Fats and Proteins Research Foundation
Report

The impact of rendered protein meal level of oxidation on shelf life and acceptability in extruded pet foods: determining sensory limits for oxidation values.

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Literature Review and Justification: The pet food industry is a $21 billion industry in the US with an estimated production of nearly 8.5 million metric tons of raw materials. Approximately 35% of that is estimated to be from rendered ingredients - much of the protein used today in pet foods is derived from rendered protein meals. Thus, rendering plays a significant role in this industry.

Holding a food such as pet food shelf-stable for this extended period of time is difficult. To do so requires the effective use of antioxidants. The most effective are the synthetic preservatives such as ethoxyquin, BHA and BHT (Gross et al., 1994). These preservatives are added to the raw ingredients at the time of production and then again during the pet food production processes to assure the food is produced from unspoiled ingredients and then once produced has enough residual preservatives to hold the food till consumed by the pet many months later. The natural antioxidants such as mixed tocopherols can be effective, but require some 10 fold more product to stabilize to a similar manner at a cost that can exceed 10 times the synthetics.

The question has been asked repeatedly when negotiating specifications between the renderer/broker of these protein meals and the pet food manufacturer as to what protein meal peroxide value is acceptable. The answer to this has typically been a fairly ambiguous number (e.g. Peroxide value of less than 10 meq/kg) based minimally on human food or livestock feed data and largely on personal bias and conjecture. This project will take on an approach based on the actual sensory properties of pet food samples. Descriptive sensory analysis enables quantification of the aroma and flavor properties of food and non-food products. Rancidity-related sensory attributes have been detected and evaluated in pet foods (Di Donfrancesco et al., 2012; Lin et al., 1998). Furthermore, when pet food is served to the pet, the owner is the mediator and an evaluator of the pet food acceptability. In case the pet food exhibits off-aromas that are related to an unacceptable product, the pet food may not be served to the pet. One of the objectives of this project was to determine the level of oxidation in pet foods after which the foods are not accepted by pet owners. The combination of results from the initial project (peroxide values, hexanal content) and this continuation project (rancidity related attributes levels and acceptability) enables us to determine the actual acceptable levels of oxidation, antioxidants, and shelf-life of pet foods manufactured with rendered meals.

Objectives:

1. Determine the effect of incorporating increasing levels of oxidation in rendered protein meals used to produce extruded pet food on **sensory** properties related to oxidation in finished product.
2. Determine the effect of increasing rancid ingredients on pet owner acceptability (liking) of extruded pet foods.
3. Determine the sensory standard for rancidity that could be allowed in a rendered protein meal without negative affecting acceptability (liking) of the finish pet food.

Materials and Methods

Samples

Rendered protein meal (approximately 1,000 lbs each) from beef (meat and bone meal) and poultry (poultry byproduct meal) that has been ground and preserved with ethoxyquin, mixed tocopherols, or unpreserved was collected and extruded into dry pet foods as shown below.

Sample	Rendered protein meal	Antioxidant	Sample Code
1	Beef	None	BOD-AM0
2	Beef	Ethoxyquin	BOD-AMET
3	Beef	Mixed tocopherols	BOD-AMMT
4	Poultry	None	COD-AM0
5	Poultry	Ethoxyquin	COD-AMET
6	Poultry	Mixed tocopherols	COD-AMMT

Shelf-life:
Samples of dry foods were collected in whirl pack bags (200-300 g) with a pin-hole for air exchange labeled time 0 in duplicates. Samples for ambient (RT) storage were collected for 0, 3, 6, 9 and 12 months.
The control, and both antioxidant treatment (ethoxyquin and mixed tocopherols) samples for both poultry and beef meal at all timepoints were subjected to descriptive sensory analysis (total n=30).

Descriptive sensory analysis

Six highly trained panelists received orientation on dry pet food before proceeding with sensory tests. Each test sample was served in a ~100 ml plastic cup for flavor and texture evaluation, and in a medium snifter covered with a watch glass for the evaluation of aroma attributes. Stale, oxidized oil, rancid, and cardboard aroma and flavor attributes as well as fracturability characteristic and other important attributes for these samples were evaluated according to lexicon developed by Di Donfrancesco et al. (2012) for pet food. For the evaluation a numeric scale of 0-15 with 0.5 increments where 0 represents none and 15 extremely high was applied to each attribute to provide a measure of intensity. The samples were evaluated in duplicate in a randomized order.

Consumer acceptance

Acceptance of the experimental pet foods was tested using a Central Location Trial (CLT). Both beef and poultry protein meal without antioxidant were selected for consumer study. Total of 106 pet owners who feed their pets dry food were recruited from the consumer database at the Sensory Analysis Center. The pet owners were screened for dog or cat ownership, and information about the breed, and diet of the dog(s) and cat(s), and owner demographic information. The pet owners had to be willing to participate in this study and have no allergies. During the Central Location Trial, conducted at the Sensory Analysis Center, selected blind-coded samples were served to the pet owners monadically in a randomized order. The pet owners were asked to evaluate their overall liking, appearance liking, and aroma liking on a 9-point hedonic scale (1 – dislike extremely, 9-like extremely). In addition questions about their feeding behavior, dog/cat food storage, and dogs/cats, were also included. The pet owners were reimbursed for their time.

This study will not give us information about pet liking of the foods. Pet owners are the ones making the decision of whether to serve the pet food to the pet or not. Because of this, and because of sample amount limitations, this study was asking for the pet owner opinion.

Data Analysis:

Data for each sensory attribute was analyzed by a two-way ANOVA mixed effect model, liking scores from consumer study were analyzed by a one-way ANOVA mixed effect model (SAS version 9.4, The SAS Institute Inc., Cary, NC,USA) using PROC GLIMMIX to determine significant differences among samples on each attribute and liking score. For all significant attributes and liking score, the sample effects were assessed using pair-wise comparisons based on SAS least square (LS) means. The criteria for significance was $p<0.05$.

Penalty Analysis for Just-about-right attributes was performed using XLSTAT version 2015.3.01 (Addinsoft, New York, NY, USA).

Partial Least Square Regression (PLSR) was used to create External Preference Mapping by regressing descriptive attributes and consumer liking data to explore the drivers of liking for dry pet food. PLSR was performed using XLSTAT version 2015.3.01 (Addinsoft, New York, NY, USA)..

The correlation between descriptive sensory data (aroma attributes), consumer acceptability and instrumental data were determined by Pearson's correlation coefficient. The correlation analysis was performed using XLSTAT version 2015.3.01 (Addinsoft, New York, NY, USA).

Results

Descriptive sensory analysis

Beef (meat and bone meal)

The mean intensity scores of 12 sensory characteristics for pet food prepared from beef (meat and bone meal) are shown in Table 1 and Table 2.

The result in Table 1 showed that all evaluated aroma attributes were significantly different across samples. Samples preserved with ethoxyquin (BOD-AMET) at the storage time of 3 months had significantly higher stale and cardboard aroma characteristics than other samples. The intensity of oxidized oil and rancid aroma of samples with all 3 different treatments (BOD-AMO, BOD-AMMT, and BOD-AMET) tended to increase when the storage time increased. Interestingly, samples preserved with mixed tocopherols (BOD-AMMT) at the storage time of 12 months were the samples that had the highest intensity on oxidized oil and rancid aroma attributes. Control sample (unpreserved) was the one that seemed to have the smallest changes on those two aroma characteristics over storage time.

In Table 2, four out of six evaluated flavor attributes were significantly different across samples. Sample with no preservatives (BOD-AM0) which had been kept for 12 months had the highest intensity of stale flavor. The results of flavor attributes in Table 2 moved in the same direction with the results in Table 1. Samples preserved with mixed tocopherols (BOD-AMMT) that had been kept for 12 months had significantly higher oxidized oil, rancid, and metallic flavor than other samples.

The results showed that samples preserved with antioxidant (ethoxyquin and mixed tocopherols) did not show significant improvement on maintaining quality of samples compared to control sample (unpreserved) based on sensory characteristics. However, changes in significantly different sensory characteristics (oxidized oil, rancid etc.) over storage time were minimal and not necessarily directional (Figure 1 and Figure 2). Consumers may not detect these minimal changes in samples. Therefore, consumer acceptance study was conducted in order to determine whether the minimal changes in sensory characteristics affect the pet owner acceptability of the finished product or not.

Table 1 Mean intensity scores of aroma and texture attributes for beef meal pet food samples

Sample	Storage Time (month)	Aroma				Texture
		Oxidized Oil	Stale	Cardboard	Rancid	Fracturability
BOD-AM0	0	2.29 fg	2.04 e	2.50 c	0.46 def	6.75
BOD-AM0	3	2.42 def	2.25 cde	2.58 bc	0.08 fg	6.83
BOD-AM0	6	2.00 g	2.04 e	2.58 bc	0.33 efg	6.96
BOD-AM0	9	2.46 cdef	2.38 bcd	2.67 bc	1.04 bc	6.67
BOD-AM0	12	2.63 bcde	2.42 bc	2.67 bc	0.71 cde	6.92
BOD-AMMT	0	2.29 fg	2.13 de	2.54 c	0.17 fg	6.71
BOD-AMMT	3	2.29 fg	2.29 bcde	2.71 bc	0.17 fg	6.75
BOD-AMMT	6	2.21 fg	2.29 bcde	2.67 bc	0.17 fg	6.58
BOD-AMMT	9	2.75 bc	2.42 bc	2.71 bc	0.92 bc	6.71
BOD-AMMT	12	3.21 a	2.54 b	2.79 b	1.63 a	6.58
BOD-AMET	0	2.33 ef	2.42 bc	2.71 bc	0.08 fg	7.04
BOD-AMET	3	2.67 bcd	2.88 a	3.04 a	0.67 cde	6.96
BOD-AMET	6	2.25 fg	2.46 bc	2.50 c	0.00 g	6.79
BOD-AMET	9	2.50 cdef	2.33 bcd	2.67 bc	0.79 cd	6.63
BOD-AMET	12	2.83 b	2.46 bc	2.58 bc	1.33 ab	6.83
p-value		*0.0320*	*0.0044*	*0.0136*	*0.0004*	*0.4755*

Note: Scores are based on a 0-15-point numeric scale with 0.5 increments.
Samples with different letters within column are significantly different from each other ($p \leq 0.05$).

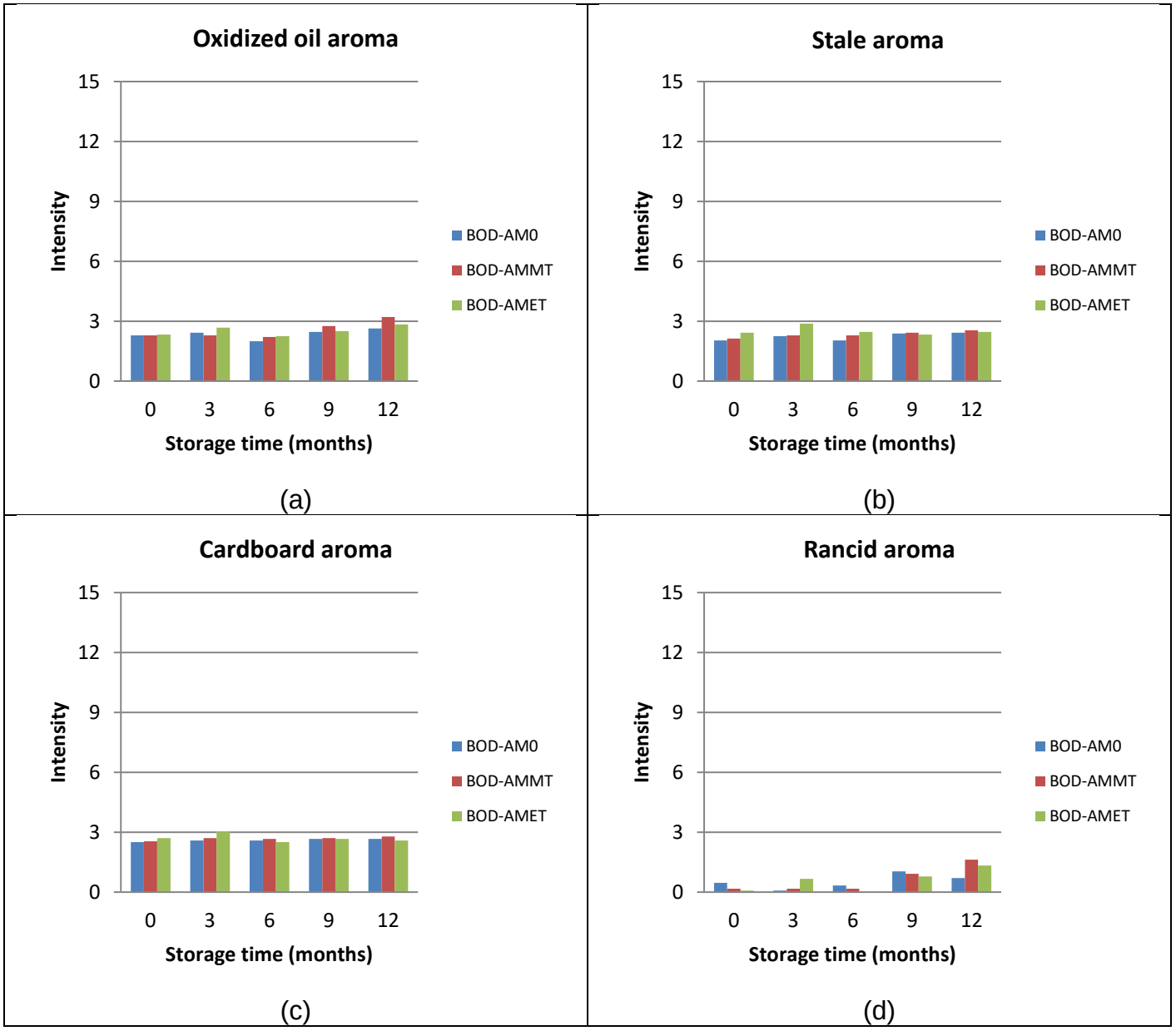


Figure 1: Bar graph of significantly different aroma attributes of dog food prepared from beef (meat and bone meal); (a)-Oxidized oil aroma; (b)-Stale aroma; (c)-Cardboard aroma; (d)-Rancid aroma

Table 2 Mean intensity scores of flavor attributes for beef meal pet food samples

Sample	Storage Time (month.)	Flavor						
		Oxidized Oil	Stale	Cardboard	Rancid	Sour	Bitter	Metallic
BOD-AM0	0	2.50 cd	2.42 bcde	2.67	1.25 bcd	1.54	2.92	1.17 abc
BOD-AM0	3	2.21 e	2.29 de	2.71	0.17 g	1.46	2.88	1.38 a
BOD-AM0	6	2.50 cd	2.33 de	2.63	1.25 bcd	1.50	2.92	1.17 abc
BOD-AM0	9	2.54 bcd	2.58 abc	2.92	1.04 de	1.54	2.92	1.17 abc
BOD-AM0	12	2.75 ab	2.67 a	2.67	1.21 cd	1.58	2.92	1.38 a
BOD-AMMT	0	2.46 cd	2.25 e	2.71	0.67 ef	1.54	2.79	1.04 bc
BOD-AMMT	3	2.54 bcd	2.25 e	2.83	0.42 fg	1.29	2.96	0.88 c
BOD-AMMT	6	2.38 de	2.33 de	2.88	0.38 fg	1.46	2.83	0.92 c
BOD-AMMT	9	2.42 de	2.38 cde	2.75	1.13 d	1.38	2.79	1.17 abc
BOD-AMMT	12	2.79 a	2.50 abcd	2.71	1.83 a	1.63	2.71	1.42 a
BOD-AMET	0	2.42 de	2.29 de	2.79	0.21 g	1.50	2.88	1.38 a
BOD-AMET	3	2.67 abc	2.63 ab	3.00	0.50 fg	1.54	2.83	1.29 ab
BOD-AMET	6	2.38 de	2.38 cde	2.67	0.38 fg	1.38	2.88	1.04 bc
BOD-AMET	9	2.54 bcd	2.50 abcd	2.71	1.67 ab	1.63	2.75	1.29 ab
BOD-AMET	12	2.75 ab	2.46 abcde	2.79	1.63 abc	1.33	2.75	1.00 bc
p-value		*0.0164*	*0.0394*	*0.2088*	*<0.0001*	*0.2206*	*0.8249*	*0.0032*

Note: Scores are based on a 0-15-point numeric scale with 0.5 increments.
Samples with different letters within column are significantly different from each other ($p \leq 0.05$).

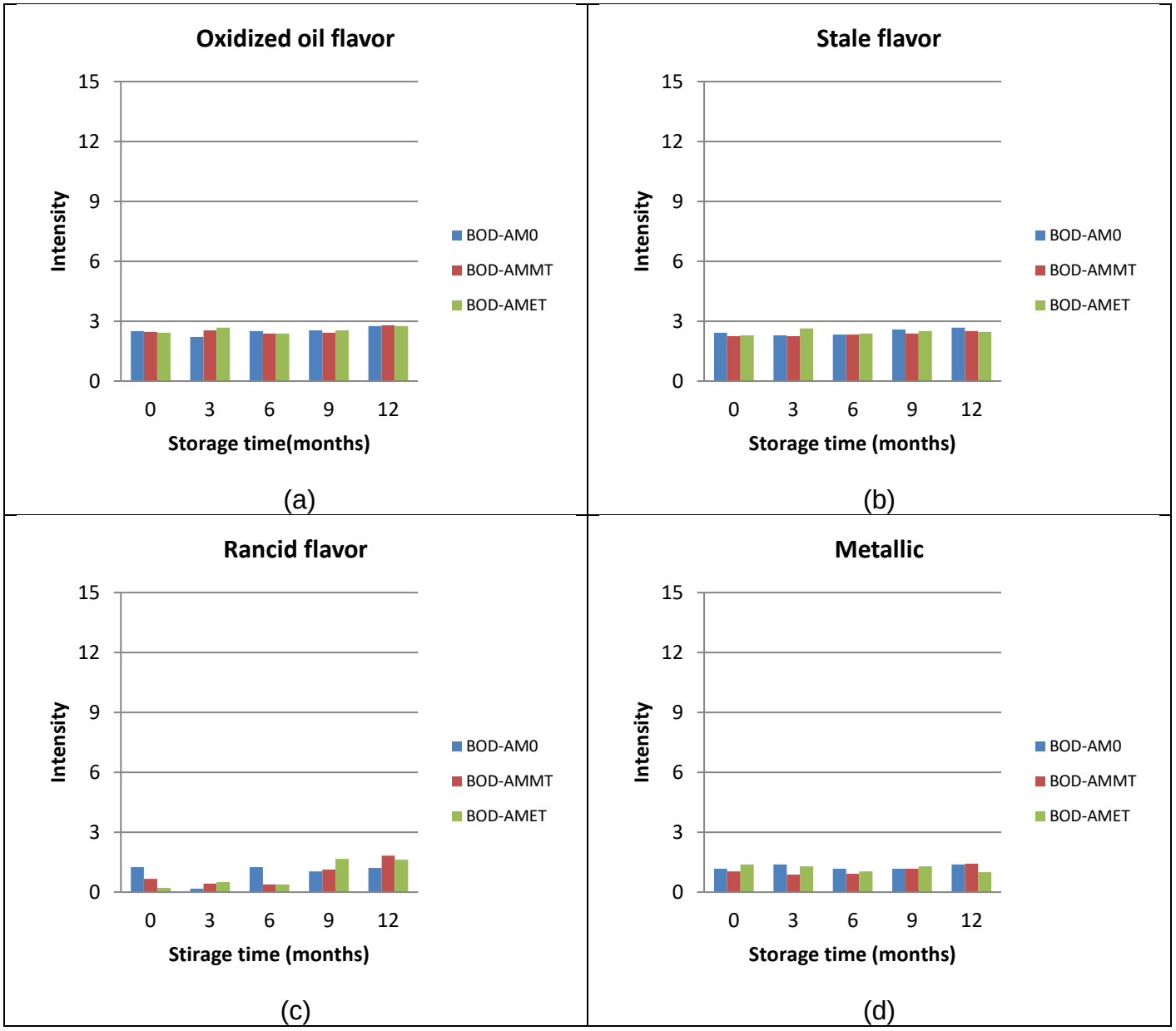


Figure 2: Bar graph of significantly different flavor attributes of dog food prepared from beef (meat and bone meal); (a)-Oxidized oil flavor; (b)-Stale flavor; (c)-Rancid flavor; (d)-Metallic

Poultry (poultry byproduct meal)

The mean intensity scores of 12 sensory characteristics for pet food prepared from poultry (poultry byproduct meal) are shown in Table 3 and Table 4.

The result from Table 3 showed that three out of four evaluated aroma attributes were significantly different across samples. Control sample (COD-AM0) which had been kept for 12 months had significantly higher oxidized oil, stale and rancid aroma than other samples. It was clearly seen that control or unpreserved sample (COD-AM0) had a significant increase in the intensity of oxidized oil, stale and rancid aroma over storage time. On the other hand, there were no significantly different aroma characteristics over storage time for sample preserved with antioxidant (COD-AMMT and COD-AMET).

The result from Table 4 was also in the same direction with result in Table 3. Two out of six flavor attributes were significantly different across samples. The intensity of oxidized oil and rancid flavor were significantly increased over storage time for Control sample (COD-AM0). Control sample which had been kept for 12 months had the highest intensity on oxidized oil and rancid flavor. There were minimal changes on oxidized oil and rancid flavor over storage time for samples preserved with antioxidant (COD-AMMT and COD-AMET).

The sensory analysis result showed that antioxidants added to pet food prepared from poultry byproduct meal played an important role to maintain sensory characteristics of samples over storage time.

Consumer acceptance study was conducted in order to determine whether the increasing in off-note sensory characteristics affect the pet owner acceptability of the finished product or not.

Table 4 Mean intensity scores of aroma and texture attributes for poultry meal pet food samples

Sample	Storage time (month)	Aroma				Texture
		Oxidized Oil	Stale	Cardboard	Rancid	Fracturability
COD-AM0	0	2.29 c	2.38 bcd	2.75	0.33 c	4.08 c
COD-AM0	3	2.50 bc	2.33 bcd	2.54	0.33 c	5.46 b
COD-AM0	6	2.88 b	2.50 ab	2.54	1.17 b	5.88 ab
COD-AM0	9	2.50 bc	2.33 bcd	2.58	1.00 bc	6.42 a
COD-AM0	12	4.13 a	2.75 a	2.83	4.21 a	6.29 a
COD-AMMT	0	2.38 c	2.25 bcde	2.54	0.63 bc	6.38 a
COD-AMMT	3	2.38 c	2.46 bc	2.46	0.33 c	6.38 a
COD-AMMT	6	2.46 c	2.33 bcd	2.54	0.83 bc	6.00 ab
COD-AMMT	9	2.38 c	2.17 de	2.50	0.29 c	6.54 a
COD-AMMT	12	2.50 bc	2.21 cde	2.42	0.71 bc	6.08 ab
COD-AMET	0	2.25 c	2.00 e	2.33	0.46 bc	5.96 ab
COD-AMET	3	2.13 c	2.29 bcd	2.38	0.58 bc	6.25 a
COD-AMET	6	2.33 c	2.25 bcde	2.50	0.46 bc	6.42 a
COD-AMET	9	2.13 c	2.17 de	2.63	0.50 bc	6.17 ab
COD-AMET	12	2.17 c	2.21 cde	2.21	0.88 bc	6.04 ab
***p*-value**		*<0.0001*	*0.0528*	*0.0739*	*<0.0001*	*<0.0001*

Note: Scores are based on a 0-15-point numeric scale with 0.5 increments.
Samples with different letters within column are significantly different from each other ($p \leq 0.05$).

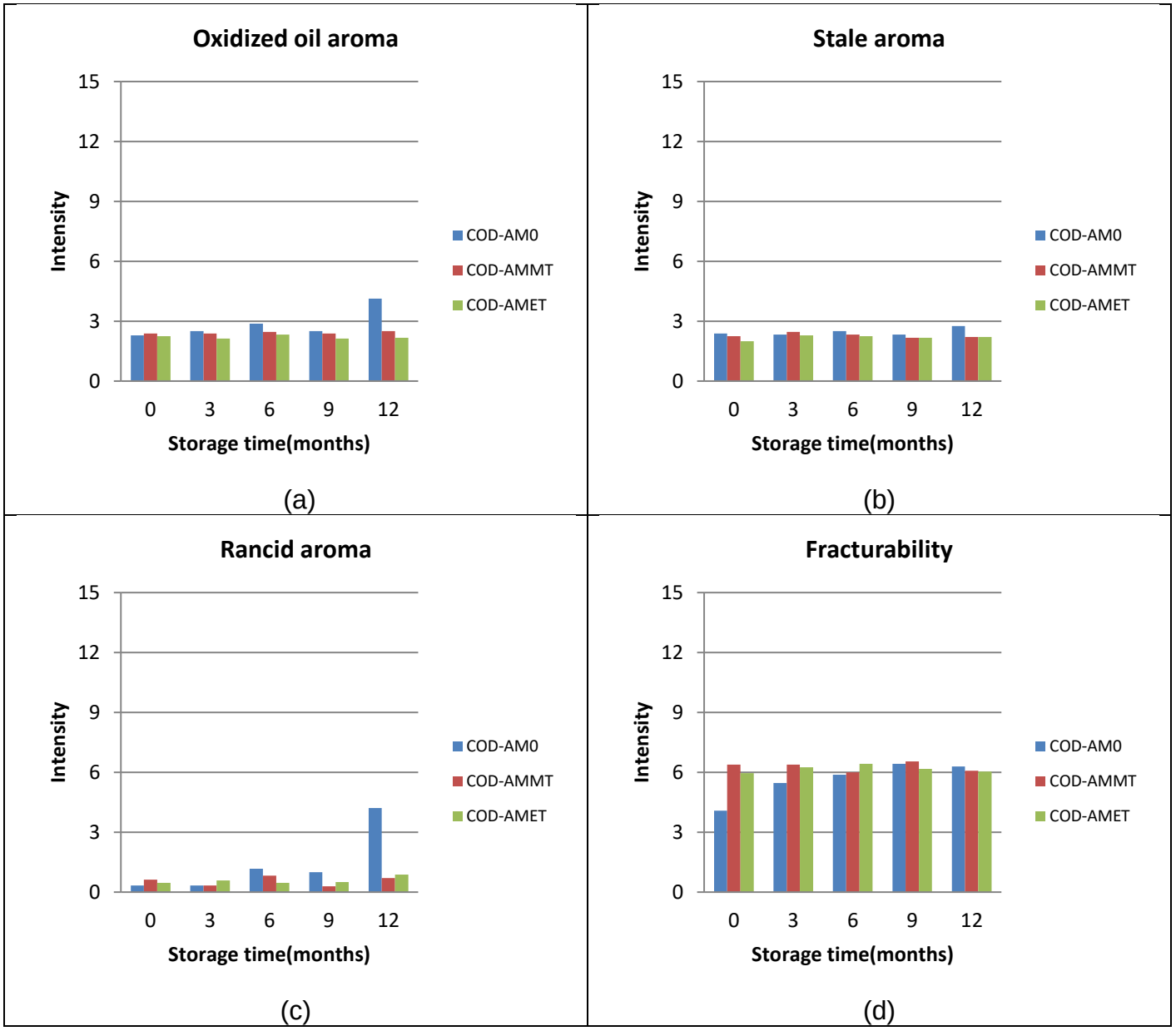


Figure 3: Bar graph of significantly different aroma and texture attributes of dog food prepared from poultry meal; (a)-Oxidized oil aroma; (b)-Stale aroma; (c)-Rancid aroma; (d)-Fracturability

Table 4 Mean intensity scores of flavor attributes for poultry meal pet food samples

Sample	Storage Time (month)	Flavor						
		Oxidized Oil	Stale	Cardboard	Rancid	Sour	Bitter	Metallic
COD-AM0	0	2.29 de	2.33	2.83	1.04 efg	1.63	2.92	0.92
COD-AM0	3	2.54 cde	2.29	2.50	1.58 cde	1.67	2.92	1.08
COD-AM0	6	3.08 b	2.42	2.75	2.83 b	1.75	3.17	1.04
COD-AM0	9	2.71 bc	2.50	2.96	1.17 ef	1.67	3.21	1.25
COD-AM0	12	3.96 a	2.83	2.83	3.92 a	1.67	3.21	1.38
COD-AMMT	0	2.50 cde	2.38	2.63	1.88 cd	1.75	2.92	1.00
COD-AMMT	3	2.42 cde	2.38	2.63	1.17 ef	1.54	2.67	0.83
COD-AMMT	6	2.75 bc	2.50	2.96	1.92 c	1.79	3.13	1.25
COD-AMMT	9	2.50 cde	2.46	2.67	1.08 efg	1.58	2.83	1.00
COD-AMMT	12	2.75 bc	2.50	2.75	1.13 efg	1.58	3.13	1.33
COD-AMET	0	2.17 e	2.13	2.38	0.79 fg	1.58	3.00	1.13
COD-AMET	3	2.63 cd	2.25	2.54	1.25 def	1.67	2.88	0.75
COD-AMET	6	2.42 cde	2.25	2.75	1.00 efg	1.67	2.92	1.21
COD-AMET	9	2.29 de	2.29	2.75	0.50 g	1.38	3.08	0.88
COD-AMET	12	2.46 cde	2.21	2.67	1.50 cde	1.50	2.88	1.21
p-value		*<0.0001*	*0.1916*	*0.1148*	*<0.0001*	*0.7205*	*0.1458*	*0.3876*

Note: Scores are based on a 0-15-point numeric scale with 0.5 increments.
Samples with different letters within column are significantly different from each other ($p \leq 0.05$).

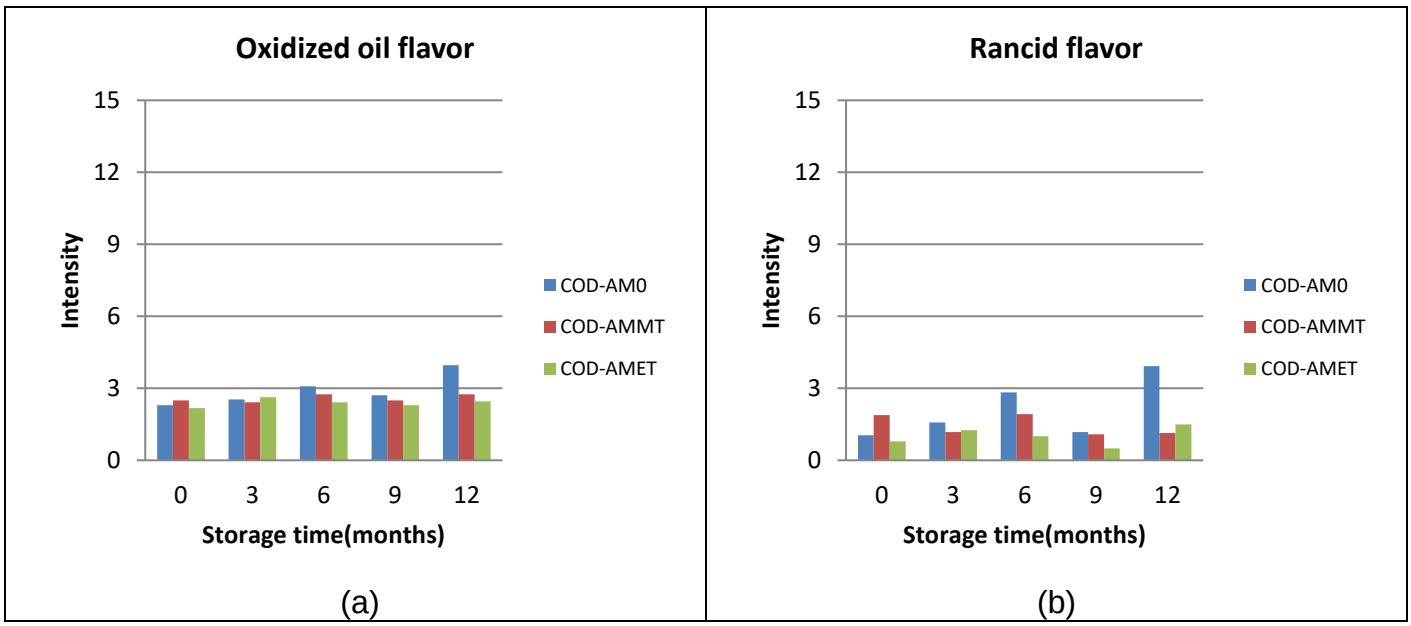


Figure 4: Bar graph of significantly different flavor attributes of dog food prepared from poultry meal; (a)-Oxidized oil flavor; (b)-Rancid flavor.

Consumer Acceptance

The results from descriptive analysis showed that the differences of rendered protein meal samples without antioxidants over storage time were more pronounced and directional, especially for poultry by product meal samples. Therefore, rendered protein meal samples (for both beet and poultry meal) without antioxidants were chosen for consumer study.

A total of 106 pet owners passed the screener (dog or cat owners; feed their pets with dry food; responsible or share responsibility of purchasing dry food; responsible or share responsibility of pet feeding). The consumers were asked to evaluate their overall liking, appearance liking, and aroma liking on a 9-point hedonic scale (1 – dislike extremely, 9-like extremely) for all samples (Appendix A). The demographic information is shown in Appendix B. The participants in this study either had dogs (58.4%), cats (20.8%) or both cats and dogs (20.8%). While dog owners tended to have more purebred dog than mixed breed, cat owners owned more mixed breed cats than purebred ones (Appendix C). The top 3 dry dog food brands the owners most often fed their dogs were Hill's Science Diet, Purina Beneful, and IAMS. The top 3 dry cat food brans the owners most often fed their cats were IAMS, Hill's Science Diet, and Meow Mix (Appendix D).

Most of pet owners stored their dry pet food in air tight containers (49.1%) or resealed in the original package (34.9%). The majority of them spent less than $100 on pet food per month and normally finished a package of dry pet food within 1 month (68.9%) (Appendix E).

Pet owner acceptance

Beef (meat and bone meal)

The mean scores for overall liking, appearance liking and aroma liking were shown in table 5. The results showed that storage time did not affect pet owners' acceptance (overall, appearance, or aroma liking) on dry pet food prepared from beef meal. The sensory descriptive data from these samples showed small changes in both aroma and flavor characteristics. These small changes might be too small for pet owners to detect the differences resulted in no significantly difference on all liking scores. However, we noticed that the average liking scores for all samples were in the range of "slightly dislike – neither like nor dislike". This meant pet owners were not particularly fond of these samples, even the fresh sample (0 month).

Table 5 The mean scores for overall liking, appearance liking, and aroma liking for beef meal samples for each storage time point. Ratings given on a 9-point scale from dislike extremely to like extremely.

Time point (month)	**Overall liking**	**Appearance liking**	**Aroma liking**
0	4.97	4.66	5.08
3	4.81	4.47	5.05
6	4.73	4.61	4.99
9	4.87	4.67	5.22
12	5.13	4.92	5.29
p-value	*0.5227*	*0.4398*	*0.7108*

The majority of pet owners said the things that made them like this sample was the size which was just about right for their pets. On the other hand, they didn't like this sample because it looked too dry, had

a bland color and had low intensity in appetizing smell (e.g. meaty). A lot of them reported that they found hair and white pieces in the sample which did not appeal to them (Figure 5 and 6). Therefore, the low liking score that consumers gave to all samples might be due to the unpleasant appearance and low intensity in appetizing aroma.

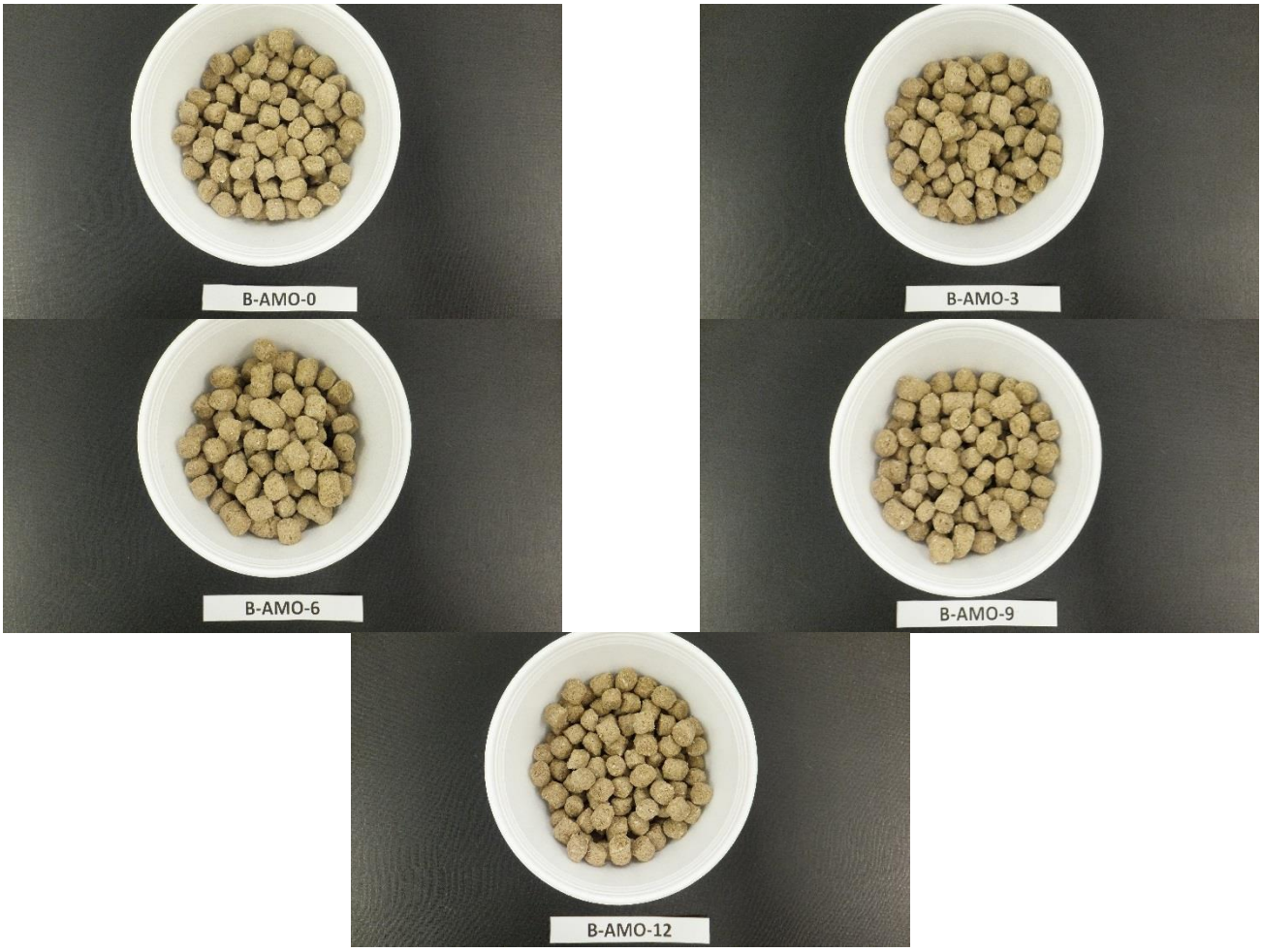


Figure 5 Samples prepare from beef meal stored at 0, 3, 6, 9, and 12 months.

Figure 6 Hair and white pieces found in dry dog food prepared from beef meal.

Poultry (poultry byproduct meal)

Table 6 showed the average of liking scores for poultry meal samples at each storage time point. There were no significant differences between samples for appearance liking. On the other hand, there was a significant difference between samples for overall liking and aroma liking. There was a significant decrease in aroma liking score for samples with a longer shelf-life. The decrease in overall liking score when sample stored for a long time was mainly due to the change in aroma of the sample.

Table 6 The mean scores for overall liking, appearance liking, and aroma liking for poultry meal samples at each storage time point. Ratings given on a 9-point scale from dislike extremely to like extremely.

Time point (month)	**Overall liking**	**Appearance liking**	**Aroma liking**
0	5.52 a	5.42	5.53 a
3	5.39 a	5.23	5.35 ab
6	5.32 a	5.23	5.22 ab
9	5.24 a	5.25	5.01 bc
12	4.95 b	5.05	4.79 c
p-value	*0.0013*	*0.0797*	*0.0004*

The result from descriptive analysis (Table 3) showed that there were significant differences in aroma and flavor across samples. The changes for oxidized oil and rancid aroma intensity were big, especially when samples kept for 9 months and sample kept for 12 months (more than 1.5 point). These changes were enough for consumers to detect the differences between samples and resulted in significant decrease in overall and aroma liking score.

The liking scores for the fresh samples (0 month) prepared from poultry meal were a little bit higher than the one prepared from beef meal. The liking scores for the fresh sample were in the range of "Like slightly – Neither like nor dislike". The main reason for higher liking score was no detection of hair or white pieces in the sample. However, some consumers still mentioned that they didn't like samples due to the unappealing color. The pictures for samples with different time point were shown in Figure 7.

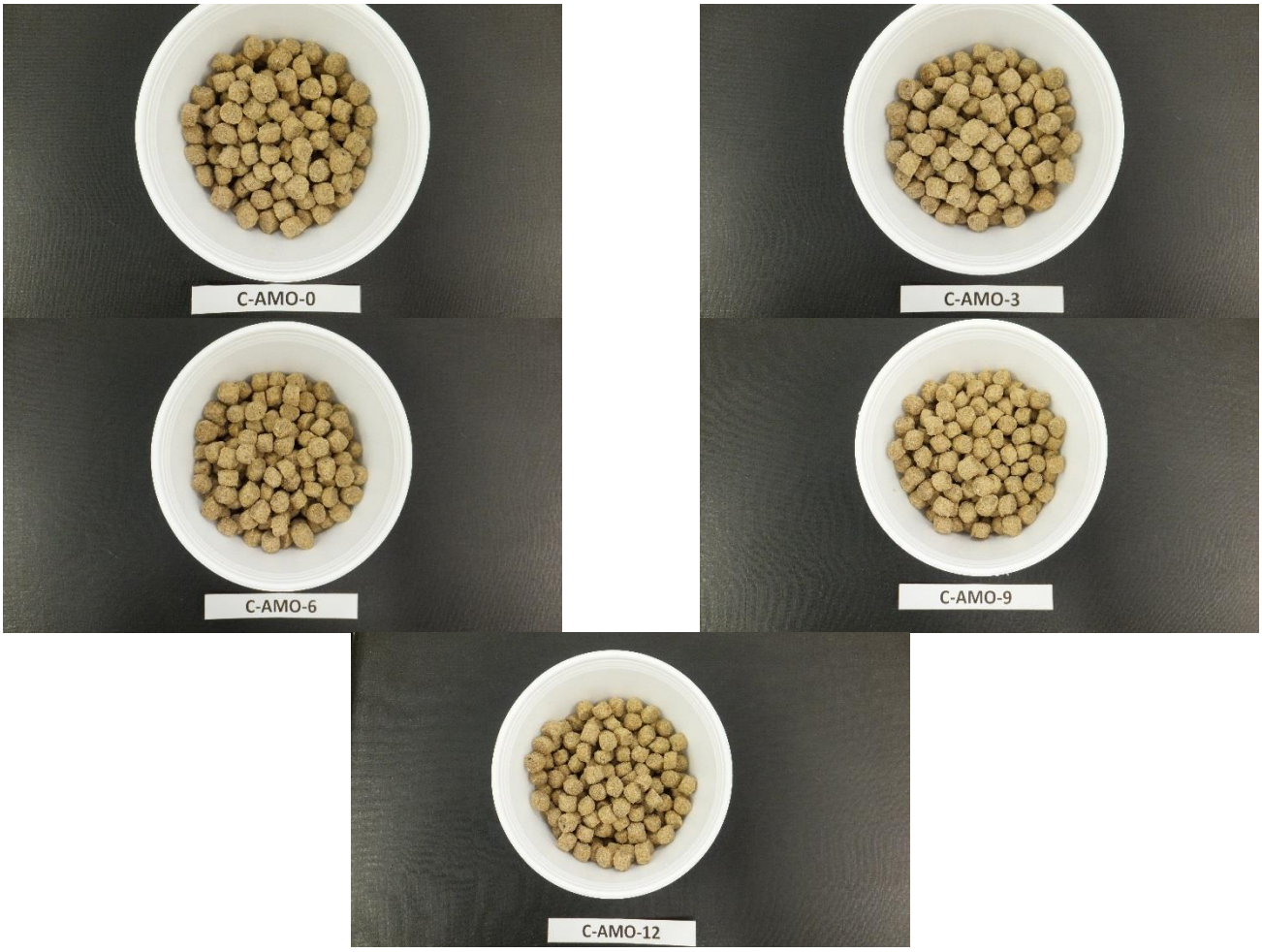


Figure 7 Samples prepared from poultry meal stored at 0, 3, 6, 9, and 12 months.

Penalty analysis

Beef (meat and bone meal)

Penalty analysis was conducted to determine whether the consumers "penalized" the samples for having too high or too low aroma characteristics. Table 7 showed that there was a significant drop in aroma liking score at $p<0.05$ for the beef meal samples that had too low aroma. This may be related to consumer expectations – the consumers expect a commercial dog or cat food to have certain aroma characteristics that are clearly perceivable.

Table 7 Mean drop in aroma liking associated with aroma attribute for beef meal samples

Variable	Level	Frequencies	%	Mean drops	p-value	Significant
	Too little	44	41.51%	0.685	**0.048**	**Yes**
AromaJAR	JAR	45	42.45%			
	Too much	17	16.04%	2.139		

From liking data in Table 5, aroma liking scores slightly increased for beef meal samples over time point. Pet owners might have their expectation about aroma of samples. When they evaluated the fresh sample that had a lower intensity of aroma than they expected, they tended to decrease their liking scores. Pet owners were more likely to give a little bit higher score for samples that had been stored for longer time, although these samples had been reported to have higher off note characteristics. The intensity of the off note characteristics might be too low for them to recognize as a "bad" aroma, but might only be enough for them to say that the aroma of the samples is not too low for them. Therefore, pet owners tended to give higher score for samples that they thought to have a higher aroma overall.

Poultry (poultry byproduct meal)

The results from Table 8 showed that pet owners strongly penalized the poultry meal samples when those had a too intense aroma ($p=0.020$). Based on the descriptive data from Table 4, the off note characteristics (oxidized oil and rancid) were obviously increased when samples had been stored for 12 months. The high intensity of the off note characteristics might have exceeded consumer's acceptability and lead the consumer to consider these higher intensity as an unpleasant aroma for the dry pet food. Therefore, the higher intensity of off note characteristics resulted in the lower liking score of the samples.

Table 8 Mean drop in aroma liking associated with aroma attribute for poultry meal samples

Variable	Level	Frequencies	%	Mean drops	p-value	Significant
	Too little	28	26.42%	0.536	0.240	No
AromaJAR	JAR	56	52.83%			
	Too much	22	20.75%	1.166	**0.020**	**Yes**

Drivers of liking

Beef (meat and bone meal)

The external preference mapping in Figure 8 combined descriptive sensory analysis data with consumer overall liking scores for beef meal samples. The samples with higher intensity in off note characteristics (samples kept for 9 and 12 months) seemed to be preferred by pet owners. Pet owners tended to give lower liking score for fresh sample and the ones kept for 3 and 6 months due to the lower in overall aroma intensity. However, there were no significant differences in liking scores across samples. This result agreed with the penalty analysis result. Noted that higher intensity in off note characteristics for beef meal samples kept for 9 and 12 months might not have been strong enough for consumers to detect and hence was not considered as an undesirable aroma.

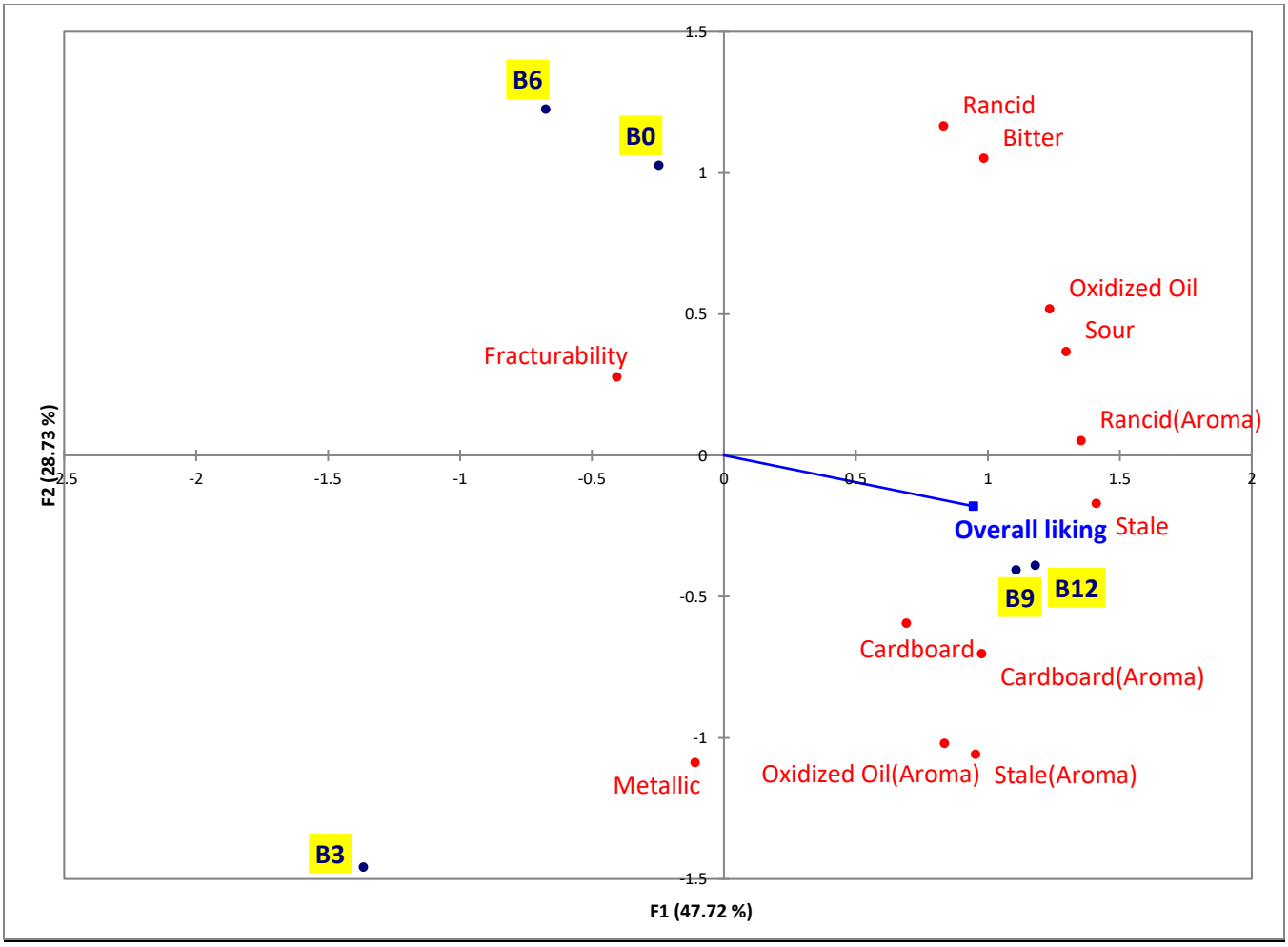


Figure 8 External preference mapping of beef meal samples from 5 different time points and average overall liking from 106 pet owners. Black dots represent samples from specific time points; red dots represent sensory attributes.

Poultry (poultry byproduct meal)

The external preference mapping in Figure 9 showed that samples with lower intensity in off note characteristics seemed to be preferred by pet owners. The descriptive sensory data showed the dramatic increase in off note characteristics, especially oxidized oil and rancid attributes. The increase in off note intensity in samples kept for 12 months was high enough for consumers to detect the differences. Moreover, these intensities tended to exceed their acceptability and this resulted in significantly lower liking scores for the samples kept for 12 months.

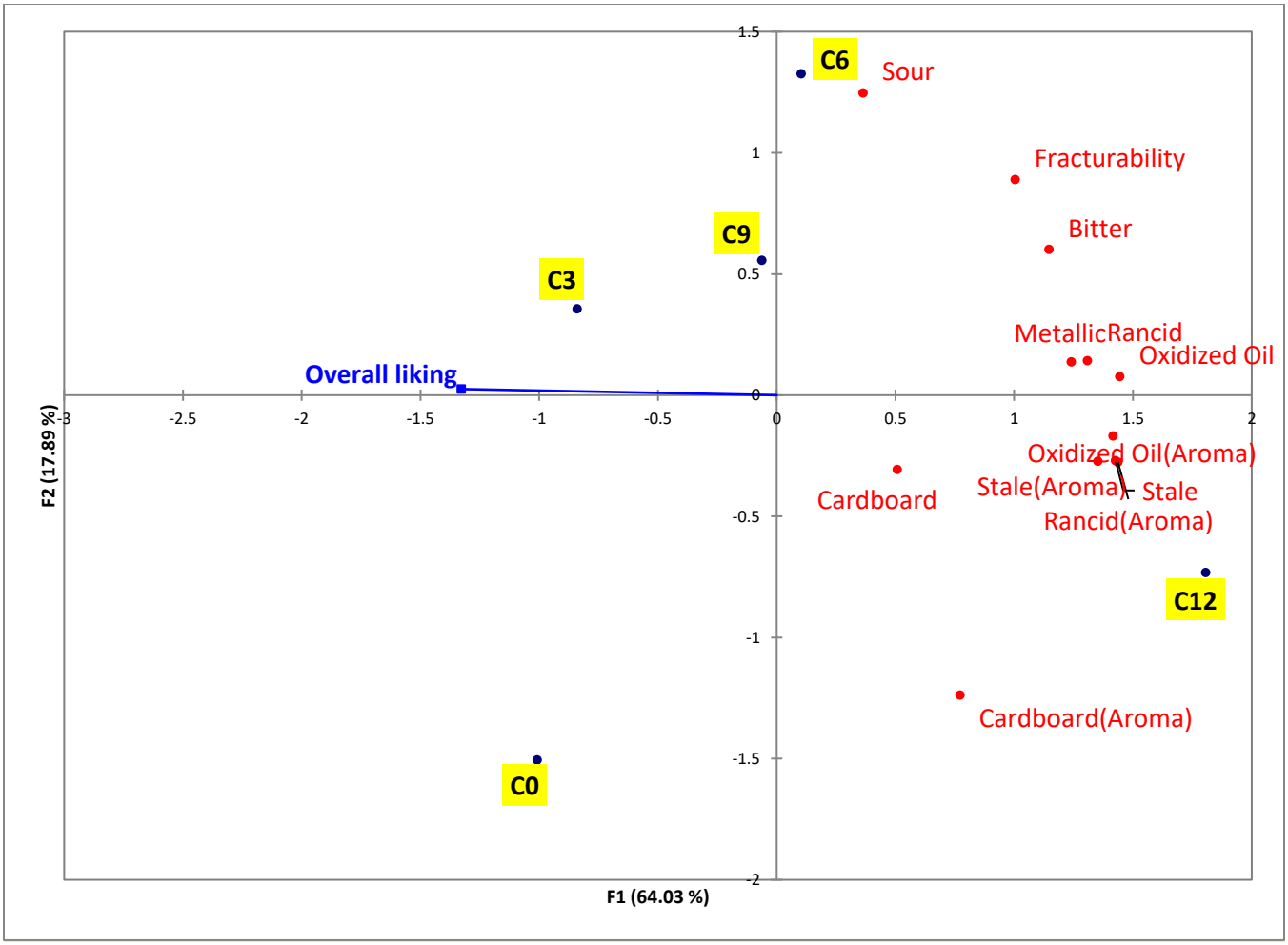


Figure 9 External preference mapping of poultry meal samples from 5 different time points and average overall liking from 106 pet owners. Black dots represent samples from specific time point; red dots represent sensory attributes.

Determination of acceptable levels of oxidation

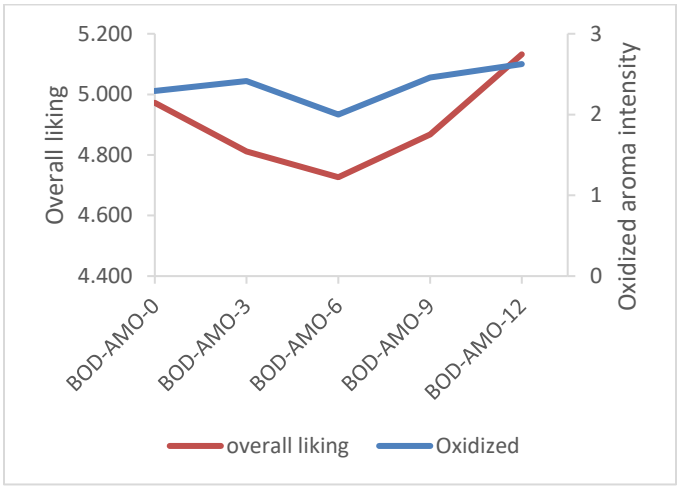
Beef (meat and bone meal)

Correlation among descriptive sensory characteristics, pet owners' acceptability, and instrumental data were presented in table 9. Interestingly, there were no significant correlation between all of those measurements (p>0.05). Volatile compound such as hexanal might have changed to other volatile compounds during storage period which gave researchers the hard time to determine level of oxidation based on chemical measurements and thus resulted in low or lack of correlation between chemical and sensory measurements. In addition, there was low development of oxidation levels over storage time for this diet and it was too low intensity for consumers to detect and considered as undesirable characteristics. Pet owners' liking score for this diet over storage time was mainly influenced by other factors besides oxidative-related characteristics such as their expectation to get more noticeable aroma from the samples.

Table 9 Correlation and significant between aroma attributes, consumer acceptability, and instrumental data for beef meal diet without antioxidant (BOD-AMO).

Variables	Hexanal	Peroxide Value(PV)	Anisidine Value (AV)	Overall liking
Oxidized	-0.627	0.471	-0.012	0.760
Stale	-0.210	0.597	-0.169	0.503
Cardboard	0.231	0.691	-0.292	0.241
Rancid	0.263	0.363	-0.850	0.408
Hexanal		-0.116	-0.541	-0.686
Peroxide Value(PV)			0.126	0.623
Anisidine Value (AV)				0.034
Overall liking				

Figure 10 was aimed to visualize the correlation between consumers' overall liking and each rancidity-related measurements (both sensory and chemical). Overall, there was no clear direction/pattern at what point consumer acceptability started significantly decline since there were no correlation between overall liking and other measurements in this diet. The correlation between consumer acceptability and descriptive sensory data/ chemical data might be clearly seen if we kept samples for more than 12 months at ambient temperature.



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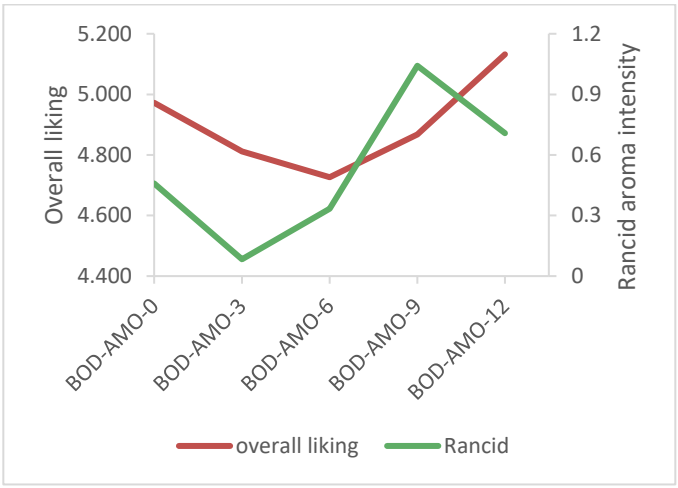

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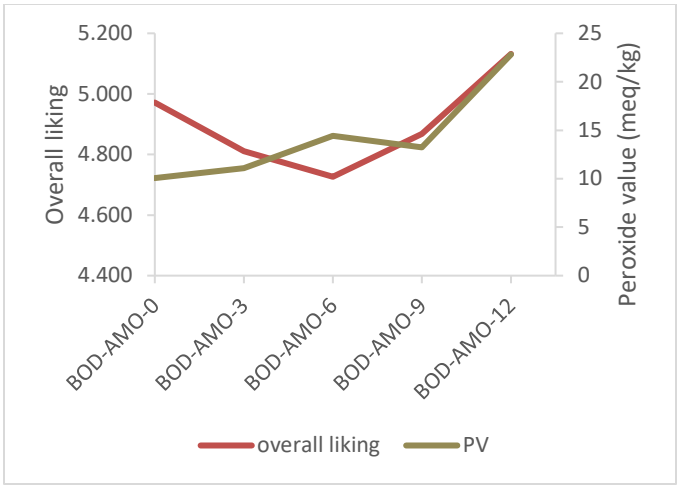

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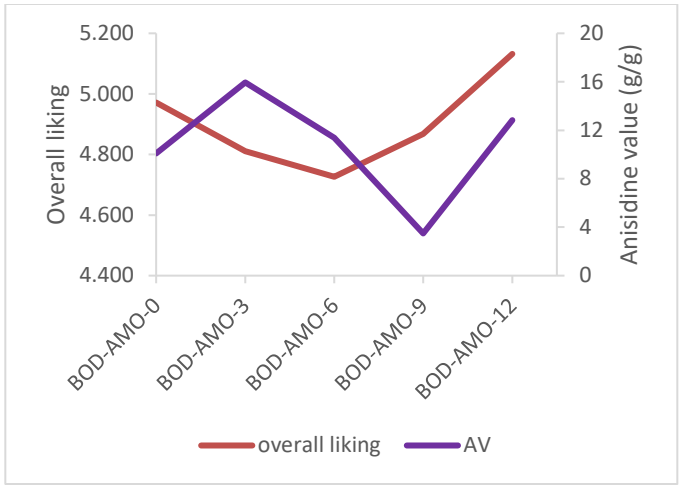

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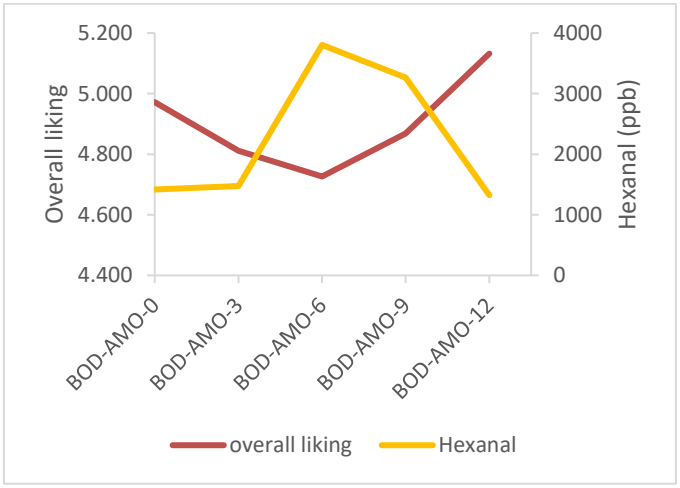


(e)

Figure 10 Line graphs of overall liking score against descriptive sensory data and instrumental data of beef meal diets without antioxidant (BOD-AMO) for each time point (a)-Overall liking *vs* Oxidized aroma; (b)-Overall liking *vs* Rancid aroma; (c)-Overall liking *vs* Peroxide value; (d)-Overall liking *vs* Anisidine value; (e)-Overall liking *vs* Hexanal

Poultry (poultry byproduct meal)

Table 10 showed the correlation between aroma attributes, consumer acceptability and chemical data for poultry meal without antioxidant diet. Anisidine value (AV) had strong positive correlation with most aroma attributes except cardboard aroma. On the other hand, consumer acceptability had strong negative correlation with oxidized and rancid aroma, implying that pet owners' acceptability of the product would be decreased when the intensity of rancid-related characteristics increased. Correlation between chemical measurements and consumer acceptability was not found in this study.

Aroma and flavor that human recognize normally come from the combination of several chemical compounds. Therefore, using only specific or single compound to determine the level of oxidation perceived by human might not be the most accurate method. Since pet food owners are the ones who make decision on purchasing food for their pets, measuring and setting acceptable level of oxidation by using human sensory analysis along with chemical analysis may be more appropriate method rather than depending on chemical characteristics of the products only.

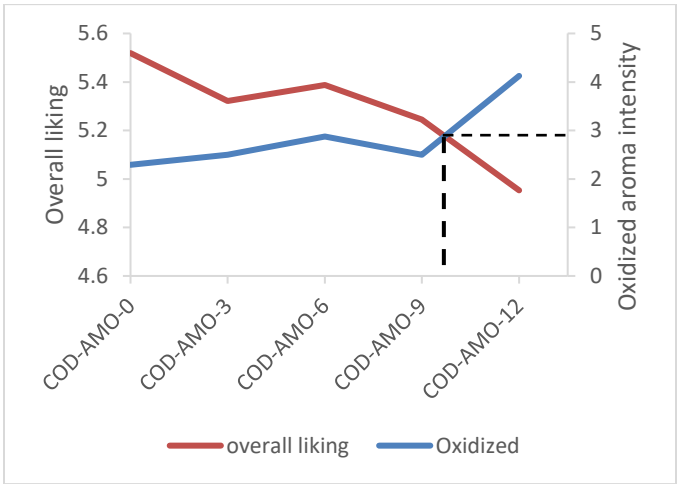
Table 10 Correlation and significant between aroma attributes, consumer acceptability, and instrumental data for poultry meal samples without antioxidant (COD-AMO).

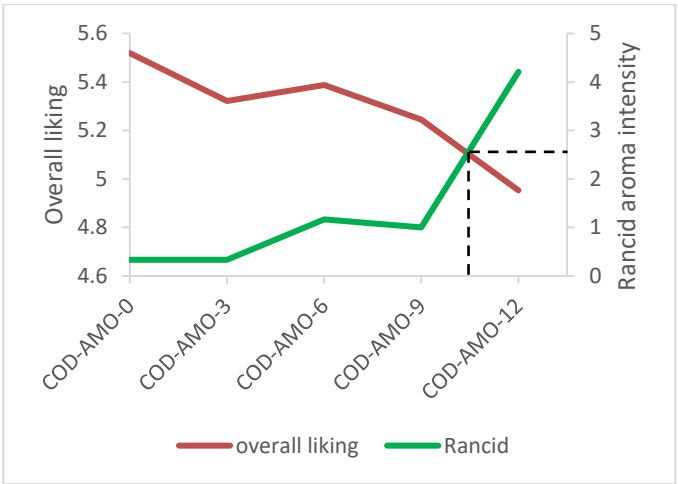
Variables	Hexanal	Peroxide Value(PV)	Anisidine Value (AV)	Overall liking
Oxidized	0.012	0.522	**0.918**	**-0.882**
Stale	-0.084	0.426	**0.929**	-0.747
Cardboard	-0.414	-0.024	0.797	-0.441
Rancid	0.081	0.578	**0.899**	**-0.903**
Hexanal		0.859	-0.363	-0.251
Peroxide Value(PV)			0.164	-0.666
Anisidine Value (AV)				-0.732
Overall liking				

Values in bold are different from 0 with a significance level alpha=0.05

Figure 11 showed pet owners' acceptability against the intensity of rancidity-related attributes and chemical compounds over storage period of poultry diet (COD-AMO). The high development of oxidation level overtime for this sample provide us a clear direction to determine the oxidation level that could be presented in a rendered protein meal without negative affecting acceptability of the finish pet food. The 2 graphs between overall liking and rancidity-related attributes (oxidized and rancid aroma) agreed that during 9 to 12 months of storage period, the oxidation level was high enough to decrease the acceptability of finished product. The result showed that the oxidation level at the time that gave oxidized or rancid aroma intensity higher than 2.5 (based on scale from 0 to 15) would negatively affect consumers' acceptability of the product.

Considering the graphs between overall liking and chemical measurements, the limit of oxidation level seemed to be inconsistent. While peroxide value and hexanal showed that the limit of oxidation level for consumers would occur during 6 to 9 storage period, anisidine value showed that it should occurred somewhere between 9 to 12 months of storage period. This finding confirmed the conclusion that setting the level of oxidation limit for human by using only chemical data might not always accurate.





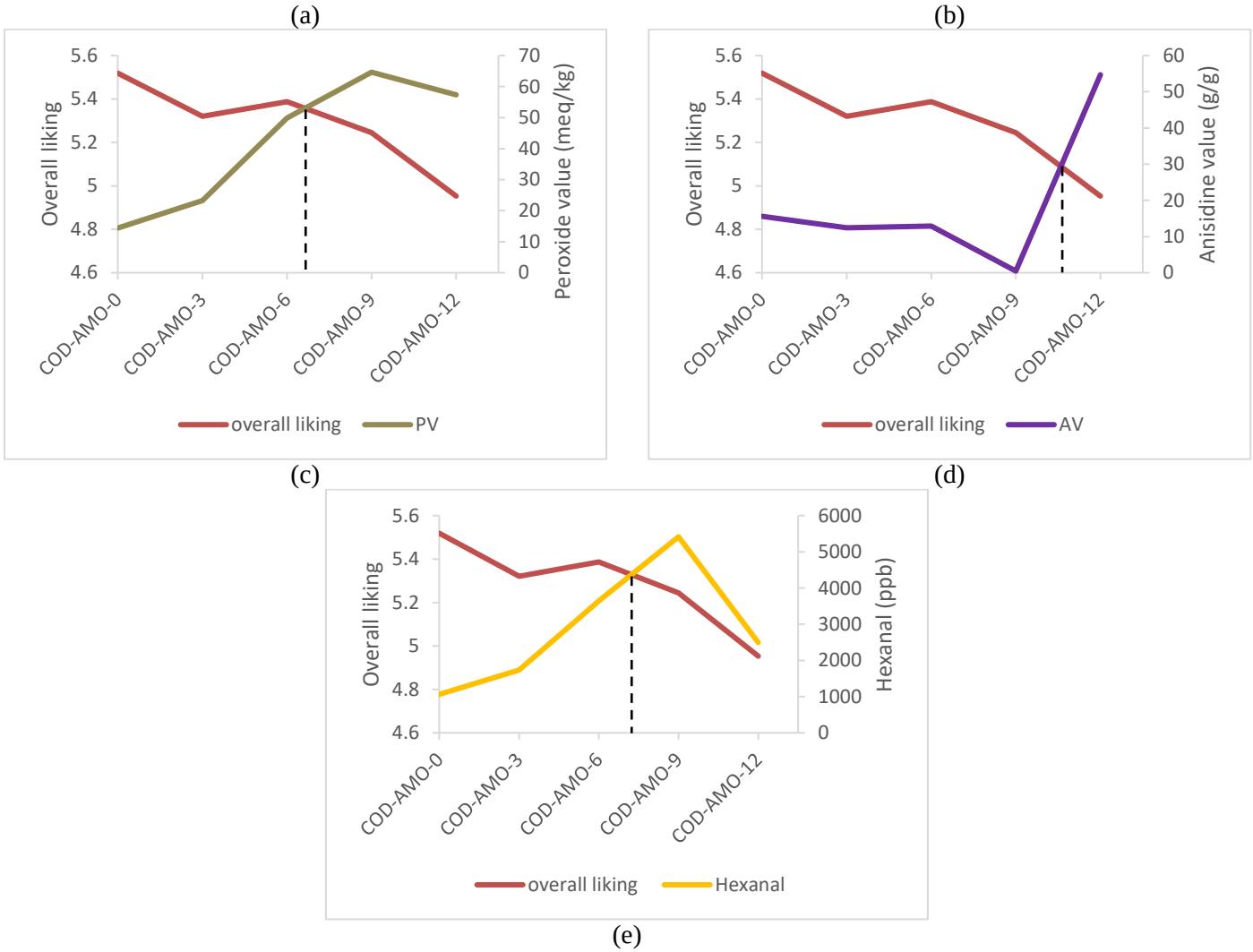


Figure 11 Line graphs of overall liking score against descriptive sensory data and instrumental data of poultry meal diets without antioxidant (COD-AMO) for each time point (a)-Overall liking *vs* Oxidized aroma; (b)-Overall liking *vs* Rancid aroma; (c)-Overall liking *vs* Peroxide value; (d)-Overall liking *vs* Anisidine value; (e)-Overall liking *vs* Hexanal.

Conclusions

Descriptive sensory analysis detected significant changes in pet food aroma and flavor characteristics for both the beef meal samples and the poultry byproduct meal samples. However, for poultry byproduct meal samples the differences were more pronounced and directional. Antioxidants did improve the stability of poultry meal samples but this was not clearly seen in beef meal samples.

The consumer study showed no differences in consumer liking for beef meal samples. This may have been

caused by the low levels of aromatics of the samples. On the other hand, the noticeable increase in aroma characteristics in poultry meal samples over storage time did have an effect on consumer liking. Consumers tended to give lower liking score for samples with either too low or too intense in aroma, but too intense aroma had more negative impact to sample liking. Besides aroma, appearance was another factor to be considered. Beef meal samples tended to have appearance characteristics (such as bone pieces and hair) that the consumers disliked. This was not as pronounced in poultry meal samples.

Sensory profile created by human could be used successfully as a powerful and predictive indicator of acceptable levels of oxidation for consumers (purchasers) due to the strong correlation between sensory profile and consumers' acceptability.

Appendix A

Attributes, definitions, and references

AROMA

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).

Stale: The aromatic impression that is flat, dull and lacks freshness.
Reference: Tortilla white flour = 2.0(a)
Preparation: Serve 4 pieces of 1" square in each medium snifter (a)

Cardboard: The aromatic associated with cardboard or paper packaging. The intensity rating is only for the 'cardboardy' character within the reference.
Reference: Tortilla white flour = 2.5(a)
Cardboard = 7.5 (a)
Preparation: 2" cardboard square in 1/2 Cup of water. Serve in a medium snifter.

Rancid: A somewhat heavy aromatic characteristic of old, oxidized, decomposing fat and oil. The aromatics may include painty, varnish, or fishy.
Reference: Microwaved Wesson vegetable oil (4 min at high) = 2.5(a)
Microwaved Wesson vegetable oil (5 min at high) = 5.0(a)
Preparation: -Microwave 1 ½ cups oil on high power for 4 minutes. Let cool and Serve ¼ cup in a 12 oz brandy snifter covered with a watch glass.
-Microwave 1 ½ cups oil on high power for 5 minutes. Let cool and Serve ¼ cup in a 12 oz brandy snifter covered with a watch glass.

- Microwave 1 ½ cups oil on high power for 5 minutes. Let cool and Pour into 1 oz cups. Serve covered.

TEXTURE

Fracturability: The force with which the sample ruptures. Evaluate on the first bite with the molars.
Reference: Cheerios = 4.0
Wheaties = 7.5

FLAVOR

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.

Stale: The aromatic impression that is flat, dull and lacks freshness.
Reference: Tortilla white flour = 2.0(f)
Preparation: Serve 4 piece of 1" square in 3.25 oz cup (f)

Cardboard: The aromatic associated with cardboard or paper packaging. The intensity rating is only for the 'cardboardy' character within the reference.
Reference: Tortilla white flour = 3.0(f)
Mama Mary's Pizza Crust = 3.0 (f)
Preparation: Totilla-Serve 4 pieces of 1" square in 3.25 oz cup (f)
Cut pizza crust into 2" square piece and place in 3.25 oz Cups

Rancid: A somewhat heavy aromatic characteristic of old, oxidized, decomposing fat and oil. The aromatics may include painty, varnish, or fishy.
Reference: Microwaved Wesson vegetable oil (4 min at high) = 3.0 (f)
Microwaved Wesson vegetable oil (5 min at high) = 5.0(f)
Preparation: -Microwave 1 ½ cups oil on high power for 4 minutes. Let cool and pour into 1 oz cups. Serve covered.
- Microwave 1 ½ cups oil on high power for 5 minutes. Let cool and Pour into 1 oz cups. Serve covered.

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

Bitter: The fundamental taste factor associated with a caffeine solution.
Reference: 0.01% Caffeine Solution = 2.0
0.02% Caffeine Solution = 3.5
0.035 % Caffeine Solution = 5.0

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

Consumer study questionnaire

PLEASE OPEN THE LID OF THE BOWL AND LOOK AND SMELL THE SAMPLE.

1. Please indicate how much you **LIKE OR DISLIKE** the sample **OVERALL**.

Dislike Extremely ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Like Extremely

****

2. How much do you **LIKE** or **DISLIKE** the **OVERALL APPEARANCE** of this sample.

Dislike Extremely ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Like Extremely

****

NOW, PLEASE SMELL THE SAMPLE AS MANY TIMES AS NEEDED TO EVALUATE IT.

3. Please indicate how much you **LIKE OR DISLIKE** the **AROMA** of the sample.

Dislike Extremely ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Like Extremely

4. Please rate the **INTENSITY/STRENGTH** of **AROMA** in the sample.

☐ ☐ ☐ ☐ ☐

Not at all intense Just about Right Extremely intense

5. What do you like about this sample?

_____

6. What do you dislike about this sample?

_____

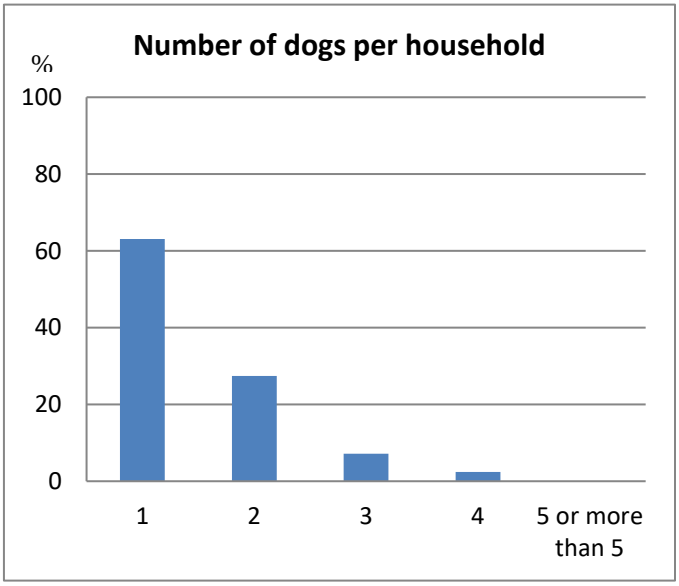
Appendix B
Demographic information representing the participants in this study (N=106)

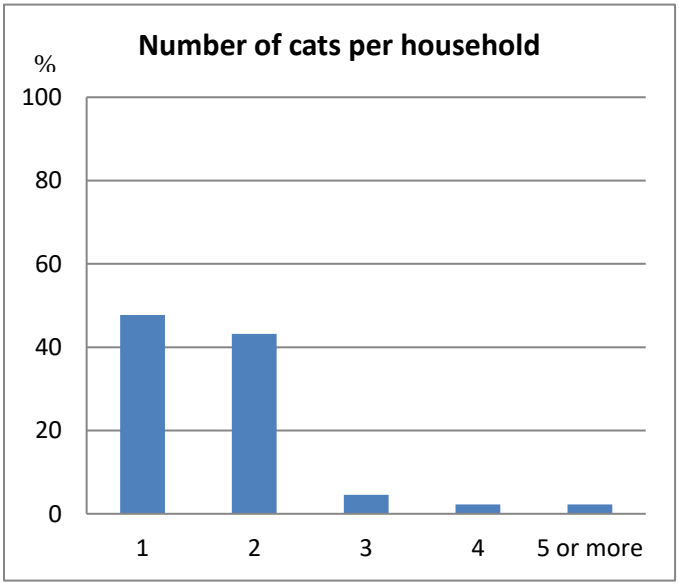
	Demographic information	Number of Participants	Percent of participants
Gender	Female	74	69.8%
	Male	32	30.2%
Age	18-24	10	9.4%
	25-34	20	18.9%
	35-44	15	14.2%
	45-54	26	24.5%
	55-64	31	29.2%
	65 or older	4	3.8%
Marital Status	Single	21	19.8%
	Married	70	66.0%
	Divorced	9	8.5%
	Domestic Partnership	6	5.7%
Number of Household members	1	16	15.1%
	2	53	50.0%
	3	12	11.3%
	4	15	14.2%
	5 or more	10	9.4%
Number of children in household	0	43	40.6%
	1	11	10.4%
	2	31	29.2%
	3 or more	21	19.8%
Education	College degree	49	46.2%
	Graduate/Professional school degree	32	30.2%
	High school degree	3	2.8%
	Some college but no degree	20	18.9%
	Some school but no degree	2	1.9%
Household Income	25,000-50,000	31	29.2%
	51,000-100,000	50	47.2%
	Less than 25,000	6	5.7%
	Over 100,000	19	17.9%

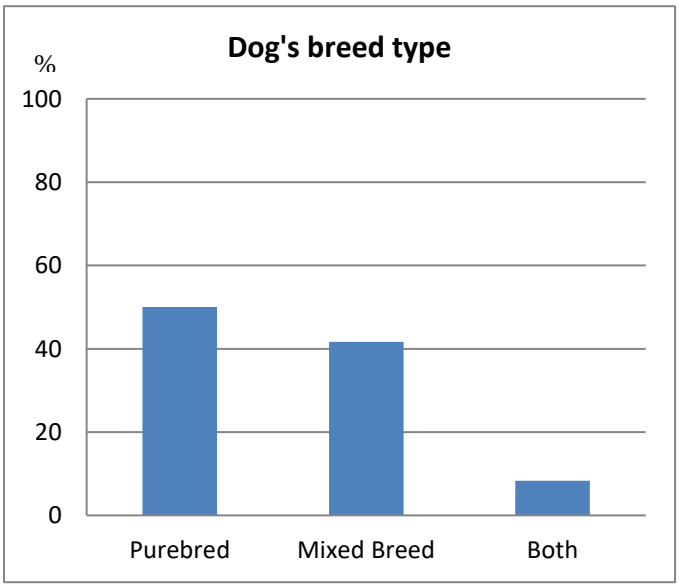
Appendix C

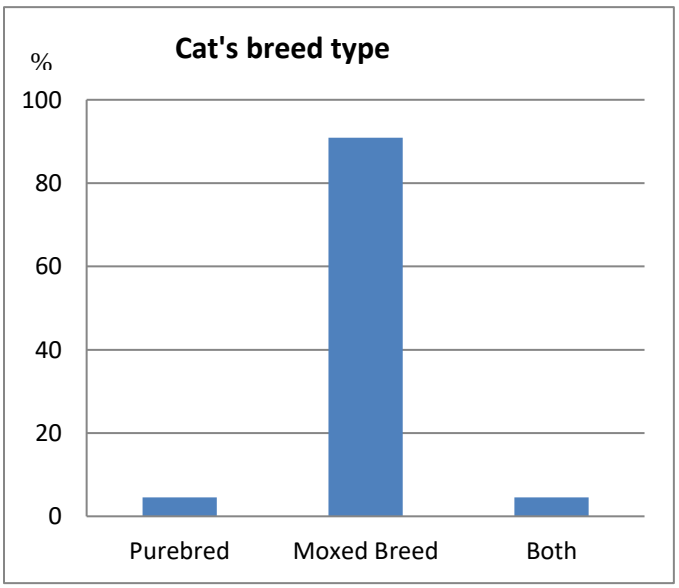
Pet information owned by participant in this study (N=106)

Pet	Number of participants	Percent of participants
Dog	62	58.4
Cat	22	20.8
Both Dog and Cat	22	20.8



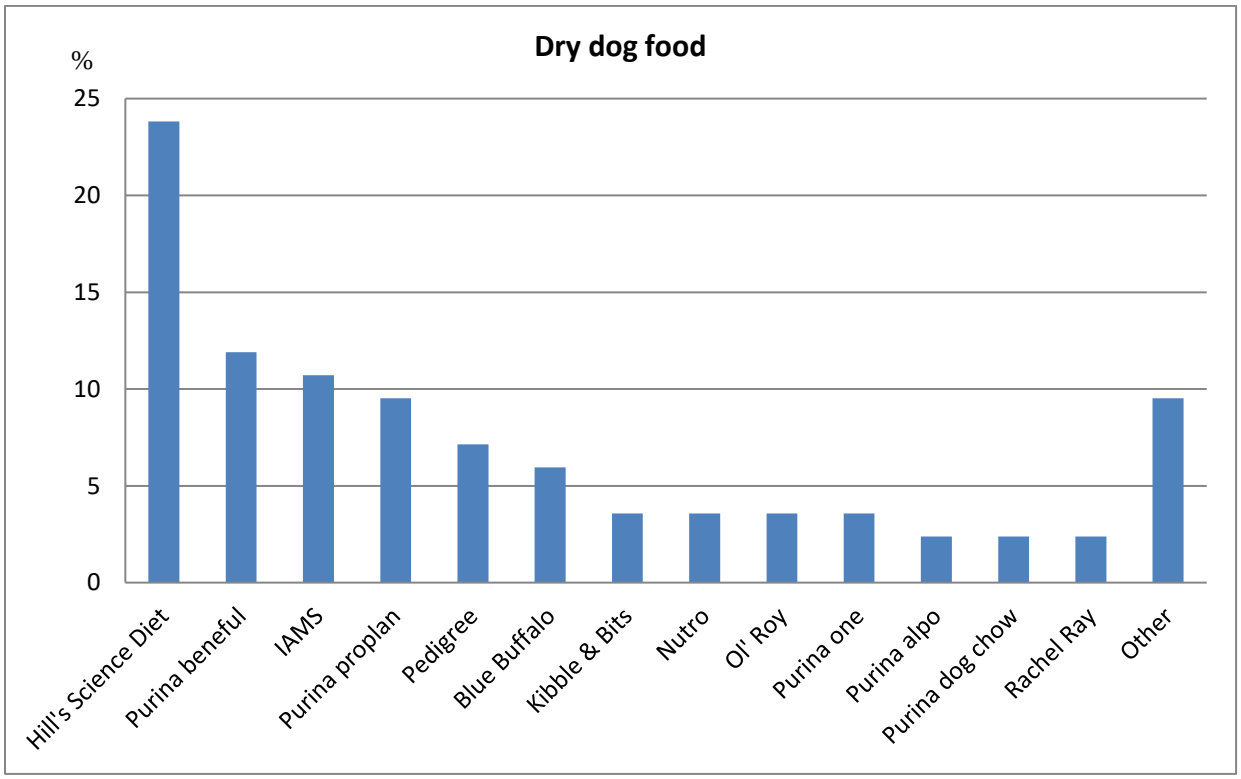


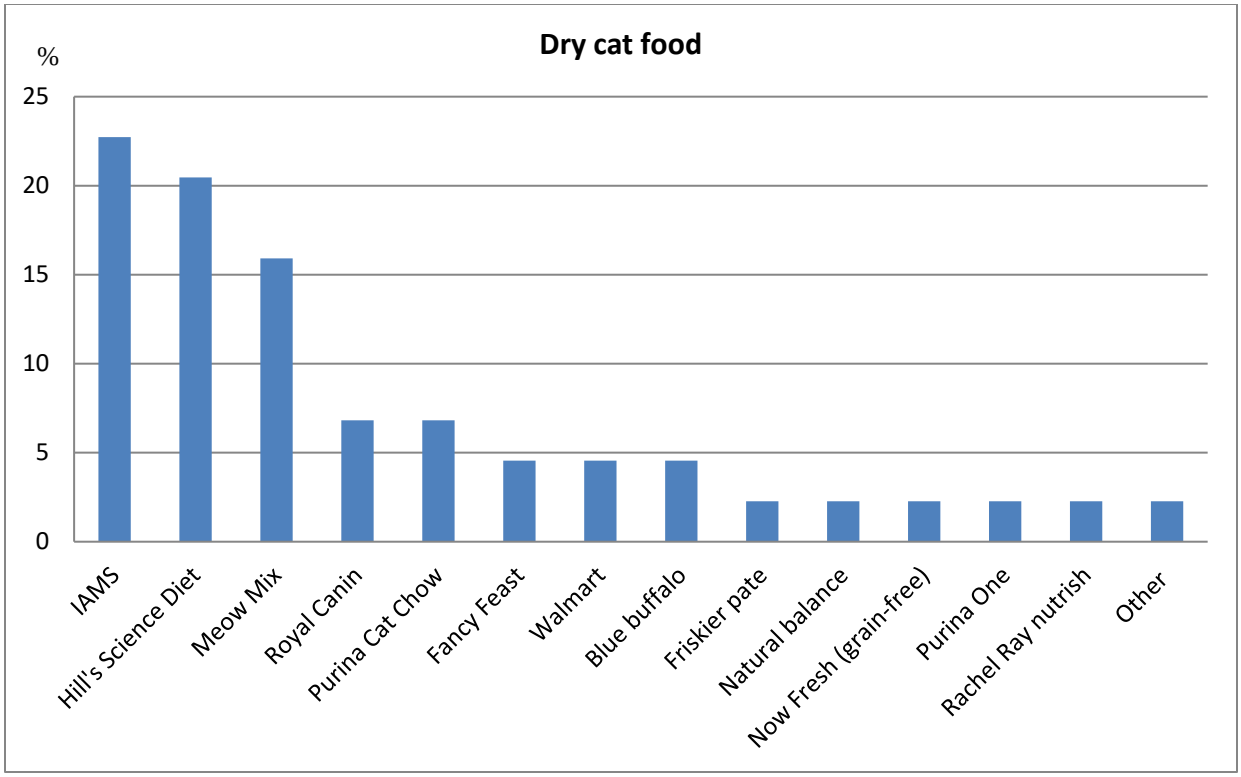




Appendix D

Dry pet food brand the owner often feed their pets





Appendix E

Pet food storage and feeding behavior (N=106)

	Feeding information	Number of Participants	Percent of participants
Method for storing dry pet food	Airtight containers	52	49.1%
	In the original packaging LEFT OPEN to the air	14	13.2%
	In the original packaging RESEALED	37	34.9%
	Other	3	2.8%
Time for finishing a package of dry pet food	1 month	73	68.9%
	1 week	14	13.2%
	3 months	17	16.0%
	6 months	2	1.9%
Money spending on pet food per month	Less than $100	92	86.8%
	$100-$300	14	13.2%