Supplementation of gluten-free sorghum flour-based pet treat with animal protein sources: effects on dough and product quality, and animal acceptance

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SPONSORING ENTITY:

Sorghum Checkoff

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 characterize the flours and evaluate the quality of baked treats when white and red sorghum replaced wheat, combined with soluble animal proteins. The experiment was conducted as a 2x4+1 factorial arrangement of treatments and was replicated three times. Two whole sorghum flours (white [WS] and red [RD]), four protein sources (none [NC], spray-dried plasma [SDP], egg protein [EP], and gelatin [GL]), and a positive control with whole wheat flour [PC] were evaluated. Higher viscosities for the sorghum flours were found. The treatment with the highest crude protein was the EP and NC the lowest. Crude fiber was similar for all the sorghum treatments; however; for PC it was higher due to wheat content. The EP and PC treatments were the most break-resistant and GL and NC the most brittle. The GL addition produced larger dimensions in the rotary molder; however, they presented a lighter weight. Biscuits produced with white sorghum and wheat had more luminosity, hue angle, chroma and color b*.Dogs did not express preferences for white or red sorghum treatments. Sensory attributes were described by the trained panelists, differences were found for the EP and PC treatments. This work indicated that RD and WS along with a soluble animal protein like GL or SDP could produce suitable baked treats for dogs. Additional refinement will be necessary to produce treats in a commercial setting.

Keywords: biscuit, dog, soluble animal proteins, sorghum, baking, treat

Introduction

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 which63.4 million households have dogs. Sprinkle (2014), based on Simmons National Consumer Survey (2014) reported that 81.6% of the dogs' owners buy some type of treat with some regularity, also, APPA (2019) determined that owners spend $76 annually on food treats. Treats are products that are not provided to fulfill the nutritional demands of an animal, instead, they are mainly dispensed as a sort of reward.

The development of new products and the quick access to information propel the rapid growth in the human and pet food industry. This has led to the addition or substitution of ingredients and the implementation of new processing methods. In addition, consumers are more aware of the ingredients in their own food, as well as the foods they buy for their pets. Therefore, they demand foods that contribute health benefits.

Sorghum is one of the most widely produced grains in the world. It represents a great alternative to healthy food trends in human and pet diets. Sorghum is a rich source of vitamins such as thiamin, riboflavin, vitamin B6, biotin and niacin and is known to have antioxidant and antiradical activities (Anglani, 1998; Hagerman et al., 1998). Furthermore, it contains a slow starch digestibility that creates satiety, making it a functional food for the diabetic population (Ratnavathi, 2019). However, because it is a naturally gluten-free grain its dough is at a disadvantage with the mass of grains that have gluten since this protein provides better water absorption capacity, cohesiveness, viscosity, and elasticity (Wieser, 2007).

In the pet food industry, there have been studies reported with this grain in treats. Pezzali, Aldrich & Koppel(2019) developed granola bars for dogs with promising results. In their work, they used five binders (corn syrup, spray-dried plasma, gelatin, albumin, and egg product) to increase the cohesiveness of the grains in the bars for dogs. For baked dog treats (biscuits) most are made with wheat. In part because of the functional properties that gluten in the wheat has the texture and durability of the products.

Proteinaceous ingredients have been studied extensively, especially for gluten-free human products in order to improve the cohesivity and other functional properties in doughs that don't contain gluten. For example, Crockett, Ie, & Vodovotz (2011)added soy protein isolate and egg white solids in gluten-free bread. They found that higher levels of soy protein and egg white solids increased dough stability. Rodriguez Furlán et al.(2015)studied bovine plasma added to gluten-free bread and reported that textural properties were improved with homogenous and smaller air cells. Han et al.(2019) also used egg white in gluten-free batter. They concluded that egg white increased the elasticity of the batter and improved the physical properties of the final bread. The use of these ingredients may add nutritional value, enhance the physical properties, and create a new market alternative for companies supplying them.

However, there are no studies evaluating the acceptability of sorghum treats in dogs, and the use of protein binders in sorghum dough as potential ingredients for this application. Additionally, gluten-free foods are one of the top human market trends. Consequently, the development of innovative pet food goods that claim gluten-free ingredients and alternative processing methods represents an outstanding opportunity to meet the demand for pet owners.

Objectives

1- To determine the effects of producing baked treats with white and red sorghum flour rather than wheat flour

2- To determine the effects of soluble animal protein ingredients in baked dog treats produced with white and red sorghum flour on product quality, product stability, animal acceptance and descriptive sensorial attributes.

Material and Methods

Materials. 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^{TM} MPI Porcine Plasma, Sonac, Maquoketa, IA); egg protein (OvaBind®, Isonova, Spencer, IA); and gelatin (Pro-Bind Plus 50, Sonac, The Netherlands).

Experimental Design. The experiment was conducted as a 4x2+1 factorial arrangement of treatments in which four protein sources (Innomax^{TM} MPI 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.

Statistical Analysis. The data processing, analysis of variance and means separation was performed using the statistical analysis software Minitab® 18. For the means separation Tukey's honest significance test was applied, and means were considered significantly different at a $P < 0.05$.

Formula Development. Initially the formulas were intended to be isonitrogenous for the treatments that included the soluble animal proteins. However, it became evident during a preliminary experiment that the functionality of the proteins differed regarding the product quality. Thus, formulas were modified to adjust the soluble animal proteins in a manner that would create treats 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 further aid in meeting the objectives for obtaining a consistent dough (Table 1).

Treat Production. Three batches of 15 kg each were produced at a pilot research facility (Cookie Cracker Laboratory in the American Institute of Baking Pilot Plant; Manhattan, KS, U.S.A). 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 was obtained prior to transferring the dough into the feeder bin above the rotary moulder (70 PSI Weidenmiller)used to make the bone shaped treats (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 an convection oven for ~20-25 minutes at 190°C (Table 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 target moisture content was less than 10% and Aw less than 0.65. The trays plus the treats were weighed again to determine the evaporation loss rate, and these were allowed then to cool to room temperature. The treats 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 1).

Flour Quality

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, U.S.A.).

Total, Digestible and Resistant Starch Analysis. Starch in each source was evaluated by duplicate per each replicate using a digestible and resistant starch assay procedure (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) and amyloglucosidase (AMG)solution] under shaking in a water bath at 37°C for 20 minutes (Rapid 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. RDS, SDS and TDS were calculated based on the absorbance at 510 nm against a reagent blank. For the RS, a 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, repeating this procedure 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. An 8 mL of 1.0 M sodium acetate buffer (pH 3.8) and 0.1 mL of amyloglucosidase (AMG) was added and 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 x 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. RS was calculated based on the absorbance at 510 nm against a reagent blank.

Pasting Profile Analysis. Whole wheat, whole white sorghum and whole red sorghum flours were evaluated by quintuplet with a Rapid Visco-Analyzer (RVA, Perten Instruments AB, Hargersten, Sweven) according to AACC International Method 76-21.01 ICC Standard No 162 (Table 3). For the sample preparation 3.50 g of flour were mixed with approximately 25 ml of deionized water (corrected to 14% moisture content) into a canister, the slurries were mixed with a glass rod to avoid flour sedimentation, a paddle was placed into the canister and this fitted to the RVA. Peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BDV), final viscosity (FV), setback viscosity (SBV), peak time (Pt) and pasting temperature (PT)were obtained and analyzed through its software (Thermocline for Windows).

Treat Analysis

Proximate Analysis. Biscuits 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, U.S.A.).

Total Digestible and Resistant Starch Analysis. Starch from each source was evaluated by duplicate per each replicate using a digestible and resistant starch assay procedure (Megazyme International Ltd, Wicklow, Ireland). The procedure details are the same as explained in the flour quality section, with the exception that the samples were ground to pass a 0.5 mm screen.

Texture Analysis. Biscuits were evaluated regarding their texture with a TA.XT2 Texture Analyzer using the bone-style dog biscuits protocol (Texture Technologies Corporation, Hamilton, MA, U.S.A.) with minor modifications (Table 4). A total of 20 biscuits were randomly selected per each size and analyzed within each replicate. Individually, bones were placed over the three-point bend ring and then they were cut in the middle of the upper holes with the probe. Hardness and fracturability were analyzed through its software (Exponent Connect).

Dimension Analysis. Length, width and thickness for 20biscuits per each size and replicate were measured with a digital caliper (Fisher Scientific). Three different width measurements were taken per biscuit (1 for the body and 2 for the end-tips), weight was obtained with an analytical scale.

Color Analysis. External surface color was evaluated with a CR-410 chroma meter (Konica Minolta Sensing Americas, Inc.) calibrated with a white standard plate. A white cup was evenly filled with the bones, making sure the top surface of the cup was covered. The chroma meter was placed over the bones, 6 measurements were taken by replicate. For each treatment the results were presented in a triple stimulus scale (L* a* b*), where L* goes from 0 being black colorto 100 white color, a* goes from -60 to 0 for green color and from 0 to +60 for red color, and b* from -60 to 0 for blue color and

0 to +60 for yellow color. The hue angle and chroma were calculated from the a* and b* values, using the following formulas:

Hue angle = tan -1 (b*/a*) Chroma = $\sqrt{(\mathrm{a}\,*)^2 + (\mathrm{b}\,*)^2}$

Animal Evaluation. The biscuit "liking," and order of preference was evaluated according to the preference ranking proposal for dogs developed by Li et al.(2017)at Kansas State University. The experiment was conducted at the Large Animal Research Center (LARC) in five different phases with 5-day length for each, and conducted under the Kansas State University Institutional Animal Care and Use Committee approved protocol #4277. The test started with an initial acclimation phase, where commercial dog treats (Milk-Bone Flavor Snack Dog Biscuits) were provided. This was followed by the white sorghum treatments evaluation, the red sorghum treatments evaluation (both compared with the positive control), and a last ranking phase comparing white and red biscuits with the positive control. Before the last phase, the white sorghum treatments were reevaluated due to a lack of dogs' response on the first trial (Table 5).

For this evaluation, biscuits for all replicates were blended into a unique sample. For the preference ranking test ~3-5 g of a treat piece was placed into a numbered hollow rubber toy (Kong®) and presented to one dog at the time. Twelve Beagle dogs (4 females and 8 males) were used for this study for 25 days. They received 2 main feedings per day (0800 and 1100) prior to starting the trial at ~1600. Daily, 5 different treats, in a randomized order, were offered to the dogs. Each dog was first allowed to sniff the toy+treat individually and then the 5 toys+treats were evenly distributed on the floor at a corner of the experimental room. The room was away from all other dogs and the space for testing consisted of a small pen measuring approximately 1.5x1.5 m. The time was recorded from the moment the dog was release until it ate each treat. Each empty toy+treat was removed from the floor and its number (sample identification) was recorded. The order of selection was analyzed accordingly by Friedman analysis of variance, and means were separated using Tukey's honest significance test, with a significance level $P < 0.05$. The data were analyzed with statistical analysis software (Minitab® 18).

Microbiological Analysis. The treats were evaluated for total coliforms and salmonella prior to the human sensory panel descriptive evaluation. 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.

Descriptive Evaluation. The descriptive analysis was conducted at Kansas State University Sensory Analysis and Consumer Behavior Center. Five highly trained panelists scored the intensity of appearance, aroma, flavor, texture/mouthfeel, and aftertaste attributes. A consensus method intensity scores was 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 dried cat and dog food. They were trained on techniques and practice in attributes identification, terminology development, and intensity scoring.

For this evaluation, biscuits from different replications were blended into a composite. Each sample was assigned randomly a 3-digit code. For appearance, flavor, texture/mouthfeel, and aftertaste evaluation one small biscuit was served in a 3.25oz. cup and provided individually to each panelist. For the aroma evaluation, one large biscuit was crushed and served 1 Tbsp. in a medium glass snifter, two panelists shared a snifter. For cleaning-out, hot towels, cucumber, and water were provided to each panelist. The evaluation was divided into three sections, in 1-day orientation the panelists smelled and tasted the samples to generate the attributes according to Di Donfrancescoet al. lexicon (2012). Then, the panelists evaluated three treatments per day during a 3-day period, finally a 1-day side-by-side evaluation was conducted to confirm the given scores. The attributes evaluated for appearance were brown, tan, color uniformity, surface roughness and surface crack. Aroma was measured for overall intensity, grain, musty/dusty, toasted, cardboard, stale and sweet aromatics. For flavor descriptions grain, cardboard, leavening, starchy, toasted and sweet aromatics were assessed. For texture/mouthfeel initial crispiness, hardness, fracturability, gritty, cohesiveness of mass and particles (residuals) were evaluated. Finally, aftertaste was assessed for levels of grain, cardboard, starchy, toasted and sweet aromatics. A Principle Component Analysis (PCA) was conducted using XLSTAT software, and radar charts to visualize the relationships among treatments and attributes.

Results and Discussion

Treats Production

The dough temperature during production fluctuated from ~24-26°C and was not significantly different among treatments ($P>0.05$). The final dough weight depended on the water added during mixing (used to obtain an ideal undeveloped dough matrix). It ranged from 13.65 kg and 15.23 kg. Generally, the white sorghum treatments needed slightly less water added compared to the red sorghum treatments, very likely resulting in reduction of total dough weight. For the PC, WS-NC, RD-NC treatments the added water was intentionally maintained at the same levels evaluated in the preliminary trials, with the goal to produce a target quantity of 15 kg. For treatment RD-EP, it was observed that the total weight surpassed the 15 kg due to the doubling of egg protein which was necessary to achieve a good undeveloped dough for molding, and the red sorghum treatment needed more water (Table 6).

For the evaporation loss it was found that the treatments which had the higher losses were the negative control ones (~16-17%) mainly because these products didn’t have any protein to bind the free water. Additionally, these products were baked longer because they were manually made which resulted in thicker and larger biscuits with less surface area to release the internal moisture. On the other hand, the products that had the lower evaporation loss were the egg protein treatments (~9-11%), which might be due to the higher protein (Ovabind®) inclusion relative to the other treatments. However, because of the well-known protein functionalities and excellent water retention when different forces, pressure, or heat are applied (Zayas, 1997) there were no statistical differences in the moisture content or water activity (P>0.05) among all treatments after baking (Table 6).

Flour Quality

Proximate Analysis. The analysis of proximate constituents was performed on single replicates samples within the same batch, so no statistics are presented. On an absolute basis, the moisture content of whole wheat flour was higher than white whole grain sorghum flour or red whole grain sorghum flours. This was factor that limited external water addition to achieve an undeveloped dough suitable for release from the moulder. The crude protein content of wheat flour was the highest, followed by the red sorghum flour with white sorghum flour the lowest. In addition, the sorghum flours had higher crude fat compared to the wheat flour. The opposite rank was observed for crude fiber and ash (Table 7).

Total, Digestible and Resistant Starch Analysis. The total digestible starch of the sorghum flours was greater (P<0.05) than for wheat flour. However, the white sorghum had more (P<0.05) slowly digestible starch than red sorghum or wheat. The resistant starch was relatively low for all flours; however, the wheat flour had less resistant starch than either sorghum. The lower values for TDS and RD in wheat flour may be the result of less total starch content (Table 8).

Pasting Profile Analysis. Based on the RVA sequence analysis, the pasting curves of the flours were divided into four regions (Figure 2). Significant differences were found among all the flours tested (P<0.05). When increasing the temperature from 50 °C to 95 °C it was found that the pasting temperature (PT) was higher for the whole white sorghum flour compared to the whole wheat flour, however, the red sorghum flour was not different to the other flours (Table 9). These results were greater than those reported by Onyango et al. (2010) who suggested that the gelatinization temperature for sorghum starches were in a range of 71-80 °C. In the second region, when keeping the temperature at 95 °C, the peak viscosity (PV) for the whole white sorghum flour was the highest followed by whole red sorghum flour and whole wheat flour. According to Ragaee& Abdel-Aal(2006) the higher pasting viscosity or water-holding capacity can be driven by a higher starch content, which was confirmed by our study based on the total starch calculated and the guaranteed carbohydrate analyses reported by the flour suppliers. Also, it was suspected that the higher PV was due to smaller particle size of the sorghum flours (<150 μm) in accordance to what was reported by Bolade et al. (2009) when evaluating maize flour at different particles sizes. The peak time (Pt= time at PV) was not statistically different among the whole wheat or whole white flour, which required more time to form a paste structure (granules absorbing and swallowing water) than whole red sorghum flour (Table 9). This differences might confirm that starch properties exhibit differences depending on the cultivar, amylose and amylopectin ratio, amylopectin chain length distribution, swelling power, starch concentration and environmental conditions suggested by(Ahmed, 2017).

The trough viscosity (TV) for the whole white sorghum flour was the highest, followed by the whole red sorghum flour and whole wheat flour; surprisingly, the TV that was expected to be gotten in the 95°C holding period, fell on the third region, when cooling the samples from 95 °C to 50 °C. The breakdown viscosity (BDV= PV-TV) was higher for whole wheat flour and whole white sorghum in comparison to the whole red sorghum (Table 9). These results might suggest that the whole red sorghum had better tolerance to deformation under and shear stress and high temperature applied because it had the lowest BDV. These findings are aligned with what it was reported by Ragaee & Abdel-Aal(2006) who found that the whole sorghum grain exhibited better ability to withstand heat and shear when compared to soft wheat, hard wheat, barley, millet, and rye.

The final viscosity (FV) and setback viscosity (SBV= FV-TV) differed among all flours, with the whole white sorghum flour greater than the whole red sorghum flour and whole wheat flour (Table 9). This means that the rate of retrogradation and syneresis for the wheat flour was lower when cooling with the sample held at 50°C. This can also be attributed to more amylopectin content in comparison to the sorghum flours based on inferences of Rincón-Londoño et al. (2016) who reported corn starch rich in amylose and amylopectin. Keeping in mind that amylose-amylopectin ratio was not evaluated in our study.

Finally, the sorghum flours pasting profiles had similar patterns with a sharper peak in Region 4 compared to the whole wheat flour (Figure 2). The curves were similar to those reported by Ragaee & Abdel-Aal(2006); although, the values for (cP) in our study were near 100% higher. While our the plots differed from those reported by Pezzali et al.(2019)when evaluating white sorghum and red sorghum flours. This may be due to different type of cultivars or the difference in a whole ground flour versus a refined flour from decorticated sorghum used in their study.

Biscuit Quality

Proximate Analysis. For all the treatments the dry matter was >90%. The crude protein for the red sorghum treatments was ~2% higher than the white sorghum treatments when compared among the same animal proteins. The EP treatments was the highest protein due to double the amount of egg protein inclusion, followed by the GL and the SDP treatments. Each was greater than the PC treatment. The crude fat was very similar among treatments with the exception of WS-GL that were ~6% above the other treatments. This may be due to the shorter mix time which could have created inconsistent shortening distribution. The crude fiber was similar among the sorghum treatments (< 1.36%), and only the PC treatment had a slightly higher value due to its original content from whole wheat flour. The ash content was comparable for all the treatments (2.0-2.5%). The exception was the SDP treatments that were higher than the other treatments. This is likely due to the higher inorganic material (ash) in Innomax TM MPI Porcine Plasma. But this is speculation as it was not tested (Table 10).

Total, Digestible and Resistant Starch Analysis. The higher RDS was found in the PC and NC treatments, whereas the lowest values belonged to the SDP and EP treatments. For the TDS the NC treatments retained higher digestible starch values. This may be attributed to the higher levels of carbohydrates in these experimental treatments. Conversely, the RD-EP treatment had the lowest TDS value. The RS values were close among all the treatments. When comparing the protein used, they did not differ except for the RD-GL that contained less resistant starch. Finally, the total starch was slightly higher for the white sorghum treatments due to the original content of the flour.

Texture Analysis. The hardness is the maximum force (kg) needed to break each biscuit until it fractures and falls into two pieces. Additionally, the fracturability or distance(mm) at the point of break is the resistance of the sample to bend. The hardness for large and small treats required the same force regardless of the treat size. However, protein had an effect; whereas the WS-EP was similar to PC when comparing the small treats. The EP and PC were the most resistant to breaking followed by the SDP. The GL and NC did not differ and were the less resilient treatments. Numerically, the small biscuits were harder than the large ones: PC (~15%), WS-SDP (~1%), RD-SDP (~2%), WS-GL (~6%) and RD-GL (~13%), with the exception for WS-NC (~11%) and RD-NC (~15%). From these findings we can infer that the size had a greater effect when producing treats with wheat and red sorghum than for those with white sorghum (Table 12 & Table 13).

The fracturability followed a slightly different pattern; wherein the EP and PC treatments were the most resistant to bending regardless of the size. On the large biscuits, the GL treatments were the most brittle, while in the small biscuits all the treatments were similar with low resistance to bending (Table 12& Table 13).

Dimension Analysis. The shape of treats can play a role in purchasing decisions. In 2014, 47% dog owners purchased bone-shape treats (Beaton, 2015). Taking measurements of the treats allowed us to identify the uniformity and the differences that protein along with the type of flour can have on expansion and shape. When evaluating the large treats, the NC treatments were the heavier, longer, wider and thicker in comparison to the other treatments. This was likely due to their lower functional protein and the difficulty to form a full shape in the rotary molder. To acquire sufficient treats of reasonable shape and consistency we were forced to manually sheet and cut the treats. This resulted in larger and more variable treats for the final evaluation. While this was not the intent of the experiment and not a desirable outcome for true evaluation, it does demonstrate that the proteins were required to provide adhesion of the flour and create a product suitable for evaluation. This situation was similar in the NC small treats, with the only difference that the tip width was the smallest because of the mold size (Table 14 & Table 15).

When comparing the large biscuits produced in the rotary molder, it was found that GL addition allowed a slightly larger and wider biscuit, whereas the other treatments were not different for any dimension. In the small treats, the width at the center for the PC treatment was similar to the GL and greater than the other treatments. The tip width of the EP treatments was the smallest. This might be due to a higher water binding and lower dough elasticity for this treatment. During production, the tips were damage due to the need to manually extract them from the molds. For the thickness, PC treatment was the highest value. This may be due to the viscoelastic properties of gluten, which might have been enhanced by the addition of the baking soda and molasses. Wherein, this combination likely produced CO_2 which was trapped inside the gluten matrix (Lauterbach & Albrecht, 1994; Ortolan & Steel, 2017). Finally, the weight of the treats was a factor of all the treat dimensions. However, the GL treatments that had a low weight compared with its despite the dimensional measures. From the treats produced on the rotary molder the PC treatments were the heavier, while the EP were numerically the lowestin the large treats (Table 14 & Table 15).

Color Analysis. The lightness (L*) values for wheat and white sorghum flour, or when combined with GL were highest (Table 16). Conversely, the combination that produced darker biscuits were EP. This was most likely due to the naturally pigmented pericarp of each flour and Maillard (browning) reactions during baking. Maillard reaction occurs between reducing sugars and free amino acids (especially lysine) and peptides (D. Manley, 2011). Since EP treatments had higher protein levels, we also expected to have greater browning effects. Additionally, the milk powder in the formulations contained lactose which would have acted as reducing sugars.

The a* positive value coordinates belong to the red spectrum, and higher values indicate more intense reddish colors. As expected, the red sorghum treatments had higher values, except for the NC which was lower intensity red. Additionally, the b* positive coordinate measures the yellow spectrum and higher values indicate more intense yellow. Higher values were observed for PC, WS-SDP and WS-GL (Table 16). Based on these results, we could infer that these two parameters were dependent on the flour and protein addition which likely produced a Maillard reaction. The more intense of which could also be the result of baking time. According to Knerr et al.(2001) as the Maillard reaction occurs a quick development of a yellow color then turns into dark brown during prolonged heating.

Finally, the hue angle and chroma provides a better understanding of the color relationship. The hue angle is measured from 0° to 360° and is divided into four quadrants. The first quadrant (0°-90°) covers red to yellow, the second (90°-180°) covers from yellow to green, the third (180°-270°) from green to blue, and the fourth (270°-360°) from blue to red. Our results were in the first quadrant. Visually the biscuits were more yellow than red. This observation was corroborated with the hue angles closer to 90°. Moreover, chroma defines the perception of an object's efficiency to reflect or transmit light. Higher chroma means that the object can transmit more saturated light. For both parameters the trend was similar with higher values belonging to the white sorghum and wheat treatments (Table 16).

Animal Evaluation

With the aim to understand the dogs' food preferences, it is important to analyze the combination of attributes such as taste, smell, and texture of the biscuits. The results presented were from 10 dogs (two of the original 12 lost interest during the study). Lower rank values indicate a preference over higher rank values. In the white sorghum evaluation, the PC, SDP, and EP treatment were similar to each other and preferred ($P<0.05$) over NC and GL. In the red sorghum evaluation, there were no differences between treatments (Table 17).

This was unexpected for the red sorghum and could be due to harder texture noted above which could have hindered the interest in these products. This was pattern was visually perceived in the study when the dogs chose these products in which the animals were attracted the stronger aroma of EP, for example; but, refused to eat them.

Even though there were not statistical differences for the moisture within the treatments, we noticed a preference for the products with higher moisture. Our treats were low moisture and Brito et al. (2010) recommended not to go below 7% of moisture and Aw 0.4. Perhaps this corroborates their findings.

The average and total time that took the dogs to complete the white sorghumphase was shorter than for the red sorghum. The treatments that took longer time were the RD-GL followed by the RD-NC, and the RD-EP (Table 18). This may be associated with the astringent flavor that has been reported for sorghum, especially when the pericarp is darker (House, Osmanzai, Gomez, & Monyo, 1995). But, this should be evaluated more fully in a study to evaluate this aspect singly.

Based on these results, it decided that an analysis comparing the proteins SDP and GL from white and red sorghum versus the positive control was merited. These treatments were selected based on their similar protein values and considering the difficulties observed for the dogs in eating the EP treatments due to their hard texture.

The ranking results for the combined phase did perform as expected. There were no differences among the treatments (P>0.05). Numerically the SDP treatments tended to have the smallest values (highest preference), followed by the GL and then the PC. The white sorghum results were also lower values (higher preference) within the same protein source (Table 19).

In contrast to what occurred in the independent phases, the total phase time and average time in the combined phase was shorter for the red sorghum when compared with the white sorghum treatments (Table 20). However, if we compared the times overall, they decreased by ~40-60% most likely due to dogs increasing acclimation as the studies progressed.

Descriptive Evaluation

Similarities and differences were found among the treatments depending on the attribute evaluated. The RD-EP treatment was separated from the other products and received the highest scores in aroma (musty/dusty, overall intensity, stale, toasted, and grain). It also had the highest initial crispiness, fracturability, hardness, and residuals when the texture was evaluated. Its predominant flavor was starchy and its appearance was brown. The WS-EP treatment was also separated from the other treatments and had high scores in toasted aroma, hard texture, and surface roughness. The PC treatment was also separated from the other treatments and its principal attributes were toasted aroma, cohesiveness of mass, hard texture, and surface roughness. These three treatments had the lower scores in surface cracks and were not characterized with sweet aromatic flavors. All the remaining treatments were closer in the PCA and shared attributes such as grain flavor, sweet aromatic aromas, and starchy aftertaste (Figure 3).

Conclusions

It was feasible to produce white sorghum and red/burgundy grain sorghumflour dog biscuits with similar characteristics to the positive control made with whole wheat flour. However, a correct amount of the dried soluble animal-based proteins is required to have good production flow, and acceptable physical biscuit attributes.

To achieve product optimum comparable to wheat-flour it will require some additional refinements in the levels of the soluble proteins and the consistency of the whole sorghum flours to assure that a product will meet all the needs of constituents along the value chain.

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Table 1. Ingredient composition of control and experimental diets.

	Treatments								
Ingredient	**PC**	**WS-NC**	**WS-SDP**	**WS-EP**	**WS-GL**	**RD-NC**	**RD-SDP**	**RD-EP**	**RD-GL**
Whole wheat flour	56.300	0	0	0	0	0	0	0	0
Whole red sorghum flour	0	0	0	0	0	48.591	53.352	51.206	52.566
Whole white sorghum flour	0	48.591	53.487	52.374	53.295	0	0	0	0
Corn gluten meal	14.070	13.500	9.660	9.425	9.505	13.500	9.636	9.215	9.375
Spray dried plasma	0	0	4.830	0	0	0	4.818	0	0
Egg protein	0	0	0	9.048	0	0	0	8.846	0
Gelatin	0	0	0	0	4.087	0	0	0	4.031
Salt	0.560	0.496	0.544	0.530	0.535	0.496	0.542	0.518	0.528
Molasses	4.500	3.974	4.350	4.245	4.281	3.974	4.340	4.150	4.222
Baking soda	0.280	0.280	0.298	0.299	0.302	0.280	0.283	0.275	0.280
Nonfat dry milk	1.760	1.552	1.699	1.658	1.672	1.552	1.695	1.621	1.649
Water	19.700	29.118	22.409	19.764	23.644	29.118	22.617	21.571	24.706
Sodiumbisulfite	0.0023	0.0020	0.0022	0.0022	0.0022	0.0020	0.0022	0.0021	0.0022
Inactive dry yeast	0.0023	0.0020	0.0022	0.0022	0.0022	0.0020	0.0022	0.0021	0.0022
Allpurpose shortening	2.825	2.484	2.719	2.653	2.675	2.484	2.712	2.594	2.639
Total (%)	**100.000**	**100.000**	**100.000**	**100.000**	**100.000**	**100.000**	**100.000**	**100.000**	**100.000**

PC: positive control; WS-NC: white sorghum negative control; WS-SDP: white sorghum + spray dried plasma; WS-EP: white sorghum + egg protein; WS-GL: white sorghum + gelatin; RD-NC: red sorghum negative control; RD-SDP: red sorghum + spray dried plasma; RD-EP: red sorghum + egg protein; RD-GL: red sorghum + gelatin

Table 2. Production parameters for baked dog treats.

Treatment	Baking		Mixing (minutes)		
	Temp. (°F)	Time (min)	Dry-speed 1	Wet-speed 1	Wet-speed 2
PC	375	25	1	2	6
WS-NC	375+150	30+10	1	2	6
WS-SDP	375	20	1	2	6
WS-EP	375	20	1	2	4.5
WS-GL	375	20	1	2	4.5
RD-NC	375+150	25+10	1	2	6
RD-SDP	375	20	1	2	6
RD-EP	375	20	1	2	4.5
RD-GL	375	20	1	2	6

Table3. Flour pasting AACC International Method 76-21.01, ICC Standard No 162

Time	Type	Value	Units
0:00:00	Temp	50	°C
0:00:00	Speed	960	rpm
0:00:10	Speed	160	rpm
0:01:00	Temp	50	°C
0:04:42	Temp	95	°C
0:07:12	Temp	95	°C
0:11:00	Temp	50	°C
0:13:00	End		
Idle Temperature: 50°C ± 1°C			
Time Between Readings: 4 s			

Table 4. Bone-style dog biscuits protocol (Texture Technologies Corporation, Hamilton, MA, U.S.A.), modified in situ.

Tool	Conditions	
Bend	Adjustable TA-92. Three-point bend ring	
	Distance	19 mm apart
Probe	TA-42 knife blade with 45° chisel-end	
	Force	15 g
	Distance travelled	5.0 mm
	Descent speed	2.0 mm/sec
	Widhdrew speed	5.0 mm/sec

Table 5. Phases and treatments evaluated for the ranking test

Phase	Treatments
Acclimation (Milk-Bone Dog Biscuits)	Bacon, Turkey, Chicken, Sausage, Beef
White Sorghum (1st attempt)	PC, WS-NC, WS-SDP, WS-EP, WS-GL
Red Sorghum	PC, RD-NC, RD-SDP, RD-EP, RD-GL
White Sorghum (repeat)	PC, WS-NC, WS-SDP, WS-EP, WS-GL
White VS. Red Sorghum	PC, WS-SDP, RD-SDP, WS-GL, RD-GL

Table 6. Production parameters outputs for all the treatments.

Treatment	Dough temp. (°C)	Dough weight (kg)	Evaporation loss (%)	Moisture (%)	Aw
PC	24.33 ± 1.16	14.97 ± 0.20 ab	12.73 ± 2.09 b	7.64 ± 1.13	0.41 ± 0.07
WS-NC	26.10 ± 1.15	14.87 ± 0.05 ab	17.29 ± 2.76 a	4.72 ± 2.86	0.32 ± 0.27
WS-SDP	24.66 ± 0.58	13.81 ± 0.16 c	12.36 ± 2.33 b	5.49 ± 2.62	0.29 ± 0.25
WS-EP	26.00 ± 1.73	13.65 ± 0.20 c	9.53 ± 1.40 c	7.95 ± 3.63	0.35 ± 0.24
WS-GL	24.33 ± 1.53	13.69 ± 0.22 c	13.55 ± 2.17 b	6.26 ± 2.80	0.31 ± 0.20
RD-NC	25.10 ± 2.15	14.84 ± 0.05 ab	16.31 ± 2.15 a	5.46 ± 2.06	0.28 ± 0.20
RD-SDP	25.00 ± 0.00	14.64 ± 0.04 b	13.04 ± 1.88 b	6.73 ± 1.62	0.24 ± 0.04
RD-EP	25.00 ± 1.00	15.23 ± 0.09 a	10.89 ± 1.66 bc	6.56 ± 1.03	0.35 ± 0.17
RD-GL	24.00 ± 1.73	14.85 ± 0.01 ab	12.58 ± 2.53 b	5.97 ± 2.36	0.22 ± 0.08
PooledStDev	1.373	0.137	2.294	2.376	0.189
p-value	0.584	< 0.001	< 0.001	0.781	0.989

a-c: Means with different superscripts within a column represent statistical difference ($P<0.05$)

Table 7. Proximate analysis of whole wheat flour, and white and red/burgundy grain sorghum flours expressed on dry basis.

Whole flour	Moisture (%)	Crude Protein, DMB (%)	Crude Fat, DMB (%)	Crude Fiber, DMB (%)	Ash, DMB (%)
Wheat	11.39	14.45	2.29	1.34	1.85
White sorghum	9.78	8.58	3.54	1.19	1.62
Red sorghum	9.17	11.23	3.70	1.08	1.24

Table 8. Rapid digestible starch, slow digestible starch, total digestible starch, resistant starch and total starch of whole wheat flour, and white and red/burgundy grain sorghum flours expressed on dry basis.

Whole flour	Dry Matter (%)	RDS (%)	SDS (%)	TDS (%)	RS (%)	TS (%)
Wheat	88.61	21.93 ± 0.42 c	32.99 ± 0.86 c	66.80 ± 1.10 b	0.23 ± 0.01 c	66.98 ± 1.02 b
White sorghum	90.22	24.49 ± 1.50 b	46.73 ± 1.20 a	80.97 ± 2.63 a	0.46 ± 0.01 b	81.33 ± 2.61 a
Red sorghum	90.83	29.26 ± 0.95 a	41.23 ± 1.42 b	78.33 ± 1.46 a	0.56 ± 0.03 a	78.75 ± 1.23 a
PooledStDev		1.055	1.182	1.848	0.018	1.765
p-value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

a-c: Means with different superscripts within a column represent statistical difference ($P<0.05$)

Table 9. Pasting profile analysis of whole wheat flour, and white and red/burgundy grain sorghum flours expressed on dry basis.

Whole flour	**Pasting Temp. (°C)**	**Peak time (min)**	**Peak viscosity (cP)**	**Trough viscosity (cP)**	**Breakdown viscosity (cP)**	**Final viscosity (cP)**	**Setback viscosity (cP)**
Wheat	87.99 ± 0.04 b	6.01 ± 0.06 a	1874.2 ± 54.8 c	1135.6 ± 30.4 c	738.6 ± 25.6 a	2647.8 ± 64.9 c	1512.2 ± 37 c
White sorghum	88.64 ± 0.36 a	5.88 ± 0.13 a	2540.8 ± 27.3 a	1880.8 ± 82.3 a	660.0 ± 77.3 a	4906.0 ± 325 a	3026 ± 360 a
Red sorghum	88.21 ± 0.39 ab	5.66 ± 0.11 b	2217.2 ± 40.2 b	1673.4 ± 41.5 b	543.8 ± 27.8 b	4319.6 ± 73.9 b	2646.2 ± 84 b
PooledStDev	0.308	0.105	42.273	56.045	49.708	196.223	214.379
p-value	0.018	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

a-c: Means with different superscripts within a column represent statistical difference ($P < 0.05$)

Table 10. Proximate analysis of pet treats expressed on dry basis.

Treatment	**Dry Matter (%)**	**Crude Protein (%)**	**Crude Fat(%)**	**Crude Fiber(%)**	**Ash (%)**
PC	92.36 ± 1.13	12.59 ± 0.03 f	6.37 ± 0.32 b	1.72 ± 0.08 a	2.20 ± 0.08 b
WS-NC	95.28 ± 2.86	8.35 ± 0.13 h	6.86 ± 0.12 b	1.24 ± 0.16 b	2.25 ± 0.10 b
WS-SDP	94.51 ± 2.62	13.29 ± 0.09 e	6.95 ± 0.49 b	1.15 ± 0.24 bc	2.98 ± 0.03 a
WS-EP	92.05 ± 3.63	17.88 ± 0.10 b	6.40 ± 0.05 b	0.80 ± 0.09 c	2.50 ± 0.24 b
WS-GL	93.74 ± 2.80	13.75 ± 0.15 e	7.64 ± 0.18 a	1.25 ± 0.11 b	2.29 ± 0.25 b
RD-NC	94.54 ± 2.06	10.19 ± 0.22 g	6.54 ± 0.14 b	1.36 ± 0.12 ab	2.33 ± 0.10 b
RD-SDP	93.27 ± 1.62	15.15 ± 0.26 d	6.46 ± 0.21 b	0.99 ± 0.10 bc	3.10 ± 0.09 a
RD-EP	93.44 ± 1.03	19.83 ± 0.18 a	6.81 ± 0.12 b	1.19 ± 0.04 bc	2.41 ± 0.18 b
RD-GL	94.03 ± 2.36	15.95 ± 0.35 c	6.67 ± 0.15 b	1.04 ± 0.18 bc	2.22 ± 0.04 b
PooledStDev	2.376	0.191	0.233	0.137	0.143
p-value	0.781	< 0.001	< 0.001	< 0.001	< 0.001

a-h: Means with different superscripts within a column represent statistical difference (P< 0.05)

Table 11. Rapid digestible starch, slow digestible starch, total digestible starch, resistant starch and total starch of pet treats expressed on dry basis.

Treatment	**Dry Matter (%)**	**RDS (%)**	**SDS (%)**	**TDS (%)**	**RS (%)**	**TS (%)**
PC	92.36 ± 1.13	46.52 ± 0.57 a	14.28 ± 1.56 d	63.11 ± 0.87 bcd	0.55 ± 0.06 a	63.66 ± 0.83 bcd
WS-NC	95.28 ± 2.86	49.14 ± 2.45 a	19.33 ± 4.27 c	72.44 ± 1.43 a	0.64 ± 0.12 a	73.12 ± 1.46 a
WS-SDP	94.51 ± 2.62	36.45 ± 2.86 cd	25.29 ± 2.08 a	66.35 ± 1.61 b	0.56 ± 0.15 a	66.92 ± 1.52 b
WS-EP	92.05 ± 3.63	33.10 ± 2.95 e	23.20 ± 3.85 abc	62.19 ± 4.80 cd	0.53 ± 0.13 ab	62.73 ± 4.76 cd
WS-GL	93.74 ± 2.80	37.47 ± 2.47 c	25.60 ± 3.93 a	65.37 ± 4.00 bc	0.51 ± 0.10 ab	65.89 ± 4.09 bc
RD-NC	94.54 ± 2.06	42.46 ± 2.90 b	24.48 ± 3.61 ab	71.40 ± 2.95 a	0.57 ± 0.11 a	71.98 ± 2.88 a
RD-SDP	93.27 ± 1.62	33.77 ± 1.84 de	25.62 ± 2.98 a	60.68 ± 4.44 d	0.53 ± 0.10 ab	61.21 ± 4.42 d
RD-EP	93.44 ± 1.03	34.27 ± 2.90 de	20.48 ± 3.52 bc	56.30 ± 3.01 e	0.41 ± 0.06 bc	56.71 ± 2.97 e
RD-GL	94.03 ± 2.36	37.74 ± 1.42 c	23.21 ± 2.61 abc	65.87 ± 1.72 bc	0.37 ± 0.03 c	66.25 ± 1.73 bc
PooledStDev	2.376	2.393	3.273	3.095	0.103	3.082
p-value	0.781	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

a-e: Means with different superscripts within a column represent statistical difference ($P < 0.05$)

Table 12. Hardness (kg) and fracturability (mm) of large pet treats.

	Large treats	
Treatment	**Hardness (kg)**	**Fracturability (mm)**
PC	10.03 ± 3.11 b	1.15 ± 0.39 ab
WS-NC	0.80 ± 0.27 d	0.62 ± 0.17 c
WS-SDP	4.92 ± 1.69 c	0.58 ± 0.17 cd
WS-EP	14.26 ± 5.97 a	1.22 ± 0.46 a
WS-GL	1.89 ± 0.53 d	0.47 ± 0.12 d
RD-NC	0.82 ± 0.25 d	0.65 ± 0.17 c
RD-SDP	5.16 ± 1.68 c	0.64 ± 0.15 c
RD-EP	12.76 ± 5.53 a	1.00 ± 0.32 b
RD-GL	1.86 ± 0.51 d	0.46 ± 0.13 d
PooledStDev	2.896	0.255
p-value	< 0.001	< 0.001

a-d: Means with different superscripts within a column represent statistical difference (P< 0.05)

Table 13. Hardness (kg) and fracturability (mm) of small pet treats.

	Small treats	
Treatment	**Hardness (kg)**	**Fracturability (mm)**
PC	11.51 ± 3.28 b	1.16 ± 0.35 a
WS-NC	0.71 ± 0.25 d	0.56 ± 0.18 b
WS-SDP	4.95 ± 1.74 c	0.56 ± 0.15 b
WS-EP	11.96 ± 5.25 b	1.11 ± 0.45 a
WS-GL	2.00 ± 0.73 d	0.50 ± 0.14 b
RD-NC	0.70 ± 0.23 d	0.63 ± 0.14 b
RD-SDP	5.24 ± 2.13 c	0.63 ± 0.24 b
RD-EP	13.52 ± 3.70 a	1.05 ± 0.23 a
RD-GL	2.11 ± 0.66 d	0.55 ± 0.18 b
PooledStDev	2.569	0.247
p-value	< 0.001	< 0.001

a-d: Means with different superscripts within a column represent statistical difference (P< 0.05)

Table 14. Dimension results (mm) and weight (g) of large pet treats.

Treatment	**Large treats**				
	Weight (g)	**Length (mm)**	**Width-center (mm)**	**Width-tips (mm)**	**Thickness (mm)**
PC	10.15 ± 0.58 b	65.44 ± 2.00 cd	19.58 ± 0.88 bc	26.15 ± 0.94 de	10.98 ± 0.82 a
WS-NC	15.51 ± 1.72 a	75.20 ± 2.61 a	23.68 ± 2.77 a	33.26 ± 1.68 a	10.54 ± 1.18 b
WS-SDP	9.71 ± 0.53 bc	65.54 ± 0.55 cd	19.14 ± 0.45 cd	26.40 ± 0.61 d	9.14 ± 0.49 de
WS-EP	9.13 ± 1.14 cd	65.16 ± 1.01 cd	18.48 ± 0.94 d	25.62 ± 1.22 f	9.98 ± 0.53 c
WS-GL	9.36 ± 0.38 cd	67.43 ± 0.85 b	20.03 ± 0.57 b	27.23 ± 0.57 c	9.54 ± 0.36 d
RD-NC	15.54 ± 1.81 a	74.52 ± 3.63 a	23.40 ± 2.17 a	32.34 ± 1.61 b	10.34 ± 1.20 bc
RD-SDP	9.47 ± 0.51 c	66.02 ± 0.46 c	18.86 ± 0.36 cd	26.49 ± 0.42 d	8.97 ± 0.54 e
RD-EP	8.85 ± 0.78 d	64.54 ± 0.93 d	18.83 ± 0.65 cd	25.75 ± 0.78 ef	9.46 ± 0.39 d
RD-GL	9.22 ± 0.50 cd	67.38 ± 0.79 b	20.01 ± 0.56 b	27.30 ± 0.54 c	9.54 ± 0.42 d
PooledStDev	0.996	1.758	1.319	1.017	0.733
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

a-f: Means with different superscripts within a column represent statistical difference (P< 0.05)

Table 15. Dimension results (mm) and weight (g) of small pet treats.

Treatment	Small treats				
	Weight (g)	Length (mm)	Width-center (mm)	Width-tips (mm)	Thickness (mm)
PC	8.44 ± 0.35 b	48.36 ± 1.44 cd	19.75 ± 0.69 b	27.14 ± 0.80 bc	10.94 ± 0.66 b
WS-NC	9.92 ± 1.31 a	53.18 ± 1.43 a	19.03 ± 0.96 cd	25.89 ± 1.87 e	11.51 ± 1.38 a
WS-SDP	7.95 ± 0.39 c	48.27 ± 0.68 cd	19.28 ± 0.40 c	27.29 ± 0.41 b	9.19 ± 0.36 e
WS-EP	7.81 ± 0.80 cd	47.87 ± 0.82 d	18.80 ± 0.94 d	26.47 ± 1.05 d	10.14 ± 0.42 c
WS-GL	7.63 ± 0.21 cd	49.56 ± 0.59 b	20.10 ± 0.58 ab	28.15 ± 0.49 a	9.68 ± 0.35 d
RD-NC	9.53 ± 1.41 a	53.35 ± 1.18 a	18.95 ± 0.97 cd	25.67 ± 2.12 e	11.00 ± 1.02 b
RD-SDP	7.79 ± 0.31 cd	48.45 ± 0.52 c	19.06 ± 0.38 cd	27.31 ± 0.40 b	9.30 ± 0.44 de
RD-EP	7.55 ± 0.66 cd	47.29 ± 0.60 e	19.07 ± 0.53 cd	26.70 ± 0.71 cd	9.54 ± 0.38 de
RD-GL	7.50 ± 0.46 d	49.50 ± 0.60 b	20.16 ± 0.48 a	28.15 ± 0.55 a	9.65 ± 0.43 d
PooledStDev	0.771	0.941	0.695	1.107	0.693
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

a-e: Means with different superscripts within a column represent statistical difference (P< 0.05)

Table 16. Color results of pet treats.

Treatment	L*	a*	b*	Hue Angle	Chroma
PC	54.61 ± 1.34 a	6.86 ± 0.65 c	22.69 ± 0.66 a	73.21 ± 1.36 c	23.71 ± 0.73 a
WS-NC	54.38 ± 3.80 a	5.96 ± 0.95 d	21.74 ± 0.66 bc	74.73 ± 1.95 b	22.55 ± 0.87 b
WS-SDP	50.81 ± 1.02 b	7.12 ± 0.73 c	22.57 ± 1.04 a	72.52 ± 1.10 c	23.67 ± 1.19 a
WS-EP	47.87 ± 2.19 cd	7.43 ± 0.73 bc	21.33 ± 1.18 c	70.79 ± 1.64 d	22.60 ± 1.23 b
WS-GL	54.59 ± 2.71 a	5.45 ± 0.91 d	22.23 ± 0.75 ab	76.28 ± 1.84 a	22.90 ± 0.92 ab
RD-NC	53.34 ± 2.11 a	6.97 ± 0.37 c	17.44 ± 0.78 de	68.21 ± 0.54 e	18.78 ± 0.85 d
RD-SDP	46.62 ± 2.10 d	7.87 ± 0.38 b	17.47 ± 0.58 de	65.75 ± 0.89 f	19.16 ± 0.63 d
RD-EP	42.77 ± 0.86 e	9.74 ± 0.42 a	18.25 ± 0.67 d	61.91 ± 0.43 g	20.68 ± 0.77 c
RD-GL	49.91 ± 1.34 bc	7.21 ± 0.22 bc	17.42 ± 0.44 e	67.52 ± 0.34 e	18.86 ± 0.49 d
PooledStDev	2.128	0.645	0.781	1.261	0.882
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

a-g: Means with different superscripts within a column represent statistical difference (P< 0.05)

Table 17. Ranking scores, median and mean for independent white sorghum and red/burgundy treatments phases.

	White Sorghum			Red Sorghum		
Treatment	**Rank**	**Median**	**Mean**	**Rank**	**Median**	**Mean**
PC	145	3.0	2.90 ± 1.31 bc	142	2.6	2.84 ± 1.52 a
NC	185	4.0	3.70 ± 1.34 a	164	4.0	3.28 ± 1.46 a
SDP	142	2.6	2.84 ± 1.33 bc	141	2.8	2.82 ± 1.34 a
EP	118	2.0	2.36 ± 1.34 c	142	3.0	2.84 ± 1.38 a
GL	160	3.4	3.20 ± 1.46 ab	161	3.6	3.22 ± 1.38 a
Chi-Square	19.50			4.21		
p-value	0.001			0.379		

a-c: Means with different superscripts within a column represent statistical difference ($P< 0.05$)

Table 18. Ranking and mean times for independent white sorghum and red/ burgundy treatments phases.

	White Sorghum		Red Sorghum	
Treatment	**Phase time**	**Mean time**	**Phase time**	**Mean time**
PC	0:19:28.73	0:00:23.37	0:22:57.81	0:00:27.56
NC	0:20:27.94	0:00:24.56	0:26:49.08	0:00:32.18
SDP	0:19:38.69	0:00:23.57	0:20:14.84	0:00:24.30
EP	0:18:53.71	0:00:22.67	0:25:24.46	0:00:30.49
GL	0:18:10.66	0:00:21.81	0:28:22.21	0:00:34.04
Total time (hh:mm:ss.0)	1:36:39.73	0:00:23.20	2:03:48.40	0:00:29.71

Table 19. Ranking scores, median and mean for combined white sorghum and red/burgundy treatments.

Treatment	White Sorghum vs. Red Sorghum		
	Rank	Median	Mean
PC	184	3.8	3.34 ± 1.44 a
WS-SDP	151	2.6	2.74 ± 1.48a
RD-SDP	153	2.2	2.78 ± 1.46 a
WS-GL	165	3.0	3.00 ± 1.28 a
RD-GL	172	3.4	3.12 ± 1.38 a
Chi-Square	5.45		
p-value	0.244		

a: Means with similar superscripts within a column represent no statistical difference (P> 0.05)

Table 20. Ranking and mean times for combined white sorghum and red/burgundy treatments.

Treatment	White Sorghum vs. Red Sorghum	
	Phase time	Mean time
PC	0:11:55.39	0:00:13.01
WS-SDP	0:13:30.64	0:00:14.74
RD-SDP	0:11:26.90	0:00:12.49
WS-GL	0:12:59.54	0:00:14.17
RD-GL	0:12:21.92	0:00:13.49
Total time (hh:mm:ss.0)	1:02:14.39	0:00:13.58

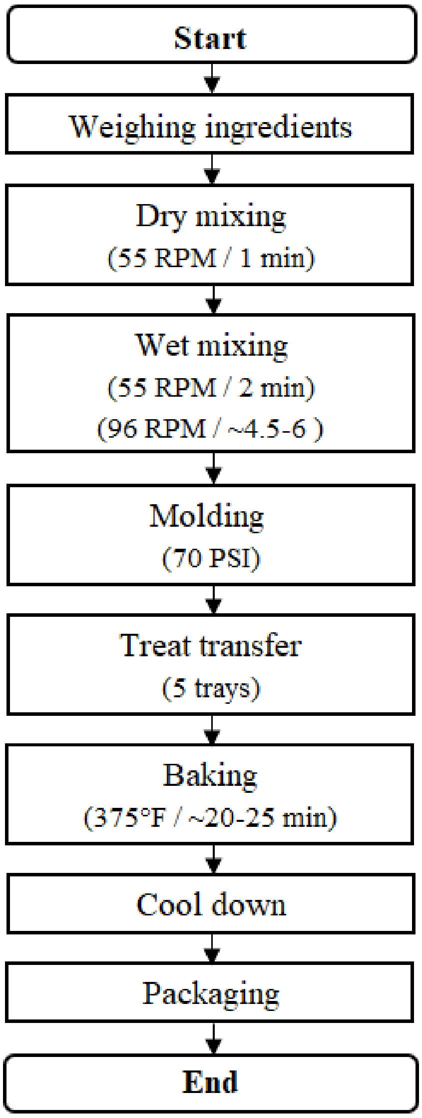

Figure 1. Flow chart of baked dog treats

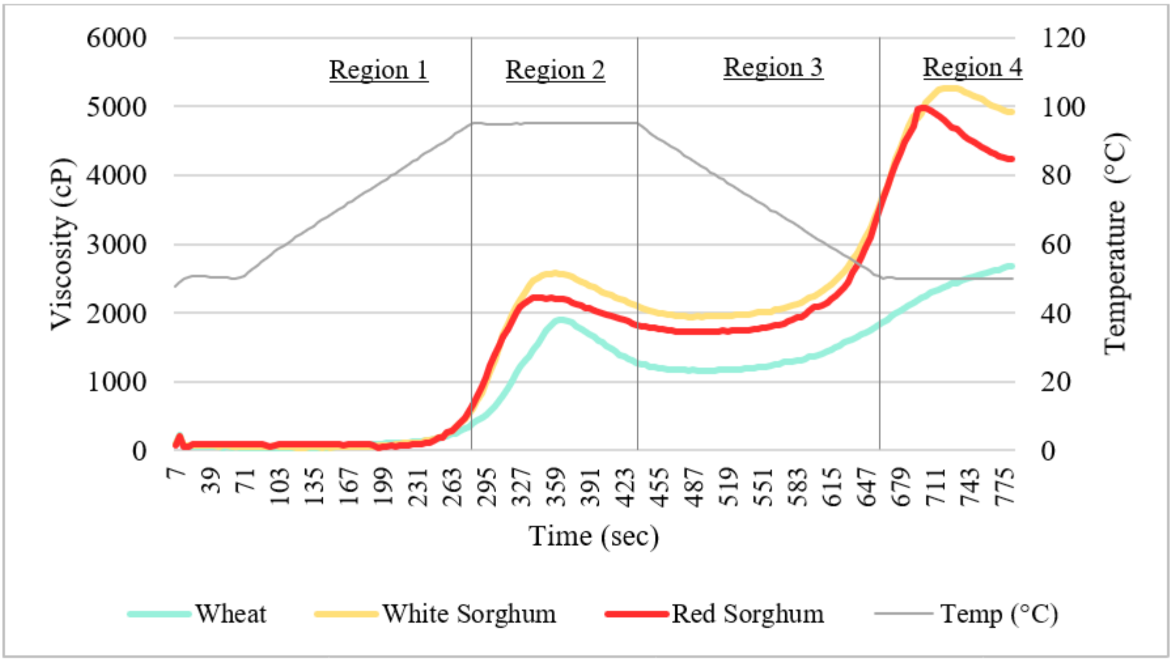

Figure 2. Pasting performance of whole wheat flour, white whole grain sorghum flour and red/burgundy whole grain sorghum flour.

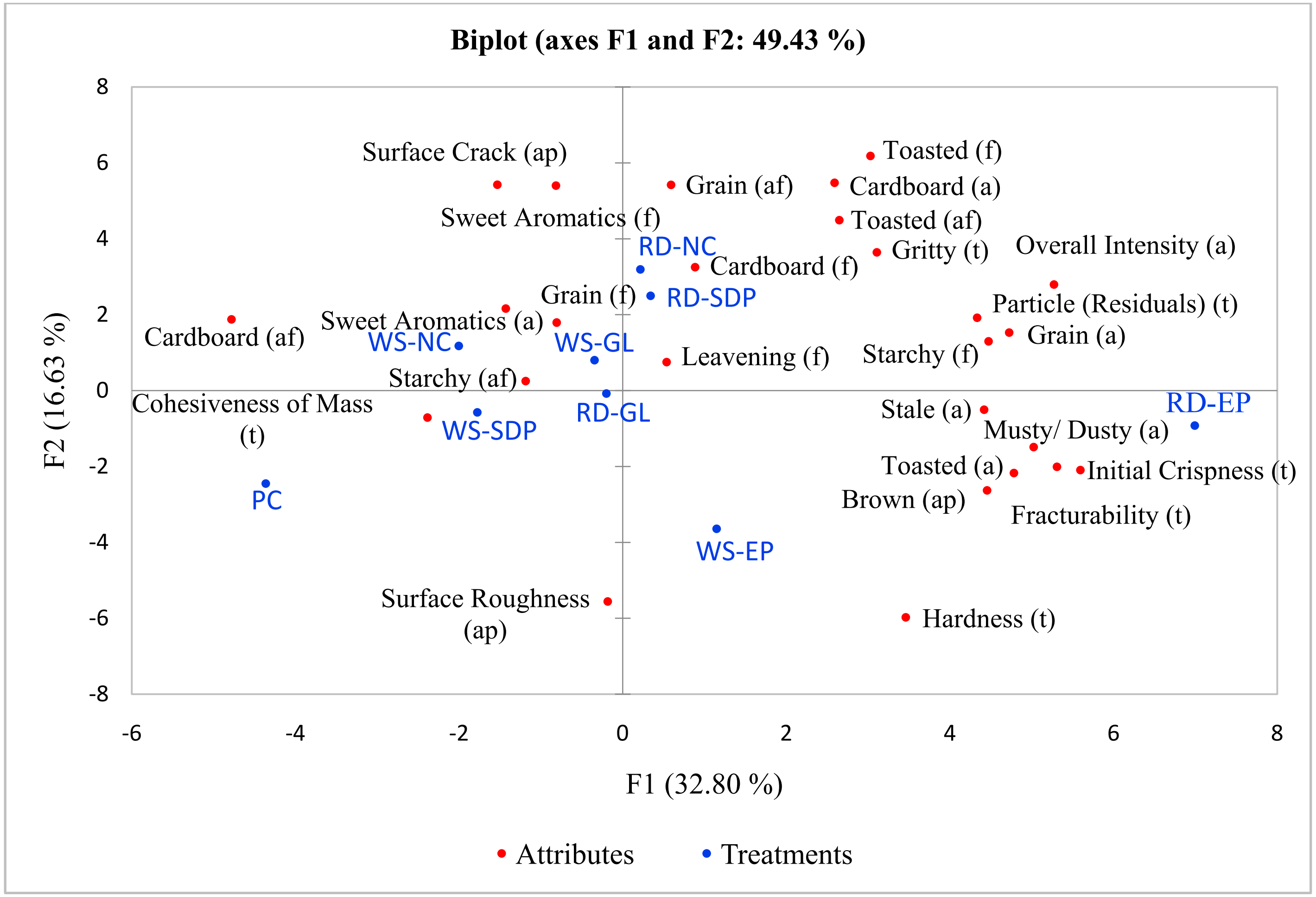

Figure 3. Principal component analysis (PCA) of appearance, aroma, flavor, texture and aftertaste attributes from dog treats.