United Sorghum Checkoff Program

Project Report

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Sustainability, oxidative stress mitigation and sensory characteristics of sorghum-based canine diets designed for the international market

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EXECUTIVE SUMMARY

Use sorghum in high value food applications is increasing and its benefits such as lower glycemic index and higher satiety potential are being studied extensively. One other major benefit often cited with regard to grain sorghum is its sustainable production system. This characteristic will be prove to be of increasing value in the U.S., as environmental regulations are put in place following the same trend as in Europe. Use of downstream sustainable processing technologies, such as extrusion, will further accentuate the sustainability attribute of sorghum-based pet food. This study is the first to quantify the CO_2 footprint during processing of extruded food products, in this case for sorghum-based dry expanded pet foods. A novel computer-based model was developed for detailed energy analysis of the extrusion process, based on which the CO_2 footprint was calculated. The model predicted CO_2 footprint in the range of 553-693 lb/ ton of manufactured pet food product and total greenhouse gas emission of 558-598 lb CO_2 equivalent/ton. The CO_2 footprint of corn based dog food (588 lb/ ton) was slightly higher than the average for sorghum based diets (575 lb/ton) at the same process intensity (high thermal energy). Sorghum based diets had lower gelatinization (69-76% for red sorghum and 68-78% for white sorghum), as compared to corn based control diet that had a gelatinization of 92%. This pointed to potential resistant starch and consequentially prebiotic effect in the former. The higher bulk density and textural hardness of the sorghum-based diets pointed to a more appealing product for canines as compared to the corn-base diet. The sensory attributes of all diets such as appearance, aroma, flavor and texture were characterized using a trained sensory panel. The sensory textural attributes such as firmness, fracturability and initial crispness mirrored the instrumental hardness data. The in-vivo studies with dogs demonstrated that diets formulated with white or red sorghum are nutritional adequate for adult dogs, without negatively affecting nutrient digestibility or causing any signs of gastrointestinal discomfort or intolerance. Coefficients of nutrient digestibility for the nutrients were high, and comparable with premium commercial canine diets. Fecal concentrations of short-chain fatty acids (e.g., acetate, propionate, and butyrate) indicate that white and red sorghum diets had comparable hindgut fermentation of C diet. Even though no statistical difference was not observed for fecal butyrate concentration, a numerical increase of this SCFA was noted in fecal samples of dogs fed red sorghum and white sorghum. This is an important finding as butyrate is a major energy source for colonocytes and is important to support gut health.

PART – 1. FORMULATION, PROCESSING, PHYSICO-CHEMICAL CHARACTERIZATION AND ENERGY/ SUSTAINABILITY ANALYSIS

A pilot-scale single screw extrusion system was used for production of dry expanded dog food based on red and white grain sorghum and corn as control for the purpose of – a) process analysis at different levels of energy input to aid in sustainability analysis, b) physico-chemical characterization of dog food kibbles, c) sensory characterization of the sorghum based dog food, d) obtaining product for in vivo studies with dogs.

Balanced standard dog food diets were formulated (Table 1), mixed and ground to particle size of 1mm using a hammer mill.

Table 1. Formulationfor dry expanded dog food based on red and white sorghum, and corn (control diet).

Ingredient	**%**
Red sorghum, white sorghum, corn	30.00
Chicken By Product Meal	56.83
Corn Gluten Meal (60% CP)	6.09
Beet Pulp	4.06
Salt	0.91
Potassium Chloride	0.71
Premix Min/Vit.*	0.60
Choline chloride	0.51
Mold inhibitor	0.20
Antioxidant	0.08

Both red and white sorghum diets were processed on a Wenger X-20 single screw extruder at three levels of process intensities involving varying levels of thermal and mechanical energy input, which are referred in the report as high thermal (Hi or H), medium thermal (Md or M) and low thermal (Lo or L). This represented a 2x3 factorial design with 6 treatments. Variation in energy input was obtained via preconditioner steam injection and extruder screw speed. The control corn diet was processed at the optimum energy level (high thermal energy) resulting in a total of 7 treatments for further analyses.

Process sustainability quantification requires thorough energy usage analyses. For this purpose, a novel computer based mathematical model was developed and implemented for mass and energy balance and thermodynamic evaluation of the extrusion process. A brief description of the model and energy calculations is provided below.

Specific mechanical energy and specific thermal energy calculations

SME was calculated for each diet using the standard equation.STE is calculated from energy balance analysis of the process that incorporates mechanical energy, thermal energy contributions including that of process steam, energy contribution from various other material streams and the any heat of reaction (example, starch gelatinization). The energy balance was carried out as described by Riaz (2000):

Preconditioner energy balance: $Q_R + Q_W + Q_S = Q_{Pc} + Q_{SL} + Q_{HL} + \sum\Delta h$

Extruder energy balance: $Q_P + Q_W + Qsme + Qbarrel = Qex + Q_{SL} + \sum\Delta h$

where:
Q_R = Raw material heat capacity
Q_{HL} = Preconditioner heat loss by convection
Qb_{arrel} = Extruder heat loss by convection
Q_W = Water input heat
Qsme = Mechanical energy amount
Q_S = Steam input heat
Qte = Thermal energy amount
Q_p = Preconditioner product heat
Qex = Extruder product heat
Q_{SL} = Steam loss heat
$\sum\Delta h$ = Reaction energy

The amount of heat (Q) associated with any material stream (except steam) was obtained from the formula: Q = m.c.T

where:
m = mass flow rate
c = specific heat capacity
T = temperature

The total specific energy was obtained by the sum of the SME and STE. The model calculated net specific mechanical energy input range of 58-101 kJ/kg) and thermal energy input range of 67-262 kJ/hr depending on treatment and process intensity, and a thermal energy percentage of 40.4-79.0% (Figures 1-3). The highest thermal energy process (STE of 218-262 kJ/kg) had the lowest mechanical energy (SME of 75-92 kJ/kg), and vice versa.

The energy data was used to calculate the CO_2 footprint based on constants currently used by the Leonardo Academy, a charitable 501(c)3 nonprofit dedicated to advancing sustainability and environmental stewardship. Results indicated that extrusion process for the dog food treatments had CO_2footprint in the range of 553-693 lb/ ton of manufactured product and total greenhouse gas emission of 558-598 lb CO_2equivalent/ton (Figures 4-5). The CO_2 footprint of corn based diet (588 lb/ ton) was higher than the average for sorghum based diets (575 lb/ton) at the same process intensity (high thermal energy), although this difference is only slight and is dependent on process variations.

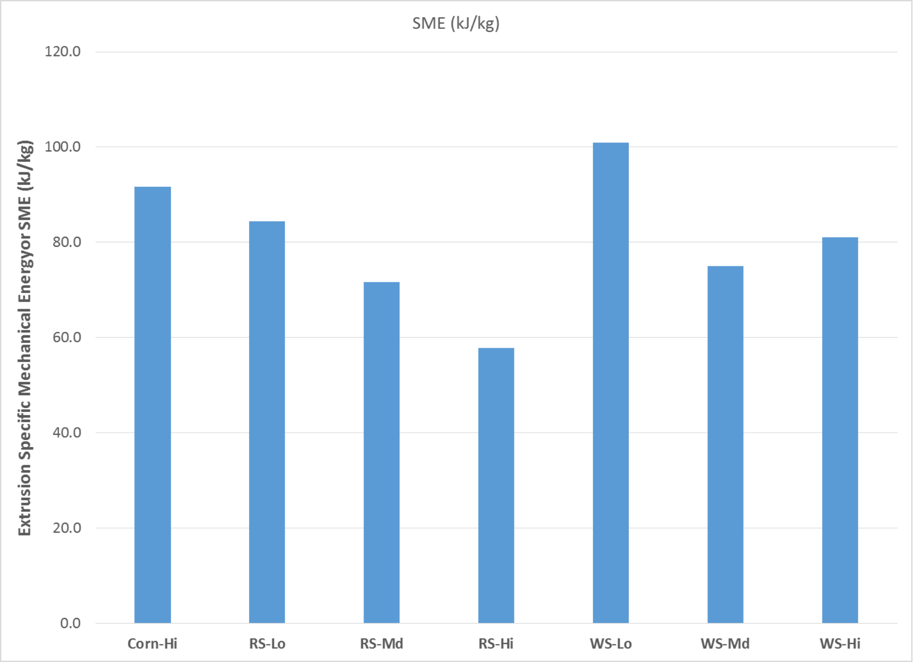


Figure 1. Energy analysis during extrusion process - specific mechanical energy (SME) input. RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

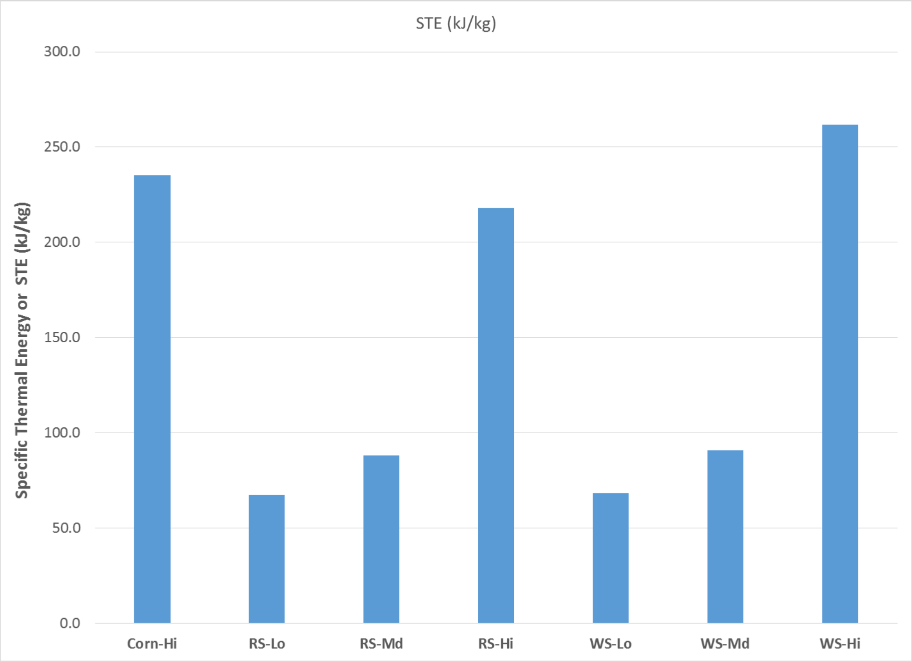


Figure 2. Energy analysis during extrusion process – specific thermal energy (STE) input. RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

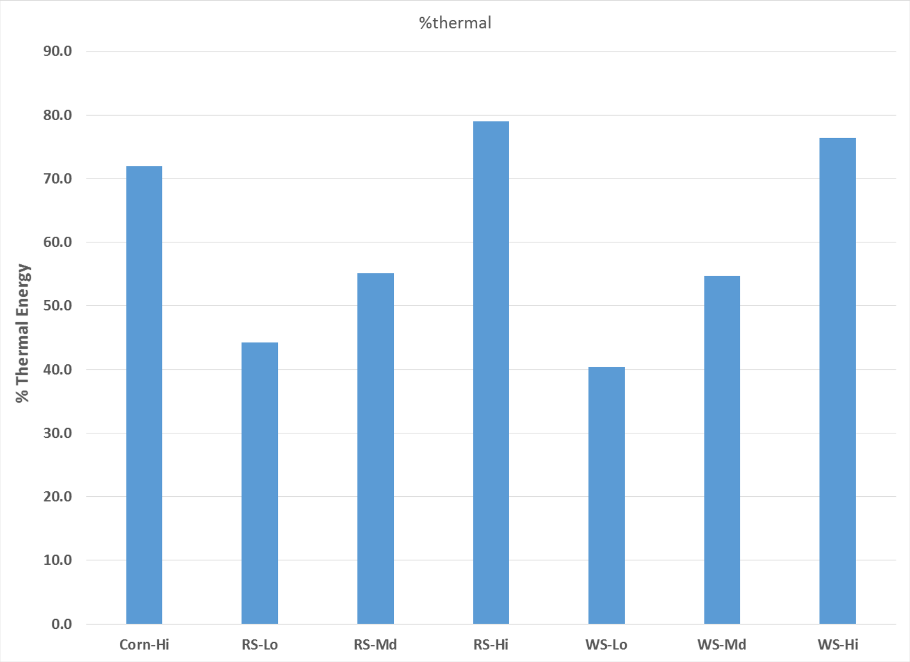


Figure 3. Energy analysis during extrusion process – percentage of thermal energy input in relation to total energy input into product. RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

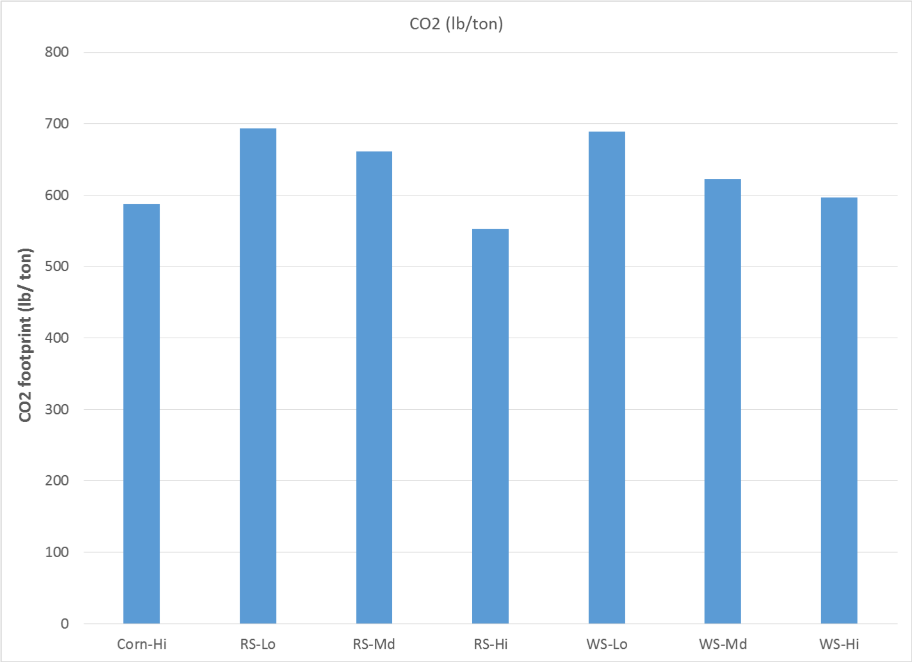


Figure 4. Sustainability analysis of extrusion process – CO_2 emission per ton of product. RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

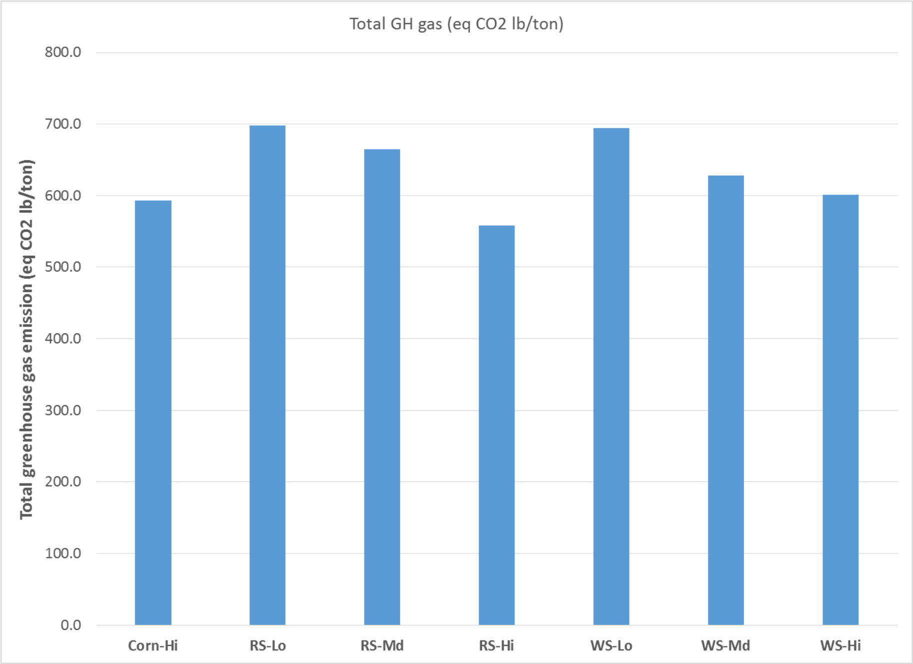


Figure 5. Sustainability analysis of extrusion process – total greenhouse emission per ton of product (expressed in equivalent CO_2 footprint). RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

Product Physico-Chemical Properties

Processing treatments had a substantial impact on product quality. Starch gelatinization of (glucoamylase enzymatic test) ranged from 68-92%. In general, sorghum based diets had lower gelatinization (69-76% for red sorghum and 68-78% for white sorghum), as compared to corn based control diet that had a gelatinization of 92%. This pointed to potential resistant starch and consequentially prebiotic effect in sorghum based diets.

Table 2. Percentage starch gelatinization using glucoamylase enzymatic test. ; Lo, Md and Hi = low, medium and high thermal energy input during processing.

Treatment	% Gelatinization
Corn-Hi	92.0
RS-Lo	69.2
RS-Md	72.8
RS-Hi	75.6
WS-Lo	68.2
WS-Md	71.0
WS-Hi	77.9

Bulk density of extruded kibbles before drying ranged from 393-578 g/L and after drying from ranged from 354-577 g/L) (Figures 6-7). Bulk density for sorghum final product was higher (red sorghum 371-577 g/L and white sorghum 383-461 g/L) than the corn based control that had a bulk density of 354 g/L. The former represented an ideal range for the optimum texture preferred by canines. The expansion ratio and piece density data (Figure 8-9) exhibited the same trends as bulk density. The peak crushing force (measured using a TA-XT2 texture analyzer under compression mode using a flat probe) of dog food kibbles ranged from 19.1-28.2 kg (Figure 10). As expected from the product density and expansion data, the sorghum-based diets had a harder texture (20.1-28.2 kg for red sorghum and 21.6-31.9 kg for white sorghum) as compared to the control corn diet (19.1 kg).

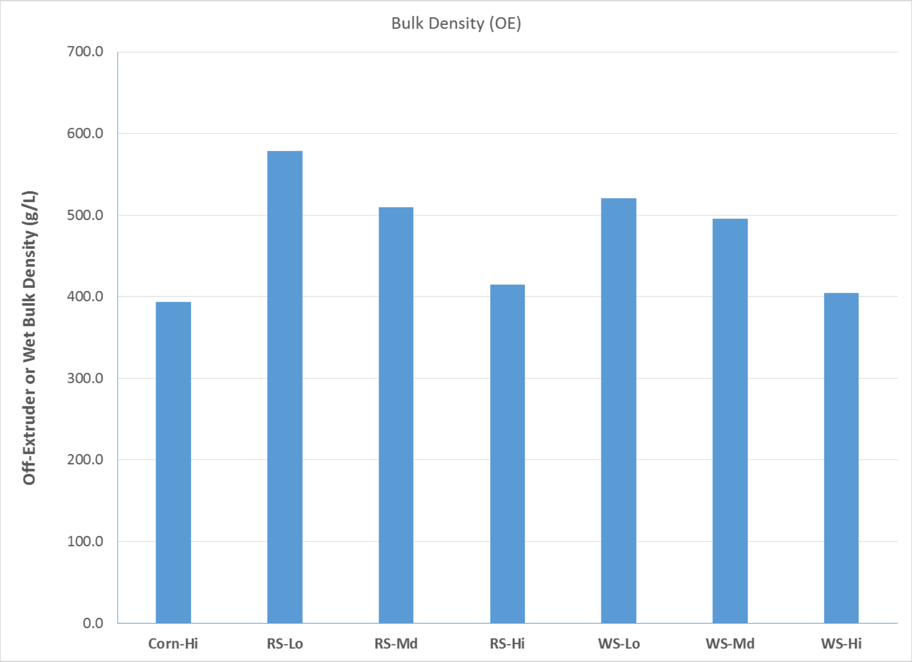


Figure 6. Dog food kibble bulk density before dryer. RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

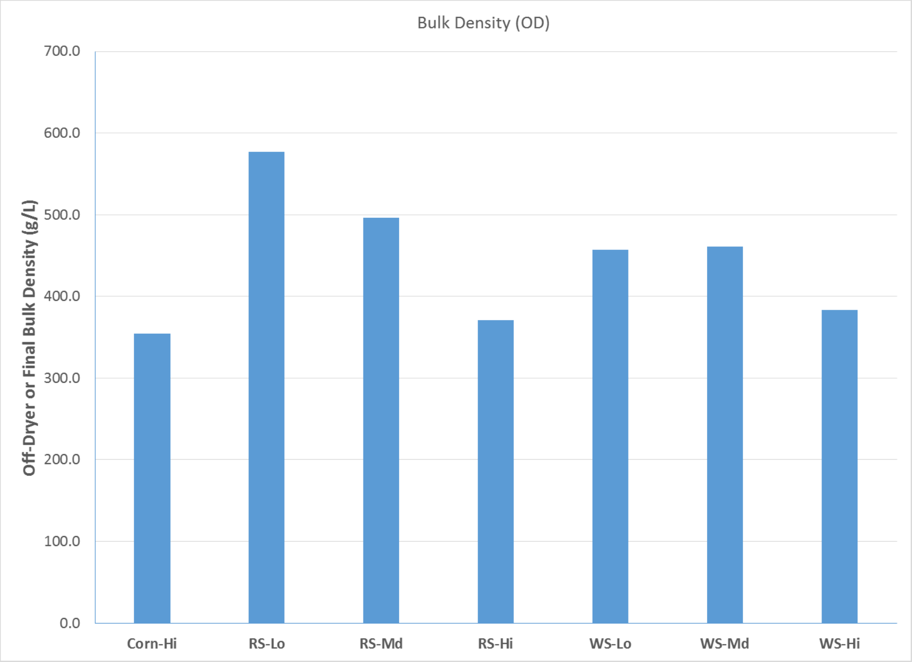


Figure 7. Dog food kibble bulk density after dryer. RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

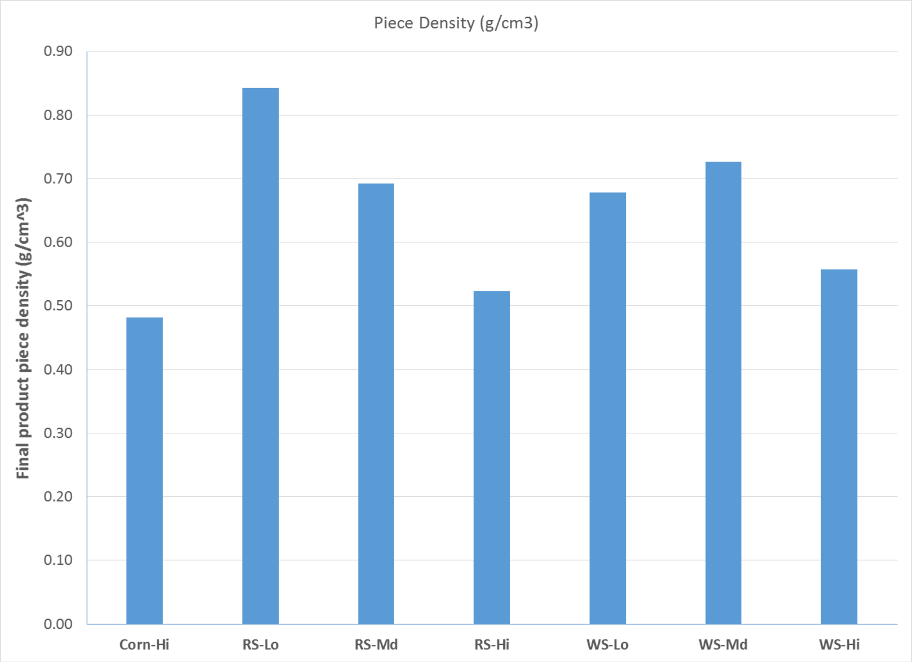


Figure 8. Dog food kibble piece density after dryer. RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

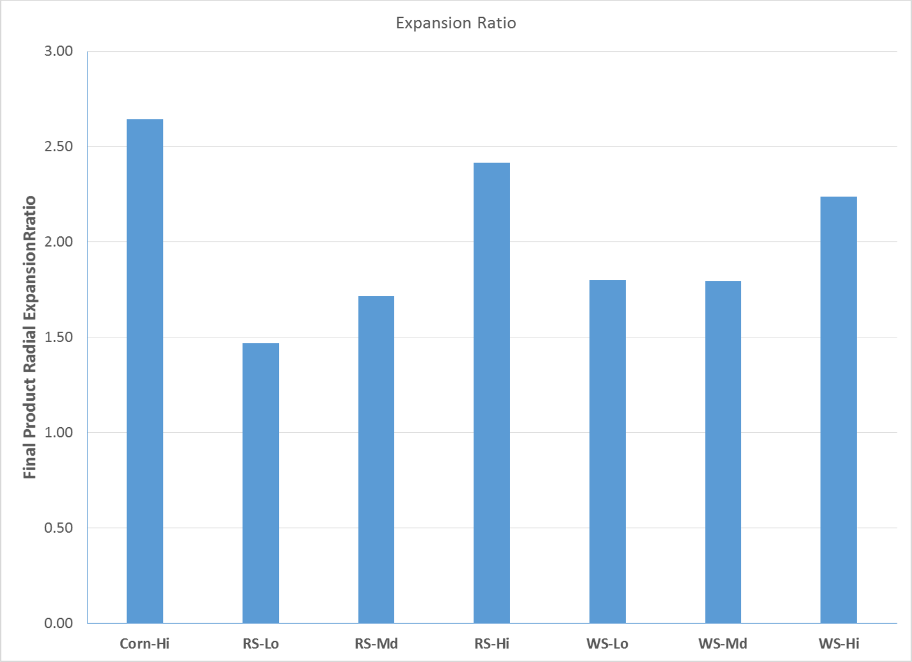


Figure 9. Dog food kibble radial expansion ratio after dryer. RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

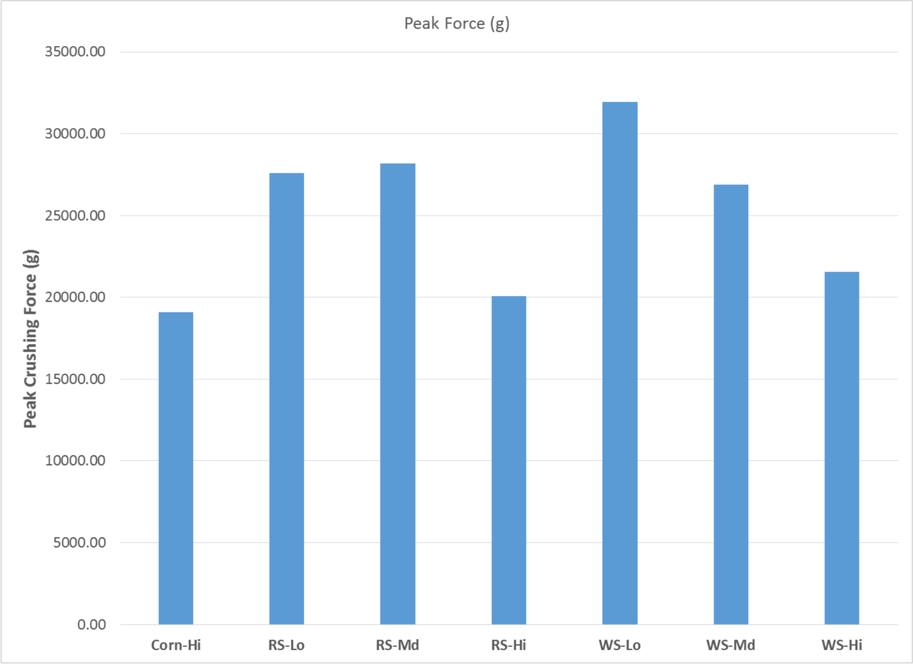


Figure 10. Dog food kibble peak crushing force or hardness (using Texture Analyzer TA-XT2). RS = red sorghum based diets; WS = white sorghum based diets; Lo, Md and Hi = low, medium and high thermal energy input during processing.

PART – 2. SENSORY ANALYSIS

Descriptive sensory analysis and gas chromatograph based study was conducted to investigate sensory and volatile differences exist between white sorghum, red sorghum and control pet food samples made with different energy input.

A total of 7 dry pet food samples were used to evaluate appearance, aroma, flavor, and texture. There were 3 white sorghum samples made using low, medium and high energy input (WSL, WSM, WSH), 3 of red sorghum samples made using high, medium and low energy input (RSL, RSM, RSH), and 1 control sample made using corn and high energy input (CORN). The samples were dry extruded dog food produced by the Department of Grain Science and Industry facilities and sent to the Center for Sensory Analysis and Consumer Behavior for testing. The parameters of the extruder processing conditions for these samples are in the appendix 1 of the report. The samples were stored at room temperature until analysis.

Total of 5 highly trained panelists participated in this project. The panelists rated the intensity of aroma and flavor attributes such as grain, barnyard, oxidized oil, and cardboard; basic taste such as bitter and sour; texture attributes such as hardness and fracturability, and appearance characteristics such as brown color and porous (Di Donfrancesco et al. 2012). For the evaluation a numeric scale of 0-15 with 0.5 increments where 0 represents none and 15 extremely high was applied to each attribute to provide a measure of intensity. The samples were evaluated in triplicate in a randomized order. The definition and reference sheet can be found in Appendix 1 of the report.

The panelists had deionized water, mozzarella cheese, cucumber slices, unsalted crackers, wash clothes and warm deionized water for palate cleansing. The sorghum samples (5g) were placed in medium covered glass snifters and 3.25 oz plastic cups labeled with a random three-digit code and served to the descriptive panel for evaluation.

Gas chromatography mass spectrometry (GC-MS)

The extraction method for studying the aroma profile in the samples was headspace solid phase micro extraction (HS-SPME), similar to what was done by Koppel et al. (2013). The samples were ground in a coffee grinder for 10 sec. About 0.5 g sample was weighed in a 10 mL screw-cap vial equipped with a polytetrafluoroethylene/silicone septum. Exactly 0.99 mL distilled water was added to the ground sample in the vial. The internal standard was 0.01 mL 100ppm 1, 3-dichlorobenzene dissolved in methanol (Fisher Scientific; Pittsburgh, PA, USA). A 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane fiber was exposed to the sample headspace. After sampling, the analytes were desorbed from the SPME fiber coating to the GC injection port at 270°C for 3 min in splitless mode. The isolation, tentative identification, and semi-quantification of the volatile compounds were performed on a gas chromatograph (Varian GC CP3800; Varian Inc., Walnut Creek, CA, USA), coupled with a Varian mass spectrometer (MS) detector (Saturn 2000). The GC-MS system was equipped with a Carbowax (Crossbond®

Carbowax ® polyethylene glycol) column (Restek, U.S., Bellefonte, PA, USA; 30 m × 0.25 mm × 0.5 µm film thickness). The samples were analyzed in triplicate.

Data analysis

Data was analyzed with a 1-way ANOVA mixed effect model (SAS 9.4 Version) to determine significant differences among samples on each attribute. For all significant attributes, the sample effects were assessed using pair-wise comparisons based on least square (LS) means. The criteria for significance was $p<0.05$. Principal components analysis was conducted in this study using XLstat 2017 version.

Appearance

The differences between samples for appearance are shown in Table 3 and Figure 11. With the energy input increasing, both white sorghum and red sorghum samples decreased in brown color scores, and increased in porous scores. To compare with the control sample, CORN, the energy input affects appearance attributes more than sorghum type.

Table 3. Mean intensity scores for appearance attributes.

Sample	**Brown**	**Porous**	**Fibrous**	**Flecks (Yes/No) Color (Frequency from high to low)**
WSL	6A	0.95D	1.3	Cream, White, Tan, Black
WSM	4.5CD	1.95BCD	1.1	Cream, White, Tan, Black
WSH	4D	3.15AB	1.4	Cream, White, Black
RSL	5.7AB	1.55CD	1.3	Cream, Black, Red, White
RSM	5.05BC	1.75CD	1.05	Cream, Black, Red, Tan
RSH	3.9D	2.75ABC	0.95	Cream, Black, White, Brown
CORN	4.15D	3.35A	1	Cream, White, Black, Tan
p-Value	<0.05	<0.05	0.35	

*intensity was measured on a scale from 0 to 15 with 0.5 point increments.

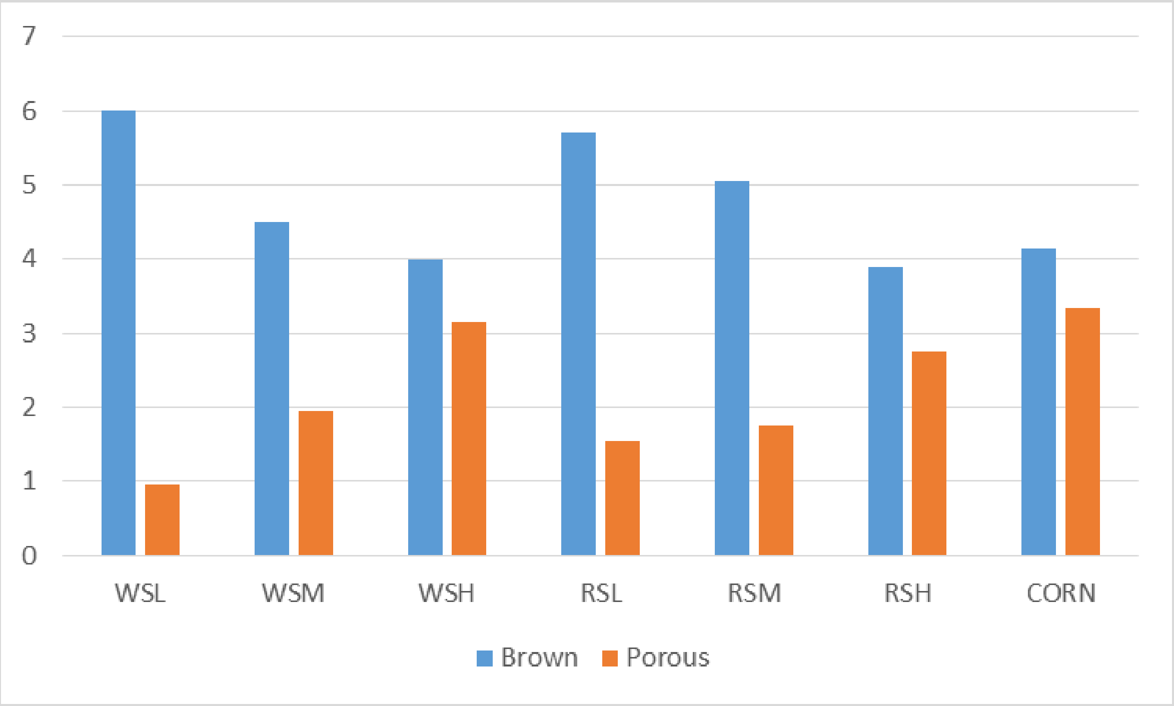


Figure 11. Bar graph of significantly different appearance attributes.

Aroma

Barnyard and Liver aromas were the only significantly different attributes among all these 7 samples (Table 4). RSM Liver aroma was most similar with CORN sample.

The only sample to differentiate significantly from other samples in barnyard aroma was WSL (Figure 12). This means we could use the medium energy input of white sorghum or low energy input of red sorghum to produce pet foods with similar barnyard and liver aroma characteristics as the control sample.

Table 4. Mean intensity scores for aroma attributes.

Sample	Vitamin	Barnyard	Liver	Grain	Cardboard	Fish	Oil Heated	Oxidized Oil	Hay-like	Musty/Dusty	Toasted
WS-Lo	2.75	4.8A	1.35ABC	3.35	3.5	0.15	3.25	0	0.85	3.4	2.65
WS-Md	2.5	4.1AB	0.55C	3.55	3.5	0	3.05	1.5	0.85	3.1	2.6
WS-Hi	2.5	3.75B	0.85BC	3.35	3.05	0	3.3	0	0.95	3	2.2
RS-Lo	2.7	3.4B	1.6AB	3.45	3.55	0	3.45	0	0.9	2.9	2.6
RS-Md	2.3	3.45B	1.05ABC	3.2	3.45	0.15	3.15	0.2	0.4	2.8	2.15
RS-Hi	2.35	3.45B	1.75A	3.5	3.6	0.15	3.3	0	0.8	3	2.45
Corn-Hi	2.75	4.1AB	1.05ABC	3.2	3.65	0	2.85	0.35	0.65	3.25	2.5
p-Value	<0.05	<0.05	<0.05	0.47	0.15	0.28	0.16	0.07	0.32	0.15	0.20

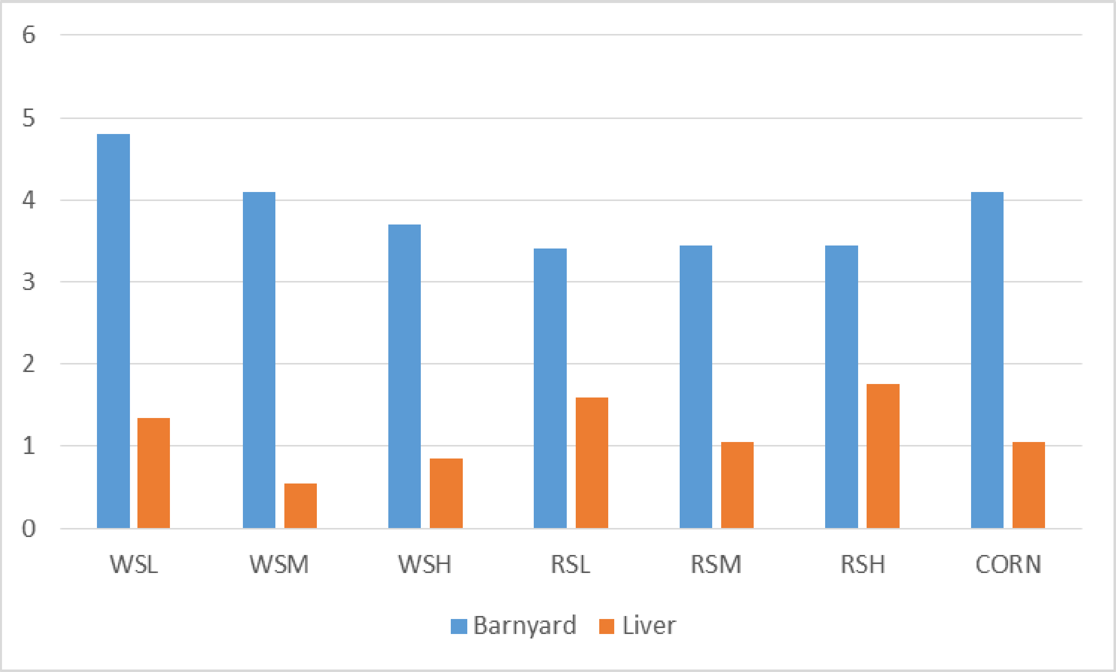


Figure 12.Bar graph of significantly different aroma attributes (p<0.05).

Flavor

Only three flavor attributes presented significant differences among samples: Liver, Barnyard, and Stale (Table 5, Figure 13). Sample CORN had the highest level in all these three attributes. The WSL and WSM both are grouped together with CORN in all these three attributes, which meant that in flavor profile WSL and WSM did not have significant differences when compared to CORN sample.

Table 5. Mean intensity scores for flavor attributes.

Sample	Vitamin	Liver	Barnyard	Grain	Heated Oil	Oxidized Oil	Cardboard	Stale	Sour	Salt	Bitter	Sweet	Metallic
WSL	2.8	1.95AB	4.45AB	3.95	3.7	0.7	3.8	1.6A	1.65	3.05	7.35	0	2
WSM	3.05	2.35AB	4.3ABC	4.6	3.15	0.45	3.95	1.4AB	1.9	2.7	7.05	0	1.95
WSH	2.9	1.55B	3.9ABC	4.25	3.45	0.6	3.8	1.05AB	1.8	3.1	6.95	0.15	1.95
RSL	2.55	2.15AB	3.85BC	3.8	3.15	0.55	3.6	1.7A	1.4	2.75	7.05	0	2.05
RSM	2.7	2.1AB	3.5C	3.8	3.15	0	3.45	0.6AB	1.3	2.7	6.9	0	1.85
RSH	3	2.5A	3.85BC	4.2	3.25	0.5	3.5	0.6AB	1.45	2.9	6.9	0	1.75
CORN	2.8	1.8AB	4.75A	4.45	3.65	0.7	4.15	1.35AB	2	3.25	7.15	0.9	1.7
p-Value	0.07	<0.05	<0.05	0.15	0.27	0.11	0.10	<0.05	0.18	0.11	0.27	0.07	0.29

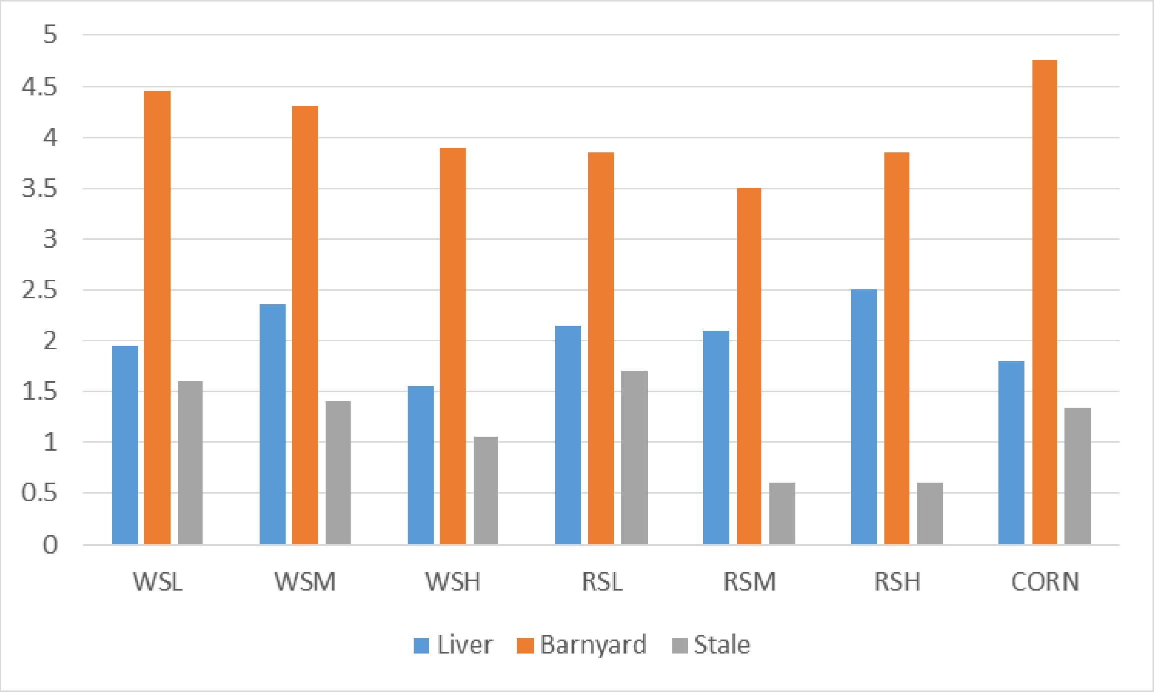


Figure 13.Bar graph of significantly different flavor attributes.

Texture

All five texture attributes were significantly different between samples ($p<0.05$). The energy input seemed to cause the change in texture. The same level energy input had similar texture no matter the material (red sorghum or white sorghum) of samples (Table 6, Figure 14).Sample WSL and RSL didn't show any significant differences in all these five attributes, and showed lowest level in Gritty and Fracturability attributes. These samples also had highest levels in Cohesiveness and Firmness attributes. They didn't have Initial Crispness attribute. The low energy input may cause the sorghum sample less crispness.Sample WSM and RSM didn't present any significant differences in all these five attributes, and showed lowest level in Gritty or middle level in Initial Crispness and Fracturability. They had the highest level in Cohesiveness and Firmness. Sample Corn, WSH and RSH had the similar attributes level in all these five attributes. The Initial Crispness, Fracturability and Gritty had the highest level. The Cohesiveness and Firmness had the lowest level.

Table 6. Mean intensity scores for texture attributes

Sample	**Initial Crispness**	**Fracturability**	**Gritty**	**Cohesiveness**	**Firmness**
WSL	0	2.35CD	2.6B	8.45A	10.45A
WSM	1.6B	3ABCD	3.2AB	9.25A	9.05A
WSH	7.5A	4.2ABC	3.55A	5.75B	5.45B
RSL	0	1.05D	2.8AB	10.15A	9.35A
RSM	1.3B	2.75BCD	2.75AB	9.55A	9A
RSH	6.75A	4.65AB	3.1AB	6.15B	5.6B
CORN	7.75A	5.05A	3.2AB	4.85B	6.05B
p-Value	<0.05	<0.05	<0.05	<0.05	<0.05

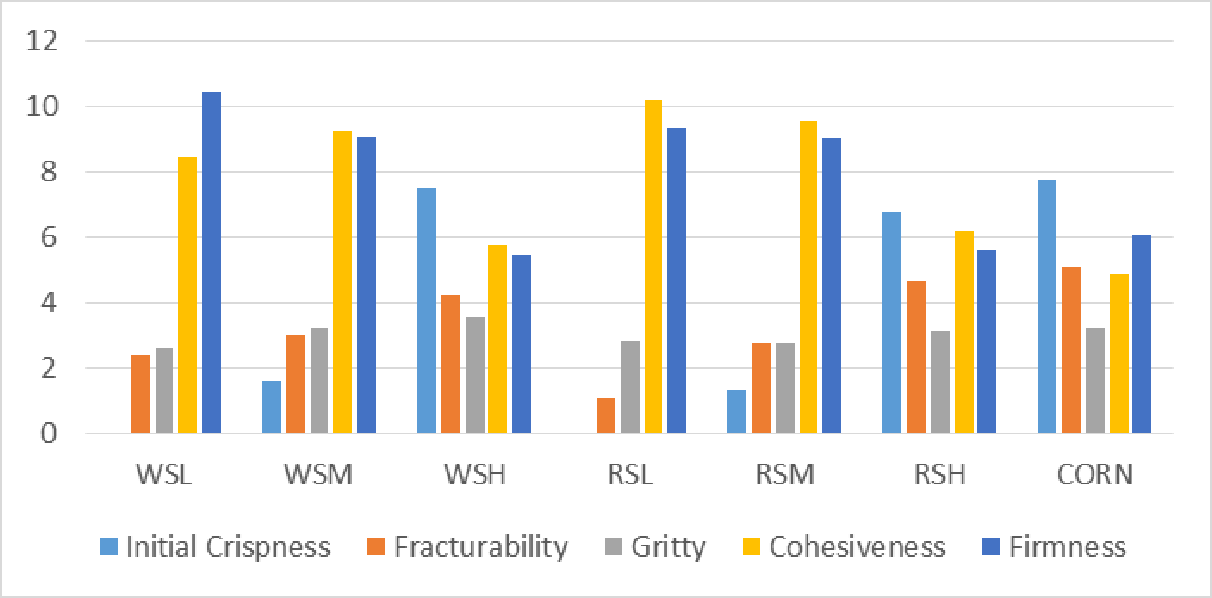


Figure 14. Bar graph of significantly different texture attributes.

GC-MS

The results for volatile compounds for all samples are shown in Table 7. Total of 19 volatile compounds were found in all these 7 samples. Volatile compounds Butanedial, Pentanal, Hexanal, and 2-pentylfuran were found in all these 7 samples. The volatile compound Hexanal was the highest concentration in both control samples and sorghum samples. The volatile compounds Butanedial, Hexanal, and Propanoic acid were more prominent in all 7 samples. The volatile compounds found in the some of the sorghum samples, but not in the control were Tetrahydrofuran, 2-Butanone, 2-methylbutanal, 3-methyl-1-butanol, 1-Pentanol, 2,3-Octanedione, 1-methylbutyl-oxirane, Furfural, and 3-Furaldehyde. The volatile compounds that were found at a higher concentration in the control sample were 3-Carene, 5-methylhexanal, 2-pentylfuran, and 1-Octen-3-ol.1-Pentanol, Butanedial, Pentanal, Hexanal, 2-pentylfuran, 1-Octen-3-ol, 5-methylhexanal, 2,3-octanedione and Furfural have also been mentioned in previous studies on pet foods (Koppel et al 2013, Koppel et al. 2014; Di Donfrancesco et al. 2017).

Table 7. Volatile compounds for all 7 samples.

Retention time (min)	MW	Formula	Match name	CORN / (ppm)	RSL/ (ppm)	RSM/ (ppm)	RSH/ (ppm)	WSL/ (ppm)	WSM /(ppm)	WSH/ (ppm)
3.84	72	C4H8O	Tetrahydrofuran	0 D	0 D	1.778 A	0 D	1.604 B	0 D	1.343 C
4.64	72	C4H8O	2-Butanone	0 D	2.259 A	0 D	0 D	1.662 B	0.7403 C	2.040 A
4.87	86	C5H10O	2-methylbutanal	0 D	5.809 A	5.135 BA	0 D	2.501 C	0.4516 D	4.243 B
4.97	86	C4H6O2	Butanedial	35.12 B	28.21 B	25.37 B	80.83 A	15.48 B	17.11 B	18.38 B
6.55	86	C5H10O	Pentanal	12.62 BA	5.479 C	5.487 C	17.35 A	2.763 C	6.836 BC	11.79 BA
7.45	136	C10H16	3-Carene	1.486 A	0 C	0 C	0 C	0 C	0.7832 B	0 C
9.5	100	C6H12O	Hexanal	69.48 BA	35.34 DC	36.02 DC	91.48 A	12.08 D	37.41 DC	58.60 BC
12.66	114	C7H14O	5-methylhexanal	6.296 A	2.942 B	3.423 B	4.084 B	0C	3.703 B	3.647 B
13.34	88	C5H12O	3-methyl-1-butanol	0 B	0 B	0 B	0 B	4.199 A	0 B	0B
13.93	138	C9H14O	2-pentylfuran	6.817 A	1.323 B	1.309 B	1.781 B	1.622 B	3.349 BA	1.671 B
14.67	88	C5H12O	1-Pentanol	0 C	2.519 BA	2.105 BA	3.211 A	3.360 A	0 C	1.885 B
16.71	142	C8H14O2	2,3-Octanedione	0 B	0 B	0 B	0 B	0 B	0 B	3.302 A
17.58	114	C7H14O	1-methylbutyl-oxirane	0 B	0 B	0 B	0 B	2.043 A	0 B	0 B
20.11	128	C8H16O	1-Octen-3-ol	5.920 A	5.456 BA	0 C	4.615 B	0 C	0 C	0C
20.43	60	C2H4O2	Acetic acid	5.029 BA	4.116 BC	5.488 BA	0D	6.371 A	2.745 C	4.226 BC
20.68	96	C5H4O2	Furfural	0 B	0 B	0 B	0 B	0 B	0.5492 A	0.5533 A
20.71	96	C5H4O2	3-Furaldehyde	0 B	0.858 A	0 B	0 B	0 B	0 B	0 B
22.47	74	C3H6O2	Propanoic acid	29.36 A	31.15 A	35.30 A	6.217 C	0 C	17.50 B	27.13 BA
24.67	88	C4H8O2	Butanoic acid	5.503 A	5.545 A	5.774 A	0 C	5.596 A	2.505 B	4.451 A

Principal Components Analysis (PCA)

Principal components 1 and 2 explained 60.28% of the variation among the samples (Figure 15). Samples WSM and CORN were positioned close in map, and all the white sorghum samples positioned closer to CORN samples than red sorghum to CORN sample. This suggests that white sorghum samples are more similar to the control sample in their sensory characteristics.

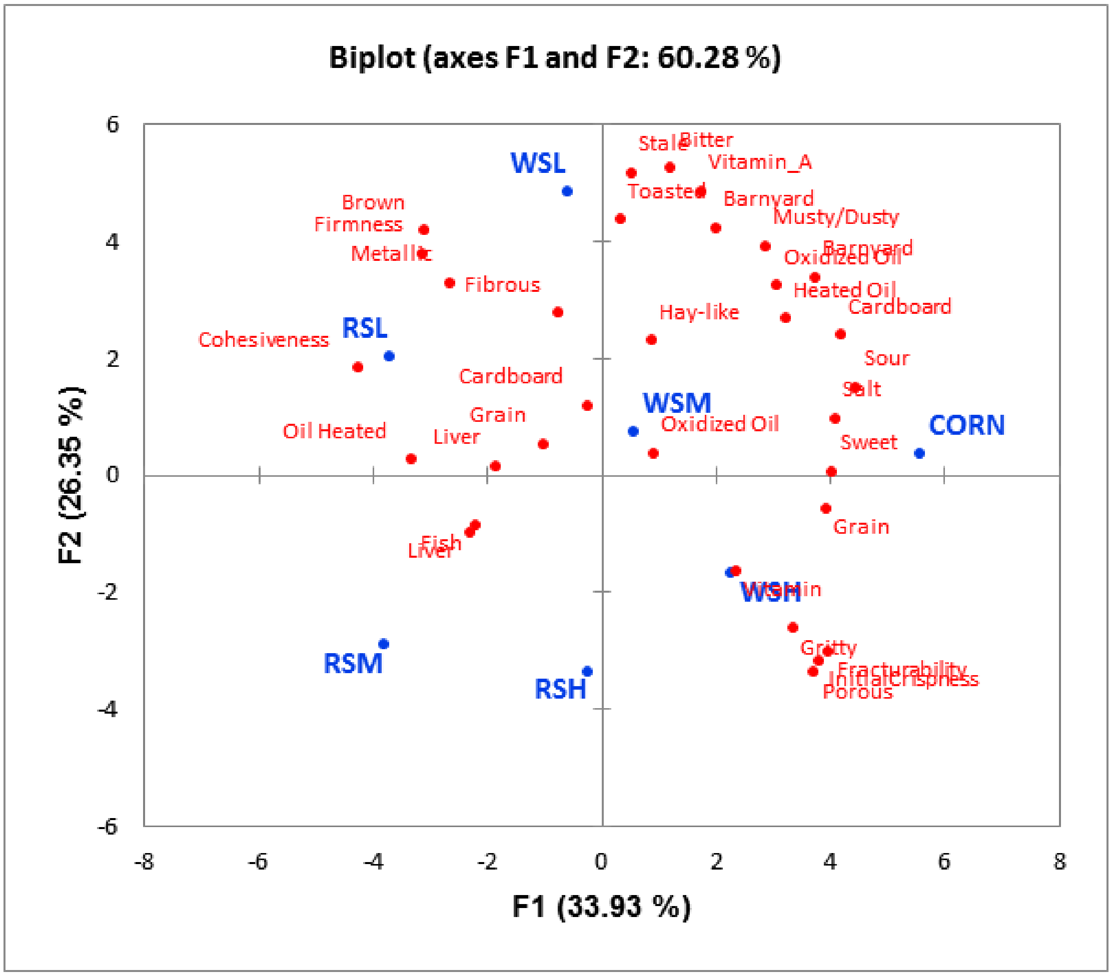


Figure 15. Principal Components Analysis of all samples based on descriptive sensory analysis attributes.

From a sensory standpoint, sorghum seems like a potential substitute for corn in pet food. In this study, we analyzed 32 sensory attributes, and 20 out of 32 attributes didn't present any significant differences between the samples. We could use sorghum at low or medium energy input, instead of corn with higher energy input. Significant differences were found for texture attributes among the samples. Especially for Initial Crispness, Cohesiveness and Firmness attributes only high energy input samples (WSH and RSH) presented the same characteristics as CORN sample. Based on the results listed above, it seems white sorghum might be a good substitute for corn in pet food formulations.

PART – 3. IN-VIVO ANIMAL STUDIES

All animal procedures were approved by the University of Illinois Institutional Animal Care and Use committee. Nine female dogs were used in a replicated 3x3 Latin square design. Each period consisted of 10d of diet adaptation and 4d of fecal and urine collection. Dogs were housed individually (1.2m x 1.8m) with nose to nose contact with dogs in adjacent runs and visual contact with all dogs in room. Dogs were randomly assigned to one of the three experimental diets and were fed to maintain BW and body condition score, which were measured once a week during the experimental period. Water was available ad libitum and feeding was done twice daily at 0800 and 1600 hours. Dogs had access to their assigned food until the next feeding time when food refusals, if present, were collected and recorded. During the collection phase, dogs were housed individually in metabolic cages and given the same access to food and water.

Diets

Three diets containing 30% of corn (C), 30% white sorghum (WS), or 30% red sorghum (RS) were formulated to meet or exceed the AAFCO (2017) nutritional requirements for adult dogs. Diets were manufactured at Kansas State University (KSU – Manhattan, KS, USA). All three experimental diets had similar ingredient composition, except for the carbohydrate source (Table 1).

Sample Collection

Throughout the 4-d of total fecal and urine collection, all feces were collected and scored using the following 5-point scale: 1 = hard, dry pellets; small hard mass; 2= hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured. All individual fecal samples identified by dog and period were stored in -20C freezer until analysis. Similarly, total urine output was collected simultaneously with fecal collection periods. The volume ad weight of acidified urine samples (10 mL of 2N HCI) were recorded and approximately 25% of each sample was saved for further analysis. Urine samples from each dog by period were stored in separate containers and frozen at -20C.

One fresh fecal sample from each dog was collected within 15 minutes (min) of defecation and analyzed for dry matter **(DM),** phenols and indoles, short-chain fatty acids **(SCFA),** branched-chain fatty acids **(BCFA)** and microbiota. A pH reading, fecal score, and total sample weight also were taken. Dry matter was measured by drying approximately 2 grams **(g)** of feces in duplicate in a 105 C oven until all moisture was removed. Approximately 2 g of feces in duplicate were stored in plastic tunes covered in parafilm and frozen at -20 C for subsequent indole and phenol analyses. Finally, 5 g of sample were stored in Nalgene bottles containing 5 milliliters (mL) of 2N hydrochloric acid and frozen at -20 C to determine SCFA and BCFA concentrations.

After overnight fasting, 5 mL of blood were collected via jugular venipuncture from each dog at the end of each experimental period. BD Vacutainer serum separator tubes and ECTA tubes

(Becton, Dickinson and Company, Franklin Lakes, NJ) were used for serum chemistry and complete blood count analyses, respectively. These analyses were conducted by the Clinical Pathology Laboratory at the University Of Illinois College Of Veterinary Medicine (Urbana, IL).

Chemical Analyses

Fecal samples from each dog and period were pooled and dried in a 57 C oven before grinding in the Wiley Mill with a 10 mesh (2mm) screen size and used for subsequent laboratorial analyses. Dry matter, organic matter **(OM)**, and ash were determined for the diets and feces using the Association of Official Analytical Chemists 1975 procedure (#942.05). Acid-hydrolyzed fat **(AHF)** of the diet and feces were done, following methods of the American Association of Cereal Chemists (Method 30-14), the official Methods of Analysis of AOAC International (2002), and Budde et al. (1952). Crude protein **(CP)** analysis was done by measuring total nitrogen using a LECO TruMac (model 630-300-300) and following the Official Method of AOAC International (2002). Gross energy of diets and feces were measured using a Parr 6200 calorimeter (Parr Instrument Company, Moline, IL).

Short chain fatty acids and BCFA concentrations were analyzed using a gas chromatograph with a glass 6’x1/4’ ODx4mmID column and 10%SP1200/1%H3PO4 on 80/100 Chrom-WAW, following the methods of Erwin et al. (1961), Supleco Inc. (1975) and Goodall and Byers (1978).

Statistical Analysis

Data were analyzed using SAS, version 9.4, using MIXED model procedures. The statistical model included the fixed effect of diet and the random effect of animal. Data normality was checked using the UNIVARIATE procedure of SAS. All treatment least-square means were compared with each other and the Turkey adjustment was used to control for experiment-wise error. P-value less than 0.5 were considered statistically different, whereas p-values less than 0.10 but greater than 0.05 were considered to represent a trend.

Results

All three diets were formulated targeting a similar nutrient profile and to be isonitrogenous and isocaloric (Table 1). Analyzed chemical composition of the experimental diets revealed that all diets were isocaloric and had similar nutrient profile (Table 8).

Daily food intake (DMB), fecal output g/day (as is), fecal output g/day (DMB) and fecal score did not differ ($P > 0.05$) among treatments (Table 9). Likewise, apparent total tract digestibility of DM, OM, CP, AHF and digestible energy were not affected ($P > 0.05$) by treatments.

Fecal pH did not have significant difference ($P > 0.05$) among treatments fed (Table 10). Fecal concentrations of acetate, propionate, and butyrate did not differ among all treatments ($P > 0.05$). Similarly, fecal concentration of valerate was greater in dogs fed C ($P < 0.05$) compared with dogs fed RS diets, with WS being not significantly different than either C or RS (P > 0.05; Table 10).

Serum chemistry profiles of dogs fed diets containing WS, RS, or C were within reference range for adult dogs and did not differ among each other (Table 11). Likewise, complete blood count results were normal among all dogs and dietary treatments (data not shown).

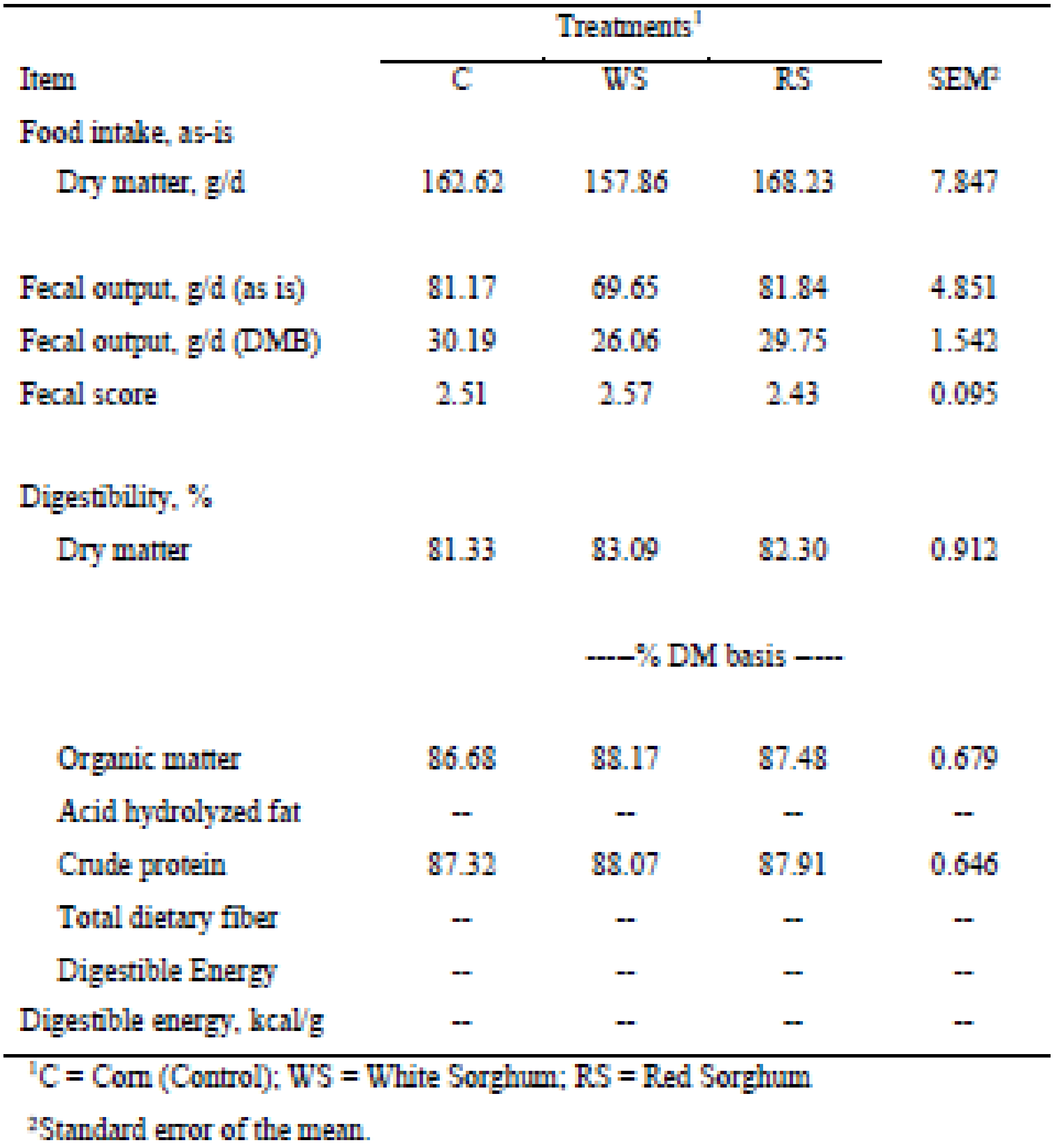
Overall, the data gathered herein demonstrate that diets formulated with up to 30% of white or red sorghum are nutritional adequate for adult dogs, without negatively affecting nutrient digestibility or causing any signs of gastrointestinal discomfort or intolerance. Coefficients of nutrient digestibility for the nutrients were high, and comparable with premium commercial canine diets. Fecal concentrations of short-chain fatty acids (e.g., acetate, propionate, and butyrate) indicate that WS and RS diets had comparable hindgut fermentation of C diet. Even though no statistical difference was not observed for fecal butyrate concentration, a numerical increase of this SCFA was noted in fecal samples of dogs fed RS and WS. This is an important finding as butyrate is a major energy source for colonocytes and is important to support gut health.

Table 8. Chemical composition of treatments.

	Treatments[1]		
Item	C	WS	RS
Dry matter, %	94.24	94.09	94.19
		----- % DM basis -----	
Organic matter	89.67	89.90	89.45
Ash	10.33	10.10	10.55
Acid hydrolyzed fat	18.04	18.92	18.13
Crude protein	44.96	44.70	44.23
Total dietary fiber	--	--	--
Soluble dietary fiber	--	--	--
Insoluble dietary fiber	--	--	--
Gross energy, kcal/g	5.34	5.36	5.32

[1] C = Corn (Control); WS = White Sorghum; RS = Red Sorghum

Table 9. Digestibility results for adult canines.

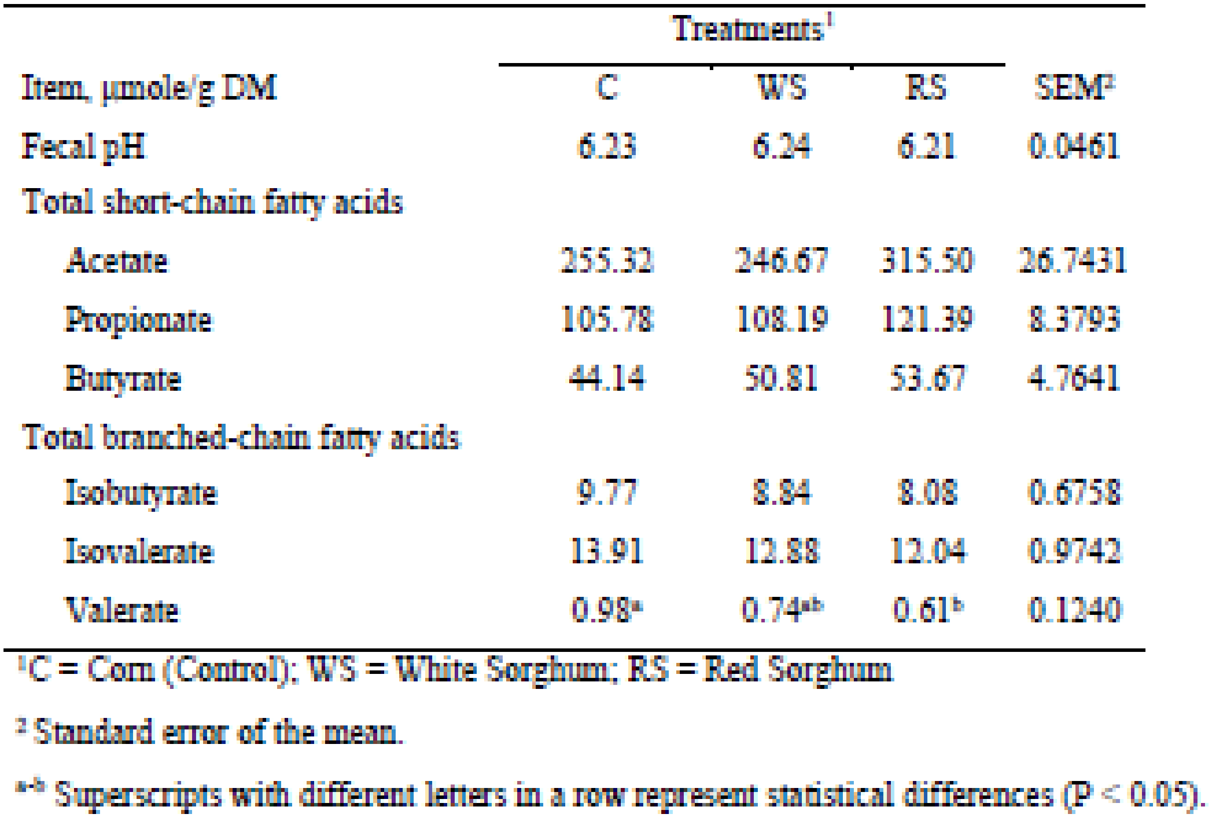


	Treatments[1]			
Item	C	WS	RS	SEM[2]
Food intake, as-is				
Dry matter, g/d	162.62	157.86	168.23	7.847
Fecal output, g/d (as is)	81.17	69.65	81.84	4.851
Fecal output, g/d (DMB)	30.19	26.06	29.75	1.542
Fecal score	2.51	2.57	2.43	0.095
Digestibility, %				
Dry matter	81.33	83.09	82.30	0.912
		----% DM basis ----		
Organic matter	86.68	88.17	87.48	0.679
Acid hydrolyzed fat	--	--	--	--
Crude protein	87.32	88.07	87.91	0.646
Total dietary fiber	--	--	--	--
Digestible Energy	--	--	--	--
Digestible energy, kcal/g	--	--	--	--

[1] C = Corn (Control); WS = White Sorghum; RS = Red Sorghum

[2] Standard error of the mean.

Table 10. Fecal fermentation end products for adult canines.



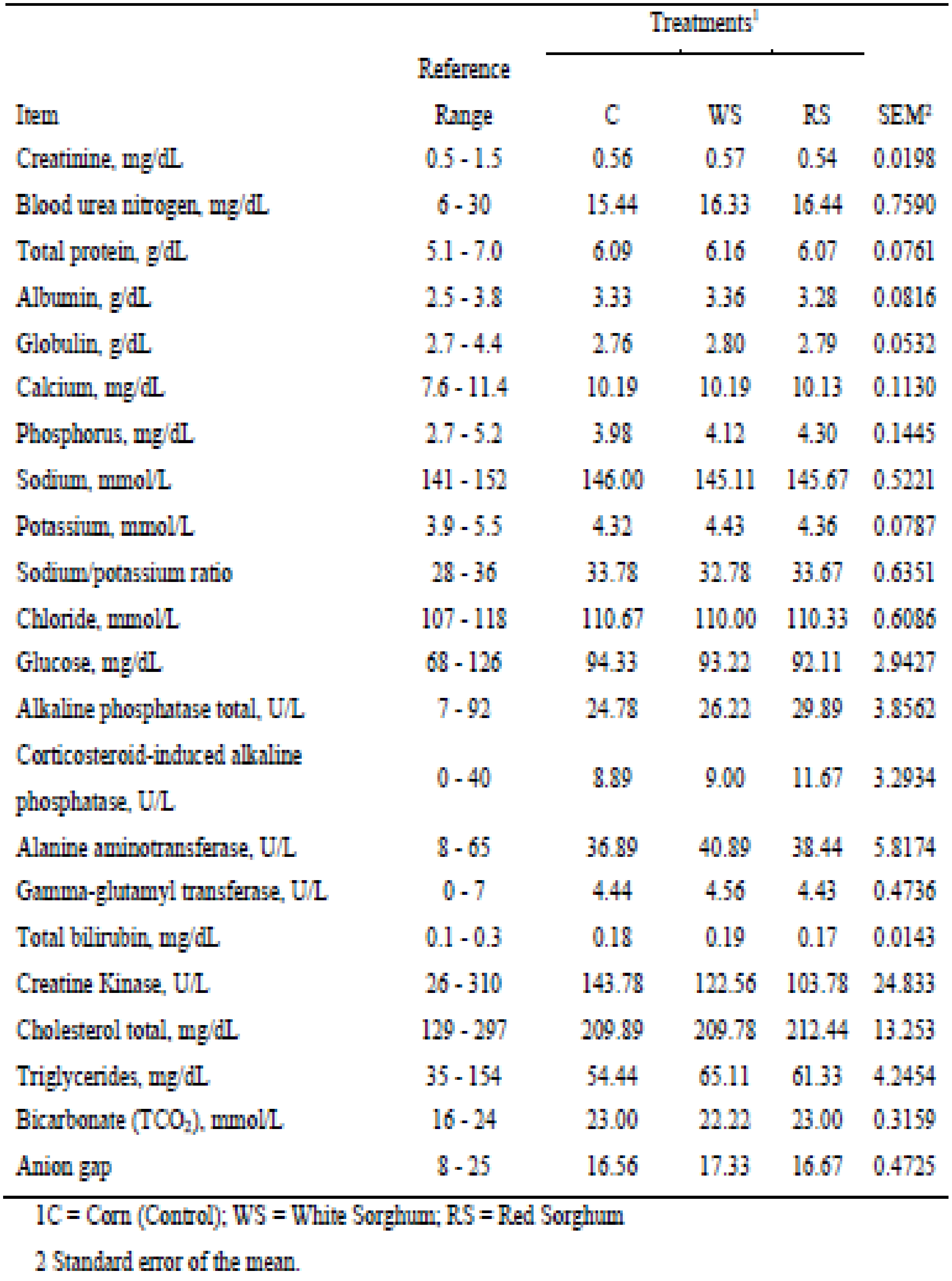
	Treatments[1]			
Item, µmole/g DM	C	WS	RS	SEM[2]
Fecal pH	6.23	6.24	6.21	0.0461
Total short-chain fatty acids				
Acetate	255.32	246.67	315.50	26.7431
Propionate	105.78	108.19	121.39	8.3793
Butyrate	44.14	50.81	53.67	4.7641
Total branched-chain fatty acids				
Isobutyrate	9.77	8.84	8.08	0.6758
Isovalerate	13.91	12.88	12.04	0.9742
Valerate	0.98^{a}	0.74^{ab}	0.61^{b}	0.1240

[1] C = Corn (Control); WS = White Sorghum; RS = Red Sorghum

[2] Standard error of the mean.

[a-b] Superscripts with different letters in a row represent statistical differences ($P < 0.05$).

Table 11. Fasted serum chemistry profile for adult canines.



	Reference	Treatments[1]			
Item	Range	C	WS	RS	SEM[2]
Creatinine, mg/dL	0.5 - 1.5	0.56	0.57	0.54	0.0198
Blood urea nitrogen, mg/dL	6 - 30	15.44	16.33	16.44	0.7590
Total protein, g/dL	5.1 - 7.0	6.09	6.16	6.07	0.0761
Albumin, g/dL	2.5 - 3.8	3.33	3.36	3.28	0.0816
Globulin, g/dL	2.7 - 4.4	2.76	2.80	2.79	0.0532
Calcium, mg/dL	7.6 - 11.4	10.19	10.19	10.13	0.1130
Phosphorus, mg/dL	2.7 - 5.2	3.98	4.12	4.30	0.1445
Sodium, mmol/L	141 - 152	146.00	145.11	145.67	0.5221
Potassium, mmol/L	3.9 - 5.5	4.32	4.43	4.36	0.0787
Sodium/potassium ratio	28 - 36	33.78	32.78	33.67	0.6351
Chloride, mmol/L	107 - 118	110.67	110.00	110.33	0.6086
Glucose, mg/dL	68 - 126	94.33	93.22	92.11	2.9427
Alkaline phosphatase total, U/L	7 - 92	24.78	26.22	29.89	3.8562
Corticosteroid-induced alkaline phosphatase, U/L	0 - 40	8.89	9.00	11.67	3.2934
Alanine aminotransferase, U/L	8 - 65	36.89	40.89	38.44	5.8174
Gamma-glutamyl transferase, U/L	0 - 7	4.44	4.56	4.43	0.4736
Total bilirubin, mg/dL	0.1 - 0.3	0.18	0.19	0.17	0.0143
Creatine Kinase, U/L	26 - 310	143.78	122.56	103.78	24.833
Cholesterol total, mg/dL	129 - 297	209.89	209.78	212.44	13.253
Triglycerides, mg/dL	35 - 154	54.44	65.11	61.33	4.2454
Bicarbonate (TCO_2), mmol/L	16 - 24	23.00	22.22	23.00	0.3159
Anion gap	8 - 25	16.56	17.33	16.67	0.4725

1C = Corn (Control); WS = White Sorghum; RS = Red Sorghum

2 Standard error of the mean.

FUTURE WORK

All project objectives have been completed except Objective 4 related to global metabolic profiling and nutrient digestibility. The in-vivo trials with dogs has been completed at University of Illinois with encouraging results related to colonic fermentation as documented in the report, and other analyses are ongoing and will be completed in the next few months. Specifically the following results will be forthcoming fecal indole and phenol concentrations and fecal microbial community analysis. In addition, UoI has undertaken an extra study (not paid through this grant) to investigate digestibility of extruded red and white sorghum using a cecectomized rooster assay model to analyze TMEn and standardized amino acid digestibility in roosters. Results of that study are also forthcoming.

REFERENCES

Di Donfrancesco, B., Koppel, K. and Chambers, E. 2012. An Initial Lexicon for Sensory Properties of Dry Dog Food. Journal of Sensory Studies 27, 498-510, DOI: 10.1111/joss.12017.

Di Donfrancesco, B., Koppel, K. 2017. Sensory characteristics and volatile components of dry dog foods manufactured with sorghum fractions. Molecules 22, doi:10.3390/molecules22061012.

Koppel, K., Adhikari, K. and Di Donfrancesco, B. 2013. Volatile compounds in dry dog foods and their influence on sensory aromatic profile. Molecules 18, 2646-2662, DOI: 10.3390/molecules18032646.

Koppel, K., Gibson, M., Alavi, S. and Aldrich, G. 2014. The Effects of Cooking Process and Meat Inclusion on Pet Food Flavor and Texture Characteristics. Animals: an open access journal from MDPI 4, 254-271, DOI: 10.3390/ani4020254.

Association of American Feed Control Officials (AAFCO). 2016. Official Publication. Association of American Feed Control Officials, Inc. Champaign, IL.

Association of Official Analytical Chemists (AOAC). 2006. Official methods of analysis. 17thed. Assoc. Off. Anal. Chem. Gaithersburg, MD.

Awika, J. M., and L. W. Rooney. 2004. Sorghum phytochemicals and their potential impact on human health. Phytochem.65:1199-1221.

Budde, E. F. 1952. The determination of fat in baked biscuit type of dog foods. J. Assoc. Off. Agric. Chem. 35:799-805.

Carciofi, A. C., F. S. Takakura, L. D. De-Oliveira, E. Teshima, J. T. Jeremias, M. A. Brunetto, and F. Prada. 2008. Effects of six carbohydrate sources on dog diet digestibility and post prandial glucose and insulin response. Anim. Physiol. Anim. Nutr. 92:326-336.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, et al. 2010. QIIME allows analysis of high280 throughput community sequencing data. Nat. Methods. 7:335–336.

Caporaso, J. G., C. L.Lauber, W. A. Walters, et al. 2012. Ultra-high–throughput microbial

community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6:1621–1624.

de Godoy, M. R. C., K. R. Kerr, and G. C. Fahey, Jr. 2013. Alternative dietary fiber sources in companion animal nutrition. Nutrients. 5:3099-3117.

Erwin, E. S., G. J. March, and E. M. Emergy. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1771.

Flickinger, E. A., E. M. W. C. Schreijen, A. R. Patil, H. S. Hussein, C. M. Grieshop, N. R. Merchen, and G. C. Fahey, Jr. 2003. Nutrient digestibilities, microbial populations, and protein catabolites as affected by fructan supplementation of dog diets. J. Anim. Sci.81:2008-2018.

Food and Drug Administration. 2016. Food labeling: revision of the nutrition and supplement facts labels. Fed. Regist. 81(103):33741-33999.

Goodall, S. R. and F. M. Byers. 1978. Automated micro method for enzymatic L(+) and D(-) lactic acid determinations in biological fluids containing cellular extracts. Anal. Bioch. 89:80-89.

Lozupone C, Knight R. UniFrac: A new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005; 71(12):8228–35.

Prosky, A., N. G. Asp, T. F. Schweizer, J. W. Devries, and I. Furda. 1992. Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. J.AOAC. 75:360-367.

Sorghum Checkoff. 2018. Sorghum: A smart commercial pet food ingredient http://www.sorghumcheckoff.com/assets/media/feedingguides/2018_04_17_ComPetFoodGuidePrint_web.pdf Accessed: July, 2018.

Supleco, Inc, 1975. GC separation of VFAC2-C25. Bull. 304 No. 7498, Bellefonte PA. Supleco, Inc.

Twomey, L. N., D. W. Pethick, J. B. Rowe, M. Choct, J. R. Pluske, W. Brown, and M. C.

Laviste. 2002. The use of sorghum and corn as alternative to rice in dog foods. J. Nutr. 132:1704S–1705S.

United States Department of Agriculture – Foreign Agriculture Service. 2018 data. Available from: https://apps.fas.usda.gov/psdonline/circulars/grain.pdf

Swanson, K. S., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, J. Chow, B. W. Wolf, K. A.

Garleb, and J. Fahey, George C. 2002. Fructooligosaccharides and Lactobacillusacidophilus Modify Gut Microbial Populations, Total Tract Nutrient Digestibilities and

Fecal Protein Catabolite Concentrations in Healthy Adult Dogs. J. Nutr. 132:3721–3731.

Appendix 1

Sensory Definitions and References

Hot water, Hot towel for cleansing, Cracker, Cucumber, Cheese
Serve references at room temperature

Appearance

Brown: Light to dark evaluation of brown color of product.
Reference: Pantone Coated Plus Series 465C =2.0
Pantone Coated Plus Series 464C =4.0
Pantone Coated Plus Series 463C =6.0

Porous: Presence of pores/air bubbles on surface of the mass
Reference: Cheerios = 8.0
Preparation: Cheerios in a 3.25 oz. cup

Flecks: Presence of flecks on the product surface
Yes No Color:

Fibrous: The perception of visible fibers and filaments on the product
Reference: Celery Stem = 4.5
Preparation: Cut celery stem into half inch, and put 4 of it in 3.25 oz.cup

AROMA

Vitamin: The aromatics associated with a just opened bottle of vitamin pills (generally thought to be oxidized thiamin)
Reference: Nature Made Super B-Complex capsule = 4.0(a)
Preparation: Grinded vitamin pill, and serve 0.2g in a medium snifter.

Barnyard: Combination of pungent, slightly sour, hay-like aromatics associated with farm animals and the inside of a barn.
Reference: White pepper in Swanson Chicken Broth 99% Fat Free (0.90g /300ml) = 6.0 (a)
Preparation: Steep 0.90 g of ground white pepper in 300 ml of Swanson Chicken Broth at 180 F for 15 min. (Don't start timing until the temp. goes up to 180F). Filter the solution and let cool for 10 min. Serve ¼ cup in each medium snifter (aroma).

Liver: Aromatics associated with cooked organ meat/liver.
Reference: Grill beef liver = 6.0 (a)
Preparation: Pan-fry beef liver until an internal temperature of 160F. Chop and serve 1 Tablespoon in each medium snifter.

Grain: The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice and oats.
Reference:Cereal Mix (dry) =5.0 (a)

Preparation:Mix 1 cup of each General Mills Rice Chex, General Mills Wheaties and Quaker Quick Oats. Put in a blender and “pulse” blend into small particles. Place 1 Tablespoon in each medium snifter (a)

Cardboard: The aromatic associated with cardboard or paper packaging. The intensity rating is only for the 'cardboardy' character within the reference.
Reference: Cardboard = 7.5 (a)
Preparation: 2" cardboard square in 1/2 Cup of water. Serve in a medium snifter.

Fish An overall impression of fishy aromatics and processed flavor associated with canned fish such as salmon and tuna.
Reference: Nature Made Fish oil pill: 7.0 (a)
Preparation: Cut 1 pill into half and pour the fish oil into a medium snifter.

Oil, heated The aromatics commonly associated with heated oil.
Reference: Wesson Vegetable Oil = 7.0 (aroma)
Preparation: 1/3 cup oil heated for 2 min on high power in the microwave oven. Served 1/3 cup oil in medium individual snifters covered with a watch glass.

Oxidized oil: The aromatics associated with aged oil and fat. May also be defined as rancid or painty at higher levels.
Reference: Microwave Oven Heated Vegetable Oil = 6.0 (a)
Preparation: Add 300ml of oil from a newly purchased and opened bottle of Wesson Vegetable Oil to a 1000ml glass beaker. Heat in the microwave oven on high power for 3 minutes. Remove from the microwave and let sit at room temperature to cool for approximately 25minutes. Then heat another three minutes, let cool another 25 minutes, and heat for one additional 3 minutes interval. Let beaker sit on counter uncovered overnight.
Serve in 1 oz cups, covered.

Hay-like Brown/green, dusty aromatics associated with dry grasses, hay, dry parsley and tea leaves.
Reference: Harts Hay = 7.0 (aroma)
Preparation: Serve 5g Harts Hay in big snifter, covered

Musty/Dusty A dry aromatic associated with stored dry grain.
Reference: Kretschner Wheat Germ = 5.0 (aroma)
Preparation: Serve 1 tablespoon wheat germ in medium snifter, covered

Toasted: A moderately browned/baked impression
Reference: General Mills Cheerios = 6.0 (a)
Preparation: Serve 5g crushed General Mills Cheerios in medium snifter

FLAVOR

Vitamin: The aromatics associated with a just opened bottle of vitamin pills (generally thought to be oxidized thiamin)
Reference: General Mills Wheaties = 2.5 (f)
Preparation: Wheaties in a 3.25 oz cup

Liver: Aromatics associated with cooked organ meat/liver.
Reference: Grill beef liver = 7.5 (f)
Preparation: Pan-fry beef liver until an internal temperature of 160F. Cut into ½” square, serve 4 pieces in 3.25oz cup.

Barnyard: Combination of pungent, slightly sour, hay-like aromatics associated with farm animals and the inside of a barn.
Reference: White pepper in Swanson Chicken Broth 99% Fat Free (0.90g /300ml) = 8.0 (f)
Preparation: Steep 0.90 g of ground white pepper in 300 ml of Swanson Chicken Broth at 180 F for 15 min. (Don’t start timing until the temp. goes up to 180F). Filter the solution and let cool for 10 min. Serve ¼ cup in each medium snifter (aroma).

Grain: The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice and oats.
Reference:Cereal Mix (dry) = 8.0 (f)
Preparation:Mix ½ cup of each General Mills Rice Chex, General Mills Wheaties and Quaker Quick Oats. Put in a blender and “pulse” blend into small particles. Serve in a 1 oz. cup (f)

Heated oil: Aromatics commonly associated with heated vegetable oil
Reference: Wesson Vegetable oil = 8.0 (f)
Preparation: Heat 1/3 cup of oil on high power for 2 ½ minutes in the microwave oven. Let cool and serve in 1oz cup

Oxidized oil: The aromatics associated with aged oil and fat. May also be defined as rancid or painty at higher levels.
Reference: Microwave Oven Heated Vegetable Oil = 7.0 (f)
Preparation: Add 300ml of oil from a newly purchased and opened bottle of Wesson Vegetable Oil to a 1000ml glass beaker. Heat in the microwave oven on high power for 3 minutes. Remove from the microwave and let sit at room temperature to cool for approximately 25minutes. Then heat another three minutes, let cool another 25 minutes, and heat for one additional 3 minutes interval. Let beaker sit on counter uncovered overnight.

Serve in 1 oz cups, covered.

Cardboard: A flat flavor note associated with cardboard or paper packaging that may be associated with a stale characteristic

Reference: Mission Tortilla white flour = 4.0 (f)

Preparation: Serve 5 pieces 1/2” in 3.25 oz cups

Stale: Aromatic impression that is flat, dull, and somewhat dry and lacks fullness and cleanness.

Reference: Mama Mary's Pizza Crust = 4.0 (flavor)

Preparation: Cut pizza crust in square 1x1", serve in 3.25oz cups.

Sour: The fundamental taste factor associated with a citric acid solution.
Reference:0.015% Citric Acid Solution = 1.5

0.050% Citric Acid Solution = 3.5

Salt: A fundamental taste factor of which sodium chloride is typical.
Reference:0.15% NaCl Solution = 1.5
0.25% NaCl Solution = 3.5

Bitter: The fundamental taste factor associated with a caffeine solution.
Reference: 0.02% Caffeine Solution = 3.5
0.035% caffeine = 5.0
0.07% Caffeine Solution = 10.0

Sweet: A fundamental taste factor of which sucrose is typical.
Reference: 1% Sucrose Solution = 2.0

Metallic: An aromatic and mouth feel associated with tin cans or aluminum foil.
Reference: 0.10% Potassium Chloride Solution = 1.5

TEXTURE/FEEL

Initial Crispness: The intensity of audible noise at first chew with molars.
Reference: General Mills Cheerios = 8.0
General Mills Wheaties = 10.5
Preparation: Serve in a 3.25 oz cup.
Serve in a 3.25 oz cup.

Fracturability: The force with which the sample ruptures. Evaluate on first bite down with the molars.
Reference: General Mills Cheerios (one piece) = 4.0
General Mills Wheaties (one piece) = 7.5

Preparation: Serve in a 3.25 oz. cup.

Serve in a 3.25 oz. cup.

Gritty: The perception of small, hard, sharp particles reminiscent of sand or granules in pairs.

Reference: Malt-O-Meal = 2.0

Preparation: Stir 1 cup of water and 3 Tbsp. of Malt-O-Meal. Cook for 1 minute in the microwave. Stir. Cook 1 minute more in the microwave. Serve 1 Tbsp. of malt-O-Meal in 3.25 oz. cup.

Cohesiveness: The degree to which the sample deforms prior to breaking apart when compressed once between the molar teeth (least to most).

Reference: Wheaties = 4.0
Cheerios = 7.0
Rice Krispies Treat= 10.0

Preparation: Serve in 3.25 oz cups
Serve in 3.25 oz cups
Cut one bar into four pieces. Serve in 3.25 oz cups.

Firmness: The force required to bite completely through the sample with the molar teeth. Evaluate on first bite down with the molars.

Reference: General Mills Cheerios = 4.0
Thomas English Muffin= 7.5
Plain Bagels (top) = 10.0

Preparation: Serve a slice of cheese
Slice into two and cut into four pieces.
Serve in 3.25 oz cups.
Slice the bagel and cut into four equal parts. Serve one part in 3.25 oz.