Process and uses of alternative carbohydrate sources in pet foods and treats

by

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Abstract

The pet food industry has been rapidly growing for years. This growth has been driven by new product development which emphasized new ingredients and food forms. Starch sources are prominent ingredients in both complete & balance diets, and in snacks & treats. The overall objective of this project was to evaluate the performance of uncommon starch sources in dry expanded pet food, and in a novel dog treat. First, two dry expanded dog diets were formulated containing different types of starches: an ancient grain (AG; including spelt, millet and sorghum), and a grain-free diet (GF, including peas, potatoes, and tapioca starch). Experimental diets were evaluated for their impact on the extrusion process and nutrient utilization by dogs. A greater specific mechanical energy (141 vs. 117 kJ/kg) and in-barrel moisture (38.2 vs. 30.3 %) input were observed for GF compared to AG. The GF kibbles were more expanded (3.5 vs. 3) and harder (6.36 vs. 3.12 kg) than the AG. Apparent total tract digestibility of most nutrients was similar between AG and GF with exception of total dietary fiber which was 32% greater for dogs fed GF ($P<0.05$). Second, we evaluated the use of white and red sorghum flour (WSF and RSF, respectively) as potential ingredients for production of extruded crisps. Nutritional analysis revealed a higher protein (9.95 vs. 8.22 %) and a lower starch content (83.81 vs. 88.15 %) for WSF compared to RSF. Pasting properties were similar between WSF and RSF ($P>0.05$), but RSF exhibited higher initial (66.56 vs. 63.34 °C) and peak gelatinization (73.89 vs. 72.42 °C) temperatures. However, these differences did not influence the extrusion process. The WSF and RSF were extruded under similar processing parameters which resulted in expanded crisps with similar characteristics ($P>0.05$). Last, the use of sorghum crisps and soluble animal protein binders were evaluated for their effect on a cereal bar application for dogs. The experiment was conducted as a 3 x 5 factorial arrangement of treatments with three sources of crisp (rice crisp,

RC; white sorghum crisp, WSC; and red sorghum crisp, RSC) and five sources of binder (corn syrup, CS; spray dried plasma, SDP; gelatin, GL; albumin, AL; and egg product, EP). Texture properties of each dietary treatment were evaluated, and dog's preferences were assessed by a preference ranking test. Regarding textural properties, a significant binder by crisp source interaction was observed ($P<0.05$); wherein, the cereal bar produced with CS and RC presented the highest toughness. Cereal bars were well accepted by dogs and WSC cereal bars produced with SDP were preferred over those produced with EP ($P<0.05$). These studies demonstrate that starch sources may behave differently during extrusion processing, and can impact nutrient utilization and dog preference in complete & balanced diets, and in snacks & treats application. Characterization of raw materials and processing are essential to the development of new products that optimize both animal nutrition and processing conditions.

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List of Abbreviation

AG   Ancient grain experimental diet

AL   Albumin

BV   Breakdown viscosity

CF   Crude fat

CP   Crude protein

CS   Corn syrup

DM   Dry matter

EP   Egg product

FV   Final viscosity

GF   Grain free experimental diet

GL   Gelatin

HRFM   Hal ross flour mill

IBM   In-barrel moisture

l_{sp} Specific length

OM   Organic matter

PT   Pasting temperature

PV   Peak viscosity

RC   Rice crisp

RSC   Red sorghum crisp

RSF   Red sorghum flour treatment

SBV   Set back viscosity

SDP	Spray-dried plasma
SEI	Sectional expansion index
SME	Specific mechanical energy
STE	Specific thermal energy
T_c	Conclusion temperature
TDF	Total dietary fiber
T_o	Onset temperature
T_p	Peak temperature
TV	Trough viscosity
wb,	Wet basis
WSC	White sorghum crisp
WSF	White sorghum flour treatment
ΔH	Enthalpy of gelatinization.

Acknowledgements

Chapter 1 - Literature review

1. Pet Food Industry

Dogs and cats are some of the most popular pets owned in the United States. According to the American Pet Products Association's 2017-2018 National Pet Owners Survey, there are more than 60 and 47 million households owning dogs and cats, respectively. The pet industry has shown an enormous growth in the past several years, and play an important role in the the United States economy. In 2018, it was estimated that Americans spent $72.13 billion in the pet market which represents a 2% growth compared to the year before (APPA, 2018). The pet market can be broken down into five major categories: pet food, supplies/over-the-counter medicine, veterinarian care, live animal purchases, and pet services.

The pet food segment accounts for most of the sales within the market, representing more than 40% of the total in 2018 (APPA, 2018). The pet food segment can be further segregated in two major categories: nutritionally complete & balanced diets, and snacks & treats. In order to be classified as complete & balanced, the product must contain all the nutrients required by the animal and in the correct ratios. On the other hand, snacks & treats are mainly used as a reward or training tool, with no need to meet the animals' nutritional requirements (AAFCO, 2018). Complete & balanced products make up the greatest share of the pet food market sales, and are mainly expanded products. Extrusion is the main process used to produce these dry expanded pet food. This is a continuous process where the material is plasticized and cooked by a combination of moisture, pressure, temperature, mechanical shear, and thermal energy (Smith, 1976).

Snacks & treats also have an important and established place in the pet food market. Although most of the treats are produced by baking, there is a plethora of products in the market

produced through alternative processes. Interestingly, there is a significant lack of published data regarding processing conditions and animal acceptance of snacks & treats.

The pet food industry is directly impacted by the owner-pet relationship. Unlike a few decades ago where dogs were kept mostly for utility, today they are considered part of the family. This transition often described as "humanization" of pets has shifted pet food product types and composition. More than ever before, human food trends are influencing by the pet food industry as pet owners want to feed their pets similar food to what they are eating. Complete & balanced diets need to meet the animals' nutritional requirements, and also meet the pet owners' expectation. In the same way, snacks & treats are produced to achieve pet owner's expectation as they are offered to pets because they are a demonstration of affection and love.

Trends towards a more natural and healthy food have edged upward in the pet food industry (Sprinkle, 2018). In many cases pet owners seek pet food claims that address the same health concerns faced in their own diet. Within these trends, the grain-free claim has become very popular. Many pet owners perceive grains such as corn and rice as unhealthy for their pets, and consider the grain-free claim as a healthier alternative. In 2017, grain-free pet food sales increased 10% in U.S. pet specialty, and accounted for 53% of new pet food products (Phillips-Donaldson, 2018). Legumes and tubers like peas, chickpeas, potatoes, and tapioca are some of the main replacements for traditional grains in these diets.

These novel ingredients are important for developing new products and for keeping pet food companies competitive in the market. However, pet food marketing may have outpaced the science (Freeman et al., 2018), as many of these novel ingredients are being added in pet products with little to no research regarding their impact on processing conditions and animal health. Although most of legumes and tubers commonly included in grain free diets have been

part of the human diet with little issue for decades, their impact on dog nutrition is not well established. Grains, legumes, and tubers are considered important carbohydrate sources. However, each ingredient has a unique nutritional composition and physical structure which may directly impact processing conditions and animal health. Thus, exploring the use of a grain-free carbohydrate sources compared to grain sources on processing conditions and animal health is vital to better manage their use in the pet products.

2. Traditional Pet Food Performance

Cereal grains have been widely used in commercial pet food since they were first extruded in 1954 (Kirk *et al.*, 2008). They are considered a major carbohydrate source and are used in pet food because of their nutritional value, and their functionally in the extrusion process. Common grains used in pet food are cereals such as corn, rice, wheat, and oats, as well as some pseudo cereals such as sorghum, and millet. A new classification within grains that has risen in popularity is the term ancient grain. Although there is no official definition to establish what constitutes an ancient grain, they are typically considered to have been cultivated for centuries with little genetic modification. Some ancient grains have been considered functional foods by the human food industry as their consumption can potentially improve cardiovascular, and gastrointestinal health (Tang and Tsao, 2017).

Despite the fact that cereal grains have been used for decades in human and pet food, some consumers remain skeptical about feeding them to dogs. Leading concerns and claims are: 1) they are considered "fillers" and have little nutritional value; 2) their quality and safety are questionable; and 3) they are a major cause of food allergy (LaFlame, 2014). None of which are supported by the scientific literature. As marketing experts often say "perception is reality." So, changing this perception may be difficult.

Although dogs do not have a dietary requirement for carbohydrates *per se*, they do have a metabolic need for glucose. Cereal grains provide this glucose and are an inexpensive energy source in dog diets. They also contain essential amino acids, fatty acids, and vitamins (LaFlamme *et al*., 2014). As an example, cereal grain proteins are a rich source of methionine (Samaranayaka, 2016), an essential amino acid for dogs (NRC, 2006). Furthermore, carbohydrate-rich ingredients provide dietary fiber, which is important to gastrointestinal health (Sivaprakasam, *et al*., 2016). Due to the high nutritional value and the low cost, many cereal grains are preferred ingredients compared to some animal proteins (Beloshapka *et al*., 2016). In addition, some cereal grains such as sorghum have potential health benefits that should be explored.

Some sorghum varieties are rich in phytochemicals such as phenolic acids and condensed tannins. Although these compounds may impact protein digestibility (Duodu *et al*., 2002), they are known to have antioxidant and antiradical activities (Hagerman *et al*., 1998). Alvarenga and Aldrich (2018) assessed the antioxidant capacity of dogs fed a control diet – based on rice, wheat, and corn – relative to diets formulated with milled sorghum fractions. They observed that dogs fed whole sorghum and the sorghum flour containing diets had similar antioxidant capacity to those fed the control diet, but dogs fed a sorghum mill-feed diet rich in bran had a higher circulating antioxidant potential.

Besides being important sources of nutrient in pet diets, cereal grains are highly functional ingredients for extrusion processing. They provide starch to the diet and are thereby classified as structure forming materials according to Guy (2001). The structure-forming materials provide the matrix for an extruded product by forming a melt fluid from biopolymers (Maskan and Altan, 2012). This contributes to binding properties, and product expansion, and

texture. Typical starch levels in pet foods range from 20 to up 65% (Riaz and Rokey, 2012). Although raw starches are poorly digestible by dogs, extrusion can improve carbohydrate utilization. The thermo-mechanical action during this process leads to gelatinization of starch, which makes it highly digestible. Extruded dog diets having rice, corn, and sorghum as carbohydrate sources resulting 100% fecal starch digestibility (Twomey *et al.*, 2002). Carciofi et al (2008) also found high starch digestibility for grain-based dog foods. Interestingly, particle size of cereal grains can directly affect starch digestibility. For example, Bazolli et al (2015) observed that a smaller particle size is required for sorghum and corn-based dog diets to provide proper starch gelatinization and digestibility, while rice is more easily digestible even if coarsely ground.

Different cereal grains can have an impact on overall digestibility of a dog food. Dog diets containing sorghum and corn as carbohydrate sources had a lower organic matter digestibility compared to a rice-based dog diet (Carciofi *et al.*, 2008). Further, Kore et al (2009) observed a lower digestibility of dry matter, organic matter and total carbohydrates when rice was replaced by sorghum in a dog food. When comparing millet to sorghum, a similar digestibility for most nutrients occured (Kore *et al.*, 2009; Fortes *et al.*, 2010). Results of protein digestibility of sorghum-based diets compared to rice and corn-based diets are inconsistent. While a study by Murray et al (1999) reported a reduced protein digestibility in a sorghum-based dog diet compared to a corn-based diet, some authors have observed the opposite (Twomey *et al.*, 2002). Unfortunately, the sorghum variety used in these studies was not specified by the authors. It is widely recognized that different sorghum varieties have different nutritional composition and tannin content. This can explain in part the inconsistent outcomes. Overall, a

lower digestibility observed in sorghum diets might be also explained by its higher fiber content and the presence of condensed tannins (Carciofi *et al.*, 2008).

Although highly nutritious and well accepted by dogs, some outbreaks of pet mycotoxicosis over the years have created some controversy about the safety and quality of cereal grains in the pet food industry (Garland and Reagor, 2001; Stenske *et al.*, 2006). The exposure of dogs to food contaminated with mycotoxins, which are secondary metabolites produced from normal metabolism of some fungi, can lead to acute and/or chronic mycotoxicosis. This is a pathology that can threaten the animals health even leading to death. These substances are highly stable at extreme physical conditions such as high temperature and moisture. Thus, they survive extrusion and drying processes used to manufacture dry pet food. Usually, corn is the source of mycotoxin contamination in pet food, and aflatoxins have been the most common cause of mycotoxicosis (Boermans and Leung, 2007). However, rice and wheat are widely used in pet food and are also susceptible to fungal growth (Maia and Siqueira, 2002), and a source of mycotoxin contamination. In 1998, 55 dogs died in Texas after eating a dog food contaminated with high levels of aflatoxin (Bingham *et al.*, 2004). Similarly, over 100 dogs were involved in an aflatoxin contamination event involving multiple dog food products from the same brand in 2005-2006, leading to illness and death (Stenske *et al.*, 2006; Newman *et al.*, 2007). Although there are no recent reports and recalls of pet food contaminated with mycotoxins, they can still be detected in some commercially available dog foods. Gazzotti et al (2015) found the presence of different mycotoxins in dog foods in Italy with a higher concentration of ochratoxin A in standard foods compared to premium foods. Many of these mycotoxins were found below the safe level established in Europe, however, the effects of chronic exposure to low doses are still unknown. Although pet food companies in the US are

required by law to monitor incoming raw materials, sampling error can lead to false negatives as mycotoxins are note uniformly distributed in foodstuffs.

Another health concern of many pet owners regarding cereal grains in their pet diets is their allergenic potential. Although food allergy is of significant concern, they are an uncommon reaction accounting for less than 10% of dermatological allergies in dogs (Verlinden *et al*., 2006). Food allergy is a hyper-sensitivity reaction to one or more food components, leading to dermatological and gastrointestinal symptoms. Proteins and glycoproteins are the major offending antigens (Laflamme *et al*., 2014). Although cereal grains contain proteins and can elicit a hyper-sensitivity response in dogs, the most common food allergens are animal proteins. Beef was reported as the most common food allergen in dogs followed by dairy, wheat, eggs, and chicken (Verlinden *et al*., 2007). Wheat accounted for 15% of the identified cases of food allergy in dogs. In the case reports in the literature, corn and rice are uncommon dietary allergens (Paterson, 1995), and are not a major concern when food allergy is being diagnosed.

Even though rice is not in the spotlight when talking about food allergies, commercial rice and lamb-meal diets have been associated with taurine deficiency in dogs (Tôrres *et al*., 2003). Taurine is needed for heart health, but it is not considered an essential dietary amino acid for dogs as they can theoretically synthetize adequate quantities when sulfur amino acids (methionine and cysteine) are present in sufficient dietary amounts (Malloy *et al*., 1981). It has been assumed that the diets involved with taurine deficiency did not provide adequate amounts of sulfur amino acids. This may have resulted in insufficient production of taurine by the dog. Low availability of sulfur amino acids in the lamb-meal, and depletion of taurine by soluble fiber present in the rice bran were also considered complicating factors for taurine deficiency in the previous episodes.

Although cereal grains have been linked to mycotoxins outbreaks, perception of allergens, and specific nutritional imbalances related to taurine, they are still widely used and considered safe. However, some pet owners still avoid pet food containing these ingredients opting for grain free foods. This category has grown precipitously over the past two decades until now they constitute a major portion of the market (Plantz, 2017). However, this may be reaching a plateau, and they market is looking for new options. The "ancient grains" claim may change pet owner's negative perception of grains due to their perceived health benefits. Said another way, this may be a new alternative to the grain free diets in the pet food market. Ancient grains are typically considered those that have been cultivated for centuries with little genetic modification. Nevertheless, there is little published research regarding the use of ancient grains in pet food. In current times, sorghum, millet, and spelt, are some of the most popular ancient grains used in pet food industry.

Spelt is a hulled specie of wheat considered one of the oldest cultivated grains in the world (Solarska *et al*., 2012). It is mostly used as an alternative feed grain (Herbek, 2012). However, its popularity as a food grain is rapidly growing due to its nutritional value. Spelt has higher protein, lipid, and vitamin B contents compared to traditional wheat (Escarnot *et al*., 2012). Although the use of spelt has been investigated mostly in baking application such as bread (Marques *et al*., 2007; Ranhotra *et al*., 1995; Zieliński *et al*., 2008), there is no published information regarding its use as a dietary ingredient in dog foods.

Millet is a warm-season cereal known for its good protein quality and high contents of phytochemicals (Shahidi and Chandrasekara, 2013). It is used in the United States and Canada mainly for feed and bird seeds. However, it is also found in dry, canned, and treat dog products. Among these categories, millet is mostly used in dry foods, being found in almost 6 % of dry dog

foods in the market. (PetfoodIndustry, 2017). The digestibility and metabolizable energy of some extruded carbohydrates sources for dogs were evaluated by Fortes et al (2010). The authors found a similar metabolizable energy and digestibility for millet, sorghum, high oil maize, and for the reference diet, which was based on maize and poultry by-product meal.

Compared to millet and spelt, sorghum is more commonly used as a dietary ingredient in pet food. This grain is found in more than 130 pet food products available in the market (SorghumCheckoff, 2017). The higher prevalence of sorghum in pet products compared to other ancient grains might be a result of its importance and popularity around the world. Sorghum is ranked among the five most important crops in the world (Agrama and Tuinstra, 2003), being known for its versatility - used as grain, forage, a sweet crop - and for its high tolerance to drought and high temperatures. The United States has been the world's top producer of sorghum, and Kansas leads the nation in production of the grain (USDA, 2016). Published research has evaluated the use of sorghum as a sole carbohydrate source in dog food. However, most of products claimed as "ancient grain" are formulated with two or combination of grains.

Moreover, ancient grains are promising ingredients for pet treat application due to their impact on animals health. Among the ancient grains listed above, sorghum has a greater potential in the treat market as it is already commonly included in complete and balanced diets. Today, very little sorghum can be found in pet treats. Low inclusion of sorghum in pet treats may be due to the lack of innovation and market exposure to sorghum application in treats. New ideas on how to include sorghum in a dog treat can present an application and opportunity to the pet food industry to include sorghum in novel treats. A recent study reported that extruded sorghum flour reduced adipogenic genes, chronic inflammation, and weight gain in obese rats (Arbex *et al*., 2018). Extruded sorghum flour may act similarly in dogs creating a new market for this grain in

a pet treat application. However, extrusion process of sorghum flour was not evaluated by the previous authors. Establishing processing conditions is essential to insert a new product in the market. Moreover, there are few studies characterizing the process for milling sorghum, and the quality of sorghum flour. Composition and functionally of sorghum flour can be different depending the sorghum variety (Palavecino *et al*., 2016), and may provide for different applications in the pet food industry.

3. Grain-Free Pet Food Performance

Pet humanization has driven the pet food industry towards products perceived as healthy and natural by pet owners. Considering that grains are perceived as unhealthy ingredients by some, grain free diets have become a major portion of the pet food industry. These diets are formulated using legumes and tubers as the major replacements for cereal grains. Legumes and tubers have also been consumed by humans for centuries with little to no side effects. However, human diets are usually composed of a wide range of ingredients, unlike the dog, who is fed a single diet that should contain all nutrients required by the animal. The total replacement of cereal grains in pet food by alternative ingredient sources such as legume and tubers may represent a challenge for animal nutritionists and processing operators.

Legumes are produced mainly for their seeds that are harvested at maturity, and are considered valuable sources of energy and protein (Jezierny *et al*., 2010). Peas, chickpeas, and lentils are some common sources of legumes added in grain-free diets. They are found in nearly 5, 11.5, and 7.8% of the dry dog food recipes, respectively (Plantz, 2017). On the other hand, tubers are a main energy source, but contribute little to dietary protein. Examples of tuber used in pet foods are potato, and tapioca starch.

Literature regarding the use of legumes and tubers on extrusion of pet food is scarce. Most of the published research has evaluated their use in extrusion of human food or as sole ingredients to evaluate its functionally. Tuber starches such as potato and tapioca are considered excellent binders, and usually result in a smoother kibble surface even when added at low levels (Riaz, 2007). These starch sources also tend to gelatinize at a lower temperature compared to cereal grains, which leads to a greater swelling power in the precense of water. The granule size of the starch source also reflects on their behavior during extrusion. Potato starch has larger oval granules compared to other starch sources (Swinkels, 1985), which leads to high melt viscosity when combined with water and heat, and early melting in the extruder (Della Valle *et al*., 1995). Furthermore, the higher swelling power observed in potato starch may be due to the presence of negatively charged phosphate groups bind to the starch molecule (Swinkels, 1985).

Legumes, on the other hand, have higher protein and lower carbohydrate content, and are not considered to be as substantial of a structure forming material compared to cereal grains and tubers. However, plant and vegetable proteins are highly functional ingredients in extrusion. They have excellent water absorption and binding characteristics. As a result, increasing levels of these ingredients require addition of more moisture in the process (Riaz, 2007). The effect of dehulled faba beans on extrusion of dry dog food was investigated by Alvarenga and Aldrich (2019). The authors reported that increasing levels of dehulled faba beans required higher water addition in the process, and resulted in a linear decrease in the specific mechanical energy. Consequently, kibbles became less expanded and harder as dehulled faba bean levels increased.

In relation to their nutritional quality, legumes are a rich sources of carbohydrates, B vitamins, and minerals (Tiwari *et al*., 2011). Although a good source of carbohydrates, legumes produce a moderate glycemic response in dogs. Carciofi *et al* (2008) observed a lower glycemic

index and lower digestibility in dogs fed pea and lentil-containing diets compared to those fed a grain-based diet. Similar results were found by Bednar *et al* (2001) who attributed these results to higher concentration of fiber and a lower proportion of rapidly digestible starch in legumes compared to cereal grains.

Moreover, legumes are an important protein contributor to grain free diets having about twice the crude protein content of cereal grains (Bednar *et al*., 2001). The quality of a protein source depends on its amino acid composition and availability, and the presence of antinutritional factors. Although legumes are a rich source of lysine, they are deficient in sulfur amino acids – methionine and cysteine (Gatel, 1994). This can be explained by the fact that the main storage protein in legumes – globulin – has a lower content of sulfur amino acids (Gueguen and Baniel, 1990). Methionine and cysteine are essential amino acids for the dog, and they are not only building blocks of protein in the body, but they are also play key roles in biological functions (Zong *et al*., 2018). Thus, when formulating a grain free diet for dogs one should play close attention to the sulfur amino acid content.

In 2018, the Food and Drug Administration (FDA) issued a warning letter (FDA, 2018) regarding a possible link between grain free diets and dilated cardiomyopathy (DCM). This condition is characterized by dilatation of the heart chamber, and decreased contractility of the heart muscle. Consequently, the heart is unable to normally pump blood to the body, which can lead to sudden death. Some large dog breeds are genetically predisposed to DCM (Freeman *et al*., 2001; Vollmar *et al*., 2013), however, non-susceptible dog breeds that were eating grain free and "boutique diets" developed the pathology, which led to some concern about these diets.

Normal myocardial function is dependent on circulating taurine concentrations. Taurine is a non-essential amino-sulfone produced by the body from sulfur amino acids (Brosnan and

Brosnan., 2006;). Some grain free diets might be deficient in bioavailable methionine and cysteine. This could result in lower levels of taurine synthesis, and consequently impair heart health. Furthermore, unlike cereal grains, legumes are a rich source of soluble fiber such as oligosaccharides, which might impact taurine status due to losses from fermentation in the gut (Ko and Fascetti, 2016).

Smalls amounts of oligosaccharides can be beneficial for the animal, but they can become an issue at high concentrations. These components are indigestible by dogs due to the lack of α-1,6 galoctosidase in the small intestine (Mohan *et al*., 2016). High amounts of oligosaccharides can lead to excessive fermentation in the colon, resulting in soft stools, and even diarrhea (Saini, 1989; Mul and Perry, 1994). Furthermore, studies in rats have associated soluble fiber with higher production of bile acids (Garcia-Diez *et al*., 1995), and to lower activity of hepatic enzymes involved in taurine biosynthesis (Ide, 1998). Corroborating evidence was observed in a recent study by Ko and Fascetti (2016) which reported lower concentration of blood taurine in dogs fed a soluble fiber from beet pulp compared to those fed insoluble fibers from cellulose.

Overuse of legumes and tubers in pet food may present nutritional challenges. However, grain free diets formulated with moderate levels of these ingredients may behave similarly to grain-based diets. Evaluation of novel ingredients is essential in order to prevent nutritional unbalances. Meeting the minimum nutritional requirements by the dog established by the Official Publication of The Association of American Feed Control Officials (AAFCO, 2018) should not be the only tool used to evaluate dog food quality. When developing a new product, it is also essential to take into account nutrient availability, nutrient interactions, and how these may be affected by processing conditions (Mansilla *et al*., 2019). There is little to no published research evaluating the nutritional or process effects of grain free pet diets. Thus, exploring the

use of a grain-free diet compared to grain-based diet on processing conditions and nutrient utilization could generate valuable information for the pet food industry.

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Chapter 2 - Effect of ancient grains and grain-free carbohydrate sources on extrusion parameters and nutrient utilization by dogs

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Highlights

- The GF diet required greater SME and IBM input during extrusion

- Digestibility of TDF was higher for dogs fed GF than those fed AG

- Dogs fed the GF diet had a higher wet fecal output and lower fecal DM

 Abstract

The aim of this study was to evaluate the impact of ancient grain and grain free carbohydrate sources on extrusion process, nutrient utilization, and palatability in dogs. Two dog diets were formulated with same proportions of carbohydrates: 1) Ancient grain diet (AG) with spelt, millet and sorghum; and 2) Grain free diet (GF) which had potato, peas and tapioca starch. Experimental diets were extruded over five replicates in a completely randomized experimental design. Digestibility was carried out with 12 dogs in a switch back experimental design. The GF diet required greater specific thermal energy and in-barrel moisture input ($P<0.05$) than AG in order to produce kibbles out of the extruder with similar bulk density ($P>0.05$). After drying, GF kibbles were less dense and more expanded, but harder than AG kibbles ($P<0.05$). Dogs preferred GF over AG in the palatability assessment. Apparent nutrient digestibility of dry matter, organic matter, gross energy, crude protein, and crude fat were not affected by treatment ($P>0.05$). However, total dietary fiber (TDF) digestibility was 31.9% greater for dogs fed GF ($P<0.05$). Moreover, wet fecal output was higher, and fecal dry matter was lower for dogs under GF ($P<0.05$). The results demonstrated that GF and AG diets behaved differently during extrusion, but were similarly utilized by dogs, with exception of TDF. Thus, fiber content of grain-free diets should be monitored in order to maximize fecal quality.

Abbreviations

AG, ancient grain experimental diet; CP crude protein; CF, crude fat; DM, dry matter; GF, grain free experimental diet; l_{sp}, specific length; IBM, in-barrel moisture; OM, organic matter; SEI, sectional expansion index; SME, specific mechanical energy; STE, specific thermal energy; TDF, total dietary fiber,

1. Introduction

Dogs and cats are some of the most popular pets owned in the United States, and are found in more than 60 and 47 million households in country, respectively (APPA, 2018). This directly impacts the pet supply industry which was worth $69.51 billion in 2017. Humanization of pets has shifted the pet food industry towards a diet perceived as healthy for pet owners. Within these trends, the "grain-free" and the "ancient grain" claims have become popular as many pet owners consider traditional cereal grains to be unhealthy for their companion animals (Laflamme, 2014).

Ancient grains are typically considered those that have been cultivated for centuries with little genetic modification, such as sorghum, millet, quinoa, chia, and spelt. Some of these grains have perceived health benefits (Tang and Tsao, 2017), which might open them for consideration as alternatives to the grain-free diets in the market. These grain-free diets are commonly formulated with tubers and legumes such as potato, peas, and tapioca starch as replacements for conventional grains. Although tubers, legumes, and grains are all carbohydrate sources, each class has a unique nutritional composition which impacts their processing (Riaz, 2007), and nutrient utilization (Fortes *et al.*, 2010). The effects of pea, lentil, sorghum, and traditional grains

on dog food digestibility have been assessed by previous authors (Carciofi *et al*., 2008). However, these ingredients were evaluated as sole carbohydrate sources, and were included in high levels. While this is valuable information, most commercial dog diets are formulated with a combination of carbohydrate sources.

Evaluation of these ingredients in a commercial dog food scenario may provide important information to maximize processing, and ensure proper nutrition for dogs. To our knowledge, there is no published study comparing an ancient grain and a grain free dog food that were formulated with a combination of different carbohydrate sources. Thus, the objective of our study was to evaluate a grain-free diet compared to ancient grain diet on processing conditions and nutrient utilization by dogs.

2. Material and methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and use Committee at Kansas State University under protocol #3883.

2.1 Diet formulation and production

Two dog maintenance diets were formulated containing the same proportion of carbohydrate sources: an ancient grain diet (AG) with spelt, millet and sorghum, and a grain free diet (GF) which had potato, peas, and tapioca starch (Table 2.1). This extrusion trial was designed as a completely randomized design with diets produced over five replicates. Experimental diets were produced in a pilot scale single-screw extruder (X-20, Wenger Manufacturing, Sabetha, KS, USA) with the following extruder profile: zone one – single flight small and steam lock; zone two – single flight and small steam lock; zone three – single flight and medium steam lock; zone four – large steam lock and uncut cone screw. Two circular dies (4.0 mm diameter) were used to produce standard size kibble for dogs. Similar bulk density out

of the extruder (~ 415 g/L) was set as the targeted parameter during production, and adjustments

of processing conditions were allowed in order keep product bulk density as specified above.

After processing stabilization, experimental diets were produced in cycling order over five

replicates each. Processing parameters and material out of the extruder were collected for each

replicate. Extruder mass flow rate was measured by collecting material out of the extruder in a

bucket for one minute. Product bulk density was measured using a one litter cup. Specific

mechanical energy (SME) was calculated according to the equation below:

$$\text{SME} \left(\frac{\text{kJ}}{\text{kg}}\right) = \frac{\frac{\tau - \tau_O}{100} * \left(\frac{N}{N_r}\right) * P_r}{m} \qquad (1)$$

Where τ is the % torque, or motor load, τ_o is the no-load torque (34%), N is the screw speed in

rpm, Nr is the rated screw speed (508 rpm), Pr is the rated motor power (37.3 kW), and m is the

total mass flow in kg/s. In-barrel moisture (IBM) was calculate as described below:

$$\text{IBM} (\%) = \frac{mf * Xf + mps + mpw + mes + mew}{mf + mps + mpw + mes + mew} \qquad (2)$$

Where mf is the dry feed rate, Xf is moisture content of the feed material, mps is the steam

injection rate in the preconditioner (kg/h), mpw is water injection rate in the preconditioner

(kg/h), mes is water injection rate in the extruder, mes is the steam injection rate in the extruder

(kg/h), and mew is the rate of water injected in the extruder.

The kibbles were dried in a double pass oven drier (Series 4800, Wenger Manufacturing,

Sabetha, KS) at104°C for 8 min each pass targeting a moisture content below 10%. Bulk density

of dried kibbles was measured in each replicate. Dried product was coated with chicken fat (4%),

dry palatant (1%), and titanium dioxide (0.4%), which was added as an external marker to

estimate apparent total tract digestibility.

2.2 Kibble characteristics

Kibble samples out of the drier were collected in each replicate in order to evaluate final product macrostructure characteristics. Twenty kibbles of each replicate were randomly selected, and their diameter, length, and weight were assessed to calculate piece density (ρ), sectional expansion ratio (SEI), and specific length (l_{sp}) as following:

$$\rho = \frac{4m_e}{\pi * l_e * d_e^2} \tag{3}$$

$$SEI = \frac{d_e^2}{d_d^2} \tag{4}$$

$$l_{sp}(m/kg) = \frac{l_e}{m_e} \tag{5}$$

A TA-XT2 Texture Analyzer (Texture Technologies Corporation, Hamilton, MA, U.S.A.) was used to determine kibble hardness and toughness. A total of fifteen kibbles per treatment were randomly selected for evaluation. A compression test was performed using a 25 mm cylindrical probe at a pre-test speed of 2 mm/s, test speed of 1 mm/s, a post-test speed of 10 mm/s, and strain level of 90%. The first peak fracture force was taken as a measure of hardness. Toughness was defined as the total energy required to break the sample at the specified strain level, and it was calculated as the total area under the fracture curve.

2.3 Palatability assessment

Experimental diets with no external addition of chicken fat (4%), dry palatant (1%), and titanium dioxide (0.4%) were used to assess palatability. The two-bowl method (Griffin, 2003) was performed with a trained dog panel consisting of twenty Beagle dogs at a commercial kennel

(Summit Ridge Farms, Susquehanna, PA). For two consecutive days, experimental diets were presented simultaneously to the dogs in separate bowls once a day for 30 minutes. Bowl position was switched in the next day to prevent side bias. The amount of food offered in each bowl exceeded the dog's daily energy requirement to allow leftovers. First choice (FC; first product eaten by the animal) was recorded by technicians, and intake ratio (IA) was calculated according to the formula below:

$$IA = \frac{consumption\ of\ diet\ A}{(consumption\ of\ diet\ A + consumption\ of\ diet\ B)} \qquad (6)$$

2.4 Digestibility assessment

The digestibility trial took place at the Large Animal Research Center (LARC) at Kansas State University where twelve castrated Beagle dogs (8 males, 4 females) of similar age, and initial body weight (12.56 kg $\pm$ 1.34, mean $\pm$ SD.) were used. Dogs were individually housed in cages (1.83m x 1.20m) equipped with an acrylic-mesh floor and a pan underneath to allow separation of feces and urine. All cages were located in a temperature (22-23°C) light-controlled (16h light:8h dark cycle) building. Food was provided twice daily (0800 and 1630 h) to maintain body weight. Food leftover was weighed at each meal, and food consumption was recorded. Daily metabolizable energy was calculated as an average for inactive dogs (ME, kcal/day= 95 x $BW^{0.75}$) according to the National Research Council (NRC, 2006). Body weight and body condition score (BCS) were measured biweekly, and food amount was adjusted accordingly. The BCS was assessed using a 1 to 9 points scale, were a score 1 represented an extremely cachectic animal, and a score 9 an extremely obese dog. A score 4 or 5 was considered ideal.

The study was conducted as a switch back design consisting of two periods of 9 days of acclimation to the diet followed by 5 days of fecal collection. Dogs were randomly assigned to experimental diets. Each dog received both diets at the end of the second period, and served as

its own control. After the 9 days of acclimation, feces were collected and scored on a 5-point scale increment wherein: 1 = watery; liquid that can be poured; 2 = soft, unformed stool; assumes shape of container; 3 = softer stool; retains shape; 4 = hard, formed stool; 5 = very hard, dry pellets. A 3.5 to 4 score was considered ideal. Fecal samples were stored in individual plastic bags, and frozen at -15°C until further analysis.

2.5 Digestibility Calculation

Feces were placed in an aluminum pan, and dried in an electric oven (Cat 52755-20, Matheson Scientific, Morris Plains, NJ) at 55°C until constant weight was achieved (24h-48h). Following drying, feces were ground through a 1-mm screen in a fixed blade laboratory mill (Retsch, type ZM200, Haan, Germany). Concentration of titanium was determined in fecal and food samples according to Myers et al. (2004). Absorbance values were read at 410 nm using a microplate reader (Synergy H1, Biotek, Winooski, VT, USA) Apparent total tract nutrient digestibility (ATTD) was calculated using the following equation:

$$\text{Nutrient digestibility} = \frac{[1-(\%Ti\ in\ food * \%nutrient\ in\ feces)]*100}{(\%Ti\ in\ feces * \%\ nutrient\ in\ food)} \tag{7}$$

2.6 Nutrient Analysis

Dry matter (DM; AOAC 930.15), organic matter (OM; AOAC 942.05), crude protein (AOAC 990.03), and fat by acid hydrolysis (AOAC 954.02) were analyzed in fecal and food samples in a commercial laboratory (Midwest Laboratories, Omaha, NE, U.S.A). Total dietary fiber (TDF; AOAC 985.29) was analyzed using a commercial kit (TDF-100A; Sigma-Aldrich; Saint Louis, MO, U.S.A.). Nitrogen-free extract was calculated by difference. Gross energy was determined with a bomb calorimeter (Parr Instrument Company, Moline, IL, USA)

Extrusion conditions, kibble macrostructure, and digestibility data were analyzed using the GLIMMIX procedure in SAS (SAS Inst. Inc., Cary, NC). For the digestibility experiment, diet was used as fixed effect, and animal nested within sequence was used as random effect. Means were separated using Fisher's LSD, and a probability of $P < 0.05$ was accepted as significant. In the palatability trial, first choice and intake ratio were analyzed using Chi^2 test and 2-way ANOVA, respectively.

3. Results

3.1 Diet formulation and production

Diets contained similar concentrations of most nutrients (Table 2.1). A lower CF content and a higher TDF content were reported for GF compared to AG diet (CF, 12.5 and 15.8%; TDF, 10.7 and 6.9 %, respectively). Similar bulk density out of the extruder was achieved through adjustment of the following parameters: preconditioner feed rate and steam were increased ($P<0.05$), and extruder screw speed was decreased ($P<0.05$) for AG compared to GF diet, respectively (Table 2.2). The IBM and SME input were 25.9 and 22.6% greater ($P<0.05$) for GF compared to AG, respectively. In addition, AG diet was processed at a faster knife speed (2381 vs. 1904 RPM) and had a lower mass flow rate compared to GF ($P<0.05$). Cone head pressure at the extruder barrel was greater ($P<0.05$) for AG than GF. Water injection into the preconditioner and into the extruder did not differ between diets.

3.2 Kibble characteristics

The bulk density of AG was greater (P<0.05) than GF diet after drying (389 vs. 367 g/L; Table 2.2). Accordingly, AG kibbles were heavier ($P<0.05$), and exhibited higher piece density ($P<0.05$) and lower sectional expansion index ($P<0.05$) compared to GF kibbles. Interestingly,

hardness was greater for GF compared to AG kibbles (6.36 vs. 3.12 $P<0.05$). Specific length and toughness did not differ between treatments.

3.3 Palatability and digestibility assessment

Food intake was similar between diets (Table 2.3). Dogs fed GF had a greater wet fecal output compared to those fed AG (69.57 vs. 59.60 g/d), and 15% decrease in fecal dry matter ($P<0.05$). No differences were observed among treatments for defecations per day, and fecal score. The IR results indicated a significant preference of dogs for GF over AG diet (IR of 0.84), and a first approach by dogs (37 vs. 3 times). No differences were observed for DM, OM, CP, CF, and gross energy digestibility between AG and GF (Table 2.3). Total dietary fiber digestibility was 32% greater for dogs fed the GF when compared to those fed AG.

4. Discussion

The first aim of this study was to evaluate the effects of ancient and grain free carbohydrate sources on extrusion parameters, and kibble characteristics. It was not our intention to evaluate single ingredients, but rather the overall effect of dog diets formulated with different carbohydrate sources in order to simulate the performance of commercial diets. Thus, only two diets were tested in this study. The results reported herein demonstrated that a similar bulk density out of the extruder could be achieved for AG and GF with minor processing adjustments. Processing difficulties have been reported during extrusion of tuber starches, mainly potato starch (Della Valle *et al*., 1995). Thus, to gain better control during the process, material feed rate into the preconditioner was 50.6 % lower for GF compared to AG. No challenges were faced during extrusion of experimental diets, which demonstrates that material feed rate can be used as a tool to closely monitor the process. Nevertheless, this may not be translated to a commercial scale where production needs to be at its maximum efficiency. Future studies may consider

keeping a constant feed rate in order to describe the challenges that might be experienced during extrusion of dog diets.

Steam injection into the preconditioner was decreased during extrusion of GF. Steam is the main source of thermal energy in the process due to vapor condensation on particle surfaces (Riaz, 2007), and impacts preconditioner temperature the most. As tubers and legumes gelatinize at lower temperatures when compared to cereal grains (Mishra *et al*, 2006; Waldt and Kehoe, 1959), steam was decreased in order to prevent complete gelatinization of these starches in the preconditioner. Moreover, tubers are known for their high swelling power compared to cereal grains (Swinkels, 1985). Greater swelling power indicates that more water is being bound by starch molecules, resulting in higher resistance to shear force and greater final viscosity (Wang *et al*., 2011). Further, potato starch has a high swelling power due to the high content of phosphate groups bound to amylopectin. The repulsive force between phosphate groups weakens the bonding within the starch crystalline domain, thus increasing hydration of starch granules (Galliard and Bowler, 1987). In order to decrease viscosity and increase material flow within the extruder barrel, screw speed was increased for GF. Under high shear condition, the viscosity of starch pastes decrease as the molecules are progressively oriented in the direction of flow, and the hydrogen bonds between amylose-amylopectin-water are ruptured (Cornell, 2004). The higher screw speed resulted in a more fluid mash inside the extruder for GF, and may explain the lower cone head pressure and motor load observed for this treatment. In an attempt to achieve similar kibble length, the knife speed was set at a lower RPM for GF due to the lower feed rate established for this treatment.

The SME and IBM are critical parameters for extrusion process, and are influenced by processing variables. The SME can be defined as the amount of frictional/mechanical energy

input per unit feed input or mass, and it is transferred to the dough due to the friction action between the material against the extruder screw and barrel. As a result, higher screw speed leads to higher SME (Riaz, 2007). Due to lower feed rate and higher screw speed, the SME input was higher for GF compared to AG. Domingues (2016) also reported an increase in SME with inclusion of potato starch in dog diets. The higher energy requirement during extrusion of potato starch and other tubers is a result of their high melt viscosity and early melting in the extruder compared to cereal grains (Vale et al, 1995).

On the other hand, IBM represents the moisture as a percentage of the total mass inside the system. Water acts as a plasticizer during extrusion (Guy, 2001) decreasing material viscosity, and friction between material and extruder. Consequently, an increase in moisture content within the system leads to a decrease in SME (Riaz, 2007). For example, Pacheco et al (2018) reported an inverse relationship between IBM and SME. However, this was not observed in our study. The lower feed rate and higher screw speed in GF compared to AG had a greatest impact on SME rather than IBM.

Water and steam addition into the system, as well as feed rate have an impact on IBM. Although steam input into the preconditioner was lower for GF, the IBM was higher for this treatment due to its lower feed rate. The difference in raw material composition of experimental diets used in our study required changes in processing conditions. As mentioned above, tubers required more water during processing as a result of their high swelling power, which also explains the higher IBM during extrusion of GF diet. Our findings are in agreement with Senouci and Smith (1986), who also reported a higher water addition during potato starch extrusion.

Processing conditions as well as raw material may impact kibble macrostructure, and consequently diet palatability. Although bulk density out of the extruder was similar for both

diets, it was lower for GF after drying. The higher IBM observed in GF may have resulted in a product out of the extruder with greater moisture content. This excess of water was probably removed during drying, resulting in a decreased bulk density. During extrusion, the material is under a high-pressure and high-temperature environment (Riaz, 2007). Upon exiting the die, the melted mash is exposed to ambient pressure and temperature, causing it to expand and solidify. The extruded material can expand both longitudinally and radially. These variables are assessed by l_{sp} and SEI calculations, respectively. To evaluate overall expansion of an extrudate, one should calculate piece density, as it considers both radial and longitudinal expansion. In our study, l_{sp} was similar among treatments, but a higher SEI was observed for GF kibbles. Thus, the lower bulk and piece density reported in GF kibbles are mainly due to their greater SEI. Also, bulk density has an inverse relationship with SME (Riaz, 2007). Consequently, the greater SME input in GF resulted in lower bulk and piece density of the final product compared to the AG.

Furthermore, SME can also impact cell structure, and consequently product texture. Hardness is a mechanical property commonly used in pet food to access product texture, and it is characterized by the material resistance to deformation. The higher input of SME during extrusion of GF led to harder kibbles. A smaller and more uniform cell structure is observed in extrudates as SME increases. Smaller cell walls reinforce each other, thus requiring more force to break the kibble (Dunsford *et al*., 2002). It was previously reported that increasing levels of potato starch in a dog diet required higher SME, and resulted in harder kibbles with greater number of cells (Domingues, 2016). Toughness is another way to assess product texture in which the total force to completely disintegrate the kibble is evaluated. In the current study, toughness was similar between treatments, although with the large numerical differences. Large variation in toughness was reported in previous studies (Alvarenga *et al*., 2018; Alveranga and Aldrich,

2019), and was attributed to the non-uniform air cells in the extrudate. Specific thermal energy (STE) can also influence kibble characteristics. Unfortunately, it was not possible to calculate this parameter in the current study. Not only processing conditions, but also raw material can impact final product characteristics. Potato and tapioca dried starch films have a greater internal and tensile strength compared to maize and wheat starch (Swinkels, 1985). However, since processing conditions were not kept constant between treatments in this study, it is difficult to evaluate the single effect of raw materials on final product traits.

The second aim of this study was to evaluate the effect of grain free and ancient grain carbohydrate sources on palatability and nutrient utilization by dogs. Uncoated diets were used to assess palatability. Although commercial diets typically undergo a coating step, where fat and other ingredients may be applied topically, our intention was to evaluate the intrinsic effects of ingredients on palatability alone. In our study, dogs exhibited a preference towards the GF diet. While sorghum, which was included in AG, has been associated with bitter and astringent notes (Kobue-Lekaleke *et al.*, 2007), Donfrancesco and Koppel (2017) reported that these characteristics can be reduced in the final product after extrusion. Moreover, sorghum has been widely used in dog diets with no reports of refusal. Thus, some component present in GF may be more attractive and palatable for the dogs. In another study, diets with higher inclusion of potato starch were also preferred by dogs (Domingues, 2016). Tubers release ribonucleotides after cooking as RNA is degraded. Ribonucleotides are precursors for umami compounds, and act as flavor enhancers (Jansky, 2010). Thereby, tubers might have an important impact on palatability. However, further studies should investigate the sensory characteristics of these ingredients, and their correlation with dog food palatability. It is noteworthy that processing conditions also play a role in palatability, and they were not kept constant between experimental diets. The higher

SME input for GF might have enhanced the flavor compounds in the diet due to higher degree of cooking. In accordance, Trivedi and Benning (2003) reported that dogs preferred diets processed with higher SME input. However, Dunsford et al (2002) showed that dogs had a higher preference towards a more thermally cooked diet while no preferences were observed by Pacheco et al (2018) when different levels of SME and STE were evaluated. In our study, the experimental diets were formulated with different raw materials, and were extruded under different conditions. Thus, a combination of factors may be playing a role on palatability beyond the SME:STE ratio.

Although dogs showed a higher preference towards GF in the palatability trial, no signs of refusal were observed for the AG diet during the digestibility study. However, diets were coated with fat and palatant before digestibility assessment, and this may improve overall diet acceptability or mask any off flavors. In our study, the ATTD of most nutrients was not affected by different starch sources. Carciofi et al (2008) reported a lower ATTD of DM, OM, and CP for dogs fed a diet containing pea compared to those fed cereal grain based diets. This was not observed in the present study. However, GF contained tapioca starch which was reported by the same author to be more digestible than those containing corn, sorghum, lentil, and pea. High digestibility for tapioca starch in dogs was also reported by Kamalu (1991). Furthermore, dried potato was reported to be highly digestible by dogs (Kendall and Holme, 1982). Despite these previous reports, the overall GF digestibility was similar for all nutrient measured with the exception of TDF. It must be noted though that only one level of grain free carbohydrate source was tested in this study. Evaluation of increasing levels of grain free carbohydrate sources may provide a better understanding of the impact of these ingredients on nutrient utilization.

The greater digestibility of TDF for GF may indicate a higher large intestinal fermentation of the fibers present in this diet compared to those in AG. This result is in accordance with other studies that also found a high digestibility of TDF for legume-based diets compared to grain-based ones (Carciofi *et al*., 2008). Legumes such as peas contain greater concentration of soluble fibers compared to cereal grains, which results in a higher TDF value for those ingredients (de-Oliveira *et al*., 2012; Bednar, 2000). Most soluble fibers are indigestible by the dog due to the lack of specific enzymes, but are readily fermented in the lower intestinal tract (Jezierny *et al*.,2010). Consequently, TDF digestibility increases as these fibers are converted to fermentative end products by colonic bacteria.

Moreover, fermentation of soluble fibers by colonic bacteria produces gases and short chain fatty acids (SCFA). Although SCFA can improve gut health and reduce inflammation (Sivaprakasam *et al*., 2016), their overproduction can attract water and sodium to the lumen due to their osmotic power and result in increasing fecal moisture content (Binder, 2010). Unfortunately, fecal pH and SCFA were not determined in the current study. These results could have provided a more concrete understanding with regards to lower bowl fermentation. Dogs fed GF diet exhibited higher wet fecal output, and lower fecal DM probably due to fermentation of soluble fibers in the lower gut. Carciofi et al (2018) also reported a lower fecal DM in dogs fed diets containing peas and lentil. Similar results were observed by Fahey et al (1990) who reported a linear increase in wet fecal weight as percentage of dietary beet pulp increased. Intriguingly, fecal score and number of defecations per day were not statistically different. High variation within treatments may explain the lack of significance; but, it is worth noting that dogs fed GF diet had on average one more defecation per day compared to those fed AG.

5. Conclusion

The two classes of carbohydrate sources behaved differently during extrusion; prompting changes to the processing parameters in order to produce diets with similar bulk densities. The GF diet had a greater energy and moisture requirement during extrusion compared to AG. This resulted in more expended and harder kibbles for GF over AG. Dogs exhibited similar digestibility of most nutrient, besides for TDF, which had a higher disappearance for those dogs fed GF. Moreover, a greater fecal output, which had higher moisture content, was observed for dogs fed the GF diet. Grain free and ancient grain carbohydrate sources were well utilized by dogs, but close attention should be given to the fiber content of GF diets in order to maximize fecal quality.

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Table 2-1 Ingredient composition of experimental rations processed by extrusion

Item	Treatments[1]	
	AG	GF
Ingredient composition (% on as-fed basis)		
Hydrolyzed pork protein	44.39	44.39
Potato, white	-	17.74
Peas, green	-	27.74
Tapioca starch	-	7.74
Spelt	17.74	-
Millet	17.74	-
Sorghum	17.75	-
Salt	0.51	0.51
Potassium Chloride	0.32	0.32
Choline Chlorine, 60% dry	0.25	0.25
Vitamin Premix	0.25	0.25
Dicalcium Phosphate	0.25	0.25
Calcium Carbonate	0.25	0.25
Trace mineral premix	0.18	0.18
Fish oil, Menhaden	0.13	0.13
Taurine	0.13	0.13
Natural antioxidant	0.13	0.13
Chemical composition (% on DM-basis)		
Moisture	5.41	5.49
Crude Protein	37.00	38.00
Crude Fat	15.8	12.5
Total dietary fiber	6.91	10.07
Insoluble fiber	5.25	3.85
Soluble fiber	1.66	6.22
Nitrogen-free extract (calculated)	34.22	35.34
Ash	4.24	4.33

[1]AG = Ancient Grain; GF = Grain-free.

Table 2-2 Processing parameters and kibble traits of dog diets formulated with different carbohydrate sources

Item	Treatment[1]		SEM	*P*=
	AG	GF		
Raw material				
Feed rate, kg/hr	166	82	2.24	<.0001
Preconditioner				
Temperature, °C	91.6	71.8	2.56	0.001
Steam injection, kg/h	14.46	5.5	0.61	<.0001
Water injection, kg/h	5.06	4.98	0.04	0.242
Extruder				
Water injection, kg/h	12.68	12.04	0.36	0.189
Extruder Screw Speed, RPM	442	637	0.84	<.0001
Knife Speed, RPM	2382	1905	73.84	0.002
Motor load	49	41	0.55	<.0001
Cone head pressure, PSI	460	188	16.59	<.0001
Mass flow, kg/hr	0.046	0.024	0.002	0.002
Bulk density, g/L	418	427	6.04	0.346
Other data				
Specific mechanical energy, kJ/kg	115	141	7.37	0.038
In-barrel moisture %	30.3	38.2	0.54	<.0001
Kibble traits				
Bulk density, g/L	389	367	3.84	0.0036
Weight, g	0.16	0.1	0.01	0.0004
Piece density, g/cm^3	0.6	0.54	0.01	0.0094
Specific length, cm/g	4.41	4.24	0.08	0.1744
Sectional expansion index	3.03	3.5	0.073	0.0052
Hardness, kg	3.12	6.36	0.22	<0.001
Toughness, kg*mm	2427	1778	220	0.0716

[1]AG = Ancient Grain; GF = Grain-free.

Table 2-3 Apparent total tract digestibility determined by estimates of fecal output using titanium dioxide as an external marker of dogs fed ancient grains and grain free diets

Item	Treatment[1]		SEM	$P=$
	AG	GF		
Feed intake and fecal characteristics				
Feed intake, g/day	151.0	149.0	4.17	0.664
Wet fecal output, g/day	59.6	69.57	2.66	0.007
Fecal DM, %	33.9	28.8	0.45	<.0001
Defecations per day	1.44	2.56	0.47	0.074
Fecal Score	3.15	3.33	0.07	0.099
ATTD[2], %				
Dry Matter	85.8	85.8	0.53	0.939
Organic Matter	87.7	87.0	0.48	0.259
Energy	87.5	87.3	0.48	0.660
Crude Protein	88.1	87.2	0.48	0.155
Crude Fat	93.1	93.6	0.21	0.133
Nitrogen-free extract	96.2	96.1	0.39	0.815
Total Dietary Fiber	39.3	51.8	2.33	0.026

[1]AG = Ancient Grain; GF = Grain-free; [2]Apparent total tract digestibility.

Chapter 3 - Characterization of white and red sorghum flour and their potential use for production of extrudate crisps

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Highlights

- Flour yield was similar between white and red sorghum

- White and red sorghum flour had subtle differences in nutritional composition and gelatinization temperatures

- White and red sorghum flour behaved in a similar manner during extrusion, and extrudates had similar macrostructure and textural characteristics

Abstract

Consumers are searching for alternative foods that are nutritious and provide health benefits. In the human food industry, the wheat-free market sales have increased over the years due to awareness of wheat allergy. Sorghum is a gluten-free grain with great potential to address shortcoming in this market. The objective of this study was to evaluate the milling process and flour quality of white and red sorghum, and evaluate extrusion as a potential process to produce sorghum crisps. White and red sorghum grain were milled into flour in three production cycle replicates. Flour quality was evaluated by determination of nutritional composition, pasting, and thermal profile. Extrusion processing of white and red sorghum flour was performed in three replicate-days and macrostructure of final product was evaluated. White and red sorghum yielded similar flour content. Chemical analysis revealed a higher protein and lower starch content for white sorghum than red sorghum flour ($P<0.05$), but their pasting properties did not differ. Initial and peak gelatinization temperatures were higher ($P<0.05$) for red sorghum compared to white sorghum flour. This difference did not impact the extrusion conditions necessary to produce sorghum crisps. Both white and red sorghum flour were extruded under similar extrusion parameters, and resulted in similar macrostructure characteristics of extrudate crisps. In conclusion, although the differences in nutritional and thermal properties observed did

not require changes to extrusion parameters in order to produce sorghum crisps with similar characteristics.

Graphical abstract

Graphical abstract is presented in Figure 3.2.

Abbreviations

BV, breakdown viscosity; FV, final viscosity; HRFM, hal ross flour mill; IBM, in-barrel moisture; PT, pasting temperature; PV, peak viscosity; RSC; red sorghum crisp; RSF, red sorghum flour treatment; SBV, set back viscosity; SEI, sectional expansion index; SME, specific mechanical energy; TV, trough viscosity; T_c, conclusion temperature; T_o, onset temperature; T_p peak temperature; wb, wet basis; WSC, white sorghum crisp; WSF, white sorghum flour treatment; ΔH, enthalpy of gelatinization.

*Keywords***: s**orghum; milling, flour quality, extrusion, expanded crisp.

1. Introduction

Growth in the human and pet food industry is driven by the addition of new products into the market. This often necessitates the use of new ingredients, and the development of new food forms, and processes. More than ever consumers are demanding foods that provide optimal health benefits for themselves and for their pets. For example, the wheat-free food market has edged up due to consumers concern regarding celiac disease, gluten sensitivity, and wheat allergy (Liu *et al*., 2012). Although these are diagnosed in a small percentage of the human (Weiser and Koehler, 2008) and dog population (Verlinden *et al*., 2007), gluten-free foods now

account for an important part of the market. This represents an opportunity for evaluation of novel wheat-free, gluten-free, and alternative ingredient recipes.

Sorghum is the fifth most important cereal crop grown in the world and the third most important in the United States (US. Grains Council, 2012). It has a great potential for the gluten-free market and as a healthy alternative ingredient. Some sorghum varieties are rich in phytochemicals such as phenolic acids and condensed tannins which are known to have antioxidant and antiradical activities (Hagerman *et al*., 1998). Sorghum can be milled into flour which can be used as a major ingredient for many food applications. The use of sorghum flour has been evaluated in different systems such as cookies (Morad *et al*., 1984), breads (Schober *et al*., 2005), noodles (Liu *et al*., 2012), and tortillas (Winger et al., 2014). A recent study reported that extruded sorghum flour reduced adipogenic genes, chronic inflammation, and weight gain in obese rats (Arbex *et al*., 2018). Development of extruded sorghum flour crisps may create a new market for this grain in the human and pet food industry. However, limited information is available on extrusion of sorghum flour. Establishing food safety and the processing conditions necessary to create a consistent extrudate is essential to insert the product into the market. Moreover, characterization of the milling process and flour quality will aid in understanding the functionalities of the raw material and provide meaningful information to the industry regarding process optimization. Thus, the objective of this study was to characterize the milling process and flour quality of white and red sorghum, to determine the extrusion parameters necessary to create consistent white and red sorghum crisp, and to evaluate the presence of *Salmonella* in the process and final products.

2. Material and methods

2.1 Grain procurement and milling

White and red sorghum (2017 crop year) were sourced from a local farmer (Kearny County, KS, U.S.A) and milled at Hal Ross flour mill (HRFM, Kansas State University, Manhattan, KS, U.S.A). Each sorghum was converted to flour each of three days totaling three replicates per treatment. Before milling, grains were cleaned, then tempered for 19.5 h with moisture increased to 16.5 ± 1.5 % (wb). The flour milling process consisted of 5 break (BK) passages, 2 sizing passages, 6 reduction passages, one quality and one tailing passage, and four purification passages. The first BK was set to net 25% release while the second and third break were set to net 75% release. Reduction roll settings were adjusted according to previous experiments (Alvarenga *et al.*, 2018). Flour mill flow sheet can be found in Figure 3.1. Flour yield was calculated as follow:

$$Flour\ yield\ (\%)\ \frac{total\ flour\ weight\ (kg)}{total\ sorghum\ to\ the\ mill\ (kg)} \tag{1}$$

White and red sorghum grain samples were collected out of the storage silo, and the tempering bin on each milling day. The moisture content of unground grain was determined using the drying oven method: 10 g in a drying oven at 130 °C for 18 h (ASABE S352.2, 1997).

2.2 Flour characterization

Upon process stabilization within each replicate production cycle, flour samples were collected every 30 minutes totaling three subsamples per day for each sorghum variety. Subsamples were composited and prepared for analysis as described below.

2.2.1 Nutrient analysis

Flour samples were evaluated for moisture (AOAC 930.15), crude protein (AOAC 990.03), crude fat (AOAC 2003.05), ash (AOAC 942.05), total dietary fiber (AOAC 991.43, mod), and minerals including calcium, phosphorus, potassium, magnesium, sodium, sulfur, copper, iron, manganese, and zinc (AOAC 985.01; mod) at a commercial laboratory (Midwest Laboratories, Omaha, NE). Total starch and damaged starch were analyzed with commercial kits (Megazyme International Ltd, Wicklow, Ireland).

2.2.2 Pasting profile

Flour pasting properties were determined using a Rapid Visco Analyser (RVA, Perten Instruments AB, Hargersten, Sweven) following AACCI Method 76-21.01. Wherein, the flour sample (3.5 ± 0.1 g) was placed in an aluminum canister with distilled water (25 ± 0.1 ml) and moisture was adjusted up to 14%. The paddle was placed into the cannister, and the assembly was inserted into the RVA. The pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), set-back viscosity (SBV), and peak time were (Themocline for Windows software).

2.2.3 Thermal properties

Thermal transition temperatures of gelatinization for white and red sorghum flours were assessed using a differential scanning calorimeter (DSC; Q100, TA Instruments, New Castle). Flour samples were weighed (7 ± 2 mg) in an aluminum pan with addition of distilled water (2:1, water/flour, wt/wt). Samples were heated from 10°C to 140°C at a rate of 10°C/min. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures, and the enthalpy of gelatinization (ΔH) were determined.

2.3 Extrusion process

Red sorghum flour (RSF) and white sorghum flour (WSF) were processed over three days using a single screw extruder (model E525, ExtruTech, Inc., Sabetha, KS, USA). Each day was considered a replicate totaling three replicates per treatment. Preconditioner shaft speed was set at 145 RPM with paddle configuration as follow: 1 beater in 45° forward, 49 beaters in neutral, 12 beaters in 45° reverse, 8 beaters in neutral, and 1 wiper. Extruder screw profile can be found in Figure 3.3. Extruder screw speed and knife speed were set a 425 and 1,500 RPM, respectively. One wearplate with twelve cylinder openings (1.1mm x 4.7 mm) was used. The extrudates were dried in a dual pass dryer for 7 min at 87°C. The operator was allowed to modify processing parameters in order to achieve similar bulk density out of extruder (~ 100g/L) for both treatments. Upon process stabilization, processing parameters were collected every 15 minutes, as well as product samples out of the extruder and out of the dryer. The total mass flow out of the extruder was calculated by summing the feed rate (kg/hr), steam injection (kg/hr), and water injection (kg/hr) into the system. Product bulk density was measured using a one-liter cup. Specific mechanical energy (SME) was calculated as follow:

$$\text{SME}\left(\frac{kJ}{kg}\right) = \frac{\frac{\tau - \tau_O}{100} * \left(\frac{N}{N_r}\right) * P_r}{m} \tag{2}$$

Where τ is the % torque, or motor load, τ_O is the no-load torque (34%), N is the screw speed in rpm, N_r is the rated screw speed (508 rpm), P_r is the rated motor power (37.3 kW), and m is the total mass flow in kg/s. In-barrel moisture (IBM) was calculated as described below:

$$\text{IBM}(\%) = \frac{mf * Xf + mps + mpw + mes + mew}{mf + mps + mpw + mes + mew} \tag{3}$$

Where mf is the dry feed rate, Xf is moisture content of the feed material, mps is the steam injection rate in the preconditioner (kg/h), mpw is water injection rate in the preconditioner

(kg/h), mes is water injection rate in the extruder, mes is the steam injection rate in the extruder

(kg/h), and mew is the rate of water injected in the extruder.

2.4 Extrudate characteristics

Sixty extrudates from each replicate were randomly selected to assess macrostructure

characteristics. Length and width were measured using a digital caliper, and weight of the same

extrudates was recorded using an analytical scale (EX324N; OHAUS Corporation, Parsippany,

NJ, U.S.A). Diameter was defined as the distance between the two parallel planes restricting the

extrudate perpendicular to that direction, and thus length of the extrudate was considered the

diameter for calculating sectional expansion index (SEI) according to the formula bellow:

$$\text{SEI} = \frac{d_e^2}{d_d^2} \tag{4}$$

Where d_e is extrudate diameter, and d_d is die diameter.

Extrudate hardness and crispness were determined using a Texture Analyzer (Model TA-

XT2; Texture Technologies Corporation, Hamilton, MA, U.S.A.). Twenty one extrudates from

each replicate were randomly selected for mechanical property assessment. Extrudates were kept

in a drying over overnight at 40°C to equilibrate moisture content. The first peak fracture force,

and the number of positive peaks were taken as a measure of hardness and crispness,

respectively. A compression test was performed using a 25 mm cylindrical probe at a pre-test

speed of 2 mm/s, test speed of 2 mm/s, a post-test speed of 10 mm/s, and strain level of 90%.

2.5 Microbiological testing

Five sites at the HRFM were tested for Salmonella before and after production. Samples

were collected with a sponge-stick pre-soaked in 10 mL buffered peptone water (BPW; 3M, St

Paul, MN) in an area of 5X5 inches. Samples were brought to the laboratory within one hour

from collection. Each sample was enriched with 50 mL of BPW (1:6 sample/ BPW, wt/wt), and

incubated at 37°C for 24 hours. Flour and extrudate samples from each sorghum variety were also tested for *Salmonella* in each replicate. A portion of sample (25g) was mixed with 225 ml of BPW and incubated for 20-24 h at 37°C. After the incubation, environmental, flour and extrudate samples were proceeded for *Salmonella* isolation and identification according to the standard culture method from Bacteriological Analytical Manual (BAM, 2011).

2.6 Statistical design and analysis

The study was designed as a completely randomized block designed with day of production as a blocking factor. Total of three replicates per treatment were achieved. Analysis of variance was conducted using the GLIMMIX procedure in statistical software (SAS 9.4 Inst. Inc., Cary, NC). Sorghum variety was used as a fixed effect while day was considered a random effect. Means were separated using Fisher's LSD, and a probability of $P < 0.05$ was accepted as significant.

3. Results

3.1 Milling

Tempering time was sufficient to bring the grain moisture from 13.74 + 0.41 to 15.90 $\pm$ 0.99 % (wb), and from 13.38 $\pm$ 0.31 to 15.21 $\pm$ 0.73 % (wb) for WS and RS, respectively. The flour yield was similar (*P*<0.05) for WS and RS (59.03 $\pm$ 8.676 and 57.02 $\pm$ 2.67 %, respectively). A lower concentration of total starch was observed for WSF versus RSF (83.81 and 88.15%, respectively). No differences were observed for damaged starch and ash content between WSF and RSF (*P*>0.05).

3.2 Flour characterization

Nutritional composition of sorghum flours is reported on dry matter basis in Table 3.1. A higher content of crude protein (9.95 and 8.22%) was observed for WSF compared to RSF,

respectively. Total potassium and copper concentration were greater for WSF ($P>0.05$). No differences were observed for the other nutritional parameters between WSF and RSF ($P>0.05$). Although visual differences were noted in the RVA profile graphic (Figure 3.4), pasting variables were not significantly different between WSF and RSF ($P>0.05$; Table 3.2). On the other hand, temperatures of gelatinization were different between WSF and RSF (Table 3.3). The results revealed a 3.22°C and a 1.47°C increase ($P<0.05$) in To and Tp, respectively for RSF while Tc and ΔH were not different from WSF.

3.3 Extrusion process and extrudate characteristics

Bulk density out of the extruder was within target specifications, and similar ($P>0.05$) for white sorghum crisps (WSC) and red sorghum crisps (RSC; Table 3.4). Extrusion conditions were kept constant between WSF and RSF ($P>0.05$), and no significant differences between the treatments were observed for variables listed. Accordingly, WSC and RSC exhibited similar values for length, width, weigh, and SEI ($P>0.05$), and for textural properties ($P>0.05$).

3.4 Microbiological testing

Environmental, flour, and extrudate samples were negative for *Salmonella* as all samples failed to produce typical colonies on selective agars (xylose lysine desoxycholate and bismuth green sulfa).

4. Discussion

The white and red sorghum varieties did not require adjustments during milling to yield similar flour content. The flour yields reported herein are higher than those obtained in laboratory scale by Alvarenga et al (2018) and Moraes et al (2015), demonstrating that commercial milling scale is more efficient than laboratory milling. The flour yield reported in our study was lower compared the those observed by Alvarenga et al (2018) when red sorghum

was milled at commercial scale. The authors milled red sorghum at HRFM and obtained 69.2% yield for flour. Whereas Alvarenga et al (2018) produced their flour in one long production test while in our study each sorghum variety was milled on three short term replicate-days. Shorter milling runs in the current study probably compromised efficiency leading to low flour yield. However, this was necessary to achieve replicates and generate data regarding variation around the process. To our knowledge, this is the first study to report the milling of sorghum in replicates allowing statistical comparison between grain variety.

Characterization of different sorghum flours is important to determine the most suitable application for each ingredient according to its nutritional and physiochemical properties. In the current study, white sorghum and red sorghum produced in western Kansas in 2017 were evaluated. However, it is noteworthy that hybrids within each sorghum variety may have different nutritional and physiochemical properties. The evaluation of WSF and RSF derived from a higher range of grain hybrids produced in Kansas would provide a better characterization of the local grain market. However, it is challenging to produce processing replicates with a high number of treatments. Thus, it was decided to first evaluate one hybrid from each white sorghum and red sorghum to better characterize the milling process.

The nutritional differences between WSF and RSF agree with other studies that also reported differences in nutritional composition among sorghum hybrids (Shober *et al*., 2005; Liu *et al*., 2012; Winger *et al*., 2014; Palavecino *et al*., 2016). The range for protein contents (8.22 to 9.95 %) are comparable to those found in the literature (Shober *et al*., 2005; Liu *et al*., 2012; Winger *et al*., 2014; Palavecino *et al*., 2016). The higher protein content measured for WSF was probably due to genetic factors. Total starch, damaged starch, and ash flour content are directly related to the milling process. The total starch results were higher than those of Liu et al (2012)

and Winger et al (2014) who reported starch concentration of 78.63% and 72.5%, respectively. The same authors observed a lower damaged starch content (2.7 – 6.89 %) compared those reported in the present study. The severity of the milling process (Frederick, 2009), as well as the grain quality, and its preparation can also affect the formation of damaged starch (Arya *et al*., 2015). Harder and larger kernels require more energy input to mill the grain resulting in more deterioration of the starch molecule (Martin *et al*., 2007). Ash is also an important aspect to consider when evaluating flour quality. It is an indication of germ and bran contamination during milling (Kim and Flores 1999). The ash content observed for WSF and RSF were similar to those of previous studies (Liu *et al*., 2012; Winger *et al*., 2014; Palavecino *et al*., 2016). The similar content of damaged starch and ash between WSF and RSF suggest that the grain hybrids used in this study would not require different milling conditions in order to produce sorghum flour. Even though ash content was similar between WSF and RSF, differences in mineral content were observed. The WSF had a higher concentration of total potassium and total copper than RSF. To our knowledge, this is the first study to report and compare mineral content in sorghum flour hybrids. Although differences in chemical composition were observed for WSF and RFS in this study, Palavecino et al (2016) evaluated twenty sorghum varieties and did not attribute the high degree of variability in chemical composition among hybrids due to color of sorghum grain. The nutritional differences between WSF and RSF observed in our study might be due to genetic and environmental factors rather than color of the grain. This can also partially explain the inconsistent results from the literature regarding nutritional composition of sorghum hybrids.

The RVA profile of a sample reflects its physicochemical property (Shibanuma *et al*., 1996). The pasting characteristics observed for WSF and RSF are comparable to those found by

Palavecino et al., 2016. Viscosity parameters are usually a function of starch properties. However, flour samples are composed of other nutrients that can affect their viscosity profile. Although RSF and WSF exhibited different starch and protein content, they resulted in similar pasting properties. The RSF had a higher numerical value for PV and SBV, and PT. Boundries et al (2009) observed higher PV for red sorghum flour compared to white sorghum flour and attributed this difference due to environmental and genetic conditions. Statistically differences were not observed in the current study due to high variably within treatments. This suggests that a larger number of samples may be required to better characterize pasting properties of sorghum flour. Thermal properties of starches can be assessed by DSC, and are influenced by a number of factors such as degree of crystallinity, amylose, and amylopectin structure (Wang *et al*., 2011). These parameters provide a better understanding of thermal properties; however, none of these were assessed in this study. Gelatinization temperatures and enthalpy for WSF and RSF are within the range reported in the literature (Palavecino *et al*., 2016; Boudries *et al*., 2009) The RSF exhibited higher *To* and *Tp* compared to WSF which is in agreement with results obtained by Boudries et al (2009). This may be due to higher degree of crystallinity and higher amylose content for RSF, which hinders water absorption and heat penetration by starch molecules due to a better packing structure. Moreover, the higher PT observed in the pasting profile for both sorghum flour varieties compared to their *To* are in agreement with Palavecino et al (2016). The PT temperature is defined by the initial increase in viscosity due to granule disruption while the *To* indicates the start of granule swelling. This indicates that starch particles gelatinize before the increase in viscosity (Liang and King, 2003).

Extrusion is a high temperature, short time process in which food materials are thermo-mechanically cooked under a combination of temperature, pressure, moisture and mechanical

shear (Riaz, 2007). In the current study, extrusion was evaluated as a potential process to produce extruded crisps from WSF and RSF. It was our intention to remove bran during the milling of WS and RS in order to decrease the fiber content and produce well expanded crisps with a smooth texture. Insoluble fibers can rupture the cell walls and prevent air bubbles from expanding (Anton *et al*., 2009). They also have a better affinity to water than starch, restricting water loss at the die and compromising expansion. (Bisharat *et al*., 2013). The higher starch and lower protein content of RSF compared to WSF did not require different extrusion process parameters. The IBM reported in the current study is higher than those reported in the literature for production expanded products. Mesquita et al (2013) evaluated the effect of moisture content on extrusion of sour cassava starch and flaxseed flour blends up to 20% moisture, and they achieved a good product when moisture was added at 12%. A similar feed moisture content (16 – 21%) was reported by Baik et al (2004) when evaluating extrusion of barley flour for production of expanded cereals. However, the previous authors did not have a pre-conditioner in advance of the extrusion process. The high steam addition into the pre-conditioner resulted in high IBM in our study. This was required to hydrate the material and improve its fluidity as the small particle size of flour can impair flow and cause clumping. The lubricating effect of water can decrease shear, and thereby reduce SME input and product expansion. However, both WSC and RSC expanded well, and had a high SEI. This may be due to the high temperature at the end of the barrel, high screw speed, and aggressive screw configuration. Baik *et al* (2004) and Ačkar *et al* (2018) reported lower SEI of barley flour expanded cereal and corn extrudates, respectively. The different experimental setups and equipment used in these reported studies make it difficult to directly compare results among studies. Although not statistically different, extrusion of RSF exhibited higher SME and led to a less dense product. This variance may be a result of

inconsistent feed rate into the system. This could have been assessed my measuring material flow rate out of the extruder rather than calculating it. Steam losses and inconsistent delivery of material were not considered when mass flow rate was assessed in this study. This could potentially overestimate actual mass flow rate. A lower feed rate for RSF into the system may have resulted in the higher numerical SME, even though it was not statistically different from WSF.

Hardness and crispness are important texture measurements to be assessed in expanded products. They are related to expansion and cell structure developement within the starch matrix during extrusion (Smith and Hardacre, 2011). In this study, hardness was defined as the peak force required to disintegrate the extrudate, and crispness was defined as the number of positive peaks during deformation of the material by the probe. Hardness of the product has been positively correlated with addition of water into the system (Liu *et al*., 2000). On the other hand, increasing feed moisture content was associated with decreased crispness of rice extrudates (Ding *et al*., 2005). As both WSC and RSC were produced under the same processing conditions, no differences were observed between texture properties. Duizer and Winger (2006) evaluated hardness and crispness of corn-based crisps using a trained sensory panel, and with a bite force apparatus. In their study, a negative relationship was observed between the maximum bite force and crispness of extrudates. The authors also found a higher value for hardness and crispness compared to our results. This could be due to the different methodologies used between studies. The use of trained panelists to evaluate product characteristics could have provided more detailed information regarding sensory and textural aspects of white and red sorghum crisps. For example, detailed properties of crispness such as sound production during crushing the product

could have been assessed. Future studies should consider the use of trained panelists in addition to evaluation with a texture analyzer to yield a better understanding of sensory properties.

Microbiological assessment of flour mill and end products – flour and crisp – was performed to assure food safety. An outbreak of Salmonella Typhimurin Phage Type 42b was reported in New Zealand due to contamination of raw wheat flour with these bacteria (McCallum *et al*,, 2013) . Thus, it is important to monitor microbial contamination during processing. In the current study, environmental and food samples were all negative for Salmonella indicating that the production chain of sorghum crisp was completely free from this group of bacteria. It is noteworthy that extrusion is considered a kill step due to its high temperature, but extruded product may be contaminated post extrusion during the drying and bagging steps. Purchasing grains from trustworthy supplies, and monitoring processing conditions are examples of practices to prevent contamination in the process.

5. Conclusion

Characterization of milling process of different sorghum hybrids, as well as flour quality are essential to establish the product in the market. In the current study the milling of red and white sorghum yielded similar flour content, but differences were observed between chemical composition and thermal properties of respective flours. White and red sorghum flour required similar extrusion conditions in order to produce expanded sorghum crisp, thereby extrudates exhibited similar macrostructure and textural characteristics. Sorghum crisps derived from extrusion of white and red sorghum flour are promising products to boost the use of sorghum in the market.

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Table 3-1 Nutrient analysis on dry matter basis of red and white sorghum flour milled at Hall Ross Flour Mill (mean $\pm$ SD[1])

Property	White Sorghum Flour	Red Sorghum Flour
Moisture, %	11.96 $\pm$ 0.19	12.28 $\pm$ 0.47
Dry matter, %	88.04 $\pm$ 0.19	87.72 $\pm$ 0.47
Crude Protein, %	9.95[a] $\pm$ 0.27	8.22[b] $\pm$ 0.16
Crude Fat, %	2.69 $\pm$ 0.36	3.03 $\pm$ 0.23
Total Starch, %	83.81[b] $\pm$ 0.63	88.15[a] $\pm$ 2.07
Damaged Starch, %	9.40 $\pm$ 1.54	8.43 $\pm$ 0.66
Total Dietary Fiber, %	2.47 $\pm$ 0.15	2.47 $\pm$ 0.15
Ash, %	1.26 $\pm$ 0.27	1.01 $\pm$ 0.20
Sulfur (total), %	0.09 $\pm$ 0	0.09 $\pm$ 0.055
Phosphorus (total), %	0.38 $\pm$ 0.03	0.30 $\pm$ 0.02
Potassium (total), %	0.37[a] $\pm$ 0.02	0.32[b] $\pm$ 0.03
Magnesium (total), %	0.16 $\pm$ 0.006	0.13 $\pm$ 0.03
Calcium (total), %	0.017 $\pm$ 0.006	0.017 $\pm$ 0.006
Iron (total), %	25.20 $\pm$ 1.91	28.93 $\pm$ 4.20
Manganese (total), ppm	20.17 $\pm$ 1.35	14.73 $\pm$ 3.52
Copper (total), ppm	2.37[a] $\pm$ 0.23	1.73[b] $\pm$ 0.21
Zinc (total), ppm	13.20 $\pm$ 1.28	13.13 $\pm$ 2.37

[a–b] Means with different superscripts within a row indicate significant difference ($P < 0.05$)
[1] Standard deviation

Table 3-2 Pasting characteristics of white and red sorghum flour (mean $\pm$ SD[1])

Property	White Sorghum Flour	Red Sorghum Flour
Peak Viscosity, cP	3769 $\pm$ 225	4021 $\pm$ 181
Trough Viscosity, cP	1535 $\pm$ 78	1678 $\pm$ 111
Breakdown Viscosity, cP	2234 $\pm$ 196	2343 $\pm$ 136
Final Viscosity, cP	3291 $\pm$ 120	3534 $\pm$ 139
Set Back Viscosity, cP	1570 $\pm$ 552	1856 $\pm$ 61
Peak time, min	4.76 $\pm$ 0.11	4.66 $\pm$ 0.11
Pasting Temperature,°C	66.23 $\pm$ 11.20	69.57 $\pm$ 6.25

[1] Standard deviation

Table 3-3 Thermal properties of white and red sorghum flour (mean ± SD[1])

Thermal property	White Sorghum Flour	Red Sorghum Flour
Initial temperature, °C	63.34[a] ± 0.45	66.56[b] ± 0.92
Peak temperature, °C	72.42[a] ± 0.87	73.89[b] ± 0.64
Conclusion temperature, °C	90.86 ± 2.81	89.01 ± 1.83
Enthalpy, J/g	6.49 ± 0.34	6.36 ± 0.04

[a–b] Means with different superscripts within a row indicate significant difference ($P < 0.05$)
[1] Standard deviation

Table 3-4 Processing data of extruded white and red sorghum flour (mean ± SD[1])

Extruder property	White Sorghum Flour	Red Sorghum Flour
Pre-conditioner		
Water, kg/h	11.43 ± 0.46	12.13 ± 0.34
Steam, kg/h	59.11 ± 2.48	59.41 ± 2.87
Temperature, °C	48.15 ± 2.12	47.28 ± 0.89
Extruder		
Motor load, A	33.93 ± 0.47	34.63 ± 0.81
Die Temperature, °C	141.48 ± 21	133.95 ± 12
Mass Flow, kg/h	159.34 ± 0.64	160.04 ± 0.62
Other data		
SME[2], kJ/kg	114.30 ± 3.33	131.30 ± 5.67
IBM[3] (%)	40.71 ± 0.72	40.99 ± 0.82
Bulk density OE[4], g/L	108.11 ± 23.50	95.08 ± 19.47
Bulk density OD[5], g/L	106.70 ± 21.46	92.16 ± 23.73
Extrudate traits		
Length, mm	12.27 ± 1.25	12.37 ± 1.62
Width, mm	3.98 ± 1.06	4.08 ± 1.23
Weight, g	0.029 ± 0.01	0.033 ± 0.03
SEI	6.88 ± 1.39	7.04 ± 1.64
Hardness, N	12.95 ± 4.92	14.34 ± 4.11
Crispness[6]	46.62 ± 21.06	44.38 ± 17.38

[1] Standard deviation; [2] Specific Mechanical Energy; [3] In-Barrel Moisture; [4] Out of the Dryer; [5] Out of the Extruder; [6] Number of positive peaks.
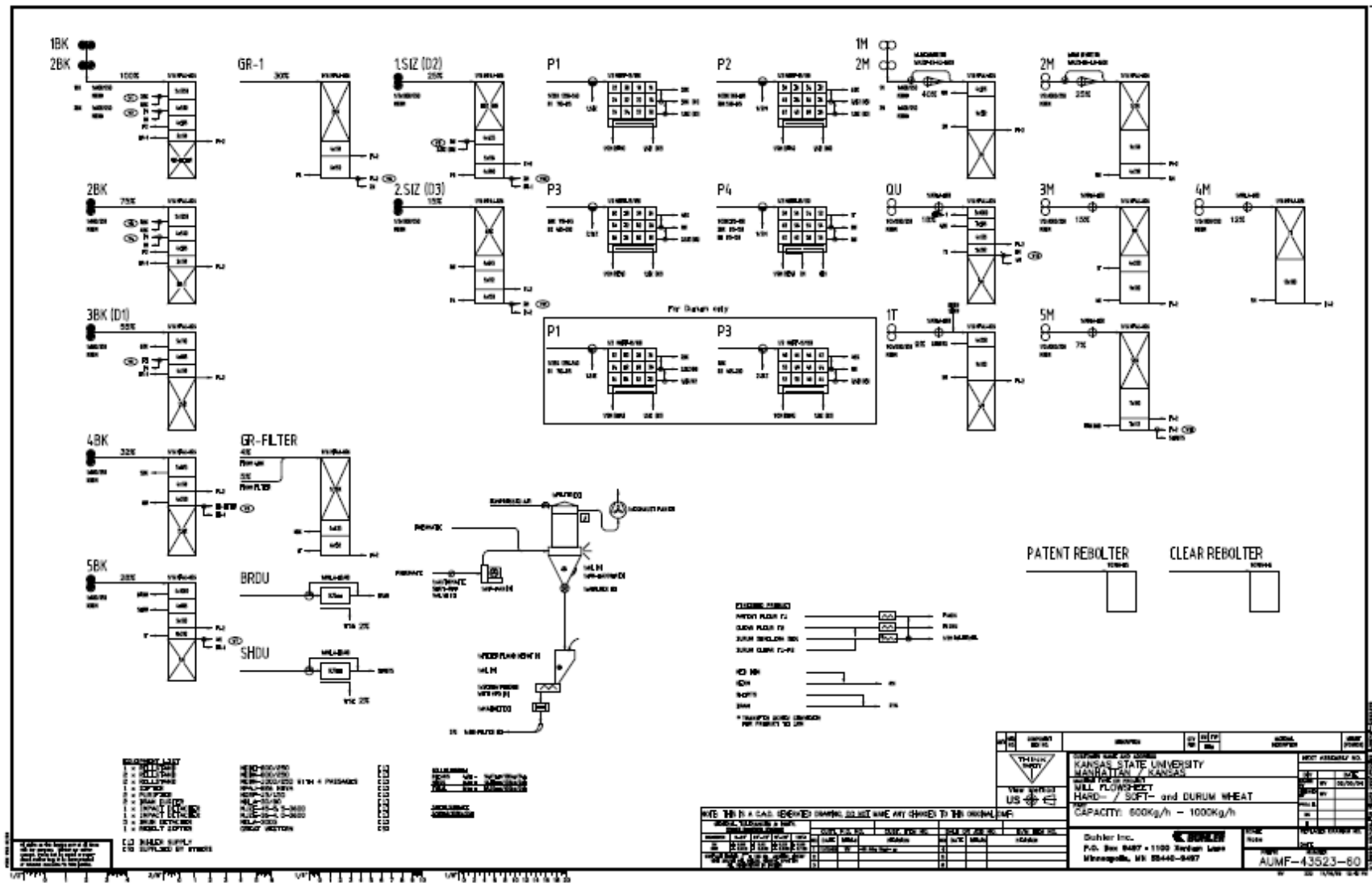
Figure 3-1 Hall Ross Flour Mill Flow Sheet
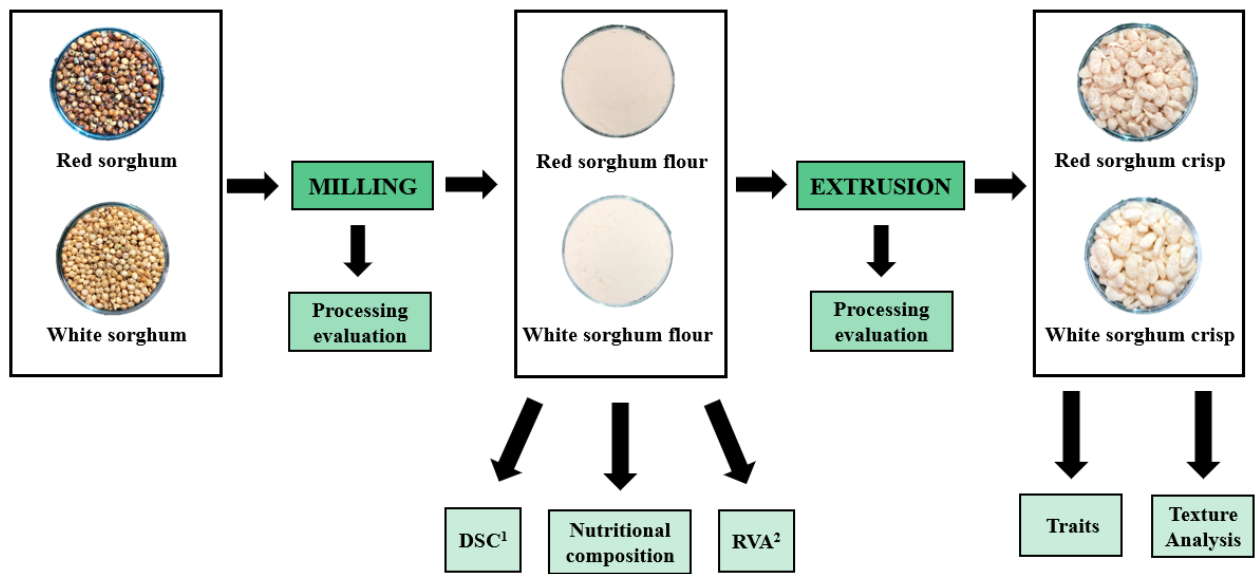
[1]Differential Scanning calorimetry

[2]Rapid Visco Analyzer

Figure 3-2 Graphical abstract
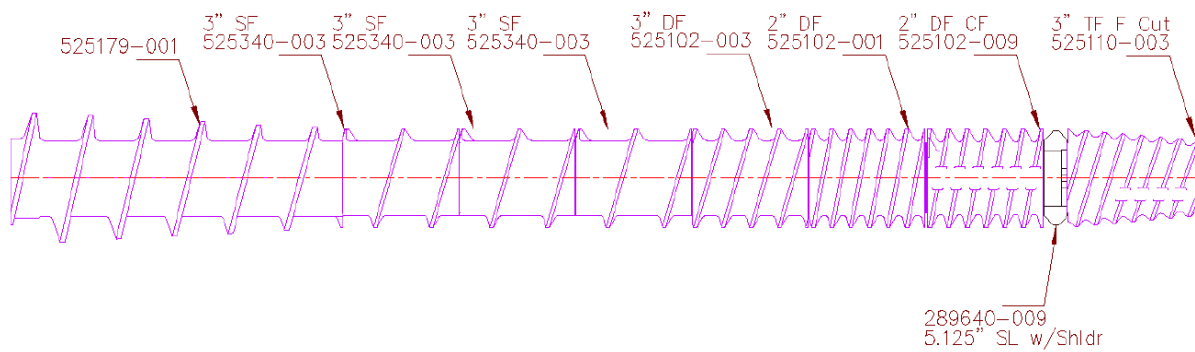
Figure 3-3 Extruder screw profile
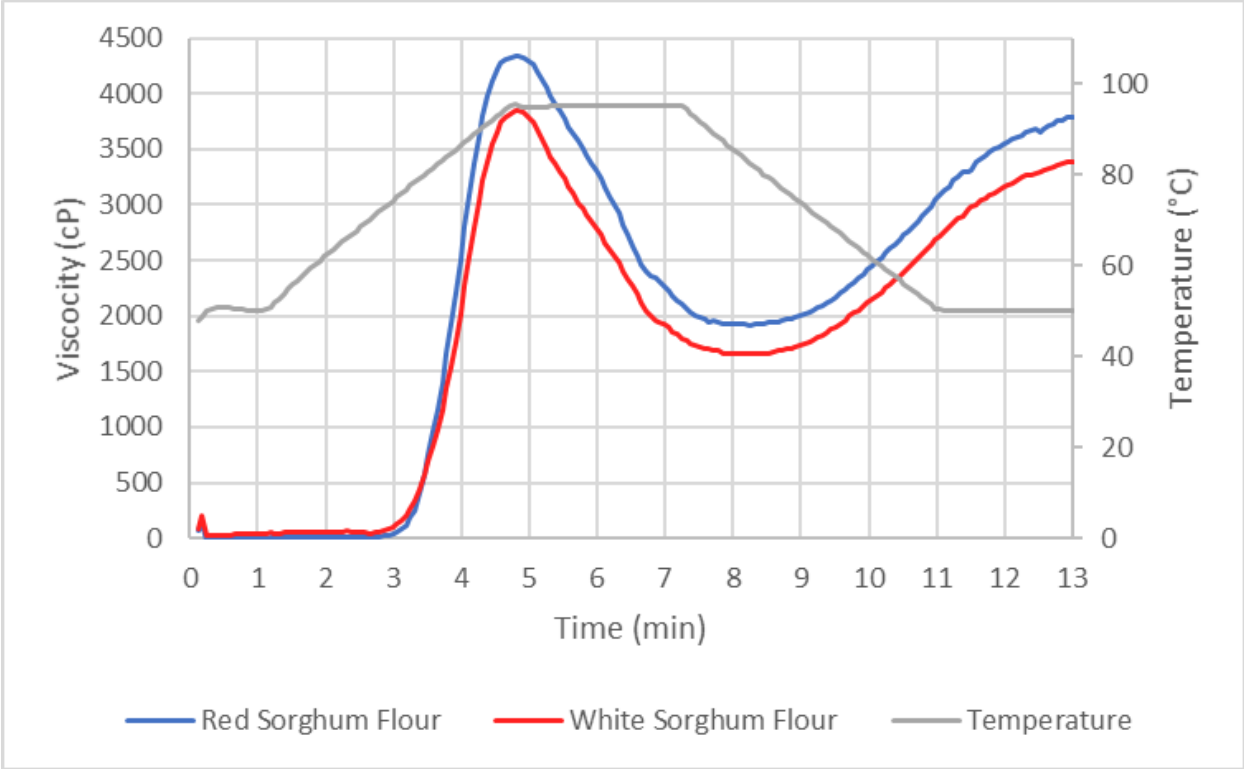
Figure 3-4 RVA pasting profile of white and red sorghum flour

Chapter 4 - The use of protein binders and sorghum crisps as potential ingredients in cereal bar for dogs

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Abstract

Humanization of pets has driven the pet food industry trends towards products perceived as healthy by pet owners. Cereal bars are a popular health snack alternative in the human food industry, but their use as dog treats has not been investigated so far. The objective of this study was to evaluate the inclusion of different protein binders and sorghum crisps in cereal bar for dogs, and their effect on product texture and dog preference. Fifteen cereal bar recipes were developed in a 3 x 5 factorial arrangement in which three crisp sources (rice crisp, RC; white sorghum crisp, WSC; and red sorghum crisp, RSC) and five sources of binder (corn syrup, CS; spray dried plasma, SDP; gelatin, GL; albumin, AL; and egg product, EP) were evaluated. Textural properties and nutritional analysis of each treatment were assessed, and the dog preference was evaluated through a preference ranking test. The WSC and RSC had to be ground prior inclusion into the recipe due to their low density. Cereal bars produced with protein binders had a higher protein content compared to those with CS. Interaction effects between binder and crisp source were found regarding hardness and toughness ($P<0.05$). Cereal bars were well accepted by dogs which preferred WSC cereal bars produced with SDP over those produced with EP. No other preferences were observed when cereal bars where compared. This study suggests that protein binders may replace CS in a cereal bar application with no negative impact on product texture and animal preference, and that sorghum crisps may need to have lower bulk density to improve their use as ingredients for cereal bars.

Abbreviations

AL = albumin; CS = corn syrup; EP = egg product; GL = gelatin; RC = rice crisp; RSC = red sorghum crisp; SDP = spray-dried plasma; WSC = white sorghum crisp.

1. Introduction

The pet food industry was worth over $69 billion in 2017 with a market growth projected to be over $72 billion in the next year (APPA, 2018). This rapidly growing market is driven by development of new products which involve addition of new ingredient, and food forms. The pet food segment can be nutritionally segregated in two major categories: complete & balanced, and snacks & treats. Although complete & balanced products compose the greatest share of the market sales, snacks & treats represent an important part of this market. More than ever, pets are considered part of the family and these products are offered as a demonstration of affection and love.

The humanization of pets has greatly impacted the pet food industry trends. Human food trends are being translated to the pet food industry as pet owners demand pet food claims that reflect their own dietary choice. Trends towards a more healthy and natural food have gained popularity in the pet food industry (Sprinkle, 2018). Cereal bars were introduced in the human food industry several decades ago as a healthy snack alternative (Bower and Whitten, 2000). These products still remain popular, and they may represent a potential alternative to healthy treats for dogs. However, a previous study revealed that the chemical composition of some cereal bars in the market were similar to those from confectionary (Boustani and Mitchell, 1990). This may not be perceived as healthy by pet owners, and it could potently exclude the product from this claim.

Most cereal bars are composed of a combination of several ingredients that are bound by the addition of sugar syrups. This result in high levels of carbohydrate in the final product. Although

carbohydrates are well utilized by dogs, many pet owners perceive them as unhealthy and seek protein-rich food forms for their pets. Proteinaceous ingredients have been used in the gluten-free industry as potential binders (Crockett *et al.*, 2011; Furlan *et al.*, 2015; Han *et al.*, 2019), and may be a replacement for sugar syrup for manufacturing cereal bars. Rice crisps are also commonly used in cereal bar recipes. The replacement of rice crisps for nutraceutical ingredients such as sorghum may add value to the product. Sorghum is rich in phytochemicals that are known to have antioxidant and antiradical activities (Hagerman et al., 1998). The replacement of sugar syrup and rice crisps for soluble animal protein binders and sorghum crisps, respectively, may improve the nutritional quality of cereal bars and add value to the product for the pet market. To date, there are no studies evaluating acceptability of cereal bars by dogs, and the use of protein binders and sorghums crisps as potential ingredients for this application. Thus, the objective of this study was to evaluate the inclusion of different proteinaceous binders and sorghum crisps in cereal bar for dogs, and their effect on product texture and dog preference.

2. Material and Methods

2.1 Cereal bar production

This experiment was conducted as a 3x5 factorial arrangement for simultaneous evaluation of three sources of crisp (rice crisp, RC; white sorghum crisp, WSC; and red sorghum crisp, RSC) and five sources of binder (corn syrup, CS; spray dried plasma, SDP; gelatin, GL; albumin, AL; and egg product, EP). The RC and the CS were used as positive control from the crisp and binder effect, respectively. The WSC and RSC used in this study were produced from previous experiment at Kansas State University (Pezzali et al 2019; Chapter 3). The RC was sourced from a local manufacture (Cereal Ingredients Inc., Leavenworth, KS). The SDP (Innomax Porcine Plasma), GL (Pro-Bind Plus 50), and AL (Innomax MPI) were acquired from Sonac ®

(Maquoketa, IA), and the EP (Ovabind -RSD 80) was acquired from IsoNova ® (Springfield, MO). Last, CS (Light Corn Syrup, Kroger ®) was acquired from a local grocery market.

Fifteen dietary treatments were developed (Table 4.1) and produced in 800 g batches. Dry ingredients (oatmeal, crisp, coconut flakes, corn starch, dried blueberry, flaxseed, pepitas seeds from pumpkin, wheat germ, salt, and palatant) were manually mixed in a stainless-steel bowl. Prior to mixing, the WSC and RSC were manually ground in order to decrease particle size. The agglomerating syrup was added onto the dry ingredients, and the mixture was kept under constant stirring until uniformly coated. The syrup was composed of corn syrup or one of the protein sources. Protein sources required hydration prior addition into the mix and were preparead as follow: SDP (1:1.76 ingredient/water, wt/wt), GL (1:2.5 ingredient/water, wt/wt), AL (1:1.18 ingredient/water, wt/wt), and EG (1:1.47 ingredient/water, wt/wt). The GL and AL were hydrated under heat. The final mixture was transferred to a cookie sheet covered with parchment paper, and baked for 20 minutes at 163°C in a convection oven (MEA 21-93-E; Garland Commercial Industries, PA). After baking, the dough was cut in approximately 4cm x 4cm square pieces. Treatments that contained one of the protein ingredients in the agglomerating syrup were dried overnight at 55°C in a convection oven (212041, HotPack, PA) to achieve moisture content below 10%. Experimental treatments were produced as described above over three replicate-days.

2.2 Nutritional analysis

For each dietary treatment, a sample of 50 g from each day of production were composited and ground through a 1-mm screen in a fixed blade laboratory mill (Retsch, type ZM200, Haan, Germany). Composite samples were analyzed for dry matter (AOAC 930.15), crude protein (AOAC 990.03), fat by acid hydrolysis (AOAC 954.02), crude fiber (AOCS Ba 6a-05), and ash

(AOAC 942.05) in a commercial laboratory (Midwest Laboratories, Omaha, NE, U.S.A).
Nitrogen-free extract was calculated by difference.

2.3 Textural properties

Hardness and toughness of cereal bars were assessed using a Shimadzu EZ-SX Texture
Analyzer (Shimadzu Scientific Instruments, Inc, KS). Five cereal bar square pieces were
randomly selected for each replicate, totaling fifteen samples per treatment. A compression test
was performed using a toothed pushrod B probe at a speed of 1.67mm/sec. Hardness was defined
as the highest peak fracture force. Toughness was defined as the total energy required to break
the sample as it was calculated as the total area under the fracture curve.

2.4 Preference ranking test

The digestibility trial took place at the Large Animal Research Center (LARC) at Kansas
State University where twelve castrate Beagle dogs were used. Dog's preference was evaluated
under a preference ranking procedure according to Li et al (2017). The experiment consisted of
10 d (5-d acclimation and 5-d data collection). Each dog was presented simultaneously with five
cereal bar treats in a rubber puzzle toy (Kong®). Dogs were allowed to smell the Kongs then
each was placed randomly in the left corner of the 1.5 m X 1.5 m test room. The order in which
the cereal bar was extracted and consumed by the dog was considered as the preference ranking
order. This ranged from 1 to 5, where 1 was considered the most preferred, and 5 the least
preferred.

First, the effect of protein source on dog's preference was evaluated. To do so, three ranking
tests were performed in which the treatments having the same crisp source and differing in the
binder source were compared. Second, the effect of crisp source on dog's preference was
assessed using a modified preference ranking test (Li et al., 207). The SDP protein source was

selected for evaluation. Three cereal bar treats produced with the SDP but differing in crisp source were presented to the dogs. Preference was assessed as described above.

2.5 Statistical analysis

Textural properties were analyzed by analysis of variance using GLIMMIX procedure in statistical software (9.4 SAS Inst. I., Cary, NC). The effects of crisp and binder source, and their interaction were analyzed. Day of production was considered as a random effect. Preference ranking data was also analyzed by analysis of variance using GLIMMIX procedure in statistical software (9.4 SAS Inst. I., Cary, NC). Dog and day were considered as random effects in the preference ranking test. Means were separated using Tukey test, and significance level was set at 0.05.

3. Results

3.1 Cereal bar evaluation

After a series of development tests, the fifteen cereal bar recipes were successfully produced (Figure 4.1). Protein sources required different processing conditions in order to create a consistent agglomeration syrup. The GL and AL were hydrated under heat. An attempt to hydrate SDP and EP under heat was performed, but these protein sources started to cook, therefore this step was not implemented. Corn starch was added to all proteinaceous agglutination syrup with exception of the SDP (Table 4.1). Chemical composition of cereal bars is reported in Table 4.1. Cereal bars produced with proteinaceous agglutination syrups had CP content an average 19.72% greater than those produced with corn syrup, while NFE content was reduced in 23.38%. Minor differences were observed for fat, CF, and DM.

Interaction effects between binder and crisp source were found with respect to hardness and toughness (Table 4.2). The CS-RC presented the highest toughness value followed by CS-RSC

($P<0.05$). Compared to these, the EP-RC, EP-WSC, EP-RSC, AL-RC, AL, RSC, SDP-WSC, and SDP-RSC treatments exhibited lower toughness($P<0.05$). In regard to hardness, the highest numerical value was observed for GL-WSC while the lowest hardness was observed for CS-RSC.

3.2 Preference ranking test

No signs of refusal were observed throughout the four different preference tests performed. Dogs showed no preference for RC cereal bars produced with different agglutination syrups (Table 4.3). On the other hand, dogs preferred WSC cereal bars produced with SDP over those produced with EP ($P<0.05$). When RSC cereal bars were presented to dogs, a preference for SDP compared to GL was observed ($P<0.05$). In the last preference ranking test where crisp source was evaluated, one dog was unable to perform the test and was removed from the study. Crisp source did not impact dog's preference for cereal bars produced with SDP.

4. Discussion

The objective of our study was to evaluate the effect of the use of protein binders, and white and red sorghum crisps in cereal bars on textural properties, and on dog preference. There is still a lack of research regarding processing condition and animal acceptance of snacks & treats. To date, this is the first study to investigate the use of these ingredients in a novel dog treat application. The results reported herein suggest that it is possible to use protein sources as a replacement for corn syrup without compromising product integrity and animal acceptance. In order to form an agglomeration syrup that effectively binds the dry ingredients, hydration of protein sources was performed differently according to their protein type. Each protein source has a unique amino acid composition, sequence, and molecular weight. These have a direct impact on the protein functionally, thereby it was expected to find differences on the

development of proteinaceous agglutination syrups. Gelatin has been used in a wide range of applications such as food, cosmetic, and pharmaceutical due to its gel-forming properties (Gómez-Guillén et al., 2011). Spray-dried plasma (Furlan et al., 2015) and egg white (Crockett et al., 2011) are commonly used in gluten-free applications due to their foam-stabilizing activity. This was the first attempt to use SDP, GL, AL, and EP as binder sources in cereal bar applications. Our study indicates that these protein sources can effectively work as binding agents for cereal bars. It was not our intention to investigate the best inclusion level of each protein source. Future studies may evaluate one single protein source at a time to identify the adequate inclusion level in order to maximize product quality and animal acceptance. Processing conditions such as baking time and temperature should also be investigated as each protein source may have different processing requirements in order to perform at its best.

The use of white and red sorghum crisps was also evaluated as replacements for rice crisps. The sorghum crisps used in this research were produced from a previous study (Pezzali et al 2019; Chapter 3) while the rice crisp was acquired from a commercial source. The rice crisps were more dense (348 g/L) compared to the white and red sorghum crisps (107 and 92 g/L, respectively). This compromised aggregation of ingredients due to lower area of contact. In order to include the same mass of rice and sorghum crisps in the recipe, they had to be manually ground to decrease particle size, and improve aggregation. Thus, sorghum crisps may be produced under different extrusion conditions to decrease expansion, and increase density in order to improve their use as an ingredient for cereal bars. Unfortunately, we were not able to acquire crisp sources from the same source. This would have prevented the variance within crisp source characteristics. A recent study showed that extruded sorghum flour can promote health benefits in obese rats (Arbex *et al.*, 2018). Extruded sorghum crisps might provide similar results

in obese dogs. Thereby, they are promising ingredients to be added in a "healthy" treat application. Farinazzi-Machado et al (2012) investigated the effect of cereal bars produced with 39% quinoa flakes on health parameters in humans, and observed a reduction of total cholesterol, triglycerides, and low density lipoprotein cholesterol that after a 30 days treatment. This suggests that the consumption of snacks & treats can impact health status. Thus, the effect of extruded sorghum crisps on animal health should be investigated to determine ideal inclusion levels, and daily consumption of treats to maximize their nutraceutical properties.

Textural characteristic of the product is an important aspect to be considered as it can influence product quality and animal acceptance. In general, addition of proteins in bread application increase product hardness (Furlan et al., 2015). For example, inclusion of egg albumin in a gluten-free bread formula improved first bite and masticatory hardness (Toufeili et al., 1994). Although the baking science behind bread production is different than the mild baking step used for development of cereal bars, we still expected that the use of protein binders would increase hardness compared to a sugar syrup. In the current study, hardness was influenced by the interaction effect of binder and crisp source. The inclusion of RC and GL likely resulted in harder products. Although it was our intention to cut the final product in 4cmX4cm square pieces, not all pieces had the same dimensions. This can greatly influence the evaluation of product texture. Furthermore, other ingredients besides binder and crisp sources could have influenced product hardness as cereal bars were not formulated with fixed amounts of each ingredient. Most of the hardness values observed in our study are in accordance with Torres et al (2011). These authors obtained hardness values between 9.4 and 19 kg for cereal bars. The toughness of cereal bars was assessed as the total energy required to break the sample. The cereal bar formulated with CS and RC showed a greater toughness value compared to the other

treatments. The small particle size of RC combined with binding effect from CS probably resulted in higher aggregation of ingredients, thereby a greater energy was required to disintegrate the sample.

The preference liking test was used to assess dog's preference. In the protein source evaluation, dogs showed a preference to SDP compared to EP in cereal bars produced with WSC. Although the null hypothesis was rejected in the preference raking test with RSC (P=0.0337), no significant differences were observed between treatments when pairwise comparisons were evaluated using the Tukey adjustment for control of type I error. In the preference liking test, aroma is initially the only factor being evaluated. Dogs have the chance to associate the aroma profile with the texture and flavor after the treats are presented for five days. However, there is no guarantee that dog will be able to make this association. The SDP was perhaps more aromatic than the other protein sources. However, the effect of other ingredients may have played a role in the dog's preference as well. In the evaluation of crisp sources, dogs did not show preference towards one of the crisp sources presented over another. Sorghum has been associated with bitter and astringent notes (Kobue-Lekaleke *et al.*, 2007), but the extrusion process performed to produce sorghum crisps probably reduced these characteristics (Donfrancesco and Koppel 2017), resulting in no differences between RC, WSC, and RSC. Additional sensory evaluation such as descriptive analysis of cereal bars can provide detailed sensory attributes which may help understand the dogs' preference. It is important to note that the size of the cereal bars was not consistent between treatments, and dogs may have based their preference on the ease of extracting the treats from the puzzle toy. Furthermore, it is noteworthy that kennel-dogs and home-dogs may not behave similarly, and may present different results regarding food preference (Griffin et al., 1984). An in-home preference test may better represent

a "real-world" setting, and provide additional insights on dog's preference for cereal bars developed in this study.

5. Conclusion

This study indicates that spray-dried plasma, gelatin, albumin and egg product can act as binder agents in a cereal bar application without compromising product texture and animal acceptance. Replacement of rice crisps for white and red sorghum crisps did not cause differences on animal preferences. However, density of sorghum crisps should be adjusted in order to keep product quality. Combination of different protein binder and crisp source can impact product texture, thereby interaction of ingredients must be considered during product development. Thus, protein binder and sorghum crisps are potential ingredients to replace sugar syrup and rice crisps, respectively, in cereal bar treat application for dogs.

Acknowledgments

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Table 4-1 Ingredient and chemical composition of cereal bars

Item	Corn Syrup			Spray-Dried Plasma			Gelatin			Albumin			Egg Product		
	RC	WSC	RSC	RC	WSC	RSC	RC	WSC	RSC	RC	WSC	RSC	RC	WSC	RSC
Dry ingredients, %															
Oatmeal	24.8	24.8	24.8	30.6	30.6	30.6	31.2	31.2	31.2	30.1	30.1	30.1	30.1	30.1	30.1
Rice crisp	14.9	-	-	18.4	-	-	18.7	-	-	18.0	-	-	18.0	-	-
White sorghum crisp	-	14.9	-	-	18.4	-	-	18.7	-	-	18.0	-	-	18.0	-
Red sorghum crisp	-	-	14.9	-	-	18.4	-	-	18.7	-	-	18.0	-	-	18.0
Coconut flakes	4.95	4.95	4.95	6.13	6.13	6.13	6.24	6.24	6.24	6.01	6.01	6.01	6.01	6.01	6.01
Corn starch	4.95	4.95	4.95	6.13	6.13	6.13	6.24	6.24	6.24	6.01	6.01	6.01	6.01	6.01	6.01
Dried blueberry	3.54	3.54	3.54	4.38	4.38	4.38	4.46	4.46	4.46	4.29	4.29	4.29	4.29	4.29	4.29
Flaxseed	3.54	3.54	3.54	4.38	4.38	4.38	4.46	4.46	4.46	4.29	4.29	4.29	4.29	4.29	4.29
Pepita seed	3.54	3.54	3.54	4.38	4.38	4.38	4.46	4.46	4.46	4.29	4.29	4.29	4.29	4.29	4.29
Wheat germ	2.83	2.83	2.83	3.50	3.50	3.50	3.57	3.57	3.57	3.44	3.44	3.44	3.44	3.44	3.44
Salt	0.35	0.35	0.35	0.44	0.44	0.44	0.45	0.45	0.45	0.43	0.43	0.43	0.43	0.43	0.43
Palatant	0.35	0.35	0.35	0.44	0.44	0.44	0.45	0.45	0.45	0.43	0.43	0.43	0.43	0.43	0.43
Agglutination syrup, %															
Corn syrup	36.4	36.4	36.4	-	-	-	-	-	-	-	-	-	-	-	-
Spray-dried plasma	-	-	-	21.3	21.3	21.3	-	-	-	-	-	-	-	-	-
Gelatin	-	-	-	-	-	-	17.8	17.8	17.8	-	-	-	-	-	-
Albumin	-	-	-	-	-	-				20.9	20.9	20.9	-	-	-
Egg white	-	-	-	-	-	-	-	-	-	-	-		20.9	20.9	20.9
Corn Starch	-	-	-	-	-	-	1.91	1.91	1.91	1.84	1.84	1.84	1.84	1.84	1.84
Chemical composition, %															
Dry Matter	92.0	92.5	92.5	90.8	91.7	91.9	92.5	90.3	89.2	93.2	92.9	92.3	90.4	92.6	92.0
Crude Protein	9.79	9.88	9.34	29.1	29.0	28.3	27.8	28.0	28.6	31.3	32.0	32.3	28.6	29.2	28.5
Fat	11.2	9.9	11.2	12.0	12.0	11.8	12.1	12.1	12.6	11.6	11.4	11.4	12.4	11.9	11.5
NFE	68.6	70.5	69.7	44.8	45.9	46.7	49.0	46.9	44.5	47.1	46.0	44.5	44.8	47.2	47.6
Crude Fiber	0.53	0.28	0.33	0.72	0.68	1.13	0.83	0.80	1.23	0.20	0.82	1.14	1.42	1.39	1.59

Table 4-2 Effect of binder and crisp source on textural properties of cereal bar for dogs

Dietary treatment		Textural Properties	
Binder	*Crisp*	Hardness (kg)	Toughness (kg*mm)
Corn Syrup	RC	23.63^{ab}	118^a
	WSC	12.69^{cde}	28.98^{bc}
	RSC	11.61^e	48.77^b
Spray-Dried Plasma	RC	20.26^{abcd}	18.54^{bc}
	WSC	15.59^{bcde}	13.19^c
	RSC	14.66^{cde}	15.59^c
Gelatin	RC	17.42^{bcde}	20.68^{bc}
	WSC	26.14^a	42.51^{bc}
	RSC	20.45^{abc}	29.99^{bc}
Albumin	RC	13.78^{cde}	16.96^c
	WSC	16.74^{bcde}	23.06^{bc}
	RSC	11.93^{ed}	15.04^c
Egg product	RC	21.07^{abc}	23.34b^c
	WSC	16.07^{bcde}	16.39^c
	RSC	17.75^{abcde}	16.27^c
SEM		1.9093	6.3684
P=		<.0001	<.0001

Different letters following the means in the same column indicate a significant difference (*P*<0.05).

Table 4-3 Effect of binder and crisp source on rank order preference in dogs (1-most preferred, 5-least preferred)

Ranking test	Treatments[1]					SEM	*P=*
	CS	SDP	GL	AL	EP		
1. RC	3.12	2.76	3.35	2.73	2.78	0.1953	0.0973
2. WSC	2.80	2.68	3.17	2.75	3.41	0.1938	0.0337
3. RSC	2.90^{ab}	2.40^b	3.62^a	2.90^{ab}	3.06^{ab}	0.1884	0.0004
	RC		WSC		RSC		
4. SDP	1.84		1.98		2.12	0.1290	0.3312

Different letters following the row in the same column indicate a significant difference (*P*<0.05).

[1]AL = albumin; CS = corn syrup; EP = egg product; GL = gelatin; RC = rice crisp; RSC = red sorghum crisp; SDP = spray-dried plasma; WSC = white sorghum crisp.
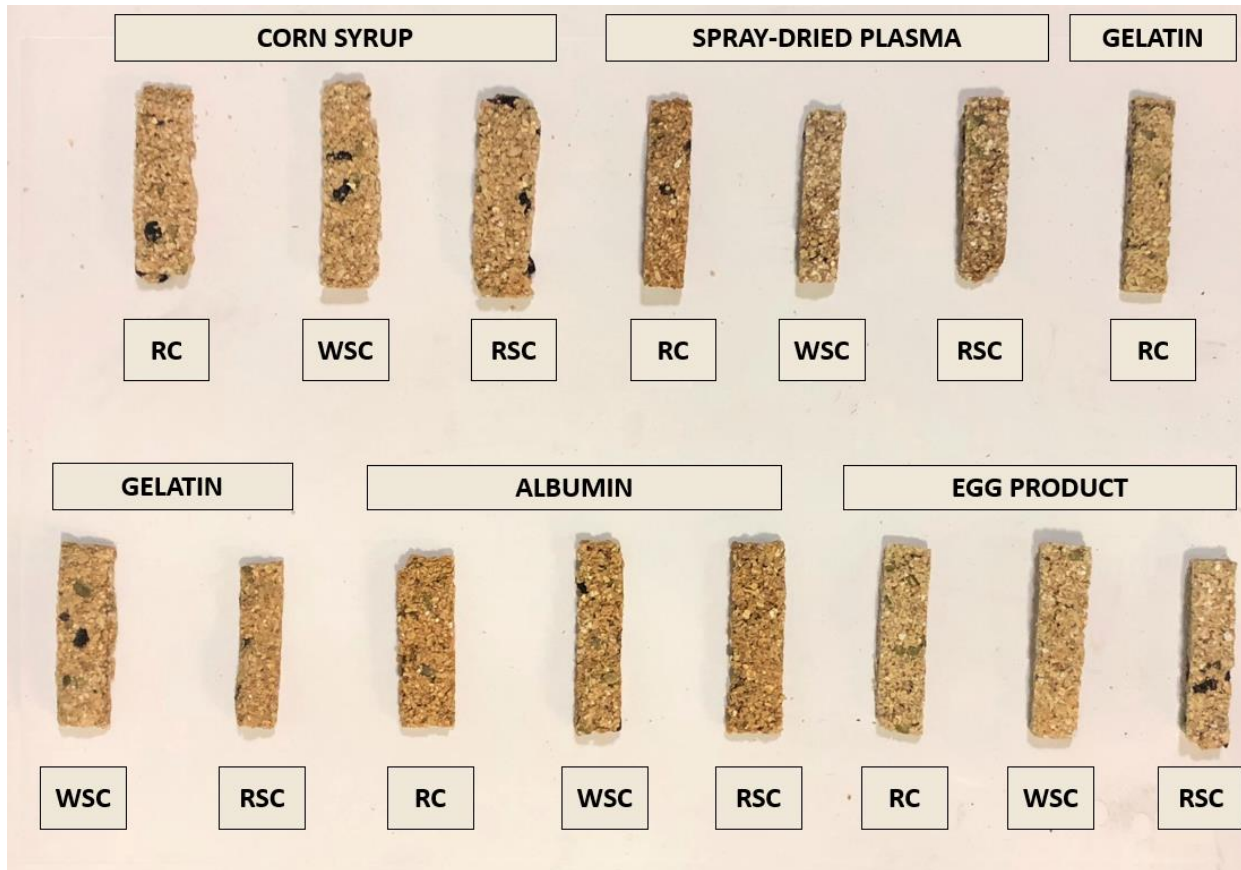
Figure 4-1 Cereal bars produced with different binder and crisp sources

Appendix A - Additional measure of ATTD

Table A.1. Apparent total tract digestibility determined by total fecal collection of dogs fed ancient grains and grain free diets (N=12)

Item	Ancient Grains	Grain Free	SEM	P=
Dry Matter, %	86.97[a]	85.87[b]	0.2571	0.0013
Organic Matter, %	88.66[a]	87.00[b]	0.2493	<.0001
Energy, %	88.48[a]	87.25[b]	0.2399	0.0003
Crude Protein, %	89.03[a]	87.14[b]	0.3211	<.0001
Crude Fat, %	93.64	93.57	0.1030	0.6516
NFE (calculated), %	96.53	96.19	0.3256	0.3369
Crude Fiber, %	42.70[a]	20.25[b]	1.0171	<.0001