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# Evaluation of the partial substitution with cricket flour (*Acheta domesticus*) on the physicochemical, protein and starch digestibility characteristics of corn tortillas.

**A thesis presented by**

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**Dedication**

I want to dedicate this thesis to my parents Martha and Victor who have always supported me-

To my sister Andrea who has always believed in me and helped me to find my passion for food.

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**Evaluation of the partial substitution with cricket flour (*Acheta domesticus*) on the physicochemical, protein and starch digestibility characteristics of corn tortillas.**

**By**

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**Abstract**

Edible insects have gained attention as an alternative and sustainable source of protein. One of the challenges is making it attractive for consumers; for this reason, it is often mixed with other ingredients. This thesis research aimed to evaluate how the addition of cricket flour (Acheta domesticus) chemical, nutritional, and digestibility characteristics of corn tortillas. Nixtamalized corn flour (NCF) tortillas with 5%, 7.5%, and 10% A. domesticus flour (AD) were formulated, elaborated, and characterized. Addition of AD caused significant visual differences in samples in terms of color and particle content. Addition of AD increased protein and lipid content while decreasing carbohydrate content. AD addition impacted carbohydrate composition by increasing the fiber portion and reducing the starch content. Furthermore, starch composition was also affected, AD increased RS (resistant starch) content. The in vitro starch digestibility was affected with the substitution of AD, which was strongly negatively correlated (R=-0.99) with the substitution percentage of AD. The presence of amylose-lipid complex, a type of resistant starch, was observed in differential scanning calorimetry, with a second transition at temperatures above 100ºC. Principal component analysis (PCA) showed an important cluster between lipid content and resistant starch content which also supports DSC findings. The lowest glycemic index (GI) in vitro was observed in tortillas substituted at 10% with AD. In vivo, results differed, and the lowest GI value was observed in the samples with 7.5% of AD.

**Keywords:** *Acheta domesticus,* Glycemic index, resistant starch, digestibility.

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# 1. Introduction

As the world population rises, food demand is projected to double in 2050 (Grafton et al., 2015). However, current agri-food systems are the leading cause of the global climate crisis; thus, innovation is required to increase the efficiency of food production (Zhang, et al., 2018). To assure food safety in the future, various foods have been proposed as alternative sources, including insects. The Food and Agriculture Organization (FAO) has been promoting insect consumption since 2003, which has led to an increase in the interest in this novel food; however, it's not something new. Insect consumption is a practice that has been around for centuries, especially in Asia, Latin America, and Africa. Still, with the modernization of food systems, it has slowly disappeared from human diets or has become a cultural and occasional food (Murefu et al., 2019). The 5 most common orders of insects consumed around the world are beetles, belonging to the Coleoptera order (31%), Lepidoptera, which includes caterpillars (18%); Hymenoptera, comprised of bees, ants, wasps 14%); Orthoptera, which contains crickets and grasshoppers (13%); Hemiptera, containing leafhoppers, planthoppers, and cicadas (10%) (Food and Argriculture Organization of the United Nations, 2013). Currently, most of the consumed insects are gathered in the wild and processed in small-scale facilities. Further development of new technologies and know-how are required to scale-up production in a way that ensures sustainability, standardization, and quality. Each insect species has different sensorial characteristics and requirements for its development; therefore, it is important to take these variables into consideration when deciding to farm and commercialize an insect species (Govorushko, 2019). In Mexico, the Orthoptera order is well known because chapulin species (*Sphenarium purpurascenes*) is an important part of the gastronomic culture in the southern region; however, an efficient farming system has not been developed on a large scale for this species (Ramos-Elorduy, 2009). On the other hand, *Acheta domesticus* belongs to the same order as *S. purpurascens*, but it has been demonstrated to be a scalable species being able to be economically farmed (Food and Argriculture Organization of the United Nations, 2013).

# 2. Literature review

## *2.2 Acheta domesticus*

*A. domesticus,* also known as house cricket, is native to Asia. However, due to their short life cycle, ease of handling, and availability, they have been widely used for rearing since 1950, mainly as pet food. They have an optimum development in temperatures ranging from 28 to 30ºC. (Clifford et al., 1977). For this reason, they are commonly found in tropical climates.

### *2.2.1 Physiology and metabolism*

Crickets are hemimetabolous, meaning they have an incomplete metamorphosis with three stages: egg, nymph, and adult. Their entire life cycle ranges from 40 to 60 days, depending on rearing conditions. Insects are poikilothermic*,* meaning that they don't generate heat through metabolic or physiological processes. For this reason, temperature highly affects their development and warmer temperatures accelerate their life cycle (Clifford et al., 1977). Crickets are omnivorous and can feed on a range of sources. They have mandibulate mouthparts, which chew and bite on food. Digestion begins in the pre-oral cavity, which contains teeth that grind food. The pre-oral cavity also contains saliva with amylase that breaks down starch; invertase which converts sucrose into glucose and fructose; chitinase, which breaks down chitin. Food is later passed to the pharynx and esophagus to reach the midgut. In the midgut, final carbohydrate and protein digestion occur by secreted digestive enzymes; columnar cells absorb most nutrients. The midgut lacks a cuticular lining, but it is covered by a peritrophic matrix (PM) composed of chitin, carbohydrates, and proteins; the PM has a selective permeability allowing nutrients and enzymes to pass while inhibiting the pathogenic bacteria and parasites from entering. The gastric caeca are located in the midgut and it aids in nutrients absorption by increasing surface area and concentrating digestive enzymes. Food bolus is then passed to the

hindgut, where osmoregulation takes place, and recovery of nutrients, such as amino acids, that were not absorbed in the midgut is now absorbed. Hindgut has an alkaline pH, and it is also in charge of fermenting and hosting several microorganisms (Figure1). (Marc J. Klowden, 2013).

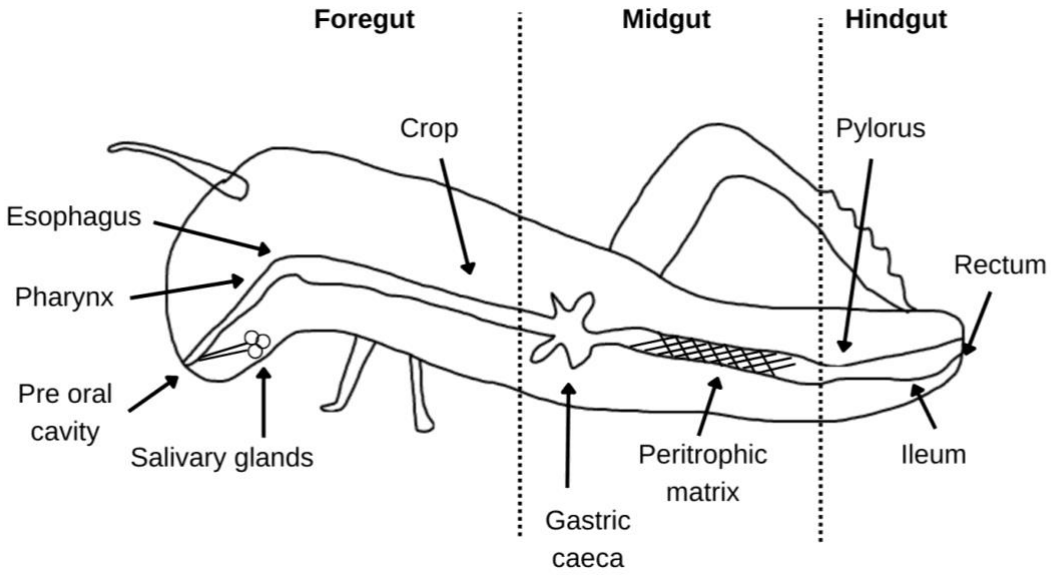


**Figure 1.** Digestive system of insects.

The fat body is a key organ for metabolism; it surrounds the digestive system and is also distributed throughout the body. Trophocytes are the primary cells of the fat body; they store proteins, lipids, glycogen, and trehalose. The fat body is responsible of administering nutrients obtained from digestion, and in response to hormonal signaling, it controls the growth of the insect. In opposition to mammals, insects use trehalose instead of glucose as the main source of energy. Trehalose is a disaccharide formed of two glucose units, however, it has a slower rate of diffusion, as compared to glucose, therefore it can be maintained at higher concentrations in the hemolymph and has a reduced osmotic impact as compared to glucose. On average, the trehalose concentration present in the hemolymph of insects is 25-10mM. The fat body converts glucose from food to trehalose, and depending on the requirements, it stores it or distributes it to the organs. As soon as glucose enters the fat body, it is phosphorylated and through the condensation

of UDP-glucose, the disaccharide containing two glucose units is formed (Figure 2). (Marc J. Klowden, 2013)

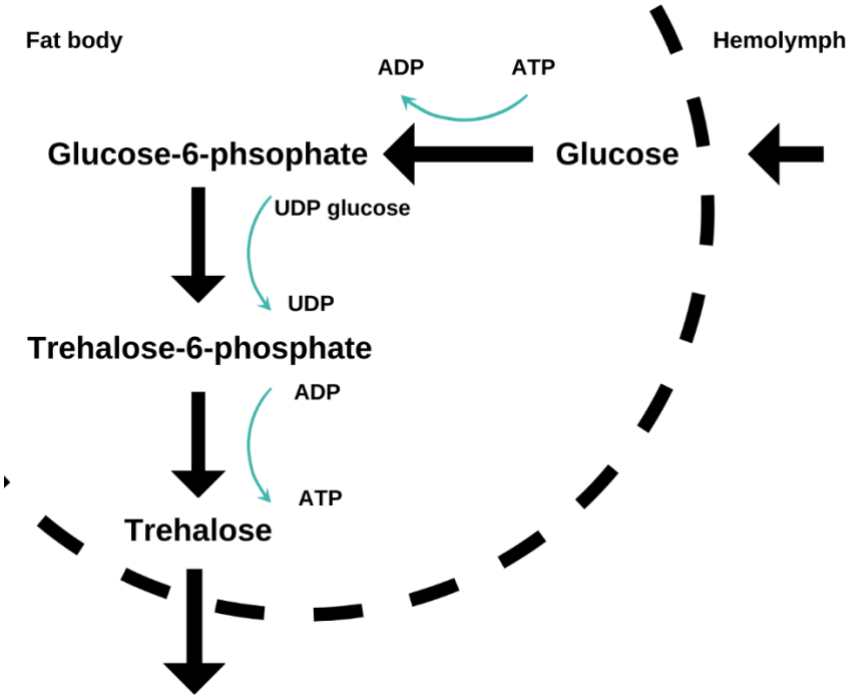


**Figure 2.** Conversion of glucose intro trehalose in the fat body

Furthermore, the fat body can produce anti-microbial peptides to protect the insect, harbor microbial symbionts and detoxify urates. To maintain all the organs in place, insects have an external covering called an exoskeleton, mainly composed of proteins (≥ 50%), chitin (10-45%), lipids, minerals, and phenolic compounds. The exoskeleton is composed of three layers (Figure 3): epicuticle, exocuticle and endocuticle which vary in composition. For instance, epicuticle is rich in lipids in order to create an hydrophobic structure to protect the insect to the exterior, whereas endocuticle and exocuticle do not contain lipids. (Marc J. Klowden, 2013).

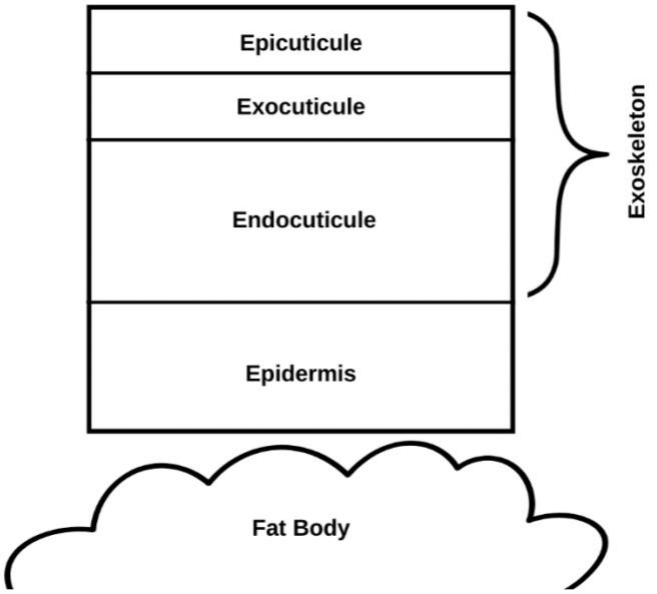


**Figure 3.** Exoskeleton structure of insects.

### *2.2.2 Environmental impact*

Their metabolic differences with traditional livestock sources give them the unique advantage that they require less energy and, consequently, fewer resources for their production (Premalatha et al., 2011). Current insect rearing requires less water, land, feed than traditional livestock. Furthermore, greenhouse gas and ammonia emissions are lower in insects than in traditional animal raising. Feed conversion ratio (FCR) relates the kilograms of feed with the kilograms of live weight gained; crickets have an FCR of 1.7, while beef has an FCR of 10. Crickets can be farmed vertically, increasing the efficiency in land use, which results in 10 times less land than cattle (Baiano, 2020). As for water usage, experimental data in a cricket farm in Thailand showed that the amount of water required to produce one kilogram of feed was on average 2 liters, whereas cattle require up to 15,000 liters (Halloran et al., 2017).

### *2.2.3 Consumer perception*

Although our ancestors widely consumed insects, culture and religion have shifted society's perspective toward them (Murefu et al., 2019). Currently, society's perspective about insects is related to fear, disgust, danger, disease, and pests. It is challenging to get consumers to taste insects due to the prejudices; however, changing peoples' perspectives can be achieved by offering a good visual presentation of insect-based products and good taste. Several studies have shown that promoting environmental and health benefits of insect consumption and reducing the perceived risk are key to increasing consumers' acceptance (Orsi et al., 2019).

### *2.2.4 Production*

Production can be divided into three parts: farming, harvesting, and post-harvesting. Farming consists of growing the crickets from the egg stage to adults in controlled conditions to increase the growth rate, minimize possible contamination, and decrease the mortality rate within the population. Afterwards, adults are harvested, and a separation from the rearing substrate and waste must be done to ensure quality. Finally, post-harvest includes the devitalization of the adults and, afterward, thermal treatment for ensuring food safety (Turck et al., 2021). A lot of variables can affect the final characteristics of the cricket flour. It has been observed that diet has a high impact on the final composition; therefore, it is important to standardize the feed source (Sorjonen et al., 2019). The devitalization method also impacts the final color, digestibility, composition, and quality of the final product. It has been observed that specific post-harvesting thermal treatments might increase protein digestibility by causing protein denaturation resulting in an unfolding of the structure, which is more susceptible to enzymes. Furthermore, a thermal treatment reduces microbial count (Singh et al., 2020).

### *2.2.5 Nutritional Value of Insects*

*Protein*

Insects are well known for their high protein content. Protein content on insect flours depends on intrinsic variables such as species and extrinsic variables such as rearing and processing conditions. For this reason, in literature, protein varies from 40 to 70%. Protein comes mainly from the muscles, fat body, hemolymph, and exoskeleton. While separating proteins in crickets according to their molecular weight, they can be categorized into three main fractions: hemocyanin (75 kDa), actin and arginine kinase (42,41 kDa), cuticle proteins (14.32 kDa). Hemocyanin is the protein in charge of delivering oxygen to cells. Actine and arginine kinase are transferase enzymes key for insect metabolism. Cuticle proteins form the exoskeleton and usually interact with chitin (Brogan et al., 2021). It is important to evaluate the possible functionality of cricket proteins for the food industry, which can be done by determining their structure according to the solubility. For cricket flour, it has been reported that 31.5% of the proteins are albumins, 30.6% globulins, 13.7% glutelins, and 24.2% prolamins (Stone et al., 2019). As for nutrition, crickets have been found to contain all essential amino acids, being especially rich in leucine (4.83 g/100 g) and lysine (3.90 g/100 g). It has been noted that chitin might increase the nitrogen content in samples, resulting in an overestimation of protein, content and conversion factors of 5.57 have been proposed. Chitin as well is thought to impact protein digestibility. However, the European Food Safety Authority (EFSA) stated that the 6.25 standard factor should be used (Turck et al., 2021). Despite these possible limitations, in vitro protein digestibility in rats has shown promising results. Evaluating the amino acid composition and nitrogen retained, protein digestibility corrected amino acid score (PDCAAS) average for cricket flour was 81% (Poelaert et al., 2018).

*Lipids*

Crickets are rich in lipids with an average content of 15.4%, from which 40.3% are polyunsaturated fatty acids (PUFA), 18% are monounsaturated fatty acids (MUFA), and the rest are saturated fatty acids (SFA). The principal unsaturated fatty acids in crickets are linoleic acid (omega-6) and oleic acid (omega-9). In contrast to mammals, linolenic acid can be synthesized de novo from oleic acid, obtained from the diet, by the action of the enzyme desaturase (Marc J. Klowden, 2013) (Blomquist et al., 1991). PUFAs are the healthiest lipids, which nutritionally gives cricket flour an advantage; however, in the technological sense, PUFAs are more susceptible to oxidation (Singh et al., 2020).

*Carbohydrates*

The principal carbohydrate in cricket flour is chitin. A polysaccharide is made up of N-acetylglucosamine units linked by β-(1-4)-N-acetyl-d-glucosamine bonds. It has a similar structure to cellulose, but with the difference, it has an amine and a hydroxyl group. It is the second biopolymer with the most abundant in nature. It is commonly found in crustaceans, fungi, yeast, and insects. Chitin is insoluble in most organic solvents; therefore, commercially, it is usually used in the form of chitosan, which refers to deacetylated chitin. To obtain chitosan from chitin, a strong alkali is used to chemically modify the structure (Ibitoye et al., 2018). Chitin, on the other side, due to its lack of solubility, is not widely used; however, it also has anti-microbial and hydrating properties (Elieh-Ali-Komi et al., 2016). Both chitin and chitosan are considered a fiber since they were believed to be undigestible by humans, however recent studies have found that humans contain two types of chitinases in their digestive system although it is unknown until what degree humans can digest it (Tabata et al., 2018). In nature, it is rare to find pure chitin or chitosan. It is usually found in different degrees of deacetylation or acetylation. Chitin is defined as the structural form that has a degree of acetylation of 0.90 or higher, whereas chitosan has a degree of deacetylation higher than 0.65. Crickets contain an estimated chitin and chitosan content of 4.3-7.1% and 2.4-5.8%, respectively

(Ibitoye et al., 2018). Cricket consumption has been shown to impact human gut microbiota, specifically increasing the probiotic strain *Bifidobacterium animalis*; this increase is related to chitin and chitosan, which can be used as a carbon source by beneficial bacteria (Stull et al., 2018). The other carbohydrate in crickets is mainly glycogen and trehalose, which are the principal energy sources of insects. However, they are usually found in small quantities (Kim et al., 2019)

*Other Nutrients*

Apart from the macronutrients, insects have been found to be a source of minerals and vitamins. In terms of the recommended daily amount, 100 grams of crickets contain 198% of zinc, 33.77% of magnesium, 35.90% of manganese, 17.69% of calcium, and 10% of iron (Köhler et al., 2019). Vitamin B12 can only be found in animal sources, and cricket flour has been demonstrated to be an important source, where 100 grams contain almost double the recommended daily amount (Turck et al., 2021)

### *2.2.6 Safety*

Insect consumption is not yet a massive practice; therefore, there is a lack of regulations and industry standards for production and commercialization. However, there is a rising interest in this food source, leading to the European Food Safety Authority (EFSA) to publish a scientific opinion about Acheta domesticus reared under controlled conditions in a closed farming system with good manufacturing practices and Hazard Analysis Critical Control Points principles. In terms of diseases, insects are susceptible to infection by virus, being only pathogenic to insects and not to humans. To de-contaminate, a thermal treatment is often used to ensure their safety. The EFSA reported no genotoxicity nor sub chronic toxicity in the cricket flour analyzed. Mycotoxins and heavy metals were found in cricket flour analyzed in small quantities below the permitted limit (Turck et al., 2021). As with any product destined for human consumption, there are risks involved,

such as those mentioned above, but a quality control system and adequate processing should ensure their safety.

### *2.2.7 Allergenicity*

Several cases of anaphylactic reactions have been reported after the consumption of insects. Using a proteomic-based approach, 46 allergenic proteins, such as arginine kinase, glutathione S-transferase, and tropomyosin, were found in *A. domesticus*. Most of these proteins are also found in crustaceans and mollusks (Barre et al., 2021). For this reason, it is suspected that insects might promote the same immune response in persons who are allergic to crustaceans. It is also possible to have additional allergens, which could be passed on to crickets by feed or rearing substrate; therefore, it is important to consider the production process (Turck et al., 2021).

## *2.3 Corn Tortillas*

Corn tortillas are an ancient food widely consumed by the Aztec and Mayan cultures in the regions. They are usually made with nixtamalized or lime-cooked corn. Nixtamalization changes the characteristics of corn, enhancing the nutritional profile and modifying the sensory characteristics. It is suspected that nixtamalization started 4,500 years ago (Serna-Saldivar & Chuck-Hernandez, 2018).

### *2.3.1 Nixtamal production*

Lime cooking is also known as nixtamalization, and it is a process that significantly softens the corn kernel. Corn kernels are soaked in a solution of water and calcium oxide at a concentration of 1% based on the kernel's weight. Temperature is then raised to 100ºC

for 15-30 minutes, and then kernels continue to soak for 8-16 hours. During this process, calcium hydroxide breaks down the hemicellulose, softening the kernel and removing the pericarp. During this process, calcium adds a significant quantity of bioavailable calcium important for human nutrition and increases niacin bioavailability and decreases phytic acid concentration. Nevertheless, phenolic compounds, vitamins, and proteins might be leaked (Serna-Saldivar & Chuck-Hernandez, 2018). Although it is limited, the alkaline pH and temperature cause a partial gelatinization. It was observed that during nixtamalization, the amount of resistant starch increased in comparison to the raw kernel. This increase can be driven since lime cooking promotes interaction between groups and the formation of new structures (Campus-Baypoli et al., 1999). Afterward, kernels are washed to remove the alkaline solution and pericarp. To obtain masa, kernels are ground in stone mills. During this process, particle size is reduced, and the mechanical movement kneads the masa making it more cohesive and flexible. During this process, previously damaged starch is released from the granules. This masa can be dried to produce nixtamalized corn flour or used directly to make tortillas (Serna-Saldivar & Chuck-Hernandez, 2018).

### *2.3.2 Tortilla consumption*

Corn tortillas are a staple food in Mexico and Central America. In Mexico, the average consumption per capita is 5 to 7 tortillas; in 2014, up to 7.4 million tons of corn tortillas were consumed. Originally, tortillas were home-cooked, but now up to 90% of tortillas consumed in Mexico are industrialized (CEDRSSA, 2014). Due to migrations and globalization, tortillas can now be found around the globe, in the USA, Russia, Korea, China, Australia, and European countries (Serna-Saldivar & Chuck-Hernandez, 2018).

### *2.3.3 Production*

Corn tortillas are made up of 3 basic ingredients: corn, lime, and water. Some additives might be used to increase shelf life, improve nutritional flavor, and optimize sensorial characteristics. In Mexico, it is reported that 50% of tortillas are produced with nixtamalized corn flour and 50% with fresh nixtamalized corn (CEDRSSA, 2014). Depending on the raw material chosen, the final characteristics of tortillas might vary. White dent corn is the variety most used; however, it is also possible to mix it with yellow corn. It is important to select the grains to ensure quality, verifying they are free from insects; they are not broken and have no mold spoilage. To produce tortillas from nixtamalized corn flour, it is important to hydrate the flour and knead to obtain a desirable and machinable masa. To form tortilla disks, masa passes through a rolling machine that creates a sheet of masa and transforms it into a disk with a cutter. Once the disk is formed, it is now cooked in a conveyor belt, heated by direct exposure to fire. Conveyor belts can be in two or three stages, making sure both sides cook the tortilla. On average, tortillas are cooked at temperatures that range from 280-302ºC for 30 seconds to 1 minute. During this process, most of the starch is gelatinized, proteins are denatured, Maillard reaction takes place, water is lost, and the microbial count is reduced (Serna-Saldivar & Chuck-Hernandez, 2018). After being cooked, tortillas must be cooled to avoid condensation in the packaging and prevent spoilage. In this step, starch retrogradation takes place, and amylose and amylopectin branches rearrange (Campus-Baypoli et al., 1999).

### *2.3.4 Nutritional value*

Tortillas are rich in carbohydrates, followed by fiber and protein. Lipid content in tortillas is minimum.

*Protein*

On average, lime-cooked corn tortillas have a protein content of 10.1%, which has shown a digestibility of 85%. (Bressani et al., 1990)(Serna-Saldivar & Chuck-Hernandez, 2018). Tortillas lack the essential amino acid, lysine, and have low tryptophan levels (Acevedo-Pacheco & Serna-Saldívar, 2016). Corn proteins are mainly prolamins (50%), glutelins (40%), and the rest are albumins and globulins. Prolamin fraction is formed by three types of zeins that are responsible for having a significant impact on the nutritional profile and functional properties due to their abundance (Serna-Saldívar, 2019c). Overall, tortilla protein quality is considered low and has demonstrated and estimated PDCAAS value of 36% (Acevedo-Pacheco & Serna-Saldívar, 2016) .

*Lipids*

Most of the lipids in tortillas come from the germ that was not removed in the process. The oil in the germ is mainly polyunsaturated fatty acids principally, linoleic acids (omega-6), and oleic acid (omega-9). From the total lipids found in corn, on average, 2.7% are phytosterols which are compounds found in plants that have beneficial effects on cardiovascular health (Serna-Saldívar, 2019b).

*Carbohydrates*

The most important macronutrient in corn tortillas are carbohydrates, principally composed of starch; this makes tortillas a great source of energy. Tortillas have been shown to have significantly higher levels of resistant starch in comparison to raw kernels (Campus-Baypoli et al., 1999). Dent white corn, the one used for tortillas, is typically comprised of 27% of amylose and 73% of amylopectin; for this reason, this same proportion is assumed to be found in tortilla samples (Rausch et al., 2019). The non-starchy polysaccharides found in tortillas are mainly cellulose, hemicellulose, lignin, and arabinoxylans which form part of the dietary fiber fractions. Sugar content in corn and,

therefore, in tortillas is low, but fructose, glucose, sucrose, and maltose can be found in minor quantities (Serna-Saldívar, 2019a).

*Other nutrients*

During lime cooking, calcium content is increased; it is estimated that up to 50% of the calcium consumed by Mexican comes from tortillas. Still, they are not a significant source of other minerals such as zinc or iron. Tortillas can supply some B-complex vitamins, such as niacin, but levels of other vitamins are low since most are lost during processing (Serna-Saldivar & Chuck-Hernandez, 2018).

### *2.3.5 Quality parameters*

Texture is a key parameter for determining the quality of tortillas. Fresh tortillas are meant to be flexible and able to roll and fold without any visible cracking. During storage, a series of phenomena such as retrogradation and moisture loss happen; for this reason, aged tortillas tend to become rigid and brittle. A parameter commonly evaluated to assess quality is rollability (Suhendro, E. L., Almeida-Dominguez, H. D., L. W. Rooney, Waniska, 1998). Colorimetry is also widely used to assess the quality of tortillas; darker colors might indicate browning caused by higher temperatures, whereas lighter colors might indicate undercooking. Blisters in tortillas are considered a defect; therefore, a visual examination to analyze the frequency and number of blisters is commonly used to evaluate tortilla quality (Serna-Saldivar & Chuck-Hernandez, 2018).

## *2.4 Carbohydrate and protein digestion*

### *2.4.1 Digestive tract*

The human digestive system consists of the mouth, esophagus, stomach, small intestine, and large intestine. During the first stage, food is ingested through the mouth, where food

particle size is reduced by mastication. In this stage, salivary glands release saliva with two main purposes: lubricate the food bolus to make it easier to move through the digestive tract; digest starch through α-Amylase. It has been studied that up to 75% of the starch found in bread or potatoes can be digested in the mouth. In this step, the pH is slightly alkaline with maximum values of 8.0. The esophagus is lined with smooth muscle, and skeletal muscle, which is under the control of the vague nerve, and this organ has the functions of swallowing the food bolus and passing it from the mouth to the stomach. Once the food bolus arrives at the stomach, any residue of α-Amylase is inactivated by the acidic pH found in the stomach, which can have values near 2.0.(Margaret E. Smith & Dion G. Morton., 2010)

The stomach has the primary function of storing ingested food and regulating its release into the small intestine by mixing the food with digestive juices (hydrochloric acid, intrinsic factor, pepsin, and mucus). Pepsin degrades proteins into peptides; it is estimated that 20% of total protein digestion occurs in the stomach. Chyme is passed through muscle contractions to the small intestine, where it mixes with pancreatic juice, bile, and juices from the intestine. The pancreas plays a key role in small intestine digestion since it secretes pancreatic juice, a major digestive secretion. Pancreatic juice is mainly formed by an alkaline solution that creates a neutral or slightly alkaline environment to ensure digestive enzymes work properly. The enzymes found in pancreatic juice are the following: trypsin, chymotrypsin, carboxypeptidase, elastase, phospholipase A, lipase, α-Amylase, glycosidase, ribonuclease, and deoxyribonuclease. This mixture of proteins digests proteins, lipids, and carbohydrates break into simpler units. In addition, it is also in charge of liberating insulin and glucagon, important hormones that impact metabolism and nutrient absorption. In the pancreas, alpha cells produce glucagon, and beta cells produce insulin. Somatostatin, a hormone, regulates the activity of alpha and beta cells, hence regulating insulin and glucagon production. Ingesting a meal activates a complex signaling pathway throughout the body that involves the digestive system and other organs, such as the one present in the nervous system. The vague nerve affects digestion and depending on the different stimuli; it can be divided into three phases: cephalic, gastric, and intestinal. The cephalic phase can begin when we see or smell food even

before it has entered the mouth. The vague nerve prepares to eat food and launches a signaling pathway to prepare the body for ingesting a meal. The gastric phase begins once food reaches the stomach, and finally, the intestinal phase happens once food reaches the duodenum (Margaret E. Smith & Dion G. Morton., 2010)

*Glucose metabolism*

Digestible carbohydrates such as starch, glycogen, and sugars are the main energy source. On the other hand, non-digestible carbohydrates such as cellulose are a source of energy for the microbiota in the colon. Two enzymes play a key role in carbohydrate digestion: α-Amylase, which breaks down α-1,4 bonds, and intestinal glycosidase, which breaks down α-1,6 bonds. These two enzymes can degrade amylose and amylopectin found in starchy foods and glycogen found in animal sources to produce a mixture of glucose and maltose. However, other enzymes also degrade small sugars, such as maltase, sucrase, and lactase. Monosaccharides (glucose, fructose, galactose) found in diet or produced as byproducts of carbohydrate digestion are absorbed into the bloodstream by two mechanisms, passive diffusion, and membrane-associated transporters. Afterward, they are transported to the liver, where fructose and galactose are transformed into glucose, the main energetic molecule, and released again to the bloodstream. As glucose blood levels increase, insulin is released, signaling the cells to increase the glucose absorption into the cell for energy production. Consequently, blood glucose levels drop, and the secretion of insulin stops. Excess glucose is stored in the liver and muscle tissue as glycogen and adipose tissue as fat. (Margaret E. Smith & Dion G. Morton., 2010)

*Protein metabolism*

Proteins are large molecules composed of amino acids and are responsible for supplying the body with the 8 essential amino acids. Proteolytic enzymes can be divided into

endopeptidases and exopeptidases, depending on the cleavage site. After protein digestion, the results are mostly short peptides formed of 4 to 2 units and single amino acids. These products enter the epithelial cells in the liver by various transport mechanisms depending on their polarity. Once in the cytosol, they become degraded to amino acids by enzymatic action. Proteins in the liver are oxidized to ketoacids to provide energy for the liver. Excess protein in the liver will be converted into triacylglycerol. Any amino acid that was not absorbed by the liver will be stored in the muscle for protein synthesis. Amino acids, present in the blood, increase the rate at which insulin is secreted. Arginine, leucine, and alanine increase have been shown to impact the most due to their charge since they can depolarize beta cells membrane (Margaret E. Smith & Dion G. Morton., 2010)

### *2.4.2 Starch Fractions*

Starch is the most important molecule for energy storage in plants, and it is stored in the form of granules. Since their main function is to store energy, they are extremely insoluble in water at room temperature. For humans, it is an important energy source, comprising on average 30% of the diet. Starch is composed of two molecules, amylose and amylopectin. Regular maize starch is composed of 25% of amylose and 75% of amylopectin. Amylose is a linear molecule composed of single glucose molecules linked by α-1,4 bonds. Amylopectin is a branched molecule composed as well by α-1,4 bonds and contains α-1,6 bonds in the branches. Amylopectin has a more complex structure and a higher molecular weight when compared to amylose. Depending on the length, amylopectin branches can be cataloged as A, B, or C chains. Short amylopectin chains are believed to be organized in a double helix structure which gives the starch a crystalline area. Long amylopectin and amylose chains, on the other hand, form the amorphous region, which tends to have a lower density. These two regions give native starch granules birefringence under polarized light, which can be observed in the form of a Maltese cross. The double helixes formed by short amylopectin chains can have two polymorphisms, A and B. Maize starch presents A-type polymorphisms, which strongly affect the amount of water it can bind; usually, A binds less water than B-type. These

ordered structures in native starch can become disrupted by melting or gelatinization; however, gelatinization is more common due to the required conditions (T. L. Wang et al., 1998).Gelatinization of starch granules occurs in high moisture environments, usually above 60% and heat. Depending on the starch source, gelatinization temperatures can range from 65-85ºC. Native starch granules absorb water and swell, which is reversible before reaching starch gelatinization; however, once the starch granule breaks as a consequence of the heat, the amylose and amylopectin interact in the continuous phase and later set into a more stable form when temperature decreases (Donmez et al., 2021). Amylase class enzymes can rapidly digest native starch; however, starch is rarely consumed in this form; most starchy products are processed and cooked before consumption, which disrupts the starch granules and a subsequent rearrangement of the starch components. Therefore, processed starch tends to be more resistant to digestive enzymes (Wang et al, 1998). Depending on how fast starch is broken down into glucose by digestive enzymes, it can be cataloged in three types: rapidly digested starch (RDS), slowly digested starch (SDS), and (RS). RDS is starch that can be digested within 20 minutes, SDS is the starch fraction digested between 20 and 120 minutes, and RS is the fraction of starch that was not digested within 120 minutes. RS can be cataloged into five types depending on its nature. RS-1 is the starch that is physically inaccessible by protecting the cell wall or by other food matrix components that do not allow access to digestive enzymes. RS-2 is found in native starch found in green bananas or potatoes, and its caused by crystalline structures which are not susceptible to enzyme degradation. RS-3 refers to the structures formed by the retrogradation of gelatinized starch. RS-4 type is formed when starches are chemically modified to be undigestible; some examples are esterification and cross-linking. RS-5 refers to the amylose-lipid complex that forms when amylose helixes wrap around lipid ligands (Li et al., 2021).

### *2.4.3 Glycemic Index*

Glycemic index (GI) measures the response of blood glucose levels to food. It is defined as the incremental area under the curve for the blood glucose response of 50 grams of

digestible carbohydrates in a determined food and expressed as the percent of the response for 50 grams of digestible carbohydrates of standard food. It was first developed in the early 1980s y Jenkins et al. (1981) to evaluate postprandial glucose levels. According to the GI value of foods, they can be cataloged as low (<55), medium (55≤GI<70), and high (GI≥70) glycemic index foods (Jenkins et al., 1981). Several studies have demonstrated that ingesting lower GI foods tends to have beneficial effects on the body. A meta-analysis demonstrated that a diet that consisted of lower GI foods lowered Glycated hemoglobin (HbA1c) and fasting blood glucose levels which can aid in the management of glucose and insulin-related diseases (Ni et al., 2022).

### *2.4.4 Protein structure and digestibility*

Amino acids are the units that form proteins and peptides in nature over 300 amino acids have been reported; however, 20 amino acids are the ones most found in foods. Their structure can be cataloged in 5 groups depending on the side chain type: aromatic, non-polar aliphatic, polar uncharged, positively charged, and negatively charged. Each amino acid has different charges and polarity, which will impact the protein's final structure. Proteins are classified into 4 levels, where the primary structure refers to the sequence of amino acids that comprise a protein. The primary structure also indicates the position of disulfide bridges and posttranslational modifications such as methylation, glycosylation, and phosphorylation. The secondary structure defines how the primary structure bends mainly due to the hydrogen bonds between C=O and N-H groups. Depending on the structure formed, it can be cataloged as α-helix or β-structures such as β-sheet, β-hairpin, and β-turns. Tertiary structure refers to how a protein folds in a three-dimensional space, which is mostly defined by the polarity of the amino acids; non-polar amino acids tend to arrange in the center of the protein while polar proteins tend to be on the surface. Tertiary structure defines the final size and shape of the protein. Quaternary structure is generally present in protein complexes, and it describes how two or more protein monomers arrange. Depending on the final structure, proteins can be classified as globular, membrane or fibrous. From these three categories only, globular proteins are soluble. Depending on their solubility in different solvents they can be classified as

albumins (soluble in water), globulins (soluble in salt), prolamins (soluble in alcohol), and glutelin (soluble in acids or bases). Secondary, tertiary, and quaternary structures can be disrupted by a variety of techniques, including changes in temperature and pH. This phenomenon is also called denaturation (Yada, 2018). Each one of these characteristics will define how susceptible a protein is to digestive enzymes.

It has been observed that denatured proteins tend to be more digestible compared to proteins in their native state since the primary structure is more exposed and accessible to proteolytic enzymes. Enzymes can break down primary structures and release small peptides or amino acids (Joye, 2019).

### *2.4.5 Molecular interactions and impact on the digestibility*

Food is a complex matrix that rarely contains only one component; usually, it is composed of various micro and macronutrients, which will impact the final characteristics in terms of digestibility. Various factors such as the material compositions and interaction between other components such as fiber, lipid, proteins can affect starch hydrolysis (De La Rosa-MilÍan et al., 2017).

*Fiber*

It has been reported that non-starchy polysaccharides, which tend to be non-digestible, increase the food bolus viscosity and can act as a physical barrier reducing the diffusion of digestive enzymes towards the substrate. It has also been reported that some NSP's are able to bind to α-Amylase reducing its activity (Yi & Li, 2022).

*Lipids*

The addition of lipids to a starchy matrix has been reported to form amylose-lipid complexes (ALC). The molecular interaction involves an amylose molecule that generally

has a random coil structure morphing into a helix structure due to lipids binding into the center of the helix (Figure 4). This rearrangement of the amylose chain affects the glycosidic bonds torsion, increasing digestion resistance. Usually, long and straight hydrocarbon chains are more prone to the formation of stable ALC structures; for this reason, palmitic and stearic acids are commonly used to produce these structures. ALC type I is an amorphous structure that forms at 60ºC and dissociates at 95-105ºC. ALC type II is a semi-crystalline structure that forms at temperatures of 90ºC and dissociates above 100ºC (Li et al., 2021).

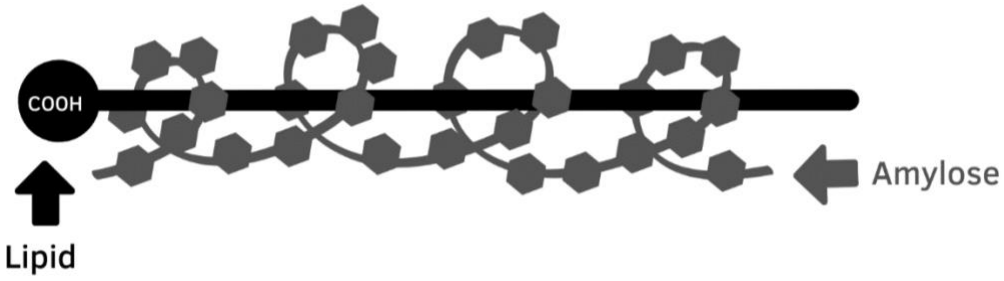


**Figure 4.** Representation of a single stranded amylose lipid complex

*Proteins*

Proteins can interact with starch structures and impact digestibility. Globulins are rich in cysteine, which has been reported to rearrange and form disulfide bridges after being denatured by heat. During this rearrangement, proteins can form a matrix that traps gelatinized starch in its interior, reducing the overall accessibility and digestibility of starch. It has also been reported that protein-starch interactions can form a complex through non-polar interactions and hydrogen bonds that result in a resistant structure (Yi & Li, 2022).

## 3. Justification

Through the partial substitution with *Acheta domesticus,* the nutritional profile of corn tortillas can be improved. By evaluating three levels of substitution of Acheta *domesticus* in corn tortillas, changes in the physicochemical and digestibility characteristics due to new molecular interactions can be evaluated.

## 4. Objectives

*General Objective*

- Evaluate cricket (*Acheta domesticus*) flour functionality in corn tortilla formulations, and production to evaluate physicochemical, nutritional, quality and digestibility characteristics.

*Specific Objectives*

- Develop functional formulations to produce corn tortillas partially substituted with cricket (*Acheta domesticus*) flour.
- Assess quality parameters in each of the formulations.
- Evaluate changes in starch fractions and the response to enzymatic digestion *in vitro* and *in vivo*.
- Evaluate how the addition of cricket (*Acheta domesticus*) flour to corn tortillas impacts protein digestibility in vitro.
- Determine the presence of undigestible starch structures by differential scanning calorimetry.

# 5. Methodology

## *5.2 Sample preparation*

### *5.2.1 Materials*

Nixtamalized corn flour (NCF) Maseca Extra Premium (Molinos Azteca S.A. de C.V.) was used to produce corn tortillas. Dried and milled *A. domesticus* (AD) was obtained from a local producer located at Querétaro, México (Griyum PASMX S.A. de C.V).

### *5.2.2 Formulation*

For flour analyses, 100% NCF and 100% AD were evaluated as well as composite flours containing 5,7.5 and 10% of AD. For tortilla samples, three substitution levels of AD were evaluated. Tortilla control sample (TC) contained 100% NCF. NCF was substituted with AD at different percentages; 5% (T5), 7.5% (T7.5), and 10% (T10) dry basis. It has been previously reported by other authors that substitution in bakery products with insect flour tends to be acceptable up to 10% (Kowalski et al., 2022). For the masa preparation,120 grams of water were added for every 100 grams of dry ingredients, this amount of water quantity was determined by machinability of the masa. Good machinability was considered when tortillas were able to form and detach from the roller without sticking and without any signs of breakage.

### *5.2.3 Tortilla production*

Ingredients were weighed and the masa was mixed in a stainless-steel bowl manually for 5 minutes until a homogeneous texture was formed. To ensure even water distribution, masas were allowed to rest for 10 minutes before prepared into tortillas. Masa was placed on the rolling machine (Figure 5), with three cooking tiers (Tortimaq-TR15). Tortillas cutter formed tortillas into 16 cm diameter circles with 1.2 mm thickness. Cooking temperature

of the heating plaques was set at 245 ± 5 ºC and tortillas were cooked for 1 minute, this temperature was constant throughout the three tiers. Afterwards, tortillas were cooled at room temperature 25ºC before being assessed for rollability and before being packed in polypropylene bags and refrigerated at 4ºC for further analysis.

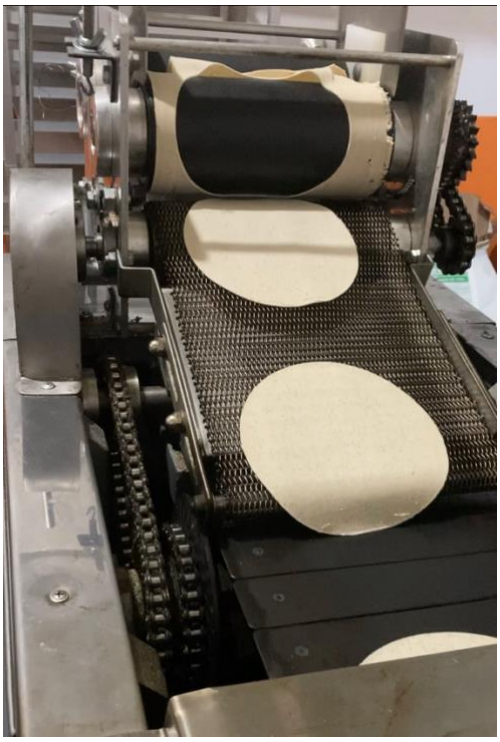

**Figure 5.** Production of tortillas in a rolling machine with three cooking tiers.

### *5.3 Sample characterization*

#### *5.3.1 Water absorption and solubility index of flours*

Water absorption index (WAI) and water solubility index (WSI) were evaluated according to the method reported by Anderson and Griffin (Anderson, R.A. Conway, H.F., Peplisnki, 1970) for composite flours. Briefly, 2.5 grams of flour was suspended on 30 ml of water at 30ºC in a 50 ml centrifuge tube. For 30 minutes the tube was stirred intermittently. Afterwards the tube was centrifuged at 3000 x *g* for 10 minutes. Supernatant was separated and water was evaporated. The final weight of the supernatant was used for the WSI. The remaining gel on the tube was weighted and used for the WAI. The analyses were performed in flour treatments containing 100% NCF, 100% AD, and substitution

percentages of 5, 7.5, and 10% AD, and were calculated with the following equations (1, 2):

$$\text{WAI} = \frac{sediment\ weight}{dry\ weight\ sample} \quad (1)$$

$$\text{WSI\%} = \frac{dry\ solids\ supernatant\ weight}{dry\ weight\ sample}\ x\ 100 \quad (2)$$

### *5.3.2 Mixolab masa characterization*

Composite flour rheological characteristics were analyzed using the Mixolab (Mixolab 2, Chopin Technologies). The analyses were performed in samples containing 100% NCF, 100% AD, and substitution percentages of 5, 7.5, and 10% AD. The Mixolab analysis was performed by duplicate using the protocol reported by Espinosa-Ramírez et al (Espinosa-Ramírez et al., 2020) .

### *5.3.3 Proximal composition of tortillas*

Crude protein, crude fat, and ash contents were estimated by the AOAC methods 925.10, 992.15, 922.06, and 923.03, respectively. Carbohydrate content was calculated by difference.

### *5.3.4 Total starch and fiber fractions of tortillas*

Total dietary fiber (TDF) content, insoluble (IDF) and soluble (SDF) fiber fractions, were quantified using the Megazyme kit K-TDFR based on the AOAC 991.43 Method. Total starch was determined with Total Starch (AA/AMG) Assay Kit according to the AOAC Method 996.11 (Megazyme, Wicklow, Ireland). Free sugar content was estimated from a 0.5 g sample, homogenized with 80% ethanol solution, the sample was stirred for 20

minutes at room temperature (25°C) and centrifuged at 1000 *g* in order to extract glucose, fructose, sucrose and trehalose. Afterwards,1 ml aliquots of the supernatant was withdrawn, and its total carbohydrate content was estimated with the phenol-sulfuric method (Dubois et al., 1956).

### *5.3.5 Rollability evaluation of tortillas*

Waniska subjective rollability procedure was performed on each of the samples (Suhendro, et al., 1998). Fresh tortillas were rolled around a 2 cm wood cylinder, and a score from 1-5 was given, where 1 is completely broken, and 5 is without any breakage. Tortillas were stored for 7 days at 4ºC, mimicking normal storage conditions. For the evaluation of stored tortillas, samples were left out for the refrigerator for 20 minutes allowing them to reach room temperature before performing the test.

### *5.3.6 Color analysis of tortillas*

Color was analyzed using Color Coll App and Nix Color Sensor to measure CIELAB units. Tortilla samples were placed on a white background with the same lightning to perform the analysis, measurement was obtained by quintuplicate. Results were expressed in CIE units as L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). Results were obtained by quintuplicate as indicated by the method. The difference in color (∆ E) was obtained by evaluating the difference between AD substituted samples with the control tortilla using the following formula (3):

$$\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - L_2)^2 + (b_1 - b_2)^2} \quad (3)$$

### *5.3.7 Image analysis of tortillas*

ImageJ software (Image J ver1.53, National Institute of Health USA) was used to measure the diameter and particle content on tortilla samples. Images were taken in a white background under the same lightning. A known distance using a scale was used in the pictures to obtain a transformation of pixels/cm. For diameter, a line was drawn from opposite extremes of the cooked tortilla to obtain diameter measurements. Particle count was evaluated in an image of 400 x 400 pixels and were converted to 8-bit images. Results were obtained by quintuplicate as indicated by the method.

### *5.3.8 Thermal characteristics of flours and tortillas*

Thermal characteristics of tortillas were determined with a differential scanning calorimeter (Diamond DSC, Perkin Elmer, Nortfolk, VA, USA), using the conditions and procedure described by De La Rosa-Millán et al (2017) . Onset temperature (*To*), peak temperature (*Tp*), conclusion temperature (*Tf*), and gelatinization enthalpy (ΔH) were calculated with the Pyris software (Perkin Elmer, Norwalk, CT, USA). Additionally, to assess the influence of lipids and their interaction with starch molecules due to the inclusion of AD flour, the temperature used was up to 120 °C, in order to evaluate corresponding peaks to amylose lipid complexes.

### *5.3.9 In vitro starch digestion and predicted glycemic index of tortillas*

The *in vitro* starch digestion fractions were determined according to the protocol of Englyst (Englyst et al., 1992) with modifications. That simulates the different stages of starch digestion. For this, tortilla samples (100 mg) were hydrated with 5 ml of deionized water at 37°C for 20 min, with vortex every 5 minutes. Then 2 ml of pepsin dispersion (5.21 mg/ml) was added under a pH of 7.0, and incubated in a shaking water bath (37°C, 200 strokes/minute) for 30 minutes. The pepsin reaction was stopped by adding a 1 ml of

1N HCl-KCl solution with continuous stirring for 15 minutes. Then, 2 ml of 0.5M sodium acetate buffer (pH 5.2) was added and homogenized, and 1 ml of an enzyme solution (pancreatin, amyloglucosidase, and invertase) and five glass beads (7 mm in diameter) were added to each tube, which was incubated at the above conditions. After 20 and 120 minutes of reaction, aliquots of 0.1 ml were taken and immediately mixed with 1 ml of 80% ethanol. The glucose content was quantified with the glucose oxidase/peroxidase reagent. Starch classifications based on the rate of hydrolysis were rapidly digestible starch (RDS) (digested within 20 minutes), slowly digestible starch (SDS) (digested between 20 and 120 minutes), and resistant starch (RS) (undigested after 120 minutes). Starch fractions were calculated as a percentage of the total starch content.

The method described by Granfeldt, Bjorck, Drews & Tovar (Granfeldt et al., 1992) was employed to evaluate the in vitro rate of starch hydrolysis in cooked tortillas. Samples were digested with pepsin (P7000) followed by a starch digestion performed by Porcine pancreatic α-amylase (Type VI-B) and fungal amyloglucosidase (A7095) at 30, 60, 90, 120 and 180 minutes, from which starch hydrolysis was estimated. The hydrolysis index (HI) of each material was calculated from the ratio between the area under the hydrolysis curve compared with a reference sample (white bread). From the HI value obtained, pGI was then calculated with the following equation (4), reported by Goñi (Goñi et al., 1977)

$$\mathrm{pGI} = 39.71 + 0.540\ (\mathrm{HI}) \qquad (4)$$

*5.3.10 In vivo glycemic response of tortillas*

The Food and Agriculture Organization method for measuring glycemic index was used in order to determine glycemic response of tortilla samples as an exploratory study (FAO & WHO, 1997). Five subjects were recruited as volunteers for the *in vivo* glycemic response study in the city of Querétaro (Mexico). Each subject gave written informed consent for the study. The inclusion criteria were that subjects should be healthy males or females, aged between 26 and 55 years old. The exclusion criteria were the use of

medications affecting glucose tolerance (excluding oral contraceptives), the presence of diseases or drugs influencing digestion or absorption of nutrients, major medical or surgical events requiring hospitalization within the past 3 months, known diabetes mellitus and related conditions, and food allergy towards any component present in the tortillas. Due to the abnormal results (>2SD) obtained in the first replica of one of the participants, data from that replica was excluded. It was later confirmed that during that replica the subject was on a special diet, after the diet was over, the next replica was performed. Each subject had a total of 8 visits two for testing the reference food (TC) and six days for testing the samples with AD. Each sample was tested two times on different days. All samples used for the test were fresh, being prepared 1 hour before consumption. Subjects had an 8-10 hour fast before the test and test were performed at the same hour of the day. 50 grams of available carbohydrates from each sample were given. Test products were consumed with a standard cup of black coffee without any sugar, milk or creamer. Contour Plus glucometer, test strips and lancets were used. Capillary blood samples were taken before the meal and 15,30,45,60,90,120 minutes after sample consumption. Incremental area under the curve was calculated for each sample tested using the trapezoidal method, any area under the baseline (fasting point) was ignored. The glycemic index was calculated as a percentage of the reference food (TC).

### *5.3.11 In vitro protein digestion of tortillas*

The protocol of Hsu was used to estimate the protein digestibility of tortilla samples (Hsu et al., 1977). First, 50 ml of an aqueous suspension of the materials, considering a protein amount of 6.25 mg of protein/ml, was prepared for each sample. These solutions were adjusted to pH 8.0 with 0.1N HCl or NaOH. A multienzyme solution of trypsin at 1.6 mg/mL (15 units/mg), chymotrypsin at 3.1 mg/ml (60 units/mg), and peptidase at 1.3 mg/mL (40 units/mg) was adjusted to pH 8.0 and maintained in an ice bath until use. The multienzyme solution (5 ml) was added to the protein suspension and incubated at 37°C in a water bath with continuous magnetic stirring for 10 minutes, pH was monitored for 10 minutes, the recorded values were used to estimate the in vitro protein digestibility. The free amino nitrogen (FAN) content was determined according to the approved AOAC

Method 945.30 (1980) to determine presence of small peptides and aminoacids in the samples.

### *5.3.12 Statistical analysis*

All procedures were performed by triplicate, unless otherwise specified. A one-way analysis of variance (ANOVA) was used for the statistical analysis of the results. Means were compared using Tukey's test to detect significant differences between AD substituted samples and control samples. ($p \leq 0.05$). To evaluate variable interaction among tortilla components, a principal component analysis (PCA) was conducted. Statistical analyses were carried out using Minitab (version 19.20201.0, Minitab, LLC).

## 6. Results and discussion

### *6.2 Raw material characterization*

### *6.2.1 Material proximal composition*

The proximal composition of Nixtamalized corn flour (NCF) and *A. domesticus* flour (AD), was provided by the suppliers (Table 1). NCF is mainly composed of carbohydrates with 77.0% and a protein content of 8.30%. AD flour is high in protein and lipids with a content of 65% and 14.97%, respectively.

**Table 1.** Proximal composition of NCF and AD as provided by supplier**.**

| Material | Moisture | Protein | Lipids | Carbohydrate | Ash |
|---|---|---|---|---|---|
| NCF | 8.96 | 8.30 | 4.35 | 77.00 | 1.39 |
| AD | 7.00 | 65.07 | 14.97 | 8.40 | 4.95 |

NCF-Nixtamalized corn flour; AD- *A. domesticus* flour.

### *6.2.2 Water absorption and solubility index of flours*

Water absorption index could indirectly reveal interactions among the molecules within the food system (Rojas-Molina et al., 2020). The NCF flour showed a significantly higher WAI than AD flour ($P \leq 0.05$) (Table 2); this was expected and could be explained by the presence of damaged starch in the NCF flours, which tend to absorb higher amounts of water. In composite flours, the WAI increased as AD content increased, which was unexpected due to the low water absorption of AD flour, which demonstrates that mixtures have a different behavior. Therefore, its hypothesized that this behavior could be due to a possible interaction between molecules of components in both materials. It has been reported that starch-protein interactions can bind water, similar results were obtained by Aboge et al (2021). The WAI value for AD was lower than previously reported. Stone et al. (2019) found an average value of 1.76 g/g for cricket flour as compared to the 1.45 g/g obtained which could be caused by the difference in suppliers and composition of AD used. AD flour had a significantly higher ($P \leq 0.05$) WSI than NCF, which indicates the presence of more soluble components (Table 2). WSI indicates the presence of small particles that can be dissolved in water, such as free sugars, low molecular weight dextrins, soluble fiber, and albumins (Oikonomou & Krokida, 2011). The WSI increased as the substitution percentage of AD increased. It has been reported that AD protein content has 31.5% of albumins, which are water-soluble and can be responsible for increasing the WSI (Stone et al., 2019). This is coherent with the results obtained for free sugar and soluble fiber content, were AD samples showed a higher proportion of both this and could be impacting the WSI (Table 5).

**Table 2.** Water solubility (%) and water absorption index (w/w) in composite flours containing AD and NCF.

| Treatment | WSI% | WAI(w/w) |
|---|---|---|
| AD | 11.51 ± 0.00$^{a}$ | 1.45 ± 0.02$^{b}$ |
| NCF | 5.43 ± 0.00$^{c}$ | 2.23 ± 0.17$^{a}$ |
| 5% | 5.53 ± 0.00$^{c}$ | 2.04 ± 0.16$^{a}$ |
| 7.5% | 5.76 ± 0.00$^{bc}$ | 2.11± 0.17$^{a}$ |
| 10% | 5.94 ± 0.00$^{b}$ | 2.15± 0.06$^{a}$ |

Values for WSI and WAI are the average of three replicates ± standard deviation. Different superscripts letters in the same column are significantly different (P ≤ 0.05). NCF-Nixtamalized corn flour; AD- *A. domesticus* flour; 5%,7.5% and 10% represent the substitution percentage with *A. domesticus* in dry basis.

### *6.2.3 Mixolab masa characterization of flours*

Mixolab equipment is used to determine dough consistencies at different temperatures to evaluate dough characteristics. Mixolab was set at a target torque at C1 of 1.001 ± 0.0015 Nm however it was observed that in samples with AD this value increased (Table 3). C1 is related to the capacity to absorb water and results in C1 have the same tendency as results obtained for WAI which indicates that as AD substitution increases samples can hold more water (Table 2). The consistency drop in C2 has been related to protein weakening when the temperature is increased. The presence of AD not only increased protein content but even improved protein force in the masa resulting in higher values of C2 as compared NCF. C3 is associated with gelatinization of starch. In this case, a 5% substitution with AD had an increased torque, and 7.5% had no effect, but at 10%, the substitution had a dilutive effect. This decrease in starch gelatinization at 10% could be explained by the high lipid content (Table 4), which has been shown to reduce proper starch gelatinization and the dilution of the starch content (Igual et al., 2020). Hot gel stability, which relates to the difference between C3 and C4, a drop in consistency for 7.5

and 10% AD flour samples can be observed. However, 5% AD substituted sample showed improved hot gel stability as compared to NCF. C4 and C5 were increased for all treatments with AD. C4 is linked to the amylolytic activity, which showed that AD samples were more resistant to this enzyme possible because the components of AD were providing resistance towards this enzymatic degradation. C5 is linked to starch retrogradation and gelling, AD samples showed higher values which supports the result that AD samples had lower values of rapid digestion starch, and possible interaction between molecules results in a more resistant gel (Codina et al., 2010).

**Table 3.** Mixolab parameters of composite flours.

| | Mixolab parameters | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **C1** | **C2** | **C3** | **C4** | **C3-C4** | **C5** |
| AD | - | - | - | - | - | - |
| NCF | $1.08^{b}$ | $0.62^{c}$ | $0.85^{b}$ | $0.73^{b}$ | $0.13^{a}$ | $1.19^{b}$ |
| 5% | $1.72^{a}$ | $0.85^{a}$ | $1.05^{a}$ | $0.89^{a}$ | $0.16^{a}$ | $1.46^{a}$ |
| 7.5% | $1.71^{a}$ | $0.83^{ab}$ | $0.84^{b}$ | $0.92^{a}$ | $-0.08^{b}$ | $1.46^{a}$ |
| 10% | $1.63^{a}$ | $0.78^{b}$ | $0.75^{c}$ | $0.97^{a}$ | $-0.22^{c}$ | $1.42^{a}$ |

Values for the Mixolab parameters are the average of two replicates. Different superscripts letters in the same column are significantly different ($P \leq 0.05$). C-Control sample; AD- *A. domesticus* flour; 5%,7.5% and 10% represent the substitution percentage in dry basis.

### *6.3 Tortilla characterization*

#### *6.3.1 Proximal composition of tortillas*

Significant differences ($P \leq 0.05$) were found in the proximal composition of samples (Table 4), AD enriched samples had an increase crude fat content which has expected due to the high crude fat content in AD (Table 1). The lipid composition found in AD flour depends on the rearing conditions and on processing treatments; however, it has been reported that the lipid composition of AD flour with similar processing conditions is ≈

44.7% polyunsaturated fatty acids, 35.7% of saturated fatty acids, and 19.6% of monounsaturated fatty acids (Singh et al., 2020). High temperatures are known to impact on AD flour, increasing lipid oxidation; therefore, it is important to consider the possibility of a decreased shelf-life stability and formation of rancid flavor and smells in products enriched with AD that are subjected to a thermal treatment. (Singh et al., 2020). The protein content also increased in AD samples which was expected. Furthermore, carbohydrate content decreased. The standard 6.25 conversion factor was used for protein quantification, which has been suggested that it might result in an overestimated value due to the nitrogen present in chitin (Ritvanen et al., 2020). However, the current regulation regarding AD flour provided by the EFSA states that the conversion factor of 6.25 should be used (Turck et al., 2021).

**Table 4.** Chemical composition of tortilla samples.

| **Chemical components(%)** | **TC** | **T5** | **T7.5** | **T10** |
|---|---|---|---|---|
| Crude protein | $5.36 \pm 0.56^{d}$ | $6.31 \pm 0.36^{c}$ | $7.43 \pm 0.45^{b}$ | $8.96 \pm 0.26^{a}$ |
| Crude fat | $5.16 \pm 0.43^{d}$ | $6.06 \pm 0.27^{c}$ | $8.90 \pm 0.75^{b}$ | $10.68 \pm 0.92^{a}$ |
| Ash | $3.11 \pm 0.34^{b}$ | $3.87 \pm 0.14^{a}$ | $4.16 \pm 0.65^{a}$ | $4.65 \pm 0.75^{a}$ |
| Carbohydrates by difference | $86.37 \pm 0.65^{a}$ | $83.76 \pm 0.87^{b}$ | $79.51 \pm 0.43^{c}$ | $75.71 \pm 0.38^{d}$ |

Values are the average of three replicates ± standard deviation. Different superscripts letters in the same row are significantly different ($P \leq 0.05$). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.*

### *6.3.2 Total starch and fiber fractions of tortillas*

It was observed that total starch decreased in AD enriched samples while TDF and FS increased (Table 5). The increase in IDF can be due to the chitin content in AD, an insoluble polysaccharide that has shown prebiotic effects (Stull et al., 2018). It has been previously reported that addition of AD increases fiber content in cereal products

(Kowalczewski et al., 2021). FS method extracts ethanol soluble carbohydrates such as glucose, sucrose, and trehalose. Afterwards, with the addition of sulfuric acid, sugars are broken down into monosaccharides and content of reducing sugars can be determined. Trehalose, is a disaccharide formed of two glucose units, and it is the principal storage carbohydrate found in crickets; therefore, this sugar might be responsible for increasing free sugar content in AD samples (Visanuvimol & Bertram, 2011).

**Table 5.** Carbohydrate fractions of tortilla samples.

| Carbohydrate fractions (%) | TC | T5 | T7.5 | T10 |
|---|---|---|---|---|
| TS | 75.39 ± 0.44$^{a}$ | 71.23 ± 0.50$^{b}$ | 65.26 ± 0.69$^{c}$ | 59.46 ± 0.88$^{d}$ |
| IDF | 1.76 ± 0.16$^{a}$ | 3.43 ± 0.09$^{b}$ | 5.21 ± 0.58$^{c}$ | 6.85 ± 0.92$^{d}$ |
| SDF | 2.56 ± 0.11$^{a}$ | 3.88 ± 0.15$^{b}$ | 4.05 ± 0.17$^{b}$ | 4.51 ± 0.07$^{c}$ |
| FS | 6.66 ± 0.12$^{a}$ | 7.32 ± 0.23$^{a}$ | 8.12 ± 0.41$^{b}$ | 9.26 ± 0.36$^{c}$ |

Values are the average of three replicates ± standard deviation. Different superscripts letters in the same row are significantly different (P ≤ 0.05). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus*. TS-total starch, IDF- insoluble dietary fiber, SDF-soluble dietary fiber, FS-free sugars.

### *6.3.3 Rollability of tortillas*

Rollability is an important parameter for evaluating quality in tortillas since tortillas are meant to be soft and easily rolled without any visible cracking. If a tortilla does not meet this expected characteristic, the consumer will most likely not accept it (Suhendro et al., 1998). During the subjective rollability method, it was observed that TC and T5 samples performed equally up to day 5 of storage showed no visible signs of damage (Table 6). However, on day 7 of storage, T5 showed no damage. T10 showed the most deterioration during storage (Figure 6). The texture of food depends on the composition of the samples; however, during storage, water is lost, and retrogradation of starches occurs, resulting in a hardening of the structures and decreased flexibility (Rojas-Molina et al., 2020). In this

sense, lipids may retard retrogradation by inhibiting the formation of double-helical structures of amylose, which might explain why the T5 sample had the highest score on day 7. On the other hand, T10 had the lowest rollability score which could be linked to the gelatinization properties of this sample observed in the Mixolab in the C3 parameter (Table 3). It was observed that T10 showed the lowest value in C3 which relates to the increase in viscosity due to starch gelatinization. This could have a negative impact on the overall texture of the final product. It could also be caused by the higher protein content which has been reported to affect retrogradation, specifically albumins and globulins and have shown to promote it (Yamaguchi et al., 2019). For optimizing tortilla quality a substitution of 5% is recommended, on the other hand adding more than 7.5% is discouraged due to the loss of flexibility in the final product.

**Table 6.** Subjective rollability scores in tortilla samples evaluated in fresh samples (D0) and during 7 days of storage at 4ºC.

| Treatment | D0 | D3 | D5 | D7 |
|---|---|---|---|---|
| TC | 5.00 ± 0.00$^{a}$ | 5.00 ± 0.00$^{a}$ | 5.00 ± 0.00$^{a}$ | 4.00 ± 1.00$^{a}$ |
| T5 | 5.00 ± 0.00$^{a}$ | 5.00 ± 0.00$^{a}$ | 5.00 ± 0.00$^{a}$ | 4.30 ± 0.60$^{a}$ |
| T7.5 | 5.00 ± 0.00$^{a}$ | 5.00 ± 0.00$^{a}$ | 5.00 ± 0.00$^{a}$ | 4.00 ± 1.00$^{a}$ |
| T10 | 5.00 ± 0.00$^{a}$ | 5.00 ± 0.00$^{a}$ | 4.70 ± 0.60$^{a}$ | 3.30 ± 0.60$^{a}$ |

Values are the average of 3 replicates ± standard deviation. Different superscripts letters in the same column are significantly different (P ≤ 0.05). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.* D0-day zero; D3-day three; D5-day five; D7-day seven. A score of 1 is completely broken and 5 is without any breakage.


**Figure 6.** Tortillas after subjective rollability evaluation after 7 days of storage. TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.*

### *6.3.4 Image and color analysis of tortillas*

Substitution of AD resulted in significant changes in color ($\Delta E > 5$) in all samples (Table 7). As more AD was added, lightness values significantly decreased ($P \leq 0.05$), which translates into darker tortillas (Figure 7). In corn tortillas, darker colors are usually associated with higher fiber content due to the bran (Rojas-Molina et al., 2020). The darker color in cereal-based products tends to be perceived by the consumer as healthier options (Kowalczewski et al., 2021).

**Table 7.** Color characteristics of tortillas samples in CIELAB units.

| Treatment | L* | a* | b* | $\Delta E$ |
|---|---|---|---|---|
| TC | $59.26 \pm 0.82^{a}$ | $0.42 \pm 1.16^{b}$ | $26.37 \pm 0.41^{a}$ | |
| T5 | $52.38 \pm 3.44^{b}$ | $2.43 \pm 0.86^{a}$ | $23.41 \pm 1.12^{b}$ | 7.71 |
| T7.5 | $49.78 \pm 3.06^{bc}$ | $2.15 \pm 0.56^{a}$ | $24.18 \pm 1.20^{b}$ | 9.84 |
| T10 | $46.07 \pm 4.20^{c}$ | $3.54 \pm 0.95^{a}$ | $24.75 \pm 0.89^{ab}$ | 13.60 |

Values are the average of five replicates ± standard deviation. Different superscripts letters in the same column are significantly different ($P \leq 0.05$). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.*

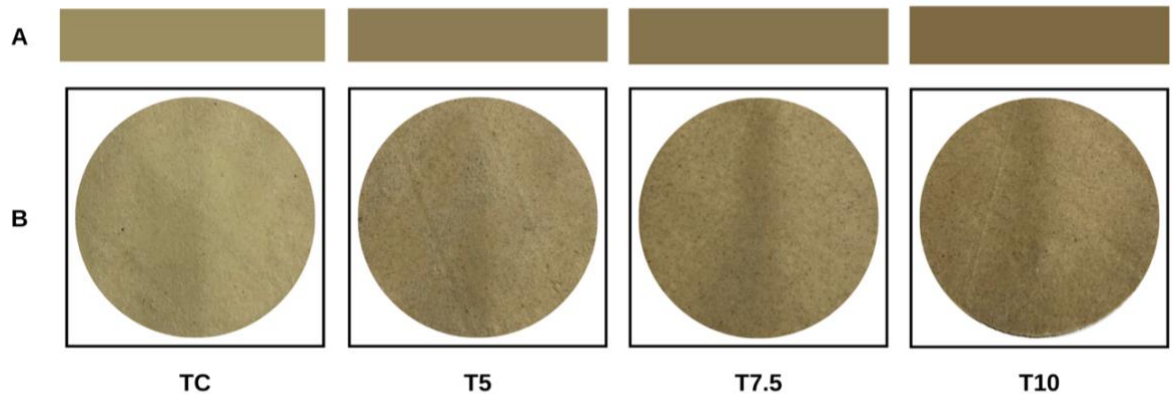


**Figure 7.** A) color of tortilla samples obtained in CIELAB. B) Images of tortilla samples after being cooked. TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.*

It was observed that a change in diameter occurred after cooking mainly due to the rearrangement of molecules due to the high temperatures used (Table 8). TC showed the highest change in diameter, whereas T7.5 sample, showed the lowest. This could be caused by a possible interaction of molecules from NSF and AD that result in a different behavior. It has been reported that protein-starch interactions can form gels capable of binding water resulting in an increased volume of the product (Cappeli et al.,2020) (Donmez et al., 2021); this is coherent with the results seen in the Mixolab C3-C4 parameters (Table 3), where AD substituted showed a more stable gel.

**Table 8.** Diameter before and after cooking of tortilla samples. Particle count in cooked tortilla samples

| Treatment | $D_o$ (cm) | $D_f$ (cm) | Δ D(cm) | Particles/cm$^2$ |
|---|---|---|---|---|
| TC | 16 | 13.91 ± 0.31$^b$ | -2.09 | 11.94 ± 8.31$^a$ |
| T5 | 16 | 14.94± 0.40$^a$ | -1.06 | 32.81 ± 12.14$^a$ |
| T7.5 | 16 | 15.31 ± 0.43$^a$ | -0.69 | 49.34 ± 9.34$^a$ |
| T10 | 16 | 14.71± 0.56$^a$ | -1.29 | 115.74 ± 32.68$^b$ |

Values are the average of five replicates ± standard deviation. Different superscripts letters in the same column are significantly different (P ≤ 0.05). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.* $D_o$- initial diameter. $D_f$ -final diameter. ΔD-change in diameter. *All tortillas were formed in the same mold which had 16 cm of diameter.

The particles/cm$^2$ in the tortillas increased as the substitution percentage of AD increased (Figure 8), 68.5% of the proteins contained in AD flour are insoluble in water, as well as the chitin; both components could be responsible for this increase (Stone et al., 2019). Only significantly different particle count values were obtained for T10 samples.

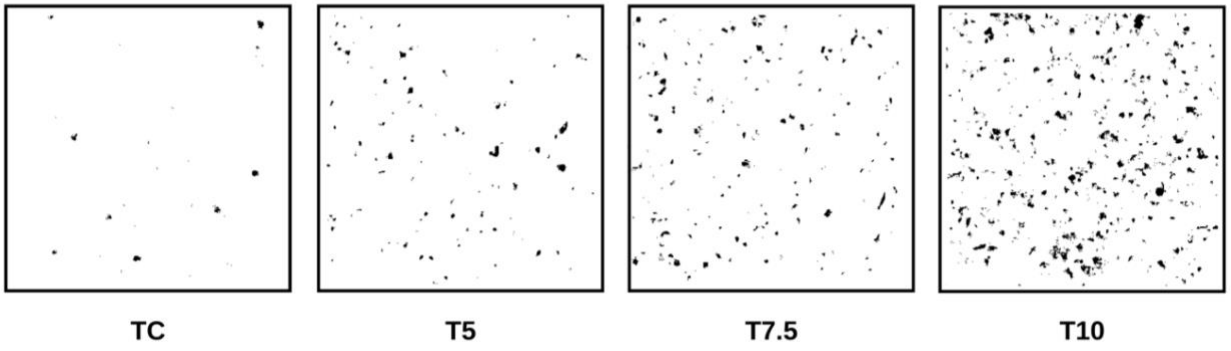


**Figure 8.** Image J particle count of tortilla samples. 400x 400 pixels; 8-bit (inverting LUT);156K. TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.*

### *6.3.5 Thermal characteristics of flours and tortillas*

To understand the impact of the ingredient composition on tortillas, both AD and NCF flours were analyzed as well as al tortilla samples. The cricket flour showed a first peak transition temperature of 90.2 ºC (Figure 9), which has been related to protein denaturation (Contreras Jiménez et al., 2020).

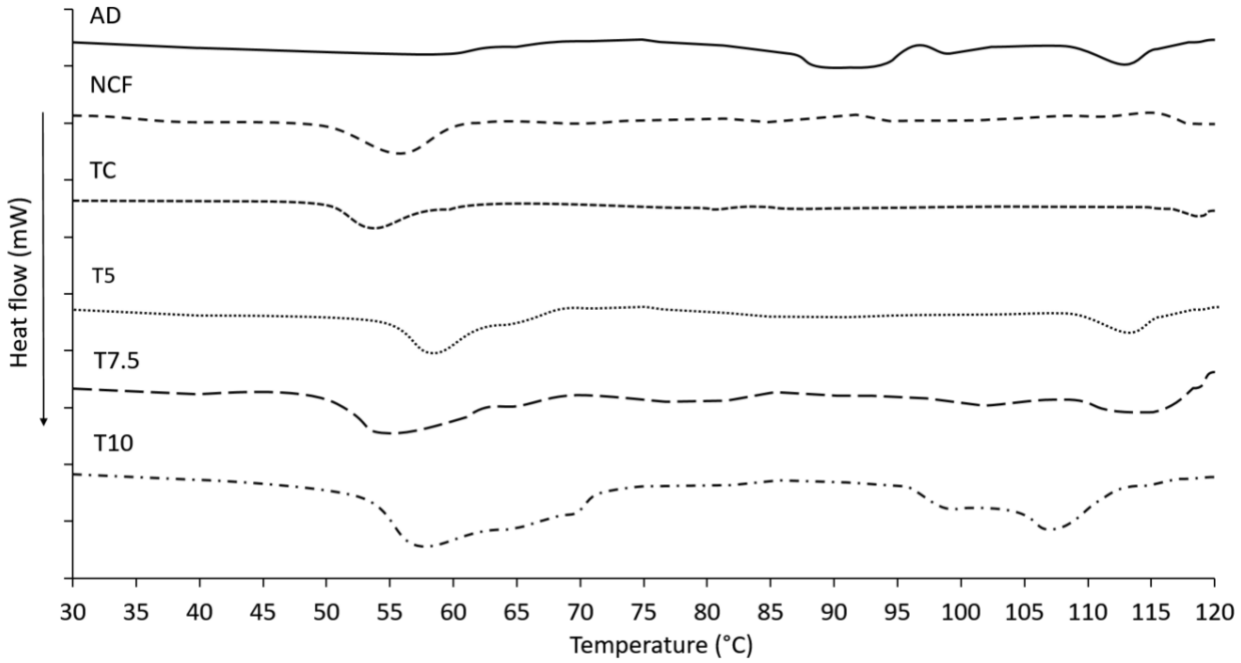


**Figure 9.** Differential scanning calorimetry of flours and tortilla samples. Nixtamalized corn flour (NCF); *Acheta domesticus* flour (AD;) TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.*

Regarding NCF, it showed the first transition above 55 °C (Table 9), that previous studies have related with gelatinization of remnant granules (Campus-Baypoli et al., 1999). In tortilla samples, the first transition occurred at temperatures above 60°C, which can be related to the rupture of remnant starch molecules. Furthermore, this transition can be associated with the disruption of residual crystalline amylopectin structures (Campus-Baypoli et al., 1999). It was observed that AD added tortilla samples showed significantly higher gelatinization peak temperatures (P≤0.05); The larger enthalpy values indicate that

such amylopectin clusters preserved a more organized structure, increasing its thermal resistance.

**Table 9.** Calorimetric parameters of flours and tortilla samples first transition

| | First transition | | | | |
|---|---|---|---|---|---|
| **Treatment** | $T_o$ (ºC) | $T_p$ (ºC) | $T_f$ (ºC) | $T_f$-$T_o$ | Δ$H$ (J/g) |
| **AD** | 88.42 ± 0.92$^{a}$ | 90.26 ± 0.31$^{a}$ | 93.30 ± 0.92$^{a}$ | 4.93 ± 0.71$^{d}$ | 4.33 ± 0.42$^{d}$ |
| **NCF** | 58.41 ± 0.52$^{b}$ | 61.42 ± 0.81$^{d}$ | 65.43 ± 0.72$^{d}$ | 7.01 ± 0.84$^{c}$ | 11.22 ± 0.94$^{a}$ |
| **TC** | 55.44 ± 0.31$^{c}$ | 60.31 ± 0.85$^{de}$ | 63.20 ± 0.30$^{e}$ | 6.91 ± 0.33$^{c}$ | 6.96 ± 0.47$^{c}$ |
| **T5** | 57.22± 0.64$^{cd}$ | 60.13 ± 0.53$^{e}$ | 65.63 ± 0.90$^{d}$ | 7.92 ± 0.61$^{c}$ | 8.92 ± 0.22$^{b}$ |
| **T7.5** | 55.32 ± 0.72$^{d}$ | 63.32 ± 0.12$^{c}$ | 67.33 ± 0.03$^{c}$ | 12.03 ± 0.46$^{b}$ | 9.31 ± 0.74$^{b}$ |
| **T10** | 55.83± 0.91$^{cd}$ | 65.42 ± 0.61$^{b}$ | 70.21 ± 0.03$^{b}$ | 14.40 ± 0.22$^{a}$ | 10.33 ±0.41$^{ab}$ |

Values for DSC are the average of three replicates ± standard deviation. Different superscripts letters in the same column indicates that means are significantly different (P ≤ 0.05). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.* $T_o$-Onset temperature; $T_p$-Peak temperature; $T_f$-Final temperature; ΔH-Enthalpy.

A second transition occurred above 110 °C in AD substituted samples (Table 10), which is related to amylose-lipid complexes, specifically type II (S. Wang et al., 2020). Type II amylose-lipid complex has a semi-crystalline structure that forms when there is an interaction of lipids and starch at temperatures above 90ºC, they are catalogued as resistant starch type V. In this sense, the palmitic and stearic acids are the lipids more prone for the formation of this complex, which *A. Domesticus* presents in 26% and 9% respectively (Li et al., 2021) (Singh et al., 2020).

**Table 10.** Calorimetric parameters of flours and tortilla samples second transition

| | Second transition | |
|---|---|---|
| **Treatment** | $T_p$ **(ºC)** | **Δ*H* (J/g)** |
| AD | 109.93 ± 0.91[c] | 2.42 ± 0.20[b] |
| NCF | - | - |
| TC | - | - |
| T5 | 115.36 ± 0.32[a] | 2.25 ± 0.80[b] |
| T7.5 | 112.37 ± 0.91[b] | 3.42 ± 0.78[b] |
| T10 | 107.43 ± 0.21[d] | 6.22 ± 0.27[a] |

Values for DSC are the average of three replicates ± standard deviation. Different superscripts letters in the same column indicates that means are significantly different (P ≤ 0.05). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.* $T_o$-Onset temperature; $T_p$-Peak temperature; $T_f$-Final temperature; ΔH-Enthalpy.

### *6.3.6 In vitro starch digestion of tortillas*

The in vitro starch digestion fractions showed an overall decrease in rapidly digested starch (RDS) (Table 11) in samples containing AD (P ≤ 0.05), while slowly digested starch (SDS) and resistant starch (RS) fractions increased. All samples contained RS regarding the formulation, which can be attributed to retrogradation of starch which corresponds to resistant starch type 3. However, the RS content is highly correlated to the percentage of substitution (R=0.99) which showed that tortilla samples substituted with AD had a significant increase. It has been reported that the addition of lipids to a starchy matrix affects digestion due to the formation of the amylose-lipids complex, which could be observed in DSC results (Figure 8). This amylose-lipid complex is responsible for the increase of RS in AD tortilla samples. However, the increase in RS can also be attributed to type 1 RS, which relates to the starch that is physically inaccessible to enzymes. In this sense, cysteine-containing proteins like globulins, which make up for 30.6% of the total protein fraction in AD flour, can form disulfide bonds after being cooked and form a matrix

that traps gelatinized starch making it less accessible for digestive enzymes (Stone et al., 2019). Furthermore, the fiber content AD samples (Table 5), may act as a physical barrier and block the interaction between enzymes and substrates. Fibers are also known to increase the viscosity of the food, consequently decreasing the diffusion of the enzyme towards the substrate (Yi & Li, 2022). Similar results were found when testing the addition of other insect flours from *Gryllodes sigillatus* and *Tenebrio molitor* (Zielińska et al., 2021). The predicted glycemic index (pGI) decreased in AD tortilla samples which was expected due to the reduction of RDS. A linear behavior could be observed where pGI decreased as AD content increased (R=-0.98)

**Table 11.** *In vitro* starch and protein digestion characteristics of tortilla samples.

| **Starch Digestion Characteristics (%)** | **TC** | **T5** | **T7.5** | **T10** |
|---|---|---|---|---|
| Starch | | | | |
| RDS | 68.45 ± 0.21$^{a}$ | 60.17± 0.33$^{b}$ | 53.26 ± 0.99$^{c}$ | 41.31 ± 0.12$^{d}$ |
| SDS | 28.19 ± 0.46$^{a}$ | 32.43 ± 0.21$^{b}$ | 38.26 ± 0.83$^{c}$ | 41.55 ± 0.63$^{d}$ |
| RS | 3.36 ± 0.75$^{a}$ | 7.40± 0.32$^{b}$ | 8.48± 0.02$^{c}$ | 17.14 ± 0.02$^{d}$ |
| HI | 71.06 ± 0.90$^{a}$ | 63.62 ± 0.85$^{b}$ | 56.51 ± 0.44$^{c}$ | 50.18 ± 0.63$^{d}$ |
| pGI | 78.72 ± 0.39$^{a}$ | 74.64 ± 0.86$^{b}$ | 70.74 ± 0.52$^{c}$ | 67.26 ± 0.26$^{d}$ |

Values are the average of three replicates ± standard deviation. Different superscripts letters in the same row are significantly different (P ≤ 0.05). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus;* RDS-rapidly digested starch; SDS-slowly digested starch; RS- resistant starch; HI-hydrolysis index; pGI-predicted glycemic index.

### *6.3.7 In vivo* glycemic *response of tortillas*

It was observed that consumption of TC sample was responsible for generating the largest glucose peaks in capillary blood with an average value of 60.75 mg/dL, while T7.5

was the sample that showed the smallest increase with an average value of 42.75 mg/dL (Figure 10). However statistical differences were only observed at minute 45, being sample T10 different and at minute 60 being samples T7.5 and T10 statistically different (Table 12). This reduction in glucose levels can be caused by the decrease in rapidly digested starch (RDS) (Table 10) and the increase of fiber content in AD substituted samples (Table 5). Despite tortillas enriched with AD flour had significantly higher values of free sugars (Table 5) as compared to TC, they still had a lower glycemic response (Table 12). As mentioned before, trehalose is the main sugar found in edible insects. It has been reported that trehalose cannot be completely digested nor absorbed by humans, furthermore it has been shown to reduce postprandial glucose levels (Yoshizane et al., 2020).

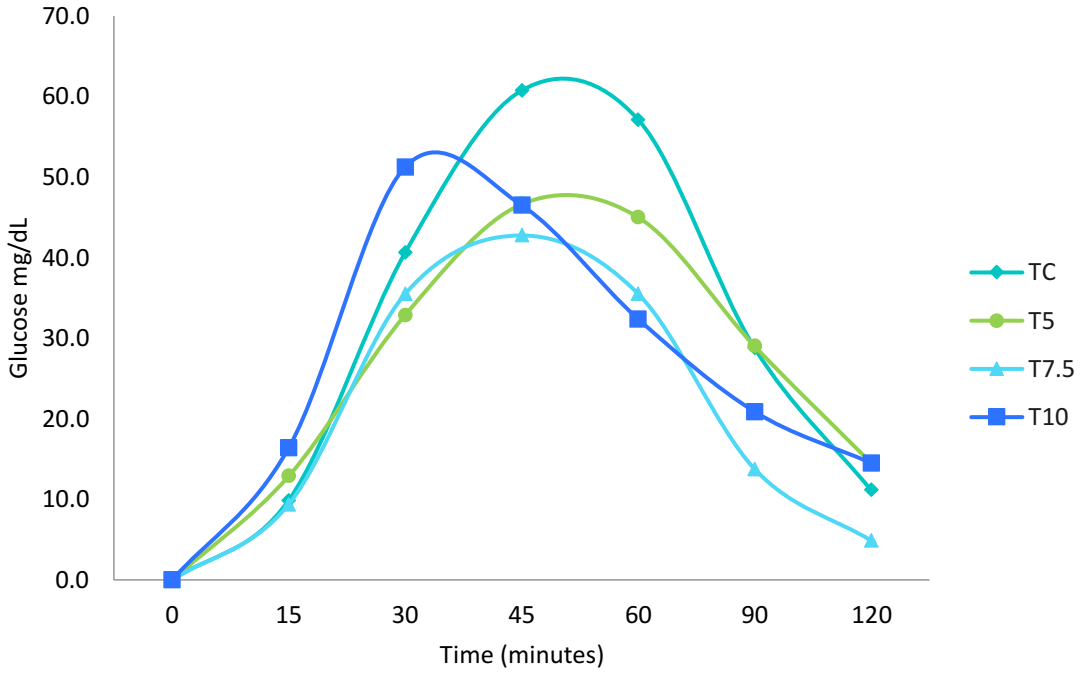


**Figure 10.** Post prandial blood glucose levees after ingestion of 50 grams of carbohydrates. Results are the average of 5 data values from different subjects with two replicates each. TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus.*

It can be observed that postprandial glucose levels at minute 45 are significantly lower ($P \leq 0.05$) for T7.5 and at minute 60 for T7.5 and T10 (Table 12). When evaluating the in vivo glycemic index, it can be observed that AD enriched tortilla samples tend to have lower values, still the differences are not statistically significant due to the large standard deviation within samples. This was expected since it was evaluated *in vivo* and therefore the variation and number of factors that can influence the results will tend to have a larger variability compared to *in vitro* results. As compared to *in vitro* results, *in vivo* glycemic index did not have a linear behavior (R=-0.37).

**Table 12.** Post prandial blood glucose levels after ingestion of 50 grams of carbohydrates

| **Time (minutes)** | **TC** | **T5** | **T7.5** | **T10** |
|---|---|---|---|---|
| 15 | 9.88 ± 11.93$^{a}$ | 12.88± 15.42$^{a}$ | 9.38 ± 8.86$^{a}$ | 16.38 ± 9.75$^{a}$ |
| 30 | 40.63 ± 14.54$^{a}$ | 32.88 ± 18.57$^{a}$ | 35.50 ± 16.27$^{a}$ | 51.25 ± 16.49$^{a}$ |
| 45 | 60.75 ± 10.74$^{a}$ | 46.63 ± 11.41$^{ab}$ | 42.75 ± 9.63$^{b}$ | 46.50 ± 12.15$^{ab}$ |
| 60 | 57.12 ± 12.74$^{a}$ | 45.00 ± 20.63$^{ab}$ | 35.50 ± 9.37$^{b}$ | 32.38 ± 16.30$^{b}$ |
| 90 | 28.75 ± 20.62$^{a}$ | 29.00 ± 10.99$^{a}$ | 13.75 ± 10.89$^{a}$ | 20.88 ± 12.10$^{a}$ |
| 120 | 11.13 ± 14.56$^{a}$ | 14.50 ± 11.88$^{a}$ | 4.88 ± 14.91$^{a}$ | 14.50 ± 9.26$^{a}$ |
| **Digestion characteristics** | **TC** | **T5** | **T7.5** | **T10** |
| GI | 100± 32.5$^{a}$ | 87.5± 22.6$^{a}$ | 65.2 ± 17.7$^{a}$ | 82.9 ± 26.1$^{a}$ |

Values are the average of 5 data values from different subjects with two replicates each ± standard deviation. Different superscripts letters in the same row are significantly different ($P \leq 0.05$). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5, GI-glycemic index.

### *6.3.8 In vitro protein digestion of tortillas*

Samples with AD showed higher in vitro protein digestion than the control sample, reaching a digestibility of 91.36% (Table 13). It was observed that digestibility increased ($P \leq 0.05$) with the substitution percentage which is similar to the results obtained by

Aboge et al. (2021) when evaluating AD flour in a porridge of wheat, maize and soy flour. Other authors have found that thermal processes and combination with starchy ingredients increase protein digestibility of insect flour. Enriched snacks with edible insect flour had a protein digestibility of 90.2%, which was higher than the values of 54%-66% reported for the raw material (Azzollini et al., 2018). In this sense, it has been observed that thermal processing results in proteins unfolding in AD flour which increases enzyme accessibility and increases digestibility (Singh et al., 2020). It has been reported that in pure insect flours subject to thermal treatment, proteins unfold and might be susceptible to form disulfide bonds resulting in less digestible structures (Manditsera et al., 2019). Therefore, the presence of starch for increasing protein digestibility in insect-based foods can be considered essential. It is hypothesized that as proteins unfold and starch granules gelatinize, new structures form and inhibit the formation of disulfide bridges within proteins. Such changes promote a rearrangement of starch, lipids, and proteins structures within the mixture, leading to a more digestible system in terms of proteins and less digestible in terms of starch. AD added tortilla samples had higher FAN levels, which could be an indicator that AD flour or the processing and rearrangement of molecules in AD tortillas resulted in more free nitrogen compounds such as small peptides and aminoacids. Despite chitin being a source of nitrogen, it does not contain free amino groups, however its deacetylated version, chitosan does. In this case FAN could also be an indicator of the presence of chitosan (Prochazkova et al., 1999).

**Table 13.** Protein in vitro digestion and free amino nitrogen of tortilla samples.

| **Characteristics (%)** | **TC** | **T5** | **T7.5** | **T10** |
|---|---|---|---|---|
| Protein Digestion (%) | 72.26 ± 0.17[a] | 80.33± 0.05[b] | 85.24 ± 0.86[c] | 91.36 ± 0.55[d] |
| FAN | 1.23± 0.14[a] | 1.75 ± 0.11[b] | 2.00 ± 0.10[b] | 2.32 ± 0.09[c] |

Values are the average of three replicates ± standard deviation. Different superscripts letters in the same row are significantly different (P ≤ 0.05). TC-tortilla control; T5-tortilla with 5% of *Acheta domesticus*; T7.5-tortilla with 7.5% of *Acheta domesticus;* T10-tortilla with 10% of *Acheta domesticus;* FAN- Free amino nitrogen.

### *6.4 Statistical analysis*

The principal component analysis (PCA) helped find relationships on how the impact of substitution levels affected the variables and how they interacted. The first and second components (PC1 and PC2) were responsible for 98.9% of the cumulative variance. PC1, predicted glycemic index (pGI), accounted for 93.3% of the total variance. Score plot shows the influence of the two main components on its overall behavior, and the substantial difference when AD flour was used, being the T10 treatment the one with most differences (Figure 11A). The loading plot shows (Figure 11B) shows that pGI is strongly affected by the amount of rapidly digestible starch present in the sample (RDS). Another significant cluster shows a strong relationship between lipid and resistant starch (R=0.916), which supports the DSC results that indicate the formation of resistant start type V (amylose-lipid complex). There is a strong relationship between the amylose-lipid complex enthalpy, that could be relate to the FAN content or small peptides. It has been reported that amylose-lipid complexes formation can be facilitated by protein molecules (S. Wang *et al*., 2020). Additionally, the insoluble fiber content and slowly digestible starch are strongly correlated (R=0.996) which can be observed in the PCA.

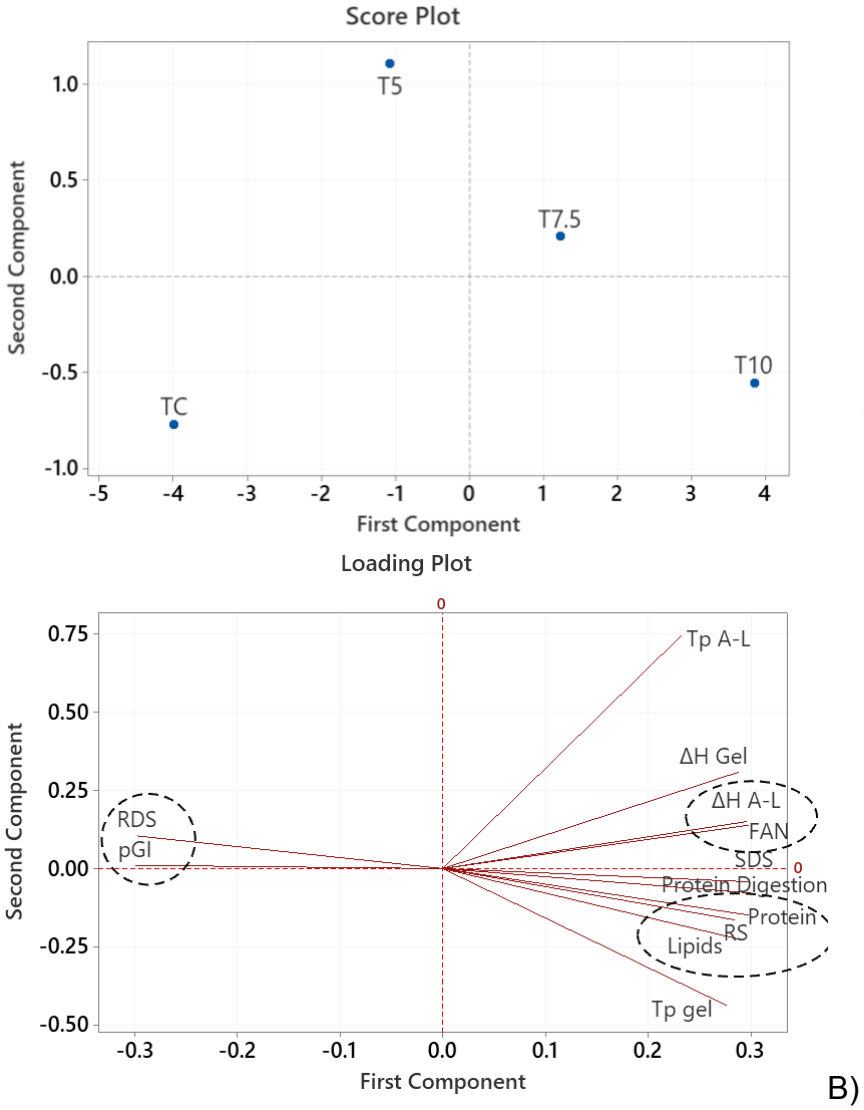


**Figure 11.** Principal component analysis of digestion and thermal parameters in tortillas added with cricket flour samples. A) Score plot, B) Loading plot.

## 7. Conclusions

NCF and AD have significant differences in terms of composition which had an impact on functional properties. NCF is rich in starch which gives this flour the capacity to hold large amounts of water and gelatinize in presence of heat, resulting in a flexible product. In contrast, AD is rich in lipids and proteins but lacks starch which resulted in a lower water absorption index. However, in composite flours, water absorption increased as AD increased, therefore it can be concluded that this behavior was driven by molecular interactions between both materials. These molecular interactions result in a different functional properties and characteristics of the final products.

Substitution with AD flour significantly modified visual characteristics of tortillas such as color and particle count, overall giving a darker color and increasing particle content which might affect consumer perception of the final products. Substitution of 5% showed a positive impact in rollability leading a possible technological advantage and functionality for AD flour.

Addition of AD significantly increased lipid and protein content whilst decreasing available starch fraction in the tortillas. Furthermore, starch digestibility and starch composition were strongly affected by the substitution percentage with AD, indicating molecular interactions such as the formation of amylose-lipid complexes which was corroborated by a DSC study. As a result, resistant starch content increased in AD substituted samples. It was observed that the increase of RS was positively correlated with the AD substitution percentage.

As expected, due to the high RS content, T10 showed the lowest pGI in vitro. As for protein digestibility, in vitro data demonstrated that presence of AD significantly increased digestibility. It can be concluded that protein and starch digestibility will depend on how each component is transformed during the process and the final structure. It is important to consider how the addition of an ingredient to a formula can impact different characteristics.

## 8. Proposals for future research

Based on results it would be of great interest to strengthen the structural investigation of the amylose lipid complex by using FTIR or an X-ray diffraction study. Furthermore, it would be of great interest to the effect of the addition of AD with other cereal mixtures and other food products. Furthermore, a consumer acceptance test to evaluate the overall likeability of the formulations would be necessary to assess viability of the product. It would be suggested to evaluate in a larger scale the impact of AD in blood glucose and insulin levels. Finally, the impact on protein digestibility could be measured *in vivo,* due to the promising results obtained in this research.

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## Appendix A

### Abbreviations and acronyms

| | |
|---|---|
| AD | *Acheta domesticus* |
| PM | Peritrophic matrix |
| FCR | Feed conversion ratio |
| kDA | Kilodaltons |
| g | Grams |
| EFSA | European Food Safety Authority |
| PDCAAS | Protein digestibility corrected amino acid score |
| PUFA | Polyunsaturated fatty acids |
| MUFA | Monounsaturated fatty acids |
| SFA | Saturated fatty acids |
| RDS | Rapidly digested starch |
| SDS | Slowly digested starch |
| RS | Resistant starch |
| GI | Glycemic index |
| NSP | Non starchy polysaccharide |
| ALC | Amylose lipid complex |
| NCF | Nixtamalized corn flour |
| mm | Millimeters |
| cm | Centimeters |
| C | Celsius |
| WAI | Water absorption index |
| WSI | Water solubility index |
| AOAC | Association of Official Agricultural Chemists |
| TDF | Total dietary fiber |
| IDF | Insoluble dietary fiber |

| | |
|---|---|
| SDF | Soluble dietary fiber |
| *g* | G force |
| T | Temperature |
| To | Onset temperature |
| Tp | Peak temperature |
| Tf | Conclusion temperature |
| H | Enthalpy |
| HI | Hydrolysis index |
| SD | Standard deviation |
| mL | Mililiters |
| mg | Miligrams |
| ANOVA | Analisis of variance |
| PCA | Principal component analysis |
| FAN | Free amino nitrogen |
| FTIR | Fourier transformed infrared |
| UDP | Uridine diphosphate |

## Curriculum Vitae

Alejandra Alvarez Barajas was born in Jalisco on March 28th of 1995. She always enjoyed food and studied a Bachelor's Degree in Food Engineering at ITESM campus Querétaro, graduating in December 2018. Passionate for seeking a more sustainable path for the food industry is that she gained interest for edible insects. In 2020 she begins a Master of Science in Biotechnology. She participated at the *Cereals & Grains Association* international congress in November 2021. She also was a part of the 52 Edition of *Congreso de Investigación y Desarrollo* at Tecnológico de Monterrey.

This document was typed using Microsoft Word by Alejandra Alvarez Barajas