Development of a preference ranking procedure with dogs

by

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Abstract

Palatability of pet food is an important factor influencing food purchase decision of pet owners. In industry, single- or two-bowl methods are traditionally used to determine food acceptance or preference by pets but shortcomings exist to these methods. The first objective of this study was to propose and develop a preference ranking procedure. Preliminary testing consisted of five phases each lasting five days. Each day twelve beagles were presented 5 treats encased in identical rubber toys ("kongs"). The order of selection was considered as the ranking of preference. The five phases consisted of training, testing lab-baked treats formulations with five varieties of fats, starches and proteins, and commercial foods. The dogs generally ranked 1-2 flavors above others, indicating this procedure could be a more efficient method to determine preference since more samples can be evaluated simultaneously. The second objective was to validate this procedure by following the same process as the preliminary test. The results from phases 2 to 4 showed a similar pattern. For phase 5, various treat formulations were tested by combining the most to least preferred ingredients in each category. The results proved that the ranking of the formulations resembled the preference of the dogs for individual ingredients. Therefore, this procedure was concluded to be reliable. The third objective was to use descriptive sensory analysis to study the sensory characteristics of the treats and gain insights on the drivers of dogs' preference. Five highly trained panelists profiled the aroma of the treats and the data was analyzed with the preference results collected from the dogs. The external preference maps showed that fish and meaty aromatics tended to be liked by the dogs and grain and musty/dusty aromatics appeared to be disliked. The last objective of this study was to further explore the applications of this procedure by studying the effect of toy/puzzle toy of the treat and ingredient dosage/ratio. With the same dogs, Styrofoam cups (puzzle toy alternative) and kongs were evaluated separately with the same treats. The results collected with Styrofoam cups were similar but less discriminating than kongs. It potentially suggested that the difficulty level of the toy can affect the significance of the dogs' preferences. No significant preference was observed when testing the treats with different ratio of

the most and the least preferred protein sources, although the human descriptive panel was able to provide different profiles for the samples. In conclusion, the preference ranking procedure is a reliable test method but more research is necessary to further explore applications.

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Chapter 1 - Literature Review

Pet Industry

The American Pet Products Association (APPA, 2016) published a survey indicating that the market size of the pet industry has increased in the past decade; the annual sales of 2017 were estimated at nearly 70 billion of dollars with an increasing trend from the past years. Within the pet industry, the pet food category had the greatest share of dollars, followed by supplies/over-the-counter medicine, veterinary care, live animal purchases, and other services. The domestic dog (Canis familiaris) is one of the most popular pet choices in the Unites States with a total of 89.7 million dogs owned by 60.2 million households.

Pet owners expressed highly positive attitudes toward their companion animals (cats/dogs) with more than half claiming their pet was treated like a member of the family, and/or made their house feel like a home (Mintel, 2017). The human-animal bond, the bond between humans and their pets, has been studied and positive effects due to the presence of companion animals for human health and emotional well-being have been reported (Friedmann, Son & Tsai, 2000; Friedmann & Tsai, 2006; Friedmann& Son, 2009; Risley-Curtiss, 2010). Pets may also provide coping mechanism and source of security to some (Archer, 1997). Further, compared to non-pet owners, having a pet may reduce loneliness (Krause-Parello & Gulick, 2013), stress and depression (Friedmann & Tsai, 2006), cardiovascular disease (Archer, 1997). Because of the importance of companion animals in human society, the pet industry has been growing steadily.

Sensory Analysis

Sensory analysis was defined by Stone & Sidel (2004) as a scientific method used to evoke, measure, analyze, and interpret the responses to a product as perceived through the senses of sight, smell, touch, taste, and hearing. The three types of sensory analysis methods for humans include descriptive analysis, discrimination analysis, and hedonic analysis (Lawless & Heymann, 2010). Descriptive analysis is used to describe the sensory characteristics of a product to compile a sensory profile. Discrimination tests are normally conducted to determine if the samples are similar or different. Hedonic analysis is conducted with consumers (panelists) who express their opinions of whether they like or dislike the product, how much they like/dislike the product, and the reasons for liking or preference. For animals, palatability tests such as the preference test and acceptability test are commonly used (Koppel, 2014) to assess the animal's opinion of food based on their sensory perceptions.

Descriptive Analysis

Descriptive analysis can be conducted with either trained or untrained panelists using different methods. Methods such as Flavor Profile, Texture Profile and Quantitative Descriptive Analysis (QDA®) are commonly used with a trained panel when the consistent understanding of the attributes is critical. Methods such as Free-Choice Profiling, Flash Profiling, and Napping are normally used for untrained panels when no attributes are used to initiate the evaluation. However, in some cases, panelists are asked to provide descriptors (Lawless & Heymann, 2010).

Descriptive sensory analysis contains qualitative and quantitative components. Qualitative components are the descriptive terms that define the product's sensory profile, also known as attributes, descriptors, character note, etc. (Johnsen, Civille, Vercellotti, Sanders & Dus, 1988). Quantitative components are the intensities being assigned to each attribute by the panelists expressed by either group consensus or individual evaluation. For shelf-life studies, when the minor changes of the attributes need to be monitored over time, individual evaluation is normally replicated to enhance the sensitivity of this method (Chanadang, Koppel & Aldrich, 2016; Lee & Chambers, 2010). For descriptive profiling, the consensus method is sometimes applied in order to compile a sensory profile that was agreed on by all panelists (Chambers, Lee, Chun & Miller, 2012; Cherdchu, Chambers & Suwonsichon, 2013; Di Donfrancesco, Koppel & Chambers, 2012; Koppel, Chambers, Vázquez-Araújo, Timberg, Carbonell-Barrachina & Suwonsichon, 2014; Koppel, Anderson & Chambers, 2015a). In this method, panelists first evaluated the sample individually, then the panel leader led a discussion and the group reached an agreement on the intensity of each attribute.

Sensory Analysis and Pet Food

Human descriptive analysis, consumer studies with owners, and palatability tests with pets are sensory analysis tools that are useful for the development of pet food products (Koppel, 2014).

Application of Descriptive Analysis

Animals are unable to describe the sensory properties of the food products. Although humans have different perceptions of sensory stimuli, a descriptive profile compiled by a human trained panel would be helpful for product development purposes and to compare descriptive data with pet palatability and consumer acceptance data, in order to potentially determine the drivers of a subject’s liking.

A lexicon of dry dog food was published by Di Donfrancesco et al. (2012). Even with only the commercial products available in the U.S. market, more than 70 attributes that describe the appearance, aroma, flavor, and texture characteristics of dry dog food products were generated and defined with references. This lexicon showed the complexity of pet food. The result of this research reported that attributes including barnyard, brothy, brown, grain, soy, vitamin, and off-flavors like oxidized oil, cardboard, and stale were commonly detected in commercial dry dog food products. The sensory characteristics of dog food have been evaluated by trained human panelists in a number of other studies (Chanadang et al., 2016; Koppel, et al., 2014; Koppel, Monti, Gibson, Alavi, DiDonfrancesco & Carciofi., 2015b).

Consumer Research with Owners

Although the animals are the actual consumers of pet food products, pet owners are the ones that make purchase decisions. Understanding the pet owners' attitude towards their pets and the pets' diet selection can potentially provide information for product development. Consumer studies with pet owners are typically about demographic (age, income, etc.), pet status, and relationship with pets (Koppel, 2014; Merle, 2009). Di Donfrancesco, Koppel, Swaney-Stueve & Chambers (2014) studied the consumers' overall liking, aroma and appearance liking of dry dog foods and also the consumers' prediction of their dogs' liking, the cost of the sample, and purchase intent. The result showed that the appearance of dry dog foods affects the overall liking more than aroma, especially color liking.

Palatability Testing (Consumer research with animals)

Preference tests and acceptability tests are the typical palatability tests for pets (Koppel, 2014). Preference tests are normally performed with more than one sample simultaneously, and the subjects need to show a preference of one over another by the frequency, time, and amount of consumption. The preference is sometimes defined as “the difference between the strength of motivation to obtain or avoid one resource or stimulus and the strength of motivation to obtain or avoid another” (Kirkden & Pajor, 2006; p.31). The traditional two-bowl test is a widely used method for preference tests. For acceptability tests, normally only one sample is presented to the subject, and the subjects’ eating behavior indicates if the sample is acceptable. For the one-bowl method, the amount of the consumption is the indicator of the acceptance of the food.

External Preference Mapping

The external preference map has been commonly used as a visualizing tool to analyze and explain the relationship between descriptive and consumer data (Cadena, Cruz, Faria & Bolini, 2012; MacFie & Thomson, 1994; Sasaki, Ooi, Nagura, Motoyama, Narita, Oe et al., 2017). This mapping method focuses on the differences among the sensory profiles of products. The first two dimensions of the multivariate analysis of sensory data are extracted, then the liking score of each consumer is regressed on top (Worch, 2013). External preference maps allow predicting the potential drivers of liking for the group of consumers considered. Linear, circular, elliptic, or quadratic regression models can be used for the creation of an external preference map (Danzart, 2009).

Palatability Testing

Palatability is an all-encompassing term that covers all perceptions (appearance, aroma, flavor, texture, temperature, size, etc.) derived at the time a food is being consumed (Kitchell, 1978). The term "acceptance" is also commonly used when the palatability of the food is being evaluated. Acceptance was defined as food being palatable enough to be eaten in the amount that fulfils the subjects' needs in order to maintain the subject's body weight in a neutral state (Hand, Thatcher, Remillard, Roudebush & Novotny, 2010; Vondran, 2013) or as the subjects voluntarily consume the food (Thombre, 2004; Vondran, 2013). High palatability of the food indicated that the food is more likely to be consumed by the subjects.

Since "pets prefer taste" is one of the most important factors that triggers pet owners' purchase behavior for pet food (Mintel, 2016), palatability is one of the most important measures of performance in the pet food industry. To determine the palatability of the products, the pet foods are often tested with animals. The palatability tests are generally categorized as consumption and non-consumption tests (Griffin, 2003), consumption tests, such as one-bowl "acceptance" method and two-bowl forced-choice methods, are most commonly used. Non-consumption tests included autonomic or conditioned response tests, and instrumental or operant conditioning tests (Aldrich & Koppel, 2015).

In reality, various breeds and types of dogs have different feeding behaviors from each other (Bradshaw, 1991) and many other factors can influence the dogs' liking of food, including the individual variations of the animals, the previous exposure to diets, experience, etc. (Rofe & Anderson, 1970). Palatability tests for animals are generally conducted as a sensory analysis method to provide guidance for the product development of pet food.

Single-Bowl Test

In the single-bowl test, animals are presented with one type of food in a measured amount, for a specified period of time, to measure acceptability (Tobie, Péron & Larose, 2015). Food is offered one or more times per day, and the amount can be adjusted based on previous feedings (by measuring leftovers). At the end of each period, the remaining food is quantified and compared with the starting amount (Aldrich & Koppel, 2015). Speed of consumption can also be used to quantify acceptability (Tobie et al., 2015). Multiple animals are often used, and studies often last 5 days or longer. Food can be switched back and forth or sequentially through different items.

An advantage of this test is that it evokes the home environment, in which the animal must either eat the food that is served or not at all. This can be helpful for determining acceptability and digestibility. The cost of the monadic test (single product evaluation) is also generally low, and can be used as a worst-case test for a product. However, it does not draw any conclusions about preference or liking, and cannot rank multiple samples (Aldrich & Koppel, 2015).

Two-Bowl Test

This type of test is a commonly used industry standard for measuring palatability (Araujo, Studzinski, Larson & Milgram., 2004). Many studies were conducted using this method (Ferrell, 1984; Rashotte, Foster & Austin, 1984; Smith, Rashotte, Austin & Griffin, 1984; Vondran, 2013). In this two-bowl test, each animal is presented with two distinct food samples at once, essentially forcing the animal to choose one over the other. The animal fasts overnight, and the samples are presented in the morning. As with the single-bowl test, the two bowls are provided for a set period of time (often 15 to 30 minutes, or until one of the samples has been completely consumed). Each bowl is filled with enough food to satisfy the daily nutritional and caloric needs of the animal (Houpt, Hintz & Shepherd, 1978). At the end of the test, the amounts of food consumed from each bowl are quantified and compared. Two-bowl tests may be conducted in multiples of two-day periods, with the side of each food (right or left) being varied to avoid bias (Aldrich & Koppel, 2015).

The results of the two-bowl test can be compared directly by weight (grams of food consumed) or as an intake ratio, where the amounts of each food consumed are expressed proportionally to the total food consumed. Where the intake ratio is less than one third or greater than two thirds, the animal is said to prefer one food over the other. This can be helpful for comparing similar foods (Aldrich & Koppel, 2015).

A disadvantage of the two-bowl test is that it cannot be used to determine whether or not the animal likes both of the foods. Furthermore, if the intake ratio was not sufficient to draw a conclusion about preference (i.e. if the foods were judged to be equivalent to each other), the study will not be able to tell which of the foods was preferred over the other. While this type of test may be helpful for research and development or competitive product evaluation, it may not be conclusive when used for similar foods, such as in quality control testing. It also cannot determine which aspects of a particular food are liked by the animal (Aldrich & Koppel, 2015).

Operant Conditioning

One variation on the two-bowl method is the operant lever-press test, in which the animal is presented with two levers. Pressing each lever produces a different type of food as a reward, with sample position being varied or alternated over the course of testing to avoid left-right bias. Test sessions can either be timed or stopped after a certain quantity of food has been dispensed.

Like the two-bowl test, this can be used to assess preference. However, as reported by one study (Rashotte et al., 1984), it requires animals to continue testing until a two-part stability criterion is met across three days. In that study, it took an average of just over 11 sessions. Unlike the standard two-bowl test, this method isolates the food types from each other, preventing their aromas from mixing, but it requires lengthy initial training of test subjects. However, an advantage of operant lever-press testing is that it forces the animal to make a choice before being satiated, and several different types of food can be tested in a day (Houpt & Hintz., 1978).

Cognitive Palatability Assessment Protocols (CPAP)

CPAP testing is a non-consumption test in which palatability is measured by associating pleasure (the reward of food) with an object. In one such discrimination-based study involving Beagle dogs, testing was conducted in four phases including preference and association testing, discrimination training, discrimination stabilization, and reversal (Araujo et al., 2004). The first phase was used to establish object preference. In this phase, each object was associated with a type of food or no food. The object that was chosen by a dog most frequently was noted as the object preferred by that dog. Following preference testing, association testing was conducted to allow the dogs to become familiar with a distinct type of food associated with each non-preferred object.

In the second phase, each dog was presented with the same three objects until they responded to one of them. While the two non-preferred objects were each placed on top of a different associated food as established in the first phase, the preferred object was never associated with food, in order to eliminate bias. This also served as a control, with the expectation that responses to the preferred object would approach zero over the duration of testing. Each trial ended when a non-preferred object was moved and the associated food was consumed, or the preferred object was moved. Over the course of trials, the positions of the objects were randomized. In the third phase, the above procedure was repeated, with responses being associated with food preference.

Finally, in the reversal phase, the strength of the dogs' food preferences was tested by changing the object associations. Reversal was broken down into days for association, discrimination training, and stabilization. During the first association day, the preferred object for each dog was paired with the non-preferred food. During the second day, the object previously associated with the non-preferred food was paired with the preferred food. The remaining phases were then conducted in the same way as described previously. At the end of the study, two-bowl testing was also done for comparison.

CPAP testing can be used effectively to compare palatability of similar foods, with few test subjects. The aforementioned study, for example, involved just thirteen beagle dogs, while successfully identifying a preference for one of the foods presented. It was also found that CPAP was less sensitive to the effects of prior feeding and satiation than the two-bowl test (Araujo et al., 2004).

Behavior-Based Prediction of Consumption

Thompson, Riemer, Ellis & Burman (2016) published a new method to assess the palatability of pet food. This method included a pre-exposure session and a testing session. Eighteen individual pet dogs participated in this study individually in a dedicated training area. During the pre-exposure session, the dog was given three pieces of each sample in two separate bowls. In the testing session, at first, one piece of each sample was placed in a separate bowl and the dog was offered the bowls one at a time, with the other bowl hidden by the experimenter behind her back. The dog was allowed to consume the sample. Then the dog was led back to a start-point while the experimenter placed one piece of the same sample as before in each of the bowls. The bowls were then covered with a wire cover so the dog could smell and see the sample but not consume it. The amount of time the dog spent investigating each cover was considered indicative of the dog's preference. Additionally, the results confirmed a restricted-intake consummatory test conducted with puzzle feeders.

From these results, it was reasonable to believe the method could assess the palatability of foods. The advantage of this method was that, for each test, the dogs only consumed up to 8 pieces or less (3 pieces during pre-exposure, 1 piece during pre-evaluation for each sample) of sample, which avoids over-eating, and it is potentially more efficient than traditional methods since more tests can be conducted in a short period of time. However, this method involved three steps: letting the dog taste the samples (pre-exposure), showing the dogs the samples again with one piece, then recording the time that the dog investigates the covered bowls. This seems to be relatively complicated and time-consuming on the whole. Two researchers (handler of the dogs and experimenter) are essential to operate this method, so it is also labor-intensive.

Human Interpretation of Canine Behavior

Analysis of canine behavior during preference testing is often necessary to eliminate uncertainty or draw deeper conclusions about the research. Because humans and dogs cannot communicate verbally with each other, it is helpful to use behavioral cues to judge canine response. It has been found that significant behavioral and physiological differences exist between dogs housed in kennels and those living in a traditional home setting, which may suggest underlying differences in stress levels (Beerda, Schilder, Hooff, Vries & Mol, 1999). While overall activity levels may be reduced, an increased diversity in movement behaviors may be observed in kennel dogs (Part Kiddie, Hayes, Mills, Neville, Morton & Collins, 2014). However, due to the difference in the feeding behaviors of different breeds and types of dog (Bradshaw, 1991), the behavior cues can vary as well.

Summary of Existing Methods

All existing palatability test methods only evaluate 1 to 2 samples per session (Table 1.1). However, in the pet food industry, the palatability of more than two samples need to be evaluated in most cases. For the consumption tests, the samples are typically the animals' main meal of the day so only one session can be conducted each day. Also, it's possible that the animals consumed the samples because of their hunger instead of their true liking/preference so these tests may not show accurate results of the palatability of the samples. The animals are not given a choice of not consuming the samples. Due to the low efficiency and existing forced-choice effects, it is necessary to development a new method that can evaluate more samples in a shorter time and reduce the forced-choice effects by not making the testing samples as the animals' main meal of the day.

Table 1.1 Summary of existing palatability tests

Method	Single-bowl	Two-bowl	Operant lever-press	Cognitive palatability assessment protocol	Behavioral prediction
Number of samples tested	1	2	2	2	2
Number of animals	30+	~10/20	5	5-6	18
Number of days for test	5+	5-6/2-4	11+	10-20	1
Number of days for training	✗	2-7	unknown	5+	<1
Risk of over-eating	✓	✓	✓	✗	✗
Main meal	✓	✓	✗	✗	✗
Time per test session	1h	15-30min	16-26min	unknown	unknown

Validation of New Methods

In every methodology development process, the validation of the method is necessary before determining if the method can be used for further testing. The purpose of a validation test is to determine the performance of the developed subject (method, equipment, theory, etc.) in accordance with its intended use (Dispas, Lebrun, Ziemons, Marini, Rozet & Hubert, 2014).

There are several options for validation tests. Results collected from different resources or conditions are often compared. When the results are in agreement, the developed subjects are considered valid. For instance, Braghieri, Piazzolla, Carlucci, Monteleone, Girolami & Napolitano (2012) conducted a validation test with different subjects/panelists as the initial study. To determine if the developed reference samples for beef evaluation are reliable, another panel evaluated the same samples with the reference samples developed. The results from both panels were aligned, so the reference samples could potentially be reliable references for beef evaluation. In addition to the utilization of different subjects, different methods (Moelich, Muller, Joubert, Næs & Kidd, in press) and data analysis (Dahl, Tomic, Wold & Næs, 2008) can also be options for validation tests to explore the reliability of the developed subjects.

Nutrient Requirements for Dogs

Pet food can be divided into dry, semi-moist, and wet categories. Pet food that contains less than 20% moisture level is considered dry pet food, and the moisture level for semi-moist pet food is 20-65%. When the moisture level is above 65%, the food is considered canned or wet pet food (AAFCO, 2016). Dry pet food products are mostly produced by baking, pelleting, or extrusion, and the majority are extruded (Gibson, 2015). Baking is mostly used to produce treats. Extrusion is also used to produce semi-moist food with a lower amount of air incorporated in the extrusion process (Dzanis, 2003). Canned pet food is normally cooked, canned, and then sterilized (Dzanis, 2003).

Just like for humans, essential nutrients for dogs include water, proteins, carbohydrates, fats, vitamins, and minerals (Hussein, 2003). Since most dogs only are fed one type of food, in order to fulfil nutritional needs, the selection of ingredients and the formulation of pet food are important and challenging. Pet food generally consists of meat, meat byproducts, fat, cereal grains, vitamins, minerals, etc. (Gibson & Alavi, 2013). However, not only because of the nutritional needs of the pets, but also because of the requests from the pet owners, the pet food industry has been constantly looking for new ingredients to improve products. Fibers (e.g. structural carbohydrates), while not one of the essential nutrients, were studied for their potential value in pet food. Fibers were found to be beneficial for the development of a reduced energy diet due to low energy availability (Mcnamara, 2014), the digestive process, and the glycemic response of pets (dog and cats) (Campbell, 2009; De Goboy, Kerr & Fahey., 2013). The natural food ingredient trend has also been growing for humans as well as their pets (Buff, Carter, Bauer & Kersey, 2014). Some pet owners are feeding their dog or cat vegetarian diets, even if they are technically natural carnivores (Knight & Leitsberger, 2016).

Fat

Due to its high energy density, dietary fat takes a big portion of dogs' energy budget. Triglycerides are the main component of dietary fat. Plant-based fat like vegetable oil normally contains lower saturated fat than animal-based fat sources like chicken fat. Some vegetable oils such as corn, soybean and safflower oil are especially beneficial for dogs due to the high content of linoleic acid, which is an essential polyunsaturated fatty acid for dogs (Hussein, 2003). Dogs are highly attracted to fat (Manabe, Matsumara & Fushiki, 2010) so fat also is used to assist the palatability of the product. Factors such as the processing/freshness and the ratio of a mixture of the fat may have influences on dogs' preference for the diet (Fragua, Barroeta, Manzanilla, Codony & Villaverde, 2015; Verbrugghe, Hesta, Gulbrandson & Janssens, 2007).

Protein

Twenty-two amino acids are required for dogs to synthetize the various body protein structures. Ten out of twenty-two amino acids are essential, which must be provided by diet, including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine (Hussein, 2003). The required amino acids can be provided adequately by the meat and meat byproduct ingredients in pet food products. However, some plant-based protein sources have been used in some pet food products as well due to the pet owners' desire to feed vegetarian diets to their pets. Several palatability tests have been done to explore the types of meat-based protein that attract the dogs. For instance, Houpt & Hintz (1978) determined that dogs preferred beef, pork, and lamb over chicken, liver, and horsemeat when the meat is served as cooked ground meat mixed with a bland diet. Plant-based protein source is generally considered less palatable than animal-based protein source (Houpt & Smith, 1981).

Carbohydrates

The main carbohydrate source in commercial products is the starch from cereal grains such as corn, wheat, sorghum, barley, or rice. Most dietary starch is highly used by the dog's metabolism because the gelatinization process, which happens during baking, canning or extrusion, expands the starch molecule, decreases the hydrogen bonding between the glucose units, and then enhances starch digestibility (Hussein, 2003). Research about the effect of carbohydrates on the palatability of dog food is very limited.

Research Objectives

Limited numbers of tests are available for researchers in the pet food industry to examine the palatability of pet food products. Most of the existing methods can only evaluate up to two samples per test, and the presence of the forced-choice effect can potentially alter the results.

The first objective of this study was to propose, develop, and validate a procedure for a new palatability test that uses dogs' motivation for preferred flavors to interpret the order of consumption as the rank of preference. This procedure gives researchers the option to evaluate multiple samples (up to 5) per test with less influence of the forced-choice effect, compared to other existing tests. The validation process indicated the reliability and consistency of the procedure.

The second objective was to determine the aromatic sensory profile of the samples for ranking tests and combine descriptive analysis results with the preference ranking procedure results in order to explore the drivers of dogs' liking. This portion of the study can potentially provide insights about what aroma characteristics are liked or disliked by the dogs.

The last objective of this study was to further test this procedure with different puzzle toys for the samples and different ratio/dosage of the ingredients to determine how or if the puzzle toys and ratio of ingredients would affect the results.

The pet food industry could benefit from this preference method in terms of gaining better insights into "liking" and maximizing outcomes with fewer resources than traditional methods require. The preference ranking procedure allows for insight into preference information based on direct multiple comparisons of single ingredient aromatics and flavor. These advantages make it imperative to further test the method with larger sets of animals with actual developmental products to determine its helpfulness for decision making in practice during the research and development of products.

References

AAFCO (2016) Model regulations for pet food and specialty pet food under the model bill. In: Stan Cook, section editor. Association of American Feed Control Officials, Inc. pp 138-202.

Aldrich, G.C., & Koppel, K. (2015). Pet Food Palatability Evaluation: A Review of Standard Assay Techniques and Interpretation of Results with a Primary Focus on Limitations. *Animals*, **5(1)**, 43-55.

APPA. (2016). Pet industry market size and ownership statistics. American Pet Products Association, Inc. Greenwich, CT. Available online: http://www.americanpetproducts.org/press_industrytrends.asp (accessed on 12 July 2017).

Archer, J. (1997) Why do people love their pets? *Evolution and Human Behavior*, 18, 237-259.

Araujo J.A., & Milgram N.W. (2004). A novel cognitive palatability assessment protocol for dogs. *Journal of Animal Science*, ***82***, 2200-2206.

Araujo, J.A., Studzinski, C.M., Larson, B.T., & Milgram, N.W. (2004). Comparison of the cognitive palatability assessment protocol and the two-pan test for use in assessing palatability of two similar foods in dogs. *American Journal of Veterinary Research*, **65(11)**, 1490-1496.

Beerda, B., Schilder, M.B., Bernadina, W., Van Hooff, J.A., De Vries, H.W., & Mol, J.A. (1999) Chronic stress in dogs subjected to social and spatial restriction. II. hormonal and immunological responses. *Physiology Behavior*, **66(2)**, 243-254.

Bradshaw, J.W.S. (1991). Sensory and experiential factors in the design of foods for domestic dogs and cats. *The Proceedings of the Nutrition Society*, **50**, 99–106.

Braghieri, A., Piazzolla, N., Carlucci, A., Monteleone, E., Girolami, A., & Napolitano, F. (2012). Development and validation of a quantitative frame of reference for meat sensory evaluation. *Food Quality and Preference*, **25(1)**, 63-68

Buff, P. R., Carter, R. A., Bauer, J. E., & Kersey, J. H. (2014). Natural pet food: A review of natural diets and their impact on canine and feline physiology. *Journal of Animal Science*, **92**, 3781-3791

Cadena, R., Cruz, A., Faria, J., & Bolini, H. (2012). Reduced fat and sugar vanilla ice creams: Sensory profiling and external preference mapping. *Journal of Dairy Science*, **95(9)**, 4842-4850.

Campbell, K.L., & Campbell, J.R (2009). Chapter 9: Feeding and Nutrition of Dogs and Cats. In Companion Animals. Their Biology, Care, Health, and Management, 2nd ed.; Pearson Education Inc.: Upper Saddle River, NJ, USA, 2009; pp. 253–299.

De Godoy, M.R.C., Kerr, K.R., & Fahey, G.C. Jr. (2013) Source dietary fiber sources in companion animal nutrition. *Nutrients*, **5**, 3099–3117.

Chambers, EIV, Lee, J., Chun, S., & Miller, A. (2012). Development of a lexicon for commercially available cabbage (baechu) kimchi. *Journal of Sensory Studies*, **27**,511–518

Chanadang, S., Koppel, K., & Aldrich, G. (2016). The impact of rendered protein meal oxidation level on shelf-life, sensory characteristics, and acceptability in extruded pet food. *Animals*, **6(8)**, 44.

Cherdchu, P., Chambers, EIV, & Suwonsichon, T. (2013). Sensory lexicon development using trained panelists in Thailand and the United States: soy sauce. *Journal of Sensory Studies*, **28**, 248–255

Dahl, T., Tomic, O., Wold, J. P., & Næs, T. (2008). Some new tools for visualising multi-way sensory data. *Food Quality and Preference*, **19(1),** 103-113.

Danzart, M. (2009). Evaluation sensorielle. Manuel méthodologique, Lavoisier, Paris, pp. 431-439

Di Donfrancesco, B., Koppel, K., & Chambers IV, E. (2012). An initial lexicon for sensory properties of dry dog food. *Journal of Sensory Studies*, **27**, 498-510.

Di Donfrancesco, B., Koppel, K., Swaney-Stueve, M., & Chambers IV, E. (2014). Consumer acceptance of dry dog food variations. *Animals*, **4**, 313-330.

Dispas, A., Lebrun, P., Ziemons, E., Marini, R., Rozet, E., & Hubert, P. (2014). Evaluation of the quantitative performances of supercritical fluid chromatography: From method development to validation. *Journal of Chromatography A*, **1353**, 78-88

Dzanis, D. (2003) Section VII: Petfood types, quality assessment and feeding management. In Petfood Technology 1st ed.; Kvamme, J.L., Philips, T.D., Eds.; Watt Publishing Co.: Mt. Morris, IL, USA, 2003; pp. 68-73

Ferrell, F. (1984). Preference for sugars and nonnutritive sweeteners in young beagles. *Neuroscience and Biobehavioral Reviews*, **8**, 199–203.

Fragua, V., Barroeta, A., Manzanilla, E., Codony, R., & Villaverde, C. (2015). Evaluation of the use of esterified fatty acid oils enriched in medium-chain fatty acids in weight loss diets for dogs. *Journal of Animal Physiology and Animal Nutrition*, **99(S1)**, 48-59.

Friedmann, E., Son, H., & Tsai, C. (2000). The animal-human bond: Health and wellness. *Handbook on animal-assisted therapy: Theoretical foundations and guidelines for practice*, pp. 41-58.

Friedmann E., & Tsai C-C., (2006) The animal-human bond: health and wellness. *Handbook on animal assisted therapy: theoretical foundations and guidelines for practice.* 2nd edition, Sand Diego (CA): Academic Press, 95-117

Friedmann, E., & Son, H. (2009). The human–companion animal bond: how humans benefit. *Veterinary Clinics of North America: Small Animal Practice*, **39(2)**, 293-326.

Gibson, M. (2015) Physico-chemical and shelf-life between baked and extruded pet foods. K-rex. Electronic These and Dissertation.

Gibson, M., & Alavi, S. (2013) Pet food processing-Understanding Transformations in Starch during extrusion and baking. *Cereal Foods World*, **58**, 232-236

Griffin, R.W. (2003) Section IV: Palatability. In *Petfood Technology*, 1st ed.; Kvamme, J.L., Philips, T.D., Eds.; Watt Publishing Co.: Mt. Morris, IL, USA, 2003; pp. 176-193

Hand, M. S., Thatcher, C. D., Remillard, R. L., Roudebush, P., & Novotny, B. J., (2010) Small Animal Clinical Nutrition 5th Edition, Copyright 2010, 2000, 1987, 1984, 1983 by Mark Morris Institute, Chapter 8 157-190.

Houpt, K. A., & Hintz, H. F. (1978). Palatability and canine food preference. *Canine Practice*, **5(6)**, 29-35.

Houpt, K. A., Hintz, H. F., & Shepherd, P. (1978). The role of olfaction in canine food preferences. *Chemical Senses*, **3**, 281-290.

Houpt, K. A., & Smith, S. L. (1981). Taste preferences and their relation to obesity in dogs and cats. *Canadian Veterinary Journal*, **21**, 77-81.

Hussein, S. (2003) Section II Nutrition. In *Petfood Technology*, 1st ed.; Kvamme, J.L., Philips, T.D., Eds.; Watt Publishing Co.: Mt. Morris, IL, USA, 2003; pp. 2-4

Johnsen, P. B., Civille, G. V., Vercellotti, J. R., Sanders, T. H., & Dus, C. A. (1988). Development of a lexicon for the description of peanut flavor. *Journal of Sensory Studies*, **3(1),** 9-17.

Kitchell, R.L. (1978). Taste perception and discrimination by the dog. *Advances in veterinary science and comparative medicine.* **22**, 287-314.

Kirkden, R.D. & Pajor, E.A. (2006). Using preference: motivation and aversion tests to ask scientific questions about animals feelings. *Applied Animal Behaviour Science*, **100**, 29–47.

Knight, A. & Leitsberger, M. (2016). Vegetarian versus Meat-Based Diets for Companion Animals. *Animals*, **6(9)**, 57

Koppel, K. (2014). Sensory analysis of pet foods. Retrieved from http://krex.ksu.edu

Koppel, K., Anderson, E. L., & Chambers, E. (2015a). Influence of processing on pomegranate (Punica granatum L.) juice flavor and aroma. *Journal of the Science of Food and Agriculture*, **95(5)**, 1066-1071.

Koppel, K., Chambers, E., Vázquez-Araújo, L., Timberg, L., Carbonell-Barrachina, A. & Suwonsichon, S. (2014). Cross-country comparison of pomegranate juice acceptance in Estonia, Spain, Thailand, and United States. *Food Quality and Preference*, **31**, 116–123

Koppel, K., Monti, M., Gibson, M., Alavi, S., Donfrancesco, B. D., & Carciofi, A. C. (2015b). The effects of fiber inclusion on pet food sensory characteristics and palatability. *Animals*, **5(1)**, 110-125.

Krause-Parello, C. A., & Gulick, E. E. (2013). Situational factors related to loneliness and loss over time among older pet owners. *Western Journal of Nursing Research*, **35(7)**, 905-919.

Lawless, H. T., & Heymann, H. (2010). Sensory evaluation of food. Second Edition. Springer, New York, USA.

Lee, J., & Chambers, D. H. (2010). Flavors of green tea change little during storage. *Journal of Sensory Studies*, **25(4)**, 512-520.

Thomson, D. M., & MacFie, H. J. H. (1994). Measurement of food preferences. Blackie Academic. pp. 137-166.

Manabe, Y., Matsumura, S., & Fushiki, T. (2010). Preference for high-fat in animal foods. *Fat Detection: Taste, Texture, and Post Ingestive Effects*. Boca Raton, FL. Chapter 10.

Mcnamara, J.P. (2014) Chapter 3: Glucose and Fatty Acids: Providers of Body Structure and Function. In Principles of Companion Animal Nutrition, 2nd ed.; Pearson Education, Inc.: Upper Saddle River, NJ, USA, 2014; pp. 27–47.

Merle, C. (2009). What pet owners really think. Proceedings of the North American Veterinary Conference, *Vet Tech*, 249

Mintel. (2016) Pet Food - US - August 2016. Retrieved June 15, 2017 from Mintel Reports database. Available from: http://academic.mintel.com

Mintel. (2017) America's Pet Owners - US - July 2017. Retrieved July 12, 2017 from Mintel Reports database. Available from: http://academic.mintel.com

Moelich, E. I., Muller, M., Joubert, E., Næs, T., & Kidd, M. (in press). Validation of projective mapping as potential sensory screening tool for application by the honeybush herbal tea industry. *Food Research International*.

Part, C. E., Kiddie, J. L., Hayes, W. A., Mills, D. S., Neville, R. F., Morton, D. B., & Collins, L. M. (2014). Physiological, physical and behavioural changes in dogs (canis familiaris) when kenneled: Testing the validity of stress parameters. *Physiology & Behavior*, **133**, 260-271

Rashotte, M. E., Foster, D. F., & Austin, T. (1984). Two-pan and operant lever-press tests of dogs' preference for various foods. *Neuroscience and Biobehavioral Reviews*, **8(2)**, 231-237.

Risley-Curtiss, C. (2010). Social work practitioners and the human-companion animal bond: a national study. *Social Work*, **55(1)**, 38-46.

Rofe, P.C., & Anderson, R.S. (1970). Food preference in domestic pets. *The Proceedings of the Nutrition Society*, **29**, 330–335.

Sasaki, K., Ooi, M., Nagura, N., Motoyama, M., Narita, T., Oe, M., et al. (2017). Classification and characterization of Japanese consumers' beef preferences by external preference mapping. *Journal of the Science of Food and Agriculture*, **97(10)**, 3463-3462

Smith, J.C., Rashotte, M.E., Austin, T., & Griffin, R.W. (1984). Fine-grained measures of dogs' eating behavior in single-pan and two-pan tests. *Neuroscience and Biobehavioral Reviews*, **8(2)**, 243-251.

Stone, H., & Sidel, J. L. (2004). Sensory evaluation practices, Third Edition. Academic, San Diego.

Thombre, A. G., (2004) Oral delivery of medications to companion animals: palatability considerations. *Advanced Drug Delivery Reviews*, **56**, 1399-1413.

Thompson, H., Riemer, S., Ellis, S. L., & Burman, O. H. (2016). Behaviour directed towards inaccessible food predicts consumption—A novel way of assessing food preference. *Applied Animal Behavior Science*, **178**, 111-117.

Tobie, C., Péron, F., & Larose, C. (2015). Assessing Food Preferences in Dogs and Cats: A Review of the Current Methods. *Animals*. **5(1)**, 126-137.

Verbrugghe, A., Hesta, M., Gulbrandson, K., & Janssens, G., (2007). The effect of salmon oil freshness on the palatability of dog foods. *Vlaams Diergeneeskundig Tijdschrift*. **76(3),** 201-207.

Vondran, J. (2013) A two-pan feeding trial with companion dogs: considerations for future testing. K-Rex. Electronic Theses and Dissertation.

Worch, T. (2013). PrefMFA, a solution taking the best of both internal and external preference mapping techniques. *Food Quality and Preference*, **30(2)**, 180-191.

Chapter 2 - Development of a preference ranking test with dogs

Abstract

In the pet food industry, single-bowl or two-bowl methods traditionally are used to determine food acceptance or preference by pets. To increase efficiency of preference testing, a preference ranking procedure is proposed. The ranking procedure includes simultaneous presentation of 5 samples of edible treats. This increases the efficiency and reduces the time of preference testing. A preliminary test of the procedure with twelve beagle dogs was conducted. Each animal was presented with five treats, in an identical, coded rubber puzzle toy or “Kong®”. Five phases were included in the test, and each lasted 5 days. Phase 1 included training with commercial treats. Phases 2 to 4 used lab-baked treats with five different ingredients in each category (fats, starches, and proteins, respectively) of a base recipe. Phase 5 included testing with commercial foods. The order and time of treat selection by dogs was recorded. Results showed this small sample of animals generally ranked 1-2 flavors above others, indicating that this procedure could be a more efficient method to determine preference than traditional test methods since more samples can be evaluated simultaneously. More research is needed to verify the method.

Introduction

Companion animals are an important part of modern Western culture (Maust-Mohl, Fraser & Morrison, 2012). There are nearly 65% of US households that own a pet, and higher numbers of households own dogs than any other companion animals, including cats, reptiles, birds, horses, small animals and fish (APPA, 2016). The bond between humans and their pets, known as the human-animal bond has been studied and one prominent realization has been that the presence of companion animals benefits human health and emotional well-being (Friedmann, Son & Tsai, 2000; Friedmann & Son, 2009; Risley-Curtiss, 2010). The pet industry has been growing steadily in part because of this.

Within pet-related costs, owners spent the most on pet food (approximately $28.23 billion), followed by supplies and nonprescription medicines in 2016 (APPA 2016). Palatability is one of the most important factors that influences purchasing decisions of pet owners (Boya, Dotson & Hyatt, 2015). Even if pet owners do not usually taste the pet food themselves, they are able to determine whether or not their pets like the food by observing their food consumption behavior.

The consumption of food is also used for testing methods in the industry to evaluate the acceptability/palatability of products (Aldrich & Koppel, 2015). These include a single-bowl monadic test, a two-bowl forced choice test, and operant testing. Some researchers also utilize the traditional two bowl forced choice test and modify it in order to fit their objective and needs (Fragua, Barroeta, Manzanilla, Codony & Villaverde, 2015; Hewson-Hughes, Hewson-Hughes, Colyer, Miller, McGrane & Hall, 2012; Verbrugghe, Hesta, Gulbrandson & Janssens, 2007). However, none provides an indication of why a choice is preferred. In addition, operant testing depends on specialized training for both technician and dog. Few tests provide an indication of liking. For nonverbal primates, and companion animal methods that rely upon their motivation to solve a puzzle may provide a clue into liking. Existing tests are easy to operate but have limitations caused by the nature of forced-choice tests that can potentially alter and (or) cover the true acceptability or palatability of the foods (Aldrich & Koppel, 2015).

A method that relies upon the dogs' own motivation to extract food from a toy based on taste/aroma preference and to do so in order of rank is needed to truly understand liking when evaluating the palatability of foods. This would give researchers an option to better pinpoint elements in pet diets that are preferred (or not preferred). Therefore, the objectives of this study were (1) to determine the effects of using the dogs' motivation for preferred flavors via extracting food from a rubber toy; (2) Use the effects to develop a ranking procedure to determine their liking; (3) further evaluate whether the method could be used as a routine testing measure for research and product development.

Part I. The proposed preference ranking procedure for dogs

Even if the single-bowl monadic test and the two-bowl forced choice test are commonly used, these are not able to provide information about the liking/disliking on a particular option (Aldrich & Koppel, 2015). To explore the drivers of dogs liking, this preference ranking method was proposed under the theory of dogs' preference on aroma/flavor would provide motivation to extract food from a toy "Kong," to obtain the food they prefer. Similar food extraction procedures, also called the "Kong test" have been used in other studies (Plueckhahn, Schneider & Delfabbro, 2016).

In this proposed preference ranking procedure twelve dogs will be the subjects of this study and each of them will participate in the test individually in a testing room, which will be separated from their living area to avoid the variations from the noise, smell, and interaction of other dogs. The limited space in the test room will also help the dogs to stay focused.

The proposed test compares 5 foods or treats simultaneously. The proposed test is composed of 5 days of testing, and during each testing day each of the dogs will be presented with 5 hollow rubber toys ("Kongs") that each have a treat inserted. The idea is, that the dogs will try to extract the food or treat from the toys based on the aroma characteristics of the treat. The food with preferred aromatics would be extracted first, and the least preferred as last. The test will be repeated for 5 consecutive days, so that the dogs would be able to connect the aroma with the flavor of the food or treat. The time and order of extraction will be recorded, so these can be potentially used to access the fatigue and interest level of the dogs throughout the study. For example, if the dogs started to slow down and lose interest on the task, it may indicate that the dogs are fatigued and fewer samples should be studied in each test.

We propose that there be a conditioning/training phase for the dogs to learn this test. This would include 5 days of testing with samples that are different in their aroma characteristics. The training phase would include a qualification test. The dogs would need to pass certain qualifications, such as extract all treats/food from toys, complete the test in a reasonable time, and general interest in the testing overall. Not all animals may be suited to conduct and complete the test. The testing should take place in a room that is a different from the general living area. A confined area of approximately 1.5 m × 1.5 m should be set-up in the testing room. Each of the dogs will be taken to this room individually in a randomized order each day.

The samples used in this test should be of the same size and shape, and stored at ambient conditions, or as needed. The samples would be inserted into hollow rubber Kongs, and laid out on the testing surface in a randomized order. Next, the dog would be allowed to smell the Kongs, and the timer would be started. The time it takes for the dog to extract the first food or treat is recorded, as well as the coding on the Kong. This would be registered as the most preferred food or treat. The dog is allowed to continue testing without pausing the test, until all treat or foods have been extracted from the toys.

The toys would be washed with detergent and hot water, and rinsed with purified water daily in order to cleanse the toys thoroughly of any residual foods or treats and animal matter. Ethanol and other organic solvents should be avoided in the cleaning procedure to avoid rubber deterioration.

The order of preference ranks would be analyzed using analysis of variance.

The idea of this new method is to potentially evaluate more samples at a time and reduce the effect from the force-choice methods. In order to reduce the effect from the forced choice nature of some methods, the dogs will be fed the same amount of food during the day as when they are not doing this procedure. If the dogs were not interested in the treats, they have the choice of not consuming any treats instead of consuming the treats due to hunger.

The limitation of the procedure outlined by this study is that aroma will initially be the only factor being evaluated. This means that the preference results will be based on the preference of dogs for a particular aroma (or aroma intensity), instead of a complete profile including texture and flavor. Repeating the procedure for 5 days should provide some opportunity for the dogs to begin to associate an aroma with a taste and texture. Even if animals choose foods based on the aroma, when the actual flavor does not reflect the aroma, the consumption behavior is unlikely to happen (Houpt & Hintz., 1978; Houpt & Smith, 1981). When using this proposed procedure, it is important for the flavor to deliver the same perception as the aroma. However, the procedure of including the choice of toys and even the format of the test can be modified based on the desired information to be gathered from the study. For example, if appearance is the main factor being evaluated during the preference ranking study, a clear, air-tight container (e.g. Tupperware) could be used in place of the solid rubber toy.

Part II. A preliminary test

A preliminary test was conducted in order to determine the feasibility of the proposed ranking procedure This research was approved by the Institutional Animal Care and Use Committee (IACUC #3722).

Materials and Methods

Subjects

Twelve young adult (2 to 4 years old) beagle dogs (4 females and 8 males) with an average weight of 11 kg ± 1.2 kg were housed at Kansas State University Large Animal Research Center under ambient environmental conditions (20°C; 60% R.H.). Dogs were housed in pairs or as individuals throughout the study in dog runs (7.8 square meter inside run with an attached 18 square meter outdoor run). During the study, the dogs were fed a standard maintenance diet twice daily at 7 AM and 11 AM in amounts to maintain body weight. The food was removed following the 11 AM feeding to increase motivation and interest of the dogs for the Kong test. Testing started at 4:30 PM daily and was completed at 6 PM. As individuals, each dog was led to the testing space in an adjacent room to their pens. The room was a quiet environment without distractions from external factors like barking and smells from other dogs. With the room, the testing space was partitioned with a metal fence to form a 1.5 m × 1.5 m square space, which allowed the dogs to be contained and promote focus on the task.

Methodology

To start the test, the dogs were introduced to the five treats contained in hollow rubber toys by two researchers. The researchers led each dog to sniff the rubber toys that contained the different treats by placing each rubber toy in front of the nose of each dog individually. The toys were composed of rigid rubber in an abbreviated cylinder with a hole through the length of the interior where the treat can be hidden (kong; Kong™, Golden, CO, US). Each of the 60 identical toys were labeled sequentially and distinctly with numbers 1 to 60. The toys were washed with hot water and soap twice and air-dried prior to use and after each day of testing to eliminate odor and other residue (hair, saliva, etc.) from the tests. After all 5 toys were sniffed by the dog randomly, the dog was led by one researcher to the start-point located approximately 2 m away from the toys. The toys were set up by the other researcher in a row on the floor with a distance of approximately 10 cm apart from each other. One researcher stayed in the fence with the dog and the other one stayed outside with a timer and a chart to record the time and order of the consumption of each treat. The timer was started and the dog was released to approach and extract the treats from the toys. The order of the treats being set-up on the floor and the assigned number of the toy for each treat were completely randomized for each dog on each day. After the dog extracted and consumed the treat from each toy, the toy was removed by the researcher in order to prevent the dog from "revisiting" the empty toy. The order and time for each dog to obtain and consume each treat was recorded. The order of the treats being extracted and consumed by the dog was considered as preference ranking order of the dog. The treat obtained by the dog first was considered most preferred, and the treat obtained last by the dog was considered least preferred.

After the dog finished the test and led back to the pen, another dog was led in the same room to start the test. The study was conducted in five sequential days to allow dogs to associate aroma with flavor and confirm rank order.

Five phases were included in this preliminary test. For each phase, the same test with the same treats repeated for five days as five replications. For the first five days of the study (phase 1), which was the training/practicing phase, the dogs were introduced to the test procedure using commercial dog treat products (three different products), followed by 3 phases of evaluation of different fat, starch, and proteins, and a final phase to evaluate commercial foods with combinations of ingredients marketed to be consistent with the ingredients tested in previous phases. The preliminary study contained five days of testing: (1) training, (2) study of fat, (3) study of protein, (4) study of starch, and (5) study of complex commercial food. The training phase was conducted to familiarize the dogs with the task (obtaining treats from the toys), learn the techniques to obtain the treat from toy, and habituate the dog to the testing environment. The purpose of studies of fat, protein and starch is to discover if the dogs could potentially express their preference on particular compounds. The study with complex commercial food is to potentially determine if the dogs were able to show their preference on the final products existing in the market. Each phase will be repeated for five sequential days.

During the first period, three commercial dog treat products were used for training/practicing. From day 1 to day 4, the dogs were allowed to explore the testing space prior to and during the test in order to get comfortable with the environment. On the last day of this phase, a qualification test was conducted in order to qualify the ability of the dogs to discern differences within the confines of the study protocol. The test consisted of 5 segments: 1) if the dog smelled all the toys, 2) the dog's initial interest in the test, 3) if the dog retained interest, 4) the ability of the dog to get the treats out, and 5) if the dog needed any assistance. Each dog was scored on a scale from1-5 (1-Didn't do the task at all, 5- did the task perfectly) in each of the categories. In order for the dog to be considered "qualified" for the study, the dog had to score a minimum of 3 in each category, for a total score of at least 15. The dogs that did not meet the requirements for the test were still allowed to complete the study, but the data of later phases collected from them were used with prejudice. No preference data was collected in this phase, since the purpose of this phase was simply to introduce the procedure to the dogs and to work out timing for the procedure. However, qualitative observations were made to provide insights on the subjects' progress of adapting the procedure of the method. For phases 2 to 5, five different treats were ranked by the dogs, respectively. Behavior cues such as sniffing, exploration of the room, interaction with researcher, and treat extraction techniques were observed in this phase. In phases 2 to 4 the dogs completed the ingredients evaluation, while phase 5 was a final test with commercial complex foods.

Samples

In phase 1, the treats included a generic dog treat (Mini dog biscuits; Great Choice, Phoenix, AZ, US), and two premium oven-baked treats (Classic Original Assortment, Classic P-Nuttier; Old Mother Hubbard Baking Co., Tewksbury, MA, US). Three of the toys were filled with a random selection from the general dog treat biscuits, and the other two were filled with a biscuit from each of the premium oven-baked treats.

In phases 2 to 4, the treats used in each phase were made one day prior to the first day of the testing in the Department of Grain Science and Industry laboratory at Kansas State University for the each of the five days of evaluation (Table 2.1). To make the treats, the dry ingredients were combined first and the shortening/fat was cut in to the dry ingredients. Then the liquid ingredients were mixed and slowly added into the dry ingredients. Next, the dough was mixed by hand until the ingredients were combined. Then the dough was rolled into a 10 mm thick sheet and cut into 15 mm*30 mm rectangles. These were placed on a cookie sheet and baked in a convection oven at 175°C for 15 minutes, treats were flipped half-way through the baking cycle. Once cooled the treats were stored in plastic bags (Ziploc, S.C. Johnson, Racine, WI, US) until used in the evaluation.

For each of the phases 2 to 4, one ingredient in each recipe was altered with different options within the ingredient category (Table 2.1). In phase 2, fats and oils ingredients were evaluated: fish oil (Omega Protein, Houston, TX, US), chicken fat (IDF Inc., Springfield, MO, US), lard (Armour, Phoenix, AZ, US), butter (Land O' Lakes, Minneapolis, MN, US), and vegetable shortening (Crisco, Orrville, OH, US). In phase 3, different meat (proteins) were evaluated. These included beef (70:30 ground beef, Dillons, Hutchinson, KS, US), chicken (Dillons, Hutchinson, KS, US), fish (Salmon, Dillons, Hutchinson, KS, US), tofu (Dillons, Hutchinson, KS, US), and chicken liver (Tyson, Springdale, AZ, US). In phase 4 starch and grain ingredients were evaluated, including whole wheat flour (Gold Medal, Minneapolis, MN, US), chickpea flour (Garbanzo Bean Flour, Bob's Red Mill, Milwaukie, OR, US), potato flour (Bob's Red Mill, Milwaukie, OR, US), corn starch (Argo, Cordova, TN, US), and tapioca flour (Bob's Red Mill, Milwaukie, OR, US). In phase 5, a final test with commercial complex dog food products was conducted. For this phase, the following diets were used: Authority Skin, Coat + Digestive Health Support: Fish and Potato (F/P), Authority Healthy Weight + Joint Support: Turkey and

Chickpea (T/C), Authority Adult: Chicken and Pea (C/P), Authority Adult: Chicken and Rice (C/R), and Simply Nourish Adult: Lamb and Oatmeal (L/O) (Petsmart, Phoenix, AZ, US).

Table 2.1 Ingredient composition for treats evaluated by dogs in phases 2-4.

Ingredient	**Phase 2 % composition**	**Phase 3 % composition**	**Phase 4 %composition**
Whole wheat flour (Starch source replacement)	10.08	24.33	***28.82
White (all purpose) flour	40.03	24.33	2.88
Corn meal	12.59	12.16	0.00
Salt	0.50	0.49	0.58
Baking soda	0.25	0.24	0.29
Dry milk	1.51	1.46	1.73
Sodium bisulfate	0.002	0.002	0.002
Dry yeast	0.002	0.002	0.002
Shortening (Fat source replacement)	*7.56	7.30	8.65
Molasses	4.03	3.89	4.61
Water	23.10	16.06	26.51
Meat (protein source replacement)	0.00	**9.73	0.00

*Fat replacement included vegetable shortening, fish oil, chicken fat, butter, or lard.
**Protein replacement included fish, beef, chicken, liver, or tofu.
***Starch replacement included whole wheat flour, tapioca flour, potato flour, corn starch, or chickpea flour.

Data Analysis

The ranked data from each of these phases was analyzed by a Friedman analysis of variance (Lawless & Heymann, 2010) using the XLStat version 2010.6.02 (Addinsoft, New York, NY, US). The time differences of each phase were analyzed by one-way ANOVA with SAS® statistical software (SAS® version 9.3, SAS Institute Inc., Cary, NC, US) using PROC GLIMMIX to determine significant differences among samples. The statistical significance of differences was defined as $p \leq 0.05$.

Results and Discussion

Phase 1

During the training phase, the dogs showed a wide variety of behaviors and changes over the five-day period. In the first two days, most dogs spent a long time (approximately 3 minutes) exploring the room. On the third day of the study, a fence was placed in the room in order to create a 1.5 m × 1.5 m testing space with the corner of the room to help restrict the dogs from roaming. With the addition of the fence, more dogs were able to complete the test in a shorter amount of time. Most dogs were able to improve their treat extraction ability in order to obtain treats and decreased their time to complete the test. However, a few dogs did not improve and constantly needed to be redirected to the task. They might have been too distracted by equipment and scents in the room other than the treats, or were not food-motivated. Another observation was, except for the first dog, the smell from the previous dogs possibly distracted some of the male dogs, they spent more time sniffed around and had repeated urination behavior throughout the 5 phases of the study. The urination behavior was proposed as scent-markers when novel odor or objects are present (Kleiman, 1966).

For the qualification test, eight out of twelve dogs that were able to complete the test were considered as qualified (Table 2.2). A few dogs showed low to no interest in the toys and treats by avoiding them at introduction, they did not sniff the toys, and ignored those after being released; often taking only a quick sniff and then walking away, etc. The dogs not completing the test tended to have more behaviors of distraction with other subjects, such as sniffing around the testing space, or jumping to interact with researchers. After the treats were given to the dogs that did not complete the test, some of the dogs did not consume or even sniff the treats. In that case, the reason for the failure to finish the test could have been lack of interest in the treats. This could be due to the treats themselves not possessing enough attractive flavor or aroma for the dogs, or the dogs may not have been hungry enough to be motivated. Another potential explanation for their behaviors was that the environment in which the test was conducted could have been too unfamiliar for the dogs. These were research Beagle dogs with a high level of energy and curiosity and with limited social interaction with experimenters prior to their exposure to this test. Depending on the personality of the dogs, they might have been too excited/curious about the new environment, or they might not have felt secure enough to perform the test. Over the course of the 5 phases in this study, participation by the disqualified dogs was inconsistent. While they initially seemed unfit to contribute to the study, their behavior sometimes proved differently. For this reason, their data was included in the results of each phase.

Table 2.2 Outcome of qualification test conducted at the end of phase 1. Dogs had to have an individual test score greater than 3 and an overall score greater than 15 to qualify without prejudice.

Dog	**Smells all rubber toys**	**Initial interest**	**Retains interest**	**Ability to get treats out**	**Needs assistance**	**Score**
F1T	5	5	5	5	5	25
F1P	4	5	4	4	4	21
F2L	3	2	1	3	2	11
F2C	5	5	3	5	4	22
M4W	1	1	1	1	1	5
M5P	5	5	5	5	5	25
M5T	3	5	4	5	5	22
M6J	3	3	3	3	3	15
M6P	3	3	3	3	3	15
M7F	3	1	1	1	1	7
M7D	2	1	1	2	1	7
M8C	5	5	5	5	5	25

Phase 2

Dogs are highly attracted to fat (Manabe, Matsumara & Fushiki, 2010) so fats are commonly used in dog food in part for their flavor. Studying the preference of common fat ingredients can potentially benefit the success of a dog food development. The preference ranking of the dogs during phase 2 using fats and oils indicated a preference ($p<0.05$) for the fish oil and butter relative to the lard treatment (Table 2.3). The vegetable shortening and chicken fat were intermediate in preference ranking and similar to the two extremes ($p>0.05$). Without the additional data from the disqualified dogs, there was no pairwise significant difference in preference between the treats ($p = 0.023$). In a previous report, both butter and fish oil contributed to a relatively strong overall aroma for the treats, which potentially attracted the dogs (Houpt, Hintz & Shepherd, 1978b). Surprisingly, chicken fat and lard as animal based fats, were not preferred relative to the vegetable shortening when it is commonly assumed that dogs prefer animal fats over vegetable fat sources. However, Verbrugghe et al. (2007) suggested that the processing/freshness of the fat may have an influence on the preference of the diet. A modified two-pan method with three diets served simultaneously were used in their study, and they considered the amount consumed of each diet to indicate preference. Their results indicated that the relative intake of the basal diet with chicken lard was higher than the basal diet with non-rapid-harvest salmon oil. The relative intake of the diet with rapid-harvest salmon oil did not differ from either chicken lard or non-rapid harvest salmon oil diet. Fragua et al. (2015) compared the preference for different ratios of esterified fatty acid oils (EAOs) with a modified free choice two-pan method in two diets and the dogs were allowed to consume both diet with preference determined by the amount of consumption. They reported that dogs preferred the basal diet with soya bean-canola (80%) and coconut (20%) EAOs relative to the basal diet with soya bean-canola (60%) and coconut (40%) EAOs, and the basal diet with soya bean-canola (60%) and coconut (40%) EAOs over the basal diet with only soya bean-canola EAOs. This may indicate that a change in the ratio of the vegetable oil sources could affect the preferences of dogs. Perhaps, more animal fat and vegetable fat/oil sources should be included in future studies to expand the range of ingredients in the evaluation.

Table 2.3 The effect of flavor treatments on rank order preference in dogs.

Phase	Treatments				
2: Fats and Oils	Fish Oil 2.48[a]	Butter 2.54[a]	Vegetable Shortening 3.21[ab]	Chicken Fat 3.22[ab]	Lard 3.53[b]
3: Proteins	Liver 2.49[a]	Chicken 2.62[ab]	Fish 3.19[ab]	Tofu 3.28[ab]	Beef 3.42[b]
4: Starches	Corn 2.29[a]	Wheat 2.62[ab]	Tapioca 3.28[b]	Potato 3.33[b]	Chickpea 3.48[b]
5: Complex Food	F/P **1.84[a]**	C/R **2.95[b]**	L/O **3.28[b]**	T/C **3.37[b]**	C/P **3.56[b]**

[abc] within a row, samples with unlike letters were significantly different ($p<0.05$).
F/P: Authority Skin, Coat + Digestive Health Support: Fish and Potato; C/R: Authority Adult: Chicken and Rice; L/O: Simply Nourish Adult: Lamb and Oatmeal; T/C: Authority Healthy Weight + Joint Support: Turkey and Chickpea, C/P: Authority Adult: Chicken and Pea

Phase 3

In the evaluation of proteins, dogs showed a preference ($p<0.05$) for the chicken liver relative to the beef treatment (Table 2.3). Chicken, fish, and tofu were intermediate and similar to the two extremes. This result disagreed with Houpt & Smith (1981) as their research indicated that dogs preferred beef over chicken and liver. The difference could be a result of processing method and/or the effects of other ingredients. While fish oil was preferred over the other samples in Phase 2, this did not carry over to fish flesh in this phase. Perhaps the fish flesh itself was not as aromatic as the fish oil. Dog food choices are initially based on the intensity of food aromatics (Houpt, Hintz & Shepherd, 1978). Alternatively, the palatability of dog food may be affected by the type of fish substances (Folador, Karr-Lilienthal, Parsons, Bauer, Utterback, Schasteen, et al., 2006). The lack of preference between the beef and the tofu was unexpected, as dogs are generally thought to prefer animal proteins over plant based ones (Houpt & Smith, 1981). Results differing from our initial hypothesis may be due to dose, process, species, and (or) aging of the ingredients. Future preference ranking studies could include the addition of more plant based proteins to determine if a wider array of sources might also rank above animal based proteins. No significant difference in preference was found among the treats without the additional data from the disqualified dogs ($p=0.079$).

Phase 4

In phase 4, the evaluation of starches showed that the dogs preferred ($p<0.05$) the cornstarch treatment in comparison to the tapioca flour, potato flour, and chickpea flour treatments (Table 2.3). The tapioca, potato, and chickpea treatments were intermediate and similar to each other, and the whole wheat flour treatment was intermediate and similar to both extremes. Existing research indicated that grain-added products have more overall aromatics than grain-free products (Koppel, Adhikari & Di Donfrancesco, 2013). The samples made with corn flour and whole wheat flour contained more grain components than all other samples, so the results partially supported these previous observations. However, no existing research was found on preference of dogs relative to starch sources. This indicated a new area that should be explored in more depth, especially considering the wide array of new products in the market that are based on non-traditional starch sources, e.g., grain free. With only the data from the qualified dogs, corn was preferred over chickpea treatment but the wheat, tapioca and potato treatments were similar with both corn and chickpea treatments ($p<0.05$).

Phase 5

In the evaluation of complex foods, the dogs showed a preference ($p<0.05$) for F/P over C/R, L/O, T/C, and C/P, though there was no difference ($p>0.05$) between C/R, L/O, T/C, and C/P (Table 2.3). The result remained the same with or without the inclusion of disqualified dogs ($p<0.05$). The F/P diet included deboned whitefish and fishmeal as the first and second ingredients listed. Even if the results suggested neither fish nor potato flour was preferred in phases 3 and 4, the variations contributed by the type and amount of other ingredients, usage of palatant and process method may have enhanced the differences on preference. Commercial pet food companies commonly use external palatants, usually in addition to fats or oils, to spray onto the kibbles for the purpose of increasing palatability and intake consistency. However, the usage and type of palatant for the particular diets in this phase was unknown. For this phase, comparing the preference on any particular ingredients was not the main purpose, that the dogs were able to choose their most preferred diet was the most important finding. To verify the results from phases 2 to 4 in a complex food format, it is important to control the variations, different combination of tested ingredients can be used with the same formula in order to compare the preference on the combinations of the ingredients.

Length of tests

Overall, the total elapsed time for each dog to complete the test was less than 5 minutes. The average time that the dogs spent at each rubber toy/treat decreased substantially from phase 2 to phase 5 ($p\text{-value}<0.0001$; from average of 29.3sec to 8.5 sec; Figure 2.1). Since the average time the dogs spent on each toy decreased, the overall time for the study also decreased by phase 5 (from average of 2.45 min to 0.75 min). The reduction of time can be explained by several observations during the study (Figure 2.2). The first was that the commercial products were smaller than the treats used in phase 2 to 4 and the shape were round instead of rectangles. This may decrease the difficulty of obtaining the treats and reduce the time needed. The second reason was an increased efficiency by the dogs to complete the task. After repeating the test several times, the dogs learned the technique for obtaining the treats and thereby reduced the amount of time necessary to obtain their reward from each toy. The dogs were also able to develop their own methods to obtain the treats from the rubber toys. This adaptive learning was seen for example by some dogs that picked the toys up with their mouth and dropped and (or) threw the toys onto the floor effectively forcing the treats to fall out. Other dogs flipped the toys upside down to cause the treats to fall out. Another dog was observed several times ramming into the line of toys to eject the treats from the force of impact. Thirdly, after being exposed to the testing room regularly, the dogs became familiar with the environment (testing space) and the time spent on exploring the testing space declined. This accounted for the largest part of the time they spent on the study in phase 1 and 2. The fourth reason for time reduction was that dogs may have become more familiar with the researchers and spent less time interacting with them.

The time the dogs spent between consuming treats did not increase linearly. Obtaining and consuming the first toy/treat after being released by the researcher took significantly less time than the treats chosen later (Figure 2.3). The time between the third chosen treat and forth chosen treat was significantly shorter than the time between the first treat chosen and second treat chosen. The time for choosing the fourth treat until choosing the fifth treat was the longest ($p\text{-value}<0.0001$) (Figure 2.3). It appeared that the decision to choose the first treat was faster than later decisions. This relative time difference between consuming samples was constant in each phase and across all data. The relatively short time the dogs spent obtaining the first four treats indicated that the dogs remained engaged in the study. The relatively long time the dogs spent to obtain the fifth treat might indicate fatigue or loss of interest. This tends to confirm that five treats were the optimum number of samples to include in this test. However, it could also because the last treat was not appetizing to the dog.

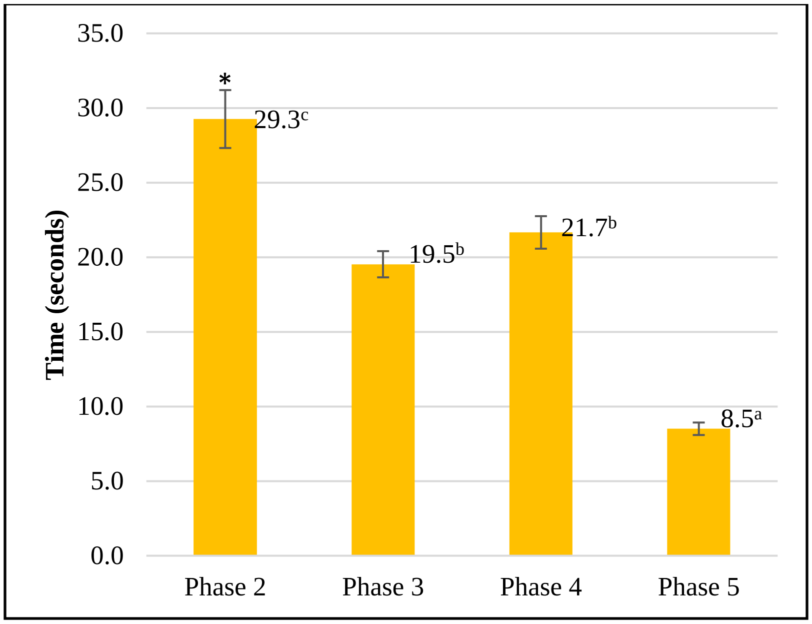

Figure 2.1 Average time spent on each toy from phase 2 to 5 in seconds (p<0.0001)
*Standard error

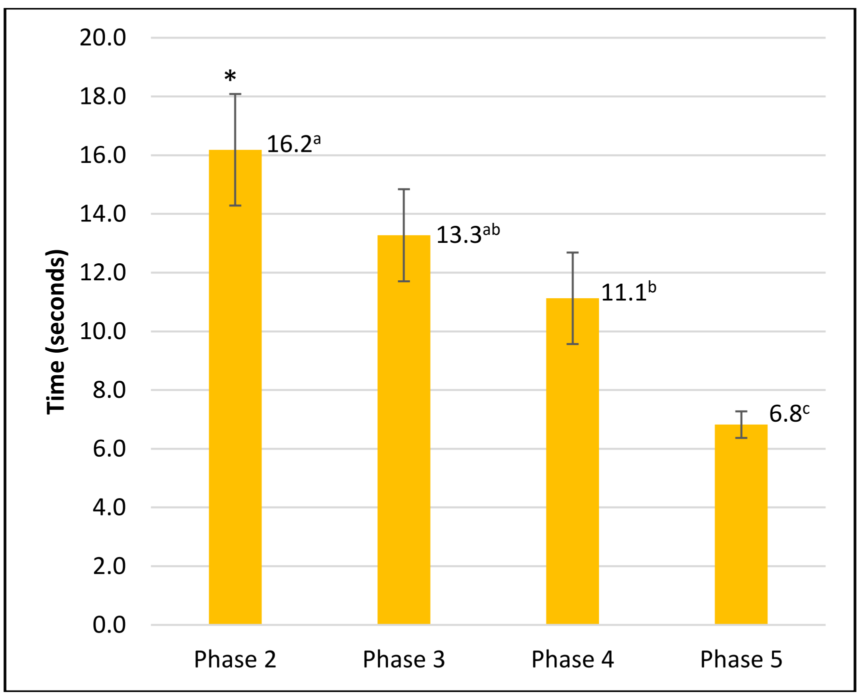

Figure 2.2 Average time to obtain the first rubber toy in seconds (p<0.0001)
*Standard error

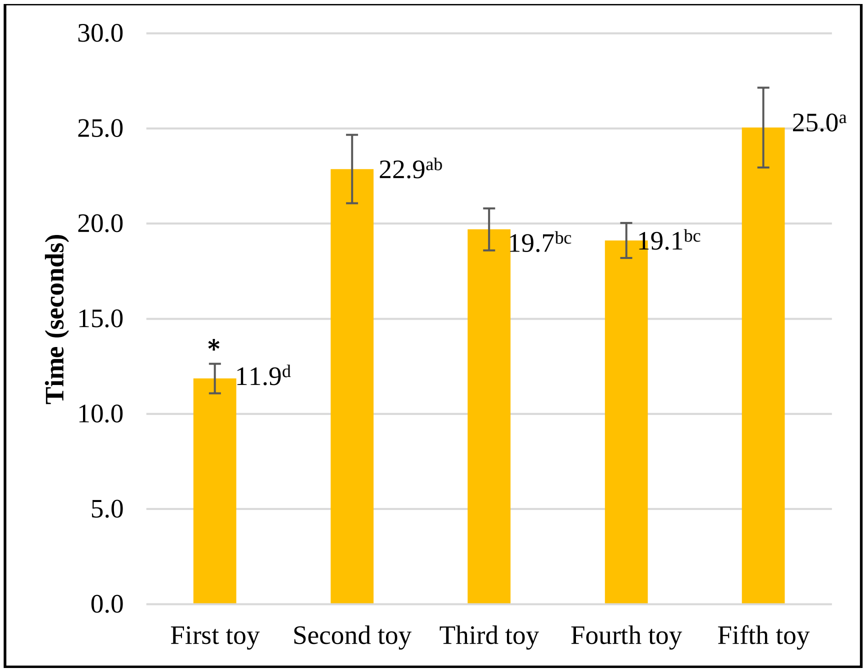

Figure 2.3 Average time spent on each toy in seconds (p<0.0001)

*Standard error

Test considerations

To understand preferences of pets, there are several existing methods with advantages and disadvantages. Single-bowl/One-bowl/One-pan test, also known as a monadic test, is one of the most common testing methods used in the pet food industry, typically for home-use-test (HUT) with pet-owner dogs (Aldrich & Koppel, 2015). In this model, the testing subjects (animals) are provided a pre-weighed bowl of food to consume as the main meal of the day, and then the food left in the bowl is weighed to measure the amount of food consumed (food intake). The food intake indicates the acceptability/palatability of the food (Griffin, 2003). This test is suitable for HUT because the procedure is similar to the dogs' normal feeding routine as no second choice is given for each meal (Aldrich & Koppel, 2015). However, this method may not provide the most consistent test result since there are many variations such as the level of hunger and mood can affect the amount of food being consumed that day. In addition, the subjective interpretation of the owners may also affect the results (Tobie, Peron & Larose, 2015). The other industry standard method is the two-bowl forced choice test, also known as split-plate test, normally used with kennel dogs. In this model, the animals are simultaneously given two foods in which to choose. The one that is consumed completely or of which is consumed more when the test ends indicates that it is preferred by the animals (Smith, Rashotte, Austin & Griffin, 1984). However, the dog only has two choices, and they are forced to choose one, which may not truly reflect their preference. Another test method known as the cognitive palatability assessment protocol (CPAP) first published by Araujo & Milgram (2004) was studied and compared with the two-bowl method by Araujo, Studzinski, Larson & Milgram (2004). For this method, three objects are given to the subjects at the same time and the subject chooses one out of three. One of the objects does not contain food as reward while the other two do, subjects receive the food to associate with the object they choose. The result showed this method was more consistent than the two-bowl method. Additionally, when the subjects were fed before the test, the ability to detect preferences was reduced in two-bowl test. Even if the result indicated the CPAP is a more accurate method than two-bowl method, again, the dogs were forced to make a choice among the three samples even if they did not have a preference. Rashotte, Foster & Austin (1984) also studied the comparison between two-bowl/two-pan test and the operant lever-press test. The operant lever-press test was a box with two response-levers and the pre-weighed foods were dispensed with automated feeder into bowls at a floor-level under the levers (Green & Rashotte, 1984). The two-bowl test and the operant lever-press test did not provide the same conclusion when evaluating the same products and both tests can only evaluate two samples at a time. The proposed preference ranking procedure may be a helpful method in the toolbox of evaluating dog preferences for foods or treats as it evaluates 5 samples simultaneously, thus making for a more efficient tool. At the same time, the method is not based on consuming a daily ration of food and thus may be limited to be used with treats instead of food meant for daily nutritional needs.

Thompson, Riemer, Ellis & Burman (2016) proposed a new approach to assess dogs' food preference, which was a non-consummatory test. The non-consummatory test was conducted by first giving the dog three pieces of each food sample to taste, then placing one piece of food samples in separate bowls for the dog to sniff or consume, then if the sample was consumed, the experimenter refilled the bowls and placed them under two wire covers so the dog was able to see and smell the samples but not touch/taste them. The time of investigation and behavior patterns of the dogs toward each cover/sample indicated their preference. After comparing with the consummatory test result, both tests showed similar results. This method was also tested with different populations of dogs (pet vs. shelter dogs) and received consistent results. This method can avoid the dogs' over-eating and potentially be more efficient than traditional methods, the dogs would not need to consume a large amount of samples in each session so probably more sessions can be conducted each day. Even if there was no discussion about the forced-choice effect on this method, because of the set-up of the study, the forced-choice effect was likely minimized since the dogs were given a third choice of not investigating any of the treats. However, only two samples can be evaluated in each session with this initial procedure. Similar with the preference ranking procedure, two researchers, or an experimenter and a handler, are necessary in order to conduct this test which can possibly be labor intensive and costly.

Preference ranking studies such as the procedure proposed can be advantageous for product development by evaluating preferences for several different protocols or ingredients. As demonstrated above, even if the dogs did not provide significant ranking of each treat using the preference ranking method, they were able to show preference for one or two over the others. In traditional single-bowl or two-bowl tests, which are usually conducted as the main meal of the day, hunger can potentially influence judgement of preference for the samples. Because of this, preference ranking procedure was not part of the dogs' daily food allowance, hunger influence was eliminated during the test, producing a potentially more reliable result. In addition, both traditional methods are forced-choice test methods, when in reality, the subjects may not have a preference among the samples. The preference ranking procedure is still a forced-choice method but since the dogs already received their daily allowance of food, they had a choice not to consume any of the treats.

The preference ranking procedure can potentially be more efficient than traditional preference determination methods. In the single-bowl method, the amount of food being consumed determines the palatability of the food; testing 5 different samples of food would require a large number of dogs or a long period of time for testing. It would also require a large amount of food for testing. In the two-bowl method, only two samples can be compared in each test. In order to compare multiple samples a large number of tests are necessary. In order to test 5 samples using a 2-bowl test we would need to conduct 10 tests. If the proposed method is reliable, using a reduced number of preference ranking tests may increase efficiency while still achieving the desired objectives.

As mentioned, one of the limitations of this method is that aroma is initially the first factor that determine the dogs' choice. However, after comparing the data analysis from the five days of each phase respectively, the significant differences did not appear until the last day of phase 3 and 4, and the significant difference of treats in phase 5 was found from day 2 to day 4. The insignificance on day 5 of phase 5 could potentially be due to fatigue. In order to determine the optimal number of days for this procedure, further evaluation is needed. It seemed that the dogs were able to learn and remember the treats but may need a longer period of exposure to the treats as all sensory aspects have potential influence on the acceptability/palatability of the food (Taylor 2014; Kitchell, 1978). Further, no additional sensory analysis, consumer analysis, or volatile characterization of the samples took place in this study. These additional analyses may help in understanding why some of the treat aromatics were preferred over others. In addition, the method needs to be validated via a repeat study. Additional studies need to determine the limits of this method as far as different ingredients and ingredient combinations and quantities are concerned as well as a larger number of dogs may need to be considered in the test. Moreover, a comparison to single-bowl test, two-bowl test, and liking test may help characterize the applicability of this proposed ranking procedure better.

Conclusion

A preference ranking procedure that includes simultaneous evaluation of 5 samples inserted in toys and that would be conducted in 5 repetitions over 5 days, was proposed. This method was proposed to assess the dogs' liking on specific compounds and potentially discover the drivers of dogs liking on dry dog food/treats. In order to determine feasibility of the proposed test, a preference ranking procedure was conducted with five phases including training, evaluation with three different categories of ingredients, and evaluation of commercial food. The preliminary test results showed that the dogs were able to generally compare dogs' preference on five samples simultaneously. The dog was able to pick out the most preferred samples (one or two) out of the five samples provided. This method may possibly be a more efficient and reliable way to determine the preference of dogs for foods than traditional methods because of the higher number of samples that can be tested. However, even for a company placing more confidence in the single-bowl or two-bowl method, it would still be informative for comparison purposes to determine if the preference ranking procedure provides a similar result. The reliability of the results from this method are not clear at this point, so a validation study is recommended. Once the method is validated, evaluating the aromas of treats with a human sensory panel and existing lexicon (Di Donfrancesco, Koppel & Chambers, 2012) could also help researchers to determine the aroma factors that influence dog choices and begin to unravel the elements for liking.

References

Aldrich, G.C., & Koppel, K. (2015). Pet Food Palatability Evaluation: A Review of Standard Assay Techniques and Interpretation of Results with a Primary Focus on Limitations. *Animals*, **5(1)**, 43-55.

APPA. (2016). Pet industry market size and ownership statistics. American Pet Products Association, Inc. Greenwich, CT. Available online: http://www.americanpetproducts.org/press_industrytrends.asp (accessed on 05 April 2017).

Araujo J.A., & Milgram N.W. (2004). A novel cognitive palatability assessment protocol for dogs. *Journal of Animal Science*, ***82***, 2200-2206.

Araujo, J.A., Studzinski, C.M., Larson, B.T. & Milgram, N.W. (2004). Comparison of the cognitive palatability assessment protocol and the two-pan test for use in assessing palatability of two similar foods in dogs. *American Journal of Veterinary Research*, **65(11)**, 1490-1496.

Boya, U. O., Dotson, M.J., & Hyatt, E. M. (2015). A comparison of dog food choice criteria across dog owner segments: An exploratory study. *International Journal of Consumer Studies*, *39(1)*, 74-82.

Di Donfrancesco, B., Koppel, K., & Chambers IV, E. (2012). An initial lexicon for sensory properties of dry dog food. *Journal of Sensory Studies*, **27**, 498-510.

Folador, J., Karr-Lilienthal, L., Parsons, C., Bauer, L., Utterback, P., Schasteen, C., Bechtel, P. & Fahey, G. (2006). Fish meals, fish components, and fish protein hydrolysates as potential ingredients in pet foods. *Journal of Animal Science*, **84(10)**, 2752-2765.

Fragua, V., Barroeta, A., Manzanilla, E., Codony, R., & Villaverde, C. (2015). Evaluation of the use of esterified fatty acid oils enriched in medium-chain fatty acids in weight loss diets for dogs. *Journal of Animal Physiology and Animal Nutrition*, **99(S1)**, 48-59.

Friedmann, E., & Son, H. (2009). The human–companion animal bond: how humans benefit. *Veterinary Clinics of North America: Small Animal Practice*, **39(2)**, 293-326.

Friedmann, E., Son, H., & Tsai, C. (2000). The animal-human bond: Health and wellness. *Handbook on animal-assisted therapy: Theoretical foundations and guidelines for practice*, pp. 41-58.

Green, P. L., & Rashotte, M. E., (1984). Demonstration of basic concurrent-schedules effects with dogs: Choice between different amounts of food. *Neuroscience and Biobehavioral Reviews*, *8(2)*, 217-224.

Griffin, R.W. (2003) Section IV: Palatability. *In Petfood Technology*, 1st ed.; Kvamme, J.L., Philips, T.D., Eds.; Watt Publishing Co.: Mt. Morris, IL, USA, 2003; pp. 176-193

Hewson-Hughes, A. K., Hewson-Hughes, V. L., Colyer, A., Miller, A. T., McGrane, S. J., Hall, S. R., et al. (2013). Geometric analysis of macronutrient selection in breeds of the domestic dog, *canis lupus familiaris*. *Behavioral Ecology*, **24(1)**, 293-304.

Houpt, K. A., Hintz, H. F. (1978). Palatability and canine food preference. *Canine Practice*, **5(6)**, 29-35.

Houpt, K. A., Hintz, H. F., & Shepherd, P. (1978). The role of olfaction in canine food preferences. *Chemical Senses*, **3**, 281-290.

Houpt, K. A., & Smith, S. L. (1981). Taste preferences and their relation to obesity in dogs and cats. *Canadian Veterinary Journal*, **21**, 77-81.

Kitchell, R.L. (1978). Taste perception and discrimination by the dog. *Advances in Veterinary Science and Comparative Medicine.* **22**, 287-314.

Kleiman D.G. (1966). Scent marking in the Canidae. *Symposium of the Zoological Society of London*, **18,** 167–177.

Koppel, K., Adhikari, K., & Di Donfrancesco, B. (2013). Volatile compounds in dry dog foods and their influence on sensory aromatic profile. *Molecules*, **18(3)**, 2646-2662.

Lawless, H. T., & Heymann, H. (2010). Sensory evaluation of foods: principles and practices, 2nd edition, pp. 501-503. Springer, New York, NY, USA.

Manabe, Y., Matsumura, S., & Fushiki, T. (2010). Preference for high-fat in animal foods. *Fat Detection: Taste, Texture, and Post Ingestive Effects*. Boca Raton, FL. Chapter 10.

Maust-Mohl, M., Fraser, J., & Morrison, R., (2012). Wild minds: What people think about animal thinking. *Anthrozoös*, **25(2)**, 133-147.

Plueckhahn, T.C., Schneider, L.A., & Delfabbro, P.H. (2016). Assessing lateralization in domestic dogs: Performance by *Canis familiaris* on the Kong test. *Journal of Veterinary Behavior,* **15**, 25-30.

Rashotte, M. E., Foster, D. F., & Austin, T. (1984). Two-pan and operant lever-press tests of dogs' preference for various foods. *Neuroscience and Biobehavioral Reviews*, **8(2)**, 231-237.

Risley-Curtiss, C. (2010). Social work practitioners and the human-companion animal bond: a national study. *Social Work*, **55(1)**, 38-46.

Smith, J.C., Rashotte, M.E., Austin, T. & Griffin, R.W. (1984). Fine-grained measures of dogs' eating behavior in single-pan and two-pan tests. *Neuroscience and Biobehavioral Reviews*, **8(2),** 243-251.

Taylor, J. Good taste: Petfood palatability update (2014). *Petfood Industry*, 2014, 9, 40-51.

Thompson, H., Riemer, S., Ellis, S. L., & Burman, O. H. (2016). Behaviour directed towards inaccessible food predicts consumption—A novel way of assessing food preference. *Applied Animal Behavior Science*, **178**, 111-117.

Tobie, C., Péron, F., & Larose, C. (2015). Assessing Food Preferences in Dogs and Cats: A Review of the Current Methods. *Animals*. **5(1)**, 126-137.

Verbrugghe, A., Hesta, M., Gulbrandson, K., & Janssens, G., (2007). The effect of salmon oil freshness on the palatability of dog foods. *Vlaams Diergeneeskundig Tijdschrift*. **76(3),** 201-207.

Chapter 3 - Validation of a preference ranking procedure with dogs

Abstract

The growth of the number of pets and the pet food industry is continuous. A preference ranking procedure for dogs was proposed by Li et al. (Chapter 2) in order to create a more efficient method to study the palatability of food products for dogs. This method was developed based on the assumption that 1) dogs would be more motivated to solve a puzzle for foods that they preferred, and 2) the order in which the dogs obtained the treats from the puzzles would indicate the ranking of their preferences. This study included a validation test that was conducted with the same twelve dogs to prove the proposed method was reliable. The validation followed the same procedure as the preliminary test for most part. The results from phases 2 to 4 showed a similar pattern with the preliminary test. The results from phase 5 proved that the ranking of the combination of the ingredients reflected the preference of the dogs for individual ingredients. As a result, this method was concluded to be reliable.

Introduction

In the US, more than half of the households own at least one companion animal and the dog is one of the most popular choices (APPA, 2016). Dog owners spent the most on pet food compared to all other pet-related costs (APPA, 2016). Palatability of the food has a high impact on their purchasing intensions (Boya, Dotson &Hyatt, 2015).

To fulfil pet owners' desire of seeing their pets consume the food, manufacturers often study the palatability of the food products to guide product development. The most commonly used methods are single-bowl and two-bowl method. Single-bowl method measures the amount of food consumed by the subjects/pets and determines how palatable the food is (Griffin, 2003; Aldrich & Koppel, 2015). The two-bowl method which is a forced choice method, compares which food was consumed in the greater quantity when two food choices are offered simultaneously (Smith, Rashotte, Austin & Griffin, 1984; Aldrich & Koppel, 2015). However, both tests have limitation such as the presence of forced choice which can possibly alter the true result (Aldrich & Koppel, 2015) or the limited number of samples being evaluated. In order to develop a new method that uses pet's motivation for preferred flavors to explore dogs' preference, a preference ranking procedure for dogs was proposed and a preliminary test was conducted (Chapter 2). The result from the preliminary test showed this method was capable for providing general information regarding ingredient preferences of dogs. However, the results have not been validated.

Validation is necessary for many development processes and it can be conducted in different formats. Normally the validation study is conducted by comparing the results collected from different resources or conditions as the initial study such as method (Moelich, Muller, Joubert, Næs & Kidd, in press), data analysis (Dahl, Tomic, Wold & Næs, 2008), subjects

(Braghieri, Piazzolla, Carlucci, Monteleone, Girolami & Napolitano, 2012), time, etc. The purpose of a validation test is to determine the performance of the developed subject (method, equipment, theory, etc.) in accordance to its intended use (Dispas, Lebrun, Ziemons, Marini, Rozet & Hubert, 2014). In order to explore the reliability of the preference ranking procedure, a validation test was needed.

Therefore, the objectives of this study were to duplicate the preliminary test and determine whether the method is reliable and consistent.

Materials and Methods

The subjects and test procedures were the same as described in Chapter 2, and are described along with modifications below. This research was approved by the Institutional Animal Care and Use Committee (IACUC #3722).

Subjects

Twelve young adult (2 to 4 years old) beagle dogs (4 females and 8 males) with an average weight of 11 kg ± 1.2 kg that were used in the previous preliminary test (Chapter 2) participated the validation study. They were housed at Kansas State University Large Animal Research Center (Manhattan, KS, USA) under ambient environmental conditions (20°C; 60% R.H.). Dogs were housed in pairs throughout the study in dog runs (7.8 square meter inside run with an attached 18 square meter outdoor run). During 25 days of study period, a standard maintenance diet in amounts to maintain body weight were fed to the dogs twice daily at 7 AM and 11 AM and removed following the 11 AM feeding. The removal of the diet was expected to increase motivation and interest of the dogs for the ranking test. The test took place from 4.30PM to 6PM daily. Each dog was led to the testing space individually to a room adjacent to their pens. The room was a noise-free and smell-free environment which eliminated the distraction from the barking and smell of the other dogs. A testing space was partitioned with fences to form a 1.5 m x 1.5 m square space in the room which allowed the dogs to be contained and promote focus on the task.

Methodology

At the beginning of the test, the researchers led one of the dogs to the testing space and introduced them to the five treats contained in hollow rubber toys. The toys were composed of rigid rubber in an abbreviated cylinder with a hole through the length of the interior where the treat can be hidden (kong; Kong™, Golden, CO, US). The dog was directed to sniff the rubber toys that contained the different treats by having the rubber toys held in front of their nose. After all 5 toys were sniffed by the dog, one researcher led the dog to the start-point located in the opposite corner of the testing space approximately 2 m away from the toys. The other researcher placed the toys in a row on the floor approximately 10 cm apart from each other. The order of the treats being set-up on the floor and the assigned number of the toy for each treat were completely randomized. One researcher stayed within the fence with the dog to remove the empty toy after each treat had been extracted by the dog during the test and the other researcher remained outside the fence to record the time and order of the consumption of each treat. The timer was started when the dog was released to approach and extract the treats from the puzzle-toys. The order of the treats being chosen (extracted and consumed) by the dog was considered the ranking order of preference. The first treat consumed by the dog was considered most preferred, and the treat consumed last by the dog was considered least preferred. If the last treat was not consumed, it was also considered as least preferred. After the dog finished the test and was returned to their pen, another dog was led to the room to start their test. The order of the dogs led to the testing space remained the same to eliminate external distractions such as unfamiliar odors. The test was repeated for five sequential days for each phase to allow dogs to associate aroma with flavor and confirm the rank order. Each of the 60 toys were labeled sequentially and distinctly with numbers 1 to 60. After each day of testing, the rubber toys were washed with hot water and soap twice and air-dried overnight prior to use to eliminate odor and other residue (hair, saliva, etc.).

When the dog was not interested in the task, researchers encouraged/guided the dog in order to continuously train them. When guiding was needed, the data was not included in analysis due to potential existence of bias during the test. Within 30 seconds from releasing the dog, if the dog was not interested on the task, researchers tapped the toy on the floor to create noise and attract the dog. If after 1 minute from releasing the dog they were not on task, researchers removed the treat from the toy and allowed the dog to sniff the treat in order to attract them with the aroma. Then the treat was placed back in the toy once the dog showed interest and the test continued. If the dog was not able to start/continue the task 2 minutes after being released or having extracted the treat, the test was ended.

Five phases were included in this validation test. For each phase, the same test with the same treats was repeated for five days as five replications. The first four phases were consistent with the preliminary test. For the first five days of the study (phase 1), which was the training/practicing phase, the dogs were introduced to the test procedure using five commercial dog treat products, followed by 3 phases of evaluation of different fat, starch, and proteins, and a final phase to evaluate complex diet with combinations of ingredients tested in previous phases.

During the first period, three commercial dog treat products were used for training/practicing. On the last day of this phase, a performance evaluation was conducted in order to monitor the ability of the dogs to discern differences within the confines of the study protocol. The test consisted of six segments including if the dog needed assistance to sniff toys prior to test, if the dog sniffed toys while choosing the treats, if the dogs needed guidance from researchers during the study (attract dogs to kongs with noise, letting the dog sniff the treat), and if the dog showed interests towards toys and treats. Each dog was recorded on if they did or did not exhibit the behavior mentioned in each category. Each category contributed 1 point, positive or negative, depending on the nature of the behavior. When the dog scored more than 4, their ability of performing the test was considered acceptable. No preference data was collected in this phase, since its purpose was simply to introduce the procedure to the dogs and to work out timing for the procedure. However, qualitative observations such as the technique to extract the treat and urinating behavior were noted to provide insights on the subjects' progress in adapting to the procedure. For phases 2 to 5, five different treats were ranked by the dogs, respectively. In phases 2 to 4 the dogs completed the ingredients evaluation, while phase 5 was a final test with a complex diet.

Samples

Generic dog treats (CON; Great Choice, Phoenix, AZ, US), and two premium baked treat (PRE; Old Mother Hubbard Baking Co., Tewksbury, MA, US) were used in phase 1 (training). Three of the toys were filled with a random selection from the CON biscuits which contained three different colored biscuits, and the other two were filled with a biscuit from each of the PRE treats.

In phases 2 to 5, the treats used in each phase were made the day prior to the first day of each phase in Center for Sensory Analysis and Consumer Behavior at Kansas State University for the each of the five days of evaluation (Table 3.1).

__Table 3.1 Ingredient composition for treats evaluated by dogs in phases 2-5.__

Ingredient	**Phase 2 % composition**	**Phase 3 % composition**	**Phase 4 %composition**	**Phase 5 %composition**
Whole wheat Flour (Starch source replacement)	10.08	24.33	***28.82	***25.84
White (all purpose) flour	40.03	24.33	2.88	25.84
Corn meal	12.59	12.16	0.00	0.00
Salt	0.50	0.49	0.58	0.52
Baking soda	0.25	0.24	0.29	0.26
Dry milk	1.51	1.46	1.73	1.55
Sodium bisulfate	0.002	0.002	0.002	0.002
Dry yeast	0.002	0.002	0.002	0.002
Shortening (Fat source replacement)	*7.56	7.30	8.65	*7.75
Molasses	4.03	3.89	4.61	4.13
Water	23.10	16.06	26.51	23.77
Meat (protein source replacement)	0.00	**9.73	0.00	**10.34

* Fat replacement included vegetable shortening, fish oil, chicken fat, butter, or lard
** Protein included fish, beef, chicken, liver, or tofu
*** Starch included whole wheat flour, tapioca flour, potato flour, corn starch, or chickpea flour

The treats were made by first combining the dry ingredients and then cutting the shortening/fat in to the dry ingredients. Then the liquid ingredients were mixed together and slowly added into the dry ingredients. The dough was then mixed by hand until the ingredients were combined and the dough was formed. The dough was rolled into a 10 mm thick sheet and cut into 15 mm*20 mm rectangles. The adjustment of size was made because the treats sometimes stuck in the kongs in the preliminary tests, researchers needed to extract the treat and place it back in the kong and some dogs changed their decision of choice within that period of time. The treats with smaller size was able to be extracted freely. The treats were then placed on a baking sheet with parchment paper and baked in a convection oven at 175°C for 15 minutes. The treats were stored at room temperature (20°C; 60% R.H.) in gallon-size plastic bags (Ziploc, S.C. Johnson, Racine, WI, US) once cooled and were stored until use in the evaluation.

For each of the phases 2 to 4, within each recipe, one ingredient was exchanged with different options within the ingredient category (Table 3.1). In phase 2, fats and oils were the selected categories of ingredients to be evaluated: fish oil (Omega Protein, Houston, TX, US), chicken fat (IDF Inc., Springfield, MO, US), lard (Armour, Phoenix, AZ, US), butter (Land O' Lakes, Minneapolis, MN, US), and vegetable shortening (Crisco, Orrville, OH, US). In phase 3, different protein sources were evaluated. These included beef (70:30 ground beef, Dillons, Hutchinson, KS, US), chicken (Dillons, Hutchinson, KS, US), fish (Salmon, Dillons, Hutchinson, KS, US), tofu (Dillons, Hutchinson, KS, US), and chicken liver (Tyson, Springdale, AZ, US). Starch and grain ingredients were evaluated in phase 4, including whole wheat flour (Gold Medal, Minneapolis, MN, US), chickpea flour (Garbanzo Bean Flour, Bob's Red Mill, Milwaukie, OR, US), potato flour (Bob's Red Mill, Milwaukie, OR, US), corn starch (Argo, Cordova, TN, US), and tapioca flour (Bob's Red Mill, Milwaukie, OR, US). In phase 5, a final test with treats made with combined ingredients from the previous three phases were prepared. In the evaluation of food mixtures, the combinations of the ingredients compared in the previous three phases were studied. The most preferred ingredients in each category (fat, protein and starch) were combined with a basal diet recipe as sample 1. The second most preferred ingredients were combined as sample 2 while the third and fourth most preferred ingredient combination were corresponded to sample 3 and sample 4. The least favored ingredients from each category was combined as sample 5. In this study the five combinations (fat, protein, and starch) were: (1) fish oil, liver, and potato flour; (2) butter, fish, and wheat flour; (3) chicken fat, chicken, and corn starch; (4) shortening, beef, and tapioca flour; (5) lard, tofu, and chickpea flour.

Data Analysis

The ranked data from each of these phases was analyzed by a Friedman analysis of variance (Lawless & Heymann, 2010) using the XLStat version 2010.6.02 (Addinsoft, New York, NY, US). The time differences of each phase were analyzed by one-way ANOVA with SAS® statistical software (SAS® version 9.3, SAS Institute Inc., Cary, NC, US) using PROC GLIMMIX to determine significant differences among samples. The statistical significance of differences was defined as $p \leq 0.05$.

Results and Discussion

Phase 1

Compared to the preliminary test, some main differences were observed. First, the dogs discovered the proper techniques to extract the treat out of the puzzle within the first two days of the training phase. The common techniques used included: (1) bouncing the puzzle by picking it up and dropping it on the floor, (2) rolling the puzzle by pushing it, and (3) reaching inside puzzle with tongue movement. In most cases, combinations of the three techniques were efficiently used. On the last day of the training phase, the dogs who completed the task finished within 3 minutes from being released (Table 3.2). Second, unlike during the preliminary test, most dogs were able to start the test without guidance or attracting them to the puzzles. Since the dogs were the same animals used in the preliminary test it is possible that the memory from the procedure from before was maintained during the 12 month period between tests and suggests this method is capable of extensive long-term and intermittent use. If this method is continuously used on a regular basis, training periods may not be necessary since the dogs would gain the ability of extracting treats/food from the puzzle from the initial training process. As a result, if the relative long training period was a concern for time and technician investment using this method an extensive re-training session may not be necessary. Thirdly, for the validation test, the dogs did not spend as much time sniffing and exploring the testing room as in the preliminary test which may indicate that they became more familiar with the space and were less stressed and curious about the environment.

Based on the experience from the preliminary test, the 1.5m x 1.5m test space formed at the beginning of the study may also contribute to the reduction of time during training process. There were some similar patterns of behavior as in the preliminary test with dogs who failed to complete the test. For example, these dogs ignored the puzzles/treats, interacted with researchers, and sniffed unrelated objects. Most of the dogs who qualified in the preliminary test also showed acceptable performance in this validation test with one exception. The possible reason for the one dog to perform differently in the two tests may potentially be associated with the change of living environment from being housed individually to being housed in pairs which can potentially result in stress.

Table 3.2 The performance evaluation result at the end of phase 1.

DOG	Need Assistance to Sniff Toys Prior To Test (Yes=0, No=1)	Sniff Toys While Choosing (Yes = 1, No=0)	Need Guidance Towards Toys (Yes=0, No=1)	Need to Sniff Treats Directly (Yes=0, No=1)	Interest in Toys (Yes = 1, No=0)	Interest in Treats (Yes = 1, No=0)	Total	Length of Study (Seconds)
F1T	1	1	1	1	1	1	6	90.0
F1P	1	1	1	1	1	1	6	71.0
F2C	1	1	0	0	1	1	4	175.0
F2L	0	0	0	0	0	0	0	n/a*
M4W	0	0	0	0	0	0	0	n/a
M8C	0	1	1	1	1	1	5	98.0
M5P	1	1	1	1	1	1	6	84.0
M5T	0	0	0	0	0	0	0	n/a
M6J	0	1	0	1	1	1	4	180.0
M6P	0	0	1	1	1	1	4	140.0
M7D	0	0	0	0	0	0	0	n/a
M7F	0	0	0	0	0	0	0	n/a

*n/a - Subject did not complete test.

Phase 2

The preference ranking during phase 2 using fats and oils indicated a preference ($p<0.05$) for the fish oil relative to the shortening and lard treatment (Table 3.3). Butter was preferred over lard, but not over chicken fat and shortening. The chicken fat was intermediate in preference ranking and similar to the two extremes. Overall the results supported the preliminary test results and showed more significant differences since the preliminary test only determined the fish oil treatment was preferred over lard. This result supported the theory that butter and fish oil aroma potentially attracted the dogs (Houpt, Hintz & Shepherd, 1978). As was observed in the preliminary test, the chicken fat and lard were not preferred relative to the vegetable shortening. When the data from each day was analyzed separately, no significant preference was observed within duration of the phase.

Table 3.3 The effect of different treatments (fat, protein, starch and complex food) in rank order preference in dogs

Phase	Treatments				
2: Fats and Oils	**Fish Oil** 2.18^{a}	**Butter** 2.72^{ab}	**Vegetable Shortening** 3.32^{bc}	**Chicken Fat** 3.10^{abc}	**Lard** 3.68^{c}
3: Proteins	**Liver** 1.64^{a}	**Chicken** 3.12^{b}	**Fish** 2.57^{b}	**Tofu** 4.32^{c}	**Beef** 3.35^{b}
4: Starches	**Corn** 3.04^{b}	**Wheat** 2.79^{b}	**Tapioca** 3.47^{bc}	**Potato** 1.44^{a}	**Chickpea** 4.27^{c}
5: Complex Food*	**Sample 1** 1.35^{a}	**Sample 2** 2.78^{b}	**Sample 3** 2.80^{bc}	**Sample 4** 3.66^{cd}	**Sample 5** 4.41^{d}

^{abc} indicates that within a row, samples with unlike letters were significantly different ($p<0.05$)
*Sample 1: fish oil, liver, potato flour; Sample 2: butter, fish, wheat flour; Sample 3: chick fat, chicken, corn starch; Sample 4: shortening, beef, tapioca flour; Sample 5: lard, tofu, chickpea flour.

Phase 3

In this study, dogs showed a preference ($p<0.05$) for the chicken liver relative to the all four other treatments and fish, chicken and beef were preferred over tofu (Table 3.3). In the preliminary test, chicken, fish, and tofu were preferred similarly which was unexpected. The result from the validation test showed that animal-based protein was preferred over plant-based protein. This agrees with the research of Houpt & Smith (1981). However, since this result was slightly different from the preliminary test, more replications or tests with other plant-based protein source should be considered in order to determine if animal-based protein sources would actually be generally preferred over plant-based protein sources. Also, the quality of the ingredients and production lots can also potentially affect the results. For each individual day, only day 2 showed that the sample prepared with chicken liver was significantly preferred over the samples with chicken and tofu.

Phase 4

The result of phase 4 was the most unexpected. The evaluation of starches showed that the dogs preferred ($p<0.05$) the potato flour treatment in comparison to the wheat flour, corn starch, tapioca flour and chickpea flour treatments (Table 3.3). The wheat flour and corn flour treatments were preferred over chickpea flour. This result was dramatically different from the preliminary test due to the switch of the position between potato flour and corn flour. Even if grain-added products have more overall aromatics than grain-free products (Koppel, Adhikari & Di Donfrancesco, 2013), the different age of the raw ingredients in the potato flour sample may have changed the intensity of overall aromatics. No existing research was found that would explain if the dogs were attracted to overall aromatics intensity or a specific aroma note in dog food. To further examine the result, additional research should be conducted. On day 5, the dogs significantly preferred the sample prepared with potato flour over sample with tapioca flour and chickpea flour. The preference ranking was not significant for the other four individual days.

Phase 5

The result indicated that sample 1 was significantly preferred over samples 2 to 5 while sample 2 was preferred over sample 4 and 5, sample 3 was preferred over sample 5 ($p<0.0001$;Table 3.3). This result further supported the assumption that the dogs were able to differentiate the ingredients and express their preferences on the types of ingredients as a combination in a similar fashion to the individual ingredients. For each individual day, significant differences on the preferences were observed on day 2, day 3 and day 5. On day 2, sample 1 was only preferred over sample 4 while, on day 3, sample 1 was preferred over both sample 4 and 5. Sample 1 was preferred over sample 2, 4 and 5 on day 5.

Length of tests

After the training phase, the total elapsed time for each dog to complete the test was less than 3 minutes. The reduction of average time was not as obvious like during the preliminary test since the time spent on the beginning of the evaluation phase (phase 2) during validation test was equal or less than phase 2 to 4 in the preliminary test (Figure 3.1). One explanation that phase 5 of the preliminary test was so short could be that the size of the commercial food samples was smaller and the shape was different from the baked treats used in phase 2 to 4. This could result from extracting process being easier and smoother. In the validation test, the dogs did not spend much time exploring the testing environment and may indicate their familiarity with the testing space was maintained, or they may have participated in other studies in that space. Instead of a dramatic decrease in the length of each phase in the validation test, the average length of phase 5 was significantly shorter than phases 3 and 4 (p=0.0003). This generally agreed with the preliminary test except that for phase 2 a shorter adjustment period was needed due to the previous test. However, the differences in the average times across phases was less than 5 seconds; thus, it is reasonable to conclude that once the training was completed, the speed of completing the tests was relatively stable and consistent.

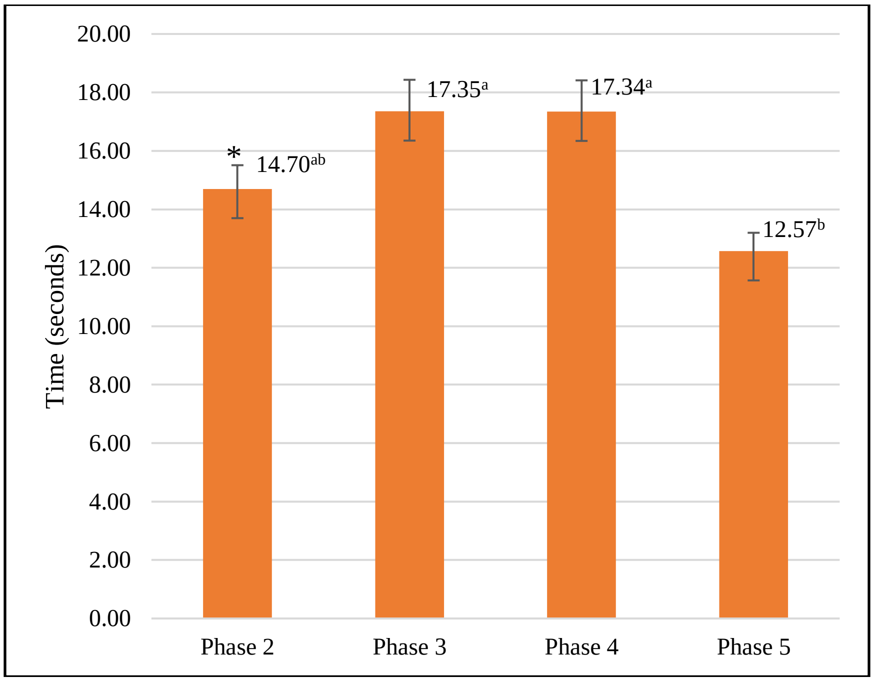

Figure 3.1 Average time spent on each toy from phase 2 to 5 in seconds (p=0.0003)

*Standard error

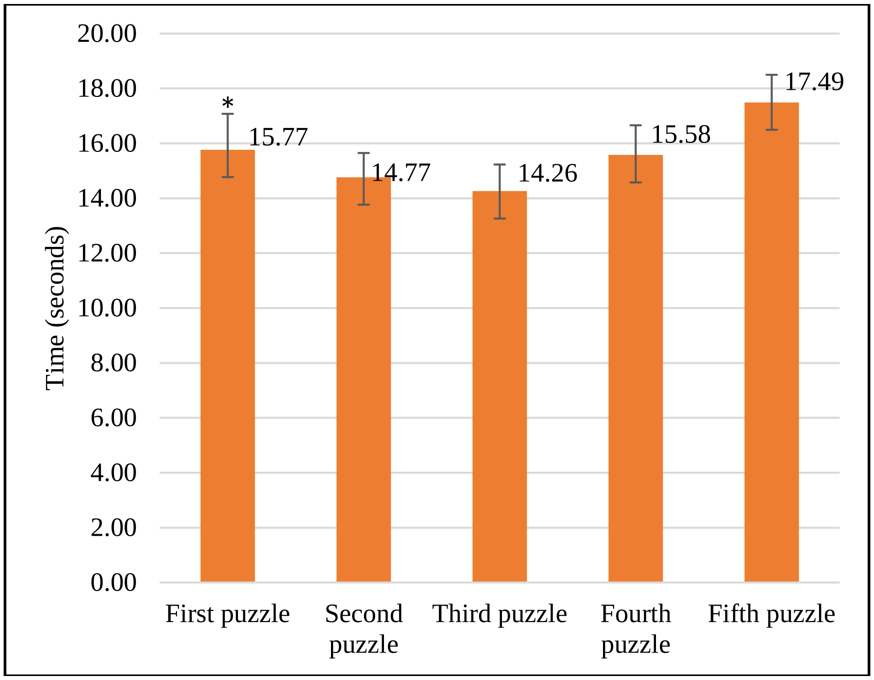

Figure 3.2 Average time spent on each toy in seconds (p=0.1030)
*Standard error

The time the dogs spent between consuming treats were relatively stable (p=0.1030). There was a slight decrease in time between sample from the first toy to the third toy, and a slight increase from the third toy to fifth toy. Obtaining and consuming the first toy/treat after being released by the researcher did not take less time than other treats when comparing to the preliminary test, and the time spent on each toy was more stable than during the preliminary test (Figure 3.2). This could be the result of the increased dog confidence and relaxation levels, and this may have decreased the excitement and stress leading to more focus to complete the test. The dogs were using their own pace to finish the study which possibly helped the decision-making process. For the validation test, the differences in preferences were generally more significant when comparing the preliminary to the validation test and this may be a result of a more relaxed decision-making process.

The composition of the treat could also be a factor that influences the dogs' choices. It is possible that the impact of one category of ingredients (fat/protein/starch) was more substantial on the dogs liking than the other categories. When comparing a high-fat diet to a high-carbohydrate diet, the high-fat diet resulted in higher voluntary dry matter intake and higher calorie intake than the high-carbohydrate diet (Schauf, Salas-Mani, Torre, Bosch, Swarts & Castrillo., 2016). Further application of the preference ranking method with dogs should be conducted to determine what the impact of the ratios of different ingredients might be. Also, a sensory descriptive study with the dog food lexicon published by Di Donfrancesco, Koppel & Chambers (2012) should be conducted in order to determine how the ratios of the ingredients might affect the individual aromatics of the dog food and the overall intensity of the aromatics. With proper statistical analysis, the results from the preference ranking procedure and the descriptive analysis can potentially determine what aromatic notes tend to drive dog liking. Further, additional validation studies need to be conducted using dogs who live in pet owners' homes in order to determine whether the preference ranking procedure can be used to make conclusions beyond the controlled laboratory and upon ingredient composition alone.

One limitation of the preference ranking procedure would be that this method is relatively labor-intensive when compared to the one-pan and two-pan methods which do not necessarily require constant presence of researcher during the tests. In the ranking test, two researchers were required to operate and manage the entire study. During the testing process, one researcher was needed to stay outside of the testing space to record the order and time of the treats being extracted and consumed. The other researcher had to stay in the testing space to remove the puzzle after the treats being extracted to avoid the dogs "revisiting" the empty puzzles. However, the observations from the researchers can potentially provide more insights on the behaviors of the dogs and ensure the accuracy of the tests. Another limitation would be the forced-choice effect is still present even if the treats were not the dogs' main meal of the day and the dogs were given a choice not to consume any treats. Only completed test results were collected for data analysis and the nature of a ranking test is still a force-choice test.

From the results of the preliminary test and the validation test, the preference ranking procedure method is reliable and potentially suitable for continuous use for different studies. The dogs were able to be re-trained to maintain the speed and techniques after a 12 month break period from the initial testing proved this method is extendable. The results from the validation test in general were similar to the results from the preliminary test and showed even more differences among samples thereby proving this method is reliable. Preference ranking studies such as this one also have the advantage of being highly efficient as more samples can be evaluated at the same time. Even if the preference ranking procedure is still a forced-choice test, the fact that the treats was not part of the dogs' daily food allowance, and the hunger influence was eliminated during the test, and they had multiple choices, and a choice not to consume any of the treats.

Conclusion

A validation test for the preference ranking procedure was conducted after the completion of the preliminary test with five phases including training, evaluation with three different categories of ingredients, and evaluation of food with combinations of preferred/not preferred ingredients. The training, evaluation with three different categories of ingredients phases repeated the same procedure and materials as the preliminary test. The preliminary test showed this method could compare dogs' preference on five samples simultaneously and provide a general conclusion on which ingredient was preferred or liked over the other. This validation test which was a semiduplication proved this method is reliable because overall it led to similar results with the preliminary test and the validation even provided more clear-cut results.

References

Aldrich, G.C., & Koppel, K. (2015). Pet Food Palatability Evaluation: A Review of Standard Assay Techniques and Interpretation of Results with a Primary Focus on Limitations. *Animals*. **5(1)**, 43-55.

APPA. (2016). Pet industry market size and ownership statistics. American Pet Products Association, Inc. Greenwich, CT. Available online: http://www.americanpetproducts.org/press_industrytrends.asp (accessed on 08 June 2017).

Boya, U. O., Dotson, M.J., & Hyatt, E. M. (2015). A comparison of dog food choice criteria across dog owner segments: An exploratory study. *International Journal of Consumer Studies*, **39(1)**, 74-82.

Braghieri, A., Piazzolla, N., Carlucci, A., Monteleone, E., Girolami, A., & Napolitano, F. (2012). Development and validation of a quantitative frame of reference for meat sensory evaluation. *Food Quality and Preference*, **25(1)**, 63-68.

Dahl, T., Tomic, O., Wold, J. P., & Næs, T. (2008). Some new tools for visualising multi-way sensory data. *Food Quality and Preference*, **19(1),** 103-113.

Di Donfrancesco, B., Koppel, K., & Chambers IV, E. (2012). An initial lexicon for sensory properties of dry dog food. *Journal of Sensory Studies*, **27**, 498-510.

Dispas, A., Lebrun, P., Ziemons, E., Marini, R., Rozet, E., & Hubert, P. (2014). Evaluation of the quantitative performances of supercritical fluid chromatography: From method development to validation. *Journal of Chromatography A*, **1353**, 78-88

Griffin, R.W. Section IV: Palatability. *In Petfood Technology*, 1st ed.; Kvamme, J.L., Philips, T.D., Eds.; Watt Publishing Co.: Mt. Morris, IL, USA, 2003; pp. 176-193

Houpt, K. A., Hintz, H. F., & Shepherd, P. (1978). Palatability and canine food preference. *Canine Practice*, **5(6)**, 29-35.

Houpt, K. A., & Smith, S. L. (1981). Taste preferences and their relation to obesity in dogs and cats. *Canadian Veterinary Journal*, **21**, 77-81.

Koppel, K., Adhikari, K., & Di Donfrancesco, B. (2013). Volatile compounds in dry dog foods and their influence on sensory aromatic profile. *Molecules*, **18(3)**, 2646-2662.

Lawless, H. T., & Heymann, H. (2010). Sensory evaluation of foods: principles and practices, 2nd edition, pp. 501-503. Springer, New York, NY, USA.

Moelich, E. I., Muller, M., Joubert, E., Næs, T., & Kidd, M. (in press). Validation of projective mapping as potential sensory screening tool for application by the honeybush herbal tea industry. *Food Research International*.

Schauf, S., Salas-Mani, A., Torre, C., Bosch, G., Swarts, H., & Castrillo, C. (2016) Effect of sterilization and of dietary fat and carbohydrate content on food intake, activity level, and blood satiety–related hormones in female dogs. *Journal of Animal Science*, **94(10)**, 4239-4250.

Smith, J.C., Rashotte, M.E., Austin, T. & Griffin, R.W. (1984). Fine-grained measures of dogs' eating behavior in single-pan and two-pan tests. *Neuroscience and Biobehavioral Reviews*, **8(2),** 243-251.

Chapter 4 - Determination of drivers of dogs' preference for treats

Abstract

Palatability of pet food is one of the most important factors that influences pet owners' food purchase decisions. A preference ranking procedure with dogs has been proposed and validated as a method to compare dogs' preference on food for up to five samples (Chapter 2, 3). The objectives of this study are to use descriptive sensory analysis to (1) compile profiles for the treats used in the development of the ranking method in order to gain insights on what aromatics characteristics possibly affected the dogs' preference; (2) study the shelf-life of the treats to determine if significant changes took place during the five days of evaluation. Five highly trained panelists profiled the aroma of the samples and the data was analyzed with the preference results collected from the dogs. The external preference maps showed that fish and meaty aromatics tended to be liked by the dogs and attributes such as grain and musty/dusty appeared to be disliked by the dogs. The shelf-life study indicated oxidized oil, cardboard, stale and rancid aromatics changed in some samples throughout the five days evaluation period which may have affected preferences of the dogs.

Introduction

In the past five years, the annual sales in the pet industry increased nearly 16 billion dollars in the U.S. market and pet food had the highest share of the total pet-related expenses (APPA 2016). The top four factors that affect pet owners' purchase-decision for pet food are the price, natural ingredients, country of origin (U.S.) and taste (Mintel 2016). When pets enjoy the taste of the food, the pet owners would be more likely to make the decision of repurchasing the same product.

Several studies have explored what ingredients/formulation/process method that pets prefer (Fragua, Barroeta, Manzanilla, Codony & Villaverde, 2015; Houpt, Hintz & Shepherd, 1978; Houpt & Smith, 1987, Manabe, Matsumara & Fushiki, 2010; Verbrugghe, Hesta, Gulbrandson & Janssens, 2007) but no study has determined what factors drive pets' liking. To do so, a sensory test would need to be conducted (Houpt et al., 1978). The existing methods include the one-pan method (Griffin, 2003) and two-pan method (Smith, Rashotte, Austin & Griffin, 1984), that have been commonly used (Aldrich & Koppel, 2015). The cognitive palatability assessment protocol (CPAP) (Araujo & Milgram, 2004) and the operant lever-press test (Green &Rashotte, 1984; Rashotte et al., 1984) have also been proposed and compared with the previous two methods. However, even if those methods were able to compare the preferences of the subjects (dogs), the limitations include: (1) all of the tests method are essentially a forced-choice method which may not reflect the subjects' true preferences (Chapter 2), (2) and with these methods only up to two samples can be evaluated at a time, efficiency is low and may be costly for the industry. As a result, a preference ranking procedure was proposed and validated (Chapter 2 and 3). This method provides the opportunity to evaluate up to five samples at a time and with less bias from the forced-choice decisions.

With the results from the preference ranking procedure (Chapter 2), the researchers discovered that the dogs indeed did have preferences over the test samples but there are many questions that still need to be answered. To start, it is difficult to determine if the subjects were attracted to a specific aromatic note or they were simply choosing the sample that smelled most intensely. Human descriptive panel has been used to evaluate pet food products (Chanadang, Koppel & Aldrich., 2016; Koppel, Gibson, Alavi & Aldrich, 2014b; Koppel, Monti, Gibson, Alavi, Di Donfrancesco & Carciofi, 2015b) and a descriptive lexicon for dry dog food was developed to assist researchers to understand sensory characteristics of dry dog food (Di Donfrancesco, Koppel & Chambers, 2012). In order to answer the questions about the effects of the aromatics properties of the samples on the dogs' consumption decision, a human descriptive sensory analysis may be an appropriate method to use. By comparing the sensory profile provided by human panelists and the preference scores of the dogs, a rapid, quantitative and predictive indication of the effects of ingredient and processing changes of products may be enabled, as suggested by Pickering (2008).This type of method has been used for human consumer studies in order to discover what attributes potentially drive consumers' liking and external preference mapping has been commonly used as a visualizing tool to analyze and explain the results regarding what sensory characteristics positively/negatively affected consumer's liking for specific products like ice cream and beef (Cadena, Cruz, Faria & Bolini, 2012; Sasaki, Ooi, Nagura, Motoyama, Narita, Oe et al., 2017).

In this study, the aromatic characteristics of the commercial samples and the lab-made treat samples that were used in the development of the preference ranking procedure for dogs were studied. The objectives of this study were to: (1) determine the aromatic sensory profile of the samples for ranking tests; (2) describe the sensory property changes of the lab-made treats throughout the five days testing period; (3) combine the descriptive analysis result with the preference ranking procedure result to explore the drivers of dogs' liking.

Materials and Methods

Samples

A preference ranking procedure was developed and validated to provide option for palatability testing (Chapter 2, 3). The procedure evaluated five samples tested simultaneously over five consecutive days. The order in which the dog extracted and consumed a flavored treat from a puzzle toy was considered the rank of dogs' preferences. After the training phase, the preference of different sources of fat, protein, starch, and the combinations of them were studied in four phases. During the process of the preference ranking procedure for dogs' development, three commercial products including Great Choice dog treats assorted flavors (Phoenix, AZ, US), Classic Original Assortment and P-Nuttier by Old Mother Hubbard Baking Co. (OMH) (Tewksbury, MA, US) were used in the training phase.

In phases 2 to 4, baked treats were made in Center for Sensory Analysis and Consumer Behavior at Kansas State University one day prior for the descriptive evaluation since all treats for dog studies were also made one day prior or the first day of the evaluation (Table 4.1). The treats were made by first combining the dry ingredients and then cutting the shortening/fat in to the mix. Then the liquid ingredients were mixed respectively and slowly added into the dry ingredients, and then mixed by hand until the ingredients were combined until formed a dough. The dough was rolled into a 10 mm thick sheet and cut into 15 mm*20 mm rectangles. These were placed on a baking sheet with parchment paper and baked in a convection oven at 175°C for 15 minutes. One the treats cooled down to room temperature, the treats were stored in gallon-size plastic bags (Ziploc, S.C. Johnson, Racine, WI, US) until used in the evaluation.

Table 4.1 Ingredient composition for treats evaluated by dogs in phases 2-5.

Ingredient	**Phase 2 % composition**	**Phase 3 % composition**	**Phase 4 %composition**	**Phase 5 %composition**
Whole wheat Flour (Starch source replacement)	10.08	24.33	28.82***	25.84***
White (all purpose) flour	40.03	24.33	2.88	25.84
Corn meal	12.59	12.16	0.00	0.00
Salt	0.50	0.49	0.58	0.52
Baking soda	0.25	0.24	0.29	0.26
Dry milk	1.51	1.46	1.73	1.55
Sodium bisulfate	0.002	0.002	0.002	0.002
Dry yeast	0.002	0.002	0.002	0.002
Shortening (Fat source replacement)	7.56*	7.30	8.65	7.75*
Molasses	4.03	3.89	4.61	4.13
Water	23.17	16.06	26.51	23.77
Meat (protein source replacement)	0.00	9.73**	0.00	10.34**

* Fat replacement included vegetable shortening, fish oil, chicken fat, butter, or lard
** Protein included fish, beef, chicken, liver, or tofu
*** Starch included whole wheat flour, tapioca flour, potato flour, corn starch, or chickpea flour

Table 4.2 Attributes, definition and references for descriptive analysis

Attribute	Definition	Reference**
Overall aroma intensity	The overall impression of aromatics that may or may not include meat or grain like aromatics.	Grain mix = 5.0 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. Place 1 Tablespoon in snifter.
Barnyard	Combination of pungent, slightly sour, hay-like aromatics associated with farm animals and the inside of a barn.	White pepper in Swanson Chicken Broth 99% Fat Free (0.90g /300ml) = 6.0. Preparation: Steep 0.90 g of ground white pepper in 300 ml of Swanson Chicken Broth at 180 F for 15 min. Filter the solution and let cool for 10 min. Serve ¼ cup in snifter.
Toasted	A moderately browned/baked impression	General Mills Cheerios crushed = 7.0. Preparation: ¼ cup crushed Cheerios in snifter
Baked	A medium brown aromatic with a cooked impression associate with baked products.	Crushed shredded wheat = 5.0 Preparation: Crush shredded wheat and serve 1 tablespoon in snifters.
Brown	A sharp, caramel, almost-burnt aromatic (a part of the grain complex).	Crushed Toasted Grape nuts = 5.5. Preparation: Place 1/2 cup Grape Nuts onto parchment lined cookie sheet. Toast at 350°F for 7 minutes. Crush with mortar. Cool and serve 1 Table spoon in snifter.
Grain	The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice and oats.	Cereal Mix (dry) =5.0. 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. Place 1 Tablespoon in snifter.
Soy	Flavor associated with soybeans or soy products.	Soy nuts (Hy-Vee Bulk) = 4.5. Preparation: Serve 1 Teaspoon in snifter.
Vitamin	The aromatics associated with a just opened bottle of vitamin pills (generally thought to be oxidized thiamin)	Nature Made Super B-Complex capsule = 10.0. Preparation: Place 1 vitamin pill in snifter.
Musty/Dusty	A dry aromatic associated with stored dry grain.	Kretschner Wheat Germ = 5.0. Preparation: Serve 1 tablespoon wheat germ in snifter.
Fish	An overall impression of fishy associated with fresh, died, or canned fish.	Nature Made Fish oil pill: 8.0. Preparation: Place the liquid content of 1 pill in a snifter.
Meaty	A measure of how much a sample is recognized as distinctly animal muscle tissue, including poultry, seafood/fish, and beef.	Canned Swanson Beef Broth 99% fat free = 5.0. Preparation: Place 1 Tablespoon in snifter.
Liver	Aromatics associated with cooked organ meat/liver.	Beef liver = 6.0. Preparation: Pan-fry beef liver until an internal temperature of 160F. Chop and serve 1 Tablespoon in snifter.

Oxidized Oil*	The aromatic associated with aged or highly used oil and fat.	Microwave Oven Heated Wesson Vegetable Oil = 6.0. 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. Repeat three times. Let beaker sit on counter uncovered overnight. Serve 1 Tablespoon of the oil in snifter.
Cardboard*	The aromatic associated with cardboard or paper packaging. The intensity rating is only for the 'cardboardy' character within the reference.	Cardboard = 7.5. Preparation: 2" cardboard square in 1/2 Cup of water. Serve in snifter.
Stale*	The aromatics associated with wet cardboard that is characterized by a lack of freshness.	Mama Mary's Pizza Crust = 4.5. Preparation: Serve 1 piece of 2" crust square in snifter.
Plastic*	An aromatic associated with plastic polyethylene containers or food stored in plastic	Ziploc bag = 3.5. Preparation: Serve 1 snack size Ziploc bag in snifter.
Rancid*	A somewhat heavy aromatic characteristic of old, oxidized, decomposing fat and oil. The aromatics may include painty, varnish, or fishy.	Microwaved Wesson vegetable oil (4 min at high) = 2.5. Preparation: Microwave 1.5 cups oil on high power for 4 minutes. Let cool and Serve ¼ cup in snifter. Microwaved Wesson vegetable oil (5 min at high) = 5.0. Preparation: Microwave 1.5 cups oil on high power for 5 minutes. Let cool and Serve ¼ cup in snifter.
Overall Sweet	The perception of the combination of sweet taste, sweet aromatics, caramelized, brown sugar, honey, maple syrup etc.	Lorna Doone Cookie = 5.5. Preparation: Serve 1 crushed cookie in in snifter.
Vegetable complex	A general term to describe a combination of cooked vegetable aromatics that may include celery, carrot, potato or other vegetables.	Swanson vegetable broth = 6.0. Preparation: Place 1 tablespoon of broth in snifter.

*Attributes evaluated for shelf-life study.

** All references were served in medium snifters covered with watch glass at room temperature.

For each of the phases 2 to 4, in each recipe, one ingredient was altered with different options within the ingredient category (Table 4.2). In phase 2, fats and oils were the selected categories of ingredients to be evaluated: fish oil (Omega Protein, Houston, TX, US), chicken fat (IDF Inc., Springfield, MO, US), lard (Armour, Phoenix, AZ, US), butter (Land O' Lakes, Minneapolis, MN, US), and vegetable shortening (Crisco, Orrville, OH, US). In phase 3, different protein sources were evaluated. These included beef (70:30 ground beef, Dillons, Hutchinson, KS, US), chicken (Dillons, Hutchinson, KS, US), fish (Salmon, Dillons, Hutchinson, KS, US), tofu (Dillons, Hutchinson, KS, US), and chicken liver (Tyson, Springdale, AZ, US). Starch and grain ingredients were evaluated in phase 4, including whole wheat flour (Gold Medal, Minneapolis, MN, US), chickpea flour (Garbanzo Bean Flour, Bob's Red Mill, Milwaukie, OR, US), potato flour (Bob's Red Mill, Milwaukie, OR, US), corn starch (Argo, Cordova, TN, US), and tapioca flour (Bob's Red Mill, Milwaukie, OR, US). In phase 5, a final test with treats made with combined ingredients from in the last three phases was conducted. The protein, fat and starch ingredients were combined based on the rank of the ingredients from the last four phases with the ingredients from the same sources. For instance, the most preferred ingredients from the previous three phases were combined in the same sample, the least favored ingredients were combined as another sample. In this study the five combinations (fat, protein, starch) were: (1) fish oil, liver, potato flour; (2) butter, fish, wheat flour; (3) chicken fat, chicken, corn starch; (4) shortening, beef, tapioca flour; (5) lard, tofu, chickpea flour. Most ingredients were purchased from local grocery store (Dillon's, Manhattan, KS) and all ingredients were human food grade.

Shelf-life

Approximately 40 treats were stored in plastic bags (Ziploc, S.C. Johnson, Racine, WI, US) under ambient environmental conditions (approximately 20°C; 60% R.H.) and labeled with their respective treatment and production date for shelf-life study. The main sensory aromatic characteristics that reflect aging were studied over a period of 5 days. From day 1 to day 5 after the day of production, the important sensory characteristics were studied in duplicates. The samples evaluated by the descriptive panel were not the same batch that was used for the dog study due to the scheduling of the tests. However, the procedure and ingredients were consistent with what the dogs received.

Panelists

The descriptive analysis and shelf-life study was conducted with 5 highly trained panelists from the Center for Sensory Analysis and Consumer Behavior in Kansas State University. All panelists had completed 120h of general descriptive sensory analysis training with a variety of different food products. Each panelist participated in this study had more than 1,000h of testing experience with a large range of food products. The majority of the panelists had experience working with pet food products. Panelists received one 1.5 hour orientation session using samples included in the study in order to finalize the attribute lists and familiarize the samples. Since the samples were similar in terms of formulation and process method, no further orientation was conducted for shelf-life study.

Each evaluation session was 1.5h. Only the aromatics of the samples were evaluated (Table 4.2) since that was the main factor that affected the dogs' preference decision. One piece of each sample was served in a covered medium snifter for aroma evaluation. The attributes list was developed based on the existing dry dog food lexicon published by Di Donfrancesco et al. (2012). The panelists chose the attributes that were more applicable for the samples used in this study and also added the overall aroma intensity due to the question about if the dogs were attracted to the more intense smell or a specific aromatic note (Chapter 2). The shelf-life attributes selected (rancid, oxidized oil, stale, cardboard, etc.) were evaluated by other study to monitor the quality change during storage time (Chanadang et al. 2016).

A numeric scale from 0 to 15 (0-none, 15-extrmemly high) with 0.5 increments was used for evaluation. For the descriptive profiling of the samples, the consensus method was applied in order to compile a descriptive profile that was agreed by all panelists. Similar approach was used by Koppel et al. (2014a, 2015a), Cherdchu, Chambers & Suwonsichon (2013), Di Donfrancesco et al. (2012) and Chambers, Lee, Chun & Miller (2012). Panelists first evaluated the sample individually, panel leader then led a discussion and reached an agreement on the intensity of each attribute. For the shelf-life study, individual evaluation was used in duplicate due to the sensitivity of this method (Chanadang et al., 2016; Lee & Chambers, 2010). The individual evaluation is able to capture minor differences and determine if the difference is significant ($p<0.05$). The order of samples was completely randomized. The testing was approved by Kansas State University Internal Review Board for Protection of Human Subjects (IRB #8634).

Data Analysis

The shelf-life data was analyzed by repeated-measures analysis over time with SAS® statistical software (SAS® version 9.3, SAS Institute Inc., Cary, NC, US) using PROC GLIMMIX to determine significant differences across the five days evaluation periods and samples. In the model, sample, day of evaluation and their interaction were considered as fixed effects while replication and panelist were considered as random effects. The statistical significance of differences was defined as $p \leq 0.05$. The significant effects of the day of evaluation were determined by Fishers protected Least Significant Difference (LSD).

The ranked data collected in the dog study was converted into preference scores of 1-5. The first treat the dogs chose was considered as the highest preference score (5). The last one chosen was given the lowest preference score (1). Then the preference ranking data from the previous ranking test (Chapter 3) was analyzed with the descriptive aroma profiles with XLStat version 2015.3.01(Addinsoft, New York, NY, USA) using descriptive data (X-matrix) and dog ranking data (Y-matrix) to create external preference mapping to potentially discover the drivers of liking for dogs. The attributes which were scored as zero for all samples in each phase was excluded from the map.

Results and Discussion

Descriptive profiling and External Preference Mapping

Commercial products

Total of 13 aromatic attributes were identified and quantified in the samples tested (Figure 4.1). Di Donfrancesco et al. (2012) stated that barnyard was one of the most common attributes in the commercial products in the U.S. market. Barnyard aromatics were detected in all commercial samples. Comparing the three commercial treats, the overall aroma intensity of the OMH classic assortment and the Great Choice samples were lower than the P-nuttier sample. Slightly stale notes presented in the OMH classic assortment and the Great Choice samples. The low aroma intensity and stale notes could be due to the different time point of evaluation. The P-Nuttier was evaluation 3 day after opening while the other two were tested after 2 months of opening. The P-Nuttier sample was the only sample had vegetable complex note detected among all commercial and lab-made samples. The OMH Classic Assortment was the only commercial sample that did not have vitamin and musty/dusty note and the Great choice dog treat was the only sample that contained meaty aromatics.

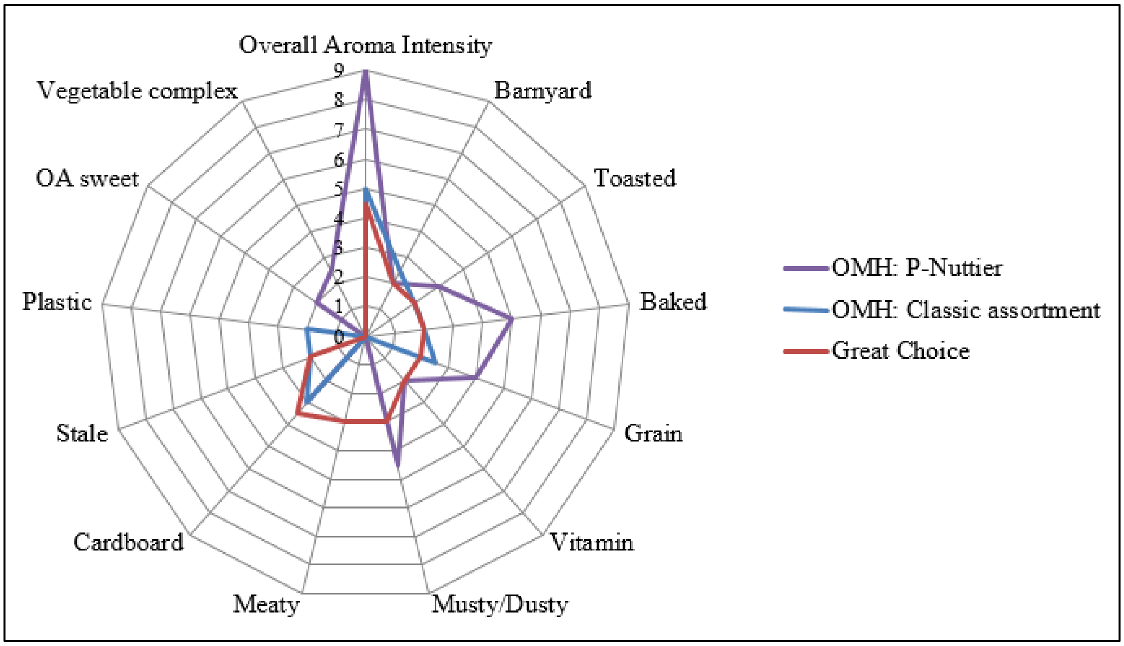

Figure 4.1 Spider plot for samples used in phase 1

*Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high.

Fat sources

The samples used in phase 2 were made with fat sources (lard, butter, fish oil, chicken fat and vegetable shortening). Many attributes showed a difference of more than 2 points across samples including overall aroma intensity, toasted, baked, brown, musty/dusty, fish, meaty, cardboard, and overall sweet aromatics (Figure 4.2). The sample prepared with fish oil had the highest overall aroma intensity among other samples and was the only sample in which fish and meaty aromatics were detected. Due to the definition of the meaty aroma, seafood/fish aroma is considered as meaty together with chicken and beef which determined the sample prepared with fish oil was the only sample had meaty aroma. The sample prepared with butter was the highest on baked, musty/dusty, cardboard and overall sweet aromatics and lowest on brown and overall aroma intensity together with the lard sample. The external preference map (Figure 4.3) showed that the dogs appeared to prefer the samples with fish or meaty aromatics and dislike the samples with toasted and grain aromatics. Sandgruber & Buettner (2012) evaluated fish oil supplements with human descriptive panel and the panelists described the fish oil samples with attributes including fatty, fish-like, geranium leaf-like, grassy-green, malty, metallic, oily, rancid, rancid-oil-like, seawater-like and tang-like. Most of those attributes were not identified in the baked sample prepared with fish oil expect fish-like.

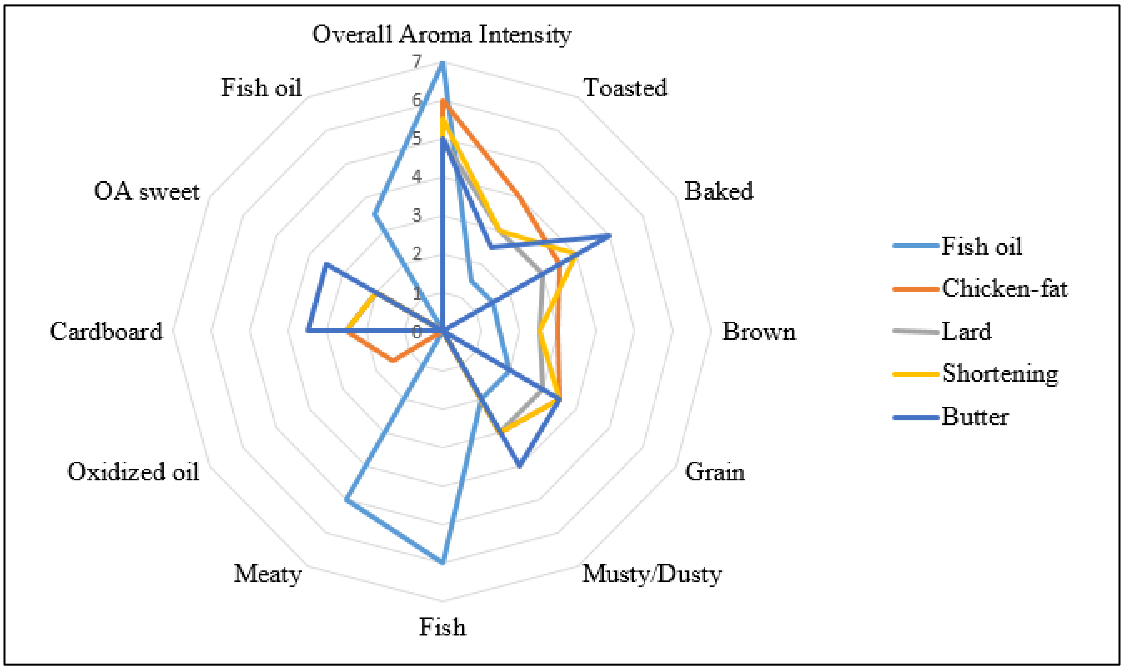

Figure 4.2 Spider plot for fat source samples

*Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high.

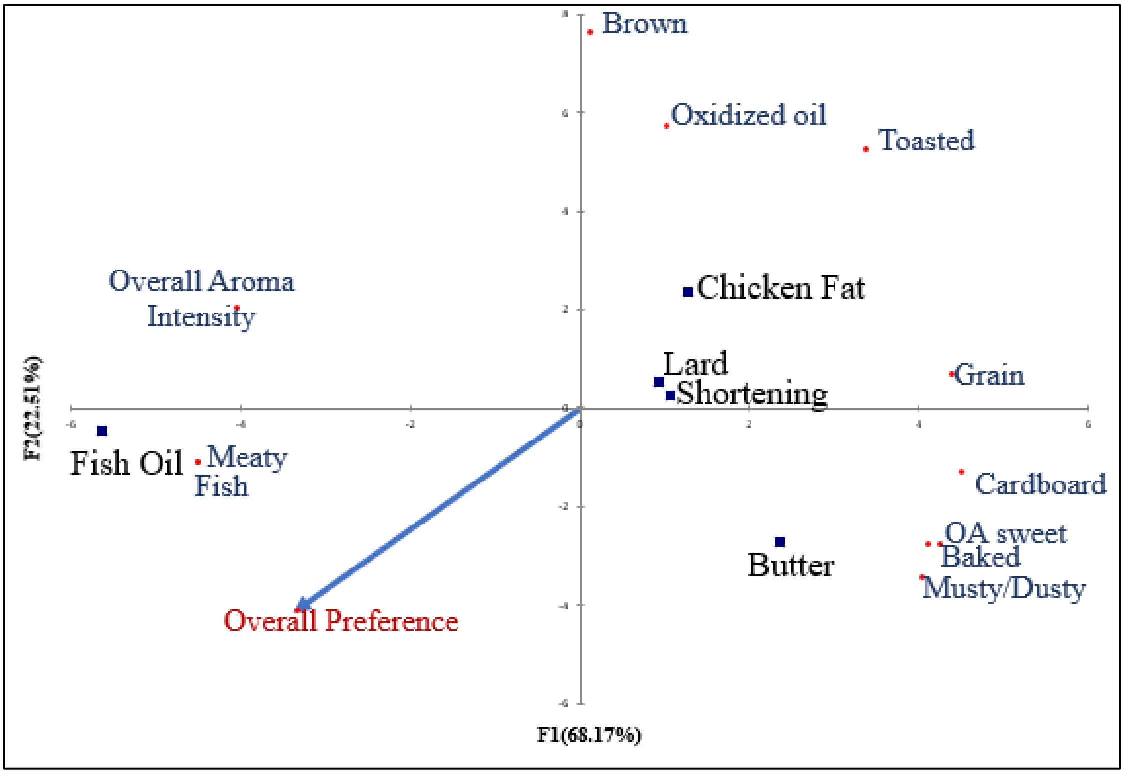

Figure 4.3 External Preference map of fat source samples

Protein sources

The sample with beef was scored highest on overall aroma intensity, toasted, baked, brown, grain, musty/dusty and overall sweet (Figure 4.4). None of the samples were noted as meaty except the sample prepared with fish meat due to the definition of meaty. The intensity of meaty and fish aromatics was lower than the samples prepared with fish oil in phase 2. Surprisingly the liver aromatics of the liver sample did not appear after baking. The sample prepared with tofu, the only plant-based protein source, had the second highest overall aroma intensity and toasted, grain, and musty/dusty aromatics and it was one of the highest samples on baked, cardboard and overall sweet. Soy aromatic was not detected in the sample prepared with tofu. The external preference map (Figure 4.5) indicated that brown aromatics were preferred over cardboard, grain and musty/dusty aromatics. However, even if brown aromatics appeared to be preferred by the dogs, the sample with beef was not the most preferred sample. The musty/dusty and grain aromatics may have affected the overall liking on this particular sample.

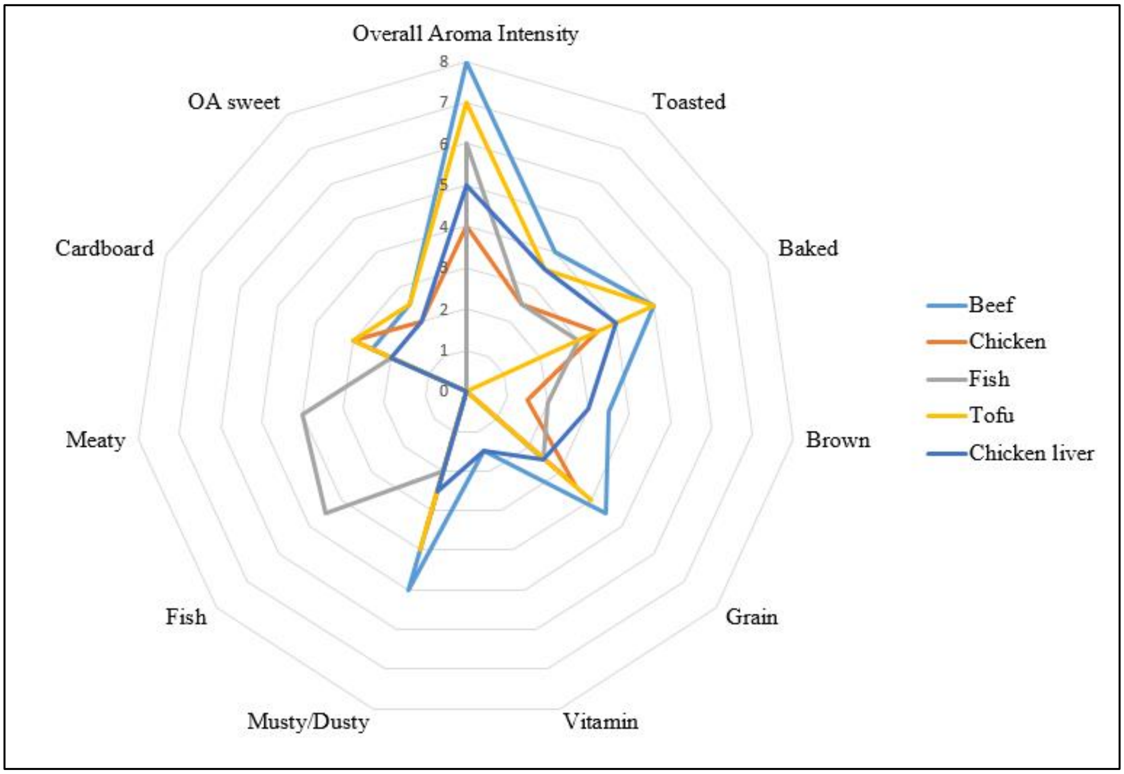

Figure 4.4 Spider plot for protein source samples
*Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high

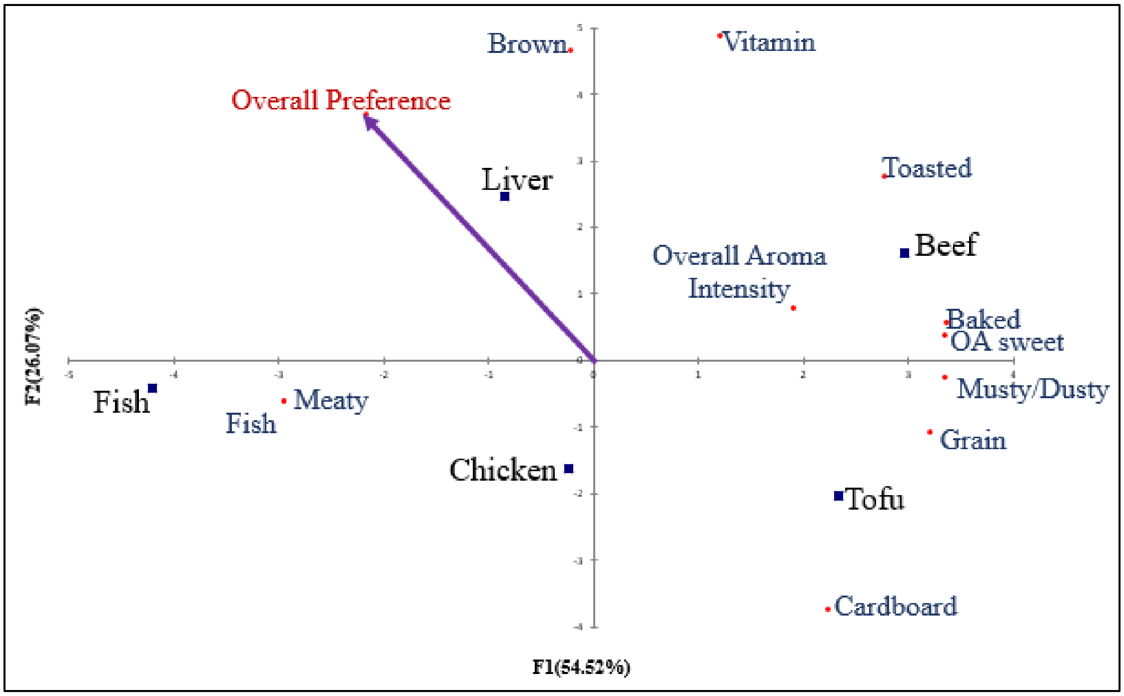

Figure 4.5 External Preference map for protein source samples

Starch sources

Other than overall aroma intensity, only seven attributes were detected from the samples in this phase. The attributes included toasted, baked, brown, grain, musty/dusty, cardboard, and overall sweet aromatics. The sample prepared with potato flour had the highest intensity on overall aroma, toasted, baked, brown, grain, musty/dusty and overall sweet aromatics (Figure 4.6). The corn starch sample was scored the lowest on overall aroma intensity, grain aromatics with the absence of cardboard, brown and overall sweet aromatics. The external preference map (Figure 4.7) indicated that the samples with toasted, musty/dusty, grain and overall sweet aromatics was preferred over cardboard and baked aromatics. The map also indicated the sample prepared with tapioca flour was least liked by the dogs. The profile of the tapioca flour and the corn starch flour were fairly similar, the difference of attributes intensities was less or equal to 0.5 point for all attributes except that the tapioca flour sample was noted to have cardboard aromatics. This agreed with the trend appeared on the external preference map. However, limited attributes were applied to the starch-alternated samples which indicated that the samples were relatively simple.

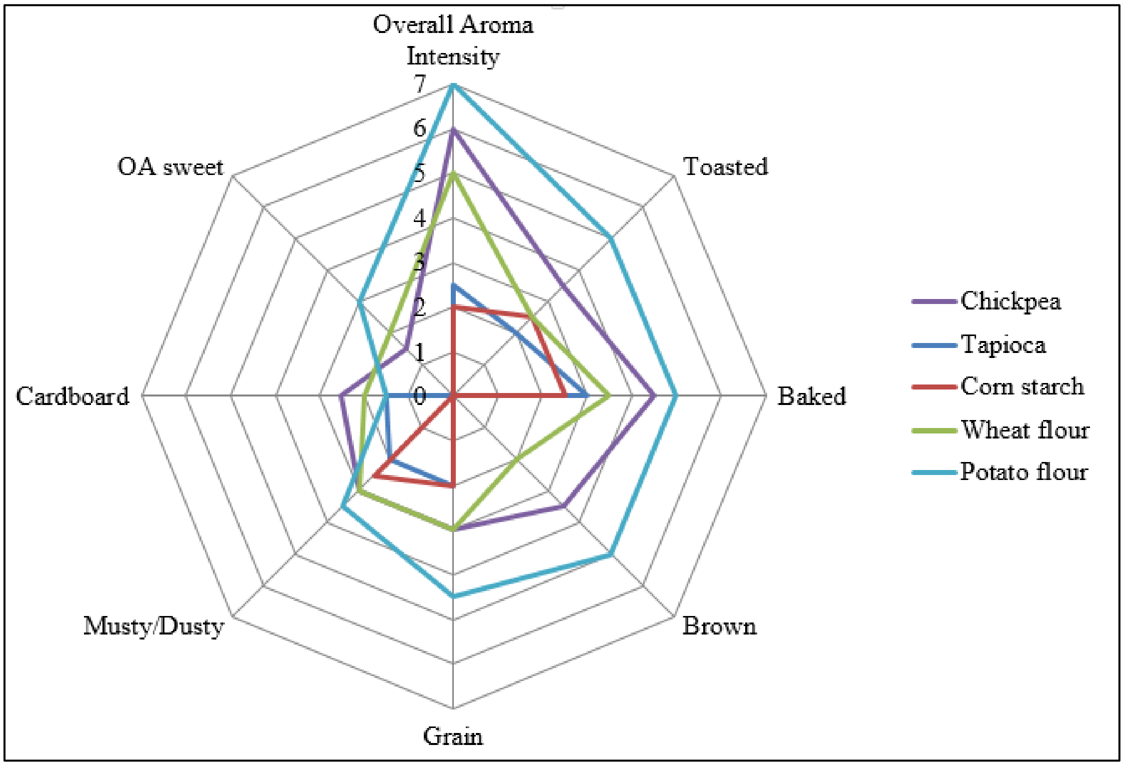

Figure 4.6 Spider plot for Starch source samples

*Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high

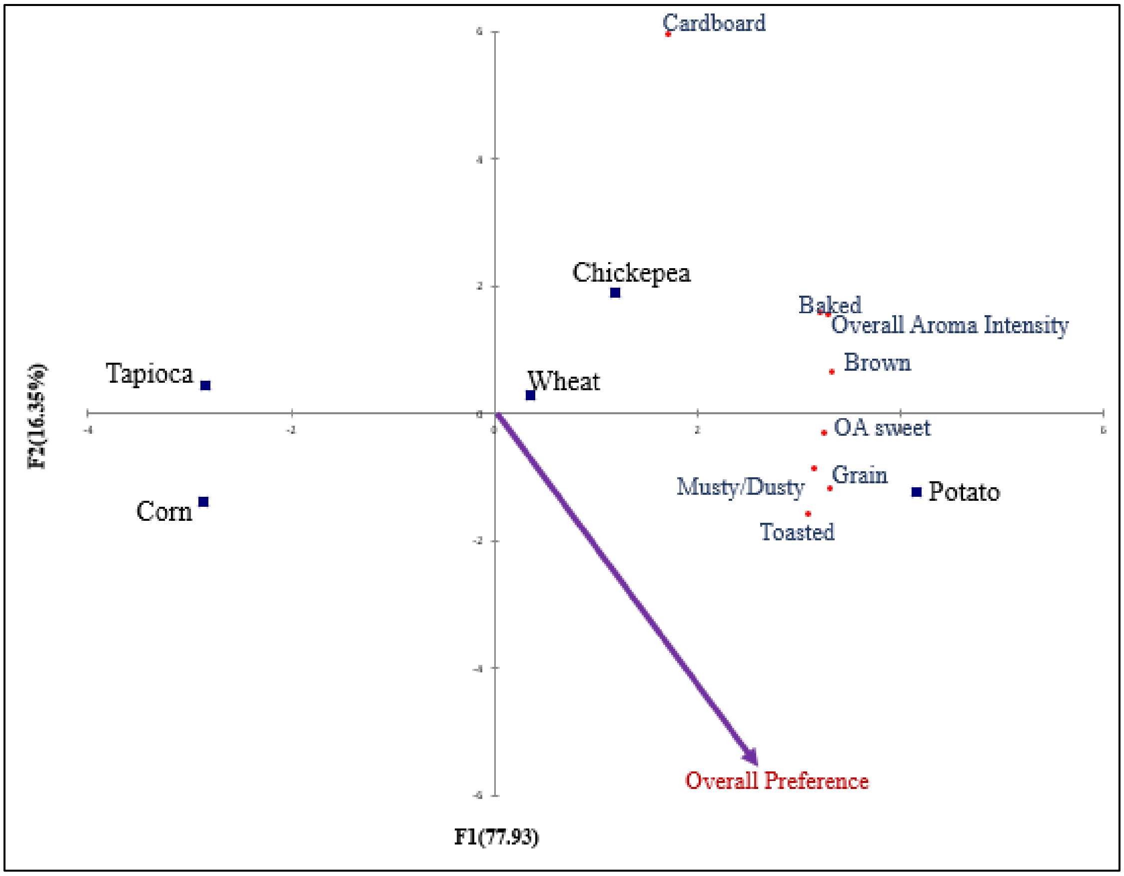

Figure 4.7 External Preference map of starch source samples

Complex foods

The overall aroma intensity of sample 1 made with fish oil (fat source), chicken liver (protein source) and potato flour (starch source) was much higher than the other samples (Figure 4.8). It was also scored the highest on fish and meaty and lowest on toasted, grain, musty/dusty, cardboard and overall sweet. Sample 5 which was made with lard, tofu and chickpea flour was the lowest on overall aroma intensity but higher in toasted, baked and brown aromatics than other samples. Sample 2 made with butter, fish, wheat flour, sample 3 made with chicken fat, chicken, corn starch and sample 4 made with vegetable shortening, beef and tapioca flour had the same overall aroma intensity but were different on specific attributes such as grain, musty/dusty, fish/meaty, toasted and baked. For the complex diet, the external preference map (Figure 4.9) showed that attributes such as overall aroma intensity, fish and meaty aromatics were potentially the drivers of liking and attributes such as toasted, baked, musty/dusty, overall sweet and cardboard were disliked by the dogs.

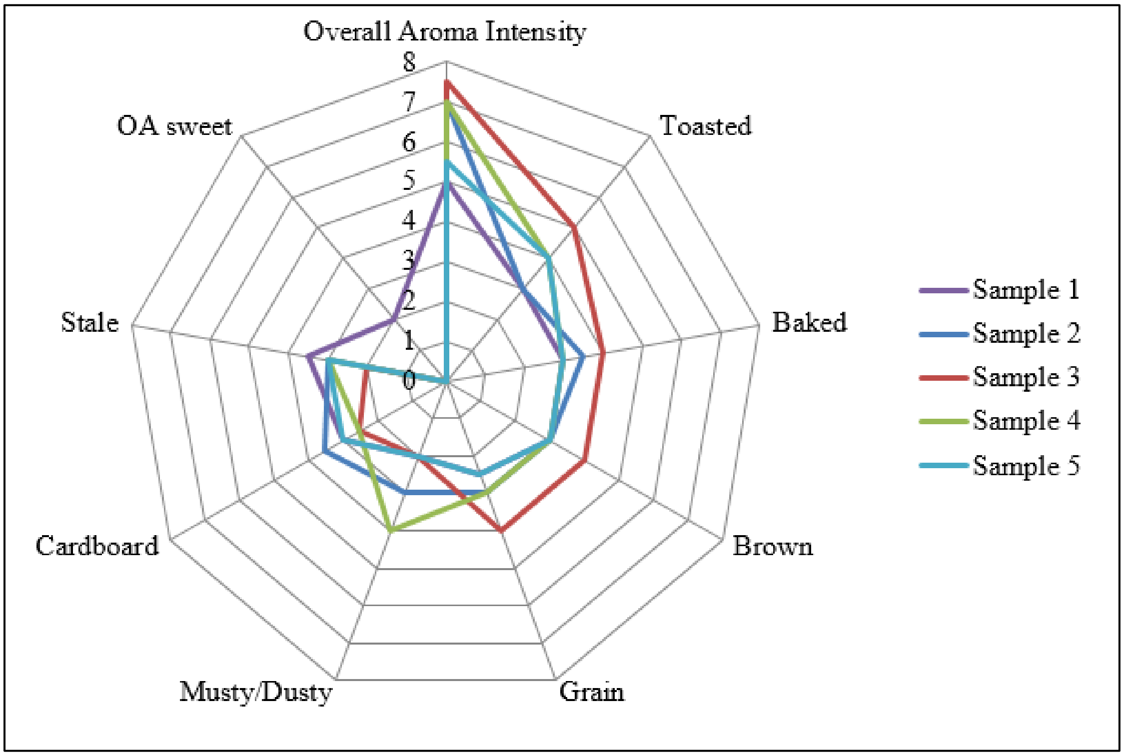

Figure 4.8 Spider plot for Complex diet

*Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high.

**Sample 1: fish oil, liver, potato flour; Sample 2: butter, fish, wheat flour; Sample 3: chick fat, chicken, corn starch; Sample 4: shortening, beef, tapioca four; Sample 5: lard, tofu, chickpea flour.

Unlike in the commercial products, barnyard aromatics were not detected in the treats used for phases 2 to 5. This suggested that the samples prepared in the lab may not be the perfect representative for the products in the market. For the other attributes, the intensity of the commercial samples generally fell in the range of intensity of the other treats.

Considering all the external preference maps created in each phase, the maps generally agreed that attributes such as fish and meaty aromatics were generally associated with dogs' liking. Brown was also preferred with the absence of meaty/fish aromatics. Grain, musty/dusty, and cardboard generally appeared to be disliked by the dogs. Based on the external preference maps, overall aroma intensity did not appear to be the factor that drives dogs' liking. Even if dogs' food choices are initially based on the intensity of the aromatics (Houpt & Hintz, 1978), the samples with the highest aroma intensity were not always preferred by the dogs. However, this map would not be applied to all dogs since the subjects were all beagle dogs, and different breeds and types of dogs' feeding behaviors differ from each other (Bradshaw, 1991). Other than that, many other factors can influence the dogs' liking including the individual variations of the animals, the previous exposure to diets, experience, etc. (Rofe & Anderson, 1970). In conclusion, the results from the study could be utilized as a reference for future studies but it may not predict what attributes all dogs prefer.

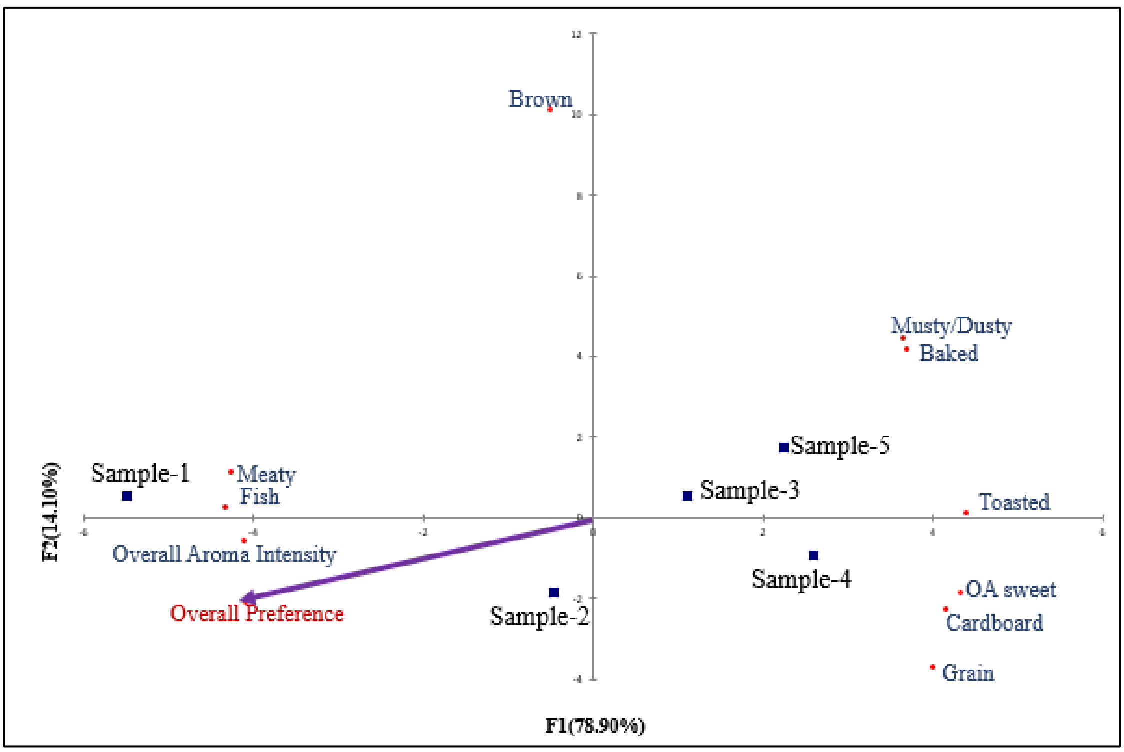

Figure 4.9 External Preference map for complex diet

* Sample 1: fish oil, liver, potato flour; Sample 2: butter, fish, wheat flour; Sample 3: chicken fat, chicken, corn starch; Sample 4: shortening, beef, tapioca flour; Sample 5: lard, tofu, chickpea flour.

Shelf-life

Fat sources

None of the samples developed rancid aromatic throughout the five days period. The fat source × day of evaluation interaction was significant in all attributes ($p \leq 0.05$) identified in samples which showed that the fat sources respond to the storage time differently (Table 4.3). For the five fat sources, significant differences were present in all attributes except plastic and rancid. The oxidized oil aromatics of the sample prepared with chicken fat on the fifth day of the study was the only sample that scored an average more than one point in the whole study. Sample prepared with fish oil and lard had slight oxidized oil note between the first and last day of the evaluation. Even if fish oil is considered highly prone to oxidation due to the high PUFA (Sullivan & Budge, 2012), the sample with fish oil did not show a dramatic increase in any of the attributes expect the stale aromatics on the fourth day of the evaluation. Stale aromatics significantly changed within the five days period for all samples which cardboard aromatics of samples varied on samples. The sample prepared with chicken fat was significantly higher on cardboard and stale on most days than all other samples. The sample prepared with fish oil was the lowest on cardboard and stale for most days. The stale aromatics had some significant changes through five days, the intensity of it was higher on day 2 and day 4 comparing to day 1 and 3 for most samples. The intensity of stale of butter, fish oil and lard were the highest on day four while shortening and chicken fat had the highest stale note on day 5. Since the dogs showed no significant preference on each individual day (Chapter 3), the effect of the change in aroma profile on the preference of the dogs was unknown.

Table 4.3Mean intensity scores[1] of attributes for fat sources samples over shelf-life.

Aroma attributes	Day of evaluation	Fat sources					p-level[2]		
		Butter	Chicken fat	Fish oil	Lard	Shortening	Fat source(F)	Day of evaluation(D)	F × D
Oxidized Oil	1	0.00	$0.00^{B,3}$	0.00^{B}	0.00	0.00	<0.0001	<0.0001	<0.0001
	2	0.00	0.20^{B}	0.20^{B}	0.00	0.00			
	3	0.00	0.00^{B}	0.00^{B}	0.00	0.00			
	4	$0.00^{b,4}$	$0.00^{B,b}$	$0.90^{A,a}$	0.40^{ab}	0.00^{b}			
	5	0.00^{b}	$2.40^{A,a}$	$0.00^{B,b}$	0.00^{b}	0.00^{b}			
Cardboard	1	2.25^{c}	2.80^{a}	2.00^{c}	2.45^{b}	2.50^{b}	<0.0001	0.2415	<0.0001
	2	2.15^{cd}	3.00^{a}	1.85^{d}	2.45^{bc}	2.85^{ab}			
	3	2.25^{b}	2.65^{a}	1.85^{c}	2.40^{ab}	2.35^{ab}			
	4	2.20^{b}	2.65^{a}	1.85^{c}	2.55^{a}	2.80^{a}			
	5	2.40^{b}	2.10^{c}	2.10^{c}	2.80^{a}	2.45^{b}			
Stale	1	$0.15^{C,b}$	$1.05^{C,a}$	$0.00^{C,b}$	$0.80^{C,a}$	$0.00^{D,b}$	<0.0001	<0.0001	<0.0001
	2	$0.00^{C,c}$	$2.50^{A,a}$	$1.05^{B,b}$	$0.85^{C,b}$	$1.05^{BC,b}$			
	3	$0.35^{C,b}$	$1.80^{C,a}$	$0.40^{C,b}$	$1.35^{BC,a}$	$0.35^{CD,b}$			
	4	$2.75^{A,a}$	$2.60^{A,a}$	$2.30^{A,a}$	$2.75^{A,a}$	$1.60^{AB,b}$			
	5	$1.50^{B,c}$	$2.85^{A,a}$	$0.40^{C,d}$	$1.95^{B,bc}$	$2.35^{A,ab}$			

1. Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high).
2. Probability of significant effects due to Fat source (F), Day of evaluation (D) and interaction effects (F x D).
3. Average for each parameter with different upper case letters (A-D) in the same column are significantly different ($p \leq 0.05$) between days within sample.
4. Averages with different lower case letters (a-d) in the same row are significantly different ($p \leq 0.05$) between sample within day.

Protein sources

No rancid aromatic was detected in any sample during the five days evaluation period. The interaction effect was significant for oxidized oil, cardboard, and stale aromatics ($p \leq 0.05$; Table 4.4). Only oxidized oil showed significant differences regarding the day of the evaluation. For all samples, the intensity of oxidized aromatic on the fifth day was lower than or equal to the highest intensity in the previous four days. However, only the first day beef sample and the third day fish sample had oxidized oil notes more than 1.5 points which was low on a scale from 0 to 15. All attributes were significantly different across samples within days. The sample prepared with tofu maintained the lowest oxidized oil intensity throughout the study. Liver developed higher cardboard aromatics than beef and fish samples on the fifth day of the evaluation while chicken sample had the lowest cardboard note on day 4. The fish sample had the highest stale note in the first two days and then the stale aromatic of the liver sample increased and became the highest. Fresh chicken liver is a rich source of essential nutrients and highly prone to spoilage as fresh and microorganism growth (Gill, 1988; Papazoglou, Tsiraki & Savvaidis, 2012), the baked products using fresh chicken liver as an ingredient may also have a shorter shelf-life. The second day of the evaluation was the only individual day the dogs' preference was significant (Chapter 3). The oxidized oil aromatic was the only attribute that differentiated ($p<0.05$) the sample prepared with liver and the samples with chicken fat and tofu. The slightly higher oxidized note may have attracted the dogs and therefore the liver sample was significantly preferred over the chicken fat sample and tofu sample.

Table 4.4 Mean intensity scores[1] of attributes for protein sources samples over shelf-life.

Aroma attributes	Day of evaluation	Protein sources					p-level[2]		
		Beef	Chicken	Fish	Liver	Tofu	Protein source(P)	Day of evaluation(D)	P × D
Oxidized Oil	1	$1.63^{A,3,a,4}$	$0.19^{BC,b}$	$0.00^{B,b}$	$0.00^{B,b}$	0.00b	<0.0001	<0.0001	<0.0001
	2	$0.75^{BC,ab}$	$0.75^{AB,ab}$	$1.10^{A,ab}$	$1.45^{A,a}$	0.40b			
	3	$1.20^{AB,a}$	$0.20^{BC,b}$	$1.70^{A,a}$	$0.30^{B,b}$	0.15b			
	4	$0.00^{C,b}$	$1.00^{A,a}$	$0.20^{B,b}$	$0.40^{B,ab}$	0.00b			
	5	0.60^{BC}	0.00^{C}	0.20^{B}	0.00^{B}	0.00			
Cardboard	1	2.25	2.88	2.56	2.63	2.75	0.0034	0.1111	0.0003
	2	2.40	2.25	2.35	2.15	2.45			
	3	2.40^{abc}	2.10^{c}	2.65^{a}	2.55^{ab}	2.25^{bc}			
	4	2.40^{a}	1.85^{b}	2.45^{a}	2.60^{a}	2.65^{a}			
	5	1.90^{b}	2.40^{ab}	2.20^{b}	2.85^{a}	2.75^{a}			
Stale	1	2.31^{b}	3.00^{ab}	3.38^{a}	2.31^{b}	3.06^{ab}	<0.0001	0.366	<0.0001
	2	2.40^{b}	2.20^{b}	3.50^{a}	2.65^{ab}	2.45^{b}			
	3	2.75	2.30	2.75	2.85	2.75			
	4	2.20^{c}	2.45^{bc}	3.15^{ab}	3.35^{a}	3.15^{ab}			
	5	2.05^{c}	2.25^{c}	3.05^{b}	3.95^{a}	3.05^{b}			

1. Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high).
2. Probability of significant effects due to Protein source (P), Day of evaluation (D) and interaction effects (P x D).
3. Average for each parameter with different upper case letters (A-C) in the same column are significantly different ($p \leq 0.05$) between days within sample.
4. Averages with different lower case letters (a-c) in the same row are significantly different ($p \leq 0.05$) between sample within day.

Starch sources

Similar with the fat and protein phase of the study, no rancid aromatic was detected in the samples made with different starch sources for all five days (Table 4.5). The interaction of starch sources and day of evaluation was significant for the other three aroma attributes including oxidized oil, cardboard and stale ($p \leq 0.05$). Those attributes showed significant differences on all starch sources and day of evaluation as well ($p \leq 0.05$). It was found that, when oxidized oil aromatics were detected, it was scored in a low intensity (average of panelists≤1.0) except the second day of potato flour (2.45 point in average). The oxidized oil aromatic was not detected in any sample on the fourth and fifth day of the evaluation. At the end of the study, the sample prepared with chickpea flour had the lowest intensity on cardboard and stale aromatics. The wheat flour sample had the highest cardboard note and the potato flour sample had the highest stale note. On the fifth day of the evaluation, the stale note of the sample prepared with potato flour was significantly higher than the samples prepared with chickpea flour and tapioca flour which possibly result the potato flour sample being preferred over the chickpea flour and tapioca flour samples (Chapter 3).

Table 4.5 Mean intensity scores[1] of attributes for starch sources samples over shelf-life.

Aroma attributes	Day of evaluation	Starch sources					p-level[2]		
		Chickpea	Corn	Potato	Tapioca	Wheat	Starch source(S)	Day of evaluation(D)	S × D
Oxidized Oil	1	$0.95^{A,3,a,4}$	$0.90^{A,a}$	$0.70^{B,ab}$	0.00^{b}	0.15^{b}	<0.0001	<0.0001	<0.0001
	2	$0.55^{AB,b}$	$0.00^{B,c}$	$2.45^{A,a}$	0.00^{c}	0.00^{c}			
	3	0.20^{BC}	0.00^{B}	0.50^{BC}	0.20	0.00			
	4	0.00^{C}	0.00^{B}	0.00^{C}	0.00	0.00			
	5	0.00^{C}	0.00^{B}	0.00^{C}	0.00	0.38			
Cardboard	1	$2.45^{A,ab}$	1.95^{b}	2.90^{a}	$2.15^{A,b}$	2.50^{ab}	0.0003	0.0247	0.0283
	2	2.45^{A}	2.40	2.80	2.40^{A}	2.25			
	3	2.20^{AB}	2.05	2.15	2.05^{AB}	2.20			
	4	2.55^{Aa}	2.10^{ab}	2.30^{a}	$1.60^{B,b}$	2.40^{a}			
	5	$1.81^{B,c}$	2.13^{bc}	2.63^{ab}	$2.23^{A,abc}$	2.69^{a}			
Stale	1	$2.25^{A,bc}$	1.85^{c}	$3.30^{AB,a}$	$0.40^{BC,d}$	$2.61^{A,b}$	<0.0001	<0.0001	<0.0001
	2	2.35^{A}	2.40	2.65^{BC}	1.85^{A}	2.25^{AB}			
	3	$2.10^{AB,a}$	1.45^{a}	$2.10^{C,a}$	$0.35^{C,b}$	$0.40^{B,b}$			
	4	$0.35^{C,d}$	2.00^{b}	$3.00^{AB,a}$	$1.10^{B,cd}$	$1.60^{C,bc}$			
	5	$1.44^{B,bc}$	1.31^{c}	$3.50^{A,a}$	$2.00^{A,bc}$	$2.19^{AB,b}$			

1. Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high).
2. Probability of significant effects due to Starch source (S), Day of evaluation (D) and interaction effects (S x D).
3. Average for each parameter with different upper case letters (A-C) in the same column are significantly different ($p \leq 0.05$) between days within sample.
4. Averages with different lower case letters (a-d) in the same row are significantly different ($p \leq 0.05$) between sample within day.

Complex diet

Unlike the other three studies, all four aroma attributes including rancid aromatic was significantly different in the interaction of complex diet and day of evaluation as well as within the day within each sample, and the complex diet samples ($p \leq 0.05$; Table 4.6). Most samples showed a dramatic decrease of oxidized oil aromatic from day 1 to day 5 of the study but not sample 2 and 5. At the beginning of the study, sample 1 was significantly higher oxidized oil note than sample 2, 4 and 5 but the aromatic was not detected on the last day of the study. Sample 2 had the highest oxidized oil note at the end of the study. The cardboard aromatic of sample 1, 2, and 3 were significantly lower than day 1. Sample 4 and 5 had the highest cardboard note on day 5 among all samples. For stale aromatic, all sample experienced a decrease from day 1 to day 5 expect sample 5 which had no significant change in the five days of evaluation. Sample 1 was the only sample scored higher than 1 point in average in rancid aromatic out of all samples on any day. The rancid note of sample 1 on the fifth day was much higher than all other samples. On day 2, sample 1 was preferred over sample 4 by the dogs (Chapter 3) and sample 1 was scored significantly higher than sample 4 on oxidized oil and stale aromatics which agreed with the theory of oxidized oil and stale note potentially increased dogs' preferences on the treats. On day 3, sample 1 was preferred over sample 4 and 5 (Chapter 3), the intensity of oxidized oil, and rancid aromatics of sample 1 was much higher than sample 4 and 5 but lower on cardboard and stale aromatics. Rancid could also be an attribute that attracted the dogs since none of the samples from the previous phases developed rancid aromatics, only the result in this phase can potentially associate rancid aromatics with the dogs' preference. On day 5, the dogs preferred sample 1 over sample 2, 4 and 5 (Chapter 3) but sample 1 was the lowest on all attributes except for rancid. Rancid may have more effect on the dogs' preference over oxidized oil and stale.

Even if the intensity of some of the attributes changed throughout the study, the highest score in most attributes were less than 3.5 points which is generally considered low on a 15-point scale except for the rancid note of sample 1 on the fifth day of complex diet evaluation. However, dogs' olfactory perception is assumed to be more sensitive than humans, and thus these changes in the aromatics might affect the dogs' preferences. The decrease of oxidized oil aromatics throughout the testing period was unexpected. Most of the shelf-life studies indicated that rancidity-related notes such as rancid and oxidized generally increased through time (Chanadang et al., 2016). This study might need to be repeated to validate the results.

Rancidity generally indicates lipid oxidation which is commonly detected and evaluated in pet foods (Chanadang et al., 2016; Lin, Hsieh, Heymann & Huff, 1998; Di Donfrancesco et al., 2012; Pickering, 2009). Antioxidants are commonly added in commercial extruded pet food in order to preserve the sensory characteristics and nutrient quality of the products (Chanadang et al., 2016). Baked dog food tends to show lower level of aromatics that indicates rancidity than extruded dog food (Koppel et al., 2014b). Without any antioxidant added, the samples used in phases 2 to 5 of this study potentially were less likely to develop rancidity-related sensory notes comparing to if the samples were extruded.

Overall a plastic aromatic was not detected which indicated that storing the treats in Ziploc bags did not contribute any plastics aromatics. Jin, Kim, Song, Kim, Lee, Hur et al. (2013) studied the effects of Ziploc bags, vacuum bags, and modified atmosphere packaging on the sensory properties of low-grade ground beef and determined that the samples stored in Ziploc bag did not have significantly more off-odor than other samples. Ziploc bag seemed to be an acceptable storage material for the baked treats used in this study.

Table 4.6 Mean intensity scores[1] of attributes for complex diet samples over shelf-life.

Aroma attributes	Day of evaluation	Complex					p-level		
		Sample1[5]	Sample2	Sample3	Sample4	Sample5	Complex diet(C)	Day of evaluation(D)	C × D
Oxidized Oil	1	3.00^{A,3,a,4}	1.95^{AB,b}	2.65^{A,a}	1.70^{A,b}	0.40^{BC,c}	<0.0001	<0.0001	<0.0001
	2	2.35^{B,a}	1.20^{BC,b}	2.35^{A,a}	0.85^{B,b}	0.90^{AB,b}			
	3	1.20^{C,ab}	1.80^{AB,a}	0.90^{B,bc}	0.20^{C,cd}	0.00^{C,d}			
	4	0.00^{D,ab}	0.40^{C,ab}	1.05^{B,a}	0.00^{C,b}	0.50^{BC,b}			
	5	0.00^{D,c}	2.20^{A,a}	0.40^{B,c}	0.00^{C,c}	1.40^{A,b}			
Cardboard	1	2.65^{A,ab}	2.40^{A,b}	3.00^{A,a}	2.60^{A,b}	2.50^{b}	0.0003	0.0003	0.0111
	2	2.25^{A}	2.15^{A}	2.30^{B}	2.50^{A}	2.45			
	3	0.40^{B,b}	2.40^{A,a}	2.70^{AB,a}	2.55^{A,a}	2.30^{a}			
	4	1.25^{AB,c}	2.15^{A,ab}	2.40^{B,a}	1.60^{B,bc}	2.50^{a}			
	5	0.80^{B,b}	1.00^{B,b}	1.60^{C,ab}	2.50^{A,a}	2.50^{a}			
Stale	1	2.55^{A,b}	2.25^{A,b}	3.20^{A,a}	2.60^{A,ab}	1.30^{c}	0.0043	<0.0001	0.006
	2	1.70^{AB,a}	0.65^{B,bc}	0.70^{C,abc}	0.35^{B,c}	1.40^{ab}			
	3	1.10^{BC,c}	2.50^{A,ab}	2.70^{AB,a}	2.25^{A,ab}	1.80^{bc}			
	4	0.85^{BC,b}	0.70^{B,b}	2.20^{AB,a}	2.20^{A,a}	1.35^{b}			
	5	0.25^{C,c}	0.60^{B,bc}	1.00^{C,abc}	2.00^{A,a}	1.45^{ab}			
Rancid	1	0.00^{C}	0.00	0.00^{B}	0.00^{B}	0.00	<0.0001	0.0043	<0.0001
	2	0.00^{C}	0.00	0.00^{B}	0.00^{B}	0.00			
	3	2.15^{B,a}	0.00^{b}	0.00^{B,b}	0.00^{B,b}	0.00^{b}			
	4	0.95^{BC,a}	0.00^{b}	0.00^{B,b}	0.00^{B,b}	0.00^{b}			
	5	6.50^{A,a}	0.30^{b}	0.95^{A,b}	1.00^{A,b}	0.00^{b}			

1. Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high).
2. Probability of significant effects due to Complex diet (C), Day of evaluation (D) and interaction effects (C x D).
3. Average for each parameter with different upper case letters (A-D) in the same column are significantly different ($p \leq 0.05$) between days within sample.
4. Averages with different lower case letters (a-d) in the same row are significantly different ($p \leq 0.05$) between sample within day.
5. Sample 1: fish oil, liver, potato flour; Sample 2: butter, fish, wheat flour; Sample 3: chicken fat, chicken, corn starch; Sample 4: shortening, beef, tapioca flour; Sample 5: lard, tofu, chickpea flour.

Conclusion

The aroma characteristics of the samples used in the development and validation of the preference ranking procedure for dogs were studied in order to gain insights about the factors drive dogs' liking. A limited number of attributes were utilized to profile the samples. In general, the fish and meaty aromatics appeared to be liked by the dogs and attributes such as grain, musty/dusty and toasted were disliked by the dogs. However, these results need to be used with caution due to the small sample variety. Some samples developed low oxidized oil, cardboard and stale aromatics throughout the five days period of the study which may have affected the preference of the dogs.

References

Aldrich, G.C., & Koppel, K. (2015). Pet Food Palatability Evaluation: A Review of Standard Assay Techniques and Interpretation of Results with a Primary Focus on Limitations. *Animals*, **5 (1)**, 43-55.

APPA. (2016). Pet industry market size and ownership statistics. American Pet Products Association, Inc. Greenwich, CT. Available online: http://www.americanpetproducts.org/press_industrytrends.asp (accessed on 08 June 2017).

Bradshaw, J.W.S. (1991). Sensory and experiential factors in the design of foods for domestic dogs and cats. *The Proceedings of the Nutrition Society*, **50**, 99–106.

Cadena, R., Cruz, A., Faria, J., & Bolini, H. (2012). Reduced fat and sugar vanilla ice creams: Sensory profiling and external preference mapping. *Journal of Dairy Science*, **95(9)**, 4842-4850.

Chambers EIV, Lee J, Chun S, & Miller A, Development of a lexicon for commercially available cabbage (baechu) kimchi. *Journal of Sensory Studies*, **27**,511–518 (2012).

Chanadang, S., Koppel, K., & Aldrich, G. (2016). The impact of rendered protein meal oxidation level on shelf-life, sensory characteristics, and acceptability in extruded pet food. *Animals*, **6(8)**, 44.

Cherdchu P, Chambers EIV, & Suwonsichon T, Sensory lexicon development using trained panelists in Thailand and the United States: soy sauce. *Journal of Sensory Studies*, **28**, 248–255 (2013).

Di Donfrancesco, B., Koppel, K., & Chambers IV, E. (2012). An initial lexicon for sensory properties of dry dog food. *Journal of Sensory Studies*, **27**, 498-510.

Fragua, V., Barroeta, A., Manzanilla, E., Codony, R., & Villaverde, C. (2015). Evaluation of the use of esterified fatty acid oils enriched in medium-chain fatty acids in weight loss diets for dogs. *Journal of Animal Physiology and Animal Nutrition*, **99(S1)**, 48-59.

Gill, C. (1988). Microbiology of edible meat by-products. In: Pearson AM, Duston TR, editors. Advances in meat research. Westport, Conn.: Avi Publishing p. 47.

Griffin, R.W. Section IV: Palatability. In *Petfood Technology*, 1st ed.; Kvamme, J.L., Philips, T.D., Eds.; Watt Publishing Co.: Mt. Morris, IL, USA, 2003; pp. 176-193

Houpt, K. A., & Hintz, H. F. (1978). The role of olfaction in canine food preferences. *Chemical Senses*, **3**, 281-290.

Houpt, K. A., Hintz, H. F., & Shepherd, P. (1978). Palatability and canine food preference. *Canine Practice*, **5(6)**, 29-35.

Houpt, K. A., &Smith, S. L. (1981).Taste preferences and their relation to obesity in dogs and cats. *The Canadian Veterinary Journal*, **21**, 77-81.

Jin, S., Kim, I., Song, Y., Kim, D., Lee, C., Hur, I., et al. (2013). Effect of packaging methods on quality characteristics of low-Grade beef during aging at 16C. *Journal of Food Processing and Preservation*, **37(6)**, 1111-1118.

Koppel, K., Anderson, E. L., & Chambers, E. (2015a). Influence of processing on pomegranate (Punica granatum L.) juice flavor and aroma. *Journal of the Science of Food and Agriculture*, **95(5)**, 1066-1071.

Koppel, K., Chambers, E., Vázquez-Araújo, L., Timberg, L., Carbonell-Barrachina, A. & Suwonsichon, S. (2014a). Cross-country comparison of pomegranate juice acceptance in Estonia, Spain, Thailand, and United States. *Food Quality and Preference*, **31**, 116–123

Koppel, K., Gibson, M., Alavi, S., & Aldrich, G. (2014b). The effects of cooking process and meat inclusion on pet food flavor and texture characteristics. *Animals*, **4(2)**, 254-271.

Koppel, K., Monti, M., Gibson, M., Alavi, S., Donfrancesco, B. D., & Carciofi, A. C. (2015b). The effects of fiber inclusion on pet food sensory characteristics and palatability. *Animals*, **5(1)**, 110-125.

Lee, J., & Chambers, D. H. (2010). Flavors of green tea change little during storage. *Journal of Sensory Studies*, **25(4)**, 512-520.

Lin, S., Hsieh, F., Heymann, H; & Huff, H. (1998). Effects of lipids and processing conditions on the sensory characteristics of extruded dry pet food. *Journal of Food Quality*, **21**, 265–284.

Manabe, Y., Matsumura, S., & Fushiki, T. (2010). Preference for high-fat in animal foods. Fat Detection: Taste, Texture, and Post Ingestive Effects. Boca Raton, FL. Chapter 10.

Mintel. (2016) Pet Food - US - August 2016. Retrieved June 15, 2017 from Mintel Reports database.

Pickering, G.J. (2008). Optimizing the sensory characteristics and acceptance of canned cat food: Use of a human taste panel. *Journal of Animal Physiology and Animal Nutrition*, **93**, 52–60.

Papazoglou, S., Tsiraki, M., & Savvaidis, I. N. (2012). Effect of thyme oil on the preservation of Vacuum-Packaged chicken liver. *Journal of Food Science*, **77(8)**, M473–M480

Rofe, P.C., & Anderson, R.S. (1970). Food preference in domestic pets. *The Proceedings of the Nutrition Society*, **29**, 330–335.

Sandgruber, S., & Buettner, A. (2012). Comparative human-sensory evaluation and quantitative comparison of odour-active oxidation markers of encapsulated fish oil products used for supplementation during pregnancy and the breastfeeding period. *Food Chemistry*, **133(2)**, 458-466.

Sasaki, K., Ooi, M., Nagura, N., Motoyama, M., Narita, T., Oe, M., et al. (2017). Classification and characterization of Japanese consumers' beef preferences by external preference mapping. *Journal of the Science of Food and Agriculture*, **97(10)**, 3463-3462

Smith, J.C., Rashotte, M.E., Austin, T. & Griffin, R.W. (1984). Fine-grained measures of dogs' eating behavior in single-pan and two-pan tests. *Neuroscience and Biobehavioral Reviews*, **8(2),** 243-251.

Sullivan, J. C., & Budge, S. M. (2012). Fish oil sensory properties can be predicted using key oxidative volatiles. *European Journal of Lipid Science and Technology*, **114(5)**, 496-503.

Verbrugghe, A., Hesta, M., Gulbrandson, K., & Janssens, G., (2007). The effect of salmon oil freshness on the palatability of dog foods. *Vlaams Diergeneeskundig Tijdschrift*, **76(3),** 201-207.

Chapter 5 - The effect of Puzzle Toy and Ratio of Ingredients in the Preference Ranking Procedure with Dogs

Abstract

A preference ranking procedure with dogs was proposed and validated in order to provide an alternative evaluation method to study food preference of dogs (Chapter 2 and 3). While evaluating samples made with five different ingredients (fat, protein, or starch sources), the dogs were able to choose the preferred samples with this method. The objectives of this study were to further explore the applications of this method by studying the effect of dosage/ratio of the ingredients and the toy/puzzle toy of the treats, as well as gaining insights on what sensory properties possibly affected the dogs' preference with descriptive profiles of the treats by human descriptive panel. With the same dogs, Styrofoam cups and rubber toys were evaluated separately with the same treats. Most dogs were able to extract the treats from the Styrofoam cups and complete the study after becoming familiar with the cups in two days of training. The results collected with Styrofoam cups were less discriminating than results from testing with a rubber toy. It potentially suggested that the difficulty level of the toy can affect the significance of the dogs' preferences. The different ratio of the most and the least preferred protein sources (chicken liver and tofu) determined by the validation test of this method was ranked by the dogs but no significant difference was found. However, the human descriptive panel was able to provide different profiles for the samples. In conclusion, different puzzle toy might affect the result slightly due to the difficulty levels and further tests are needed in order to determine if the preference ranking procedure was suitable for the studies regarding dosage of ingredients.

Introduction

The annual sales of the pet industry increased from 38.5 billion dollars (2006) to 66.75 billion dollars (2016) in the last decade, the annual sales of 2017 was also estimated at $69.36 billion (APPA, 2016). The constant growth of the pet industry indicated the importance of pets as a social element in modern society. The pet food industry is competitive since the pet owners spent the most on pet food over any other pet-related expenses (APPA, 2016). In order for a pet owner to make the decisions to purchase/repurchase the pet food, the food has to be palatable enough for the pets so they would consume it. As a result, the palatability of the pet food is certainly one of the most important factors, as well as other factors such as price, natural, country of origin, etc.(Mintel, 2016).

To study the palatability of pet food, several palatability test methods have been developed and often utilized in the pet food industry. The most commonly used methods are the single-bowl method and two-bowl method (Aldrich & Koppel, 2015; Griffin, 2003; Smith, Rashotte, Austin & Griffin, 1984). Li, Smith, Aldrich & Koppel (Chapter 2) proposed a preference ranking procedure that relies upon the dogs' own motivation to extract a treat from a toy based on taste/aroma preference to explore the possibility of using the subjects' order of consumption to determine the rank of their preference of up to five samples. This method could potentially be more efficient due to the higher number of samples tested. So far, only one type of toy was studied to conceal the treats. However, the toys/puzzle toys need to be changed for different test or different subjects. For instance, if the test is conducted as a home-use-test (HUT) with a variety of breed of dogs, the size of the puzzle toy needs to be suitable for the size of different dog. Currently, most palatability tests were conducted with uncovered bowls especially when the amount of consumption is used to determine the palatability of the food (Griffin, 2003; Smith et al. 1984). Some other tests use bowls/pans together with or as part of other equipment (cage, lever, box, etc.) depending on the procedure of the study (Green & Rashotte, 1984; Thompson, Riemer, Ellis & Burman, 2016). In those cases, the other equipment was used to prevent the subject from consuming the food, to increase the difficulty of retrieving the food, or take the other samples away after the preferred sample is chosen by the dogs. The rubber toy used in the original preference ranking procedure is one of the most common dog toys that can hold a treat. However, more types of puzzle toys need to be explored in order to determine the flexibility of procedure and make this procedure more complete.

Sensory analysis with a human descriptive panel is widely utilized as a tool to access the drivers of liking in food products in conjunction with consumer preference results (Cadena, Cruz, Faria & Bolini, 2012; Sasaki, Ooi, Nagura, Motoyama, Narita, Oe et al., 2017). The sensory characteristics of pet food has been evaluated by human descriptive panels (Chanadang, Koppel & Aldrich., 2016; Lee & Chambers, 2010; Koppel, Gibson, Alavi & Aldrich., 2014; Koppel, Monti, Gibson, Alavi, Donfrancesco & Carciofi, 2015) and Di Donfrancesco, Koppel & Chambers (2012) developed a descriptive lexicon for dog food in all sensory aspects (appearance, aroma, flavor, and texture). With the sensory profiles given by the human descriptive panel and the preference result collected from the subjects, it is possible to determine the drivers of the subjects' liking.

In order to determine if the preference ranking procedure is effective while using different puzzle toys, other forms of puzzle toys need to be studied. Also, to explore the application of this method, additional tests with specific treat formulations need to be studied. Therefore, the objectives of this study were to (1) determine the effects of puzzle toy/toy on the preference results, (2) explore if the method is effective when evaluating the dosage/ratio of ingredients; (3) further study the drivers of the subjects' liking.

Materials and Methods

The research was approved by the Institutional Review Board for Protection of Human Subjects (IRB #9634) and by the Institutional Animal Care and Use Committee (IACUC #3722).

Subjects

Similar to the preliminary test and the validation test (Chapters 2 and 3), the twelve young adult (2 to 4 years old) beagle dogs (4 females and 8 males) with an average weight of 11 kg ± 1.2 kg were housed at Kansas State University Large Animal Research Center (Manhattan, KS, USA) under ambient environmental conditions (20°C; 60% R.H.) during participation in this study. Dogs were housed in pairs throughout the study in dog runs (7.8 square meter inside run with an attached 18 square meter outdoor run. The standard maintenance diet in amounts to maintain body weight were fed to the dogs twice daily at 7 AM and 11 AM and removed following the 11 AM feeding throughout the study. The removal of the diet was expected to increase motivation and interest of the dogs for the ranking test. The test took place from 4.30PM to 6PM daily. Each dog was led to the testing space individually in an adjacent room to their pens. The room was a clean, noise-free and smell-free environment which eliminated distractions from the barking and smell of the other dogs. A testing space was partitioned with a metal fence to form a 1.5m x 1.5m square space in the room which allowed the dogs to be contained and promote focus on the task.

Methodology

Two types of toys were evaluated as puzzle toys in this study including the same rubber toy for phase 1 and 3 (kong; Kong™, Golden, CO, US) and a 118.3ml Styrofoam cup (DART Container Corporation, Mason, MI, US) upside down with a whole on the bottom of the cup, placed on top of an upside down matching lid (phase 2 and 4). Four phases were included in this test. For each phase, the same test with the same treats repeated for five days as five replications. Prior to the first phase of the study, the dogs were trained for two days in order to get familiar with the new toy. During the training, the dogs practiced with the Styrofoam cups with the five treats being evaluated in phase one in each cup followed the same testing procedure.

The first phase of this study took place as the last phase of the validation test, where the dogs evaluated the complex diet made with different combinations of the ingredients based on the dogs' preferences in the previous evaluation (Table 5.1). For phase 2 evaluation, phase 1 was repeated with Styrofoam cups instead of rubber toys. The results from both phases were compared in order to explore the effect from the puzzle toys and determine if the puzzle toys can be alternated with this test method when testing the same samples. The same applied for phases 3 and 4 of this study. In both phase 3 and 4, the dogs evaluated the five samples made with different ratio of protein sources, rubber toys were used in phase 3 and phase 4 repeated phase 3 with Styrofoam cup.

To start the test, the researchers led one of the dogs to the testing space and introduced the five treats contained in toys to them. The dog was directed to sniff the puzzle toys that contained the different treats by having the rubber toys held in front of its nose. After all 5 puzzle toys were sniffed by the dog, one researcher led the dog to the start-point located over the opposite corner of the testing space which was approximately 2 m away from the puzzle toys. The other researcher placed the puzzle toys in a row on the floor with approximately 10 cm apart from each other. Since the dogs may be influenced by the order or the position of the toy being placed on the floor (Fiset & LeBlanc, 2006; Péter, Topál, Miklósi & Pongrácz,2016), the start-point of the dogs was directly behind the researcher who set up the toys, the dogs were not able to observe the order or the position of each puzzle toy. The order of the treats being set-up on the floor and the assigned number of the toy for each treat were completely randomized. One researcher stayed in the fence with the dog to remove the empty puzzle toy after each treat had been extracted by the dog during the test and the other one stayed outside the fence to record the time and order of the consumption of each treat. The timer was started while the dog was released to approach and extract the treats from the puzzle toys. The order of the treats being chosen (extracted and consumed) by the dog was considered the ranking order of preference of the dog. The treat consumed by the dog first was considered most preferred, and the treat consumed last by the dog was considered least preferred. If the last treat was not consumed, it was also considered as least preferred. After the dog finished the test and led back to the pen, another dog was led in the same room to start the test. The order of the dogs led to the testing space remained the same to eliminate external distractions such as unfamiliar odors. The test was repeated for five sequential days for each phase to allow dogs to associate aroma with flavor and confirm the rank order. Sixty clean rubber toys (Kongs) were used per day, each of the 60 rubber toys were labeled sequentially and distinctly with numbers 1 to 60 and the Styrofoam cups were labeled from 1 to 5, 12 cups for each number. After each day of testing, the rubber toys were washed with hot water and soap twice and air-dried overnight prior to use to eliminate odor and other residue (hair, saliva, etc.). The Styrofoam cups were disposed after testing and clean new cups were labeled for each day for each dog.

When the dog was not interested in the task, researchers encouraged/guided the dog in order to continuously train them. When guiding was needed, the data was not included in analysis due to potential existence of bias during the test. Within 30 seconds from releasing the dog, if the dog was not interested on the task, researchers either banged toy or tapped toy on the floor to make noise and attract the dog. After 1 minute from releasing the dog and the dog was not on task, researchers took out the treat from the toy and had the dog sniff the treat in order to attract them with the odor. The treat was placed back in the toy once the dog showed interest and the test continued. If the dog was not able to start/continue the task 2 minutes after released or extracting the treat, the test ended. This procedure was applied to both types of puzzle toys.

Samples

In all phases, the treats used in each phase were made the day prior to the first day of each phase in Center for Sensory Analysis and Consumer Behavior at Kansas State University for the each of the five days of evaluation (Table 5.1). The treats were made by first combining the dry ingredients and then cutting the shortening/fat in to the dry ingredients. Then the liquid ingredients were mixed respectively and slowly added into the dry ingredients. The dough was then mixed by hand until the ingredients were combined and the dough was formed. The dough was rolled into a 10 mm thick sheet and cut into 15 mm*20 mm rectangles. These were placed on a baking sheet with parchment paper and baked in a convection oven at 175°C for 15 minutes. The treats were stored at room temperature (20°C; 60% R.H.) in gallon-size plastic bags (Ziploc, S.C. Johnson, Racine, WI, US) once cooled until used in the evaluation.

For phases 1 and 2, the combinations of the ingredients used in the validation test were studied (Chapter 3). The five combinations of fat, protein, and starch were: (1) fish oil, liver, and potato flour; (2) butter, fish, and wheat flour; (3) chicken fat, chicken, and corn starch; (4) shortening, beef, and tapioca flour; (5) lard, tofu, and chickpea flour. The samples were tested with the rubber toy in phase 1 and with Styrofoam cups as a new format of toy in phase 2 (Table 5.1). For phases 3 and 4, to determine if this method was helpful for evaluating the effect of ingredient dosage, the samples with different ratio of most preferred protein source (liver) and least preferred protein source (tofu) determined by the validation test (Chapter 3) were evaluated. The ratios of chicken liver (Tyson, Springdale, AZ, US) and tofu (Dillons, Hutchinson, KS, US) included 3:0, 2:1, 1.5:1.5, 1:2, and 0:3 liver/tofu.

Table 5.1 Ingredient composition for treats evaluated by dogs.

Ingredient	Phase 1 and 2 %composition	Phase 3 and 4 % composition
Whole wheat flour (Starch source replacement)	25.84***	24.33
White (all purpose) flour	25.84	24.33
Corn meal	0.00	12.16
Salt	0.52	0.49
Baking soda	0.26	0.24
Dry milk	1.55	1.46
Sodium bisulfate	0.002	0.002
Dry yeast	0.002	0.002
Shortening (Fat source replacement)	7.75*	7.30
Molasses	4.13	3.89
Water	23.77	16.06
Meat (protein source replacement)	10.34**	9.73(liver: tofu = 3:0, 1:2,1.5:1.5, 2:1,0:3)

* Fat replacement included vegetable shortening, fish oil, chicken fat, butter, or lard
** Protein included fish, beef, chicken, liver, or tofu
*** Starch included whole wheat flour, tapioca flour, potato flour, corn starch, or chickpea flour

Descriptive profiling

To gain insights on the aroma characteristics of the treats prepared with different ratios of protein sources (chicken liver and tofu), the aroma descriptive profiles of the samples with different ratios of protein sources were compiled with a highly trained descriptive human panel (5 panelists) from the Center for Sensory Analysis and Consumer Behavior in Kansas State University (Manhattan, KS, US). All panelists had completed 120h of general descriptive sensory analysis training with a variety of different food products. Each panelist participated in this study had more than 1,000h of testing experience with a large range of food products. The majority of the panelists had adequate experience working with pet food products. With a consensus method as all panelists must reach an agreement through discussion on the intensity and provide only one score for each attribute, a numeric scale from 0 to 15 (0-none, 15-extrmemly high) with 0.5 increments was applied for the compilation of descriptive aroma profiles of the samples (Koppel et al., 2014; Koppel, 2015; Cherdchu, Chambers & Suwonsichon, 2013; Di Donfrancesco et al., 2012; Chambers, Lee, Chun & Miller, 2012). After a 1.5h orientation, the aroma profiles of the samples used in phase 3 and 4 were evaluated in one 1.5h session. For each panelist, one piece of sample was placed in a medium snifter which was labeled with three-digit blind codes for aroma evaluation. The definitions and references remained the same from Chapter 4 (Table 5.2) which was based on the dry dog food lexicon developed by Di Donfrancesco et al. in 2012.

Data Analysis

The ranked data from each of these phases was analyzed by a Friedman analysis of variance (Lawless & Heymann, 2010) using the XLStat version 2010.6.02 (Addinsoft, New York, NY, US). The statistical significance of differences was defined as $p \leq 0.05$.

The ranked data collected from the dogs in the first phase was converted into preference scores of 1-5 to be analyzed with the descriptive analysis results of the same samples from the validation test (Chapter 2). The first treat the dogs chose was considered as the highest preference score (5). The last one chosen was given the lowest preference score (1). Then the data was analyzed with the descriptive profiles with XLStat version 2015.3.01(Addinsoft, New York, NY, US) to create external preference mapping to potentially discover the drivers of liking for dogs. The attributes which were scored as “0” for all samples in each phase were excluded from the map.

Table 5.2 Attributes, definition and references for descriptive analysis

Attribute	Definition	Reference**
Overall aroma intensity	The overall impression of aromatics that may or may not include meat or grain like aromatics.	Grain mix = 5.0 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. Place 1 Tablespoon in snifter.
Barnyard	Combination of pungent, slightly sour, hay-like aromatics associated with farm animals and the inside of a barn.	White pepper in Swanson Chicken Broth 99% Fat Free (0.90g /300ml) = 6.0. Preparation: Steep 0.90 g of ground white pepper in 300 ml of Swanson Chicken Broth at 180 F for 15 min. Filter the solution and let cool for 10 min. Serve ¼ cup in snifter.
Toasted	A moderately browned/baked impression	General Mills Cheerios crushed = 7.0. Preparation: ¼ cup crushed Cheerios in snifter
Baked	A medium brown aromatic with a cooked impression associate with baked products.	Crushed shredded wheat = 5.0 Preparation: Crush shredded wheat and serve 1 tablespoon in snifters.
Brown	A sharp, caramel, almost-burnt aromatic (a part of the grain complex).	Crushed Toasted Grape nuts = 5.5. Preparation: Place 1/2 cup Grape Nuts onto parchment lined cookie sheet. Toast at 350°F for 7 minutes. Crush with mortar. Cool and serve 1 Table spoon in snifter.
Grain	The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice and oats.	Cereal Mix (dry) =5.0. 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. Place 1 Tablespoon in snifter.
Soy	Flavor associated with soybeans or soy products.	Soy nuts (Hy-Vee Bulk) = 4.5. Preparation: Serve 1 Teaspoon in snifter.
Vitamin	The aromatics associated with a just opened bottle of vitamin pills (generally thought to be oxidized thiamin)	Nature Made Super B-Complex capsule = 10.0. Preparation: Place 1 vitamin pill in snifter.
Musty/Dusty	A dry aromatic associated with stored dry grain.	Kretschner Wheat Germ = 5.0. Preparation: Serve 1 tablespoon wheat germ in snifter.
Fish	An overall impression of fishy associated with fresh, died, or canned fish.	Nature Made Fish oil pill: 8.0. Preparation: Place the liquid content of 1 pill in a snifter.
Meaty	A measure of how much a sample is recognized as distinctly animal muscle tissue, including poultry, seafood/fish, and beef.	Canned Swanson Beef Broth 99% fat free = 5.0. Preparation: Place 1 Tablespoon in snifter.
Liver	Aromatics associated with cooked organ meat/liver.	Beef liver = 6.0. Preparation: Pan-fry beef liver until an internal temperature of 160F. Chop and serve 1 Tablespoon in snifter.

Oxidized Oil*	The aromatic associated with aged or highly used oil and fat.	Microwave Oven Heated Wesson Vegetable Oil = 6.0. 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. Repeat three times. Let beaker sit on counter uncovered overnight. Serve 1 Tablespoon of the oil in snifter.
Cardboard*	The aromatic associated with cardboard or paper packaging. The intensity rating is only for the 'cardboardy' character within the reference.	Cardboard = 7.5. Preparation: 2" cardboard square in 1/2 Cup of water. Serve in snifter.
Stale*	The aromatics associated with wet cardboard that is characterized by a lack of freshness.	Mama Mary's Pizza Crust = 4.5. Preparation: Serve 1 piece of 2" crust square in snifter.
Plastic*	An aromatic associated with plastic polyethylene containers or food stored in plastic	Ziploc bag = 3.5. Preparation: Serve 1 snack size Ziploc bag in snifter.
Rancid*	A somewhat heavy aromatic characteristic of old, oxidized, decomposing fat and oil. The aromatics may include painty, varnish, or fishy.	Microwaved Wesson vegetable oil (4 min at high) = 2.5. Preparation: Microwave 1.5 cups oil on high power for 4 minutes. Let cool and Serve ¼ cup in snifter. Microwaved Wesson vegetable oil (5 min at high) = 5.0. Preparation: Microwave 1.5 cups oil on high power for 5 minutes. Let cool and Serve ¼ cup in snifter.
Overall Sweet	The perception of the combination of sweet taste, sweet aromatics, caramelized, brown sugar, honey, maple syrup etc.	Lorna Doone Cookie = 5.5. Preparation: Serve 1 crushed cookie in in snifter.
Vegetable complex	A general term to describe a combination of cooked vegetable aromatics that may include celery, carrot, potato or other vegetables.	Swanson vegetable broth = 6.0. Preparation: Place 1 tablespoon of broth in snifter.

*Attributes evaluated for shelf-life study.

** All references were served in medium snifters covered with watch glass at room temperature.

Results and Discussion

Preference ranking test with dogs

Training phase with Styrofoam cups

The Styrofoam cups were set up upside down on the lid during testing. The treat was placed on the lid under the cup. Due to the set-up of the puzzle toy, the dogs needed to remove the cup from the lid by sliding/pushing the cup. Most of the dogs were able to extract the treats from the cups easily on the second day of training. However, on the first day, almost all dogs showed behaviors such as ears held back, panting, retreating from toys, etc. which indicated their excessive fear towards the new toy as an unfamiliar object (Jones & Gosling, 2005; Siracusa, 2014). Especially when the dogs moved the lids on the floor and the friction between the lid and the floor produced a loud sharp noise, most of the dogs backed off. Some of them slowly approached the toys again and extracted the treats. One of the dogs acted terrified after and was not willing to approach the toys. Siracusa (2014) treated a dog with excessive fear when exposed to new people, animals and objects by arranging safe environment for the dog, using a leash, food-enhanced toys and drugs (Clomipramine and Alprazolam). The dog showed improvement eight weeks after the behavior evaluation and became less likely to be aroused by outdoor stimuli. This indicated that it is possible to treat the dogs in order to accept new objects/toys. However, due to the high cost of resources essential for the treatment of the dogs with excessive fear, toys that are noise-free are recommended.

Comparison of puzzle toys

With the Styrofoam cups, sample 1(fish oil, liver, potato flour) was ranked significantly higher than the other samples by the dogs while the four other samples were similar with each other in terms of preference (Table 5.3). Unlike phase 1 (Chapter 3) which evaluated the samples with rubber toys, the data of phase 2 did not demonstrate the preference differences between samples 2 (butter, fish, and wheat flour) and sample 4 (vegetable shortening, beef and tapioca flour) and 5 (lard, tofu and chickpea flour), and between sample 3 (chicken fat, chicken, and corn starch) and sample 5. Sample 2 was significantly preferred over sample 4 and 5, and sample 3 was significantly preferred over sample 5 in the last phase of validation test. Since sample 1 appeared to be the most preferred sample in both phases, both puzzle toys delivered similar result but the rubber toys separated the preference of the samples more than the Styrofoam cups. The average time the dogs spent on each toy decreased from 12.57 seconds to 8.23 seconds when changing the rubber toy to the Styrofoam cups (Table 5.3). The difference of time with the two puzzle toys possibly indicated that the difficulty of extracting treats from Styrofoam cups was lower than from rubber toys. The difficulty level of the toy might have also affected the preference on the samples. This method was developed under the assumption that the subjects' desire towards the treats would convert into motivation to put effort into extracting the treats, less difficulty level may reduce the effort needed.

Ratio effect on preference

The sample prepared with chicken liver appeared to be the most preferred and the sample prepared with tofu was the least preferred when the effect of protein source ingredients was studied in the validation test (Chapter 3). However, the preference difference did not appear when the ratio/dosage of the most preferred protein source and the least preferred protein source was studied with either toy (Table 5.3). One potential explanation was, that after more than 30 days of evaluation/training, the dogs might be fatigued or lost motivation to make their choices based on their preferences. Another possible reason was that the method may not be suitable for the evaluation of ratio/dosage effect since the samples were very similar in terms of ingredients. This method was not able to determine the dogs' preference on the treats with different ratio of protein sources. However, it would be helpful to conduct more tests to explore the possible application of this method on other ingredient categories or dosages.

Table 5.3 The effect of flavor treatments in rank order preference in dogs

Phase	**Treatments (Rank 1st to 5th)**					**Average time spent on each toy (seconds± standard error)**
2: Complex diet with cups[1]						
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	*p-value*	8.23±1.08
1.91[a]	2.82[b]	3.01[b]	3.58[b]	3.64[b]	*<0.0001*	
3: Protein dosage study with rubber toy[2]						
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	*p-value*	*9.7±0.47*
3.01	2.80	3.23	2.61	3.27	*0.112*	
4: Protein dosage study with cups[2]						
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	*p-value*	*2.6±0.12*
2.84	3.20	3.06	3.06	2.86	*0.724*	

[abc] indicates that within a row, samples with unlike letters were significantly different ($p<0.05$)
[1]. Sample 1: fish oil, liver, potato flour; Sample 2: butter, fish, wheat flour; Sample 3: chicken fat, chicken, corn starch; Sample 4: shortening, beef, tapioca flour; Sample 5: lard, tofu, chickpea flour.
[2]. Sample 1: Liver: tofu = 3:0; Sample 2: Liver: tofu = 2:1; Sample 3: Liver: tofu = 1.5:1.5; Sample 4: Liver: tofu = 1:2; Sample 5: Liver: tofu = 0:3.

For the existing palatability tests, different puzzle toys are used for a variety of reasons. For the palatability tests that evaluate how palatable the food is by the amount of consumption, such as traditional one-bowl method or two-bowl methods, uncovered bowls/pans are commonly used (Smith et al., 1984). However, the preferences ranking procedure was developed based on the assumption that the dogs would put more effort to extract the treats if they prefer the flavor/aroma of the sample, with open bowls/pans, the dogs would easily obtain the treats and potentially lose the motivation of choosing the treat based on their preference. The palatability test conducted with a box with two response-levers and the pre-weighed foods are dispensed with automated feeder into bowls at a floor-level under the levers is the operant lever-press test (Green & Rashotte, 1984), this test is a variation of the two-bowl test. The puzzle toy for the food in this method is relatively complicated but it can potentially be modified and applied to the preference ranking procedure. Not only the toy/puzzle toys that are commonly used with dogs (bowls, kongs, etc.), other containers such Tupperware and boxes can also be potential food puzzle toys for testing (Chapter 2). More forms/sizes of toys/puzzle toys should be tested with the preference ranking procedure since the objectives and subjects could be different in various studies.

Descriptive profiling and External Mapping

External preference map for phase 2

The descriptive profile of the samples indicated that sample 1 (fish oil, chicken liver, potato flour) had the highest intensity on fish and meaty and lowest on toasted, grain, musty/dusty, cardboard and overall sweet aromatics and sample 5 (lard, tofu, chickpea flour) was low on overall aroma intensity, fish and meaty aromatics (Chapter 4). Sample 2 (butter, fish, wheat flour), 3 (chick fat, chicken, corn starch) and 4 (shortening, beef, tapioca flour) were similar in overall aroma intensity but different in specific attributes. The external preference map (Figure 5.1) suggested that aroma attributes such as meaty, fishy and overall aroma intensity potentially drove the dogs' liking which was in agreement with the external preference map analyzed with the aroma profile in phase 1 and the dogs' preference results with rubber toy (Chapter 4). The relative locations of the samples were also extremely similar with the previous map. These maps validated that the preference of dogs collected with different toy/puzzle toy provided similar results.

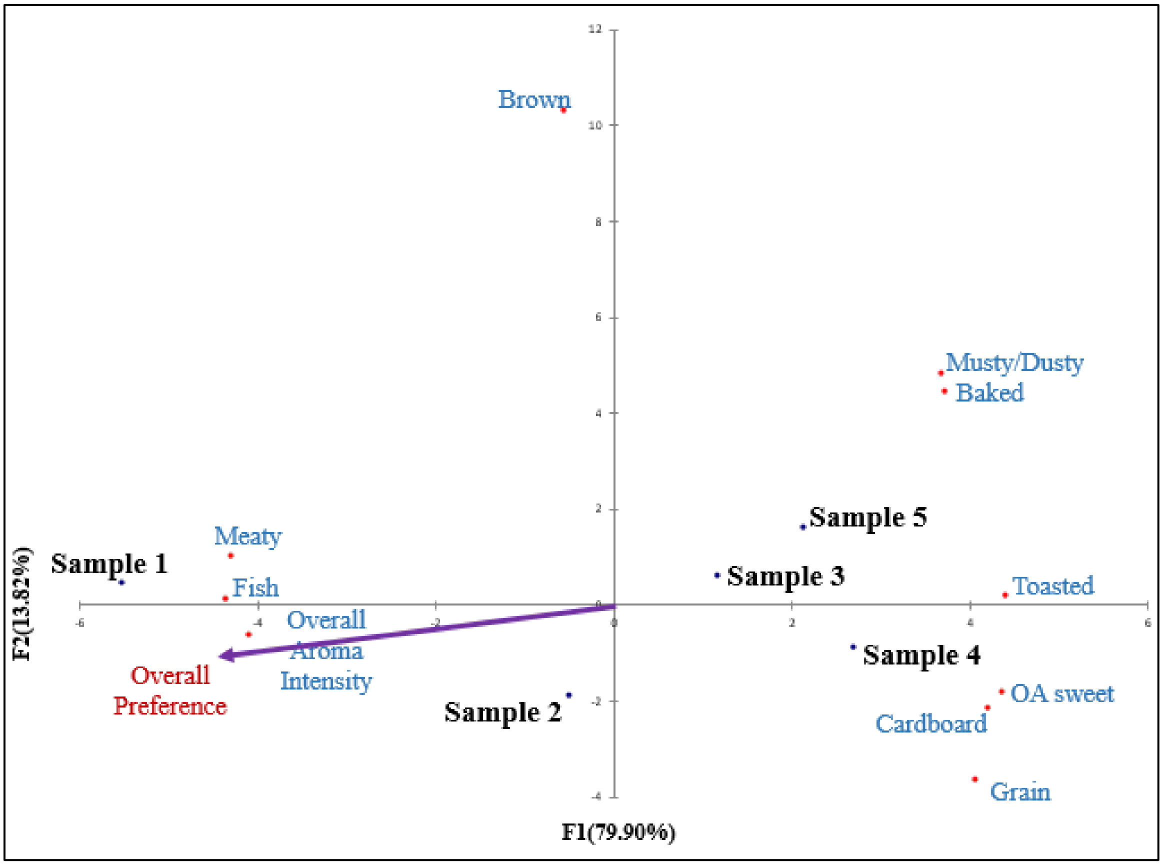

Figure 5.1 External Preference map for Phase 2

* Sample 1: fish oil, liver, potato flour; Sample 2: butter, fish, wheat flour; Sample 3: chicken fat, chicken, corn starch; Sample 4: shortening, beef, tapioca flour; Sample 5: lard, tofu, chickpea flour.

Descriptive profile for phase 2 and 3

Even if the dogs did not show a significant preference difference on the samples with different ratio of protein sources, the aroma profile compiled by the human descriptive panelists showed some differences across samples (Figure 5.2). Attributes including overall aroma intensity, toasted, musty/dusty and overall sweet aromatics showed differences of equal to or more than two points across samples. Sample 1 (liver: tofu= 3:0) appeared to be the lowest on overall aroma intensity, toasted and musty/dusty aromatics, and it was the only sample scored on overall sweet aromatics. The sample with the highest overall aroma intensity and toasted aromatic was sample 3(liver: tofu= 1.5:1.5). Sample 4 (liver: tofu= 1:2) had higher musty/dusty note than all other samples. No liver aromatics were detected in any of the samples even if liver is commonly used in the commercial pet food products especially palatant.

Although dogs are generally assumed to be more sensitive with the aromatics than human, the descriptive panel was able to differentiate the samples by providing different descriptive profiles while the dogs did not show significant difference on their preference. The reason is difficult to conclude. As mentioned, the dogs might be too fatigued or lost motivation to make their choices even if the samples were different from each other, or this procedure was not the ideal method to evaluate five samples with similar ingredients.

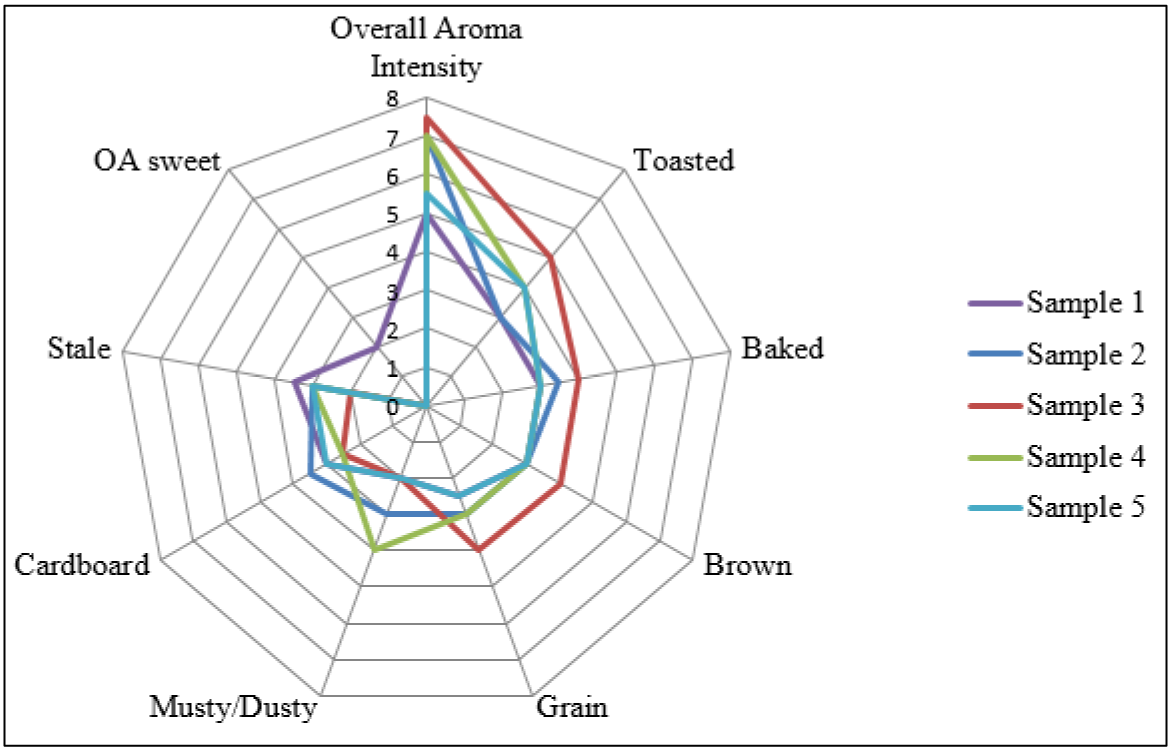

Figure 5.2 Spider plot for sample with different ratio of protein sources
*Scores are based on a 0-15-point numeric scale with 0.5 increments (0 = none and 15 =extremely high.
**Sample 1: Liver: tofu = 3:0; Sample 2: Liver: tofu = 2:1; Sample 3: Liver: tofu = 1.5:1.5; Sample 4: Liver: tofu = 1:2; Sample 5: Liver: tofu = 0:3.

Many pet owners are interested in feeding their companion animals vegetarian diets due to the personal moral philosophy, ethical concern of animal welfare, or health and the environment

(Brown, 2009). Some studies showed evidence that cats and dogs maintained on vegetarian diets may be healthy but owners should regularly monitor urinary acidity and adjust their animal's diet/additives if urinary alkalinisation appears (Knight & Leitsberger, 2016). Soybean as one the most common plant-based protein source had been studied as an ingredient of pet food for its nutritional value (Carciofi, de-Oliveira, Valério, Borges, de Carvalho, Brunetto & Vasconcellos, 2009; Clapper, Grieshop, Merchen, Russett, Brent & Fahey, 2001; Knight & Leitsberger, 2016) but not a lot of palatability tests have been conducted. In this test, tofu was used to represent plant-based protein. Even if the sample was the least preferred sample in the preliminary test and validation test comparing to other animal-based protein source (Chapter 2 and 3), the higher ratio of the tofu versus liver was not disliked by the dogs comparing with higher amount of liver.

There are many studies that can be conducted, in addition to testing different puzzle toys and dosages of ingredients, there are more opportunities to explore the appropriate application of the preference ranking procedure. For instance, the dogs participated in this procedure were kennel dogs which were housed in a research facility. It would be interesting to conduct studies with this procedure with home-housed dogs to determine if it is appropriate for home-use-test settings. Also, by evaluating the same samples, the results collected from kennel dogs and home-dogs could be compared to determine if the dogs produce similar results.

Conclusion

The effect of puzzle toy/toy and dosage/ratio of the protein sources were studied. The same treats evaluated in the last phase of the validation test were tested with another format of toy—Styrofoam cups and demonstrated that altering the form of the toy could potentially result slightly different result. The results collected with rubber toys appeared to be more discriminated but the most preferred sample was consistent with both toys. The treats prepared with multiple ratio selections of chicken liver and tofu were not significantly preferred over one another with either toy/puzzle toy even if the human descriptive panel determined the aroma profile of the samples were somewhat different on overall aroma intensity, toasted, musty/dusty and overall sweet aromatics.

References

Aldrich, G.C., & Koppel, K. (2015). Pet Food Palatability Evaluation: A Review of Standard Assay Techniques and Interpretation of Results with a Primary Focus on Limitations. *Animals*, 5(1), 43-55.

APPA. (2016). Pet industry market size and ownership statistics. American Pet Products Association, Inc. Greenwich, CT. Available online: http://www.americanpetproducts.org/press_industrytrends.asp (accessed on 08 June2017).

Brown, W.Y. (2009). Nutritional and ethical issues regarding vegetarianism in the domestic dog. *Recent Advance in Animal Nutrition Australia*, **17**, 137–143.

Cadena, R., Cruz, A., Faria, J., & Bolini, H. (2012). Reduced fat and sugar vanilla ice creams: Sensory profiling and external preference mapping. *Journal of Dairy Science*, **95(9)**, 4842-4850.

Carciofi, A.C., de-Oliveira, L.D., Valério, A.G., Borges, L.L., de Carvalho, F.M., Brunetto, M.A. & Vasconcellos, R.S. (2009). Comparison of micronized whole soybeans to common protein sources in dry dog and cat diets. *Animal Feed Science and Technology*, **151**, 3-4

Chambers, EIV, Lee, J., Chun, S., & Miller, A. (2012). Development of a lexicon for commercially available cabbage (baechu) kimchi. *Journal of Sensory Studies*, **27**,511–518

Chanadang, S., Koppel, K., & Aldrich, G. (2016). The impact of rendered protein meal oxidation level on shelf-life, sensory characteristics, and acceptability in extruded pet food. *Animals*, **6(8)**, 44.

Cherdchu, P., Chambers, EIV, & Suwonsichon, T. (2013). Sensory lexicon development using trained panelists in Thailand and the United States: soy sauce. *Journal of Sensory Studies*, **28**, 248–255

Clapper, G. M., Grieshop, C. M., Merchen, N. R., Russett, J. C., Brent, J. L., & Fahey, G. C. (2001). Ileal and total tract nutrient digestibilities and fecal characteristics of dogs as affected by soybean protein inclusion in dry, extruded diets. *Journal of Animal Science,* **79**, 1523-1532.

Di Donfrancesco, B., Koppel, K., & Chambers IV, E. (2012). An initial lexicon for sensory properties of dry dog food. *Journal of Sensory Studies*, **27**, 498-510.

Fiset, S. & LeBlanc, V. (2007).Invisible displacement understanding in domestic dogs (Canis familiaris): the role of visual cues in search behavior, *Animal Cognition*, **10(2)**, 211-224.

Griffin, R.W. Section IV: Palatability. *In Petfood Technology*, 1st ed.; Kvamme, J.L., Philips, T.D., Eds.; Watt Publishing Co.: Mt. Morris, IL, USA, 2003; pp. 176-193

Jones, A. C., & Gosling, S. D. (2005). Temperament and personality in dogs (Canis familiaris): a review and evaluation of past research. *Applied Animal Behaviour Science*, **95(1)**, 1-53

Lawless, H. T., & Heymann, H.(2010). Sensory evaluation of foods: principles and practices, 2nd edition, pp. 501-503. Springer, New York, NY, USA.

Knight, A., & Leitsberger, M. (2016). Vegetarian versus Meat-Based Diets for Companion Animals. *Animals*, **6(9)**, 57.

Koppel, K., Gibson, M., Alavi, S., & Aldrich, G. (2014). The effects of cooking process and meat inclusion on pet food flavor and texture characteristics. *Animals*, **4(2)**, 254-271.

Koppel, K., Monti, M., Gibson, M., Alavi, S., Donfrancesco, B. D., & Carciofi, A. C. (2015). The effects of fiber inclusion on pet food sensory characteristics and palatability. *Animals*, **5(1)**, 110-125.

Mintel. (2016) Pet Food - US - August 2016. Retrieved June 15, 2017 from Mintel Reports database.

Péter, A., Topál, J., Miklósi, Á. & Pongrácz, P. (2016). I saw where you have been— The topography of human demonstration affects dogs' search patterns and perseverative errors. *Behavioral Processes,* **125**, 51-62

Sasaki, K., Ooi, M., Nagura, N., Motoyama, M., Narita, T., Oe, M., et al. (2017). Classification and characterization of Japanese consumers' beef preferences by external preference mapping. *Journal of the Science of Food and Agriculture*, **97(10)**, 3463-3462

Siracusa, C. (2014). Animal Behavior Case of the Month. *Journal of the American Veterinary Medical Association*, **244(1)**, 1260-1262

Smith, J.C., Rashotte, M.E., Austin, T. & Griffin, R.W. (1984). Fine-grained measures of dogs' eating behavior in single-pan and two-pan tests. *Neuroscience and Biobehavioral Reviews*, **8(2)**, 243-251.

Thompson, H., Riemer, S., Ellis, S. L., & Burman, O. H. (2016). Behaviour directed towards inaccessible food predicts consumption—A novel way of assessing food preference. *Applied Animal Behavior Science*, **178**, 111-117.

Appendix A - Ballot for Descriptive profiling in Chapter 4 and 5

Panelist: ______ **Sample code:** ________ **Date** __________

AROMA

Overall aroma Intensity	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	**10**	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Barnyard	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	**8**	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Toasted	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	**7**	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	**14**	14.5	15
Cooked	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	**8**	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	**14**	14.5	15
Brown	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	**6**	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Grain	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	**5**	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Soy	0	0.5	1	1.5	2	2.5	3	3.5	4	**4.5**	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Vitamin	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	**10**	10.5	11	11.5	12	12.5	13	13.5	**14**	14.5	15
Dusty/Earthy	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	**8**	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Fish	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	**10**	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Meaty	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	**5**	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Liver	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	**5**	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Musty	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	**8.5**	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Oxidized Oil	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	**6**	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Cardboard	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	**7.5**	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Stale	0	0.5	1	1.5	2	2.5	3	3.5	4	**4.5**	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Plastic	0	0.5	1	1.5	2	2.5	3	**3.5**	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Rancid	0	0.5	1	1.5	2	**2.5**	3	3.5	4	4.5	**5**	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15

Appendix B - Ballot for Shelf-Life study in Chapter 4

Panelist: ______ **Sample code:** ________ **Date** __________

<u>AROMA</u>

Oxidized Oil	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	**6**	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Cardboard	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	**7.5**	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Stale	0	0.5	1	1.5	2	2.5	3	3.5	4	**4.5**	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Plastic	0	0.5	1	1.5	2	2.5	3	**3.5**	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Rancid	0	0.5	1	1.5	2	**2.5**	3	3.5	4	4.5	**5**	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15

Appendix C - Ranking and time data collection ballot in Chapter 2, 3 and 5

Phase	Day	Dog	1st Kong	Time	2nd Kong	Time	3rd Kong	Time	4th Kong	Time	5th Kong	Time
		F1P										
		F1T										
		F2C										
		F2L										
		M4W										
		M5P										
		M5T										
		M6J										
		M6P										
		M7D										
		M7F										
		M8C										

Appendix D - SAS Code for Shelf-life Analysis in Chapter 4

```
proc glimmix data= fat;
class sample rep panelist;
model oxidized=sample/ddfm=sat;
random panelist rep;
lsmeans sample/pdiff lines;
run;
```