

Sensory analysis and acceptability of pet food

by

Brizio Di Donfrancesco

Laurea Triennale, University of Bologna, Italy, 2007
Laurea Specialistica, University of Bologna, Italy, 2010

AN ABSTRACT OF A DISSERTATION

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Department of Food, Nutrition, Dietetics and Health
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Abstract

The pet food industry represents a competitive and growing part of the food industry that is constantly looking for innovation to differentiate products in the market. In recent years, the pet food market has undergone a humanization trend that has transformed pet owners into parents. In the light of this trend, pet owner acceptance has become even more crucial to product developers as the owners are the ones who make purchasing decisions. Performing descriptive sensory analysis on pet foods utilizing a human panel can assist in understanding the sensory characteristic of products. Knowing the sensory profile of pet food can then be useful in product development, in order to relate the descriptive data with palatability data from pets and to understand specific sensory attributes that drive pet liking. At the same time descriptive analysis can help understand what drives consumer acceptance of the products. The first objective of the research was to develop a sensory lexicon that could assist researchers and sensory professionals working in the pet food industry to describe appearance, aroma, flavor, and texture characteristics of dry dog food. More than seventy sensory terms were identified, defined, and referenced. The second objective was to utilize this sensory lexicon to understand relationships between sensory properties of products and pet owners' liking. Results indicated that appearance played a major role in driving consumer liking of dry pet food. The next objective of the research was to understand sensory qualities and acceptance of extruded dry dog food manufactured with different fractions of red sorghum through some of the developed concepts. Sorghum is an important crop to Kansas that represents the first producer in USA. Sorghum characteristics such as a low glycemic index and antioxidant properties make it a perfect fit for pet food industry. A process such as extrusion may then help improve some negative characteristics such a lower digestibility that has been associated with sorghum in the past. Descriptive sensory analysis was performed and results indicated that aroma and flavor

profile of the sorghum diets were not dissimilar to the ones of a control diet manufactured with rice, wheat, and corn, grains that are typically used by the pet food industry. Acceptance of pet owners was then assessed through a Central Location Test involving 105 consumers. The whole sorghum diet resulted to be the most liked sample by consumers, at the same level of the control diet. The next objective was then to understand how the experimental diets would be accepted by pets compared in a home situation. Thirty dogs were fed the diets in their own household environment over 20 consecutive days. No differences in acceptance for the diets were found. The last portion of the research was to determine volatile compounds present in the four diets and try to identify possible relationship with the sensory properties of the samples. Thirty-six compounds were identified with aldehydes being the most abundant volatiles group. Several relationships with sensory characteristics of samples were found.

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Major Professor
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Chapter 1 - Literature Review

Sensory Analysis

Sensory analysis has been defined by Stone and Sidel (2004) as a scientific method used to evoke, measure, analyze, and interpret those responses to product as perceived through the senses of sight, smell, touch, taste, and hearing. More specifically, sensory analysis utilizes controlled testing conditions such as specific test designs, repetitions of measurements, the use of individual test booths, or samples labeled with random numbers, in order to minimize biases. In sensory analysis numerical data are collected in order to determine relationships between product characteristics and human perceptions. When collecting data, concepts like precision, accuracy, sensitivity, and avoidance of false positives are crucial. Statistical analysis is then performed in order to understand the source of variation in the data and ensure that the relationships observed are 'real' and not due to uncontrolled sources of variation in human response. Examples of uncontrolled source of variation are the physiological sensitivity to sensory stimuli proper of each individual, the respondents' motivation, or the degree of familiarity with the tested product. Finally, the results need to be interpreted, according to factors such as the research objectives and experimental conditions, in order to draw meaningful conclusions. The application of sensory analysis can be various but the central reason to perform sensory testing is to reduce the risk and uncertainty in decision making (Lawless and Heymann, 2010)

Types of Sensory Tests

Discrimination Tests

Discrimination tests are used to understand whether two type of products are perceived as different. These tests can be performed to try to determine if a sensory difference exists between two products. Two samples can indeed differ for chemical composition but still be perceived as similar by humans. Discrimination tests can also be performed to test for similarity, in order to determine whether two products are similar enough to be used interchangeably (Meilgaard *et al.*, 2007).

These type of tests are usually conducted when there are only two samples to test. Several types of discrimination tests exist, such as paired comparison tests, triangle tests, and duo-trio tests.

For these tests, statistics of frequencies and proportions of right and wrong answers are considered. If the proportion of correct choices is above the proportion expected by chance (test-specific) then inference about the samples being perceptibly different is drawn. Each test has a different chance of probability. For example, in the triangle test, the sample will be considered different when the long-run probability of making a correct selection, when a difference is perceived between the samples, is larger than one in three, the chance of probability specific for the triangle test.

Discrimination tests can be used, for instance, in product development when reformulation of products or processing changes are made with the goal of having consumers not detecting the difference. In other cases, a company may want the product to be perceived different, because of a claimed 'improved' formulation (Lawless and Heymann, 2010).

Descriptive Sensory Analysis

When the detailed description of sensory product characteristics, such as appearance, aroma, flavor, and texture is required, descriptive analysis techniques are used. Descriptive methods consist in the detection and the description of qualitative and quantitative sensory aspects of the product by a panel of trained judges. Product can share the same qualitative characteristics but be very different for the intensity of each of the sensory notes. Typically, 5-10 subjects are used to perform descriptive analysis. However, when testing for products of mass production, such as beer or soft drinks, a higher number of panelists can be utilized since for this products even the smallest difference can have a significant impact (Meilgaard *et al.*, 2007).

Several descriptive analysis methods, such as Flavor Profile, Quantitative Descriptive Analysis (QDA), and the Spectrum are available. It is important to select the method that provides the best description together with the best discrimination for the specific products or product categories, and the specific test objectives. Often existing methods are modified according and customized based on the specific testing needs.

In the Flavor Profile method, a panel of four to six trained panelists analyze the aroma and flavor characteristics of a product. Intensities, order of appearance, and aftertastes of each attribute are indicated. The degree to which different flavor or aroma characteristics are balanced and blended together in a product (amplitude) is also typically indicated with this method (Meilgaard *et al.*, 2007). After a training phase, panelists develop reference samples and define a common terminology to be used during the evaluation. Panelists first evaluate products individually and then report their results to a panel leader who leads a discussion to reach a consensus profile for each of the samples.

In Quantitative Descriptive Analysis (QDA), data are not generated through consensus and panel leaders do not actively participate in the evaluation. Unstructured line scales are used to describe the intensity of the sensory attributes. During a preliminary training phase panelists are exposed to different variation of product that will be tested. Panelists will then decide reference standards and develop a standardized vocabulary to describe the sensory attributes present in the product. Each panelist evaluates products individually, typically in isolated booths. In QDA, panelists replicate their evaluations and analysis of variance (ANOVA) and multivariate analysis techniques can be utilized to analyze the data (Lawless and Heymann, 2010).

In the Spectrum method, developed by Gail Civille, panelists score the intensity of the different attributes using pre-learned references and absolute intensity scales in order to obtain a sensory profile of the products that can be universally understandable and utilizable. In order to do this, lists of standard attribute names, called lexicons, and a set of standards to define a scale of intensity (typically from 0 to 15) are provided (Meilgaard *et al.*, 2007).

Hedonic Tests

With this type of testing researchers want to understand whether and how much a product is liked or disliked by consumers. In preference tests consumers are given a choice, usually between two samples, and asked to select the sample they prefer the most. This type of hedonic tests is very simple but they did not provide any information about the magnitude of consumers' liking or disliking.

This information can be provided by acceptance tests where a rating scale is adopted to quantify the degree of liking or disliking of products by respondents. A typical scale used to quantify the degree of liking or disliking of a product is the 9-point hedonic scale, developed at the Quartermaster Food and Container Institute of the U.S. Army in order to measure food preferences of soldiers (Peryam and Girardot, 1952). The nine phrases used in this scale range from 'dislike extremely' to 'like extremely'. Other types of scales can also be used for hedonic responses, such as line scales, magnitude estimation, or pictorial scales, such as facial scales. The line scales used in acceptance testing are unstructured line scales that can include anchor terms on each end (like and dislike). When using magnitude estimation, respondents are free to use any number and asked to consider the proportions between samples. Another type of scale, commonly used in areas such as product development, is the just-about-right scale (JAR) (Rothman and Parker, 2009). With the adoption of this scale respondents are asked to express both a hedonic and an intensity judgement. It consists of a center point, labeled 'just-about-right' and end anchors points labeled differently depending on the attributes involved, such as 'too weak' and 'too strong', or 'not sweet enough' and 'too sweet'. When used in product development, this scale can help to understand directions to take about specific 'key' attributes taking into consideration what consumers may expect and what level of a given sensory characteristic they would like to have in the product.

A number of participants between 75 and 150 is typically used for acceptance tests (Lawless and Heymann, 2010). However, different sample sizes are reported in literature when performing acceptance tests and several studies have been conducted to estimate the ideal number of consumers to utilize in this type of tests (Hough *et al.*, 2006, Gacula and Rutenbeck, 2006). When performing acceptance tests, evaluation of samples is usually not replicated by same consumers.

Central location tests (CLT) are tests conducted in a central location. A central location to conduct the testing may be, for instance, a sensory lab, a shopping mall, a retail outlet, or a school, depending on the type of test that need to be conducted and the type of consumers that need to be involved in the test. A CLT test setting provides a good control over testing conditions and sample preparation. On the other hand, this type of setting can also limit consumers' interaction with products compared to an in-home testing scenario.

Home use tests (HUT), offer several advantages, such as the fact that consumers take the product home, and can use the product over a period of time, on several occasions, and can interact with packaging, close to an everyday usage situation. On the other hand, HUTs are expensive and their setting and administration can be time consuming (Lawless and Heymann, 2010).

Sensory Analysis and Pet Food

The pet food industry represents an important and constantly growing sector of the food industry. In the last decade, in the United States alone, sales of pet supplies increased from \$38 billion of dollars to an estimated \$63 billion of dollars in 2016. Sales of pet food are estimated \$24 billion of dollars for 2016 (APPA, 2016). This industry is constantly searching for new ingredients and new ways to add value and differentiate products in the market. Recently, a humanization trend has transformed pet owners into ‘parents’ and pets into ‘children’. This is evidenced by 95% of pet owners in the United States considering their pets as part of the family compared to 88% in 2007 (Nielsen, 2016). This growing humanization trend impacts consumer choices and the pet food industry new products, to promote a stronger connection between human food and pet food. Several human food trends have been observed in the pet food segment, such as “gluten free”, “grain free”, a “low glycemic index”, “antioxidant enriched”, “naturally preserved”, or “non-GMO”. For their pets, consumers are moving from “high quality” product expectations toward more ‘humanized’ products. The trend suggests that they want the pet food they purchase to possess the same healthy benefits they find in their human food (Nielsen, 2016).

According to the pet food trade magazine, pet owners are willing to spend more money for pet food products if they can have the best product for their pets (Taylor, 2013). Even tradeoffs with other indulgences. For example, in a survey, pet owners indicated that they would be willing to give up on things such as their Netflix subscription or chocolate, if on a strict budget, in order to purchase high quality food for their pets (Nielsen, 2016). A survey conducted in August 2011 indicated that more than one fifth (21%) of the dog owners in the United States spent an average of \$100 dollars or more per month on their dogs (Phillips-Donaldson, 2011).

With pet food, like for other food categories such as the baby food industry, the actual consumer of the products is not the one that selects and makes the purchasing decision about the product. With acceptance of pet food by owners playing such a big role in the success of a product, emphasis on the sensory characteristics of products can represent an important aspect when trying to understand how to improve pet owner acceptance criteria. Sensory characteristics such as appearance and aroma of pet food can influence consumers' liking of pet food (Di Donfrancesco *et al.*, 2014). In addition, sensory analysis can be utilized to understand a given pet food products palatability for pets.

Descriptive sensory analysis of pet food products can be conducted using a human panel. Although cats and dogs perceive sensory stimuli differently than humans, gathering the descriptive sensory profile of a product, that pets cannot otherwise provide, can be useful for product development purposes and to compare descriptive data with pet palatability and consumer acceptance data. Comparing the sensory data and pet acceptance of the products can help understand what kind of sensory properties of the food might drive pet liking. Moreover, comparing descriptive data gathered with a human sensory panel may also assist in understanding what drives pet owners liking of a product. Previous studies, although not numerous, have utilized human panels to evaluate this product category (Pickering, 2008; Pickering, 2009; Lin *et al.*, 1998; Koppel *et al.*, 2013; Koppel *et al.*, 2015; Di Donfrancesco *et al.*, 2012).

Palatability Testing

Palatability can be described as the perception derived while the food is consumed. Palatability takes into account the perceived flavor and appearance by the animal, and the temperature, size, texture, and consistency of the food. Prior experience with a specific food can also play a role affecting palatability (Kitchell, 1978; Bradshaw, 2006). According to the National Research Council (NRC 2006) palatability is defined as “physical and chemical properties of the diet which are associated with promoting or suppressing feeding behavior during the pre-absorptive or immediate post absorptive period”. Another aspect that needs to be taken into account when discussing palatability is the interaction between pet owners and pets (Aldrich and Koppel, 2015). Palatability tests can be consumption or non-consumption tests (Griffin, 1984). The consumption type of testing is more common and consists of measuring food intake by the animal. A consumption test can investigate the acceptance of a specific food by the animal, such as with monadic or single-bowl (one-pan) test, or the preference of one food over another using two-bowl (two-pan, paired stimulus, split plate, or *versus* test) or forced choice preference test (Thombre, 2004; McArthur *et al.*, 1993; Tobie *et al.*, 2015).

Single Bowl Test

In the single bowl test food intake is measured, after a specified time interval, by the difference from the initial amount of food provided to the animal less the amount leftover. One or multiple feedings per day are planned and it is usually repeated for 5 or more days for a specific food. After a first sample is served, other samples can be presented to the animal for the same period of time. The different intake values relative to the different foods showed to the animal are then compared.

This test resembles the type of feeding experience a pet would have in the household environment. As the name suggests, an acceptability test can help to understand whether particular samples are considered acceptable or unacceptable by the animal. But, it does not provide any information about preferences and degree of liking. So, this type of test is not recommended for situations that need to provide flavor direction when developing a product, or to collect data in order to back-up a marketing claim. The number of animals necessary for this type of test can be as low as 8-10. Acceptance tests can be performed using trained kennel pets or using untrained pets in a home-use-test setting (HUT), and different breeds and pet size can be utilized (Aldrich and Koppel, 2015). However, it must be noted that animals and in the home and kennel might not respond to foods in the same way (Griffin *et al.*, 1984).

Two-bowl Test

In the two-bowl type of test two food samples are presented to the animal at the same time, offering the animal a choice. Aromatic characteristics will drive the animal toward one bowl or the other and researchers will collect this type of information in addition to the amount of food consumed from each bowl. As in the one-bowl test the two samples are available to the animal for a limited interval of time, usually 15-30 min. The amount of food sufficient to satisfy the animal daily caloric intake is placed in each of the two bowls. The consumption for each food and the total consumption out of the two bowls is measured in order to understand whether one food or the other is consumed in a greater proportion. In the past years, the number of animals used for this type of test used to be about 10 for a 5-6 days test period. In more recent years, the use of about 20 animals for 2-4 test days is preferred since it provides more true observations of the experimental unit. After an adaptation phase to the test environment conditions, animals used for this type of test should be

validated through an obvious test and a null test. In the obvious test the animal is provided two foods that are clearly different with one intentionally more flavorful than the other. In this way it is possible to verify whether the animal is able to discriminate between foods. In the null test the same food is provided to the animal in each of the two bowls. This test can help to understand eventual side-biases of the animal and the degree of the bias.

Other than the first approach, first bite, total consumption, and consumption ratio, it can also be possible to calculate the preference ratio. Animals that show to have a preference for one food over the other are 'counted' for one of the two food providing a Food 1: No Choice: Food 2 ratio (Aldrich and Koppel, 2015).

The two-bowl test is the most common type of test for palatability evaluation for pet food (McArthur *et al.*, 1993; Hutton, 2002).

This test is well suited for developing new products and testing whether the animal has a preference. However, the two-bowl test does not indicate what it might be liked or disliked by the animal for a specific food and the outcome for a food is dependent on the food that is placed next to it in the test.

Alternative approaches and complementary methods

To add information to the data gathered from the two main type of tests (acceptance and preference) described above, other methods can be adopted to better understand palatability.

In the Liking test (Becques and Niceron, 2014) several criteria, considered indicators of animal satisfaction by pet owners, were identified. A single-bowl approach is utilized in order to be closer to in-home feeding conditions. Adjusted amount of food depending on individual needs are utilized in order to have the animal finishing the bowl infrequently. Several indicators such as consumption

ratio, percentage of finished bowls (bowls with less than 1g remaining for cats or more than 97.5% of the individual ration consumed for dogs), refusals (quantity consumed equal to 0), and consumption speed are considered in order to understand additional aspects related food enjoyment by pets. Moreover, the comparison of consumption with the reference level of consumption of each pet is considered. In this case the reference consumption is calculated with a specific formula. With the “kinetics” approach it is possible to obtain several additional information performing both one-bowl and two-bowl tests. Measures of the cumulative amount of food eaten on a moment-by-moment basis are taken (fine-grained measurement). This provides quantitative information about individual feeding styles such as slow eaters versus fast eaters, the way animals distribute their food consumption when performing a choice test, or the initial disruptive effects of a new diet (Smith *et al.*, 1984). The kinetics approach can also be used to measure other indicators such as or the attractiveness of a sample differentiating between an immediate attractiveness of the sample and the consumption over time. This is gathered through the measurement of average time before the first visit, average consumption per feeding events, and number of passages without consumption (Tobie *et al.*, 2015).

Another alternative type of test is the Cognitive Palatability Assessment Protocol (CPAP), developed by Araujo and Milgram (2004). This is an object discrimination learning task procedure that allows the animal to express a preference for a food without any food intake. In this method three objects are made available for the animal to interact. Two of these objects are paired with two different foods. The animal will develop a preference for an object and the associated food and when the pairs are switched by the researcher, the animal has to learn the new association in order to continue receiving the preferred food with the previous object-food associations. Advantages of this approach are that it can provide indication regarding preferences in the long-

or short-term without having to take into account nutritional and caloric effects. This method has been shown to be less sensitive to prior feeding and satiation when compared to other type of tests such as a two-bowl test (Araujo *et al.*, 2004).

A method used to quantify the actual hedonic aspect of a food is the concurrent schedule paradigm (CSP). A lever pressing apparatus, or human-animal interaction such as nose touching, are utilized to understand the strength of the motivation to eat by an animal (Rashotte and Smith, 1984).

Studying pet behavior when they are exposed to food is also another approach that can be used to understand palatability. Observation of the olfactory exploration can provide useful information about differences in the palatability perceived by pets. The study of postures and its correlation with food intake have also been considered (Becques *et al.*, 2014).

Pet Food

A classification criterion for the multitude pet food products types in the market is outlined by the Association of American Feed Control Officials (AAFCO) model pet food regulations (AAFCO, 2016). The simplest classifications are those based on the moisture level:

- Less than 20% - “dry” pet food
- 20% or more but less than 65% - “semi-moist” pet food
- 65% or more – “canned” or “wet” pet food

Dry Pet Food

Extruded pet food

Most of the pet food products (more than 60%) are produced by extrusion (Gibson, 2015). During this process ingredients are mixed, moved in a preconditioner and then conveyed to an extruder. The food material is cooked by heat and pressure while it is forced along a shaft assisted by screws. Food is then forced out through a die at the end of the extruder barrel. The die can have different shapes and size depending on the type of products desired. Extruded food is then cut off the die by a knife positioned at end of the extruder. The knife cutting speed can effect size and shape as well. A drying and cooling phase follows. For instance, in this study a dual pass dryer-single pass cooler (Model 480; Wenger Mfg, Sabetha, KS, U.S.A.) was utilized for this stage. Dry or liquid ingredients, such as fat, can be then sprayed onto the pet food surface following drying; in most cases using a rotating tumble drum. At this stage the final moisture level is usually around 8-10%. Incorporation of air bubbles during extrusion can contribute to the food expansion and impact the product density (Dzanic, 2003).

Extrusion accounts for 80% of the dry pet food production whit baking and pelleting representing the other two major types of production processes (Gibson, 2015). While baking involves just thermal energy, extrusion involves both mechanical and thermal energies, making it possible to have 90% to 100% of gelatinization in extruded kibbles (Gibson and Alavi, 2013). Starch gelatinization observed in baked pet food kibbles was $\leq 60\%$. Together with and increased starch (gelatinization) and protein denaturation, extrusion may also yield a higher degree of amylose-fat complex formation. Amylose-lipid complexation (Pilli, 2011) may decrease the level of free or

unbound fat, susceptible to oxidation. Moreover, the amylose-lipid complexation slows starch digestibility which may represent a health benefit for dogs (Muoki, 2011)

Studies (Gibson, 2015) have also shown that extrusion reduces and kills bacteria in animal feed, but there are no studies showing the same effect on animal feed by baking processing. However, in studies comparing sensory properties of extruded and baked samples the authors observed that baked samples were lighter in color and had lower levels of attributes related to rancidity (Koppel *et al.*, 2014).

Gibson (2015) observed that an increased amounts of total energy input during extrusion increased starch gelatinization, with lower level of piece density, and larger kibble expansion ratio. There was an observed decrease in the amount of aerobic bacteria via Aerobic Plate Count and *Salmonella* spp. with an increase of total energy input into the extrusion system.

As mentioned in the previous paragraph, extrusion processing can increase starch digestibility to 90-95% for dog food diets (Gibson, 2013; Murray *et al.* 1999; Twomey *et al.* 2002) due to an increased starch gelatinization that makes more readily accessible to starch degrading enzymes (Twomey, 2003). Murray *et al.* (2009) found that the rapidly digestible starch in sorghum increased from 36.8% to 90.3% (% Dry Matter basis; DMB) after high-temperature extrusion (124 to 140 °C) and resistant starch in sorghum decreased from 45.6% to 2.7% (DMB) after high-temperature extrusion. Other studies observed a 98% starch digestibility when feeding dogs extruded sorghum diets (Carciofi *et al.* 2008).

Particle size can have an impact on starch gelatinization during extrusion of sorghum diets. Putarov *et al.* (2014) used two types of sorghum (Red and White) milled at three different particle sizes (0.5, 0.8 and 1.0 mm) in a premium dog food formulation. Diets were extruded using two different Specific Thermal Energy (STE):Specific Mechanical Energy (SME) ratios: High STE:SME

(300RPM/85-90°C) and Low STE:SME (400RPM/75-80°C). Specific mechanical energy is a scale-independent measure of the mechanical energy put into the extrudate. More specifically, SME provides a measure of the energy going into the extrusion system per unit mass in the form of work from the motor (FoodExtrusion.org). The thermal energy, transferred through the barrel wall (or generated by viscous dissipation), generates an increase in the temperature with a subsequent phase change such as moisture evaporation or melting of solid material (Janssen *et al.*, 2002). Bulk density, inversely related to kibble expansion during extrusion, increased with particle size and was higher for the diets extruded at Low STE:SME. Starch gelatinization was affected by particle size (93%, 85% and 82% of starch gelatinization for 0.5, 0.8 and 1.0 mm particle size respectively) but not by process conditions. A higher starch gelatinization was observed for white sorghum when compared to red sorghum and a control rice diet (89%, 85% and 80%, respectively) (Putarov *et al.*, 2014).

Baked pet food

Baking is less common than extrusion when manufacturing dry pet food products, although it was commonly used in the past. It is mostly used when manufacturing treats, but sometimes it can be used to produce complete feeds. The first step in baking is the forming of a dough that is then flattened out by rollers to form a sheet. The desired shapes are cut out from this sheet and then baked and cooled into a tunnel oven. Baking can allow for a larger varieties of shapes of the final product than extrusion, representing one of the reason it is used for treats production. At the same time, to keep shape and rigidity of products, a lower flexibility in the ingredients that can be used is required. During baking there is not air incorporation in the same way that occurs during

extrusion with baked pieces having a higher density and hardness than extruded products (Dzanic, 2003).

Semi-moist Pet Food

Semi-moist products are also produced using an extruder but they differ from the dry extruded products in the final moisture content that is usually around 30%, and in the lower amount of air incorporated in the extrudate. Because of the higher moisture content and consequent concerns about products being more susceptible to mold and bacterial growth, humectants are used to bind the free water and reduce its availability for microorganisms. One of the main characteristics of this kind of products is the very high pliability that makes it possible to create a wide variety of shapes. For instance, semi-moist products can be shaped to resemble meat chunks, hamburgers patties, or shaped like whole sausages that can be sliced by the pet owner. Hybrid blends of dry and semi-moist products can be called “semi-dry” or “soft-dry” (Dzanic, 2003).

Canned pet Food

Canned food has a higher content of moisture with more than 65% and usually no more than 78% is allowed, except for products such as “stews” or “in gravy” (AAFCO, 2016). To make these products, ingredients are first ground, mixed, and then lightly cooked. Cans are then filled, hermetically sealed, and then subjected to a sterilization process through additional heating, in order to avoid microbial contamination. Formulation of canned food allows a high level of flexibility in the choice of the ingredients, usually a mixture of grains and animal products with a larger portion of meat, fish, or poultry compared to dry or semi-moist products. This usually translates into a lower carbohydrate and higher protein and fat content product than dry foods.

Since fat is not added as a separate ingredient, but with the animal-source component, there may be a higher variability in fat content from batch-to-batch when compared to dry and semi-moist pet food (Dzanic, 2003).

Pets diet requirements

One characteristic that differentiates pet food from human food is that with pet food, one type of food has to satisfy all the daily nutritional requirement of a pet. To meet carbohydrates, protein, fat, and macronutrients requirements, a variety of ingredients such as grains, meat products, fat, and micro-ingredients (vitamin and minerals) are combined in a complex food matrix (Gibson and Alavi, 2013).

When considering protein requirements of pets, both dogs and cats need 22 amino acids to synthesize the various body protein structures. In dogs, 13 amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine are essential and must be present in the diet in a sufficient amount depending on the animal needs. Ten amino acids are considered essential for cats (Hussein, 2003a; Hussein, 2003b). Most of the commercial dog food products supply proteins above the minimum requirements. Thus, protein deficiencies in adult dogs is usually not expected (Kallfelz, 1989). However, in particular life stages such as gestation, lactation or during particular heavy work conditions, the choice of low quality and poor formulated diets can lead to protein deficiency (Case, 1999). Protein requirements can vary depending on factors such as protein quality, protein digestibility, energy intake, age, and reproduction status. For adult dogs, a minimum requirement of 18% crude protein (CP) on DMB was adopted by AAFCO for maintenance, and a minimum of 22% CP for growth and reproduction (AAFCO, 2016).

For adult cats, higher protein requirements are adopted by AAFCO: a minimum of 26% CP on DMB for maintenance, and a minimum of 30% CP on DMB for growth and reproduction stages. Although no specific requirements are adopted for carbohydrate levels in dogs and cats diets, these pets can utilize carbohydrates when provided in a proper form. Most of the dog and cat food products contain carbohydrates in the form of starch from cereal grains such as corn, wheat, sorghum, barley, and rice. Glucose derived from carbohydrate digestion is utilized together to glucose produced endogenously (Hussein, 2003a; Hussein, 2003b).

Fats are important in pets' diets for several reasons such as because of their high energy density, because of their role as carriers of fat-soluble vitamins, the improvement of food palatability, and because they can provide desirable texture to the food. Low fat levels in the diets can lead to energy shortage and decreased palatability of diets with consequent intake reduction. A minimum level of 5% fat on DMB for dogs, and a minimum of 9% fat on DMB for cats, are recommended by AAFCO for maintenance (Hussein, 2003a; Hussein, 2003b).

From a nutritional perspective, if a product is well formulated in order to satisfy the nutritional requirements, the form of the food is not relevant. However, it might be relevant for the pet owner and have an influence on pet preferences (Dzanic, 2003).

Sorghum

Sorghum nutrient profile

Sorghum contains 10.62% of protein, more than corn, with lysine and threonine representing the first and second limiting amino acids in dogs (Aldrich, 2015). However the content of these amino acids in sorghum is slightly lower than in corn (Wall and Paulis, 1976). Fat content in sorghum is 3.46%, slightly lower than corn, and responsible for a slightly lower metabolizable energy. Linoleic acid (C18:2n6), an essential fatty acid for both dogs and cats, represents more than half of the fatty acids and oleic acid (C18:1n9), not an essential fatty acid for pets, accounts for one third of the fatty acids profile in sorghum. The omega-3 linolenic acid (C18:3n3) represents less than 3% of the total fatty acids. Most of the fiber portion (6.7%) is insoluble with appreciable amounts of lignin in the seed coat. When compared to corn, sorghum contains a higher level of phosphorus, potassium, iron, and a lower sodium content. The vitamin content is similar to several other cereal grains (Sorghum Checkoff Program, 2016).

Sorghum is rich in phytochemicals with potential impact on human health such as tannins, phenolic acids, anthocyanins, phytosterols, and policosanols. Health benefits associated with these fractions, such as antioxidant activity, are comparable to the ones associated with fruits (Awika, 2004). Studies have shown that sorghum consumption reduces the risk of certain types of cancer in humans. All sorghum contains phenolic compounds but the amount in each cultivar, together with color, appearance, and nutritional quality, is influenced by genotype and the growing environment (Dykes, 2005). Color of grain sorghum can be influenced by several factors such as the genetics of pericarp color, pericarp thickness, presence or absence of a testa, color and thickness of the testa, and the endosperm color (Rooney and Miller, 1981). Condensed tannins are

present in cultivars with a pigmented testa but the color alone is not a good indicator of tannin content (Boren, 1992).

Digestibility

Together with the sensory characteristics and acceptance of products investigated in this dissertation, digestibility represents another fundamental factor that cannot be ignored when discussing the role of sorghum in pet food production. Below a review of several studies on sorghum digestibility in pets, in order to provide a background to the reader on this important aspect.

Bednar *et al.* (2001) observed that the starch composition differs between grains and legumes and has an impact on digestibility in dogs. The portion of rapidly digestible starch was higher in sorghum, brewer's rice, and corn than in legumes such as peas and lentils.

In 2002, a study by Twomey *et al.* with dogs investigated nutrient digestibility and effect on fecal quality of diets containing rice (49% inclusion), sorghum (46% inclusion), and corn (51% inclusion). A difference was observed in the fecal score among the treatments with rice resulting in higher fecal score (looser feces). All the fecal scores were within the ideal range according to the Waltham Fecal Scoring System, indicating that the sorghum and corn diets did not have a negative effect on fecal quality. Starch digestibility was not different among diets and each resulted in 100% fecal starch digestibility. The authors indicated that this was probably due to the extrusion process that gelatinized the starch in the sorghum and corn diets making them more digestible. Fecal protein, fat, and gross energy digestibility coefficients were higher for the rice treatment compared with the other diets. Thus, rice diet had a higher digestible energy content than sorghum and corn. Even if nutrient digestibility of rice was higher than the other diets, nutrient digestibility

of all the diets was above the average digestibility values for commercial dog foods according to the National Research Council nutrients requirements of dogs.

Carciofi *et al.* (2008) investigated the effect of six extruded diets with different starch sources, including sorghum, on total tract apparent digestibility and glycemix and insulinemic response in dogs. The experimental diets (and relative inclusion of the starch source) were: cassava flour (42.49%), corn (53.49%), sorghum (59.27%), brewer's rice (45.66%), lentils (69.53%), and pea (66.35%). Sorghum used in this study had a low tannin content (0.57%). To obtain balanced diets containing similar percentages of starch, fat, calcium and phosphorus, additional ingredients such as isolated soybean protein were added. All dogs consumed the experimental diets with no episodes of vomiting, diarrhea, or meal refusal. Dogs fed sorghum-based diets ingested more protein than those receiving lentil-based diets and ingested less fat than dogs fed the cassava flour diet. Protein digestibility was higher for dogs fed brewer's rice than sorghum, corn, pea, and lentil diets. Starch digestibility was >98% for all the diets (brewer's rice and cassava flour had the highest digestibility and pea and lentils the lowest). Digestibility of total dietary fiber (TDF) was higher in sorghum, pea, and lentil diets than in brewer's rice, corn, and cassava flour. There was no observed difference in fecal scores among the treatments. Fecal dry matter content was higher for dogs fed brewer's rice, sorghum, and corn than for those fed pea diet.

In the same study, mean plasma glucose concentrations in dogs fed cassava flour and corn diets were lower than those in dogs consuming the other treatments. At 180 min after consumption of brewer's rice, cassava flour, and corn diets, the dogs mean plasma glucose concentrations were not different from basal values. While, after 300 min plasma glucose concentration for dogs fed sorghum, pea, and lentil diets remained above basal values. The post-prandial insulin response curve of the sorghum diet did not return to baseline during the 300 min observation time. The total

Area Under the Curve (AUC) of insulin (0 to 300 min) was smaller for dogs fed cassava flour than those fed with sorghum. The AUC \geq 30 min was greater after ingestion of sorghum than brewer's rice or cassava flour. This study showed that diets containing sorghum, lentils, or pea can add a positive effect on dogs' health delaying and lowering the glycemic and insulinemic response.

In 2009 Kore et al. conducted a study to investigate the digestibility of samples made of sorghum, pearl millet, and corn as alternatives to rice in dry dog food. Each of the diets contained 70.5% of the specific grain, 25% extruded soya, 1.8% soya oil, 0.3% salt, 1.3% dicalcium phosphate, 1% calcium carbonate, vitamins and minerals. Dry matter (DM) digestibility was significantly lower for dogs fed corn, pearl millet, and sorghum when compared to rice. Protein and fat digestibility were similar for rice, sorghum, and corn. The fecal DM was significantly lower when dogs were fed the rice diets compared to corn, pearl millet, or sorghum. Silva Junior et al. (2005) also reported a lower DM digestibility coefficient in dogs when replacing rice (87%) with corn (83%) or sorghum (81%).

Murray et al. (1999) observed a reduced protein digestibility in dogs when corn-based diets (crude protein digestion 86.5%) were replaced with sorghum diets (Total Tract Digestion; TTD, 83.3%). In this study the TTD (84.9%) for dogs fed the rice-based diet was comparable to dogs fed either corn or sorghum diets. The dogs fed sorghum, corn, and rice diets had 44.2%, 43.6, and 44.1%, respectively, of the specific starch inclusion.

A study by Twomey *et al.* (2002) found a lower protein digestibility in dogs fed sorghum diets (85%) and corn diets (83%) when compared to rice diets (87%). In this study, the inclusion rate for the different starch sources was 49% for rice, 51% for corn, and 46.1% for sorghum.

Another study by Fortes *et al.* (2010) compared the nutritional value of broken rice, sorghum, high oil corn, corn germ, rice bran, and millet. The ingredient composition of the reference diet (g/kg,

as-fed basis) was: 667.7g of corn; 178g of poultry by-product meal; 62.1g of corn gluten meal (600g crude protein/kg); 36g of poultry fat; 30.4g of dried hydrolysed bovine liver; 13.3g of dicalcium phosphate; 3.4g of calcium carbonate; 5.6g of vitamin and mineral premix; and 3.6g of sodium chloride. For each of the test diets 700g of the reference diet and 300g of the test ingredient were combined. The total (dry matter), protein, and starch digestibility was higher for the reference diet, the high oil corn, sorghum, millet, and broken rice diets than corn germ and rice bran. The sorghum had a similar metabolizable energy content compared to the high oil corn, millet, and broken rice diet, and lower ME than the corn germ diet. Production of feces by dogs was similar for those fed sorghum, high oil corn, broken rice, millet, corn germ, and rice bran. Overall, for dog diets containing sorghum, high oil corn, broken rice, and millet had a better digestibility and greater metabolizable energy than wheat bran, corn germ, or rice bran.

An *in vivo* trial in dogs by Kansas State University (Alavi, 2016) in 2016 showed that digestibility of sorghum diets (inclusion around 50% of the food) was not different for dogs fed rice and corn diets. The fiber level of the rice diet was corrected including more beet pulp, since rice has less fiber than sorghum, to obtain similar nutrient composition among diets. This correction is not often performed in published studies even if it may have a role in some of the differences observed in the studies. For fecal production and quality, the study also showed no differences among the diets for fecal score, fecal moisture content, and fecal production. Moreover, the feces were very close to ideal for all of the diets. Fecal pH was lower for feces of dogs fed with red and white sorghum diets than those fed with rice and corn diets. Postprandial glucose analysis was not different for glucose mean, glucose maximum, incremental increase, and peak concentration among diets.

A difference in starch digestibility between different diet sources can be related to the different cereal type, a different starch-protein interaction, physical granule form, starch type, digestion

inhibitors and, probably the most important, processing methods. In corn and sorghum, a starch-protein interaction may interfere with endogenous enzymatic digestion in the gastrointestinal tract (Kore, 2009). The starch-protein matrix formation is also related to the method of processing and it is an accepted fact that processing a dog food diet by extrusion increases the starch digestibility to 90-95% (Murray *et al.* 1999). Most probably this is because it facilitates starch gelatinization, making the starch almost completely digestible (Twomey, 2003). Carciofi *et al.* (2008) reported that dogs fed extruded diets containing sorghum, cassava flour, brewer's rice, and corn had starch digestibility coefficient >98%.

Fecal quality is an important factor for owners. Twomey *et al.* (2003) observed that the addition of a feed enzyme containing a mixture of carbohydrates into a sorghum diet fed to dogs improved fecal scores to equal feces from dogs fed a rice-based diet. The enzyme caused a slight softening of the feces without making them diarrheic. The enzyme was sprayed on the dry diet at a level of 1,000 ml/t and the diets used in the experiment contained 55.2% sorghum, 53.5% corn, and 52.1% rice. However, the lower fecal score for dogs fed corn and sorghum without enzyme addition were all within 'ideal' according to the Waltham scale.

Corsato-Alvarenga (2016), incorporated whole sorghum, sorghum flour, and enriched mill-feed into extruded dog foods and compared the results to a control diet containing rice, corn, and wheat and observed that dry matter digestibility was similar between the control diet and the whole sorghum diet, but slightly less than for the flour diet (89.9, 88.9 vs 92.0%, respectively). For dogs fed the mill-feed diet, dry matter digestibility was the lowest among samples (78.5%). Organic matter, energy, and crude protein digestibility followed a similar pattern. The sorghum flour diet provided a slight improvement to digestion coefficients and the author suggested that this might represent the opportunity for new uses for "easy to digest" products. The mill-feed diet showed

the highest amount of wet feces excreted followed by whole sorghum diet, then the control diet, and the lowest amount was observed for the flour diet. The amount observed for the mill-feed diet was almost three times higher than that of flour diet (95.4 vs 32.6 g/d). The number of defecations per day was similar among the control, whole sorghum, and flour diets. With more feces excreted daily and more defecations per day one may suspect a higher moisture level and perhaps softer stools. However, the mill-feed diet had the highest fecal scores (3.91 on a 5-point scale in which 4 is firm dry feces). Dogs fed the control also had the lowest fecal score.

Sensory characteristics of sorghum pet food

Phenolic compounds can be responsible for bitterness and astringency in food and beverages. In sorghum, phenolic compounds such as tannins, anthocyanins, and phenolic acids are mainly found in the bran. Type and level of phenolic compounds in different varieties of sorghum are influenced by both genetics and environmental factors. Kobue-Lekalake et al. (2007) investigated the sensory characteristics of sorghum that contained different levels of total phenolic compounds, condensed tannins versus those which were tannin-free. Bran infusions and cooked sorghum grains were used in the study. Astringency and bitterness, from descriptive sensory analysis, were present to some extent in all the sorghum cultivars analyzed. The bran infusions made with tannin sorghum were perceived more bitter and more astringent than those made with tannin-free sorghum, that were perceived sweeter and cloudy. The cooked sorghum from white sorghum had a harder endosperm and it was less chewy than the other sorghums used. An unexpected result was that, for the cooked sorghum grains, the bitterness and astringency of one of the tannin sorghum cultivars (NS 5511) with more than twice the total phenol level was comparable to those of tannin-free sorghums

suggesting that not all sorghums containing condensed-tannin have undesirable sensory characteristics.

In a study done in 2016 at Kansas State University (Alavi, 2016), extruded sorghum diets, manufactured with white and red sorghum (inclusion 50%), and a control diet based on corn and rice were fed to 36 adult dogs (both male and female, of various races) kennel dogs. No statistically significant difference was observed in the intake of these diets.

Other studies, such as Kore et al. (2009) showed that the mean daily DM intake by dogs as a whole (g day⁻¹) and relative to their body weight (g kg⁻¹ BW) of rice (140.2 ± 5.2 and 22.9 ± 1.5), maize (129.2 ± 2.0 and 21.9 ± 1.7), pearl millet (122.4 ± 10.2 and 19.8 ± 3.0), and sorghum (140.7 ± 2.9 and 22.3 ± 1.5) diets were not significantly different. Carciofi et al. (2008) and De-Oliveira et al. (2008) also obtained similar results when feeding corn, sorghum, and brewer's rice extruded diets for dogs and cats. This results might indicate the absence of off-notes in sorghum diets that might be perceived as undesirable by dogs. However, dogs can tolerate bitterness better than cats (Bradshaw, 1991) and palatability tests using cats might show different results.

Several studies showed that digestibility performances of extruded sorghum diets can be similar to that of other grains with good fecal quality and a lower glycemic index as well. However, some studies showed a possible lower protein digestibility of sorghum when compared to rice or corn. Extrusion can increase starch digestibility of sorghum diets up to 0.98% in dogs. Moreover, extrusion might limit the presence of undesirable sensory characteristics in sorghum extrudates. Cardoso *et al.* (2015) showed that proanthocyanidins, which can be responsible for higher bitterness and astringency, were reduced after extrusion.

Volatile Aromatic Composition

Descriptive analysis indicated that pet foods often represent a category of very complex products. Several type of ingredients such as different types of grains, meat sources, oils (animal and vegetable), minerals, vitamins, antioxidants, preservatives and other types of additives can be included in pet food formulation. Because of this complexity the study of aromatic composition of pet food can provide important information in order to understand the product (Koppel *et al.*, 2013). Several studies exist that investigated the volatile aromatic composition of food such as grains or meat ingredients that can be part of in pet food products as raw materials (Busko *et al.*, 2010; Lammers *et al.*, 2011; Parker *et al.*, 2000).

The analysis of volatile compounds composition of different types of grains such as corn, rye, wheat, barley, and rice, has been conducted using solvent extraction techniques but solid-phase microextraction (SPME) has become a widely utilized technique. Advantages of SPME are that it is fast, solvent-free and it can assist in the simplification of complex sample matrixes. The concentration of the fiber and the availability of different coatings impart sensitivity and selectivity to this technique (Kaseleht *et al.*, 2010; Waters, 2016).

During the extrusion of grain flours, it is possible to individuate two main reactions that lead to the formation of volatile compounds: Maillard reactions and lipid degradation. During the Maillard reaction, where several reactions between reducing sugars, amino acids, and their respective degradation occurs, mostly desirable notes such as toasted grains aroma notes are generated. From a compound generation standpoint, a common category of compounds generated during Maillard reaction are Strecker aldehydes, by decarboxylation and deamination of amino acids (Parker *et al.*, 2000). Off-flavors, associated with compounds such as hexanal and pentanal, are instead often the

products of lipid degradation reactions. The volatile compounds produced by lipid degradation have been extensively described and are mostly represented by aliphatic aldehydes, alcohols, and ketones derived from fatty acids. Extrusion conditions can have an influence on both these type of reactions (Parker et al., 2000).

Research Objectives

The first objective of this research was to develop a sensory lexicon that could assist researchers and sensory professionals working in the pet food industry to describe sensory characteristics of dry pet food products such as appearance, aroma, flavor, and texture.

The second objective was to utilize the previously developed sensory lexicon to understand relationships between sensory properties of products and pet owners' liking.

The studies addressing the first two objectives (Chapter 2 and Chapter 3) can be considered the first part of the research with conclusions that can be applied to the whole dry pet food category.

Results from this first part made it possible to work on the second part of the research that investigated specific samples manufactured by the researchers to understand how different incorporations of a specific raw material (sorghum) might have an impact on sensory properties and acceptance of the finished product. Sorghum is an important crop to Kansas that represents the first producer in USA. Sorghum characteristics such as a low glycemic index and antioxidant properties make it a perfect fit for pet food industry. A process such as extrusion may then help improve some negative characteristics such digestibility and sensory properties.

In this direction, the next objectives of the research were to understand sensory qualities and acceptance of extruded dry dog food manufactured with different fractions of red sorghum.

Descriptive sensory analysis of samples using a highly trained descriptive panel was performed and acceptance of products (appearance and aroma) by pet owners was then assessed through a Central Location Test involving 105 consumers. The next objective was then to understand how the experimental diets would be accepted by pets compared in a home situation. A one-bowl palatability test was held using 30 dogs fed each of the four diets in their home by their owners for 20 days.

The last objective of the study was to understand the volatile compounds composition of the sample to understand if different volatiles and different concentration of volatiles was present in the diets. Moreover, relationships between volatiles in the samples and sensory characteristics were investigated.

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Chapter 2 - An Initial Lexicon for Sensory Properties of Dry Dog Food

Abstract

A sensory lexicon for human description of the flavor, aroma, texture and appearance characteristics of dry dog food was developed using a consensus profile method. Twenty-one products, available in the U.S market, were studied. A five-member highly trained descriptive sensory panel identified, defined and referenced more than 70 sensory attributes for this product category. The lexicon established included attributes common to most of the samples such as barnyard, brothy, brown, grain, soy, vitamin, the off-flavors oxidized oil, cardboard, and stale, and attributes appropriate for only a few products such as liver, fish, burnt, brown spice, garlic, celery, clove, and smoky. The product category often showed a blended sensory profile and overall impact was evaluated to better discriminate among the products.

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Introduction

Pet food production represents a competitive and economically relevant part of the food processing industry. As with human food, the development of new products must take into account both nutritional and palatability aspects. Although it may be assumed that dogs prefer meat, such as beef and pork, over cereal diets this may not necessarily be the case for domesticated dogs. A number of factors such as the dog's gender, reproductive status, weight and relationship to owners, the physical and social environment as well as the content of the dog's meals, seem to be related to some flavor preferences of pet dogs. Pet dogs have more variable preferences than cats according to their owner's subjective evaluation (Houpt, 1981). The pet food industry offers a wide range of products in order to satisfy the disparate pet's and owner's requirements.

Dogs, like cats, use both taste and smell in the detection and selection of food. There seems to be some plasticity in the link between odor and food since meaty odor alone will not sustain interest in a bland food for dogs (Bradshaw, 1991). Smell and taste are considered the first two chemosensory systems. The third chemosensory system, the vomeronasal organ appears to be involved only in the perception of social odors (Hart and Leedy, 1987). Dogs tolerate bitter taste better than cats and bitterness can be accepted by dogs. Another difference with cats is that dogs, like humans, are sensitive to mono- and disaccharides, particularly fructose and sucrose, and some alternative sweeteners, while cats are not. Cats and dogs respond to sour taste and in part to the umami taste. Both of them show a lower sensitivity to NaCl compared to other species (Bradshaw, 1991).

Although taste and olfactory perceptions are not completely the same between dogs and humans, sensory data from humans can be useful to assist with pet food formulation. Humans, as pet owners

make decisions on the purchase of pet food products. In addition, the comparison between flavor profiles created by human panelists and data obtained from pet acceptance and preference trials may enable a more rapid, quantitative and predictive indication of the effects of ingredients and processing changes on the products (Pickering, 2008). Some companies from the pet food industry conduct in-house tasting trials using human testers (Pickering, 2009). Lin *et al.* (1998) assessed the effects of extrusion parameters (fat type, fat content and initial moisture content) of some sensory characteristics of extruded pet food during storage using a human sensory panel. In addition, Pickering (2008, 2009) studied dry and canned cat food products by descriptive analysis techniques traditionally used for human food. Behavioral studies such as preference and acceptance tests with animals can be conducted to improve the product development process but they are expensive, time consuming and yield limited and often equivocal data (Booth, 1976). Moreover, factors such as individual animal variation, previous diet, prior experience and lateral bias complicate the protocol of tests using animals (Rofe and Anderson, 1970). The objective of the current study was to develop a lexicon that could describe the appearance, texture, aroma and flavor characteristics of dry dog food.

Materials and Methods

Samples

Twenty-one dry dog food products were used in this study. All of the products were available and made in the U.S.A except for one manufactured in Canada (#373). The samples were chosen from an initial list of approximately two hundred products found in local stores. Product selection aimed

to evaluate a sufficiently wide range of formulas and peculiar characteristics declared by the manufacturer in an effort to provide a reasonable representation of the category.

The chosen samples differed in size, shape, and color. Some samples were specifically for adult dogs, some for all ages of dogs, and others for specific age categories such as puppy or mature dogs. Two of the samples (#698, #373) were specific for small breeds. Some of the samples were grain free (#220, #509, #586, #874, #373, and #819). Other samples were described as products useful for specific functions such as oral care (#117), weight control (#767), sensitive skin (#675), or healthy mobility (#280). Several products had particular ingredient compositions such as organic (#495), low fat (#952), or simple formulas with limited ingredients in order to avoid allergies, intolerances, or to make digestion easier (#740, #481, #254, and #874). One sample was sold specifically as a low cost formulation (#824). Other samples were chosen because the manufacturer declared valuable flavors and ingredients such as salmon (#905), duck (#190, #481), lamb (#586), venison (#740), or steak (#120) on the package. One sample (#120) consisted of three different kibbles (#120 a, #120 b, #120 c) and was evaluated as a whole for aroma, but individually for other sensory aspects. Another sample (#190) contained two different kibbles. However, one kibble was infrequent in the product; thus, only the most representative kibble was evaluated. The products were obtained 1 week before testing and were stored according to the instructions given on the packages. All samples were within the “best by” code date on the package. In addition, all sample lots were checked to ensure that the samples had not been subject to a product recall.

Sample Preparation

All of the samples were served without further manipulation except for one freeze-dried sample (#586), that was prepared according to the instructions given by the manufacturer on the package (water was added in the amount sufficient to cover the product 4 minutes before serving). Each sample was served in a ~100 ml plastic cup for appearance, texture, and flavor evaluation, and in a medium snifter covered with a watch glass for the aroma evaluation. The amount of product in the snifter was 3 g. Samples were prepared 30 minutes prior to the testing (water was added to sample #586 4 min before evaluation) and were coded with three-digit random numbers.

Panelists

Five highly trained panelists from the Sensory Analysis Center, Kansas State University (Manhattan, KS, USA) participated in this study. The panelists had completed 120 h of general descriptive analysis panel training with a variety of food products. This training included techniques and practice in attribute identification, terminology development, and intensity scoring. Each of the panelists had more than 1,000 h of testing experience with a variety of food products and had conducted studies using the consensus method used in this study. For this project the panelists received further orientation to dry dog food using samples that may or may not be included in the study.

Terminology Development and Description

The descriptive terminology was developed initially using only eight of the dry dog food products selected by the researchers through smelling and tasting to represent a range of samples. Various descriptive references were provided for the panelists. Some references were proposed by the

panelists, based on previous work and experience, whereas other references were added to the lexicon during this initial lexicon development phase. Six 1.5 h orientation sessions were held to establish the initial attributes and descriptive references. Recent studies by Suwonsichon *et al.* (2012); Adhikari *et al.* (2011); Civile *et al.* (2010), Koppel and Chambers (2010); and Talavera-Bianchi *et al.* (2010) have used similar attribute determination and description procedures.

The panel was aware that this terminology development phase was not final. If new attributes appeared during testing, panelists knew that additional descriptive terminology and references could be added during the testing phase by agreement among the panel. This consensus profile method is particularly useful in lexicon development studies because new attributes can be easily added, defined, and referenced when they appear in products the panelists are seeing for the first time.

Sample Evaluation Procedure

All of the samples were evaluated for appearance, flavor, texture, and aroma using the lexicon developed. Twenty sessions, 2 h each, were held for the evaluation phase. One to two samples were evaluated during each session in order to reduce the carryover of flavors. All of the samples were coded with three-digit random numbers and the order in which the products were evaluated was randomized. When the evaluation of a sample was split into two different sessions, appearance and texture were evaluated first and aroma, flavor and aftertaste in the next session. Panelists added attributes to the descriptive terminology when new characteristics were found in the sample they tested.

A descriptive scale of 0-15 with 0.5 increments where 0 represents none and 15 extremely high, was used to measure intensity. Each panelist individually assigned intensities to the attributes

present in the sample according to the appearance, aroma, flavor and texture references included in the lexicon. Next, the panel leader led a discussion to determine the consensus scores for each attribute. In case a new attribute emerged, a discussion focused on the appropriateness, definition, references and evaluation technique of the attribute. The panelists also were asked to determine order of appearance regarding the first three aroma and flavor attributes.

During testing we noted that dry dog foods were highly blended products with low flavor scores that did not necessarily have a wide range. Thus, two additional sessions were held to evaluate “overall impact” of the flavor in order to have an additional parameter that might differentiate the samples. Panelists were asked to evaluate the flavor peak during the whole mastication time, indicating the dominant flavor note for each product.

Panelists were asked to chew 1 kibble for flavor and texture evaluation, and 2 kibbles in the case of small-sized kibbles. The panelists were instructed to swallow once and expectorate all other times during tasting. The panelists were provided with apple slices, unsalted crackers, purified water, and toothbrushes (for gentle tooth brushing when needed) for palate cleansing between evaluations. The testing room was at 21 ± 1 C and $55 \pm 5\%$ relative humidity.

Data Analysis

The UnscramblerX version 10.2 (Camo Software, Norway) was used for Principal Component Analysis (PCA). A PCA was conducted for appearance and texture attributes and separately for flavor, aroma and aftertaste. Attributes that were scored in 12 or fewer products (Table 2-1) were excluded from the PCA analysis because they tend to force the multivariate statistics into separating unique attributes at the expense of understanding relationships among common overarching characteristics. Using this criterion allowed us to study the overall pattern of common

attributes in the dry dog food products in this study. It places uncommon, but potentially characterizing attributes into a separate “basket” that must be examined further by the researchers on a case by case basis to determine suitability to the lexicon.

Results and Discussion

Lexicon development

At the end of the orientation phase, the eight samples tested (#819, #495, #767, #120a, b, and c, #117, #220, #586, and #373) resulted in a first list of 107 attributes. The panel leader initiated a discussion in order to eliminate synonyms and to combine or split attributes to better define the characteristics, to modify definitions, and to check pertinence with tested products. As a result, the panelists started sample evaluations using a lexicon that included 72 attributes. Fifteen attributes were associated with appearance, 46 attributes concerned aroma and flavor, and 11 attributes concerned texture.

An example of an attribute that was split into additional attributes was “uniformity of appearance”, which was divided into “color uniformity”, “shape uniformity”, and “size uniformity”. An example of attributes that were merged was herb and grass which became “green”, with a corresponding definition and references which better explained the concept the panel needed to convey for the products evaluated. Attributes that the panel considered redundant or unnecessary were foam-like, rancid, and iron because fracturability, oxidized oil, and metallic, respectively described the samples better. For texture attributes the panelists eliminated sandy and oily residual because gritty and oily mouthfeel, respectively, described the samples more precisely. The firmness attribute was

first excluded from the initial lexicon, but was then included back because the definition of the hardness attribute was not suitable for spongy samples that could be compressed but not bitten completely through.

Because of the blended nature of the samples sometimes the panel indicated the need to use an overall term instead of specific individual characteristic. For example, spice complex and brown spice were used instead of individual attributes such as cinnamon or clove because it was impossible to score the specific attributes. Spice complex was associated with onion, garlic, cumin, black pepper and paprika whereas the brown spice was characterized as cinnamon, clove, and nutmeg notes. However, when possible, the panelists identified the single spices present in the samples. In the same manner, the panel included a vegetable complex attribute to describe cooked vegetable aromatics; although when it was possible they used the specific vegetable attributes such as celery and carrot. The dried fish, fish oil and fishy attributes were unified in the fish attribute while attributes like smoky and ashy were kept separated. The attribute mouthcoat-fatty was modified into mouthcoat in the final lexicon, with the possibility for the panelists to specify the kind of mouthcoating present in the sample, is possible. The panel unified attributes such as meaty, beefy, and Bologna-like into the more generic attribute meaty. This happened probably because although the manufacturers highlight the presence of beef, lamb, beef, chicken, and duck as well as venison meats, the result of this often extreme mixture is a blended rather than fractionated flavor. In these conditions it was difficult to distinguish individual meat flavors.

Some attributes were suggested or found only by a single panelist. For example, tongue burn, tongue numbing, tongue tingle, and heat burn were suggested by only a single panelist and the rest of the panel did not agree with these attributes. It was decided not to include these in the initial lexicon because they could be added later in the testing phase if needed.

The size, shape, and flecks attributes were not given an intensity score. Instead the panelists were asked to check one between multiple choices. For example, in the initial lexicon the samples were described as round, x-shaped, chunk, nugget, oval, and miscellaneous for the shape attribute. The panelists evaluated the presence or absence of flecks and also specified the colors of the flecks. For the size attribute the panelists were asked to give an evaluation about the perception of kibble size, from small to large.

Sample evaluation

Some attributes that were not included in the initial lexicon and were added to the lexicon during the evaluation resulting in a final lexicon of 72 attributes (Table 2-2). Some were specific for only one sample; some were used for more than one. For example, the appearance attribute red brown color (sample #120 b), flavor attributes carrot (#586) and musty (#120 b, #254), texture attributes firmness and springiness (#120 b) and the attribute overall impact which was evaluated for all the samples. The musty attribute was added because two samples (#120 b and #254) were characterized by a damp note not present in the definition of the musty/dusty attribute. Some of the attributes (cumin, anise, green, and petroleum-like for flavor, and crumbliness for texture) were included in the initial lexicon, but not scored during evaluation, therefore they were not included in the final lexicon (Table 2.2).

There were flavor and aroma attributes common to almost all of the samples: barnyard, brothy, brown, cardboard, cooked, grain, vitamin and for flavor the stale attribute. The most common aftertaste attributes among the samples were bitter, cardboard, and barnyard.

Some attributes were characteristic for only one or a small number of samples (Table 2-1). However, frequency should not be equated with importance. For example, starchy, onion, garlic,

and vegetable complex aroma attributes, as well as carrot, celery, starchy, and vegetable complex flavor attributes were present only in sample #586 and were among the highest scored attributes in this sample. The vegetable complex was evaluated for samples #120 c and #586. In sample #586 vegetable complex was also the dominant note when overall impact was evaluated. Fish attribute was among the highest level of flavors in sample #905 and the highest level for aroma in samples #509 and #373, representing also the dominant note in the latter.

Table 2-1. Aroma and flavor attributes scored in 12 or fewer dry dog food samples

Table 2-1A: AROMA	
Attribute	Samples #
Ashy	824
Black pepper	220
Burnt	698, 280, 740
Clove	495
Dusty/earthy	767, 509, 117, 698, 740, 905, 120 a, 120 c, 380
Earthy	645, 586
Fermented	280, 645, 254
Fish	373, 698, 819, 952, 905
Garlic	586
Hay-like	495, 120 c
Liver	767, 190, 509, 698, 819, 905
Meaty	190, 509, 698, 280, 220, 874, 120 a, 120 b, 120 c, 120 overall
Musty	254
Musty/dusty	190, 824, 819, 952, 874, 495, 120 b, 120 overall, 380
Oily	373, 824, 220, 874
Onion	586
Oxidized oil	509, 117, 698, 481, 819, 645, 905, 120 b, 120 c, 120 overall,
Plastic	120 overall
Pungent	280, 254
Soy	509, 824, 280, 740, 495, 120 a, 120 b, 120 c, 120 overall
Smoky	740, 220, 874, 120 b, 120 overall
Spice brown	874, 495, 254
Spice complex	698, 824, 280, 740, 220, 874, 495, 586
Starchy	586
Vegetable complex	586

Table 2-1B: FLAVOR	
Attribute	Samples #
Ashy	280
Black pepper	767, 373, 190, 117, 586
Burnt	280, 740, 874
Carrot	586
Celery	586
Clove	495
Dusty/earthy	373, 190, 481, 220
Egg	120 b
Fermented	280, 645, 254, 380
Fish	767, 373, 509, 698, 819, 952, 905
Garlic	586
Hay-like	767, 117, 824, 495, 120 c
Liver	767, 373, 190, 509, 117, 698, 819, 952, 740, 905, 874, 495
Meaty	190, 824, 280, 740, 220, 874, 120 a, 120 b, 120 c, 586
Metallic	190, 509, 698, 280, 905, 874, 495, 120 b, 254
Musty	120 b, 254
Musty/dusty	509, 120 b
Oil	874
Onion	586
Plastic	120 b
Smoky	740
Spice brown	767, 495
Spice complex	767, 373, 190, 117, 698, 280, 740, 220, 645, 120 a, 586
Starchy	586
Straw-like	190, 481
Sweet	509, 824, 740, 220, 874, 495, 120 b, 586
Vegetable complex	120 c, 586

The panelists were asked to indicate which three aroma and flavor notes appeared first in the samples (Table 2-3). For aroma the barnyard attribute was the first note in seven samples (#373, #509, #819, #220, #645, #254, and #380). The brown (#190, #698, and #740) and grain (#767, #117, and #824) aroma attributes were the first notes in three samples. Meaty was the first note in #120 and all of the individual kibbles of that sample. Two samples (#481, #905) had oxidized oil as the first aroma note. According to the ingredient list sample #905 contained real North Atlantic salmon as first ingredient but, for aroma, the fish attribute appeared as the second note after the oxidized oil. This was probably due to the high percentage of polyunsaturated fats which are

subject to oxidation. This assumption also holds true for sample #952 where herring is listed in the ingredients and was characterized first by the fish note, and oxidized oil as the second note. Other attributes present as the first note were fermented (#280), vegetable complex (#586) and spice brown (#495).

Table 2-2. Sensory attributes for appearance, aroma, flavor, texture, and amplitude included in the final version of the lexicon for dry dog food

APPEARANCE		
Attribute	Definition	Reference
Brown color	Light to dark evaluation of brown color of product.	Porter Paint Sample 6720 – 2 = 10.0 Porter Paint Sample 6840 – 2 = 4.0
Green color	Light to dark evaluation of green color of product.	Porter Paint Sample 6996 – 4 = 10.0
Red Brown color	Light to dark evaluation of reddish brown color of product.	Porter Paint Sample 6048 – 5 = 12.0
Color uniformity	A measurement describing uniformity between kibbles regarding color (%).	Completely different = 0% Totally similar = 100%
Shape uniformity	A measurement describing uniformity between kibbles regarding shape (%).	Completely different = 0% Totally similar = 100%
Size uniformity	A measurement describing uniformity between kibbles regarding size (%).	Completely different = 0% Totally similar = 100%
Fibrous	The perception of visible fibers and filaments on the product.	Post Shredded Wheat = 12.0
Flecks	Presence of flecks on the product surface (presence-absence, describing color)	
Grainy	The perception of small round particles that appear to be relatively harder than the surrounding product.	Malt-O-Meal Original = 12.0 Preparation: stir 1 cup of water and 3 tablespoon of Malt-O-Meal. Cook for 1 minute in microwave. Stir. Cook 1 minute more.
Oily	The amount of oil perceived on the product surface.	Wall Mart Party peanuts = 2.5
Porous	Presence of pores/air bubbles inside the mass.	General Mills Cheerios = 8.0
Shape	Shape of the kibble (multiple choices).	
Size	Size of the kibble (multiple choices).	
Starchy	Visual perception of starch lumps.	2% Argo corn starch in water = 6.0. Preparation: 5 g of corn starch in 250 ml of water. Boil 1 minute and allow to cool
Surface roughness	Presence of indentations/bumps on surface; smooth to rough.	General Mills Cheerios = 4.0 General Mills Wheaties = 8.0
Wet moist	The visual perception of product moistness	Water = 15.0; Bush`s pinto beans (canned) = 12.0. Malt-O-Meal = 6.0 Preparation: drip the beans and rinse with de-ionized water. Prepare Malt-O-Meal as described for grainy reference.

AROMA and FLAVOR		
Attribute	Definition	Reference
Ashy	Bark-like lingering aromatics associated with a cold campfire	McCormick Hickory Seasoning Salt = 5.0 (aroma). Preparation: place 1 teaspoon in a medium snifter, covered.
Barnyard	Combination of pungent, slightly sour, hay-like aromatics associated with farm animals and the inside of a barn.	White pepper in canned Swanson chicken broth 99% fat free. 0.90 g of pepper/ 300 ml of broth = 8.0 (aroma). 0.45 g of pepper in 300 ml of broth = 4.0 (flavor). Preparation: steep the ground white pepper in 300 ml of broth at 180 °F for 30 min. Filter the solution and let cool for 10 min. Serve ¼ cup in a medium covered snifter (aroma).
Brothy	The aromatic sensation associated with boiled meat, soup or stock.	Swanson chicken broth 99% fat free = 4.0 (aroma); 5.5 (flavor). Preparation: 1 tablespoon at room temperature in a medium covered snifter (aroma).
Brown	A sharp, caramel, almost-burnt aromatic (a part of the grain complex).	Bush's pinto beans (canned) = 6.0 (aroma); 3.0 (flavor). Preparation: drip the beans and rinse with de-ionized water. Place 1 tablespoon in a medium covered snifter (aroma).
Burnt	The sharp/acrid flavor note associate with over roasted beef muscle, something over baked or excessively browned in oil.	Alf's Red Wheat Puffs (2 pieces in the mouth) = 8.0 (aroma); 3.0 (flavor). Place 1 tablespoon in a medium covered snifter (aroma)
Cardboard	The aromatic associated with cardboard or paper packaging. The intensity rating is only for the 'cardboardy' character within the reference.	Cardboard = 7.5 (aroma). Preparation: place a 2 inches cardboard square in ½ cup of water. Serve in a medium covered snifter.
Carrot (raw)	Sweet, musty, slightly green, woody aromatics associated with uncooked carrots.	Carrot (raw) = 8.0 (flavor). Serve ¼ inch cubes of raw carrot.
Celery	Green, viney, slightly bitter, somewhat sweet aromatics associated with fresh celery.	Fresh celery = 9.0 (aroma). Preparation: place 1 teaspoon of thinly sliced fresh celery stalk in a medium covered snifter.
Clove	A pungent, brown spicy aromatic.	LorAnn Gourmet clove leaf oil = 12.0 (aroma). Preparation: 1 drop oil on a cotton ball and place in a medium covered snifter.
Cooked	Musty, brown, metallic, earthy aromatics associated with the peel of a baked potato.	Potato peel = 5.0 (aroma). Preparation: bake one large potato 8 minutes in microwave on high. Serve 3 g of peel in a medium covered snifter.
Dusty/earthy	The light dusty, musty aroma associated with harvested grains and dry brown soil.	Potato peel = 8.0 (aroma) Preparation: as described for cooked reference.
Earthy	Musty, somewhat sweet, full aromatics commonly associated with decaying vegetative matter and damp black oil.	Geosmin (4,000 ppm) = 9.0 (aroma). Preparation: 1 drop solution on a cotton ball in a medium covered snifter.

Attribute	Definition	Reference
Egg	A general term used to describe the aromatics of cooked whole egg	Chopped egg white = 5.5 (flavor)
Fermented	A combination of aromatics that are sweet, slightly brown, overripe and somewhat sour.	Blackberry WONF 3RA654 (full strength) = 7.0 (aroma). Preparation: 1 drop oil on a cotton ball in a medium covered snifter.
Fish	An overall impression of fishy aromatics and processed flavor associated with canned fish such as salmon and tuna.	Starkist chunk light tuna in water = 10.0 (aroma); 7.5 (flavor). Preparation: 1 tablespoon of drained tuna in a medium covered snifter.
Garlic	The musty, slightly brown, sweet, pungent aromatics associated with garlic.	McCormick garlic powder = 9.5 (aroma); McCormick garlic powder in Swanson beef broth 99% fat free = 7.5 (flavor). Preparation: serve ½ teaspoon of garlic powder in a medium covered snifter (aroma). Add ½ teaspoon of garlic powder to 7.25 oz of beef broth and bring to a boil. Remove from heat and let cool.
Grain	The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice and oats.	Cereal mix (dry) = 5.0 (aroma); 8.0 (flavor). 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.
Hay-like	Brown, sweet aromatics associated with dry grasses, hay, dry parsley and tea leaves, somewhat barnyard-like.	McCormick parsley flakes = 7.5 (aroma); 7.5 (flavor). Preparation: 1 teaspoon in a medium covered snifter (aroma).
Liver	Aromatics associated with cooked organ meat/liver.	Brauschweiger liver sausage = 5.0 (aroma); 10.0 (flavor)(taste and swallow). Preparation: 1 teaspoon of liver sausage in a medium covered snifter (aroma)
Meaty	A measure of how much sample is recognized as distinctly animal muscle tissue.	Swanson beef broth 99% fat free (canned) = 5.0 (aroma); 6.0 (flavor). Preparation: serve 1 tablespoon in a medium covered snifter (aroma).
Metallic	The impression of slightly oxidized metal, such as iron, copper, and silver spoons.	0.10 % potassium chloride solution = 1.5 (flavor)
Musty/dusty	Dry, dirt-like aromatic associated with dry, brown soil	Kretshner Wheat Germ = 5.0 (aroma). Preparation: 1 tablespoon in a medium covered snifter.
Musty	An aromatic that has a damp, earthy character similar to fresh mushrooms or raw potato.	Fresh chopped mushrooms = 8.5 (aroma); 8.5 (flavor). Preparation: 1 teaspoon of chopped mushrooms in a medium covered snifter.
Oily	The aromatic associated with heated oil.	Safflower Oil = 10.0 (flavor). Heat 1/3 cup in microwave on high for 2 1/2 min

Attribute	Definition	Reference
Onion	The aromatics commonly associated with dehydrated onion and characterized as sweet, slightly brown and slightly pungent.	McCormick onion powder = 9.5 (aroma); McCormick onion powder in Swanson beef broth 99% fat free = 7.5 (flavor). Preparation: serve ½ teaspoon of onion powder in a medium covered snifter (aroma). Add ½ teaspoon of onion powder to 7.25 oz. of beef broth and bring to a boil. Remove from the heat and let cool (flavor).
Oxidized Oil	The aromatic associated with aged or highly used oil and fat.	Wesson Vegetable Oil = 6.0 (aroma). Preparation: add 300ml of oil from a newly purchased bottle of Wesson vegetable oil to a 1000ml glass beaker. Heat in the microwave oven on high power for 3 minutes. Remove from microwave and let sit at room temperature to cool for approximately 25 minutes. Then heat another 3 minutes, let cool another 25 minutes, and heat for one additional 3-minute interval. Let beaker sit on counter uncovered overnight. Serve 1 tablespoon in a medium covered snifter.
Pepper, black	Spicy, pungent, musty and woody aromatic characteristics of ground black pepper.	McCormick pure ground black pepper = 13.0 (aroma); McCormick pure ground black pepper in Wyler's Chicken Bouillon = 3.5 (flavor); McCormick black pepper (0.16g) in 1 cup tomato juice = 5.0 (flavor). Preparation: place ½ teaspoon of pepper in a medium covered sniffer (aroma). Dissolve 2 bouillon cubes in 2 cups of water that has been heated in microwave for 3 minutes on high power. Stir 0.23 g of ground black pepper into the prepared bouillon (flavor). Add 0.16 g of black pepper to 1 cup of Campbell's tomato juice. Process (puree 1 min) and microwave on high power for 1 min and 20 seconds (flavor).
Plastic	An aromatic associated with plastic polyethylene containers or food stored in plastic.	Ziploc Bag = 3.5 (aroma). Preparation: place 1 small Ziploc bag in a medium covered sniffer.
Pungent	The sharp physically penetrating sensation in the nasal cavity.	Majestic Mountain Sage Orange Brazil Essential Oil = 5.0 (aroma). Preparation: serve 1 drop of essential oil in 1 tablespoon of 3% sucrose solution in a medium covered sniffer.
Smoky	An aromatic associated with meat juices and fat dripping on hot coats which can be acrid, sour, burnt, etc.	Wright's Natural Hickory Seasoning in water = 9.0 (aroma); Wright's Natural Hickory Seasoning Liquid Smoke in water = 5.0 (flavor). Preparation: put ¼ teaspoon seasoning in 100 ml of water, in a large covered sniffer (aroma). Mix 0.3 ml of Hickory Seasoning Liquid Smoke in 1 quart of distilled water (flavor).
Soy	Flavor associated with soybeans or soy products.	Soy nuts (Hy-Vee Bulk) = 4.5 (aroma), 4.5 (flavor). Preparation: serve 1 tablespoon in a medium covered sniffer.

Attribute	Definition	Reference
Spice complex	The aromatics commonly associated with a spice complex with paprika, onion, garlic, cumin and black pepper.	Spice complex = 8.5 (aroma), 4.5 (flavor). Preparation: add 0.25 g of paprika powder, 0.25 g of onion powder, 0.25 g of garlic powder, 0.12 g of cumin powder and 0.12 of black pepper powder in 4 cups of hot distilled water. Serve 1 tablespoon in a medium covered snifter (aroma).
Brown Spice	The aromatics commonly associated with brown spices.	Spice brown complex = 10.5 (aroma), 10.5 (flavor). Preparation: stir 0.25 g of cinnamon powder, 0.25 g of all spice powder, 0.25 of nut meg powder, 0.125 g of cloves powder. Serve 1/4 teaspoon of powder in a medium covered snifter (aroma).
Stale	The aromatics associated with wet cardboard that is characterized by a lack of freshness.	Mama Mary's pizza crust = 4.5 (aroma), 4.0 (flavor). Preparation: serve 1 piece of 2 inches crust square in a medium covered snifter.
Starchy	Aromatics associated with starch and starch based ingredients.	2% Argo Corn Starch in water = 4.0 (aroma). Preparation: add 5 g of corn starch in 250 ml of water. Boil 1 minute and allow to cool. Serve 1/4 cup in a medium snifter. Cover.
Straw-like	Somewhat sweet, dry, slightly dusty aromatics with the absence of green; associated with dry grain stems.	Lecithin powder = 7.0 (aroma). Preparation: place 1 teaspoon of powder in a medium covered snifter.
Toasted	A moderately browned/baked impression.	General Mills Cheerios crushed = 7.0 (aroma); Post Shredded Wheat = 3.5 (flavor); General Mills Cheerios = 7.0 (flavor). Preparation: place 1/4 cup of crushed Cheerios in a medium covered snifter (aroma).
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 (aroma), 7.0 (flavor). Preparation: place 1 tablespoon of broth, at room temperature, in a medium covered snifter (aroma).
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 (aroma); General Mills Wheaties = 2.5 (flavor) Preparation: place 1 vitamin pill in a medium covered snifter (aroma).
Bitter	The fundamental taste factor associated with a caffeine solution.	0.01% caffeine solution = 2.0; 0.02% caffeine solution = 3.5; 0.035 % caffeine solution = 5.0
Salt	A fundamental taste factor of which sodium chloride is typical.	0.20 % NaCl solution = 2.5; 0.25% NaCl solution = 3.5
Sour	The fundamental taste factor associated with a citric acid solution.	0.015% citric acid solution = 1.5; 0.050% citric acid solution = 2.5
Sweet	A fundamental taste factor of which sucrose is typical.	1% Sucrose Solution = 1.0

TEXTURE		
Attribute	Definition	Reference
Cohesiveness of mass	The maximum degree to which the mass holds together during mastication.	Carrot = 2.0 (5-7 chews). Preparation: use 1/2" slices of uncooked, unpeeled and fresh carrot.
Fibrous	The perception of fibers and filaments in the product after 3-5 chews.	Post Shredded Wheat = 10.0
Firmness	The force required to completely compress the sample with the molar teeth. Evaluate on first bite down with the molars.	Kellogg's Rice Krispies Treat = 3.5 Starburst Fruit Candy (chew ½ piece) = 13.0
Fracturability	The force with which the sample ruptures. Evaluate on first bite down with the molars.	General Mills Cheerios (one piece) = 4.0 General Mills Wheaties (one piece) = 7.5
Graininess	The perception of small round particles that is relatively harder than the surrounding product after 5-7 chews.	Malt-O-Meal Original = 9.0. Preparation; see grainy appearance reference
Gritty	The perception of small, hard, sharp particles reminiscent of sand or granules in pears after 5-7 chews	Malt-O-Meal Original = 2.0; Jiffy Corn Bread Mix = 5.0. Preparation: see grainy appearance reference for Malt-O-Meal preparation. Prepare the muffins according to package directions, using Dillon's whole milk.
Hardness	The force required to bite completely through the sample with molar teeth. Evaluate on first bite down with the molars.	General Mills Wheaties (one piece) = 7.5 Wal Mart Party Peanuts = 9.5
Initial crispness	The intensity of audible noise at first chew with molars.	General Mills Cheerios = 8.0 General Mills Wheaties = 10.5
Mouthcoat	Any mouth coating perceived after swallowing such as chalky, waxy, or oily.	Kraft Philadelphia Fat Free Cream Cheese = 3.0
Oily mouthfeel	The amount of oily coating appearing generally throughout the mouth during mastication.	Nabisco Lorna Doone = 12.0 Cool Whip = 5.0
Powdery	The feeling of un-dissolved starch in vegetable product such as potatoes and hominy	Mashed Potato Flakes (not cooked) = 5.0
Springiness	The degree to which sample returns to its original height when compressed once partially with molar teeth and slowly released.	Kraft Miniature Marshmallows = 7.0 Bar S Beef Hot Dog = 4.0
AMPLITUDE		
Overall Impact	The maximum overall sensory impression during whole mastication.	Post Shredded Wheat = 3.5; Nabisco Wheat Thins Crackers = 5.5; Nabisco Chicken in a Biskit = 6.5; Ruffles Loaded Chill & Cheese Flavored = 9.0; Herr's Babyback Ribs = 11.0

Table 2-3. Order of appearance during evaluation for aroma and flavor attributes.

Sample #	1 st AROMA attribute	1 st FLAVOR attribute
767	Grain	Grain
373	Barnyard	Barnyard
190	Brown	Grain
509	Barnyard	cardboard
117	Grain	Sour
698	Brown	Liver
824	Grain	Cardboard
481	Oxidized oil	Oxidized oil
280	Fermented	Barnyard
819	Barnyard	Fish
952	Fish	Fish
740	Brown	Brown
220	Barnyard	Broth
645	Barnyard	Barnyard
905	Oxidized oil	Fish
874	Meaty	Brown
495	Spice brown	Grain
120 a	Meaty	Broth
120 b	Meaty	Meaty
120 c	Meaty	Grain
120 overall	Meaty	-
254	Barnyard	Grain
380	Barnyard	Barnyard
586	Vegetable complex	Starchy (potato)

For flavor the most often recurring flavor attributes as the first flavor note were grain, in five samples (#767, #190, #495, #120c, and #254), barnyard (#373, #645, #380, #280), and fish (#819, #952, #905). Cardboard flavor attribute was the first note in two samples (#509, #824) as well as brothy (#220, #120a), and brown (#740, #874). Sour taste (#117) along with liver (#698), oxidized oil (#481) and meaty (#120b) flavors were presented as the first note in only one sample.

Samples with higher overall impact scores had barnyard (#117, #380), fish (#373) and barnyard and fermented (#280) as strongest notes. The lowest impact scores were characterized only by liver (#767) and barnyard (#952). The flavor attribute barnyard was present in eleven samples as the strongest note (#509, #117, #824, #280, #952, #220, #645, #120a, #120c, #254, and #380). The attribute liver characterized four samples (#190, #767, #819, and #905), oxidized oil three samples (#698, #481, and #495) and brown two samples (#740, #874). Attributes fermented (#280), fish (#373), vegetable complex (#586), spice complex (#586), bitter (#874), and meaty (#120b) were present as strongest

flavor note in only one sample. For some samples (#280, #874, and #586) panelists detected two dominant notes at the same level.

There was some inconsistency in flavor among the different kibbles of the same product. This was noted for fish and liver flavors as in some kibbles liver flavor and in others fish flavor was stronger (#905, #698). One sample (#220) was inconsistent about the presence of liver flavor in some kibbles. Some samples showed low uniformity, in appearance, especially regarding shape and size, e.g. samples 120b and 586.

In the lexicon developed in this study flavor attributes were detected, defined, and referenced for the first time that we found for dry dog food. Similar studies with cat food products were carried out by Pickering (2008, 2009). For dry cat food twenty flavor and four texture attributes were developed in the earlier study. Some of those, such as fish, spicy, soy, meaty, burnt, hardness, and grittiness are also included in the lexicon of the present study. Lin *et al.* (1998) studied the effects of lipids and processing conditions on the sensory characteristics of extruded dry pet food and used attributes such as cooked cereal, fatty, painty, and cardboard odors and oiliness and smoothness for appearance. They showed that the off-aromas painty and cardboard odors represent the aroma of lipid oxidation and that they are inversely correlated to cooked cereal aroma. One of the reasons for this is the interference of strong animal fat odor with the cooked cereal aroma. Extruded pet food is often increased in fat and energy content by the process of spraying hot fat on the surface of the product (Lin *et al.* 1998). It is possible to associate painty, an aromatic associated with oxidized oil (Civille and Lyon, 1996; Lin *et al.*, 1998) and cooked cereal attributes with the oxidized oil and grain attributes detected in the present study. These attributes, along with cardboard, are fundamental to describe the oxidation effects on the sensory properties of the products. Moreover, ingredient lists of the samples in this study showed that the fat added to products is often poultry fat. This kind of fat usually has a higher

percentage of unsaturated fatty acids which is less stable against oxidation, than other kinds of fats used for this purpose, like beef tallow. Thus the importance of a wide and complete lexicon can be explained in regard with quality control aspects and shelf life of dog food, such as tracking the impact of alternative ingredients on the sensory properties.

Principal Component Analysis (PCA)

Principal Components (PC) 1-4 for texture and appearance are shown in figures 2-1 and 2-2. The first four PCs explain 81% of the variation among samples. PC1 is positively loaded with grainy appearance and negatively with fracturability and initial crispness texture attributes. PC2 shows positive loading for porous appearance and gritty texture attribute. PC3 is positively loaded with surface roughness, porous and grainy appearance attributes and negatively loaded with gritty texture attribute. PC4, explaining 12% of the variation, is positively loaded with grainy and fibrous appearance and negatively with porous appearance and mouthcoat attribute.

Principal components 1-4 for flavor, aroma and aftertaste attributes are shown in figures 2-3 and 2-4. The first four PCs explain 59% of the variation among the sample set. These percentages suggest there is considerable variability in flavors and aromatics in this product category. PC1 is positively loaded with oxidized oil flavor and cardboard aroma and negatively loaded with brown aroma. PC2 separates samples according to the higher presence of aftertastes like barnyard, sour and bitter versus those where these aftertastes are lower. PC3 is positively loaded with barnyard aroma and oxidized oil flavor and negatively with soy flavor and cardboard aftertaste. PC4, which accounts only for 9% of the variation in the data, is defined by its positive loading for cardboard, stale and toasted aromatics. These PCs make it possible to identify key differentiating dimensions such as oxidized oil

flavor and cardboard aroma versus brown, brothy and toasted aroma that can assist in evaluating acceptance or potential shelf-life of the products.

Overall impact was evaluated in addition to the descriptive sensory analysis often carried out in similar studies (Koppel and Chambers, 2010; Suwonsichon *et al.* 2012). The overall impact score gave additional information useful to draw an overall pattern of products. It confirmed the diffusion of barnyard flavor attribute among the samples as a dominant characterizing note in this product category. In addition, this score highlighted the blended nature of the samples. Highly scored attributes in specific samples could not necessarily represent the dominant notes for that sample because of other overpowering attributes in samples with complex compositions. For example, sample #824 was characterized by the dominant barnyard note throughout the whole mastication. However, this sample was evaluated higher in bitter taste, cardboard, grain, and stale flavor attributes in the descriptive evaluation. Sample #767 had liver flavor as the dominant note, although bitter taste was evaluated at a higher level, and liver and barnyard attributes at the same level as the dominant note.

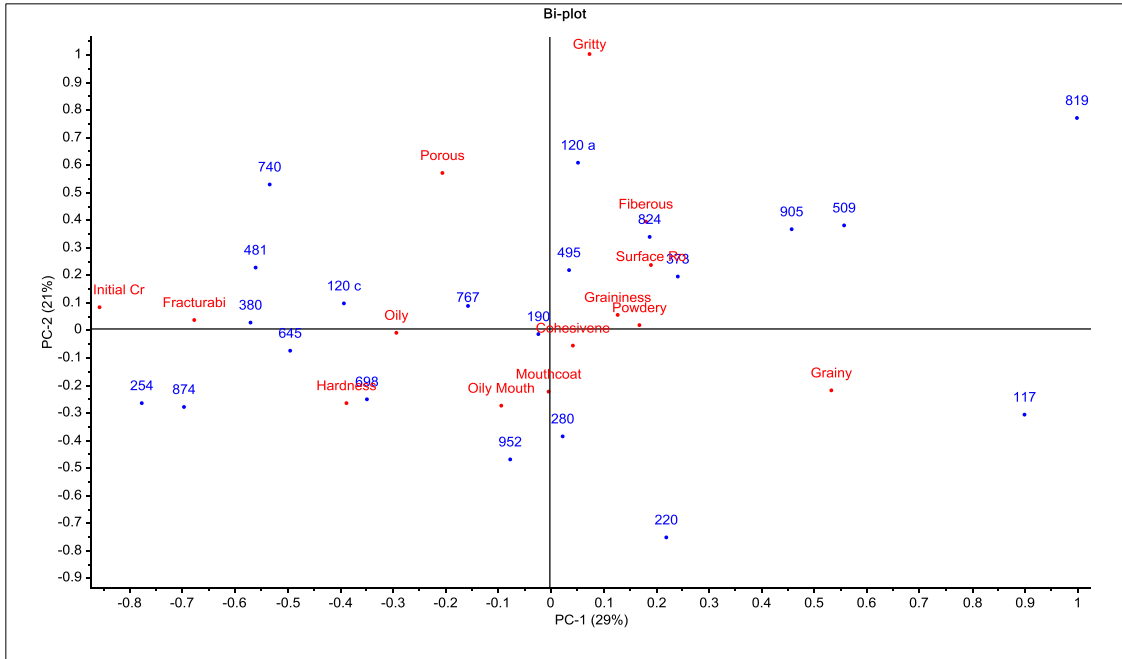


Figure 2-1. Principal Components (PCs) 1 and 2 loading plot (texture and appearance).

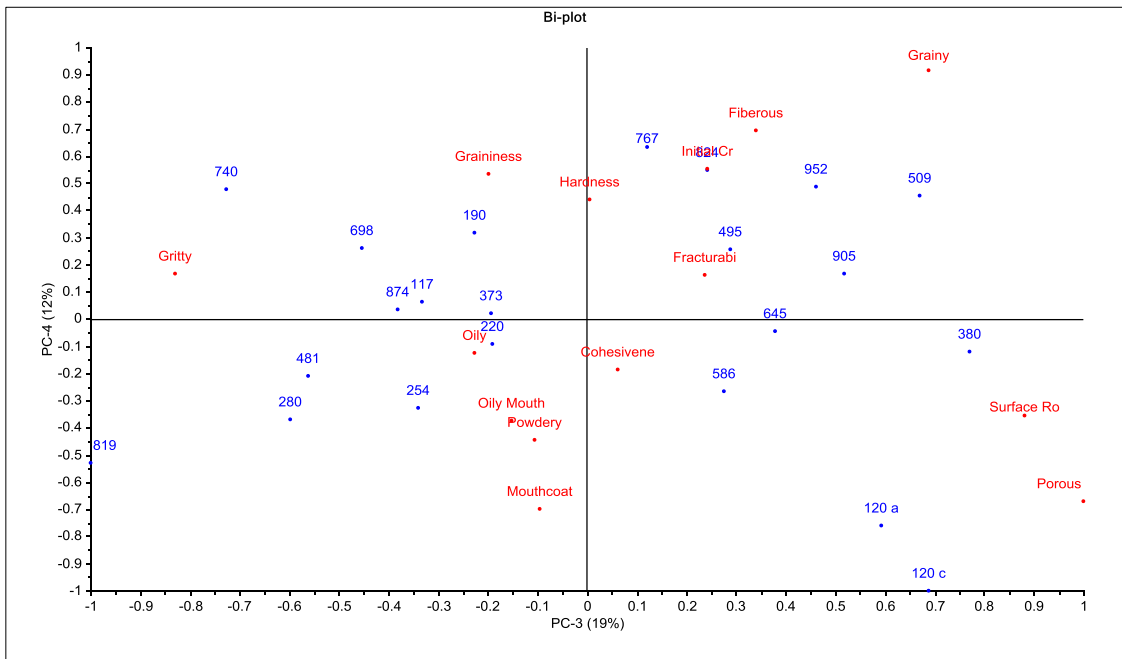


Figure 2-2. PCs 3 and 4 map of products (texture and appearance).

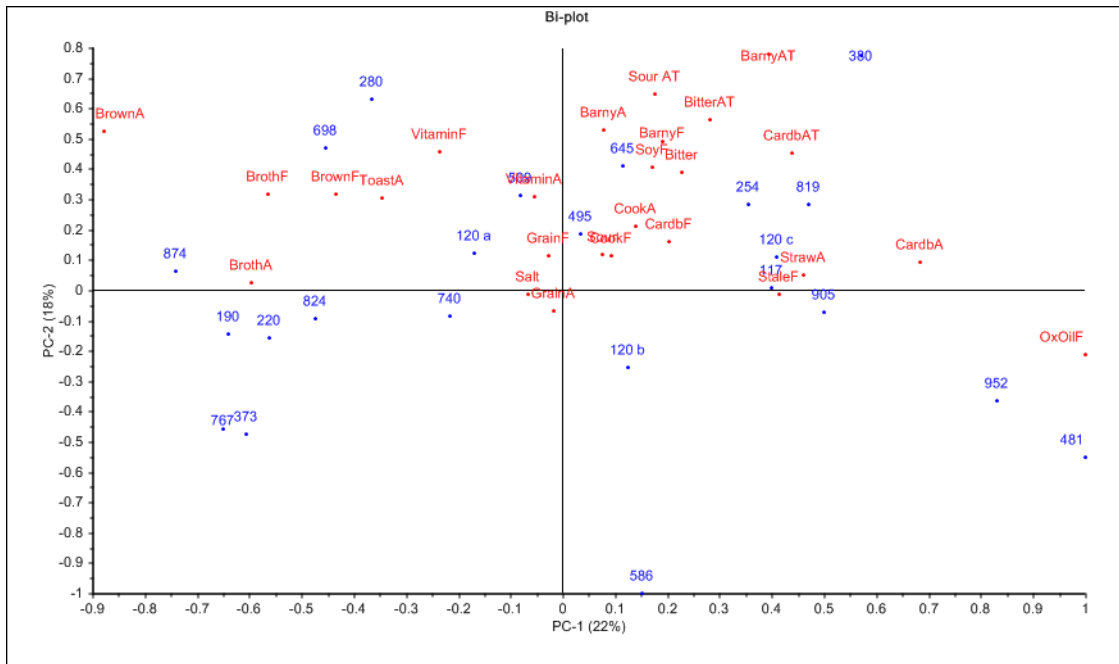


Figure 2-3. PCs 1 and 2 loading plot (aroma, flavor, and aftertaste). a - aroma; AT - aftertaste; no suffix – flavor.

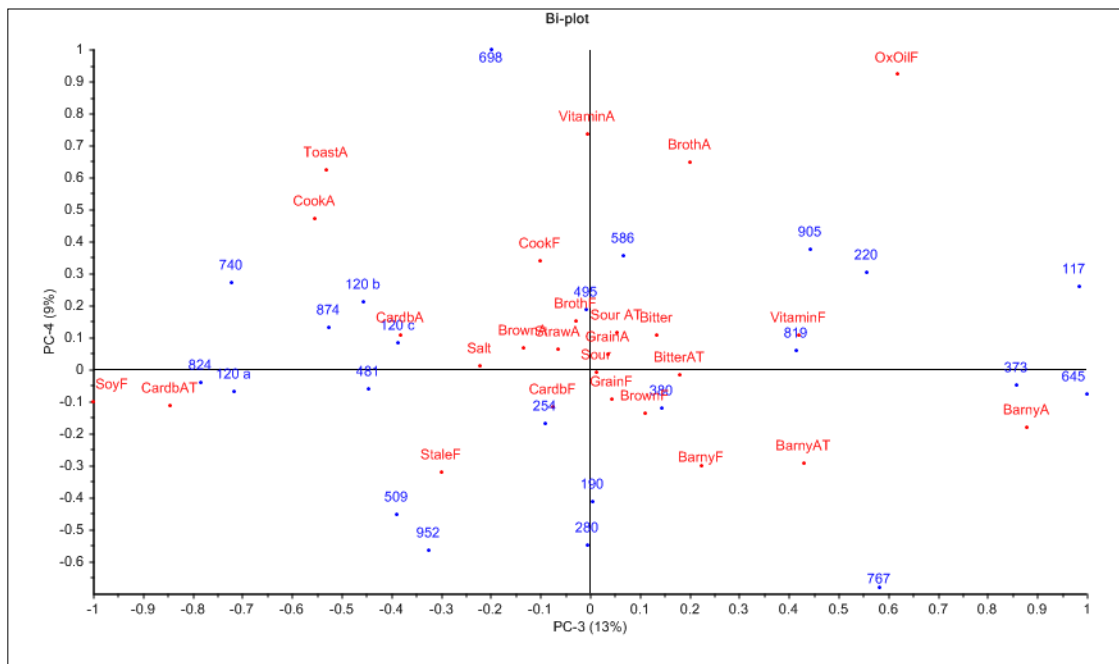


Figure 2-4. PCs3 and 4 loading plot (aroma, flavor, and aftertaste). a - aroma; AT - aftertaste; no suffix – flavor.

Conclusions

Seventy-two sensory attributes were selected, defined and referenced by a highly trained descriptive panel to describe the appearance, aroma, flavor, and texture characteristics of dry dog food products commonly sold in the U.S.A. This study highlighted the blended nature of this product category and its variability, with few attributes higher than the others in each product, reflecting the often complex composition of samples. Changes of quality may negatively influence both the purchase of the food by owners and consumption by the pets. A lexicon to measure appearance, flavor and texture of dry dog food can be helpful for pet food industries for product development and assessment and to understand ways to increase palatability. Once relationships can be determined of sensory properties to acceptance, a human sensory panel could be used as an alternative to or an integral part of preference tests with animals and their owners. This study was intended to be a first step for future studies on the subject, but clearly more work needs to be done.

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Chapter 3 - Consumers' Acceptance of Dry Dog Food

Abstract

The objectives of this study were to compare the acceptance of different dry dog food products by consumers, determine consumer clusters, and identify the characteristics of dog food that drive consumer acceptance. Eight dry dog food samples available in the local market were evaluated by dog owners. In this study consumers evaluated overall liking, aroma, and appearance liking of the products. Consumers were also asked to predict purchase intent, their dog's liking, and cost of the samples. Moreover, Just About Right (JAR) questions were answered for aroma and appearance. The results indicated that appearance of the sample, especially the color, influenced pet owner's overall liking more than the aroma of the product. Some of the overall liking clusters were related to income, age, gender, or education, indicating the main role of the individual consumer in the acceptance of dog food products.

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Introduction

Pet food buyers, despite economic recession challenges and money-saving trends, seem to be more than willing to spend money on pet food. A recent survey (Taylor, 2013) revealed nearly one-third of consumers “preferred to shop at pet product retailers that offer the best products available, even if they are more expensive”. Another survey on pet owners showed that 21% of US dog owners spend an average of US\$ 100 or more per month on their dogs (Phillips-Donaldson, 2011). Pet food industry benefits from this consumer behavior and is focusing even more on premium products. In the pet food market this means natural ingredients and focus on health issues.

Factors such as brand and packages contribute to the formation of expectation, the selection process, and the purchase intention for a product by consumers (Gadioli *et al.*, 2012). Brand represents a signal of quality especially for those products that can only be experienced after purchase. In categories where several products are available for the consumer, such as the pet food category, brand can help in the selection process (Cuellar and Claps, 2013). Moreover, considering that pet food will not be directly consumed by owners, and then a complete feedback cannot be provided, branding can hold a much higher importance. The package in which pet food is contained, conveys information on the particular properties of ‘premium’ products specific for particular health problems or life stages, and the information on natural and healthy ingredients. The latter has been increasing with humanization of pets by owners. When brand and packaging information are removed, only drivers related to sensory quality of food contribute to the consumer’s acceptance for a food product.

A wide variety of dry dog food products are available in the U.S. market and sensory properties vary from one product to another for appearance, aroma, flavor, and texture as shown by recent studies.

Di Donfrancesco *et al.* (2012) showed that some common aroma attributes, such as barnyard, grain, brothy, oxidized oil, cardboard, and stale, and are present among this category. Other aroma notes such as meaty, liver, or fishy are more product-specific. Dry dog food products also showed to be different for appearance characteristics such as surface roughness, oily appearance, porousness, and fibrousness.

The objectives of this study were to 1) compare different dry dog food products for consumers' acceptance, 2) determine consumer clusters and their relation to age, income, gender, or education, and 3) identify drivers of consumers' liking and purchase intent.

Materials and Methods

Samples

Eight samples were purchased from local pet stores, grocery stores, and discount stores. All samples were within the "best by" date on the package and all sample lots were checked to ensure they had not been subject to a product recall. The products were purchased one week before testing and were stored at room temperature, as stated by package directions.

Samples were different in brand, type of kibble, price, and presence of specialty ingredients (Table 3-1). Sample A (3 kibbles) and sample C (4 kibbles) were each composed by multiple kibbles, differing in size, shape and color. Sample B and D had formulas claimed to be for mature dogs. One sample (E) was chosen for its extremely affordable price compared with the other products. Sample G claimed to contain real salmon as the first ingredient. Sample F was a "grain-free" and "easy to digest" product while sample H was a low-fat product indicated for weight management of dogs.

These specific samples were chosen for this study based on the aroma and appearance characteristics observed in a previous study. Samples showed small (D, G), medium (A, B, E, F, H), and large sizes (one kibble type of sample A). Some samples were nugget shaped (A, B, E, G, H), others were oval shaped (kibble in sample A), 'o- shaped' (D), square shaped (F), or they showed a miscellaneous shape like the kibble in sample A. The choice of some of the samples was based on previous descriptive analysis studies in order to have a wide range of aroma profiles within the sample set. Some samples had higher fishy notes (G, H) while others, such as sample E, had higher grain notes. Sample D had high oxidized oil aroma levels and sample A had plastic aroma notes. Some samples (F and D) presented higher meaty notes than the other while sample G had higher levels of liver aroma. All samples were within the “best by” date on the package and all sample lots were checked to ensure they had not been subject to a product recall. The products were purchased one week before testing and were stored at room temperature, as stated by package directions.

Descriptive Sensory Analysis

Six highly trained panelists from the Sensory Analysis Center, Kansas State University (Manhattan, KS) analyzed the samples for aroma and appearance attributes. Each of the panelists had more than 120 h of descriptive analysis panel training with a variety of food products. The training included techniques and practice in attribute identification, terminology development, and intensity scoring. Moreover, each assessor had more than 1,000 h experience with a variety of food products, including dried dog food, and had conducted studies using the consensus method used in this study. Attribute intensities were evaluated on a scale where 0 = none and 15 = very high. Samples were first evaluated individually by panelists, then the final aroma and appearance profile was developed after a discussion led by the panels leader to determine the consensus score for each attribute. The same

procedure was recently used in other studies by Koppel *et al.* (2013), Suwonsichon *et al.* (2012), and Adhikari *et al.* (2011). The lexicon used in descriptive profiling was the same developed and used by Di Donfrancesco *et al.* (2012).

Samples C and A were evaluated for aroma attributes in this study as these samples were constituted of multiple kibbles, different in shape and color.

Each sample was prepared 30 minutes prior to the testing and was served in a ~100 ml plastic cup for appearance evaluation. For aroma evaluation, 3 g of sample was weighed in a medium snifter, and covered with a watch glass. Samples were coded with three-digit random numbers. The testing room was at 21 ± 1 °C and $55 \pm 5\%$ relative humidity. For appearance, the attributes evaluated were brown color, color uniformity, shape uniformity, size uniformity, surface roughness, porous, oily, grainy, and fibrous. For aroma, panelists evaluated ashy, barnyard, broth, brown, cardboard, cooked, dusty/earthy, fish, grain, liver, meaty, musty/dusty, oily, oxidized oil, soy, smoky, spice brown, spice complex, stale, starchy, straw-like, toasted, and vitamin.

Consumer Study

A Central Location Test (CLT) was conducted in the Sensory and Consumer Research Center (Olathe, KS, US), Kansas State University. Participants ($n = 100$, men = 30, women = 70) were recruited from the Kansas City area. Consumers were recruited via e-mails and fliers and were screened for age (>18 years old), dog ownership, not to work in the pet food industry, and to be personally responsible for at least 50% of dog food purchases. Among recruited consumers, 66% had only one dog in the household, 29% had two dogs, and 5% had 3 dogs. Demographic consumer characteristics are shown in Table 3-2.

For the evaluation each of the panelists used a tablet computer and computer questionnaires were administered by Compusense *at hand* software (Compusense Inc., Guelph, Ontario, Canada).

Number of consumers for each single session varied from 6 to 8. The samples were served monadically in a randomized order in a brown paper bag, lined with a plastic bag, containing approximately 900 grams of sample. Bags were labeled with three-digit random codes. All samples were served to all participants during a 1-hour session. Participants were asked to pour the sample into a ceramic pet bowl and then rate *overall liking*, *aroma liking*, *appearance liking*, *color*, *size*, *shape*, *uniformity*, and *oily appearance liking*. In addition, the participants were asked to indicate how much they thought their dog would have liked the sample. A 9-point hedonic scale was used, where 1 = *dislike extremely* and 9 = *like extremely*. Pet owners were also asked about *aroma*, *oily appearance*, *uniformity shape*, *size*, and *color* intensities on a 5-point Just-About-Right (JAR) scale where 1 indicated “too weak”, 3 “just about right”, and 5 “too strong”. Moreover, participants predicted purchase intent (5 = *definitely would purchase*, 4 = *probably would purchase*, 3 = *may or may not purchase*, 2 = *probably would not purchase*, 1 = *definitely would not purchase*), their dog’s liking, and the cost of each sample (1 = *not at all expensive* to 5 = *very expensive*). Consumers were also asked to describe their likes and dislikes for each sample.

Table 3-1. Dry dog food products utilized in the study and ingredients

Sample	Additional information	Ingredients *
A	Multiple kibbles, speciality recipe	Beef, soybean meal, soy flour, animal fat, brewers' rice, soy protein concentrate, corn gluten meal, ground yellow corn, glycerin, poultry by-product meal, ground wheat, animal digest, pearled barley, calcium carbonate, calcium phosphate, salt, grilled sirloin steak flavor, dried green beans, dried potatoes, sulfur, Vitamin E supplement, choline chloride, zinc sulfate, ferrous sulfate, added color (Red 40, Blue 2, Yellow 5, Yellow 6), niacin, wheat flour, potassium chloride, L-Lysine monohydrochloride, vitamins, minerals, garlic oil, C-5900
B	Mature dogs	Whole grain corn, chicken by-product meal, animal fat, soybean mill run, flaxseed, chicken liver flavor, lactic acid, corn gluten meal, potassium chloride, l-lysine, choline chloride, vitamin E supplement, iodized salt, vitamins, calcium carbonate, dicalcium phosphate, minerals, l-tryptophan, taurine, glucosamine hydrochloride, l-carnitine, chondroitin sulfate, phosphoric acid, beta-carotene, rosemary extract.
C	Multiple kibbles	Corn, soybean meal, beef & bone meal, ground wheat, animal fat (BHA used as preservative), wheat middlings, corn syrup, water sufficient for processing, animal digest (source of roasted flavor), propylene glycol, salt, apple, hydrochloric acid, potassium chloride, caramel color, vegetable blend (peas, carrots & green beans), sorbic acid, sodium carbonate, minerals, choline chloride, vitamins, calcium sulfate, titanium dioxide (color), red 40 lake, yellow 5, red 40, BHA, blue 2 lake, yellow 6 lake, blue 1, DL-methionine, yellow 6.
D	Small breed, aging care	Chicken meal, rice, brown rice, corn gluten meal, chicken fat, barley, natural chicken flavor, dried beet pulp (sugar removed), rice flour, dried egg product, anchovy oil, dried brewers' yeast, potassium chloride, flaxseed, calcium carbonate, fructo-oligosaccharides (FOS), salt, choline chloride, sodium tripolyphosphate, DL-methionine, vitamins, taurine, salmon meal, trace minerals, glucosamine hydrochloride, tea (green tea extract), L-carnitine, chondroitin sulfate, marigold extract (<i>Tagetes erecta</i>), rosemary extract.
E	Affordable cost	Ground corn, wheat middlings, de-fatted rice bran, meat and bone meal, animal fat, salt, potassium chloride, animal digest, corn gluten meal, coline chloride, minerals, vitamins.
F	Easy to digest, grain free	Chicken, potatoes, chicken meal, pea protein, peas, sweet potatoes, poultry fat (preserved with mixed tocopherols), apples, pumpkin, natural flavor, tapioca starch, tomato pomace, salt, potassium chloride, choline chloride, vitamins, minerals, citric acid (used as a preservative), <i>Yucca schidigera extract</i> , rosemary extract.
G	Real salmon	Salmon, brewers' rice, ground whole grain sorghum, potato, ground whole grain barley, chicken meal, fish meal, chicken fat, dried egg product, dried beet pulp, natural flavor, brewers' dried yeast, potassium chloride, salt, sodium hexametaphosphate, calcium carbonate, dl-methionine, choline chloride, fructooligosaccharides, minerals, vitamins, beta-carotene, rosemary extract.
H	Low fat	Turkey, chicken, barley, brown rice, potato, rice, pea fiber, chicken meal, herring, natural flavors, chicken fat, flaxseed, apple, carrot, herring oil, sunflower oil, egg, cottage cheese, alfalfa sprouts, pumpkin, dried chicory root, l-carnitine, vitamins, minerals, direct fed microbials (dried <i>Lactobacillus acidophilus</i> fermentation product, dried <i>Lactobacillus casei</i> fermentation product, dried <i>Enterococcus faecium</i> fermentation product), lecithin, rosemary extract

*Vitamin and mineral lists of all samples (Except for sample B) are not all-inclusive.

Table 3-2. Participants’ demographics – gender, age, consumers’ income (US dollars), number of dogs owned

Gender	Male		Female			
Consumers %	30%		70%			
Age	18-24	25-34	35-44	45-54	55-64	>55
Consumers n.	2	25	22	35	12	4
Income (USD)	<25,000	25,000 – 49,000	50,000 – 74,000	75,000 – 100,000	>100,000	
Consumers n.	3	14	26	33	24	
Education level	Some high school	High school degree	Some college	College degree	Some graduate studies	Graduate degree
Consumers n.	1	3	14	44	10	27
Number of dogs	One	Two	Three	Four or more		
Consumers n.	66	29	5	0		

Data Analysis

To compare differences among samples, consumers’ data were analyzed using SAS® statistical software. (Version 9.3, SAS Institute Inc., Cary, NC). Significant differences ($p = 0.05$) in products were determined using Fisher’s Least Significant Difference (LSD). Clusters among consumers, according to their *overall liking* score (Ward’s clustering procedure) and significant differences within clusters, by analysis of variance (ANOVA). Unscrambler software (The Unscrambler X version 10.2, Camo Software AS, Oslo, Norway) was also used to map consumer flavor liking scores with descriptive sensory analysis data using Internal Preference Mapping and to plot a liking map of samples fitted with consumers’ liking. Compusense *at hand* (Compusense Inc., Guelph, Ontario, Canada) was used to collect consumer scores and analyze JAR data. For this data, scores 1-2 were

grouped “too low” and scores 4-5 were grouped as “too high”. Scores of 3 were considered as “just about right”.

Results

Descriptive Data

The aroma attributes common to all of the samples were barnyard and cooked (Table 3-3). Aroma attributes such as broth, grain, musty/dusty, straw-like, and vitamin, were detected in seven samples out of eight while brown and cardboard were present in six samples. Other attributes found were oxidized oil (B, D, G, H, A), toasted (C, D, E, F, A), meaty (C, D, F, A), soy (C, D, E, A), spice complex (C, D, E, F), stale (B, C, E, A), smoky (C, F, A), oily (C, E, F), fish (G, H), dusty/earthy (B, G), vegetable complex (C, A), burnt (D), liver (G), spice brown (F), starchy (D) and plastic (A). All the scores were included in a low intensity range (0 – 5), indicating the blended and complex nature of the product category where few attributes have a moderate (5 – 10) intensity range (Di Donfrancesco *et al.*, 2012). Descriptive analysis confirmed the specific characteristics expected to be present in the samples, such as the fishy notes in samples G and H, or the higher meaty notes in samples D and F. Sample D had the highest levels of barnyard, broth, burnt, and cooked as well as meaty, musty/dusty, oxidized oil, soy, spice complex, starchy, straw-like, toasted, and vitamin. Sample F presented the highest levels for brown and meaty, together with smoky and spice brown notes. Sample H had higher levels for both fish and oxidized oil, and any presence of attributes such as broth or brown, present in most of the samples. Plastic aroma was only present in sample A, probably due processing or packaging processes. Sample C showed grain, smoky, oily, meaty,

vegetable complex, and brown notes as well as the highest levels for stale aroma among the sample set. Oxidized oil aroma was not detected in this sample.

Table 3-3. Aroma profile from descriptive analysis (0-15 scale).

Sample #	A	B	C	D	E	F	G	H
Attribute								
Ashy	0	0	0	0	1.5	0	0	0
Barnyard	3.0	5.0	3.0	5.5	3.0	2.5	3.0	2.5
Broth	2.5	2.5	3.0	3.5	2.5	3.0	2.5	0.0
Brown	3.0	2.0	2.5	0.0	3.0	4.0	3.0	0.0
Burnt	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0
Cardboard	3.0	4.0	2.5	0.0	3.0	0.0	3.0	3.0
Cooked	2.0	3.0	2.5	5.0	2.0	3.0	3.0	2.0
Dusty/Earthy	0.0	2.0	0.0	0.0	0.0	0.0	2.0	0.0
Fish	0.0	0.0	0.0	0.0	0.0	0.0	4.0	4.0
Grain	3.0	2.5	3.0	0.0	4.0	2.5	3.0	3.0
Liver	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0
Meaty	3.5	0.0	3.0	4.0	0.0	4.0	0.0	0.0
Musty/Dusty	2.0	2.0	2.0	3.0	3.0	2.5	0.0	2.5
Oily	0.0	0.0	2.5	0.0	2.0	2.0	0.0	0.0
Oxidized Oil	2.5	2.5	0.0	5.0	0.0	0.0	3.5	3.0
Plastic	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Soy	2.5	0.0	1.5	3.0	2.5	0.0	0.0	0.0
Smokey	2.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0
Spice Brown	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
Spice Complex	0.0	0.0	2.5	3.0	1.5	1.0	0.0	0.0
Stale	2.5	2.0	2.5	0.0	2.0	0.0	0.0	0.0
Starchy	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0
Straw-like	1.5	2.0	2.0	2.5	0.0	2.0	2.5	2.0
Toasted	1.5	0.0	1.5	4.0	2.5	2.0	0.0	0.0
Vegetable Compl.	2	0.0	2.0	0.0	0.0	0.0	0.0	0.0
Vitamin	2.0	2.5	2.0	3.0	2.5	2.5	2.0	0.0

Samples G and F were the ones characterized by a darker brown color while sample H was lighter (Table 3-4). Sample E showed the lowest shape and size uniformity within the samples set. Samples such as B, G, and E, presented a higher surface roughness than the other samples. Samples F and D showed low scores for both grainy and fibrous appearance.

Table 3-4. Appearance profile from descriptive analysis (0-15 scale) *.

Sample # Attribute	A	B	C	D	E	F	G	H
Color Uniformity**	nd	99%	nd	70%	99%	98%	98%	98%
Shape Uniformity**	nd	93%	nd	60%	80%	95%	85%	95%
Size Uniformity**	nd	0.95%	nd	80%	80%	90%	90%	98%
Surface Roughness	nd	6.5	nd	2.0	6.0	2.0	5.0	3.0
Porous	nd	6.0	nd	2.0	4.0	2.0	6.0	3.5
Oily	nd	3.0	nd	5.0	2.0	3.0	2.5	2.0
Grainy	nd	4.0	nd	2.0	4.5	1.5	6.0	4.0
Fibrous	nd	2.0	nd	2.0	2.0	1.5	4.5	2.0

*samples not shown Data relative to appearance of sample composed by multiple different kibbles (#120 and #513) are not showed

Consumer Study Results

Acceptability and intensity scores

Sample C, one of the two samples composed by multiple kibbles, was the sample that earned the highest overall liking score as well as appearance, shape, uniformity, oily appearance, and color liking scores (Table 3-5). Liking score for color in this sample represented the highest liking score observed in the study (average score >7). Color intensity of kibbles in sample C was perceived by most of the consumers (79%) as ‘just about right’ (Table 3-6). Moreover, contrarily to the other sample with

multiple different kibbles (A), perceived as being low uniform in shape by a large portion of consumers (54%), sample C was perceived as ‘just about right’ for shape uniformity by 77% of consumers. When asked to predict purchase intent and product price, consumers indicated this sample as the one they were more willing to buy despite this sample was perceived as the most expensive product within the sample set (Fig. 3-1). This could be explained by the positive correlation shown between perception of quality by consumers and price (Rao and Monroe, 1989) and known that a product is more expensive, consumer rates it better than the product perceived as less expensive (Plassmann et al., 2008). Consequently, a low price perception is translated into a lower acceptability by consumers because of expected lower product quality. In this study the highest predicted price was 3.75 on a scale where 3 = *neither expensive nor inexpensive* and 5 = *very expensive*. Pet owners also predicted that sample C would be the most appreciated product by their dogs. The low cost sample (E), characterized by a nugget shape and a moderate brown color, earned the second highest overall liking and appearance liking score, despite almost 1 point of difference from sample C for both the attributes (Table 3-5). Sample E also had the highest score for size liking and the second highest score for shape and uniformity liking (average scores >6). The aroma profile of this sample indicated the highest level for grain notes among samples. Samples E and C were the only samples earning an average score higher than 5 for aroma liking.

The least overall liked product was sample F, dark brown and square shaped and with the highest oily appearance among samples, from descriptive analysis. It showed the lowest liking scores for size, shape, and oily appearance. This sample earned the lowest predicted ‘dog liking’ score, but was close for this attribute to other samples such as G (nugget shaped and dark brown) or H (nugget shaped and light brown). The sample perceived as having the lowest cost was the sample that earned the lowest score for color liking too (sample H).

Mean aroma liking scores fell in a range between '*dislike slightly*' and '*like slightly*'. This score tendency indicates possible consumer clusters. The slightly most liked sample for aroma was sample C. The less liked for aroma was sample D.

When asked to comment their likes and dislikes for the products, consumers focused on overall appearance of samples, with the number of comments more than the double than for other aspects such as aroma or specific appearance characteristics. For instance, consumers' comments indicates that sample C was appreciated mostly because the variety of colors, shapes, sizes, and textures. This variety was associated by some consumers to a variety in ingredients and flavors, with someone perceiving it as an indicator of healthiness or freshness of the product. Consumers' comments also indicated that in minor part this sample was appreciated because of a mild and not too strong aroma. For sample F, the least overall liked, most of the comments pointed out on the too small size of the sample, with another part of the comments also indicating the smell and the too dark color as the reasons of because this sample was not liked. The o-shaped sample (D), one of the least liked among the set, earned comments about its similarity with a known breakfast cereal product. For some consumers this was a good aspect, while some other, the most, considered this shape odd for a dog food product, more fitting with cat food, or even dangerous because the possibility by a child to mistake the product for a breakfast cereal. From the comments results for sample A, composed by multiple kibbles, some consumer appreciated its variety of colors and size but, another part of consumers highlighted the low uniformity of the sample together with kibble shapes considered too strange, especially for one of the kibble, big sized, springy and with an irregular shape, perceived by consumers like a chunk of meat imitation.

Table 3-5. Consumers' liking scores (1-9 hedonic scale): overall, kibbles appearance, aroma, color, size, shape, uniformity, oily appearance, and predicted dog liking by owners.

Sample #	A	B	C	D	E	F	G	H
Overall liking	5.26bc**	5.70b	6.77a	5.15bc	5.79b	4.54c	4.83c	5.01bc
Appearance liking	5.24bcd	5.82bc	6.99a	5.24bcd	6.12ab	4.53d	4.86d	4.92cd
Aroma liking	5.00abc	4.69bc	5.56a	4.37c	5.27ab	4.63bc	4.69bc	4.97abc
Color liking	5.97bc	6.20bc	7.05a	6.29ab	6.32ab	5.02de	5.41cd	4.40e
Size liking	5.16bc	6.14a	6.32a	5.52ab	6.42a	4.14d	4.28cd	6.40a
Shape liking	4.98cd	6.55ab	6.84a	5.37cd	6.59ab	4.91d	5.80bc	6.43ab
Uniformity liking	5.02c	6.22ab	6.77a	5.77bc	6.25ab	5.5bc	5.69bc	6.08ab
Oily appearance liking	5.59ab	5.29b	6.08a	5.56ab	5.31b	5.26b	5.38b	5.48ab
Dog liking *	5.76b	5.99b	6.87a	5.85b	5.70b	5.28b	5.40b	5.30b

*predicted by consumers; **Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$).

Table 3-6. Response to aroma, color, appearance, size, and uniformity intensity as 'Too low', Just About Right (JAR), or 'Too high' by % of consumers.

Intensity	AROMA		COLOR			OILY APPEARANCE			SIZE		UNIFORMITY				
	Too low	JAR	Too high	Too dark	JAR	Too light	Too low	JAR	Too oily	Too small	JAR	Too large	Too low	JAR	Too high
A	19	48	33	31	58	11	26	73	1	16	46	38	54	44	2
B	9	43	48	6	72	22	11	61	28	6	56	38	5	67	28
C	6	61	33	14	79	7	12	76	12	5	67	28	20	77	3
D	5	34	61	21	72	7	8	65	27	43	53	4	7	69	24
E	11	50	39	2	70	28	34	64	2	24	71	5	8	70	22
F	11	37	52	60	39	1	9	68	23	75	25	0	6	68	26
G	13	43	44	46	52	2	18	68	14	76	24	0	9	65	26

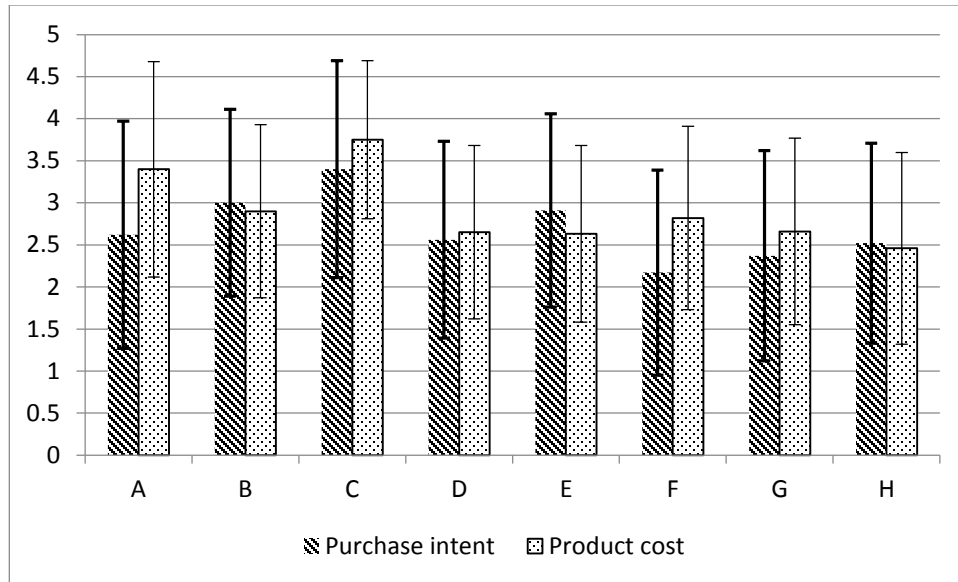


Figure 3-1. Predicted cost (5 = very expensive to 1 = not at all expensive) and purchase intent (5 = definitely would purchase, 3 = may or may not purchase, 1 = definitely would not purchase by consumers).

Consumer Clusters

Cluster analysis for overall liking detected six clusters among consumers participating in the study (Table 3-7). Individual consumer variation showed to have a greater role in the clusters composition rather than income, age, gender, and education that showed few relations with clusters. Cluster 4 (n=21) included consumers that moderately liked the majority of the samples (average scores >6). Sample C, was one of the most liked samples in all the clusters except in cluster 6 (n=18). Sample E, the low cost product, was the most liked sample in cluster 4 and cluster 6. Consumers in cluster 1 (n=19) liked samples B, C, D, and E (scores >6) and disliked samples A, F, and G (scores <4). In this cluster, it is possible to observe one of the few relations between clusters and demographic data, because it did not include any of the consumers having an income lower than \$50,000/year (n=17). In cluster 2 (n=17), pet owners liked samples A and C, the two samples composed by multiple kibbles, and disliked sample F. Consumers in cluster 3 liked sample C and disliked sample B and E. Cluster

5 (n=10) included consumers that liked samples A, B, and C and disliked samples G and H, the two samples that had fish aroma notes. Sample G earned in this cluster the lowest score among clusters (score <2). This sample was indeed one of the most liked in cluster 6 together with sample E (scores >6 for both samples). Multiple kibbles products were less appreciated in Cluster 6 than in the other clusters.

Table 3-7. Consumer clusters according to overall liking.

Sample#	Cluster 1 (n=19)	Cluster 2 (n=17)	Cluster 3 (n=15)	Cluster 4 (n=21)	Cluster 5 (n=10)	Cluster 6 (n=18)
A	3.7 d	7.8 a	4.8 c	6.0 b	7.3 a	2.8 d
B	6.8 a	5.6 bc	3.6 d	6.3 ab	6.6 ab	5.2 c
C	7.7 ab	7.8 ab	7.0 bc	6.3 c	8.1 a	4.4 d
D	6.2 a	4.0 c	5.9 ab	6.5 a	4.5 bc	3.3 c
E	6.2 ab	5.3 bc	4.1 d	6.9 a	4.5 cd	6.7 a
F	3.7 cd	3.2 d	5.3 b	6.6 a	3.0 d	4.6 bc
G	3.8 b	4.6 b	4.7 b	6.5 a	1.4 c	6.2 a
H	5.8 a	5.5 ab	4.3 b	5.8 a	2.5 c	4.8 ab

*Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$).

Drivers of liking

As shown in the liking map (Fig. 3-2), overall liking was greatly influenced by the appearance of the samples. Among the different specific appearance characteristics, color liking was similar to appearance liking. Characteristics like size, shape, and uniformity liking were less correlated to pet owners' overall liking. It is also possible to notice that prediction of dog liking was highly correlated with the consumers' overall liking. On the other hand, aroma did not greatly drive consumer's overall liking as appearance did, as correlation analysis result indicated (Table 3-8). The internal preference

map indicates a low correlation between aroma descriptive data and consumers' liking, with most of the samples spread over the margin of the plot, far from the aroma descriptive attributes (Fig. 3-3).

Table 3-8. Correlation between consumers' overall liking and the other liking scores.

Correlation <i>r</i>	Aroma liking	Appearance liking	Color liking	Size liking	Shape liking	Uniformity liking	Oily appearance liking	Dog liking
Overall liking	0.76178	0.99288	0.80871	0.70515	0.72067	0.75433	0.69734	0.92756
p-value	0.028	<.0001	0.0151	0.0507	0.0437	0.0306	0.0545	0.0009

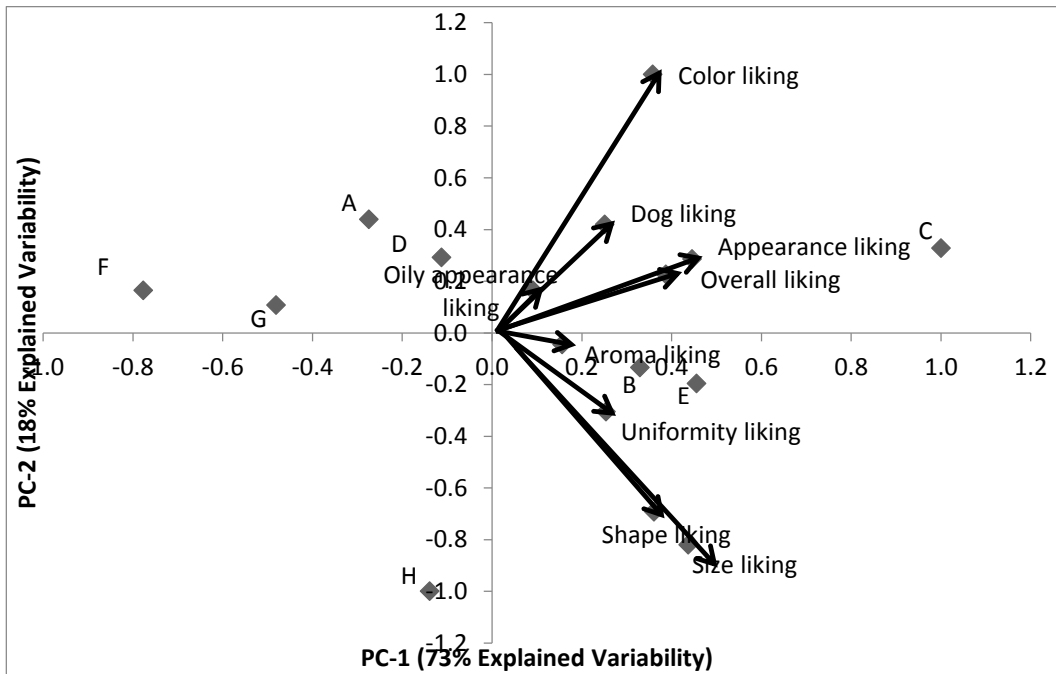


Figure 3-2. Liking map of samples fitted with consumer liking data.

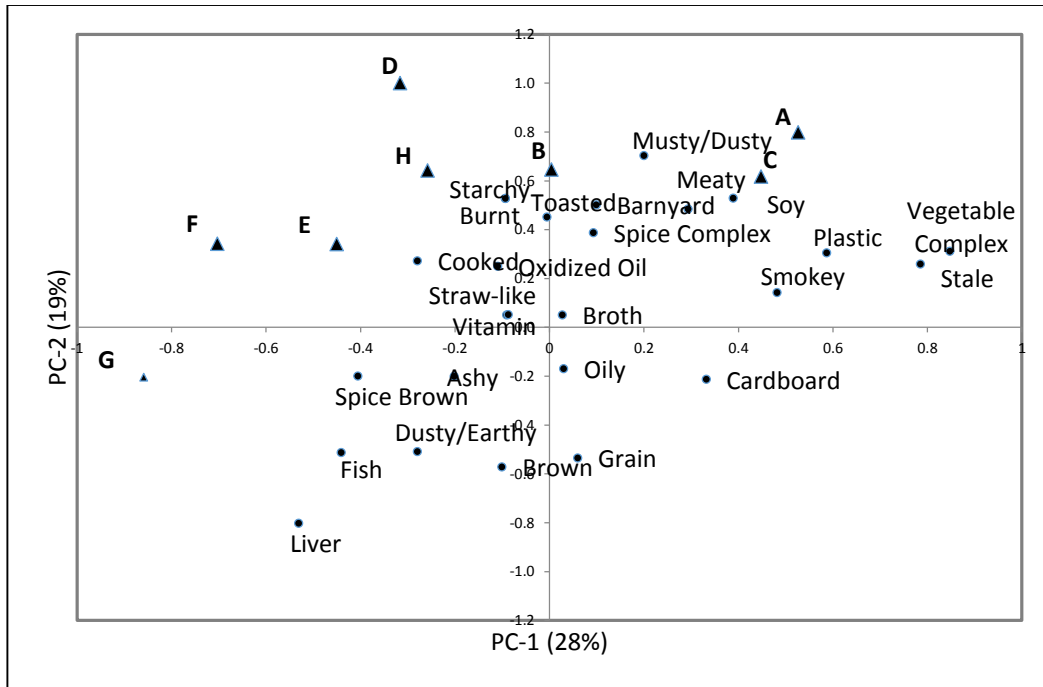


Figure 3-3. Internal preference mapping of samples overall liking scores by consumers with descriptive aroma intensity scores.

Discussion

Descriptive analysis showed the chosen samples were different from each other offering sufficiently wide range of characteristics to study in the samples. This enabled analysis of these different characteristics with the aim of understanding the aspects that drive consumers' acceptance of dry dog food. At the same time, some similarities among products helped better discriminate if a characteristic influenced the consumer's liking or not. An example of this was the influence of appearance rather than aroma on overall liking for the sample composed by multiple different colored and shaped kibbles (C). It was also characterized by aroma attributes such as smoky, broth, grain, meaty, toasted, and musty/dusty. This sample was the most liked among consumers while sample F,

with similar aroma characteristics was the least overall liked among sample set. This indicates the importance of visual stimulation in overall liking of dry dog foods. When asked about color intensity, sample F was perceived by 60% of consumers as too dark. Specific characteristics of samples also highlighted the lower influence of aroma on pet owners liking scores. For instance, samples with the highest scores for off-flavors, such as oxidized oil aroma (sample D) did not result in being less liked. The results indicated that multiple kibbles in a product are not enough to drive consumer's liking, and an optimal combination in color, shape, size, and oily appearance has to be pursued. While sample C was highly liked, the other sample with multiple kibbles (A) was one of the least liked by consumers, earning the lowest uniformity liking scores among the samples, with almost 2 points of difference from sample C. Both the samples composed by multiple kibbles were perceived as the most expensive samples in the set.

Consumers' intensity and liking scores indicated that a 'too small' kibble size may not be appreciated by pet owners. Samples G and F were perceived as too small by consumers (respectively 76% and 75%) and consequently earned the lowest score for size liking.

Previous studies have shown that appearance plays a major role, among sensory characteristics, in determining consumers purchase intent (Herrera-Corredor *et al.*, 2007)

Moreover, for new products and products that consumers have not previously tasted, appearance is even more important in driving consumer preference (Rocha *et al.*, 2012). The sample perceived as less expensive by consumers was one of the most expensive in the market (F). This product was grain free and claimed to favor an easy digestion, and was produced with several natural ingredients, received the lowest scores for appearance liking, size liking, shape liking, and oily appearance liking as well as for overall liking. The low cost sample (E) was not perceived to be expensive by consumers

but neither was the one with the lowest cost among sample set (sample H) as shown in Fig. 3-2. Sample E, having a lower cost than other samples in the market, earned some of the highest scores for attributes such as aroma liking and appearance liking. The low nutritional was probably counterbalanced in consumer's perception by the appearance (not too dark and uniform brown color, high surface roughness, moderate porousness, fibrousness, and low oily appearance) and a high level of grain aroma notes with absence of oxidized oil aroma.

Results indicate that the prediction of dog food liking follows, for most of the sample, pet owners' liking trend. The sample earning the lowest score for dog liking was the same one earning the lowest score for overall liking, size, shape, oily appearance, and overall appearance liking.

From intensity scores given by consumers it is possible to observe that a 'too strong' aroma was not appreciated by consumers. Sample D had higher intensity scores (close to moderate) for several aroma attributes, compared to low scores of the other samples. It was perceived by 61% of consumers as having a 'too strong' aroma intensity and earned the lowest score for aroma liking. Results also indicate that the sample where the grain aroma note was absent, was the one earning the lowest aroma liking score by consumers.

Conclusions

Eight dry dog food samples, with different appearance and aroma characteristics, were evaluated by a descriptive panel and by pet owners. The study indicated that consumers' liking was greatly influenced by the appearance of the products. Aroma of dry dog food seems to have a minor role to drive consumers liking and their willingness to purchase the product respect to appearance. Pet

owners, when brand is excluded, tend to associate visually appealing products with higher price. If pet owners like appearance and color of a product, they also think their dog will like the product. Product darkness seems to have a negative relation with pet owners' liking. A product perceived as too dark was poorly liked. Perception of uniformity also had an important role in driving the appearance liking. A product with variety in colors, shapes and sizes is not liked by pet owners if the uniformity is perceived as too low. Regardless of the liking score, samples with variety of colors, size, and shape are perceived as more expensive by consumers. Products with too strong aroma are not appreciated. Moreover, a low cost product, with few nutritional value and low ingredient quality, seems to be appreciated if appearance does not impact negatively consumers' perception and pleasant aroma notes, such as grain, are present, and off-flavors are reduced. This information is useful for the pet food industry and researchers in understanding characteristics of dry dog food driving consumer's acceptability.

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Chapter 4 - Descriptive Sensory Analysis and Consumer Acceptance of Dry Dog Food Manufactured with Fractions of Sorghum

Abstract

Sorghum is a key world grain and Kansas is the top producer in the US. To increase the use of sorghum, industries such as the pet food industry have been targeted. However, to succeed it is important to understand characteristics of sorghum such as sensory properties, pet owners' acceptance, and pet acceptance of the final products. The objectives of this study were to 1) develop a sensory profile for dry dog foods containing sorghum, and 2) assess consumer acceptance of the dog food products. Three samples containing different sorghum fractions (mill-feed, flour, and whole sorghum) and a control sample containing mixed grains (rice:wheat:corn) were manufactured. A trained human descriptive sensory panel described the sensory characteristics of the samples. In addition, a total of 105 pet owners evaluated the samples for appearance, color, aroma, and overall liking. Differences among samples from the descriptive analysis were small and related to a few appearance and texture characteristics. The consumer panel found the whole sorghum and the control samples to be most liked overall in appearance and color. No difference was found in aroma liking when compared with the similar aroma profiles of the samples. The slight difference in liking scores seems to be caused by the different appearance of the samples. The whole sorghum diet was accepted at the same level as the control diet and the results suggest that improvement in appearance of the other sorghum samples will improve consumer acceptance to that of the control.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important crop in the world after wheat, corn, rice and barley. The United States is the largest producer of sorghum in the world with 597 bushels produced in 2015 (Sorghum Checkoff Program, 2016). Within the USA, Kansas is the top producer. Sorghum, also called milo, originates from Northeastern Africa spreading then to India, China and Australia. Sorghum tolerates arid climates with lower moisture and rainfall requirements when compared to other crops such as rice, corn, and soybeans (Aldrich, 2015) and it is one of the most efficient crops in conversion of solar energy and use of water, and therefore considered environmentally friendly (Sorghum Checkoff Program, 2016). Currently it is primarily used for livestock feed and ethanol production but its popularity is increasing in the food industry. Opportunities to increase the use of sorghum may come from targeting industries such as pet food manufacturing. The pet food industry is an economically important part of the food processing industry, with more than \$23 billion in sales for 2015 and more than \$24 billion estimated for 2016 in the United States alone. It is an industry constantly searching for new and alternative ingredients (APPA, 2016). The increasing relationship between pets and their owners leads to adoption of human trends (e.g. organic, hypoallergenic, and increased vegetable consumption) by owners for their pets diet. Because of a limited name recognition by consumers and lack of nutritional data and acceptance data by both owners and pets (Alavi *et al.*, 2015) the current use of sorghum by the pet food industry is limited. However, labeling claims such as gluten free and non-GMO, as sorghum currently is, together with a better understanding of sorghum digestibility and sensory characteristics may contribute to increase its use, especially in pet food specialty markets. Moreover, sorghum is rich in phytochemicals such as condensed tannins, anthocyanins, phytosterols, and policosanols with high antioxidant activity and potential impact on human health

(Awika, 2004). Most of the dry pet food produced is processed by extrusion because the adaptability of this process and the functional characteristics that it can impart to the products such as improving texture, detoxifying and sterilizing (Cheftel, 1986). Among these effects, extrusion may also modify other sensory characteristics such as taste, flavor, and appearance by increasing friability, crispness, and hardness when compared to baked pet food products (Koppel *et al.*, 2014b).

However, to succeed it is important to understand sorghum's characteristics such as the sensory properties and pet owners' acceptance of the final products. Although using a human panel is not frequent to investigate sensory characteristics of pet food such as appearance, aroma, texture, and flavor, a few studies have been conducted adopting descriptive or hedonic analysis (Koppel, 2014; Di Donfrancesco *et al.*, 2015; Pickering, 2008; Pickering, 2009; Lin *et al.*, 1998). Evaluating flavor characteristics of pet food products, despite humans having a different flavor perception when compared to dogs or cats, can assist in product development when comparing descriptive sensory data to palatability results conducted with pets. Sensory characteristics also have an influence on pet owners' liking and purchase intention. Assessing human consumer liking of characteristics such as appearance and aroma of pet food products can be an important step when developing a successful product. Previous studies (Laflamme *et al.*, 2008) also observed that 31% of dog owners observe their pets eating sessions, indicating a high exposure of pet owners with the pet food product. As with parents with infants, this exposure to the food product together with behavioral feedback coming from the pet, contribute to the perceived pet food acceptability by pet owners.

The objectives of this study were to 1) develop a sensory profile for dry dog food containing different sorghum fractions, 2) assess consumer acceptance of the dog food products.

Material and methods

Samples

Milling process

Red sorghum used in the study was selected from locally grown supplies in the Manhattan, Kansas area during the 2013 and 2014 crop year. This sorghum contained a pigmented testa with condensed tannins, rich in polyphenolic acids that infer antioxidant properties. Sorghum was first cleaned of impurities such as straw, weed seeds, soil particles and dust. Then, most of the sorghum used in the study to manufacture samples was milled in April 2015 at the Hal Ross Flour Mill (HRFM; Kansas State University, Manhattan, KS, USA) in order to separate flour, bran (mill-feed) and germ. Sorghum moisture was conditioned with water to increase the moisture level to 16% from an initial 14% to promote the separation of the endosperm component from the germ and the hull. The milling process separated the different sorghum components according to particle size and consisted of grinding, sifting and purification steps. The grinding process consisted of 5 break passages that removed the endosperm from the bran portion and successively collected in a bin. A purification step followed, where the bran was cleaned from any residual endosperm particles with the use of purifiers during the sifting process. The clean endosperm was then ground into flour. Germ was also collected and flattened into large flakes. To produce the whole sorghum meal used to manufacture the whole sorghum diet (WSD), the remaining portion was ground using a hammer mill (#16 standard sieve – 1.191 mm). After been ground, the sorghum was passed through a sifter sized with a 560 micron screen (Corsato-Alvarenga, 2016).

Diet formulations

Four samples containing different sorghum fractions: whole sorghum (WSD), sorghum flour (FD), sorghum bran enriched mill-feed diet (MF) and the control diet (CD) made with corn, wheat, and brewers' rice in a ratio of 1:1:1, were extruded in the Kansas State University facilities. The MF diet was composed of bran, shorts (finer bran), red dog (leftovers of the last flour cloth in the mill) and some coarse flour (Corsato-Alvarenga, 2016). Other than sorghum, rice, wheat, and corn, the diets also contained chicken by-product meal, beet pulp, corn gluten, calcium carbonate, potassium chloride, salt, dicalcium phosphate, choline chloride (60% dry), natural antioxidant (dry), trace minerals Premix, and Vitamin Premix in order to have iso-nutritional diets (Table 4-1).

Rendered chicken fat was provided from IDF (Springfield, MO, USA) and it was preserved with a commercial antioxidant added by the seller (BHA, propyl gallate, and citric acid). The additional ingredients were acquired from a local mill that supplies ingredients for pet food production (Fairview Mills L.P., Seneca, KS, USA). The diets were formulated in order to be iso-nutritional for carbohydrate, lipid, protein, and mineral content (Table 4-2).

Table 4-1. Experimental diets composition: control (rice:wheat:corn) (CD), whole sorghum (WSD), sorghum flour (FD) and sorghum mill-feed (MF).

Ingredients, %	CD	WSD	FD	MF
Brewers' rice	21.21	-	-	-
Corn	21.21	-	-	-
Wheat	21.21	-	-	-
Whole sorghum	-	64.69	-	-
Sorghum flour	-	-	62.31	-
Sorghum mill-feed	-	-	-	67.65
Chicken by-product meal	20.94	20.02	21.00	20.00
Chicken fat	5.34	5.52	5.52	3.29
Beet Pulp	4.00	4.00	4.00	4.00
Corn gluten meal	3.00	3.00	3.00	3.00
Calcium carbonate	0.75	0.35	0.23	0.67
Potassium chloride	0.49	0.52	0.64	0.19
Salt	0.46	0.45	0.46	0.43
Dicalcium phosphate	0.87	0.95	1.19	0.24
Choline chloride	0.20	0.20	0.20	0.20
Vitamin premix	0.15	0.15	0.15	0.15
Trace mineral premix	0.10	0.10	0.10	0.10
Natural antioxidant (dry)	0.07	0.07	1.21	0.08
Natural antioxidant (liquid)	0.02	0.02	0.02	0.01

Table 4-2. Nutrient composition analysis of diets (as fed). Control diet (CD), whole sorghum diet (WSD), sorghum flour diet (FD), sorghum mill-feed diet (MF) (Proximate analysis, Midwest Laboratory, Inc., Omaha, NE, USA)

Nutrient	CD	WSD	FD	MF
Moisture, %	7.20	6.46	6.44	9.56
Dry matter, %	94.67	94.31	95.08	94.29
Organic matter, %	91.55	93.10	93.10	92.42
Protein (crude), %	21.30	21.70	21.00	23.10
Fat (acid hydrolysis), %	12.80	10.60	10.20	9.80
Fiber (crude), %	0.57	1.69	1.07	3.13
Ash, %	8.45	6.90	6.90	7.58
Calcium, %	1.54	1.52	1.34	1.54
Phosphorus, %	0.85	0.94	0.86	0.88
Potassium, %	0.55	0.66	0.62	0.60
Magnesium, %	0.09	0.13	0.10	0.18
Sodium, %	0.28	0.28	0.27	0.24
Sulfur, %	0.28	0.28	0.26	0.28
Copper, ppm	15.50	16.60	15.00	16.50
Iron, ppm	168.00	177.00	156.00	181.00
Manganese, ppm	30.80	24.20	18.60	37.00
Zinc, ppm	132.00	141.00	131.00	144.00

Mixing, grinding, and extrusion processes

The mixing, grinding, and extrusion steps were conducted at the Bioprocessing and industrial Value Added Program (BIVAP) facilities at Kansas State University, Manhattan KS, USA. After being weighed with a digital scale ingredients were placed in a 227 kg paddle mixer. Micro ingredients (<1% inclusion) were first mixed together and then added to the rest of the ingredients

in the mixer. Ingredients were mixed for 5 minutes and then finely ground through a hammer mill with an 840 μ m screen size to facilitate the next extrusion phase.

For the extrusion of all the diets, a single screw extruder (Model X-20; Wenger Mfg, Sabetha, KS, USA) with a standard pet food screw profile was utilized. The adopted screw profile consisted of inlet screw, single flight full-pitch screw, small shear lock, single flight full-pitch screw, small shear lock, single flight screw, medium shear lock, double flight single pitch screw, large shear lock and double flight cut cone screw. After extrusion the kibbles were conveyed to a dual pass dryer/single pass cooler (Model 4800; Wenger Mfg, Sabetha KS, USA) set at 99°C in order to obtain a final moisture level lower than 10%. After the drying phase, the kibbles were transported in a coating tunnel where the addition of chicken fat occurred. Finally, the extruded diets were manually collected and placed in ~9 kg poly-lined Kraft-paper bags (Corsato-Alvarenga, 2016).

Diameter of five kibbles from each diet was measured before the coating step (Corsato-Alvarenga, 2016). All of the diets had diameter sizes statistically different ($P < 0.05$) from each other. The larger diameter was measured for sample FD (15.3mm) followed by the CD sample (14.2mm), the WSD sample (13.2mm) and the MF sample (11.0mm). Pictures of the samples are shown in figures 4-1 to 4-4.



Figure 4-1. Control sample (CD) made with corn, wheat, and brewers' rice in a ratio of 1:1:1.



Figure 4-2. Sorghum flour sample (FD).



Figure 4-3. Mill-feed sample (MF) composed of bran, shorts (finer bran), red dog (leftovers of the last flour cloth in the mill) and some coarse flour.



Figure 4-4. Whole sorghum sample (WSD).

Descriptive Sensory Analysis

Five highly trained panelists from the Sensory Analysis Center, Kansas State University (Manhattan KS, USA) analyzed the four sample diets for appearance, aroma, flavor and texture characteristics. Each of the sensory panelists had more than 120 hours of general descriptive sensory analysis panel training. The panel training included techniques and practices in attribute identification, terminology development and intensity scoring. In addition, each panelist had experience with a variety of different food products including dried pet food, for both cats and dogs.

An initial list of attributes from a sensory lexicon developed by Di Donfrancesco *et al.* (2012) was utilized. The intensity of each attribute was evaluated on a 0-15 scale where 0 = none and 15 = very high. For color uniformity, shape uniformity, and size uniformity that were evaluated for the appearance of the kibbles, panelists indicated uniformity % (0-100) among kibbles of the same sample. Panelists evaluated samples individually. Each sample diet was served in a ~100 ml plastic cup for appearance, flavor and texture evaluation. For aroma evaluation, 3 grams of each sample were placed in a medium glass snifter covered with a watch glass. Cups and snifters were coded with three-digit random numbers. The testing room was maintained at 21 ± 1 °C and $55 \pm 5\%$ relative humidity. Appearance attributes evaluated were brown color, porous, oily, grainy, fibrous, surface roughness, color uniformity, shape uniformity and size uniformity. The aroma and flavor attributes evaluated were barnyard, brothy, brown aromatics, cardboard, dusty, earthy, grain, liver, meaty, musty, oxidized oil, stale, toasted, vitamin. In addition, bitter, salt, sour, sweet, and metallic were also part of the pool of attributes. Aftertaste descriptors included barnyard, liver, brown, grain, cardboard and bitter. Cohesiveness of mass, graininess, gritty, firmness, fracturability, hardness, and initial crispness, were evaluated for texture (Di Donfrancesco *et al.*, 2012).

Consumer study with pet owners

A Central Location Test (CLT) was conducted in the Sensory Analysis Center at Kansas State University (Manhattan KS, USA) to assess pet owners' acceptance of the diets. Consumers ($n = 105$, men = 25, female = 80) were recruited from the Manhattan, Kansas area. Part of the participants were recruited from the Sensory Analysis Center database and part were recruited through advertisements in the local newspaper, and on Kansas State University campus. Participants were screened for their age (>18 years old), dog ownership (had to own a dog), and direct involvement in the pet food purchase (had to be responsible or share responsibility of purchasing dog food). Furthermore, the participants had to be involved in feeding the dog (had to be the person responsible for feeding the dog or share responsibility with another person). They also had to feed their dog with dry dog food. Fifty percent of the participants had 1 dog in their household and 52% had a dog between 2 and 5 years old. The majority of the pet owners who took the test had a small size dog, weighing between 4 and 10 kg (42%). Participant demographics are shown in Table 4-3.

Table 4-3. Demographics (%) of the pet owners in the CLT: gender, age, income, education level, number of dogs, dog age, dog size, amount of money spent monthly for dog food, dog food purchasing place, and factors considered when buying dog food. (N=105)

Gender	Male				Female			
Consumers %	24				76			
Age	18-24	25-34	35-44	45-54	55-64	65 and older		
Consumers %	8	21	12	32	24	2		
Income	Less than \$25,000		\$25,000-\$50,000	\$51,000-\$100,000		Over \$100,000		
Consumers %	1		30	50		19		
Education Level	High School		Some college	College		Graduate/Professional school degree		
Consumers %	9		21	42		33		
Number of dogs	1 dog		2 dogs	3 dogs		4 dogs		
Consumers %	50		30	14		6		
Dogs age*	2-5 years old		6-10 years old		>10 years old			
Consumers %	52		40		28			
Dog size*	Very small < 4kg	Small 10kg	4-	Medium 10.5 – 25Kg	Large 25.5 – 45Kg	Very Large >45Kg		
Consumers %	14	42		37	37	2		
Money spent for dog food (monthly)	Less than \$100			About \$100		\$100-\$150		
Consumers %	83			16		1		
Where do they buy dog food?*	Online	Clinic/ Veterinary	Small markets in living area	Supermarkets/C onvenience stores	Pet shops/Pet stores	Other		
Consumers %	6	17	3	50	44	15		
Important factor considered when buying dog food*	Improves health	Brand	Price	Appearance	Dogs likes it	Ingredients	Specific health problems	Other
Consumers %	67	31	53	17	65	52	22	10

*Multiple selections allowed

Data were collected using a tablet computer and questionnaires were administered through RedJade software (RedJade ®, Redwood Shores, CA, USA). No more than 12 participants attended one session, for a total of 15 sessions. Samples were served monadically and in a randomized order. About 127 grams of sample were placed in a Styrofoam (Dart Container Corporation, Mason, MI, USA) ~240 ml bowl, covered with a plastic lid and served to participants. Pet owners were then instructed to remove the lid from the bowl, look at the kibbles and evaluate

the appearance of the samples first. After the appearance evaluation they were asked to evaluate the aroma of the diets. Specifically, consumers were asked to rate overall liking, overall appearance liking, color liking, aroma liking, and strength of aroma. A 9-point hedonic scale was used for the liking questions where 1 = dislike extremely and 9 = like extremely and a 5-point Just-About-Right (JAR) scale was used to score the intensity of aroma where 1 indicated “too weak”, 3 “just about right”, and 5 “too strong”.

Data Analysis

Analysis of variance (ANOVA) was performed (SAS version 9.4, The SAS Institute Inc., Cary, NC, US) using PROC GLIMMIX for sensory characteristics (using sample as a fixed effect and panelist and replicate as random effects) and for liking scores by consumers (using samples as a fixed effect and consumers as a random effect). Fisher’s Protected Least Significant Difference (LSD) post-hoc means separation was used to determine specific significant ($P < 0.05$) differences among the diets. Principal Component Analysis (PCA) (Correlation matrix) of the descriptive analysis data, Penalty analysis of the JAR data (20% threshold for population size), Cluster Analysis (Agglomerative hierarchical clustering, Euclidean distance, Ward’s agglomeration method) of consumers based on overall liking scores, and Correlation Analysis (Similarity/Dissimilarity matrices, Pearson correlation coefficient) between acceptance parameters were conducted using XLSTAT software (Addinsoft, New York, NY, USA).

In order to understand how the sensory characteristics of the samples may have been related to the owners’ liking and acceptance of samples, XLSTAT software was also used to perform a Partial Least Squares Regression analysis (PLSR). PLSR was conducted to create an external preference map using descriptive data (X-matrix) and consumer data (Y-matrix). The analysis included

appearance and aroma attributes from the descriptive analysis since consumers were asked to smell and look at the samples when asked to indicate their overall liking score for the samples. Moreover, only significantly different descriptive sensory attributes were included in the analysis. They included oily, fibrous, grainy, surface roughness appearance, brown color, and toasted aroma attributes.

Results

Descriptive Analysis

Sensory profile

Results from descriptive sensory analysis showed that significant differences ($P < 0.05$) among samples were mostly found for appearance attributes. Flavor and aroma profiles of samples were significantly different for only a few attributes (toasted aroma, musty and dusty flavor, barnyard and brown aftertaste). Generally, the intensities for most attributes were in the low range of the scale (0-4.5). Similar findings have been found by Di Donfrancesco et al. (2012) and Chanadang *et al.* (2016) when analyzing sensory characteristics of dry dog food samples.

Mill-feed diet (MF) had a darker brown color when compared to the other diets, while control (CD) and sorghum flour (FD) diets had the lightest kibbles (Table 4-4). The sensory color characteristics of the final products resembled the color characteristics of the raw ingredients used as the carbohydrate source. The sorghum diets were darker than the rice, wheat, and corn diet and the refined sorghum flour had the lightest color within the sorghum fractions utilized. The darker color of the MF diet can also be explained by a higher concentration of bran when compared to

the whole sorghum utilized for the WSD sample. MF was found to have a less oily appearance compared to the other samples. FD sample showed lower intensity for grainy appearance among the sample set, while the whole sorghum diet (WSD) had the highest grainy appearance score. FD sample also had the lowest surface roughness and fibrous appearance. This lower grainy appearance characteristic of the flour diet was consistent with the raw material used to manufacture this sample, a refined flour, versus whole grains and bran fractions that were used for the other diets. Porousness was not found to be significantly different among samples. Shape uniformity was also significantly different among samples. The FD sample was perceived as having the lowest uniformity of shape (94.53%) from one kibble to another.

The aroma profile was found to have little differences among samples with scores lower than 2.0 (on a scale from 0 to 15) except for barnyard aroma scores (2.3 – 2.73) (Table 4-5). Toasted aroma was found to be the only attribute that was significantly different among the samples. The MF sample had the highest value for the toasted aroma (1.70 score).

Low scores were also observed for the flavor notes of the samples. The only samples with a score > 2.0 were barnyard, cardboard, and bitter. Sensory attributes were similar for flavor as well, with the only significant difference found in the mill-feed (MF) sample that showed a higher dusty flavor and a musty note (Table 4-6). No differences were found for bitterness or astringency.

The aftertaste notes showed some differences among diets (Table 4-7). The FD sample was lower than the other samples for barnyard aftertaste and the MF sample showed the highest brown aftertaste. In descriptive sensory analysis brown is often utilized to describe - a 'sharp, caramel, almost-burnt aromatic (a part of the grain complex)' (Di Donfrancesco *et al.*, 2012).

Within texture attributes hardness was significantly different among samples (Table 4-8). Sample FD was the hardest sample. Samples also showed a marginally significant difference for grittiness (p-value=0.0557) and fracturability (p-value=0.0667). MF diet was found to be the lowest for both of these attributes.

Table 4-4. Descriptive analysis for appearance of control (CD), whole sorghum (WSD), flour (FD) and sorghum mill feed (MF) containing diets with a trained human sensory panel.

Item	CD	WSD	FD	MF	p-value
Brown color	8.77 ^d	10.40 ^b	9.43 ^c	11.23 ^a	<.0001
Fibrous	0.80 ^a	0.70 ^a	0.13 ^b	0.50 ^{ab}	0.0201
Grainy	2.30 ^b	2.57 ^a	1.57 ^c	2.17 ^b	<0.0001
Oily	2.00 ^{ab}	2.13 ^a	2.20 ^a	1.87 ^b	<0.0001
Porous	2.13	2.33	2.13	2.50	0.1578
Surface roughness	2.20 ^a	2.27 ^a	1.90 ^b	2.20 ^a	0.0503
Color Uniformity (%)	98.47	98.07	98.13	97.06	0.7764
Shape Uniformity (%)	97.27 ^a	97.20 ^a	94.53 ^b	97.93 ^a	0.0130
Size Uniformity (%)	97.80	97.73	97.20	98.67	0.2503

Table 4-5. Descriptive analysis for aroma of control (CD), whole sorghum (WSD), flour (FD) and sorghum mill feed (MF) containing diets with a trained human sensory panel.

Item	CD	WSD	FD	MF	p-value
Barnyard	2.4	2.4	2.3	2.73	0.1561
Broth	1.5	1.17	1.33	1.33	0.2838
Brown	1.20	1.27	1.17	1.53	0.2208
Cardboard	1.93	1.73	1.80	1.87	0.5732
Dusty	1.07	1.07	1.27	1.27	0.4968
Earthy	0.10	0.07	0.07	0.07	0.9783
Grain	1.83	1.67	1.73	1.97	0.5456
Liver	0.10	0.10	0.37	0.40	0.2419
Meaty	0.93	0.90	0.70	0.80	0.6293
Musty	0.00	0.07	0.00	0.00	0.3997
Oxidized Oil	0.80	0.37	0.50	0.70	0.2208
Stale	0.43	0.20	0.37	0.27	0.6103
Toasted	1.43 ^{ab}	1.20 ^b	1.23 ^b	1.70 ^a	0.0288
Vitamin	0.40	0.30	0.50	0.33	0.6474

Table 4-6. Descriptive analysis for flavor of control (CD), whole sorghum (WSD), flour (FD) and sorghum mill feed (MF) containing diets with a trained human sensory panel.

Item	CD	WSD	FD	MF	p-value
Barnyard	2.90	2.83	2.87	3.03	0.7425
Bitter	3.70	3.93	3.67	3.93	0.1728
Broth	1.97	1.83	1.67	1.97	0.1480
Brown	1.83	2.00	2.03	2.23	0.1995
Cardboard	2.27	2.07	2.17	2.23	0.2790
Dusty	1.17 ^b	1.40 ^b	1.33 ^b	1.70 ^a	0.0063
Earthy	0.37	0.47	0.20	0.37	0.4397
Grain	2.30	2.33	2.17	2.30	0.7810
Liver	1.20	1.20	1.03	1.23	0.7934
Meaty	1.30	1.37	1.03	1.37	0.4366
Metallic	0.37	0.50	0.30	0.77	0.0946
Musty	0.00 ^b	0.00 ^b	0.00 ^b	0.30 ^a	0.0209
Oxidized Oil	1.27	1.17	1.03	1.27	0.7721
Salt	2.00	1.97	1.93	1.93	0.9238
Sour	1.70	1.73	1.60	1.73	0.7611
Stale	0.47	0.30	0.27	0.60	0.3853
Sweet	0.13	0.07	0.07	0.07	0.8134
Toasted	2.07	1.77	1.90	1.93	0.4406
Vitamin	1.10	0.93	0.80	1.07	0.3917

Table 4-7. Descriptive analysis for aftertaste of control (CD), whole sorghum (WSD), flour (FD) and sorghum mill feed (MF) containing diets with a trained human sensory panel.

Item	CD	WSD	FD	MF	p-value
Barnyard	2.90 ^a	2.63 ^a	2.20 ^b	2.87 ^a	0.0023
Bitter	3.43	3.53	2.90	3.07	0.1766
Brown	1.10 ^b	1.30 ^{ab}	1.03 ^b	1.57 ^a	0.0282
Cardboard	2.03	1.80	1.80	2.00	0.5706
Grain	1.53	1.77	1.73	1.60	0.7277
Liver	1.23	1.13	0.80	0.93	0.2504
Sour	1.17	1.00	1.07	1.00	0.6582

Table 4-8. Descriptive analysis for texture of control (CD), whole sorghum (WSD), flour (FD) and sorghum mill feed (MF) containing diets with a trained human sensory panel.

Item	CD	WSD	FD	MF	p-value
Cohesiveness (mass)	1.80	1.83	1.73	1.83	0.8805
Fracturability	8.97	8.77	8.90	8.37	0.0667
Graininess	8.30	8.33	8.37	8.00	0.6733
Gritty	6.00 ^a	5.97 ^a	5.50 ^{ab}	5.37 ^b	0.0557
Hardness	8.70 ^b	8.67 ^b	9.07 ^a	8.63 ^b	0.0330
Initial crispness	11.07	10.83	11.13	10.70	0.1518

Central Location Test

Acceptability and Aroma Intensity scores

Consumers' acceptance was significantly different among samples for overall liking (6.08 - 6.59 average range), overall appearance (6.00 – 6.67 average range), and color liking (6.12 – 6.59 average range) (Table 4-9). Aroma liking was not different among samples. This is in agreement with the sensory profile from descriptive sensory analysis that showed there was little difference among samples for aroma. Control (CD) and whole sorghum (WSD) samples were the most liked overall and for the appearance of the kibbles. Correlation analysis (Table 4-10) showed that overall liking was highly related to overall appearance and color liking and less related with aroma. For color, the sample with the darkest kibbles (MF) was the least liked. Differences in liking, even if statistically significant, were not extremely large and all the average scores were >6.00 and <7.00, indicating that all of the samples were somewhat liked even if at a different degree.

Table 4-9. Consumer panel (N=105 from 500 screened) of dog owner's evaluation of "liking" (1-9 hedonic score; 1 = dislike to 9 = likes extremely) control (CD), whole sorghum (WSD), flour (FD) and sorghum mill feed (MF) containing diets.

	CD	WSD	FD	MF	p-value
Overall Liking	6.44 ^a	6.59 ^a	6.17 ^b	6.08 ^b	0.0003
Overall Appearance	6.60 ^a	6.67 ^a	6.23 ^b	6.00 ^b	<0.0001
Liking					
Color Liking	6.46 ^a	6.59 ^a	6.34 ^{ab}	6.12 ^b	0.0119
Aroma Liking	5.89	6.09	5.91	5.91	0.4231

*Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing then same letter were significantly different ($p \leq 0.05$).

From the Correlation Analysis it was possible to observe that overall liking, appearance liking, and color liking were highly correlated ($r > 0.95$) with each other while aroma liking showed a lower correlation with all of them ($r < 0.7$)

When asked about aroma intensity, 63% and 60% of participants indicated that the WSD and MF sample, respectively, had a ‘just about right’ aroma strength. Overall, samples were not perceived as having a ‘too strong’ aroma. Samples CD and FD were both perceived by 38% of consumers as having a ‘too weak’ aroma (Table 4-10). Penalty analysis, a method used in sensory analysis to understand how a specific attribute, as perceived by consumers, can penalize the consumer liking was performed. The results indicated that for sample MF the penalty was statistically significant showing that consumers penalized the samples when they considered the aroma too weak (mean drop 0.93). The mean drop for sample FD when the sample was too strong was 1.01 but the % of cases was 8.57% (<20%). Therefore, it was not considered significant with the 20% threshold for population size established for the analysis. Overall the penalty was not significant for sample FD. For sample WSD, this sample was penalized when perceived ‘too weak’ by consumers (mean drops 0.75, 29.52% of cases).

Table 4-10. Response to aroma as ‘Too low’, Just About Right (JAR), or ‘Too high’ by % of consumers.

	CD	WSD	FD	MF
Too Weak (%)	38	30*	38	30*
Jar (%)	57	63	53	60
Too Strong (%)	5	8	9	10

*Penalty analysis statistically significant ($P < 0.05$)

Cluster Analysis

Cluster analysis was conducted to understand if specific segments of consumers were detectable based on their acceptance of samples. From the analysis, three clusters were differentiated. Dendrogram in Figure 4-5 displays graphically the clusters dissimilarities. In Table 4-11 the average overall liking scores of samples for each cluster are shown. Cluster 3, on the left of the dendrogram, is completely separated from cluster 1 and 2. Cluster 3, included 43 participants (Table 4-11) and was characterized by consumers and overall tended to assign higher scores (> 7.00) to all the sample sets. Having a cluster of “likers” that liked all the samples and consumers that assign lower scores overall was also observed in previous studies on consumers’ acceptance of dry dog food (Di Donfrancesco *et al.*, 2014) or on consumer acceptance of other type of food (Koppel *et al.* 2014^a, Oupadissakoon *et al.*, 2009). An opposite trend can be observed in cluster 1 ($n = 43$) where consumers tended to give lower scores throughout the group of samples (< 6.00). Cluster 2 was the smallest cluster composed by 19 participants that assigned intermediate scores between cluster 1 and cluster 3 but at the same time extremely disliked the mill feed sample (MF) assigning 4.11 to the overall liking score, which was the lowest among clusters for this sample. As mentioned in the previous paragraph related to the sensory characteristics of samples, these consumers might have disliked the darker brown color of this sample more than others.

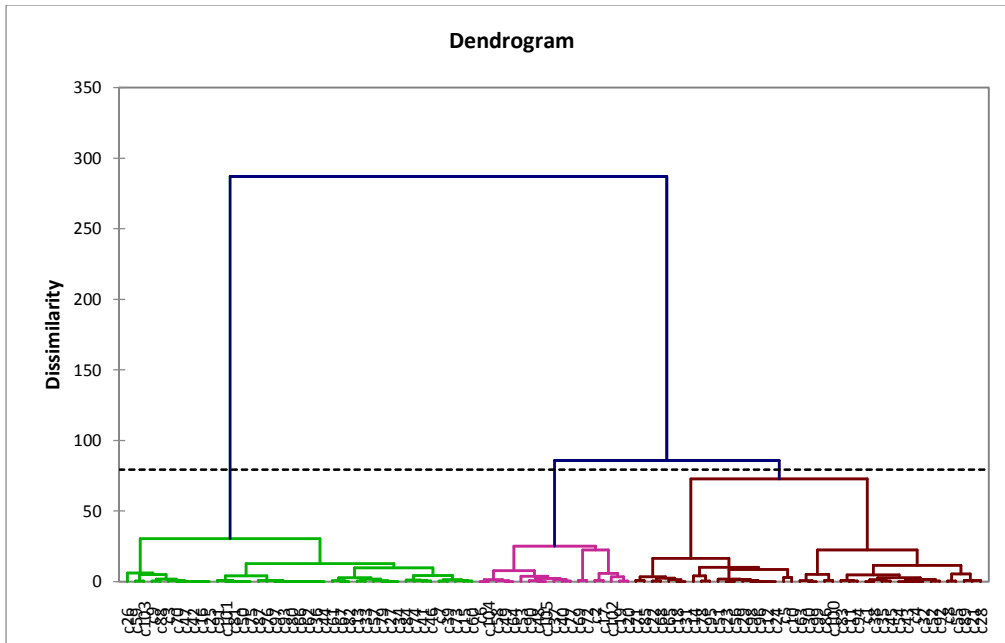


Figure 4-5. Dendrogram from Cluster Analysis showing three dissimilarities among three main clusters. From left to right cluster 3, 2, and 1 are shown.

Table 4-11. Average overall liking scores of samples for each cluster.

Cluster	MF	CD	FD	WSD	n
1	5.74 ^a	5.44 ^{ab}	5.16 ^b	5.67 ^a	43
2	4.11 ^b	6.47 ^a	6.53 ^a	6.63 ^a	19
3	7.28 ^{ab}	7.42 ^a	7.02 ^b	7.49 ^a	43

Descriptive and Consumer data association

To compare and understand the relationship between overall liking and sensory characteristics of samples Partial Least Squares regression analysis was performed (Figure 4-6). In the graphic it is possible to observe that sample CD and WSD were closer to the overall liking point. It was also shown that these samples were closer to attributes such as fibrous, grainy, and surface roughness appearance. This indicated these sensory characteristics of samples may have driven consumers' overall liking. In the graphic oily appearance was not close to the liking point, indicating that it negatively influenced consumers' acceptance scores when not associated with other appearance attributes influencing the liking mentioned above. For sample MF, the darkest brown color may have contributed to the lower acceptance. In the graphic this sample is close to both brown color and toasted aroma. However, the toasted aroma, higher for sample MF, probably did not contribute to the lower liking of this sample. Moreover, aroma was not related to the overall liking and according to this finding, even if the MF sample had a characteristic (higher toasted aroma) that could be expected to be pleasant to consumers, it did not increase the liking score of the sample. The darkest brown color of the MF kibbles might have reduced the overall liking.

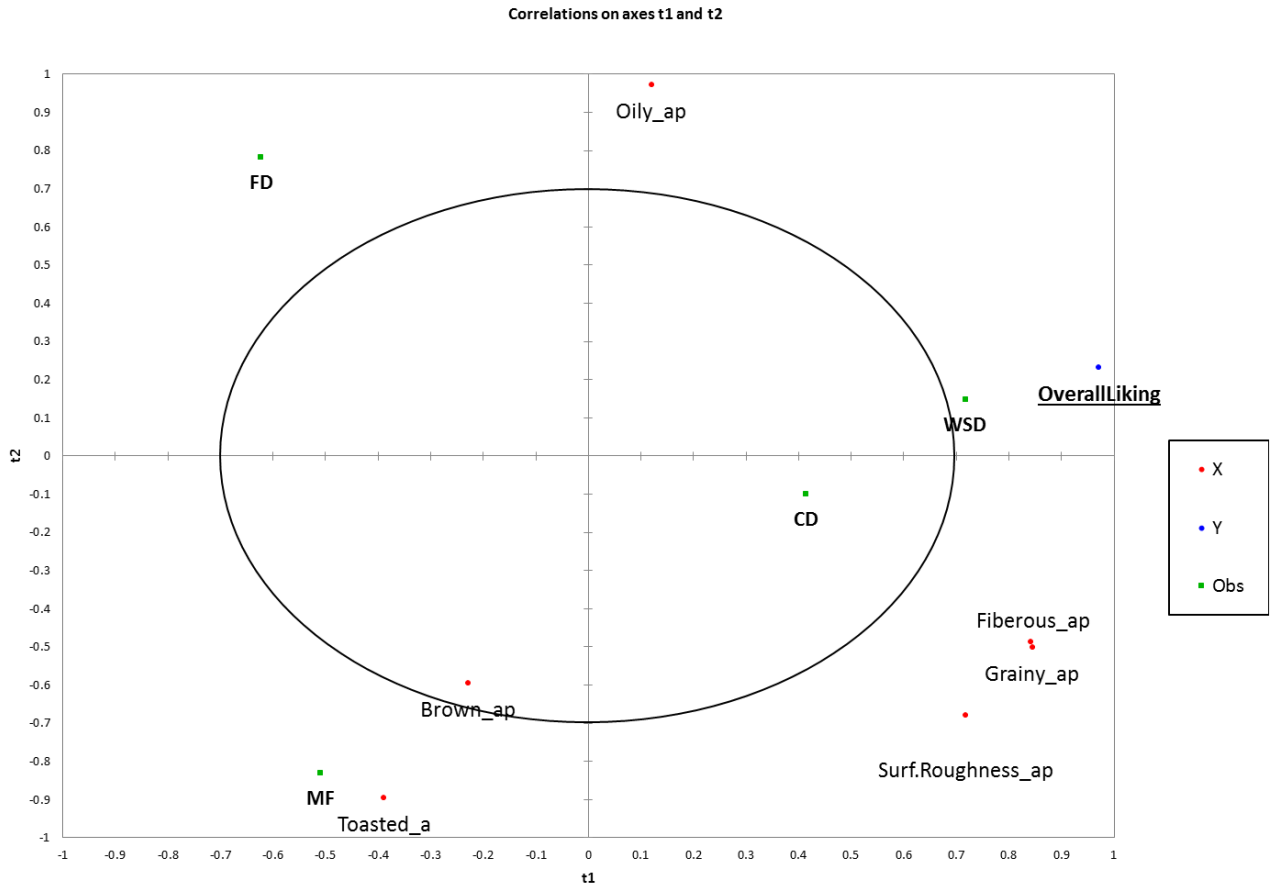


Figure 4-6. Partial Least Square Regression. Dependent variable (Y): Overall liking score (from Consumer panel), Explanatory variable (X): Descriptive sensory analysis (from human trained panel). Observations: Control (CD), whole sorghum (WSD), flour (FD) and sorghum mill feed (MF) containing diets.

The fact that the appearance characteristics of samples influenced the overall liking more than the aroma characteristics in this study, the data is in agreement with a previous study that observed the same pattern (Di Donfrancesco *et al.*, 2014).

Discussion

Regarding the descriptive analysis results, similar low scores in dry dog food products have been noted by Di Donfrancesco et al. (2012) who described, from a sensory analysis standpoint, dry dog food products as a highly blended category with low flavor scores and often narrow ranges. However, this should also be interpreted by taking into account the specific methodology adopted, with 3g of sample used for aroma analysis and the specific anchors on the scale used. Sensory attributes were similar for flavor as well, with the only significant difference represented by the mill-feed (MF) sample that showed a higher dusty flavor and a musty note (Table 4-6).

When considering the overall aroma as perceived by consumers, rather than the single attributes intensity from descriptive analysis, there was some difference in the way the aroma was perceived, as highlighted by the penalty analysis. Penalty analysis performed on the JAR data showed for example that for sample WSD, the perception of the sample as having a 'too weak' overall aroma penalized in some way the consumers' overall liking. This can provide an indication about the fact that a stronger overall aroma may increase the liking for sample WSD.

The samples analyzed in the study were not perceived to be different for bitterness or astringency. Studies have indicated sorghum can cause bitter and astringent notes (Kobue-Lekalake et al., 2007). Results from this study showed that extrusion can limit these characteristics in the final product. This data is in agreement with findings from Cardoso *et al.* (2015) who showed that proanthocyanidins, which can be responsible for higher bitterness and astringency, were reduced after extrusion. Further studies need to show how a reduced bitterness and astringency may contribute to the acceptance of extruded products by dogs. Dogs tolerate bitter taste better than

cats (Bradshaw, 1991) but this does not mean that lower bitterness could add value to the product and increases palatability.

Consumer liking scores indicated that the appearance aspect was the major factor for the pet owners' acceptance of the samples. Di Donfrancesco *et al.* (2015) showed similar results in a consumer study conducted on commercial dry dog food products indicating that, even if it might be expected, both aroma and appearance play a role in consumer acceptance of the products. Aroma had more of a minor role than appearance. Aroma did not have an impact on pet owners' acceptance of the different sample in this study, however, it has to be stated that the samples in this study did not particularly differ for aroma characteristics. Therefore, this might be another explanation for the aroma not having a role in determining consumer liking for these samples.

Clustering also highlighted the different segments that can be present among pet owners' in regards to the way they perceive and like pet food characteristics. Results showed some consumers more than others considered a darker color of samples while others just overall liked or disliked samples. This aspect can be crucial for the pet food industry and a better understanding of this can help to more precisely target pet owners with the appropriate product.

Not many studies have compared sensory characteristics of dry dog food products and owners' acceptance and there was no literature found about this type of investigation made with dry dog food manufactured with sorghum. This study represents a first step for future research opportunities to understand the sensory properties of pet food manufactured with a major inclusion of grain sorghum. Limitations of this study might be the low sensory variety of the samples. A higher sensory variety might have helped to better understand what was driving the consumer liking. A larger number of samples might have also contributed to a better comparison between consumer and descriptive data. When manufacturing a larger sample set, the addition of samples

containing palatants used in commercial products might improve the variety of sensory characteristics of the samples. This would help to understand how the sensory properties and consumer acceptance might vary with different formulations. Moreover, the information presented in this paper needs to be compared with results from palatability tests with pets, testing the animal acceptance and studies testing the digestibility of dry dog food with grain sorghum inclusion.

Conclusions

The results from this study indicated extruded samples manufactured with different sorghum fractions differed between each other and when compared to a control sample, not containing sorghum. The differences were mostly for appearance characteristics such as color, graininess or texture characteristics such as hardness. However, aroma and flavor profiles of samples were very close without a higher bitterness reported for sorghum samples. This aspect can definitely be interpreted as a good signal for an increased use of extruded products containing sorghum in the pet food market. Moreover, pet owners' acceptance of products indicated the whole sorghum diet was liked at the same level as the control diet. This was mostly due to the appearance of the products. Another interesting finding was that the other two samples manufactured with sorghum were not too close from the most liked samples. These results indicate there is the possibility of a higher consumer acceptance if appearance characteristics are improved. This study indicates the potential for an increased use of sorghum, regardless of component, in dry dog food and future studies will need to investigate the acceptance of extruded dog food manufactured with sorghum by pets.

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Chapter 5 - Pet food acceptance of dry dog food manufactured with sorghum

Abstract

Pet food palatability assessment represents an important part of product development in the pet food industry and crucial for the success of a product in the market. The one-bowl technique is where the food intake is used to investigate pet acceptance for a specific food. The objective of this study was to understand pet acceptance between sorghum diets and compared to a control. Thirty dogs of different size and breed were fed three dry dog food diets containing different sorghum fractions and one control diet containing wheat, rice, and corn. Diets were formulated to be iso-nutritional for macronutrients and mineral content with about 60% grain. Each sample was fed for 5 days for a total of 20 days of testing. Results indicated that no difference was observed among diets, and sorghum samples were accepted at the same level as the control diet during the test.

Introduction

The pet food industry is a growing sector of the food industry, constantly looking for innovation and new ingredients, with an estimated \$24 billion dollars in sales for 2016 in the USA (APPA, 2016). Nutritional properties, palatability, and owners' liking are characteristics that determine the success of a product in the market. This industry, in the last few years, has been strongly influenced by humanization of pets, with dogs and cats perceived more as members of the family and pet owners becoming closer to parental figures. This led to an increased role of owners 'liking' for product success as compared to the past. Owners' acceptance can be influenced by sensory properties of products such as appearance and aroma (Di Donfrancesco et al., 2014) together with acceptance of ingredients on the label. However, palatability testing remains an important step in product development for a pet food product and it is often the crucial element for the success of a product in the market (Aldrich and Koppel, 2015). Palatability is not only about the taste of a food but it deals with other factors such as aroma, mouthfeel, ingestive behaviors, form of the food, and frequencies of feeding (Kvamme, 2003).

Different techniques are adopted to evaluate palatability. Two main methods are used to assess the palatability of a pet food product. The one bowl or single-bowl test is used to assess the acceptability of a product measuring pets' food intake. A second method, the two-bowl test is adopted to determine the preference of one product when compared to another while still measuring pets' food intake.

The types of pet panels that can be used to conduct palatability testing can be constituted by 'expert' trained pets in pet centers or untrained pets fed in an in-home test setting (Tobie *et al.*, 2015). The two panels can provide different types of information and they are both used when

developing a product. An expert pet panel can be more accurate because the pets are trained to the testing protocol and perform palatability tests on a daily basis but the training can be intensive with animals that need to be exposed to a different variety of food. An in-home test can provide additional useful information such as pet behaviors when interacting with the meal, and feedback about pet food diets from pet owners' perspective after being exposed to the diets and observing their dog consuming them for several test days.

The objective of this study was to understand if pets' acceptance of dry dog food diets manufactured with different sorghum fractions was different from the acceptance of a control diet not containing sorghum but other grain ingredients such as wheat, rice, and corn.

Materials and methods

Samples

Red sorghum purchased from 2013 crop from local grower in the Manhattan, KS area and it was milled at the Hal Ross flour mill at Kansas State University (Manhattan, KS, USA) to produce mill-feed, flour, and ground whole sorghum. Samples were mixed, ground, and extruded at the Bioprocessing and Industrial Value Added Program (BIVAP) facilities at Kansas State University, Manhattan, KS, USA (Di Donfrancesco, 2016; Corsato-Alvarenga, 2016).

As described in Chapter 4, three diets used in this study contained different sorghum fractions: whole sorghum (WSD), sorghum flour (FD), and sorghum mill-feed (MF). A control diet (CD) was made with corn, wheat, and brewers' rice in a ratio of 1:1:1. The MF diet was composed by

bran, shorts (finer bran), red dog (leftovers of the last flour cloth in the mill) and some coarse flour (Corsato-Alvarenga, 2016). Other than the different starch portions specific for each diet, samples also contained common ingredients such as chicken by-product meal, beet pulp, corn gluten, calcium carbonated, potassium chloride, salt, dicalcium phosphate, choline chloride (60% dry), natural antioxidant (dry), trace minerals Premix, and Vitamin Premix (Table 4-1 – Chapter 4).

Rendered chicken fat (IDF, Springfield, MO, USA) was preserved with BHA, propyl gallate, and citric acid. The rest of the ingredients were acquired from a local mill (Fairview Mills L.P., Seneca, KS, USA). Diets contained different starch sources but were formulated in order to be iso-nutritional for macronutrients (carbohydrate, lipid, protein), and mineral content. Milling, mixing, grinding, and extrusion of samples are described in Chapter 4.

Recruitment of pet owners

The dogs used in the palatability study were screened according to specific criteria. The first step was to create and implement a database with pet owners from the local (Manhattan, KS, USA) area willing to participate to consumer and palatability studies managed by the Sensory Analysis Center at Kansas State University. Previous consumers present in the database were contacted and asked about their pet ownership. Moreover, potential participants were also recruited through flyers distribution, advertising on a local newspaper and on the Kansas State University online bulletin.

Screening

Pet owners present in the database were sent an online screening questionnaire using Compusense at-hand software (Compusense Inc., Guelph, ON, Canada). First, they were asked about the type

of pets they owned (participation criteria – dog owner). The first parameter considered in the screener was the age of the dog that could be available for the study. Dogs younger than 2 years old and older than 10 years old were not accepted for participation in the test. This was because after a certain age (>10 years) results may be impacted for problems related to teeth, sensory perception of food, or other problematics. In addition, dogs younger than 2 years old were not used since different metabolic requirements might have required a different food amount relative to baseline calculations and the problems this would present when comparing intakes among treatments.

For gender, the goal was to have a ratio close of 1:1 female and male animals. Dog size was also considered when recruiting pets. Only dogs between 4 kg and 45 kg were considered for the study. The initial goal was to have a homogenous group of dog sizes in the study.

Dogs enrolled in this study had to be in a good health status with no allergies and not fed specific prescription diets at the time of selection to be able to be eligible. To be included in the palatability study dogs had to be normally fed exclusively with dry dog food, and not wet dog food. Dogs fed with both dry and wet dog food were excluded from the project. Another factor related to feeding habits of dog considered during the recruitment phase was the time the dog was fed daily. Since the study required one meal per day, only animals that usually were fed once a day were accepted. Moreover, owners were asked whether the dog was able to eat in a quiet place, without interruption by other pets or children, during the study. Lastly, they were asked about their eventual availability for a palatability study where they had to feed their dog four different diets for a total of twenty days. Pet owners selected were provided monetary compensation for participation. Out of the 500 pet owners screened 30 qualified and were recruited for the study.

One-bowl Test

The experiment was conducted as a completely randomized design where each diet was served monadically (as the sole food item in a single bowl) for 5 days to each dog for a total of 20 days of testing. Data were collected from each individual dog and if more than one dog was present in the household, the dog participating in the test had to be separated from the others. The test design is shown in Appendix A.

At the beginning of the study each participant picked up the first two diets from the Sensory Analysis Center. Samples were contained in a ~3.8 liter Ziploc bag (S.C. Johnson & Son, Inc. Racine, WI, USA) marked with diet feeding order number, diet code, owner's name and test day when the diet had to be fed to dogs. The amount of food placed in each bag was calculated based on the body weight indicated by the owner. Dogs daily metabolizable energy (ME) requirements were calculated as an average for laboratory kennel dogs or active pet dogs: $130 \cdot BW^{0.75}$ (National Research Council, 2006).

Owners were instructed to pour the content of a bag into the bowl normally used to feed their pet and leave the food available for a maximum of 30 minutes. After that time the sample was removed. Participants were instructed to collect the leftover food and place it back in the original bag marked with the diet number and test day. The bags were then returned to the Sensory Analysis Center and the food weighed and recorded.

If the dog was usually given a few treats during the day, owners were allowed to continue this practice, but were instructed to not give more than the usual daily amount. Dogs were not required to be hungrier than usual during the test, but at the same time an increased amount of treats could decrease their appetite for the experimental diets.

At the end of the second diet, after 10 test days of testing, participants returned the bags containing leftover food and picked-up their third and fourth diet. They were also asked a few questions about their opinion regarding the two diets they had given their pet. This was done in the form of a short survey (Appendix D). After the completion of test days 11-20, dog owners had to return the bags with any leftovers, return the questionnaire they were given at the beginning of the test, and complete the survey (Appendix D) related to the last two diets.

After receiving the bags containing the leftover food, the leftover dog food was weighted and intake was calculated according to formula (1).

$$100 - \left(\frac{\textit{leftover food}}{\textit{initial amount of food}} * 100 \right) \quad (1)$$

For each dog, leftovers from each test day were weighed and the intake related to each day was calculated using the formula above (1). The daily intake for each day for each diet and each dog were analyzed using ANOVA.

Consent Form

Participants were asked to read and sign a consent form in which they agreed to participate in the feeding tests for 20 days and were appraised that they could drop out of the study at any time if the dog showed any sign of sickness related to the food. It was also stated that the diets were formulated to meet the nutritional requirement of their dogs, that the researchers were not responsible in any way for health problems occurring during the test time period, and that if the dog was allergic to any product similar to those provided in the study they should not have

participated in the test. It was also communicated that the monetary compensation (\$120) was provided only after completing all 20 days of testing. The complete consent form is shown in Appendix B. The research was approved by the Institutional Review Board for Protection of Human Subjects (IRB #7761) and by the Institutional Animal Care and Use Committee (IACUC #3603). Consent form is shown in Appendix B.

Questionnaire

A questionnaire with instructions and diet codes specified for each test day and dates was provided to each pet owner. Owners were first instructed about pouring the food into the food bowl and starting the timed process in order to remove the bowl after 30 minutes. The dog owners were then asked to make a written record of the behavior of their dog right before the meal while they were pouring the food into the bowl, during the meal consumption and right after the meal. If the dog finished all of the food provided in the bowl they had to indicate the time it took the dog to finish. The questionnaire is shown in Appendix C. Instructions about treat provision at different times from the meal, if that was a common practice with their dog, was also provided. After the second diet a summary table was provided where participants could check the test-days completed, indicating whether they provided treats to the dog and the time elapsed before complete consumption of the diet (if the whole portion was eaten). This summary table was intended to remind owners to provide the required information that they might have forgotten to answer on the single test day questionnaire pages.

Post-study Survey

After completion of the first two diets, the participants were asked to fill in the first part of a short survey in a waiting room before receiving the bags with the next 10 day of food rations. For each of the two diets, they were asked a few open-ended questions such how they felt about feeding the diet to their dog; whether they would have fed that diet if currently available on the market (motivating the answer); what they liked about the specific diet, and what they disliked.

When participants came in to the SAC, after completion of the entire testing procedure to return the leftover diets and receive the monetary compensation, they were provided the second part of the survey. Other than the questions related to the diets noted above, they were also asked open-ended questions about their thoughts on the use of sorghum in pet food product, and about aspects they personally liked and disliked about the diets. The complete survey is shown in Appendix D.

Data Analysis

Analysis of variance (ANOVA) was performed (SAS version 9.4, The SAS Institute Inc., Cary, NC, US) using the PROC GLIMMIX procedure for mixed models. Means were separated by Fisher's Least Significant Difference (LSD) post-hoc to determine statistical significant ($P < 0.05$) differences between the diets for intake using diet as a fixed effect and dogs and test-days as random effects. Each daily intake value was used as a different replication for each diet and each dog.

An ANOVA was also performed to determine if there were differences ($P < 0.05$) for the intake among the different dog sizes, genders, age and (or) household composition.

Results

Dog panel composition

Male dogs represented 47% of the panel and females 53% (Table 5-1). Dogs older than 6 years old comprised of 67% of the panel while the remaining 33% were from 2 to 5 years old. One third of the panel weighed from 4 to 10 kg, one third from 11 to 20 kg, and another third of the dogs weighed more than 20 kg. Within the latter category most of the dogs weighed less than 30kg with only 1 dog weighing 40.8 kg.

Table 5-1. Dog panel (n=30 dogs) utilized for the one-bowl palatability test characteristics: dogs' gender, age, and size.

Dog Gender	Male	Female	
%	47	53	
Dog Age	2-5	6-10	
%	47	53	
Dog size	4-10 kg	11-20 kg	>20 kg
%	33.3	40.0	27.0

Food intake

Food intake values were not different ($p = 0.179$) among dietary treatments (Table 5-2). The variability among dogs was high, (Standard Error of the Mean = 4.1044) with some dogs eating most of the food provided and a few eating a small amount of sample. However, the test-day seemed to not have an effect on the intake. The expectation was that in the first test-days with a new diet the dogs might be more suspicious and thus intake might be lower. On average, the range food intake was 53-58% (Table 5-2). A total of five dogs ate less than 40%. Four of these dogs were small size dogs (< 5kg). Eight dogs consumed more than 70% of the diets overall. From

feedback received from a portion of dog owners, some dogs were usually fed an amount of food lower than the ratio calculated and provided for the test. The feedback indicated some animals became full before the bowl was completely empty. Only one dog ate 100% of all of the diets (female, 9.1 kg). Eighty-eight times the diet was completely finished by the dogs.

Comparing the intake of the different size of dogs, the overall intake combining all of the four diets was not significantly different among sizes (Figure 5-1). There was also no difference when comparing the intake for different diets by dog size. Figure 2 shows a lower intake value for small dogs consuming the FD diet but the difference was not significant. No difference in overall intake was also observed for the different dog gender, age or number of people living in the household (Figure 5-3, 5-4, and 5-5).

A table with the average intakes for each dog and diets is shown in the Appendix E.

Table 5-2. Dogs' food average intake % for each of the diets (WSD, MF, FD, and CD) (SEM: Standard Error of the Mean).

Sample	Intake (%)
MF	57.50
CD	57.06
WSD	55.62
FD	53.44
p-value	0.1729
SEM	4.1044

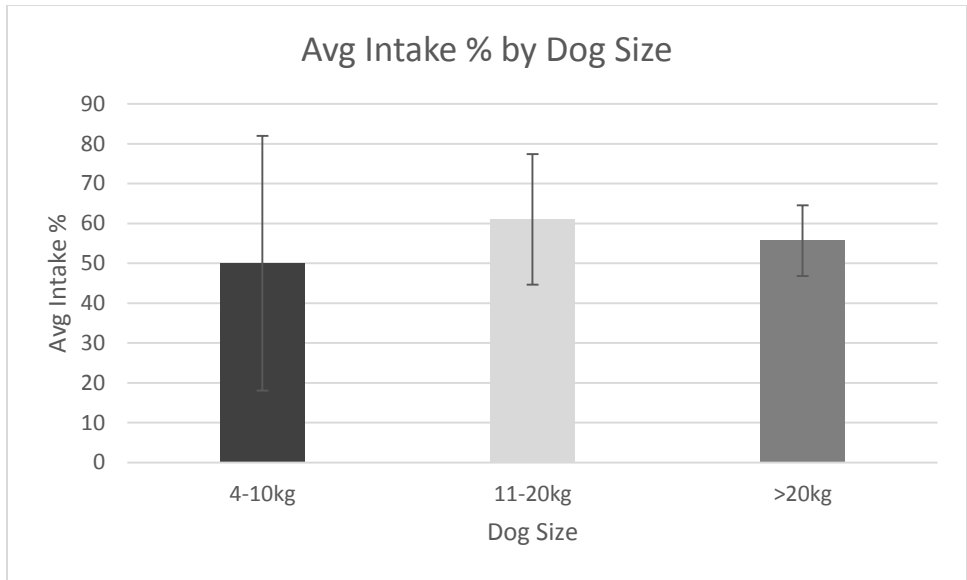


Figure 5-1. Dogs' average total intake % by different dog size (intake of all diets combined).

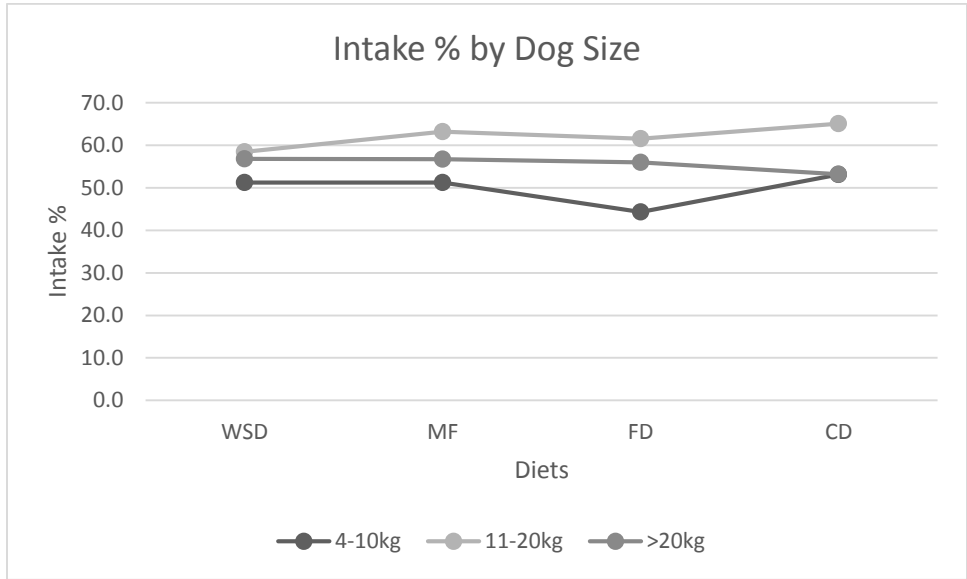


Figure 5-2. Dogs' average intake % for each of the diets (WSD, MF, FD, and CD) of the different dog size groups.

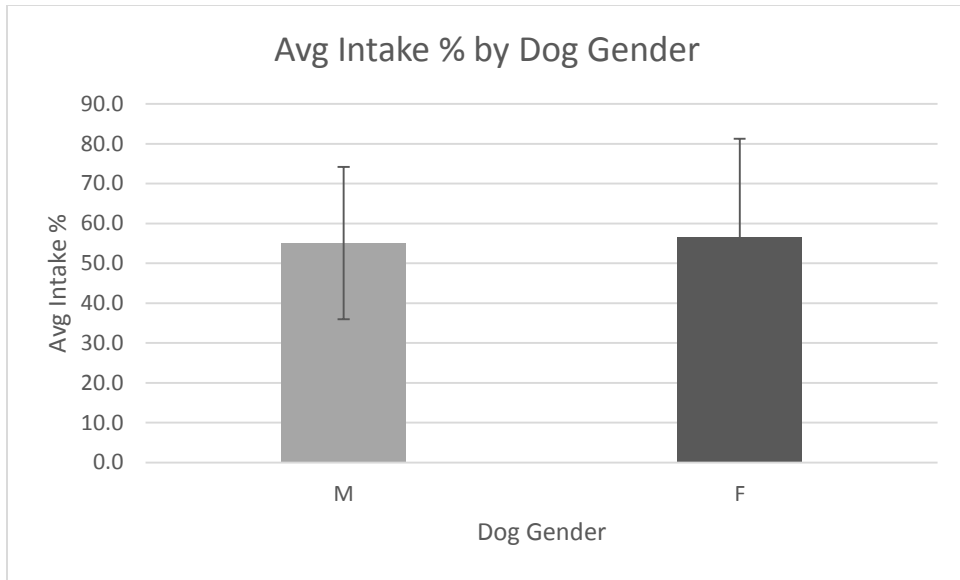


Figure 5-3. Dogs’ average total intake % by dog gender (intake of all diets combined)

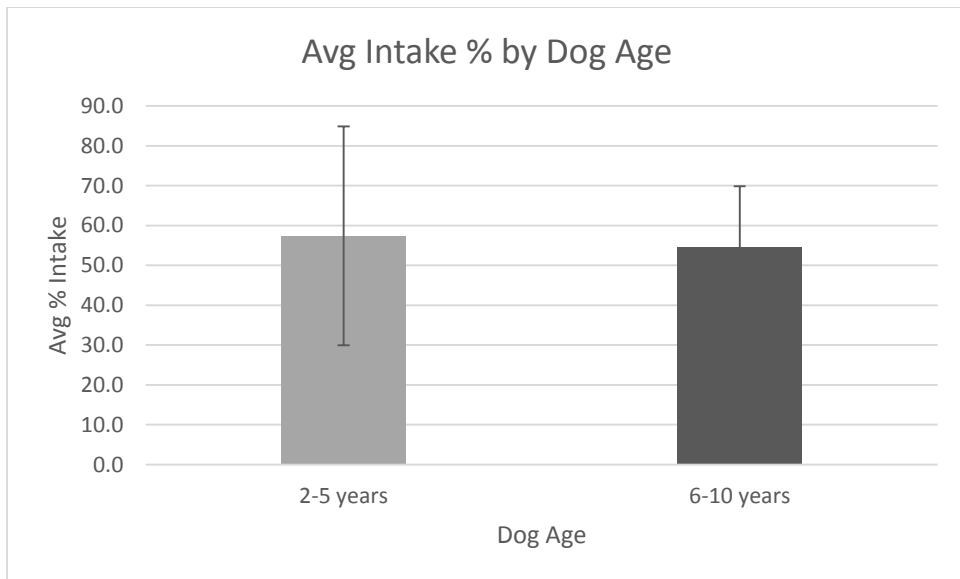


Figure 5-4. Dogs’ average total intake % by dog age (intake of all diets combined).

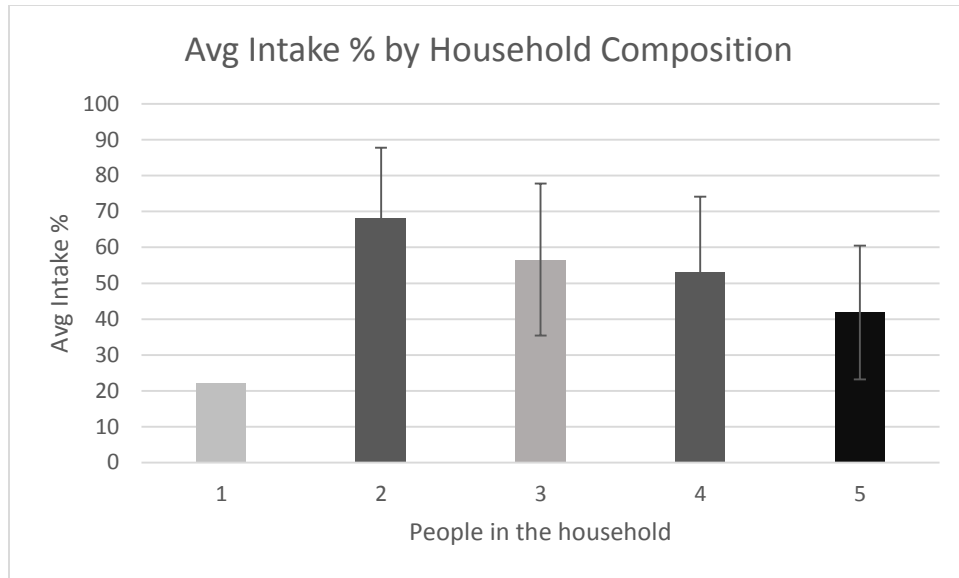


Figure 5-5. Dogs’ average total intake % by people composing the household where the dogs were fed (intake of all diets combined).

Note: only 1 household was composed by 1 person

Questionnaire

The participants were instructed to fill-in a questionnaire while watching the dog eating the experimental diets each day of the test. Of the behaviors observed the majority were not linked to a particular diet, but were common among dogs. For instance, common behaviors were observed before the meal and while the owner was pouring the food in the bowl. These observations were excitement associated with tail wagging, jumping, licking lips, salivating and in some cases barking. For some dogs a suspicious approach to the new food, sometimes consisting of sniffing the sample and showing no interest. After the meal, lying down and sleeping were behaviors also common across most of the dogs and diets.

There were some incidents. For example, during the meal consumption for diet WSD there was an episode of vomiting (female, 8kg, 6-10 years old). The owner observed mostly water with some

kibbles present (two times, the same test day, after 12 and 14 minutes from the beginning of the meal). The WSD diet also provoked defecating on the floor in another dog (female, 4.5kg, 2-5 years old). This was considered by the owner unusual episode for the dog. In another instance a dog eating the WSD diet consumed cat feces (male, 5kg, 6-10 years old), an act dog had never done before. No vomiting episodes were reported for the other diets; although, diets MF and FD provoked some cases of stomach upsetting in the form of gas. Some additional thirstiness in four dogs was reported. One dog (male, 4.5kg, 2-5 years old) was thirsty after all of the diets except diet MF. Two dogs appeared thirsty after the consumption of the MF diet and diet CD was associated with thirstiness in 3 out of the four dogs.

One dog (female, 11kg, 2-5 years old) had issues chewing diets FD and CD and the same dog was observed “loud crunching” the MF diet (not specifically mentioned as an issue). Another dog (female, 8kg, 6-10 years old) was observed to crunch and chew each kibble of the FD diet thoroughly. For one owner the crunchiness of diet FD was considered a positive thing. One dog (female, 10kg, 2-5 years old) consumed FD diet on the side of her teeth, according to the owner it seemed she did not want to touch the kibbles with her tongue. The CD sample also provoked an episode of loud chomping which was associated with coughing and spitting out some kibbles. This was interpreted by the owner as the diet may be tough to chew. A female dog (7kg) also defecated in the kennel after consuming the CD diet which was not usual for this pet.

Post-study Survey

Pet owners completed a survey in the middle and at the end of the test. At the end of the test the survey was taken online. Shape, size and color were often mentioned as liking factors for diet WSD, with one owner perceiving WSD and CD as good quality products with a natural looking

appearance. Owners also liked easiness to chew (mentioned by 3 participants), lack of smell (mentioned by 6 participants) and 'weird' aroma notes. One participant liked the size and texture consistency of the WSD diet. One owner also perceived the diet as easy to digest by the dog. Reasons for participants to dislike this diet were that their dog simply did not seem to like it (6 participants), the size was considered too big by two owners for the size of the dog (15 kg and 10 kg). A low appearance variety was also mentioned by a participant as making the kibble 'boring' but this low variety can be considered a factor common to all four diets.

Diet MF was also liked by owners because of the size (five mentions), good shape (four mentions), and absence of strong odors and off aroma notes (five mentions). The color of this diet was perceived by a participant as 'rich' and by another participant indicating "without artificial color". It was also noted by two participants that the size, and smell of the food (one participant) were consistent and did not vary between individual portion containers. The fact that a dog consumed less when eating this diet was perceived as a positive factor by one owner because the dog needing to lose weight. This diet was also liked (one mention) because it looked like a 'normal' dog food on the market. Among the factors negatively influencing consumers liking of the MF diet we found the color was considered boring by one participant who indicated a preference for variety of color and considered variety of shapes an 'extra bonus' when purchasing dry dog food products. One owner did not like the smell of this diet.

The FD diet was appreciated by some consumers for the color (five owners), smoothness of the kibbles (one owner) and the shape (four owners). The shape was described by one owner as more disc-shaped when compared to the other diets and considered to have a "non-strange shape with a healthy look. The sample was perceived as uniform in size, color and texture (one owner). A pleasant smell and the absence of strong aromas was also considered a positive factor (seven

mentions). For this sample one participant also noted the size as too large for the 10 kg dog. The absence of a meaty flavor (one mentions) was also listed as a negative factor together with causing gas (three mentions) and making the dog thirsty (one mention).

The CD sample was liked for a round and smooth appearance (one owner), with a more interesting shape for another owner. It was perceived as a nutritious food (one owner), looking healthy and with no artificial colors (one owner). Two participants mentioned the easiness to chew by their dog. The aroma was also mentioned as contributing to the liking (seven mentions). Among the factor considered negative, the lighter color as compared to the other diets was perceived as not dark or meaty enough. Lack of variety was mentioned for this diet as well. The fact that owners liked this diet just because the dog liked it was mentioned nine times; more than any other diet.

One common liking factor for all of the diets was when dogs liked the diets the owner liked the diet too. Seeing the dog happy while eating a diet was in some cases listed as the main factor for the owner to like the diet, no matter what appearance or aroma it might have.

When asked if they would feed a diet to their dog, if available on the market, more than one participant answered that it would depend on the price (three owners) and ingredients (four owners) of the product. Overall, each of the diets received a majority of positive responses from the owners that actually answered this specific question in the survey. The attitude of participants toward use of sorghum in pet food was overall positive, as long as the diet followed the proper nutritional requirements. Participants that indicated a negative response mentioned possible allergies of their dog, or they just did not know much about sorghum in order to express an opinion.

Discussion

In this study we observed several dogs not eating all of the diet while others consumed all of the diet. This contributed to an increase in the data variability with regards to the nature of a home use test (HUT) where untrained dog panels are utilized. Several dogs consumed less of the experimental diets compared to the usual amount of food provided as the customary diet. This intake reduction during the experiment was sometimes also accompanied by a suspicious behavior toward the new food or disinterest for it. This may be related to neophobia, a mechanism thought to be evolved to reduce exposure to danger. A study also showed that dogs may have a lower interest for novel objects, in this case food, when compared to other canids (Canidae family) such as wolves (Moretti *et al.*, 2014).

Tobie *et al.* (2015) described a lower reliability for quantitative data occurring when conducting tests using untrained dog panels. An untrained dog panel is not exposed to a variety of foods. Griffin *et al.* (1984) showed that, when testing canned food, home-dogs and kennel dogs performed similar but when testing semi-moist and dry food, differences were observed. In that study the causes of these differences were related to a difference in feeding history prior to the test.

With in-home tests, there is also a lower control on testing conditions and the possible introduction of biases by dog owners. For these types of tests, a higher number of dogs, when compared to what is recommended for an expert dog panel (at least 30 dogs), should be recruited to improve the test reliability (Tobie *et al.*, 2015). Palatability can also be influenced by factors such as temperature of food, socialization and group feeding (Kvamme, 2003). All these factors may play an important role when conducting home-use tests where the pet owners feed their dogs without a constant control by researchers.

Furthermore, an opposite dog behavior was also observed. Some of the dogs were extremely excited by the introduction of the new diets and high intake values were recorded. Together with genetic factors (Kvanmme, 2003), food preference can be affected by previous exposure to a particular flavor in dogs (Bradshaw, 1991). When the new food is not rejected, but preferred over a usual diet, we can refer to a novelty effect or neophilia.

If a HUT provides less control over the test protocol and utilizes non trained pets, it may also offer advantages. For example, the location where the test is conducted represents the environment where food is normally consumed (Vondran, 2013; Pound *et al.*, 2000). A main reason to choose a HUT over a CLT with a trained kennel panel is that this kind of setting creates an opportunity to gather precious feedback about owners and dogs, in a ‘real life’ situation (Tobie *et al.*, 2015).

Previous studies using kennel dog food palatability testing of sorghum versus other types of grain based diets have been conducted. These studies reported a good performance of this grain ingredient with no difference in DM intake when compared to other starch sources (Kore *et al.*, 2009, Murray, 1999, Fortes *et al.*, 2010). In the current experiment the diets did not contain any additional flavor added to the formulation such as processed meat, yeast or oil-based flavors. Additional flavors may have an effect and increase the food intake by dogs. Kibbles were only coated with chicken fat and this can still be considered a good flavor enhancement, although added for calories in this case. The addition of fat was done in order to produce diets similar to actual commercial product available on the market normally consumed by dogs. Providing diets too different from a typical commercial diet could have misrepresented the ‘real life’ HUT setting.

A previous consumer study regarding dry dog food acceptance by pet owners showed different segments of consumers may perceive a variety of color differently. Some consumers tend to like a higher variety while others prefer products with a lower variety (Di Donfrancesco *et al.*, 2014).

In this study, some pet owners liked the natural color of the diets and some would have preferred more color variety. The same thing was observed for the shape of the kibbles. Most of the liking factors dealt with the appearance of the samples or the smell of the sample (a mild smell was liked). However, another key factor for consumers liking the sample was simply related to the fact that their dog liked the diets. Belk (1996) has shown that there are other factors such as pet owner's personality, lifestyle, and families that influence the pet food selection. Tesfom (2010) indicated that owners take buying healthy dog food more seriously than buying healthy human food for themselves and that brand loyalty plays a major role in the pet food selection. This may represent a limit when asking consumers about their purchase intention of a blind unbranded pet food product.

Conclusions

These results showed that diets manufactured with different fractions of red sorghum performed equally in an HUT palatability acceptance study where dogs were fed by their owners and consumed the diets in their usual environment. The control diet made with starch sources typically used by the pet food industry such as corn, rice, and wheat did not perform better than the sorghum diets. Qualitative data from pet owners also provided important feedback about the way these extruded sorghum diets might be perceived with possible strengths and weaknesses. This preliminary study represents an encouraging first step in understanding how the use of grain sorghum can be increased in the pet food production sector. A limitation to the study was the limited number of dogs constituting the panel when considering the problems associated with an in-home testing environment. The collection of owners' feedback with exclusively open ended

questions and with the creation of an extremely fragmented data set was also a limiting factor when trying to interpret and quantify consumer opinions. Providing participants a limited set of alternatives established by researchers such with CATA questions would most likely improve the quality of the information and optimize the work-load interpreting the data and the power in drawing meaningful conclusion.

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Chapter 6 - Volatile constituents and effect on sensory characteristics of dry dog food manufactured with red sorghum

Abstract

Descriptive sensory analysis and gas chromatography-mass spectrometry (GC-MS) with modified headspace solid-phase microextraction (SPME) method was performed on three extruded dry dog food diets manufactured with different fractions of red sorghum and a control diet containing corn, brewer's rice, and wheat as grain source. The aroma and flavor profile of samples was similar with small differences such as higher toasted aroma notes, and musty and dusty flavor in the mill-feed sample. A total of 39 compounds were tentatively identified and semi-quantified. Aldehydes were the major group present in the samples. Total volatile concentration was low reflecting the mild aroma of the samples. Partial Least Squares regression was performed to identify correlations between sensory characteristics and instrumental data. Possible relationships, such as hexanal and oxidized oil, and broth aromatics were identified. Volatile compounds were also associated with earthy, musty, and meaty aroma and flavor notes. This study showed that extruded dry dog food manufactured with different red sorghum fractions has a similar aroma and flavor profile with similar volatile compounds composition.

Introduction

Pet food industry represents an important segment of the food industry with a significant growth in the recent years, with an estimated \$24 billion of dollars in sales for 2016, in the USA only (APPA, 2016). This industry is constantly searching for new ingredients and, because of humanization of pets observed in the last few years, there are trends in human food being adopted in pet food products.

Palatability of pet food products play an important role for success on the market but consumer acceptance of aroma and appearance of kibbles plays an important and increasing role as well (Di Donfrancesco *et al.*, 2014). Descriptive sensory analysis has been used to understand sensory characteristics of dry and canned pet food products (Koppel, 2014; Di Donfrancesco *et al.*, 2012, Pickering, 2008; Lin, 1998).

A wide variety of ingredients is utilized for different type of pet foods, such as dry, canned, or semi-moist products. With pet food any given product must satisfy all the nutritional requirements of the pet. For this reason, pet food is often a complex product. This can be reflected in the aromatic composition of products too. Ingredients utilized in dry dog food includes meat ingredients such as poultry, beef, and pork products, fresh or rendered, representing an important portion of the formulations. A variety of grains are also utilized as starch sources, mainly rice, wheat, and corn. Other grains, such as barley and oat, are also utilized in smaller amounts. Sorghum represents one of the least utilized starch ingredients in pet foods (Willard, 2003). Red sorghum is poorly utilized by the pet food industry because of the condensed tannin content that can impart higher bitterness to products (Herbert, 2003; Kobue-Lekalake *et al.*, 2007) and perhaps because consumers do not recognize this grain.

The United States is one of the main producers of sorghum and Kansas is the leading producer in the USA. Because of its health benefits, such as its antioxidant properties, together with more environmentally sustainable agricultural practices (Vazquez-Araujo *et al.*, 2012) and potential marketing claims, sorghum could have a big potential for an increased use in human food and the potential to become the next novel carbohydrate ingredient utilized by the pet food industry. To understand if an increased utilization of sorghum is possible by this industry, it is essential to understand aspects like the sensory characteristics of pet food manufactured with sorghum and its fractions.

Because of the complexity of pet food, the study of the aromatic composition can provide important information in order to understand the product (Koppel *et al.*, 2013). Several studies exist that investigated the volatile aromatic composition of food such as grains or meat ingredients that can be part of in pet food products as raw materials (Busko *et al.*, 2010; Lammers *et al.*, 2011; Parker *et al.*, 2000).

The analysis of volatile compounds composition of different types of grains such as corn, rye, wheat, barley, and rice, has been conducted using solvent extraction techniques but solid-phase microextraction (SPME), used in the current study, has become a widely utilized technique

The objectives of this study were to 1) detect the volatile compounds composition of pet foods manufactured with sorghum fractions and compare those to the control, and 2) understand the possible relationship between instrumental and descriptive sensory analysis data.

Materials and Methods

Samples

Red sorghum was purchased from local grower from 2013 crop in the Manhattan, KS area and was milled at the Hal Ross flour mill at Kansas State University (Manhattan, KS, USA). Extruded diet kibbles were manufactured with different red sorghum fractions at the Bioprocessing and Industrial Value Added Program (BIVAP) facilities at Kansas State University, Manhattan, KS, USA (Chapter 4 and 5; Corsato-Alvarenga, 2016).

The three sorghum diets used in this study contained different sorghum fractions, specifically whole sorghum (WSD), sorghum flour (FD), and sorghum mill-feed (MF). A control diet (CD) was also manufactured with corn, wheat, and brewers' rice in a ratio of 1:1:1. The MF diet was composed of bran, shorts (finer bran), red dog (overs of the last flour cloth in the mill) and some coarse flour (Corsato-Alvarenga, 2016). Ingredients common to all the four diets were chicken by-product meal, beet pulp, corn gluten, calcium carbonated, potassium chloride, salt, dicalcium phosphate, choline chloride (60% dry), natural antioxidant (dry), trace minerals Premix, Vitamin Premix (Table 1). These ingredients were acquired from a local mill that supplies ingredients for pet food production (Fairview Mills L.P., Seneca, KS, USA). Diet ingredients are shown in Table 6-1.

Rendered chicken fat was provided from (IDF Springfield, MO, USA) was preserved with BHA, propyl gallate, and citric acid. Diets were formulated to be iso-nutritional for macronutrients (carbohydrate, lipid, protein) and minerals content. Milling, mixing, grinding, and extrusion of samples are described in previous chapters (4 and 5) and Corsato-Alvarenga (2016).

Table 6-1. Experimental diets composition. Control (CD), whole sorghum (WSD), sorghum flour (FD) and sorghum mill-feed (MF).

Ingredients, %	CD	WSD	FD	MF
Brewers' rice	21.21	-	-	-
Corn	21.21	-	-	-
Wheat	21.21	-	-	-
Whole sorghum	-	64.69	-	-
Sorghum flour	-	-	62.31	-
Sorghum mill-feed	-	-	-	67.65
Chicken by-product meal	20.94	20.02	21.00	20.00
Chicken fat	5.34	5.52	5.52	3.29
Beet Pulp	4.00	4.00	4.00	4.00
Corn gluten meal	3.00	3.00	3.00	3.00
Calcium carbonate	0.75	0.35	0.23	0.67
Potassium chloride	0.49	0.52	0.64	0.19
Salt	0.46	0.45	0.46	0.43
Dicalcium phosphate	0.87	0.95	1.19	0.24
Choline chloride	0.20	0.20	0.20	0.20
Vitamin premix	0.15	0.15	0.15	0.15
Trace mineral premix	0.10	0.10	0.10	0.10
Natural antioxidant (dry)	0.07	0.07	1.21	0.08
Natural antioxidant (liquid)	0.02	0.02	0.02	0.01

Extraction procedure of volatile aroma constituents

Headspace-solid phase microextraction (HS-SPME) was the extraction method used to determine the aroma profile of the three dry dog food diets manufactured with red sorghum and the control diet (Koppel *et al.*, 2013). A mortar and pestle was utilized to grind the samples and then 0.5g was weighed and placed in a 10 ml screw-cap vial equipped with a polytetrafluoroethylene/silicone septum. Successively 0.99 ml of distilled water was added to the ground sample in the vial. Adhikari *et al.* (2010) indicated that, when conducting SPME analysis, the addition of water or other surface-active compounds to solid samples is recommended in order to improve the transport of compounds from the sample to the gaseous phase. The internal standard utilized was 0.01 ml of 1,3-dichlorobenzene dissolved in hexane (mixture of isomers, optima grade, Fisher Scientific; Pittsburgh, PA, USA) with a final concentration in the sample of 20 µg/kg. Vials were equilibrated in an autosampler (Pal system, model CombiPal, CTC Analytics, Zwingen, Switzerland) for 10 min at 40 °C and then agitated at 250 rpm. Following equilibration, a 50/30 µm divinylbenzene/carboxen/polydimethyl-siloxilane fiber was utilized because of the high capacity of trapping compounds in food products, according to Ceva-Antunes *et al.* (2006). The fiber was exposed to the sample headspace for 30 min at 40 °C. After the sampling the analytes were desorbed from the SPME fiber coating to the injection port of gas chromatography (GC) at 270 40 °C for 3 min in splitless mode.

Chromatographic Analysis

Isolation, tentative identification, and semi-quantification of the volatile compounds were performed on a gas chromatograph (Varian GC CP3800; Varian Inc., Walnut Creek, CA, USA) coupled with a Varian mass spectrometer (MS) detector (Saturn 2000). The GC-MS system was

equipped with a Stahlwax (Crossbond® 5% Carbowax polyethylene glycol) column (Restek, U.S., Bellefonte, PA, USA; 30 m × 0.25 mm × 0.5 µm film thickness). The initial temperature of the column was 40 °C and it was held at this temperature for 4 min; the temperature was then increased by 5 °C per minute to 240 °C, and held at this temperature for 10 min. All the samples were analyzed in triplicates.

To identify most of the compounds two different methods were utilized: 1) mass spectra, and 2) Kovats indices (NIST/EPA/NIH Mass Spectral Library, Version 2.0, 2005), for pure chemicals. When only based on mass spectral data the identification was considered tentative. The retention times for a C7-C40 saturated alkane mix (Supelco Analytical, Bellefonte, PA, USA) was used to determine experimental Kovats indices for the volatile compounds detected. Chemicals that were used to confirm the volatiles were: 2-nonen-1-ol, 1-nonen-3-ol, isovaleraldehyde, hexanal, octanal, furfural, 5-methyl-2-furaldehyde, and 6-methyl-5-hepten-2-one (Sigma Aldrich, St. Louis, MO).

Descriptive Analysis data for regression

In the first part of the study (Chapter 4) a sensory panel of five highly trained panelists evaluated the diets for aroma, flavor, and aftertaste characteristics. Each panelist had more than 120 hours of general descriptive sensory analysis panel training. The panel training included techniques and practices in attribute identification, terminology development, and intensity scoring. In addition, each panelist had experience with a variety of different food products including dried pet food, for both cats and dogs.

The testing room was maintained at 21 ± 1 °C and $55 \pm 5\%$ relative humidity. The aroma and flavor attributes evaluated were barnyard, brothy, brown aromatics, cardboard, dusty, earthy, grain, liver, meaty, musty, oxidized oil, stale, toasted, vitamin. In addition, bitter, salt, sour, sweet, and metallic

were also part of the pool of attributes. For the aftertaste the descriptors considered were barnyard, liver, brown, grain, cardboard and bitter.

Data analysis

Analysis of variance (ANOVA) was performed (SAS version 9.4, The SAS Institute Inc., Cary, NC, USA) using PROC GLIMMIX and Fisher's Least Significant Difference (LSD) post-hoc means separation to determine statistical significance ($P < 0.05$) difference between the diets for sensory characteristics using sample as fixed effect and panelist and replicate as random effects. In addition, Partial least square regression (PLSR) was performed to determine the correlation between instrumental data from the chromatographic analysis (X-matrix) and descriptive sensory data (Y-matrix). Other studies (Vazquez-Araujo *et al.*, 2011; Lee *et al.*, 2011) also utilized the same approach when determining correlations between volatile compounds sensory characteristics of food. To perform PLSR, XLSTAT software was utilized (Addinsoft, New York, NY, USA).

Results and discussion

Dry Dog Food Volatile Composition

A total of 37 aromatic compounds were tentatively identified among the four dry dog food diets. The concentration of each of the compounds, grouped by chemical family, and the total concentration of each chemical group are reported in Table 6-2. The compounds were grouped as alcohols (six compounds), aldehydes (eleven compounds), alkanes (eleven compounds), alkenes

(two compounds), carboxylic acids (two compounds), furans (one compound), hydroxy acid (one compound), ketones (four compounds), and terpenes (one compound).

Total concentration of volatiles was similar among the four samples (Table 6-2) (1.78–2.09 $\mu\text{g}/\text{kg}$, average 1.89 $\mu\text{g}/\text{kg}$). All the diets contained grains (about 60% of the total ingredients) and other common ingredients such as chicken fat, beet pulp, and corn gluten meal. A similar total content of volatile among samples might have been expected. In a study that identified volatile compounds of commercial extruded dry dog food products from the market, Koppel *et al.* (2013) found an average of 22.07 $\mu\text{g}/\text{kg}$ (10.60–41.34 $\mu\text{g}/\text{kg}$) for grain added samples and 13.63 $\mu\text{g}/\text{kg}$ (8.24–17.37 $\mu\text{g}/\text{kg}$) for grain-free samples. These volatile concentrations are much higher than the ones found in the present study. The lower concentrations might be due to sample formulations without any additional flavors and palatants used in this study.

Aldehydes were the most abundant group detected in the samples, accounting for almost 50% of the total volatile compounds in each diets. Differences among the samples were found for aldehydes content. The WSD sample had the highest concentration (0.98 $\mu\text{g}/\text{kg}$) of aldehydes, followed by the MF sample (0.91 $\mu\text{g}/\text{kg}$), CD sample (0.85 $\mu\text{g}/\text{kg}$), and FD (0.72 $\mu\text{g}/\text{kg}$). Aldehydes have been shown to play a major role in odor contribution even if present in low concentration, since they have low thresholds, in the range of few micrograms per liter of water (Lammers *et al.*, 2011). Hexanal, the main product of oxidation of linoleic acid (Belitz *et al.*, 2001), was the most abundant compound among the aldehydes, with a concentration range varying from 0.33 $\mu\text{g}/\text{kg}$ in the FD sample to 0.55 $\mu\text{g}/\text{kg}$ in the MF sample. Odors notes associated with hexanal have been described as leaf-like (Rychlik *et al.*, 1998), greenish (Specht and Baltes, 1994) green, grass-like, and green tomato (Bryant *et al.*, 2011).

Other studies that identified volatile compounds in grains, such as oats (Lampi *et al.*, 2015) also found hexanal to be the abundant compound in the samples; Lwande and Bentley (1987) identified volatile compounds present in sorghum seedlings (*S. bicolor*, Serena cultivar) and listed hexanal as the third most abundant compound preceded by (Z)-3-Hexen-1-ol and (Z)-3-Hexen-1-ol acetate. Both these compounds were not identified in the extruded samples in the current study.

Other aldehydes such as benzaldehyde, isovaleraldehyde, 5-methylhexanal, furfural, and benzeneacetaldehyde were present at lower concentrations. Benzaldehyde can be a thermal reaction product of hexanal and deca-2, 4-dienal (Lasekan *et al.*, 1997) and it has been identified in extruded cereals (Pfannhauser, 1993), cooked rice (Buttery, 1988) and dry dog food products (Koppel *et al.*, 2013).

The group with the second highest concentration of volatiles was represented by the alkanes. Alkanes can be formed during lipid oxidation from division of saturated alkoxy radicals. Aliphatic hydrocarbon compounds have high odor thresholds and do not usually play a major role in odor contribution (Wettasinghe *et al.*, 2000). Sample FD had the highest concentration of total alkanes (0.55 µg/kg) while sample MF showed the lowest (0.48 µg/kg). Specifically, sample FD had the highest content of 2,6-Dimethylheptadecane (0.17 µg/kg).

Alcohols and ketones were also detected in smaller concentrations in all of the samples. Alcohols are formed by decomposition of the secondary hydroperoxides of fatty acids (Liu *et al.*, 2012). They are generally associated with fruity, floral, and grassy notes in cereal. The two alcohol compounds with the highest concentration were 2-nonen-1-ol and 1-nonen-3-ol (0.07–0.09 µg/kg).

The total alcohols concentration was lower in the MF sample compared to the other samples.

Hydroxy and carboxylic acids such as butyric acid, hexanoic acid, and 2,3,5-trimethoxymandelic acid (di-TMS) were also identified in all of the diets even if all of these were present at very low concentrations (butyric acid at 0.01 µg/kg in all of the samples, hexanoic acid with a range of 0.01-0.03, and di-TMS with a range of 0.02-0.03 µg/kg). In this study WSD (0.06 µg/kg) sample had the highest concentration of carboxylic acids while sample MF (0.02 µg/kg) had the lowest.

Hexanoic acid has been shown to be the major volatile compound in oat extrudates during extensive lipid oxidation, even more than hexanal, usually adopted as lipid oxidation indicator (Lampi *et al.*, 2015).

During the extrusion of grain flours, it is possible to individuate two main reactions that lead to the formation of volatile compounds: Maillard reactions and lipid degradation. During the Maillard reaction, where several reactions between reducing sugars, amino acids, and their respective degradation occurs, mostly desirable notes such as toasted grains aroma notes are generated. From a compound generation standpoint, a common category of compounds generated during Maillard reaction are Strecker aldehydes, by decarboxylation and deamination of amino acids (Parker *et al.*, 2000). Off-flavors, associated with compounds such as hexanal and pentanal, are instead often the products of lipid degradation reactions. The volatile compounds produced by lipid degradation have been extensively described and are mostly represented by aliphatic aldehydes, alcohols, and ketones derived from fatty acids. Extrusion conditions can have an influence on both these type of reactions (Parker *et al.*, 2000).

Koppel *et al.* (2013) identified volatile compounds in extruded commercial dry dog food, with and without grain and found that aldehydes were the major volatile group in the wide variety of samples analyzed. The most abundant compounds found in that study for grain based food were hexanal followed by benzaldehyde. Pyrazines, such as 2,5-Dimethyl pyrazine, was found in some of the

samples, both grain based and grain free. However, pyrazines were not detected in the samples analyzed in this current study.

Table 6-2. Content ($\mu\text{g}/\text{kg}$) of aroma compounds in the MF (Mill-feed), CD (Control Diet), FD (Flour Diet), and WSD (Whole Sorghum Diet). KI (Exp): experimental Kovats index, KI (Lit): Kovats index from literature.

Sample				MF		CD		FD		WSD	
Code	Compound	KI (Exp)	KI Lit.	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
<i>Alcohols</i>											
C1	Z-10-Pentadecen-1-ol	905	N/A	0.01	0.00	0.01	0.00	0.02	0.00	0.01	0.00
C2	4-Methyl-1-pentanol	N/A	851a	0.01	0.00	0.03	0.01	0.01	0.00	0.01	0.00
C3	2-nonen-1-ol	1218	1051a	0.07	0.01	0.09	0.02	0.09	0.02	0.09	0.02
C4	1-nonen-3-ol	1237	1072b	0.07	0.02	0.07	0.01	0.07	0.01	0.09	0.00
C5	3-furanmethanol	N/A	866b	0.01	0.00	0.02	0.01	0.01	0.00	0.02	0.01
C6	Butanol, 4-(hexyloxy)-	1409	N/A	0.01	0.00	0.01	0.00	0.01	0.00	0.02	0.00
Total Alcohols				0.18		0.24		0.21		0.25	
<i>Aldehydes</i>											
C7	Isovaleraldehyde (3-methylbutanal)	N/A	936b	0.06	0.02	0.08	0.01	0.07	0.02	0.08	0.01
C8	Hexanal	N/A	800c	0.55	0.09	0.39	0.09	0.33	0.04	0.53	0.12
C9	5-Methylhexanal	1325	1150a	0.06	0.01	0.07	0.03	0.04	0.01	0.05	0.03
C10	Octanal	1191	1004c	0.04	0.01	0.05	0.01	0.04	0.01	0.05	0.00
C11	2- Heptenal (Z)-	1434	951d	0.02	0.01	0.02	0.00	0.01	0.00	0.02	0.00
C12	2-Octenal	1247	1060d	0.02	0.00	0.01	0.00	0.01	0.00	0.02	0.00
C13	Furfural	N/A	852c	0.03	0.01	0.06	0.02	0.06	0.01	0.05	0.01
C14	Benzaldehyde	1538	1522b	0.07	0.01	0.10	0.01	0.09	0.02	0.10	0.01
C15	5-Methyl-2-furaldehyde	N/A	964b	0.01	0.01	0.03	0.00	0.02	0.00	0.03	0.01
C16	Benzeneacetaldehyde	1306	1043d	0.02	0.01	0.04	0.00	0.02	0.00	0.04	0.00
C17	Cinnamaldehyde	1356	1232b	0.02	0.00	0.01	0.01	0.02	0.01	0.02	0.01
Total Aldehydes				0.91		0.85		0.72		0.98	

Table 6-2 (continue) Content (µg/kg) of aroma compounds in the MF (Mill-feed), CD (Control Diet), FD (Flour Diet), and WSD (Whole Sorghum Diet). KI (Exp): experimental Kovats index, KI (Lit): Kovats index from literature.

Code	Sample Compound	KI Exp.	KI Lit.	MF		CD		FD		WSD	
				Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
<i>Alkane</i>											
C18	2,2,3-Trimethyldecane	650	1104a	0.02	0.01	0.03	0.00	0.03	0.00	0.02	0.00
C20	3,4-Dimethyldecane	876	936a	0.03	0.01	0.03	0.00	0.04	0.00	0.03	0.01
C21	2,6-Dimethylheptadecane	N/A	N/A	0.11	0.03	0.12	0.02	0.17	0.03	0.13	0.04
C23	5-Ethyl-2,2,3-trimethylheptane	1013	924a	0.15	0.02	0.15	0.03	0.14	0.11	0.15	0.06
C25	Hydroxylamine, O-decyl	1055	1100a	0.04	0.00	0.04	0.01	0.04	0.02	0.05	0.02
C26	2,3 Dimethyldecane	0	N/A	0.11	0.04	0.11	0.08	0.12	0.02	0.13	0.05
C27	Nonadecane	N/A	1900a	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
C28	4-Chloro octane	1283	1149a	0.01	0.00	0.02	0.01	0.01	0.00	0.01	0.00
Total Alkane				0.48		0.50		0.55		0.52	
<i>Alkene</i>											
C29	3-Dodecene, (E)	1202	N/A	0.03	0.01	0.02	0.00	0.02	0.01	0.02	0.01
C30	3,7-Dimethyl-1-octene	1503	963a	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
				0.04		0.04		0.03		0.03	
<i>Carboxylic acid</i>											
C31	Butyric acid	N/A	821b	0.01	0.00	0.02	0.01	0.02	0.01	0.03	0.01
C32	Hexanoic acid	N/A	973a	0.01	0.00	0.02	0.00	0.02	0.00	0.03	0.00
Total Carboxylic acid				0.02		0.03		0.04		0.06	0.09
<i>Furans</i>											
C33	2-Pentylfuran	1146	1001b	0.12	0.04	0.14	0.03	0.14	0.04	0.13	0.02
<i>Hydroxy acid</i>											
C34	2,3,5-Trimethoxymandelic acid, di-TMS	N/A	N/A	0.02	0.01	0.02	0.00	0.02	0.00	0.03	0.00
<i>Ketones</i>											
C35	2,3-Octanedione	1207	N/A	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
C36	6-Methyl-5-hepten-2-one	1214	1341a	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
C37	(E,E)-3,5-Octadien-2-one	1274	1049d	0.02	0.00	0.02	0.00	0.02	0.00	0.02	0.00
C38	3,5-Octadien-2-one	1288	1049d	0.01	0.01	0.02	0.00	0.02	0.00	0.02	0.00
Total Ketones				0.05		0.06		0.06		0.08	
<i>Terpenes</i>											
C39	Camphene	1157	1097b	0.01	n.d	0.01	0.00	0.01	0.00	0.01	0.00
Total				1.81		1.89		1.78		2.09	

a: Pubchem.ncbi.nlm.nih.gov; b: The Pherobase; c: Goodner, 2008, d: The Flavornet

Partial Least Squares Regression

The Partial Least Square regression (PLSR) map with sensory aroma and flavor attributes related to instrumental data is shown in figure 6-1.

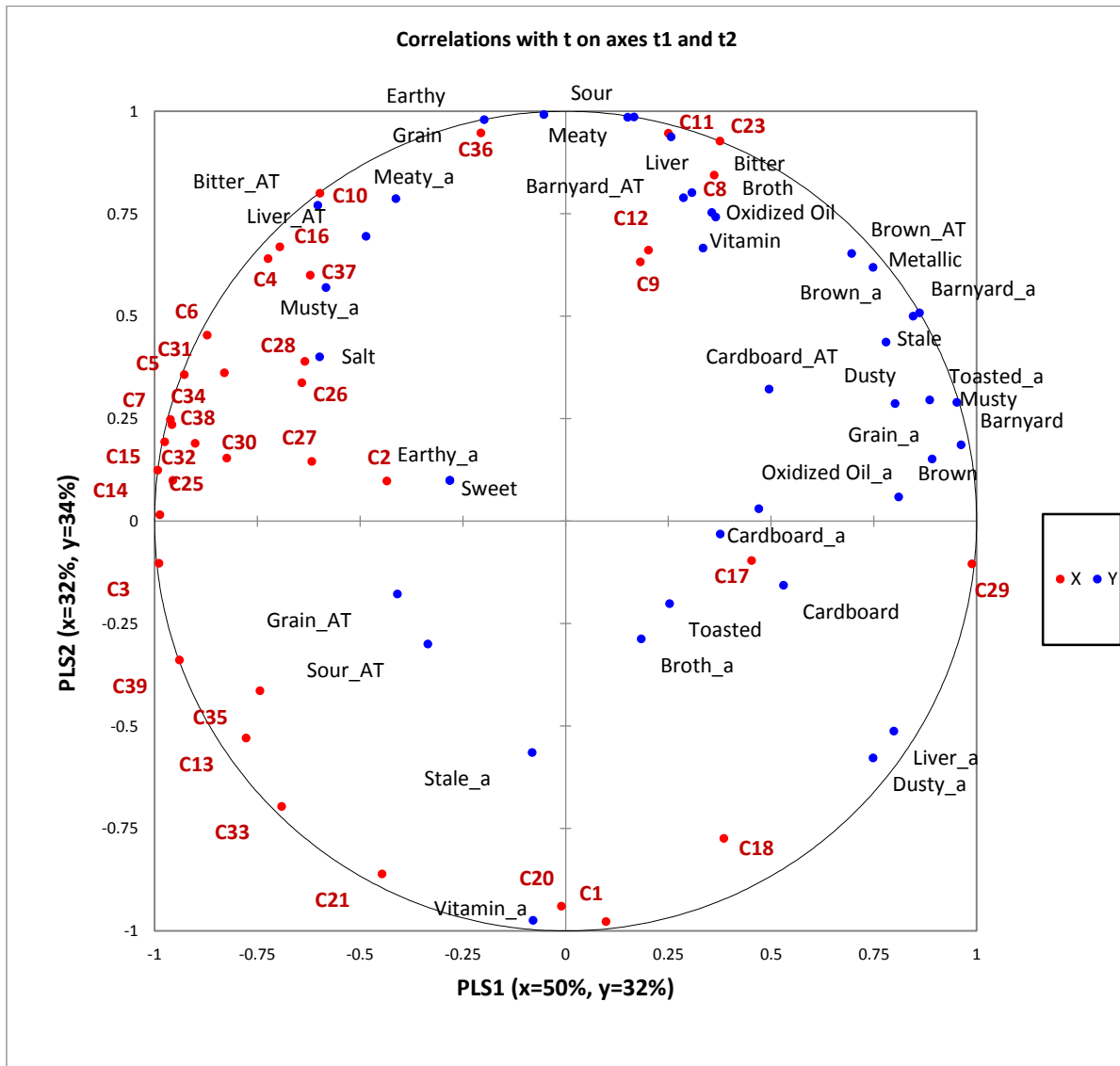


Figure 6-1. Partial Least Squares Regression factors 1 (x=50%, y=32%) and 2 (x=32%, y=34%). X-matrix = chromatographic analysis and Y-matrix = descriptive sensory data. Red dots (C): volatile compounds from the chromatographic analysis; Blue dots: sensory attributes from the descriptive sensory data (no suffix: flavor; a suffix: aroma; AT suffix: aftertaste).

The first 2 Partial Least Squares factors explained 66% of the Y-matrix (descriptive data) variability and 82% of the X-matrix (instrumental data) variability.

Several correlations were observed between sensory characteristics of the diets and volatile compounds detected. Compounds such as Z-10-Pentadecen-1-ol (C1), and 3,4-Dimethyldecane (C20) were related to vitamin aroma. Further, octanal (C10) was correlated to different attributes in the samples ($r = > 0.85$), such as meaty aroma, grain, and earthy. From the literature (The Pherobase) this compound has been associated with boiled meat, stewed, and rancid notes. For the same compound we also find association with aromatic notes such as green, citrus, and flower (The Pherobase). Bryant *et al.* (2011) described octanal to be also associated with citrus, fruity, floral, and fatty notes.

From the PLSR (Figure 6-1), benzeneacetaldehyde (C16), also known as phenyl acetaldehyde, was also associated with meaty aroma. However, from literature this compound has previously been associated with green, clover, honey, and cocoa notes (The Good Scents Company).

Hexanal (C8), has been described to be associated with green notes, and fat and tallow odors (The Pherobase) and to be related to lipid oxidation (Lampi *et al.*, 2015). Vazquez-Araujo *et al.* (2011) also described this compound to be associated with fatty, fruity, and green. In the PLSR map (Figure 6-2) it is possible to observe that hexanal was actually related to oxidized oil and broth flavor in the samples.

Musty aroma was correlated to volatiles such as 1-nonen-3-ol (C4) and (E,E)-3,5-octadien-2-one (C37). Different types of musty notes can be identified when performing descriptive analysis. These include musty dry, musty wet, and earthy/damp notes (Vazquez-Araujo *et al.*, 2011). The type of musty note that was described in the descriptive analysis portion of this study was defined

as ‘an aromatic that has a damp, earthy character similar to fresh mushrooms or raw potato’ (Di Donfrancesco *et al.*, 2012). The compound 1-nonen-3-ol has been actually described in literature as having earthy, mushroom, and green notes (The Pherobase). The compound (E,E)-3,5-octadien-2-one has been also associated in literature with mushroom, woody, fresh, and sweet notes.

Cinnamaldehyde (C17) was close to toasted and cardboard aromatics contributing to the flavor of the samples. In literature this volatile has been described as associated with notes such as cinnamon, spicy, and sweet aromatics (The Pherobase). Cinnamaldehyde, derived from the cinnamon tree, is the aldehyde responsible for flavor and aroma typical of cinnamon. Other than being used as food additive, cinnamaldehyde is also used as a fungicide in agriculture practices (Paranjape *et al.*, 2015). The cinnamaldehyde concentration was slightly lower in the CD samples compared to the others.

The PLSR map also shows that earthy aroma was associated with 4-Methyl-1-pentanol (C2), an alcohol that showed the highest concentration, although still low, in the CD sample (0.03 µg/kg). In literature this compound has been associated with nutty aroma notes (The Good Scent Company).

Conclusions

Thirty-seven aromatic compounds were tentatively identified and semi-quantified in three extruded dry dog food samples manufactured with different red sorghum fractions and a sample manufactured with a combination of wheat, corn, and rice as grain sources. The total concentration of volatile compounds was similar across the different diets, as well as the concentration of the

different volatile compounds groups. This result reflects the similar sensory profile indicated by descriptive sensory analysis. Aldehydes represented the major compound in the samples. The PLSR showed some association between sensory characteristics from the descriptive analysis and volatile compounds such as hexanal with oxidized oil and broth flavor, and octanal with meaty aroma. The total concentration of volatile detected in these samples was low compared with other studies that analyzed commercial dry dog food with a higher variety of ingredients and added flavors. Future studies using samples containing a higher variety in sensory characteristics will help to better understand relationships between sensory characteristics and volatile compounds in extruded dry dog food manufactured with different types of grains.

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Chapter 7 - Conclusions

A sensory lexicon specific for the dry dog food category was developed and more than seventy sensory terms were identified, defined, and referenced. Some terms were specific for a few type of foods while others such as grain and barnyard were recurrent in several products within the category. When using the sensory lexicon together with consumer hedonic data it was possible to understand the relationships between sensory properties of products and pet owners liking. Results indicated that appearance played a major role in driving consumer liking of dry pet food. The next objective of the research was to understand sensory qualities and acceptance of extruded dry dog food manufactured with different fractions of red sorghum through some of the developed concepts. Descriptive sensory analysis was performed and results indicated that aroma and flavor profile of the sorghum diets were not dissimilar to the ones of a control diet manufactured with rice, wheat, and corn, grains that are typically used by the pet food industry. Acceptance of pet owners was then assessed through a Central Location Test involving 105 consumers. Results indicated that the whole sorghum diet was to be the most liked sample by consumers, at the same level of the control diet. Results also indicated that the appearance was the driver of liking for consumers. When conducting a HUT to understand how the experimental diets would be accepted by pets compared in a home situation, no differences in acceptance for the diets were found. When analyzing the volatile compounds present in the four diets show that thirty-six compounds were identified with aldehydes being the most abundant volatiles group. Several relationships with sensory characteristics of samples were also found.

This study was intended to be a first step in trying to understand the best way to apply sensory analysis to pet food products to better understand relationship between sensory characteristics of

products, consumer acceptance and pet acceptance of products. The information reported in the study may provide useful guidance for future research and for sensory professionals and product developers working in the pet food industry.

**Appendix A - One-bowl Home Use Test Design (CD: Control Diet,
WSD: Whole Sorghum Diet, FD: Sorghum Flour Diet, MF: Mill-feed Diet) –**

Chapter 5

Dog	Diet 1 (Day 1-5)	Diet 2 (Day 6-10)	Diet 3 (Day 11-15)	Diet 4 (Day 16-20)
1	FD	CD	WSD	MF
2	CD	MF	FD	WSD
3	MF	WSD	CD	FD
4	WSD	FD	MF	CD
5	MF	WSD	FD	CD
6	WSD	CD	MF	FD
7	FD	MF	CD	WSD
8	CD	FD	WSD	MF
9	FD	WSD	CD	MF
10	WSD	MF	FD	CD
11	MF	CD	WSD	FD
12	CD	FD	MF	WSD
13	MF	CD	FD	WSD
14	WSD	FD	CD	MF
15	CD	WSD	MF	FD
16	FD	MF	WSD	CD
17	WSD	MF	CD	FD
18	MF	FD	WSD	CD
19	CD	WSD	FD	MF
20	FD	CD	MF	WSD
21	WSD	CD	FD	MF
22	FD	WSD	MF	CD
23	CD	MF	WSD	FD
24	MF	FD	CD	WSD
25	MF	WSD	CD	FD
26	WSD	FD	MF	CD
27	CD	MF	FD	WSD
28	FD	CD	WSD	MF
29	MF	WSD	FD	CD
30	FD	MF	CD	WSD

Appendix B - Example of consent form provided to participants –

Chapter 5

Informed Consent Statement

Sensory Analysis Center

Kansas State University

Ice Hall

Manhattan, KS 66502

1. I, (print) _____, agree my dog to participate in a feeding research study for the Sensory Analysis Center at Kansas State University.
2. I understand that the purpose of this research is to participate in a test where my dog will be fed four different dry dog food diets made with usual commercial ingredients meeting the nutritional requirements of my dog. All samples will be also tested to avoid any microbiological issue.
3. I understand that I will be participating in this research project for 20 days, during a period approximately comprised from September 9th to September 29th 2015 period.
4. For this test, I will receive \$120 when I complete all the days of testing.
5. I understand that if my dog is allergic to any product similar to those in the study formulations I should not participate in the study. Although there are no reasons why the dogs would get sick from the study, if any anomaly behavior or health problem sign may be showed during the study, the owner may withdraw from the research anytime. Compensation will not be provided if the study is not completed.
6. Kansas State University will not be responsible for any health problem not directly related to the study samples consumption that the dog may show during the study. A good dog health status is required and owners are asked in this regards before the beginning of the study
7. Owners may receive, through previous communication, a visit by researchers that may want to check that the provided instruction is followed.

8. I understand that the information I provide will be treated as research data and will in no way be associated with me for other than identification purposes, thereby assuring confidentiality of my performance and responses.
9. I understand that I do not have to participate in research, and that if I choose not to participate there will be no penalty, but I will not receive the \$120 compensation.
10. I understand that I may withdraw from this research at any time, but I will not receive compensation.
11. If I have any questions concerning this study, I understand that I may contact _____

12. If I have questions about my rights as a consumer or about the manner in which this research was conducted, I may contact _____

Signature

Date

Appendix C - Example of questionnaire with test instruction and behavior annotation – Chapter 5

Pet Feeding Study Instruction

FIRST PART

Day 1 September 5th (Diet 1)

- Pour the content of the bag “Day 1 – Sep 5” in the bowl that you normally use to feed your dog
- Start counting the time and leave the bowl available for 30 minutes. **Do not add water to the food.**
- Take note of any particular behavior the dog showed while you were pouring the food and before the dog started to eat (use space below).
- Take note of any particular behavior the dog showed while eating the food (use space below).
- After 30 minutes take the bowl away and put any leftover back in the original plastic bag.
- Take note of any particular behavior the dog showed right after finishing the food (use space below).
- If during the day (at a different time from the main meal) you usually give your dog a few additional treats you can do it (the same amount you usually give to the dog) during the test as well. We don't want the dog to be hungrier than usual during the test. **You don't have to feed the dog treats during the test if you usually don't do it.**
- If the dog eats all the food in the bowl please take note of the time it took for the dog to finish the food before the 30 minutes.

Behavior:

- Before starting to eat and while the food was poured in the bowl:

- While eating:

- At the end of the meal:

Time required to finish all the food in the bowl: _____ minutes

Additional notes:

Appendix D - Example of pet owners' survey – Chapter 5

Pal Test – Group 2 9/18/15 Participant name and code: _____

Diet 3

Would you feed this dog food to your dog if it was available on the market? Why?

What did you like about this dog food?

What did you dislike about this dog food?

Diet 4

Would you feed this dog food to your dog if it was available on the market? Why?

What did you like about this dog food?

What did you dislike about this dog food?

What are your thoughts about using grain sorghum in pet food?

Appendix E - Average Food Intake (%) for each diet and each dog –

Chapter 5

Each dog was served a different amount of food according to different daily metabolizable energy (ME) requirements, calculated as an average for laboratory kennel dogs or active pet dogs: $130 \cdot BW^{0.75}$ (National Research Council, 2006).

Dog ID	Gender	Size	Age	WSD intake (%)	MF intake (%)	FD intake (%)	CD intake (%)
1	M	40.8	6-10 years	49.3	49.6	55.3	59.1
2	F	4.4	2-5 years	14.0	14.4	15.0	14.0
3	M	5.0	6-10 years	31.5	11.8	16.7	28.4
4	F	10.0	2-5 years	41.1	49.5	48.4	55.4
5	M	11.8	6-10 years	39.2	34.0	n.d*	n.d*
6	F	8.2	6-10 years	58.6	33.6	25.0	49.1
7	M	5.0	2-5 years	10.3	8.9	6.4	16.7
8	M	22.7	6-10 years	64.5	68.0	72.5	79.5
9	M	4.5	2-5 years	30.9	48.6	29.2	46.1
10	F	16.3	2-5 years	70.7	90.4	69.3	70.4
11	M	27.2	2-5 years	55.5	49.5	40.3	50.5
12	F	16.8	2-5 years	67.8	73.2	51.6	47.8
13	F	6.8	6-10 years	82.6	95.3	60.6	83.0
14	M	4.5	2-5 years	94.3	100.0	97.4	85.6
15	F	27.2	6-10 years	56.1	75.9	56.9	46.2
16	M	20.4	6-10 years	47.0	60.0	33.6	57.7
17	F	20.4	2-5 years	28.6	44.7	44.0	43.4
18	F	22.7	6-10 years	44.9	65.6	51.4	37.6
19	M	18.1	2-5 years	61.3	67.0	91.8	66.2
20	M	22.7	6-10 years	39.5	47.1	41.4	43.3
21	F	25.2	6-10 years	65.8	60.4	54.8	49.9
22	F	15.9	2-5 years	67.0	54.7	53.8	58.8
23	F	5.0	6-10 years	48.9	50.0	44.4	53.0
24	F	15.9	6-10 years	65.0	84.0	70.9	57.7
25	F	18.1	6-10 years	53.4	52.2	57.0	48.3
26	M	24.9	6-10 years	78.8	37.8	75.4	59.6
27	F	9.1	2-5 years	100.0	100.0	100.0	100.0
28	M	19.1	6-10 years	48.0	55.4	100.0	100.0
29	M	20.4	2-5 years	100.0	100.0	69.8	100.0
30	F	11.8	2-5 years	53.8	43.0	35.5	65.9

*Note: One pet owner (dog ID 5) did not submit the leftover related to the last two diets