United Sorghum Checkoff Program

Final Report

September 19, 2018

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

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EXECUTIVE SUMMARY

There is potential for use of grain sorghum in pet food products as a less expensive and more sustainable alternative to other cereal ingredients such as corn and rice. Sorghum can also provide nutritional benefits related to slower digestibility of starch or lower glycemic response, which can aid in premium products targeted towards obese, diabetic and geriatric (or old) pets. Digestibility can especially be retarded by controlling the particle size of the grain sorghum used in the diets, and this was the focus of this study, besides evaluation of sensory attributes, and in vivo studies with cats for determining palatability, colonic fermentation by-products and gut microbiota or pre-biotic effect of sorghum-based diets. An initial extensive experiment design was used to screen for processing factors that had the maximum impact. Based on the results, the second extrusion experiment involved formulation of six premium cat food diets using red and white grain sorghum with three particle sizes (0.5mm, 1.0mm and 1.6mm) and also two control diets formulated with brewers rice and corn. The formulations were extruded to make dry expanded kibbles. The finished diets were evaluated for starch gelatinization, in vitro digestibility and other physico-chemical attributes. Palatability trials with cats indicated that it is possible to utilize red or white sorghum varieties as main ingredients in premium extruded cat diets, allowing better results in palatability when compared to diets that included corn or rice as cereal sources. When sorghum varieties were compared, white sorghum presented better consumption than red sorghum. Cats maintained body weight and general health, presented adequate intake and produced feces in normal quantities and scores when fed sorghum-based diets, with no differences found to corn or brown rice-based diets. The intake of diets based on coarse grind sorghum reduced fecal pH, probably by bacterial intestinal fermentation, indicating a potential prebiotic effect that could be important for intestinal health, encouraging additional research. The sensory attributes of the products were evaluated by descriptive sensory analysis and HS-GCMS to understand the differences on sensory perceptions and the volatiles composition. This research provided guidelines in order to formulate the best palatants to mask any undesirable notes and enhance desirables ones. Significant differences among the eight cat food samples were found on appearance attributes of brown, fibrous and texture/mouthfeel attributes of fracturability and gritty. No significant difference was found in terms of aroma and flavor attributes, and this provides scientific evidence in using grain sorghum as a promising ingredient to replace rice and corn without impact on sensory perception. GC-MS results complemented the sensory tests results. Thirty aromatic compounds were tentatively identified and semi-quantified in the eight dry cat food samples manufactured with different red sorghum, white sorghum, rice and corn. The volatile compounds composition of the eight dry cat foods were found to be similar. Dry cat food kibbles produced with the four different ingredients were also coated with seven sources of fat. To compare the difference brought by the coating on sorghum based cat food products, descriptive sensory analysis and HS-GCMS were applied to understand the differences on aromatic profiles and the volatiles composition. Results indicated that fat/oil coating with strong aroma profile (fish & salmon oil) could change the overall perception of the dry cat food a lot by exposing different dominant aroma notes.

PART – 1. FORMULATION, PROCE SSING AND PHYSICO-CHEMICAL CHARACTERIZATION

A pilot-scale Wenger X-20 single screw extrusion system was used for production of dry expanded cat food based on red and white grain sorghum and corn and rice as control for the purpose of – a) physico-chemical characterization of cat food kibbles, c) sensory characterization of the sorghum based dog food, d) obtaining product for in vivo and palatability studies with cats.
Balanced standard cat food diets were formulated (Table 1), mixed and ground using a hammer mill.

The first experiment was conducted with an extensive 2x3x3 full factorial design consisting of two different grain sorghum varieties (red and white), three levels of raw material particle grinding size (0.5mm, 1.0mm and 1.6mm) and three levels of specific thermal to mechanical energy (STE:SME) ratios (low, medium and high). A control corn and rice based cat food diet was also produced. This consisted of a total of 20 treatments. The effect of particle size on the product physico-chemical attributes was significant. In-vitro digestibilities of red or white sorghum based diets (87.6-87.9%) were slightly lower than corn or rice based diets (88.4-90.9%) when controlled for the same raw ingredient particle size (1.0 mm) and processing conditions (high STE: SME). In general, the digestibilities of red sorghum based diets (81.1-89.6%) were similar to those based on white sorghum (79.2-90.2%). A substantial decreasing trend was observed with increase in particle size, but STE: SME ratios did not have much impact on in-vitro digestibility.

Therefore after the initial screening based on in-vitro digestibility, a second extrusion experiment was conducted with a narrower 2x3 full factorial design for sensory, palatability and in vivo studies with cats. The experimental design consisted of two different grain sorghum varieties (red and white) and three levels of raw material particle grinding size (0.5mm, 1.0mm and 1.6mm). A control corn and rice based cat food diet was also produced. This consisted of a total of 8 treatments. Water and steam flow in the preconditioner, and extruder shaft speed were varied to achieve the desired preconditioner temperature and specific thermal to mechanical energy ratios . The in-barrel moisture was approximately 28% (wb). The target specifications for the cat food kibbles were 5.5-6.5 mm diameter, 2-3mm thickness and 350-450g/L bulk density.

The net specific mechanical energy input in the extrusion process ranged from 124-217 kJ/kg (Figure 1). In general, for the sorghum diets highest SME (203-205 kJ/kg) was observed for the medium grind treatments and lowest SME (124-143 kJ/kg) for the coarsest grind treatments.

Table 1. Ingredient composition of experimental diets for cats formulated with different cereal sources.

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

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

Product Physico-Chemical Properties

Processing treatments had a substantial impact on product quality. Starch gelatinization of (glucoamylase enzymatic test) ranged from 72-92%. In general at the same particle size of 1.0mm, sorghum based diets had lower gelatinization (86% for red sorghum and 84% for white sorghum), as compared to corn and rice based control dieta that had a gelatinization of 90 and 89%, respectively (Table 1). The coarser ground (1.6mm) sorghum based diets had an even lower starch gelatinization level (72-77%). This pointed to potential resistant starch and consequentially prebiotic effect in sorghum based diets.

Table 2. Percentage starch gelatinization using glucoamylase enzymatic test. RS = red sorghum based diets; WS = white sorghum based diets; 0.5, 1.0 and 1.6 = fine (0.5mm), medium (1.0mm) and coarse (1.6mm) grind.

Treatment	% Starch gelatinization
Corn1.0	89.9
Rice1.0	89.0
RS0.5	92.0
RS1.0	85.7
RS1.6	77.0
WS0.5	91.1
WS1.0	84.2
WS1.6	72.4

Bulk density of extruded cat food kibbles before drying ranged from 349-396 g/L and after drying from ranged from 347-403 g/L) (Figures 2-3). Bulk density had an increasing trend with the grind size, which was expected as coarser grind represents higher surface area and less hydration and heat transfer. The expansion ratio and piece density data (Figure 4-5) exhibited the same trends as bulk density. The peak crushing force of cat food kibbles (measured using a TA-XT2 texture analyzer) under cutting mode using a knife proble ranged from 1.8-2.4 kg and compression mode using a flat probe ranged from 15.6-19.5 kg (Figure 6-7).

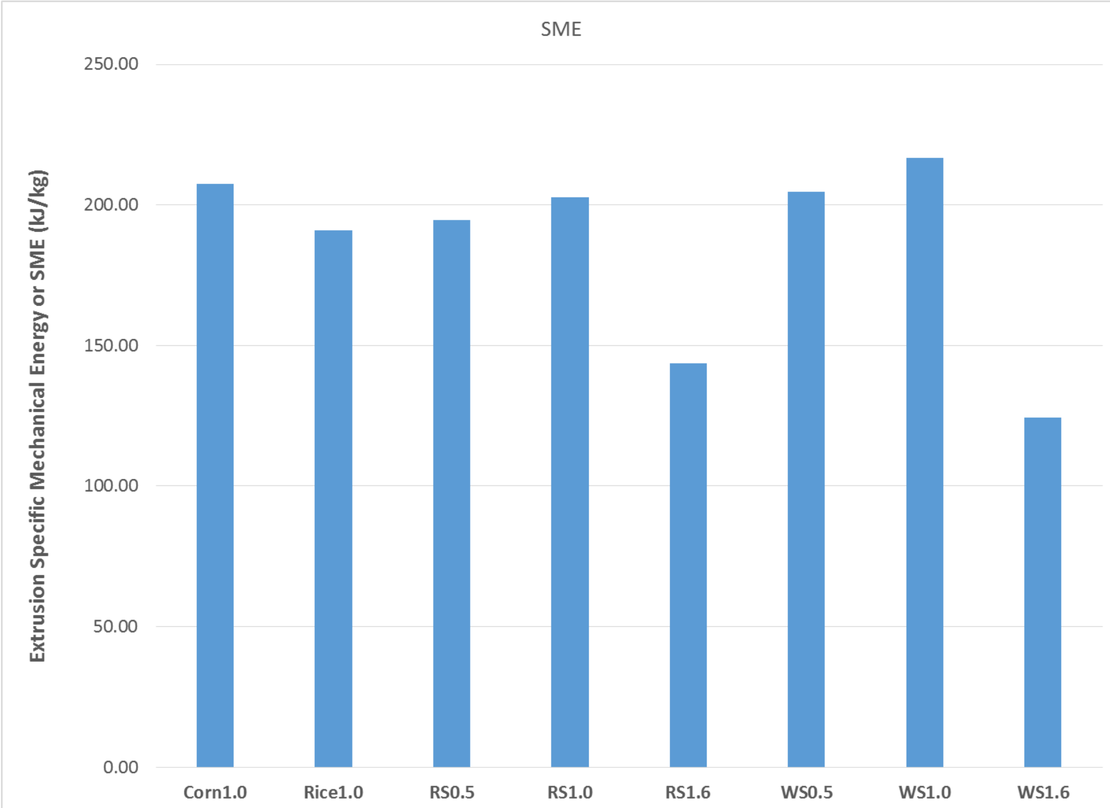


Figure 1. Extrusion specific mechanical energy (SME) input. RS = red sorghum based diets; WS = white sorghum based diets; 0.5, 1.0 and 1.6 = fine (0.5mm), medium (1.0mm) and coarse (1.6mm) grind.

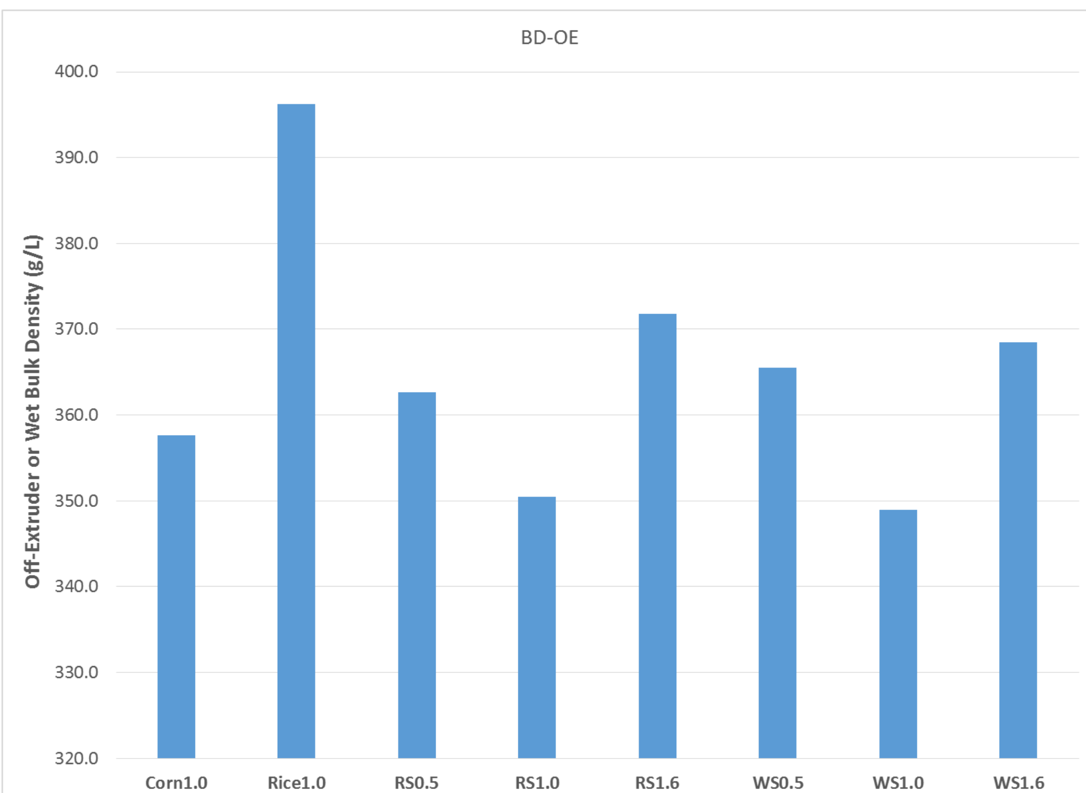


Figure 2. Wet product bulk density. RS = red sorghum based diets; WS = white sorghum based diets; 0.5, 1.0 and 1.6 = fine (0.5mm), medium (1.0mm) and coarse (1.6mm) grind.

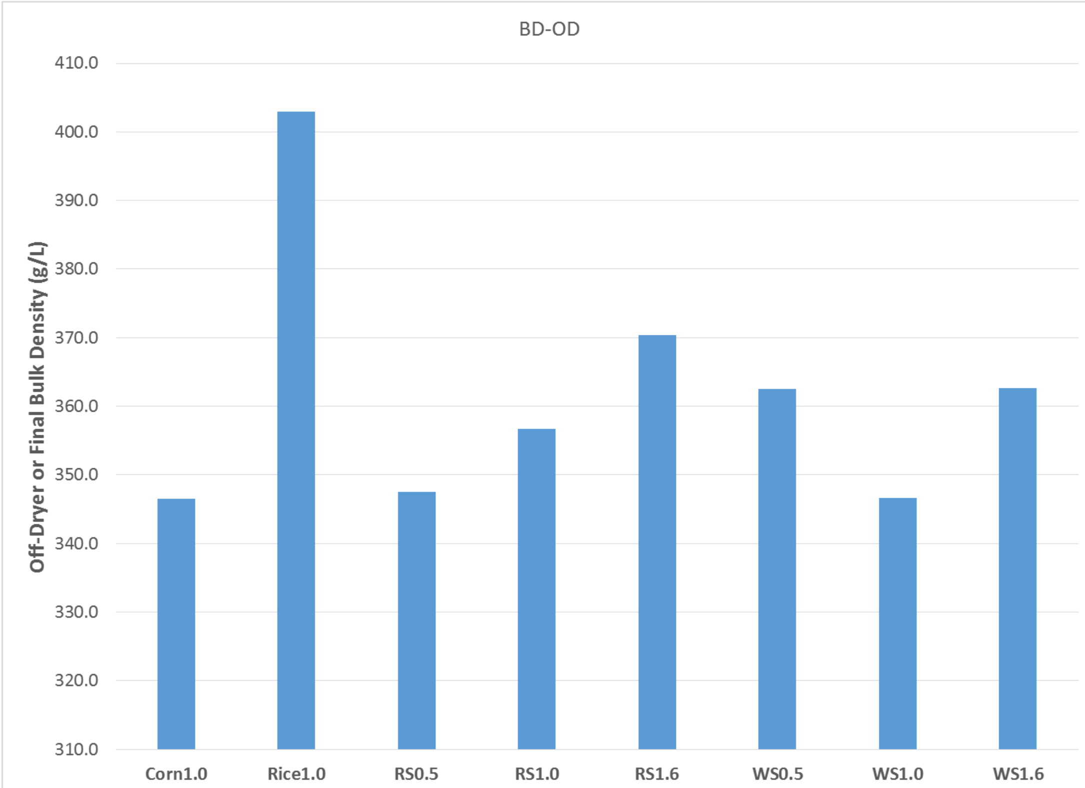


Figure 3. Final product bulk density. RS = red sorghum based diets; WS = white sorghum based diets; 0.5, 1.0 and 1.6 = fine (0.5mm), medium (1.0mm) and coarse (1.6mm) grind.

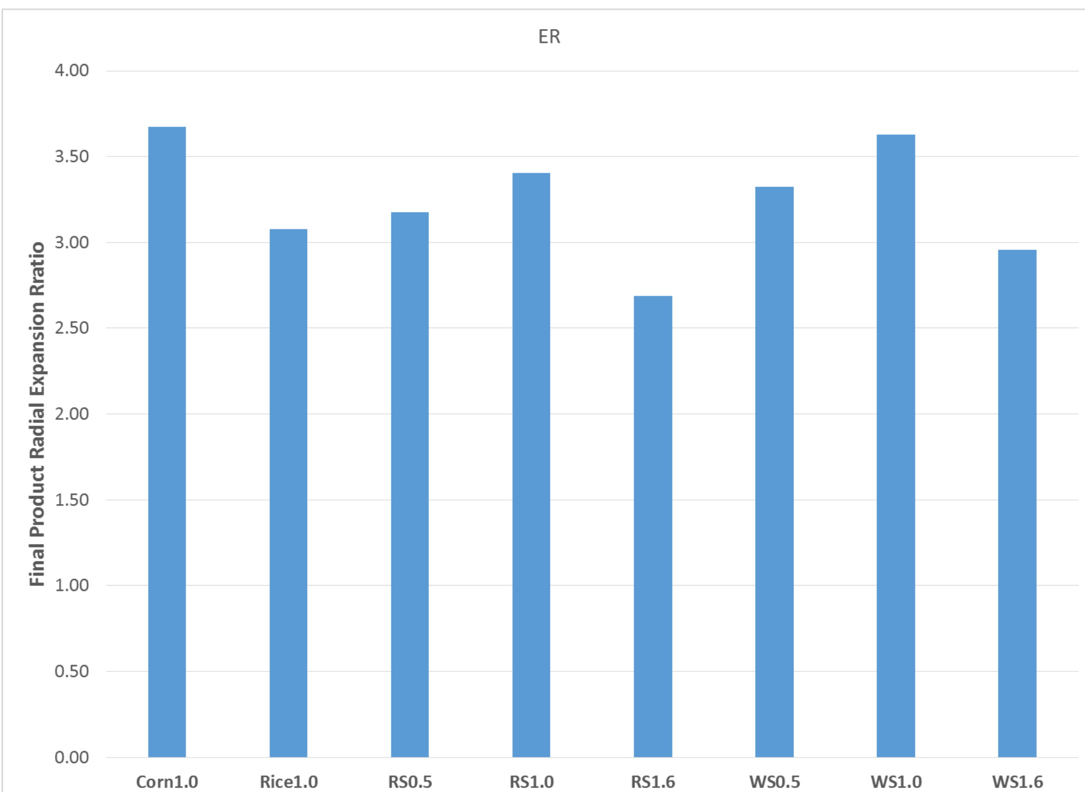


Figure 4. Final product expansion ratio. RS = red sorghum based diets; WS = white sorghum based diets; 0.5, 1.0 and 1.6 = fine (0.5mm), medium (1.0mm) and coarse (1.6mm) grind.

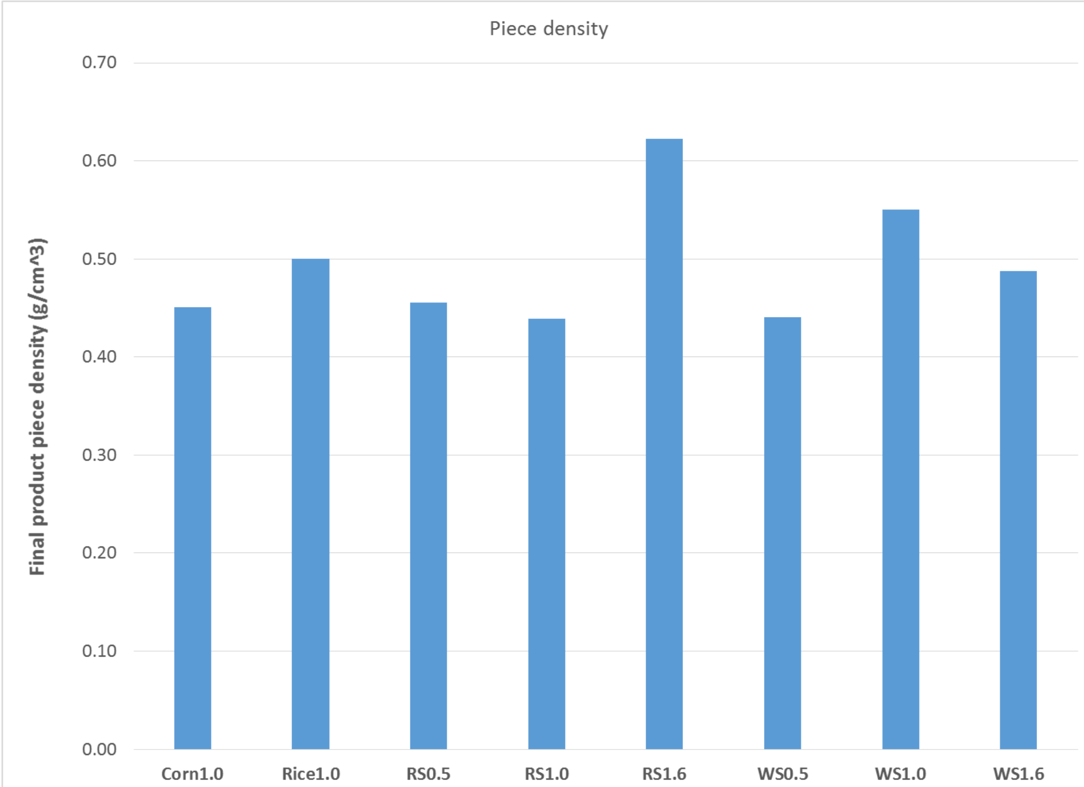


Figure 5. Final product piece density. RS = red sorghum based diets; WS = white sorghum based diets; 0.5, 1.0 and 1.6 = fine (0.5mm), medium (1.0mm) and coarse (1.6mm) grind.

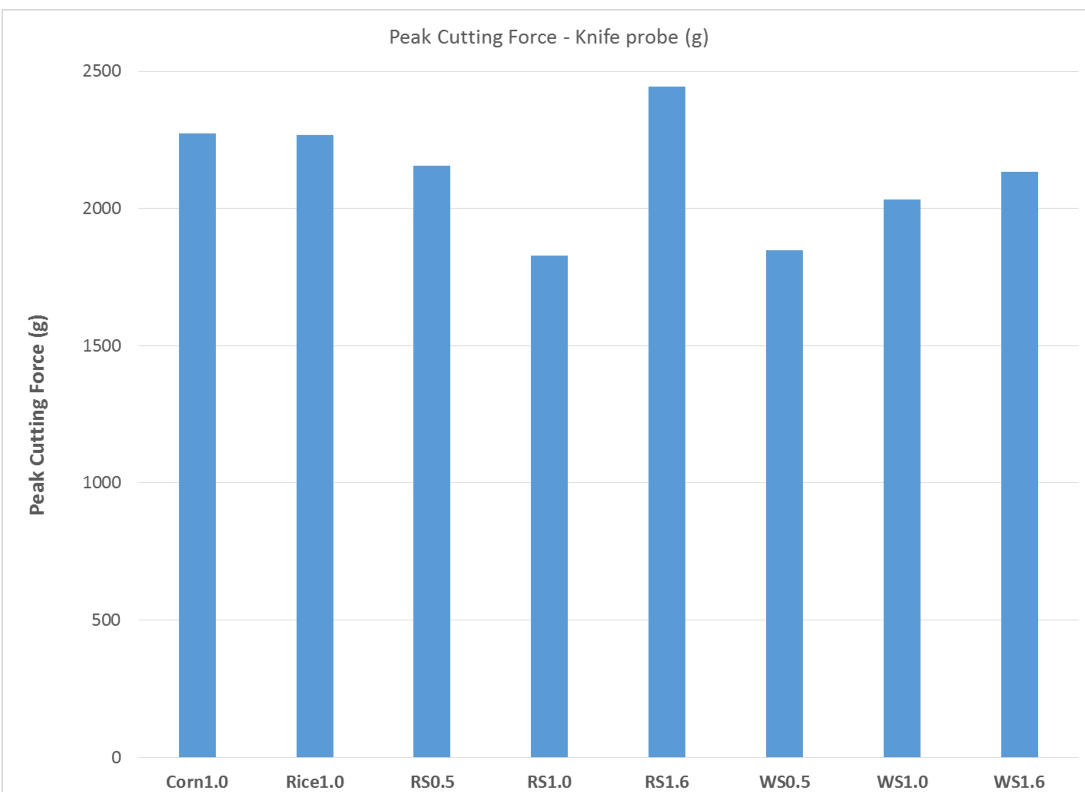


Figure 6. Final product peak cutting force. RS = red sorghum based diets; WS = white sorghum based diets; 0.5, 1.0 and 1.6 = fine (0.5mm), medium (1.0mm) and coarse (1.6mm) grind.

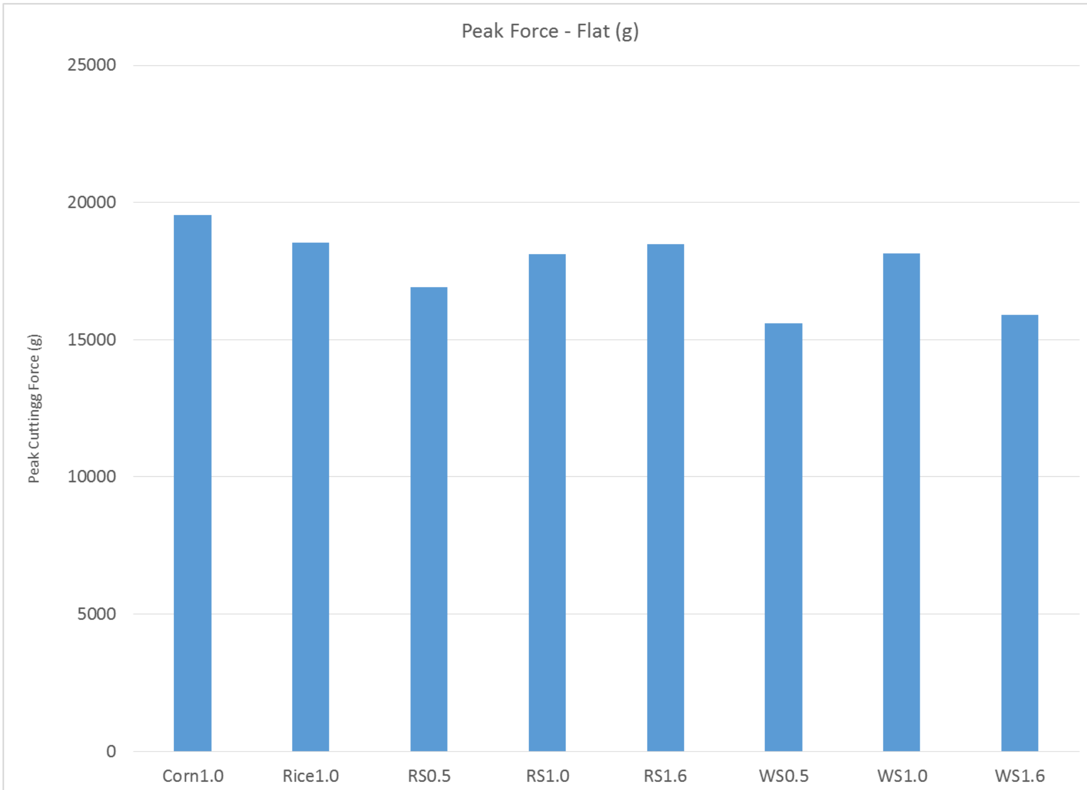


Figure 7. Final product peak crushing force. RS = red sorghum based diets; WS = white sorghum based diets; 0.5, 1.0 and 1.6 = fine (0.5mm), medium (1.0mm) and coarse (1.6mm) grind.

Part – 2. Sensory Analysis

Obj I: Dry cat food produced in similar formula and process, but with different ingredients: rice, corn, red sorghum, white sorghum were compared by using descriptive sensory analysis. Meanwhile, red sorghum and white sorghum with three different level of grind process have been tested, respectively. The purpose is to help to understand the differences between kibbles with different ingredients. So to give guides on formulate the best palatants to mask any undesirable notes and enhance desirables ones.

Samples

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

Sample code	Main Ingredient
Corn 1.0	Corn
Rice 1.0	Rice
RS 0.5	Red Sorghum
RS 1.0	Red Sorghum
RS 1.6	Red Sorghum
WS 0.5	White Sorghum
WS 1.0	White Sorghum
WS 1.6	White Sorghum

Panelists

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

Sample Evaluation

Cat food samples were labeled with 4-digit random codes, were served at room temperature to panelists for evaluation in a sensory lab. Panelists were given Toothbrushes, mozzarella, crackers, cucumbers, tomato juice, and hot water for neutralizing the effect of preceding cat food samples. The panelists evaluated intensities of each cat food for attributes covering sensory characteristics of appearance, aroma, flavor and texture/mouthfeel. Panelists have to taste two kibbles per bite. Intensities were scored on a 15-point numerical scale divided into half-point increments, with 0 meaning "none" and 15 meaning "extremely strong".

Test Design

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

Data Analysis

Three-way MANOVA with 2-way interaction, combing two multivariate statistical measures: Wilks` lambda (Λ) and Pillai`s criterion were used in this work to apply significance tests relating to the differences across dimensions of the dependent variables. If the MANOVA is significant, a series of 3-way ANOVA with 2-way interaction are conducted to evaluate the significance levels of the individual attributes. When there was a significant effect of sample, along with its interaction with judge or replication term, the impact of this interaction was measured using pseudomixed model.

Results

Table 1 and Table 2 contains respectively the MANOVA results for testing both the main effects and interactions. The two statistical measures (Wilk`s Λ and Pillai`s criterion) gave similar significance value for each source of variation, except on Judge*Product. Results show that there were a significant effect of replication, judge, product, replication*judge and Replication*Product toward the dependent variables as a group. There was an exception for the significance level of Judge*Product interaction, in which Wilks` Λ indicated a significant difference ($p < 0.05$), where Pillai`s criterion did not. Since Pillai`s criterion is considered to be the most powerful and most robust in statistics (Gregory Carey, 1998), Pillai`s trace was used in this study. To conclude, the significant effects according to MANOVA were: replication, judge, product, Replication*Judge, Replication*Product.

Table 1. Multivariate tests of Wilks` Λ for group differences in sensory attributes of cat food

Source of Variation	Wilks` Λ	approx F	Pr(>F)
Rep	0.048759	3.7901	6.767e-07 ***
Judge	0.000002	28.1951	< 2.2e-16 ***
Product	0.000572	2.2111	1.429e-08 ***
Rep:Judge	0.000096	2.5769	8.716e-13 ***
Rep:Product	0.000008	1.7059	6.356e-08 ***
Judge:Product	0	1.1655	0.01998 *

Table 2. Multivariate tests of Pillai`s Criterion for group differences in sensory attributes of cat food

Source of Variation	Pillai`s Criterion	approx F	Pr(>F)
Rep	1.5249	3.5658	1.451e-06 ***
Judge	3.7618	18.7196	< 2.2e-16 ***
Product	3.8738	1.6063	0.0002481 ***
Rep:Judge	4.9746	2.1923	2.762e-10 ***
Rep:Product	6.3998	1.3099	0.0017238 **
Judge:Product	9.5606	1.0769	0.1183991

After obtaining a significant multivariate test for the main effects and interaction, univariate F tests (ANOVAs) for each variable were examined for each variable to interpret the respective effect. Table 3 presents the tabulation of significant value of each effects, taken from ANOVA tables, toward every sensory attributes.

Eight attributes (Brown, Porous, Fibrous, Liver (a), Oxidized oil (a), Liver (f), Fracturability (T/M), and Gritty (T/M)) were significantly different across the cat food ($p<0.05$). Moreover, there were significant product*Judge interaction for attributes of Porous, Fibrous and Gritty, and product*replication interaction for Porous, Liver (a), Oxidized oil (a) and Liver (f), which led to the analysis of pseudomixed model.

The pseudomixed model analysis ruled out Porous, Liver (a), Liver (f) and oxidized oil (a) from post-hoc analysis. For attributes of Porous there was a significant interaction between product and judge, which deemed the effect of product unimportant. For attributes of Porous, Liver (a), and oxidized oil (a) and Liver (f), there were a significant interaction between product and replication, which deemed the effect of product unimportant as well.

Table 3. Significant value for main effects and their two-way interaction of every sensory attributes

Attributes	Rep	Judge	Product	R*J	R*P	J*P	Effect of cat food
Brown	0.0001906 ***	0.1475789	< 2.2e-16 ***	0.7083701	< 2.2e-16 ***	0.8464389	Significant
Porous	1.935e-05 ***	6.116e-16 ***	0.002566 **	5.717e-05 ***	0.001103 **+	0.018601 *+	Nonsignificant
Fibrous	1.627e-06 ***	6.693e-14 ***	6.868e-07 ***	0.000215 ***	0.051027	0.016815 *+	Significant
Vitamin (a)	0.03831 *	3.106e-07 ***	0.42489	0.04766 *	0.84465	0.73435	Nonsignificant
Liver (a)	0.223807	0.07102	0.008531 **	0.298729	0.005097 **+	0.112943	Nonsignificant
Grain (a)	0.6069068	4.916e-12 ***	0.7695265	0.0006938 ***	0.0810274	0.6828794	Nonsignificant
Oxidized Oil (a)	0.419908	< 2.2e-16 ***	0.021801 *	0.05413	0.008832 **+	0.565014	Nonsignificant
Cardboard (a)	0.3930853	1.743e-13 ***	0.8287934	0.0003388 ***	0.1654647	0.2927735	Nonsignificant

Vitamin (f)	0.03719 *	8.347e-08 ***	0.20481	0.18082	0.25011	0.76565	Nonsignificant
Liver (f)	0.0359074 *	4.453e-13 ***	0.0312785 *	0.0062666 **	0.0001586 ***+	0.1810971	Nonsignificant
Grain (f)	1.061e-05 ***	3.496e-12 ***	0.391705	0.000229 ***	0.428718	0.704738	Nonsignificant
Heated Oil (f)	0.004979 **	< 2.2e-16 ***	0.560727	0.040460 *	0.904866	0.088855	Nonsignificant
Oxidized Oil (f)	0.2568	<2e-16 ***	0.3866	0.6003	0.3655	0.1609	Nonsignificant
Cardboard (f)	0.009934 **	5.276e-14 ***	0.116794	0.167509	0.281567	0.443369	Nonsignificant
Sour (f)	0.013729 *	5.247e-11 ***	0.059694	0.000552 ***	0.534373	0.165417	Nonsignificant
Salt (f)	0.9115	<2e-16 ***	0.2498	0.2731	0.3097	0.5798	Nonsignificant
Bitter (f)	0.0003518 ***	1.001e-05 ***	0.4232645	0.0003599 ***	0.0165639 *	0.307658	Nonsignificant
Metallic (f)	0.5218	<2e-16 ***	0.8074	0.9701	0.6088	0.8802	Nonsignificant
Initial Crispness (T/M)	0.043275 *	0.005723 **	0.451759	0.415019	0.760682	0.376129	Nonsignificant
Fracturability (T/M)	0.3140284	0.0002932 ***	0.0099551 **	0.0302535 *	0.4589437	0.1906307	Significant
Denseness (T/M)	0.0001097 ***	0.0123650 *	0.5358412	0.065632	0.0446774 *	0.9872928	Nonsignificant
Gritty (T/M)	0.02842 *	< 2.2e-16 ***	5.913e-09 ***	3.040e-06 ***	0.08786	0.03214 *+	Significant
Liver (at)	0.12788	3.325e-16 ***	0.120644	8.421e-05 ***	0.001351 **	0.237014	Nonsignificant
Grain (at)	0.158748	0.012963 *	0.143052	0.000106 ***	0.643245	0.945494	Nonsignificant
Oxidized Oil (at)	0.0444 *	<2e-16 ***	0.9418	0.1607	0.6363	0.1303	Nonsignificant
Cardboard(at)	0.98341	< 2e-16 ***	0.37089	0.03693 *	0.72041	0.4494	Nonsignificant
Bitter (at)	0.0062565 **	1.939e-10 ***	0.931263	0.0003444 ***	0.2857694	0.9278733	Nonsignificant

*indicate significance at p<0.05, ** indicate significance at p<0.01, ***indicate significance at p<0.001

+ Tested for pseudomixed model effect

The Result of LSD analysis toward significant attributes Brown, Fibrous, Fracturability (T/M) and Gritty (T/M) are shown in Table 4, combining the rest mean scores for each products.

Table 4. Mean of attributes for every cat food

Product	Brown	Porous	Fibrous	Vitamin (a)	Liver (a)	Grain (a)	Oxidized Oil (a)
Corn 1.0	6.1 b	7.0	1.2 cde	2.4	3.4	3.0	0.7
Rice 1.0	6.0 bc	6.0	1.8 ab	1.9	3.0	2.9	0.8
RS 0.5	6.0 bc	6.5	1.3 cd	2.2	3.3	3.0	1.0
RS 1.0	6.1 b	6.6	1.5 bc	2.4	3.4	3.4	1.1
RS 1.6	6.6 a	7.1	2.2 a	2.5	3.9	3.1	1.4
WS 0.5	5.4 d	6.2	0.9 de	2.1	3.1	3.1	0.9
WS 1.0	5.2 e	6.5	0.8 e	2.3	3.3	3.1	0.8
WS 1.6	5.9 c	7.0	2.1 a	2.3	3.3	3.0	0.8

Product	Cardboard (a)	Vitamin (f)	Liver (f)	Grain (f)	Heated Oil (f)	Oxidized Oil (f)	Cardboard (f)
Corn 1.0	2.3	2.4	4.0	3.1	0.7	1.2	2.4
Rice 1.0	2.3	2.4	3.5	2.8	0.6	1.2	2.4
RS 0.5	2.4	2.4	3.7	3.2	0.8	1.0	2.5
RS 1.0	2.2	2.5	4.2	3.4	0.7	1.7	2.4
RS 1.6	2.3	2.6	4.1	3.3	0.9	1.2	2.5
WS 0.5	2.4	2.3	3.6	3.3	0.6	1.2	2.2
WS 1.0	2.3	2.2	3.7	3.2	0.5	1.2	2.4
WS 1.6	2.3	2.2	3.9	3.2	0.6	1.4	2.5

Product	Sour (f)	Salt (f)	Bitter (f)	Metallic (f)	Initial Crispness (T/M)	Fracturability (T/M)	Denseness (T/M)
Corn 1.0	0.5	1.0	4.6	0.9	9.0	6.8 ab	5.0
Rice 1.0	0.7	1.0	4.7	0.7	8.5	6.8 ab	5.1
RS 0.5	0.8	1.4	4.2	0.9	8.6	6.0 c	4.7
RS 1.0	0.7	1.2	4.7	0.7	9.1	7.3 a	5.3
RS 1.6	0.5	0.9	4.5	0.6	9.1	7.4 a	4.8
WS 0.5	0.8	1.3	4.6	0.7	8.7	6.3 bc	5.1
WS 1.0	0.3	1.1	4.7	0.8	9.4	6.9 ab	5.2
WS 1.6	0.4	1.1	4.7	0.8	9.2	6.7 abc	4.8

Product	Gritty (T/M)	Liver (at)	Grain (at)	Oxidized Oil (at)	Cardboard(at)	Bitter (at)
Corn 1.0	6.1 b	3.8	2.5	1.2	1.9	4.7
Rice 1.0	5.6 cd	3.6	2.4	1.1	1.8	4.7
RS 0.5	5.2 de	3.8	2.8	1.3	1.9	4.6
RS 1.0	5.9 bc	4.0	2.6	1.3	1.9	4.7
RS 1.6	6.5 a	4.0	2.7	1.3	1.9	4.6
WS 0.5	4.9 e	3.5	2.5	1.1	1.7	4.5
WS 1.0	5.5 cd	3.4	2.6	1.1	2.2	4.6
WS 1.6	6.3 ab	3.9	2.3	1.2	1.8	4.9

Note: Different superscript for every column indicate significant difference among cat food ($p<0.5$) separated by LSD analysis.

Attribute Brown indicates the light to dark evaluation of brown color of product. Basically, all the white sorghum cat food have the lowest brown color. Red sorghum 1.6 cat food has the darkest brown color. Following by RS 1.0 and Corn 1.0 with slight lighter brown color. RS 0.5 and Rice 1.0 have the medium brown color.

Attribute Fibrous indicating the perception of visible fibers and filaments on the product. RS 1.6 and WS 1.6 have the highest score on Fibrous. Following by Rice, RS 1.0, RS0.5 and Corn, WS 0.5 and WS 1.0 have the lowest score on Fibrous.

Attribute Fracturability (T/M) is a texture / mouthfeel indicator on the force with which the sample ruptures. The attribute was evaluate on first bite down with the molars. Red sorghum 1.6 & 1.0 need more force to rupture than white sorghum 1.0, corn and rice. RS 0.5 and WS 0.5 need the lowest force for rupture.

Attribute Gritty (T/M), describe the perception of small, hard, sharp particles reminiscent of sand or granules in pairs after 5-7 chews. RS 1.6 and WS 1.6 have highest perception of Gritty. On the opposite, RS 0.5 and WS 0.5 have the lowest score. In the middle, the perception of Gritty from high to low follow the order by Corn, RS 1.0, Rice and WS 1.0.

Overall, RS1.0 and WS 1.0 were most close to Rice 1.0 and Corn 1.0 in terms of sensory characteristics. They were chosen for next step experiment in Part II.

Discussion

Overall, the 8 cat food were found different in sensory perception in terms of appearance and texture. No significant different have been found on aroma and flavor attributes across the 8 cat food samples. This study provided evidence on showing the grain sorghum produced dry cat food have no differences with rice and corn in in terms of bitterness or astringency. Indicating a promising opportunity replace rice and corn with red Sorghum and white sorghum without impact on taste and aroma of the cat food. Significant differences were found on appearance attributes of Brown and Fibrous, and texture/mouthfeel attributes of Fracturability and Gritty, which could be the focus for further process optimization for the project.

Obj II: Dry cat food kibbles produced with four different ingredients were coated with seven sources of fat in order to compare the difference brought by the coating on sorghum based cat food products.

Samples

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

Kibbles coated with fat (10% in weight) were prepared. Prepare the fat oil: butter, lard and chicken fat need to be melted in microwave before coating. First weigh the needed kibbles and fat. After spread the melted fat oil in the panner evenly first, pour the kibbles in the panner. The kibbles will be coated with the panner (Rollermac) in two minutes with set rotation rate at 16rpm and set temperature at 60 ◦C. Next, lower the temperature to room temperature for kibbles` cooling and evenly coating in the panner in another 15min.
The coated kibbles were stored in glass jars and tested after aging one week in room temperature.

Coating fat Record:

Name	Chicken fat A 802	Chicken fat B 801	Salmon fish oil	Fish oil	Sunflower oil	Unsalted Butter	Lard
Brand	ADF	ADF	Lortscher	Lortscher	Lortscher	Kroger	Morrell

Panelists

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

Sample Evaluation

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

Test Design

Totally the cat food were evaluated in three different sessions: orientation, consensus evaluation and side by side evaluation. In Orientation, six samples (CA RS, Fish oil WS, Sunflower Rice, Butter corn, Lard RS, Salmon fish WS) representative in different sources of ingredients and coating fat were randomly tested by the panelists to build up the attributes for evaluation. In consensus evaluation session, a completely randomized design was used by Red jade to determine the serving order for the cat food. Nine or ten coated cat foods were tested in each of three 2h sessions. Next, three sessions in each of 2h were conducted for side by side evaluation, each session include tasting 8 or 12 samples. Between the first two sessions, a brake of 15min were taken. Unlike in consensus evaluation session, the samples were evaluated in pure monadic. During the side by side evaluation, the samples were presented to the panelist simultaneously. Panelists need to taste the samples following the randomized order. All the panelists will need to validate their consensus data, discuss if they would like to change the data any more. So to finalize the consensus data.

Data Analysis

The means from the 11 attributes were obtained directly from the consensus evaluation. No preliminary work using MANOVA/ANOVA checking if all of them were significantly affected by the different types of the coated cat food. The means from all the 11 attributes were retained and used to build the PCA model. To determine the number of PCs to be retained, Kaiser`s rule and Scree plot was applied in the work.

Results

For identifying pattern and gaining insight into inter-correlation, this work built PCA model from a correlation matric using a dataset resulted from descriptive analysis of 28 coated cat food, and 11 sensory attributes.

Kaiser recommended retaining only PCs with eigenvalues (Table 5) exceeding one for correlation matrix (Latin et al., 2003). Meanwhile scree plot (Figure 1) can be used as a relative judgement by retaining only PCs above an “elbow” – point after which the remaining eigenvalues decline in linear fashion in the curve. It was decided to use the first 3 PCs, accounting for 44.42%, 18.11% and 11.76% of the total variance.

Table 5. Eigenvalues of each PCs extracted from correlation matrix

	Correlation Matrix		
Principal component	eigenvalue	% of variance	cumulative percentage of variance
comp 1	4.89	44.42	44.42
comp 2	1.99	18.11	62.54
comp 3	1.29	11.76	74.30
comp 4	0.85	7.76	82.06
comp 5	0.66	6.01	88.07
comp 6	0.59	5.34	93.41
comp 7	0.35	3.22	96.63
comp 8	0.22	2.02	98.64
comp 9	0.11	0.97	99.61
comp 10	0.04	0.34	99.96
comp 11	0.00	0.04	100.00

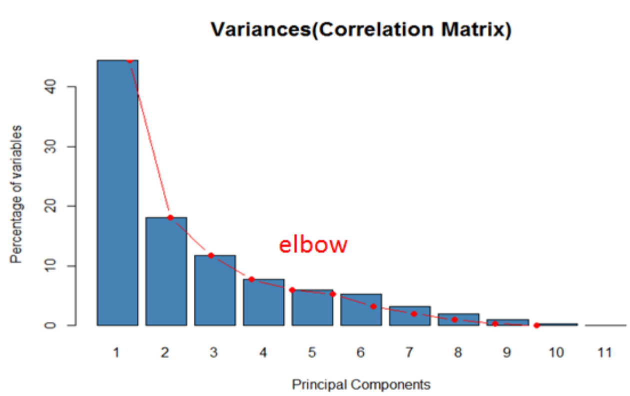


Figure 1. Scree plot of eigenvalues

The biplots of variables and individuals were complemented with contribution value, indicating how much the variables contributing to the PC dimensional space (Figure 2 & 3).

All the four kinds of cat food coated with fish oil and salmon oil (except salmon rice), showed high attribute intensities for aroma attributes of oxidized oil, fish and hay-like, which composed most of the variation of the data shown in PC 1. In contrast, cat food coated with Butter (except Butter RS1.0) and CA RS1.0 were characterized by aroma attributes of Grain and heated oil.

PC 2 contrasted between cat food from Butter RS 1.0, Sunflower RS 1.0 and CB Rice, which is perceived high intensity in aroma attributes of liver and vitamin and appearance attributes of Brwon, versus cat food of WS 1.0 coated with chicken fat B, chicken fat A and Lard, which are perceived as higher intensity in appearance of attribute Porous. Moreover, PC3 contrasted between cat food of CA RS 1.0 which is most fibrous, versus Lard corn, which is perceived as cardboard.
Other cat food did not separate clearly based on the 3 PCs, indicating that they have average scores of attributes, hence lower correlation with the attributes.

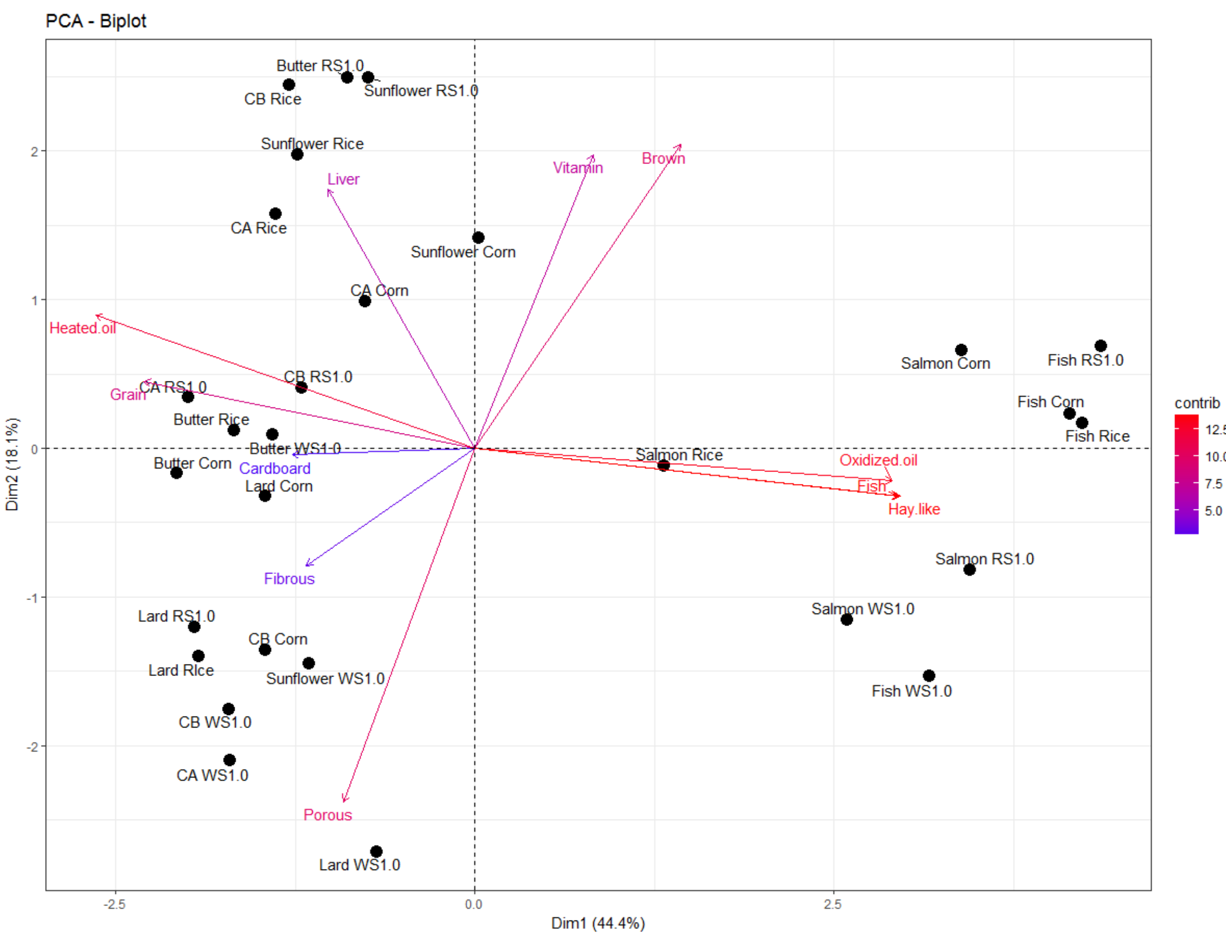


Figure 2. Biplot of variables and individuals from correlation matrix: PC1 vs. PC2

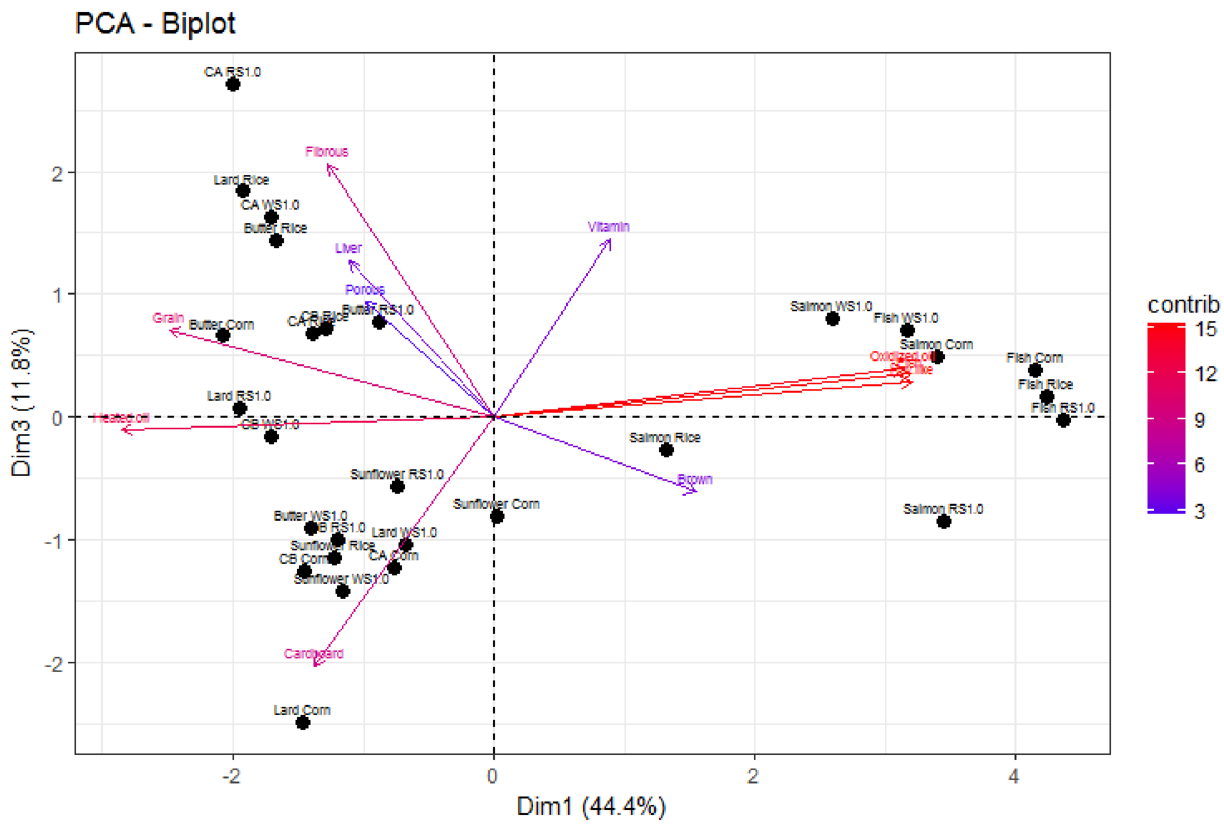


Figure 3. Biplot of variables and individuals from correlation matrix: PC1 vs. PC3

Discussion

In general, the 28 coated dry cat food could be grouped into four groups based on their aroma profile and appearance characteristics. Process of coating with fish oil and salmon oil dominantly separated the coated cat food (no matter produced with which ingredients) with the other ones by higher intensity in aroma attributes of oxidized oil, hay-like and fish notes.

Most of the cat food produced of white sorghum (Sunflower WS1.0, CB WS1.0, CA WS1.0 and Lard WS1.0) were grouped at the bottom of the mapping with highest intensity on porous and least intensity on brown.

Three of the cat food coated with butter (except Butter RS1.0) and CA RS1.0 were characterized with higher aroma intensity of Heated oil and Grain.

Three of the cat food produced of Rice were close in the left top of the mapping due to their higher intensity in aroma attributes of liver, vitamin and darker brown color.

This study show that dry cat food coated with a strong aroma fat oil could distinguish the products very much in terms of overall sensory perception. In the opposite, fat oil with relatively weak aroma intensity like sunflower oil, lard and chicken fat did not distinguish the coated dry cat food with the others. Butter could bring relatively more aroma to the coated dry cat food, therefore, distinguish the cat food out a bit more than sunflower oil, lard and chicken fat. The relative close position of the coated cat food produced of rice in the map on the left top of the mapping, indicating ingredient red sorghum may bring stronger liver aroma to them. Therefore, if the coated fat did not have a strong aroma profile, this factor of fat coating may not impact a lot as much as the ingredient factor did on the overall sensory perception. Similar effect was not observed in the part I experiment (samples were not found significant different in terms of Liver and Vitamin attributes). However, worth an attention for further study.

Another dominant sensory character impact the overall perception was appearance, specifically, Porous and Brown in this case. They separated the coated dry cat food produced of white sorghum out versus the others, indicating the importance of appearance and texture on coated cat food.

Part – 3. Gas Chromatography Studies on Volatiles

Objectives of the study:
Obj I: Understand the volatile compounds composition of dry cat food manufactured with different grain ingredients grinded in different levels: red sorghum, white sorghum, rice and corn.
Obj II: Understand the impact of seven different kinds of fat coating on the volatiles composition of dry cat food manufactured with 4 different grain ingredients.

Key findings:
Obj I:
Thirty aromatic compounds were tentatively identified and semi-quantified in the four dry cat food samples manufactured with different red sorghum, white sorghum, rice and corn. The volatile compounds composition of the four dry cat foods manufactured with different grain ingredients were found similar. Aldehydes represented the main aromatic groups identified. Volatile compounds: Hexanal, 3-Methylbutyraldehyde, Benzaldehyde, 2-Pentylfuran and 1-Octen-3-ol were found relatively high in the quantity among the four kinds of dry cat food.
Part II:
43 volatile compounds were tentatively identified among the 28 coated kibble samples, with 12 new compounds newly defined compared to uncoated kibbles. Coating with fish, salmon and butter oil could be distinguishable, due to specific volatiles with fishy odor were found and high concentration volatiles detected. Coating with chicken fat, sunflower oil, lard did not impact much on the volatile composition of the kibbles compared to uncoated ones, due to the close positions of the products obtained in PCA mapping. Meanwhile, most of the newly detected volatiles were recorded have the odor characteristics of fatty, oily and greasy, indicating similar aromatic perception could be obtained among uncoated kibbles and kibbles coated with the chicken fat, sunflower oil and lard.

Materials and Methods

SPME extraction

SPME integrated sampling, extraction, concentration and sample introduction into a single solvent –free step was invented by Pawliszyn and co-workers in 1989 (Vas, 2004). SPME has been routinely used in combination with gas chromatography (GC) and GC/ mass spectrometry (GC/MS) and successfully applied to a wide variety of compounds, especially for the extraction of volatile and semi-volatile organic compounds from environmental, biological and food sample (Vas, 2004). Kinetics of the SPME extraction process depends on a number of parameters (film thickness, agitation of the sample). Next step is transfer of the analyte from the fiber into the chromatograph. In this case, thermal desorption of the analyte takes place in the hot GC injector. The choice among sampling and chromatography depends mainly on the polarity and volatility of the analytes. Bipolar mixed fiber coatings were widely applied for effective extraction of the volatiles. Different thickness of the coating could affect both the equilibrium time and sensitivity of the method. Agitation of the sample matrix will be able to improve the transportation of analytes from the sample phase to the fiber

Obj1

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

Sample code	Main Ingredient	Production date
Corn 1.0	Corn	07/29/2017
Rice 1.0	Rice	07/30/2017
RS 0.5	Red Sorghum	07/30/2017
RS 1.0	Red Sorghum	07/29/2017
RS 1.6	Red Sorghum	07/29/2017
WS 0.5	White Sorghum	07/30/2017
WS 1.0	White Sorghum	07/30/2017
WS 1.6	White Sorghum	07/30/2017

Obj2

Four cat food (Corn 1.0, Rice 1.0, RS 1.0, WS 1.0) produced in similar grinding grade with different ingredients including rice, corn, red sorghum and white sorghum were coated with seven different source of fat (Chicken fat A, Chicken fat B, Sunflower oil, Salmon oil, Fish oil, Butter and Lard) using a panner to mimic the industrial production of coated kibble. Same batch of the cat food used as in Obj I.

Kibbles coated with fat (10% in weight) were prepared. Butter, lard and chicken fat needed to be melted in microwave before coating. The needed kibbles and fat was weighed. The melted fat oil in the panner was spread and the kibbles were poured in the panner. The kibbles were be coated with the panner (Rollermac) in two minutes with set rotation rate at 16rpm and set temperature at 60 ◦C. Next, lower the temperature to room temperature to cool the kibbles for another 15min. The coated kibbles were stored in glass jars and tested after aging one week at room temperature.

Coating Fat Record

Name	Brand	Ingredients
Chicken fat A 802	ADF	Chicken fat
Chicken fat B 801	ADF	Chicken fat, BHA, Propyl Gallate, and Citric acid
Salmon fish oil	Lortscher	Salmon oil, Natural antioxidant
Fish oil	Lortscher	Menhaden Fish oil, Natural antioxidant
Sunflower oil	Lortscher	Sunflower Oil, Natural antioxidant
Unsalted Butter	Kroger	Pasteurized cream (from milk), Natural Flavorings
Lard	Morrell	Lard, BHT, BHA

Volatile compounds extraction

Same volatiles extraction methods were used for both Obj I & II. Solid phase microextraction (SPME) was applied to extract the volatiles of the eight dry cat food samples manufactured with rice, corn, red sorghum and white sorghum. The extraction method followed the approach in Di Donfrancesco and Koppel`s research on studying dry dog food (Di Donfrancesco, 2017). The samples were ground into powder, then 0.5g + 0.02g of each sample were weighted and placed in a 10mL screw-cap vial (Supelco Analytical, Bellefonte, PA, USA) equipped with a polytetrafluoroethylene / silicon septum (Supelco Analytical, Bellefonte, PA, USA). Next, 0.99mL of distilled water was added to each of the ground sample in the vial, followed by adding 0.01mL of 100ppm 1,3-dichlorobenzene dissolved in methanol (Sigma Aldrich, St. Louis, MO, USA) as the internal standard. Therefore, the final concentration of the internal standard in the sample is 20ug/kg.

Vials were equilibrated for 10 min at 40°C and then agitated at 250rpm by using an autosampler (Pal system, model CombiPal, CTC Analytics, Zwingen, Switzerland). Next, a 50/30 um divinylbenzene / carboxen / dimethyl-siloxilane fiber (Supelco Analytical, Bellefonte, PA, USA) was utilized to extract from the headspace of the vials for 30min at 40°C. After sampling, the analytes were desorbed from the SPME fiber coating to the injection port of gas chromatography (GC) at 270°C for 3min in splitless mode.

GC-MS analysis

Same GC-MS analysis methods were applied for both Obj I & II. GC-MS analysis followed the approach built up by Di Donfrancesco and Koppel`s research on dry dog food (Di Donfrancesco, 2017). Isolation, tentative identification, and semi-quantification of the

volatile compounds were performed on a gas chromatograph (Varian GC CP3800; Varian Incl., Walnut Creek, CA, USA) coupled with a Varian mass spectrometer (MS) detector (Saturn 2000). The GC-MS system was equipped with a Stabilwax (Crossbond® 5% Carbowax polyethylene glycol) column (Restek, U.S., Bellefonte, PA, USA; 30m *0.25mm * 0.5µm film thickness). The initial temperature of the column was 40°C and it was held at the temperature for 4min; the temperature was then increased by 5°C per minute to 240°C and held at this temperature for 10min. All of the samples were analyzed in duplicate.
To identify the compounds, two different methods were utilized: (1) mass spectra, and (2) Kovats indices (NIST Mass Spectral Library, Version 2.0, 2005, Gaithersburg, MD, USA). It was considered to be tentative identification for pure chemicals when only mass spectral data could be obtained. To calculate the Kovats indices, C7-C16 saturated alkane mix (Supelco Analytical, Bellefonte, PA, USA) was used to determine experimental Kovats indices for the volatile compounds detected. Fifteen chemicals were additionally used to confirm the volatiles detected under same detection conditions. They were Isobutyraldehyde, 3-Methylbutyraldehyde, Pentanal, Hexanal, Heptanal, Octanal, 6-Methyl-5-Hepten-2-one, Nonanal, 1-Octen-3-ol, 1-Heptanol, Furfural, 2-Ethylhexanol, Benzaldehyde.

Statistical Analysis

The average concentration for the 43 compounds identified in the 28 coated kibbles and 8 uncoated were retained and used to build the PCA model conducted in RStudio (version 3.4.1) with package of SensoMineR, to understand the impact of coating on kibbles` volatiles composition. To determine the number of PCs to be retained, Kaiser`s rule and Scree plot was applied in the work.

Results

Obj I (see Appendix 1 for Table 1 with GCMS volatiles data)

A total of 31 aromatic compounds were tentatively identified among the eight kinds of dry cat food produced with different ingredients (Table.1), including 12 aldehydes, 6 alcohols, 5 ketones, 4 carboxylic acids, 2 furans, 1 alkanes and 1 disulfides.

Aldehydes comprised the largest group of compounds detected in the dry cat foods. Four of the aldehydes have been reported detected in previous two dry dog food researches, including: Hexanal, Furfural, Benzaldehyde and Octanal. Three of the aldehydes: (E)-2-Hexenal, Heptanal and Nonanal have been reported in dry dog food research (Koppel, 2013) and another two aldehydes: Isovaleraldehyde and Cinnamaldehyde have been reported in the study of 2017 (Di Donfrancesco, 2017). Cinnamaldehyde have been reported have a pungent, spicy, cinnamon note.

All the eleven aldehydes were identified in the four kinds of dry cat food, except than Isobutyraldehyde and (E)-2-Hexenal were not detected in dry cat food produced by Red sorghum. The composition of the four dry cat foods were close, with the same four aldehydes accounts the most in quantity: Hexanal, 3-methylbutyraldehyde, Benzaldehyde and Cinnamaldehyde.

Among the 6 identified alcohol compounds, 1-Pentanol and 1-Octen-3-ol have been identified in the dry dog food research (2013) and 4-Methyl-1-pentanol has been confirmed in the study in 2017. All the six alcohols could be found in dry cat food produced with corn. Volatile compound 1-Heptanol could only be found in dry cat food produced with corn. Except that, (E)-5-Octen-1-ol was not detected in dry cat food produced with White sorghum. 1-Octen-3-ol was found highest in the quantity in alcohol compounds group among all the four kinds of dry cat food.

Among the 5 identified ketone compounds, 6-methyl-5-hepten-2-one and 3,5-Octadien-2-one have been detected in the previous two studies on dry dog food (2013, 2017). 2-Heptanone was identified in the study in 2013, and 2,3-Octanedione was detected in the research of 2017. All the five identified ketones could all be found in the 4 kinds of dry cat food.

None of the four carboxylic acids detected in this study have been reported in the previous dry dog food study. Among them, Propionic was relatively highest in the quantity in the group and it is the only carboxylic acids defined in all the four kinds of dry cat food. Valeric acid could only be found in dry cat food produced with rice and corn. Methyl Cinnamate could only be found in dry cat food produced with white sorghum.

Among the 2 identified furan compounds, 2-Pentylfuran was detected in the previous two dry dog food study. Both of the two identified furans were found in all the four kinds of dry cat food, with 2-Pentylfuran relatively high in quantity.

2,4-Dimethylhexane was not reported in the previous two dry dog food study and it has been detected in all the four dry cat food.

Dimethyl Disulfide was reported in the dry dog food study in 2013. It has been found in all the four kinds of dry cat food. It is reported have the aroma of garlic-like.

Obj 2 (see Appendix 1 for Table 1 with GCMS volatiles data)

In total, 43 volatile compounds were tentatively identified among the 28 coated kibble samples (Table 2). It covered the same 7 compounds groups defined in the 8 kibble samples without coating, including 14 aldehydes, 3 furans, 9 ketones, 10 alcohols, 4 carboxylic acids, 2 alkanes and 1 sulfur compound.

Compared to the volatile compounds identified in the non-coated kibbles, 12 new compounds were tentatively defined, including 2 aldehydes (Benzeneacetaldehyde, (E, E)-2,4-Heptadienal), 1 fruan (2-Ethylfuran), 4 ketones (3-Octen-2-one, (E,Z)-3,5-octadien-2-one, Nona-3,5-dien-2-one), Gamma-undecalactone), 4 alcohols (1-Penten-3-ol, 6-methyl-5-Hepten-2-ol, 1-Hexadecanol, Phenol, 2,4,6-tris (1-methyl)) and 1 alkane (Nonadecane).

Among the 12 compounds identified in the coated kibbles. Benzeneacetaldehyde has been detected in the kibbles coated with chicken fat, salmon, butter and lard. (E, E)-2,4-Heptadienal were detected in all the kibbles coated with fish oil and two of the kibbles coated with salmon oil. It was reported has a greasy, oily, fatty and green odor note (Goodscentcompany website). (E, E)-2,4-Heptadienal suggested the kibbles coated with fish oil a characterized greasy, oily, fatty note.

2-Ethylfuran was detected in kibbles coated with chicken fat A, salmon oil, fish oil, butter and lard. It has been reported has an odor description of chemical, burnt, earthy and malty (Goodscentcompany website). With a small quantity detected, it may come from the oil coating process.

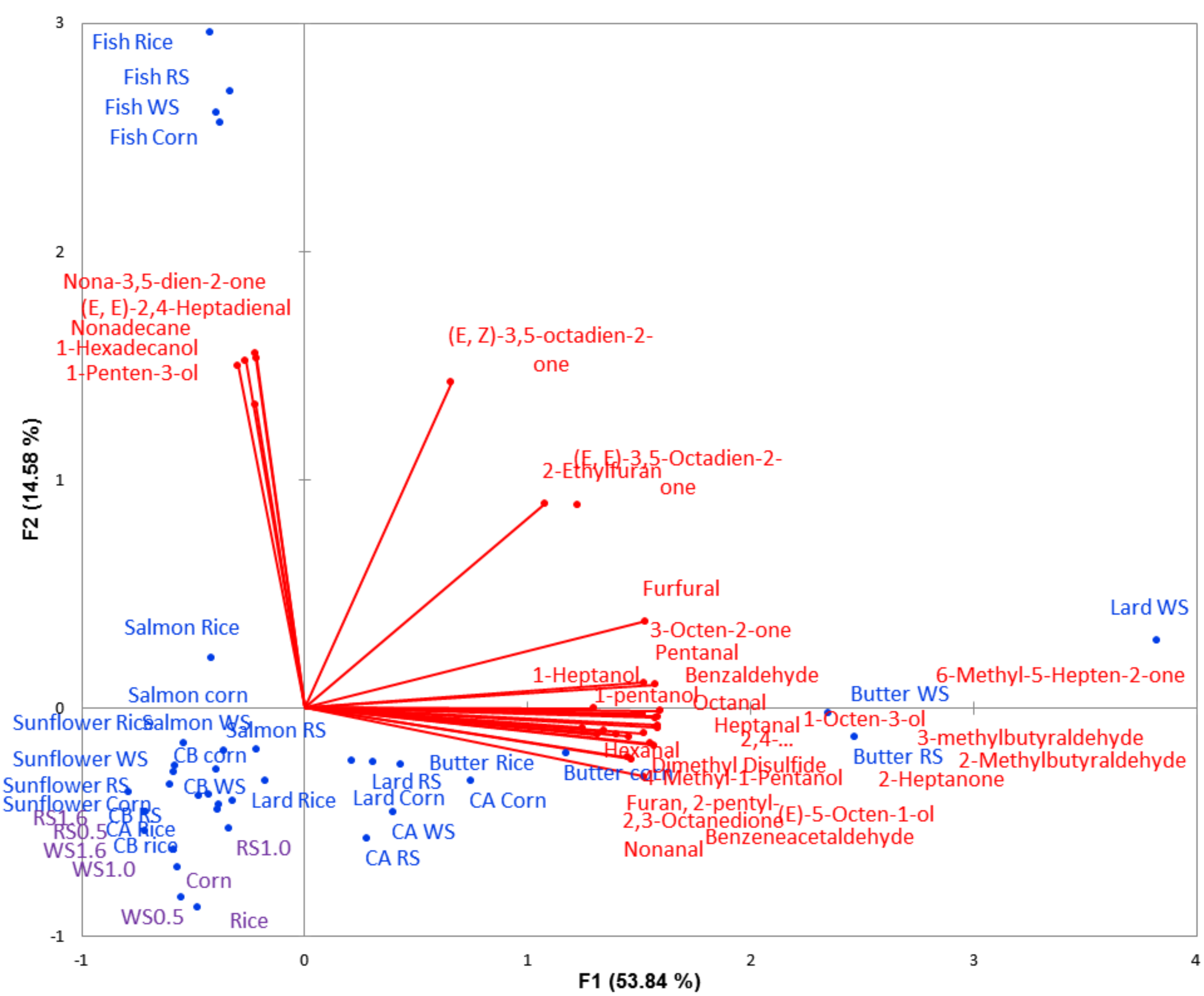
Four ketones were detected in the coated kibbles. 3-Octen-2-one and (E,Z)-3,5-Octadien-2-one have been detected in almost all the kibbles coated with fats. Nona-3,5-dien-2-one was only detected in kibbles coated with fish oil. Gamma-undecalactone were only detected in two Red sorghum and white sorghum made kibbles coated with chicken fat A. 3-Octen-2-one has been reported has earthy, oily, mushroom type odor (Goodscentscompany website). (E,Z)-3,5-Octadien-2-one was recorded has odor notes of fatty, fruty, hay, green and herbal. The odor of 3-Octen-2-one and (E,Z)-3,5-dien-2-one indicating these two ketones may come from the oil coating process. Nona-3,5-dien-2-one has been reported as a natural volatile found in cooked asparagus (Goodscentscompany website). Further analysis will be needed to define which aromatic note this compound may bring to the kibbles coated with fish oil. Gamma-undecalactone was reported has an odor note of fatty, coconut, creamy, peach, lactonic and fruity, indicating it coming from the oil coating process as well.
With the newly detected alcohols, 1-hexadecanol could only be detected in the kibbles coated with fish oil. Pubmed record showed 1-hexadecanol has faint odor or odorless (Pubmed website). 1-Penten-3-ol could only be found in the kibbles coated with fish & salmon oil. FEMA recorded 1-Penten-3-ol has a flavor profile of butter, fish, green, oxidized and wet earth (FEMA website). Phenol, 2,4,6-tris (1-methylethyl)- was detected in the kibbles coated with lard. 5-Hepten-2-ol, 6-methyl- were detected in the kibbles coated with the salmon oil, fish oil, butter and lard. It was described has the odor of powerful, fatty, green and citrus in Pubmed (Pubmed website).

One new alkane compound Nonadecane was detected in the kibbles coated with fish and sunflower oil. Nonadecane has been reported occurrence in natural apple seed, coconut seed oil, papaya seed oil and sunflower seed (Goodscentscompany website).
The newly detected volatiles found in the kibbles coated with different types of oil were mostly have the odor characteristic of fatty, oily and greasy, except for the kibbles coated with fish and salmon oil where volatiles of fishy notes were found as well. This indicated the coating process with other types of oil may not differentiate much the kibbles regarding aromatic perception.

For gaining insight into how the coating with different fat impact on the volatiles composition, this work built PCA model from a correlation matric using a dataset resulted from 28 coated kibbles (Part II experiment) and 8 uncoated kibbles (Part I experiment), totally 43 compounds. It was decided to use the first 2 PCs, accounting for 68.42% of the total variance.

By looking into the PCA Biplot, the kibbles coated with fish oil clear distinguished out compared to the other kibbles in terms of relatively higher quantity in volatiles of Nona-3,5-dien-2-one, (E,E)-2,4-Heptadienal, Nonadecane, 1-Hexadecanol and 1-Penten-3-ol. Except that Nonadecane was detected in kibbles coated with sunflower oil, all the five volatiles were only detected in kibbles coated with fish and salmon oil. PC1 contrasted from Kibbles coated with butter and Kibble coated with Lard made of White soghurm, which is relatively high in concentration of the other volatiles, versus the other kibbles either coated with less flavor oil and the kibbles uncoated. The PCA mapping did not differentiate the uncoated kibbles with the kibbles coated with sunflower oil, chicken fat and lard in terms of the volatile composition, which indicating similar aromatic profiles may be perceived among them.

Biplot (axes F1 and F2: 68.42 %)



Overall, this work indicated that oil coating could impact the volatile composition of kibbles in a degree, depending on oil type used. Coating with fish oil, salmon oil and butter oil could distinguish the coated kibbles with uncoated ones, due to higher concentration of volatiles were detected. Volatiles with specific fishy characteristics were detected in kibbles coated with fish and salmon oil, indicating an impact on the aromatic perception with fishy notes delivered to the coated kibbles.

Coating with chicken fat, sunflower oil, lard and may not impact much on the volatile composition of the kibbles compared to uncoated ones. This conclusion could be supported by the PCA modeling: the uncoated kibbles and the kibbles coated with sunflower oil, chicken fat, and lard were close in their positions in the PCA mapping, indicating their similar volatiles composition and concentration levels. Meanwhile, with the newly detected volatiles in these coated kibbles, most of them have the odor description of fatty, greasy and oily, not other types of the odor may impact on aromatic perception of uncoated kibbles. This indicated the less impact on aromatic perception by coating kibbles with sunflower, chicken and lard oil.

<u>Note:</u> See Appendix I for Tables 1 and 2 with data on GCMS Volatile Contents in Coated and Uncoated Cat Food Kibbles (attached word document).

PART – 4. ANIMAL STUDIES

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

Definition of the study approach

Before starts the study with cats, a pilot experiment was conducted with the aim to compare the effect of two processing variables on in vitro digestibility of the organic matter: the effect of thermal energy application on extrusion and the effect of raw material particle size. To evaluate this, 4 diets were formulated (ingredient composition shown on Table 1), with corn, brown rice, white sorghum or red sorghum as the cereal source. A factorial arrangement of treatments was used, with 2 sorghum varieties (white and red) x 3 screen sieve sizes (0.4, 1.0, and 1.6mm) x 3 applications of specific thermal energy (low, medium, high) plus 2 control foods (corn and brown rice), adding up 20 treatments. Foods was prepared as described latter and extruded to obtain the specified conditions for thermal energy application. After preparation, foods were evaluated for the in vitro digestibility of the organic matter, as a screening for the possible effects of the different grinding and extrusion conditions on cats. The in vitro digestibility of organic matter (OM) was determined as described by Hervera et al. (2007) in samples collected after the dryer. The incubation conditions simulate two steps of the digestion process, digestion in the stomach and in the small intestine, using an enzymatic system with pepsin and pancreatin, respectively. As a result, the specific thermal energy application showed only small effect on the *in vitro* digestibility for the sorghum based diets, however grinding appeared be an interesting process to explore, since coarser grinding (1.6mm) reduced the *in vitro* digestibility, maybe with a potential prebiotic effect as an increase in resistant starch (**Figure 1**). Based on these results the approach for the *in vivo* study focused on different mean geometric diameter of the different varieties of sorghum.

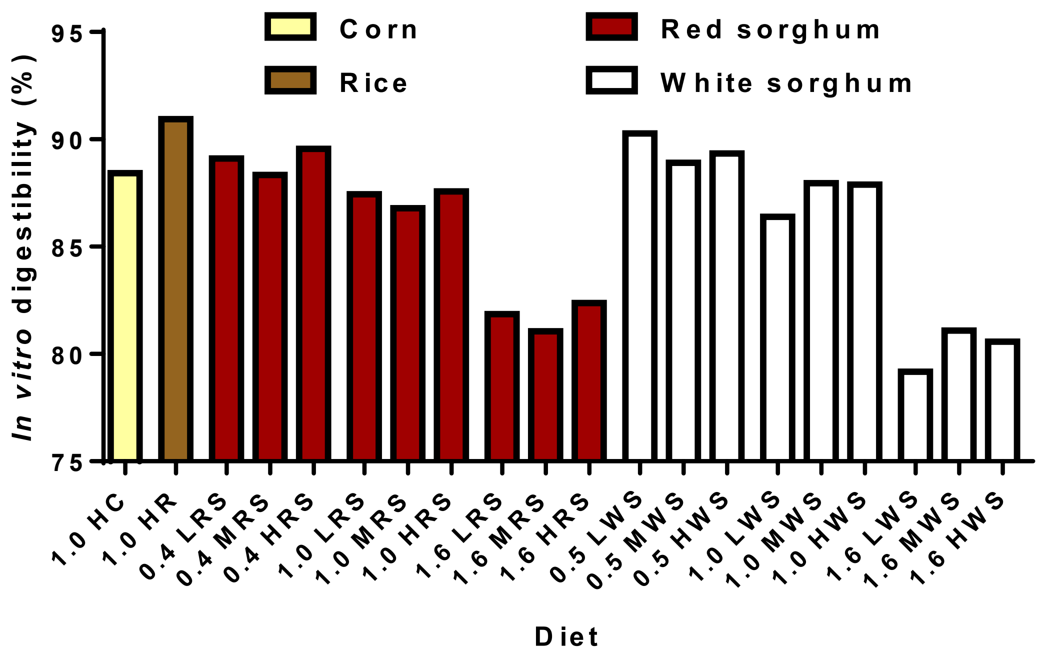


Figure 1. *In vitro* digestibilities of the organic matter of cat foods prepared with different cereal sources, ground with different screen sieve sizes, and extruded with different specific thermal energy applications: 0.4, 0.5, 1.0, 1.6 = screen sieve sizes (mm); L, M, H = low, medium or high specific thermal energy application during extrusion; C, R, RS, WS = corn, brown rice, red sorghum or white sorghum as carbohydrate source.

Study design and diets (based on raw material particle size)

The study followed a 2 x 3 factorial arrangement of treatments, composed by 2 sorghum varieties (red or white) ground in 3 different particle sizes, more 2 controls (brown rice and corn), totaling 8 complete diets. Different varieties of low tannin sorghum were used as the primary carbohydrate source for two of the formulations. As controls, two other formulations based on rice or corn were used. To formulate the diets, samples of brown rice, corn, white sorghum, red sorghum, beet pulp, and poultry by-product meal were purchased and analyzed for dry matter, ash, crude protein, acid-hydrolyzed fat and total dietary fiber, following AOAC (2012) methods. Based on the results, four separate diets were balanced to present similar nutrient composition following the recommendations of AAFCO (2016) for cat maintenance (Table 1).

Animals and in vivo study design

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

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

During the adaptation period, from 4 p.m. to 8 a.m. all cats were restricted to individual stainless steel metabolic cages (0.80 m x 0.80 m x 1.0 m), with experimental foods and water available. From 8 a.m. to 4 p.m., the cats were kept in a collective cattery of 50 m^2 for exercise and socialization, where they had access to water but not to food. During the fecal collection periods, cats were restrained all time to their cages. The daily amount of food was individually defined according to the energy requirements for cat maintenance, as 100 kcal/$kg^{0.67}$/day (NRC, 2006). Food metabolizable energy content was estimated from their chemical composition (NRC, 2006). Each day, food was weighed and divided into 2 similar portions, and given at 9 a.m. and 4 p.m. Refused foods were collected and weighed, and the consumption recorded.

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

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

Food palatability test

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

Results

At the palatability comparisons red and white sorghum-based diets presented higher consumption than corn (**Figure 2 and 3**) or brown rice based (**Figure 4 and 5**) foods ($P<0.05$). This is an important outcome for the industry, since generally is attributed to sorghum palatability issues due to it tannin content. In fact, our data suggested higher preference for cats to extruded diets based on sorghum, showing that the modern sorghum varieties, very low in tannin is in fact palatable and readily consumed, when incorporated in properly balanced formulations for felines. No special needs for palatability technologies was verified, and the use of a standard amount of a commercial palatant was enough to cats eat the foods, preferring the sorghum than brown rice or corn, more traditional cereals.

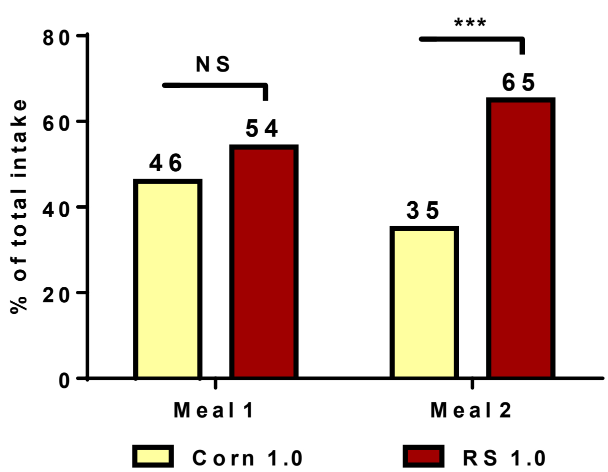


Figure 2. Proportion of consumption between diets based on corn (1.0 mm) or red sorghum (1.0 mm) to cats. NS = not significant; ***P<0.01.

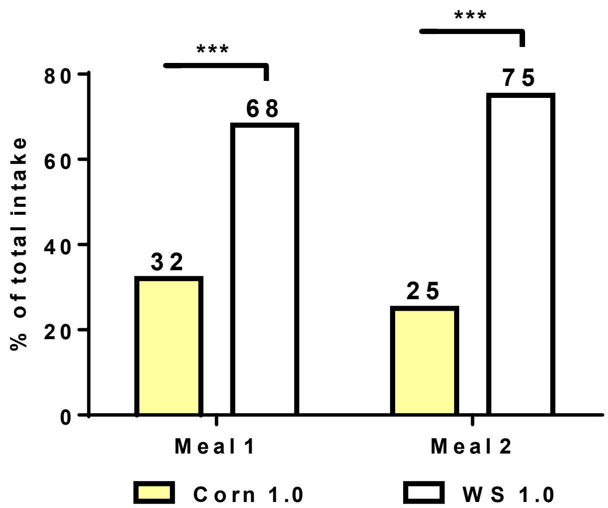


Figure 3. Proportion of consumption between diets based on corn (1.0 mm) or white sorghum (1.0 mm) to cats. ***P<0.01

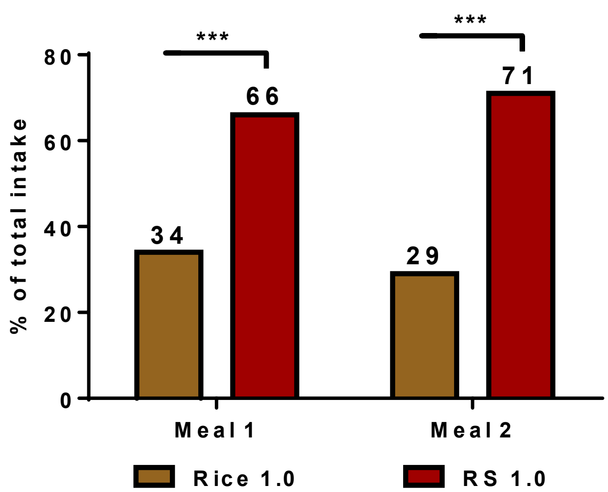


Figure 4. Proportion of consumption between diets based on rice (1.0 mm) or red sorghum (1.0 mm) to cats. ***P<0.01.

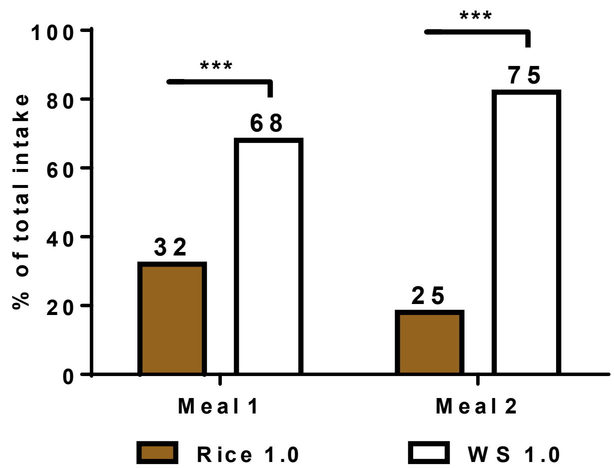


Figure 5. Proportion of consumption between diets based on rice (1.0 mm) or white sorghum (1.0 mm) to cats. ***P<0.01.

Between the two sorghum varieties, the food based on white sorghum (1.0mm) presented higher consumption than red sorghum formulation (1.0mm) effect consistent on the two evaluated meals (**Figure 6**; P<0.05).

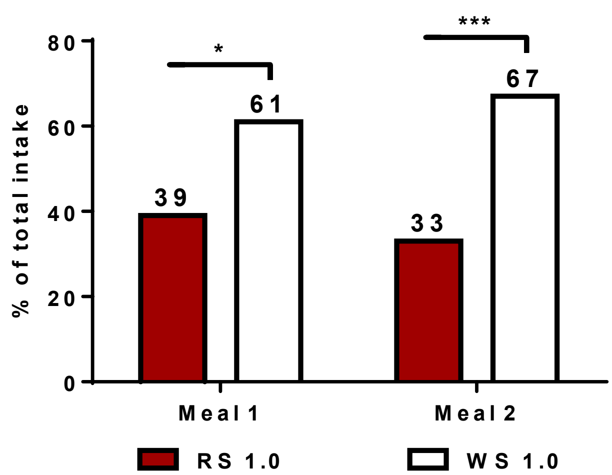


Figure 6. Proportion of consumption between diets based on red sorghum (1.0 mm) or white sorghum (1.0 mm) to cats. *P<0.05. ***P<0.01.

Inside each sorghum variety, for diets based on red sorghum the coarse ground food (1.6mm) was preferred by cats than the finely ground (0.5mm), as presented on **Figure 7** (P<0.01). However, the raw material particle size did not change food intake for diets based on white sorghum (**Figure 8**).

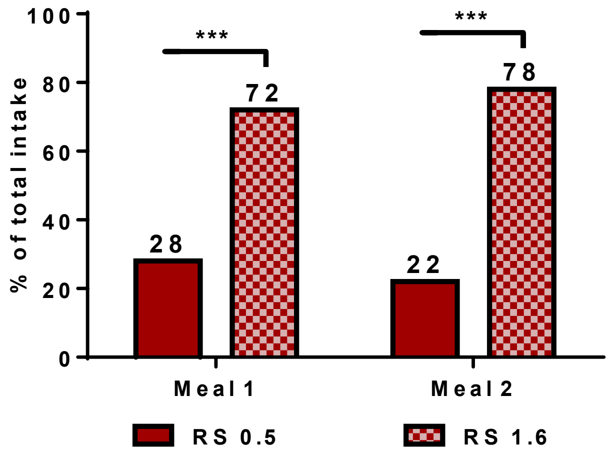


Figure 7. Proportion of consumption between diets based on red sorghum ground with 0.5 mm or 1.6 mm screen sieve sizes. ***P<0.01.

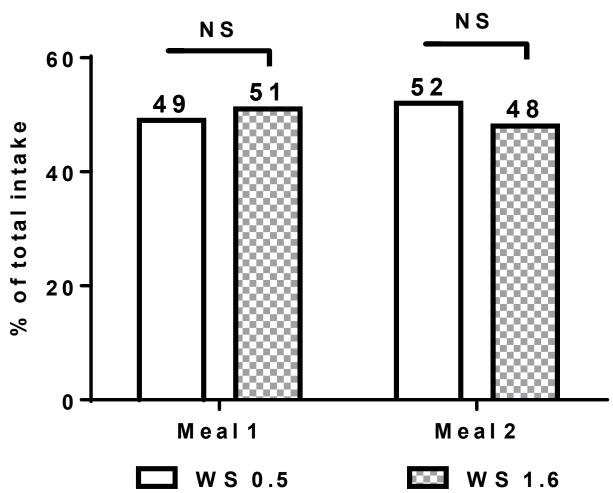


Figure 8. Proportion of consumption between diets based on white sorghum ground with 0.5 mm or 1.6 mm screen sieve sizes. NS = not significant.

All these results on palatability are very relevant for the sorghum utilization on premium cat foods. It was possible to verify not only preference for sorghum over brown rice and corn, but also that white sorghum is more palatable than red sorghum and that no special grind is necessary to produce palatable foods based on sorghum. Particle size reduction is a relevant cost on food preparation, and as coarse particles may be equally preferred, or even more palatable than foods prepared with fine grind sorghum, the industry do not need to specially consider the grinding step or add unnecessary cost on food preparation.
On the digestibility study cats readily eat the diets. During the 30 days when only the experimental foods were fed, the body weight of the animals remained constant, as the initial (4.24±0.05 kg) and final body weight (4.23±0.06 kg) was similar (P=0.785). No episodes of diet rejection or reduced consumption were observed, as well as no episodes of

vomiting of soft stools were verified. The food intake to achieve constant body weight (**Table 2**) also did not differ between diets (P=0.844).

The fecal score did not differ between food (P=0.774), and remained on the adequate, or ideal score for cats. Feces production was also similar between foods (P=0.595), showing that all cereals exhibited similar digestibility and resulted on the production of the same amount of feces. These results demonstrate that cats have a good utilization of red and white sorghum-based foods, and premium formulations based on these cereals not only are palatable but are also adequately used by their digestive tract resulting in the maintenance of body weight, general health, and allows the production of adequately formed feces. Grinding also did not change feces production or score (P>0.05), showing that there are no special needs regarding to raw material particle size to produce sorghum-based foods for cats.

For the same raw material particle size, the consumption of corn, red sorghum, or white sorghum-based foods resulted in similar feces pH (P>0.05). The brown rice diet, however, induced greater feces acidification on cats in comparison with the corn diet (P<0.05). Important effect of particle size was verified for the red and the white sorghum-based foods, when fed foods produced with coarser cereals, the feces of the cats had lower pH (P<0.05). These are relevant findings, although coarse ground sorghum-based formulations did no interfere on palatability or feces formation, their consumption increased the fermentative activity on cat's colon, and the correspondent higher formation of short-chain fatty acids may explain the verified reduction on feces pH. This implied alteration on fermentative products, in association with the lower pH verified on feces suggest a prebiotic effect of these diets. It is suggested that potential health benefits could be explored for cats for the consumption of diets based on coarse ground sorghum.

FUTURE WORK

All objectives of the study have been completed except for those related to in vivo digestibility trials with cats. The feeding trials are complete and analyses are ongoing. Part of the data have already been obtained and presented in this report including partial food intake, fecal traits and amount (score, pH, DM) results. These will be completed with a final set of data by September-end. Results of nutrient digestibility and fermentation products (based on feces) will be complete by mid-November. The extended time will allow 9 different analyses in duplicate on 64 fecal samples (the total number of analyses are around 1,152). The analysis of gut microbiome is expected to be completed by December, and all the in vivo results will be compiled by December-end.

Table 2. Food intake and characteristics of the feces of cats fed extruded diets based on different cereal sources, grind to different raw material particle sizes (0.5mm, 1.0mm, 1.6mm).

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

[1] RS = red sorghum; WS = white sorghum

[2] Standard Error Mean (n = 8 cats per food)

[3] Scored according to the following system: 0=liquid stools; 1=soft, unformed stools; 2=soft, poorly formed stools, which assume the shape of the container; 3=soft, wet and formed stools that retains shape; 4=well formed and consistent stools, which do not adhere to the floor; 5=dry, hard stools, with reduced volume.

[a, b] Means not sharing a common superscript differ (P<0.05).

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<u>**Appendix I**</u>

GCMS Volatile Contents in Coated and Uncoated Cat Food Kibbles

(see attachment)

<u>**Appendix II**</u>

Sensory References and Definitions

Toothbrushes, mozzarella, crackers, cucumbers, tomato juice, and hot water for cleansing
Serve references at room temperature
Tasting instructions: Take 2 kibble per bite

<u>**APPEARANCE**</u>

Brown: Light to dark evaluation of brown color of product.
Reference: Pantone Coated Plus Series 466C =2.0
Pantone Coated Plus Series 465C =4.0
Pantone Coated Plus Series 464C =6.0

Porous: Presence of pores/air bubbles inside 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: Post Shredded Wheat = 12.0
Preparation: Serve in 3.25 oz. cup

<u>**AROMA**</u>

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 = 10.0 (a)
Preparation: Place 1 vitamin pill in a medium snifter.

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)

Oxidized Oil: The aromatic associated with aged or highly used oil and fat.
Reference: Microwave Oven Heated Wesson 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 microwave and let sit at room temperature to cool for approximately 25 minutes. Then heat another 3 minutes, let cool another 25 minutes, and heat for one additional 3 minute interval. Let beaker sit on counter uncovered overnight. Serve 1 Tablespoon of the oil in a medium snifter, covered (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.

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: 1 Tsp of 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 1oz cup.

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 aromatic associated with aged or highly used oil and fat.
Reference: Microwave Oven Heated Wesson Vegetable Oil = 6.0 (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 microwave and let sit at room temperature to cool for approximately 25 minutes.

Then heat another 3 minutes, let cool another 25 minutes, and heat for one additional 3 minute interval. Let beaker sit on counter uncovered overnight.

Cardboard: The aromatic associated with cardboard or paper packaging. The intensity rating is only for the 'cardboardy' character within the reference.
Reference: Kroger Gluten Free Low Sodium Rice Cake = 2.5
Preparation: Cut one rice cake into 6 pieces like a pizza and place two slices in each 3.25 oz. cup.

Sour: The fundamental taste factor associated with a citric acid solution.
Reference: 0.015% Citric Acid Solution = 1.5
0.050% Citric Acid Solution = 2.5

Salt: A fundamental taste factor of which sodium chloride is typical.
Reference: 0.15% NaCl Solution = 1.5
0.2% NaCl Solution = 2.5

Bitter: The fundamental taste factor associated with a caffeine solution.
Reference: 0.02% Caffeine Solution = 3.5
0.035 % Caffeine Solution = 5.0
0.05% Caffeine Solution = 6.5

Sweet: A fundamental taste factor of which sucrose is typical.
Reference: 1% Sucrose Solution = 1.0

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

<u>TEXTURE/FEEL</u>

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.

Denseness: The compactness of the product when compressed once between the tongue and palate. Size and ratio of air cells to solid product evaluated during compression of sample with molars on the first bite.
Reference: General Mills Cheerios = 4.0
Malted Milk Ball = 6.0
Preparation: Place five Malted Milk Balls in each 3.25 oz. cup

Gritty: The perception of small, hard, sharp particles reminiscent of sand or granules in pairs after 5-7 chews

Reference: Malt-O-Meal = 2.0
Jiffy Corn Bread Mix = 5.0

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. Prepare the muffins according to package directions, using Dillon's whole milk. Serve half muffin in a 3.25 oz. cup.

AFTERTASTE

Evaluate 15 seconds after expectorating

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 1oz cup.

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)

Oxidized Oil: The aromatic associated with aged or highly used oil and fat.
Reference: Microwave Oven Heated Wesson Vegetable Oil = 6.0 (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 microwave and let sit at room temperature to cool for approximately 25 minutes. Then heat another 3 minutes, let cool another 25 minutes, and heat for one additional 3 minute interval. Let beaker sit on counter uncovered overnight.

Cardboard: The aromatic associated with cardboard or paper packaging. The intensity rating is only for the 'cardboardy' character within the reference.
Reference: Kroger Gluten Free Low Sodium Rice Cake = 2.5
Preparation: Cut one rice cake into 6 pieces like a pizza and place two slices in each 3.25 oz. cup.

Bitter: The fundamental taste factor associated with a caffeine solution.
Reference: 0.02% Caffeine Solution = 3.5
0.035 % Caffeine Solution = 5.0
0.05% Caffeine Solution = 6.5

Table 1. Content (μg/kg) of aroma compounds in the eight dry cat food samples.

Retention time (min)	Compound	KI Exp.	KI Lit.	WS0.5	WS1.0	WS1.6	Rice	Corn	RS0.5	RS1.0	RS1.6
				Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Aldehydes											
3.204	Isobutyraldehyde*	817	817a	0.23 ± 0.01	0.16 ± 0.00	0.12 ± 0.00	0.18 ± 0.05	0.16 ± 0.03	n.d.	n.d.	n.d.
4.928	2-Methylbutyraldehyde	919	917a	0.78 ± 0.01	0.8 ±0.07	0.61 ± 0.06	0.62 ± 0.16	0.72 ± 0.02	0.62 ± 0.09	1.06 ± 0.32	0.43 ± 0.01
5.042	3-Methylbutyraldehyde*	924	920a	2.09 ± 0.04	2.14 ±0.15	1.5 ± 0.11	1.71 ± 0.48	1.71 ± 0.01	1.46 ± 0.23	2.84 ± 0.92	1.17 ± 0.12
6.632	Pentanal*	986	985a	0.3 ± 0.01	0.32 ±0.04	0.24 ± 0.02	0.35 ± 0.11	0.25 ± 0.01	0.21 ± 0.01	0.42 ± 0.12	0.21 ± 0.01
9.58	Hexanal*	1086	1088b	3.96 ± 0.12	4 ±0.33	3.04 ± 0.13	5.32 ± 0.47	3.83 ± 0	3.37 ± 0.43	4.79 ± 0.18	2.77 ± 0.08
12.732	Heptanal*	1190	1190a	0.52 ± 0.09	0.35 ±0.07	0.3 ± 0.02	0.35 ± 0.2	0.27 ± 0.11	0.28 ± 0.02	0.49 ± 0.27	0.28 ± 0.04
15.814	Octanal*	1294	1291b	0.31 ± 0.00	0.23 ±0.02	0.18 ± 0.01	0.59 ± 0.45	0.22 ± 0	0.2 ± 0.01	0.27 ± 0.08	0.15 ± 0.01
16.013	(E)-2-Hexenal	1301	1264a	0.21 ± 0.00	0.20 ± 0.00	0.05 ± 0.00	0.23 ± 0.04	0.06 ± 0.01	n.d.	n.d.	n.d.
18.74	Nonanal*	1399	1398a	0.63 ± 0.07	0.56 ±0.02	0.36 ± 0.03	0.7 ± 0.29	0.43 ± 0	0.37 ± 0.03	0.63 ± 0.14	0.34 ± 0.03
20.796	Furfural *	1480	1432b	0.07 ± 0.00	0.07 ±0.01	0.04 ± 0.00	0.08 ± 0.04	0.06 ± 0.01	0.06 ± 0.00	0.11 ± 0.01	0.06 ± 0.02
22.365	Benzaldehyde*	1540	1525b	1.67 ± 0.05	2 ±0.17	1.39 ± 0.04	1.69 ± 0.52	1.4 ± 0.06	1.44 ± 0.09	2.19 ± 0.22	0.89 ± 0.13
33.799	Cinnamaldehyde	N/A	2015-2084a	1.58 ± 0.37	2.08 ±0.43	0.65 ± 0.03	1.83 ± 0.11	0.8 ± 0.01	0.66 ± 0.11	3.1 ± 0.13	0.75 ± 0.03
Furans											

3.905	Tetrahydrofuran	864	868a	0.65 ± 0.00	0.67 ±0.07	0.12 ± 0.01	0.71 ± 0.19	0.11 ± 0.01	0.1 ± 0.00	0.93 ± 0.26	0.09 ± 0.01
14.011	2-Pentylfuran	1234	1239a	1.51 ± 0.1	1.34 ±0.3	0.84 ± 0.04	1.44 ± 0.17	1.38 ± 0.03	1.36 ± 0.29	1.45 ± 0.10	0.38 ± 0.01
Ketones											
4.701	2-Butanone	908	908a	0.44 ± 0.01	0.46 ±0.05	0.08 ± 0.01	0.44 ± 0.13	0.06 ± 0	0.07 ± 0.00	0.55 ± 0.21	0.05 ± 0.01
12.678	2-Heptanone*	1188	1187a	0.38 ± 0.03	0.29 ±0.03	0.21 ± 0.01	0.38 ± 0.27	0.35 ± 0.06	0.19 ± 0.01	0.46 ± 0.22	0.18 ± 0.03
23.41	3,5-Octadien-2-one	1580	1578a	0.1 ± 0.00	0.13 ±0.01	0.09 ± 0.01	0.1 ± 0.04	0.13 ± 0.01	0.11 ± 0.02	0.21 ± 0.08	0.11 ± 0.01
16.818	2,3-Octanedione	1331	1335a	0.22 ± 0.01	0.26 ±0.01	0.17 ± 0.01	0.26 ± 0.04	0.18 ± 0.01	0.14 ± 0.03	0.34 ± 0.03	0.17 ± 0.00
17.201	6-Methyl-5-Hepten-2-one*	1345	1341a	0.22 ± 0.02	0.26 ±0.00	0.13 ± 0.01	0.21 ± 0.06	0.18 ± 0	0.17 ± 0.01	0.27 ± 0.05	0.13 ± 0.00
Alcohols											
14.696	1-pentanol	1258	1258a	0.09 ± 0.00	0.1 ±0.01	0.1 ± 0.01	0.13 ± 0.05	0.14 ± 0.01	0.08 ± 0.01	0.17 ± 0.04	0.09 ± 0.01
17.585	4-Methyl-1-Pentanol	1359	1338a	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.05	0.16 ± 0.01	0.11 ± 0.02	0.58 ± 0.46	0.15 ± 0.01
20.11	1-Octen-3-ol*	1454	1454a	0.68 ± 0.03	0.8 ±0.03	0.79 ± 0.05	0.85 ± 0.29	0.73 ± 0.03	0.56 ± 0.02	1.12 ± 0.30	0.62 ± 0.00
20.309	1-Heptanol*	1461	1460a	n.d.	n.d.	n.d.	n.d.	0.06 ± 0	n.d.	n.d.	n.d.
21.192	2-Ethylhexanol*	1494	1494a	0.21 ± 0.00	0.07 ±0.00	n.d.	0.24 ± 0.07	0.25 ± 0.01	n.d.	0.09 ± 0.02	n.d.
24.312	(E)-5-Octen-1-ol	N/A		n.d.	n.d.	n.d.	0.06 ± 0.02	0.07 ± 0.01	0.04 ± 0.01	0.08 ± 0.03	0.05 ± 0.00
Carboxylic acids											
22.621	Propionic acid	1550	1554a	0.27 ± 0.04	0.31 ±0.00	0.46 ± 0.04	0.44 ± 0.07	0.4 ± 0	0.25 ± 0.06	0.64 ± 0.28	0.28 ± 0.01
24.757	Valeric acid	N/A	1686-1766a	n.d.	n.d.	n.d.	0.25 ± 0.04	0.18 ± 0.00	n.d.	n.d.	n.d.

34.383	Methyl Cinnamate	N/A	2019-2105 a	0.15 ± 0.01	0.18 ±0.02	0.11 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.
37.673	Dimethyl Terephthalate	N/A		0.04 ± 0.00	0.05 ±0.01	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	n.d.	n.d.	n.d.
Alkanes											
2.94	2,4-DimethylHexane	795		0.09 ± 0.02	0.06 ± 0.00	0.08 ± 0.01	0.06 ± 0.00	0.07 ± 0.02	0.09 ± 0.00	0.06 ± 0.01	0.04 ± 0.00
19.354	1,3-Dichlorobenzene (IS)	1424	1418 a	19.3 ± 0.04	20.23 ±0.41	20.38 ± 0.35	20.16 ± 0.48	19.59 ± 0.55	18.42 ± 0.44	20.11 ± 0.52	19.78 ± 0.16
Sulfur compounds											
9.331	Dimethyl Disulfide	1079	1077 a	0.06 ± 0.00	0.06 ±0.01	0.07 ± 0.00	0.05 ± 0.00	0.08 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	0.05 ± 0.01

KI Exp.: experimental Kovats index

KI Lit.: Kovats index from the literature

*: validated by pure compounds analyzed under same GC conditions.

n.d.: not detected

a: Pubchem

b: Flavornet

Table 2. Content (μg/kg) of aroma compounds in the coated kibbles (1/4)

Retention time (min)	Compound	KI Exp.	KI Lit.	CA RS	CA WS	CA Rice	CA Corn	CB RS	CB WS	CB rice	CB corn
				Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Aldehyde (14)											
3.204	Isobutyraldehyde *	817	817 a	n.d.	n.d.	n.d.	n.d.	n.d.	0.18 ± 0.1	0.15 ± 0.04	n.d.
4.928	2-Methylbutyraldehyde	919	917 a	1.38 ± 0.45	0.15 ± 0.01	0.06 ± 0.03	2.17 ± 0.59	0.67 ± 0.03	0.65 ± 0.17	0.56 ± 0.03	0.83 ± 0.34
5.042	3-methylbutyraldehyde *	924	920 a	4.09 ± 1.47	4.46 ± 0.13	1.91 ± 0.71	5.25 ± 0.83	2.17 ± 0.27	2.08 ± 0.77	1.71 ± 0.2	2.39 ± 0.8
6.632	Pentanal *	986	985 a	1.12 ± 0.42	1.4 ± 0.39	0.69 ± 0.19	1.44 ± 0.27	0.61 ± 0.09	0.58 ± 0.15	0.66 ± 0.05	0.7 ± 0.28
9.58	Hexanal *	1086	1088 c	13.57 ± 4.87	17.73 ± 1.41	9.18 ± 1	19.8 ± 5.05	7.64 ± 1.12	7.81 ± 2.34	8.04 ± 0.54	7.65 ± 2.61
12.732	Heptanal *	1190	1190 a	1.16 ± 0.43	1.33 ± 0.8	0.68 ± 0.08	1.33 ± 0.44	0.72 ± 0.08	0.75 ± 0.26	0.75 ± 0.19	0.6 ± 0.22
15.814	Octanal *	1294	1291 c	0.5 ± 0.25	0.89 ± 0.66	0.35 ± 0.11	0.65 ± 0.14	0.31 ± 0.03	0.46 ± 0.12	0.54 ± 0.09	0.32 ± 0.12
16.013	(E)-2-Hexenal	1301	1264 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18.74	Nonanal *	1399	1398 a	0.95 ± 0.38	0.98 ± 0.25	0.45 ± 0.14	1.12 ± 0.17	0.51 ± 0.08	0.49 ± 0.12	0.58 ± 0.02	0.57 ± 0.17
20.796	Furfural *	1480	1432 c	0.18 ± 0.04	0.19 ± 0.03	0.1 ± 0.03	0.22 ± 0.03	0.09 ± 0.02	0.09 ± 0.04	0.08 ± 0.01	0.09 ± 0.03
21.559	(E, E)-2,4-Heptadienal	1508	1508 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22.365	Benzaldehyde *	1540	1525 c	3.26 ± 1.36	4.18 ± 0.34	1.6 ± 0.43	4.15 ± 0.84	1.59 ± 0.23	1.51 ± 0.45	1.56 ± 0.16	1.73 ± 0.57
25.224	Benzeneacetaldehyde	N/A	1648 a	0.66 ± 0.24	0.64 ± 0.11	0.31 ±	0.95 ±	0.3 ±	0.32 ±	0.28 ±	0.46 ±

						0.05	0.11	0.05	0.09	0.04	0.06
33.799	Cinnamaldehyde	N/A	2015 - 2084 a	0.98 ± 0.04	0.99 ± 0.19	0.35 ± 0.06	0.86 ± 0.2	0.28 ± 0.03	0.24 ± 0.07	0.24 ± 0.02	0.25 ± 0.04
Furans (3)											
3.905	Tetrahydrofuran	864	868 a	n.d.	n.d.	n.d.	n.d.	0.59 ± 0.1	0.55 ± 0.15	n.d.	n.d.
5.899	2-Ethylfuran	959	960 a	n.d.	0.11 ± 0.02	0.04 ± 0.02	0.12 ± 0.05	n.d.	n.d.	n.d.	n.d.
14.011	Furan, 2-pentyl-	1234	1239 a	2.38 ± 0.94	2.48 ± 0.28	1.25 ± 0.28	3.91 ± 2.99	1.02 ± 0.03	0.98 ± 0.15	1.19 ± 0.27	1 ± 0.42
Ketones (9)											
4.701	2-Butanone	908	908 a	0.12 ± 0.04	0.14 ± 0.02	0.07 ± 0.02	0.19 ± 0.03	0.17 ± 0.04	0.16 ± 0.05	0.21 ± 0.13	0.08 ± 0.02
12.678	2-Heptanone *	1188	1187 a	0.68 ± 0.24	0.77 ± 0.17	0.32 ± 0.08	0.98 ± 0.26	0.24 ± 0.04	0.31 ± 0.12	0.36 ± 0.01	0.36 ± 0.16
16.818	2,3-Octanedione	1331	1335 a	0.32 ± 0.13	0.3 ± 0.06	0.18 ± 0.05	0.26 ± 0.05	0.17 ± 0.02	0.13 ± 0.04	0.17 ± 0.03	0.16 ± 0.08
17.201	6-Methyl-5-Hepten-2-one *	1345	1341 a	0.4 ± 0.12	0.45 ± 0.06	0.19 ± 0.07	0.47 ± 0.09	0.2 ± 0.02	0.2 ± 0.06	0.18 ± 0.01	0.22 ± 0.09
19.193	3-Octen-2-one	1417	1411 a	n.d.	0.38 ± 0.1	0.17 ± 0.04	0.54 ± 0.04	n.d.	0.16 ± 0.03	0.16 ± 0.02	0.22 ± 0.04
22.136	(E, Z)-3,5-octadien-2-one	1531	1529 a	0.37 ± 0.1	0.37 ± 0.03	0.18 ± 0.02	0.51 ± 0.11	0.22 ± 0.04	0.19 ± 0.02	0.2 ± 0.02	0.21 ± 0.05
23.41	(E, E)-3,5-Octadien-2-one	1580	1578 a	0.42 ± 0.21	0.39 ± 0.02	0.2 ± 0.05	0.47 ± 0.04	0.2 ± 0.03	0.17 ± 0.05	0.18 ± 0.01	0.22 ± 0.08
24.379	Nona-3,5-dien-2-one	N/A	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
35.696	Gamma-undecalactone	N/A	2210 - 2300 a	1.44 ± 0.93	1.09 ± 0.63	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alcohols (10)											

12.041	1-Penten-3-ol	1169	1169 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14.696	1-pentanol	1258	1258 a	0.58 ± 0.22	0.47 ± 0.19	0.34 ± 0.11	0.78 ± 0.05	0.35 ± 0.05	0.29 ± 0.09	0.34 ± 0.03	0.37 ± 0.12
17.585	4-Methyl-1-Pentanol	1359	1338 a	0.33 ± 0.12	0.21 ± 0.01	0.11 ± 0.03	0.55 ± 0.06	0.1 ± 0.07	0.1 ± 0.02	0.12 ± 0.01	0.27 ± 0.07
20.11	1-Octen-3-ol *	1454	1454 a	1.9 ± 0.61	1.82 ± 0.25	1.09 ± 0.28	2.58 ± 0.36	0.98 ± 0.1	0.86 ± 0.23	1.03 ± 0.05	1.06 ± 0.36
20.309	1-Heptanol *	1461	1460 a	0.14 ± 0.04	0.14 ± 0.03	0.08 ± 0.01	0.17 ± 0.06	0.06 ± 0.01	0.08 ± 0.02	0.09 ± 0	0.11 ± 0.04
21.192	2-Ethylhexanol *	1494	1494 a	n.d.	n.d.	0.1 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.
24.16	5-Hepten-2-ol, 6-methyl-	N/A	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24.312	(E)-5-Octen-1-ol	N/A	N/A	0.19 ± 0.06	0.18 ± 0.03	0.09 ± 0.01	0.19 ± 0.02	0.14 ± 0.02	0.07 ± 0.02	0.11 ± 0.02	0.09 ± 0.02
27.093	1-Hexadecanol	N/A	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30.837	Phenol, 2,4,6-tris(1-methylethyl)-	N/A	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carboxylic acids (4)											
22.621	Propionic acid	1550	1554 a	0.92 ± 0.53	1.05 ± 0.1	0.45 ± 0.24	1.13 ± 0.13	n.d.	n.d.	n.d.	0.54 ± 0.16
24.757	Valeric acid	N/A	1686 - 1766 a	0.46 ± 0.13	0.49 ± 0.02	0.19 ± 0.06	0.43 ± 0.05	n.d.	0.1 ± 0.04	0.18 ± 0.05	0.22 ± 0.06
34.383	Methyl Cinnamate	N/A	2019 - 2105 a	1.53 ± 0.23	1.54 ± 0.39	0.45 ± 0.07	1.04 ± 0.24	0.34 ± 0.03	0.3 ± 0.07	0.27 ± 0.01	0.29 ± 0.06
37.673	Dimethyl Terephthalate	N/A	N/A	0.1 ± 0.04	0.08 ± 0.02	0.07 ± 0.02	0.16 ± 0.03	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.02
Alkanes (3)											
2.94	2,4-DimethylHexane	795	N/A	0.22 ± 0.1	0.28 ± 0.08	0.12 ± 0.05	0.21 ± 0.09	n.d.	0.08 ± 0.03	n.d.	n.d.

19.354	1,3-Dichlorobenzene *	1424	1418 a	20.08 ± 0.42	20.15 ± 0.75	19.9 ± 0.48	20.19 ± 0.6	20.4 ± 0.2	20.11 ± 0.6	20.27 ± 0.5	20.3 ± 0.53
25.938	Nonadecane	N/A	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfur compounds (1)											
9.331	Dimethyl Disulfide	1079	1077 a	0.15 ± 0.09	0.15 ± 0.04	0.06 ± 0.02	0.17 ± 0.04	0.07 ± 0	0.08 ± 0.03	0.07 ± 0.01	0.08 ± 0.02

Table2. Content (μg/kg) of aroma compounds in the coated kibbles (2/4)

				Salmon RS	Salmon WS	Salmon Rice	Salmon corn	Fish RS	Fish WS	Fish Rice	Fish Corn
Retention time (min)	Compound	KI Exp.	KI Lit.	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Aldehyde(14)											
3.204	Isobutyraldehyde *	817	817 a	0.25 ± 0.07	n.d.	0.15 ± 0.03	0.19 ± 0.07	0.23 ± 0.03	0.18 ± 0.04	0.12 ± 0.04	0.16 ± 0.01
4.928	2-Methylbutyraldehyde	919	917 a	0.76 ± 0.16	0.74 ± 0.19	0.59 ± 0.09	0.9 ± 0.24	0.73 ± 0.08	0.63 ± 0.16	0.49 ± 0.1	0.73 ± 0.03
5.042	3-methylbutyraldehyde *	924	920 a	2.32 ± 0.58	2.32 ± 0.57	1.78 ± 0.28	2.61 ± 0.8	2.14 ± 0.24	1.85 ± 0.43	1.52 ± 0.34	2.04 ± 0.09
6.632	Pentanal *	986	985 a	0.73 ± 0.2	0.76 ± 0.21	0.75 ± 0.09	0.81 ± 0.28	0.75 ± 0.1	0.86 ± 0.23	0.78 ± 0.17	0.77 ± 0.04
9.58	Hexanal *	1086	1088 c	7.81 ± 1.63	7.94 ± 1.69	8.45 ± 0.93	9.18 ± 2.61	6.12 ± 0.77	6.22 ± 1.05	6.87 ± 1.35	6.64 ± 0.1
12.732	Heptanal *	1190	1190 a	0.77 ± 0.17	0.78 ± 0.25	0.64 ± 0.13	0.6 ± 0.2	0.57 ± 0.11	0.58 ± 0.08	0.5 ± 0.05	0.53 ± 0.05
15.814	Octanal *	1294	1291 c	0.33 ± 0.08	0.29 ± 0.05	0.33 ± 0.03	0.34 ± 0.1	0.22 ± 0.04	0.24 ± 0.07	0.23 ± 0.06	0.24 ± 0.02
16.013	(E)-2-Hexenal	1301	1264 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18.74	Nonanal *	1399	1398 a	0.54 ±	0.49 ±	n.d.	0.61 ±	n.d.	n.d.	0.32 ± 0.1	0.36 ±

				0.16	0.08		0.24				0.03
20.796	Furfural *	1480	1432 c	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.01	0.13 ± 0.05	0.18 ± 0.02	0.16 ± 0.03	0.17 ± 0.04	0.17 ± 0.02
21.559	(E, E)-2,4-Heptadienal	1508	1508 a	n.d.	0.04 ± 0	0.04 ± 0.01	n.d.	0.24 ± 0.02	0.22 ± 0.01	0.29 ± 0.02	0.26 ± 0.06
22.365	Benzaldehyde *	1540	1525 c	1.78 ± 0.41	1.95 ± 0.5	1.56 ± 0.2	2.02 ± 0.75	1.61 ± 0.21	1.74 ± 0.7	1.53 ± 0.37	1.8 ± 0.12
25.224	Benzeneacetaldehyde	N/A	1648 a	0.53 ± 0.11	0.38 ± 0.07	0.22 ± 0.07	0.47 ± 0.17	n.d.	n.d.	n.d.	n.d.
33.799	Cinnamaldehyde	N/A	2015 - 2084 a	0.2 ± 0.03	0.22 ± 0.04	0.18 ± 0.01	0.22 ± 0.08	0.18 ± 0.01	0.15 ± 0.03	0.16 ± 0.03	0.15 ± 0.01
Furans (3)											
3.905	Tetrahydrofuran	864	868 a	n.d.	n.d.	n.d.	0.16 ± 0.05	1.08 ± 0.12	0.92 ± 0.2	n.d.	n.d.
5.899	2-Ethylfuran	959	960 a	n.d.	0.03 ± 0	0.28 ± 0.37	0.04 ± 0.02	0.17 ± 0.03	0.21 ± 0.03	0.23 ± 0.02	0.2 ± 0.02
14.011	Furan, 2-pentyl-	1234	1239 a	0.72 ± 0.11	0.69 ± 0.04	0.82 ± 0.13	0.9 ± 0.21	0.73 ± 0.08	0.52 ± 0.18	0.96 ± 0.01	0.75 ± 0.25
Ketones (9)											
4.701	2-Butanone	908	908 a	0.11 ± 0.04	0.12 ± 0.03	0.12 ± 0.02	0.13 ± 0.05	0.41 ± 0.04	0.35 ± 0.09	0.06 ± 0.02	0.06 ± 0.01
12.678	2-Heptanone *	1188	1187 a	0.34 ± 0.04	0.37 ± 0.07	0.26 ± 0.03	0.35 ± 0.1	0.27 ± 0.07	0.27 ± 0.04	0.28 ± 0.12	0.41 ± 0.2
16.818	2,3-Octanedione	1331	1335 a	0.16 ± 0.04	0.14 ± 0.02	n.d.	0.11 ± 0.03	0.16 ± 0.03	0.11 ± 0.02	0.11 ± 0.01	0.11 ± 0.01
17.201	6-Methyl-5-Hepten-2-one *	1345	1341 a	0.2 ± 0.03	0.21 ± 0.04	0.18 ± 0.03	0.22 ± 0.06	0.19 ± 0.01	0.2 ± 0.06	0.18 ± 0.02	0.14 ± 0.05
19.193	3-Octen-2-one	1417	1411 a	n.d.	0.19 ± 0.02	0.14 ± 0.01	0.23 ± 0.06	0.18 ± 0.01	0.17 ± 0.05	0.14 ± 0.02	0.19 ± 0.01
22.136	(E, Z)-3,5-octadien-2-one	1531	1529 a	0.44 ± 0.06	0.4 ± 0.03	0.47 ± 0.02	0.5 ± 0.16	1.54 ± 0.08	1.38 ± 0.26	1.6 ± 0.25	1.68 ± 0.2

23.41	(E, E)-3,5-Octadien-2-one	1580	1578 a	0.29 ± 0.09	0.28 ± 0.06	0.29 ± 0.04	0.37 ± 0.14	0.71 ± 0.17	0.68 ± 0.27	0.74 ± 0.24	0.7 ± 0.06
24.379	Nona-3,5-dien-2-one	N/A	N/A	n.d.	n.d.	n.d.	n.d.	0.06 ± 0	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
35.696	Gamma-undecalactone	N/A	2210 - 2300 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alcohols (10)											
12.041	1-Penten-3-ol	1169	1169 a	0.06 ± 0.01	n.d.	0.06 ± 0.01	n.d.	0.24 ± 0.04	0.22 ± 0.07	0.2 ± 0.06	n.d.
14.696	1-pentanol	1258	1258 a	0.37 ± 0.09	0.33 ± 0.13	0.31 ± 0.01	0.35 ± 0.16	0.33 ± 0.03	0.22 ± 0.05	0.18 ± 0.03	0.26 ± 0.07
17.585	4-Methyl-1-Pentanol	1359	1338 a	0.22 ± 0.06	0.15 ± 0.03	0.11 ± 0.01	0.29 ± 0.11	0.18 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.21 ± 0.01
20.11	1-Octen-3-ol *	1454	1454 a	1.04 ± 0.19	1.02 ± 0.21	0.99 ± 0.09	1.29 ± 0.43	1.07 ± 0.08	0.97 ± 0.27	0.99 ± 0.22	1.05 ± 0.04
20.309	1-Heptanol *	1461	1460 a	n.d.	0.06 ± 0.02	0.07 ± 0.01	0.11 ± 0.04	0.07 ± 0	0.07 ± 0.02	n.d.	n.d.
21.192	2-Ethylhexanol *	1494	1494 a	0.17 ± 0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24.16	5-Hepten-2-ol, 6-methyl-	N/A	N/A	n.d.	0.08 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	0.08 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
24.312	(E)-5-Octen-1-ol	N/A	N/A	0.15 ± 0.03	0.08 ± 0.01	0.08 ± 0.01	0.1 ± 0.03	0.07 ± 0.01	0.05 ± 0	0.06 ± 0.01	0.07 ± 0.01
27.093	1-Hexadecanol	N/A	N/A	n.d.	n.d.	n.d.	n.d.	0.09 ± 0.03	0.1 ± 0.04	0.13 ± 0.05	0.14 ± 0.01
30.837	Phenol, 2,4,6-tris(1-methylethyl)-	N/A	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carboxylic acids (4)											
22.621	Propionic acid	1550	1554 a	0.35 ± 0.02	0.45 ± 0.09	0.37 ± 0.07	0.55 ± 0.33	0.51 ± 0.09	0.39 ± 0.21	0.41 ± 0.05	0.35 ± 0.14

24.757	Valeric acid	N/A	1686 - 1766 a	n.d.	0.14 ± 0.01	0.11 ± 0.02	0.16 ± 0.08	0.17 ± 0.06	0.12 ± 0.05	0.15 ± 0.04	0.13 ± 0.03
34.383	Methyl Cinnamate	N/A	2019 - 2105 a	0.24 ± 0.03	0.25 ± 0.03	0.2 ± 0.03	0.29 ± 0.12	0.24 ± 0.02	0.2 ± 0.06	0.2 ± 0.04	0.22 ± 0.01
37.673	Dimethyl Terephthalate	N/A	N/A	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Alkanes (3)											
2.94	2,4-DimethylHexane	795	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14 ± 0.01	n.d.
19.354	1,3-Dichlorobenzene *	1424	1418 a	20.44 ± 0.28	19.73 ± 0.55	19.99 ± 0.22	20.35 ± 0.38	20.37 ± 0.29	20.01 ± 0.22	20.28 ± 0.17	20.38 ± 0.05
25.938	Nonadecane	N/A	N/A	n.d.	n.d.	n.d.	n.d.	0.52 ± 0.18	0.51 ± 0.26	0.68 ± 0.27	0.75 ± 0.05
Sulfur compounds (1)											
9.331	Dimethyl Disulfide	1079	1077 a	0.06 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.07 ± 0.03	n.d.	0.07 ± 0.02	0.06 ± 0	0.06 ± 0

Table2. Content (µg/kg) of aroma compounds in the coated kibbles (3/4)

				Sunflower RS	Sunflower WS	Sunflower Rice	Sunflower Corn	Butter RS	Butter WS	Butter Rice	Butter corn
Retention time (min)	Compound	KI Exp.	KI Lit.	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Aldehyde(14)											
3.204	Isobutyraldehyde *	817	817 a	n.d.	0.16 ± 0.09	0.1 ± 0.01	0.15 ± 0.04	n.d.	0.76 ± 0.02	n.d.	n.d.
4.928	2-Methylbutyraldehyde	919	917 a	0.65 ± 0.15	0.64 ± 0.22	0.43 ± 0.05	0.66 ± 0.15	3.92 ± 2.2	3.72 ± 0.29	1.43 ± 0.3	2.41 ± 1.89
5.042	3-methylbutyrald	924	920 a	1.85 ± 0.37	1.82 ± 0.72	1.35 ± 0.24	1.85 ± 0.42	10.91 ±	10.59 ±	4.11 ±	4.55 ±

	ehyde *							1.79	1.26	0.66	1.29
6.632	Pentanal *	986	985 a	0.56 ± 0.14	0.66 ± 0.26	0.59 ± 0.1	0.5 ± 0.14	3.41 ± 0.72	3.83 ± 0.69	1.8 ± 0.29	2.32 ± 1.69
9.58	Hexanal *	1086	1088 c	6.46 ± 1.33	5.2 ± 2.14	6.66 ± 1.26	6.38 ± 0.06	38.01 ± 2.74	37.64 ± 4.35	21.12 ± 2.59	32.85 ± 2.37
12.732	Heptanal *	1190	1190 a	0.42 ± 0.08	0.47 ± 0.19	0.45 ± 0.08	0.31 ± 0.19	4.88 ± 2.11	2.78 ± 0.29	1.7 ± 0.11	1.54 ± 1.04
15.814	Octanal *	1294	1291 c	0.21 ± 0.06	0.29 ± 0.11	0.28 ± 0.04	0.2 ± 0.04	1.85 ± 0.45	1.63 ± 0.45	0.86 ± 0.16	1 ± 0.7
16.013	(E)-2-Hexenal	1301	1264 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18.74	Nonanal *	1399	1398 a	0.33 ± 0.06	0.43 ± 0.16	0.42 ± 0.05	0.33 ± 0.08	3.05 ± 0.68	2.18 ± 0.39	1.08 ± 0.31	1.5 ± 1.08
20.796	Furfural *	1480	1432 c	0.08 ± 0.02	0.08 ± 0.03	0.07 ± 0.02	0.09 ± 0.04	0.41 ± 0.23	0.33 ± 0.06	0.2 ± 0.05	0.27 ± 0.17
21.559	(E, E)-2,4-Heptadienal	1508	1508 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22.365	Benzaldehyde *	1540	1525 c	1.33 ± 0.29	1.53 ± 0.6	1.46 ± 0.33	1.35 ± 0.33	7.59 ± 1.45	8.03 ± 1.79	3.39 ± 0.41	4.68 ± 1.73
25.224	Benzeneacetaldehyde	N/A	1648 a	n.d.	n.d.	n.d.	n.d.	1.14 ± 0.54	1.59 ± 0.32	n.d.	n.d.
33.799	Cinnamaldehyde	N/A	2015 - 2084 a	0.11 ± 0.02	0.12 ± 0.03	0.11 ± 0.02	0.09 ± 0.01	0.41 ± 0.17	0.44 ± 0.07	0.18 ± 0.03	0.25 ± 0.12
Furans (3)											
3.905	Tetrahydrofuran	864	868 a	n.d.	n.d.	n.d.	n.d.	1.68 ± 0.88	n.d.	n.d.	n.d.
5.899	2-Ethylfuran	959	960 a	n.d.	n.d.	n.d.	n.d.	0.32 ± 0.18	0.31 ± 0.06	0.17 ± 0.05	0.15 ± 0.12
14.011	Furan, 2-pentyl-	1234	1239 a	0.38 ± 0.11	0.41 ± 0.07	0.64 ± 0.13	0.6 ± 0.05	7.18 ± 0.5	5.65 ± 0.94	3.24 ± 0.81	3.29 ± 1.48
Ketones (9)											
4.701	2-Butanone	90	908	0.06 ±	0.07 ±	0.06 ±	0.06 ±	0.72	0.78	n.d.	n.d.

		8	a	0.01	0.03	0.01	0.01	± 0.4	± 0.12		
12.678	2-Heptanone *	1188	1187 a	0.24 ± 0.05	0.27 ± 0.1	0.24 ± 0.08	0.17 ± 0.12	1.81 ± 1.06	1.94 ± 0.19	0.78 ± 0.22	1.31 ± 1.29
16.818	2,3-Octanedione	1331	1335 a	0.12 ± 0.04	0.07 ± 0.03	0.09 ± 0.03	0.08 ± 0	0.7 ± 0.4	0.78 ± 0.1	0.42 ± 0.06	0.51 ± 0.35
17.201	6-Methyl-5-Hepten-2-one *	1345	1341 a	0.18 ± 0.04	0.19 ± 0.06	0.14 ± 0.02	0.16 ± 0.03	1.01 ± 0.54	1.16 ± 0.22	0.44 ± 0.06	0.63 ± 0.41
19.193	3-Octen-2-one	1417	1411 a	0.17 ± 0.03	0.16 ± 0.03	n.d.	0.14 ± 0.01	1.16 ± 0.66	1.01 ± 0.21	0.34 ± 0.06	0.51 ± 0.2
22.136	(E, Z)-3,5-octadien-2-one	1531	1529 a	0.23 ± 0.05	0.19 ± 0.03	0.19 ± 0.02	0.16 ± 0.01	1.12 ± 0.61	0.82 ± 0.19	0.33 ± 0.05	0.49 ± 0.26
23.41	(E, E)-3,5-Octadien-2-one	1580	1578 a	0.19 ± 0.05	0.18 ± 0.06	0.74 ± 1.01	0.16 ± 0.04	0.96 ± 0.52	0.77 ± 0.16	0.39 ± 0.05	0.62 ± 0.39
24.379	Nona-3,5-dien-2-one	N/A	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
35.696	Gamma-undecalactone	N/A	2210-2300 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alcohols (10)											
12.041	1-Penten-3-ol	1169	1169 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14.696	1-pentanol	1258	1258 a	0.33 ± 0.09	0.28 ± 0.14	0.24 ± 0.06	0.24 ± 0.06	n.d.	1.58 ± 0.47	0.73 ± 0.12	1.19 ± 0.88
17.585	4-Methyl-1-Pentanol	1359	1338 a	0.17 ± 0.04	0.1 ± 0.04	0.09 ± 0.02	0.19 ± 0.06	1.02 ± 0.54	0.59 ± 0.15	0.23 ± 0.06	0.7 ± 0.47
20.11	1-Octen-3-ol *	1454	1454 a	0.94 ± 0.22	0.89 ± 0.26	0.87 ± 0.14	0.81 ± 0.22	5.11 ± 0.66	4.16 ± 0.82	2.1 ± 0.19	3 ± 1.91
20.309	1-Heptanol *	1461	1460 a	0.08 ± 0.02	0.07 ± 0.03	0.07 ± 0.01	0.07 ± 0.01	0.37 ± 0.18	n.d.	n.d.	0.26 ± 0.18
21.192	2-Ethylhexanol *	1494	1494 a	n.d.	n.d.	n.d.	n.d.	0.24 ± 0.12	n.d.	n.d.	n.d.
24.16	5-Hepten-2-ol, 6-methyl-	N/A	N/A	n.d.	n.d.	0.05 ± 0	0.04 ± 0.01	n.d.	0.28 ±	0.16 ±	0.28 ±

									0.07	0.03	0.18
24.312	(E)-5-Octen-1-ol	N/A	N/A	0.07 ± 0.02	0.07 ± 0.03	0.09 ± 0.02	0.06 ± 0.02	0.46 ± 0.27	0.29 ± 0.1	0.17 ± 0.02	0.27 ± 0.16
27.093	1-Hexadecanol	N/A	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30.837	Phenol, 2,4,6-tris(1-methylethyl)-	N/A	N/A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carboxylic acids (4)											
22.621	Propionic acid	1550	1554 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24.757	Valeric acid	N/A	1686 - 1766 a	0.14 ± 0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.46 ± 0.28
34.383	Methyl Cinnamate	N/A	2019 - 2105 a	0.15 ± 0.03	0.16 ± 0.04	0.13 ± 0.03	0.13 ± 0.03	n.d.	n.d.	n.d.	n.d.
37.673	Dimethyl Terephthalate	N/A	N/A	0.04 ± 0	0.04 ± 0.01	0.04 ± 0	0.03 ± 0	n.d.	0.21 ± 0.06	0.08 ± 0.02	0.13 ± 0.1
Alkanes (3)											
2.94	2,4-DimethylHexane	795	N/A	n.d.	n.d.	0.07 ± 0.01	0.05 ± 0.02	0.85 ± 0.51	0.56 ± 0.09	0.44 ± 0.08	0.37 ± 0.27
19.354	1,3-Dichlorobenzene *	1424	1418 a	19.91 ± 0.44	20.25 ± 0.37	20.38 ± 0.38	20.4 ± 0.37	20.33 ± 0.56	19.69 ± 0.3	20.27 ± 0.5	20.03 ± 0.3
25.938	Nonadecane	N/A	N/A	0.14 ± 0.05	0.11 ± 0.01	0.06 ± 0.02	0.04 ± 0	n.d.	n.d.	n.d.	n.d.
Sulfur compounds (1)											
9.331	Dimethyl Disulfide	1079	1077 a	0.06 ± 0.02	0.07 ± 0.02	0.05 ± 0	0.05 ± 0.01	0.38 ± 0.23	0.38 ± 0.09	0.16 ± 0.01	0.23 ± 0.17

Table2. Content (µg/kg) of aroma compounds in the coated kibbles (4/4)

				Lard RS	Lard WS	Lard Rice	Lard Corn

Retention time (min)	Compound	KI Exp.	KI Lit.	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Aldehyde(14)							
3.204	Isobutyraldehyde *	817	817 a	0.41 ± 0.24	1.42 ± 0.11	0.2 ± 0.03	0.37 ± 0.14
4.928	2-Methylbutyraldehyde	919	917 a	0.15 ± 0.06	5.29 ± 0.21	0.79 ± 0.16	1.39 ± 0.69
5.042	3-methylbutyraldehyde *	924	920 a	4.46 ± 2.15	17.15 ± 1.13	2.18 ± 0.2	3.5 ± 0.02
6.632	Pentanal *	986	985 a	1.28 ± 0.6	4.28 ± 0.72	0.81 ± 0.03	1.07 ± 0.5
9.58	Hexanal *	1086	1088 c	16.97 ± 8.34	20.64 ± 4.51	10.22 ± 0.46	13.31 ± 0.25
12.732	Heptanal *	1190	1190 a	1.23 ± 0.84	4.24 ± 0.97	0.71 ± 0.08	0.78 ± 0.31
15.814	Octanal *	1294	1291 c	0.66 ± 0.4	1.99 ± 0.38	0.47 ± 0.1	0.53 ± 0.16
16.013	(E)-2-Hexenal	1301	1264 a	n.d.	n.d.	n.d.	n.d.
18.74	Nonanal *	1399	1398 a	0.93 ± 0.48	2.96 ± 0.19	0.58 ± 0.05	0.73 ± 0.35
20.796	Furfural *	1480	1432 c	0.15 ± 0.07	0.47 ± 0.05	0.08 ± 0.01	0.13 ± 0.06
21.559	(E, E)-2,4-Heptadienal	1508	1508 a	n.d.	n.d.	n.d.	n.d.
22.365	Benzaldehyde *	1540	1525 c	3.02 ± 1.32	12.49 ± 0.37	2.07 ± 0.25	2.78 ± 0.02
25.224	Benzeneacetaldehyde	N/A	1648 a	n.d.	2.92 ± 0.42	0.37 ± 0.01	0.57 ± 0.26
33.799	Cinnamaldehyde	N/A	2015-2084 a	0.16 ± 0.07	0.53 ± 0.07	0.09 ± 0.01	0.15 ± 0.07
Furans (3)							
3.905	Tetrahydrofuran	864	868 a	n.d.	n.d.	n.d.	n.d.
5.899	2-Ethylfuran	959	960 a	0.09 ± 0.05	0.26 ± 0.06	0.04 ± 0.01	0.08 ± 0.03
14.011	Furan, 2-pentyl-	1234	1239 a	1.91 ± 1.26	4.94 ± 2.17	1.5 ± 0.2	1.91 ± 0.59
Ketones (9)							
4.701	2-Butanone	908	908 a	0.15 ± 0.06	0.51 ± 0.05	0.09 ± 0.02	0.12 ± 0.05
12.678	2-Heptanone *	1188	1187 a	0.65 ± 0.29	2.28 ± 0.54	0.41 ± 0.06	0.57 ± 0.28
16.818	2,3-Octanedione	1331	1335 a	0.29 ± 0.17	0.78 ± 0.08	0.15 ± 0.02	0.18 ± 0.07
17.201	6-Methyl-5-Hepten-2-one *	1345	1341 a	0.4 ± 0.2	1.37 ± 0.36	0.24 ± 0.02	0.32 ± 0.12
19.193	3-Octen-2-one	1417	1411 a	0.39 ± 0.19	1.19 ± 0.29	0.23 ± 0.01	0.37 ± 0.15

22.136	(E, Z)-3,5-octadien-2-one	1531	1529 a	0.39 ± 0.2	1.23 ± 0.04	0.22 ± 0.02	0.31 ± 0.15
23.41	(E, E)-3,5-Octadien-2-one	1580	1578 a	0.34 ± 0.17	1.28 ± 0.25	0.24 ± 0.04	0.35 ± 0.17
24.379	Nona-3,5-dien-2-one	N/A	N/A	n.d.	n.d.	n.d.	n.d.
35.696	Gamma-undecalactone	N/A	2210-2300 a	n.d.	n.d.	n.d.	n.d.
Alcohols (10)							
12.041	1-Penten-3-ol	1169	1169 a	n.d.	n.d.	n.d.	n.d.
14.696	1-pentanol	1258	1258 a	0.61 ± 0.22	2.16 ± 0.43	0.42 ± 0.04	0.52 ± 0.2
17.585	4-Methyl-1-Pentanol	1359	1338 a	0.36 ± 0.17	0.65 ± 0.17	0.14 ± 0.02	0.38 ± 0.16
20.11	1-Octen-3-ol *	1454	1454 a	2.14 ± 1.05	6.09 ± 0.58	1.37 ± 0.15	1.74 ± 0.76
20.309	1-Heptanol *	1461	1460 a	0.17 ± 0.09	0.48 ± 0.1	0.11 ± 0.02	0.15 ± 0.06
21.192	2-Ethylhexanol *	1494	1494 a	n.d.	n.d.	n.d.	n.d.
24.16	5-Hepten-2-ol, 6-methyl-	N/A	N/A	0.13 ± 0.05	0.46 ± 0.04	0.11 ± 0.01	0.14 ± 0.06
24.312	(E)-5-Octen-1-ol	N/A	N/A	0.16 ± 0.07	0.46 ± 0.11	0.12 ± 0.03	0.15 ± 0.06
27.093	1-Hexadecanol	N/A	N/A	n.d.	n.d.	n.d.	n.d.
30.837	Phenol, 2,4,6-tris(1-methylethyl)-	N/A	N/A	0.13 ± 0.07	0.63 ± 0.09	0.07 ± 0.01	0.13 ± 0.07
Carboxylic acids (4)							
22.621	Propionic acid	1550	1554 a	0.72 ± 0.37	2.06 ± 0.74	0.45 ± 0.18	0.7 ± 0.57
24.757	Valeric acid	N/A	1686-1766 a	n.d.	0.82 ± 0.14	0.17 ± 0.07	0.22 ± 0.14
34.383	Methyl Cinnamate	N/A	2019-2105 a	n.d.	n.d.	n.d.	n.d.
37.673	Dimethyl Terephthalate	N/A	N/A	0.08 ± 0.04	0.28 ± 0.03	0.05 ± 0.01	0.07 ± 0.04
Alkanes (3)							
2.94	2,4-DimethylHexane	795	N/A	0.16 ± 0.1	0.65 ± 0.16	0.13 ± 0.02	0.16 ± 0.07
19.354	1,3-Dichlorobenzene *	1424	1418 a	20.05 ± 0.51	20.06 ± 0.57	19.74 ± 0.37	20.36 ± 0.21
25.938	Nonadecane	N/A	N/A	n.d.	n.d.	n.d.	n.d.
Sulfur compounds (1)							
9.331	Dimethyl Disulfide	1079	1077 a	0.17 ± 0.09	0.46 ± 0.15	0.09 ± 0.01	0.11 ± 0.05

KI Exp.: experimental Kovats index

KI Lit.: Kovats index from the literature

*: validated by pure compounds analyzed under same GC conditions.

n.d.: not detectable

a: Pubchem

b: Flavornet