# Supplementation of gluten-free sorghum flour-based dog treats with soluble animal proteins

by

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# Abstract

Pet treats are given to dogs to strengthen pet and owner ties and as a reward. Most treats available on the market are baked and based on wheat. Alternatively, sorghum is a gluten-free grain that provides antioxidants and has slow starch digestibility. Sorghum might be used to produce dog treats as an alternative for pet owners looking for healthy foods. However, because it lacks gluten, functional proteins to help with binding are required. The objective of this study was to determine the effect of adding soluble animal proteins to whole sorghum flours in lieu of whole wheat on the physical, nutritional, and preference of rotary molded baked dog treats. The experiment was conducted in triplicate as a 2x4+1 augmented factorial arrangement of treatments. Two whole sorghum flours (white [WWS] and red [WRS]), four protein sources (none [NC], spray-dried plasma [SDP], egg protein [EP], and gelatin [GL]), and a control with whole wheat flour [WWF-GTN] were evaluated. Higher crude protein and lower total starch (TS), total digestible starch (TDS), resistant starch (RS), peak viscosity (PV), total viscosity (TV), and setback viscosity (SBV) were found in WWF as compared to the WWS or WRS ($P<0.05$). A similar final dough temperature (24 -26°C) was achieved across treatments. The dough moisture, dough weight, and evaporation rate were influenced by the water-binding ability of the proteins and the water added. The WRS treatments were heavier ($P<0.05$) than the WWS. Due to differing water addition to achieve a machinable dough, the moisture fluctuated from 27.23% to 36.39%, wherein the NC treatments had the highest moisture, followed by GL, SDP, WWF-GTN, and EP ($P<0.05$). The NC treatments had the highest evaporation rates (17.35% and 16.31%), and the EP treatments the lowest (9.66% and 11.7%) for WWS and WRS, respectively ($P<0.05$). The dog biscuits had similar dry matter (>92.0%), Aw (<0.65), and caloric content (3.40-3.54 Kcal/g). However, the EP treatments had the highest crude protein (>17.8%) and the NC treatments the lowest (<10.2%;

$P<0.05$). The ash for the SDP treatments was higher (>2.9%; $P<0.05$) than all others. The values for rapidly digestible starch (RDS) and RS increased after baking. The SDP, EP, and GL treatments had comparable RDS values but lower than WWF-GTN or NC ($P<0.05$); nonetheless, no differences across treatments occurred for RS. Also, the TDS and TS declined due to dilution from the added protein sources with no protein or cereal ingredient main effect. The texture of the sorghum treatments was enhanced by the proteins added. The EP were the hardest treatments, followed by those with SDP and GL ($P<0.05$). The NC treatments were very brittle and were not comparable in dimensions or texture to the other treatments since they had to be sheeted and cut because they would not extract intact from the die roll. The thickness of large and small WWF-GTN biscuits was greater ($P<0.05$) compared to the sorghum treatments. The color of the biscuits was influenced by the proteins and cereals used, being lighter ($P<0.05$) for those with WWS, WWF, and GL or NC. The dogs did not exhibit a preference between WWF-GTN, WWS, or WRS treats when evaluated together. However, when evaluating WWS treats, the dogs preferred WWF-GTN, and those which included SDP and EP ($P<0.05$). The dogs had difficulty eating the EP treatments due to their hard texture. When assessed by a trained sensory panel variation across treatments for appearance and texture was high. The WRS and WWS biscuits with SDP or EP resulted in a darker appearance, while NC biscuits had more surface cracks. Initial crispness, hardness, and fracturability were greater for EP than all other protein-containing treatments. The WWF-GTN was in between the sorghum treatments regarding hardness. The sensory panel identified the predominant flavor and aftertaste as grainy. Volatile assessment for hexanal among all treatments was <1.0 mg/kg except for the EP treatments which ranged from 2.0 to 19.3 mg/kg over the duration of the evaluation (112 days at 30°C - 60% RH). This work indicated that WWS and WRS coupled with soluble animal proteins like GL or SDP could produce suitable baked treats

for dogs comparable to wheat. Additional refinement will be necessary to produce treats in a commercial setting.

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# Chapter 1 - Literature Review

## 1. Pet Food Industry and its History

The Pet Food industry saw its beginnings in the 1800s when humans began feeding their pets with processed goods instead of leftovers. Since then, it has evolved, and in the past decade experienced unprecedented growth. According to Nielsen Global Connect (2019), between 2007 and 2017, annual pet food expenditure per household increased by 36%. Furthermore, the global pet food market projection for 2025 is expected to reach US$ 113.08 billion (Grand View Research, 2019). Globally, the United States is the country that leads the world with 31% of the pet food production with a total of 517 pet food manufacturing facilities that generate more than 398,000 jobs in the U.S. as reported by IFEEDER (Semple, 2018). For 2020, according to the American Pet Products Association (APPA) estimation, the forecast for pet food and pet treats sales represents 4% growth from a previous 6% in 2019, which may reach US$ 38.4 billion (APPA, 2019).

Over time, pets have become a fundamental part of the family nucleus. The 2019-2020 APPA National Pet Owners Survey states that 67% of U.S. households (~85 million families) own a pet, from which 63.4 million households have dogs (APPA, 2019). People have included dogs in their daily life because of companionship and emotional wellbeing. Studies show that dogs are linked with a reduction in heart attack prevalence, blood pressure, cholesterol levels, anxiety, and depression of their owners (CDC, 2020; LaMotte, 2019).

Since 1860, the pet food industry has changed immensely. The production has advanced from biscuits for dogs made of wheat, vegetables, beetroot, and beef blood (Palika, 2009) to more functional diets. Today, this industry uses a wide range of over 500 ingredients, from major commodity crops to specialty fruits, vegetables, beef, poultry, seafood, and rendered products that guarantee complete nutrition for dogs and cats (IFEEDER, 2020).

An increase in pet ownership has corresponded with a growing tendency to treat pets as family members and encourage purchasing of safe, convenient, and sustainable premium pet food products marketed with special benefits, ingredients, or processing technologies. This has been emphasized by the quick access to information, generational changes, and greater purchasing power (Euromonitor, 2020; Kestenbaum, 2018) that makes consumers more aware of the ingredients in their own diet, as well as the food they buy for their pets, in many cases regardless of cost. According to APPA (2019), cat or dog owners annually spend between US$ 200-300 on pet food. Additionally, Packaged Facts (2017) reported that 92% of dog owners buy treats with some regularity, spending $76 annually on treats (APPA, 2019). Unprecedented events, such as COVID-19, have led to a shift in purchasing patterns. During the second and third week of March, all pet food categories spiked in sales due to stocking up; however, dry food sales declined by up to 15% below last years' performance after this period. Interestingly, pet treats continued to grow at 9% over the same period, according to the Nielsen Global Connect (Simpson, 2020). This behavior could result from pet owners indulging their pets as they spent more time with them or because people were adopting new pets.

## 2. Pet Treats and Snacks

Pet treats and snacks are products that are not intended to meet the complete nutritional needs of an animal; rather, they are mainly provided as a reward to indulge or train pets, and preferably, should not exceed 10% of the dog's daily energy requirements (PFMA, 2015). The treats category is an expanding segment in the pet market valued at US$ 5.4 billion in 2014, with sales expected to reach US$ 6.7 billion at retail within the U.S. in 2019 (Packaged Facts, 2015, 2019). Globally, treat sales are projected to reach US$ 31.37 billion by 2021 (Technavio Research, 2017). According to AAFCO (2012), these products must specify in their label “snack or treat.” Snacks usually refer to edible products with high-quality ingredients. Whereas treats are typically considered a tool for the reinforcement of positive behavior in pets, and this category encompasses edible and chewable products (Transparency Market Research, 2020b). Despite this distinction, the word “treat” is often used interchangeably. The treats and snacks classification is extensive; it includes crunchy, soft, freeze-dried and jerked meats, dental and edible bones, animal bones and hooves, rawhide, and pig ears (Stregowski, 2019).

In an attempt to better understand the pet owners’ purchase behavior, there have been several studies and surveys conducted. According to 2015 Packaged Facts data, among the whole category of treats, the most preferred are bone-shaped (47%), followed by kibble-shaped (39%), stick-shaped (29%), and wafer-shaped treats (28%), with some owners buying more than one option. Additionally, 77% of dog owners purchased crunchy and soft snacks, selecting those over dental chews, rawhides, or jerky treats. Similarly, dog treat purchasing of functional formulations was predominant when dental/oral care was addressed (36%), followed by calming or motion sickness (14%), joint health (12%), and skin coat (10%) (Sprinkle, 2015). In a different study conducted with 2,217 dog owners, it was reported that 32% gave their dogs just one treat; 52% from 2 to 5 treats; 10% from 6 to 10; 2% from 11 to 15; 2% more than 15 treats per day; and 2% did not provide an answer (Morelli et al., 2020). Currently, the treats and snacks preferred in the market are raw, natural, organic, U.S. sourced, with functional claims, limited ingredients, exotic proteins, and clean labels that resemble human foods (Sprinkle, 2019).

### *2.1 Crunchy treats*

Crunchy treats are baked products with a hard texture; they represent the oldest commercialized pet products and remain one of the most popular pet treat categories. One benefit of the crunchy treats is that their texture could help prevent plaque and calculus buildup on teeth. According to Samuelson & Cutter (1991), regular and tartar control biscuits slowed tartar accumulation for dogs eating canned food when the treats were offered daily when compared to a control group that was fed food alone. Industrially, these products are made by mixing grain flour with water to produce a dough. This dough can be shaped by rotary molding and stamping, by sheeting and cutting, by extruding and cutting, or on a smaller scale by manually filling into molds (Gallagher, 2008). Most pet food reports state that wheat flour is the most widely used ingredient in dog treats because its starch and gluten contribute to the appealing texture and flavor of biscuits (Case et al., 2011). According to Nielsen Data, the U.S. total ingredient quantities used in dog treats listed in descending order are wheat flour (120,515 tons), meat and bone meal (42,268 tons), wheat bran (42,162 tons), chicken (29,100 tons), and other animal by-products (21,857 tons). Water and sorghum were used in lesser proportions (13,113 tons and 466 tons, respectively; IFEEDER, 2020). In this context, sorghum represents a use of only 0.4% relative to wheat.

## 3. Rotary-molded Biscuits

The name “biscuit,” adopted by the human and pet food industries, derives from the Latin (*bis coctus*) meaning twice cooked. Biscuit generally refers to food made of wheat flour that is baked and dried in a slow oven. In the United States, these products are also known as “cookies or crackers” (Davidson, 2019c). In rotary molded biscuits, the dough is forced into molds on a rotating roll, extracted from the cavity after rolling a half-turn, and placed on a web for baking (Wiley-VCH, 2017). As in many other industries, the principles applied when producing short dough human biscuits can be transferable to dog treats; however, some process settings and formulation constituents need to be adjusted according to the targeted attributes.

Rotary-molded biscuits are produced from short doughs. Short doughs have a firm, crumbly consistency and have minimal gluten development. In other words, the dough is cohesive for molding and forming without excessive stickiness but has a short and cuttable texture (Arendt & Dal Bello, 2011). This is highly desirable because it provides higher density and toughness to the products. It also helps preserve the shape because it reduces the shrinkage after molding and during baking (Cauvain & Young, 2009a; Manley, 2011b). The water used in the mixing of the short doughs is reduced compared to bread production. This is mainly to minimize gluten development and ensure hard-eating qualities because of minimal evaporation during baking (Cauvain & Young, 2009a). Nonetheless, it improves the homogenization of all ingredients and controls the dough temperature (Hazelton et al., 2003; Lauterbach & Albrecht, 1994a). High amounts of fat and sugar can be added in treats intended for human consumption to provide lubrication, create soft textures, reduce gluten development, and promote clean die extraction (Gallagher, 2008; Manley, 2011b; Pallottini, 2013). However, lower quantities are added when producing pet treats to obtain harder products. Before molding, some bakers may allow the dough to stand for at least 30 minutes to reduce stickiness (Davidson, 2019a).

Besides the cereal source, which is the main ingredient, other components are included in pet treat production. Cornmeal provides a denser structure and prevents baked products from sticking (Bakerpedia, 2020). Nonfat dry milk generates browning of the product. It also contributes to texture, flavor, mouthfeel and prolongs shelf-life because of its water-binding capacity (All American Foods Inc., 2020; Van Boekel, 1998). Molasses is used as a humectant; it is a hygroscopic ingredient that slows down the tendency of product dryness; it also enhances the palatability, flavor, and color-forming because of Maillard reaction (Davidson, 2019b; Seguin, 2015). In addition, processing aid ingredients are used in smaller amounts. Sodium bicarbonate or baking soda is used as an aerating agent because when heated, it reacts with the acidic materials (molasses) in the dough and releases carbon dioxide and water (Lauterbach & Albrecht, 1994a). Sodium metabisulfite is used as a dough relaxer to reduce the shrinkage of the product after baking. It also acts as a reducing agent in the dough. For example, in wheat-containing formulas it breaks the disulfide bonds in the gluten matrix and creates a more extensible and less elastic dough (Cauvain & Young, 2009b; Davidson, 2019b). Inactive yeast is dead yeast, which has been pasteurized and sterilized. It lacks leavening and fermenting action but contributes as a dough conditioner by improving its extensibility and machineability (Lesaffre, 2015). Finally, salt is used as a flavor enhancer. It can also strengthen the dough, increase its resistance to extension, and suppress the growth of undesirable bacteria (Hazelton et al., 2003).

One of the bottlenecks which can be experienced in this process is piece extraction from the die. There can be two main causes. The dough may stick in the rotary die cavities, or the dough may form a “tail” in the extraction web due to a piece wedging in the cross-section. This condition can be improved by using low friction insert molds (i.e., bronze), coating the dies rolls (i.e., PTFE or ceramic), bigger diameter dies, and (or) a sticky extraction web (Pallottini, 2013). In contrast, if the release is too easy, the biscuits may fall apart or curl. This mainly occurs when the doughs are overly extensible or toughened (Manley, 1998). As shown in Figure 1.1, the principal control settings of a rotary molder are the gap between the forcing and the molding rolls, the position of the scraper, the pressure between the molding roll and the rubber roll, and the differential speed between the molding roll and the extraction web (Wright, 2020). It is also advisable to use finely ground ingredients since coarse or fibrous materials in the dough affect the performance of the scraper in the molder (Manley, 2011b).

Most literature found in short dough cookies or biscuits is related to human products; with very few studies published which focus on dog treats. Koya (2013) studied whole grain sorghum sheeted biscuits fortified with cowpea and compared those to commercially available wheat biscuits. She found that the protein level was comparable to wheat biscuits, whereas the mineral content was 37% higher, the phenolic content was 70% higher, and the pepsin-invitro digestibility was 76% lower than wheat biscuits. Also, Badi & Hoseney (1976) studied the physical characteristics of sweet cookies made from 100% sorghum flour. The authors determined that cookie appearance and texture were not desirable since they were denser and more compact but fragile, with a mealy and gritty appearance. They were able to reduce the grittiness of the products by increasing the pH through the substitution of sodium carbonate for sodium bicarbonate. However, they could not reduce the fragility of the cookies; therefore, they included wheat flour in the recipes. Similarly, Rai et al. (2014) reported that the peak force required to puncture pearl millet: sorghum cookies was lower than that needed for wheat cookies, which meant less hardness. Moreover, Adebowale et al. (2012) studied sheeted biscuits composed of wheat flour with 5-20% of sorghum flour. These authors did not report differences in weight, thickness, width, spread ratio, or spread factor among samples.

Regarding the development of pet treats, González-Forte et al. (2014) produced sheeted dog biscuits with wheat flour or whole wheat flour with added soy. The authors included *L. plantarum* as a probiotic and evaluated the survival by in-vitro digestion when calcium alginate or starch glycerol were added as a coating. The formulation with whole wheat flour and soy had a better protective effect without coating. In addition, the physical attributes, color, and texture were considered desirable. Scaglione & Gellman (1986) patented a low-calorie rotary-molded baked treat using a blend of wheat flour with 15-30% of vegetable hulls and fish meal. They determined that a high fiber containing biscuit had a hard and brittle texture with a flat appearance; however, they concluded that rice hulls were less disruptive to the dough because of their low bulk density and swelling properties versus cellulose. Moreover, Kelly & Kelly (2001) prosecuted an invention of hard or semi-hard sheeted and extruded biscuits to control the malodorous breath in dogs. They included 35% whole wheat flour, 17% oat bran, and 8% brown rice flour as the preferable cereals. Their patent advised that other farinaceous ingredients might only be included in small proportions to not impair the flavor, texture, and effectiveness of the dog biscuits. It was concluded that the biscuits had a desirable texture for chewing by dogs and were able to reduce or completely neutralize the malodorous breath on 140 dogs fed a treat after each meal for three months.

As demonstrated in different studies, although most biscuits are made of wheat, some experiments have been conducted to evaluate other cereals, including sorghum, and comparisons to a wheat standard as the control. In short dough biscuits, the gluten network is not completely developed; instead, it is slightly formed to provide texture and cohesion for handling and shaping. Thus, substitutions in the cereal source might be thought of as desirable from a product development standpoint.

## 4. Sorghum

Sorghum is the fifth most-produced cereal in the world. Despite its origin which traces back to Northeastern Africa, the largest producer is the United States, where it is grown in the “Sorghum Belt” that extends from South Dakota, Colorado, Kansas, Oklahoma, to Texas (FAO & ICRISAT, 1996). In the U.S., Kansas is the leading state accounting for close to 60% of the total sorghum harvested in 2019 (Shahbandeh, 2020). In Africa and India, nearly 40% of sorghum production has been destined for human consumption; while elsewhere, it is primarily used for animal feed (roughage and grain) (Culliney, 2013) where it may be preferred over other cereals such as corn due to its lower cost (Ratnavathi & Komala, 2016). Comparably, in the U.S., a great deal of the sorghum is exported, used in animal feed or human food, and recently it has been increasingly redirected to the biofuels market (National Sorghum Producers, 2020). At present, sorghum is also being explored for its use in other markets such as pet foods, building materials, fencing materials, and even floral arrangements (Sorghum Checkoff, 2018).

Sorghum is a crop of great interest because it is resilient, sustainable, and tolerant to high environmental temperatures and droughts (Arendt & Zannini, 2013). It is also a rich source of dietary fiber, resistant starch, and B vitamins such as thiamine, riboflavin, vitamin B6, biotin, and niacin (Anglani, 1998; Ratnavathi & Komala, 2016). Sorghum, especially whole grains, is an important nutraceutical source due to its relatively high concentration of antioxidant phenolic compounds (Arendt & Zannini, 2013). These phenolic compounds are able to react with free radicals that otherwise would attack the DNA, lipids, and proteins (Slavin, 2004). Because of this, they have been recognized as immunomodulating, anti-inflammatory, cardioprotective, anticarcinogenic, and vasodilating components (Yahfoufi et al., 2018). Additionally, sorghum possesses slow starch digestibility that produces satiety and delays glucose uptake making it a functional food for the diabetic and obese population (Ratnavathi, 2019). Furthermore, it has been valued for its natural gluten-free properties that make it a safe source for consumers with celiac disease, gluten intolerance, or gluten sensitivity (Culliney, 2013).

There have been some concerns regarding sorghum and its bitter flavor, lower digestibility and feed efficiency for humans and animals, which are mainly attributed to its high condensed tannin levels compared to other major cereal crops (Xiong et al., 2019). The total sorghum condensed tannin concentration varies depending on the genetic background, and generally, it is present in higher proportions in darker varieties, serving as crop defense against bird predation or bacterial and fungal attack (Dykes et al., 2005; Watson, 2018). In the U.S., sorghum production as a feedstock has been almost exclusively restricted to non-tannin varieties obtained by artificial selection at breeding (Wu et al., 2012). However, high lysine traits are of great interest in improved sorghum varieties (Tuinstra, 2008).

In the pet food industry, sorghum has been increasingly utilized since this cereal provides beneficial health attributes, does not contain genetically modified organisms (GMO) or gluten, and is competitively priced. Additionally, pet owners are more conscious of sustainable and value-added ingredients within the products they purchase. There is a general belief that gluten can be harmful, so many people try to avoid it, including in pet diets. Unlike people, celiac problems are not common in dogs, except for a few cases of gluten sensitivity reported on Irish Setters (Hall & Batt, 1992). Nonetheless, the increasing demand for this type of products has created a new market niche associated with health benefits (Transparency Market Research, 2020a). According to a survey conducted by Packaged Facts, 30% of dog owners purchase functional pet treats to address various of health concerns or conditions (Sprinkle, 2019).

In 2016, sorghum was used by 15 pet food companies that manufactured more than 130 complete canine and feline diets (Sorghum Checkoff, 2016). However, despite the existence of a wide variety of commercial pet products formulated with sorghum, the total market share in the U.S. is still low. According to American Sorghum (2015), the pet food industry only accounts for around 2% of sorghum consumption. This would suggest that an understanding of how this grain can be used and research regarding physical, nutritional, and quality product and process attributes is essential to create awareness of the benefits and implications for pets and pet owners when introducing sorghum into the diets and treats. Di Donfranceso et al. (2018) researched the acceptability of dry sorghum dog food. They found no differences in animal or pet owners' acceptance between sorghum samples and commercially available dry dog food manufactured with wheat, rice, and maize. Also, Alvarenga et al. (2018) found that extruded pet foods with sorghum were nutritionally comparable to corn, rice, or wheat. Further, they concluded that mill-feed from sorghum could add even more fiber and phenolic antioxidants to the food.

Similarly, Alavi et al. (2018) found that adding white or red sorghum to extruded cat diets improved the palatability compared to corn or rice; however, no differences existed in flavor or aroma attributes according to a trained sensory panel. The authors also found that the diet intake based on coarsely ground sorghum reduced fecal pH, which was attributed to fermentation via prebiotic activity. Furthermore, Teixeira et al. (2019) studied the effects on digestibility and postprandial glycemia on adult dogs when rice was partially substituted by sorghum containing condensed tannins or when added as hydrolysable tannins. They reported no adverse effects on consumption attributed to condensed tannins or changes in the postprandial blood glucose but found decreased protein digestibility and metabolic energy from the diets with higher sorghum inclusion. Contrary to this, Alavi et al. (2018) demonstrated through in-vivo studies with dogs that diets formulated with white or red sorghum were nutritionally adequate for adult dogs, without negatively affecting nutrient digestibility or causing gastrointestinal intolerance.

Regarding sorghum use in pet treats, Pezzali et al. (2019) developed sorghum baked crisps bars for dogs with good acceptability. They used five binders (corn syrup, spray-dried plasma, gelatin, albumin, and egg product) to provide cohesion of the particulates. The authors concluded that the use of binders and the replacement of rice crisps with sorghum crisps did not alter the dog's preference. Also, Markham & Kieth (2004) created an extruded treat base made of decorticated and defatted sorghum grain. The sorghum base had either a light and puffy consistency or a dense crunchy nugget consistency depending on the extrusion temperature and pressure. The authors suggested molding this base into a treat immediately or cure it (>60% air moisture at room temperature) until it reached stability. The cured base was pulverized and combined with water, plasticizers (tapioca, gluten, gelatin, starch), glycerin, and other ingredients to create a dough that was further pushed through dies and cut into desirable shaped treats ready to bake. According to the authors, this combination controlled the hardness and texture of the products.

It has clearly been demonstrated that sorghum is readily available, versatile, and nutritional, making it worthy of interest as an alternative for healthy food trends in human and pet diets. However, because it is a gluten-free grain, its dough and physical product attributes are at a disadvantage to grains like wheat which have gluten, especially when making baked goods. Equally, Scaglione & Gellman (1986) found biscuit forming issues when gluten-containing flours were removed and gluten-free ingredients used as a replacement were not as effective for dough development or strength. Furthermore, Gallagher (2008) demonstrated that starch source substitution needs to be complemented with different protein fractions when developing gluten-free biscuits.

Proteinaceous ingredients have been added to multiple gluten-free products to improve the cohesion and binding properties of doughs. Therefore, one might suggest as a functional alternative to be incorporated into sorghum-based biscuit formulations to enhance the processing and final product features.

## 5. Soluble Animal Proteins

Vegetable and animal proteins have been extensively used as ingredients that provide dough enhancement, amino acid enrichment, and generate satiety effects (Nogueira & Steel, 2018). In the literature, there exist numerous human gluten-free studies conducted with added proteins. For example, Crockett et al. (2011) added soy protein isolate and egg white solids in gluten-free bread. They found that dough stability increased with higher levels of soy protein and egg white solids. Also, Rodriguez et al. (2015) included bovine plasma in gluten-free bread and reported that textural properties were improved by having homogenous and smaller air cells. Similarly, Han et al. (2019) used egg white in a gluten-free batter and concluded that egg white increased the dough's elasticity and improved the physical qualities of bread. Although dogs are part of the order *Carnivora*, they are considered omnivorous based on their nutrient metabolism and could utilize either vegetable or animal protein ingredients. Nonetheless, animal-proteins can be thought of as more palatable sources as they have better olfactory properties (Beaver et al., 1992; Brown, 2009; Houpt et al., 1978). A study conducted by Callon et al. (2017) measured and contrasted the rate of consumption, hesitation, and level of interest before and after consuming a vegetable or animal-based protein diet with dogs. The authors found a higher interest in dogs eating the animal-based diets after consumption; however, there were no differences in other variables. Based on previous evidence, pet treats would benefit from animal-based proteins as binders rather than vegetable sources. Animal sources from rendered meals do not have any functionality for binding; however, soluble animal proteins like spray-dried plasma, egg whites, and gelatin might provide some support to the treats as they contain high amounts of albumin, ovalbumin, and collagen, respectively (Jayathilakan et al., 2012). The use of these proteins can add nutritional value, enhance the physical product properties, and support a new market alternative for companies supplying them.

### *5.1 Spray-dried plasma*

Porcine, ovine, or bovine spray-dried plasma is produced from the blood of healthy animals approved for slaughter for human consumption. The plasma is obtained by centrifugation of whole blood and further concentrated by membranes. It is then dehydrated using spray-drying technology that retains the functional, physicochemical, and biological properties of the product (Gatnau, 1990). Spray-dried plasma (SDP) consists of protein, minerals, and water; wherein, 95% of its protein is albumins and globulins (Torrallardona, 2009). It has a high protein content and in-vitro digestibility of 70-80% and 99%, respectively (Balan et al., 2020; Bureau et al., 1999). Among its benefits, it has good water binding ability, gelling and emulsifying properties, boosts palatability, and is a good source of antibodies, immunoglobulins, and amino acids (Pérez-Bosque et al., 2016; Polo et al., 2005; Rodríguez et al., 2016).

Use of SDP in the feed industry extends to swine feed, pet food, aquafeed, ruminant, and poultry feed (Research and Markets, 2019). In the pet food industry, it has been primarily explored in wet foods. Spray dried plasma is well known for improving the texture and maintaining a high degree of cohesion between the ingredients when cooked; it also provides juiciness to the restructured meat chunks (Polo et al., 2005). Moreover, it has also been studied in dry pet food. Andrade et al. (2019) evaluated food palatability, digestibility, and blood parameters in dogs fed diets with increasing SDP levels. They concluded that SDP increased total dry matter and crude protein digestibility; at 12%, it increased total circulating leukocytes, total plasma protein, and albumin levels; however, levels above 4% decreased the palatability of the food. The literature on dog treats with SDP is very limited. Pezzali et al. (2019) developed a ranking test and determined that granola bars made with white and red sorghum crisps were well accepted by dogs; SDP was preferred over egg protein or gelatin. The authors attributed SDP preference due to a more intense aroma when compared to the other proteins.

### *5.2 Egg protein*

Egg protein is produced by drying liquid egg whites that have been previously reduced lysozyme, avidin, and glucose concentration/activity (E-CFR, 2020). Egg protein powder can have a protein of 90% based on a dry matter basis. In addition, when it is concentrated by ultrafiltration, a partial demineralization (30-50%) can be achieved. Therefore, it generally has a very high protein to mineral ratio (Lechevalier et al., 2013). It is a pasteurized and shelf-stable ingredient with a high gel strength, foaming, emulsification, and water absorption capacity (Hoppe, 2010), with an excellent amino acid bioavailability (Réhault-Godbert et al., 2019). When egg proteins solidify, they act as an adhesive, connecting ingredients or food components to each other (Alleoni, 2006).

Eggshells, whites, yolks, or whole eggs have been used in the pet food industry for many years, particularly in dog diets. According to Turk (2017), more than 25% of dry recipes and less than 5% of wet recipes contain dried eggs. In the pet treat market, there have been a few experiments reported. Spiel et al. (1987; 1989) patented a rotary molded baked simulated egg treat with a hard texture to clean pets’ teeth and gums. For the yolk portion, they used 30-40% water, 15-25% flour, 15-20% real egg yolk solids as heat coagulable proteins, 4-8% plant or animal meal, and 12-18% sugar. Similarly, the outside contained 40-50% flour, 30-40% water, 10-15% egg solids, and 10-20% meat meal, fish meal or oatmeal, along with flavorings, coloring agents, vitamins, and minerals. According to the authors, the product was baked with low heat to promote adequate and even bonding, which improved the quality of the final product. Moreover, they suggested that modified starches or vegetable gums could replace the egg. In a similar fashion, Chanioti (2019) added whole eggs and rapeseed oil to a dough made of 50% wet spent grain (75% moist) and 50% rice flour and noted a significant enhancement of dough connectivity and cohesiveness with less porosity. The dough quality obtained was adequate for molding and baking dog biscuits. In a separate trial, the authors evaluated egg powder with different combinations of flours (potato, rice, wheat, and corn flours), rapeseed oil, water, and wet spent grain, which attained acceptable color and texture. However, when an animal study was conducted, the dogs took longer to eat the products as compared to commercial brands.

### *5.3 Gelatin*

Gelatin is a soluble protein produced from the partial acid or alkaline hydrolysis of collagen, and is a fibrous protein element found in skin, cartilage, and bone. Its functional properties depend on the source, age of the animal, and type of collagen (Johnston-Banks, 1990). Gelatin has a high protein level (85%) and a pepsin digestibility close to 100%. In the feed industry, gelatin has been used as a natural binder that increases the pellet durability and stability at low dosages and represents a cost-effective ingredient with a high nutritional value (Manbeck et al., 2017). Moreover, it does not lose its functionality when heated and cooled several times in a pet treat application (Mathe & Aldrich, 2016).

In the pet food industry, there have been some attempts to process treats and high-meat extruded kibbles with gelatin, as it binds the edible components together, so they are easier to handle (Manbeck et al., 2017). Spanier (1991) patented a chewy, semi-plastic, microbiologically stable dog biscuit. It contained 12-30% of gelatin, acidulant, cereal starch, a release and taste agent, sugar, salt, and water. The dog treat had a compact form (not brittle or gummy) and long-lasting properties. The author indicated that gelatin acted as a textural and a water-soluble, binding-gelling agent. It was also mentioned that gelatin could be partially replaced with gluten without loss of chewiness, but the gelatin level should not be below 12% when gluten is used. Moreover, Seguin (2015) proposed a method to produce high meat rotary molded pet treats with raw meat (70-80% moisture) mixed with an absorbent fiber to bind the water, a minor amount of gelatin, hydrocolloid, humectants, and flavorings. These grain-free treats had an appearance similar to jerky after being baked and dried. Furthermore, Mathe & Aldrich (2016) patented a gummy treat that did not need to be cooked or baked to form the final product. It was made of >15% gelatin, a carbohydrate material such as tapioca, modified potato starch, or molasses; and an aqueous phase mainly comprises hot water. The treat also contained flavorings, glycerin, and preservatives. The mixture was left on molds until it hardened at room temperature.

Throughout the published literature and patent records, multiple reports and studies are using soluble animal proteins as binders in the development of new pet food products. However, scarce information is available regarding rotary molded baked whole sorghum dog biscuits or those using spray-dried plasma, egg whites, or gelatin. To fill this gap in our knowledge and take advantage of sorghum properties in pet foods, work should be completed to gain a better understanding. Thus, the main objective of the following research was to create and manufacture appropriate formulations with sorghum as an alternative grain to wheat and to substitute the gluten with soluble animal proteins with similar protein content (>80%). A secondary objective was to characterize the raw flours and compare the physical, nutritional, and sensorial attributes and the shelf-life of whole wheat treat to those formulated with whole sorghum (red and white), in which animal proteins were added. As validated by many researchers, the inclusion of whole flours is beneficial for the animal’s health due to the high integration of fiber and phenolic compounds. It is expected that this work will further cement the benefit and utility of sorghum and provide an innovative approach for the production of a nutritive gluten-free baked pet treat that will satisfy the growth of humanization in the pet treat category.

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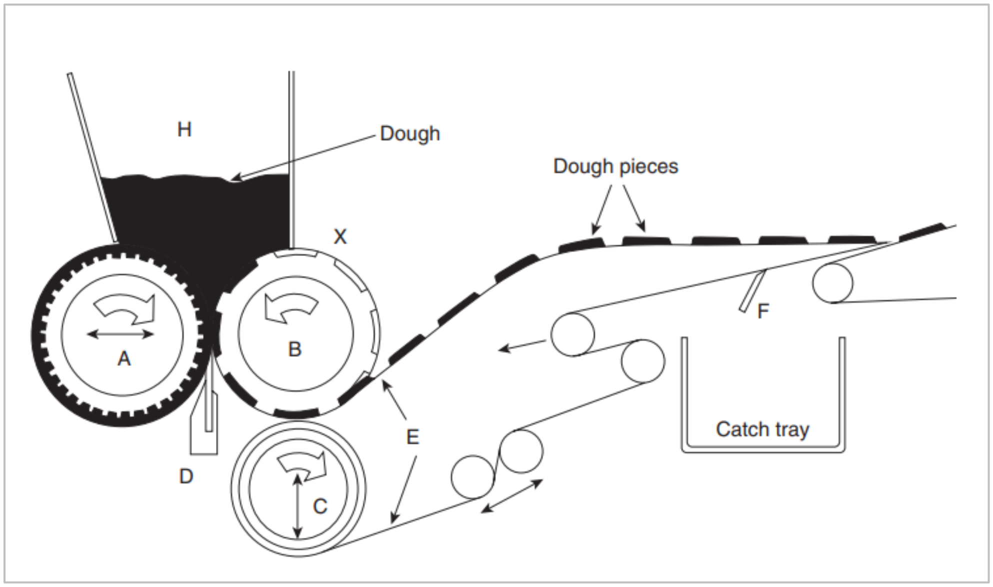

Figure 1.1 Rotary molder diagram.
Source: Subramaniam (2018). A) forcing roller (forces the dough into molder cavities). B) molder. C) rubber roller (extraction roller). D) knife. E) extraction web. F) knife (scrap the dough sticking to the web). H) dough hopper.

# Chapter 2 - Characterization of wheat, white sorghum and red sorghum flours for process optimization of sorghum-based dog treats (biscuits) supplemented with soluble animal proteins

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## Abstract

Pet treats are given to dogs to strengthen pet and owner ties and as a reward. Most baked treats are based on wheat. Alternatively, sorghum is a grain that provides antioxidants and has slow starch digestibility. However, because it lacks gluten it has processability disadvantages. The objective of this study was to determine the effect of adding soluble animal proteins to whole white sorghum (WWS) and whole red sorghum (WRS) flours on the production of rotary molded baked treats. Treats produced in the same manner with whole wheat flour (WWF) served as the control. Proximate analysis, quantification of the starch fractions, and a pasting profile analysis was performed on the flours. The biscuits were produced in triplicate using a 2x4+1 augmented factorial arrangement of treatments. With the main effects of whole sorghum flours (WWS and WRS), four types of soluble animal protein sources (none [NC], spray-dried plasma [SDP], egg protein [EP], and gelatin [GL]), and the control from wheat [WWF-GTN] were evaluated. Higher protein content and lower TS, TDS, and RS were found in WWF ($P<0.05$). The RVA of the flours foreshadowed lower biscuit quality with WWS or WRS because of higher PV, TV, FV, and SBV ($P<0.05$) compared to WWF. Similar final dough temperature was achieved. The dough moisture, dough weight, and evaporation rate were influenced by the water-binding ability of the proteins and the water added. The WRS treatments were heavier than the WWS ($P<0.05$). As a function of differing water addition rates, the dough moisture fluctuated from 27.23% to 36.39%, wherein the NC treatments had the highest moisture, followed by GL, SDP, WWF-GTN, and EP ($P<0.05$). The NC treatments had the highest evaporation rates (17.35% and 16.31%), whereas the EP treatments had the lowest (9.66% and 11.7%) for WWS and WRS, respectively. This work indicated that WWS and WRS, along with soluble animal proteins like SDP, GL, or EP provide enhancement to processing by rotary molding when adequate amounts of protein sources and water were added.

## Abbreviations

RDS, rapidly digestible starch; SDS, slowly digestible starch; TDS, total digestible starch; RS, resistant starch; TS, total starch; PV, peak viscosity; TV, trough viscosity; BDV, breakdown viscosity; FV, final viscosity; SBV, setback viscosity; PkT, peak time; PT, pasting temperature; WWF, whole wheat flour; WWS, whole white sorghum; WRS, whole red sorghum; GTN, gluten; NC, no protein; SDP, spray-dried plasma; EP, egg protein; GL, gelatin

***Keywords*****:** baking, gluten-free, rotary molder, sorghum, starch, viscogram

## 1. Introduction

The pet food industry has grown substantially with more pets becoming a fundamental part of the family nucleus. According to APPA (2019), close to 85 million families own a pet, of which 63.4 million have dogs. Currently, pet owners spend more each year to pamper their animals. Therefore, it is more common to find people providing treats to their dogs. Treats are commonly used to indulge or train pets, and according to Packaged Facts (2017) 92% of dog owners buy treats with some regularity, with global sales projected to reach US$ 31.37 billion by 2021 (Technavio Research, 2017).

A humanization trend led by treating animals as another member of the family has shifted the demand pattern and altered the supply chain. Currently, the pet food industry uses more than 500 ingredients in an attempt to satisfy different product formats, consumer preferences, processing constraints, and functional formulations (such as dental/oral care, calming or motion sickness, and joint health or digestive health/probiotics) (IFEEDER, 2020; Sprinkle, 2015, 2019).

There are multiple treat formats on the market; however, 77% of dog owners purchased crunchy and soft snacks, selecting those over dental chews, rawhides, or jerky treats (Sprinkle, 2015). The crunchy treats, known as biscuits, are formulated with wheat as the main ingredient because its gluten contributes to an appealing texture and flavor (Case et al., 2011). The gluten network forms an elastic web that gives the dough strength and allows it to be machined (Davidson, 2019c). However, in short dough biscuits the gluten network is slightly developed to provide texture and cohesion of the dough for handling and shaping in the rotary molder. Nonetheless, some gluten-free alternatives such as sorghum are also available but not often used. According to Nielsen Data, the U.S. total wheat flour used in dog treats is 120,515 tons versus only 466 tons of sorghum (IFEEDER, 2020).

Sorghum is the fifth most-produced cereal globally (FAO & ICRISAT, 1996), and in the U.S., Kansas is the leading state accounting for close to 60% of the production in 2019 (Shahbandeh, 2020). Sorghum is a rich source of dietary fiber, resistant starch, and B vitamins (Anglani, 1998; Ratnavathi & Komala, 2016). Additionally, it has slow starch digestibility and possesses high antioxidant phenolic compounds (Arendt & Zannini, 2013; Ratnavathi, 2019).

Despite the favorable nutritional profile of sorghum, its natural gluten-free properties create a processability disadvantage, especially in baked products. Nonetheless, the increasing demand for more foods similar to those consumed by people has created a new market niche associated with health benefits (Transparency Market Research, 2020a). Some research has been conducted that include proteins to emulate gluten properties and to provide dough enhancement, amino acid enrichment, and generate satiety effects (Nogueira & Steel, 2018). Nonetheless, most of this work has been studied in human products with very few applied to pet treats. A few proteins such as spray-dried plasma (Pezzali et al., 2019), egg-protein (Chanioti, 2019; Pezzali et al., 2019; Spiel et al., 1987; Spiel et al., 1989), and gelatin (Pezzali et al., 2019; Seguin, 2015; Spanier, 1991) have been included as binders with good results in treats.

To our knowledge, there is no information available regarding rotary molded baked sorghum dog biscuits or those using spray-dried plasma, egg protein, or gelatin. Thus, the objectives of this study were to characterize whole wheat and whole sorghum raw flours and to determine the effect of adding soluble animal proteins to whole white sorghum and whole red sorghum flours on the production of rotary molded baked dog treats.

## 2. Material and Methods

### *2.1 Materials*

Experimental ingredients included whole wheat flour <180 µm (Ultragrain Hard, Ardent Mills, Denver, CO); whole white and red sorghum flours <150 µm (White Whole Grain and Burgundy Whole Grain, Nu Life, Scott City, KS); spray-dried plasma (Innomax Porcine Plasma, Sonac, Maquoketa, IA); egg protein (OvaBind®, Isonova, Spencer, IA); gelatin (Pro-Bind Plus 50, Sonac, The Netherlands); cornmeal (Enriched Corn Meal Yellow, Sysco); salt (Iodized Salt, Morton Salt Inc., Chicago, IL); molasses (Rich Brown Hue [40% - #715 and 60% - #677], International Molasses Corporation, Ltd., Saddle Brook, NJ); baking soda (Pure Baking Soda, Arm & Hammer, Princeton, NJ); nonfat dry milk (Nonfat Dry Milk Classic, Sysco, Houston, TX); sodium bisulfite (Sodium Metabisulphite, LD Carlson Company, Kent, OH); inactive dry yeast (Nutritional Yeast, Bob's Red Mill Natural Foods, Milwaukie, OR); and all-purpose shortening (Premium All-Purpose Shortening, Ventura Foods, Brea, CA) (Table 2.1).

### *2.2 Statistical Analysis*

The experiment was conducted as a 4x2+1 factorial arrangement of treatments in which four protein sources (Innomax Porcine Plasma, OvaBind®, Pro-Bind Plus 50, and "none" used as a negative control), two different sorghum flours (white whole grain sorghum flour and red whole grain sorghum flour), and a positive control formulated with whole wheat flour.

The data processing, analysis of variance, and least-squares means separation was performed using the GLM procedure of the statistical analysis software (SAS 9.4 Inst. Inc., Cary, NC). Tukey's HSD (Honest Significance Difference) test was applied for the least-squares means separation and considered significant at a probability $P<0.05$. For the production variables only, three different models were used to evaluate the augmented arrangement of treatments according to Marini (2003). Wherein a one-way ANOVA comparing the nine treatments, a 2-way ANOVA to test the main effects, and a one-way ANOVA with single-degree-of-freedom contrasts to compare different groups of treatments as well as interactions of interest with a probability $P<0.05$.

## *2.3 Flour Quality*

### *2.3.1 Proximate Analysis*

Whole wheat, whole white sorghum, and whole red sorghum flours were evaluated for moisture (AOAC Method, 930.15), crude protein (AOAC Method, 990.03), crude fat by acid hydrolysis (AOAC Method, 2003.05), crude fiber (AOCS Ba 6a-05), and ash (AOAC Method, 942.05) in a commercial laboratory (Midwest Laboratories, Omaha, NE).

### *2.3.2 Total, Digestible and Resistant Starch Analysis*

The various fractions of starch were evaluated by duplicate using digestible and resistant starch assay procedures (K-DSTRS 02/19; Megazyme International Ltd, Wicklow, Ireland). Briefly, 1 g of flour was incubated with 1 mL of ethanol 95%, 35 mL of maleate buffer, and 5 mL of pancreatic α-amylase (PAA) + amyloglucosidase (AMG) solution under shaking in a water bath at 37°C for 20 minutes (Rapidly Digestible Starch- RDS), 120 minutes (Slowly Digestible Starch- SDS), and 240 minutes (Total Digestible Starch- TDS and Resistant Starch- RS).

At each time point, 1 mL of the suspended solution was removed and combined with 20 mL of 50 mM acetic acid solution and centrifuged for 10 minutes at 1500 × g. By duplicate, 0.1 mL of the supernatant was transferred to a glass tube with 3 mL GOPOD reagent. The tubes were incubated at 50°C for 20 minutes. The RDS, SDS, and TDS were calculated based on the absorbance at 510 nm against a reagent blank. For the RS, 4 mL of the suspended solution was removed and combined with 4 mL of ethanol 95%. The tubes were centrifuged at 1500 × g for 10 minutes. The supernatant solution was decanted, and the pellet was resuspended with 8 mL of ethanol 50%. The solution was centrifuged again; this procedure was repeated twice. The supernatant was decanted, and the pellet was stirred with 2 mL of cold 1.7 M NaOH in an ice/water bath for 20 minutes. Then, 8 mL of 1.0 M sodium acetate buffer (pH 3.8) and 0.1 mL of amyloglucosidase (AMG) were added, the tubes were incubated at 50°C for 30 minutes (with intermittent mixing). Since all samples had less than 10% RS, the contents were centrifuged for 10 minutes at 1500 × g. By duplicate, 0.1 mL of the supernatant was transferred to a glass tube with 3 mL GOPOD reagent. The tubes were incubated at 50°C for 20 minutes. The RS was calculated based on the absorbance at 510 nm against a reagent blank.

### *2.3.3 Pasting Profile Analysis*

Whole wheat, whole white sorghum, and whole red sorghum flours were evaluated as quintuples with a Rapid Visco-Analyzer (RVA, Perten Instruments AB, Hargersten, Sweden) according to AACC International Method 76-21.01 ICC Standard No 162. For the sample preparation, 3.5 g of flour were mixed with approximately 25 ml of deionized water (corrected to 14% moisture content) into a canister, the slurries were dispersed with a glass rod to avoid flour sedimentation, a paddle was placed into the canister and this fitted to the RVA. The sample was heated to 50°C and stirred at 960 rpm for 10 s. After this, the slurry was held at 50°C for up to 1 min, and then heated to 95°C over 3:42 min, held at 95°C for 2:30 min, cooled to 50°C over 3:48 min, and held at 50°C for 2 min while constant stirred at 160 rpm. The total test time was 13 minutes, with readings taken every 4 s. Peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BDV), final viscosity (FV), setback viscosity (SBV), peak time (PkT), and pasting temperature (PT) were obtained and analyzed through its software (Thermocline Software for Windows).

### *2.4 Formula Development*

Initially, the formulas were intended to be isonitrogenous for the treatments that included soluble animal proteins. However, during a preliminary experiment, it became evident that the functionality of the proteins differed regarding the product quality, and some biscuits would not form. Thus, the formulas were modified to adjust the soluble animal proteins to create biscuits that extracted from the rotary die and were of reasonable quality and consistency to measure the remaining effects. Further, the water addition was adjusted during production to aid in meeting the objectives for obtaining a uniform short dough (Table 2.1).

### *2.5 Biscuit Production*

Three batches of 15 kg each were produced at a pilot research facility (Cookie Cracker Laboratory, AIB International, Inc.; Manhattan, KS). Dry ingredients were mixed in a planetary mixer (Hobart Legacy HL800 Mixer) for one minute at 55 rpm, then wet ingredients were added and mixed for 2 minutes at 55 rpm plus ~4.5-6 minutes at 96 rpm. The final dough weight and temperature were obtained before transferring the dough to the feeder bin above the rotary molder (70 PSI Weidenmiller) to make the bone-shaped biscuits (2 sizes, small and large). The molded treats were manually transferred to 5 labeled trays. The trays plus the biscuits were weighed and placed in a convection oven for ~20-25 minutes at 375°F (Table 2.2). After the elapsed baking time, moisture content and water activity of randomly selected treats were analyzed with a moisture analyzer (Halogen; AOAC Method, 1999) and water activity meter (Aqualab; AOAC

Method, 1995), respectively. The trays plus the biscuits were weighed again to determine the evaporation loss rate, and these were allowed to cool to room temperature. The biscuits were weighed and placed into plastic bags labeled according to the numbered tray (1-5) and stored at room temperature in resealable mylar bags inside totes for further analysis (Figure 2.1). The dough moisture content was measured by duplicate (AOAC Method, 930.15) on reproduced formulations mixed on a Hobart N50 5 Qt. commercial countertop mixer. The total mixing time was kept constant for each treatment as on the large scale but at 136 rpm.

## 3. Results

### *3.1 Flour Quality*

#### *3.1.1 Proximate Analysis*

The analyses of proximate constituents were performed on single replicate samples within the same batch, so no statistics are presented. On an absolute basis, the moisture content of all flours was below 12%. The whole wheat flour had the highest moisture (11.39%) followed by the whole grain sorghum flours, with 9.78% and 9.17% for the white and red sorghum flours, respectively. For the nutrient composition, expressed on a dry basis, the crude protein content of wheat flour was the highest (14.45%), followed by the red sorghum flour (11.23%), with the white sorghum flour the lowest (8.58%). The sorghum flours had higher crude fat (>3.5%) compared to the wheat flour (2.29%). The opposite rank was observed for crude fiber and ash, with values that ranged from 1.08-1.34 % and 1.24-1.85%, respectively (Table 2.3).

#### *3.1.2 Total, Digestible and Resistant Starch Analysis*

The starch fractions on the flours, expressed on a dry basis, differed in terms of percentage ($P<0.05$). The TS of the whole sorghum flours was greater (>78.9%) than whole wheat flour (67%) and was primarily composed of TDS (99.3-99.7%) and a very small fraction of RS (0.36-0.71%).

Comparing the sorghum flours, the whole white sorghum flour had more SDS and less RDS than the whole red sorghum flour (46.74% and 24.49% vs. 41.24% and 29.27%), respectively, and the whole wheat flour ranked lowest (33% and 21.94%). The RS was relatively low in all flours; nonetheless, the whole red sorghum flour contained more RS (0.56%), followed by whole white sorghum (0.47%) and whole wheat flour (0.24%) (Table 2.4).

### *3.1.3. Pasting Profile Analysis*

Based on the RVA sequence, the pasting curves of the flours were divided into four regions (Figure 2.2). Differences were found among the flour profiles during heating and cooling with an excess of water. When increasing the temperature from 50°C to 95°C it was found that the pasting temperature (PT) was higher ($P<0.05$) for the whole white sorghum flour (88.64ºC) compared to the whole wheat flour (87.99ºC); however, the red sorghum flour did not differ from the other flours (88.21ºC) (Table 2.5).

In the second region, when keeping the temperature at 95°C, the peak viscosity (PV) for the whole white sorghum flour was the highest ($P<0.05$; 2540.8 cP), followed by the whole red sorghum flour (2217.2 cP) and whole wheat flour (1874.2 cP). The peak time (Pkt= time at PV), known as the time that granules absorb water and form a paste structure, was close among all samples; however, the whole wheat and whole white flour (~ 6 min) were different ($P<0.05$) than whole red sorghum flour (5.7 min) (Table 2.5).

The trough viscosity (TV) for the whole white sorghum flour was the highest ($P<0.05$; 1880.8 cP), followed by the whole red sorghum flour (1673.4 cP) and whole wheat flour (1135.6 cP). The breakdown viscosity (BDV= PV-TV) was higher ($P<0.05$) for whole wheat flour (738.6 cP) and whole white sorghum (660 cP) in comparison to the whole red sorghum (543.8 cP). The final viscosity (FV) and setback viscosity (SBV= FV-TV) of the whole sorghum flours had values nearly 100% more than the whole wheat flour, with the whole white sorghum flour values greater (*P*<0.05; 4906.4 cP and 3025.6 cP) than the whole red sorghum flour (4319.6 cP and 2646.2 cP) or the whole wheat flour (2647.8 cP and 1512.2 cP), respectively (Table 2.5). In general, the pasting profile of the sorghum flours shared a similar pattern with higher viscosities and a sharper peak in Region 4 compared to the whole wheat flour (Figure 2.2).

### *3.2 Biscuit Production*

After mixing, the dough temperature fluctuated from 24.0°C to 26.10°C and was not significantly different among treatments (*P*=0.5842). In this experiment it stayed close to room temperature. The final dough weight depended on the total water added during mixing and had a cereal and protein source effect (*P*<0.05). It ranged from 13.66 kg to 15.24 kg, with the WRS treatments heavier than the WWS. For the WWF-GTN, WWS-NC, and WRS-NC treatments, the added water was intentionally maintained at the same levels evaluated in the preliminary trials to produce a target quantity of 15 kg. However, the total weight in the WRS-EP treatment surpassed 15 kg batch size due to doubling EP. This was necessary to achieve a good undeveloped dough for molding, plus the red sorghum treatments needed more water. The resulting dough moistures fluctuated from 27.23% to 36.39%. The NC treatments had the highest moisture, followed by GL, SDP, and EP when comparing the sorghum treatments (*P*<0.05); there was no difference between white and red sorghum treatments (*P*=0.9817); however, they differed from wheat (*P*<0.05). The WWF-GTN dough moisture was lower than all other treatments (28.81%), except for EP. The NC treatments had the highest evaporation rates (16.31% and 17.35%), whereas the EP treatments had the lowest (9.66% and 11.7%) (Table 2.6).

## 4. Discussion

### *4.1 Flour Quality*

#### *4.1.1 Proximate Analysis*

According to the proximate analysis, the values in our study for whole wheat flour were near to those reported for durum wheat (*Triticum durum* L.*)* (Cruz, 1997; INRA et al., 2017b; Ocheme et al., 2018). Similarly, the values reported for whole white and red sorghum flours were similar to values previously reported for low-tannin varieties of sorghum grain (*Sorghum bicolor*, L. Moench) (INRA et al., 2017a; Zaparrart & Salgado, 1994). Nonetheless, some variation could be attributed to different agronomic practices, growing conditions, planting, and (or) harvesting times.

Based on their nutritional characteristics, wheat can be classified as hard, medium, or soft grain. Generally, hard wheat has high protein quantity and quality, possesses a vitreous endosperm, and has a highly packed starch-protein matrix. In contrast, soft wheat has a less compact starch-protein complex, less starch damage, and lower water absorption (Hazelton et al., 2003). In human products such as cookies, crackers, and short-dough biscuits, soft wheat flour (7-9% protein) is usually preferred as it produces less resistant and more extensile doughs. It also provides good tenderness and softer bite (Davidson, 2019c; Gebreselassie & Clifford, 2016; Mamat & Hill, 2017; Panghal et al., 2018). However, because dog treats were intended to be produced in our study, harder textures were desired. Thus, hard wheat was evaluated.

Gluten consists of two proteins, gliadin and glutenin in wheat, and its percentage determines the flour strength. More gliadin usually decreases the dough stability and increases the softening, cohesiveness, and adhesiveness of the dough, whereas more glutenin tends to improve the mixing characteristics of the flour and increase the hardness of the dough (Barak et al., 2014). On the other hand, **s**orghum protein can vary from 6-18%, with an average of 11%. Close to 80% of its protein is found as a prolamin protein, kafirin found in the endosperm (Arendt & Zannini, 2013). Other sorghum proteins are in the form of glutelins, albumins, and globulins (Smith, 2012). Kafirin, in the presence of oleic acid, can form viscoelastic doughs similar to those of wheat. However, the kafirin is more hydrophobic than gluten and its dough system dries rapidly and becomes stiff (Oom et al., 2008).

### *4.1.2 Total, Digestible and Resistant Starch Analysis*

The starch characterization of the flours provides a better insight into the expected caloric intake of the baked treats given that whole wheat and sorghum flours are the main farinaceous ingredients in rotary molded products. Additionally, it serves as a reference point for comparison with the starch fractions of the pet treats after they are baked and when soluble animal proteins are added to each formulation. The determination of each fraction also allows one to determine the rate and extent of digestion and glucose that will be delivered to the animal. The starch digestibility can be influenced by the size, shape, and composition of the starch granule and its structure of amylose and amylopectin (Ramadoss et al., 2019). The starch can be classified into rapidly digestible starch (RDS) which provides a fast and high rate of glucose delivery, slowly digestible starch (SDS) that provides a slow and prolonged glucose delivery, and resistant starch (RS) that in theory does not provide glucose (Englyst et al., 1992).

The RDS is a fraction digested and absorbed in the duodenum and proximal regions of the small intestine. This contrasts with RS which is not digested in the small intestine and reaches the large intestine where it can be fermented by the gut microflora. The SDS is the starch portion between RDS and RS, and it is digested along the whole small intestine (Englyst & Hudson, 1996; Zhang & Hamaker, 2009). The TDS corresponds to the total starch digested for four hours as it was claimed to correspond to the residence time of food in humans' small intestine (McCleary et al., 2020). Comparable to humans, we can expect similar transit times in dogs given that the small intestine length in *in-vivo* humans is estimated to be close to 3 m, whereas in Beagle dogs, it is between 2.25-2.90 m long (Kararli, 1995). Miyabayashi et al. (1986) reported a small intestine transit time range of 30- 120 min and a small intestine emptying time range of 180-300 min in normal Beagle dogs. However, the intestine motility can be influenced by food components, hormones, and the nervous system (Smeets-Peeters et al., 1998). Finally, TS is the sum of TDS and RS.

From the results obtained, we should expect higher energy from the starch sources of the sorghum flours compared to the wheat flour because they had more TDS. At the same time, the sorghum flours also contained higher RS that is associated with health benefits. The RS can shift the colon environment by stimulating bacterial fermentation. This fermentation can reduce the fecal pH, increase the butyrate concentration, and increase satiety (Goudez et al., 2011; Haenen et al., 2013; Peixoto et al., 2018). However, the amount of RS in an ingredient oscillates based on its origin and the processing conditions to which it has been exposed (Spears & Fahey, 2004). The lower TDS and RS values found in wheat flour may be the result of less TS content. Nonetheless, the calculated proportion of RS based on the TS was still lower in the wheat flour than the sorghum flours (0.35% vs. 0.57% and 0.71%) for WWS and WRS, respectively. The RS found in the raw flours is classified as RS1, which is physically inaccessible to digestion due to entrapment within the milled grains and the presence of intact cell walls. Moreover, some of it is expected to be heat stable that will not break down during regular cooking (Raigond et al., 2015).

### *4.1.3. Pasting Profile Analysis*

The functionality of cereal-based products is primarily dependent on starch property characteristics. Understanding this is essential to make inferences about how processes and products might perform. Wheat is generally understood as a better ingredient for cookie production, with poor cookie quality starch typically resulting from higher PV, TV, FV, and SBV as compared to starch of good cookie quality. In addition, good cookie quality starches exhibit higher PT (Devi et al., 2019). However, according to Adebowale et al. (2012), the FV should be the most common parameter used to define the quality of starch-based flour products as it shows the ability of the flour to form a viscous paste and resist shearing after cooking and cooling.

The PT is the temperature in which viscosity first increases by at least 25 cP over a 20 s period (Ragaee & Abdel-Aal, 2006), and it occurs when the starch granules absorb water and swell, creating an interaction with each other (Batey & Curtin, 2000). In our study, the PT obtained for the whole flours evaluated were higher than those reported in the literature. Some authors have declared values around 75-82.6°C for sorghum flours and a wide range of temperatures from 59.50°C to 84.3°C for wheat flours (Belton & Taylor, 2002; Majzoobi et al., 2011; Truong et al., 2017).

According to Ragaee & Abdel-Aal (2006), the higher PV, which is the maximum hot paste viscosity or water-holding capacity, can be driven by a higher starch content or differences in protein composition. This was confirmed in our study based on the total starch measured and the guaranteed carbohydrate analyses reported by the flour suppliers, wherein the white and red sorghum flours had higher starch values and less protein content. Also, Troung et al. (2017) evaluated the relationship between sorghum protein and its pasting properties among 13 sorghum grains. They concluded that sorghum proteins had a detrimental effect on PV and BDV and a significant increase of the PT. Comparing the white and red sorghums in our study, we had similar results regarding PV and BDV; however, there were no differences for the PT. Also, it was presumed that the higher PV of the sorghum flours was due to the smaller particle sizes (<150 µm) than the wheat flour (<180 µm). Bolade et al. (2009) and Liu et al. (2012) came to these conclusions when analyzing the PV and water uptake of maize flour at different particle sizes and non-tannin sorghum hybrids, respectively. In addition, the differences in the PkT might confirm that starch properties exhibited differences depending on the cultivar, amylose: amylopectin ratio, amylopectin chain length distribution, swelling power, starch concentration, and environmental conditions as suggested by Ahmed (2017).

The TV, which is the minimum hot paste viscosity, was supposed to be achieved in the 95°C holding period; however, it occurred on the third region, when cooling the samples to 50°C. The 95°C holding period is usually associated with the disruption of the starch granules and amylose leaching (Ragaee & Abdel-Aal, 2006); thus, a breakdown in the slurry viscosity was expected. Based on the BDV results, it might be thought that the whole red sorghum flour had a better tolerance to deformation under shear stress and high temperature applied, as it had the lowest BDV. These findings are aligned with Ragaee & Abdel-Aal (2006), who reported that the whole sorghum grain exhibited a better ability to withstand heat and shear than soft wheat, hard wheat, barley, millet, and rye.

The FV and SBV values indicate that the retrogradation rate and syneresis for the wheat flour were significantly lower than the sorghum flours when cooling and holding the sample at 50°C. Lower retrogradation values are commonly attributed to more amylopectin content. Rincón-Londoño et al. (2016) reported these behavioral patterns when studying corn starch rich in amylopectin. However, based on available sorghum and wheat literature, Belton & Taylor (2002) mentioned that starch from traditional sorghum varieties contains 20-30% amylose and 70-80% amylopectin. In contrast, in wheat flours the expected values were 25-28% for amylose and 72-

75% for amylopectin (Van Hung et al., 2006). This serves to partially explain our results, but it is important to keep in mind that the amylose: amylopectin ratio was not evaluated in this study.

The pasting profile curves obtained were similar to those reported by Ragaee & Abdel-Aal (2006); although, the viscosity values (cP) in our study were near 100% higher. However, when compared with Truong et al. (2017), our values were within the range of the 13 sorghum varieties they studied. Finally, the viscogram differed from those indicated by Pezzali et al. (2019) and Liu et al. (2012) when evaluating white and red sorghum flours. This may be attributed to different cultivars, hybrids, or the difference between ground whole flour vs. a refined flour from decorticated sorghum used in their study.

### *4.2 Biscuit Production*

Dough temperature can rise during mixing from friction generated by the paddles against the dough, especially when the dough stiffens. Monitoring this is vital for consistent processing and product quality. Davidson (2019b) suggests keeping the dough temperature between 18-22ºC when producing rotary molded short dough biscuits. The dough temperatures measured in our study were higher (>24ºC) and consistent among all treatments. The final temperature in short doughs can be affected by the type of fat used (Cauvain & Young, 2009b), and usually, more fat results in less water added (Davidson, 2019a). In comparison with rotary molded biscuits for people, we included considerably less fat and more water for dog treats. The flour in our study was also added at the first mixing step (with all the dry ingredients) and was mixed for longer times. This might have led to higher temperatures as more energy could have been imparted to the dough; however, the specific mechanical energy (SME) input was not determined. According to Bloksma (1985), higher final dough temperatures usually lead to lower dough viscosities. Similarly, Charun et al. (2000) observed that semisweet wheat biscuit length and dough stickiness decreased with dough temperatures higher than 35°C due to an increase in the elastic behavior of dough [storage modulus (G′)] and shrinkage. In addition, Rosell and Collar (2009) found that increases in wheat dough temperature have a negative effect on the development time and consistency of the dough. In contrast to these previous observations, we did not notice variations in the dough consistency across treatments and in the length of the rotary molded biscuits. Moreover, in a preliminary trial, we did not observe differences in the viscosity of WWF-GTN and WWS-NC doughs when measured in a modular compact rheometer MCR 72 (Anton Paar, Graz, Austria) (data not shown). This finding may be attributed to the amount of water that was added which allowed us to obtain comparable results.

The final dough weight was strictly linked to the total water included during wet mixing; thus, it was not a target parameter, but an outcome aligned with the physical characteristics of the undeveloped dough and its production functionality. The total added water was adapted according to the moisture of raw ingredients and the expected dough consistency that varied depending on the soluble animal protein used and its water holding capacity. For example, the white sorghum treatments needed slightly less water than the red sorghum treatments because of their original moisture content in the whole flour. Similarly, the NC treatments required more water to be able to be sheeted instead of shaped in the rotary molder due to their lack of gluten. According to Seguin (2015), pet treat dough for rotary molding generally has a moisture content from 30-40%, being the most favored between 30-32%. In comparison with this, all the doughs obtained were in this range, except the EP and WWF-GTN treatments, which had lower dough moisture due to higher protein water-binding and retention functionality that were potentiated by mixing forces, pressure, and heat generated (Zayas, 1997).

The moisture migrates from the center of the product to the surface by capillary action and diffusion (Hazelton et al., 2003). The differences in the evaporation losses were likely influenced by the protein content of each formulation and their water-binding capacity. This means that products with less protein content had less ability to bind the free water; therefore, they had higher evaporation rates to achieve a moisture target of less than 10%. According to Hazelton et al. (2003), approximately 46% of the total water absorbed by the dough is associated with the starch, 31% with the protein, and 23% with the pentosan concentrations. The NC treatments were baked longer because these formulations contained more water. In addition, they were manually sheeted, which resulted in thicker and larger biscuits with less surface area to release the internal moisture. The opposite effect with the EP proteins was perhaps due to the same reason. This occurred because the protein-containing ingredients absorbed and (or) retained water. This implies that they can bind water at the molecular level and thereby be unavailable as a solvent, or water can be trapped in the protein matrix and interact with polysaccharides or fat (Kneifel et al., 1991) which reduces the evaporation rate during heating.

## 5. Conclusion

The characterization of the flours identified higher protein content and less TS, TDS, and RS in the wheat flour as compared to the sorghum flours. Based on the RVA results, lower biscuit quality was expected with sorghum flours because of higher PV, TV, FV, and SBV compared to the wheat flour. Thus, it was essential to include other ingredients to emulate the response in a similar fashion to that of wheat. As gluten substitutes, soluble animal proteins SDP, EP, and GL were included with the sorghum flour formulations. Similar dough temperature and physical dough consistency were achieved among treatments. In contrast, it was observed that the dough moisture, dough weight, and evaporation rate properties were influenced primarily by the water-binding ability of the protein ingredients and the water added to obtain a functional short dough.

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Table 2.1 Ingredient composition of the experimental diets.

| Ingredient | Treatments (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | WWF-GTN | WWS-NC | WWS-SDP | WWS-EP | WWS-GL | WRS-NC | WRS-SDP | WRS-EP | WRS-GL |
| Whole wheat flour | 70.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Whole red sorghum flour | 0 | 0 | 0 | 0 | 0 | 68.6 | 69.0 | 65.3 | 69.8 |
| Whole white sorghum flour | 0 | 68.6 | 68.9 | 65.3 | 69.8 | 0 | 0 | 0 | 0 |
| Cornmeal | 17.5 | 19.1 | 12.5 | 11.8 | 12.5 | 19.1 | 12.5 | 11.8 | 12.5 |
| Spray dried plasma | 0 | 0 | 6.22 | 0 | 0 | 0 | 6.23 | 0 | 0 |
| Egg protein | 0 | 0 | 0 | 11.28 | 0 | 0 | 0 | 11.28 | 0 |
| Gelatin | 0 | 0 | 0 | 0 | 5.35 | 0 | 0 | 0 | 5.35 |
| Water (% added on top of ingredients) | 24.5 | 41.1 | 28.9 | 24.6 | 31.0 | 41.1 | 29.2 | 27.5 | 32.8 |

Other ingredients: molasses 5.6%, all-purpose shortening 3.5%, non-fat dry milk 2.2%, salt 0.7%, baking soda 0.4%, sodium bisulfite 0.003%, inactive dry yeast 0.003%. WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

Table 2.2 Production parameters for baked dog treats.

| Treatment | Mixing (min) | | | Baking | |
|---|---|---|---|---|---|
| | Dry* 55 rpm | Wet# 55 rpm | Wet 96 rpm | Temperature (°F) | Time (min) |
| WWF-GTN | 1 | 2 | 6 | 375 | 25 |
| WWS-NC | 1 | 2 | 6 | 375[a]+150[b] | 30[a]+10[b] |
| WWS-SDP | 1 | 2 | 6 | 375 | 20 |
| WWS-EP | 1 | 2 | 4.5 | 375 | 20 |
| WWS-GL | 1 | 2 | 4.5 | 375 | 20 |
| WRS-NC | 1 | 2 | 6 | 375[a]+150[b] | 25[a]+10[b] |
| WRS-SDP | 1 | 2 | 6 | 375 | 20 |
| WRS-EP | 1 | 2 | 4.5 | 375 | 20 |
| WRS-GL | 1 | 2 | 6 | 375 | 20 |

* WWF/WWS/WRS, cornmeal, SDP/EP/GL, non-fat dry milk, salt, baking soda, sodium bisulfite, inactive dry yeast. # water, molasses, all-purpose shortening. Temperature and time ([a]) combined. Temperature and time ([b]) combined. WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

Table 2.3 Proximate composition (expressed on dry matter basis) of whole wheat flour, white whole grain sorghum flour and red/burgundy whole grain sorghum flour.

| Parameter | Flours | | |
|---|---|---|---|
| | WWF | WWS | WRS |
| Moisture, % | 11.39 | 9.78 | 9.17 |
| Dry matter, % | 88.61 | 90.22 | 90.83 |
| Crude protein, % | 14.45 | 8.58 | 11.23 |
| Crude fat, % | 2.29 | 3.54 | 3.70 |
| Crude fiber, % | 1.34 | 1.19 | 1.08 |
| Ash, % | 1.85 | 1.62 | 1.24 |

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum

Table 2.4 Starch fractions (expressed on dry matter basis) of whole wheat flour, white whole grain sorghum flour and red/burgundy whole grain sorghum flour.

| **Parameter** | **Flours** | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | WWF | WWS | WRS | | |
| TS, % | 67.05 $^{b}$ | 81.44 $^{a}$ | 78.90 $^{a}$ | 0.9239 | <.0001 |
| RDS, % | 21.94 $^{c}$ | 24.49 $^{b}$ | 29.27 $^{a}$ | 0.5278 | <.0001 |
| SDS, % | 33.00 $^{c}$ | 46.74 $^{a}$ | 41.24 $^{b}$ | 0.5905 | <.0001 |
| TDS, % | 66.81 $^{b}$ | 80.98 $^{a}$ | 78.34 $^{a}$ | 0.9238 | <.0001 |
| RS, % | 0.24 $^{c}$ | 0.47 $^{b}$ | 0.56 $^{a}$ | 0.0079 | <.0001 |

a-c: Means with different lowercase superscripts within a row represent statistical difference among flours (*P*<0.05). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum. TS=total starch, RDS=rapidly digestible starch, SDS=slowly digestible starch, TDS=total digestible starch, RS=resistant starch

Table 2.5 Pasting profile analysis of whole wheat flour, whole white sorghum flour and whole red/burgundy sorghum flour.

| **Parameter** | **Flours** | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | WWF | WWS | WRS | | |
| PV, cP | 1874.2 $^{c}$ | 2540.8 $^{a}$ | 2217.2 $^{b}$ | 18.905 | <.0001 |
| TV, cP | 1135.6 $^{c}$ | 1880.8 $^{a}$ | 1673.4 $^{b}$ | 25.064 | <.0001 |
| BDV,cP | 738.6 $^{a}$ | 660.0 $^{a}$ | 543.8 $^{b}$ | 22.230 | 0.0002 |
| FV, cP | 2647.8 $^{c}$ | 4906.4 $^{a}$ | 4319.6 $^{b}$ | 87.754 | <.0001 |
| SBV, cP | 1512.2 $^{c}$ | 3025.6 $^{a}$ | 2646.2 $^{b}$ | 95.873 | <.0001 |
| PT, $^{o}$C | 87.99 $^{b}$ | 88.64 $^{a}$ | 88.21 $^{ab}$ | 0.1379 | 0.0178 |
| Pkt, min | 6.008 $^{a}$ | 5.878 $^{a}$ | 5.662 $^{b}$ | 0.0470 | 0.0008 |

a-c: Means with different lowercase superscripts within a row represent statistical difference among flours (*P*<0.05). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum. PV=peak viscosity, TV=trough viscosity, BDV =breakdown viscosity, FV=final viscosity, SBV=setback viscosity, PT=pasting temperature, Pkt=peak time

Table 2.6 Parameters obtained during production for dough and baked dog treats produced with different cereals and soluble animal proteins combinations.

| **Treatments** | **Dough temp., °C** | **Dough weight, kg** | **Dough moisture, %** | **Evaporation rate, %** |
|---|---|---|---|---|
| WWF-GTN | 24.33 | 14.98 $^{ab}$ | 28.81 $^{e}$ | 12.73 $^{abc}$ |
| WWS-NC | 26.10 | 14.87 $^{ab}$ | 36.39 $^{a}$ | 17.35 $^{a}$ |
| WWS-SDP | 24.67 | 13.81 $^{c}$ | 30.41 $^{d}$ | 12.34 $^{bc}$ |
| WWS-EP | 26.00 | 13.66 $^{c}$ | 27.23 $^{f}$ | 9.66 $^{c}$ |
| WWS-GL | 24.33 | 13.69 $^{c}$ | 33.08 $^{b}$ | 13.55 $^{abc}$ |
| WRS-NC | 25.10 | 14.85 $^{ab}$ | 35.98 $^{a}$ | 16.31 $^{ab}$ |
| WRS-SDP | 25.00 | 14.65 $^{b}$ | 30.48 $^{d}$ | 13.04 $^{abc}$ |
| WRS-EP | 25.00 | 15.24 $^{a}$ | 28.86 $^{e}$ | 11.7 $^{bc}$ |
| WRS-GL | 24.00 | 14.86 $^{ab}$ | 31.80 $^{c}$ | 12.58 $^{bc}$ |
| SEM | 0.793 | 0.079 | 0.152 | 0.937 |
| *P*-value model | 0.5842 | <.0001 | <.0001 | 0.0007 |
| **Contrasts** | | | | |
| WWS vs WRS | 0.3841 | <.0001 | 0.9817 | 0.7870 |
| WWF vs WWS | 0.3020 | <.0001 | <.0001 | 0.6405 |
| WWF vs WRS | 0.6242 | 0.3836 | <.0001 | 0.5248 |
| GTN vs NC | 0.2084 | 0.2451 | <.0001 | 0.0022 |
| GTN vs SDP | 0.6127 | <.0001 | <.0001 | 0.9749 |
| GTN vs EP | 0.2450 | <.0001 | 0.0006 | 0.0908 |
| GTN vs GL | 0.8656 | <.0001 | <.0001 | 0.7725 |
| WWF-GTN vs all | 0.4214 | <.0001 | <.0001 | 0.5611 |

a-f: Means with different lowercase superscripts within a column represent statistical difference among treatments ($P<0.05$). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

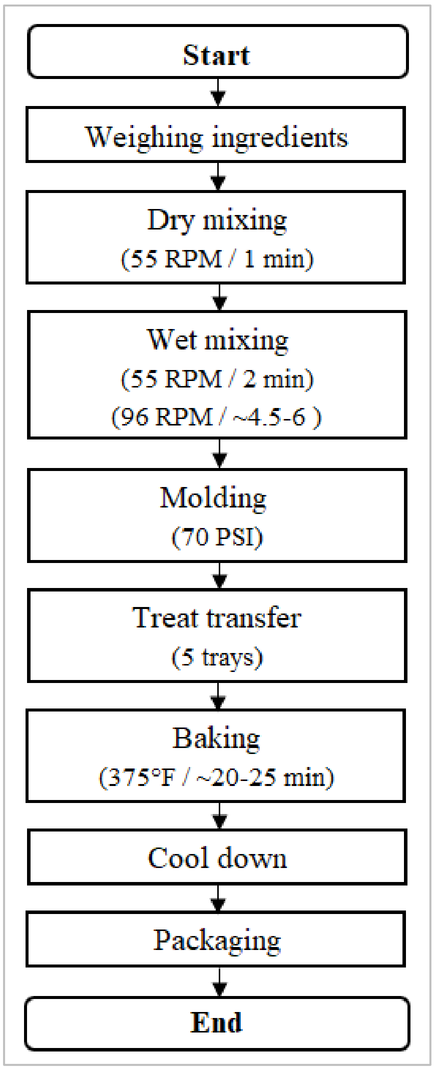

Figure 2.1 Flow chart of rotary-molded baked dog treats.

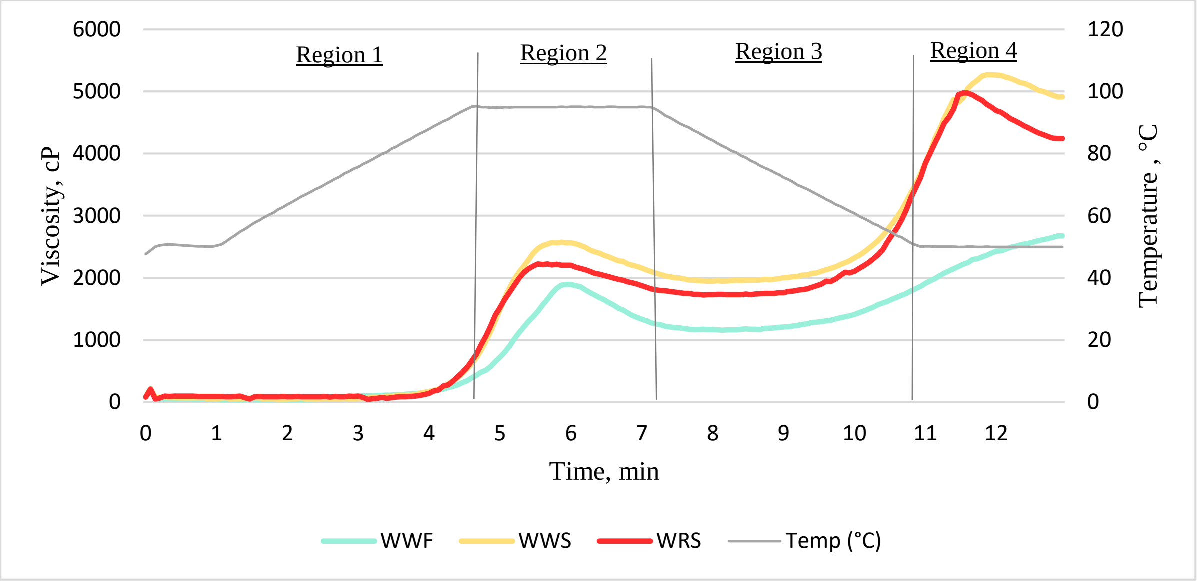

Figure 2.2 Viscogram of whole wheat flour (WWF), whole white grain sorghum flour (WWS), and whole red/burgundy grain sorghum flour (WRS).

# Chapter 3 - Physical and nutritional characterization of sorghum-based dog treats (biscuits) supplemented with soluble animal proteins

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## Abstract

Pet treats are given to dogs to strengthen pet and owner bonds and as a reward. Treat description and characterization are essential to understand the functionality of the raw materials and relate their impact on animal health and acceptance of the pet and the pet owner. The objective of this study was to determine the effect of added soluble animal proteins with whole sorghum flour in rotary molded dog treats on the physical and nutritional characteristics. The treats were produced in triplicate using a 2x4+1 augmented factorial arrangement of treatments. Two whole sorghum flours (WWS and WRS), four protein sources (none [NC], spray-dried plasma [SDP], egg protein [EP], and gelatin [GL]), and a positive control with WWF-GTN were evaluated. The data was analyzed in 1-way ANOVA, 2-way ANOVA, and 1-way with contrasts using the GLM procedure by statistical software SAS 9.4 and significance was considered at a probability $P<0.05$. A proximate analysis, quantification of the starch fractions, texture, size dimensions, and color analyses were performed on the biscuits. The dog biscuits had similar dry matter (>92.0%), Aw (<0.65), and caloric content (3.40-3.54 Kcal/g). However, the EP treatments had the highest protein (>17.8%) and the NC treatments the lowest (<10.2%). The ash for the SDP treatments was higher than all others. The values for RDS and RS increased ($P<0.05$) after baking. However, because of a formula dilution by the soluble animal proteins, the TDS and TS reduced in all treatments ($P<0.05$). The texture of the sorghum treatments was enhanced ($P<0.05$) by the protein ingredients added. The EP produced the hardest treats, followed by SDP and GL. The NC treatments had the lowest hardness and were not comparable in dimensions or texture to the other treatments ($P<0.05$). The thickness of large and small WWF-GTN biscuits was greater ($P<0.05$) compared to the sorghum treatments. The color of the biscuits was influenced by the protein sources and cereals used ($P<0.05$). This work indicated that WWS and WRS combined with

soluble animal proteins like SDP, GL, or EP allowed for the production of comparable products to those made with WWF-GTN.

## Abbreviations

RDS, rapidly digestible starch; SDS, slowly digestible starch; TDS, total digestible starch; RS, resistant starch; TS, total starch; WWF, whole wheat flour; WWS, whole white sorghum; WRS, whole red sorghum; GTN; gluten; NC, no protein; SDP, spray-dried plasma; EP, egg protein; GL, gelatin

***Keywords***: baking, dog treats, gluten-free, hardness, Maillard reaction, starch

## 1. Introduction

The pet food industry has significantly expanded because more pets are becoming a fundamental part of the family nucleus. According to APPA (2019), close to 85 million families own a pet, of which 63.4 million have dogs. It is currently more common that people are providing treats to their dogs as a means of pampering, rewarding, or for training. According to Packaged Facts (2017), 92% of dog owners buy treats with some regularity. The pet food industry has also experienced a shift in its demand patterns led by quick access to information, generational changes, and greater purchasing power (Euromonitor, 2020; Kestenbaum, 2018). These events have created more aware pet owners, especially when deciding what ingredients to include in their diet and the food they buy for their pets.

Market niches such as gluten-free and the demand for new products has intensified innovation in product lines and ingredients. The ingredients in pet foods are selected based on their nutrient content, impact on palatability, digestibility, and consumer preferences (Laflamme et al., 2014). Nowadays, most crunchy treats are formulated with wheat as the primary carbohydrate source because its gluten promotes an appealing texture and flavor (Case et al., 2011). However, other gluten-free sources such as sorghum, with important nutritional benefits (Anglani, 1998; Arendt & Zannini, 2013; Ratnavathi, 2019; Ratnavathi & Komala, 2016) can be combined with proteinaceous ingredients to promote water absorption, cohesivity, viscosity, and dough elasticity as gluten replacers (Wieser, 2007).

Establishing the processing conditions and the characteristics of the treats is necessary to create a consistent product and to understand the functionality of experimental raw materials. This characterization can also provide insight regarding the impact on animal health, as well as acceptability by the pet and the pet owner. For instance, shape and dimensions play a role in purchasing decisions. Case in point, 47% of dog owners purchased bone-shaped treats over other shapes (Sprinkle, 2015). Similarly, the texture combined with taste, aroma, size, appearance, and consistency can affect the perceived palatability (Griffin & Beidler, 1984). The texture also impacts product transportation and packaging. While color influences the acceptance of the product by the pet owner. When a biscuit is perceived to be too light or white it may appear to be undercooked, or conversely, when too dark to be overcooked or burned. Therefore, a biscuit is better perceived when brown but not dark (Scaglione & Gellman, 1986). Thus, the objective of this study was to determine the effect of added soluble animal proteins with whole sorghum flour in rotary molded dog treats on the physical and nutritional characteristics. The outcome of using soluble animal proteins (spray-dried plasma, egg protein, and gelatin) may provide meaningful insights regarding process and formula optimization in sorghum and other non-gluten containing cereals for these baked treat type products.

## 2. Material and Methods

### 2.1 Materials

Rotary-molded baked dog treats were produced at a pilot research facility (Cookie Cracker Laboratory, AIB International, Inc.; Manhattan, KS). The experimental ingredients included whole wheat flour <180 µm (Ultragrain Hard, Ardent Mills, Denver, CO), whole white and red sorghum flours <150 µm (White Whole Grain and Burgundy Whole Grain, Nu Life, Scott City, KS), spray-dried plasma (Innomax Porcine Plasma, Sonac, Maquoketa, IA), egg protein (OvaBind®, Isonova, Spencer, IA), and gelatin (Pro-Bind Plus 50, Sonac, The Netherlands). Each of the treatments also included cornmeal (Enriched Corn Meal Yellow, Sysco), salt (Iodized Salt, Morton Salt Inc., Chicago, IL), molasses (Rich Brown Hue [40% - #715 and 60% - #677], International Molasses Corporation, Ltd., Saddle Brook, NJ), baking soda (Pure Baking Soda, Arm & Hammer, Princeton, NJ), nonfat dry milk (Nonfat Dry Milk Classic, Sysco), sodium bisulfite (Sodium Metabisulphite, LD Carlson Company, Kent, OH), inactive dry yeast (Nutritional Yeast, Bob's Red Mill Natural Foods, Milwaukie, OR), and all-purpose shortening (Premium All-Purpose Shortening, Ventura Foods, Brea, CA) (Table 2.1).

### 2.2 Statistical Analysis

The experiment was conducted as a 4x2+1 augmented factorial arrangement of treatments in which four protein sources (Innomax Porcine Plasma, OvaBind®, Pro-Bind Plus 50, and "none" as a negative control), two different sorghum flours (white whole sorghum flour, and red whole sorghum flour), and a positive control formulated with whole wheat flour.

The data processing, analysis of variance, and least-square means separation was performed using the GLM procedure by statistical analysis software (SAS 9.4 Inst. Inc., Cary, NC). Tukey's HSD (Honest Significance Difference) test was applied for the least-squares means separation, with significance considered at a probability $P<0.05$. The sequence of analysis was approached according to Marini (2003). Three different models were generated: a one-way ANOVA comparing the nine treatments, a 2-way ANOVA to test the main effects, and a one-way ANOVA with single-degree-of-freedom contrasts to compare different groups of treatments as well as interactions of interest.

## *2.3 Biscuit Quality*

### *2.3.1 Proximate Analysis*

Biscuits were evaluated for moisture (AOAC Method, 930.15), crude protein (CP; AOAC Method, 990.03), crude fat by acid hydrolysis (AOAC Method, 2003.05), crude fiber (AOCS Ba 6a-05), and ash (AOAC Method, 942.05) in a commercial laboratory (Midwest Laboratories, Omaha, NE). Dry matter (DM), nitrogen-free extract (NFE), and metabolic energy (ME) were calculated using the following formulas:

$$\text{DM}_{(\%)} = 100 - \text{moisture } (\%) \tag{1}$$

$$\text{NFE}_{(\text{DM }\%)} = 100 - [\text{CP}_{\text{DM}}\,(\%) + \text{crude fat}_{\text{DM}}\,(\%) + \text{crude fiber}_{\text{DM}}\,(\%) + \text{ash}_{\text{DM}}\,(\%)] \tag{2}$$

$$\text{ME}_{(\text{Kcal/g})} = \{[3.5 \times \text{CP } (\%) + 3.5 \times \text{NFE } (\%) + 8.5 \times \text{crude fat } (\%)] \times 10\} \div 1000 \tag{3}$$

### *2.3.2 Total, Digestible and Resistant Starch Analysis*

The various fractions of starch were evaluated by duplicate within each replicate using digestible and resistant starch assay procedures (K-DSTRS 02/19; Megazyme International Ltd, Wicklow, Ireland). The procedure was similar to that explained in the flour quality section (Chapter 2, section 2.3.2), with the exception that biscuits were ground to pass through a 500 µm screen in a laboratory fixed blade impact mill (Retsch, type ZM200, Haan, Germany).

### *2.3.3 Texture Analysis*

Biscuits were evaluated regarding their texture (TA.XT2 Texture Analyzer) using the bone-style dog biscuits protocol (Texture Technologies Corporation, Hamilton, MA) with minor modifications. An adjustable bridge (TA-92N) with an opening of 19 mm and a probe (TA-42 knife blade with 45° chisel-end) were used. A total of 20 biscuits were randomly selected per each size and analyzed within each replicate. Individually, each bone was placed over the three-point bend bridge to be cut in the middle of its upper holes with the probe. The probe force was 15 g, the distance traveled 5 mm, the descent speed 2 mm/s, and the withdraw speed 5 mm/s. Hardness and fracturability were analyzed through the equipment software (Exponent Connect, Texture Technologies Corporation, Hamilton, MA).

### *2.3.4 Dimension Analysis*

Length, width, and thickness of 20 biscuits randomly selected per each size and replicate were measured with a digital caliper (Fisher Scientific, Pittsburgh, PA). Three different width measurements were taken per biscuit (1 for the center-body and 2 for the end-tips). The weight was measured with an analytical scale (Figure 3.2).

### *2.3.5 Color Analysis*

The external surface color was measured with a colorimeter (CR-410 chroma meter, Konica Minolta Sensing Americas, Inc., Japan) calibrated with a white standard plate. A white cup was evenly filled with the biscuits, making sure the top surface of the cup was covered. The chromameter was placed over the sample and six measurements were taken by replicate. The results were presented in a three-dimensional scale (L* a* b*), where L* goes from 0 being black to 100 white, a* from -60 to 0 for green and from 0 to +60 for red, and b* from -60 to 0 for blue and 0 to +60 for yellow. The hue angle and chroma were calculated from a* and b* values, using the following formulas:

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \tag{4}$$

$$\text{Chroma} = \sqrt{(a*)^2 + (b*)^2} \tag{5}$$

#### *2.3.6 Microbiological Analysis*

The biscuits for all replicates were blended into a composite and evaluated for total coliforms and Salmonella. Total coliforms were assessed with the 3M™ Petrifilm™ Coliform Count Plate (AOAC Method, 991.14), and Salmonella was analyzed through end-point PCR technology and selective agar plating using the procedure described in the Bacteriological Analytical Manual (Andrews et al., 2020).

## 3. Results

### *3.1 Biscuit Quality*

#### *3.1.1 Proximate Analysis*

For all the treatments, the results are reported on dry matter basis, which was similar among treatments (>92.0%). The crude protein fluctuated from 8.36-19.84% and there was a main effect of cereal and protein sources ($P<0.05$). The WRS treatments had approximately 2% more crude protein than the WWS treatments when comparing the same animal protein source. Regarding crude protein, the EP treatments had the highest values ($P<0.05$), followed by the GL and the SDP. The sorghum treatments, with no protein ingredients added, had the least crude protein, followed by the WWF-GTN. The crude fat ranged between 6.38-7.27%; however, higher numerical values were obtained for WWS-GL and lower for WWF-GTN ($P<0.05$). The crude fiber of the biscuits was low and relatively similar between the sorghum treatments (< 1.37%); these were lower in crude fiber than the WWF-GTN treatment (1.72%; $P<0.05$). The ash content did not vary among treatments (2.0-2.5%), except for the SDP treatment which had a greater ($P<0.05$) concentration (3.0%). The NFE (a crude measure of starch) was calculated by difference based on the proximate analysis. The results differed by cereal and protein source added ($P<0.05$); wherein, the SDP and GL treatments were comparable but lower than NC and WWF-GTN, and higher than EP. The caloric content (gross energy; GE) and Aw of the biscuits were in the range of 3.40-3.54 Kcal/g and 0.22-0.41, respectively, and were not different among treatments (Table 3.1).

### *3.1.2 Total, Digestible and Resistant Starch Analysis*

The starch fractions of the biscuits expressed on a dry matter basis varied among treatments; wherein, the WWF-GTN was similar to all treatments. However, the NC treatments had the highest TS values ($P<0.05$; > 71%) and EP the lowest (62.7% and 56.7%) for WWS and WRS, respectively. The total starch of biscuits was mainly composed of TDS (99.1-99.4%) and a very small fraction of RS (0.7-0.9%). The higher RDS was found in the WWF-GTN and NC treatments (>42.5%), whereas there was no difference among the other products (range 33.1- 37.7%). Based on the total starch content, the RDS portion in WWF-GTN and WWS-NC was (73.1% and 68.5%, respectively); all other sorghum treatments ranged from 52.8 to 60.4%. For SDS the sorghum treatments were greater ($P<0.05$; >19.3%) than WWF-GTN (14.3%). Furthermore, based on the total starch content, the SDS of WWF-GTN was 22.4% of the total starch, whereas the sorghum treatments fluctuated from 27.0- 41.9%. The RS values were low with no real differences between WWF-GTN (0.55%) and the sorghum diets (0.38%-0.64%); however, the highest and lowest values were recorded for WWS-NC and WRS-GL, respectively (Table 3.2).

### *3.1.3 Texture Analysis*

The hardness is the maximum force needed to break each biscuit until it fractures and breaks into two pieces. The hardness of large and small treats followed a similar trend regardless of the size and was strictly dependent on the protein ingredient added ($P<0.05$). There was no difference between WWS or WRS treatments within the same protein source; however, all of them differed relative to WWF-GTN ($P<0.05$). The EP and WWF-GTN treatments were the most resistant to breakage (10.04 -14.15 kg) for both, large and small treats. The SDP treatments had intermediate hardness or breaking tolerance values (4.93 - 5.24 kg) relative to the other treatments. The GL and NC treatments did not differ and were the least resistant (< 2.12 kg) to breakage force. Numerically, the force required to break the small biscuits was higher than in the large ones: WWF-GTN (14%), WWS-SDP (0.5%), WRS-SDP (1.5%), WRS-EP (6%), WWS-GL (6%), and WRS-GL (13%); except WWS-NC (13%), WRS-NC (14.5%), and WWS-EP (6%) which was higher in the large biscuits as compared to the small ones (Tables 3.3 & 3.4).

The fracturability or distance at the point of the break is equivalent to the resistance of the sample to bend. This parameter followed a slightly similar pattern to hardness and was dependent on the protein source added. There was no difference between WWS or WRS treatments when compared to the same proteinaceous ingredient; however, they differed from the WWF-GTN treatment ($P<0.05$). The fracturability values ranged from 0.47 to 1.24 mm; wherein, the EP and WWF-GTN treatments resulted in the most resistant to bending regardless of their size. All other treatments did not differ and averaged <0.66 mm (Tables 3.3 & 3.4).

### *3.1.4 Dimension Analysis*

The weight, length, and width (body and tips) obtained for the rotary molded treats were similar regardless of cereal or animal soluble protein used. The weight values ranged from 8.74 to 10.16

g, the length from 64.43 to 67.44 mm, the width-body from 18.43 to 20.04 mm, and the width-tips from 25.60 to 27.31 mm in the large biscuits. Similarly, in the small treats, the weight ranged from 7.50 to 8.45 g, the length from 47.29 to 49.56 mm, the width-body from 18.81 to 20.17 mm, and the width-tips from 26.47 to 28.15 mm. Slight variations were observed for thickness, where the WWF-GTN were thicker than other treatments ($P<0.05$) (Table 3.3 & 3.4).

When comparing the manually sheeted to the rotary molded biscuits, the large NC treatments were heavier (>60%), longer (>11%), and wider (>17%) than all other treatments ($P<0.05$). The thickness was greater (>6%) than the sorghum treatments but comparable to WWF-GTN ($P=0.1509$). This trend was also measured in small NC treats, with the only distinction that the width did not differ. The NC small treats were heavier (>23%) and longer (>7%) than rotary molded treatments. The NC thickness was greater (>13%) than the sorghum treatments but equivalent to WWF-GTN ($P=0.252$) (Table 3.3 & 3.4).

### *3.1.5 Color Analysis*

The lightness values (L*) ranged from 42.77 to 54.61 and there were a cereal and protein sources effect ($P<0.05$). The treatments with WWF and WWS resulted in similar values ($P=0.093$); however, WRS treatments were darker, with the lowest L* values ($P<0.05$). Wheat and white sorghum flours, or when combined with GL produced the highest L* (lightest color) values ($P<0.05$). Conversely, the combination that produced the darkest biscuits was EP with red sorghum flour ($P<0.05$). In the red sorghum treatments, the reduction in lightness when protein ingredients were added was more noticeable than in white sorghum treatments (Table 3.5 and Figure 3.1).

The a* positive value axis is associated with the red spectrum; thus, higher values indicate more intense reddish hues. This attribute ranged from 5.45 to 9.74 and was affected by cereal and protein ingredients ($P<0.05$). The red sorghum treatments had the highest a* values, especially when combined with EP. Likewise, the b* positive axis measures the yellow spectrum and higher values indicate more intense yellow color. This attribute ranged from 17.42 to 22.69 and was only affected by cereal type (*P*<0.05). The highest b* values were recorded for WWF-GTN and WWS treatments (Table 3.5).

The hue angle and chroma provide a better understanding of the color relationship. The hue angle is measured from 0° to 360° and it is divided into four quadrants. The first quadrant (0°-90°) encompasses from red to yellow, the second (90°-180°) from yellow to green, the third (180°-270°) from green to blue, and the fourth (270°-360°) from blue to red. Our results fell in the first quadrant and had a cereal type and protein ingredient effect (*P*<0.05). From an observational perspective, all biscuits were more yellowish than reddish. This was corroborated with the calculated hue angles closer to 90° (61.90° – 76.28°). The WRS treatments were in the lower end, especially when combined with EP. In contrast, the WWS treatments without soluble animal protein or with GL had the highest values. Moreover, chroma defines the perception of an object's efficiency to reflect or transmit light. Higher chroma means that an object can transmit more saturated light, which manifests in higher intensity colors. In our study, the values fluctuated from 18.78 to 23.71 and differed by cereal (*P*<0.05). The trend of higher values in wheat and white sorghum treatments was similar for the hue angle and the chroma parameters (Table 3.5).

### *3.1.6 Microbiological Analysis*

Biscuits from all the treatments were negative for Salmonella and coliforms <10 CFU/g.

# 4. Discussion

## *4.1 Biscuit Quality*

### *4.1.1 Proximate Analysis*

The moisture and Aw are important quality parameters of baked products since these are highly responsible for shelf-life stability. The target for these parameters was set at a maximum 10% moisture and maximum Aw of 0.65 to avoid microbial spoilage from mold growth. A similar moisture and Aw among the treatments were achieved by taking inline measurements and controlling the baking time and temperature. These parameters were influenced by the functionality (water retention and binding) of the proteins (Zayas, 1997) that determined the evaporation rate.

The EP treatments contained the highest ($P<0.05$) crude protein values due to doubling the amount of egg protein included in order to attain a consistent short dough and release the product from the rotary mold. This was not the intent of the original study design but was necessary to acquire samples for evaluation. As would be expected, the sorghum treatments with no soluble animal proteins had lower crude protein than WWF-GTN due to original protein content on the raw flours. The higher fat level on the WWS-GL treatment was not expected as all formulas contained similar shortening levels. Nevertheless, this treatment was comparable to WWS-NC, WWS-SDP, WRS-EP, and WRS-GL. Regarding the fiber, the higher value on the WWF-GTN was due to its original content from whole wheat flour. Similarly, the higher ash content in the SDP treatments was likely due to a higher inorganic material (ash) from the porcine plasma. The ash of SDP is a natural characteristic of blood plasma that maintains the body’s osmotic pressure and the result of anticoagulants addition at the time of bleeding (Polo et al., 2005). However, this parameter was not tested.

The NFE in the biscuits was increasingly diluted when proteinaceous ingredients were included in the cereal base recipe; thus, the EP treatments had the lowest NFE values, while the NC had the highest concentration. It is important to control the caloric content within a product from a product development standpoint because it can impact the perception of the product. For example, in a survey conducted by Morelli et al. (2020), it was determined that 84% of dog owners read the label when purchasing a product. Additionally, 3% said they do not buy treats because of excessive energy contribution to their pet diets. Based on the treats weight and caloric content per gram (calculated), the large treats in our study had values between 30.3-54.7 Kcal per treat and the small treats between 26.2-35.2 Kcal per treat. The NC treatments had the greatest caloric content because of their greater weight which occurred as a result of sheeting and cutting them manually rather than from being rotary molded.

### *4.1.2 Total, Digestible and Resistant Starch Analysis*

Each fraction of starch was described in the flour quality discussion (Chapter 2, section 4.1.2). The higher TS and RDS found in the NC treatments could be attributed to more carbohydrates in these experimental diets because no soluble animal proteins were included. The WWF-GTN had higher RDS than the sorghum treatments with protein ingredients added; however, it is worth emphasizing that the wheat flour had more intrinsic protein content than the sorghum flours, and the reason why TS for WWF-GTN was comparable to other treatments. According to Singh et al. (2010), differences in starch digestibility can be influenced by the textural and rheological characteristics of food, the presence of other food constituents (proteins, lipids, and non-starch polysaccharides), and the interactions occurring in them during food processing. For instance, Jenkins et al. (1987) observed that removing gluten from wheat raised the blood-glucose in people when consuming white bread compared to regular wheat flour. Therefore, these authors concluded that the starch-protein interaction can reduce the rate of starch absorption and glycemic response of a product. Comparably, in our study, this mechanism might have primarily reduced the RDS, especially in the treatments where SDP, GL, or EP were included.

During baking, the amount of SDS usually decreases as the RDS increases compared to raw products because gelatinization increases starch susceptibility to enzymatic digestion (Wang & Copeland, 2013). The baking process involves high temperatures that can lead to gelatinization; however, it is usually limited in biscuits because of the low inclusion of water and high fat levels in the dough (Leiva-Valenzuela et al., 2018). As mentioned before, our formulations contained more water and less fat compared to human biscuits. Thus, some degree of gelatinization was expected as the progressive melting of the crystalline structure of starch occurred (Wahl et al., 2012).

Even when the raw sorghum flours contained more RS than wheat flour, there were no differences in the biscuits RS. Resistant starch can act as dietary fiber, especially in animals fed diets high in protein and fat (Spears & Fahey, 2004). In our study, the increment of RS might be attributed to the retrogradation after baking, and in a lesser proportion, to the formation of amylose-lipid complexes. The retrogradation is known as a staling process that occurs after baking. In short-term storage, most amylose retrogradation occurs over minutes to hours, whereas recrystallization of amylopectin can occur over hours to days (Wang & Copeland, 2013). As a consequence, it increases the firmness and hardness of the starch (Horstmann et al., 2017). On the other hand, a formation of amylose-fat complexes can also occur during starch gelatinization. These complexes reduce the starch digestibility and retard the starch retrogradation due to limiting the surface accessibility by blocking the void sites and enzyme binding (Horstmann et al., 2017; Lau et al., 2016; Oates, 1997). Nonetheless, this phenomenon is more prevalent in high-amylose products such as tubers.

Alvarenga and Aldrich (2020) characterized the starch fractions of 20 commercial extruded pet foods (dog and cat, and grain-based and grain-free diets) and found an average of 0.95% RS in the dog diets, 0.70% RS in the cat diets, 0.83% in the grain-based diets, and 1.06% in the grain-free diets. Moreover, Beloshapka et al. (2014) fed 0, 2.5, or 5 g of RS per day to twelve Miniature Schnauzer dogs on top of a diet and found no differences in the fecal fermentative end-products, which were mainly attributed to the low proportions of RS fed to the animals. Based on these studies, we would not expect colonic benefits to the animals since our RS values were below these levels. Goudez et al. (2011) observed that size of the animal influences the RS response, with large dogs more sensitive to RS supplementation. Indeed, the authors found it important to provide low RS to large breed dogs to ensure an optimal fecal score. Thus, further research should be conducted using multiple sizes and breeds of dogs.

### *4.1.3 Texture Analysis*

The texture of the biscuits was attributed to their structure (degree of moistness/ dryness or openness/porosity) which is a consequence of ingredient combinations, their particle size, and the processing conditions (Chen & Rosenthal, 2015). The substitution of whole wheat flour by whole sorghum flours in the formulas reduced the hardness and increased the fracturability. In large part because the multifunctional properties of glutenin-gliadin found in gluten were removed. Wheat flour has been widely used in rotary-molded products since it allows for the formation of treats with excellent strength, integrity, and reduced breakage during formation, packaging, and transport (Lombard et al., 2012).

For the products containing animal proteins, a positive impact on product hardness was observed as they acted as structural agents, dough conditioners, and moisture controllers. Including protein ingredients in a gluten-free matrix elevates the protein content for low-protein flours, increases the viscoelasticity of the dough, and improves dough extensibility and machinability (Tandazo, 2013). Also, the texture and "hard-eating" qualities resulted from the reduced water inclusion that limited the evaporation during baking and minimized gluten development (Cauvain & Young, 2009a).

Another important parameter that influenced the texture formation was the baking conditions. During baking the dough undergoes chemical and physical changes such as fat-melting, loss of granular structure, protein denaturation, Maillard reactions, and dough expansion by water evaporation (Chevallier et al., 2002). The baking temperature used in this study was 375 °F (190°C) for the rotary molded treatments, and a combination of 375 °F (190°C) with 150 °F (66°C) for the manually sheeted treatments. Panghal et al. (2018) observed that wheat flour-based sugar snap cookies which were baked at 190°C had better quality for hardness and spread as compared to lower (128°C) or higher (250°C) baking temperatures. They noted that the "spread factor" was reduced at higher temperatures because of early starch gelatinization and protein coagulation. Additionally, they suggested that higher temperatures could negatively increase the density and hardness of the products because of an early escape of gases and vapors from the dough. Even when excessive hardness could be a negative factor for human products, it may be advantageous for dog products. However, no higher temperature values were evaluated in this study in part to avoid case hardening which could have impacted moisture removal from the biscuits. Furthermore, in short doughs, since a minimal gluten network is formed and there is no gluten in the sorghum treatments, the texture and rigidity of the baked biscuits would only be partially attributed to a protein-starch network and more to starch gelatinization, sugar recrystallization, and glass transition temperature after cooling (Gallagher, 2008; Hazelton et al., 2003).

Texture is an essential factor for biscuits or crunchy treats which are often offered as an aid to teeth cleaning. Teeth cleaning is important as it decelerates the development and progression of periodontal diseases in dogs, especially as they get older (Pietraniec et al., 2017). In accordance with AAFCO (2021), snacks and treats with claims associated with dental benefits are not objectionable when targeted for abrasion or mechanical action of the product. Nonetheless, it needs to be specified what attribute of the product (sharp edges or ridges) contributes to it. If too soft or too brittle, the treat would not provide enough abrasive action to clean the animal's teeth or would not adapt to the tooth surface during chewing (Scaglione & Gellman, 1986). On the contrary, if a texture is too hard for the size, breed, or animal age, it might cause discomfort and oral lesions (Pinto et al., 2020), leading to a reduction in product palatability, mouthfeel, and overall consumption. Carroll et al. (2020) noted that dental chews formulated with wheat gluten provided chewing resistance with high contact with the animal teeth. Similarly, other ingredients such as pea protein, gelatin, and fiber sources also provided a scrubbing effect during mastication of the treat.

### *4.1.4 Dimension Analysis*

Measuring biscuits allowed us to determine that there was minimal impact on dimensions regardless of animal protein or cereal used when shaped by rotary molding. Nonetheless, the NC treatments had different dimensions because cutting bone molds were used to shape them since they lacked functional proteins necessary to produce in the rotary molder. This resulted in larger and more variable treats for the final evaluation. Although this was not the intent of the experiment and not a desirable outcome for true evaluation, it clearly demonstrated that soluble animal proteins were required to provide adhesion of the flour and create a product suitable for evaluation.

The greater thickness values observed in the WWF-GTN treatment might be attributed to the viscoelastic properties of gluten. Even though fully developed gluten is not expected to be achieved in short doughs, this reaction might be catalyzed by the addition of baking soda and molasses. The combination of these ingredients likely produced $CO_2$ that was trapped inside the partially formed gluten matrix (Lauterbach & Albrecht, 1994b; Ortolan & Steel, 2017). Hazelton et al. (2003) reported that wheat pieces generally shrink in circumference before and during baking and then become thicker. In contrast, dough pieces formed with short doughs tend to retain their shape until baking, but then they spread or flow, becoming thinner. The unbaked piece dimensions were not measured in the current experiment but this potential change in dimensions would likely still occur.

The weight of the treats was tied to the dimensions of the biscuits after molding and baking. Uniform product weight can be regulated in the rotary molder by keeping a constant dough level in the hopper (Wright, 2020), verifying that all die cavities have the same depth (Pallottini, 2013), checking the position of the knife (the higher the knife the greater the amount of dough in the mold, and vice versa), adjusting the roller pressure (Ant, 2015), and keeping the dough consistency (obtained by monitoring its moisture and letting it stand in a cool area for 30 minutes) (Davidson, 2019a; Manley, 1998). Additionally, during baking, the dough piece weight can be controlled by the total moisture evaporation. In this experiment, the dough was not allowed to rest before molding; indeed, the EP doughs became so dry (firmer and less plasticized) over time that it caused difficulties with piece extraction and generated damage on the edges of the biscuits. This might be a result of the higher protein inclusion that led to higher hydration rates and water retention properties that competed with water in the dough (Atchley, 2016). To counter this, production equipment adjustments (extraction web and extraction roller) were necessary in order to obtain adequate bone-shaped treats.

### *4.1.5 Color Analysis*

The color of a product is defined by the ingredients, their quantities, and the processing conditions. For instance, the biscuits with either WWS or WWF were lighter than WRS, most likely because of the naturally pigmented pericarp in the whole flours. In addition, the luminosity (L*) was influenced by non-enzymatic browning reactions that occurred during baking. Maillard reaction occurs between reducing sugars and free amino acids (especially lysine) and peptides when heated (Manley, 2011a). It usually starts in the second baking phase (while the dough losses moisture at its maximum rate) and keeps rising in the third phase (with a lower moisture loss) (Hazelton et al., 2003). Since EP and WRS treatments had higher protein levels than WWS treatments, it was also expected to have more non-enzymatic browning, thus lowering luminosity values. Similar observations were reported by Leiva-Valenzuela et al. (2018) when evaluating the luminosity of potato and wheat starch-based biscuits with increasing levels of wheat gluten. Furthermore, the milk powder in the formulations contained lactose that acted as reducing sugar (Van Boekel, 1998).

As expected, the a* color, the WRS treatments had higher values than WWF or WWS. Moreover, the same baking time could have had a greater effect on these treatments because of their higher protein content. According to Knerr et al. (2001), as the Maillard reaction occurs, a rapid development of a yellow color exists, which then turns into reddish-dark brown when prolonged heating. Similarly, Manley (2011a) mentioned that if there exists an excessive Mallard Reaction, it may be challenging to dry the biscuit without too much darkening. Moreover, as the starch gel is heated in baked products, dextrinization also occurs, which contributes to product coloring (Davidson, 2019b). For the b* color, the WWS and WWF-GTN had greater values and did not differ regarding the added protein ingredients; hence, this parameter was associated with the pericarp of the flour used.

### *4.1.6 Microbiological Analysis*

The microbiological analyses in pet food allow us to verify the good manufacturing practices, confirm a reduction in risk to human (and animal) health, and assure pet owner safety during handling. In addition, dogs may become infected, either asymptomatically or clinically, increasing the potential exposure to humans (FDA, 2013). In this study, Salmonella and coliforms were analyzed because the biscuits were going to be further manipulated and tested by human panelists.

The Salmonella negative and coliforms detected below $10^2$ CFU/g were under the FDA and PHLS guidelines, respectively. The FDA states that pet food is considered as adulterated "Section 402(a)(1) of the FD&C Act [21 U.S.C. 342(a)(1)]" if it is contaminated with Salmonella and will not subsequently undergo a commercial process that kills it (FDA, 2013). Similarly, according to Gilbert et al. (2000), ready-to-eat products that will be consumed by humans should not exceed $10^2$ CFU/g enterobacteria or 20 CFU/g *E. coli*. Given that total coliforms were <10 CFU/g, *E. coli* was not tested.

## **5. Conclusion**

The dog biscuits had similar dry matter (>92.0%), Aw (< 0.65), and caloric content (3.40-3.54 Kcal/g). However, the EP treatments had the highest crude protein (>17.8%) and the NC treatments the lowest (<10.2%). The ash for the SDP treatments was higher than all others. The values for RDS and RS increased after formulating and baking the biscuits, attributed to a partial starch gelatinization and retrogradation of starch, respectively. However, because soluble animal proteins and extra ingredients were added into the cereal-based diet, the TDS and TS were reduced. The texture of the products was significantly enhanced by the action of the soluble animal proteins added in the sorghum treatments, with the EP producing harder treats, followed by SDP and GL. The NC treatments were very brittle and not comparable in dimensions or texture to the other treatments. The WWF-GTN had the highest thickness in both the large and small biscuits compared to the sorghum treatments and was attributed to the partial action of gluten. The color of the biscuits was influenced by the protein ingredients and pericarp of the cereals used; the higher non-enzymatic browning effect occurred on the EP and WRS treatments attributed to their higher protein content. Regarding the Salmonella and coliforms analyses, the dog biscuits were validated as safe for human consumption.

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Table 3.1 Proximate composition (expressed on dry matter basis) for dog treats combining different cereals and soluble animal proteins.

| **Treatments** | **Moisture, %** | **Dry matter, %** | **Crude protein, %** | **Crude fat, %** | **Crude fiber, %** | **Ash, %** | **Aw** | **NFE calculated, %** | **ME calculated, Kcal/g** |
|---|---|---|---|---|---|---|---|---|---|
| WWF-GTN | 7.64 | 92.36 | 12.60 $^{f}$ | 6.38 $^{b}$ | 1.72 $^{a}$ | 2.20 $^{b}$ | 0.41 | 77.11 $^{c}$ | 3.40 |
| WWS-NC | 4.72 | 95.28 | 8.36 $^{h}$ | 6.87 $^{ab}$ | 1.25 $^{b}$ | 2.26 $^{b}$ | 0.32 | 81.27 $^{a}$ | 3.54 |
| WWS-SDP | 5.49 | 94.51 | 13.30 $^{e}$ | 6.95 $^{ab}$ | 1.15 $^{bc}$ | 2.99 $^{a}$ | 0.30 | 75.61 $^{d}$ | 3.50 |
| WWS-EP | 7.95 | 92.05 | 17.89 $^{b}$ | 6.41 $^{b}$ | 0.81 $^{c}$ | 2.51 $^{b}$ | 0.36 | 72.39 $^{f}$ | 3.41 |
| WWS-GL | 6.26 | 93.74 | 13.76 $^{e}$ | 7.27 $^{a}$ | 1.25 $^{b}$ | 2.30 $^{b}$ | 0.32 | 75.42 $^{d}$ | 3.51 |
| WRS-NC | 5.46 | 94.54 | 10.20 $^{g}$ | 6.54 $^{b}$ | 1.37 $^{ab}$ | 2.33 $^{b}$ | 0.29 | 79.56 $^{b}$ | 3.50 |
| WRS-SDP | 6.73 | 93.27 | 15.16 $^{d}$ | 6.46 $^{b}$ | 0.99 $^{bc}$ | 3.10 $^{a}$ | 0.25 | 74.29 $^{e}$ | 3.43 |
| WRS-EP | 6.56 | 93.44 | 19.84 $^{a}$ | 6.82 $^{ab}$ | 1.20 $^{bc}$ | 2.41 $^{b}$ | 0.35 | 69.74 $^{g}$ | 3.47 |
| WRS-GL | 5.97 | 94.03 | 15.96 $^{c}$ | 6.68 $^{ab}$ | 1.04 $^{bc}$ | 2.22 $^{b}$ | 0.22 | 74.10 $^{e}$ | 3.50 |
| SEM | 1.372 | 1.372 | 0.11 | 0.135 | 0.079 | 0.082 | 0.108 | 0.196 | 0.049 |
| *P*-value model | 0.7809 | 0.7809 | <.0001 | 0.0028 | <.0001 | <.0001 | 0.966 | <.0001 | 0.4948 |
| **Contrasts** | | | | | | | | | |
| WWS vs WRS | 0.9392 | 0.9392 | <.0001 | 0.0175 | 0.5363 | 0.9337 | 0.5576 | <.0001 | 0.6562 |
| WWF vs WWS | 0.3313 | 0.3313 | <.0001 | 0.0040 | <.0001 | 0.0034 | 0.4882 | 0.0005 | 0.1210 |
| WWF vs WRS | 0.3549 | 0.3549 | <.0001 | 0.1181 | <.0001 | 0.0030 | 0.2920 | <.0001 | 0.1966 |
| GTN vs NC | 0.1470 | 0.1470 | <.0001 | 0.0629 | 0.0005 | 0.3757 | 0.4468 | <.0001 | 0.0631 |
| GTN vs SDP | 0.3751 | 0.3751 | <.0001 | 0.0604 | <.0001 | <.0001 | 0.3173 | <.0001 | 0.2857 |
| GTN vs EP | 0.8229 | 0.8229 | <.0001 | 0.1703 | <.0001 | 0.0194 | 0.6838 | <.0001 | 0.5175 |
| GTN vs GL | 0.3776 | 0.3776 | <.0001 | 0.0020 | <.0001 | 0.5660 | 0.3059 | <.0001 | 0.1106 |
| WWF-GTN vs all | 0.3181 | 0.3181 | <.0001 | 0.0180 | <.0001 | 0.0021 | 0.3571 | <.0001 | 0.1351 |

a-h: Means with different lowercase superscripts within a column represent statistical difference among treatments ($P<0.05$). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin, ME=metabolic energy

Table 3.2 Starch fractions (expressed on dry matter basis) for baked dog treats produced with different cereals and soluble animal proteins combinations.

| **Treatments** | **TS, %** | **RDS, %** | **SDS, %** | **TDS, %** | **RS, %** |
|---|---|---|---|---|---|
| WWF-GTN | $63.66^{bcd}$ | $46.52^{ab}$ | $14.28^{b}$ | $63.11^{bcd}$ | $0.55^{ab}$ |
| WWS-NC | $71.72^{ab}$ | $49.14^{a}$ | $19.33^{ab}$ | $71.08^{ab}$ | $0.64^{a}$ |
| WWS-SDP | $66.92^{abc}$ | $36.45^{d}$ | $25.29^{a}$ | $66.35^{abc}$ | $0.57^{ab}$ |
| WWS-EP | $62.73^{cd}$ | $33.10^{d}$ | $23.20^{a}$ | $62.20^{cd}$ | $0.53^{ab}$ |
| WWS-GL | $65.89^{abc}$ | $37.47^{cd}$ | $25.60^{a}$ | $65.37^{abc}$ | $0.51^{ab}$ |
| WRS-NC | $71.98^{a}$ | $42.46^{bc}$ | $24.48^{a}$ | $71.41^{a}$ | $0.57^{ab}$ |
| WRS-SDP | $61.21^{cd}$ | $33.77^{d}$ | $25.63^{a}$ | $60.68^{cd}$ | $0.54^{ab}$ |
| WRS-EP | $56.71^{d}$ | $34.27^{d}$ | $20.48^{ab}$ | $56.30^{d}$ | $0.41^{ab}$ |
| WRS-GL | $66.25^{abc}$ | $37.74^{cd}$ | $23.22^{a}$ | $65.87^{abc}$ | $0.38^{b}$ |
| SEM | 1.661 | 1.187 | 1.755 | 1.653 | 0.049 |
| *P*-value model | <.0001 | <.0001 | 0.0034 | <.0001 | 0.0405 |
| **Contrasts** | | | | | |
| WWS vs WRS | 0.0296 | 0.0297 | 0.9393 | 0.0338 | 0.0210 |
| WWF vs WWS | 0.1069 | <.0001 | 0.0002 | 0.1064 | 0.8583 |
| WWF vs WRS | 0.8416 | <.0001 | 0.0002 | 0.8084 | 0.1730 |
| GTN vs NC | 0.0008 | 0.6288 | 0.0023 | 0.0008 | 0.4042 |
| GTN vs SDP | 0.8444 | <.0001 | <.0001 | 0.8437 | 1.0000 |
| GTN vs EP | 0.0685 | <.0001 | 0.0025 | 0.0727 | 0.1936 |
| GTN vs GL | 0.2522 | <.0001 | 0.0002 | 0.2305 | 0.0947 |
| WWF-GTN vs all | 0.3300 | <.0001 | 0.0001 | 0.3187 | 0.5224 |

a-d: Means with different lowercase superscripts within a column represent statistical difference among treatments ($P<0.05$). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin. TS=total starch, RDS=rapidly digestible starch, SDS=slowly digestible starch, TDS=total digestible starch, RS=resistant starch

Table 3.3 Texture and dimension attributes for large-sized baked dog treats combining different cereals and soluble animal proteins.

| **Treatments** | **Hardness, kg** | **Fracturability, mm** | **Weight, g** | **Length, mm** | **Width-body, mm** | **Width-tips, mm** | **Thickness, mm** |
|---|---|---|---|---|---|---|---|
| WWF-GTN | 10.04 $^{b}$ | 1.16 $^{a}$ | 10.16 $^{b}$ | 65.45 $^{b}$ | 19.58 $^{c}$ | 26.16 $^{b}$ | 10.99 $^{a}$ |
| WWS-NC | 0.83 $^{d}$ | 0.63 $^{b}$ | 15.42 $^{a}$ | 75.19 $^{a}$ | 23.68 $^{a}$ | 33.22 $^{a}$ | 10.54 $^{ab}$ |
| WWS-SDP | 4.93 $^{c}$ | 0.63 $^{b}$ | 9.72 $^{b}$ | 65.55 $^{b}$ | 19.14 $^{c}$ | 26.40 $^{b}$ | 9.15 $^{bc}$ |
| WWS-EP | 14.15 $^{a}$ | 1.24 $^{a}$ | 9.10 $^{b}$ | 65.03 $^{b}$ | 18.43 $^{c}$ | 25.60 $^{b}$ | 9.99 $^{abc}$ |
| WWS-GL | 1.89 $^{cd}$ | 0.48 $^{b}$ | 9.34 $^{b}$ | 67.44 $^{b}$ | 20.04 $^{bc}$ | 27.21 $^{b}$ | 9.55 $^{abc}$ |
| WRS-NC | 0.82 $^{d}$ | 0.66 $^{b}$ | 15.63 $^{a}$ | 74.54 $^{a}$ | 23.41 $^{ab}$ | 32.38 $^{a}$ | 10.35 $^{abc}$ |
| WRS-SDP | 5.17 $^{c}$ | 0.65 $^{b}$ | 9.48 $^{b}$ | 66.02 $^{b}$ | 18.86 $^{c}$ | 26.38 $^{b}$ | 8.98 $^{c}$ |
| WRS-EP | 12.74 $^{ab}$ | 1.01 $^{a}$ | 8.74 $^{b}$ | 64.43 $^{b}$ | 18.80 $^{c}$ | 25.76 $^{b}$ | 9.43 $^{bc}$ |
| WRS-GL | 1.87 $^{cd}$ | 0.47 $^{b}$ | 9.23 $^{b}$ | 67.38 $^{b}$ | 20.01 $^{bc}$ | 27.31 $^{b}$ | 9.55 $^{abc}$ |
| SEM | 0.792 | 0.056 | 0.429 | 0.827 | 0.691 | 0.358 | 0.295 |
| *P*-value model | <.0001 | <.0001 | <.0001 | <.0001 | 0.0001 | <.0001 | 0.0017 |
| **Contrasts** | | | | | | | |
| WWS vs WRS | 0.6007 | 0.2275 | 0.683 | 0.7257 | 0.9183 | 0.5477 | 0.2844 |
| WWF vs WWS | <.0001 | <.0001 | 0.143 | 0.0063 | 0.3505 | 0.0001 | 0.0021 |
| WWF vs WRS | <.0001 | <.0001 | 0.2205 | 0.0103 | 0.3837 | 0.0003 | 0.0005 |
| GTN vs NC | <.0001 | <.0001 | <.0001 | <.0001 | 0.0002 | <.0001 | 0.1509 |
| GTN vs SDP | <.0001 | <.0001 | 0.2989 | 0.7422 | 0.5020 | 0.6060 | <.0001 |
| GTN vs EP | 0.0025 | 0.5971 | 0.0301 | 0.4892 | 0.2677 | 0.2909 | 0.0024 |
| GTN vs GL | <.0001 | <.0001 | 0.1116 | 0.0682 | 0.6056 | 0.0218 | 0.0009 |
| WWF-GTN vs all | <.0001 | <.0001 | 0.1572 | 0.0057 | 0.3421 | 0.0001 | 0.0006 |

a-d: Means with different lowercase superscripts within a column represent statistical difference among treatments ($P<0.05$). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

Table 3.4 Texture and dimension attributes for small-sized baked dog treats combining different cereals and soluble animal proteins.

| **Treatments** | **Hardness, kg** | **Fracturability, mm** | **Weight, g** | **Length, mm** | **Width-body, mm** | **Width-tips, mm** | **Thickness, mm** |
|---|---|---|---|---|---|---|---|
| WWF-GTN | 11.51 $^{a}$ | 1.16 $^{a}$ | 8.45 $^{abc}$ | 48.36 $^{bc}$ | 19.75 | 27.14 $^{ab}$ | 10.95 $^{ab}$ |
| WWS-NC | 0.72 $^{c}$ | 0.57 $^{b}$ | 9.93 $^{a}$ | 53.18 $^{a}$ | 19.03 | 25.89 $^{ab}$ | 11.51 $^{a}$ |
| WWS-SDP | 4.96 $^{b}$ | 0.57 $^{b}$ | 7.95 $^{bc}$ | 48.27 $^{bc}$ | 19.29 | 27.25 $^{ab}$ | 9.19 $^{c}$ |
| WWS-EP | 11.83 $^{a}$ | 1.12 $^{a}$ | 7.81 $^{c}$ | 47.88 $^{bc}$ | 18.81 | 26.47 $^{ab}$ | 10.15 $^{bc}$ |
| WWS-GL | 2.01 $^{bc}$ | 0.52 $^{b}$ | 7.63 $^{c}$ | 49.56 $^{b}$ | 20.10 | 28.15 $^{a}$ | 9.69 $^{c}$ |
| WRS-NC | 0.70 $^{c}$ | 0.63 $^{b}$ | 9.59 $^{ab}$ | 53.36 $^{a}$ | 19.01 | 25.50 $^{b}$ | 11.00 $^{ab}$ |
| WRS-SDP | 5.24 $^{b}$ | 0.63 $^{b}$ | 7.79 $^{c}$ | 48.46 $^{bc}$ | 19.06 | 27.28 $^{ab}$ | 9.30 $^{c}$ |
| WRS-EP | 13.52 $^{a}$ | 1.08 $^{a}$ | 7.55 $^{c}$ | 47.29 $^{c}$ | 19.07 | 26.71 $^{ab}$ | 9.56 $^{c}$ |
| WRS-GL | 2.12 $^{bc}$ | 0.55 $^{b}$ | 7.50 $^{c}$ | 49.51 $^{b}$ | 20.17 | 28.15 $^{a}$ | 9.65 $^{c}$ |
| SEM | 0.852 | 0.06 | 0.343 | 0.391 | 0.313 | 0.475 | 0.215 |
| *P*-value model | <.0001 | <.0001 | 0.0003 | <.0001 | 0.0418 | 0.0104 | <.0001 |
| **Contrasts** | | | | | | | |
| WWS vs WRS | 0.4019 | 0.4653 | 0.3694 | 0.8054 | 0.9290 | 0.9317 | 0.1065 |
| WWF vs WWS | <.0001 | <.0001 | 0.7661 | 0.0059 | 0.2225 | 0.7072 | 0.0033 |
| WWF vs WRS | <.0001 | <.0001 | 0.3882 | 0.0083 | 0.2432 | 0.6676 | 0.0003 |
| GTN vs NC | <.0001 | <.0001 | 0.0059 | <.0001 | 0.0738 | 0.0230 | 0.2520 |
| GTN vs SDP | <.0001 | <.0001 | 0.188 | 0.9863 | 0.1501 | 0.8366 | <.0001 |
| GTN vs EP | 0.2785 | 0.3883 | 0.0847 | 0.1247 | 0.0490 | 0.3537 | 0.0006 |
| GTN vs GL | <.0001 | <.0001 | 0.051 | 0.0244 | 0.3289 | 0.0999 | 0.0001 |
| WWF-GTN vs all | <.0001 | <.0001 | 0.5396 | 0.0049 | 0.2094 | 0.6714 | 0.0006 |

a-c: Means with different lowercase superscripts within a column represent statistical difference among treatments ($P<0.05$). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

Table 3.5 Color attributes for baked dog treats produced with different cereals and soluble animal proteins combinations.

| Treatments | L* | a* | b* | Hue angle | Chroma |
|---|---|---|---|---|---|
| WWF-GTN | 54.61 $^{a}$ | 6.86 $^{bc}$ | 22.69 $^{a}$ | 73.21 $^{ab}$ | 23.71 $^{a}$ |
| WWS-NC | 54.38 $^{ab}$ | 5.96 $^{bc}$ | 21.74 $^{a}$ | 74.73 $^{a}$ | 22.55 $^{ab}$ |
| WWS-SDP | 50.81 $^{abc}$ | 7.13 $^{bc}$ | 22.57 $^{a}$ | 72.52 $^{ab}$ | 23.66 $^{a}$ |
| WWS-EP | 47.87 $^{bcd}$ | 7.43 $^{bc}$ | 21.33 $^{a}$ | 70.79 $^{bc}$ | 22.59 $^{ab}$ |
| WWS-GL | 54.59 $^{ab}$ | 5.45 $^{c}$ | 22.23 $^{a}$ | 76.28 $^{a}$ | 22.9 $^{ab}$ |
| WRS-NC | 53.34 $^{abc}$ | 6.97 $^{bc}$ | 17.44 $^{b}$ | 68.21 $^{cd}$ | 18.78 $^{c}$ |
| WRS-SDP | 46.62 $^{cd}$ | 7.87 $^{ab}$ | 17.47 $^{b}$ | 65.76 $^{ef}$ | 19.16 $^{c}$ |
| WRS-EP | 42.77 $^{d}$ | 9.74 $^{a}$ | 18.25 $^{b}$ | 61.90 $^{f}$ | 20.68 $^{bc}$ |
| WRS-GL | 49.91 $^{abc}$ | 7.21 $^{bc}$ | 17.42 $^{b}$ | 67.53 $^{cd}$ | 18.85 $^{c}$ |
| SEM | 1.36 | 0.409 | 0.409 | 0.795 | 0.485 |
| *P*-value model | <0.001 | <.0001 | <.0001 | <.0001 | <.0001 |
| **Contrasts** | | | | | |
| WWS vs WRS | 0.001 | <.0001 | <.0001 | <.0001 | <.0001 |
| WWF vs WWS | 0.0930 | 0.4375 | 0.1295 | 0.6794 | 0.1672 |
| WWF vs WRS | 0.0005 | 0.0281 | <.0001 | <.0001 | <.0001 |
| GTN vs NC | 0.658 | 0.4466 | <.0001 | 0.0916 | <.0001 |
| GTN vs SDP | 0.0023 | 0.2167 | <.0001 | 0.0006 | 0.0011 |
| GTN vs EP | <.0001 | 0.0028 | <.0001 | <.0001 | 0.0026 |
| GTN vs GL | 0.1734 | 0.3087 | <.0001 | 0.1968 | 0.0002 |
| WWF-GTN vs all | 0.0053 | 0.4119 | <.0001 | 0.0006 | <.0001 |

a-f: Means with different lowercase superscripts within a column represent statistical difference among treatments ($P<0.05$). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

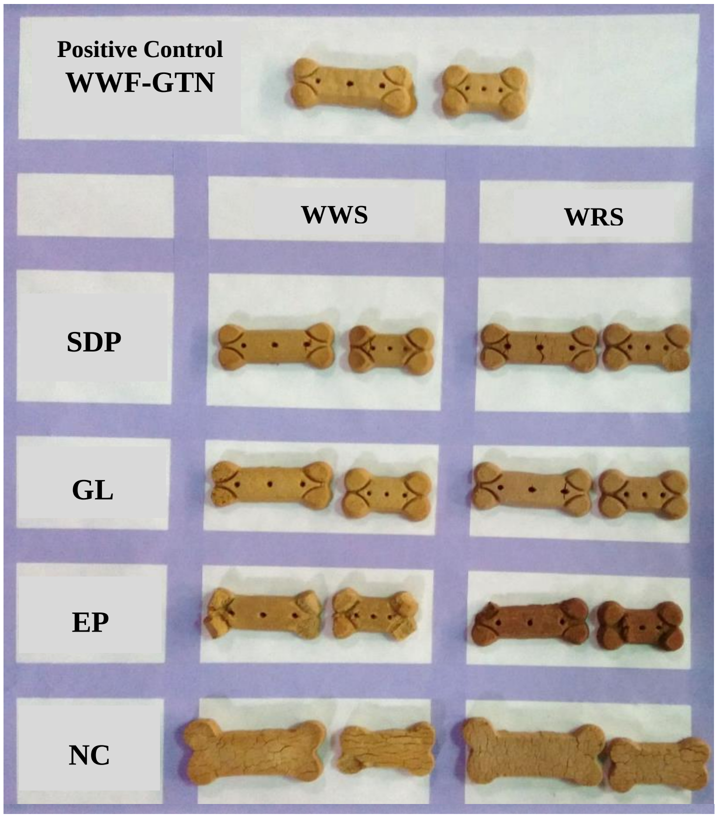

Figure 3.1 Baked dog treats produced with different cereals and soluble animal proteins combinations.

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

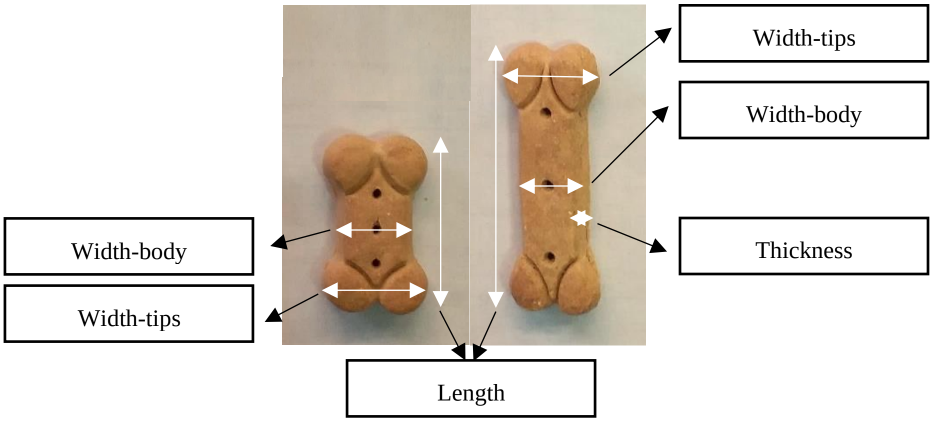

Figure 3.2 Dimension measurements of small and large baked dog treats produced with different cereals and soluble animal proteins combinations.

# Chapter 4 - Sensorial attributes, animal ranking evaluation, and shelf-life study of sorghum-based dog treats (biscuits) supplemented with soluble animal proteins

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## Abstract

The development of pet food products connects many activities that go from raw material and processing gaps to commercialization. However, one important step is validating and testing a product concept after it is manufactured. Therefore, the objectives of our study were to determine the effects of soluble animal proteins supplemented whole sorghum rotary molded dog treats on animal rank of preference, sensorial attributes, and shelf-life stability. The treats were produced in triplicate in a 2x4+1 augmented factorial arrangement of treatments. Two whole sorghum flours (WWS and WRS), four protein sources (none [NC], spray-dried plasma [SDP], egg protein [EP], and gelatin [GL]), and a positive control with wheat (WWF-GTN) were evaluated. A ranking test with twelve dogs was performed. Additionally, five highly trained panelists scored the intensity of appearance, aroma, flavor, texture/mouthfeel, and aftertaste attributes of the products. Finally, the biscuits were stored for 112 days at 30°C and 60% RH, and hexanal concentrations were measured on days 0, 28, 56, and 112. The data was analyzed using the statistical software SAS 9.4 for the animal and shelf-life evaluations with significance considered at a probability $P<0.05$; and for the descriptive sensory evaluation, a multivariate analysis on XLSTAT was performed. In the preference ranking test the dogs did not detect differences between WWF-GTN, WWS, or WRS treats when evaluated together. However, in the white sorghum evaluation with the different protein sources, the WWF-GTN, WWS-SDP and WWS-EP treatments were preferred. Because of their hard texture, the EP treatments led to some difficulties for the consumption by dogs. The panelists reported a high degree of variation in the appearance and texture across treatments. The WRS and WWS biscuits with SDP or EP were darker, while NC biscuits had more surface cracks. Initial crispness, hardness, and fracturability were very noticeable in EP treatments compared to all other sorghum treatments. The WWF-GTN was in between regarding hardness. The predominant flavor and aftertaste identified were described as grainy. The hexanal values for all biscuits were <1.0 mg/kg except for the EP treatments that had considerably higher hexanal concentrations (2.0-19.3 mg/kg) across the duration of the evaluation. This work indicated that the replacement of WWF-GTN by WWS and WRS, along with soluble animal proteins like SDP or GL would not affect the animal acceptability, sensorial attributes, and shelf-life performance. However, additional research should be conducted with EP to obtain more comparable results.

## Abbreviations

WWF, whole wheat flour; WWS, whole white sorghum; WRS, whole red sorghum; GTN, gluten; NC, no protein; SDP, spray-dried plasma; EP, egg protein; GL, gelatin; PCA, principal component analysis; AP, appearance; A, aroma; F, flavor; T, texture; A, aftertaste

***Keywords*:** beagle dogs, descriptive panel, hexanal, palatability, ranking test, shelf-life

## 1. Introduction

The development of new products involves many steps, such as identifying the product and market requirements, developing and testing the concept, defining and developing the product, sourcing from suppliers, planning the manufacturing process, and the marketing program design (Wang et al., 2012). In pet treats, it is also important to assess the acceptance by dogs, their people, and their stability throughout storage to assure nutritional quality and palatability.

Multiple approaches have been studied in dogs as methods of understanding their preferences since they cannot provide verbal feedback. For instance, food choice has been conducted as preference and acceptance tests, wherein two types of food (two-bowl test) or only one type of food (single-bowl test) is displayed (Tobie et al., 2015). The preferred food is determined by the total quantity eaten. Other researchers prefer using operant methods in which the animal is required to show a response (press a lever) and access a food (Rashotte & Smith, 1984). However, in cases where more food options are intended to be compared, and there is no intention for the animals to consume excessive quantities of food, other approaches, such as a ranking test, are applied. The ranking test is a forced-choice test that allows one to understand a preference based on multiple comparisons of ingredient aromatics and flavors, and determines attitudes towards food when offered repetitive times (Li et al., 2017). This technique of determining the acceptability of a product over other options is important considering that 44% of U.S. consumers purchase pet food and treats when their pet shows a positive attitude towards the flavor (Dornblaser, 2017). Moreover, the behavior of pets can influence their feeding time, quantity, and type of food provided (Day et al., 2009).

Similarly, human perception is essential because the owner interacts with the pet treat and the animal response. Most pet owners look for treats and snacks marketed as raw, natural, organic, U.S. sourced, with functional claims, with limited ingredients, with exotic proteins, and with clean labels that resemble human foods (Sprinkle, 2019). Moreover, the brand is also associated with quality and helps with the selection process. For instance, in a study conducted in New Zealand with 103 pet owners, 62% replied that they were loyal to a brand (Surie, 2014). The cues and attitudes of the animal towards the food can also help the owners in purchasing decision; however, because the owners do not consume pet food (typically), and feedback from the animals is partially interpreted, pet owners also consider sensorial attributes such as appearance and aroma, with color the most influential attribute (Di Donfrancesco et al., 2014). Nonetheless, the reasons behind palatable and unpalatable food can be better understood with a detailed breakdown of the sensorial attributes identified in a product by a trained panel, even though the real perceptions of taste and flavor differ from humans to dogs or cats (Koppel, 2014).

The shelf-life of the product is a period in which a product maintains acceptable quality, specific functionality, and safety (Young, 2011). Low-moisture crackers, biscuits, or treats generally have a long shelf-life due to their low water activity that prevents pathogenic and spoilage microorganism growth (Bramoulle et al., 2013). Although, loss of crispness and lipid oxidation can occur because of moisture adsorption and penetration of oxygen and (or) light (Galić et al., 2009) during long term storage. The moisture adsorption is usually controlled with suitable packaging. However, the oxidation process can still occur, and is generally the main reason for quality decline during storage (Manzocco et al., 2020). With lipid oxidation, secondary volatiles such as hexanal are produced. They can impact food quality and negatively alter the organoleptic, nutritional, and shelf-life properties of a product (Jeleń & Wąsowicz, 2011).

To assess these finished product attributes, the objectives of our study were to determine the effects of whole wheat containing dog treats versus those produced with whole sorghum supplemented with soluble animal proteins on the sensorial attributes, ranking preferences with the target species, and the shelf-life.

## 2. Material and Methods

### *2.1 Materials*

Rotary-molded baked dog treats were produced at a pilot research facility (Cookie Cracker Laboratory, AIB International, Inc.; Manhattan, KS). The experimental ingredients included whole wheat flour <180 µm (Ultragrain Hard, Ardent Mills, Denver, CO), whole white and red sorghum flours <150 µm (White Whole Grain and Burgundy Whole Grain, Nu Life, Scott City, KS), spray-dried plasma (Innomax Porcine Plasma, Sonac, Maquoketa, IA), egg protein (OvaBind®, Isonova, Spencer, IA), and gelatin (Pro-Bind Plus 50, Sonac, The Netherlands). Each of the treatments also included cornmeal (Enriched Corn Meal Yellow, Sysco), salt (Iodized Salt, Morton Salt Inc., Chicago, IL), molasses (Rich Brown Hue [40% - #715 and 60% - #677], International Molasses Corporation, Ltd., Saddle Brook, NJ), baking soda (Pure Baking Soda, Arm & Hammer, Princeton, NJ), nonfat dry milk (Nonfat Dry Milk Classic, Sysco), sodium bisulfite (Sodium Metabisulphite, LD Carlson Company, Kent, OH), inactive dry yeast (Nutritional Yeast, Bob's Red Mill Natural Foods, Milwaukie, OR), and all-purpose shortening (Premium All-Purpose Shortening, Ventura Foods, Brea, CA) (Table 2.1).

### *2.2 Animal Evaluation*

The biscuit order of preference was evaluated according to the preference ranking test for dogs developed by Li et al. (2017). The experiment was conducted at Kansas State University Large Animal Research Center (LARC) in five different phases of 5-day length each, under the Kansas State University Institutional Animal Care and Use Committee (IACUC) #4277 protocol. The test consisted of an acclimation phase in which commercial dog treats (Milk-Bone Flavor Snack Dog Biscuits, Big Heart Pet Brands Inc., San Francisco, CA) were provided. It was followed by evaluation of white sorghum treatments, red sorghum treatments (both compared to WWF-GTN), and a final ranking test comparing WWF-GTN to selected white and red sorghum treatments. The treatments for the last phase were chosen based on the results obtained in the two previous phases. The white sorghum treatments were reevaluated before the last phase due to a lack of dog responses on the first trial.

For this study, twelve Beagle dogs (4 females and 8 males) aged 5.58 ± 0.23 years old were used. They received two main feedings per day (0800 and 1100) before starting the trial at 1600 each day. Biscuits from all prior production replicates were blended into their respective composite samples. In each test, 3.0-5.0 g of biscuit was placed into a numbered hollow rubber toy (Kong®). Each dog was first allowed to sniff each toy+treat individually, then five toys+treats, in a randomized order, were evenly distributed on the floor in a corner of the experimental pen. The pen had an area of approximately 1.5m x 1.5m in a room which was separate from all other dogs. The time recording started from the moment the dog was released until it ate each treat. Each empty rubber toy was picked up from the floor and its number (sample identification) was recorded. Each dog was allowed to continue with the test until all treats had been removed from the toys.

#### *2.2.1 Statistical Analysis*

The ranking scores were analyzed with ANOVA Cochran-Mantel-Haenszel statistic, which is a generalization of Friedman's test using the FREQ Procedure by statistical analysis software (SAS 9.4 Inst. Inc., Cary, NC). Then, the rank means were separated using Tukey's HSD (Honest Significance Difference) test and considered significant at a probability of $P<0.05$ using the GLIMMIX procedure by statistical analysis software (SAS 9.4 Inst. Inc., Cary, NC).

### *2.3 Descriptive Sensory Evaluation*

Descriptive analysis was conducted at Kansas State University Center for Sensory Analysis and Consumer Behavior under the IRB protocol #5930. In this work, five highly trained panelists scored the intensity of appearance, aroma, flavor, texture/mouthfeel, and aftertaste attributes of the biscuits. A consensus method and intensity scores were used based on a 15-point scale (0= none to 15=extremely high) with 0.5 increments according to the work of Di Donfrancesco (2012). Each of the sensory panelists had more than 120 h of descriptive analysis panel training with a variety of products, including dry cat and dog food. They were trained on techniques and practices for attribute identification, terminology development, and intensity scoring.

For this evaluation, biscuits from different replicates were blended into a composite. Each sample was randomly assigned a 3-digit code. For appearance, flavor, texture/mouthfeel, and aftertaste evaluation, one small biscuit was served in a 100 mL cup and provided individually to each panelist. For the aroma evaluation, one large biscuit was crushed and served (approximately 15 g) in a medium glass snifter; two panelists shared a snifter. Hot towels, cucumbers, and water were provided to assist panelists as a cleanse. The evaluation was divided into three phases. On orientation day 1, the panelists smelled and tasted the samples to generate a lexicon of attributes according to Di Donfrancesco et al. (2012). Then, the panelists evaluated three treatments per day for a duration of 3 days. Finally, a single day side-by-side evaluation was conducted to confirm scores.

The attributes identified and generated by the trained panelists were brown, color uniformity, surface roughness, and surface cracks for the appearance. For the aroma, attributes such as overall intensity, grain, musty/dusty, toasted, cardboard, stale, and sweet aromatics were detected. The identified flavors were grain, cardboard, leavening, starchy, toasted, and sweet aromatics. Moreover, the texture/mouthfeel attributes detected were initial crispiness, hardness, fracturability, gritty, cohesiveness of mass, and particles. Finally, grain, cardboard, starchy, and toasted were perceived as aftertaste attributes (Table 4.3).

### *2.3.1 Statistical Analysis*

A multivariate analysis approach was applied to the perceived attributes using XLSTAT (Addinsoft, New York, USA) and a Principal Component Analysis (PCA) was performed to differentiate the biscuit treatments relative to the sensorial characteristics. To determine linear correlations across the attributes, Pearson correlation coefficients were used with significance considered at $P<0.05$. Radar charts were also plotted in Excel to visualize the relationships among treatments and attributes.

### *2.4 Shelf-life Evaluation*

Samples were kept frozen (-18 °C) prior to this evaluation. Approximately 50 g of biscuits per replicate were placed into a whirl-pak bag, each with four pinholes and kept in an environmental chamber at 30°C and 60% relative humidity for evaluation at 0, 28, 56, and 112 days. At each time point samples were removed and frozen (-18°C) prior to analyzing aromatic compounds. For the sample preparation biscuits were ground in a coffee grinder and 0.5 ± 0.02 g of the pulverized sample was weighed into a 10 mL screw-cap vial to which 0.99 mL of distilled water was added. The extraction of the volatiles was performed by headspace-solid phase microextraction (HS-SPME). A 50/30 μm DVB/CAR/PDMS fiber was exposed to the sample headspace for 20 minutes. The isolation, tentative identification, and semi-quantification of the volatile compounds were performed on a gas chromatograph (GC-2010 Plus; Shimadzu, Tokyo, Japan) coupled with a mass spectrometer (MS) detector (GCMS-QP2020; Shimadzu, Tokyo, Japan). The GC-MS system was equipped with an SH-Rxi-5Sil MS cross bond column (Shimadzu, Tokyo, Japan; 30 m × 0.25 mm × 0.25 μm film thickness). The column was heated from 40°C to 240°C. The ion source was set at 200°C and the mass spectrometer scanned for masses between 35 and 350 m/z. Volatile compounds were identified using the NIST library. All treatments were analyzed in triplicate. Volatiles were considered for a sample if detected in 2 or 3 of the 3 replicates. Hexanal was reported and calculated against 10 µL 100 ppm 1,3-dichlorobenzene as the internal standard.

#### *2.4.1 Statistical Analysis*

The data processing, analysis of variance, and least-squares means separation for repeated measures across time was performed using the GLM procedure of the statistical analysis software (SAS 9.4 Inst. Inc., Cary, NC). For the least-squares means separation Tukey’s HSD (Honest Significance Difference) test was applied and were considered significant when the probability was *P*<0.05. Two different models were generated: a one-way ANOVA comparing the nine treatments across day and a one-way ANOVA comparing time within treatment.

## 3. Results

### *3.1 Animal Evaluation*

The ranking results correspond to 10 dogs because two lost interest as the study was being conducted. Lower values indicate more preferred treatments. In the white sorghum evaluation, the WWF-GTN, SDP, and EP treatments were comparable and preferred (*P*<0.05) over NC. The GL was less preferred than EP but equally accepted relative to the SDP and WWF-GTN treatments. In the red sorghum evaluation, there were no differences among treatments (*P*>0.05); however, lower numerical values were associated with SDP, EP, and WWF-GTN treatments. Based on the results of the individual phases, an analysis comparing the proteins SDP and GL from white and red sorghum vs. the positive control (WWF-GTN) was merited. These treatments were selected based on their similar protein content and considering the difficulties observed for the dogs in eating the EP treatments due to their harder texture. In this last comparison, no differences were found between treatments (*P*>0.05); nonetheless, lower numerical values were observed for the sorghum treatments. Wherein the SDP treatments tended to have the lowest values, followed by the GL. Also, the white sorghum treatments had lower rank values within the same protein source (Table 4.1).

The average time the dogs took to complete the white sorghum phase was slightly shorter than the red sorghum phase (2.2%). However, opposed to what occurred in the individual phases, the average time in the combined evaluation was shorter for the WRS when compared to the WWS treatments. When average times were compared overall, they decreased from 50-150% in the final evaluation, most likely because the dogs were more acclimated to the study procedures with each phase of testing (Table 4.2).

### *3.2 Descriptive Sensory Evaluation*

Brown and surface cracks were the most differentiating appearance attributes, wherein WRS and WWS biscuits with SDP or EP resulted in a darker appearance (10.0-14.0), while NC biscuits had more surface cracks (10.0-12.0) (Figure 4.1). Aroma attributes did not vary substantially among samples except for the overall intensity that was higher for WRS-EP (7.0). Sweet aromatics were mostly imperceptible (< 2.0) for all treatments (Figure 4.2). Grainy was the most perceived flavor with values ranging from 5.0-7.0. Other flavors such as cardboard, leavening, starchy, and toasted were perceived at lower proportions (2.0-4.0), while sweet aromatics were almost unnoticed (<1.0) (Figure 4.3). Initial crispness, hardness, and fracturability were very pronounced in EP treatments (11.0-14.5) in comparison to all other sorghum treatments (4.0-9.0). The WWF-GTN treatment was higher than SDP, GL, and NC treatments regarding hardness (10.0) but had lower initial crispiness (6.0) and fracturability (5.0). All biscuits had less cohesiveness of mass and more particle residuals than the control WWF-GTN (Figure 4.4). The predominant aftertaste of all the samples was grainy with values that ranged from 4.0-6.0 (Figure 4.5).

It was found that brown appearance had a strong positive correlation with musty/dusty aroma (r=0.944) and initial crispiness (r=0.891). Moreover, aroma attributes such as grain had a strong positive correlation with the overall aroma intensity (r=0.808) and toasted aroma with stale aroma (r=0.922). Regarding the texture attributes, initial crispiness had a strong positive correlation with musty/dusty aroma (r=0.868) and biscuits fracturability (r=0.860) (Tables 4.4, 4.5 & 4.6).

An overall picture of the attributes perceived per treatment is presented in the biplot obtained by PCA (Figure 4.6). (The components F1 and F2 explained 49.43% of the variation in the dataset) wherein, hardness, toasted flavor, cardboard aroma, initial crispiness, and overall intensity aroma were the attributes that explained a large proportion of the total variation. The PCA clustered similarly perceived samples (NC, SDP, and GL) regarding their sensorial attributes with most of them in the negative quadrant of component 1 (F1). Nonetheless, the EP treatments were separated and located in the positive quadrant of component 1 (F1). The WWF-GTN treatment was not part of the main cluster; however, it was also located in the negative quadrant of component 1 (Figure 4.6).

### *3.3 Shelf-life Evaluation*

Hexanal is an aldehyde that originates from the oxidation of unsaturated fatty acids such as linoleic acid within a food matrix. Therefore, it can be used as a marker of oxidative rancidity. The values of hexanal obtained for all biscuits were relatively low in all treatments (<1.0 mg/kg) except for the EP that had considerably higher hexanal concentrations (2.0-19.3 mg/kg) across the duration of the evaluation (112 days). The hexanal concentration for the EP treatments, especially when WRS was the cereal source produced a hexanal peak that was more noticeable on day 0. Contrary to what was expected, the hexanal concentrations declined over time for the WRS-EP and WRS-GL treatments. For the rest of the treatments, the hexanal values remained relatively constant throughout the evaluation timeline (Table 4.7).

## **4. Discussion**

### *4.1 Animal Evaluation*

Throughout years of evolution, dogs have retained many ancestral eating behaviors. For instance, dogs rely heavily on olfactory senses when offered any food. Some research shows that olfactory sense is critical to discerning preferred versus non-preferred foods (Houpt et al., 1982). However, it is not well understood whether the odors of the preferred foods are more hedonically appealing (Hall et al., 2017). Also, dogs usually do not take much time masticating and savoring as they regularly eat in a gluttonous manner (Aldrich & Koppel, 2015). Dogs possess only a fraction of the taste buds in comparison to humans (Koppel, 2014). Nonetheless, dogs can detect sour, bitter, salty, sweet, and umami flavors when stimulation of these chemoreceptors occurs (Barnett, 2020). Therefore, it can be inferred that their highly developed sense of smell (>220 million olfactory receptors) contributes to a greater degree their overall flavor perception as the nose concentrates, moisturizes, and directs odorized air toward their olfactory epithelium which assures that warmed molecules are more easily detected (Castillo, 2014; Padodara & Jacob, 2014). In addition, dogs have different bite forces that increase with higher body weight and size of the skull which can also be influenced by the dog's chewing enthusiasm, personality, breed, and training (Kim et al., 2018).

Similar to humans, dogs choose food based on its palatability. This is influenced by a combination of taste, aroma, texture, size, appearance, temperature, and consistency (Griffin & Beidler, 1984). Moreover, their food preferences can also be determined by the genetics and early-life experiences (Bhadra & Bhadra, 2014). Our results could be explained by the combination of these factors, which were perceived by the animal after the various treatments were offered repeatedly. For instance, the EP treatments were numerically preferred over the other treatments, most likely because of a stronger aroma, especially when these treats were offered for the first time. However, these biscuits, particularly when combined with WRS, were quite hard and difficult to chew and consume which may have overridden the animals' interest. Therefore, dogs may have selected different treats than one might predict as the odor alone may not have been sufficient motivation to maintain a strong response across the multiple trials. Instead, the texture may have played an essential role regarding enjoyment while eating.

Lower dry matter and crude fiber of dry foods are thought to be parameters that can boost palatability and a dogs’ food preference (Alegría-Morán et al., 2019). Pétel et al. (2018) demonstrated that higher moisture can increase the elasticity and, probably, the porosity of kibbles, which may contribute to greater volatile (aroma) release. In our study, the biscuits did not differ for moisture across treatments with the average values fluctuating between the 3-8% recommended by Bramoulle (2013).

An important observation in this study was that the addition of protein sources increased the acceptance of the sorghum treats. In both individual phases, the treatments with no added soluble animal proteins had the highest numerical values (least preferred). For this reason, the NC treatments were not included in the final comparison. According to Nagodawithana et al. (2008), the hydrolysis of proteins can help enhance a product’s performance. One reason could be that the biogenic and volatile amines can influence the smell of a product. In turn this may increase product palatability given that the aroma of a food presented in the anticipatory phase of eating can increase the appetite (Zoon et al., 2016). Moreover, dogs tend to be highly sensitive to the tastes of amino acids, organic acids, and nucleotides that are mainly found in animal tissues (Case et al., 2011; Hidalgo & Takatsu, 2012).

In the preliminary phases, the finding that dogs ate the WWS faster relative to WRS was thought to be associated with the astringent flavor that has been reported for sorghum, especially when the pericarp is darker (House et al., 1995). Awika and Rooney (2004) indicated that red sorghums have significantly higher levels of extractable phenols than white sorghums. Nonetheless, a slightly different pattern was observed in the combined phase in which both WWS and WRS were analyzed. Thus, further investigation regarding this single parameter should be conducted to better understand the change.

Comparable to our study, Thompson et al. (2016) conducted a preference dog food study in two phases. In the first phase, the dogs sniffed and observed two products without being able to eat them, while in the second phase, the dogs were allowed to consume the products. The authors observed that the proportion of time spent by the dogs exploring the foods was correlated to their consumption. In the ranking test we conducted, the time allowed for sniffing each toy+treat before starting the trial was not recorded. However, the dog handler displayed each of the five treatments to the dogs for the same approximate amount of time. Nonetheless, the observation reported by Thompson et al. (2016) agreed with our study in which there was a substantial impact from aroma second to visual cues based on the dogs' selection.

### *4.2 Descriptive Sensory Evaluation*

The human sensory panel complemented the ranking test results and the physical measurements obtained by the instrumental equipment. Sorghum products have been previously evaluated regarding their sensorial attributes. For example, similar to our experiment, Chiremba et al. (2009) found comparable acceptance of red tannin-free sorghum biscuits in comparison to wheat regarding liking but not texture. In our case, the panelists found similarities across treatments regarding flavor and aftertaste as “grainy” was the predominant flavor and “overall intensity” the stronger aroma.

As discussed in the color analysis (Chapter 3, section 4.1.5) and the texture analysis (Chapter 3, section 4.1.3), the panelists identified darker hues in SDP and EP biscuits, and also for GL when combined with WRS. Visually, the NC treatments, because of their lack of added protein had more surface fissures/ cracks. Thus, the inclusion of proteinaceous ingredients corroborated once again their importance in increasing the hardness and cohesiveness from a production and consumer perspective. Similarly, as discussed in the animal evaluation (Chapter 4, section 4.1) the highly positive correlations found between initial crispiness with musty/dusty aroma and fracturability were mainly driven by the scores of the EP treatments. The panelists identified the EP treatments as very hard and difficult to bite, with values of 13.0 and 14.5 for the WWS and WRS, respectively. Peak bite forces in adult humans can go from 200 to 450 newtons (N) (Lieberman, 2011). As mentioned before, these treatments also presented eating difficulties for adult Beagle dogs. Adult dogs can have a wide range of bite forces. Lindner et al. (1995) evaluated 22 pet dogs between 7 to 55 kg and determined bite forces ranges from 13 to 1394 N with a mean of 256 N which closely resembles values reported in humans.

The sensory relationships described by the panelists regarding various attributes for color, aroma, and hardness (brown appearance with musty/dusty aroma and initial crispiness, and toasted aroma with stale aroma) may have been associated with the Maillard Reaction that occurred during baking. Among pet foods the Maillard Reaction is unique to this type of baked pet treat product and includes a group of reactions rather than a single reaction. In biscuit production, reducing sugars react with free amino acids when the product is heated during baking and promotes the brown hue formation on the surface, contributing to the texture and flavor (Leiva-Valenzuela et al., 2018).

Similarly, the predominant "grainy" flavor detected and the strong positive correlations between the "grainy" aroma and the "overall intensity" aroma could be influenced by the formulation of the products in which the main ingredient was a cereal (wheat or sorghum). According to Ma et al. (2017), high-carbohydrate (human) food is usually related to sweet taste, while the savory taste is associated with high-protein food (Griffioen-Roose et al., 2012). Savory taste refers to nonsweet taste and it is closely linked to the "umami," which is also described as a "broth-like" or "meaty" flavor (Yamaguchi & Ninomiya, 2000). In our evaluation, the sweet aromatics were only slightly perceived, whereas the savory taste was not identified. Therefore, the soluble animal proteins in the amounts added did not overshadow the predominant "grainy" taste from the high level of cereals.

Commonly, sweet and umami are well-accepted tastes by dogs and humans because they are associated with nutritive foods (Yarmolinsky et al., 2009). Despite the differences reported among species in sweet taste receptors and genes that influence the sweet taste responses (Bachmanov et al., 2011), the scores obtained from the panelists gave us a narrower idea of the attributes which existed in these products. Nonetheless, further research should be conducted to better understand these observations.

### *4.3 Shelf-life Evaluation*

Lipid oxidation is a process in which unsaturated fatty acids react with oxygen, creating intermediate products (lipid hydroperoxides) that are tasteless and odorless. These will be further decomposed into volatile compounds (aldehydes, ketones, and hydrocarbons) that can interact with food components (Mozuraityte et al., 2016). The secondary volatile products are important quality indicators because they degrade food quality and influence the organoleptic, nutritional, and shelf-life properties of a product (Jeleń & Wąsowicz, 2011).

Compounds such as peroxide value are an indicator of hydroperoxides, anisidine value is an indicator of non-volatile secondary oxidation products, free fatty acids are products of hydrolysis of the triglyceride, and organic volatiles are markers frequently used to quantify oxidative and hydrolytic rancidity (Bench, 2019; Mozuraityte et al., 2016; Velasco et al., 2010). In raw and processed cereals, hexanal is often considered as a good indicator of oxidation because of their high linoleic acid content (Gebreselassie & Clifford, 2016). Hexanal is a main product of n-6 polyunsaturated fatty acids oxidation. In cooked products it is mainly formed by autoxidation which occurs via a free-radical chain mechanism in an autocatalytic manner (Mozuraityte et al., 2016).

Oxidation can happen before and during the processing of biscuits. The oxidative stability of a product can be attributed to the ingredients, the processing, the antioxidants included, the packaging, and storage conditions (Galić et al., 2009). For this reason, some authors suggest analyzing the fat composition and level of oxidation that ingredients possess before making a product (Manzocco et al., 2020) because an ingredient with a very high oxidation level can lead to a rise in the level of primary oxidation products, and subsequently secondary oxidation products may accumulate after processing. It has been documented that there exists a high level of lipid oxidation in dough preparation due to the presence of active enzymes and oxygen available. It can also occur during baking, but in minor proportions (Caponio et al., 2008; Maire et al., 2013). Interestingly, the high baking temperatures can have a two-factor effect on a product. They can inactivate the enzymes responsible for oxidation (lipase and lipoxygenase) and also favor auto-oxidation (Maire et al., 2013). Additionally, the baking temperatures can produce Maillard Reactions Products (MRP) which to some degree are considered antioxidants (Barden & Decker, 2016). The MRP can act as oxygen scavengers or metal ion sequestrators, slowing the initial lipid oxidation and thereby hydroperoxide formation (Bressa et al., 1996).

Wheat and sorghum contain low levels of total fats that vary from (2.2-3.3%) and (3.9%), respectively. Additionally, the predominant fatty acids from wheat are linoleic (56.3%) and palmitic (24.5%); whereas, in sorghum, oleic and linoleic acids account for 84% of the total fatty acids making it highly unsaturated (Becker, 2007). In our study, the original level of hydroperoxides and secondary oxidation products was not analyzed in the ingredients before producing the biscuits. This could be why the initial hexanal level and stale aroma detected by the panelists were higher, especially in the EP treatments. Besides the WRS-GL and WRS-EP treatments that reduced hexanal content over time, most of the treatments had consistent values. This observation agreed with Mandić et al. (2013), who noted that hexanal content in refined and whole grain wheat and buckwheat crackers had values lower than 1.0 mg/kg until the sixth month. However, after that point, the values increased to > 5.0 mg/kg towards month 12 at ambient temperature (22 ± 2°C). Similarly, Sakač et al. (2016) observed a similar pattern during the first nine months of unpacked and packed gluten-free rice-buckwheat cookies stored at 23°C and 40% relative humidity for sixteen months. Nonetheless, these authors reported higher aldehydes values (2.05-3.93 mg/kg) when they combined the octanal, hexanal, and pentanal results. It is worth emphasizing that our study was conducted at a higher temperature and relative humidity and yet the biscuits had acceptable shelf-life stability. Though the cited studies all evaluated products with higher fat content (>20%) and for more extended periods.

The reduction of hexanal observed in some treatments could be explained by the possibility that some oxidative reactions involving hexanal occurred during the storage period. Similar findings were observed by Purcaro et al. (2008) who analyzed crispy bread for 12 months at 39-43 % RH. However, further evaluation should be performed characterizing the spray-dried plasma, egg protein, and gelatin level on markers of oxidation.

Another factor that can influence oxidation is the level of iron in a product due to its ability to enhance the propagation of lipid peroxidation through the redox cycling even at very low concentrations (<50 ppb). This reaction creates free radicals that further attack labile molecules (Goddard et al., 2012; Minotti & Aust, 1992). The manufacturers reported that the whole flours used in our study contained iron; thus, some oxidation was expected. Our observations were similar to what was reported by Barden (2014), in which iron did not affect the oxidation stability of treats because of the low moisture in the product which most likely reduced its diffusion.

## 5. Conclusion

The dogs did not detect differences between WWF-GTN, WWS, or WRS treats when evaluated together. However, in the white sorghum evaluation, the WWF-GTN, WWS-SDP, and WWS-EP treatments were preferred. Nonetheless, the dogs had some trouble eating the EP treatments due to their hard texture. Results from the human sensory panel complemented the interpretation of the ranking test and better-defined differences in the product appearance and acceptability. The addition of different protein sources created more noticeable variation across treatments regarding their appearance and texture. The WRS and WWS biscuits with SDP or EP resulted in a darker appearance, while NC biscuits had more surface cracks. Initial crispness, hardness, and fracturability were very pronounced in EP treatments compared to all other sorghum treatments. The predominant flavor and aftertaste were grainy, which indicated that the soluble animal proteins did not interfere with the typical cereal notes of the treats. The hexanal values were not affected when SDP or GL were included as compared to WWF-GTN (<1.0 mg/kg); however, the EP considerably increased the hexanal concentrations (2.0-19.3 mg/kg) especially at the beginning of the study and throughout the evaluation (112 days at 30°C - 60% RH). It is recommended that another ranking test and descriptive sensory be performed over time to identify rancidity notes which would help predict shelf-life stability. Also, other aldehydes typical for rancidity development should be analyzed to identify the changes in their profile over a longer period.

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Table 4.1 Rank order preference of baked dog treats produced with different cereals and soluble animal proteins combinations.

| **Treatment** | WWF GTN | WWS NC | WWS SDP | WWS EP | WWS GL | WRS NC | WRS SDP | WRS EP | WRS GL | SEM | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WWF-GTN / WWS | 2.90 $^{bc}$ | 3.70 $^{a}$ | 2.84 $^{bc}$ | 2.36 $^{c}$ | 3.20 $^{ab}$ | - | - | - | - | 0.192 | 0.0001 |
| WWF-GTN / WRS | 2.84 | - | - | - | - | 3.28 | 2.82 | 2.84 | 3.22 | 0.200 | 0.2822 |
| WWF-GTN /WWS /WRS | 3.35 | - | 2.75 | - | 3.00 | - | 2.78 | - | 3.13 | 0.190 | 0.1619 |

a-c: Means with different lowercase superscripts within a raw represent statistical difference among treatments (*P*<0.05). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

Table 4.2 Average time (mm:ss.0) of ranking phases completion of baked dog treats produced with different cereals and soluble animal proteins combinations.

| **Treatment** | WWF GTN | WWS NC | WWS SDP | WWS EP | WWS GL | WRS NC | WRS SDP | WRS EP | WRS GL | Avg phase time |
|---|---|---|---|---|---|---|---|---|---|---|
| WWF-GTN / WWS | 00:23.37 | 00:24.56 | 00:23.57 | 00:22.67 | 00:21.81 | - | - | - | - | 0:00:23.20 |
| WWF-GTN / WRS | 00:27.56 | - | - | - | - | 00:32.18 | 00:24.30 | 00:30.49 | 00:34.04 | 0:00:29.71 |
| WWF-GTN /WWS /WRS | 00:13.01 | - | 00:14.74 | - | 00:14.17 | - | 00:12.49 | - | 00:13.49 | 0:00:13.58 |

a-c: Means with different lowercase superscripts within a raw represent statistical difference among treatments (*P*<0.05). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin, mm:ss.0=minutes:seconds.hundredths

Table 4.3 Definitions of sensory attributes evaluated in baked dog treats produced with different cereals and soluble animal proteins combinations (Part 1).

| **Sensory attribute** | **Definition** |
|---|---|
| **Appearance** | |
| Brown | Light to dark evaluation of brown color of product |
| Surface roughness | Indentations/bumps on surface; smooth to rough |
| Surface crack | The perceived amount of cracks on the surface |
| **Aroma** | |
| Overall intensity | The total intensity of all types of notes perceived |
| Grain | The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice, and oats. |
| Musty/Dusty | Dry, dirt-like aromatic associated with dry, brown soil |
| Toasted | A moderately browned/baked impression |
| Cardboard | The aromatic associated with carboard or paper packaging |
| Stale | The aromatics associated with wet cardboard that is characterized by a lack of freshness |
| Sweet aromatics | Aromatics associated with the impression of sweet substance |
| **Flavor** | |
| Grain | The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice, and oats |
| Cardboard | A flat flavor note associated with cardboard or paper packaging that may be associated with a stale characteristic |
| Leavening | The flat metallic somewhat sour and bitter aromatics associated with baking soda and/or baking powder in baked flour products |
| Starchy | The flat flavor note associated with raw or processed starch-based grain products |
| Toasted | A moderately browned/baked impression |
| Sweet aromatics | Aromatics associated with the impression of sweet substance |

Table 4.3 Definitions of sensory attributes evaluated in baked dog treats produced with different cereals and soluble animal proteins combinations (Part 2).

| Sensory attribute | Definition |
|---|---|
| **Texture/Mouthfeel** | |
| Initial crispiness | The intensity of audible noise at first chew with molars |
| Hardness | The force required to bite through the sample with molar teeth (until breaking). Evaluated on first bite down with the molars |
| Fracturability | The force with which the sample ruptures. Evaluate in the first bite down with the molars |
| Gritty | The perception of small, hard, sharp particles reminiscent of sand or granules in pairs after 5-7 chews |
| Cohesiveness of mass | The degree to which the mass holds together during mastication after 5 chews. *A drink is taken before evaluation |
| Particle (residuals) | The amount of small pieces of sample remaining in mouth just after swallowing. Refers only to particulate matter on mouth surfaces other than in and between the molar teeth. |
| **Aftertaste** | |
| Grain | The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice, and oats |
| Cardboard | A flat flavor note associated with cardboard or paper packaging that may be associated with a stale characteristic |
| Starchy | The flat flavor note associated with raw or processed starch-based grain products |
| Toasted | A moderately browned/baked impression |

Table 4.4 Pearson´s correlation values for appearance and aroma attributes from baked dog treats scored by the descriptive panel.

| | **Variables** | **Appearance** | | | **Aroma** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Brown | Surface Roughness | Surface Crack | Overall Intensity | Grain | Musty/ Dusty | Toasted | Cardboard | Stale | Sweet Aromatics |
| **AP** | Brown | **1** | 0.098 | -0.515 | 0.443 | 0.437 | **0.944** | 0.467 | 0.041 | 0.298 | 0.391 |
| | Surface Roughness | 0.098 | **1** | -0.179 | -0.278 | -0.337 | -0.083 | -0.014 | -0.120 | -0.153 | -0.472 |
| | Surface Crack | -0.515 | -0.179 | **1** | -0.103 | 0.070 | -0.372 | -0.534 | 0.620 | -0.335 | -0.140 |
| **A** | Overall Intensity | 0.443 | -0.278 | -0.103 | **1** | **0.808** | 0.543 | **0.720** | 0.395 | **0.687** | -0.156 |
| | Grain | 0.437 | -0.337 | 0.070 | **0.808** | **1** | 0.607 | 0.610 | 0.316 | 0.516 | -0.071 |
| | Musty/ Dusty | **0.944** | -0.083 | -0.372 | 0.543 | 0.607 | **1** | 0.505 | 0.217 | 0.396 | 0.313 |
| | Toasted | 0.467 | -0.014 | -0.534 | **0.720** | 0.610 | 0.505 | **1** | -0.024 | **0.922** | -0.305 |
| | Cardboard | 0.041 | -0.120 | 0.620 | 0.395 | 0.316 | 0.217 | -0.024 | **1** | 0.163 | -0.158 |
| | Stale | 0.298 | -0.153 | -0.335 | **0.687** | 0.516 | 0.396 | **0.922** | 0.163 | **1** | -0.369 |
| | Sweet Aromatics | 0.391 | -0.472 | -0.140 | -0.156 | -0.071 | 0.313 | -0.305 | -0.158 | -0.369 | **1** |
| **F** | Starchy | 0.271 | -0.214 | -0.093 | 0.638 | 0.486 | 0.496 | 0.605 | 0.538 | **0.752** | -0.378 |
| | Toasted | 0.278 | -0.342 | 0.322 | 0.622 | 0.376 | 0.390 | -0.013 | **0.754** | 0.085 | 0.164 |
| | Sweet Aromatics | 0.011 | **-0.777** | 0.083 | 0.188 | 0.100 | 0.026 | -0.270 | -0.052 | -0.248 | **0.699** |
| **T** | Initial Crispness | **0.891** | 0.167 | -0.422 | **0.709** | 0.658 | **0.868** | **0.735** | 0.179 | 0.575 | 0.035 |
| | Fracturability | **0.718** | 0.363 | -0.301 | 0.626 | 0.565 | **0.753** | 0.596 | 0.379 | 0.441 | -0.240 |
| | Particle (Residuals) | **0.702** | 0.025 | 0.059 | **0.681** | 0.612 | 0.662 | 0.314 | 0.426 | 0.204 | 0.236 |
| **AF** | Cardboard | -0.611 | -0.042 | 0.157 | -0.629 | **-0.767** | **-0.755** | -0.511 | -0.212 | -0.396 | 0.224 |

AP=appearance, A=aroma, F=flavor, T=texture, AF=aftertaste. Pearson values in bold are different from 0 (*P*<0.05)

Table 4.5 Pearson´s correlation values for flavor attributes from baked dog treats scored by the descriptive panel.

| **Variables** | | **Flavor** | | | | | |
|---|---|---|---|---|---|---|---|
| | | Grain | Cardboard | Leavening | Starchy | Toasted | Sweet Aromatics |
| **AP** | Surface Roughness | 0.361 | 0.087 | 0.332 | -0.214 | -0.342 | **-0.777** |
| **A** | Cardboard | 0.178 | 0.580 | 0.271 | 0.538 | **0.754** | -0.052 |
| | Stale | -0.531 | 0.338 | 0.386 | **0.752** | 0.085 | -0.248 |
| | Sweet Aromatics | -0.113 | -0.574 | -0.557 | -0.378 | 0.164 | **0.699** |
| **F** | Grain | **1** | 0.219 | 0.222 | -0.255 | 0.425 | 0.157 |
| | Cardboard | 0.219 | **1** | 0.423 | 0.520 | 0.302 | -0.302 |
| | Leavening | 0.222 | 0.423 | **1** | 0.194 | 0.078 | -0.363 |
| | Starchy | -0.255 | 0.520 | 0.194 | **1** | 0.466 | -0.186 |
| | Toasted | 0.425 | 0.302 | 0.078 | 0.466 | **1** | 0.459 |
| | Sweet Aromatics | 0.157 | -0.302 | -0.363 | -0.186 | 0.459 | **1** |
| **AF** | Toasted | -0.024 | 0.318 | -0.119 | **0.725** | **0.719** | 0.464 |

AP=appearance, A=aroma, F=flavor, AF=aftertaste. Pearson values in bold are different from 0 (*P*<0.05)

Table 4.6 Pearson´s correlation values for texture and aftertaste attributes from baked dog treats scored by the descriptive panel.

| | **Variables** | **Texture** | | | | | | **Aftertaste** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial Crispness | Hardness | Fracturab. | Gritty | Cohesiv. of Mass | Particle (Residuals) | Grain | Cardboard | Starchy | Toasted |
| **AP** | Brown | **0.891** | 0.646 | **0.718** | 0.188 | -0.064 | **0.702** | -0.137 | -0.611 | -0.123 | 0.236 |
| **A** | Overall Intensity | **0.709** | 0.177 | 0.626 | 0.613 | -0.499 | **0.681** | 0.283 | -0.629 | 0.024 | 0.486 |
| | Grain | 0.658 | 0.070 | 0.565 | 0.454 | -0.525 | 0.612 | 0.065 | **-0.767** | -0.054 | 0.259 |
| | Musty/ Dusty | **0.868** | 0.571 | **0.753** | 0.180 | -0.115 | 0.662 | -0.170 | **-0.755** | -0.189 | 0.426 |
| | Toasted | **0.735** | 0.594 | 0.596 | 0.484 | -0.302 | 0.314 | 0.138 | -0.511 | 0.115 | 0.179 |
| **F** | Starchy | 0.442 | 0.404 | 0.576 | 0.218 | -0.187 | 0.153 | 0.000 | -0.592 | -0.214 | **0.725** |
| | Toasted | 0.303 | -0.167 | 0.385 | 0.380 | -0.186 | 0.621 | 0.297 | -0.331 | -0.249 | **0.719** |
| **T** | Initial Crispness | **1** | **0.687** | **0.860** | 0.450 | -0.341 | **0.786** | 0.032 | **-0.713** | -0.134 | 0.166 |
| | Hardness | **0.687** | **1** | **0.680** | 0.008 | -0.093 | 0.223 | -0.251 | -0.443 | -0.170 | -0.048 |
| | Fracturability | **0.860** | **0.680** | **1** | 0.399 | -0.190 | 0.624 | -0.140 | **-0.776** | -0.565 | 0.220 |
| | Gritty | 0.450 | 0.008 | 0.399 | **1** | -0.082 | 0.545 | **0.783** | 0.000 | -0.218 | 0.123 |
| | Cohesiv. of Mass | -0.341 | -0.093 | -0.190 | -0.082 | **1** | -0.481 | -0.085 | 0.443 | -0.374 | 0.166 |
| | Particle (Residuals) | **0.786** | 0.223 | 0.624 | 0.545 | -0.481 | **1** | 0.305 | -0.497 | -0.089 | 0.137 |
| **AF** | Grain | 0.032 | -0.251 | -0.140 | **0.783** | -0.085 | 0.305 | **1** | 0.472 | 0.171 | 0.096 |
| | Cardboard | **-0.713** | -0.443 | **-0.776** | 0.000 | 0.443 | -0.497 | 0.472 | **1** | 0.254 | -0.286 |
| | Starchy | -0.134 | -0.170 | -0.565 | -0.218 | -0.374 | -0.089 | 0.171 | 0.254 | **1** | -0.081 |
| | Toasted | 0.166 | -0.048 | 0.220 | 0.123 | 0.166 | 0.137 | 0.096 | -0.286 | -0.081 | **1** |

AP=appearance, A=aroma, F=flavor, T=texture, AF=aftertaste. Pearson values in bold are different from 0 (*P*<0.05)

Table 4.7 Hexanal detection (mg/kg) in baked dog treats produced with different cereals and soluble animal proteins combinations.

| **Treatment** | **Shelf-life period** | | | | SEM* | *P*-value* |
|---|---|---|---|---|---|---|
| | Day 0 | Day 28 | Day 56 | Day 112 | | |
| WWF-GTN | 0.18 $^{b}$ | 0.27 $^{b}$ | 0.18 $^{b}$ | 0.19 $^{c}$ | 0.036 | 0.2996 |
| WWS-NC | 0.98 $^{b}$ | 0.44 $^{b}$ | 0.22 $^{b}$ | 0.11 $^{c}$ | 0.430 | 0.5208 |
| WWS-SDP | 0.74 $^{b}$ | 0.45 $^{b}$ | 0.33 $^{b}$ | 0.31 $^{bc}$ | 0.113 | 0.0842 |
| WWS-EP | 7.01 $^{b}$ | 6.30 $^{ab}$ | 3.29 $^{a}$ | 2.05 $^{ab}$ | 1.806 | 0.2385 |
| WWS-GL | 0.57 $^{b}$ | 0.36 $^{b}$ | 0.21 $^{b}$ | 0.21 $^{bc}$ | 0.155 | 0.3626 |
| WRS-NC | 0.82 $^{b}$ | 0.28 $^{b}$ | 0.15 $^{b}$ | 0.20 $^{bc}$ | 0.172 | 0.0836 |
| WRS-SDP | 0.70 $^{b}$ | 0.49 $^{b}$ | 0.40 $^{b}$ | 0.37 $^{bc}$ | 0.080 | 0.0729 |
| WRS-EP | 19.37 $^{aA}$ | 9.56 $^{aAB}$ | 4.38 $^{aC}$ | 3.52 $^{aC}$ | 2.473 | 0.0068 |
| WRS-GL | 1.35 $^{bA}$ | 0.24 $^{bB}$ | 0.21 $^{bB}$ | 0.24 $^{bcB}$ | 0.243 | 0.0256 |
| SEM** | 1.477 | 1.312 | 0.516 | 0.375 | | |
| *P*-value** | <.0001 | 0.0004 | <.0001 | <.0001 | | |

a-c: Means with different lowercase superscripts within a column represent statistical difference among treatments within each day ($P<0.05$)
A-C: Means with different uppercase superscripts within a row represent statistical difference among days within each treatment ($P<0.05$). WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin
*: reference to treatments **: reference to days

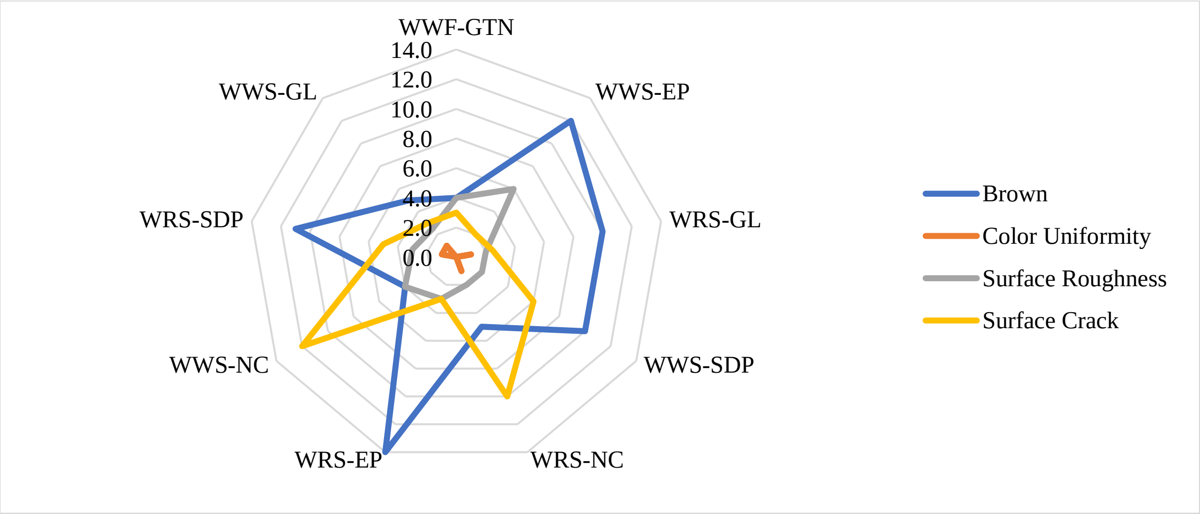

Figure 4.1 Radar chart for appearance attributes of baked dog treats produced with different cereals and soluble animal proteins combinations.
WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

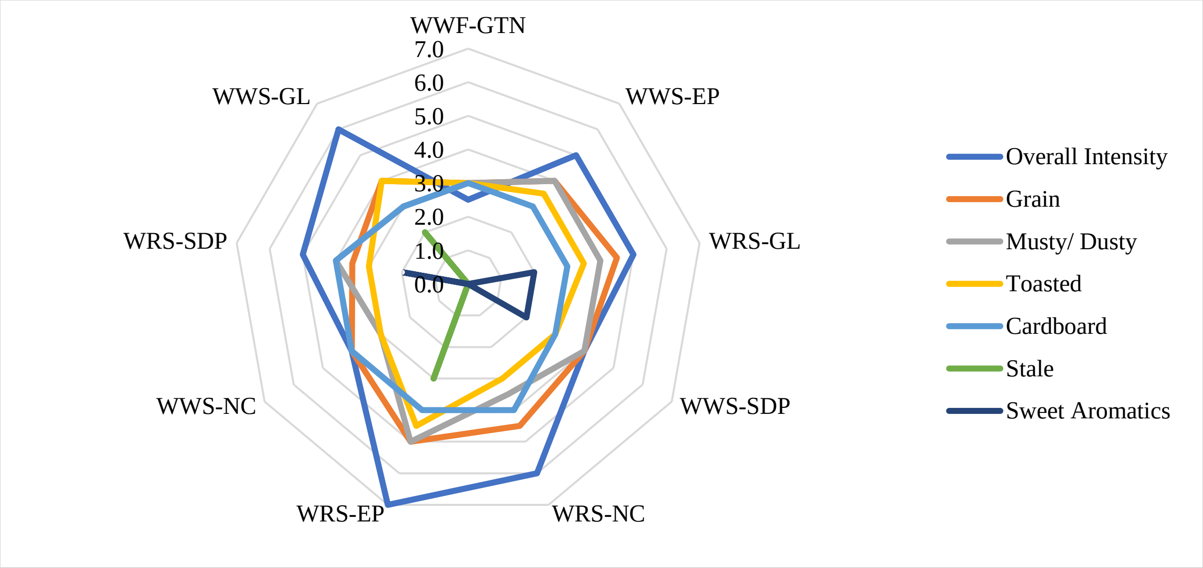

Figure 4.2 Radar chart for aroma attributes of baked dog treats produced with different cereals and soluble animal proteins combinations.

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

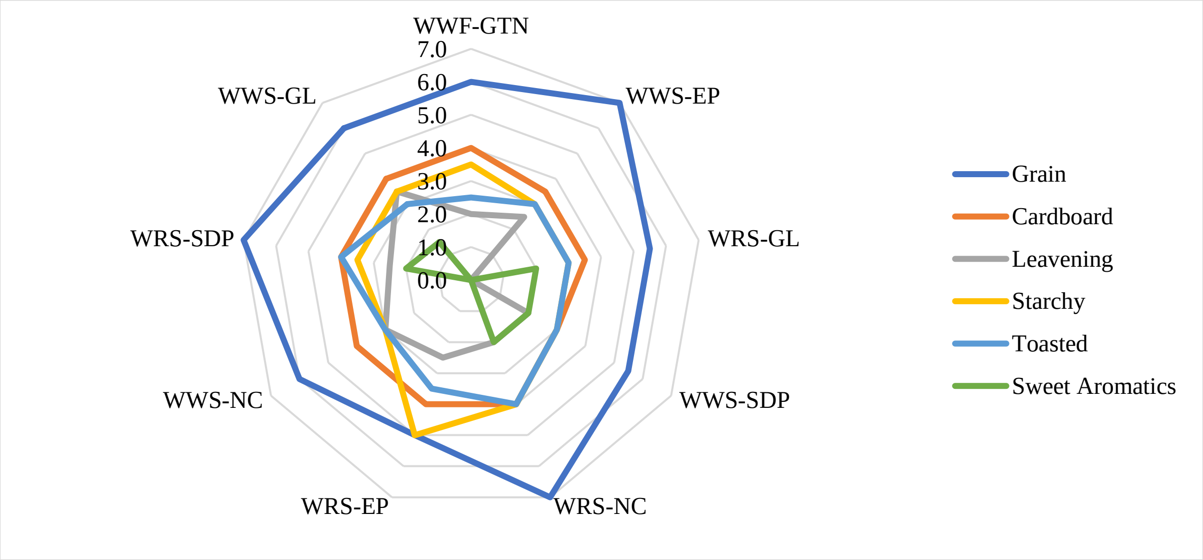

Figure 4.3 Radar chart for flavor attributes of baked dog treats produced with different cereals and soluble animal proteins combinations.

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

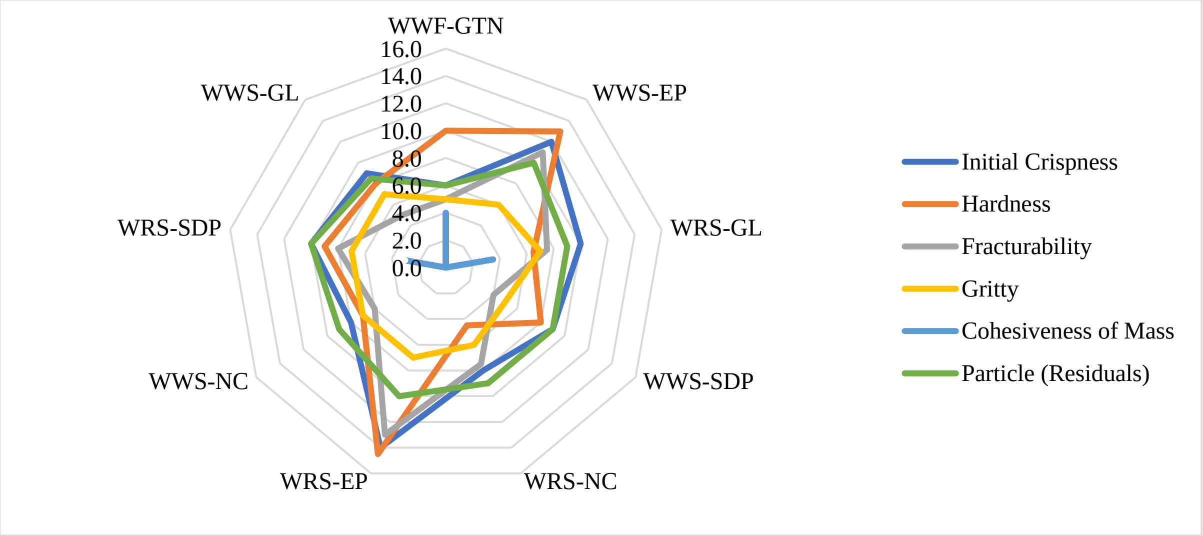

Figure 4.4 Radar chart for texture attributes of baked dog treats produced with different cereals and soluble animal proteins combinations.

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

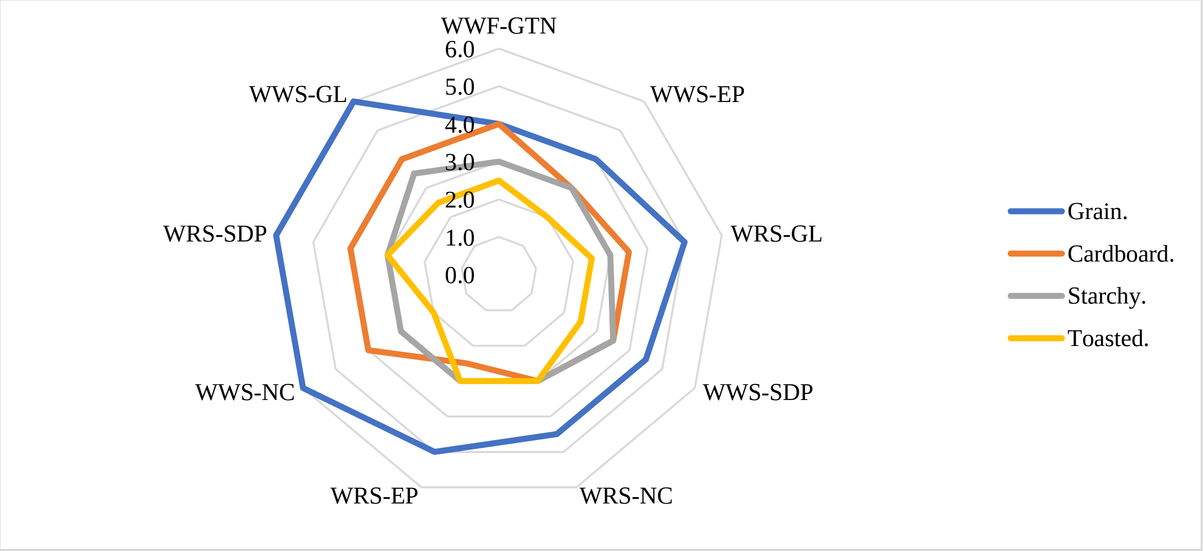

Figure 4.5 Radar chart for aftertaste attributes of baked dog treats produced with different cereals and soluble animal proteins combinations.

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

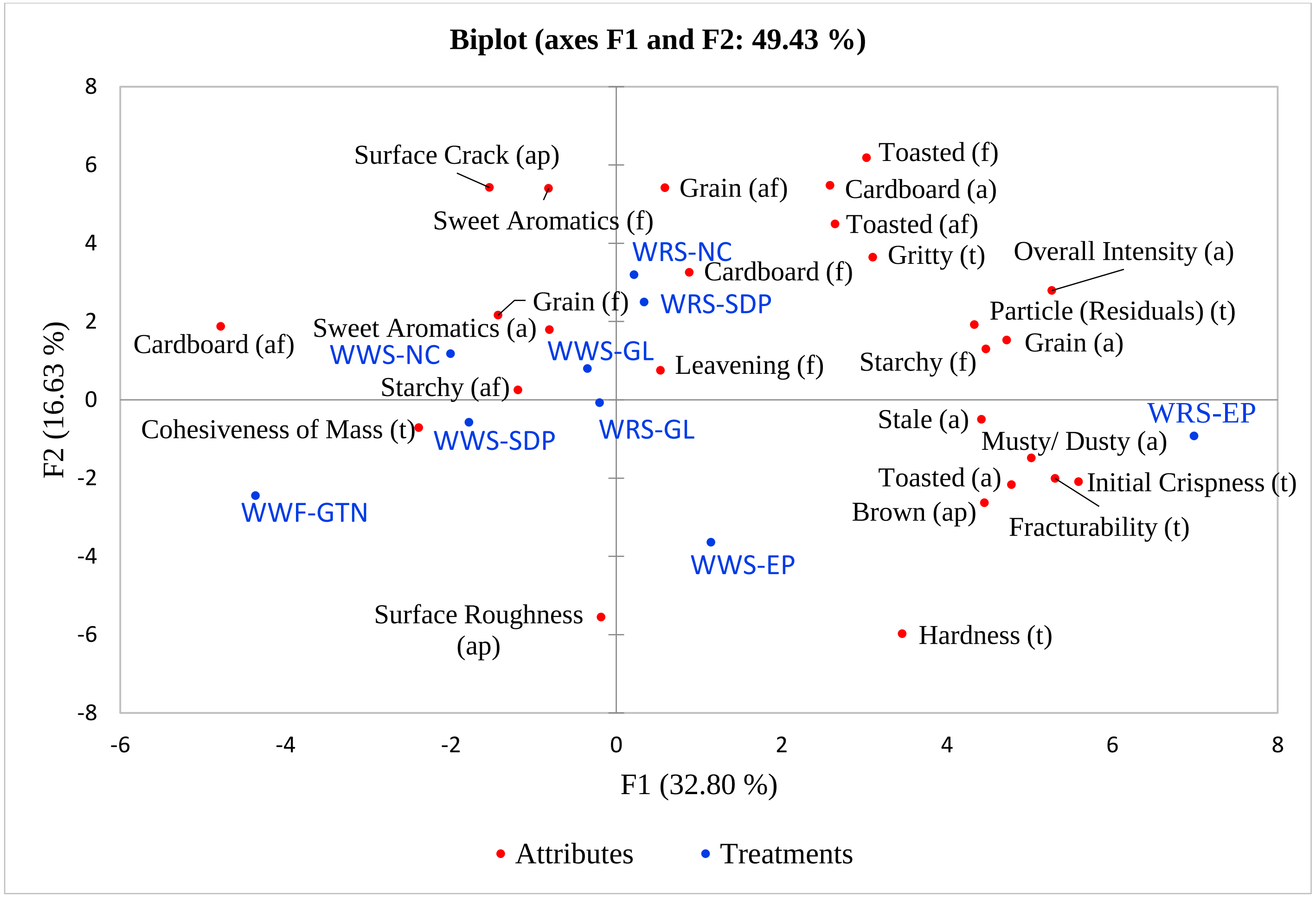

Figure 4.6 Principal component analysis (PCA) of appearance, aroma, flavor, texture, and aftertaste attributes of baked dog treats produced with different cereals and soluble animal proteins combinations.

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

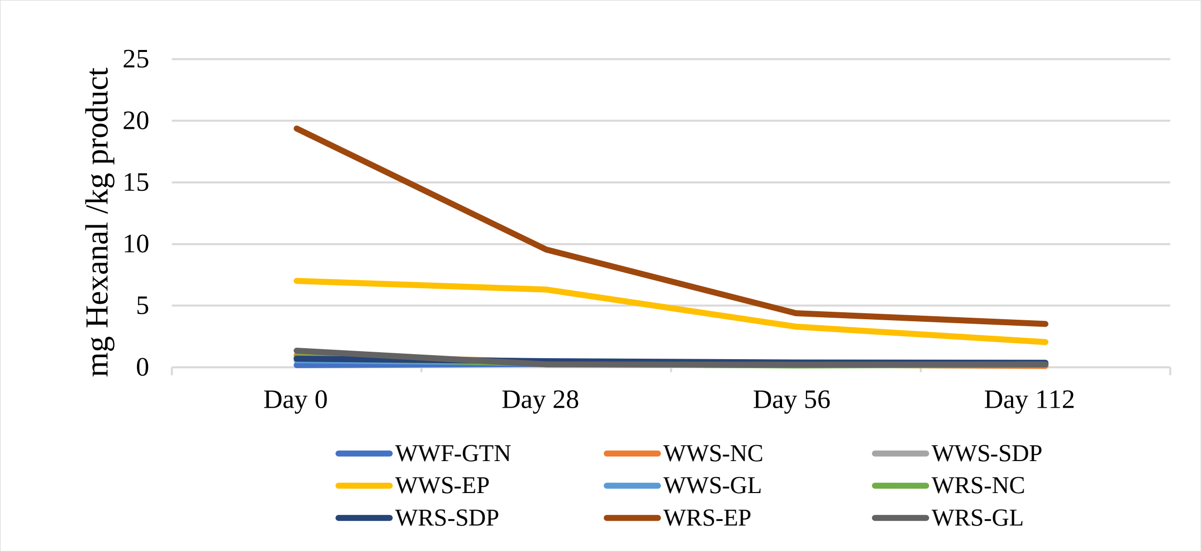

Figure 4.7 Hexanal detection (mg/kg) in baked dog treats produced with different cereals and soluble animal proteins combinations.

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin