Use of grain sorghum as the primary grain ingredient in premium extruded foods designed for cats

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Abstract

The effect of particle size and type of primary grain on processing and properties of extruded dry expanded cat food kibbles was investigated. Most of the diets were primarily sorghum-based. Two varieties of grain sorghum (white and red) milled at three grind sizes (0.5, 1.0 mm and 1.6 mm) were incorporated in a premium cat food formulation and processed using a pilot-scale single screw extruder. Corn- and brown rice-based formulas were also extruded as comparisons. Grind size and grain type (red sorghum, white sorghum, corn and brown rice) both had a significant ($p<0.05$) impact on starch gelatinization, product expansion and breaking force (F_p). The average particle size of diets was inversely correlated to extrusion specific mechanical energy ($r = -0.8517$; SME), enzymatically determined gelatinized starch content ($r = -0.8158$), piece density ($r = 0.8221$; inversely proportional to product expansion), and product F_p ($r = 0.5670$). Expansion measures such as bulk density (346.5-403.0 g/L) and sectional expansion index (3.08-3.67) indicated that the brown rice-based formulation expanded the least and corn the most, with sorghum having intermediate expansion when grind size was the same.

Specific Mechanical Energy (SME) ranged from 105.4-183.1 kJ/kg depending on grind size and grain variety and seemed to be one of the determinants of kibble properties such as expansion. Raw material physico-chemical properties such as gelatinization onset temperature (T_o; 66.7-78.3^{o}C) determined using differential scanning calorimetry and pasting temperature (T_{paste}; 76.6-81.5^{o}C) obtained from rapid visco-analyzer also appeared to have an impact on expansion, as observed from their correlations with bulk density ($r= 0.8125$ and 0.7742, respectively). These observations support that although the SME input is important as the driving force, having a matrix that has film forming or extensibility properties is also critical for product

expansion. Lower T_o and T_{paste} indicated a greater propensity for starch gelatinization and degradation, as seen from the negative correlations with respect to enzymatically obtained gelatinized starch (r= -0.5844 and -0.6085, respectively). This was the reason for connecting T_o and T_{paste} to matrix extensibility and expansion. The negative correlation of particle size with expansion was thus clearly explained, as an increase in grind size negatively impacted both the driving force for expansion and matrix extensibility. The lower surface area per unit volume of particles with higher grind size possibly led to poor heat and moisture penetration during preconditioning and extrusion, and thus negatively affected film forming ability and expansion. Similarly grain type also impacted both driving force and extensibility, and thus expansion. For example, the brown rice-based formulation, which had one of the lowest expansions of all grain varieties, also had one of the lowest SME inputs (161.2 kJ/kg) and gelatinized starch content after extrusion (30.7%).

The textural attribute of kibble F_p was found to have a positive correlation with piece density (r= 0.7059), which aligned with the theory of mechanical strength of porous matrices that dictates that the strength of cellular products is inversely proportional to cell wall size or in other words expansion.

Results show that overall, a larger particle size raw material flows faster through the extruder (at the same volumetric setting as a smaller particle size), leads to lower SME, higher Specific Thermal Energy (STE), a higher STE : SME ratio and lower overall energy consumption than smaller particle size material. The measured and also the calculated dry feed rates confirmed the observation for material flow rate above, higher throughput implies faster flow rate.

Sensory properties such as color, fracturability, fibrousness and grittiness were related with product palatability tests using cats and product properties such as peak breaking force (F_p) and degree of cooking. It was concluded the color of the final product based on differences of grain ingredient and the peak breaking force seemed to have the biggest impact on palatability and product sensory properties. In vivo digestibility and colonic fermentation results using cats were also related to product attributes.

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Chapter 1 - Introduction

Grain sorghum is a crop that is grown, historically for animal feed, worldwide due to its ability to be grown in arid climates and survive drought conditions. It is also used as a renewable energy fuel base as it produces ethanol in comparative amounts to other feedstocks while using less water for growth with approximately one-third of the US crop going to this market ("All About Sorghum," n.d.). Sorghum is also finding its way into other markets for new uses such as human food, building materials and especially pet food. The uses in human and pet food are due to its nutritive benefit related to slowing digestibility and having a lower glycemic response when compared to other grains such as corn and brown rice (Kim et al., 2020).

The pet food industry is a large and rapidly growing industry with the global pet food market reaching over $90 billion in 2018 and a 31% growth from 2013 to 2018 (Debbie Phillips-Donaldson, 2019). While dry, expanded dog and cat kibble brought in $7.8 billion dollars in 2020 (Bedford, 2021a) with a sales trend of clean label being free of GMO ingredients growing to 28.8% in 2018 (Bedford, 2019). This clean label trend is one of the many reasons that grain sorghum is growing as a pet food ingredient.

Convenience in packaging, options for types and styles of food and a healthy lifestyle for pets are three of the driving forces leading to the big sales numbers in this industry. New and novel is always at the forefront of progression, but some companies are seeing old ingredients in a new light. The market is open for new opportunities in various areas, but especially in a return to grain-included diets as premium labels are including grains in general, and ancient grains as a new twist (Tyler, 2020).

Traditional grains used in pet food include corn, brown rice, wheat, barley and rye; along with the aforementioned ancient grains, that include grains as quinoa, buckwheat and sorghum,

among others. These various grains provide starch, fiber, protein and essential amino acids and when prepared properly are highly digestible. Many ancient grains are non-GMO, with some being grown organically (Roseboro, 2017), provide good nutrition and an alternative approach to avoid traditional grains which have become unpopular with pet owners as they consider traditional grains unhealthy (Pezzali and Aldrich, 2019).

1.1 Sorghum as an Alternative Grain

Grain sorghum is one of the most important cereal crops grown in the world (Reddy et al., 2010), but has been primarily used for livestock feed in the past. Interest has grown over the last several years in using sorghum for various purposes in pet food. While it can be beneficial when parts of the sorghum seed is used after milling separation, it is usually used whole and ground to an appropriate processing size when used for pet food (Alvarenga et al., 2018).

This, of course, depends on the nutrition profile needed. Sorghum has been reported to have lower nutritive values compared to other grains, such as corn (Al-Rabadi et al., 2011; Rooney and Pflugfelder, 1986). In contrast to the above, a review by Lancheros, (2020a) stated that there was an increase in digestibility with extrusion for sorghum and corn fed to pigs. Therefore, they could possibly be used interchangeably in the diets depending on local availability of certain grains versus others. Bazolli (2015) found that the digestibility between the same grain types was similar with proper processing parameters.

1.2 Extrusion Processing of Pet Food

Extrusion is the primary, processing technique for producing dry, expanded pet food. This high temperature, medium shear, short time process results in energy transference from the

machine to the raw material. This induces a physicochemical transformation. Principally, starch cooking (gelatinization), which positively influences the formation of kibble shape and structure derived from expansion and recrystallization of the starch which results in lower product density (mass/volume). These chemical and physical changes can alter the nutritional and physical quality of the product (Riaz, Mian N., 2007; Tran et al., 2008). Extrusion combines the raw material with aids to processing like water and steam, pressure and shear to transform starch granules into viscous materials before they exit the extruder. This cooking action and gelatinization can be partial or complete. (Bazolli et al., 2015). The cooking action and energy transfer is at the heart of the extrusion process.

The transfer of energy into the product, both mechanical and thermal, is what drives the cooking process, gelatinization and expansion. (Monti et al., 2016a). The specific mechanical energy (SME) describes the amount of work imparted by the extruder to the material and is produced as a result of the friction of the material between the screw, material and barrel (Hataichanok Kantrong et al., 2018). Specific thermal energy (STE) is the energy resulting from temperature change and occurs predominately in the preconditioner, where steam is added; it can also happen in the extruder barrel, but typically to a lesser degree (Pacheco et al., 2018).

In addition to the physico-chemical aspects, mass and energy balance can be an important tool to help understand and quantify, what happens inside an extrusion system (Levine, 2014a). A basic law of thermodynamics states that mass can neither be produced nor destroyed; it is conserved. This means that for an extrusion system in steady state, $mass_{in} = mass_{out}$ (Janssen et al., 2002) and allows us to verify, by calculation, the actual dry feed rate entering the preconditioner, the extruder barrel or at any point in the system. We can also do the same for water and steam. Energy, like mass, is conserved, so an energy balance can be utilized to

account for changes in energy inputs and outflows for each treatment or process from the inlet of the hopper to the outlet of the extruder. Having this information can help food scientists and engineers improve the process as needed to improve operational efficiency and/or enhance profitability by maximizing input usage and minimizing non-product outputs, such as electrical or utility wastage (Levine, 2014a).

Inflows such as material mass and particle size are relatively easy to quantify. Outflows, on the other hand, are more complex and require the use of estimations and/or reasonable assumptions; these include the raw material masses, now with more moisture and higher temperature, and possibly excess steam and energy gains and losses through the barrel wall. The raw material that enters the preconditioner contains ingredients that will determine the total heat capacity of the material, which needs to be calculated; as well as containing starch and protein that will be cooked. With pet food production, it is typical that not all of the steam condenses. An assumption that needs to be made is the starch and protein cook levels in the preconditioner. With the knowledge of the actual inputs and sound, educated assumptions, the calculations can be accomplished (Levine, 2014a).

Most of the energy added in the preconditioner is thermal. Whereas the extruder barrel has thermal energy input and the ability to impart mechanical energy into the product. This can help bring about the desired change to the raw material. Like the preconditioner, in the extruder barrel there is a raw material component, water and possibly steam impact. Plus there is also heat entering or leaving the process as an input or an outflow. Other outflows are due to the material with a new temperature and moisture level, steam loss and convection losses as noted previously.

While the extruder barrel has similar inputs and outputs to the preconditioner, the barrel becomes more complex with the ability to heat and/or cool the barrel sections and with the addition of mechanical energy imparted to the product from the screw(s). The mechanical energy is quantified with some known parameters and a couple of calculations (Jafari et al., 2017). However, the transfer of thermal energy to and from the barrel and environment can be challenging to properly determine (Levine, 1997a) due to the different surface areas and thicknesses of the extruder barrel sections.

The extrusion of pet food, that contains plant and animal-based ingredients with a variable and complex chemical composition, offers challenges that the extrusion of polymers does not; they change with moisture, temperature and cook / denaturation. These changes affect the viscosity of the material and thus can be problematic for steady-state control of the process (Janssen et al., 2002).

When material is passing through the barrel, several things are happening simultaneously. Energy is transferred to the material from the screw and barrel by friction. Water and possibly steam are being injected into the process. Energy may flow into the product from the barrel in one section and energy flow from the product to the barrel in another section. Then the product leaves the barrel along with steam flash-off. The mechanical energy and thermal energy transferred to the material results in a temperature rise of the extrudate. A simple temperature probe, ideally an extended probe that is in the direct flow of the extrudate, will measure the temperature before the die, but obtaining the temperature after the die is much more difficult as the temperature changes rapidly due to the energy release. Experiments from several authors agree that a good estimate of this water flash temperature occurs at 85 °C (Janssen et al., 2002;

Levine, 1997b). Another energy source is from the phase change due to cooking of starch and protein, which can be estimated at 17 J/g (Janssen et al., 2002).

Specific Mechanical Energy has been mentioned before and is simple to calculate with a few known quantities. However, specific thermal energy (STE), is more difficult to calculate, but can be estimated using the temperatures before and after the die, along with the estimated temperature, inflow or outflow can be calculated (Karwe and Godavarti, 1997; Levine, 1997b).

Mass and energy balances are a useful tool to help improve the extrusion process by identifying energy inputs that may not being utilized as efficiently as possible and to help reduce extruder wear so that service life might be extended (Levine, 2014a). This helps the company improve efficiency and reduce costs. We hypothesize that a larger grind size will negatively affect the processing and ultimately, the palatability of cat food and that grain sorghum can be a suitable replacement for corn and brown rice in cat food.

This work is focused on identifying the changes in mass and energy as it applies to raw materials that differ in particle size due to different grind sizes. As mentioned earlier, the particle size can ultimately affect the palatability of cat food as well as the process. While there are a few studies that have focused on particle size in pet food diets, very few have focused on the effect of the particle size as it relates to processing using mass and energy balance, energy transfer, starch gelatinization estimation and physical kibble traits.

1.3 Physico-Chemical Properties of Extruded Pet Food

Total starch content, as well as gelatinized starch are two measures that can be used to evaluate different pet foods for quality control or against other trials or foods for research. These two measures give insight in to how much of the starch is in the products and thus, how much

may cook. The functionality of the starch can be determined by the time and temperature relationship resulting in the gelatinization temperature, onset temperature, pasting temperature and the amount of retrogradation (Zeng et al., 1997). The particle size of the raw material is an important factor in pet food production as it can affect the gelatinization of the starch and other physical traits such as density. The reduction in particle size is necessary for several reasons; whole grains are typically too large to effectively process, mixing of different size grains leads to feed non-uniformity in broilers (Behnke, 1996), an improved nutrient digestibility and improved feed efficiency in swine (Lancheros et al., 2020b) and the resulting surface area increase also helps with the processing in the form of increased hydration in the extruder. The amount of gelatinization is generally greater for material with smaller particle size than those with larger particle size (Bazolli et al., 2015).

Extrusion technology relies upon energy transference to mass induce physicochemical transformations which result in higher starch cook, which is influenced positively along with the formation of the kibbles that have higher expansion and lower density resulting in chemical and physical changes that alter the nutritional and physical quality of the product (Riaz, Mian N., 2007; Tran et al., 2008). The transfer of energy into the product, both mechanical and thermal, is what drives the cooking process, gelatinization and expansion. (Monti et al., 2016a).

In dry, pet food kibbles, cooking and expansion are the primary results of the cooking extruder. The amount of expansion depends upon three things; the feed composition, extent of cooking and the pressure differential between the extruder and the ambient environment. As the product exits the die, the pressure drop causes liquid water inside the extruder to convert to the gas phase and thus the volume increases drastically. As the water turns to steam, the starch

molecules stretch and then expand from the original size of the die from which the material exits. (Desrumaux et al., 1998).

The texture of pet food is an important trait that can affect the palatability and overall acceptability of a cat food. There are several attributes to texture which help to relate the product to animal acceptability, such as peak breaking force, fracturability, cohesiveness, springiness, gumminess, and chewiness. The F_p is simply how much force is applied before the kibble fractures and breaks; this simulates the biting action of the animal. Expansion and F_p are often negatively correlated; wherein, products which expand more from greater energy input become less hard. The main driving force for expansion of extrudates is the mechanical energy input. Two things, of many, that could lead to an increase in expansion are reducing in-barrel moisture and increasing the screw speed. Reducing the moisture would lead to an increase in melt viscosity, which in turn would increase the mechanical energy input resulting in increased expansion, while increasing the extruder screw speed would likely lead to more expansion by increasing the mechanical energy input (Agbisit et al., 2007). Texture can also be influenced by the particle size of the raw material and thus affect acceptability of the food by the pets. The change in acceptability can come in the form of mouth feel and even the way the kibble is chewed. (Monti et al., 2016a). The goal of this study was to investigate the effect of particle size and type of primary grain on processing and properties of complete and balanced extruded dry expanded cat food kibbles, with a focus on grain sorghum as an alternative carbohydrate source to the more traditional corn and brown rice.

1.4 Mechanisms Underlying Palatability and Digestibility of Cat Food

Once food is made and the process and kibble analyzed, palatability and digestion studies are often completed to determine the how the foods are consumed and utilized by the animal. A relatively new technique in pet food analysis is using human sensory panelists to quantify the sensory attributes that animals cannot directly tell us. Sensory analysis, using trained human panelists, can help fill a void of understanding in comparing pet foods without the need for using animals, which is costly (Lin et al., 1997). Sensory analysis is not able to give us information on how the animals interact with the product (taste, feel, smell), but it can help understand the sensory properties better.

Fox (2020) stated that "Palatability is the capacity of a food or an ingredient to stimulate the appetite of dogs or cats to encourage eating and satiety." It can also be defined as having a pleasant mouthfeel, aroma and taste with sensory characteristics (Koppel, 2014). This is where art and science meet to encourage a pet to consume the food that we want it to eat. Nutrition is very important but doesn't matter if the cat refuses to consume the food.

Literature relating palatability and sensory data has been difficult to find as there are only a few published studies with this focus. However, one study did link some sensory attributes to palatability. The properties of fracturability and aroma were correlated to wheat bran fiber inclusion levels and the authors concluded that as aroma intensity increased the palatability also increased. (Koppel et al., 2015).

Digestibility data is helpful to the development of pet foods as the pets depend on their owners to provide complete and nutritionally balanced foods so that good health can be maintained. Pet food manufacturers use digestibility, physical appearance and palatability data to increase the healthiness and consumption of the foods that they produce (Koppel et al., 2014).

The lack of studies involving the correlation between digestibility and physical attributes leaves a space to be filled which may help understand this topic better. There are several studies that link starch gelatinization to in vitro digestibility. Altan (2009) found that extrusion cooking increased the starch in vitro digestibility in barley, while Alonzo et.al. (2000) found the same with pea starch. Yağcı and Göğüş (2009) also found that increased gelatinization of starch led to increased digestibility when working with various food by-products.

The overall hypothesis of this study is that sorghum is a comparable grain source to corn or brown rice when it comes to pet food processing and palatability. The grind size of raw material has a big impact on processing, palatability and digestibility. Product attributes, like texture and expansion, can be related to process parameters and raw materials using analytical techniques like measured starch gelatinization, rapid-viscoamylography and other instrumental analysis. Knowing the process and physico-chemical traits in detail will allow a better understanding of the mechanisms involved with palatability and digestibility in pet food. To test these hypothesis', the following study is divided into three main objectives.

1.5 Objectives

Chapter two delves in to the processing of the above mentioned four primary grains, white sorghum, red sorghum, corn and brown rice with a focus on grain sorghum as an alternative carbohydrate source to the more traditional corn and brown rice. The objectives of Chapter 2 are, one, to develop balanced formulations for extruded dry cat food with white and red sorghum varieties as the primary grain ingredient, and brown rice- and corn-based formulations as the controls, two, mill the sorghum varieties to varying particle sizes and examine their utilization in production of dry expanded cat food kibbles using extrusion

processing, and three, to understand the thermal and mechanical energy balance in the pet food extrusion process.

Chapter three was focused on the determination of how the resulting kibble effected the physico-chemical attribute. The objective is to determine the relationship between grind size, grain type and the physico-chemical attributes from the extrusion of cat food.

Chapter four objectives were to develop a descriptive sensory evaluation using a human panel, relate this to complete palatability testing in an industry-setting cattery and to determine the effect of these experimental treatments on the in-vivo digestibility and fermentation in the colon (measurement of short chain fatty acids in feces) of sorghum-based diets and compare these results with standard brown rice- and corn-based diets using laboratory cats and four, to relate the resulting sensory data with the palatability and digestion data.

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Chapter 2 - Use of Grain Sorghum as the Primary Grain Ingredient in Premium Extruded Foods Designed for Cats

Abstract

The pet food market is growing at a rapid pace through processing advances in nutrition, packaging convenience and increasing consumer awareness of what it means to have a healthy pet. One part of this growth trend is that some pet food companies, after reducing the inclusion of grain in pet foods based on consumer pressure that "grain is bad," are now re-examining the use of grain sources in pet food. They are especially considering ancient grains, which will provide nutrition and are highly digestible. Sorghum is one of those grains getting a closer look for inclusion into pet food. Grain sorghum is one of the most important crops in the world and can be grown in arid conditions, thus making grain sorghum a good economic choice as it is readily available and therefore in good supply. The particle size of a raw material can influence the processing traits, energy transference, starch cooking, texture, digestibility and, ultimately, the palatability. Making food that is palatable is not enough, it must also be nutritious, as making a pet food that is nutritious, but not consumed, has no benefit. This study focuses on using mass and energy balances to examine differences in grain type and grind size in a premium cat food diet. Three types of primary grains in the formulas, sorghum (red and white), corn and brown rice and three levels of grind size, 0.5mm, 1.0mm and 1.6mm, were extruded through a single screw extruder at conditions likely to produce a "typical" expanded dry kibble and then dried in a dryer and cooler. Process conditions were recorded and then used to calculate the mass balance and energy balances required for the analysis. Results show that overall, a larger particle size raw material flows faster through the extruder (at the same volumetric setting as a smaller particle size), leads to lower Specific Mechanical Energy (SME), higher Specific

Thermal Energy (STE), a higher STE:SME ratio and lower overall energy consumption than smaller particle size material. The measured and also the calculated dry feed rates confirmed the observation for material flow rate above, higher throughput implies faster flow rate.

2.1 Introduction

The global pet food market reached over $90 billion in 2018 with a 31% growth from 2013 to 2018 (Debbie Phillips-Donaldson, 2019). Convenience in packaging, options for types and styles of food and a healthy lifestyle for pets are a few of the driving forces in this industry. Innovation is always progressing, but some companies are even seeing old ingredients in a new light. The market is open for new opportunities in various areas, but especially in a return to grain-included diets with premium labels including grains in general, and ancient grains as a new twist (Tyler, 2020).

Traditional grains used in pet food include corn, brown rice, wheat, barley and rye; along with the aforementioned ancient grains such as quinoa, buckwheat and sorghum. These various grains provide starch, fiber, protein and essential amino acids and when prepared properly are highly digestible. The ancient grains provide good nutrition and an alternative approach to avoid traditional grains which have become unpopular with pet owners as they consider traditional grains unhealthy (Pezzali and Aldrich, 2019).

Grain sorghum is one of the most important cereal crops grown in the world (Reddy et al., 2010), but has been primarily used for livestock feed in the past. Interest has grown over the last several years in using sorghum for various purposes in pet food. While it can be beneficial when parts of the sorghum seed is used after milling separation, it is usually used whole and ground to an appropriate processing size when used for pet food (Alvarenga et al., 2018).

This, of course, depends on the nutrition profile needed. Sorghum has been reported to have lower nutritive values compared to other grains, such as corn (Al-Rabadi et al., 2011; Rooney and Pflugfelder, 1986). In contrast to the above, a review by Lancheros, (2020a) stated that there was an increase in digestibility with extrusion of sorghum and corn in diets fed to pigs. Therefore, these grains could possibly be used interchangeably in the diets depending on local availability of certain grains versus others.

There is little doubt regarding the benefits of a preconditioner in the production of dry, expanded kibble for dogs and cats. The preconditioner is an integral part of the pet food production process; it moves material from the supply hopper to the extruder barrel, while simultaneously mixing steam and water into the dry raw materials, in other words, plasticizing. This process also preheats and starts the cooking process of extrusion of expanded cat food (Levine, 2014a; Riaz, 2000). It also helps to reduce the wear on the extruder barrel by this plasticizing process which reduces the mechanical energy required for the desired change in product (Levine, 2014a; Pacheco et al., 2018).

Extrusion is the primary medium shear processing technique for producing dry, expanded pet food. The technology has been around for decades producing human food products since the 1930's and pet foods since the early 1950's (Riaz, 2000). Starch is a primary carbohydrate source for animal nutrition and is also the binder that helps hold extruded pet foods together. This high temperature, short time extrusion process results in energy transference from the machine to induce physicochemical transformation such as starch cooking (gelatinization), which positively influences the formation of kibble shape and structure from expansion which lowers product density (mass/volume). These chemical and physical changes can alter the nutritional and physical quality of the product (Riaz, Mian N., 2007; Tran et al., 2008).

Extrusion combines the raw material with processing aids like water and steam under heat, pressure and shear to transform starch granules into viscous material before exiting the extruder. This cooking action and gelatinization can be partial or complete. (Bazolli et al., 2015).

The transfer of energy into the product, both mechanical and thermal, is what drives the rate of the cooking process, extent of gelatinization and product expansion. (Monti et al., 2016a). The specific mechanical energy (SME) describes the amount of work imparted by the extruder to the material and is produced as a result of the friction of the material between the screw, material and barrel (Hataichanok Kantrong et al., 2018). Specific thermal energy (STE) is the energy resulting from temperature change and occurs predominately in the preconditioner, of pet food extrusion, where steam is added; it can also happen in the extruder barrel, but typically at a lesser degree (Pacheco et al., 2018).

When assessing pet food and pet food processing from a scientific point of view, several physico-chemical attributes are evaluated.

In dry, expanded pet food, starch conversion (gelatinization) and expansion are the primary outcomes from the cooking extruder. The amount of expansion depends upon three things; the feed composition, extent of cooking and the pressure differential between the extruder and the ambient environment. As the product leaves the die, the pressure drop causes liquid water embedded in the molten starch matrix inside the extruder to vaporize to steam (the gas phase) which drastically increases volume. As the water turns to steam, the starch matrix stretches and then sets (re-crystallizes) expanding beyond the original size of the die from which the material exited (Desrumaux et al., 1998).

In addition to the physico-chemical attributes, mass and energy balance can be an important tool to help understand and quantify, what happens inside an extrusion system. A basic law of thermodynamics states that mass can neither be produced nor destroyed; it is conserved. This means that for an extrusion system in steady state, $mass_{in} = mass_{out}$ (Janssen et al., 2002) and allows us to verify, by calculation, the actual dry feed rate entering the preconditioner, the extruder barrel or at any point in the system. We can also do the same for water and steam. Energy, like mass, is conserved, so an energy balance can be utilized to account for changes in energy inputs and outflows for each treatment or process from the inlet of the hopper to the outlet of the extruder. Having this information can help food scientists and engineers improve the process as needed to improve operational efficiency and/or enhance profitability by maximizing input usage and minimizing non-product outputs, such as electrical or utility wastage.

There is a need for more publications in the scientific literature that are focused on the extrusion process of pet food using mass balance and energy balance to quantify the changes brought about by the altering the test ingredient or process requirements.

The particle size of the raw material is an important factor in pet food production as it can affect the gelatinization of the starch and other physical traits such as density. The particle size reduction and resulting surface area increase also helps with the processing in the form of increased hydration in the extruder. The amount of gelatinization is generally greater for material with smaller particle size than those with larger particle size. (Bazolli et al., 2015).

Outflows, on the other hand, are more complex and require the use of estimations and/or reasonable assumptions; these include the raw material (now with more moisture and higher temperature) and possibly excess steam and energy through the barrel wall. The raw material

that enters the preconditioner contains ingredients that will determine the total heat capacity of the material, which needs to be calculated; as well as containing starch and protein that will be cooked. The steam and water also possess heat capacities that must be calculated for inclusion in the mass and energy balance equations. The assumptions mentioned earlier include that at steady state, the surface of the preconditioner will be at the same temperature as the material and that, unless calculated, all steam condenses into the product (Levine, 2014a). With pet food production, it is typical that not all of the steam condenses. Another assumption to be made is the starch and protein cook levels in the preconditioner. With the knowledge of the actual inputs and sound, educated assumptions, the calculations can be accomplished.

Heat loss in the preconditioner may account for a significant amount, depending on the process and equipment size. Small preconditioners have a much higher surface area to throughput ratio than large preconditioners and thus will likely have a larger heat loss than a large preconditioner (Levine, 2014b). All of these contributions to and from the preconditioner need to be considered when performing the mass and energy balance calculations.

Most of the energy added in the preconditioner is thermal. While the extruder barrel can also have thermal energy input, it has the ability to impart mechanical energy into the product which can help bring about the desired change in the material. Like the preconditioner, in the extruder barrel there is a raw material component, water and possibly steam impact, and also heat entering or leaving the process as an input or an outflow. Other outflows are due to the material with a new temperature and moisture level, steam loss and convection losses as noted previously.

While the extruder barrel has similar inputs and outputs to the preconditioner, the barrel becomes more complex with the ability to heat and/or cool the barrel head sections and with the

addition of mechanical energy imparted to the product from the screw(s). The mechanical energy is fairly easy to quantify with some known parameters and a couple of calculations. However, the transfer of thermal energy to and from the barrel and environment can be challenging to properly calculate (Levine, 1997a) due to the different surface areas and thicknesses of the extruder barrel sections.

This study uses calculations to derive the quantity of heat lost while another study from the research literature used measurements to arrive at the STE value (Karwe and Godavarti, 1997). The measurement method relied upon specialized computer hardware and sensors to be connected to the barrel and thus would not be very flexible to compare from extruder to extruder, while calculation is applicable to most any extruded process.

The extrusion of pet food, that contains plant and animal-based ingredients which have a variable and complex chemical composition, offers challenges that the extrusion of polymers does not; they change with moisture, temperature and cook / denaturation. These changes affect the viscosity of the material and thus can be problematic for steady-state control of the process (Janssen et al., 2002).

When material is passing through the barrel, several things are happening simultaneously. Energy is transferred to the material from the screw and barrel by friction. Water and possibly steam are being injected into the process. Energy may flow into the product from the barrel in one section and energy flow from the product to the barrel in another section. Then the product leaves the barrel along with steam flash-off. The mechanical energy and thermal energy transferred to the material results in a temperature rise of the extrudate. When looking at the area just before the die exit, the extrudate is plasticized, under pressure, at a high temperature and has moisture in the liquid phase; all of this culminates with a stored energy value that is equal to the

value of energy from steam release and extrudate on the "outside" of the die. Thus, when the extrudate crosses the die face, the moisture turns to the gas phase due to the drop to atmospheric pressure and releases the stored energy along with the extrudate. This energy release can be calculated if the temperature just before the die and the temperature after the die are known.

A simple temperature probe, ideally an extended probe that is in the direct flow of the extrudate, will measure the temperature before the die, but obtaining the temperature after the die is much more difficult as the temperature changes rapidly due to the energy release. Experiments from several authors agree that a good estimate of this water flash temperature occurs at 85 °C (Janssen et al., 2002; Levine, 1997b). Another energy source is from the phase change due to cooking of starch and protein, which can be estimated at 17 J/g (Janssen et al., 2002).

Specific Mechanical Energy has been mentioned before and is fairly easy to calculate with a few known quantities. However, Specific Thermal Energy (STE), is more difficult to calculate, but can be estimated using the temperatures before and after the die, along with the estimated temperature, inflow or outflow can be calculated.

Janssen, et al (2002) conducted an energy balance study for an extruder barrel using vegetable raw material and calculated SME and STE values along with the temperature differential across the die face and possible energy associated with the phase changes for starch, protein and water. This approach allowed them to estimate the STE value as a loss or a gain in the extruder barrel, based on whether the number was negative or positive.

Riaz (2000) outlined an approach in which mass in must equal mass out using salmon feed as an example for a complete system. Mass and energy balance were calculated from moisture, carbohydrate, protein, fat and ash content of the material exiting the extruder. The heat

capacities of carbohydrates, proteins, fat, ash, water and steam, along with the flow rates for each, SME and reaction energies were used to calculate total energy inputs and outputs. The end result will enable operators to make decisions on scale and size of new equipment and where possible energy savings might be realized.

In another study by Monti (2016a), the researchers used principles and methods outlined by Riaz to calculate the net steam absorption and used the steam added in the preconditioner divided by the mass flow to arrive at a STE result. The total energy was the sum of STE and SME. They concluded that increased SME due to the increased particle size of wheat bran would cause an increase in the power consumption, which would, in turn, cause higher equipment wear.

Mass and energy balances are a useful tool to help improve the extrusion process by identifying energy inputs that may not being utilized as efficiently as possible and to help reduce extruder wear so that service life might be extended. This helps the company improve efficiency and reduce costs.

As shown by literature, few studies have focused on the effect of the particle size as it relates to processing using mass and energy balance and energy transfer. We hypothesize that a larger grind size will negatively affect the processing determined by product attributes, and ultimately, the palatability of cat food and that grain sorghum can be a suitable replacement for corn and brown rice in cat food.

This work is focused on identifying the changes in mass and energy as it applies to raw materials that differ in particle size due to different grind sizes.

2.2 Materials and Methods

2.2.1 Formulas

The diets were formulated to be nutritionally complete and balanced (AAFCO , 2018) with similar levels of starch and fiber specifically designed for cats. The formulas, with calculated protein at 34.0% and the starch calculated at 28.5 – 29.7% are shown in Table 2.1. Ingredients were purchased, sampled, and analyzed for dry matter, crude protein, fat, ash and total dietary fiber and the formulas were adjusted to the analyzed chemical composition of the raw materials. Thus, the grain inclusion changed slightly between the diets, from 34.70 % of brown rice, 41.97 % corn, 45.55 % red sorghum and 46.76 % white sorghum. Beet pulp and chicken by product meal were adjusted in the formula to produce iso-protein and iso-starch composition. The grinding steps utilized a pilot scale hammer mill (Fitzpatrick, Westwood MA, USA) for all grinding operations. The primary grains were initially ground through a 6.35mm screen to reduce them to a particle size that could be further reduced in a second grinding step. The fish oil and the dry ingredients (except for sorghum) that were used were sourced from a local supplier (Lortscher Animal Nutrition, Inc., Bern, Kansas, USA) as individual ingredients. The sorghum was sourced from a reginal sorghum processor (NU-Life Market, Scott City, KS, USA). The poultry fat (International Dehydrated Foods, Monett, MO, USA) and palatant (SPF-Diana, Hodges, NC, USA) were from primary producers.

2.2.2 Grinding and Mixing

The whole grains were pre-ground through a pilot scale hammer mill (Model D Comminuter, Fitzpatrick, Westwood MA, USA) though a 6.35 mm screen with round openings to break the kernels and make them closer to the larger of the specified size.. They were then

ground a second time, through a 1.0mm screen for the two control grains and the sorghum grains were ground through a 0.5mm, 1.0mm and a 1.6mm screen to produce the 3 experimental particle sizes required. The grain ingredients were then mixed with the other dry ingredients in a double ribbon mixer (Wenger, Sabetha, KS USA). The mixer was operated for 3 minutes to provide a suitable mixing time for mix uniformity. The complete dry formulas were post-ground through the same mill using screens with 0.51 mm, 1.05 mm and 1.65 mm round openings to obtain the maximum particle size goal of 0.5 mm, 1.0 mm and 1.6 mm, respectively.

2.2.3 Particle Size

Particle size assays were conducted using a rotating-tapping sieve system (Ro-Tap, Tyler Co., Mentor, OH, USA) and a laser diffraction system (LS 13 320 SW, Dry Powder System, Beckman Coulter, Indianapolis, IN, USA). The Ro-Tap system uses a series of sieves with increasing screen size with each tray in the stack. A representative 100g sample of material is placed on the top sieve and the shaker turned on and allowed to run for 10 minutes. The material that remains on the top of each sieve is weighed and recorded and data entered into a spreadsheet for the calculations to be performed. The spreadsheet provides an estimate of particle size distribution, variation, cumulative distribution and estimated particles per gram.

Laser diffraction particle size assay was conducted by placing a 100-gram sample into the test chamber of the test equipment (LS 13 320 SW Dry Powder System, Beckman-Coulter, Indianapolis, IN, USA) and initiating the automated test procedure. The procedure measures the manner in which laser light is diffracted off of particle particles differing in size and then provides a summary report (Lyu et al., 2020).

2.2.4 Feed Rate Calibration

The ability of the feeder screw to deliver a constant and consistent feed rate is of vital importance to carry out the various calculations required for a complete and accurate mass and energy balance analysis. The feeder system used in this study was calibrated by measuring the output from the preconditioner at steady state using the WS material, the three grind sizes (0.5mm, 1.0mm, 1.6mm) and also by material height in the feed hopper (high, medium, low). The feed rate in kg/min was measured three times and averaged within each of the three grind sizes at each of the three feed levels. During the extrusion runs, a medium hopper level was utilized as it was easy to maintain that level of fill in the hopper and as the mid-point between high and low weight in the bin. The fill can affect the mass flow rate in a volumetric feeder. Keeping the material at this level also allowed quicker return to normal feed rates as the hopper was allowed to run low and then was refilled for each raw material changeover.

Using the mass balance theory of $mass_{in} = mass_{out}$, the total solids in will equal the total solids out and lead to the raw material feed rate entering the preconditioner multiplied by the raw material dry matter percent will equal the material flow exiting the extruder multiplied by the dry matter percent exiting the extruder, which leads to the calculation method for determining the raw material feed rate, m_r (kg/hr), entering the preconditioner by equation 1:

$$m_r = m_{tot}\text{-}((1\text{-}X_{ex}))/((1\text{-}X_r)) \qquad \text{eq. 1}$$

Where m_{tot} is the mass flow rate of the material coming out of the extruder (kg/hr), X_{ex} is the moisture in the material leaving the extruder (%) and X_r is the moisture content of the raw material entering the preconditioner. The two methods, calibration and mass balance calculation, were averaged, to account for any errors in either method, to provide the final feed rate value that was used in all calculations where needed.

2.2.5 Extrusion processing

The diets were extruded through a X20/E325 single screw extruder with a preconditioner (Model 2 Differential Diameter Conditioner, Wenger, Mfg., Sabetha, KS) for the kibble production. The extruder utilized a 3.25 inch (82.55 mm) diameter barrel with internal spiral heads and a 10:1 L/D ratio. The barrel had six heads that were divided into three heating zones with set temperatures of 60°C for heads 2 and 3, 75°C for heads 4 and 5 and 90°C for head 6, the cone head, from the feed to discharge end. The screw configuration (Figure 2.1) started with a single flighted full pitch screw at the feed throat and transitioned to a double flighted half pitch cone screw element closer to the discharge end. Shear locks were placed between each screw element with increasing size from small to large. One 3.85mm circular, 36° full taper die with a 1.0 mm land length was used, along with 6 knife blades to cut the product.

The extrusion process started with material in a live bottom feed hopper. The material exited the hopper via a variable speed, volumetric, feed auger and then dropped through a transition pipe into the preconditioner. The preconditioner moved material from the inlet to the discharge while also mixing injected water and steam to provide material hydration and start the cooking process. Material was then transferred to the extruder barrel by a diverter. The extruder barrel moved material from the inlet to the exit (die) while imparting mechanical and thermal energy to the product. The die temperature data was collected about 5cm behind the die exit from a probe inserted into the product stream. After being cut with the knife, a negative pressure pneumatic system transports the wet extrudate to the dryer inlet for the drying and cooling process.

Processing conditions such as feeder screw speed, preconditioner screw speed, preconditioner water and steam addition, extruder screw speed, extruder water addition, barrel head temperatures and knife speed were kept unchanged, although some natural process variability occurred, across all treatments. This was done so that any differences in product or independent variables would be attributed to material or grind sizes. The process data is reported in Appendix 1.

2.2.6 Experimental Design

The experiment was designed as a 2x3+2 factorial with 2grian types, red and white sorghum, 3 screen sizes, 0.5 mm, 1.0 mm and 1.6 mm) with 2 comparison treatments, corn at 1.0 mm grind and rice at 1.0 mm grind making up 8 experimental units.

2.2.7 Preconditioner Mass Balance

Using the mass balance principles; wherein, total mass entering the preconditioner equals the total mass exiting the preconditioner, the input streams are raw material, steam and water. The exit streams are material and steam. Therefore, the mass of the steam lost from the preconditioner or not absorbed in the preconditioner (m_{slpc}) equals the sum of the mass of the input streams minus the mass of the material exiting the preconditioner. This was determined using the following equation:

$$m_{slpc} = (m_r + m_{spc} + m_{wpc}) - m_{pc} \qquad \text{eq. 2}$$

Where m_{spc} is the mass flow rate of the steam entering the preconditioner (kg/hr), m_{wpc} is the mass flow rate of the water entering the preconditioner (kg/hr) and m_{pc} is the mass of the

preconditioned material exiting the preconditioner (kg/hr). The mass of the material exiting the preconditioner, m_{pc}, can be calculated from mass balance of solids with the following equation:

$$m_{pc} = m_r ((1-x_{wr})/(1-x_{wpc})) \quad \text{eq. 3}$$

Where x_{wr} is the percent moisture in the raw material (%) and x_{wpc} is the moisture in the material leaving the preconditioner (%). Mass flow streams can be seen graphically in Figure 2.2.

2.2.8 Specific Thermal Energy – Preconditioning

2.2.8.1 Specific Thermal Energy – Net Steam Absorbed

Two methods were used to determine STE. The first method is based on how much steam is absorbed by the raw material.

$$STE_{pcnsa} = Q_{thermal}/m_r \quad \text{eq. 4}$$

To utilize equation 4, $Q_{thermal}$, the energy of the steam absorbed in the preconditioner, must be calculated. This is the energy flow of the steam input minus the energy lost by the steam loss and is calculated as follows:

$$Q_{thermal} = (Q_{spc}-m_{spc}*4.187*T_r)-(Q_{slpc}-m_{slpc}*4.187*T_r) \quad \text{eq. 5}$$

Where Q_{spc} is the enthalpy of steam, m_{pc} is the mass flow rate of steam (kg/hr), 4.187 is the specific heat of water (4.187 kJ/kg-°C), T_r is the temperature of the water in the raw material incoming water to the preconditioner (°C), Q_{slpc} is the steam loss in the preconditioner (kg/hr), m_{slpc} is the steam loss from the preconditioner outlet (kg/hr).

2.2.8.2 Specific Thermal Energy – Net Energy Absorbed

The second method of determining STE is calculating the energy that is absorbed by the material from the steam and water input and the energy in the raw material itself. This method uses the following equation using principles of energy balance:

$$STE_{pcnea} = C_r\,(T_{pc}-T_r)+\Delta_{hcpc} \qquad \text{eq. 6}$$

This equation uses the heat capacity of the raw material, the temperature difference between the inlet and outlet of the preconditioner and the energy required to cook starch and protein to calculate the STE in the preconditioner. Where, C_r is the specific heat of the feed material before hydration (kJ/kg/°C), T_{pc} is the final preconditioner temperature (°C), T_r is the temperature of the incoming feed material (°C) and Δh_{cpc} is the energy absorbed per unit mass of raw material in cooking of starch and protein in the preconditioner (kJ/kg), follows:

$$C_r = (X_w*C_w)+(X_c*C_c)+(X_p*C_p)+(X_f*C_f)+(X_a*C_a) \qquad \text{eq. 7}$$

Where C_r (kJ/kg°C) is calculated by summing the specific heat values of water, carbohydrates, proteins, fats and ash, multiplied by the respective amounts of each fraction in the formula. The X_w is the percent water (%), C_w is the heat capacity of water (4.187 kJ/kg°C), X_c is the percent carbohydrate (%), C_c is the heat capacity of carbohydrate (1.424 kJ/kg°C), X_p is the percent protein (%), C_p is the heat capacity of protein (1.549 kJ/kg°C), X_f is the percent fat (%), C_f is the heat capacity of fat (1.675kJ/kg°C), X_a is the percent ash (%) and C_a is the heat capacity of ash (0.837 kJ/kg°C). The heat capacity constant values are from Singh and Heldman (2009).

In addition to C_r, Δh_{cpc}, the energy put into cooking the starch and protein fractions, needs to be calculated to complete the STE_{pcnea} calculation. The formula for calculating the energy required for starch and protein cook is as follows:

$$\Delta h_{cpc} = (\%\ S_{pc\ raw}*H_c*\%\ S_{pc\ cook})+(\%\ P_{pc\ raw}*H_p*\%\ P_{pc\ cook}) \qquad \text{eq.8}$$

Where % $S_{pc\ raw}$ is the starch amount in the raw material (%) determined by proximate analysis, H_c is the enthalpy of starch (14 kJ/kg), % $S_{pc\ cook}$ is the amount of cook of the starch in the preconditioner (%) calculated by taking the total starch cook and multiplying by 35 % as an estimated amount of cook in the preconditioner from (Strahm, 2021), % $P_{pc\ raw}$ is the protein amount in the raw material (%) determined from proximate analysis, H_p is the enthalpy of protein (95 kJ/kg) and % $P_{pc\ cook}$ is the amount of cook of the protein in the preconditioner (%) and assumed to be equal to that of starch.

2.2.9 PC Wall Loss

The energy that is used to heat the PC wall is important to account for as that is one of the ways that energy can leave the system and will facilitate getting a complete and accurate energy balance. Continuing with the mass balance principles, the PC wall loss energy, Q_{wall}, uses the energy with raw material, water and steam that is entering the preconditioner as equal to the energy leaving the preconditioner with the material, steam loss, cooking loss and wall heating. The equation is as follows:

$$Q_{wall} = [(m_r*C_r*T_r)+(m_{wpc}*T_{wpc})+(m_{spc}*h_{spc})]-[(m_{pc}*C_{pc}*T_{pc})+(m_{slpc}*h_{slpc})+ (\%\ S_{pc\ raw}*H_c*\%\ S_{pc\ cook})+(\%\ P_{pc\ raw}*H_p*\%\ P_{pc\ cook})] \quad \text{eq. 9}$$

Where T_r is the temperature of the raw material going into the preconditioner (°C), h_{spc} is the enthalpy of the steam going into the preconditioner, C_{pc} is the heat capacity of the material in the preconditioner (kJ/kg°C) and T_{pc} is the temperature of the preconditioner (°C).

2.2.10 Specific Mechanical Energy

$$SME\ (kJ/kg) = ((\tau-\tau_0)*N/N_0*P_r)/m_r \quad \text{eq.10}$$

The specific mechanical energy calculation (eq. 10) is used to calculate the mechanical energy imparted to the product resulting from material in contact with the screw and the barrel as it moves down the length of the barrel, from the inlet to the discharge. Where τ is the motor load at processing speed (%), τ_0 is the motor load at no-load speed (%), N is the operating screw speed (rpm), N_0 is the rated screw speed (rpm), P_r is the rated motor power (kW) and m_r is the raw material feed rate (kg/sec).

2.2.11 Specific Thermal Energy - Extruder

The specific thermal energy absorbed by the material in the extruder is energy that is used for raising the temperature of the material and energy for cooking; it is calculated from the following equation using principles of energy balance:

$$STE_{ex} = C_r\,(T_{exit}-T_{pc}\,)+\Delta h_{cex}-SME \qquad \text{eq. 11}$$

Where, STE_{ex} is the specific thermal energy absorbed (kJ/kg), T_{exit} is the final temperature to which the product briefly equilibrates upon leaving the die (°C), T_{pc} is the final preconditioner temperature (°C) and Δh_{cex} is the energy absorbed per unit mass of raw material in cooking of starch and protein in the extruder (kJ/hr), which is calculated as follows:

$$\Delta h_{cex} = (\%\ S_{ex\ raw}*H_c*\%\ S_{ex\ cook}\,)+(\%\ P_{ex\ raw}*H_p*\%\ P_{ex\ cook}) \qquad \text{eq. 12}$$

Where Δh_{cex} is the energy absorbed per unit mass of raw material in cooking of starch and protein in the extruder (kJ/kg), $\%\ S_{ex\ raw}$ is the starch amount in the raw material (%) as determined from lab analysis, H_c is the enthalpy of starch (14 kJ/kg), $\%\ S_{ex\ cook}$ is the amount of cook of the starch in the extruder (%), which we used as one minus the starch cook in the preconditioner (%), $\%\ P_{ex\ raw}$ is the protein amount in the raw material as determined by

proximate analysis(%), H_p is the enthalpy of protein (95 kJ/kg) and % $P_{ex\ cook}$ is the amount of cook of the protein in the extruder (%), using one minus the protein cook in the preconditioner.

$$T_{exit} = ((m_{tot}*C_{tot}*85)+Q_{slex})/(m_{ext}*C_{ext}) \quad \text{eq. 13}$$

The T_{exit} temperature is the temperature of the material immediately after the die (°C), m_{tot} is the mass flow rate from the measurement method (kg/hr), C_{tot} is the specific heat of the material in the measurement method (kJ/kg/°C), 85 is the "final" product temperature (°C), Q_{slex} is the heat capacity of the steam lost at extruder, m_{ext} is the mass flow rate from the extruder (kg/hr), and C_{ext} is the specific heat of the material in the extruder (kJ/kg/°C).

2.2.12 Extrudate Mass Balance for Steam Loss (m_{slex})

The steam loss from the extruder is important to calculate so that the mass balance will be accurate and complete and is based on the total mass entering equals total mass exiting. Thus, the mass of the input streams, material form the preconditioner and water in the barrel must equal the mass of the output streams, material and steam. It is calculated by this equation:

$$m_{slex} = m_{pc} + m_{wex} - m_{tot} \quad \text{eq. 14}$$

Where m_{slex} is the mass flow of the steam lost (not absorbed) in the extruder (kJ/kg), m_{pc} is the mass flow rate of the raw material entering the extruder (kg/hr), m_{wex} is the mass flow rate of the water entering the extruder (kg/hr) and m_{tot} is the mass of the material exiting the extruder (kg/hr) and is calculated from the following:

$$m_{tot} = m_r\ ((1-x_{wr})/(1-x_{wtot})) \quad \text{eq. 15}$$

Where x_{wr} is the percent moisture in the material entering the preconditioner (%) and x_{wtot} is the percent measured moisture of the material exiting the extruder (%). This calculation is based on dry matter as it passes through the system.

2.2.13 Energy Totals

The energy input ratio and the total energy equations are helpful for comparing processes and treatments across products or across within treatments; the energy input ratio is calculated using this equation:

$$\text{Energy input ratio} = (STE_{pc} + STE_{ext})/SME \quad \text{eq. 16}$$

Where STE_{pc} is the specific thermal energy from the preconditioner (kJ/kg), STE_{ext} is the specific thermal energy from the extruder (kJ/kg) and SME is the specific mechanical energy from the extruder (kJ/kg).

The total specific thermal energy absorbed in the extrusion process is simply:

$$STE_{tot} = STE_{pc} + STE_{ex} + SME \quad \text{eq. 17}$$

Where STE_{pc} is the specific thermal energy from the preconditioner (kJ/kg), STE_{ex} is the specific thermal energy from the extruder (kJ/kg), but for positive values only, as negative STE Is not possible, and SME is the specific mechanical energy from the extruder (kJ/kg).

2.2.14 Statistics

This experiment was blocked for a time design with multiple samples taken during the steady state condition for each treatment. The product attributes for raw material physicochemical qualities, finished product physicochemical qualities and other quality measurements were assigned as dependent variables. Each measured or calculated response variable was analyzed statistically using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Analysis of variation (ANOVA) and least squared means (LSM) procedures for pair-wise comparisons were done to obtain the F-value and P-score with a significant level of $\alpha=0.05$ within a Type III SS. The type III SS level was selected as these values tend to be narrower and give less of an

opportunity to have a false negative. This same raw data was put into Excel 2013 (Microsoft, Inc. Redmond, WA, USA) to obtain the variable correlations.

2.3 Results and Discussion

2.3.1 Feed Rate

2.3.1.1 Feeder Calibration

The dry material feed rate is the key to starting and maintaining process stability. If the feed rate is not constant and consistent, then there is little chance that the rest of the process will be constant and consistent. This extrusion system used a live bottom feed bin, with a volumetric feeding system bringing material to the preconditioner inlet via a feeder screw. A volumetric system is based on the speed of the feed screw and doesn't compensate for changes in density of the material, nor does it compensate for changes in the fill level of the feed hopper above it. Therefore, it may be difficult, depending on the operating procedure, to maintain the constant and consistent feed rate that is beneficial to an extrusion process. The fill level of the material in the hopper should be kept as constant as possible so that each revolution of the screw delivers the same volume of product.

The calibration method was completed with the WS formula at all three grind sizes and all three hopper levels. The RS, corn and brown rice formulas were assumed to be an equivalent feed rate to their respective level and grind.

2.3.1.2 Mass Based Calculation

After the extrusion runs were complete and the mass and energy balances were being computed, the dry material feed rate was back-calculated. Based on moisture content of the kibble directly off of the preconditioner, known amounts of steam and water added to the preconditioner and the temperature of the material off the preconditioner the actual feed rates were determined to vary with particle size. The results of the two methods for determining the raw material feed rate are shown in Figure 2.3. There is a noticeable difference in that the calibration method ranged from 1.5-13.3% higher than the mass balance method among all treatments. This could be due to the location and time of sample collection for moisture measurement and the time required to do the weighing step for the mass flow value. The mass calculation method requires sample collection for moisture measurement and also weighing of the timed sample of the wet extrudate off the extruder. The sample collection location, meaning whether the sample was collected immediately after the discharge from the die or collected a few seconds later at the point of sample weighing could make a difference in the moisture value as the material is constantly losing moisture to the environment. The time required for sample collection and the subsequent weighing can also be a source of error for the same reason, the moisture evaporating to the environment could also be a source of weight loss.

The final values reported in Figure 2.4 are an average of the calculated feed rate and the adjusted actual feed rate as determined from the mass balance. There was a positive correlation between particle size and the feed rate (R^2=.9619, $P<0.05$. While the 1.0 mm grind size for WS and RS are slightly lower than corn and brown rice, the corn had an increased flow versus the brown rice. This increased flow with the larger particle size is likely due to the lower surface area (to mass), more area between particles and thus is a more “free flowing” material (McGuire, 2017).

2.3.2 Preconditioning Process Analysis

2.3.2.1 Mass Balance and Steam Loss

One of the primary causes of energy loss is the steam loss from the preconditioner. This is the amount of steam that is "lost" out of the preconditioner to the environment and thus, has not been absorbed by the material. This amount can vary from process to process and formula to formula, but is common to most dry, expanded pet food extrusion lines. Steam loss from the preconditioner (m_{slpc}) occurs because of excess steam that has not been condensed on to the raw material flowing through the preconditioner. This is a common component of pet food extrusion, but it is difficult to quantify as measuring it is challenging. However, we can calculate the steam loss if we use equation 2 and equation 3. Using the data from the White Sorghum 0.5 mm grind treatment, we first use equation 2 to calculate m_{pc}, the mass flow in the preconditioner, by using m_r, the mass flow rate of the raw material entering the preconditioner (119.5 kg/hr), x_{wr}, the percent moisture in the raw material (6.99/100 %) and x_{wpc}, the measured moisture of the material exiting the preconditioner (19.86/100 %). This yields a value for m_{pc} of 138.7 kg/hr. Using this information in equation 1 we can now determine the m_{slpc} with the following data; m_r as above (119.5 kg/hr), m_{spc}, the mass flow rate of the steam entering the preconditioner (14.9 kg/hr), m_{wpc} is the mass flow rate of the water entering the preconditioner (7.0 kg/hr) and m_{pc}, as calculated above (138.7 kg/hr). Thus, we get the rate of steam exiting the preconditioner of 2.7 kg/hr. Levine (1997b, 2014a) reported a similar value of 8.9 kg/hr, although a bit higher, for a sample calculation based on experimental data.

In this work by Levine (2014a), they discussed mass and energy balance, but did not calculate steam loss. Instead they made the assumption that all steam was condensed on to the

material. However, this assumption may not be correct for preconditioner temperatures higher than 90°C.

Figure 2.5 shows the steam lost, not condensed on the material, range was 0.94-5.29 kg/hr, with most values between 1.82-3.43 kg/hr. This steam loss estimation led to a percent loss of 6.3-36.7%, as noted in the figure, above the bars. Had this been statistically significant, the trend for less steam loss as particle grind size increased may have been due to the increased feed rate of the larger grind size material. This increased feed rate means that more dry material fill will increase the total surface area for steam to condense, more surface for the steam to condense means more steam condensing on the material and less steam exiting the preconditioner. The WS treatments as a whole, had less steam loss than the RS treatments. This could be from the RS having a different particle size after grinding than the WS (WS was the only treatments to be calibrated), the RS, corn and brown rice formulas were assumed to be similar to their respective WS grind size. This lack of calibration for RS could also be why the RS0.5 and RS1.0 values appear to be different also.

2.3.2.2 Heat Loss Through Preconditioner Wall

Heat lost through the preconditioner wall is an outflow of energy that certainly affects the efficiency of that process. Small preconditioners tend to lose more heat than large preconditioners due to differences in surface area per unit of mass flow (Levine, 2014b), so being able to calculate this loss might help with scale-up and troubleshooting problems.

The heat lost to the preconditioner wall is calculated by equation 9 using the following numbers; M_r, the mass flow rate of the raw material (119.5 kg/hr), C_r, the heat capacity of the raw material (1.53 kJ/kg°C), T_r, the temperature of the raw material (25.6 °C), M_{wpc}, the mass of

the water going into the preconditioner (7.0 kg/hr), C_w, the heat capacity of the raw material (4.187 kJ/kg°C), T_{wpc}, the temperature of the water going into the preconditioner (4.4 °C), M_{spc}, the mass of the steam entering the preconditioner (14.9 kg/hr), h_{spc} is the enthalpy of the steam going into the preconditioner (2661 kJ/kg°C) , M_{pc}, the mass flow rate of the material in the preconditioner (141.3 kg/hr), C_{pc}, the heat capacity of the material in the preconditioner (1.93 kJ/kg°C), T_{pc}, the temperature of the preconditioner (86.0 °C), M_{slpc}, the mass of the steam loss exiting the preconditioner (2.69 kg/hr), h_{slpc}, the enthalpy of the steam loss exiting the preconditioner (2224.2 kJ/kg°C), % $S_{pc\ raw}$, the starch amount in the raw material (40.6/100 %), H_c, the enthalpy of starch (14 kJ/kg), % $S_{pc\ cook}$, the amount of cook of the starch in the preconditioner (91.14/100 %), % $P_{pc\ raw}$, the protein amount in the raw material (33.55/100 %), H_p is the enthalpy of protein (95 kJ/kg) and % $P_{pc\ cook}$, the amount of cook of the protein in the preconditioner (35/100 %). The Q_{wall} for WS 0.5 is 8315.82 kJ/hr and dividing by the raw material flow rate (119.5 kg/hr), we get 69.61 kJ/kg going to heat the preconditioner walls.

The results are shown on Figure 2.6. Any value greater than zero indicates that the value is thermodynamically possible and can be used as a check on calculations. This calculation has seven quantities that are not measured; they are either calculated or assumed; this allows for results to vary greatly depending on the assumptions made and thus, these assumptions are important. A higher wall loss value will lead to a lower preconditioner downspout temperature, T_{pc}, because the steam is heating the walls and not heating the material. The CO treatment stands out as the single significant treatment, likely due to the way that the corn particles fracture when ground and don't allow moisture to penetrate as well as the other treatments. While not statistically different among all WS compared to RS and an overall R^2 value of 0.5242, it appears

there is a trend that RS absorbs more energy and thus allows less energy to escape through the preconditioner wall.

2.3.2.3 STE

2.3.2.3.1 STE Net Steam Absorbed

STE_{pcnsa}, the first method for determining the STE In the preconditioner is calculating the amount of steam that has been absorbed using equation 4 and 5. Equation 5 uses the following values for the WS 0.5 grind treatment; Q_{spc}, the enthalpy of steam (39648.90 kJ/kg-°C), S_{pc}, the mass flow rate of steam (14.85 kg/hr), 4.187 kJ/kg-°C, the specific heat of water, T_(pc water), the temperature of the incoming water to the preconditioner (25.58 °C), Q_{slpc}, the steam loss in the preconditioner (9232.92 kg/hr) and S_{slpc}, the steam loss from the preconditioner outlet (4.15 kg/hr). The equation leads to $Q_{thermal}$ being 29263.83 kJ/kg. Equation 4 simply uses the above calculated $Q_{thermal}$ and the mass flow rate of the raw material (119.46 kg/hr). Using these two numbers, we get STE_{pcnsa} = 244.96 kJ/kg.

When looking at the difference in WS versus RS in Figure 2.7, it is evident that the RS has lower values than WS, even while the R^2 value of 0.5116. This is feasibly due to several reasons. The RS was not directly calibrated, but instead, assumed to be the same as WS; this change in feed rate would change the calculated number. The WS has a higher STE_{pcnsa} and a lower m_{slpc}, the RS is opposite, with a lower STE_{pcnsa} and a higher m_{slpc}. The corn, with the highest STE_{pcnsa}, again, was not directly calibrated for feed rate and the particle size, even though ground through the same screen, was different than the WS. A potential disadvantage of this method is that it does not correct, or account for, the heating of the preconditioner walls or the heating of the water component.

Unlike SME, shown in Figure 2.8 and discussed later, the larger particle size does appear to have less loss in the white sorghum treatments. As the finer particle size flow rate is less, there is less fill inside the preconditioner, less fill means less bed depth (product fill), and less bed depth means that there is less material for the steam to condense upon and therefore proportionally more loss. The larger particle size therefore has a greater fill and even though there is less absorption in total due to particle size, there is more material to absorb the steam thus more absorption per unit of time.

2.3.2.3.2 STE Net Energy Absorbed

Using equations 6, 7 and 8, the second method can estimate STE_{pcnes}, using the net energy absorbed. Starting with Equation 7 to calculate the C_r, we need the following data from analysis of the material for the WS 0.5 grind treatment; X_w, the percent water in the formula (6.99/100 %), C_w, the heat capacity of water (4.187 kJ/kg°C), X_c, the percent carbohydrate (39.43/100 %), C_c, the heat capacity of carbohydrate (1.424 kJ/kg°C), X_p, the percent protein (33.55/100 %), C_p, the heat capacity of protein (1.549 kJ/kg°C), X_f, the percent fat (6.17/100 %), C_f, the heat capacity of fat (1.675 kJ/kg°C), X_a, the percent ash (5.93/100 %) and C_a, the heat capacity of ash (0.837 kJ/kg°C). The resulting C_r is 1.53 kJ/kg°C (Table 2.1).

Using equation 8, to calculate the Δh_{cpc}, the energy absorbed per unit mass of raw material in cooking of starch and protein in the preconditioner (kJ/kg),we need the following information; % $S_{pc\ raw}$, the starch amount in the raw material (40.6/100 %), H_c, the enthalpy of starch (14 kJ/kg), % $S_{pc\ cook}$, the amount of cook of the starch in the preconditioner (91.14/100 %), % $P_{pc\ raw}$, the protein amount in the raw material (33.55/100 %), H_p is the enthalpy of protein (95 kJ/kg) and % $P_{pc\ cook}$, the amount of cook of the protein in the preconditioner (35/100

%). We used 35% cook in the preconditioner as a protein cook value from Strahm (2021). The final value for Δh_{cpc} is 1431.18 kJ/kg.

Using the above C_r and Δh_{cpc} values we can use equation 6, plus the two temperature values to calculate the STE from the preconditioner. The temperature values needed are the T_{pc}, the final preconditioner temperature (95.25 °C) and T_r, the temperature of the incoming feed material (25.6 °C). The STE_{pc} for WS 0.5 is 118.54 KJ/kg. The same trend and effects are seen here as stated above, only differing total values as the calculation source values are different.

2.3.3 Extruder Process Analysis

2.3.3.1 SME

Specific mechanical energy (SME) is energy that is transferred to the product material from the extruder screw (Hataichanok Kantrong et al., 2018) and is defined as the amount of energy per unit of mass, kJ/kg.

SME input on extrusion varies due to resistance to flow and process parameters such as screw speed. It enters the extrudate as dissipated heat and can help achieve the cooking level desired of the product produced (Altan et al., 2009). In the case of extruded cat food, SME, along with STE discussed later, will help deliver the cook required for the starch and the energy required for protein denaturation. It is not unexpected that motor load is positively correlated (r=0.9683) with SME, meaning as the motor load goes up, SME also goes up. It's also expected that there is moderate positive correlation between SME and the die temperature (r=0.6166). As SME increases, the resistance to flow increases, which increases the die temperature as the die is the last point in the extruder barrel that is a flow restriction.

Figure 2.8 shows a trend that the larger grind size of 1.6 results in lower SME for both white and red sorghum varieties. While the 0.5 and 1.0 showed subtle differences, the 1.6 grind level demonstrated a drastic, and significant, reduction of almost 30% from the average of the 0.5 and 1.0 grind sizes for each sorghum variety and an R^2 of 0.7885. Alvarenga (2018) found similar results in their work with sorghum mill feed, whole sorghum and sorghum flour; although the results were not as drastic, with a 16% difference from the smaller to the larger particle size, no details or explanation was given.

Mathew (1999), working with corn in pet food found that a larger particle size resulted in lower SME, which lowered the dough viscosity and thus a lower motor load.

Figure 2.14 shows the final in barrel moisture based on measured moisture content at the preconditioner exit plus the amount of water added to the barrel. For both the WS and the RS treatments, as the grind size increased from 1.0 mm to 1.6 mm, the moisture decreased, as did the SME. This can be explained, once again, to the larger particle size having less surface area per unit mass for the moisture and energy to penetrate.

2.3.3.2 STE

The STE for the extruder is similar in form to that for the preconditioner, only we need to subtract the SME to obtain the true STE, as the SME will influence the temperature rise. Equations 11, 7 and 12 are used to calculate STE_{ex}. The specific heat of the raw material is calculated with equation 7 by knowing the proximate analysis (moisture, carbohydrate, protein, fat and ash) of the raw material, multiplying those percentages by the respective heat capacities and adding the results together. For the white sorghum 0.5 grind, the proximate and heat

capacities and total specific heat are as follows with the moisture as an example (0.1989 x 4.187 kJ/kg/°C = 0.831457 kJ/kg/°C):

From table 2.2, we calculate the C_r as 1.87 kJ/kg/°C. This gets multiplied by the temperature difference in the barrel, 69.65°C ($T_{exit} – T_{pc}$, 144.75°C – 95.25°C) and the Δh_{cpc} required for cooking the starch and protein fractions in the formula. To calculate Δh_{cpc}, Formula 12 is used and is based on 40.6% raw starch, 14 kJ/kg specific heat of starch, 59.241% starch cook (of the total starch cooked, 35% of the starch is cooked in the preconditioner and 65% is cooked in the extruder), 33.55% protein in the raw material, 95 kJ/kg specific heat of protein, 33.55% protein cook and the dry feed rate of 119.46 kg/hr. Using the above values, equation 12 gives us Δh_{cpc} = 22.248846 kJ/kg. Putting this value into formula 11, gives us 1.87 kJ/kg/°C * 49.50°C + 22.248846 kJ/kg – 205.56 kJ/kg = -90.746154 kJ/kg for STE_{ex} in the extruder. This number being negative, indicates that there is energy flowing out of the barrel, due to the heating of the barrel jackets.

Figure 2.9 shows the effect of larger particle size creating less SME and therefore creating less temperature leads to less energy leaving system. Carvalho (2010) theorized that with corn meal, larger particle size material would have less contact with the barrel wall and therefore would be less affected by barrel temperature and thus not heat as quickly as smaller particle size material. The finer particles would heat more rapidly and reach the melt transition temperature faster than coarser particles resulting in lower viscosity and hence reduce SME. Also, energy expended to reduce the size of the particles in the extruder may not be available for starch conversion.

The differences in WS and RS are likely due to material differences such as the RS having a different particle size versus assuming it is the same as the WS. If the particle size is different, then the feed rate would also be different.

2.3.3.3 Mass Balance and Steam Loss

The extruder steam loss happens at the extruder discharge as steam flash-off occurs when product exits the die. This is the result of water in the liquid phase, under pressure at a high temperature, exits the die with the extrudate; as the pressure drops to atmospheric pressure suddenly, the water turns to steam and expands in volume. This action is what stretches and expands the extrudate and the steam dissipates to the atmosphere as the temperature drops. The flash off steam can be calculated from the mass balance, using moisture measurements and the mass flow rate.

Figure 2.10 shows that the steam loss at the extruder is lowest for the 1.6 mm grind in both white and red sorghums (8.00 kJ/hr and 6.89 kJ/hr, respectively) and highest for the 0.5 and 1.0 mm grind level (9.65-11.05 kJ/kg for white sorghum and 8.30-10.45 kJ/kg for red sorghum). The corn had the highest loss among the 1.0 mm grind level at 14.97 kJ/kg with an overall R^2 value of 0.4266. These results are in general agreement with calculations done by other researchers (Levine, 1997b). This is due to the same reasons as discussed earlier on the preconditioner steam loss.

The energy associated with steam loss can be referenced by examining the steam tables for the correct combination of factors. A difficult issue with calculating the energy of the extruder barrel is accounting for the convection and radiation of heat to the environment from the extruder barrel or even the heat put into the process by the barrel heating / cooling jackets. As

barrel shape and jacketing will affect the heat loss by convection to the environment, the best way is to measure the temperature at several places, top, bottom and sides, on the surface of the barrel simultaneously. However, this is not available on most extruders as the equipment is expensive and tedious to setup properly (Karwe and Godavarti, 1997; Levine, 1997a).

Karwe (1997) did this as part of an experiment to determine heat transfer while running a corn meal based formula on a twin screw extruder. They placed several types of sensors through the barrel, in contact with the product flow to determine and compare extrudate temperatures in the extruder and also sensors on the outside of the extruder to measure the surface temperature of the barrel so a value for heat loss could be calculated. They found that the heat loss from the bottom of the barrel was greatest with the loss from the top and sides were next in order, respectively. Karwe's work was in agreement with previously published research on convection heat transfer theory. Our results are shown in Figure 2.11. Results from Karwe (1997) and Levine (1997a) note that the heat transfer coefficient can be approximated to be about 10-15 W/m2-°C.

Our results (Figure 2.12) demonstrate that as the grind size increases, the heat loss decreases. This is likely due to the SME decreasing and leading to cooler process as can be seen by a cooler die temperature. This in turn leads to a lower temperature differential between the temperature behind the die and the temperature in the surrounding environment. Janssen (2002) demonstrated a typical example and found a STE loss of about 54 kJ/kg to our 38.83 kJ/kg for the WS 0.5 grind treatment.

2.3.3.4 T_{exit} versus T_{die}

The temperature inside the extruder barrel, before the die, T_{die}, and after the die, T_{exit}, are close to the same place physically on the extruder but can differ greatly in value. The T_{die} can be measured with a temperature probe in the melt stream and is ideally placed immediately behind the die. This placement will give the last temperature before the melt exits the die. The T_{exit}, on the other hand, is the temperature right after the product crosses the die face and goes through the pressure differential from the pressure in the extruder to atmospheric pressure in the environment and has to be calculated. Equation 13 uses the following data, from WS 0.5, M_{tot} is the mass flow rate from the measurement method (136.8 kg/hr), C_{tot} is the specific heat of the material in the measurement method (1.98 kJ/kg*°C), 85 is the "final" product temperature that the product cools to after the initial flash-off (85 °C), Q_{slex} is the heat capacity of the steam lost at extruder (21466 kJ/kg*°C) , M_{ext} is the mass flow rate from the extruder (147.2 kg/hr), and C_{ext} is the specific heat of the material in the extruder (2.12 kJ/kg/°C). Solving this equation results in T_{exit}=142.3 °C. Figure 2.13 shows the T_{exit} and T_{die} results; there is a general trend towards the larger particle size having a lower temperature. A larger particle size leads to lower SME, lower SME leads to lower die temperature, and this leads to less steam loss to the environment.. Due to physics, T_{exit} should always be higher than T_{die} because there is more energy in the steam vapor upon exiting the die than the liquid vapor under pressure before the die. This makes calculating T_{exit} a useful tool for checking the assumptions made in the mass and energy balance calculations. One such assumption made in this experiment that could use revisiting is the feed rate calibration and doing a calibration for each and every grain and grind size.

The sorghum treatments shown in Figure 2.15, show a definite SME reduction with the higher grind size as compared to the smaller grind size, demonstrating that the larger particle size reduces the SME by lower temperature and thus not forming a viscous material in the extruder.

The brown rice formula shows (Figure 2.15) that it has the lowest SME of the 1.0 mm grind size along with the lowest phase transition temperature (the temperature at which the material begins for soften and flow through an orifice, measured by a capillary rheometer) and the highest moisture indicating that the brown rice formula absorbs the moisture better, makes a viscous mass and flows at a lower temperature than the other formulas, possibly due to the higher moisture. Die temperature is moderately correlated with several parameters that are temperature and moisture based, such as the cylinder steam flow (r=0.5583), which makes sense as more steam is injected into the cylinder, the temperature in the preconditioner rises and therefore has more ability to keep that temperature and drive the die temperature as well. The die temperature is also negatively correlated with the extruder water flow (r=-0.5453) due to same reasoning earlier, the increased moisture acts to cool the material and thus a lower die temperature.

2.3.4 Overall Energy Analysis

2.3.4.1 STE / SME

The STE:SME ratio is a quantity that is easy to calculate and useful for comparing processes and treatments to each other. The ratio is simply the total STE divided by the SME; equation 16 demonstrates the calculation and the results are shown on Figure 2.16. The first method uses the steam balance where STE_{pcnes}, the specific thermal energy from the preconditioner (269.77 kJ/kg), STE_{ex}, the specific thermal energy from the extruder (0.00 kJ/kg) and SME, the specific mechanical energy from the extruder (205.56 kJ/kg). The STE / SME ratio for the steam method is 1.31. The second method uses the absorption where, STE_{pcnes}, the specific thermal energy from the preconditioner (118.54 kJ/kg), STE_{ex}, the specific thermal energy from the extruder (0.00 kJ/kg) and SME, the specific mechanical energy from the

extruder (205.56 kJ/kg). The STE / SME ratio for the steam method is 0.58. The ratio will always be smaller for the absorption method as it accounts for the wall heating. As we look at the ratio and total energy, we find that the STE doesn't change that much as discussed previously; all the changes are related to SME and thus change the energy ratio and total energy calculation due to the higher flow rate of the larger particle size.

2.4 Conclusion

Grind size and to a certain extent, grain type, plays a key role in processing pet food. The differences in flow rate due to grind size can be sizeable and thus, a consistent grind size is important. Steam loss and heat loss in the preconditioner are two other important factors to consider when doing processing pet food. The larger the grind size, the higher the raw material flow, the lower the steam loss that occurred in the preconditioner and in the barrel and the lower the SME that was imparted to the product.

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Chapter 2 Figures

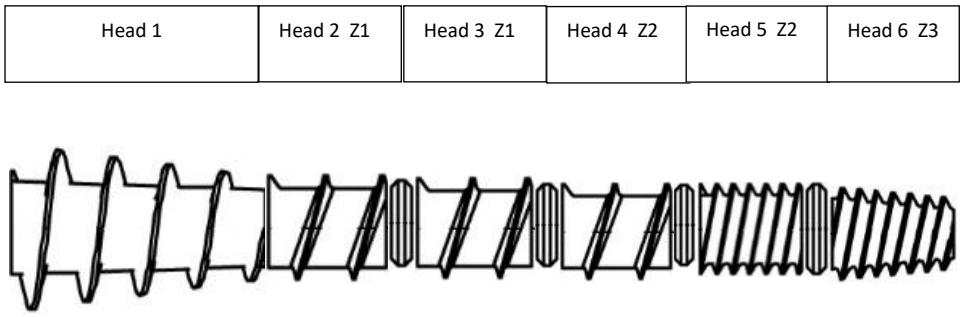


Figure 2.1 Extruder Screw Profile

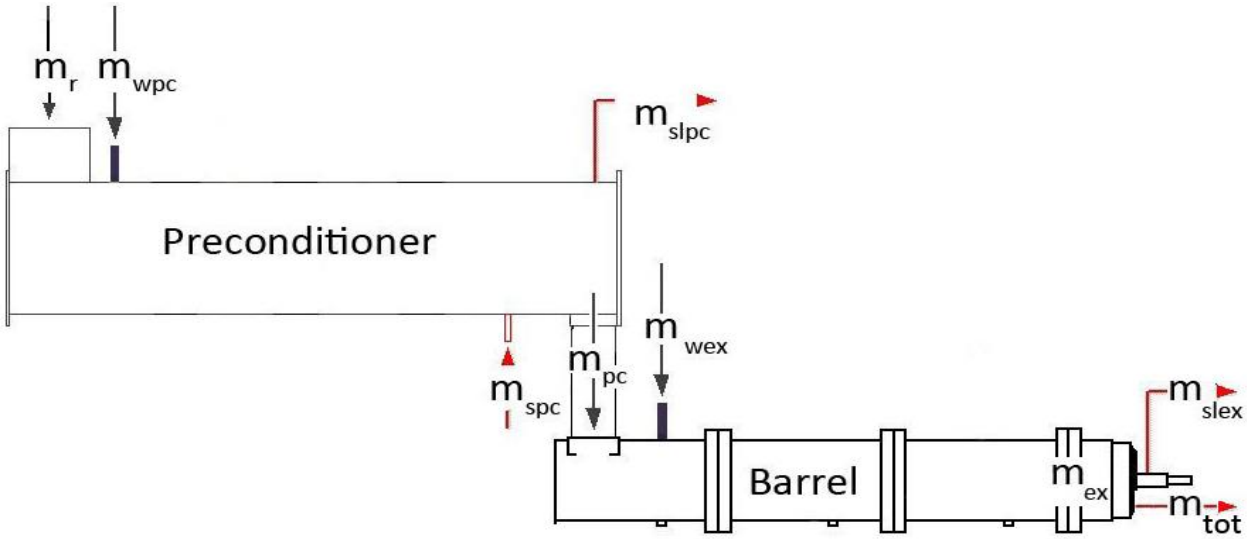


Figure 2.2 Mass Streams in Extrusion System

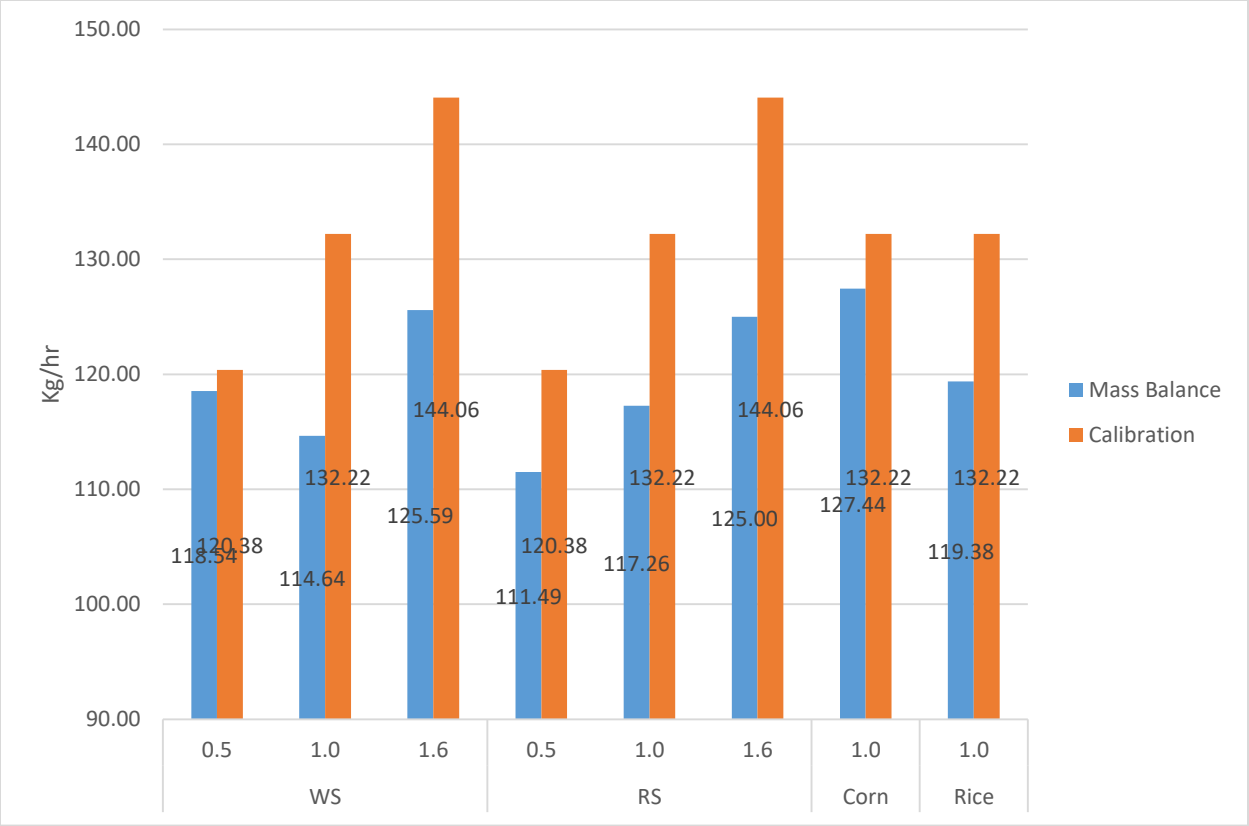


Figure 2.3 Calibrated and Calculated Raw Material Feed Rate for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

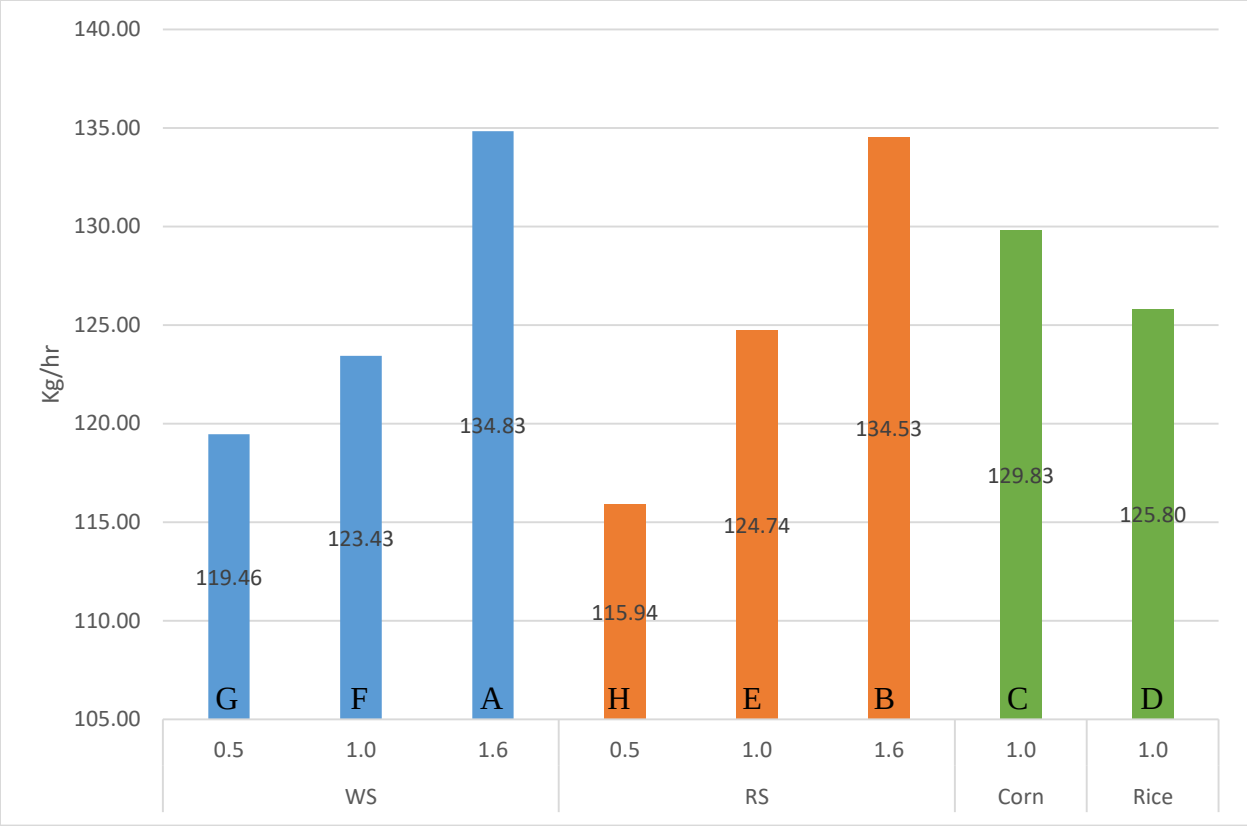


Figure 2.4 Mean Feed Rate Between the Calibration Method and the Calculation Method for Determining The Actual Feed Rate for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

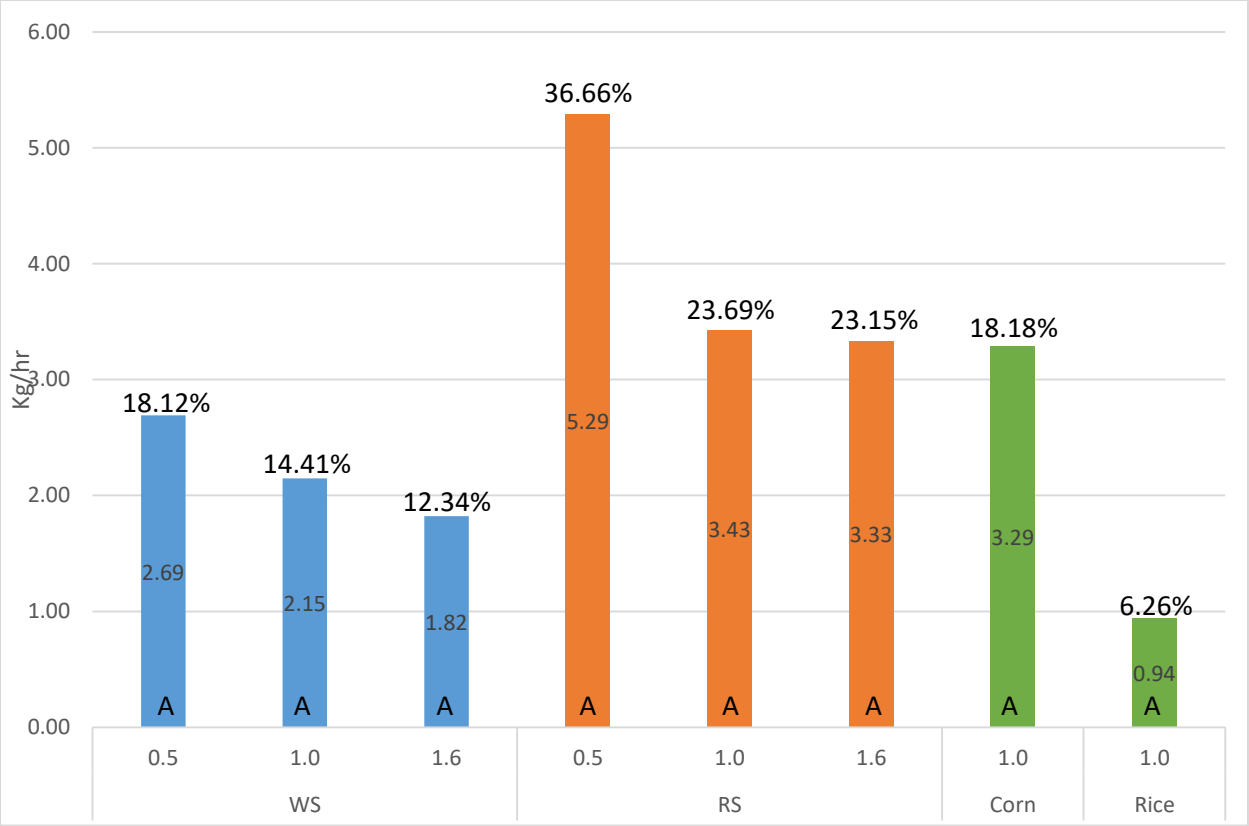


Figure 2.5 Calculated Steam Loss in the Preconditioner (m_{slpc}) for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

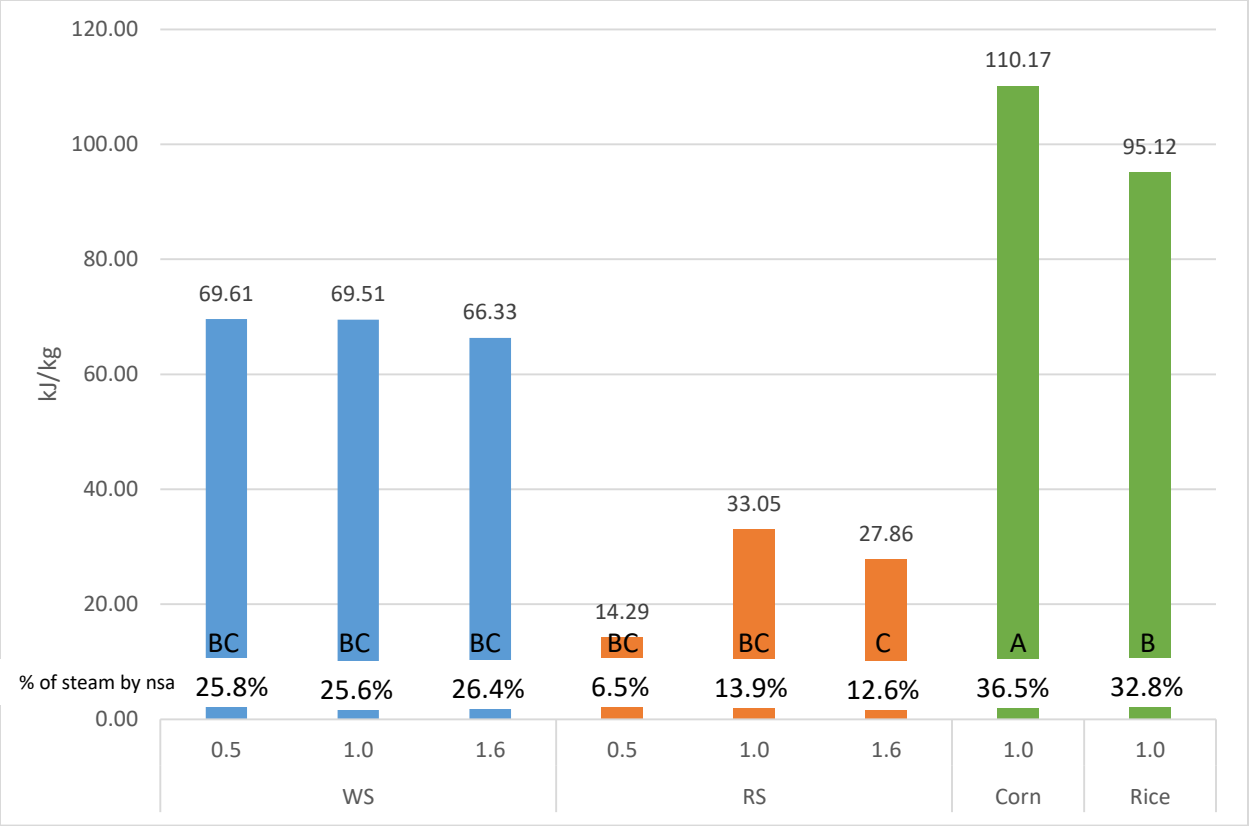


Figure 2.6 Heat Loss Through the Preconditioner Wall (Q_{wall}) for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

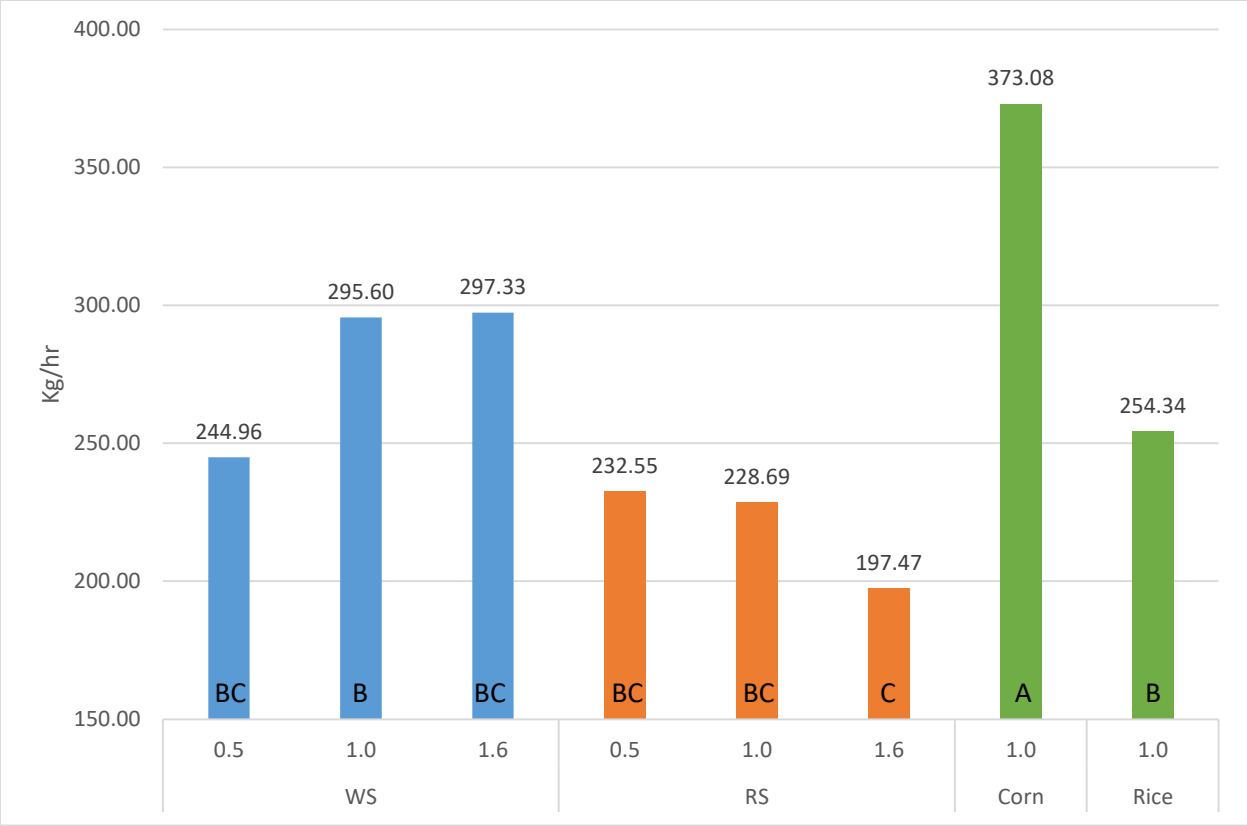
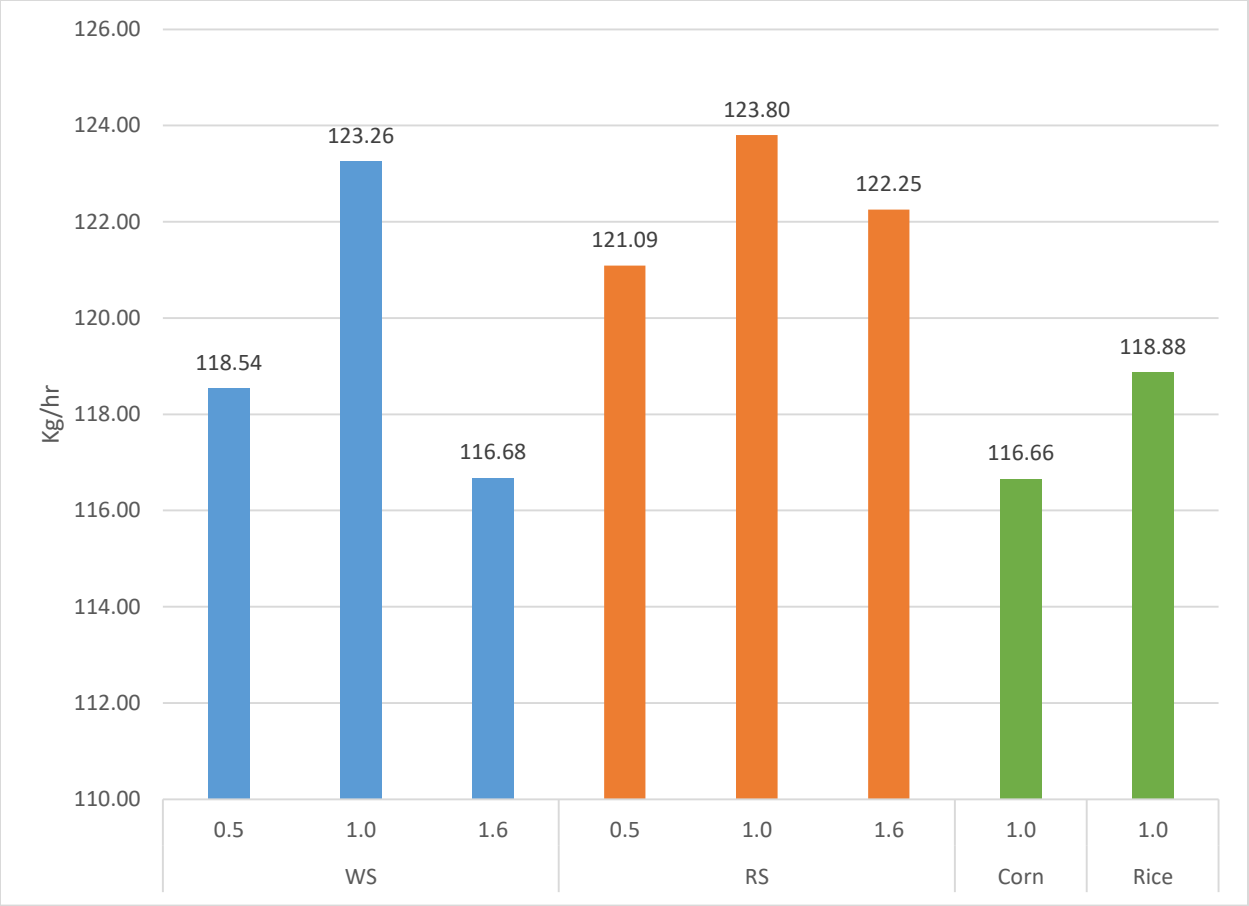


Figure 2.7 Calculated STE_{pcnsa} for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes



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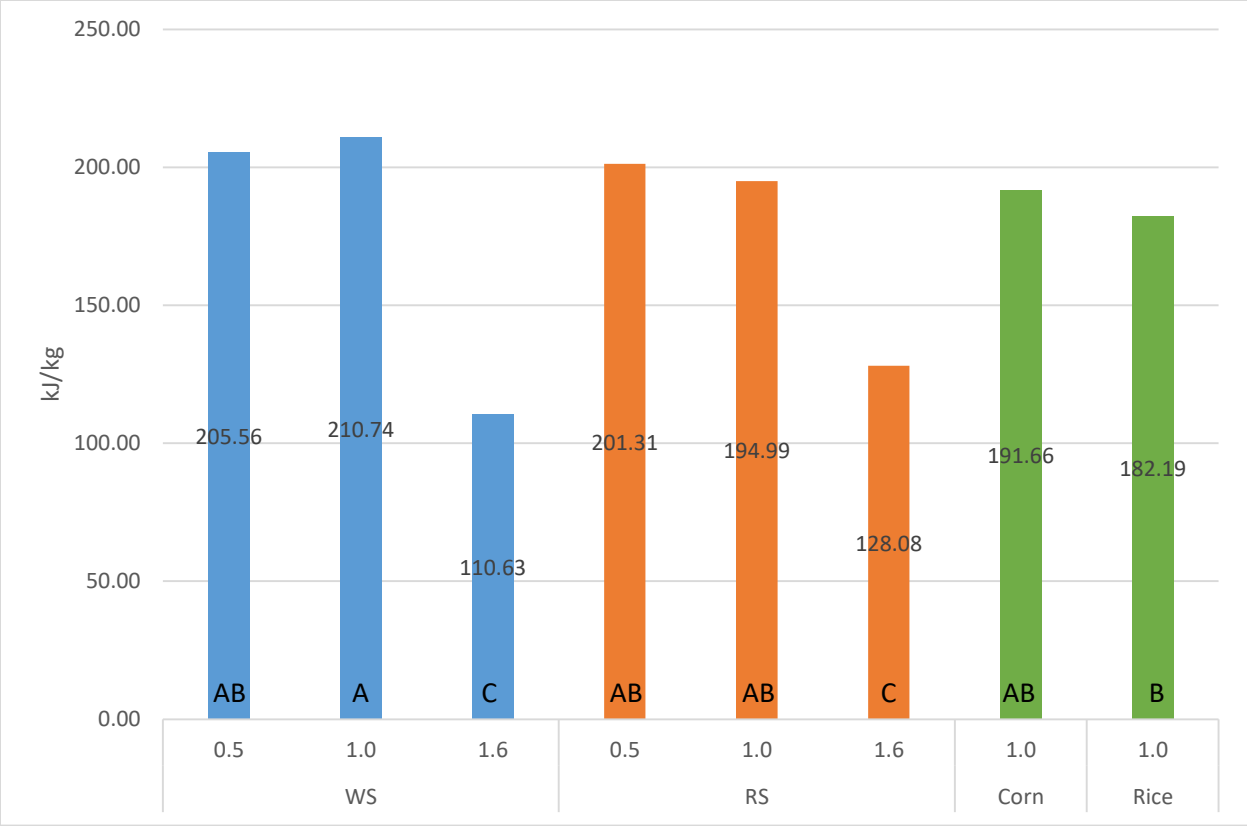

Figure 2.9 Calculated SME for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

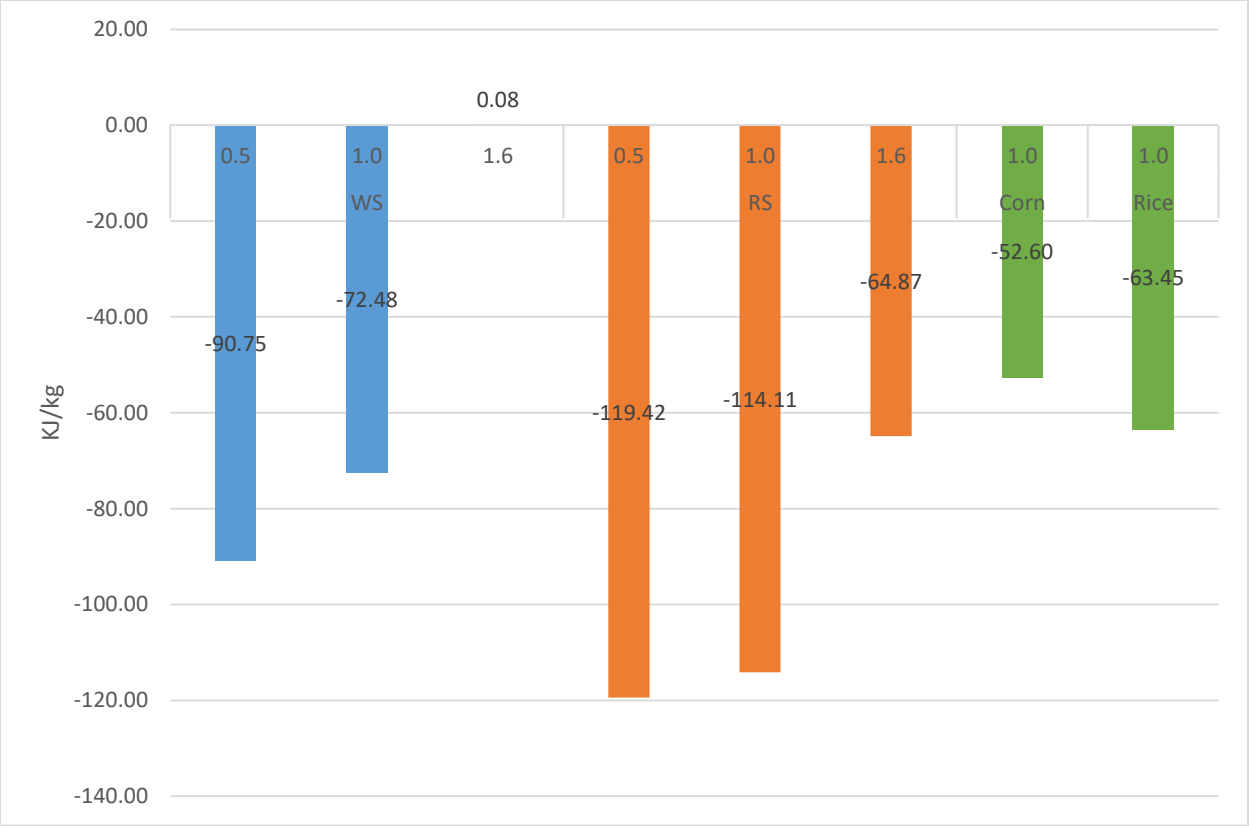

Figure 2.10 STE_{ex} for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

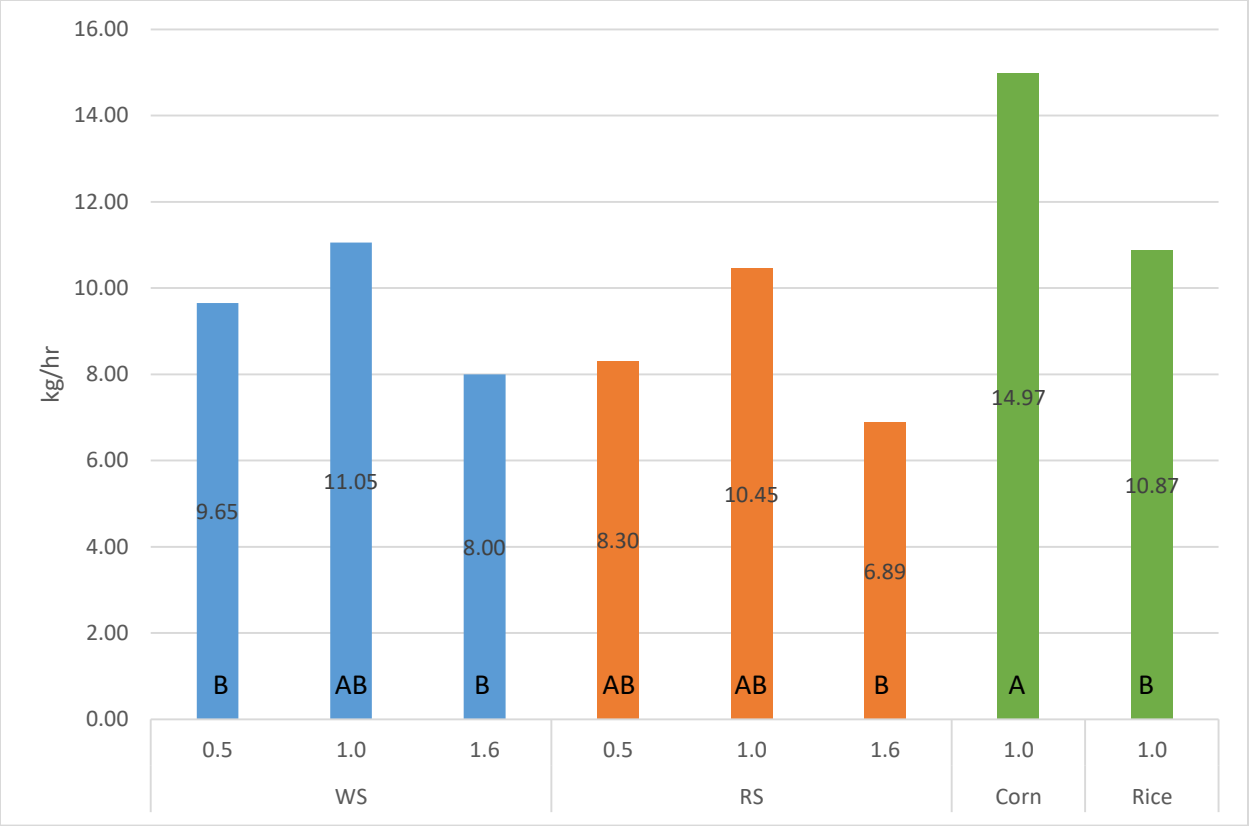

Figure 2.11 Extruder Barrel Steam Loss (m_{slex}) for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

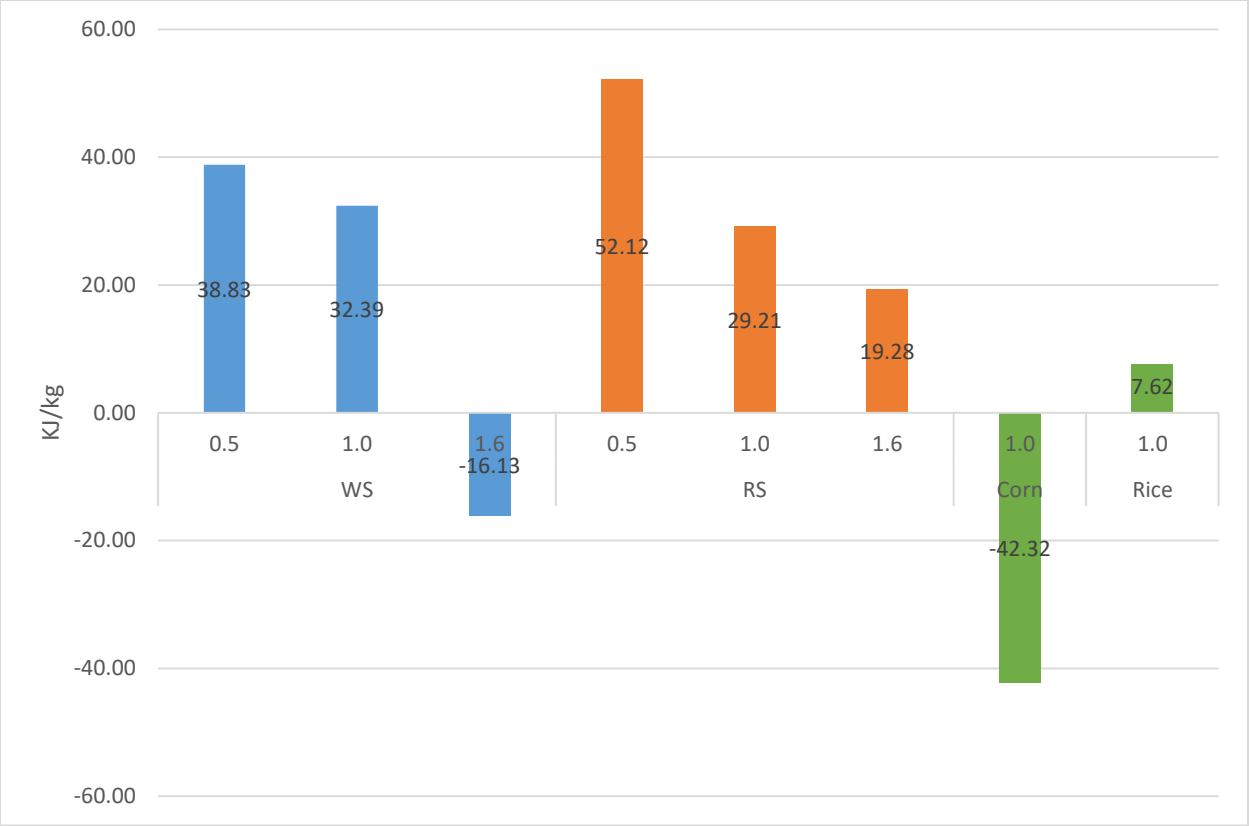

Figure 2.12 Q Loss in Barrel for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

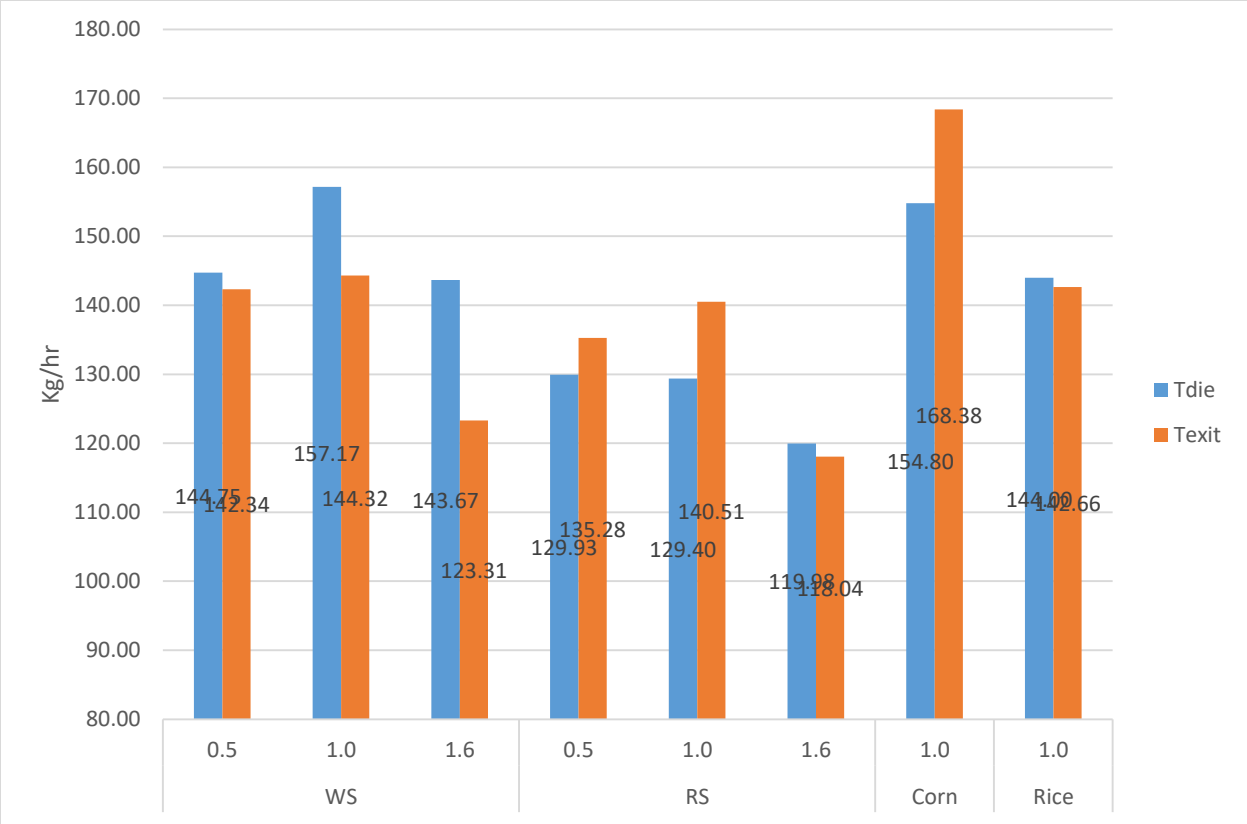

Figure 2.13 T_{exit} and T_{die} for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

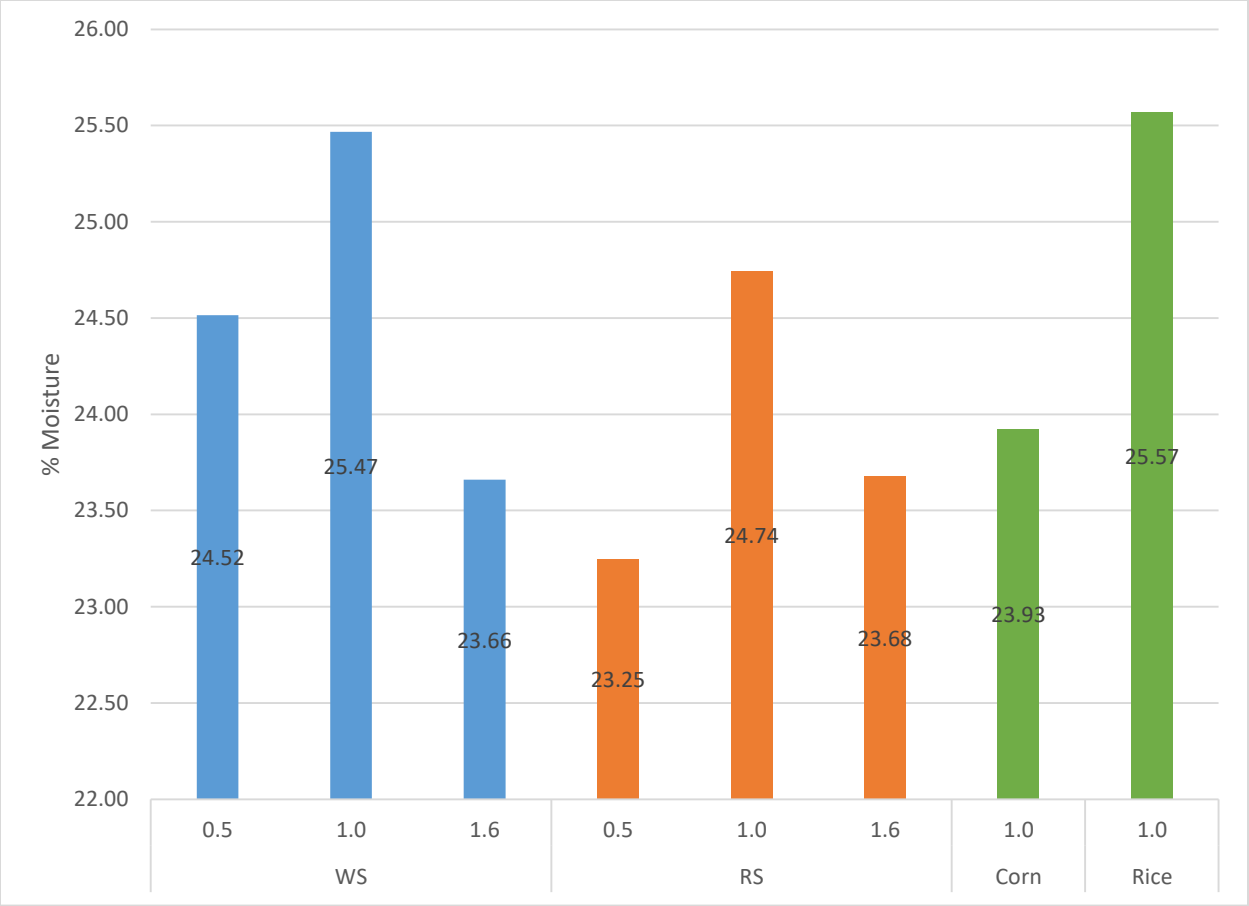

Figure 2.14 Calculated In-barrel Moisture Amounts for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

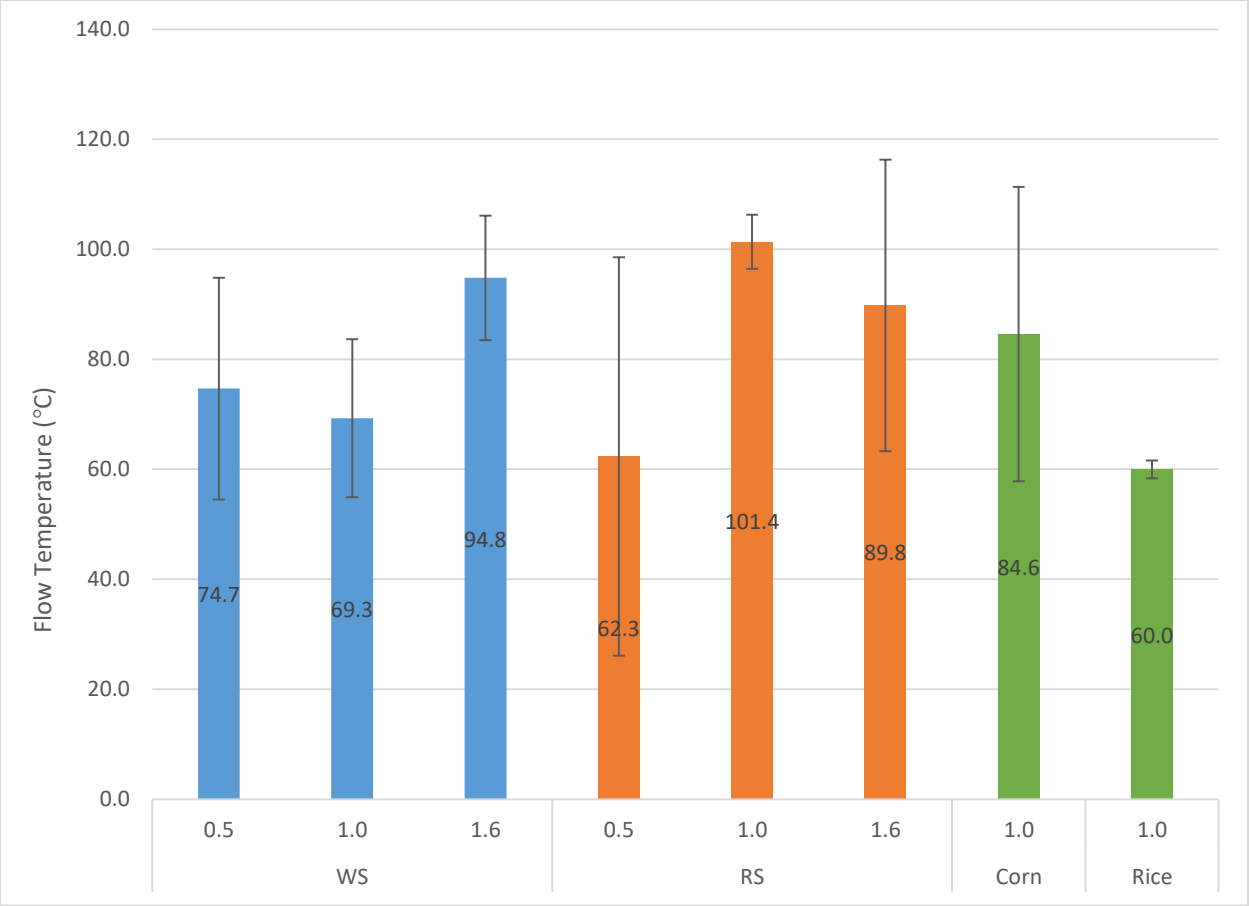

Figure 2.15 Phase Transition Analyzer Results for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

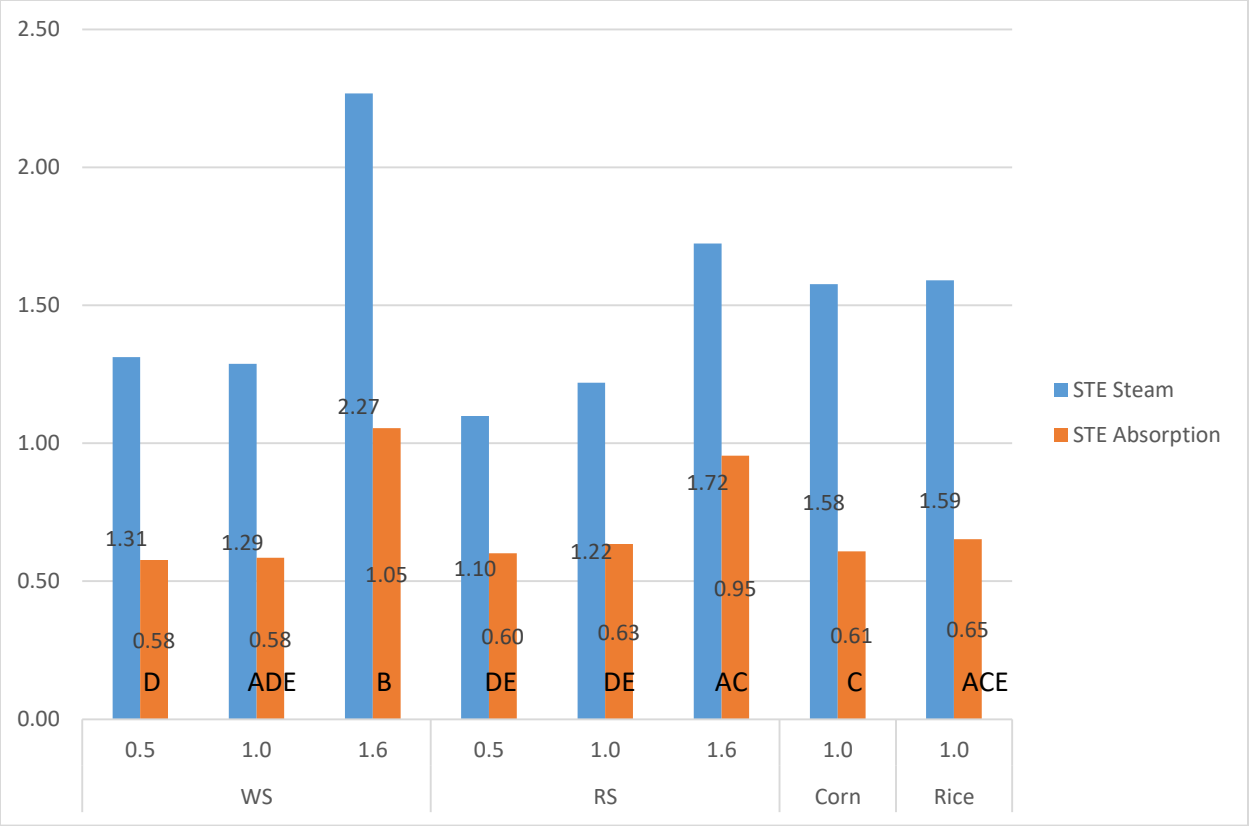

Figure 2.16 STE : SME Ratio Using Both Methods of Accounting For Steam Absorption Amount in White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

Chapter 2 Tables

Table 2.1 C_r Preconditioner Calculations Example

Component	%	Heat Capacity (kJ/kg)	C_p (kJ/Kg)
Moisture	6.99	4.187	0.292611
Carbohydrate	39.43	1.424	0.561483
Protein	33.55	1.549	0.51969
Fat	6.17	1.675	0.103348
Ash	5.93	0.837	0.049634
		Total Specific Heat:	**1.526765**

Table 2.2 C_r Extruder Calculations Example

Component	%	Heat Capacity (kJ/kg)	C_p (kJ/Kg)
Moisture	19.89	4.187	0.831457
Carbohydrate	33.33	1.424	0.474582
Protein	28.36	1.549	0.439256
Fat	5.22	1.675	0.087352
Ash	5.01	0.837	0.041952
		Total Weighted Average Specific Heat:	**1.874599**

Chapter 3 - Relationship Between Processing and Physico-Chemical Characteristics of Sorghum-Based Extruded Cat Food

Abstract

The effect of particle size and type of primary grain on processing and properties of extruded dry expanded cat food kibbles was investigated. Most of the diets were primarily sorghum-based. Two varieties of grain sorghum (white and red) milled at three grind sizes (0.5, 1.0 mm and 1.6 mm) were incorporated in a premium cat food formulation and processed using a pilot-scale single screw extruder. Corn- and brown rice-based formulas were also extruded as comparisons. Grind size and grain type (red sorghum, white sorghum, corn and brown rice) both had a significant ($p<0.05$) impact on starch gelatinization, product expansion and F_p. The average particle size of diets was inversely correlated to extrusion specific mechanical energy ($r= -0.8517$; SME), enzymatically determined gelatinized starch content ($r = -0.8158$), piece density ($r = 0.8221$; inversely proportional to product expansion), and product F_p ($r = 0.5670$). Expansion measures such as bulk density (346.5-403.0 g/L) and sectional expansion index (3.08-3.67) indicated that the brown rice-based formulation expanded the least and corn the most, with sorghum having intermediate expansion when grind size was the same.

SME ranged from 105.4-183.1 kJ/kg depending on grind size and grain variety and seemed to be one of the determinants of kibble properties such as expansion. Raw material physico-chemical properties such as gelatinization onset temperature (T_o; 66.7-78.3^{o}C) determined using differential scanning calorimetry and pasting temperature (T_{paste}; 76.6-81.5^{o}C) obtained from rapid viscoanalyzer also appeared to have an impact on expansion, as observed from their correlations with bulk density ($r= 0.8125$ and 0.7742, respectively). These observations affirmed that although the SME input is important as the driving force, having a

matrix that has film forming or extensibility properties is also critical for product expansion. Lower T_o and T_{paste} indicated a greater propensity for starch gelatinization and degradation, as seen from the negative correlations with respect to enzymatically obtained gelatinized starch (r= -0.5844 and -0.6085, respectively). This was the reason connecting T_o and T_{paste} to matrix extensibility and expansion. The negative correlation of particle size with expansion was thus clearly explained, as an increase in grind size negatively impacted both the driving force for expansion and matrix extensibility. The lower surface area per unit volume of particles with higher grind size possibly led to poor heat and moisture penetration during preconditioning and extrusion, and thus negatively affected film forming ability and expansion. Similarly grain type also impacted both driving force and extensibility, and thus expansion. For example, the brown rice-based formulation, which had one of the lowest expansions of all grain varieties, also had one of the lowest SME inputs (161.2 kJ/kg, not significant) and gelatinized starch content after extrusion (30.7%, $p<0.0002$).

The textural attribute of kibble breaking force (F_p)was found to have a positive correlation with piece density (r= 0.7059), which aligned with the theory of mechanical strength of porous matrices that dictates that the strength of cellular products is inversely proportional to cell wall size or in other words expansion.

3.1 Introduction

The global pet food market reached over $90 billion in 2018 with a 31% growth from 2013 to 2018 (Debbie Phillips-Donaldson, 2019). Convenience and healthy lifestyle for pets is one of the driving forces in this industry. Pet owners are more label conscious and looking for ingredients that sound like they are high quality and avoid products with ingredients they have heard are bad for their pets; specifically if those ingredients sound artificial or synthetic. (Tyler, 2020). New and novel is always progressing, but some are even seeing ingredients in a new light. The market is open for new opportunities in various areas, but specifically in a return to grain-included diets as premium labels are including grains, and ancient grains (Tyler, 2020).

One of the grains with renewed interest is sorghum. Grain sorghum is one of the most important cereal crops grown in the world (Reddy et al., 2010), but has been historically primarily used for livestock feed. Interest has grown over the last several years in using sorghum in varying capacities in pet food as it can be beneficial when parts of the sorghum seed is used after milling separation, it is usually used whole and ground to an appropriate processing size when used for pet food (Alvarenga et al., 2018). This, of course, depends on the nutrition profile needed. Sorghum has been reported to have lower nutritive values (Rooney and Pflugfelder, 1986) compared to other grains, such as corn (Al-Rabadi et al., 2011). However, sorghum and corn led to similar digestibility increases in pigs, according to a study by Lancheros, ((Lancheros et al., 2020b). And therefore, could possibly be used interchangeably in the diets depending on local availability of certain grains versus others.

Traditional grains used in pet food include corn, brown rice, wheat; along with the aforementioned ancient grains such as quinoa, and buckwheat and sorghum. These various grains provide protein, carbohydrates, fiber and essential amino acids and when prepared

properly are digestible. The ancient grains provide similar nutrition and an alternative to modern traditional grains that some people may consider unhealthy (Pezzali and Aldrich, 2019). When assessing pet food and pet food processing from a scientific point of view, several physico-chemical attributes must be evaluated. Starch is a primary carbohydrate which can be evaluated for animal nutrition and is also the "glue" that helps hold many extruded pet foods together. Extrusion combines the raw material with processing aids like water and steam under heat, pressure and shear to transform starch granules into viscous material before exiting the extruder. This cooking action and gelatinization can be partial or complete. (Bazolli et al., 2015).

Total starch content, as well as gelatinized starch are two measures that can be used to evaluate different pet foods for quality control or against other trials or foods for research. These two measures give insight in to how much of the starch is in the products and thus, how much may cook. Instrumental tests such as differential scanning calorimetry (DSC) and rapid visco-analysis (RVA) also give insight as to the processing changes that happen during extrusion with measurements involving the pasting time and temperature, and viscosity changes as the starch matrix is cooked. The functionality of the starch can be determined by the time and temperature relationship resulting in the gelatinization temperature, onset temperature, pasting temperature and the amount of retrogradation (Zeng et al., 1997). The particle size of the raw material is an important factor in pet food production as it can affect the gelatinization of the starch and other physical traits such as density. The size reduction in particle size is necessary for several reasons; whole grains are typically too large to effectively process, mixing of different size grains leads to feed non-uniformity in broilers (Behnke, 1996), improved nutrient digestibility and improved feed efficiency in swine (Lancheros et al., 2020b). The primary reason for the improvement in feed ingredient performance due to particle size reduction is the increase in surface area. This

increase in surface area allows for the material, primarily starches, to be more easily digested by the body. The particle size reduction and resulting surface area increase also helps with the processing in the form of increased hydration in the extruder. The amount of gelatinization is generally greater for material with smaller particle size than those with larger particle size. (Bazolli et al., 2015). It has also been found that Extrusion is the primary processing technique for producing dry, expanded pet food. The technology has been around for decades making human food since the 1930's and pet food since the early 1950's (Riaz, 2000).

This high temperature, short time processing technology causes energy transference to mass induced physicochemical transformations with higher starch cooking, which influenced positively the formation of the kibbles that showed higher expansion and lower density chemical and physical changes that alter the nutritional and physical quality of the product (Riaz, Mian N., 2007; Tran et al., 2008). The transfer of energy into the product, both mechanical and thermal, is what drives the cooking process, gelatinization and expansion. (Monti et al., 2016a). The specific mechanical energy (SME) describes the amount of work imparted by the extruder to the material and is produced as a result of the friction of the material between the screw and the material (Hataichanok Kantrong et al., 2018).

In dry, expanded pet food, cooking and expansion are the primary results of the cooking extruder. The amount of expansion depends upon three things: the feed composition, extent of cooking and the pressure differential between the extruder and the ambient area. As the product leaves the die, the pressure drop causes liquid water inside the extruder to convert to the gas phase and thus the volume increases drastically. As the water is turned to steam, the starch molecules stretch and expanded from the original size (the die from which the material exits. (Desrumaux et al., 1998).

The texture of pet food is an important trait that can affect the palatability and overall acceptability of the cat food. Overall, texture has several attributes to it, such as peak breaking force, fracturability, cohesiveness, springiness, gumminess, and chewiness; and helps to quantify the acceptability to animals. F_p is simply how much force is applied before the kibble fractures and breaks; this simulates the biting action of the animal. Expansion and F_p are often negatively correlated as, generally, when products expand more from more energy input, they become less hard. The main driving force for expansion of extrudates is the mechanical energy input. Two things that could lead to an increase in expansion are possibly reducing the in-barrel moisture and increasing the screw speed. Reducing the moisture would lead to an increase in melt viscosity, which in turn would increase the mechanical energy input and therefore increase expansion, while increasing the extruder screw speed would likely also lead to more expansion by increasing the mechanical energy input (Agbisit et al., 2007). Texture can also be influenced by the particle size of the raw material and thus affect acceptability of the food by the pets. The change in acceptability can come in the form of mouth feel and even the way the kibble is chewed. (Monti et al., 2016a). The goal of this study was to investigate the effect of particle size and type of primary grain on processing and properties of complete and balanced extruded dry expanded cat food kibbles, with a focus on grain sorghum as an alternative carbohydrate source to the more traditional corn and brown rice.

3.2 Materials and Methods

3.2.1 Formulas

The diets were formulated to be complete and balanced with similar levels of starch and fiber specifically designed for cats (AFFCO, 2018) (Table 3.1). The fish oil and the dry

ingredients (except for sorghum) were sourced from Lortscher Animal Nutrition, Inc. (Bern, Kansas, USA) as individual ingredients. The sorghum was sourced from NU-Life Market (Scott City, KS, USA). The poultry fat was sourced from International Dehydrated Foods (Monett, MO, USA) and the palatant was obtained from SPF-Diana (Hodges, NC, USA).

3.2.2 Grinding and Mixing

The whole grains were pre-ground through a Model D - Comminuter pilot scale hammer mill (Fitzpatrick, Westwood MA, USA) through a 6.35 mm screen with round openings to break the kernels and make them closer to the larger of the specified size, then they were ground again through the desired final screen for proper mixing. The next step is that the grain ingredients were mixed with the other dry ingredients in a double ribbon mixer (Wenger, Sabetha, KS USA). The mixer was operated for 3 minutes to provide a suitable mixing time. Then the complete dry formulas were post-ground through the same mill using screens with 0.51mm, 1.05 mm and 1.65 mm round openings to obtain the maximum particle size goal of 0.5mm, 1.0 mm and 1.6 mm, respectively.

3.2.3 Particle Size

The particle sizes were run both on a Tyler Ro-Tap (Mentor, OH, USA) and by laser diffraction on a Beckman Coulter LS 13 320 SW, Dry Powder System (Indianapolis, IN, USA). The Ro-Tap Method uses a series of twelve sieves, to which 100g of material, from a representative sample is placed on the top sieve and the shaker turned on and allowed to run for 10 minutes. After the time expired, the material that remained on the top of each sieve was recorded on a spreadsheet for particle size distribution, cumulative distribution and estimated particles per gram.

3.2.4 Extrusion processing

The diets were extruded through a Wenger X20/E325 single screw extruder with a Model 2 Differential Diameter Conditioner (DDC) (Wenger, Mfg., Sabetha, KS) for the kibble production. The extruder has a 3.25 inch (82.55 mm) diameter barrel, internal spiral heads and a 10:1 L/D ratio. The barrel had six heads that were divided into three heating zones with set temperatures of 60°C for heads 2 and 3, 75°C for heads 4 and 5 and 90°C for head 6, the cone head, from the feed to discharge end. The screw configuration (Figure 3.1) started with a single flighted full pitch screw at the feed throat and transitioned to a double flighted half pitch cone screw element closer to the discharge end. Shear locks were placed between each screw element with increase in size from small to large. One 3.85mm circular, 36° angle taper, with a 1.0 mm land length die was used, along with 6 knife blades to cut the product.

The extrusion process starts with material in a live bottom feed hopper. The material then leaves the hopper via a variable speed feed auger and drops through a transition into the preconditioner. The preconditioner moves material from the inlet to the discharge while also mixing injected water and injected steam to provide material hydration and start the cooking process. Material is then transferred to the extruder barrel by another transition / diverter. The extruder barrel moves material from the inlet of the barrel to the exit (die) while imparting mechanical and thermal energy to the product.

Processing conditions are shown in Appendix 1.

3.2.5 Bulk Density

Bulk density measurements, both off the extruder (OE) or wet bulk density and off the dryer (OD) were taken by filling a previously tared 1 liter cup over the rim of the cup and striking off the amount of material that was above the rim. The amount remaining filling the cup was weighed on the same scale and the weight recorded. This reading is in grams/1 liter (g/L).

$$Bulk\ Density\ \left(\frac{g}{L}\right) = \frac{mass_{bulk}}{Volume_{bulk}} \quad (1)$$

Where the mass is in grams and the known volume is in Liters.

3.2.6 Piece Density

The piece density of the dried kibble was calculated from measurements made of the individual kibble pieces. Thirty pieces of kibble randomly selected from the dried material were measured for length and 2 places for width. The two width measurements were averaged so that a final kibble width could be obtained. Each piece of kibble selected was also weighed and this amount recorded. Using the length, average width and weight of each of the thirty pieces of kibble per treatment; the volume, piece density, specific length and sectional expansion index could be calculated. The volume is calculated by dividing the average width by two to get the radius (r), squaring r and then multiplying r^2 by π and multiplying that by the kibble height.

$$\rho\left(\frac{g}{cm^3}\right) = \frac{m_e}{V_e} \quad (2)$$

Where m_e is the mass of one piece of extrudate and V_e is the volume of that piece of extrudate.

3.2.7 Void Fraction

Porosity, also called the void fraction, is the amount of space inside of the kibble that is not occupied by the material forming the cell walls. The larger this percent, the larger more

expanded the material as there is more open area inside the kibble. The volume of air inside the kibble is divided by the volume of air in the kibble plus the volume of solids in the kibble. The more practical and accurate method to find the void fraction is to use a gas pycnometer. A gas pycnometer operates by measuring the difference in gas pressure in an empty chamber to the gas pressure in a chamber of equal size that contains the sample. The difference of the amount of gas displaced is the volume of solid material. The research was done on a Quantachrome Instruments gas pycnometer (Model: Ultrapyc 1200e). Each sample was ground through a Hamilton Beach handheld electric coffee grinder for 30 seconds to minimize the closed cell structure and then weighed on a Mettler Toledo scale (Model: XP2003SDR) and recorded. After turning on the pycnometer and allowing it to run a self-test for proper operation, the sample is put into the test chamber and the weight is entered into the machine. The test begins at the press of the start button. The pycnometer runs three tests on the sample automatically and gives the resulting volume, density and standard deviation of the three tests.

$$VF\ (\%) = \frac{volume\ of\ air}{Volume\ of\ air + volume\ of\ solids} \Rightarrow VF = 1 - \frac{P}{P_s} \quad (3)$$

Where VF is the void fraction, P is the extrudate piece density and P_s is the solid density.

3.2.8 Sectional Expansion Index

The sectional expansion index is the square of the diameter of the extrudate divided by the square of the die diameter. The diameters were measured for 30 kibble pieces, SEI calculated and then averaged to find a mean SEI for the treatment.

$$SEI = \frac{D_e^2}{D_d^2} \quad (4)$$

Where D_e is the extrudate diameter and D_d is the die diameter.

3.2.9 Specific Length

Specific length is the measurement in length divided by the measurement of weight of 1 kibble. The lengths and weights were taken for 30 kibbles, specific length calculated and then averaged to find the mean specific density for the treatment.

$$l_{sp} = \frac{l_e}{m_e} \tag{5}$$

Where l_e is the extrudate length and m_e is the extrudate mass.

3.2.10 DSC

Differential Scanning Calorimetry (DSC) is used to measure gelatinization. The degree of gelatinization (DG) was also evaluated using DSC. Unlike the other 2 enzyme hydrolysis methods, the DSC method relies on measurement of enthalpy of non-processed and processed samples, and the difference between the 2 represents the extent of gelatinization. Approximately 10 mg (dry basis) of feed sample was weighed in a stainless steel pan, and deionized water (1:2, feed/water, wt/wt) was added and the sample was allowed to equilibrate overnight. Thermal scans were conducted using a Q100 DSC (TA Instruments, New Castle, DE). The measurement was performed by heating the pan in the test chamber from 10 to 160°C at a heating rate of 10°C/min. The onset, peak, and conclusion gelatinization temperatures and the enthalpy of gelatinization (ΔH) were determined. The DG was calculated as DG (%) = [(ΔH0 – ΔH1)/ΔH0] × 100, in which ΔH0 is the gelatinization enthalpy of native starch (J/g) and ΔH1 is the gelatinization enthalpy of the cooked product (J/g). A 100% DG equates to completely cooked starch no raw starch. All measurements were performed in duplicate.

3.2.11 RVA

Rapid Visco-Analyzer is a rotational rheometer that measures the resistance to rotational flow of slurries and gives an idea of starch damage and related physico-chemical properties. The standard RVA method (AACC Method 76-21.02) was used to analyze pasting properties of the raw materials and finished kibble. The method hydrated 3.5 g samples with water to obtain a solids level of 14% (w.b). The samples were constantly mixed, and the temperature was increased to 95^{o}C before cooling to 50^{o}C. The temperature, in the heating cycle, at which the viscosity of the starch begins to rise is known at the pasting temperature (T_{paste}) (Balet et al., 2019).

3.2.12 Enzymatic Gelatinization Test

Briefly, 0.5 g of sample was boiled with 25 mL distilled water for 20 min and then cooled to ambient temperature. Meanwhile, another 0.5 g of sample was hydrolyzed in 25 mL distilled water for 20 min at 25°C as a control. Next, 10 mL of acetate buffer solution was added into each tube followed by 5 mL of glucoamylase, and samples were incubated at 40°C for 70 min. Next, 5 mL of trichloroacetic acid was added to halt hydrolysis. After the sample was cooled to room temperature, distilled water was added to make a final volume of 100 mL. Free d-glucose was then measured using a glucose analyzer YSI 2700 (model 2700; YSI Inc., Yellow Springs, OH). The resulting quantity of free glucose determined in the control (G_{cold}) represents the percentage of starch that was gelatinized during processing. Meanwhile, the quantity of free glucose determined in the cooked sample (G_{boil}) represents the percentage of TS within a sample. The DG is then calculated as DG (%) = (G_{cold}/G_{boil}) × 100. Analyses were conducted in duplicate.

3.2.13 Tumble Test

The pellet durability index (PDI) is a standard for measuring the pellet durability index in diets manufactured with pellet mills, but can be used for extruded kibble as well. Measurement is as follows:, 500 grams of cooled, screened pellets are placed in a metal box with dimensions of 30 cm by 30 cm by 12 cm and containing a baffle 23 cm long, 5 cm wide and centered diagonally inside the box. This box, or can, is rotated at 50 RPM for 10 minutes, after which the pellets are removed and screened. The pellet durability index (PDI) is defined as the percentage of pellets surviving the test and retained on the sifting screen.

3.2.14 Pneumatic Test

One hundred grams of sieved pellets were tested. The sample was placed in the chamber and the machine turned on for a preset amount of time (30, 60 or 90 seconds). When complete, the fines were removed automatically from the sample and thus, the weight of the pellets recovered at the conclusion of the test is considered percent durability.

3.2.15 Texture Analysis Peak Breaking Force

The peak braking force (F_p) measurement property of the extrudates was analyzed using a texture analyzer (TA-XT2) and the Texture Exponent 32 software (both from Stable Micro Systems, Godalming, UK). Thirty randomly selected kibbles from each treatment were equilibrated to a moisture of 8.0±0.5% (wb) using a humidity chamber (30% RH, 24 h). Samples were compressed parallel to the direction of extrusion to 80% of their original length, using a

knife (or wedge) shaped cutting probe at a test speed of 2.0 mm/s. The positive peak force in g-f was recorded and then averaged for a single number representing the treatment.

3.2.16 Statistics

This experiment was blocked for a time design with sample units of sample collection with multiple samples taken during the steady state condition for each treatment. The product attributes for raw material physicochemical qualities, finished product physicochemical qualities and other quality measurements were assigned as dependent variables. Each measured or calculated response variable was analyzed statistically using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Analysis of variation (ANOVA) and least squared means (LSM) procedures for pair-wise comparisons were done to obtain the F-value and P-score with a significant level of α=0.05 within a Type III SS. The type III SS level was selected as these values tend to be narrower and give less of an opportunity to have a false negative. This same raw data was put into Excel 2013 (Microsoft, Inc. Redmond, WA, USA) to obtain the variable correlations.

3.3 Results and Discussion

3.3.1. Extrusion Processing

The specific mechanical energy (SME) input in the extrusion process is considered to be the driving force for product expansion. SME input ranged from 105.4-183.1 kJ/kg depending on grind size and grain variety. Interestingly, the average particle size of diets was inversely correlated to SME (r= -0.8517). This is likely the result of energy required for cooking that limited hydration and swelling of starch granules, lower viscosity and SME transference/absorption to mass, therefore limiting the extrusion efficiency, cooking, and

expansion. Similar trends were reported in other pet food studies using corn as a base cereal ingredient. The research by Mathew et al. (1999) showed that the coarser particle size generally led to lower SME. Their study with pet food had a higher SME range of 304-289 kJ/kg for particles ground through 0.750 mm and 1.500 mm screen, respectfully, than our study. This may be due to the Mathew formula, which was not specifically reported, having more total starch than our formula and also their use of a twin screw extruder, where we used a single screw extruder. The Mathew study also did a corn curl processing trial which showed that the same grind sizes resulted in a SME range of 630-593 kJ/kg. They, likewise, believed that the larger particle size generated a lower viscosity, thereby lowering the motor load, which lowered the SME. A second study (Garber et al., 1997), also using corn meal for snack extrusion and thus ,had the same results, the higher the particle size, the lower the SME. Their products ranged from 50 microns to 1,622 microns in five increments with a range of approximately 880 kJ/kg to 800 kJ/kg for the 400 rpm treatments (the other RPM treatments were similar) Garber's study speculated that the lowering of SME resulted in the lowering of the die temperature, which, in turn, lowered expansion. The lower temperature also caused the starch to not gelatinize as much as the higher SME products.

A study conducted by Carvhalo et al (2010) using corn meal in six different particle sizes to process direct expanded snacks, also showed a similar particle size versus SME relationship, although the SME range reported was higher than this study, as expected, for low moisture snack applications. As the average particle size decreased from 835 to 249 microns, SME increased significantly from 528-719 kJ/kg. They theorized that the difference in surface area was responsible for the transition temperature entering the smaller particle size quicker and lowering the viscosity and thus reducing the SME. Another study found similar results to Carvalho while

working with barley for expanded snack food; their results showed a trend that was contradictory to ours. Altan et al (2009) found that a larger particle size raised the SME in barley grits versus barley flour with both a medium and a severe screw profile. The average SME, for the two screw profiles, was 336 kJ/kg for barley grit and was 294 kJ/kg for the barley flour treatment. Alton came to the same conclusion as Carvalho regarding surface area and temperature absorption.

3.3.2 Physico - Chemical Analyses

3.3.2.1Raw Material Particle Size

The cumulative particle size distributions of the 8 formulations used in this study are shown in Figures 3.3, 3.4 and 3.55. The grind size (or hammer mill using 0.5, 1.0 and 1.6 mm screens) had an impact on the average particle size of the formulation, which was 202.2, 300.9 and 549.6 µm for white sorghum based diets and 199.0, 311.2 and 699.1 µm for red sorghum, respectively. The average particle size did not differ much as a result of grain variety used in the formulations, and ranged between 292.5-311.2 µm with brown rice based formulation having the lowest and red sorghum based formulation highest (Mathew et al., 1999).

As mentioned previously, each grain was pre-ground, mixed with the remaining ingredients and the final recipe was ground again using the same screen. The average particle size of the pre-ground grain before mixing and final grinding followed the same trends as for the whole formulation after final grinding. The ranges for pre-ground grain were 244.5-663.2 µm for white sorghum, 246.1-649.3 for red sorghum and 289.0-390.5 µm for all grains ground using 1.0mm screen. In general, it was apparent that final grinding using the same screen further

reduced the particle size as shown for white sorghum in Figure 3.6 (WS1.0 388.2 >> 300.9) as an example.

3.3.2.2 Raw Material Rapid Viscoanalysis (RVA)

The pasting properties of raw materials and extruded samples are indicators of how the starch transforms during extrusion process of cooking and shear. Analyzing this information can lead to a better understanding and prediction of how future processing might also affect the starch (Sandrin et al., 2018). The RVA test provides an idea of ease of gelatinization of starch and its swelling capacity and also the propensity of starch granules to break down on undergoing thermal and mechanical treatment, among other things, based on viscosity changes in any recipe in slurry form. Typically a higher raw material RVA pasting temperature (T_{paste}) suggests that the starch is more resistant to gelatinization and degradation (Sandrin et al., 2018). The raw material T_{paste} (Figure 3.7) varied between 76.6-81.5^{o}C, depending on grind size and grain variety. Grain variety had a significant impact on T_{paste} ($p=0.0066$) with corn having the lowest (76.6^{o}C), followed by red and white sorghum (78.3-79.4^{o}C) and brown rice (81.5^{o}C), when comparing diets with 1.0 mm grind. This is likely due to the shape and size of the particles after grinding. Particle size also had a significant impact on T_{paste} ($p=0.0343$), with the biggest difference within red sorghum based diets where the 1.6 mm had higher pasting temperature (81.4^{o}C) as compared to 0.5-1.0 mm grind (78.2-78.3^{o}C). Serna-Saldívar and Mezo-Villanueva (2003) found similar T_{paste} with corn and red sorghum with 76.1^{o}C and 77.1^{o}C, respectfully. They felt that the corn was easier to gelatinize and also more stable during processing (Serna-Saldívar and Mezo-Villanueva, 2003). o-Pérez et al (1999) found slightly different results between corn and sorghum in that the T_{paste} for corn and sorghum were almost identical at 74.7^{o}C for corn and

74.6ºC for red sorghum. A study by Pezzali et al (2020) reported a slightly different range for, 66.23-69.57ºC, but the differences in white sorghum versus red sorghum were close, like our results.

Raw material T_{paste} had high positive correlations with gelatinization onset and peak temperatures as obtained by differential scanning calorimetry (r = 0.8989 and 0.8108, respectively). Sandrin et.al (2018) found similar results in their work, although it was on oat-rice infant food. This supported the above assertion that a higher T_{paste} means more energy is required for gelatinization. T_{paste} also had moderate to marked negative correlations with enzymatically determined total starch and gelatinized starch in extruded products (r = -0.5201 and -0.6085, respectively), which confirmed that the formulation having higher pasting temperature had lower potential for starch granule degradation during extrusion and consequently lower film forming capacity and expansion. Film forming capacity or ability is the capability of the matrix to stretch and extend, thus defining the mechanical and functional properties of the network being examined.

3.3.2.3 Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) test provides information such as the onset temperature (T_o), peak temperature (T_{peak}) and other parameters linked to starch gelatinization based on thermal energy input as temperature is increased at a constant rate. Typically, a higher T_o implies that starch in the recipe is more resistant to gelatinization and requires more time and/ or energy for it to start gelatinizing. The raw material T_o (Figure 3.8) varied between 66.7-78.3ºC, depending on grind size and grain variety. Grain variety had a significant impact on T_o (p=0.0008) with corn having the lowest (66.7ºC), followed by red and white sorghum (72.5-

73.0^{o}C) and brown rice (78.3^{o}C), when comparing diets with 1.0 mm grind. Particle size also had a clear impact on T_o, with higher onset temperature observed for 1.6 mm grind (74.1-74.7^{o}C) as compared to 0.5-1.0 mm grind (71.7-73.0^{o}C). A study by Srichuwong et al (2017) reported similar raw material T_o for corn and red and white sorghum treatments, with corn being the lowest (66.7^{o}C) and followed by red and white sorghum (74.6-71.9^{o}C). Another study, by Pezzali et al. (2020) showed a lower range of T_o for red and white sorghum; their results were 66.57 ^{o}C and 63.34^{o}C, respectfully.

A very high correlation, ($r = 0.9657$, $p<0.0008$) was observed between raw material DSC gelatinization onset temperature and peak temperature. As discussed before, there was a high correlation between T_o and RVA raw material T_{paste}, with very similar trends with respect to grain variety and particle size. It was also evident that T_o and T_{paste} temperatures were similar, with the former being a few degrees lower. IT must be noted that both are indirect measures of phenomena related to onset of starch gelatinization, with the DSC measuring it based on thermal energy absorbed by the raw material samples and the RVA based on viscosity increase due to swelling of starch granules. The slightly higher temperatures for T_{paste} indicated that swelling of starch granules takes a little more energy to initiate than the onset of gelatinization as measured thermally. Moderate to marked negative correlation was observed between T_o and enzymatically determined total and gelatinized starch in extruded products ($r = -0.6701$ and -0.5844, respectively). This is similar to the observations with respect to RVA pasting temperature, discussed above, having the same reasons and implications related to starch film forming ability.

A peak was not found in the extruded samples as all of the starch was gelatinized during the extrusion process. These results were in agreement with other researchers who found similar results, that the starch granules were completely gelatinized during extrusion, in corn starch

extrusion (Blanche and Sun, 2004; Ozcan and Jackson, 2005) and wheat-ginseng snack production (Chang and Ng, 2011).

3.3.2.4 Enzymatically Determined Starch Gelatinization

Data for gelatinized starch content as determined by the enzymatic method (glucoamylase test) is shown in Table 3.2. Gelatinized starch had a high positive correlation (r= 0.8450) with SME input, and moderate to marked negative correlations with raw material gelatinization onset temperature (T_o) and raw material RVA pasting temperature (T_{paste}) (r= -0.5844 and -0.6085, respectively).

3.3.3 Expansion

Product expansion is a result of the combination of many factors such as the ingredients and formulation, processing conditions, and the resultant thermal and mechanical energy inputs during the extrusion process (Moraru and Kokini, 2003). Expansion and the several measurements used to quantify it are general indicators of energy the product may have received as an input and the gas holding (extensibility) capacity of the raw materials. Expansion significantly impacts product texture or the perception of hardness or softness and thus also palatability of the pet food.

The various measures of pet food expansion include those that reflect volumetric or overall expansion such as bulk density, piece density and porosity, and also parameters that represent directional expansion such as SEI and specific length.

Different measures of overall expansion, including bulk density (OE & OD), piece density and porosity, were moderately to highly correlated with each other (|r| = 0.3998, -

0.9226). These overall expansion measurements were found to be more closely related to radial expansion (SEI) as compared to longitudinal expansion (specific length). The SEI had a moderate to marked negative correlation with OE bulk density (r = -0.6263), OD bulk density (r=-0.5641) and piece density (r = -0.7266). On the other hand, specific length was found to have negligible or low correlation with all overall expansion measurements except for piece density. The marked positive correlation of specific length with piece density (r = 0.7301) indicated that as the product expanded more in the longitudinal direction, the overall expansion decreased. The reason for this is that increase in specific length appears to be accompanied by a decrease in radial expansion or SEI (r = -0.8250), meaning that longitudinal expansion occurs at the expense of radial expansion and tends to decrease the overall or volumetric expansion. Alvarenga et al (2018) found similar results with sorghum flour and whole sorghum in pet food diets in that sorghum flour had a lower bulk density.

3.3.3.1 Bulk density

The bulk density of the material off the extruder (OE) is a measurement that gives a quick, overall observation about how much the kibble has expanded and allows for a rapid measurement and indicator of how the extruder is performing and a general insight into the extrusion process. The lower the value, usually measured in g/L, the greater the expansion.

The OE bulk density ($P<0.0009$ for particle size) ranged from 349-396 g/L depending on the grain and particle size making up the formulations, while OD bulk density ranged from 347-403 g/L ($P<0.0001$ for grain type). During drying two opposite phenomena tend to occur that impact the bulk density of the product, one, moisture evaporation that has a decreasing effect and two, product shrinkage that has an increasing effect. Depending on which of these two

phenomena are predominant, the bulk density either increases or decreases. The data clearly shows that moisture loss counteracted by the shrinkage effect, leading to no or very little difference between OE and OD bulk densities.

As bulk density is an indicator of product overall expansion, the data can be explained by factors that impact expansion, primarily the driving force, including STE and SME input, and the expanding ability or extensibility of the product matrix. Both these factors seem to have a role in this study. As seen from Figure 3.9, it is evident that the corn-based formulation has the lowest bulk density (358 g/L OE and 346 g/L OD) and the brown rice-based formulation the highest (396 g/L OE and 403 g/L OD), while the former has one of the highest SME inputs (174 kJ/kg) and the latter lowest (161 kJ/kg) among treatments with the same grind size (1.0 mm). A similar study on particle size variations, only using corn meal instead of sorghum, demonstrated that as the particle size increases, the bulk density increases. In this work, Garber et al (1997) extruded corn meal in five particle sizes ranging from 50.2 microns to 1622.1 microns and measured the corresponding bulk densities in the range from 47.92 to 111.74 g/L. This corroborates that as the particle size increases, the bulk density increases because the expansion decreases.

With regard to the matrix extensibility, factors such as starch gelatinization and degradation have an important role. Typically higher level of gelatinization and degradation or breakdown of starch granules would lead to better extensibility and film forming ability and will positively impact the product expansion (Garber et al., 1997). Gelatinization onset temperature (T_O) of raw formulations from DSC had a significant effect ($P>0.0008$) with respect to the grain type. These temperatures ranged from 66.7-78.3^oC, with the corn formulation having the lowest and brown rice formulation the highest. Rice starch is known to have higher temperature and energy requirements for gelatinization (Dalbhagat et al., 2019). Higher T_O typically implies

greater energy requirement for gelatinization. Thus, it follows that a brown rice matrix will have a lower potential for extensibility, while the corn formulation will have higher potential. This aligns with the bulk density trends discussed overall. In fact, raw material T_O had a marked positive correlation with OE and OD bulk densities (r=0.6925 and 0.7959, respectively). This confirmed that higher gelatinization temperature leads to less film forming ability of the extrudate and lower overall expansion. The link between gelatinization and extrudate expansion has been elaborated in previous studies as well (Garber et al., 1997).

The RVA pasting temperature (T_{paste}) relationship among treatments also confirm these observations with a fairly high P-value for Grain size (P<0.0066) and particle size (P<.0434), with brown rice formulation having the highest (81.4^{o}C) and corn lowest (76.6^{o}C) among all the treatments. There was also a marked positive correlation between T_{paste} and the OE and OD bulk densities (r=0.6923 and 0.7742, respectively). RVA viscosity starts increasing as starch starts to gelatinize and swell, and that is why the pasting temperature is a similar measure as gelatinization onset temperature. A study by Dalbhagat et al. (2019) was based on extruded brown rice products and obtained a similar T_{paste} range of about 80^{o}C and reached a similar conclusion for brown rice; that a higher gelatinization temperature leads to increased gelatinization amount.

Interestingly, starch content data for the extruded product indicated that the brown rice based formulation had the lowest level of total starch (34%) and corn had one of the highest (40%), even though all formulations were designed to have the same starch. The enzymatic total starch (P<0.0002) has great significance with respect to grain type and also for the particle size (P<0.0343). The enzymatic test that led to these results detected less starch in the brown rice-based product possibly because there was lower tendency for it to get hydrolyzed as it was not as

much degraded. This aligns with the previous discussion regarding the brown rice formulation having a lower film forming tendency and extensibility. Indeed total starch had marked to high negative correlation with OE and OD bulk densities (r= -0.7449 and -0.8053, respectively), which supports these observations. Similarly e gelatinized starch, determined enzymatically, is significantly correlated for both grain type ($P<0.0113$) and particle size (0.0002), also is an indicator of degradation, and had marked negative correlation with OE and OD bulk densities (r= -0.3967 and -0.4725, respectively).

The particle size of the raw material is an important factor in extrusion. One important reason is that the surface area per unit volume of the raw material particles is a function of their size. Larger particle size leads to lower surface area per unit volume, which leads to lesser opportunity for hydration and penetration of energy as compared to smaller particles size. In figure 3.9 the WS and RS formulations with 1.6 mm grind had higher OE bulk density (368 and 372 g/L, respectively) and OD bulk density (363 and 370 g/L, respectively) as compared to the formulations with 0.5 or 1.0 mm grind that had an OE density of 349-366 g/L and OD density of 347-363 g/L. This clearly indicates that the larger particle size limits the hydration and energy absorption, thus reducing expansion. Garber et al (1997) also found that as the particle size increased, the expansion of corn based extrudates decreased in the radial, longitudinal and overall expansion planes due to the same reasons. A pet food study by Alvarenga et al (2018) found similar results for bulk density and SEI. Their study examined whole sorghum versus sorghum flour. As the particle size increased, bulk density decreased (334 g/L for flour and 376 g/l for whole sorghum), as did SEI (4.81 for flour and 3.56 for whole sorghum. Monti et al (2016a), theorized that an opposite effect was occurring; that the larger particle of wheat bran, used in their study, bound more water, increased the shear and thus increased the SME.

3.3.3.2 Piece density

Piece density is also a measurement of overall expansion, but is specific to individual kibble pieces, instead of several pieces that determine bulk density and is significant at P=0.0319 when related to particle size. It is useful to examine expansion at the scale of the kibble, which is what determines product properties such as texture and palatability. Piece density ranged from 0.00044 – 0.00063 g/cm^3, depending on the grain variety and formulation grind (Figure 3.10).

The piece density had a marked inverse correlation (r=-0.6399) with SME input during the extrusion process, which means that as the SME increased, the piece expansion also increased. This was an expected result, as discussed previously in the case of bulk density, and has also been reported in all kinds of extrusion based studies for expanded products (Dalbhagat et al., 2019; Garber et al., 1997). Higher SME typically not only leads to more starch gelatinization and degradation and thus film forming ability for the matrix, but also drives up the temperature of the melt and increases water vapor pressure and the driving force for expansion.

The raw material pasting temperature from RVA has a marked positive correlation with the piece density (r=0.6580), as was the case with respect to bulk density. This relationship is due to the potential of starch to gelatinize and degrade during extrusion, and also the extensibility of the matrix, as discussed earlier. A moderate inverse correlation (r=-0.5695) was found between gelatinized starch determined enzymatically and the piece density, which again points to the relationship between starch degradation and expansion.

Piece density for the white sorghum formulation with 0.5 mm grind (0.00044 g/cm^3) was lower than that for the 1.0-1.6 mm grind (0.00049-0.00057 g/cm^3). Similarly piece density for the red sorghum formulation 0.5-1.0 mm grind was lower (0.00044-0.00046 g/cm^3) than that for

the 1.6 mm grind (0.00063 g/cm^3). This implies that piece density increased or expansion decreased as the particle increased for each sorghum variety. This relationship with grind or particle size is also true for the average piece density across both sorghum varieties, which was 0.00045, 0.00050 and 0.00056 g/cm^3 for 0.5, 1.0 and 1.6 mm grinds, respectively. The decrease in surface area to volume as particle size increases and the impact of that on moisture and heat penetration during extrusion is the primary reason, as mentioned above. Monti et al (2016a) also found that the larger particle size had a higher piece density, although that work was with wheat bran, but also in pet food. Their reported results showed the larger particle size having a piece density of 0.00045 kg/m^3 and the smaller particle size having a piece density of 0.00041 kg/m^3.

3.3.3.3 Void Fraction or Porosity

Void fraction is a measure the amount of void space in the kibble. It represents the air volume (as opposed to volume occupied by solid material) as a fraction of the total kibble volume, and can have a value close to 1.0 or less depending on the product expansion. It is a measure of overall expansion just as bulk density and piece density and is significant in the research in both grain type ($P<0.0001$) and grind size ($P<0.0001$). Void fraction of the cat food kibbles in this study ranged from 0.56 to 0.69, as shown in Figure 3.11, depending on the grain variety in the formulation and the grind.

The void fraction for the 0.5mm grind white sorghum formulation (0.69) was higher than that for the 1.0-1.6 mm grind (0.60-0.66). Likewise, the void fraction for the red sorghum formulation 0.5-1.0 mm grind was higher (0.67-0.69) than that for the 1.6 mm grind (0.56). This implies that void fraction or overall expansion decreased as the particle increased for each sorghum variety. As with piece density, this relationship of void fraction with grind size is also

true for the average void fraction (see Figure 3.11) across both sorghum varieties, which was 0.68, 0.64 and 0.61 for 0.5, 1.0 and 1.6 mm grinds, respectively. Obviously, the particle size or grind has an important role in expansion as discussed before in the context of other expansion measures as the surface area to volume particles impacts heat and moisture penetration during processing. Carvalho et al. (2000) also found, as discussed before, that large particles will hydrate less due to less surface area (Carvalho and Mitchell, 2000).

3.3.3.4 Sectional Expansion Index and Specific Length

The Sectional Expansion Ratio or Index (SEI) is the quantification of expansion in the radial direction as compared to the die cross section and is highly significant in this work for grain size ($P<0.0001$) and particle size ($P<0.0001$) also. It is a directional measure of expansion, unlike the previously described measures. The SEI of the kibbles ranged from 2.69 to 3.67 depending on the grain variety in the formulation and the grind (Figure 3.12). Among the treatment with 1.0 mm grind, the corn-based formulation had the highest SEI (3.67) and brown rice the lowest (3.08). This observation is consistent with other expansion related measurements such as bulk density. The mean SEI for all formulations based on white sorghum was higher than those based on red sorghum. In fact, for each of the three grind sizes, white sorghum treatments had a higher SEI than red sorghum treatments. Particle size or grind had a similar effect on SEI as for other measures of expansion, with the 1.6 mm grind having the lowest SEI for both red and white sorghum based formulations (2.69 and 2.96, respectively). Garber et al ((1997), again, extruding corn meal, also reported that the range of particle size 50.2-1622.1 micron resulted in the expansion ratio going from 4.69 to 3.99, supporting and verifying our data trend and resulting numbers. Monti et al (2016a) also reported an analogous trend for wheat bran in pet food with

the larger particle size having a lower SEI than the smaller particle size, 2.8 and 3.1, respectively.

As for other expansion parameters, SEI had a positive correlation to SME (r=0.7830), and negative correlations with respect to the raw material pasting temperature (r=-0.7239) and gelatinization start temperature (r=-0.6487). Similarly, SEI had moderate to marked correlations with enzymatically determined total starch (r = 0.5752) and gelatinized starch (r=0.6583). All these correlations are consistent with reasons related to driving force for expansion and the matrix extensibility that were elaborated earlier.

Specific length is an indirect representation of longitudinal expansion, and is also a directional measure just like SEI although along an axis perpendicular to it and along the extrudate flow. Specific length, shown in Figure 3.13, ranged from 43.0 – 59.6 mm/g, depending on the grain variety and grind size. As was mentioned before, specific length had a high negative correlation with SEI (r=-0.8250), implying that longitudinal expansion was at the expense of radial and also overall expansion. Garber also supported our results with specific length. Again, Monti (2016a) shared similar results with the larger particle size wheat bran having a specific length of 51.4 mm/g and the smaller particle size wheat bran having a 51.7 mm/g result.

3.3.4 Kibble Texture and Integrity

Hardness of extrudates are mainly determined by their cellular structure, formed during the expansion of the extrudate, and by the phase properties and composition of the solid matrix (Moraru and Kokini, 2003; Santala et al., 2014). The overall texture quality of a product and specifically the hardness of a kibble can be of importance as it impacts the palatability of the product, which is a measure of relative preference and amount of product consumed by the

animal. Kibble integrity is also a quality attribute that is important as it could influence owner appeal. Results from three different tests that measure textural and integrity attributes are discussed below, including kibble durability as measured by the tumble test and by air impact and kibble hardness measured using texture analyzer.

3.3.4.1 Durability Using the Tumble Test

The tumble test gives an idea of durability, which is reported as the percentage of intact kibbles after mechanical agitation. This attribute is also commonly known as pellet durability index (or PDI). Higher the PDI, more durable is the kibble and likely to remain intact during handling. The tumble test PDI ranged from 94.9-96.5%, depending on grain type and grind size (Figure 3.14). When examining the impact of grain type within the 1.0mm grind, the corn-based cat food kibbles had the lowest PDI (94.9%), followed by red and white sorghum (95.9% for both), and then brown rice that had the highest PDI (96.5%). This means that the brown rice-based kibbles resisted impact the most, possibly due to a stronger cell structure that is accompanied by less overall expansion or high density. On the other hand, the corn-based product is more easily damaged as it has the weakest cell structure. This negative relationship between expansion and tumble test PDI is supported by the moderate correlations between the latter and sectional expansion index ($r = -0.5629$) and OD bulk density ($r = 0.5640$). These correlations are dominated by the varietal effect. On the other hand, grind size had very little effect on tumble test PDI, as can be seen by its narrow range for white and red sorghum based product based on different grind (95.9-96.4% and 95.8-96.1%, respectively). This general range was lower than the tumble test PDI reported by Winowiski (2000) for other animal feed products. Their PDI range was 89.1-98.4%, but compared different feed types (Winowiski et al., n.d.).

3.3.4.2 Durability Using the Pneumatic Test

The pneumatic test (also called the Holmen test) is another measurement of kibble durability, but it is different from the previously discussed durability test as it is based on air impact, or interaction and abrasion between kibbles while suspended and moving in an air stream. The results are also reported as pellet durability index. Depending on grain type and grind size, the pneumatic test PDI ranged from 82.2-97.6% (Figure 3.15). This range was lower than the pneumatic test PDI, again, reported by Winowiski (2000) for other animal feed products. Their PDI range was 68.5-96.5%, but compared different feed types (Winowiski et al., n.d.).

This is possibly due to the fact that abrasive action between kibbles, as in the pneumatic test, might lead to excessive fines rather than product breakage that is measured with the tumble test. Winowiski also used a binder in at least one formula and thus, the results may be slightly different from our ranges in both the tumble test and the pneumatic test; however, the trends are still the same in both tests. Grain type had a significant impact ($p < 0.0001$) on pneumatic test PDI, with brown rice having the least (94.5%). Grind size also significantly impacted the pneumatic test PDI ($p < 0.0001$), with the highest grind size of 1.6 mm having the lowest PDI (82.2% for white sorghum and 97.1% for red sorghum). These results clearly pointed towards the role of gelatinized starch in positively affecting binding and durability of the kibbles and thus decreasing the fines generated as a result of abrasion between pieces. As discussed previously, enzymatically determined gelatinized was found to be the lowest in brown rice-based kibbles as compared to other grain varieties and in products based on 1.6 mm grind formulations as

compared to finer grinds. The marked positive correlation between pneumatic test PDI and gelatinized starch content in the OD product (r = 0.6437) supported this relationship.

3.3.4.3 Texture Analyzer Peak Breaking Force

The peak breaking force on a texture analyzer is measured for individual kibbles as the maximum downward force applied by the knife (or wedge) shaped cutting probe as the pieces fracture. Figure 3.16 shows the F_p data of cat food kibbles. The textural attribute of kibble F_p ranged from 1827.18-2444.58 g- force, depending on grain type and grind size. It was found to have a positive correlation with piece density (r= 0.7059), which was aligned with theory of mechanical strength of porous matrices (or the Gibson-Ashby model) that dictates that the strength of cellular products is inversely proportional to cell wall size or in other words expansion. Other research papers on extrusion, such as Agbisit (2007) and Karkle (2012) found similar results that followed the Gibson-Ashby model; that smaller sized cells are harder to crush than larger cells. In other words, the more something expands, the easier it is to fracture the product. (Agbisit et al., 2007; Barrett and Peleg, 1992; Karkle et al., 2012; Manbeck et al., 2017). Agbisit (2007) worked with corn starch while Karkle (2012) and Barrett (1992) worked with corn based cereal products. Manbeck (2017) worked with a corn and brown rice pet food formulation and found that two experiments were similar in nature. One of the experiments showed that as bulk density went from 351.83 g/L to 281.67 g/L, the F_p went from 4.40 kg to 4.09 kg. The second experiment also demonstrated the same trend with the bulk density going from 345.83 g/L to 266.67 g/L while the F_p went from 4.39 kg to 4.11 kg. Both experiments showing that as expansion increases, the F_p decreases due to the cell wall becoming larger (more expansion).

3.4 Conclusion

Results from this study confirmed for dry expanded cat food not only the SME input is important as the driving force for expansion, but also having a matrix that has film forming or extensibility properties is also critical for product expansion. The SME and matrix extensibility had a strong relationship with grain type and particle size, and was explained using various physico-chemical analyses of the raw materials. The use of red and white sorghum made good kibble under similar conditions to those of corn and brown rice.

3.5 References

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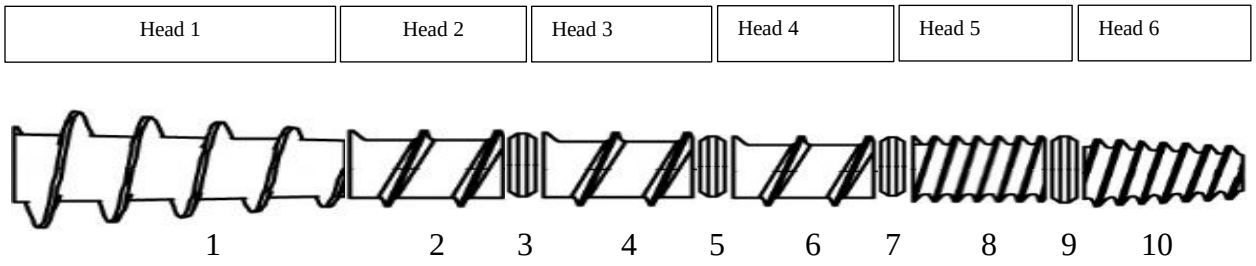
Chapter 3 Figures



1 = Inlet, single flight, full pitch screw
2 = Single flight, full pitch screw
3 = Small steam lock
4 = Single flight, full pitch screw
5 = Small steam lock
6 = Single flight, full pitch screw
7 = Medium steam lock
8 = Double flight, ½ pitch screw
9 = Large Steam Lock
10 = Double flight,1/2 pitch, uncut cone screw

Figure 3.1 Screw Profile

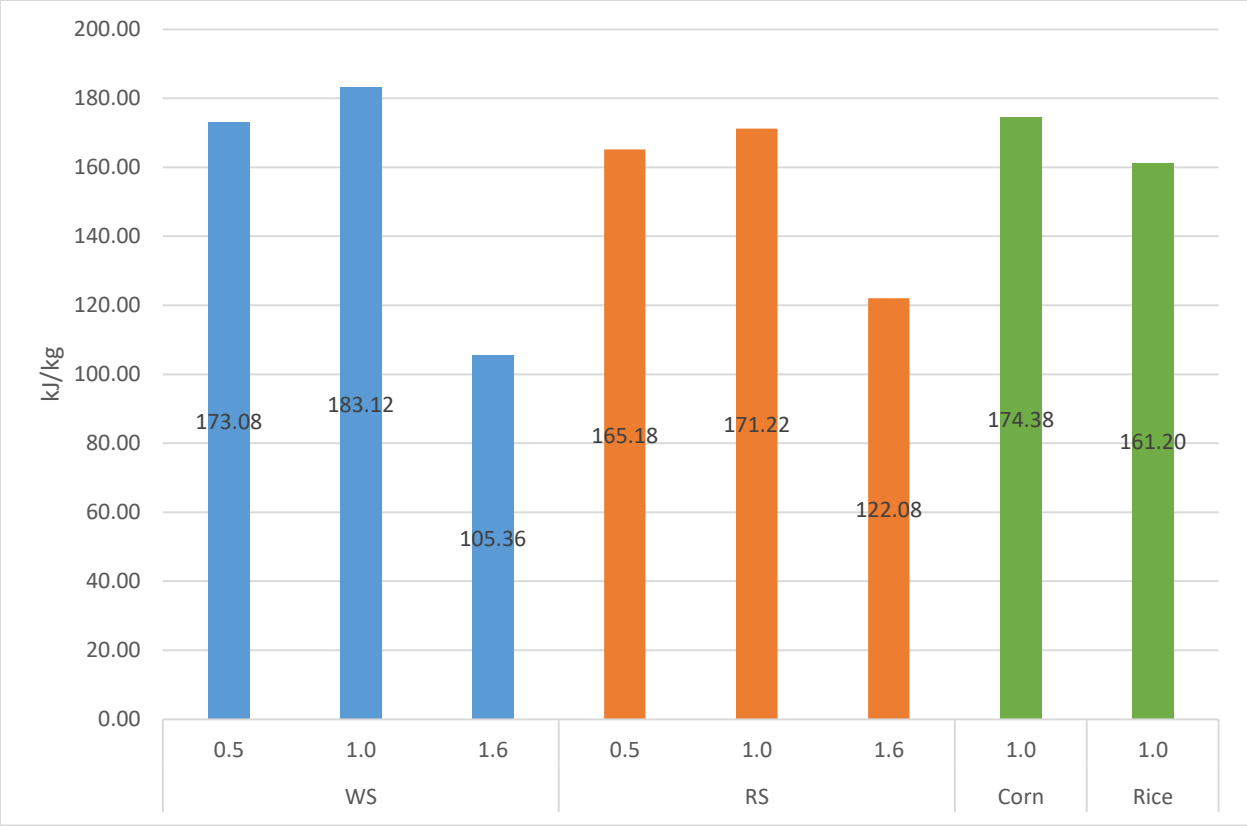


Figure 3.2 Average Calculated SME for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

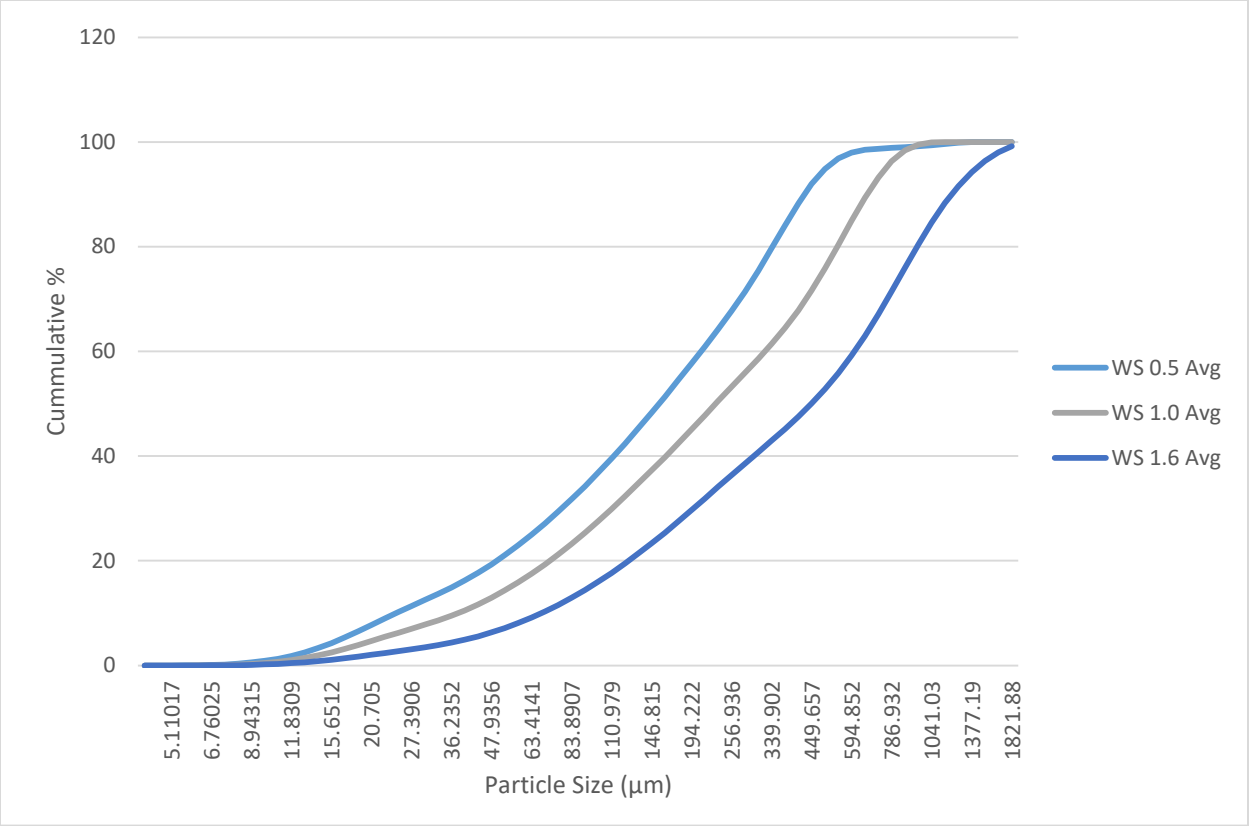


Figure 3.3 Cumulative Grind Size Average for White Sorghum (WS) Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

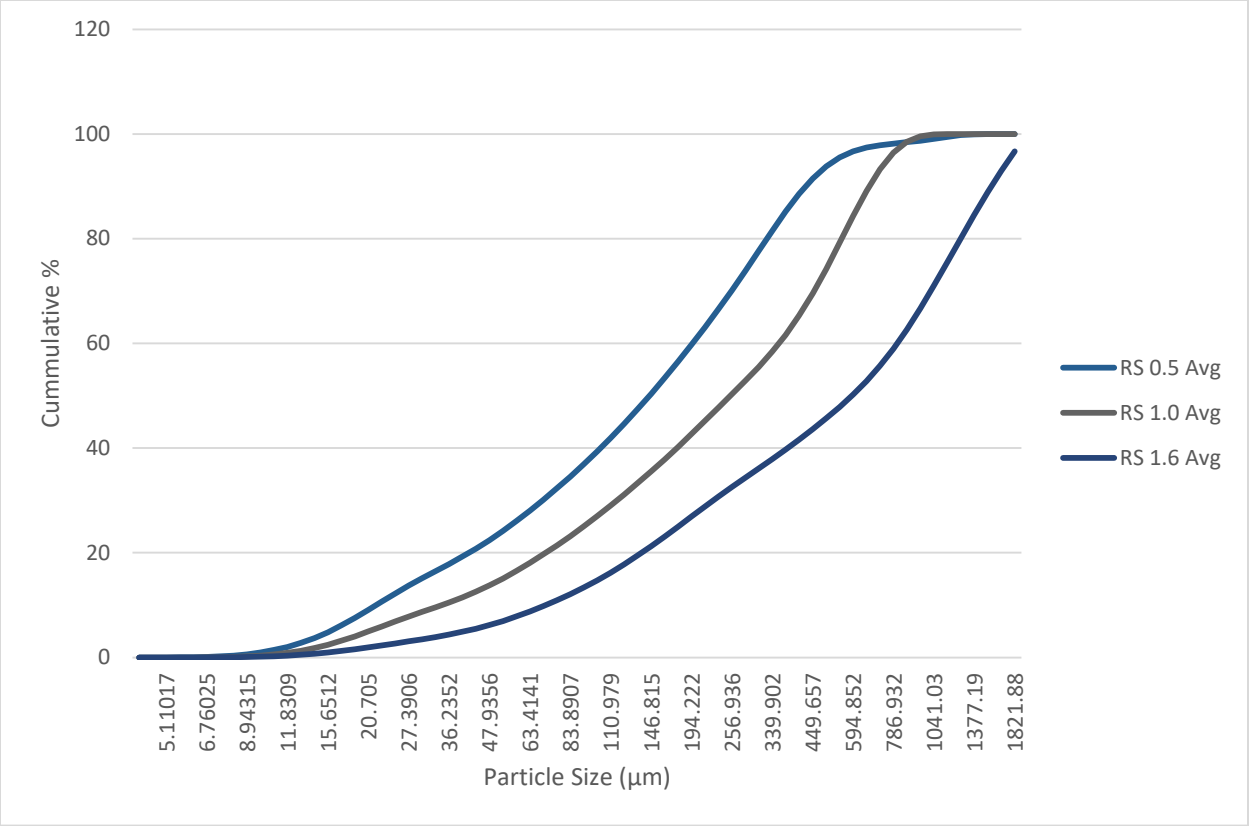


Figure 3.4 Cumulative Grind Size Average for Red Sorghum (RS) Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

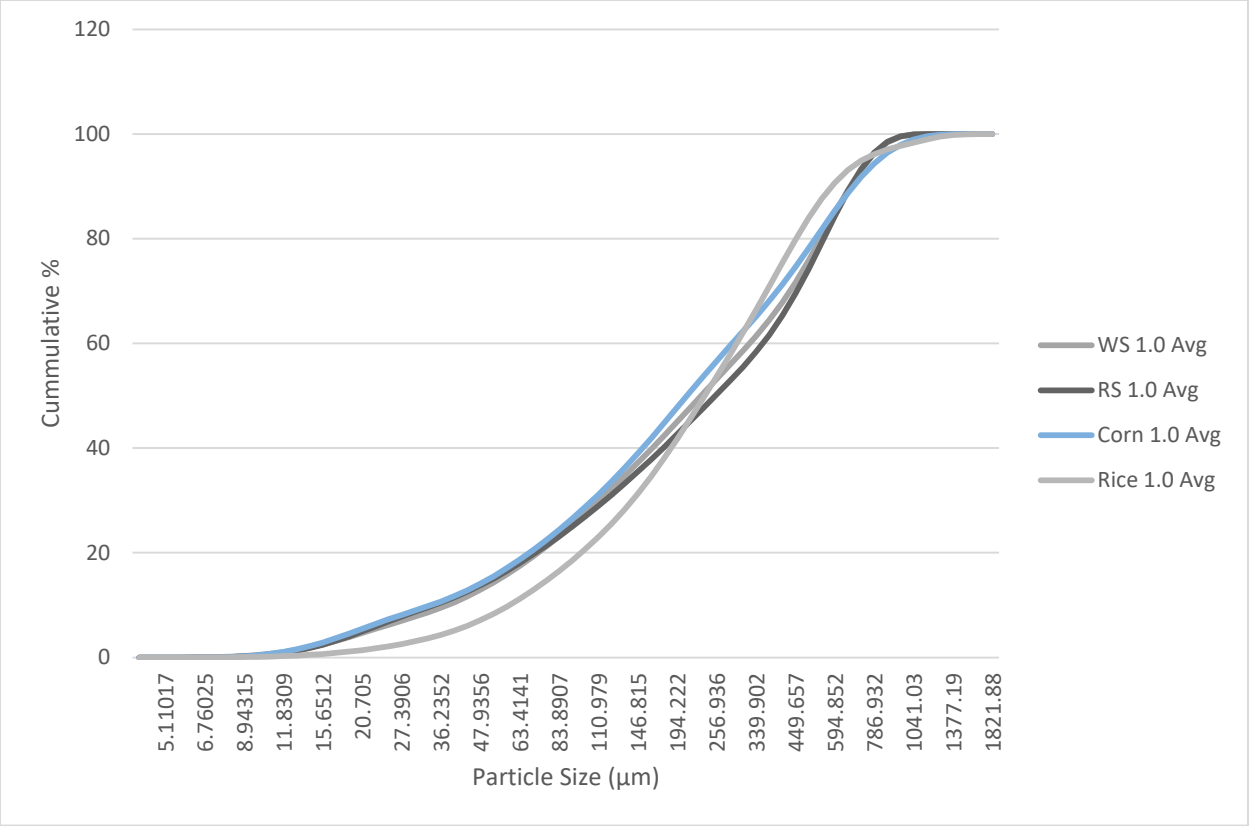


Figure 3.5 Cumulative Grind Size Average for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

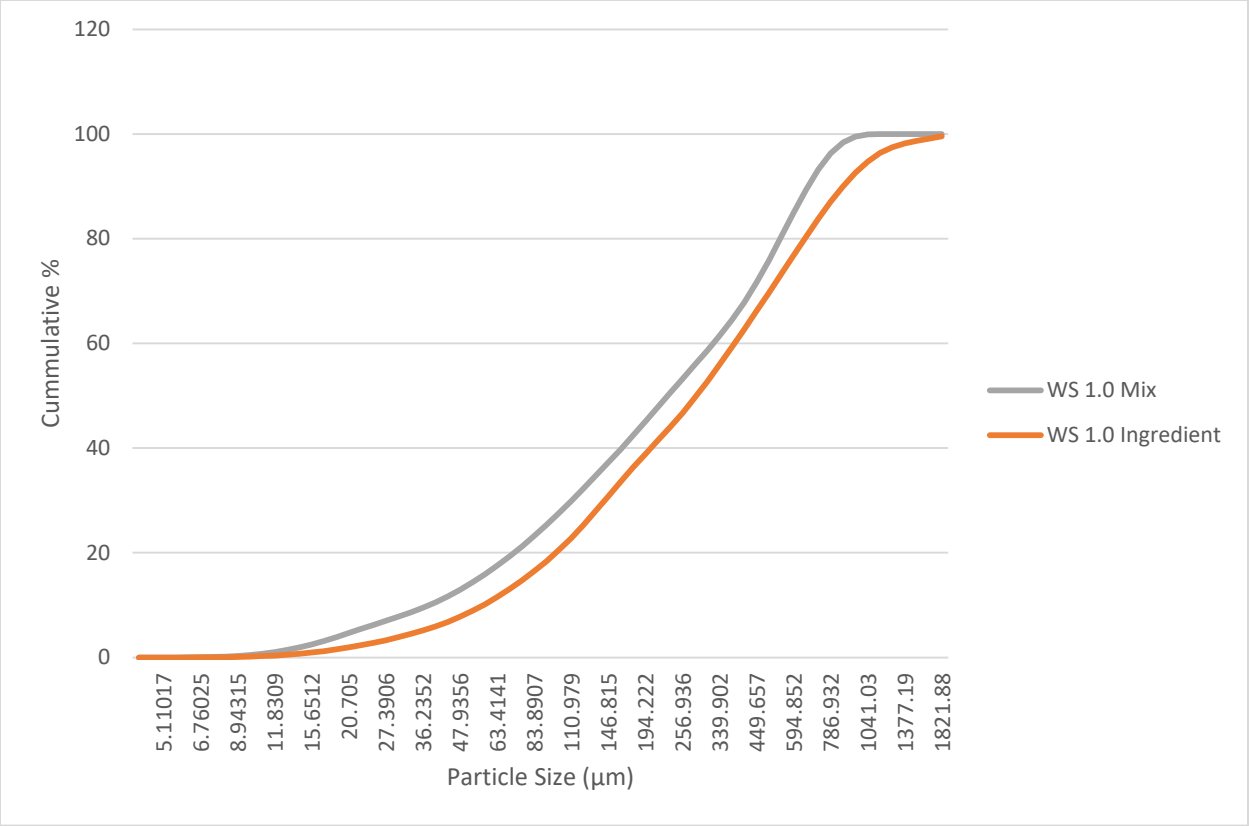


Figure 3.6 Cumulative White Sorghum (WS) Ground Through a 1.0 mm Screen Size

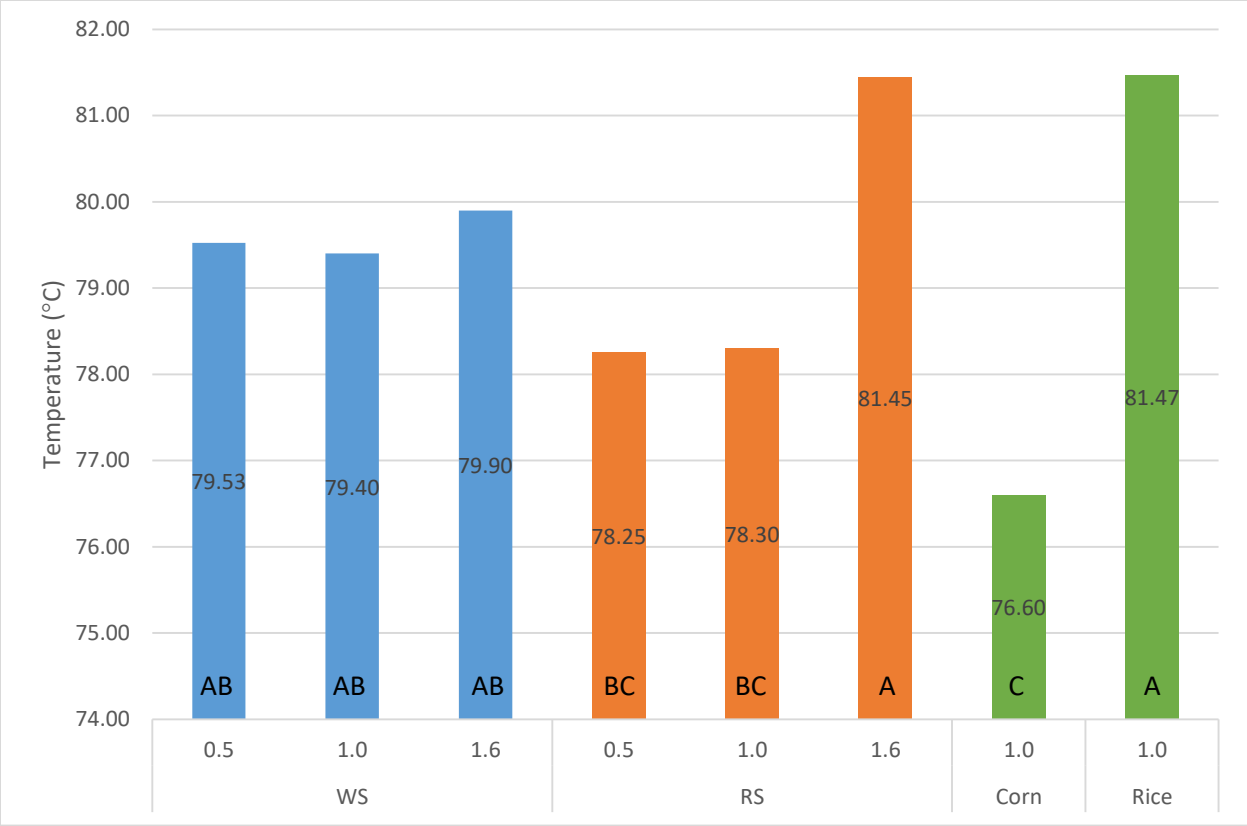


Figure 3.7 RVA Raw Material Pasting Temp (T_{paste}) for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

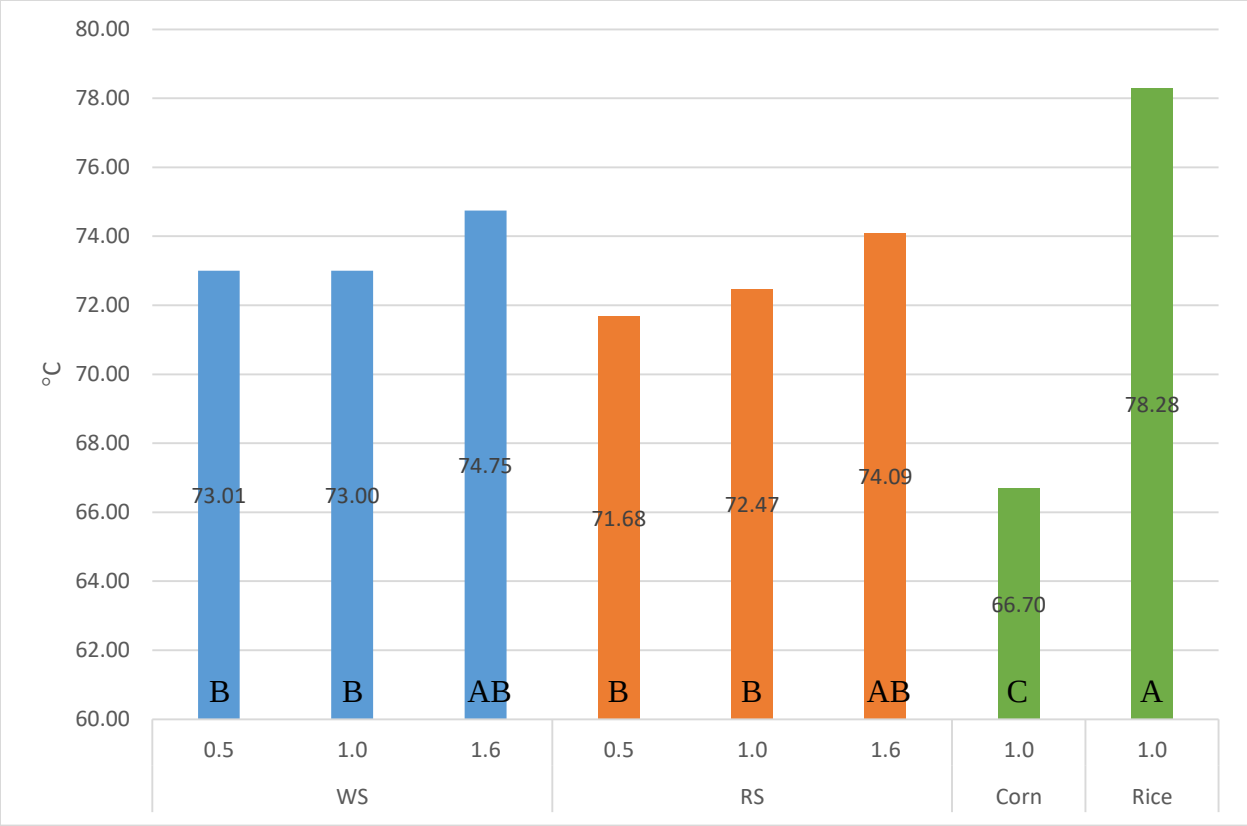


Figure 3.8 DSC Onset Temperature (T_o) for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

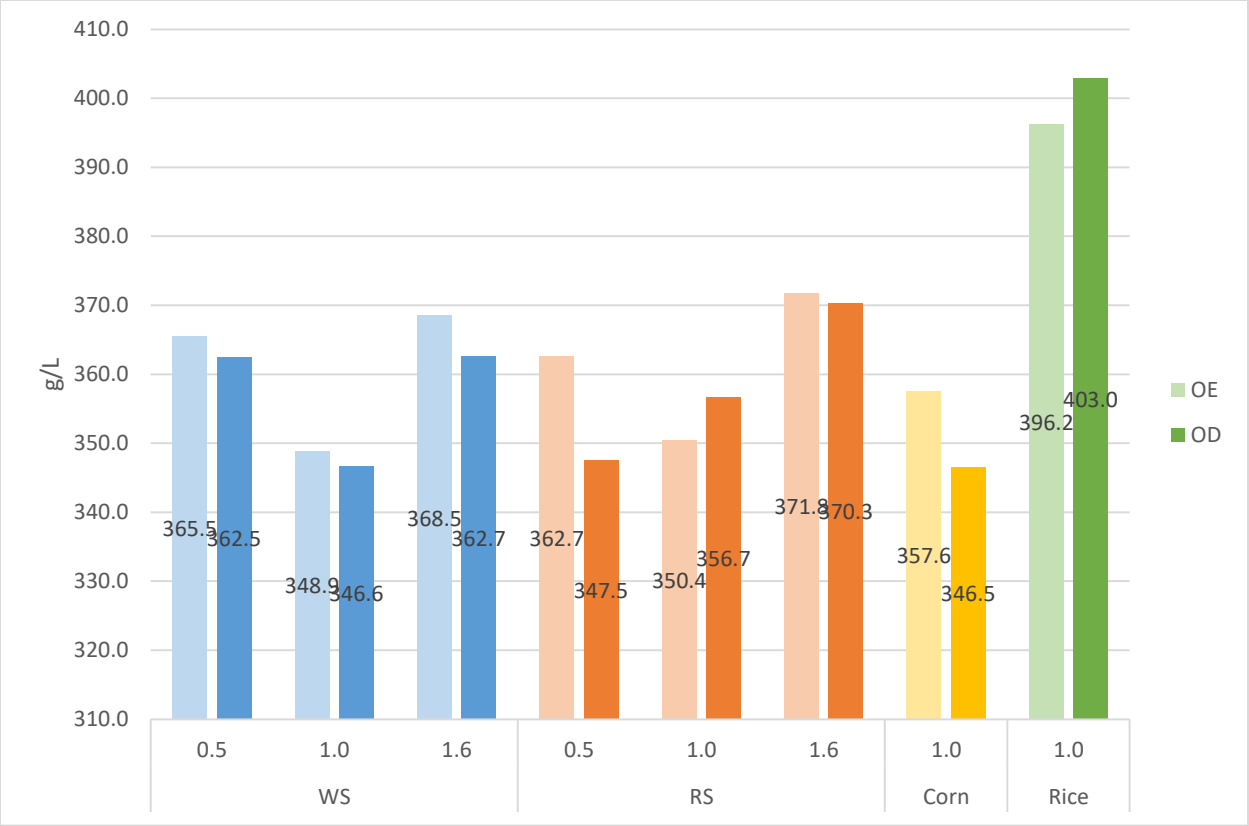


Figure 3.9 Off Extruder and Off Dryer Bulk Densities for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

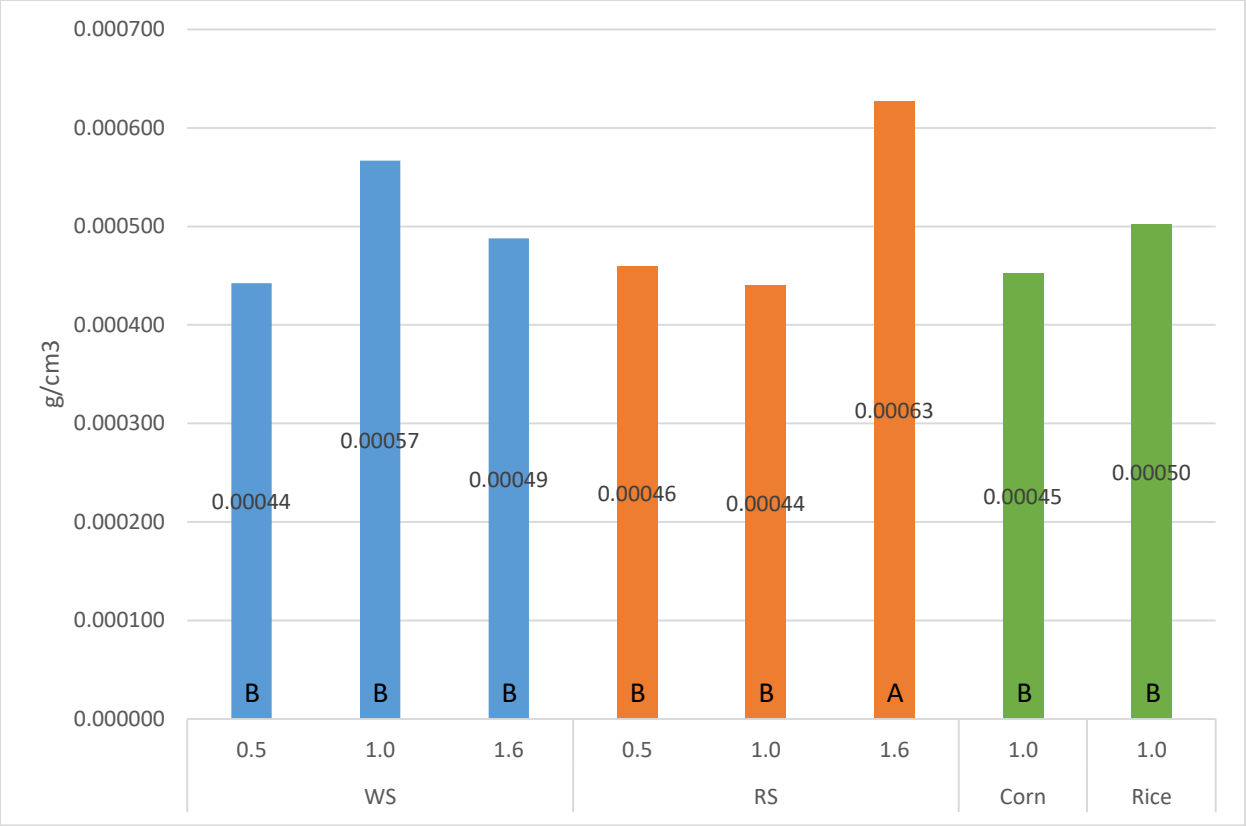


Figure 3.10 Mean Piece Density for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

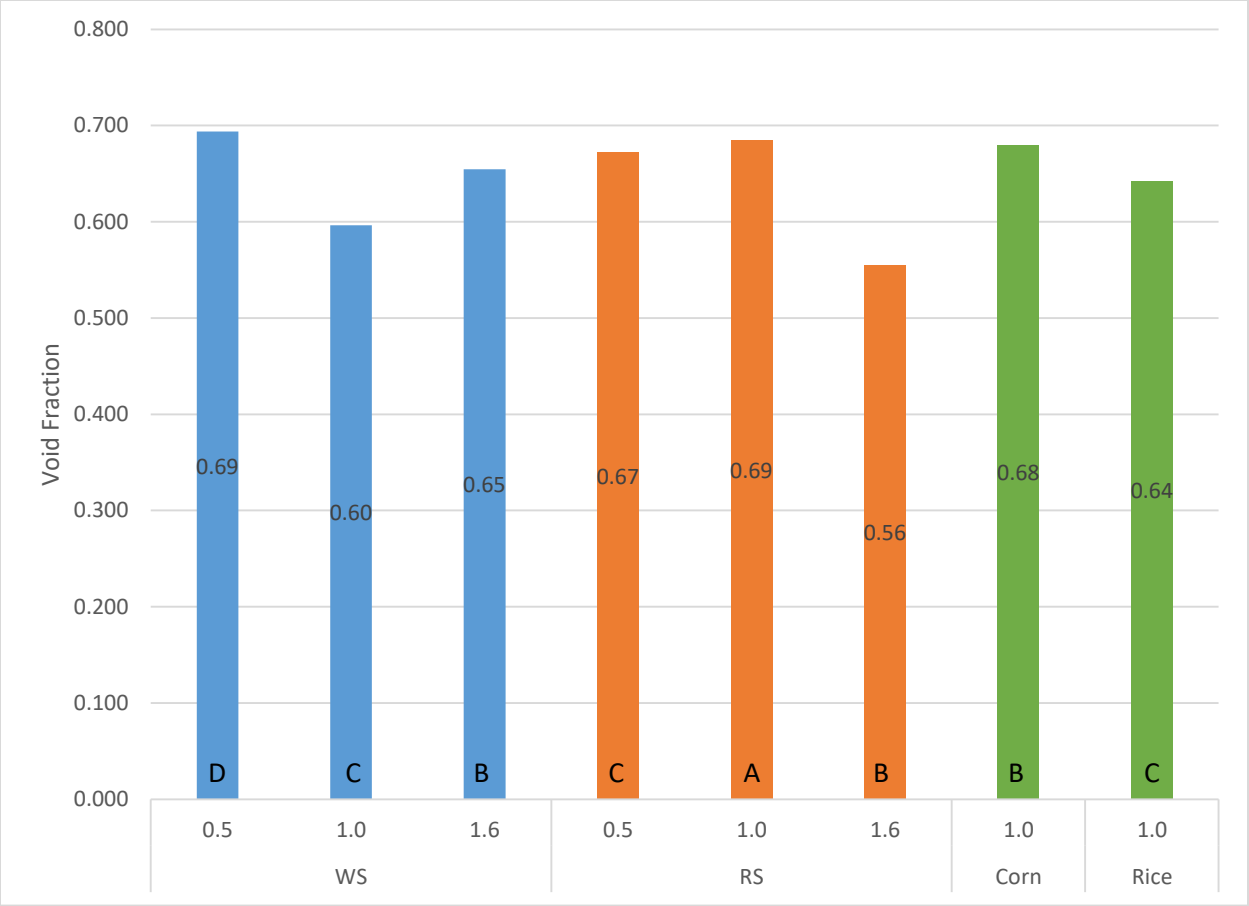


Figure 3.11 Average Void Fraction for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

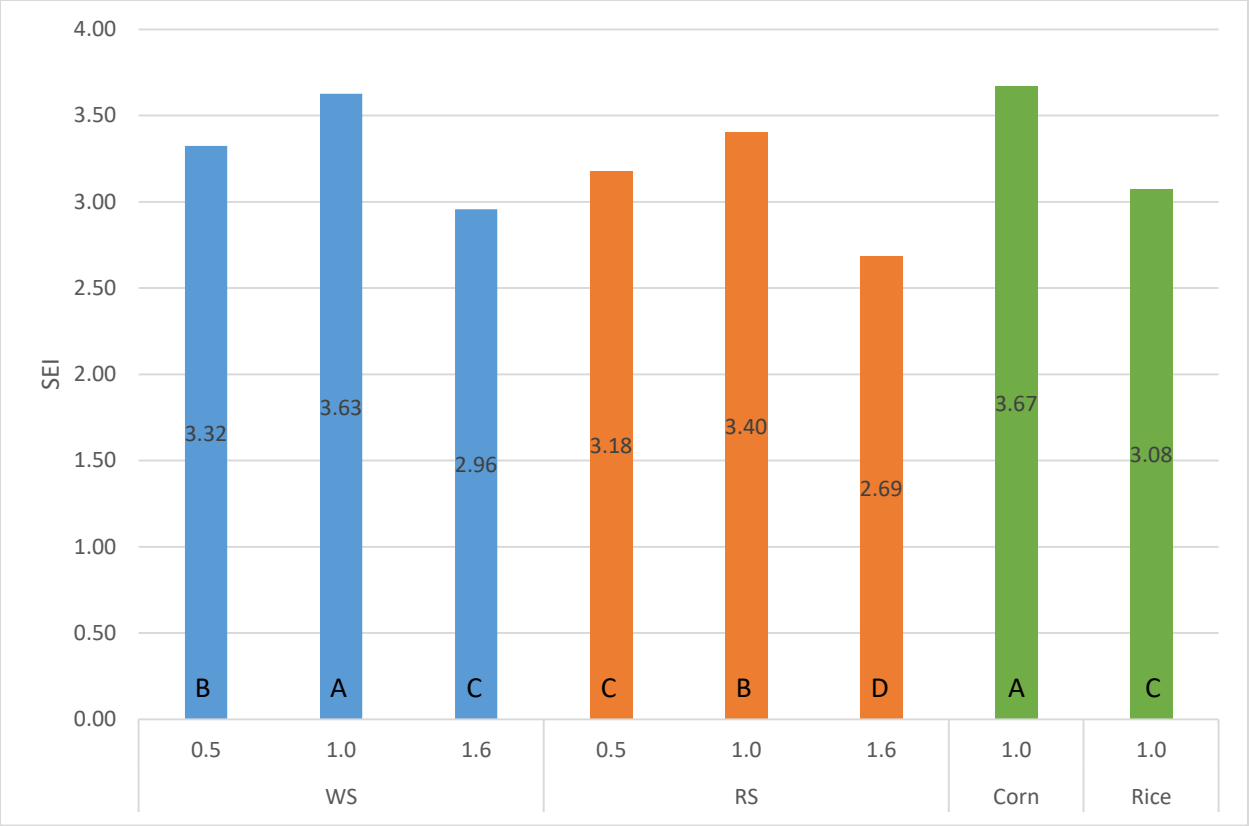


Figure 3.12 Average Sectional Expansion Index for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

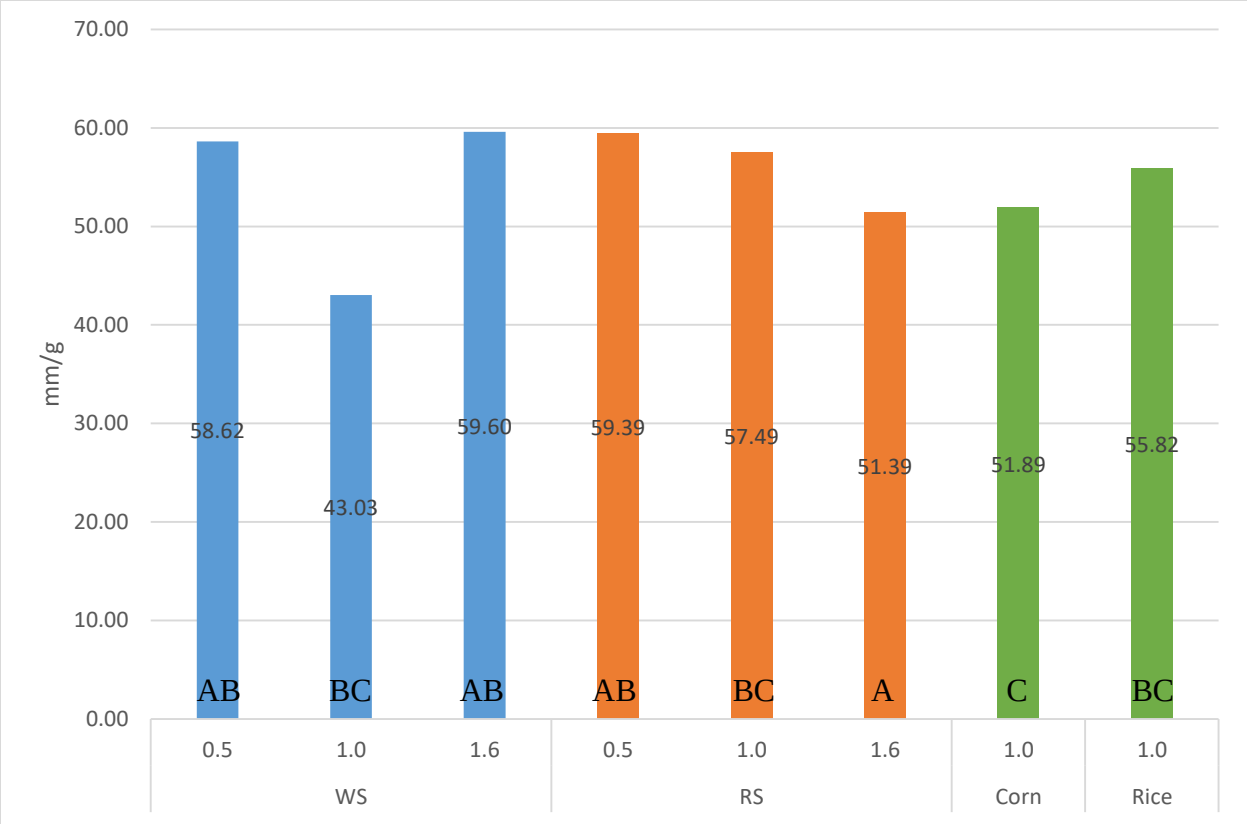


Figure 3.13 Average Longitudinal Expansion Index for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

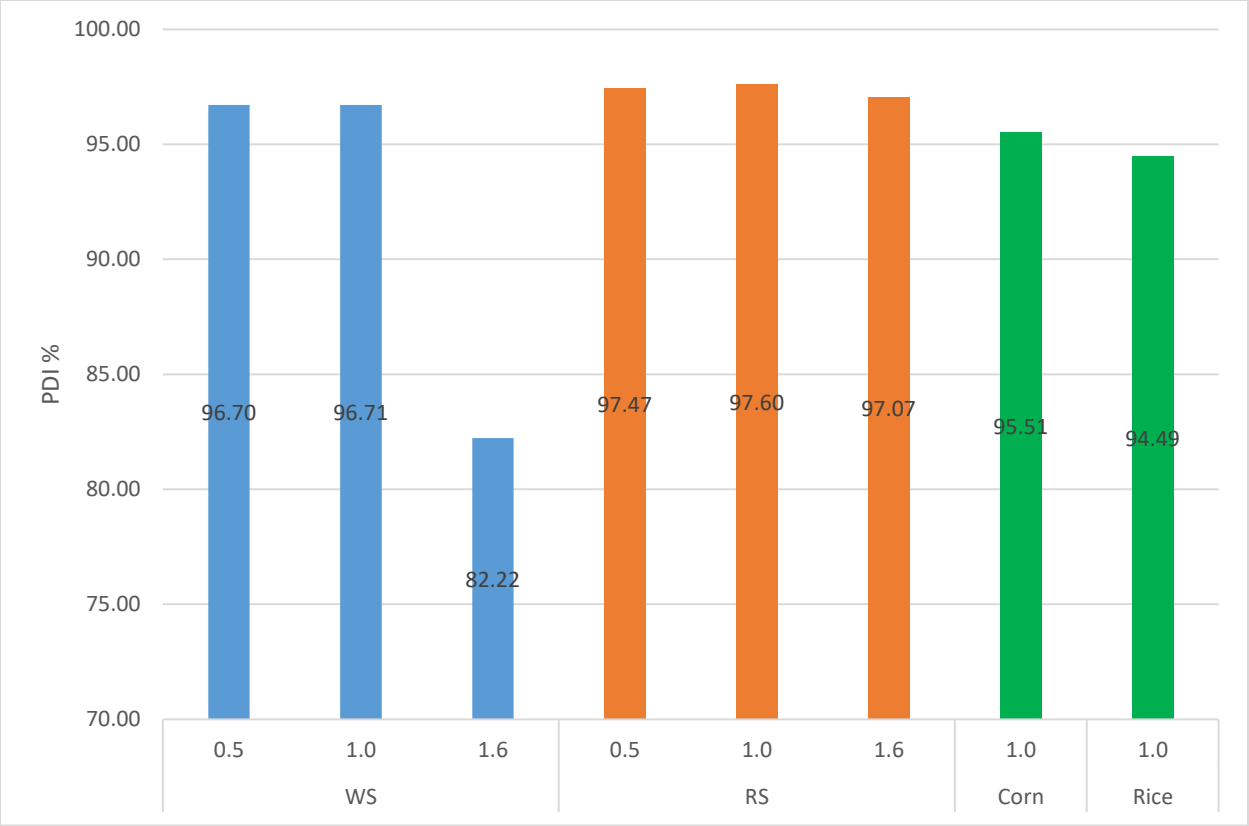


Figure 3.14 Average Tumble Test Pellet Durability Results for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

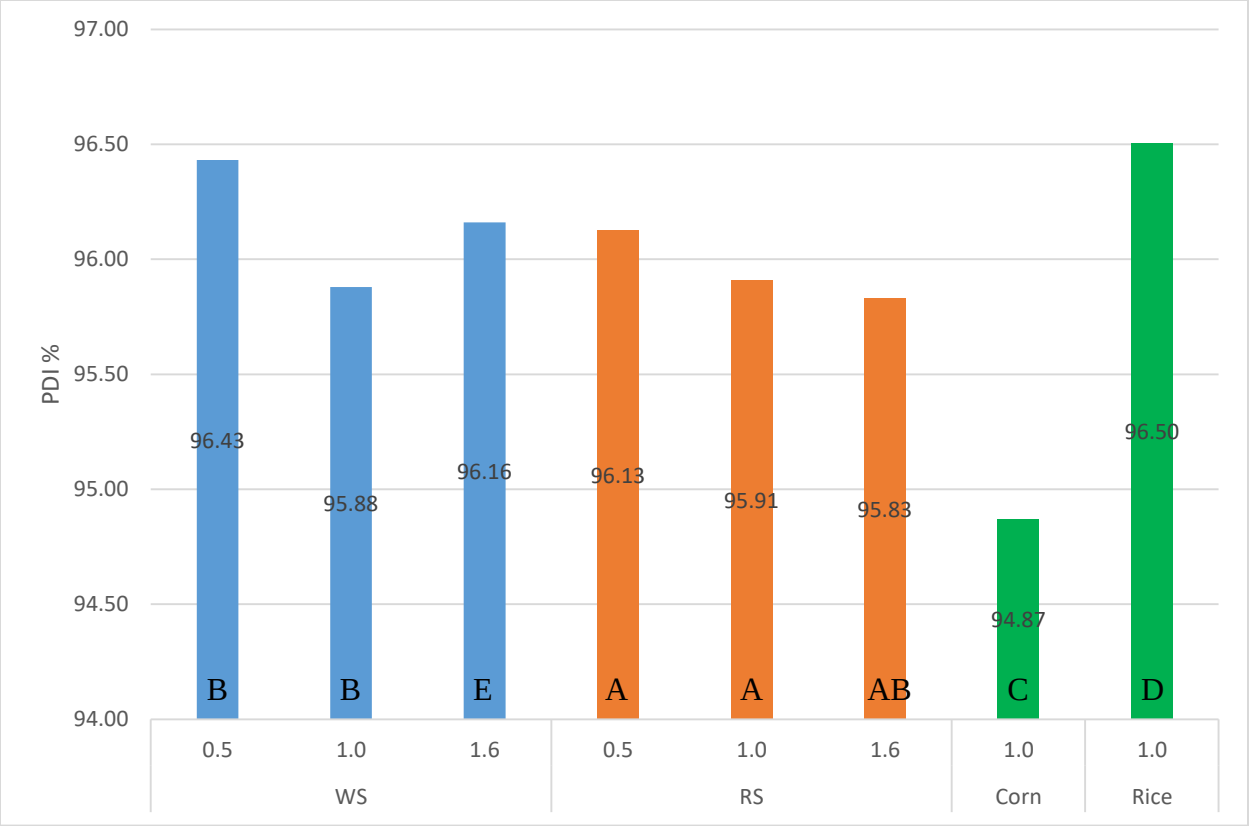


Figure 3.15 Pneumatic Test Pellet Durability Average for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

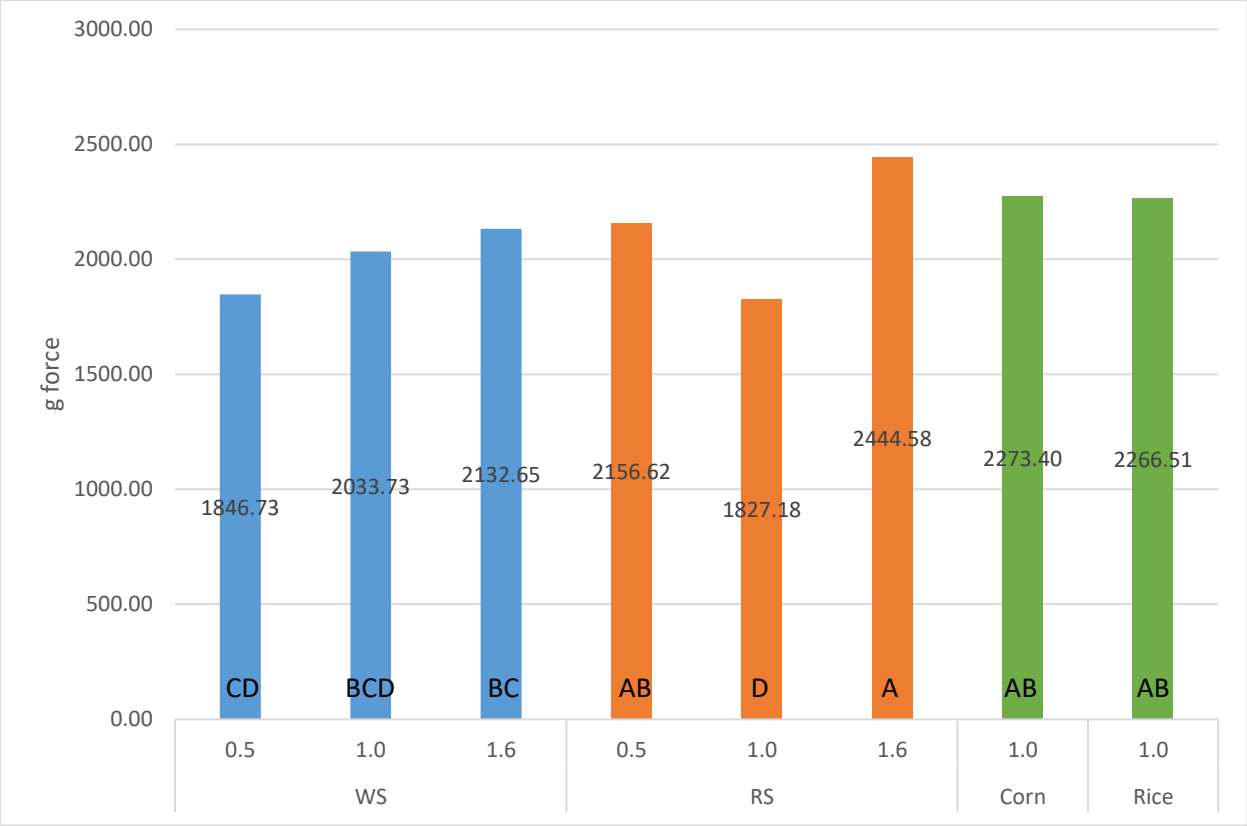


Figure 3.16 Average Texture Analysis Peak Breaking Force Test for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

Chapter 3 Tables

Table 3.1 Ingredient Composition of Experimental Diets for Cats - Formulated with Different Cereal Sources.

Item	Diets			
	Red Sorghum	White sorghum	Brown rice	Corn
Ingredients (g/kg, as-fed basis)				
Red sorghum	466.5	-	-	-
White sorghum	-	496.0	-	-
Brown rice	-	-	388.6	-
Corn	-	-	-	460.8
Poultry by-product meal	365.0	365.0	365.0	365.0
Corn gluten meal	30.0	24.1	37.0	48.6
Beet pulp	24.0	18.5	88.5	13.0
Common salt	5.0	5.0	5.0	5.0
Potassium chloride	5.0	5.0	5.0	5.0
Mineral premix[1]	1.5	1.5	1.5	1.5
Vitamin premix 0,25%[2]	2.5	2.5	2.5	2.5
Choline chloride	4.0	4.0	4.0	4.0
Taurine	1.2	1.2	1.2	1.2
Mold inhibitor[3]	1.0	1.0	1.0	1.0
Antioxidant[4]	0.5	0.5	0.5	0.5
Fish oil	0.4	0.4	0.4	0.4
Poultry fat	73.4	55.3	79.8	71.5
Palatability enhancer, liquid	20.0	20.0	20.0	20.0

[1] Supplied per kilogram of diet: Iron, 100 mg; Cupper, 9.25 mg; Manganese, 6.25 mg; Zinc, 150 mg; Iodine, 1.87 mg; Selenium, 0.13 mg.
[2] Supplied per kilogram of diet: vitamin A, 18.750 IU; vitamin D, 1.500 IU; vitamin C, 125 mg; vitamin K, 0.15 mg; thiamine, 5 mg; riboflavin, 16 mg; pantothenic acid, 35.75 mg; niacin, 62.5 mg; pyridoxine, 7.5 mg; cobalamin, 45 mcg; folic acid, 0.75 mg.
[3] Myco Curb, Kemin AgriFoods North America, Inc. Propionic acid, sodium carbonate, calcium hydroxide, amorphous silicon dioxide, lemon oil, ammonium hydroxide, benzoic acid, phosphoric acid, sorbic acid, propylparaben, methylparaben, butylated hydroxyanisole and tertiary butyl hydroquinone.
[4] Naturox, Kemin AgriFoods North America, Inc. Amorphous silicon dioxide, citric acid, natural mixed tocopherols, vegetable oil and rosemary extract.

Table 3.2 Enzymatic Starch Results (off – dryer)

SAMPLE	MOISTURE		TOTAL		GELATINIZED		% COOK	
	% wb	Average	STARCH *	Average	STARCH *	Average	%	Average
WS 0.5	5.12	5.12	40.98	40.6	36.99	36.99	90.28	91.14
WS 0.5	5.12		40.22		36.99		92	
WS 1.0	5.75	5.75	40.39	40.39	33.99	33.99	84.18	84.18
WS 1.6	6.04	6.04	37.45	37.45	27.11	27.11	72.38	72.38
RS 0.5	4.98	4.98	38.74	38.455	35.05	35.38	90.46	92.03
RS 0.5	4.98		38.17		35.71		93.6	
RS 1.0	5.9	5.9	37.39	37.39	32.04	32.04	85.7	85.7
RS 1.6	6.54	6.54	37.65	37.795	28.99	29.105	76.98	76.99
RS 1.6	6.54		37.94		29.22		77	
Corn 1.0	6.2	6.2	39.63	39.63	34.54	35.645	87.19	89.945
Corn 1.0	6.2		39.63		36.75		92.7	
Rice 1.0	5.64	5.64	33.96	34.435	29.95	30.665	88.21	89.055
Rice 1.0	5.64		34.91		31.38		89.9	

Chapter 4 - The Relationships Between Sensory, Palatability, in-vivo Digestibility of Sorghum-Based Extruded Cat Food and its Physico-Chemical Attributes and Processing

Abstract

Palatability testing with live animals in pet food is a common occurrence in the pet food industry. This allows manufacturers and researchers to make inferences as to what preferences animals might have to diet formulation, energy inputs and other physical traits that are the result of the extrusion process. Sensory analysis has, in the past, been reserved to human foods, but is finding its way into the pet food industry as researchers try to figure out what pets like. This research aimed to establish correlations between palatability, digestion and sensory analysis attributes of an extruded cat food so that information on grain type and particle grind size could be found without the use of live animal studies.

Four main formulas were extruded, each with a difference grain source, containing white sorghum (WS), red sorghum (RS), corn (CO) and brown rice (RI). In addition, three grind sizes were used for the sorghums; screen sizes used were 0.5 mm, 1.0 mm and 1.6 mm, which resulted in average particle sizes of 200 μm, 300 μm and 624 μm, respectively. The resulting kibble was tested for several physical attributes, evaluated by a trained human sensory analysis panel and fed to cats in a palatability study.

Palatability results found that generally, white sorghum was preferred to red sorghum ($P < 0.05$) and both sorghums were preferred over the corn and brown rice formulas ($P < 0.01$). This was likely due to both color preference and texture preference, with the cats preferring light

colored food over dark colored food and in the absence of color, they seemed to prefer harder texture over softer texture.

Sensory analysis identified four main categories out of twenty-seven total categories with significant differences; color brown, fibrous, fracturability and gritty. The color and fibrous attributes were ocular determined and the fracturability and gritty are physical attributes. The color brown results correlated with the palatability results well, fibrous only correlated slightly with the specific length attribute, the fracturability and, to an extent, the gritty attribute, seems to relate to texture hardness.

Digestibility results show that a courser particle size results in lower pH, increased fermentation and acted like a fiber or prebiotic to slow the digestion that may work to help reduce the obesity and promote a healthier animal.

4.1 Introduction

There are approximately 42.7 million households in the United States that have at least one cat and there are over 400 million cats worldwide (Cvetkovska, 2020). With these kinds of numbers, cat food is big business, as is evident from the amount of money spend on cat food, up to $500 per year ("Cost of Owning a Cat - HSHV.pdf," n.d.). US pet food sales have increased every year from 2011-2021 with the food and treat segment being the largest (Bedford, 2021b). A part of this segment is the dry kibble market and it is important, just like human food, to provide nutritious and palatable food for the cats to consume.

Dry cat food kibble is generally made by extrusion cooking. Extrusion cooking is a short time, high temperature process that uses a rotating screw and a stationary barrel to push material towards an opening for shape (die). It is a flexible process that can be used for human food, livestock feed and pet food to mention a few. Currently, extrusion is the primary processing technique for producing a dry expanded cat food as it has advantages over other processes, such as the ability to use both thermal and mechanical energy inputs to make a consistent and safe product. Extruders also excel at creating expansion, mixing, homogenization and pasteurization, to name a few (Riaz, Mian N., 2007).

Sensory analysis, using trained human panelists, can help fill a void of understanding in comparing pet foods without the need for using animals. Sensory analysis is not able to give us information on how the animals interact with the product (taste, feel, smell), but it can help understand the sensory properties better (Koppel et al., 2015). Another benefit of sensory analysis is that humans make the purchasing decisions for their pets and having sensory data form human panelists might be a benefit for pet food producers (Donfrancesco et al., 2012). A study by Koppel et al (2014) found reported attributes such as STE and SME had an effect on

sensory characteristics. They also found that attributes such as fracturability and hardness were affected by the thermal energy of extrusion, although they did not provide much data. Another study found that changes in the physical attribute fracturability was different between the control diets and the fiber treatments; stating that "changes in kibble texture may affect acceptability of the food by dogs" found in the sensory aspect (Monti et al., 2016b).

Fox (2020) says that "Palatability is the capacity of a food or an ingredient to stimulate the appetite of dogs or cats to encourage eating and satiety." It can also be defined as having a pleasant mouthfeel, aroma and taste with sensory characteristics (Koppel, 2014). This is where art and science meet to encourage a pet to consume the food that we want it to eat. Nutrition is very important, but doesn't matter if the cat refuses to consume the food.

As mentioned previously, palatability is getting a pet to eat the food in which we would like them to eat and like that option. A mix of aroma and taste can and will be the deciding factors on what a cat will eat. Palatability can be difficult to analyze and find definite answers as the animals cannot tell us what they like and don't like; we have to devise methods and tests to compare treatments so that we can draw scientific conclusions.

Literature correlating palatability data and sensory data has been difficult to find as there are not many studies doing this same type of work. However, one study was able to link some sensory attributes to palatability. The properties of fracturability aroma and smell were correlated to wheat bran fiber inclusion levels and concluding that as intensity increased the palatability may also increase. (Koppel et al., 2015).

In vitro digestion data is critical to the development of pet foods as the pets depend on their owners to provide complete and nutritious food so that good health can be maintained. Pet food manufacturers use digestion data to increase the healthiness of the foods that they produce.

The lack of studies involving the correlation of in vitro digestibility and physical attributes leaves a space to be filled to help understand this topic better. There are several studies that link starch gelatinization to in vitro digestibility (A. Altan et al., 2009; Yağcı and Göğüş, 2009), but none that link it to other physical traits.

This study seeks to fill a void in literature over several areas by relating product attributes, first, directly to sensory responses and second, to palatability and digestion data. Another objective is to directly compare sensory data with the palatability and digestion data.

4.2 Materials and Methods

4.2.1 Processing

The diets were formulated to be nutritionally complete and balanced diets with similar levels of starch and fiber specifically designed for cats. Ingredients were purchased, sampled and analyzed for dry matter, crude protein, fat, ash and total dietary fiber and the formulas were adjusted to the analyzed chemical composition of the raw materials. The fish oil and the dry ingredients (except for sorghum) that were used were sourced from a local supplier (Lortscher Animal Nutrition, Inc., Bern, Kansas, USA) as individual ingredients. The sorghum was sourced from a reginal sorghum processor (NU-Life Market, Scott City, KS, USA). The poultry fat (International Dehydrated Foods, Monett, MO, USA) and palatant (SPF-Diana, Hodges, NC, USA) came from primary producers.

The whole grains were ground through a pilot scale hammer mill (Model D Comminuter, Fitzpatrick, Westwood MA, USA) three different screens to achieve the average particle size of 200 μm, 300 μm, 624 μm and then extruded through a X20/E325 single screw extruder with a preconditioner (Model 2 Differential Diameter Conditioner, Wenger, Mfg., Sabetha, KS) for the kibble production.

Processing conditions such as feeder screw speed (13 rpm), preconditioner screw speed (400 rpm), preconditioner water (4.6 – 7.6 kg/hr) and steam addition (14.4 – 18.1 kg/hr), extruder screw speed (413 rpm), extruder water addition (7.6 – 8.6 kg/hr), barrel head temperatures (50-70-90 °C) and knife speed (3600 rpm) were kept unchanged, although some natural process variability occurred, across all treatments. This was done so that any differences in product or independent variables would be attributed to material or grind sizes.

4.2.2 Measured Attributes

Bulk density measurements, both off the extruder (OE) or wet bulk density, and off the dryer (OD) were taken by filling a previously tared 1 liter cup over the rim of the cup and striking off the amount of material that was above the rim. The amount remaining in the cup was weighed on the same scale and the weight recorded. This reading is in grams/1 liter (g/L).

The piece density of the dried kibble was calculated from measurements made of the individual kibble pieces. Thirty pieces of kibble randomly selected from the dried material were measured for length and 2 places for width. The two width measurements were averaged so that a final kibble width could be obtained. Each piece of kibble selected was also weighed and this amount recorded. Using the length, average width and weight of each of the thirty pieces of kibble per treatment; the volume, piece density, specific length and sectional expansion index could be calculated. The volume is calculated by dividing the average width by two to get the radius (r), squaring r and then multiplying r^2 by π and multiplying that by the kibble height.

The sectional expansion index is the square of the diameter of the extrudate divided by the square of the die diameter. The diameters were measured for 30 kibble pieces, SEI calculated and then averaged to find an average SEI for that treatment.

Specific length is the measurement in length divided by the measurement of weight of 1 piece of kibble. The lengths and weights were taken for 30 kibble pieces, specific length calculated and then averaged to find an average specific density for that treatment.

The enzymatic gelatinization test is as follows: 0.5 g of sample was boiled with 25 mL distilled water for 20 min and then cooled to ambient temperature. Meanwhile, another 0.5 g of sample was hydrolyzed in 25 mL distilled water for 20 min at 25°C as a control. Next, 10 mL of

acetate buffer solution was added into each tube followed by 5 mL of glucoamylase, and samples were incubated at 40°C for 70 min. Next, 5 mL of trichloroacetic acid was added to halt hydrolysis. After the sample was cooled to room temperature, distilled water was added to make a final volume of 100 mL. Free d-glucose was then measured using a glucose analyzer YSI 2700 (model 2700; YSI Inc., Yellow Springs, OH). The resulting quantity of free glucose determined in the control (G_{cold}) represents the percentage of starch that was gelatinized during processing. Meanwhile, the quantity of free glucose determined in the cooked sample (G_{boil}) represents the percentage of TS within a sample. The DG is then calculated as DG (%) = (G_{cold}/G_{boil}) × 100. Analyses were conducted in duplicate.

There are three tests for hardness, the tumble hardness is a standard for measuring the pellet durability index in diets manufactured with pellet mills, but can be used for extruded kibble as well. Measurement is as follows:, 500 grams of cooled, screened pellets are placed in a metal box with dimensions of 30 cm by 30 cm by 12 cm and containing a baffle 23 cm long, 5 cm wide and centered diagonally inside the box. This box, or can, is rotated at 50 RPM for 10 minutes, after which the pellets are removed and screened. The pellet durability index (PDI) is defined as the percentage of pellets surviving the test and retained on the sifting screen.

The second test is pneumatic and 100 grams of sieved pellets are tested. The sample is placed in the chamber and the machine is turned on for a preset amount of time (30, 60 or 90 seconds). When complete, the fines have been removed automatically from the sample and thus, the weight of the pellets recovered at the conclusion of the test is the actual percent durability.

The third test is a peak breaking force measurement property of the extrudates and was analyzed using a texture analyzer (TA-XT2) and the Texture Exponent 32 software (both from Stable Micro Systems, Godalming, UK). Thirty randomly selected kibbles from each treatment

were equilibrated to a moisture of 8.0±0.5% (wb) using a humidity chamber (30% RH, 24 h). Samples were compressed parallel to the direction of extrusion to 80% of their original length, using a knife (or wedge) shaped cutting probe at a test speed of 2.0 mm/s. The positive peak force was recorded and then averaged for a single number representing the treatment.

4.2.3 Sensory for Dry Expanded Cat Food

4.2.3.1 Samples

Eight cat food produced with four difference sources of ingredients combining different levels of grinding grades have been offered by Grain Science College for the sensory study. Grinding grades were indicated by the sample code in Figure 4.1.

4.2.3.2 Panelists

Five professional panelists of the Sensory Analysis Center of Kansas State University participated in this study. They are highly trained panelists had completed 120h of training in flavor and texture analysis; had completed a minimum of 1000h of general sensory testing on a wide variety of food and beverages. In addition to that pre-training, the panelists received further orientation on dried cat food before proceeding with sensory tests, totally 3h in two different session.

4.2.3.3 Sample Evaluation

Cat food samples were labeled with 4-digit random codes, were served at room temperature to panelists for evaluation in a sensory lab. Panelists were given Toothbrushes, mozzarella, crackers, cucumbers, tomato juice, and hot water for neutralizing the effect of

preceding cat food samples. The panelists evaluated intensities of each cat food for attributes covering sensory characteristics of appearance, aroma, flavor and texture/mouthfeel. Panelists have to taste two kibbles per bite. Intensities were scored on a 15-point numerical scale divided into half-point increments, with 0 meaning “none” and 15 meaning “extremely strong”.

4.2.3.4 Test Design

A completely randomized design was used by Red jade to determine the serving order for the cat food. Five or four cat foods were tested in each of five 2h sessions. Three replicate judgments of each cat food were made by each panelist over the 5 sessions held for this study. All panelists were present for all tasting sessions.

4.2.4 Sensory for Coated Dry Expanded Cat Food

4.2.4.1 Samples

Four cat food produced in same process (similar grinding grade) with different ingredients including brown rice, corn, red sorghum and white sorghum were coated with seven different source of fat (Chicken fat A, Chicken fat B, Sunflower oil, Salmon oil, Fish oil, Butter and Lard) using a panner to mimic the industrial production of coated kibble. Same batch of the cat food used as experiment Objective I.

Kibbles coated with fat (10% in weight) were prepared. Prepare the fat oil: butter, lard and chicken fat need to be melted in microwave before coating. First weigh the needed kibbles and fat. After spread the melted fat oil in the panner evenly first, pour the kibbles in the panner. The kibbles will be coated with the panner (Rollermac) in two minutes with set rotation rate at 16rpm and set temperature at 60 ◦C. Next, lower the temperature to room temperature for

kibbles` cooling and evenly coating in the panner in another 15min. The coated kibbles were stored in glass jars and tested after aging one week in room temperature.

4.2.4.2 Panelists

As in Objective I, Five professional panelists from the Sensory Analysis Center of Kansas State University participated in this study (Three panelists are the same from Objective I). In addition to that pre-training, the panelists received a 2h of orientation before progressing on this project.

4.2.4.3 Sample Evaluation

Cat food samples were labeled with 4-digit random codes, served at room temperature for panelists to evaluate in a sensory lab. Panelists were given hot towel for neutralizing the effect of preceding cat food samples. The panelists evaluated the aroma and appearance characteristics of each cat food. Intensities were scored on a 15-point numerical scale divided into half-point increments, with 0 meaning “none” and 15 meaning “extremely strong”.

4.2.4.4 Test Design

Totally the cat food were evaluated in three different sessions: orientation, consensus evaluation and side by side evaluation. In Orientation, six samples (CA RS, Fish oil WS, Sunflower Rice, Butter corn, Lard RS, Salmon fish WS) representative in different sources of ingredients and coating fat were randomly tested by the panelists to build up the attributes for evaluation. In consensus evaluation session, a completely randomized design was used by Red jade to determine the serving order for the cat food. Nine or ten coated cat foods were tested in

each of three 2h sessions. Next, three sessions in each of 2h were conducted for side by side evaluation, each session include tasting 8 or 12 samples. Between the first two sessions, a brake of 15min were taken. Unlike in consensus evaluation session, the samples were evaluated in pure monadic. During the side by side evaluation, the samples were presented to the panelist simultaneously. Panelists need to taste the samples following the randomized order. All the panelists will need to validate their consensus data, discuss if they would like to change the data any more. So to finalize the consensus data.

4.2.5 Digestibility and Palatability

The animal study was performed in accordance with the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee on Animal Use of the College of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP) – Jaboticabal Campus.

4.2.5.1 Definition of Approach

Before the actual study started with cats, a pilot experiment was conducted with the aim to compare the effect of two processing variables on in vitro digestibility of the organic matter: the effect of thermal energy application on extrusion and the effect of raw material particle size. To evaluate this, 4 diets were formulated (ingredient composition shown on Table 1), with corn, brown rice, white sorghum or red sorghum as the cereal source. A factorial arrangement of treatments was used, with 2 sorghum varieties (white and red) x 3 screen sieve sizes (0.4, 1.0, and 1.6mm) x 3 applications of specific thermal energy (low, medium, high) plus 2 control foods (corn and brown rice), adding up 20 treatments. Foods was prepared as described latter and

extruded to obtain the specified conditions for thermal energy application. After preparation, foods were evaluated for the in vitro digestibility of the organic matter, as a screening for the possible effects of the different grinding and extrusion conditions on cats. The in vitro digestibility of organic matter (OM) was determined as described by Hervera et al. (2007) in samples collected after the dryer. The incubation conditions simulate two steps of the digestion process, digestion in the stomach and in the small intestine, using an enzymatic system with pepsin and pancreatin, respectively. As a result, the specific thermal energy application showed only small effect on the in vitro digestibility for the sorghum based diets, however grinding appeared be an interesting process to explore, since coarser grinding (1.6 mm) reduced the in vitro digestibility, maybe with a potential prebiotic effect as an increase in resistant starch (Figure 1). Based on these results the approach for the in vivo study focused on different mean geometric diameter of the different varieties of sorghum.

4.2.5.2 Study Design and Diets

The study followed a 2 x 3 factorial arrangement of treatments, composed by 2 sorghum varieties (red or white) ground in 3 different particle sizes, more 2 controls (brown rice and corn), totaling 8 complete diets. Different varieties of low tannin sorghum were used as the primary carbohydrate source for two of the formulations. As controls, two other formulations based on rice or corn were used. To formulate the diets, samples of brown rice, corn, white sorghum, red sorghum, beet pulp, and poultry by-product meal were purchased and analyzed for dry matter, ash, crude protein, acid-hydrolyzed fat and total dietary fiber, following AOAC (2012) methods. Based on the results, four separate diets were balanced to present similar

nutrient composition following the recommendations of AAFCO (2016) for cat maintenance (Table 1).

4.2.5.3 Animals and In Vivo Study Design

A total of 64 healthy adult mixed-bred cats (33 males; 31 females), with a mean age of 3.44±0.46 years old, and initial body weight of 4.24±0.09 kg was included in the study. All cats were from the Laboratory of Research on Nutrition and Nutritional Diseases of Dogs and Cats "Prof. Dr. Flávio Prada", Department of Veterinary Clinic and Surgery, FCAV/UNESP - Jaboticabal Campus. The health of the cats was previously accessed by physical examinations, and hematology and serum biochemical analysis, and all were considered health.

The experiment with cats followed a randomized block design with four blocks of 16 cats each. In each block two cats were fed each diet, totaling 8 replicates per food. The blocking factor was time. Each block lasted 31 days: days 1 to 17 were used for diet adaptation; days 18 to 20 for fresh feces collection to determine pH, short-chain fatty acids, biogenic amines, ammonia and lactate; days 23 to 29 for total feces collection for digestibility and fecal score analysis; on day 31 fresh feces were collected to analyze microbiome.

During the adaptation period, from 4 p.m. to 8 a.m. all cats were restricted to individual stainless steel metabolic cages (0.80 m x 0.80 m x 1.0 m), with experimental food and water available. From 8 a.m. to 4 p.m., the cats were kept in a collective cattery of 50 m2 for exercise and socialization, where they had access to water but not to food. During the fecal collection periods, cats were restrained all time to their cages. The daily amount of food was individually defined according to the energy requirements for cat maintenance, as 100 kcal/kg0.67/day (NRC, 2006). Food metabolizable energy content was estimated from their chemical composition

(NRC, 2006). Each day, food was weighed and divided into 2 similar portions, and given at 9 a.m. and 4 p.m. Refused foods were collected and weighed, and the consumption recorded.

The digestibility test was conducted by the quantitative collection of feces method, following the AAFCO (2016) recommendations. After diet adaptation, quantitative collection of feces was performed for 7 days. Fecal samples were weighted and frozen (−20°C) as they were collected and pooled by cat. At the end of the collection period, feces were thawed, homogenized, and dried in a forced-air oven at 55°C for 72 h (Fanem, São Paulo, Brazil) and ground in a cutting mill (MOD 340, ART LAB, São Paulo, Brazil) with a 1.0 mm screen sieve size prior to laboratory analysis.

During the digestibility trial, fecal samples were scored according to Carciofi et al (2008): 0 = liquid stools; 1 = soft, unformed stools; 2 = soft, poorly formed stools, that assume the shape of the container; 3 = soft, wet and formed stools that retains shape; 4 = well-formed and consistent stools, which do not adhere to the floor; 5 = dry, hard stools, with reduced volume. Scores 3 and 4 are considered adequate. Fecal pH was determined by mixing 6 mL of MilliQ water with 2 grams of fresh feces and the pH measured in a pH meter (Model Q-400-Bd, Quimis, São Paulo, Brazil).

4.2.5.4 Fecal pH and Fermentation Products

Fresh feces samples (collected and processed within 15 min after defecation) were used to measure pH and fermentation products. The pH was determined using 2 g of diluted feces in 6 mL of MilliQ water, and the pH was measured in a pH meter with an accuracy of 0.01 (DM20, Digimed Analítica, São Paulo, SP, Brazil). For short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA) analyses, 10 g of feces were mixed in 30 mL of formic acid solution at

4.2 N (1:3 w/v) and precipitated at 4°C for 72 h, and the supernatant was centrifuged three times (5,000 G at 15°C for 15 min). The analyses were performed by gas chromatography (GC-2014, Shimadzu Corporation, Kyoto, OP, Japan) following procedures described by Erwin et al. (1961). Ammonia of the feces was assessed in the extracts prepared for SCFA using the method suggested by Fenner (1965) with modifications by Vieira (1980). The extracts were thawed at room temperature and diluted with ultrapure water (2:13 v/v), and then ammonia was distilled in a nitrogen system (Tecnal TE-036/1, Tecnal, Piracicaba, SP, Brazil).

4.2.5.5 Food Palatability

Food preference comparisons were performed at Panellis Latin America (Descalvado, Sao Paulo, Brazil) using a panel of qualified cats. Seven preference tests were performed: brown rice versus white sorghum, brown rice versus red sorghum, corn versus white sorghum, corn versus red sorghum, white sorghum versus red sorghum, red sorghum ground 0.5mm versus red sorghum ground 1.6mm and white sorghum ground 0.5mm versus white sorghum ground 1.6mm. The first choice (first product consumed) and the preferred product (product consumed in greater amount) were determined using the two-pan method (Griffin, 2003). For the study, 36 adult mixed breed cats, individually housed, were used. Cats were tested in two consecutive meals. In the morning, after 12 hours of fasting, the animals received the first meal in two pans, each containing one of the experimental foods, and were allowed to eat for 30 minutes. The position of the food bowls was changed at the evening meal. The amount of food offered in each bowl surpassed the consumption capacity of the animal to ensure that there would be leftovers to measure. After 30 minutes, the bowls were removed, the remains weighed, and the consumption

calculated by taking the difference in amount offered and amount remained. Due to the differences in body weight, the results were calculated as the relative consumption of each diet.

4.3 Results and Discussion

4.3.1 Palatability and Product Attributes – Related to Starch Cook by Ranking

Palatability, regardless if human or animal, means that it is pleasant to the palate or to the taste of the individual. Humans can tell what suits them, but animals cannot; they have to be observed and conclusions drawn from their behavior. Palatability is important for cats as is digestibility, but less essential to dogs (Tran et al., 2008). It is also a complex environment of attribute interactions that will constantly provide good palatability if each factor is maintained satisfactorily (Riaz, Mian N., 2007).

A primary focus of this work is that palatability will increase as the starch cook level increases due to the density being lighter, and possibly the aroma (Riaz, Mian N., 2007). Our results show that of the two sorghums, WS and RS were consumed in higher amounts, 54% up to 75%, over corn or brown rice as seen in Table 4.2. Figure 4.2 shows STE and SME values along with the total energy, the summation of STE and SME. WS and RS have lower STE values than corn or brown rice, 271.46 and 237.79 kJ/kg, compared to 302.18 and 289.67 kJ/kg, respectively. The SME values are, on the other hand, are higher for the WS and RS (210.74 and 194.99 kJ/kg) verses corn and brown rice (191.66 and 182.9 kJ/kg). These two trends point towards preference for foods with a lower STE : SME ratio. Comparing the kibble side by side allows judgment based on color to be completed, where, in fact, the WS appears to be the lightest in color with RS next and then RI and CO. The percent cook from enzymatic starch test, Table 4.3, reveals that

the WS (84.18) and RS (85.70) have less percent cook than the CO (89.95) and RI (89.06); again suggesting that cats prefer less thermal energy input.

Table 4.2 shows that WS is preferred over RS. Thus, cats seem to prefer food that is lighter in color when looking at how close to "brown" the kibbles are colored. In these 4 treatments at the 1.0 mm size, the WS was the lightest color, followed by RS, RI and CO, respectively. The reason for RI being darker than WS is that this specific rice type is brown rice and not a pure white rice, so the natural color is darker than a white table rice commonly used for human consumption.

When we look at the kibbles from a macro point of view, we see that if all kibbles are close to the same size and texture, then color may be the deciding factor for preference. Thus, the trend appears to be that the light color is predominate for preference and the lightest color will influence first choice. If there is no difference in color, then secondary preferences show up and other attributes become important for choice.

As we look at the effect of particle size, particle size was only significant for the RS, the WS showed no significance, as shown in Table 4.2. The RS 1.6 mm grind size was heavily preferred over the RS 0.5 mm grind size. However, when we look at the peak breaking force in Figure 4.1, we see that there is a slight trend that the 1.6 mm grind size is harder than the 0.5 mm size for both the WS and RS, although it was not statistically significant. This means that as a general rule, cats prefer the courser grind and the courser grind generally requires more force to break. Thus, pets must prefer kibble that requires more force to break, or is "harder".

When looking at the peak breaking force (F_p) in Figure 4.1, for grain type between the 1.0 mm grind treatments for WS, RS, CO and RI, the results demonstrate that the only significant difference is in the RS 1.0 mm, as it is the lowest F_p, the WS, CO and RI are all not significantly

different. From this texture test for peak breaking force, the F_p is related to expansion and the Gibson–Ashby model that states that cells that are smaller in size are more difficult to crush than cells with a larger size. The RS has the second heaviest OD bulk density, but requires the least peak breaking force to fracture the kibble. Thus, it seems that this data reinforces the earlier statement that color is the primary attribute choice and that F_p is a secondary choice attribute.

Table 4.3 shows that among the 1.0 mm grind size there doesn't seem to be a trend between sorghum type and the CO and RI for the starch data; there is also no significant difference between the four treatments. This could be due to the fact that each formula was formulated to be iso-starch and within the treatments with the same grind the different grains cooked similarly. This data does not support our hypothesis that more cook equals more palatability.

4.3.2 In Vitro Digestibility and Product Attributes

In vitro digestibility measures or estimates, chemically, the ability of the diet to be digested and to what amount. It would be a reasonable assumption that as the starch cook level increases, the digestibility would increase also due to the cooked proteins starches and aroma. There also seems to be a relationship to STE and SME, although inversely. As the STE and SME increase (Figure 4.2), the digestibility decreases, which in our case, of looking at obesity, is a positive effect. When comparing the STE : SME ratios, we see that the larger the particle size due to grinding, the less the total energy and also that the energy ratio is larger for larger grind size. This is mainly due to the larger particle size lowering the SME as discussed earlier.

The enzymatic starch cook data shows that the CO and RI treatments have more % starch cook than the WS and RS.

In vitro digestibility may also be related to overall expansion (bulk density). As the OE bulk density increases, the digestibility seems to also increase. This may be due to a less expanded product having a lower bulk density and thus smaller cell size. This smaller cell structure leads to less area for the enzymes to break down the material and the longer breakdown time leads to a fiber and pre-biotic effect for good digestion.

The fecal score (Table 4.4) shows no significant difference in score; this means that sorghum is digested no differently that corn or brown rice. Also, there was no difference on fecal score for particle size and thus there is no advantage to having a smaller grind than the 1.6 mm in this experiment attribute.

RI had an increase in fecal pH (acidification), but no difference in WS, RS, CO. This shows a slight trend with the same ranking occurring with OD bulk density. The higher the OD bulk density, the more increase of fecal pH because of the same reasoning as above, This smaller cell structure leads to less area for the enzymes to break down the material and the longer breakdown time leads to a fiber and pre-biotic effect for good digestion and thus increased pH. The results show that a larger particle size also led to lower fecal pH. This is likely due to the larger particle size having less expansion and less chance for digestive enzymes to work on the food, as stated above. The larger particle size leading to less cook, less cook leads to lower digestibility, lower digestibility leads to lower pH because there is less of a chance for the enzymes to work on the material.

The fecal concentration of acetate was higher in the RI diet than the CO treatment with the WS and RS being similar to RI. Table 4.5 shows that the intake of the diets with higher raw material particle size for WS 1.6 and RS 1.6 increased the butyric acid fecal concentration significantly. Valeric acid concentration followed a similar trend that the WS 1.6 and increased

versus the WS 0.5 and RS 0.5. Thus, the larger particle size seems to favor the increase in these short chain fatty acids that, in turn, ferment more and acts like a fiber, slowing digestion and having a positive impact on gut health. Jackson, et al. (2020) found similar results with cats fed maintenance diets based on grain that allowed production of postbiotics with fiber like benefits. The results for isobutyric and isovaleric acids, ammonia and biogenic amines did not differ between diets.

The resistant starch shown in Table 4.8 shows that as particle size increases, the amount of resistant starch increases, thus providing another avenue having a fiber like benefit and slowing the digestion process to provide increased gut health. It is interesting to note that as the particle size increases and resistant starch increases, the dry matter and organic dry matter, while not statistically significant, trend to increase that the starch gelatinization decreases and thus leads to less digestion also. These results overall, show that as the particle size increases, the organic dry matter decrease, starch gelatinization decreases and this leads to less digestion, which increases the fermentation and production of short chain fatty acids.

4.3.3 Sensory and Product Attributes

Sensory analysis is important to be able to identify product attributes which may help guide manufacturers with understanding palatability without actually using kennels and doing animal studies. Trained human panelists can actually tell us what they like and don't like about the products. Our results show that there was no real difference in aroma and flavor, but there was significant differences in color (brown), fibrous, fracturability and gritty (Table 4.7, Table 4.8). Color and fibrous are optical observations from panelists and fracturability and gritty are physical traits from panelists chewing the kibble. The brown attribute describes how light or

dark the sample is from the color brown, fibrous is an evaluation of the perception of fibers on the product, fracturability is a measure of how much force is required to break the sample and gritty is how much small, sharp, sand-like particles remain after five to seven chews.

There does not seem to be any correlation from color to any of the physical attributes measured as color of the raw material and the final kibble was out of scope for this project. However, from observing the raw material it is evident that the WS is the lightest, followed by RS, RI and CO. There was no particle size trend observed for the color attribute.

As with color, there only seems to be one attribute with a relationship to fibrous, and that is the specific length. Specific length (Figure 4.4) seems to be related to the fibrous attribute because as the specific length increases, it may appear as more fibrous due to seeing some of the particles with "more surface" to view.

The attribute fracturability seems most related to peak breaking force (Figure 4.1) because as the particle size increases, panel fracturability score increases. This matches with our data - the more expanded the product, the less hard the product, this is due to aligning with the Gibson-Ashby model as above, smaller sized cells are harder to crush. Therefore, the harder the kibble, the more fracturability increases as would be expected. Even though it takes more force to break the kibble, when it does break, it fractures in to smaller pieces.

Gritty seems most related to tumble hardness (Figure 4.5). Similar to the abrasiveness of the tumble hardness test, as the sample is tumbled during the test, the material may lose some fines that would be dislodged from the kibble. These fines might be of various sizes, but remain that size and are not further broken down. A similar action happens with the gritty test, the abrasiveness the kibble is subjected to during the chewing process, particles break off, but don't break down quickly – they stay intact though the saliva tries to break them down further in size.

4.3.4 Palatability vs Sensory

There were few relationships found with respect to palatability and sensory comparisons. As fracturability increases, the palatability increases also. This could be due to the cats preferring a harder kibble that fractures more easily.

As the gritty (sensory) increases, the palatability decreases due to the hardness and once fractured, the pieces are left are small.

As the kibble gets closer to the color brown, palatability goes down; WS is preferred over all other grains.

There does not seem to be a trend between fibrous and palatability.

4.4 Conclusion

The results show that sorghum can be used successfully as a replacement grain for corn or brown rice as it is generally more palatable than either corn or brown rice. A larger grind size is preferred over a smaller grind size when comparing treatments between the same grain type.

The grind size appears to be a secondary preference attribute to color; cats prefer a lighter color to a darker one. Grind size only appears to come into consideration if there are no color choices available.

4.5 References

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Chapter 4 Figures

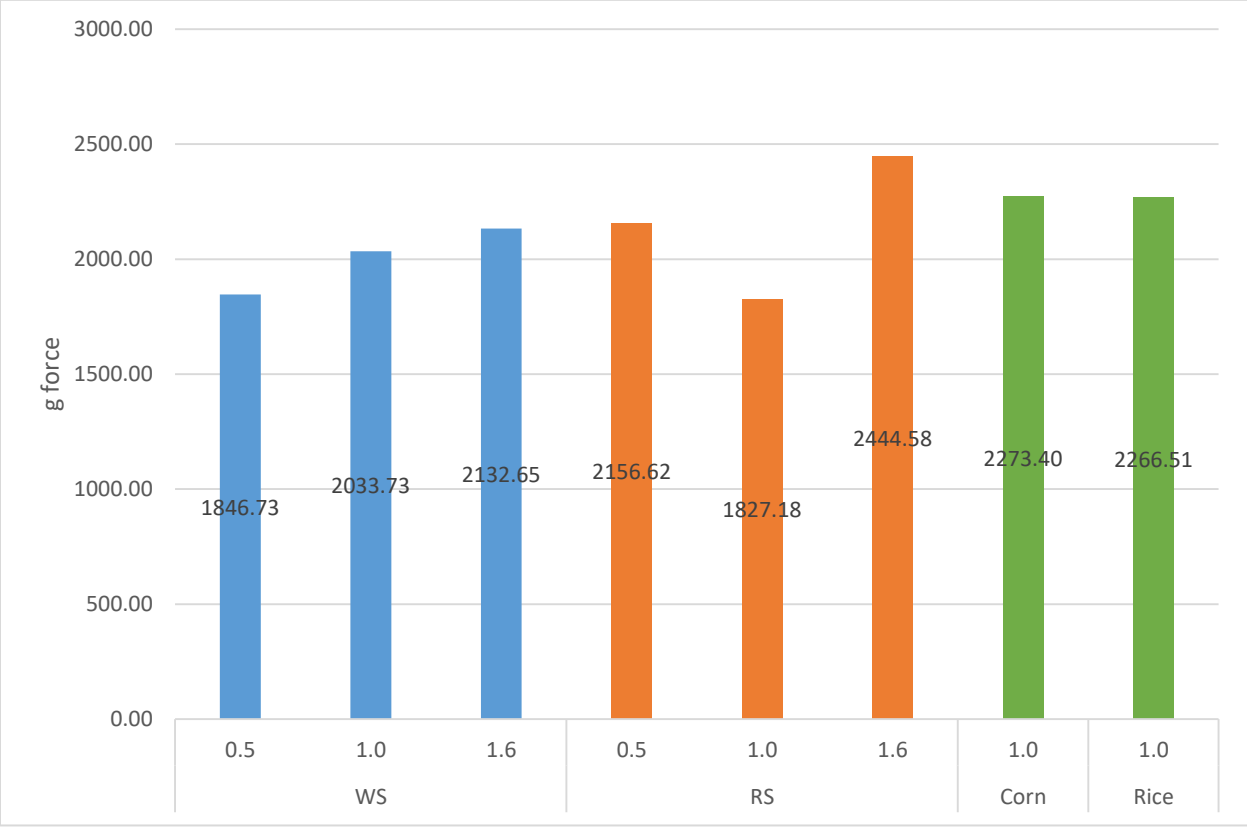


Figure 4.1 Peak Breaking Force Average for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

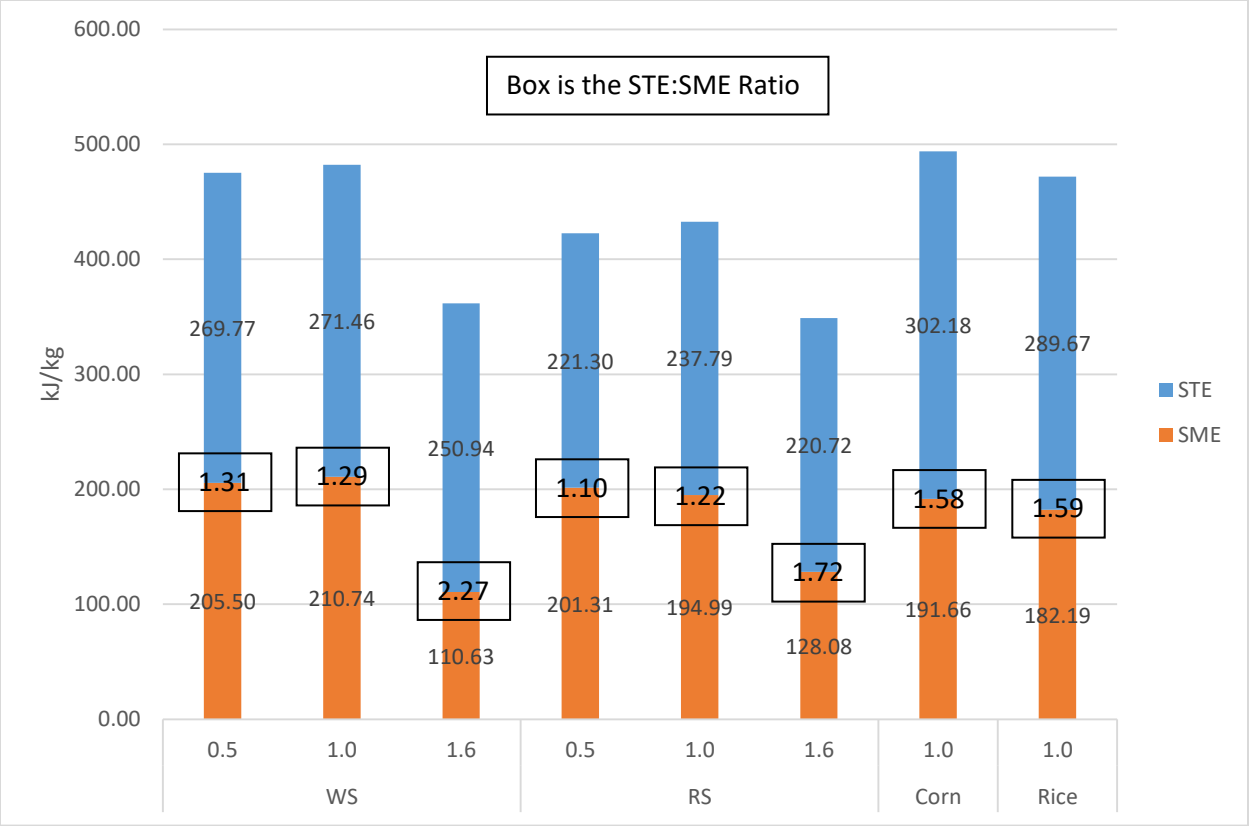


Figure 4.2 Average STE and SME Results for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

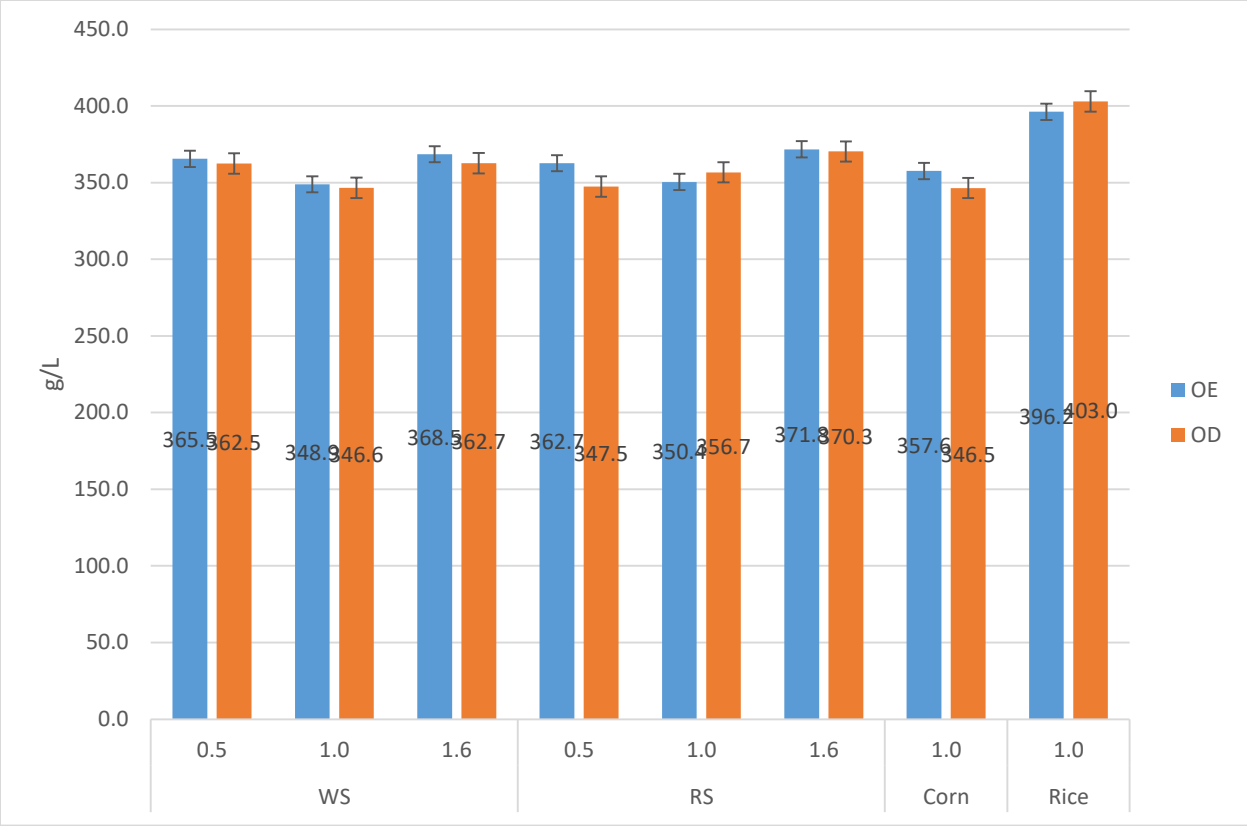


Figure 4.3 Average Bulk Density for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

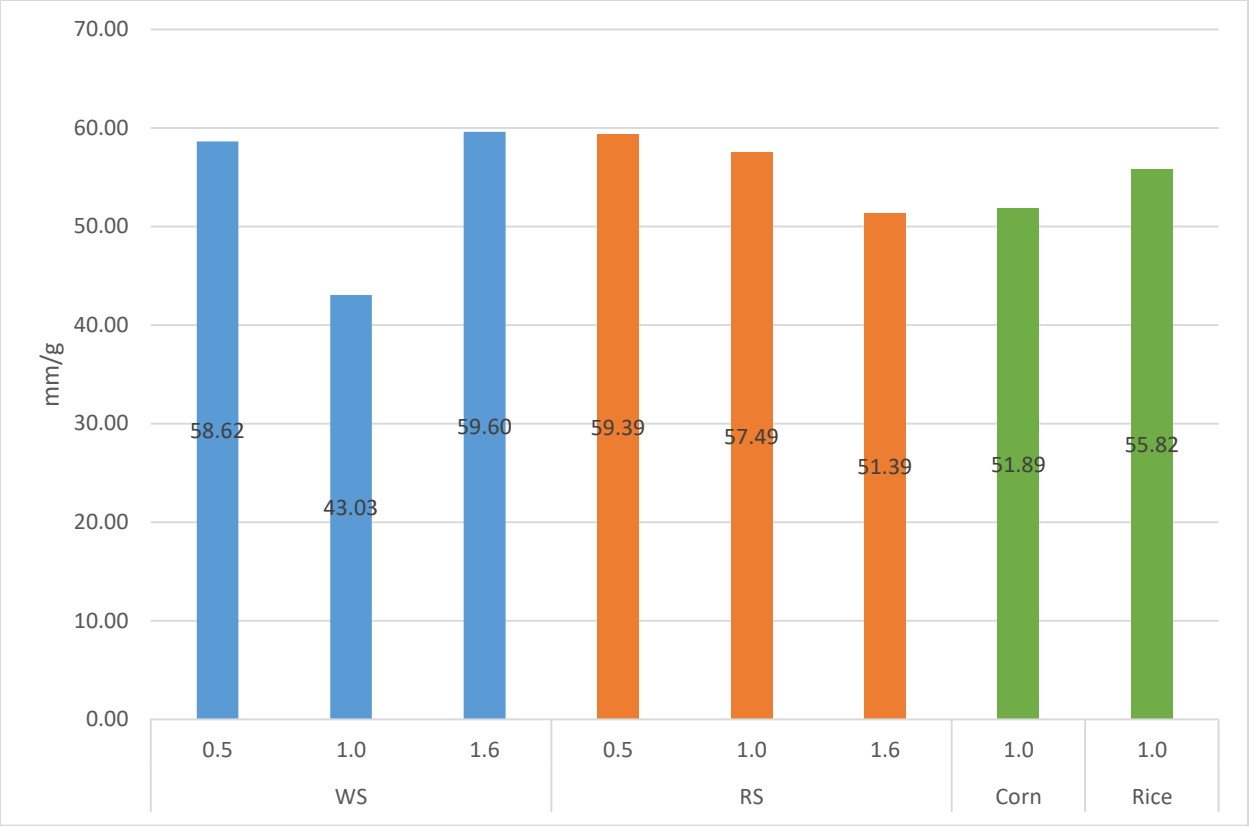


Figure 4.4 Average Specific Length for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

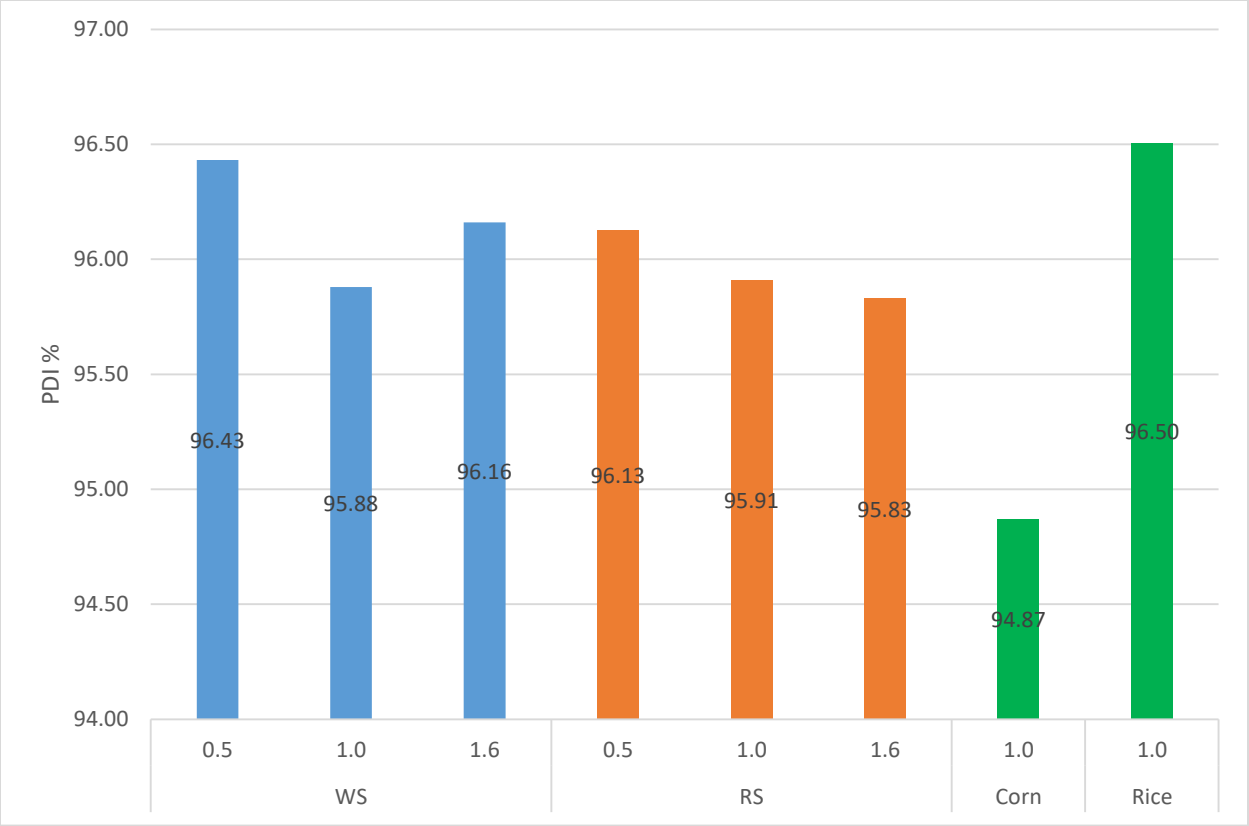


Figure 4.5 Average Tumble Hardness for White Sorghum (WS), Red Sorghum (RS), Corn (Co) and Rice Ground Through 0.5 mm, 1.0 mm and 1.6 mm Screen Sizes

Chapter 4 Tables

Table 4.1 Sample Contents and Sample Identification Key

Sample code with grind size	Main Ingredient
Corn 1.0	Corn
Brown rice 1.0	Rice
RS 0.5	Red Sorghum
RS 1.0	Red Sorghum
RS 1.6	Red Sorghum
WS 0.5	White Sorghum
WS 1.0	White Sorghum
WS 1.6	White Sorghum

Table 4.2 Consumption Results Summary of Two-Bowl Preference Test

Treatment	Meal 1	Meal 2
Corn 1.0	32^{A}	25^{A}
WS 1.0	68^{B}	75^{B}
Corn 1.0	46	35^{A}
RS 1.0	54	65^{B}
Rice 1.0	32^{A}	25^{A}
WS 1.0	68^{B}	75^{B}
Rice 1.0	34^{A}	29^{A}
WS 1.0	66^{B}	71^{B}
RS 1.0	39^{a}	33^{A}
WS 1.0	61^{b}	67^{B}
RS 0.5	28^{A}	22^{A}
RS 1.6	72^{B}	78^{B}
WS 0.5	49	52
Ws 1.6	51	48

Upper case letters denote significance at $P<0.05$.
Lower case letters denote significance at $P<0.01$,
No letters denotes no significance

Table 4.3 Average Starch Gelatinization Results from the Enzymatic Gelatinization Test

SAMPLE	Total Starch Mean	Gelatinized Starch Mean	% Cook Mean
WS 0.5	40.6	36.99	91.14
WS 1.0	40.39	33.99	84.18
WS 1.6	37.45	27.11	72.38
RS 0.5	38.46	35.38	92.03
RS 1.0	37.39	32.04	85.70
RS 1.6	37.80	29.11	76.99
Corn 1.0	39.63	35.65	89.95
Rice 1.0	34.44	30.67	89.06

Table 4.4 Food Intake and Characteristics of the Feces of Cats Fed Extruded Diets Based on Different Cereal Sources, Ground to Different Raw Material Particle Sizes (0.5mm, 1.0mm, 1.6mm). Data excerpted from Ribeiro (2020)

Item	Experimental diets[1]								SEM[2]	P-value
	Corn (1.0 mm)	Brown Rice (1.0 mm)	RS (0.5 mm)	RS (1.0 mm)	RS (1.6 mm)	WS (0.5 mm)	WS (1.0 mm)	WS (1.6 mm)		
Food intake ($g/kg^{0.67}$/d, as-is)	16.91	17.28	15.97	17.77	16.91	17.69	16.99	17.27	0.199	0.844
Fecal characteristics										
Score[3]	3.40	3.60	3.62	3.42	3.65	3.27	3.14	3.55	0.044	0.776
pH	6.38[a]	5.89[b]	6.30[a]	6.12[ab]	5.89[b]	6.26[a]	6.13[ab]	6.08[b]	0.029	<.0001
$g/kg^{0.67}$/d (as-is)	6.97	8.92	7.08	8.40	9.07	7.63	8.80	8.63	0.297	0.595

Table 4.5 Fermentation Products Concentration (mmol/kg feces in DM) in the Feces of Cats Fed Food Formulations with Different Cereal Sources and Raw Material Particle Sizes. Data excerpted from Ribeiro (2020).

Item, mmol/kg feces in DM	Experimental diet								SEM[1]	P-value
	Brown rice	Corn	Red sorghum			White sorghum				
Screen sieve size, mm	1.0	1.0	0.5	1.0	1.6	0.5	1.0	1.6		
Acetic acid	331.34[a]	200.91[b]	222.83[ab]	271.11[ab]	215.82[ab]	258.16[ab]	231.58[ab]	206.76[ab]	11.148	0.041
Butyric acid	78.36[ab]	58.87[b]	53.20[b]	74.30[ab]	117.53[ab]	53.42[b]	61.63[b]	143.44[a]	7.103	0.002
Isobutyric acid	5.54	6.25	6.15	6.04	4.78	6.57	5.54	6.16	0.229	0.606
Isovaleric acid	7.39	9.70	8.93	10.18	7.36	9.52	8.20	9.63	0.456	0.656
Valeric acid	30.46[ab]	24.68[ab]	18.95[b]	27.24[ab]	44.94[ab]	21.80[b]	26.20[ab]	49.27[a]	2.391	0.004

Table 4.6 Matter, Organic Matter, Protein and Starches. Data excerpted from Ribeiro (2020).

Item, %	Diet							
	Brown rice	Corn	Red sorghum			White sorghum		
Screen sieve size (mm)	1.0	1.0	0.8	1.0	1.6	0.8	1.0	1.6
Dry matter	93.9	94.5	94.1	93.5	93.2	93.7	93.6	93.5
Organic matter	90.6	91.2	90.9	90.5	90.8	91.5	91.4	90.9
Starch	26.5	28.6	27.4	26.7	26.2	29.9	29.4	27.5
Crude protein	35.7	35.9	36.2	35.3	35.1	34.8	35.8	36.0
Resistant starch	0.11	0.92	0.36	1.02	2.49	0.40	1.70	2.34
Starch gelatinization, %	78.7	81.4	86.7	76.7	70.4	85.8	77.2	72.6

Table 4.7 Significance Levels for the Four Significant Attributes Determined from Sensory Panel Data.

Attributes	Rep	Judge	Product	R*J	R*P	J*P	Effect of cat food
Brown	0.0001906 ***	0.1475789	< 2.2e-16 ***	0.7083701	< 2.2e-16 ***	0.8464389	Significant
Fibrous	1.627e-06 ***	6.693e-14 ***	6.868e-07 ***	0.000215 ***	0.051027	0.016815 *+	Significant
Fracturability (T/M)	0.3140284	0.0002932 ***	0.0099551 **	0.0302535 *	0.4589437	0.1906307	Significant
Gritty (T/M)	0.02842 *	< 2.2e-16 ***	5.913e-09 ***	3.040e-06 ***	0.08786	0.03214 *+	Significant

Table 4.8 Sensory Attribute Scoring Mean

Product	Brown	Fibrous	Fracturability (T/M)	Gritty (T/M)
Corn 1.0	6.1 b	1.2 cde	6.8 ab	6.1 b
Rice 1.0	6.0 bc	1.8 ab	6.8 ab	5.6 cd
RS 0.5	6.0 bc	1.3 cd	6.0 c	5.2 de
RS 1.0	6.1 b	1.5 bc	7.3 a	5.9 bc
RS 1.6	6.6 a	2.2 a	7.4 a	6.5 a
WS 0.5	5.4 d	0.9 de	6.3 bc	4.9 e
WS 1.0	5.2 e	0.8 e	6.9 ab	5.5 cd
WS 1.6	5.9 c	2.1 a	6.7 abc	6.3 ab

Chapter 5 - Conclusion

Results from this study have shown several points that are interesting and vital to further the understanding of how grain type and particle size effect process results, such as SME and STE transfer, starch gelatinization, expansion, cell structure, palatability and digestion This information may help manufacturers to change processing parameters to achieve a more narrow focus of target conditions that will help reduce obesity, improve digestive system health and improve the overall quality of life for cats.

The use of mass and energy balance principles allowed attributes that could not be measured, to be calculated. These calculation results can be used to verify processing data that then can be compared with measured data. The calculation of feed rate using mass balance compared to the calibration method showed that as particle size increased, the feed rate increased due to the larger particle size material having a lower surface area of the particles, but more area between the particles and thus, more free flowing. The method of calculating energy balance is important to confirm that all energy streams are accounted for and minimizing assumptions made will ensure good results. Particle size effects the process in various ways and must be consistent for a uniform product to be produced.

SME has long been an important calculated parameter in extrusion; it allows for the comparison between treatments and even between products. This study has confirmed that for dry expanded cat food not only the SME input is important as the driving force for expansion, but also having a matrix that has film forming or extensibility properties is also critical for product expansion. The SME and matrix extensibility had a strong relationship with grain type and particle size, and was explained using various physico-chemical analyses of the raw

materials. The use of red and white sorghum produced a quality kibble under similar processing and particle size conditions to those of corn and brown rice.

The results from processing are important in that everything should be focused on producing a food that will be consumed and be nutritious for the pet. Therefore, palatability and digestibility research work needs to be conducted to verify that the food will serve both purposes. Sensory results using human panelists identified four attributes that were significant: color (specifically brown), fibrous appearance, fracturability and grittiness. The visual attributes of color and fibrousness had very little correlation to physical attributes measured. They did point to color being a primary choice driver with color similarity primary to particle size. Relating the palatability data with sensory data, noted that cats preferred lighter color over darker color with WS being preferred and that when color was similar, they chose food with a larger particle size over the smaller particle size, 1.6 mm grind over the 0.5 mm grind.

As weight loss is an overreaching goal of this study (although not directly conducted, but inferred form the results), digestion is important metric to consider when comparing the treatments. As the particle size declined, the SME and the STE increased and the digestibility decreased, this is a positive finding from this work. This is due to the larger particle size leading to increased passage of starch to the colon (greater RS) and increasing colonic fermentation (lower pH) and thus acting as a prebiotic fiber in the digestive system, providing benefit to the animal (e.g., increased butyrate production).

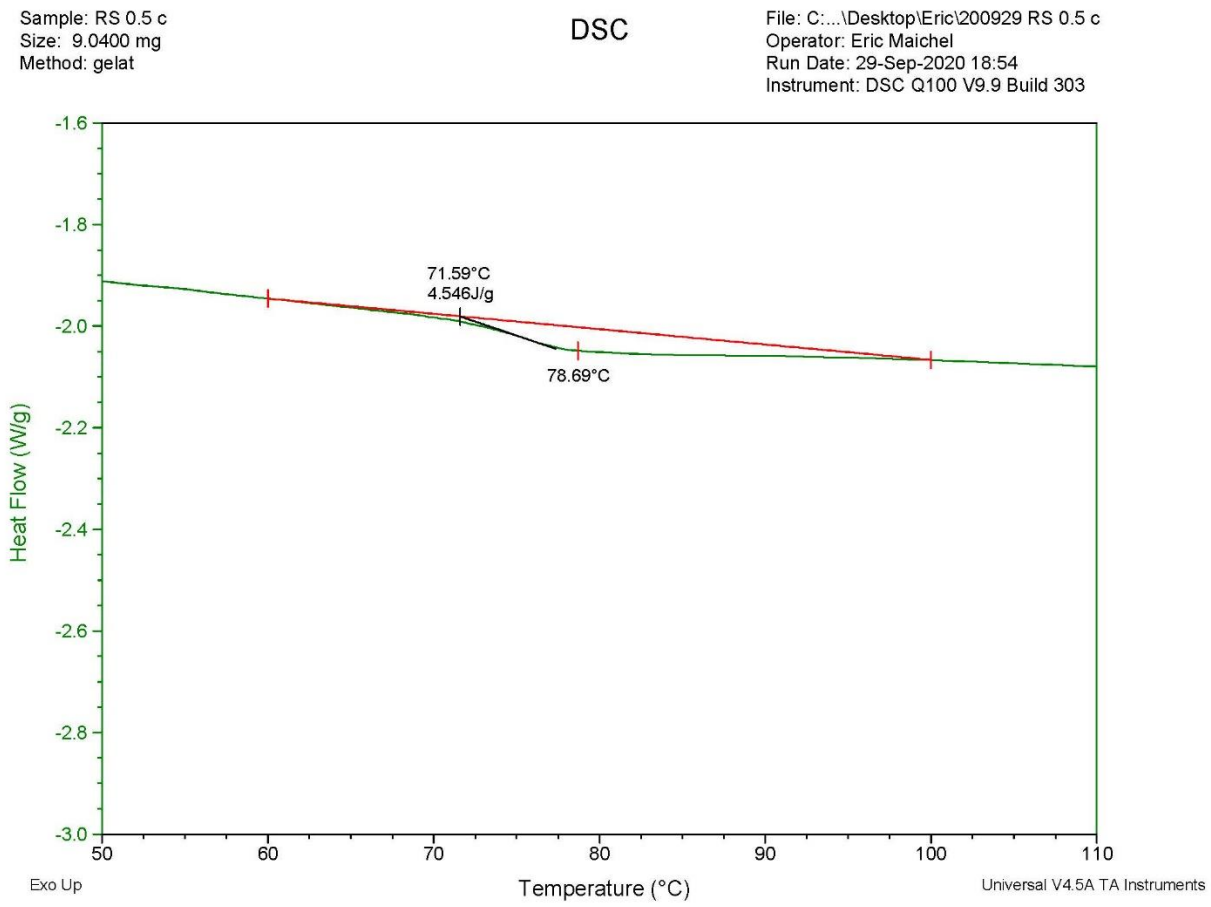
When looking at this work a whole, grain type and particle size can influence processing conditions and be used to change the palatability and digestion of established corn or brown rice diets by switching to grain sorghum. These findings indicate that there are benefits to optimizing the extrusion process instead of the current trend in manufacturing to maximize production.

Appendix A - Runsheet

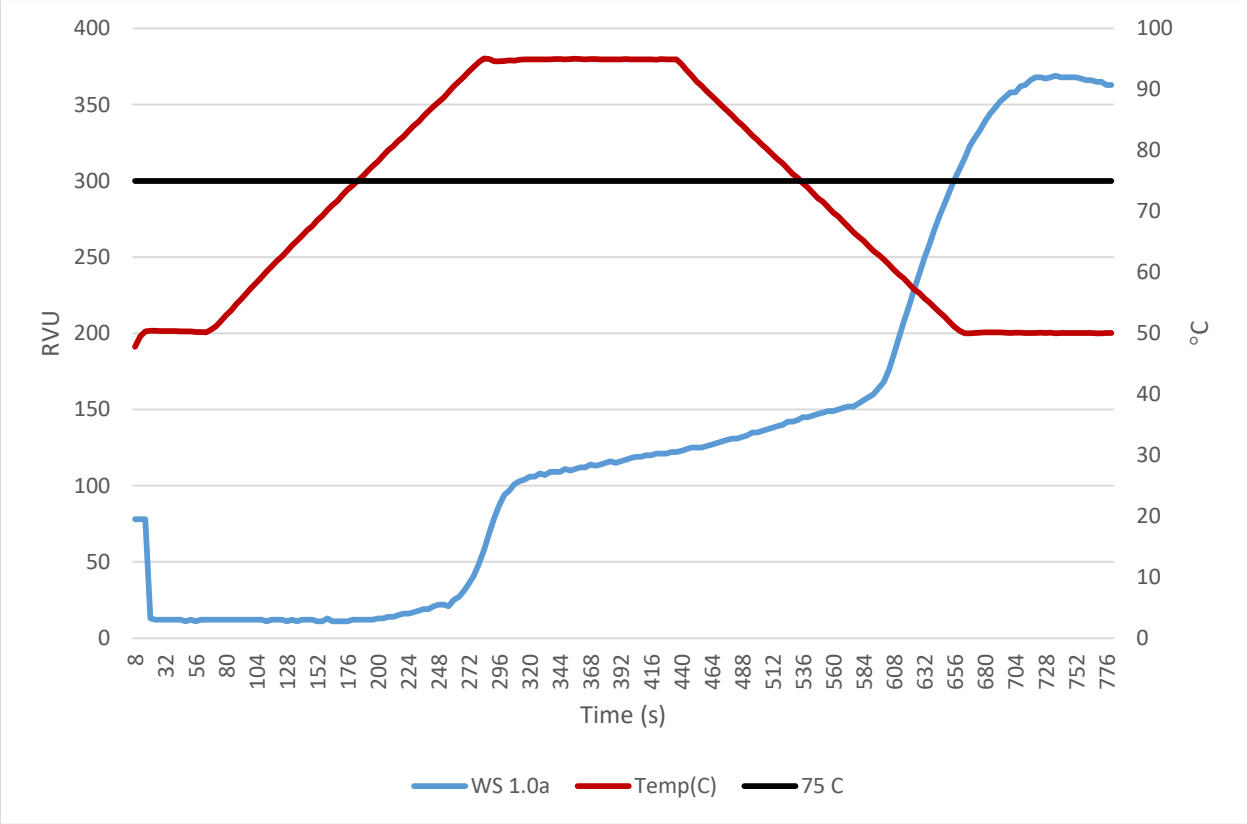
Run No.		901	902	903	904
Time Start		1600	1920	2100	1015
Time End		1900	2040	2230	1309
Raw Materials					
Density	g/L	566	519	574	559
Moisture	%	8.37	7.08	8.69	8.44
Feed Temperature	°C	26.4	25.7	26.1	26
Feed Rate	kg/hr	120	120	120	120
Feed Screw Speed	rpm / Hz	13	13	13	13
Preconditioning					
Cylinder Speed	rpm	400	400	400	400
Steam Flow	kg/hr	14.5	14.4	14.4	14.9
Water Flow	kg/hr	7.6	6.9	6.75	7.2
Water Temperature	°C				
Additive 1					
Additive 1 Rate	kg/hr				
Additive 1 Temperature	°C				
Additive 2					
Additive 2 Rate	kg/hr				
Additive 2 Temperature	°C				
Discharge Moisture	%	18.81	19.29	19.18	21.99
Discharge Temperature	°C	97.7	97	96.3	96.2
Extrusion					
Shaft Speed	rpm	413	414	414	413
Motor Load	%	56.3	55.3	49.8	57.8
Motor Power	kW				
Steam Flow	kg/hr				
Water Flow	kg/hr	8.5	8.4	8.6	8.3
Water Temperature	°C				
Additive 3					
Additive 3 Rate	kg/hr				
Additive 3 Temperature	°C				
Knife Speed	rpm	3601	3600	3600	3600
Zone 1 (Setpoint / Actual)	°C	50 / 50	50 / 50	50 / 48	50 / 50
Zone 2 (Setpoint / Actual)	°C	70 / 86	70 / 82	70 / 80	70 / 78
Zone 3 (Setpoint / Actual)	°C	90 / 89	90 / 93	90 / 94	90 / 87
Zone 4 (Setpoint / Actual)	°C	/	/	/	/
Zone 5 (Setpoint / Actual)	°C	/	/	/	/
Zone 6 (Setpoint / Actual)	°C	/	/	/	/
Die Temp	°C	129.4	129.9	120	143.7
Other Temp	°C				
Other Temp	°C				
Cone Head Pressure	psig	511	600	545	533
Other Data					
Bulk Density – OE	g/L	350	363	372	369
Moisture – OE	%	18.42	18.46	21.23	20.66
Bulk Density – OD	g/L	357	348	370	347
Moisture – OD	%	6.55	6.75	7.8	6.37
Dryer Formula	°F,T,T,T	215, 8, 8, 5	215, 8, 8, 5	215, 8, 8, 5	215, 8, 8, 5
Ambient Temperature	°F				
Relative Humidity	%				
Customer Formula		RS 1.0	RS 0.5	RS 1.6	WS 1.0
Run Rating					

Extruder Performance					
Run No.		905	906	908	909
Time Start		1400	1630	1930	2230
Time End		1531	1827	2130	23.55
Raw Materials					
Density	g/L	538	566	568	565
Moisture	%	6.99	8.01	8.16	8.11
Feed Temperature	°C	25.6	26	26	26
Feed Rate	kg/hr	120	120	120	120
Feed Screw Speed	rpm / Hz	13	13	13	13
Preconditioning					
Cylinder Speed	rpm	400	400	400	400
Steam Flow	kg/hr	14.9	14.8	15.1	18.1
Water Flow	kg/hr	7	6.8	7.1	4.6
Water Temperature	°C				
Additive 1					
Additive 1 Rate	kg/hr				
Additive 1 Temperature	°C				
Additive 2					
Additive 2 Rate	kg/hr				
Additive 2 Temperature	°C				
Discharge Moisture	%	18.16	19.14	21.11	20.86
Discharge Temperature	°C	95.3	94.3	94	92.8
Extrusion					
Shaft Speed	rpm	415	413	413	413
Motor Load	%	56.5	47.7	55	56.8
Motor Power	kW				
Steam Flow	kg/hr				
Water Flow	kg/hr	8.6	7.9	8.2	7.58
Water Temperature	°C				
Additive 3					
Additive 3 Rate	kg/hr				
Additive 3 Temperature	°C				
Knife Speed	rpm	3600	3600	3600	3600
Zone 1 (Setpoint / Actual)	°C	50 / 50	50 / 50	50 / 52	50 / 50
Zone 2 (Setpoint / Actual)	°C	70 / 78	70 / 70	70 / 106	70 / 97
Zone 3 (Setpoint / Actual)	°C	90 / 96	90 / 90	90 / 92	90 / 95
Zone 4 (Setpoint / Actual)	°C	/	/	/	/
Zone 5 (Setpoint / Actual)	°C	/	/	/	/
Zone 6 (Setpoint / Actual)	°C	/	/	/	/
Die Temp	°C	144.8	143.7	144	154.8
Other Temp	°C				
Other Temp	°C				
Cone Head Pressure	psig	515	520	568	532
Other Data					
Bulk Density – OE	g/L	366	368.5	396	357.6
Moisture – OE	%	19.49	19.35	20.64	17.49
Bulk Density – OD	g/L	363	362.7	403	346.5
Moisture – OD	%	5.89	6.68	7.93	6.95
Dryer Formula	°F,T,T,T	215, 8, 8, 5	215, 8, 8, 5	215, 8, 8, 5	215, 8, 8, 5
Ambient Temperature	°F				
Relative Humidity	%				
Customer Formula		WS 0.5	WS 1.6	Rice 1.0	Corn 1.0
Run Rating					
Extruder Performance					

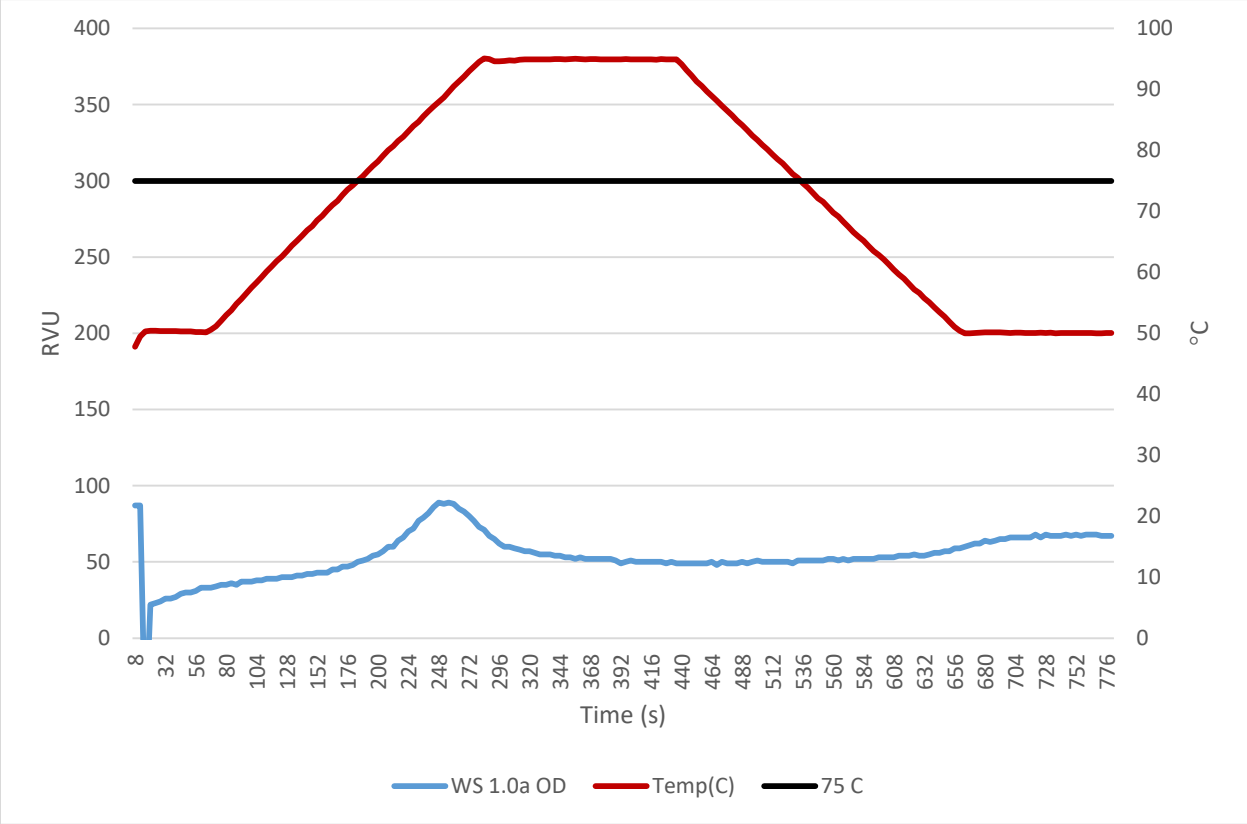
Appendix B - Representative DSC Thermogram (Raw)



Appendix C - Representative RVA Curve (Raw)



Appendix D - Representative RVA Curve (Extruded)



Appendix E - Data Compilation

Attribute	Units	White Sorghum			White Sorghum			Corn	Rice
		0.5 mm	1.0 mm	1.6 mm	0.5 mm	1.0 mm	1.6 mm	1.0 mm	1.0 mm
Feed Rate - Calibrated	kg/hr	120.38	132.22	144.06	120.38	132.22	144.06	132.22	132.22
Feed Rate - Calculated	kg/hr	128.54	114.64	125.50	111.49	117.26	125.00	127.44	119.38
Calculated Steam Loss in The PC	kg/hr	2.69	14.41	12.34	36.66	23.69	23.16	3.29	0.94
PC Wall Loss	kJ/kg	69.61	69.51	66.33	14.29	33.05	27.86	110.17	95.12
STE (PC)-NSA	kg/hr	244.96	295.60	297.33	232.55	228.69	197.47	373.08	254.34
STE (PC) - NEA	kg/hr	118.54	123.26	116.68	121.09	123.80	122.25	116.66	118.88
Specific Mechanical energy	kJ/kg	205.56	210.74	110.63	201.31	194.59	128.08	191.66	182.19
Specific Thermal Energy EX)	kJ/kg	-90.75	-72.48	1.60	-119.42	-134.11	-64.87	-52.60	-63.45
Extruder Barrel Steam Loss	kg/hr	9.65	11.05	8.00	8.30	10.45	6.89	14.97	10.87
Q Loss in Barrel	kJ/kg	38.83	32.36	-16.13	52.12	20.21	29.28	-42.32	7.62
T-die	°C	144.75	127.17	143.67	129.93	129.40	119.98	154.80	144.00
T-exit	°C	142.34	144.32	123.31	129.31	135.28	118.04	168.88	142.66
In-barrel Moisture	%	24.53	25.47	23.66	23.25	24.71	23.68	23.93	25.57
Phase Transition Analyzer	°C	74.70	69.30	94.80	62.30	101.40	59.80	84.60	60.00
STE:SME Ratio - Steam		1.31	1.29	2.27	1.10	1.22	1.72	1.58	1.59
STE:SME Ratio - Absorption		0.58	0.58	1.05	0.60	0.63	0.63	0.61	0.66
RVA Pasting Temperature	°C	79.53	79.40	79.90	78.25	78.30	81.45	76.60	81.47
DSC Onset Temperature	°C	73.01	73.00	74.75	71.68	72.47	74.09	66.70	78.28

Attribute	Units	White Sorghum			White Sorghum			Corn	Rice
		0.5 mm	1.0 mm	1.6 mm	0.5 mm	1.0 mm	1.6 mm	1.0 mm	1.0 mm
Bulk Density - Off Extruder	g/L	365.50	348.90	368.50	362.70	350.40	371.80	357.60	396.20
Bulk Density - Off Dryer	g/L	362.50	346.60	361.70	347.50	356.70	370.30	346.50	403.00
Piece Density	g/cm^3	0.00044	0.00057	0.00049	0.00046	0.00044	0.00063	0.00063	0.00050
Void Fraction		0.69	0.60	0.65	0.67	0.69	0.56	0.68	0.64
Sectional Expansion Index		3.32	3.63	2.96	3.18	3.40	2.69	3.67	3.08
Specific Length	mm/g	58.62	43.08	59.60	59.39	57.49	51.39	51.89	55.82
Tumble Test	PDI %	96.70	96.71	82.22	97.47	97.60	97.07	95.51	94.49
Pneumatic Test	PDI %	96.48	95.88	96.16	96.13	95.91	95.83	94.87	96.50
Texture Analysis	g force	1846.73	2033.73	2132.65	2156.62	1827.18	2444.58	2273.40	2266.51