are present, vitamin A is found in low levels, and vitamin E is present in some species, such as silkworms (*Bombyx mori*) (Huis et al., 2013a).

Chitin makes up the majority of carbohydrates present in insects, being approximately 10% of the dry weight. The exoskeleton is composed of chitin and protein bound together into a crystalline structure through schlerotization which has been found to incorporate heavy metals such as iron, zinc, and manganese (Muzzarelli, 2011). Chitin is thought to be indigestible by humans, but chitinase has been identified in human gastric juices, potentially allowing it to act as a dietary fiber (Paoletti, Norberto, Damini, & Musumeci, 2007). Chitin is known to lower the overall digestibility of insects, and extraction prior to processing increases its quality (DeFoliart, 1992; Shockley & Dossey, 2014).

Table 2.1 Nutritional comparison between commonly consumed insects, beef, and milk (Shockley & Dossey, 2014)

Item	Calories (kcal/kg)	Protein (g/kg)	Fat (g/kg)
Black soldier fly larvae (*Hermetia illucens*)	1,994	175	140
House cricket (*Acheta domesticus*)	1,402	205	68
Mealworm larvae (*Tenebrio molitor*)	2,056	187	134
Waxworm larvae (*Galleria mellonella*)	2,747	141	249
Silkworm larvae (*Bombyx mori*)	674	93	14
Beef (75% lean meat)	2,776	256	187
Whole Milk Powder	4,982	265	268

Chemical Analysis

The chemical analysis of insects does not have unique protocols. Instead, researchers use AOAC standard methods for food products similar to insects and adapt them when necessary.

Protein Content

The Kjeldahl test was developed by Johann Kjeldahl in 1883 to determine the level of organic nitrogen within a sample. Although it has been refined over the years, it is the AOAC's internationally recognized method for estimating protein content in foods due to its precision and reproducibility. It does not give the true protein content of a food, rather it measures the nitrogen

content of a food. This is one of the downfalls of this test, as non-protein sources of nitrogen can give false results. Additionally, a conversion factor specific to the food being measured must be used to account for the varying amounts of nitrogen found in the amino acids that make up the protein (Vaclavik & Christian, 2008).

The Kjeldahl method is divided into three steps: digestion, neutralization, and titration. Digestion with sulfuric acid converts nitrogen to ammonium sulfate. The diluted digest is neutralized then distilled with a known volume of acid. The solution is titrated, the nitrogen is calculated from the amount of titrant used, and the nitrogen is converted to protein content using a conversion factor, most commonly 6.25 (McClements, 2003).

Alternative methods of quantifying protein are those using UV-visible spectroscopy or nitrogen combustion. Some proteins naturally absorb or scatter light in the UV visible range, or can be chemically or physically modified to allow them to affect light in this region. Different tests act on different parts of the protein molecule to produce the absorption or scattering (McClements, 2003). Tests using spectroscopy must be done alongside a standard curve of known protein concentration in order to accurately calculate the correct protein quantity (Bradford, 1976).

A straightforward, quick test using spectroscopy in the visible spectra is the Bradford Method. The dye used in this test, Coomassie Brilliant Blue G-250, shifts absorption maximums from 465 nm to 595 nm upon binding to a protein. The intensity of blue color is directly correlated to the amount of protein within solution. The color appears rapidly and maintains a stable absorbance for a relatively long time, allowing ample time for samples to be tested. Other protein quantification tests are subject to interference by other compounds and sometimes carbohydrates, while being extremely time sensitive and complicated (Bradford, 1976).

Fat Content

Fat content is often measured after Soxhlet extraction. This method is applicable to many products yet is most accurately used with meat, poultry, and processed meat products with a fat content greater than 0.12%. The sample is first dried to remove moisture then a solvent is run through the product over multiple cycles to dissolve the fat away. The final sample is weighed and the difference is calculated to be the fat content (U.S. Department of Agriculture, 2016).

Physical Properties

Although it is most common for entomophagy to occur with whole insects that have only undergone one or two processing steps such as roasting, massive benefits can be gained by processing insects into a low-moisture powder. Low moisture powders are easily stored and transported, and can be incorporated into food products. All powders behave differently, and a multitude of handling equipment has been developed to best transport and store powders with different flowabilities and moisture properties (Barbosa-Canovas, Ortega-Rivas, Juliano, & Yan, 2005).

Powder Production and Properties

Food products in the solid and liquid form are converted and used as powders for ease of use and storage. Powders are solid state materials that are composed of discreet particles. The combined effect of individual particle properties leads to bulk powder properties that affect how a powder is stored and handled. Various terms are used to denote powder products, based on the size or the source of the material. “Powder” is the catch-all phrase, but includes granules (particle size 200-4000μ), flour (100-5000μ), and dust (5-100μ) (Bhandari, 2013).

Powders are created from solid or liquid products through drying, grinding, pulverization, crystallization, or precipitation. Adding a secondary grinding or milling step can reduce the

particle size even further. Particle size and shape, surface characteristics, and chemical composition contribute to the functional properties of powders such as flowability, packaging, density, ease of handling, hygroscopicity, dust forming, miscibility, and surface activity.

Powder flow describes the general movement of bulk particles among neighboring particles and/or along a wall surface (Peleg, 1977). Undesirable flow characteristics of a material can complicate the handling and storage due to cohesiveness, friction, and interlocking. Especially with fine particles, the intermolecular and cohesive forces can be greater than the forces on individual particles, so that the powders fail to flow. By understanding how gravity, friction, cohesion, and adhesion affect the movement of the powder, systems can be built to efficiently and safely handle bulk powder (Barbosa-Canovas et al., 2005).

Hygroscopicity and Moisture Isotherms

Dynamic hygroscopicity and moisture isotherms describe the relationship between a product and its water activity, equilibrium moisture content, and environmental temperature and humidity. The knowledge of how a powder will interact with its environment is important in building and using equipment, designing packaging, predicting quality and shelf-life, and transporting and storing it (Andrade, Lemus, & Perez, 2011).

A product with a high rate of hygroscopicity will absorb moisture very rapidly and require more careful packaging and handling to prevent agglomeration. If a product absorbs water quickly, inert chemicals are added to decrease hygroscopicity and improve shelf life and flow. The production process also has an effect on the the hygroscopicity rate. For example, freeze drying bitter whey protein concentrate hydrolysate significantly decreased the hygroscopicity and made it a more stable product (Ma et al., (2014).

The shape of a food isotherm indicates how water will be picked up and bound into the system. A typical isotherm can be divided into three regions based on how strongly water binds and how it is held within the food. Region A has strongly bound water due to hydrogen bonds and bonds to hydrophilic and polar groups. This water is not available for chemical reactions and has a higher enthalpy of vaporization than pure water. Region B has less firmly bound water molecules that are present in small capillaries and is considered a transition from bound to free water. Region C has the largest amount of free water, found within voids, crevices, and large categories (Andrade, Lemus, & Perez, 2011).

Brunauer (1945) developed a classification of sorption isotherms, putting them in 3 general categories, although more specific categories have been developed and described elsewhere. Type I isotherm is a convex curve, absorbing large amounts of water at low water activities. This is typical of anticaking agents, where water becomes bound onto specific absorption sites. Once these sites are filled, added water only interacts with already present water, showing a sudden increase in water activity with little increase in moisture content. Most foods follow the Type II isotherm of a sigmoidal curve. The two abrupt curves, usually 0.2-0.4 a_w and 0.65-0.75 a_w, are a result of changes in physical and chemical interactions. Type III curves are characteristic of crystalline substances and show very little moisture gain until the water activity becomes high enough to dissolve the crystal surface and allow water to interact via hydrogen bonds with surface hydroxyl groups. For these substances, surface area has a very large effect on its sorption curve and must be taken into account during processing and storage (Bell & Labuza, 2000).

Models mathematically express the relationship between water activity and moisture content at a specific temperature. Many diverse models have been developed, as models suitable

for one food are not suitable for another, and some are only accurate within certain water activity ranges. In some ranges of water activity, isotherms can be approximated to linear equations, while others require regression equations with coefficients to explain the zones mentioned above. There are a number of equations with two or three fitting parameters. This review will discuss the two most commonly used equations for food isotherms, the Brunauer-Emmett-Teller (BET) equation, and the Guggenheim-Anderson-de Boer (GAB) model, and the equation they are based off of, the Langmuir equation (Andrade, Lemus, & Perez, 2011; Bell & Labuza, 2000).

The Langmuir equation is a theoretical model based on the assumptions that the surface containing the adsorbing sites is a perfectly flat, homogeneous plane, all the sites are equivalent, and each site can only hold one molecule (Masel, 1996). This singular molecule per site is known as the monolayer and is crucial to the Langmuir model. It indicates the amount of water that is strongly absorbed into the sites and is the conceptual basis for this model (Hanaor, Ghadiri, Chrzanowski, & Gan, 2014). Extensions of this monolayer idea result in the multi-layer adsorption theories that make up the BET and GAB models which are then able to more accurately describe the sigmoidal shaped isotherms commonly observed in food products (Andrade, Lemus, & Perez, 2011).

The most widely used model in food systems is the BET equation. It is the basic interpretation of multilayers in which gas molecules adsorb onto solid layers infinitely and the Langmuir equation can be applied to each layer (Brunauer, Emmett, & Teller, 1938). In almost all cases, BET graphs are accurate only between the water activity ranges of 0.05 to 0.45, and therefore their main use is related to the estimation of surface areas (Andrade, Lemus, & Perez, 2011). The BET model is commonly arranged as

$$\frac{a_w}{(1-a_w)*m} = \frac{1}{(m_0*c)} + \frac{(c-1)}{(m_0*c)} * a_w \qquad \text{(Eq. 1)}$$

where

m is dry basis moisture content

T is temperature in °K

a_w is water activity

m_0 is monolayer moisture content

c is surface heat constant

Going beyond the BET model is the GAB model, which has the advantage of being a refinement of the Langmuir and BET theories and being applicable in water activities up to 0.9. Within this water activity range, it provides a good description of sorption behavior of almost every food product and its parameters have a concrete meaning (Prothon & Ahrné, 2004). Its multilayer adsorption differs from the BET in that it contains a third constant, k, to differentiate between the molecules in the layers and those of the pure liquid state. When k is equal to 1, the GAB equation becomes the BET equation (Andrade, Lemus, & Perez, 2011). The GAB equation is made up of two additive terms and arranged as

$$\frac{a_w}{((1-k*a_w)*m)} = \frac{1}{(m_0*c*k)} + \frac{(c-1)}{(m_0*c)} * a_w \quad \text{(Eq. 2)}$$

where

k is constant, in the range 0.6-1

a_w is water activity

m_0 is GAB monolayer moisture

c is constant (not equal to the BET c)

Color

Consumers judge fresh and processed foods by their size, shape, texture, and color among other attributes. The color is critical to product acceptance as it correlates strongly to quality

attributes that allow the consumer to reject or accept the food (Leon, Mery, Pedreschi, & Leon, 2006). When a new food is developed, the color of the product and consumers' opinion about that color plays an important role in its marketing (Pathare, Opara, & Al-Said, 2013).

Perception and interpretation of color are subjective. In addition to having different physiological factors (eye fatigue and age), each person verbally describes a color differently due to their current environment and previous experiences. In order to clearly communicate a particular color, instruments have been developed to measure reflected light and then assign a numeric value through a series of mathematical equations (*A Guide to Understanding Color Communication*, 2007; Sahin & Sumna, 2006).

"Color" is a characteristic of light as it hits objects and is measurable through its intensity and wavelength (Sahin & Sumna, 2006). Humans see color due to three types of cones present in the retina of the eyes, which interpret the intensity and wavelengths that hit them. The brain then translates this as the color of the object (Wolfe, 2016).

Spectrophotometers directly measure the reflected or transmitted light on the visual spectrum, resulting in a curve that is useful for identifying a color but not for translating to how a human would see it. Colorimeters, on the other hand, attempt to emulate the human eye's response to light and color by utilizing a three-filter system of red, green, and blue. Colorimeters are a low cost answer to numerically determining the color of an object. Because it uses a single type of light, usually incandescent or xenon, it cannot account for shifts in the appearance of the color due to the lighting changes. Spectrophotometers are designed in a way that allows them to compensate for this shift (*A Guide to Understanding Color Communication*, 2007; Mackinney & Little, 1962).

Every color can be described by its hue, chroma, and lightness value. Hue is where the color sits on a color wheel. The color wheel is a continuum of colors, where red becomes yellow which becomes green which becomes blue which becomes red and encompasses all the color possibilities between those major four. Chroma is also known as the saturation of a color. It is a spectrum with completely gray at one end and completely saturated and vivid at the other end. Lightness value is the luminous intensity of a color, with the spectrum ranging from totally black to totally white (*A Guide to Understanding Color Communication*, 2007).

The standardization of color systems was done by the Commission Internationale de l'Eclairage (CIE) in 1931 through the specification of the illuminant and equipment use to derive the hue, chroma, and lightness of an object. These values are used as coordinates in color space and include systems such as CIE XYZ, CIE L*a*b*, and CIE L*C*h˚. These values are calculated by assigning reflected light wavelengths as numbers, which are recorded as points on the visible spectrum as a spectral curve. Mathematics are then applied to map the color onto a color space that can be understood based on the average human's response to reflected light and give the CIE XYZ system. Further math must be applied to achieve the CIELAB and CIE L*c*h˚ systems. The CIE L*c*h˚ system uses a straightforward, relatable scale to indicate the lightness (L), chroma (c), and hue angle (h). The CIELAB system relates lightness the same, but a and b reference whether the color is more red or green (a+ or a-), or more yellow or blue (b+ or b-) (*A Guide to Understanding Color Communication*, 2007; Mackinney & Little, 1962).

Properties as an Ingredient in Food

Solubility

Protein solubility is the amount of protein that dissolves into a solution. Food proteins range from being completely insoluble, to partly soluble, to entirely soluble. This

physiochemical property is one of the first characteristics researched and used when determining the potential uses for a food protein (Zayas, 1997).

Solubility is influenced by the protein's composition- the amino acids, their sequence, and their arrangement of polar and nonpolar groups- and its conformation, as well as environmental factors such as ionic strength, pH, solvent, temperature, and previous treatments on the protein.

The pH of the medium plays an important role in determining the degree of solubility. The electrostatic and hydrophobic interactions of the amino acids with one another creates a sensitive balance of repulsive and attractive intermolecular and intersolvent forces. Solubility increases if electrostatic repulsion is greater than and able to overcome some of the hydrophobic interactions between protein molecules. The greater the interaction of protein molecules with the solvent, the greater the solubility. The point of least solubility is the isoelectric point (pI). At this pH, proteins have a net zero charge and attractive forces within the molecules predominate, lessening its interaction with the surrounding solvent. At pHs above and below the pI, solubility increases, creating a U-shaped solubility curve when pH is plotted on the x-axis and solubility on the y-axis. Solubility, along with yield of extraction, is greater at alkaline pH. (Santos et al., 2015; Zayas, 1997).

Salts also affect the solubility through electrostatic interactions between proteins and the solvent. Increased protein solubility can be seen at salt concentrations of .01 to 1.0 M, but it does depend heavily on the type of salt, the protein, pH, and temperature. Salt ions interact with opposing charge groups on the protein to form a double layer of ionic groups, causing more protein solvation. Solubility decreases when there is greater competition between proteins and salt ions for water (Santos et al., 2015).

Foamability

Foams are a complex two-phase system of air cells surrounded and separated by a thin continuous liquid lamellar phase. A mixture of gases, liquids, solids, and surfactants influence the size distribution of the air cells, which in turn influences the effectiveness, stability, appearance, and textural properties of the foam. Proteins play a role in contributing to the uniform distribution of small air bubbles throughout the foam structure. Well foaming proteins need to be able to stabilize foams rapidly at low concentrations, perform over the pH range found in foods, and perform with inhibitors present such as fat, alcohol, or flavoring agents (Zayas, 1997).

The most widely used foaming agents are egg white, gelatins, milk proteins, soy proteins, and gluten. These vary significantly in their properties, but all are useful in decreasing interfacial tension, increasing the viscous and elastic properties of the liquid phase, and forming strong films. Proteins reduce surface tension at the air-liquid interface by absorbing into the interface and forming a protective film around the air cells. The protein molecules undergo some unfolding and orientation changing to direct polar groups into the water and nonpolar groups toward the air. A continuous film is formed as a result of polypeptide interaction and associations through electrostatic and hydrophobic interactions and hydrogen bonds (Damodaran, 2007).

The pH of the solution plays an important role in how well proteins foam. Conversely to solubility, some proteins foam better at the pI due to their electrostatic attraction being at the maximum. Extensive electrostatic bonding allows for thick interfacial films to form, which maximizes the rigidity and stability of the foam obtained.

Protein concentration and foam inhibitors influence the foamability of the solution. A protein concentration between 2 to 8% can give maximum foam, because a denser foam with

thicker interfacial films is formed. Foam inhibitors, especially polar lipids with high surface activity, destabilize the film and rupture the air cells. Even levels as low as 0.1% impair foaming. (Yi et al., 2013; Zayas, 1997).

Emulsifying Capacity and Stability

Emulsions are defined as a dispersed system of two or more liquids that are normally immiscible, with one liquid being the continuous phase, and the other the dispersed phase. Emulsions are important in cosmetics, pharmaceuticals, paints, and food. Food emulsions are macroemulsions with droplets from 0.2 to 50 μm. They are either oil-in-water (o/w) or water-in-oil (w/o). Food emulsions are end products such as salad dressings, or ingredients that participate in forming more complex structures, like cream in ice cream. These emulsions are thermodynamically unstable systems; any stability is merely kinetic (Zayas, 1997).

Stabilizers are added to emulsions to increase their stability through reducing the interfacial tension between the two phases. Low molecular weight surfactants (mono- and diglycerides, phospholipids), and macromolecules (proteins and certain hydrocolloids) are commonly used stabilizers. Some food proteins are widely used in emulsions due to being generally recognized as safe (GRAS) and containing both hydrophilic and hydrophobic sections, making them an amphipathic molecule. Proteins are able to lower the interfacial tension at the oil-water interface and form adsorption films around the dispersed phase by uncoiling and orienting in a series of loops and tails to form the film. The stability of the emulsion and ability of proteins in solution to affect it depends on the shape, charge, and hydrophobicity of the protein composition, as well as environmental factors like pH and temperature (Vaclavik & Christian, 2008; Zayas, 1997).

Rheological Properties

The viscosity of a material and its rheology are tied together. Viscosity describes the forces within a liquid and how it reacts when it is acted upon by a force; a high viscosity fluid will not easily deform and will flow slowly compared to a low viscosity fluid. Rheology is the study of that reaction through the flow and deformation of the material.

Rheology is broken down into shear flow, shear rate, and shear stress. Shear flow is the movement of particles within the material over or past each other. This is usually illustrated as hypothetical layers sliding over one another. The gradient of the velocity at right angles to the flow is the shear rate, and the force produced or creating the flow is the shear stress. Shear stress, being force per unit area, is technically newtons per square meter, but is expressed in pascals (Pa).

When a liquid or semi-solid is deformed, thermodynamic forces work to restore it to its natural rest state. This movement from the rest state represents a storage of energy which manifests itself as an elastic force trying to return to its rest state. The measure of this elasticity is known as the storage modulus (G') and loss modulus (G''). This restoring force initially increases linearly with the distance that the material is deformed from its rest state, but eventually it deforms too much and the resulting force is non-linear. This region of linearity is known as the linear viscoelastic region and is the most stable region to run tests on due to the significant response of G' and G''.

A common rheological test for liquids and semi-solids is to apply an oscillating stress or strain to the liquid and monitor the resulting output of G' and G''. By repeating the same sinusoidal motion at the same frequency, another variable such as temperature can be applied to the liquid and the change over temperature is observed. The measure of G' and G'' indicates how

viscous the material is and if it changes over time. This is also used to determine protein denaturation and gelling behavior. A material that shows a higher G′ than G′′ will be more elastic and gel-like while a higher G′′ indicates the material is more liquid and viscous. (Barnes, 2000)

Rheology is useful for determining the temperature at which a protein denatures and testing the strength of a gel, if one is formed. If a product has strong gelling properties it can be used as an additive for meat binding, texture stabilizers, or as a fat substitute (Gao, Zhang, & Zhou, 2015).

Use in Food Products

Every decade or so insects make a resurgence into popular culture of Western countries for various reasons. This most recent resurgence may prove to be the definitive shove into the mainstream. Crowdfunding campaigns and social media have allowed multiple companies to make a profit off of insect-based ingredients and value-added products. The emergence and popularization of new technology aids this endeavor. Better forms of processing and drying allow high quality, functional powders and pastes to be produced and incorporated into well-known food products.

Freeze drying creates a high-quality product through the process of dehydrating a food through sublimation. This is great for foods with "functional" properties that can be ruined through the use of high heat, but must be preserved somehow. The food product is frozen, placed into a vacuum, and heat is applied, converting the ice within into vapor. Drying happens in two phases- free water sublimates first, then bound water desorbs out of the product. Freeze drying is widely recognized as producing the highest quality final powder due to the lack of heat applied, but it is the most expensive and slowest method. It is often held as the standard reference for

other drying methods and numerous studies have tested the characteristics of freeze dried powder vs spray dried, drum dried, or vacuum dried powders. (Ratti, 2013; Vaclavik & Christian, 2008).

Extrusion

Extrusion processing is a high-pressure process used in the manufacturing of pasta, crunchy snack foods, pet food, chewing gum, and a variety of other products. Depending on the product and the need to interchange between products, an extruder can have one or two screws that are fit into a stationary barrel. Pre-ground ingredients are introduced into the barrel, mixed with water, forced down the barrel by the screw action, and out a die or perforated plate at the end, giving them a specific design (Harper, 1981).

The high shear and temperature inside the barrel, potentially that of high heat, result in the mixing of ingredients and catalyze chemical reactions that constitute the cooking process. This process denatures proteins, induces starch gelatinization, and creates uniform final products (Gopalakrishna & Jaluria, 1992).

In order to create a puffy, crunchy snack food, a high starch ingredient such as corn must be combined with the exact right amount of water to produce a product that will expand upon coming in contact with the atmosphere after exiting the high pressure system. Too much or too little water and the product will not puff enough or collapse upon exit. The extent of expansion and the rigid, porous structure created by the starch gelatinization is called the expansion ratio and is useful for comparing ingredient formulations and specifications (Yagci & Gogus, 2009).

Extrusion processing can have a large effect on the nutritional and microbial properties of food products. The high pressure system is efficient at reducing microorganisms and certain toxins, but it has also been shown to destroy lysine and vitamin A and break down complex starches into simple starches. A review over multiple studies summed the complex relationship

extrusion processing has on food products by simply stating that the “effects of extrusion cooking on nutritional quality are ambiguous” (Singh, Gamlath, & Wakeling, 2007).

References

Andrade, R. D., Lemus, R., & Perez, C. E. (2011). Models of Sorption Isotherms for Food: Uses and Limitations. *Vitae, 18*, 2145-2660

Barbosa-Canovas, G. V., Ortega-Rivas, E., Juliano, P., & Yan, H. (2005). Bulk Properties. In G. V. Barbosa-Canovas (Ed.), *Food Powders: Physical Properties, Processing, and Functionality* (pp. 55-90). New York: Kluver Academic/Plenum Publishers.

Barnes, H. A. (2000). *A Handbook of Elementary Rheology*. Wales: The University of Wales Institute of Non-Newtonian Fluid Mechanics, Department of Mathematics.

Bell, L. N., & Labuza, T. P. (2000). *Moisture Soprtion: Practical Aspects of Isotherm Measurement and Use*. United States of America: American Association of Cereal Chemists.

Bhandari, B. (2013). Introduction to food powders. In B. Bhandari, N. Bansal, M. Zhang, & P. Schuck (Eds.), *Handbook of food powders: Processes and properties* (pp. 1-25). Cambridge, UK: Woodhead Publishing Limited.

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry, 72*, 248-254.

Brunauer, S. H., Emmett, P. H., & Teller, E. (1938). Adsorption of Gases in Multimolecular Layers. *Journal of the American Chemical Society, 60*, 309-319.

Bukkens, S. (1997). The Nutritional Value of Edible Insects. *Ecology of Food and Nutrition, 36*, 287-319.

Damodaran, S. (2007). Amino Acids, Peptides, and Proteins. In S. Damodaran, K. L. Parkin, & O. R. Fennema (Eds.), *Fennema's Food Chemistry* (4th ed.). Boca Raton, FL: CRC Press.

DeFoliart, G. (1992). Insects as human food. *Crop Protection, 11*, 395-405.

Finke, M., DeFoliart, G., & Benevenga, N. (1989). Use of a four-parameter logistic model to evaluate the quality of the protein from three insect species when fed to rats. *Journal of Nutrition, 119*, 864-871.

Finke, M. D. (2002). Complete Nutrient Composition of commercially Raised Invertebrates Used as Food for Insectivores. *Zoo Biology, 21*, 269-285.

Gao, X.-q., Zhang, W.-g., & Zhou, G.-h. (2015). Emulsion stability, thermo-rheology and quality characteristics of ground pork patties prepared with soy protein isolate and carrageenan. *Journal of the Science of Food and Agriculture, 95*, 2832-2837.

Gopalakrishna, S., & Jaluria, Y. (1992). Modeling of Starch Gelatinization in a Signle-Screw Extruder. In J. L. Kokini, C.-T. Ho, & M. V. Karwe (Eds.), *Food Extrusion Science and Technology* (Vol. 10). New York, New York: Marcel Dekker, Inc.

A Guide to Understanding Color Communication. (2007). X-rite. Grand Rapids, MI, USA.

Hanaor, D. A. H., Ghadiri, M., Chrzanowski, W., & Gan, Y. (2014). Scalable Surface Area Characterization by Electrokinetic Analysis of Complex Anion Adsorption. *American Chemical Society, 30*, 15143-15152.

Harper, J. M. (1981). *Food Extrusion* (Vol. I). United States: CRC Press, Inc.

Huis, A. v., Itterbeeck, J. v., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Edible insects: future prospects for food and feed security*. Retrieved from Rome:

Kellert. (1993). Values and Perceptions of Invertebrates. *Conservation Biology, 7*, 845-855.

Leon, K., Mery, D., Pedreschi, F., & Leon, J. (2006). Color measurement in L*a*b* units from RGB digital images. *Food Research International, 39*, 1084-1091.

Ma, J.-J., Mao, X.-Y., Wang, Q., Yang, S., Zhang, D., Chen, S.-W., & Li, Y.-H. (2014). Effect of spray drying and freeze drying on the immunomodulatory activity, bitter taste and hygroscopicity of hydrolysate derived from whey protein concentrate. *Food Science and Technology, 56*, 296-302.

Mackinney, G., & Little, A. (1962). *Color of Foods*. Westport, Connecticut: The Avi Publishing Company, Inc.

Masel, R. I. (1996). *Principles of Sorption and Reaction on Solid Surfaces*. New York: John Wiley & Sons, Inc.

McClements, D. J. (2003, October 24, 2003). Analysis of Proteins. *Analysis of Food Products.* Retrieved from http://people.umass.edu/~mcclemen/581Toppage.html

Muzzarelli, R. A. A. (2011). Chitin Nanostructures in Living Organisms. In N. S. Gupta (Ed.), *Chitin* (Vol. 34): Springer Science+Business Media.

Paoletti, M. G., Norberto, L., Damini, R., & Musumeci, S. (2007). Human gastric juice contains chitinase that can degrade chitin. *Annals of Nutrition and Metabolism, 51*(3), 244-251.

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food Bioprocess Technology, 6*, 36-60.

Peleg, M. (1977). Flowability of food powders and methods for its evaluation-a review. *Journal of Food Processing and Engineering, 1*, 303-328.

Prothon, F., & Ahrné, L. l. M. (2004). Application of the Guggenheim, Anderson and De Boer model to correlate water activity and moisture content during osmotic dehydration of apples. *Journal of Food Engineering, 61*, 467-470.

Ratti, C. (2013). Freeze drying for food powder production. In B. Bhandari, N. Bansal, M. Zhang, & P. Schuck (Eds.), *Handbook of food powders* (pp. 57-84). Cambridge, UK: Woodhead Publishing Limited.

Sahin, S., & Sumna, S. G. (2006). *Physical properties of foods*. New York: Springer.

Santos, D. O., Coimbra, J. S. R., Teixeira, C. R., Barreto, S. L. T., Silva, M. C. H., & Giraldo-Zuniga, A. D. (2015). Solubility of Proteins from Quail (Coturnix coturnix japonica) Egg White as Affected by Agitation Time, pH, and Salt Concentration. *International Journal of Food Properties, 18*, 250-258. doi:10.1080/10942912.2012.654557

Shockley, M., & Dossey, A. (2014). Insects for Human Consumption. In J. Morales-Ramos, G. Rojas, & D. I. Shapiro- Ilan (Eds.), *Mass Production of Beneficial Organisms-Invertebrates and Entomopathogens*: Elsevier.

Singh, S., Gamlath, S., & Wakeling, L. (2007). Nutritional aspects of food extrusion: a review. *Internation journal of food science and technology, 42*, 916-929.

U.S. Department of Agriculture, A. R. S. (2016). USDA National Nutrient Database for Standard Reference. 28.

Vaclavik, V. A., & Christian, E. W. (2008). *Essentials of Food Science* (Third Edition ed.). New York, NY: Springer.

Yagci, S., & Gogus, F. (2009). Development of extruded snck from food by-products: a response surface analysis. *Journal of Food Process Engineering, 32*, 565-586.

Yi, L., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Shadler, V., Van Huis, A., & van Boekel, M. A. J. S. (2013). Extraction and characterisation of protein fractions from five insect species. *Food Chemistry, 141*, 3341-3348. doi:http://dx.doi.org/10.1016/j.foodchem.2013.05.115

Zayas, J. F. (1997). *Functionality of Proteins in Food*. New York: Springer.

CHAPTER 3

EVALUATION OF CRICKET POWDER PROPERTIES

Micali, C.S., & Kerr, W.L. (2017). To be Submitted to *Food and Bioprocess Technology* .

Abstract

Two species of crickets were freeze dried into powders, *Acheta domesticus* (AD) and *Gryllodes sigillatus* (GS), and compared with a third commercially available cricket powder, CP, also made of *A. domesticus*. Powders were evaluated for physiochemical and functional properties for use as an ingredient in human food, focusing on those that would most impact product performance and acceptability. Powders made from *A. domesticus* is a more useful powder for use in food products since it is lighter in color and will not affect the color of products if incorporated, has a lower rate of hygroscopicity, and a better flowability for bulk storage.

Introduction

Insects have been consumed by many cultures, and there has been growing interest in developing insect foods or ingredients in areas of the world that do not traditionally eat them. The nutritional values vary widely between insect species and within species themselves. The metamorphic stage of an insect (caterpillar vs butterfly), the diet of the insect, and the processing that the insect has undergone influences the nutritional composition. There are some generalizations that can be made thanks to the thorough study of hundreds of insects at different life stages over many years. In general, edible insects are high in protein and fat with little to no digestible carbohydrates. Many are nutritionally comparable to other animal meat such as beef and pork. The amino acid profile of insects is equivalent or superior to soy protein, and studies have shown that the digestibility of insect powder is as high as 91% (Bukkens, 1997; Huis et al., 2013b; Shockley & Dossey, 2014).

The incorporation of insects into human food products as powders or pastes can increase consumption while circumventing some of the “ick” factor that prevents them from becoming mainstream. Cricket powders are being manufactured and sold by several existing companies,

but the heat treatment it undergoes removes some functional properties of the proteins. As converting insects into a low-moisture powder is a relatively new process, little research has been done to understand the properties of these powders. Food powders vary widely in their flowability and moisture absorbing properties, and a multitude of handling equipment has been developed to best transport, store, and mix them.

The color of a food, including powdered ingredients, is critical to product acceptance by consumers as it normally correlates strongly with quality and to what a consumer considers "normal" (Leon et al., 2006). Foods with off-putting color will negatively impact a consumer's opinion of the product and may make it more difficult to market (Pathare et al., 2013).

Food solids and liquids are turned into powders to ease handling, storage, and shipment. Raw crickets are about 70% water, but that water is trapped within tissue, just as in other animal muscle tissue. Just as water is removed from muscle tissue to make jerky or protein powder, water must be removed from the crickets to create a stable product. There are a few options for producing powders from insects. Freeze or vacuum drying may be useful for drying whole insects with minimal changes due to high temperature. Whole insects may also be dried in hot air (50-80°C) or even roasted, but these processes cause major chemical changes and likely impact the performance of the powders and the functionality of the proteins they contain. For all of these processes, the product would need to undergo an additional grinding step in order to form a powder. In addition, powdered insect ingredients may be prepared by spray drying. However, this would usually necessitate extracting much of the soluble matter, especially protein, forming a material that could be pumped and successfully pushed through the spray dryer atomizer.

Once dried, powders made from animal tissue have complex properties due to intermolecular, interparticle, and gravitational forces that affect their handling and interaction

with environmental conditions. A critical property of powders is how they move in bulk when conveyed or poured, and this is determined in large part by their tendency to cake or form clumps. The measurement of flowability is a useful tool to characterize how a powder will move when acted upon by gravity and allows processors to develop best systems for moving the powder (Barbosa-Canovas et al., 2005). In addition, consumers or processors are impacted by powder flowability issues when they try to measure, mix or disperse food powders.

The knowledge of how a powder will interact with its environment is important in building and utilizing dry equipment, designing packaging, predicting quality and shelf-life, and properly transporting and storing it (Andrade, Lemus, & Pérez, 2011). For example, freeze drying bitter whey protein concentrate hydrolysate significantly decreased the hygroscopicity and made it a more stable product (Ma et al., 2014). Interactions with moisture are especially important. Powders at low water activity are prone to absorb moisture from the surrounding humid air. Two properties are important in this respect. First, the dynamic hygroscopicity measures how quickly a powder will absorb moisture due to the water potential difference between the powder and its surroundings. In addition, moisture isotherm relations show how the equilibrium moisture content varies with the water activity of the food. While moisture content measures the mass ratio of water, water activity (a_w) is related to the chemical potential of water in the food. It has been found that a_w is a good predictor of food stability problems including those related to microbial growth, chemical changes, textural changes such as crispness, and tendency for particulate materials to clump. In addition, moisture adsorption and desorption isotherms are useful in understanding and modeling drying processes. To our knowledge there has been no other research on dynamic hygroscopicity and moisture isotherm data pertaining to insect powders.

The purpose of this study was to determine and compare the physical properties of powdered crickets for use as an ingredient in human food, focusing on those that would most impact product performance and acceptability. This study was meant to look at the entire "raw" cricket in the form of a powder and determine what functionality it holds as a product that has not undergone any extraction or separation steps. Two different types of crickets that are already being farmed in large quantities for human consumption were tested- *Acheta domesticus* (AD) and *Gryllodes sigillatus* (GS). Live crickets of both species were received and processed in-house, and a commercially produced powder made of *A. domesticus* was tested as well. We measured the color, flowability, hygroscopicity, moisture isotherm, protein content, and fat content of the powdered crickets in order to determine these properties and facilitate a greater understanding for their use in foods.

Materials and Methods

Sample Preparation

Live sub-adult *A. domesticus* (AD) were received from Armstrong's Cricket Farms (Glennville, Georgia, USA) and held for 24 h with no food before being transferred to a -40˚C freezer. After 48 h in the freezer they were processed into powder. Frozen crickets and liquid nitrogen were blended together for 5 second intervals in a standard cross-blade blender (Model 33BL12, Waring, New Hartford, Connecticut, USA). More liquid nitrogen was added between blending intervals. The cricket paste was transferred to aluminized PET bags, sealed, and placed in the -40˚C freezer until being freeze dried.

Live sub-adult *G. sigillatus* (GS) were received from Ghann's Cricket Farm (Augusta, Georgia, USA) and held for 24 h with no food. After being fasted they were combined directly

with liquid nitrogen, freezing instantaneously. They were then collected in aluminized PET bags, sealed, and held at 0˚C for less then 48 h before being freeze dried.

Both species of crickets underwent the same freeze drying process at different times. The crickets were spread into a thin layer over several metal sheets in the freeze drier (Model RD53S5, Millrock Technology, Kingston, New York, USA) and frozen first to -40˚C for 90 min. The primary drying started at -10˚C and 100 MT of vacuum for 120 min, increased to 0˚C and 150 MT for another 120˚C, and lastly increased to 20˚C at 180 MT for 600 min. The secondary drying conditions were 30˚C at 100 MT for 120 min. The whole, freeze-dried GS were ground into a powder in a cross-blade grinder (NutriBullet LLC, Pacoima, California, USA).

All Things Bugs, LLC provided several kilograms of a commercial powder (CP) for tests. This was made of *A. domesticus* and was prepared by wet grinding to obtain particles <1,000µm, air drying, and subsequent milling to obtain particles <100µm in size.

Color

Color measurements were taken of the two species of cricket powders using a chroma meter (Model CR_410 Minolta Co Ltd, Tokyo, Japan). The CIE Lab L*c*h˚ system was used, with L* representing lightness/darkness (0 is black, 100 is pure white), c* representing chroma (the higher the value the more saturated), and h˚ representing hue angle (degree of color on color wheel; red is 0˚, yellow is 90˚, green is 120˚ and blue is 240˚).

Powder flowability

Powder flowability was measured using an apparatus created at the University of Georgia as described by Jaya & Das (2004) and Varner (2014). Seven grams of cricket powder was loaded into an aluminum drum 9cm long and 12 cm in diameter. Two slits, 4 mm wide and 7 cm long were located on opposite sides of the drum. A direct-drive DC motor (BK Precision Model

1710, Yorba Linda, CA) powered the drum, rotating it at 34 rpm. The quantity of powder that dropped out of the slits onto a scale (Model ANDEK-300i, A&D Co, San Jose, CA, USA) below was recorded every 2 seconds by a Panasonic computer (Model CF-74 Toughbook, Kadoma, Osaka, Japan) plugged in to the scale. Three replications of each powder flow were measured. The total percentage of powder that came out of the drum after 5 s, 30 s, and 60 s was recorded.

Dynamic Hygroscopicity

Approximately 2 g of powder was spread evenly on a large plastic pan that was placed on top of an analytical balance inside an environmental chamber set to 22˚C and 75% relative humidity. The mass of the powder was recorded every 120 s by a laptop computer (Model CF-74 Toughbook, Panasonic, Kadoma, Osaka, Japan) attached to an analytical balance until a constant mass was reached. Three replications of each powder were measured. Percent hygroscopicity (HG) of the powder was calculated using the equation:

$$HG\ [\%] = \frac{\frac{b}{a}+w_i}{1+\frac{b}{a}} \qquad \text{(Eq. 1)}$$

where

a is the initial powder weight (g)

b is the increase in weight of powder

w_i is the initial moisture content of the sample

Plots of moisture content (MC) over time were also well-fit by an exponential equation, that is:

$$MC = Ae^{Kt} \qquad \text{(Eq. 1b)}$$

where

A is a constant

K measures the rate at which moisture changes

Moisture Isotherm

Approximately 0.6 g of powder was spread in a thin layer on a plastic weigh boat and placed on a platform suspended above a saturated salt solution within a plastic desiccator (Nalgene, Penfield, NY, USA). The saturated salt solutions and their resulting relative humidity levels were: lithium chloride, 11%; potassium acetate, 22.5%; magnesium chloride, 32%; magnesium nitrate, 57%; and sodium chloride, 75%. The samples were sealed inside the chambers and left to absorb moisture for 30 days until they reached a constant mass. The dry basis moisture content (M_{db}) of each sample was determined by the following:

$$M_{db}\left[\frac{g\ water}{g\ sample}\right] = \frac{w_f - w_s}{w_s} \quad \text{(Eq. 2)}$$

where

w_f is the total mass of wet samples

w_s is the mass of solids in the original samples

Moisture isotherms were fit to data using the Water Analyzer 97.4 program (WebbTech, Australia). Isotherms were fit to both the BET and GAB models.

The BET equation is arranged as:

$$\frac{a_w}{(1-a_w)*m} = \frac{1}{(m_0*c)} + \frac{(c-1)}{(m_0*c)} * a_w \quad \text{(Eq. 3)}$$

where

m is the dry basis moisture content

T is the temperature in °K

a_w is the water activity

m_0 is the monolayer moisture content

c is the surface heat constant

The GAB equation is arranged as:

$$\frac{a_w}{((1-k*a_w)*m)} = \frac{1}{(m_0*c*k)} + \frac{(c-1)}{(m_0*c)} * a_w \quad \text{(Eq. 4)}$$

where

k is the constant, in the range 0.6-1

a_w is the water activity

m_0 is the GAB monolayer moisture

c is the constant (not equal to the BET c)

Protein Content

Protein content analyses of AD and GS were outsourced to the University of Georgia Soil Science Extension Laboratory (Athens, GA, USA), which used standard AOAC method 981.10 for protein analysis of meat. All Things Bugs supplied a certificate of analysis for their product.

Fat Content

Fat content of AD and GS was analyzed by a Soxhlet extraction performed according to the AOAC procedure 960.39. Measurements were taken in triplicate per GS and AD and calculated according to the equation:

$$Fat\ content, percent = \frac{100(b-c)}{a} \quad \text{(Eq. 5)}$$

where

a is the the sample weight

b is the the weight of the flask after extraction

c is the the weight of the flask prior to extraction

Statistical Analyses

Statistical analyses were performed using JMP 13 (SAS Institute, Cary, NC). Two-way ANOVA was performed to determine statistical significance of independent variables. Tukey

HSD was performed for mean difference testing. Significant differences were indicated for those with $p < 0.05$.

Results and discussion

Color

The color of the three insect powders' L*c*h° colorspace was highly significantly different for all three variables and was visibly different to the human eye (Figure 3.1). Numeric colorspace values measured by the colorimeter are reported in Table 3.1. The color of the powders reflects the actual colors of the crickets prior to being processed into a powder. The two powders made from *Acheta domesticus* processed into a light tan powder with a few dark flecks, while *Gryllodes sigillatus* was a darker tan-brown powder due to the darker coloration of the insect itself. Different processing methods affected the color of the two powders made from *Acheta domesticus*. CP was slightly darker, with an L of 59.11 compared to AD's L of 67.25, due to the heat used in creating the spray-dried powder. AD and GS were freeze-dried, and the lack of heat used in this treatment meant that no additional browning occurred. With less heat-related browning, AD measured the closest h° to yellow, 86.26°, but CP had a higher c*, 16.20, meaning it was the most color saturated. GS was the darkest, least color-saturated powder, with a grey-brown color.

The color has a profound effect on people's attitude toward a food. If dark colored cricket powder is incorporated into a normally light colored food product, it might sway a consumer's opinion of the product negatively. A consumer's preconceived notion of how a food is supposed to look may cause them to turn down the item out of perceived disgust, thinking it might be spoiled or have an off-flavor (Crumpacker, 2006). On the other hand, some consumers have become concerned with the consumption of artificial colorings and look favorably on food

products that use natural coloring (Spence, 2015). Consumers are also affected by labelling and descriptive information. These non-sensory cues bias how an observer interprets the color of the product and can positively influence their flavor perception (Yeomans, Chambers, Blumenthal, & Blake, 2008).

Powder flowability

The results of the powder flowability test are displayed in Figure 3.2 and Table 3.2. There was no significant difference in the percent of powder released at 5 s, but by 30 s, all powders showed significantly different percent powder released. In general, CP had the best flowability, releasing 74% of total powder within the drum after 60 s. GS had the least flowability, only releasing 5.2% powder after 60 s. GS had so little flow that after 10 min only 11% of the seven grams total had flowed out of the drum.

The cohesiveness of GS may be due to its irregular shape, surface roughness, presence of fat, and some additional intermolecular forces. Powders with smaller particles exhibit a more cohesive nature and poor flowability, as exhibited by studies of low moisture wheat powder and dairy powders (Amagliani, O'Regan, Kelly, & O'Mahony, 2016; Janjatovic et al., 2012).

Flowability allows a powder's behavior to better be defined and understood. Understanding a powder's behavior during storage and processing is important in order to maximize efficiency (Teunou, Fitzpatrick, & Synnott, 1999). Cricket powders that use the entire insect are complex powders not easily defined. Additionally, the treatment of a powder can affect its flowability. Similarly, dairy powders have a large diversity due to the multiple derivatives and processing techniques used, leading them to have a large diversity in powder properties. Fat plays an especially large role, as skim milk powder exhibits a much greater flowability than milk powders with greater levels of fat (Benković, Srečec, Špoljarić, Mršić, & Bauman, 2013;

Fitzpatrick, Iqbal, Delaney, Twomey, & Keogh, 2004). GS has the highest fat content of the three powders (fat content discussed below). This fat likely contributes to the cohesiveness of the powder. A study of industrially spray dried milk powders showed that the surface fat content was measured to be much higher than the bulk average composition and explained why a skim milk powder with 1% fat had a similar cohesiveness and flowability to a whole milk powder with 26% fat (Kim, Chen, & Pearce, 2002).

Dynamic Hygroscopicity

The measured hygroscopicity values of GS and AD powder are shown in Figure 3.3 and Table 3.3. Both GS and AD absorbed water at nearly the same rate until about 18 min. After 18 min, the AD water absorption rate declined while the rate at which GS absorbed water remained nearly the same. At the end of 254 min (4.23 h), GS had adsorbed 0.045g more water than AD, adsorbing 0.275g of water compared to 0.230 g of water, respectively. Data were also fit using the exponential equation in Eq. 1b. The GS powder had a high K value, indicating it absorbed more water at a faster rate overall than the AD powder. This is likely due to the slight differences in processing and particle size of the two powders. Smaller molecules and particles present a greater affinity with moisture and a greater ability to agglomerate (Kurozawa, Morassi, Vanzo, Park, & Hubinger, 2009).

Moisture Isotherm

The results of the moisture isotherms are displayed in Figure 3.4. The summary of the GAB and BET models fit to the isotherms are displayed in Table 3.4. After absorbing moisture for 30 days, powders at the higher humidity were slightly darker and stuck together, but there was no great visible difference. The data of AD can be classified as a Type II isotherm, as AD is a complex powder made of protein, chitin, and fat, all with varying degrees of hydrophilic and

hydrophobic molecules. Water binds first to hydrophilic sites, such as those found on protein, then begins to fill in capillaries until the high water activity overcomes the space available and increases proportionally with the increase in moisture content (Andrade, Lemus, & Pérez, 2011; Bell & Labuza, 2000). The powder of CP gradually absorbed moisture until the water activity hit 0.75, upon which it climbed rapidly. This suggests that CP is a more crystalline powder that does not absorb water until the water activity reaches a certain point.

Protein and Fat Content

The dry basis protein and fat content of the two insect species are reported in Table 3.5. Finke (2002) found *Acheta domesticus* raised for animal consumption to contain 20.5% protein on a wet basis, whereas van Huis et al. (2013) found reports varying between 8 to 25% on a wet basis for adult crickets, with no breakdown by species. Bukkens (1997) reported a cricket species in the same family as *Gryllodes sigillatus*, *Brachytrypes membranaceus*, as having 13.7% protein on a wet basis.

Finke (2002) reported 6.8% fat on a wet basis for *Acheta domesticus*, and Bukkens (1997) reported 5.3% fat on a wet basis for *Brachytrypes membranaceus*. This study found AD to have 5.3% fat and GS to have 6.45% fat on a wet basis.

This variation in protein and fat content is expected due to the role diet plays on the nutrition of the insect, the specific life stage of the insect, and the protein quantification method. Farm-raised crickets, such as those used in this study and the Finke study, will have a more consistent protein quantity as their diet is more regulated and the life stage at which they are processed more closely managed, but wild caught insects, such as those used in the van Huis et al. (2013) and Bukkens (1997) have a varied diet and could have been caught at any point in their life.

Conclusion

Two species of crickets were freeze dried into powders, *Acheta domesticus* (AD) and *Gryllodes sigillatus* (GS), and compared with a third commercially available cricket powder, CP. The color values showed GS to be the darkest of the powders, but all had a similar hue and saturation of yellow-grey. CP and AD had similar flowability, while GS had the least flowability, likely due to interparticulate forces. GS and AD appeared to have similar rates of hygroscopicity. GS was shown to absorb water at an overall faster rate than AD. CP showed an unusual moisture isotherm, while AD showed a typical isotherm curve that was easily fit to a GAB model. All powders had comparable levels of fat and protein and differences are likely due to diet and life stage. AD is a more useful powder for use in food products since it is lighter in color and will not affect the color of products if incorporated, a lower rate of hygroscopicity, and a better flowability for bulk storage.

Figures and Tables

Figure 3.1 Color comparison of *Gryllodes sigillatus*, *Acheta domesticus*, commercial powder (left to right)

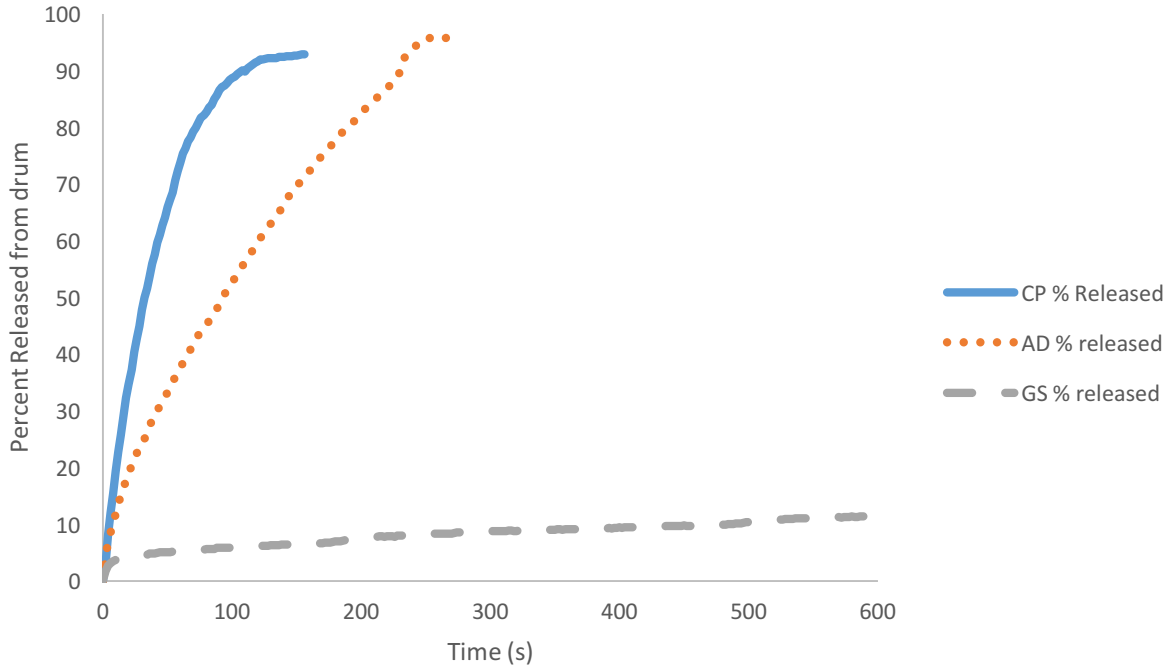

Figure 3.2 Flowability of commercial powder (CP), *A. domesticus* (AD), and *G. sigillatus* (GS)

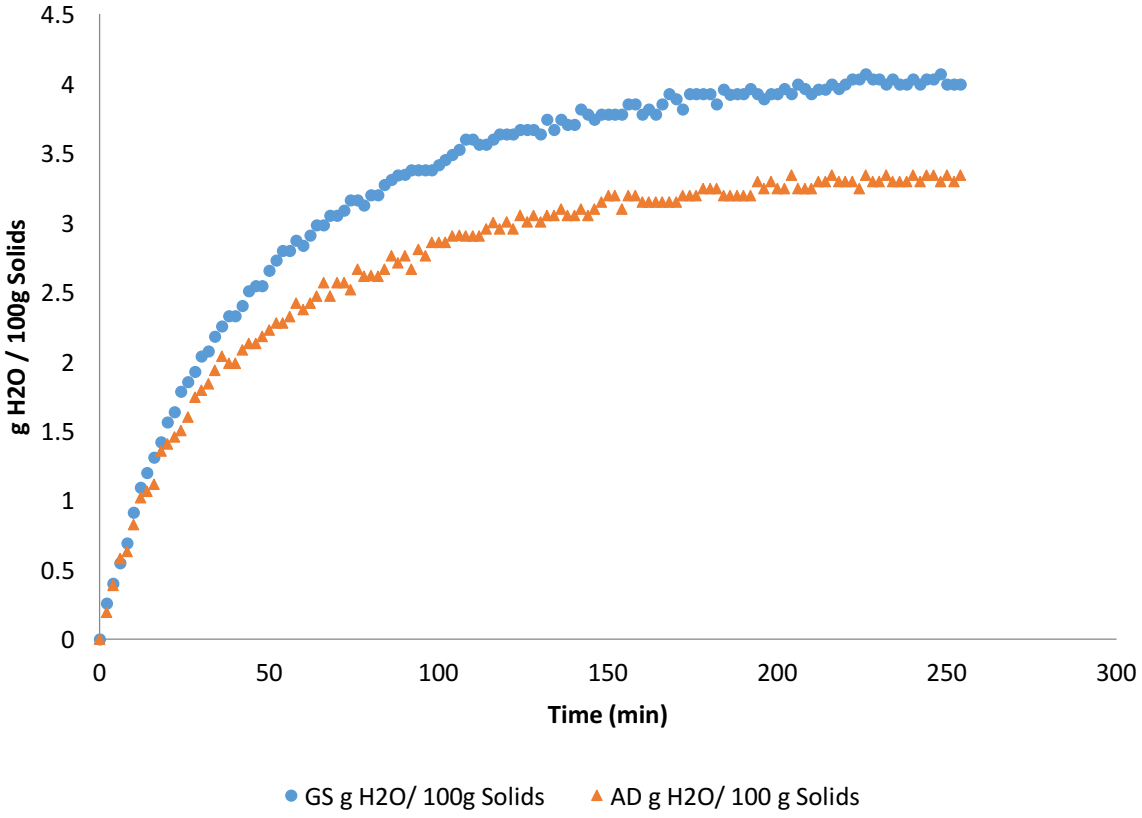

Figure 3.3 Dynamic Hygroscopicity- moisture content of *G. sigillatus* (GS) and *A. domesticus* (AD) as they adsorb moisture over time

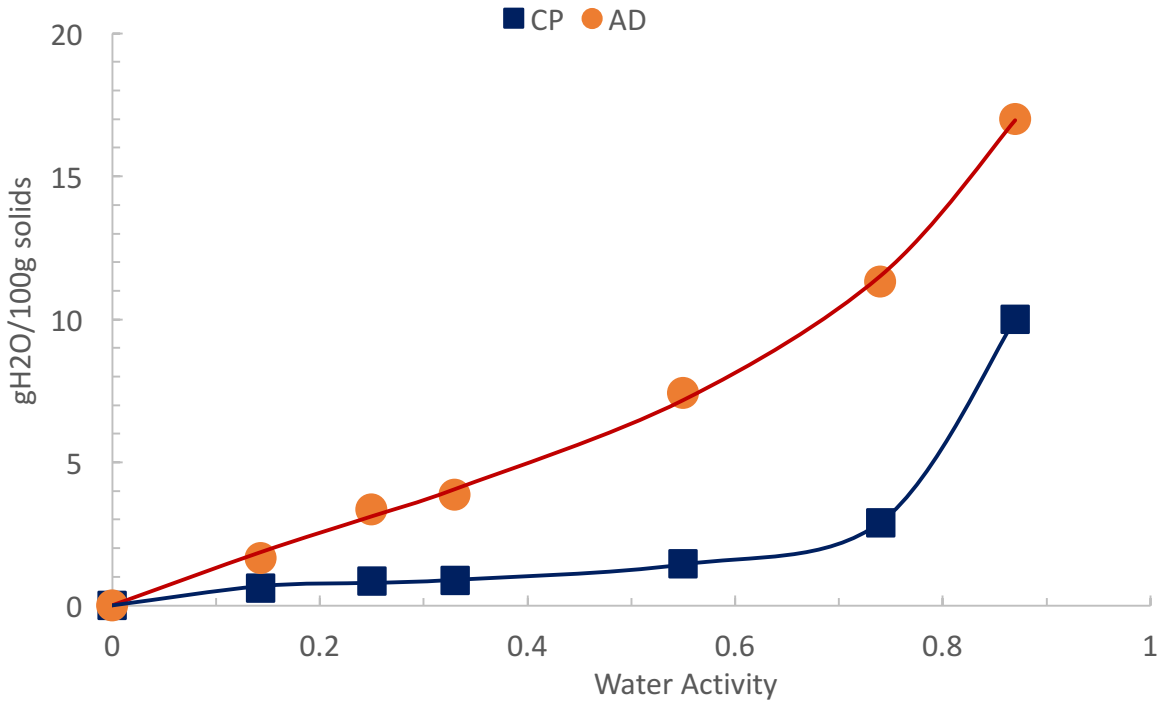

Figure 3.4 Moisture Isotherms of *A. domesticus* (AD) and commercial powder (CP) with predictive model

Table 3.1 Colorspace of three types of dried cricket powder [1,2]

Sample	L	c*	h°
Acheta domesticus	67.3[a]	15.3[a]	86.3[a]
	(0.01)	(0.01)	(0.06)
Gryllodes sigillatus	47.0[b]	13.81[b]	78.1[b]
	(0.03)	(0.01)	(0.01)
Commercial powder	59.11[c]	16.20[c]	80.64[c]
	(0.38)	(0.02)	(0.55)

[1]Mean values are presented followed by standard deviation in parentheses
[2] Mean values followed by a different superscript letter within a column are significantly different ($p < 0.05$)

Table 3.2 Powder flowability of three types of cricket powder[1,2]

Powder	% released at 5s	% released at 30s	% released at 60s	Water Activity
Commercial powder	12%[a] (2.8%)	48%[a] (2.2%)	74%[a] (5.8%)	0.36
Acheta domesticus	8.6%[a] (3.3%)	24%[b] (0%)	38%[b] (0.9%)	0.49
Gryllodes sigillatus	3.1%[a] (3.7%)	4.4%[c] (5.1%)	5.2%[c] (2.7%)	0.24

[1]Mean values are presented followed by standard deviation in parentheses
[2] Mean values followed by a different superscript letter within a column are significantly different ($p < 0.05$)

Table 3.3 Hygroscopicity equations

Powder	K (min-1)	R^2	Equation
Acheta domesticus	$1.7219x10^{-2}$	.97499	$y = 8.3058E\text{-}01e^{-1.7219E\text{-}02x}$
Gryllodes sigillatus	$2.0455x10^{-2}$	.97119	$y = 7.5881E\text{-}01e^{-2.0455E\text{-}02x}$

Table 3.4 Moisture isotherm equations

	GAB				BET		
	c	k	X^2	M_0 (g H_2O/100 g Solids)	c	M_0 (g H_2O/100 g Solids)	Sorption Heat (cal/mole H_2O)
Acheta domesticus	3.318	0.826	0.0243	5.88	1.63	5.42	287
Commercial powder	200	1.08	0.0119	0.70	21.90	0.65	1,810

Table 3.5 Protein and fat content of three types of cricket powder

Sample	% Protein Dry Basis	% Fat Dry Basis
Commercial Powder	64.00	20.00
Acheta domesticus	57.45	17.17
Gryllodes sigillatus	58.12	20.80

References

Amagliani, L., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2016). Physical and flow properties of rice protein powders. *Journal of Food Engineering, 190*, 1-9.

Andrade, R. D., Lemus, R., & Perez, C. E. (2011). Models of Sorption Isotherms for Food: Uses and Limitations. *Vitae, 18*, 2145-2660.

Barbosa-Canovas, G. V., Ortega-Rivas, E., Juliano, P., & Yan, H. (2005). Bulk Properties. In G. V. Barbosa-Canovas (Ed.), *Food Powders: Physical Properties, Processing, and Functionality* (pp. 55-90). New York: Kluver Academic/Plenum Publishers.

Bell, L. N., & Labuza, T. P. (2000). *Moisture Soprtion: Practical Aspects of Isotherm Measurement and Use*. United States of America: American Association of Cereal Chemists.

Benković, M., Srečec, S. a., Špoljarić, I., Mršić, G., & Bauman, I. (2013). Flow Properties of Commonly Used Food Powders and Their Mixtures. *Food Bioprocess Technology, 6*, 2525-2537.

Bukkens, S. G. F. (1997). The Nutritional Value of Edible Insects. *Ecology of Food and Nutrition, 36*, 287-319.

Crumpacker, B. (2006). *The Sex Life of Food: When Body and Soul Meet to Eat*. New York, NY: Thomas Dunne Books.

Finke, M. D. (2002). Complete Nutrient Composition of commercially Raised Invertebrates Used as Food for Insectivores. *Zoo Biology, 21*, 269-285.

Fitzpatrick, J. J., Iqbal, T., Delaney, C., Twomey, T., & Keogh, M. K. (2004). Effect of powder properties and storage conditions on the flowability of milk powders with different fat contents. *Journal of Food Engineering, 64*, 435-444.

Huis, A. v., Itterbeeck, J. V., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). Nutritiona Value of Insects for Human Consumption *Edible insects: future prospects for food and feed security* (pp. 67-80). Rome: Food and Agriculture Organization of the United Nations.

Janjatovic, D., Benkovic´, M., Srec˘ec, S., Jez˘ek, D., Špoljaric´, I., & Bauman, I. (2012). Assessment of powder flow characteristics in incoherent soup concentrates. *Advanced Powder Technology, 23*, 620-631.

Jaya, S., & Das, H. (2004). Effect of maltodextrin, glycerol monostearate and tricalcium phosphate on vacuum dried mango powder properties. *Journal of Food Engineering, 63*, 124-134.

Kim, E. H.-J., Chen, X. D., & Pearce, D. (2002). Surface characterization of four industrial spray-dried dairy powders in relation to chemical composition, structure and wetting property. *Colloids and Surfaces B: Biointerfaces, 26*, 197-212.

Kurozawa, L. E., Morassi, A. G., Vanzo, A. A., Park, K. J., & Hubinger, M. D. (2009). Influence of Spray Drying Conditions on Physicochemical Properties of chicken MEat Powder. *Drying Technology, 27*, 1248-1257.

Leon, K., Mery, D., Pedreschi, F., & Leon, J. (2006). Color measurement in L*a*b* units from RGB digital images. *Food Research International, 39*, 1084-1091.

Ma, J.-J., Mao, X.-Y., Wang, Q., Yang, S., Zhang, D., Chen, S.-W., & b, Y.-H. L. (2014). Effect of spray drying and freeze drying on the immunomodulatory activity, bitter taste and hygroscopicity of hydrolysate derived from whey protein concentrate. *Food Science and Technology, 56*, 296-302.

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food Bioprocess Technology, 6*, 36-60.

Shockley, M., & Dossey, A. T. (2014). Insects for Human Consumption. In J. Morales-Ramos, G. Rojas, & D. I. Shapiro- Ilan (Eds.), *Mass Production of Beneficial Organisms-Invertebrates and Entomopathogens*: Elsevier.

Spence, C. (2015). On the psychological imapct of food colour. *Flavour, 4*(21).

Teunou, E., Fitzpatrick, J. J., & Synnott, E. C. (1999). Characterisation of food powder flowability. *Journal of Food Engineering, 39*, 31-37.

Varner, A. S. (2014). *Modeling and Optimization of the Dehydration of Beets for use as a Value-Added Food Ingredient.* (Master of Science), University of Georgia, Athens, Georgia.

Yeomans, M. R., Chambers, L., Blumenthal, H., & Blake, A. (2008). The role of expectancy in sensory and hedonic evaluation: The case of smoked salmon ice-cream. *Food Quality and Preference, 19*, 565-573.

CHAPTER 4

RHEOLOGICAL AND FUNCTIONAL PROPERTIES OF CRICKET-BASED FOOD INGREDIENTS

Micali, C.S., & Kerr, W.L. (2017). To be Submitted to *Journal of Texture Studies*.

Abstract

Powders from *Acheta domesticus* and *Gryllodes sigillatus* showed similar functional properties at multiple pHs. When heated they formed weak gels, with the strongest gel occurring at pH 10. Foam capacity was higher at pH 10 as well, but it was not a stable foam, likely due to the fat and chitin present in the solution. Both showed high emulsification stability and capacity. Similar to other proteins, they are least soluble at a pH of 5, and their general solubility increases in a 0.5M NaCl solution. These properties could likely be improved through the extraction and isolation of the proteins, as the fat and chitin present interfere with the functionality, especially of foaming.

Introduction

Insects such as crickets are an important food source for many peoples, and their use and consumption is growing in many areas where insects are not traditionally consumed. While some insects are cooked and eaten intact, they may also be milled or dried to create an ingredient to be used in other foods. Insects contain on the order of 100-250 g protein per kg, and the functional properties of this component are of great importance to their successful use in new food products. Understanding how food proteins interact and react with components in a food matrix is crucial to utilizing them in the best way possible. Food proteins exhibit different levels of solubility, foamability, emulsifying capacity, and stability at different pH values

One of the most important properties of proteins as a food ingredient is the solubility, that is the amount of protein that dissolves into an aqueous solution. Food proteins vary from being completely insoluble, partly soluble, or entirely soluble. This physiochemical property is one of the first characteristics researched and used when assessing the potential uses for a food protein, as it will determine if a protein can be used in beverages or is more effective in other food systems (Zayas, 1997).

Foams are a complex two-phase system of air cells surrounded and separated by a thin continuous liquid lamellar phase. As proteins are large molecules with hydrophilic and hydrophobic regions, they play a role in contributing to the uniform distribution of small air bubbles throughout a foam structure. Well foaming proteins need to be able to form and stabilize foams rapidly at low concentrations, perform over the pH range found in foods, and perform in the presence of inhibitors such as fat, alcohol, or flavoring agents (Yi et al., 2013). Protein concentration and inhibitors influence the foamability of the solution. A protein concentration between 2 to 8% can give maximum foam, because a denser foam with thicker interfacial films is formed. Foam inhibitors, especially polar lipids with high surface activity, destabilize the film and rupture the air cells. Even levels are low as 0.1% impair foaming (Damodaran, 2007).

Emulsions are defined as a dispersed system of two or more liquids that are normally immiscible, with one liquid being the continuous phase, and the other the dispersed phase. Emulsions are important in cosmetics, pharmaceuticals, paints, and food (Zayas, 1997). Emulsions are thermodynamically unstable and will separate over time, so stabilizers in the form of proteins and other surfactants are introduced. Stabilizers are added to emulsions to increase their longevity through reducing the interfacial tension between the two phases. The stability of the emulsion and ability of proteins in solution to affect it depends on the shape, charge, and hydrophobicity of the protein composition, as well as environmental factors such as pH and temperature (Vaclavik & Christian, 2008; Zayas, 1997).

Rheological properties relate to how a food flows or deforms when subject to mechanical forces. Some food materials are more liquid in nature, and the type and concentration of proteins present influences the fluid viscosity and perceived thickness. Other foods have the properties of solids or semi-solids. With proteins, gelation can be a desirable means of building a food

viscoelastic structure. Dynamic rheological analyzers can measure the properties of these gels and the conditions under which they form. For example, dynamic rheological thermal analyses is useful for determining the temperature at which a protein denatures and testing the strength of a gel, if one is formed. If a product has strong gelling properties it can be used as an additive for meat binding, texture stabilizers, or as a fat substitute (Gao et al., 2015). Strong gels have high storage modulus (G′) as they are able to be stressed and return to their point of origin. Knowing the temperature at which the protein denatures gives insight into the best processing manner in order to keep the protein functional (Barnes, 2002). Likewise, the formation of viscous liquids, sols or gels using proteins is much determined by pH and ionic strength and these conditions can be tested in the rheometer.

As cricket powders are being considered for a variety of food applications, it is important to understand the properties that determine how they function. In addition, it is useful to measure these properties in a way in which they can be compared to other food proteins that might be considered for a food product. The purpose of this study was to evaluate the functional properties of freeze-dried cricket powder and how these manifest at different temperature and pH values.

Materials and Methods

Sample Preparation

Live sub-adult *Acheta domesticus* (AD) were received from Armstrong's Cricket Farms (Glennville, Georgia, USA) and held for 24 h with no food before being transferred to a -40˚C freezer. After 48 h they were removed from the freezer. Small batches of crickets were blended with liquid nitrogen for two 15 s intervals in a blender (Model 33BL12, Waring, New Hartford, Connecticut, USA) fitted with a cross-blade. More liquid nitrogen was added between blending

intervals. The cricket paste was transferred to aluminized plastic bags, sealed, and placed in the -40˚C freezer until being used.

Live sub-adult *Gryllodes sigillatus* (GS) were received from Ghann's Cricket Farm (Augusta, Georgia, USA) and held for 24 h with no food. After the holding period they were frozen with liquid nitrogen. They were collected in aluminized bags, sealed, and held at 0˚C for less then 48 h before being freeze dried.

Both species of crickets underwent the same freeze-drying process at different times. The crickets were spread into a thin layer over several metal sheets in the freeze drier (Model RD53S5, Millrock Technology, Kingston, NY, USA) and frozen first to -40˚C for 90 min. The primary drying started at -10˚C and 100 MT of vacuum for 120 min, increased to 0˚C and 150 MT for another 120˚C, and lastly increased to 20˚C at 180 MT for 600 min. The secondary drying was 30˚C at 100 MT for 120 min. The whole, freeze-dried GS were ground into a powder in a cross-blade grinder (NutriBullet LLC, Pacoima, CA, USA).

Rheometer Methods

The thermo-rheological properties of 30% w/v cricket powder in distilled water were assessed on a dynamic rheometer (Discovery HR-2, TA Instruments, New Castle, Delaware, USA). A 60mm cone at a 1.993056° angle (Model 511606.905, TA Instruments, New Castle, DE, USA) with a 2500μm gap on top of a plate with internal heating/cooling system was used. The sample was placed between the cone and the plate and enclosed in an insulated shell to minimize moisture and heat loss. The sample was heated from 25˚C to 85˚C at a rate of 3˚C every 10 s while continuously being sheared in oscillatory mode. The oscillations were fixed at a strain of 1% in order to stay within the previously established linear viscoelastic region. To test the dependence of these properties on pH, NaOH or HCl was used to adjust the suspension to

pH4, pH6 or pH10. These were denoted AD4, AD6 or AD10 (*Acheta domesticus* at pH4, 6, or 10) and GS4, GS6 or GS10 (*Gryllodes sigillatus* at pH4, 6, or 10).

Foam capacity and stability

Foam expansion was measured according to Knickerson (2012c). First, 15 mL of 1% cricket solution was poured into a 400 mL beaker. The solution was foamed using a homogenizer (Model 150, Fisher Scientific, Hampton, NH, USA) with the tip just immersed into the solution. The foamed solution was then decanted into a graduated cylinder. The initial serum and foam volume were recorded and then again after 30 min. Three replications of foaming properties of AD and GS were recorded each at pH4, pH6, and pH10. The pH was adjusted using NaOH and HCL. The foaming capacity and stability were calculated according to:

$$Foam\ Capacity = 100 * \frac{foam\ volume}{initial\ volume} \quad \text{(Eq. 1)}$$

$$Foam\ Stability = \ 100 * \frac{volume\ after\ 30\ minutes}{initial\ foam\ volume} \quad \text{(Eq. 2)}$$

Emulsion Capacity

Emulsion capacity was measured according to Knickerson (2012a) and (Wang & Maximiuk, 2015). Capacity was measured by combining 2g of 1% solution with 3g, 4g, 5g, or 6g of soybean oil in a 50mL centrifuge tube. This mixture was homogenized for 5 min with a homogenizer (Model 150, Fisher Scientific, Hampton, NH). The capacitance was measured using a conductivity meter (Accumet Basic, model AB30, Fisher Scientific, Hampton, NH) and recorded. The amount of oil vs capacitance was plotted and the mass of oil at which there was a dramatic decrease in capacitance was considered its emulsion capacity. Suspensions made from

the two ingredients, AD and GS, were measured at pH4, pH6, and pH 10. The pH was adjusted using NaOH and HCL. Capacity was calculated according to:

$$Emulsion\ capacity = \frac{average\ weight\ (g) of\ oil\ before\ and\ after\ inversion\ point}{weight\ \ (g)\ of\ protein} \quad \text{(Eq. 3)}$$

Emulsion Stability

Emulsion stability was measured according to Knickerson (2012b). Stability was measured by combining 5g of 1% solution with 5g of oil in a 50mL centrifuge tube. This mixture was homogenized for 5 min with a homogenizer (Model 150, Fisher Scientific, Hampton, NH) and then decanted into a graduated cylinder. The volume of the aqueous phase after 30 min was recorded. Suspensions made from the cricket powders were measured at pH4, pH6.3, and pH10. Emulsion stability was calculated according to:

$$Emulsion\ stability = 100 * \frac{V_B - V_A}{V_B} \quad \text{(Eq. 4)}$$

where

V_B is the volume of the aqueous phase before emulsification

V_A is the volume of the aqueous (serum) layer after 30 min

Solubility

Powder solubility was determined using a method similar to that described by Zhao et al. (2016). GS and AD were dispersed into either distilled water or 0.5 M NaCl to create a 10% w/v suspension. The pH was adjusted to 3, 5, 7, and 9 using HCl or NaOH. The solution was stirred, vortexed and centrifuged at 2060 g for 30 min (Eppendorf Centrifuge 5810, Hamburg, Germany). The supernatant was measured for protein content using the Bradford Method with Bovine Serum Albumin (BSA) as standard. The experiment was performed in duplicate. The protein solubility index (PSI) was calculated as:

$$PSI\ (\%) = 100 * \frac{soluble\ protein}{total\ protein} \quad \text{(Eq. 5)}$$

Statistical Analyses

Statistical analyses were performed using JMP 13 (SAS Institute, Cary, NC). Two-way ANOVA was performed to determine statistical significance of independent variables. Tukey HSD was performed for mean difference testing. Significant differences were indicated for those with $p < 0.05$.

Results and discussion

Rheometry

In order to perform subsequent tests, a linear viscoelastic region test was first done. The linear region, in which the storage modulus (G′) remains constant as shear stress was increased, was found to range from 0.001 to 1 Pa for the samples tested (Figure 4.1). Since smaller oscillations are more susceptible to environmental interference, a strain of 1% was chosen. A strain this size was found to be the most reliable in the linear region for all the samples. To double-check the appropriateness of this strain, a test temperature ramp was performed ensuring that the sinusoidal stress and strain readings had minimal noise and did not clip during the test. The effects of temperature on the cricket paste at different pH are shown in Figure 4.2 and 4.3, illustrating changes in the storage modulus and tan δ, respectively. The storage modulus (G′) measures the extent that the material stores energy like an elastic solid. The loss modulus (G″) measures energy lost through dissipation of heat. Tan delta (tan δ) is equivalent to the ratio G″/G′, and thus gives a relative measure of the energy lost versus the energy stored as the material is subject to oscillatory stresses. The tan δ of samples ranged from 0.9902˚ to 0.0950˚, indicating that all samples had higher G′ than G″ and thus had more elastic, gel-like properties rather than viscous properties.

Three distinct regions were observed during the temperature ramp, and related to changes in the protein due to denaturation. The storage modulus initially decreased as the samples were heated, reached a plateau, then increased at higher temperatures. The magnitude of these phases depended upon the pH of the sample. GD10 and GD4 had three very distinct phases, while GD6, AS6, AS4, and AS10 have subtler transitions.

The initial decrease in G′ can be attributed to a decrease in the elastic modulus as temperature increases. That is, the firmness decreases upon heating as long as there is no change in the degree of networking. Most samples showed an increase in G′ at temperatures near 50 to 60°C. AD4 and AD10 samples started with the lowest G' (0.8-3 Pa), which increased to ~10-20 Pa once the samples reached 80°C. Samples GS6 and AD6 had greater G′ at 25°C (70-80 Pa) which had increased to 300-700 Pa at 80°C. This suggests that the samples at pH 6 had a greater degree of networking at low temperature than those at pH 4 or 10. At higher temperatures, the protein can undergo denaturation which can encourage further gelation. One exception was the GS samples at pH 10. These started with a relatively high G′ (~300 Pa) at 25°C which gradually decreased to ~30 Pa when the sample reached 80°C. This suggests that the alkaline conditions were enough to destabilize the protein and cause gelation even at low temperature. The effect of increased temperature in this case was just to soften the existing network structure.

Both AD4 and AD10 had the greatest tan δ at 25°C, with values of 0.75 and 0.84 respectively, while the next highest tan δ was for AD6 at 0.37. This suggests these samples, which also had low G′ values, were more viscous in nature than the others. Other samples had tan δ values clustered below 0.4 at 25°C, and thus were more solid-like at low temperature. With the exception of GS10, samples had tan δ values that decreased upon heating and converged at temperatures above 60°C, and ending at or below 0.13. AD4 and AD10 went through the

greatest change. As noted, these started with lowest G′ and the decreasing tan δ attests that it goes through substantial increased networking with heating. The transitions began at near 40°C and continued over an approximately 20°C. In contrast, the tan δ of GS10 increased as it was heated, reaching a maximum value at 52˚C before decreasing to an ending value of 0.29. This shows a structure that was initially partially gelled, softens slightly with temperature, then forms more structure after a critical temperature is reached.

The only correlation maximum G′ values had between variables was that the unadjusted samples had the highest G′, yet none were high enough to form a firm gel. The highest G′ was observed for AD6 (958.5 Pa), and the next highest G' for GS6 (464.1 Pa). AD4, GS4, and AD10 had G′s below 50 Pa.

Little data is available on the viscoelastic properties of insect protein. Yellow mealworm protein extract (YMPE) had a G′ value of 870.8 Pa after gelation of a 20% suspension (Zhao et al., 2016). The values are somewhat lower than those found for other food proteins. A study on conditions for forming tofu found that the majority of tofu gels had a G′ of 100 to 3,000 Pa. However, acid-induced gels made from 7% protein had G′ values of nearly 100,000 Pa (Nicole, Caimeng, Eric, & Yufei, 2014). A study on additives in ground pork patties found they formed a stiff gel with G′~12,000 Pa when the mixture included soy protein isolate and carrageenan (Gao et al., 2015). While the maximum G′ for the cricket suspensions was lower than for some other food proteins, it should be noted that these values were determined from whole powders and not protein extracts or isolates. That is, chitin, lipid and other components in the cricket powder may limit full realization of a gel network.

Foam capacity and stability

Foam capacity and foam stability results are shown in Table 4.2. The cricket powder solutions showed a modest capacity for foaming for both species at the original pH and at the acidic pH of 4. Thus, AD4 and AD6 produced foams that were 20 and 37% greater than the unfoamed solution volume. For GS4 and GS6 samples, the foam volumes were 26 and 24% greater than the initial volume. Once the pH was raised to 10, the foam capacity increased significantly. Thus, AD10 and GS10 produced 109% and 113% more foam than the starting volume.

As for foam stability, samples at lower pH were more stable. Thus, AD4 and GS6 samples retained 100% of the foam volume after 30 min. For GS4 and AD6, these values were 95% and 75%. Even though samples adjusted to pH 10 created the largest foam volume, these foams were also the least stable. It is likely that at high pH changes in electrostatic interactions allow the proteins to unfold, so that they can better orient at the liquid-air interface. However, interference from fat and chitin decreased tends to decrease stability.

The ability of solutions to foam is influenced by their protein concentration and the presence of fatty acids. The protein concentration needs to be high enough to create and maintain a structure in order to generate a large volume, stable foam. In this study, the solution that was foamed was 1% in total solids, which produced a 0.58% protein solution. A higher concentration of protein would give a denser and more stable foam due to an increase in the thickness of the interfacial film. Previous studies have determined the maximum overrun of foam to be obtained at a protein concentration between 2% and 8%. Lipids present in the cricket powder may have also destabilized the foam. Very low levels of lipids, between 0.1% to 0.5% impair the foaming process. The solution of AD had a fat content of 0.17% while GS had a fat content of 0.21%.

Lipids and other water insoluble substances rupture the interfacial film and destroy the air bubbles. Defatting whey, soy, and pea proteins has been shown to increase their foam capacity significantly (Yi et al., 2013; Zayas, 1997).

Emulsion Capacity

The results of the emulsification tests are displayed in Table 4.2. All of the GS samples could emulsify 8.14 grams of oil per gram of protein, and AD 6 and AD10 could emulsify 8.33 grams of oil per gram of protein. The exception was AD4, which could emulsify 9.85 grams of oil per gram of protein. Although measured by electrical conductivity changes, the inversion could also be seen visually as the w/o emulsion was thicker and creamier than the o/w emulsion.

Emulsion Stability

Both AD and GS at all tested pHs had little or no separation of the emulsion after 30 min. The AD10 solution was the most stable, with no visible separation of components. There was very little separation for AD6 and GS10, with average changes in emulsion volume of 0.0333 mL and 0.100 mL, respectively. The least stable emulsion formed from the GS4 solution, which had an average separation volume of 0.500 mL after 30 min.

As with foams, the creation and stabilization of oil/water emulsions is improved by the inclusion of surface-active proteins. Although the properties of proteins that help create and stabilize foams and emulsions are similar, their activities in the two colloidal systems is not identical. For one thing, the emulsion presents an oil phase in which hydrophobic regions of the protein can dissolve. In addition, foams are more sensitive to rheological properties of the thin film interface. That is, incorporation of proteins helps increase the viscosity of the film and limit drainage. Proteins in the film can also present steric hindrance that stabilize the film, and protein-

protein interactions can influence this phenomenon. The emulsification stability and capacity may be further improved by extracting and isolating the protein fraction.

Solubility

The solubility of AD and GS at multiple pH is displayed in Figure 4.4. Both showed the least solubility at pH 5, which is typical for several food proteins such as soy protein isolate and whey protein. One determinant to solubility is the isoelectric point of the protein. The isoelectric point is the pH at which there is no net charge on the protein. At lower or higher pH, the protein has ionic groups that can better interact with water molecules. As previously noted, increased solubility can also help the protein act as an emulsifier or foaming agent as it increases the ability of the protein to move to the interface. Having the AD or GS in 0.5M NaCl increased their general protein solubility due to the salt ions interacting with opposing charges on the proteins. General solubility may be decreased as well due to the presence of other components such as fats and chitin (Santos et al., 2015).

Conclusion

Powders made from both *Acheta domesticus* and *Gyllodes sigillatus* have functional properties that are beneficial for food applications. When heated they do form soft gels. The gelling is pH dependent, with a stronger gel forming at more alkaline pH. The largest foam volume was created using a solution at a pH of 10, but those foams were not as stable as foams formed at lower pH. Both AD and GS were good emulsifiers, with a high emulsification stability. Similar to other food proteins, they are least soluble at a pH of 5, and their solubility increases in a 0.5M NaCl solution. These properties could likely be improved through the extraction and isolation of the proteins, as the fat and chitin present interfered with the ability to form foams.

Figures and Tables

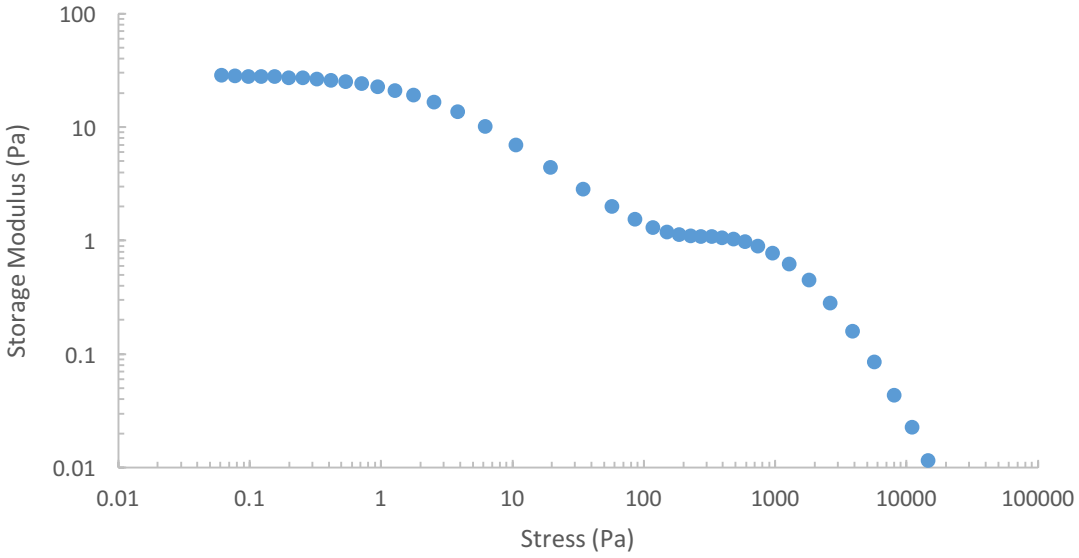

Figure 4.1 Linear Viscoelastic Regions of 30% cricket paste

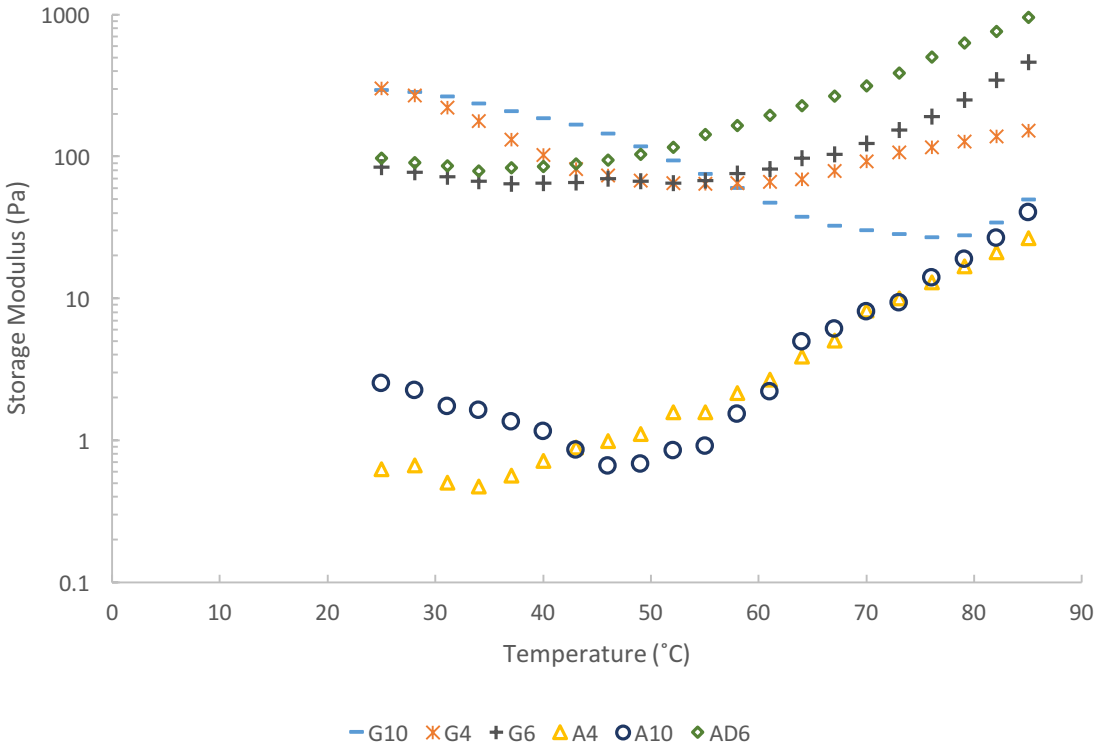

Figure 4.2 Storage modulus (G′) from temperature sweep for 30% w/v cricket paste for *G. sigillatus* and *A. domesticus* at pH 4, 6, and 10

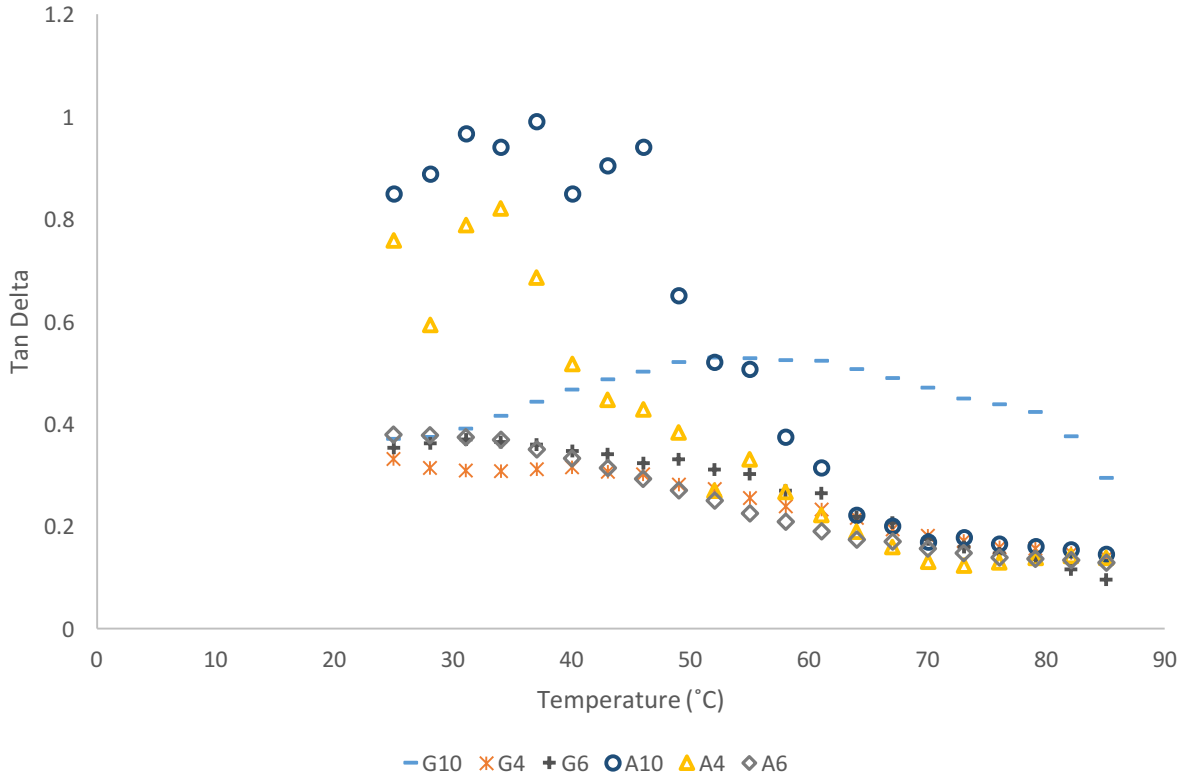

Figure 4.3 Tan delta from temperature sweep for 30% w/v cricket paste for *G. sigillatus* and *A. domesticus* at pH 4, 6, and 10

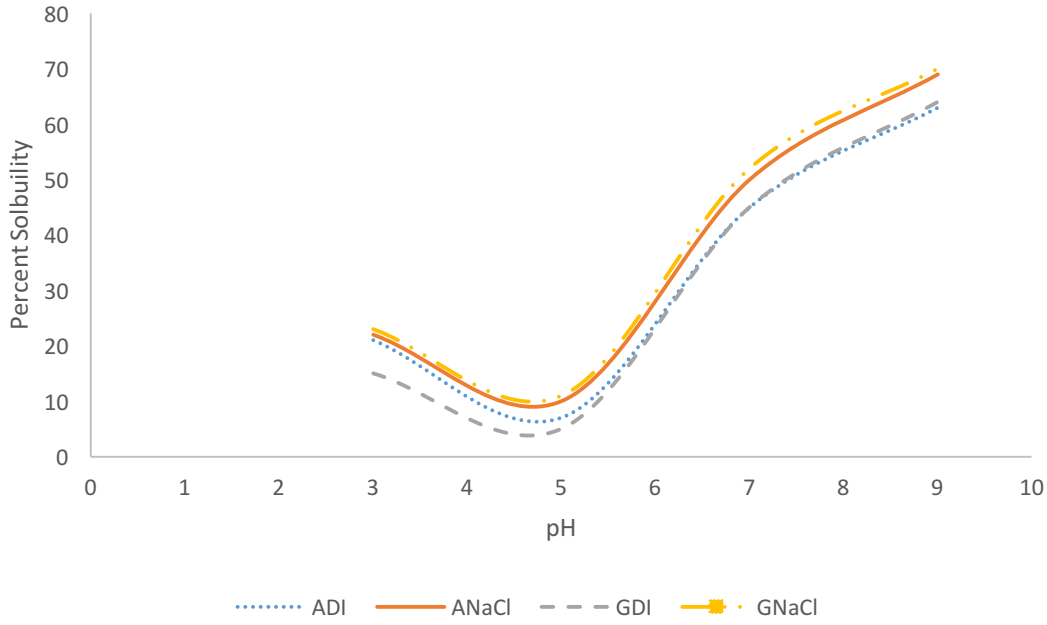

Figure 4.4 Solubility as a function of pH for *G. sigillatus* and *A. domesticus* in DI water or 0.5M NaCl

Table 4.1 Maximum G′ achieved during temperature sweep for 30% w/v cricket paste for *G. sigillatus* and *A. domesticus* at pH 4, 6, and 10

Sample	Max G' (Pa)
AD 6	958.457
AD 4	26.5146
AD 10	40.2042
GS 6	464.148
GS 4	152.111
GS 10	49.5897

Table 4.2 Functional properties for *G. sigillatus* and *A. domesticus* at pH 4, 6, and 10

Sample	Foam Capacity	Foam Stability	Emulsion Capacity (g oil/g protein)	Emulsion Stability (mL of separation)
AD6	37^{a} (9)	75^{bc} (17)	8.33^{a}	0.0333^{ac} (0.06)
AD4	20^{b} (0)	100^{a} (0)	9.85^{b}	0.183^{b} (0.03)
AD10	109^{c} (3)	69^{c} (1)	8.33^{a}	0^{c} (0.00)
GS6	24^{ab} (4)	100^{a} (0)	8.14^{c}	0.150^{b} (0.05)
GS4	26^{ab} (2)	95^{ab} (8)	8.14^{c}	0.500^{d} (0.00)
GS10	113^{c} (7)	67^{c} (2)	8.14^{c}	0.100^{ab} (0.00)

[1]Mean values are presented followed by standard deviation in parentheses
[2] Mean values followed by a different superscript letter within a column are significantly different ($p < 0.05$)

References

Damodaran, S. (2007). Amino Acids, Peptides, and Proteins. In S. Damodaran, K. L. Parkin, & O. R. Fennema (Eds.), *Fennema's Food Chemistry* (4th ed.). Boca Raton, FL: CRC Press.

Gao, X.-q., Zhanga, W.-g., & Zhoua, G.-h. (2015). Emulsion stability, thermo-rheology and quality characteristics of ground pork patties prepared with soy protein isolate and carrageenan. *Journal of the Science of Food and Agriculture, 95*, 2832-2837.

Nicole, M., Caimeng, Z., Eric, K., & Yufei, H. (2014). Salt and Acid-Induced Soft Tofu-Type Gels: Rheology, Structure and Fractal Analysis of Viscoelastic Properties as a Function of Coagulant Concentration. *International Journal of Food Engineering, 10*, 595-611.

Vaclavik, V. A., & Christian, E. W. (2008). *Essentials of Food Science* (Third Edition ed.). New York, NY: Springer.

Wang, N., & Maximiuk, L. (2015). Development of an Improved Electrical Resistance Method for Determining Emulsifying Capacity of Pulse and Soy Materials. *American Association of Cereal Chemistry, 92*, 253-257.

Yi, L., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Shadler, V., Van Huis, A., & van Boekel, M. A. J. S. (2013). Extraction and characterisation of protein fractions from five insect species. *Food Chemistry, 141*, 3341-3348. doi:http://dx.doi.org/10.1016/j.foodchem.2013.05.115

Zayas, J. F. (1997). *Functionality of Proteins in Food*. New York: Springer.

Zhao, X., Vázquez-Gutiérrez, J. L., Johansson, D. P., Landberg, R., & Langton, M. (2016). Yellow Mealworm Protein for Food Purposes - Extraction and Functional Properties. *PLoS ONE, 11*. doi:10.1371/journal.pone.0147791

CHAPTER 5

DEVELOPMENT AND EVALUATION OF A PUFFED EXTRUDED SNACK PRODUCT CONTAINING CRICKET PASTE

Micali, C.S., & Kerr, W.L. (2017). To be Submitted to *Journal of Food Engineering*.

Abstract

The physiochemical and sensory qualities of a corn-wheat-rice based puffed snack product extruded with a twin-screw extruder were evaluated with the addition of *Acheta domesticus* paste (5, 10, 15 g paste/100 g dry feed). Bulk density, packed density, expansion ratio, and protein content were significantly different for all treatments. The addition of cricket powder decreased the expansion ratio, increased the bulk density, and increased the amount of force required to snap it. Protein content increased with increased cricket paste incorporation. Incorporation of *Acheta domesticus* resulted in a darker and less color saturated product. Puffed snacks were not significantly different in likeability for appearance, flavor, and overall acceptability, but the texture of products with 5 g paste/100 g was best liked.

Introduction

Processed foods produced for snacking purposes is a steadily growing market, with an estimated 2% compound annual growth rate in the U.S. (*Savory Snacks in the United States*, 2015). Nearly 94% of Americans snack at least once daily, preferring organic snacks and products with added nutrition such as protein and vitamins. Consumers indicate a desire for these healthier snacks, and to be able to serve healthier snacks to their children, yet they snack mainly to satisfy a craving. Millenials are drawn to products with high fiber, energizing claims, or higher protein content (Topper, 2015). Snacks with a high protein content provide increased satiety and decreased appetite, reducing overall energy intake and potentially facilitating weight loss (Doyon et al., 2015).

Insects have played a role in the diet of humans around the world for millennia. Many cultures consume them as a regular part of the diet, both as a seasonal staple and a delicacy. Over the years there have been movements to encourage people in Western cultures that are less

familiar with consuming insects to acknowledge the nutritious and potentially sustainable qualities that insects can play in their diet (Shockley & Dossey, 2014).

Although the exact nutritional content depends upon the species being consumed and their diet, insects are a good source of protein and fat. Many are nutritionally comparable to other animal meats such as chicken, pork, and beef. Their amino acid profile is equivalent or superior to soy protein and the digestibility is as high as 95% (Bukkens, 1997; Huis et al., 2013a).

Extrusion is a high-pressure process used in the manufacturing of pasta, crunchy snack foods, pet food, chewing gum, and a variety of other products. Depending on the product, an extruder incorporates one or two screws that are fit into a stationary barrel. Pre-ground ingredients are introduced into the barrel, mixed with water, forced through the barrel by the screw action, and out a die or perforated plate at the end, giving the end-product a specific design (Harper, 1981). To create a puffy, crunchy snack food, a high starch ingredient such as corn must be combined with the right amount of water to produce a product that will expand after exiting the high-pressure system and contacting the atmosphere. Too much or too little water and the product will either not puff enough or collapse upon exit. The extent of expansion and the rigid, porous structure created by the starch gelatinization is called the expansion ratio and is useful for comparing ingredient formulations and specifications (Yagci & Gogus, 2009).

One avenue for expanding the use of insects is as an ingredient for value-added food products. The protein can add nutritional value and may provide useful functional properties. In addition, the food matrix may help mask or obviate characteristics that would be objectionable to some consumers. A promising approach could be to use insect ingredients as part of an extruded snack food. Incorporating insect paste or powder into a familiar puffed snack product that touts

high protein could be a means of increasing consumer appeal. In addition, high-shear extruder conditions can help modify the structure and functionality of food ingredients.

The hypothesis of this study was that a high-quality extruded snack could be developed that incorporated minimally processed cricket paste as an ingredient. To this end, several physical and sensory properties were measured to determine the effects of added cricket on product quality. In addition, extruded products were made with different levels of cricket paste to determine optimal levels.

Materials and Methods

Expanded extruded snacks were prepared based on a corn/wheat/rice blend developed by The University of Georgia's Food Processing Lab in 2016. The basic blend was fortified with 0, 5, 10 or 15 g/100 g ground cricket paste, as preliminary work suggested that greater levels inhibited expansion. Several product characteristics related to quality were determined including density, expansion ratio, color, protein and moisture content, and physical measurements associated with hardness. In addition, consumer sensory tests were conducted to assess degree of likeability of each of the samples.

Cricket paste processing

Live sub-adult *Acheta domesticus* (AD) were received from Armstrong's Cricket Farms (Glennville, GA, USA) and held for 24 h with no food before being transferred to a -40˚C freezer. After 48 h they were removed from the freezer. Small batches of crickets were blended with liquid nitrogen for two 15 s intervals in a blender (Model 33BL12, Waring, New Hartford, CT, USA) fitted with a cross-blade. More liquid nitrogen was added between blending intervals. The cricket paste was transferred to aluminized PET bags (ABC Packaging Direct, Avon, OH, USA), sealed, and stored at -40˚C until subsequent processing.

Extrusion Processing

A pilot-scale 25:1 co-rotating twin-screw extruder (Model MPF30, APV Baker Limited, Staffordshire, England) was run with a dry feed mixture that consisted of 33.2% corn flour (Aztec Milling L.P.), 33.2% wheat flour (ConAgra Mills), and 33.2% rice flour (Erawan Marketing Co., LTD, Bangkok, Thailand) with 0.25% calcium carbonate. Four different formulations were extruded. One was a control containing no additional ingredients while the others had 5%, 10%, or 15% of cricket paste added to the basic flour blend.

The screw configuration was: 4 1.5D twin lead feed screws, 7 90˚ paddles, 4 1.5D twin lead feed screws, 8 90˚ paddles, 3 1D twin lead feeds screws, 1 1.5D twin lead feed screws, 15 90˚ paddles, and 1 1D single lead discharge screw. The exit die diameter was 2.6mm. Prior to each run a dry feed rate calibration and a wet feed rate calibration were performed. The wet feed rate was set to produce an in-barrel moisture content of 19%, with a different pump setting depending on the moisture content of the individual formulations. The barrel screw speed was 100 RPM.

After extrusion, the products typically contained 8-10% moisture. Samples were further dried in an impingement oven (Model 1450, Lincoln, Food Service Products Inc, Fort Wayne, IN, USA) to reach a moisture content of 1-3%. Samples were collected prior to drying and immediately after drying to determine the change in moisture content. After drying, all samples were collected in aluminized PET bags, sealed, and stored at -80˚C.

Physical and Chemical Tests

Bulk and Packed Density

The bulk density was determined from the mass of the extrudate divided by the volume as described in (Cheng & Hansen, 2016). Extruded pieces from each treatment were lightly

placed into a tared 400mL glass beaker and weighed on an analytical balance. Measurements were replicated three times. A similar method was used to determine packed density, except the beaker was tapped on the table for 20 seconds after being filled and then additional extrudate was added until no more could fit.

Expansion Ratio

The expansion ratio was determined as the diameter of the extrudate divided by the diameter of the die opening (0.26 cm) (Yagci and Gogus, 2009). The cross sectional diameters were measured using a standard caliper. These measurements were taken at 3 different sections along 5 random samples for each treatment.

*L*c*h˚ Colorspace*

Color was measured after grinding extrudate samples for 20s in a cross-blade grinder (NutriBullet LLC, Pacoima, CA, USA) and pouring the ground sample into a petri dish to fully cover the bottom. The colorspace values were recorded in triplicate using a chroma meter (Model CR-410, Minolta, Konica Minolta Sensing, Inc, Tokoyo, Japan) under the D65 illuminant system (T~6500K). The chroma meter was calibrated against a white calibration plate (No. 13333105) supplied by Minolta.

Protein Content

Protein content of the extrudates was determined through the Kjeldahl method for determining total nitrogen of meat (AOAC method 981.10), and performed in duplicate. Each ground extrudate was transferred to a 250-mL FOSS digestion tube and 2 FisherTab CT-37 Kjeldahl tablets to which 25mL of concentrated H_2SO_4 was added. The contents were slowly heated in a BD20 digester for about an hour, then cooled and placed in the 2200 Kjeltec autodistillation unit (FOSS, Eden Prairie, MN). Several drops of methyl red/methylene blue

indicator was added to a 250mL Erlenmeyer flask which was then inserted into the receiver of the 2200 Kjeltec autodistillation unit. The unit automatically dispensed 4% (w/v) boric acid into the Erlenmeyer flask and the distillation program began. The ammonia within the digestion tube was distilled and passed through a condenser to be collected in the Erlenmeyer flask. The Erlenmeyer flask was titrated with standardized 0.1N H_2SO_4 solution (Pegg, 2016).

Moisture Content

Moisture content of samples was measured for samples exiting the extruder and after impingement drying by the use of a Halogen Moisture Analyzer (Model HR73, Mettler Toledo, Columbus, OH, USA).

Hardness

A snap test was performed by a texture analyzer to assess textural attributes of the products (Altan, McCarthy, & Maskan, 2008). A texture analyzer (Model TA-XT2, Stable Microsystems, London) fitted with a 3mm thick rounded edge blade (TA-43R) was used to determine the hardness of the extrudates. A piece of extrudate was set perpendicularly across two metal bars, suspending a section in the gap above the platform. The blade was lowered at 2 mm/s and allowed to snap the extrudate. The amount of force used to snap the extrudate was determined from the force-distance curves. The maximum force peak was identified and averaged for 5 samples of each level of cricket addition.

Sensory evaluation

A sensory panel was recruited to determine the likeability of the various products, and whether snacks with particular levels of cricket were most preferred. The consumer panel (n=50, 13M, 37F) evaluated the four samples on a 9-point hedonic scale, with 1 being “dislike extremely” up to 9 being “like extremely”. Panelists also filled out an Intent to Buy survey, with

1 being “I would never buy this” and 6 being “I would buy this very often”. Extrudate samples were snapped into 3.8 cm pieces and presented in plastic sample cups. Samples were labeled with a random code and presented in an individual random order. Panelists evaluated samples alone with no outside input. Free choice water and carrots were provided to the panelists as a palate cleanser between samples.

Statistical Analyses

Statistical analyses were performed using JMP 13 (SAS Institute, Cary, NC). Two-way ANOVA was performed to determine statistical significance of independent variables. Tukey HSD was performed for mean difference testing. Significant differences were indicated for those with $p < 0.05$.

Results and discussion

Bulk and Packed Density

Several physical properties of the extruded snacks are presented in Table 5.1. Bulk density is an important physical property related to extrudate expansion and can be an indicator of the textural properties of the extrudate. A higher bulk density means the product has less air within its structure and a smaller expansion ratio (Suksomboon, Limroongreungrat, Sangnark, Thititumjariya, & Noomhorm, 2011). The amount of cricket paste had a significant effect on the bulk density of the extrudate. The bulk density ranged from 34.92 g/L to 87.33 g/L for all samples. Samples with 0 and 5 g/100 g AD had the lowest bulk density, 34.92 g/L and 45.50 g/L, respectively. Samples with 10 and 15 g/100 g had significantly greater bulk density, 84.08 g/L and 87.33 g/L, respectively, but were not significantly different from each other.

The packed density ranged from 40.58 g/L to 99.92 g/L, and all treatments were significantly different from one another. A pattern similar to bulk density was seen, that is,

samples with 10 or 15 g/100 g AD had nearly double the packed density than those with 0 or 5 g/100 g AD.

Expansion Ratio

Expansion ratio describes the degree of puffing undergone by the product as it exits the extruder die. The expansion ratios ranged from 3.29 to 4.86 (Table 5.1). The cricket paste directly affected the expansion ratio; all four samples had significantly different expansion ratios. As the amount of cricket paste increased, the expansion ratio decreased from 4.86 to 3.29. The decrease in expansion ratio is likely due to the dilution of the total starch available to create the rigid, puffed structure and also due to the inhibitory action of the protein and fat from the paste. Additional protein may retard expansion by increasing the firmness of the extrudate as it exits the die, and lipids may retard the degree of gelatinization (Prinyawiwatkul, Resurreccion, Phillips, & Beuchat, 1995; Yagci & Gogus, 2009).

*L*c*h Colorspace*

Addition of cricket paste affected the color of the extrudates (Table 5.1). Those with 15 g/100 g AD were significantly different from the other three treatments for both L* and h˚. Most noticeably different was that extrudates with 15 g/100 g AD had a L* of 78.43 and a h˚ of 93.49, while those with 0 g/100 g AD had a L* of 83.17 and h˚ of 94.9. Samples with 15 or 10 g/100 g AD had lower c* values than those with 0 or 5 g/100 g, indicating they were less color saturated. The addition of cricket paste resulted in a darker, less color saturated extrudate, likely due to an increase in Maillard browning from the increased protein content, and dilution of carotenoid pigments originating with the corn (Altan et al., 2008).

Protein Content

The protein content (percent) of the extrudates is shown in Table 5.2. As expected, the addition of cricket paste increased the total protein for the extruded snacks. Protein content varied from 10.34 to 12.33 g/100 g, with samples from 10 and 15 g/100 g AD having significantly greater levels than those at 0 and 5 g/100 g AD. A survey of common extruded grain snacks found them to have between 6% to 10% protein content (UDSA database). Snacks higher in protein have become more attractive to consumers due to their increased satiating (Doyon et al., 2015).

Moisture Content

The addition of cricket paste increased the moisture content of the extrudate, both prior to being dried and after drying, even though the moisture content of dough in the extruder barrel was maintained at 19% for all cases (Table 5.2). The moisture content of extrudates ranged from 8.81 g H_2O/100 g (for samples containing 0 g/100 g AD) to 13.02 g H_2O/100 g (for samples containing 15 g/100 g AD). Likewise, after impingement drying the moisture content ranged from 1.91 g H_2O/100 g (for samples with 0 g/100 g AD) to 2.92 g H_2O/100 g (for samples with 15 g/100 g AD). After final drying, the difference in moisture contents were not large, and all samples were in the glassy state. It is likely that samples with higher levels of protein could better bind water, thus helping inhibit evaporation during the extrusion process. In addition, as samples with greater protein experienced less expansion, this may have reduced the rate of mass transport of water away from the product.

Hardness

The addition of cricket paste affected the hardness of extruded samples, with the max force needed to snap the product ranging from 823.97 g to 1523.65 g (Table 5.1). Samples with

10 g /100 g AD were significantly harder than the other three samples, with a maximum force of 1523.65 g. The protein and fat from the cricket influenced the starch gelatinization and structure of the extrudate. With 0 g/100 g AD and 5 g/100 g AD, the structure was rigid with a high expansion ration yet not very strong from its voluminous, airy structure. Higher amounts of cricket paste decreased the starch expansion and increased the hardness. Although the extrudates with 15 g/100 g AD had the least expansion and tightest structure, it was not significantly harder than those with 0 g/100 g AD and 5 g/100 g AD. The additional protein and fat from the cricket paste affected the starch gelatinization and made it weaker due to the dilution of the starch and diminished structure (Prinyawiwatkul et al., 1995; Yagci & Gogus, 2009).

Sensory Evaluation

Results from the consumer acceptability panel are shown in Table 5.3. The addition of cricket paste did not significantly affect the likeability of the extruded products. All scores fell between 5.20 and 7.08, indicating that the samples were more liked than disliked. For each attribute, scores ranged from: appearance (6.04 to 6.36), texture (7.08 to 6.30), flavor (6.65 to 5.20) and overall (6.10 to 5.71). The texture of samples containing 5 g/100 g AD was more liked than for the other three samples, which had similar texture scores. The texture of samples with 5 g/100 g AD appear to be the most similar to commercially produced extruded puffed snacks, and so the most familiar texture to the panelists.

The panelists' scores for sample appearance may be related to measured color values. Thus, as samples with 10 or 15 g/100 g AD were darker and less color saturated, they also had slightly lessened likeability. However, samples with 5 g/100 g AD had the greatest likeability. It has become normal for consumers to associate darker processed foods with added whole grains and fiber and find them acceptable and healthy, although excessive darkness may be associated

with overly cooked products and negatively impact a consumer's perception (Pathare et al., 2013).

Flavor scored the lowest for all the samples, with all samples receiving scores in the 5 range. This indicates that panelists did not find the flavor of the samples to be highly acceptable, yet were not opposed to it. It should be noted that this product was purposefully left without the flavor coating that is customarily added onto commercially produced puffed snacks. Leaving the extrudates without the coating allowed the panelists to rate the actual flavor of the product, and with a greater ability to note any flavor defects, rather than flavors associated with the coating. It would be expected that adding salt and/or any of several available flavor coatings would increase the flavor appeal of the product.

In general, panelists did not express a strong intention to buy any of the products. Again, it is likely these scores would be higher if there had been an agreeable flavor coating on the product and it had been presented as more of a finished product. In terms of overall likeability, samples with 5 g/100 g AD had the highest likeability. This indicates that an acceptable product that incorporates cricket paste can be produced and liked by consumers.

Conclusion

The physiochemical and sensory qualities of a corn-wheat-rice based puffed snack product extruded with a twin-screw extruder were evaluated with the addition of *A. domesticus* paste (5, 10, 15 g paste/100 g dry feed). Bulk density, packed density, expansion ratio, and protein content were significantly different for all treatments. The addition of cricket powder decreased the expansion ratio, increased the bulk density, and increased the hardness. Protein content increased with increased cricket paste incorporation. Incorporation of *A. domesticus* resulted in a darker and less color saturated product. Puffed snacks were not significantly

different in likeability for appearance, flavor, and overall acceptability, but the texture of products with 5 g paste/100 g dry feed was best liked.

Tables

Table 5.1 Physical properties of cricket extruded snacks[1,2]

Cricket Paste (g/100 g Dry Feed)	Bulk Density (g/L)	Packed Density (g/L)	Expansion Ratio	L*	c*	h (°)	Maximum Force Peak (g)
0	34.92[a]	40.58[a]	4.86[a]	83.17[a]	26.21[a]	94.91[a]	823.97[a]
	(2.1)	(1.0)	(0.20)	(0.46)	(0.18)	(0.08)	(57)
5	45.50[b]	52.75[b]	4.45[b]	82.49[a]	26.11[a]	95.05[a]	880.02[a]
	(1.6)	(1.9)	(0.20)	(0.21)	(0.03)	(0.08)	(69)
10	84.08[c]	92.67[c]	3.51[c]	82.37[a]	24.21[b]	95.36[a]	1523.65[b]
	(3.1)	(1.5)	(0.13)	(0.13)	(0.54)	(0.17)	(107)
15	87.33[c]	99.92[d]	3.29[d]	78.43[b]	24.96[b]	93.49[b]	1039.40[a]
	(3.1)	(2.1)	(0.23)	(0.45)	(0.45)	(0.19)	(152)

[1]Mean values are presented followed by standard deviation in parentheses
[2] Mean values followed by a different superscript letter within a column are significantly different ($p < 0.05$)

Table 5.2 Chemical properties of cricket extruded snacks[1,2]

Cricket Paste (g/100 g Dry Feed)	Extrudate Moisture Content (g H_2O/100 g)	Final Moisture Content (g H_2O/100 g)	Protein Content (%)
0	8.81%	1.91%	10.34[a] (0.17)
5	10.22%	2.24%	10.83[ab] (0.29)
10	10.77%	2.87%	11.31[b] (0.07)
15	13.07%	2.92%	12.33[c] (0.13)

[1]Mean values are presented followed by standard deviation in parentheses
[2] Mean values followed by a different superscript letter within a column are significantly different ($p < 0.05$)

Table 5.3 Consumer acceptability of extruded puffed cricket snacks[1,2]

Cricket Paste (g/100 g Dry Feed)	Appearance	Texture	Flavor	Overall	Intent to Buy
0	6.36[a]	7.08[ab]	5.65[a]	6.10[a]	3.26[a]
	(1.71)	(1.64)	(0.225)	(1.66)	(1.43)
5	6.58[a]	7.50[a]	5.68[a]	6.44[a]	3.29[a]
	(1.50)	(1.41)	(0.222)	(1.31)	(1.22)
10	6.04[a]	6.48[b]	5.12[a]	5.70[a]	2.82[a]
	(1.76)	(1.72)	(0.223)	(1.31)	(1.10)
15	6.04[a]	6.30[b]	5.20[a]	5.71[a]	2.88[a]
	(1.58)	(1.97)	(0.222)	(1.49)	(1.29)

[1]Mean values are presented followed by standard deviation in parentheses
[2] Mean values followed by a different superscript letter within a column are significantly different ($p < 0.05$)

References

Altan, A., McCarthy, K. L., & Maskan, M. (2008). Twin-screw extrusion of barley–grape pomace blends: Extrudate characteristics and determination of optimum processing conditions. *Journal of Food Engineering, 89*, 24-32.

Bhandari, B. (2013). Introduction to food powders. In B. Bhandari, N. Bansal, M. Zhang, & P. Schuck (Eds.), *Handbook of food powders: Processes and properties* (pp. 1-25). Cambridge, UK: Woodhead Publishing Limited.

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry, 72*, 248-254.

Brunauer, S. H., Emmett, P. H., & Teller, E. (1938). Adsorption of Gases in Multimolecular Layers. *Journal of the American Chemical Society, 60*, 309-319.

Bukkens, S. (1997). The Nutritional Value of Edible Insects. *Ecology of Food and Nutrition, 36*, 287-319.

Cheng, H., & Hansen, J. H. (2016). Extrudate expansion model in a twin-screw extrusion cooking process considering melt rheological property. *Food Bioprocess Technology, 9*, 604-611.

Doyon, C. Y., Tremblay, A., Rioux, L.-E., Rhéaume, C., Cianflone, K., Poursharifi, P., & Turgeon, S. L. (2015). Acute effects of protein composition and fibre enrichment of yogurt consumed as snacks on appetite sensations and subsequent ad libitum energy intake in healthy men. *Applied Physiology, Nutrition, and Metabolism, 40*, 980-989.

Finke, M., DeFoliart, G., & Benevenga, N. (1989). Use of a four-parameter logistic model to evaluate the quality of the protein from three insect species when fed to rats. *Journal of Nutrition, 119*, 864-871.

Gopalakrishna, S., & Jaluria, Y. (1992). Modeling of Starch Gelatinization in a Signle-Screw Extruder. In J. L. Kokini, C.-T. Ho, & M. V. Karwe (Eds.), *Food Extrusion Science and Technology* (Vol. 10). New York, New York: Marcel Dekker, Inc.

Harper, J. M. (1981). *Food Extrusion* (Vol. I). United States: CRC Press, Inc.

Huis, A. v., Itterbeeck, J. V., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). Nutritiona Value of Insects for Human Consumption *Edible insects: future prospects for food and feed security* (pp. 67-80). Rome: Food and Agriculture Organization of the United Nations.

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food Bioprocess Technology, 6*, 36-60.

Pegg, R. (2016). *Determination of Food Proteins by the Kjeldahl Method and Electrophoresis.* Instrumental Methods of Food Analysis. Experiment Procedure. Food Science and Technology, University of Georgia.

Prinyawiwatkul, W., Resurreccion, A. V. A., Phillips, D., & Beuchat, L. R. (1995). Modelling the effects of peanut flour, feed moisture content, and extrusion temperature on physical properties of an extruded snack product. *Internation journal of food science and technology, 30*, 37-44.

Savory Snacks in the United States. (2015). Retrieved from United States:

Shockley, M., & Dossey, A. (2014). Insects for Human Consumption. In J. Morales-Ramos, G. Rojas, & D. I. Shapiro- Ilan (Eds.), *Mass Production of Beneficial Organisms-Invertebrates and Entomopathogens*: Elsevier.

Singh, S., Gamlath, S., & Wakeling, L. (2007). Nutritional aspects of food extrusion: a review. *Internation journal of food science and technology, 42*, 916-929.

Suksomboon, A., Limroongreungrat, K., Sangnark, A., Thititumjariya, K., & Noomhorm, A. (2011). ffect of extrusion conditions on the physicochemical properties of a snack made from purple rice (Hom Nil) and soybean flour blend. *Internation journal of food science and technology, 46*, 201-208.

Topper, A. (2015). *Snacking Motivations and Attitudes-US*. Retrieved from

Yagci, S., & Gogus, F. (2009). Development of extruded snck from food by-products: a response surface analysis. *Journal of Food Process Engineering, 32*, 565-586.

CHAPTER 6

CONCLUSION

This research focused on utilizing whole crickets as an ingredient in food. Two species of crickets, *Acheta domesticus* and *Gryllodes sigillatus*, were received live and then processed. The first objective was to evaluate the physical properties in the freeze-dried, powder form to better understand the powder behavior. *A. domesticus* proved to have a lighter color, a better flowability, and lower hygroscopicity than *G. sigillatus* or the commercial powder. All powders had a similar protein and fat content, due to the similarity of the species and the fact that they were farm raised for consumption.

The rheological and functional properties of the two species of cricket paste were also studied in an attempt to unlock any hidden potential. Both species formed loose gels at 60˚C with the potential to form stronger gels under the right circumstances. Both species also proved to form high capacity, stable emulsions. Neither had a large foam capacity, although it did increase when the pH was raised to 10, yet was most stable at a pH of 4. Both had good solubility in distilled water and 0.5M NaCl with a pI of 5.

A fresh paste of *A. domesticus* was incorporated into a corn-wheat-rice extruded snack product to test consumer acceptability of snacks made from insects. In general, panelists did not express a strong like or dislike of the products, but samples with 5 g/100 g AD had the highest likeability rating. It is likely that with an agreeable flavor coating on the product, the scores would have been higher.

Overall, crickets have a great potential to be incorporated into the food industry. Companies can increase consumer acceptance through the incorporation of cricket paste or cricket powder into well-known, pre-existing foods that consumers are already familiar with. By freeze-drying the cricket or not drying it at all, the functional properties can be preserved and products that require emulsifiers or gelling agents can use crickets as an alternative source.