Impact of chicken proteins on canine preference as measured using sensory analysis

by

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B.S., Kansas State University, 2013

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2022

Approved by:

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Abstract

As the number of households with pets in the US continues to grow, so does the amount of pet food produced by the industry. It is important to understand the impact of feeding all these pets on our environment. Sustainability, defined from a pet food industry standpoint, is described as the ability to produce enough food for the growing pet population, while providing sufficient nutrition, and leaving the smallest environmental footprint possible. Among the rising number of pets within each household, dogs are the most popular. To provide a sustainable future, the shift to more sustainable ingredients must be discussed. Of the macronutrients needed to formulate a complete extruded canine food, protein is essential but is also the most expensive. Chicken has been shown to have the lowest impact from a carbon footprint standpoint, yet consumers have a negative perspective when it comes to any other form of chicken besides fresh chicken in their pet's food. However, chicken by-products and meals provide a quality source of dietary protein, vitamins, and minerals to create nutritionally complete dog food products. The first objective of this research was to formulate nutritionally adequate extruded dog foods utilizing four different chicken proteins as the sole animal protein source and to determine canine preference. Palatability was measured using the two-bowl test to determine if a preference between the different chicken proteins was recognized. All five experimental formulas were tested with a panel of 25 dogs for each palatability test and took place over 2 days. There was a preference ($p<0.05$) for the chicken by-product meal formula over the fresh chicken at 14%, fresh chicken at 25%, and dried chicken formulas. There was no preference when the chicken by-product meal formula was compared to the chicken meal formula. The second objective was to understand the sensory characteristics of the canine formulas through descriptive sensory analysis and consumer

acceptance. The descriptive sensory attributes for aroma and appearance between the experimental formulas were similar. Consumer data showed that the chicken by-product meal formula was the most liked in aroma, appearance and overall liking and was ranked the most preferred when measured blindly. Consumers were then provided the chicken protein ingredients for each sample and were asked to rank them again. Once consumers were made aware of the protein sources for each sample, their ranking changed to prefer the fresh chicken samples. The last objective was to determine volatile compounds present in each of the experimental formulas and to identify possible correlations to the sensory aroma attributes. The qualitative analysis tentatively identified thirteen compounds in each of the samples, consisting mostly of carboxylic acids and aldehydes. Partial Least Squares regression found some correlations between sensory aroma attributes and volatile compounds. Hexanal, heptanoic acid, 2-heptanone, and octanoic acid correlated closest to the *oxidized oil* aroma attribute while acetic acid, propanoic acid, and butanoic acid correlated closest to the *liver* aroma attribute. This research will provide insight on canine palatability of fresh chicken, dried chicken, chicken meal, and chicken by-product meal as the main animal protein source in extruded dog foods. It will also describe the sensory characteristics associated with the different chicken protein sources used, consumers' acceptance, and correlations to volatile compounds that may associate to sensory aroma attributes and possibly canine preference.

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Acknowledgements

I would like to thank my mentor, Dr. Nicholas Rozzi, for his guidance, patience, and support as I completed my graduate studies and research. I would also like to thank my major professor, Dr. Martin Talavera, and my committee members, Drs. Greg Aldrich and Sara Gragg for accommodating me as a distance graduate student and for providing their assistance along the way. Additionally, a big thank you to my colleagues at Hill's Pet Nutrition for their continuous support and words of encouragement. Finally, I want to thank my husband, Michael Shields, along with my family and friends for always believing in me and being so supportive in every way they could.

Chapter 1 - Literature Review

Pet Food Industry

Pets have a significant impact on people's lifestyles and will continue to as the humanization of pets becomes more mainstream each year. Consumers want pet food products that mimic food products they buy for themselves. Some examples of these food products include gluten-free, non-GMO, and high in protein. The 2021 American Pet Products Association (APPA) announced that over $103 billion was spent within the pet industry in 2020 (APPA, 2021). As the actual sales were broken down, it was no surprise that nearly half of this is represented by pet food sales. As the number of pets continues to rise, so does the need for more pet food. Sales growth is predicted to rise 5%, from $42 billion in 2020 to $44 billion in 2021, and will continue on a solid growth track for the next five years (APPA, 2021). The COVID-19 pandemic along with humanization and premiumization trends are driving this increase in sales (Sprinkle, 2021). Within the US society, 70% of households, about 90.5 million families, own a pet, with 69 million households owning dogs (APPA, 2021). The pet food industry also reflects this, as dog food accounts for nearly half of the total pet food sales for the US. Within the different forms of dog food, dry, wet, & semi-moist, dry food remains the dominant form representing approximately two thirds of sales (Mintel, 2021). Since the development of the extrusion process, expanded dry pet food has dominated the market every year, and represents the most economical production method used by the pet food industry.

According to the Association of American Feed Control Officials (AAFCO) the classification criterion for a "dry" pet food is to have a moisture level less than 20% (AAFCO, 2021). The most common and economical method used to produce dry pet food is via the extrusion process. Extrusion is defined by the process of forcing raw material to flow under mixing, heating, and shear through a die which can aid in the form/puff of the finished product (Rossen & Miller, 1973). **Figure 1.1** illustrates the production of extruded canine food products from start to finish. To begin the extrusion process, dry ingredients are measured then ground and mixed in the feeder/delivery system. The purpose for this is to ensure the dry ingredients have a consistent particle size before entering the preconditioner (Riaz, 2003). In the preconditioner, the ingredients are mixed, hydrated with water, and pre-cooked using steam, agitation, and through the rotation of paddles (Riaz, 2003) (Rokey et al., 2010). Thermal energy is added via the direct injection of steam and water (Riaz, 2000). Within the extruder, there can be multiple processing zones, as the material is transferred down the barrel via the screw. Each area can have a different purpose ranging from conveying, mixing, and kneading to compression of the material to generate pressure prior to leaving the die. As the mixture moves to the extruder, it progresses through each processing zone where it is worked into a dough like form (Rokey et al., 2010). The dough is cooked under high pressure and temperature as it is forced by the screw profile through the barrel of the extruder. Energy can be imparted to the material via mechanical or thermal energy. Mechanical energy, imparted by the rotating screw, increases the temperature within the barrel while thermal energy, typically imparted by steam, also assists with increasing temperature and moisture (Rokey et al., 2010). Once the cooked material has reached the end of the extruder, it is pushed through a die plate. The die plate offers restriction to material flow which causes the extruder to develop the required pressure and shear to shape the material (Rokey et al., 2010). Depending on the die plate, the mixture can be formed into different shapes while a rotating knife cuts the forms into the desired size (Riaz, 2000). The hot new kibble will then travel to the drying and cooling phase of the process. As the product leaves the die, the sudden drop in pressure will cause moisture to flash off, reducing both the temperature and moisture of the product. The kibble may also be run through a dryer which allows the finished moisture content to be 10% or less (Rokey et al., 2010). In the final step of the extrusion process, fat and/or palatant coatings can be added to aid in acceptance, palatability, and nutrition.

Domesticated dogs are part of the order *Carnivora*, however they are considered omnivorous regarding their nutrient metabolism because they need a healthy ratio of both protein and carbohydrates (Bradshaw, 2006) (Hewson-Hughes et al., 2013). Researchers have examined the eating habits of both wolves and coyotes and have compared them back to domesticated dogs. Wolves and coyotes are considered opportunistic scavengers and will hunt and eat what is available (Sheldon, 1992). Scavenging is not only limited to animal protein, but wolves and coyotes have been known to consume berries, persimmons, mushrooms, and melons (Sheldon, 1992). Typically, many free-ranging dogs consume diets high in carbohydrates and rarely hunt for protein sources as many people may assume (Hand & Lewis, 2010). More commonly, domesticated dogs “hunt” animal protein sources for sport, or fun, and not to meet their nutritional requirements. They are also opportunistic eaters and have developed anatomic and physiological characteristics that permit digestion and usage of a varied diet (Hand & Lewis, 2010). It has been speculated that these abilities have come about during the domestication process when dogs became adapted to consuming a diet closer to that of their human owners (Bosch et al., 2015).

Before the food product is made and fed, extensive work is done during the formulation process to ensure the final food product meets targeted nutritional requirements. This is important because, unlike humans, dogs consume their daily nutritional requirements from a single food. To meet these daily nutritional requirements, different ingredients are combined to deliver target levels of carbohydrates, proteins, fats, vitamins, and minerals. Dogs use carbohydrates for energy and in modern dry food formulas, these are supplied by grains such as corn, wheat, sorghum, barley, and rice. Dietary starch from these cereal grains is digested within the small intestine and converted into glucose, fat, and other compounds. Most of the dietary starch is easily digested and utilized within dog foods due to the gelatinization process of starches during extrusion (Gibson & Alavi, 2013). Gelatinization reduces the amount of resistant starch found in unprocessed grains. Indigestible complex carbohydrates, known as dietary fiber, help with gastric motility and benefit the microbiome in the dog's colon. This helps with bowel function and maintains a normal stool consistency (Hussein 2003). Proteins are used for energy and to sustain life and must be incorporated into food products either as animal or plant-based protein sources. Proteins are broken down during digestion into individual amino acids. Of the twenty-two amino acids dogs require, ten are considered essential because they are not synthesized in the body therefore these amino acids must be included in the food (Hussein 2003). The ten essential amino acids include: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine (Hussein 2003). The proteins used must supply enough of these essential amino acids to meet the dog's life stage nutritional requirements or be supplemented with crystalline amino acids. For adult dogs, AAFCO has set a minimum requirement of 18% crude protein (CP) on a dry matter basis (DMB) for maintenance (AAFCO 2021). Dietary fats, as well as specific fatty acids (omega-6 and omega-3), provide energy, are carriers of fat-soluble vitamins, help improve palatability, and provide desirable texture to the food. Omega-6 (linoleic acid) and Omega-3 (alpha-linolenic acid), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential fatty acids that are utilized in cell membranes and in hormone production (Hussein 2003). AAFCO has set a minimum of 5.5% fat on a DMB for dogs for maintenance (AAFCO 2021). Vitamins and minerals are typically added into food products as part of premixes that supplement what the other ingredients lack.

Sustainability of the Pet Food Industry

Sustainability, defined from a pet food industry standpoint, is described as the ability to produce enough food for the growing pet population, to provide sufficient nutrition, while leaving the smallest footprint possible (Meeker & Meisinger, 2015). It is important to understand the impact of increasing pet food demand and how it will affect the raw material supply and the footprint it leaves. The environmental sustainability of a food industry can be measured by several factors: land use, waste management, water use, greenhouse gas emissions, or impact to biological diversity (Wallén et al., 2004). The environmental impact of a food industry can be quantified through analysis of all stages of a product's life including the material inputs (energy and natural sources), material outputs (waste and emissions), and their associated costs. This process is also known as the life-cycle assessment (LCA) (Mogensen et al., 2009).

Acuff et al. (2021) discusses how the pet food product's life cycle can be defined by two additional attributes: diet selection and nutritional composition. Diet selection dictates the intended species, life stage, food format, and inclusion or exclusion of specific ingredients. Nutritional composition determines the level of raw materials needed to achieve the desired nutrient levels. Both attributes have a direct impact on the resources required to construct a final pet food product (Acuff et al., 2021). To provide a sustainable future, the shift towards more sustainable ingredients must be considered. Protein is both the most expensive macronutrient and one of the most resource intensive but is an essential part of a dog's nutritional requirements. As mentioned previously, adult dogs (at maintenance) require a minimum of 18% protein in their diets (AAFCO, 2021). However, consumer market trends drive for more protein within their dog's food, with some protein levels exceeding 30%. It is uncertain if protein levels far in excess of the dogs' requirements are beneficial to their health (Acuff et al., 2021). What is certain is the strain it is placing on the global demand for more protein sources across not only the pet food industry but also the human food industry (Okin, 2017) (Nijdam et al., 2012).

Most of the protein used for both the human and pet food industries comes from animal sources. There is a continuous upward trend for both quality and quantity of the type of meats or animal proteins used in pet food diets (Okin, 2017). Despite the competition with the human food industry to utilize the same animal proteins, the pet food industry has an advantage in that it can utilize various animal by-products the human food industry cannot.

Animal By-Products in Pet Food

According to AAFCO, the definition of by-products is the "clean secondary products produced in addition to the principal product" (AAFCO 2021). Many consumers presume that these secondary products provide little benefit and often avoid purchasing products with these ingredients on the label. By-products are a big topic that is often talked about in a negative light, especially from the pet owner's perspective. To help alleviate the negativity that the by-product term has on consumers, Acuff et al. (2021) proposed the term by-product be transitioned to "coproduct" in their most recent publication. However, consumers may not realize that these secondary animal protein sources provide good-quality protein and higher levels of trace minerals, (iron, zinc, calcium, and copper) when compared to muscle tissue sources (Biel et al., 2019) (Murray et al., 1997). Murray et al. (1997) concluded that animal by-products are good sources of digestible nutrients for dogs and should be used in pet food formulations. To put this into perspective, the average carcass yield is between 50% and 74% of the live animal weight for red meat, pork, and poultry products in the US, leaving a significant portion of secondary material that cannot be used for the human food industry and will need to be disposed of (Knight, 2020). When pet food producers utilize these animal by-products, it helps lessen the environmental effects associated with waste disposal, providing a more sustainable future (Gooding & Meeker, 2016). If the current trends continue, there will not be enough animal protein to support both the human and pet food industries. The process for growing livestock for protein requires fossil fuel energy inputs (fertilizers, agricultural machinery, fuel, irrigation, and pesticides) for grain, vegetable, and forage production (Swanson et al., 2013). As technology and innovation continues to advance along with livestock production efficiency, the environmental footprint will be reduced and sustainability of pet foods and pet ownership will improve (Putman et al., 2017).

Chicken By-Product Meal

Of the animal protein sources, chicken has been shown to have the lowest environmental impact, and the poultry industry continues to make improvements. Some examples of chicken by-products include cartilage, bone, and organ meat, and when rendered properly provide quality protein and trace minerals to canine foods when incorporated (AAFCO 2021). Acuff et al. (2021) compiled a data table to organize the average global warming potential estimates for select protein ingredients typically used in US pet foods. Of the protein sources recorded, the life cycle analysis (LCA) study for chicken production within the US concluded that chicken has a carbon footprint of 1.99 kg of CO_2 per 1 kg of live weight (Putman et al., 2017) while a similar LCA study in Portugal concluded that poultry by-product meal measures 0.73 kg of CO_2 per 1 kg of live weight (Campos et al., 2020) (Acuff et al., 2021). Results of additional LCA studies on chicken and poultry production are summarized in **Table 1.1**.

Research is limited regarding studies analyzing canine preference between foods formulated with chicken by-product meal versus other chicken proteins, fresh chicken, dried chicken, and chicken meal. Of the few studies available, Murray et al. (1998) compared the digestibility of nutrients of extruded dog foods formulated with fresh poultry versus poultry by-product meal as the main protein source. They concluded that fresh poultry and poultry by-products in extruded dog foods are reliable sources of highly digestible nutrients in regard to total digestibility (Murray et al., 1998). Towards the end of their study, it was questioned if the level of ingredients had a positive influence on texture or palatability. It is uncertain which chicken protein is more preferred or if there is a preference.

Sensory Analysis

Sensory analysis is defined as a scientific method used to evoke, measure, analyze, and interpret those responses to stimuli as perceived through a combination of the senses of sight, smell, touch, taste, and hearing (Stone & Sidel, 2004). By utilizing sensory analysis techniques, scientists can measure human responses to products (food and nonfood) while minimizing bias effects of brand identity and other information that may influence consumer perceptions (Lawless & Heymann, 2010). There are three main classes of sensory techniques: discrimination, descriptive, and affective. Each technique provides unique insights and thus selection of the method should be based on the question being asked. Discrimination tests are used to determine whether there is a perceptible difference between two products. Discrimination tests include the paired comparison test, the triangle test, and the duo-trio test (Lawless & Heymann, 2010).

Descriptive tests are used to describe and quantify the perceived intensities of sensory characteristics of a product while using five to twelve highly trained human panelists. There are several descriptive methods, such as Flavor Profile, Quantitative Descriptive Analysis (QDA), and Spectrum that can be used (Lawless & Heymann, 2010). Each method is unique in what it can quantify, and the desired outcome determines which method should be used. These methods can also be commonly modified based on the testing needs and available resources (Lawless & Heymann, 2010). Affective tests, also referred to as hedonic tests, are used to determine how well products are liked or disliked by consumers who are familiar with the products being tested but have no sensory analysis training. Since these hedonic tests use untrained panelists, a higher number of participants, typically ≥ 100, are used. Hedonic questionnaires ask panelists, using a 9-point scale, to quantify the degree of liking or disliking of the product or products they are analyzing (Lawless & Heymann, 2010).

Sensory Analysis in Pet Food

Sensory analysis is utilized in the pet food industry to provide information to scientists on why pets find certain products palatable or unpalatable. Pets, much like infants, do not use verbal communication to express what they like or dislike. Instead, they communicate with people through body language and vocal cues. Pets cannot describe the reasons for their choices, therefore pet parents and scientists must rely on behavioral cues. From these cues, scientists can develop a better understanding from the response of the pet to food products being compared. The pet food industry is similar to the human infant nutrition industry because the actual consumer of the product does make choices between the different options nor do any purchasing. With product acceptance being determined by two different participants, not only does the food have to be accepted by the pet, but it also must be accepted by the pet parent, underscoring the importance of sensory studies. When considering pet food sensory analysis research, Koppel (2017) (Koppel, 2017) explained the process by breaking it down into three elements: the pet owner (who buys the food), the pet (animal eating the food), and the food (physical characteristics of the food itself). For a pet food product to be successful, all three elements must work together.

There are limitations with using human panelists to perceive sensory stimuli of pet food products and to describe them from a pets' perspective. Previous studies have measured canine food preference through odor, taste, and texture. A study conducted by Houpt et al. (1978) demonstrated the importance of odor and how it correlated to dogs making a food selection within a test. The results of this study indicated that the food that had a meaty aroma was more preferred, however, the odor must be paired with the taste of meat for the preference to be sustained (Houpt et al., 1978). A dog's olfactory sense has been recorded to be as much as 10,000-100,000 times greater than that of the average human (Walker et al., 2006). However, humans' taste perception has developed more than a dog's (Koppel, 2014) (Neufield, 2012). In addition, Griffin (2003) determined the importance of evaluating texture of dog food products when testing palatability and acceptance. Although perception may be different between dogs and humans, sensory analysis can provide an increase in understanding the "why" dogs prefer some products over others.

To date, pet food sensory analysis data has been collected by companies during their product development process and kept confidential. However, each year more research is being published that contains pet food descriptive sensory analysis and pet preference testing. Sensory analysis allows for a better understanding of how varying characteristics in dog food impact the pet owner and pet acceptance of the products.

Descriptive Sensory Analysis in Pet Food

Human sensory analysis of pet food can give a better understanding of the sensory properties present and provides insight on why pets make certain food choices. Koppel, (2014) utilized descriptive sensory analysis through the evaluation of appearance, aroma, texture, and flavor characteristics of pet foods. This technique is most useful for measuring the intensities of sensory attributes of a product or multiple products. It can also be useful, when used in conjunction with acceptance testing, to determine which attributes are important to acceptance. Koppel, (2014) also describes how descriptive sensory analysis information can be used in combination with other sources of information about the product, such as ingredients, processing, and palatability data. This combination of data can help explain the effects of ingredients and/or processing on flavor and/or texture. The information gained from descriptive analysis can be used for many purposes such as marketing research, developing new products, or for quality control (Koppel, 2014).

In (2012), Di Donfrancesco et al. used descriptive sensory analysis to develop an initial lexicon to describe flavor, aroma, texture, and appearance of twenty-one extruded dog food samples. More than 70 sensory attributes were identified and defined. This development provides a baseline to describe these characteristics (appearance, texture, aroma, and flavor) characteristics of products for future studies. Di Donfrancesco et al., (2014) conducted a dry dog food study with current in market dog food products using the lexicon developed to determine attributes utilizing descriptive analysis to isolate drivers of consumers' liking. Within the eight dog food samples analyzed, descriptive sensory analysis provided attributes on a wide range of shapes, sizes, aromas, and flavors. The kibble sample consumers liked the most in this study consisted of aroma attributes such as *smoky, broth, grain, meaty, toasted*, and *musty/dusty* (Di Donfrancesco et al., 2014).

Koppel et al. (2014) used descriptive sensory analysis to identify appearance, aroma, flavor, and texture properties between different levels of meat (fresh vs meal) inclusion, processing methods (baked versus extruded), and thermal to mechanical energy ratio used during extrusion. The results of this study concluded that processing did have an impact on the products sensory characteristics, more specifically texture. Baked samples were lighter in color and resulted in lower levels of attributes associated with rancidity. Extruded samples resulted in attributes such as friable, hard, and crisp. The raw ingredients (fresh meat versus meat meals) did not consistently impact the overall product's sensory characteristics; however, the fresh meat inclusion samples affected the attributes bitterness, fishiness, and cohesiveness of mass regardless of baked versus extruded (Koppel et al., 2014).

Consumer Studies in Pet Food

Utilizing consumer input on pet food products can provide useful information when studying the acceptability and preference of products to the purchaser. Koppel (2014) explains how consumer studies provide additional understanding of how pet parents select certain food products for their pets. Consumer studies collect information from pet owners, typically through questionnaires. This information provides an understanding of consumer acceptance, and any issues products may have either during development or in the market. Typical repurchase of a product depends on the experience that relates back to the sensory properties as perceived by both the pet parent and pet (Koppel, 2014).

A few studies have investigated consumer acceptability of pet foods. Di Donfrancesco et al. (2014) conducted a consumer study to determine and identify different acceptable characteristics of dry dog food products within the US market. Results indicated that the appearance of food products had more influence than the aroma regarding overall liking. Chanadang et al. (2016) conducted a consumer acceptance study to determine the effect of oxidation within rendered protein meal formulas with and without antioxidants. The study investigated how various levels of oxidation would affect the sensory properties of pet food. The study found that an increase in aroma characteristics of samples without antioxidants added had a negative impact on consumer liking. Therefore, if the pet food exhibited any off aromas, then the pet owner labeled the pet food as unacceptable and would not offer it to their pet (Chanadang et al., 2016). Gomez Baquero et al. (2018) conducted a case study in Poland to analyze the acceptability of dry dog food visual characteristics by consumers. This study only asked consumers about the appearance of the food. The study found that consumer acceptability was positively influenced by the number of different kibbles present, color(s), shape(s), and size(s) of the product (Gomez Baquero et al., 2018). While several studies have looked at consumer acceptability of pet foods, there is still much to research in order for the pet food industry to better understand how product characteristics influence pet and pet owners’ decision making.

Palatability Testing & Canine Preference

Palatability, as defined by the National Research Council (NRC), is the “physical and chemical properties of the food which are associated with promoting or suppressing feeding behavior during the pre-absorptive or immediate post absorptive period" (NRC, 2006). It is the initial response to the food product before any metabolic effects of food intake can occur (Aldrich & Koppel, 2015). In the pet food industry, palatability is often referred to as the measure of food preference through sensory characteristics of taste, aroma, and mouthfeel (Griffin, 2003). According to Tobie et al. (2015), the hedonic properties of the food product can be defined not only by an attractive taste, smell, and mouthfeel but also through nutritional and physiologic post-ingestion effects. Of the five basic senses, dogs predominantly use taste and smell in their selection of food. Taste can be described as the sensation that is a response from stimuli on the taste buds. Flavor, a part of taste, is associated with combining the perception of taste and smell through the laryngeal cavities during consumption (Kitchell, 1978) (Bradshaw, 2006). Smell (aroma) is associated with the volatile components of food and its effects within the nasal cavity. Within a dog's nasal cavity is the olfactory epithelium, which contains olfactory receptors, and the vomeronasal organ, Jacobson's organ. This is where odor molecules interact to stimulate an olfactory sensation and where chemicals are detected (Hand et al., 2010). A dog's olfactory sense has been recorded to be as much as 10,000-100,000 times greater than that of the average human (Walker et al., 2006). Food products are a major component within pet care and, along with being nutritionally complete, must also appeal to the pet's senses.

Bradshaw, (1991) concluded that dogs respond to a range of taste sensations such as a tolerance to bitterness, response to sour and umami, exhibit sensitivity to monosaccharides and disaccharides (fructose and sucrose) and have a lower sensitivity to sodium chloride. According to Houpt & Smith, (1981) dogs prefer meat over grain, and among the animal proteins, favor: "beef>pork>chicken>lamb>horse." Additionally, they found that "dogs prefer sugar over no sugar, and canned/semi moist food over dry kibble." Houpt & Smith, (1981) indicated that food odor preferences should be paired with taste for continuous acceptance, however more work is needed in this area to determine the impact of other taste and flavor sensations.

Pet food product developers are faced with a unique challenge, as not only does the food have to be nutritionally balanced, but it must also have a desirable odor, flavor, and texture to ensure adequate consumption. There are multiple methods to measure canine palatability such as the single-bowl, two-bowl, operant conditioning, and exploratory behavior tests (Tobie et al., 2015). Food consumption is the primary indicator used in the industry to evaluate the acceptability of products (Aldrich & Koppel, 2015). The two most common ways to evaluate canine palatability are the single-bowl and the two-bowl testing methods. In a single-bowl test, a dog is presented with only one bowl for a set number of days, per the study, and is fixed for time and caloric intake. The purpose of this method is to measure the acceptability of the food. It is also used to identify products that are unacceptable due to an off-flavor, aroma, or texture (Aldrich & Koppel, 2015). In the two-bowl test, two foods are presented simultaneously. Dogs are selected for a palatability panel based on their ability to make a choice during training. Given a fixed amount of time and caloric intake, the dog will make a choice between food A and food B. Both bowls are weighed during or after the test, and the bowl with the most amount consumed is recorded as preferred. This same test is presented again the next day to the same dog, but typically with the bowls switched to avoid a side bias. Consumption of each food is recorded, and preference results are measured using the bias-free method based on the intake ratio (Griffin, 2003):

$$Intake\ Ratio\ =\ \frac{Food\ A\ Consumed}{(Total\ Consumption\ Food\ A\ +\ Total\ Consumption\ Food\ B)}$$

By calculating a ratio for each animal on each day, and then averaging those ratios, each day for each animal carries equal weight in the overall mean (Griffin 2003). The result for the group can then be expressed as the percentage of animals preferring Food A, Food B or having no preference. This ratio can also be summarized for a group of animals by reporting the average intake ratio (Hand et al., 2010). To further test palatability results, a Student's t-test is conducted using a 95% confidence interval, where the p-value is the final judgement in the categorization of the test (Basque et al., 2019) (Vondran, 2013). A p-value of less than 0.05 expresses that the intake ratio of the test is either greater than or less than 0.50, with a 95% statistical certainty. The verdict of the test is then determined by evaluating the intake ratio of the test food to determine how to interpret the results of the palatability test.

Many studies use the two-bowl test versus the single-bowl test method when a choice between two foods must be made simultaneously. The single-bowl test determines if the food is acceptable or not acceptable by the pet. However, neither test determines "what" about the food was preferred (higher intake food) or was not preferred (lower intake food). As dogs continually analyze food characteristics during consumption, positive attributes entice dogs to consume more. Palatability may also reflect a nutrient-conditioned preference and foods that provide nutritional benefits are generally liked more (Griffin 2003). Palatability is very complex and requires more research to develop a better understanding of canine preference.

These methods can be measured using trained dogs in a center location test (CLT) or untrained dogs in a home use test (HUT) (Tobie et al., 2015). Each option provides advantages and constraints when assessing food products. A trained dog panel, in a CLT, tends to be more accurate due to the amount of training needed and infrastructure available to provide reliable data at the end of each feeding. A panel of 25-30 trained dogs is typically necessary to build strong statistical data for palatability testing (Tobie et al., 2015). For a HUT an untrained dog panel provides the opportunity for additional information from the owner regarding the dog's observed behavior when interacting with the food product. Constraints to using HUT versus CLT revolve around the amount of work it takes to set up the study. This additional work is placed on the pet owner: following the feeding protocol, closely monitoring additional foods consumed, and providing clear observation notes. For these reasons, it is suggested that a panel of 100 or more untrained dogs be used, as it is necessary to ensure the reliability of data gathered from the test (Tobie et al., 2015). The comparison between trained dog panels and in-home untrained dog panels often reveals many differences, however both panels provide unique contributions to the development of new food products.

Even with the different methods to measure canine preference, it is still unclear what dogs like and if the effect of odor influences a dog's food preference. Basque et al. (2019) used a complementary approach of a two-bowl test and a dual-port olfactometer to measure odor preference. From their results they concluded that the trained panel of dogs could discriminate between products by olfaction and the dogs could express the same food preference for various products, even with different odors (Basque et al., 2019).

Canine Eating Behavior

Aside from the dogs' sensory inputs of taste and smell, other factors (breed, life stage, and lifestyle) can influence canine preference. These include behavior, environmental effects, learned food aversions, neophobia, social conditions, and other early age and recent experiences. Examples of behavioral factors that impact choice are described in multiple studies. Dalal & Hall (2019) aimed to identify whether a basic measure of behavioral persistence, the maintenance of a behavior in the presence of behavioral disruptors, was associated with the dogs' performance on an odor discrimination learning task. They found that dogs with a higher persistence performed worse and had more difficulties in learning more complex odor discrimination tasks when compared to dogs that took longer to train but maintained persistence (Dalal & Hall, 2019). Additionally, Dale et al. (2017) researched the influence of social relationships, both as a pair and in a pack, on food tolerance regarding sharing of a food source in both wolves and dogs. For both species, as a pair, wolves and dogs showed more of a strong bond together and shared amongst the food source. In a pack, both species had dominant individuals and subordinate individuals. The observed difference between the species was in the dog pack in which the subordinates stayed clear of the food source while the dominates in the wolf pack stayed near the food source and continued to feed from it (Dale et al., 2017). Dogs can also experience what is known as the "novelty effect." This means they would rather eat new food when they have been eating the same food for an extended period of time (Hand & Lewis, 2010). Neophobia, the fear of something new, can occur when a dog is unfamiliar with certain food products and becomes too timid to eat/try new food. A research study on this topic in 2006 found neophobic responses may reduce exposure to danger, constrain explorative behavior, and limit opportunities for learning and innovating (Stöwe, Bugnyar, Heinrich, et al., 2006) (Stöwe, Bugnyar, Loretto, et al., 2006). The most easily recognized behavior is food aversion. Food aversion typically leads to persistent refusal of the food that was consumed while the dog was sick (Bradshaw, 2006). With these varying factors of canine behavior, determining canine food preferences can be challenging.

Volatile Aromatic Composition of dry dog food

Instrumental analysis, such as gas chromatography (GC), paired with mass spectrometry (MS), is best suited for analysis of molecular compounds and can be useful for analyzing the chemical compounds found in the extruded dog foods. Chromatography is a separation method based on the partitioning of a solute between a mobile phase and a stationary phase (Koppel et al., 2013) (Qian et al., 2017). GC-MS is used mostly for the analysis of thermally stable volatile compounds. This method has a wide range of detectors that can be used to provide sensitivity or selectivity in analysis (Qian et al., 2017). For analysis of pet food, ingredients and volatile compounds, GC-MS paired with a fused-silica fiber is most common.

The method of solid phase microextraction (SPME) paired with GC-MS has been used to determine the aromatic compounds found in the headspace of extruded dog food samples in multiple studies (Koppel et al., 2013) (Donfrancesco & Koppel, 2017) (Yin et al., 2020). Additionally, there are multiple studies available that investigate the volatile aromatic composition of raw ingredients (grains and meats) that are commonly utilized when formulating pet food products (Wettasinghe et al., 2000) (Buśko et al., 2010) (Koppel et al., 2014) (M. Chen et al., 2017). Dry samples can be analyzed from a ground or whole state with and without the addition of water. These techniques help with the transition of polar and non-polar aromatic compounds onto the fused-silica SPME fiber to then be transferred and analyzed by the chromatograph (Meeker & Meisinger, 2015) (Pawliszyn et al., 1995). In 2013, a study conducted by Koppel et al. identified 54 aromatic compounds within the headspace of fourteen dry dog food samples using SPME GC-MS. Majority of the volatiles identified in this study were aldehydes and ketones. This study also concluded that dry dog food products have complex odor characteristics due to the many different ingredients needed to formulate a complete diet (Koppel et al., 2013). Along with SPME GC-MS analysis, the addition of an olfactometry port (GC-O) may be used to further analyze the aromatic compounds present in pet foods. Odor-active compounds that have been separated by the GC can be “sniffed” by highly trained panelists through a port connected to the instrument.

A few studies measuring the volatile compounds in fresh chicken and chicken by-products separately are available (Wettasinghe et al., 2000) (Wettasinghe et al., 2001), however, there is a lack of published research that measures the impact of different chicken protein sources utilized in dry dog foods. It is uncertain if any of these volatile compounds found in fresh chicken or chicken by-products correlate to canine preference. A deeper understanding of the correlation (or lack thereof) between volatile compounds found in chicken by-products to sensory analysis and canine preference may prove to be beneficial to pet food manufacturers. The use of these instruments provides a better understanding of the volatile compounds possibly associated with the sensory analysis attributes and canine palatability.

Summary/Research Objective

As the pet food industry continues to grow, the drive for pet food manufacturers to continuously improve the sustainability of their businesses is persistent. This begins with formulation, ingredient selection and utilizing more sustainable ingredients. Previous research states that chicken by-product meal provides quality protein and trace minerals to extruded canine formulas, and the digestibility of chicken by-product meal versus fresh chicken is similar. Consumer’s acceptance of by-products is lacking and there is not much research regarding canine preference for chicken proteins in extruded food products.

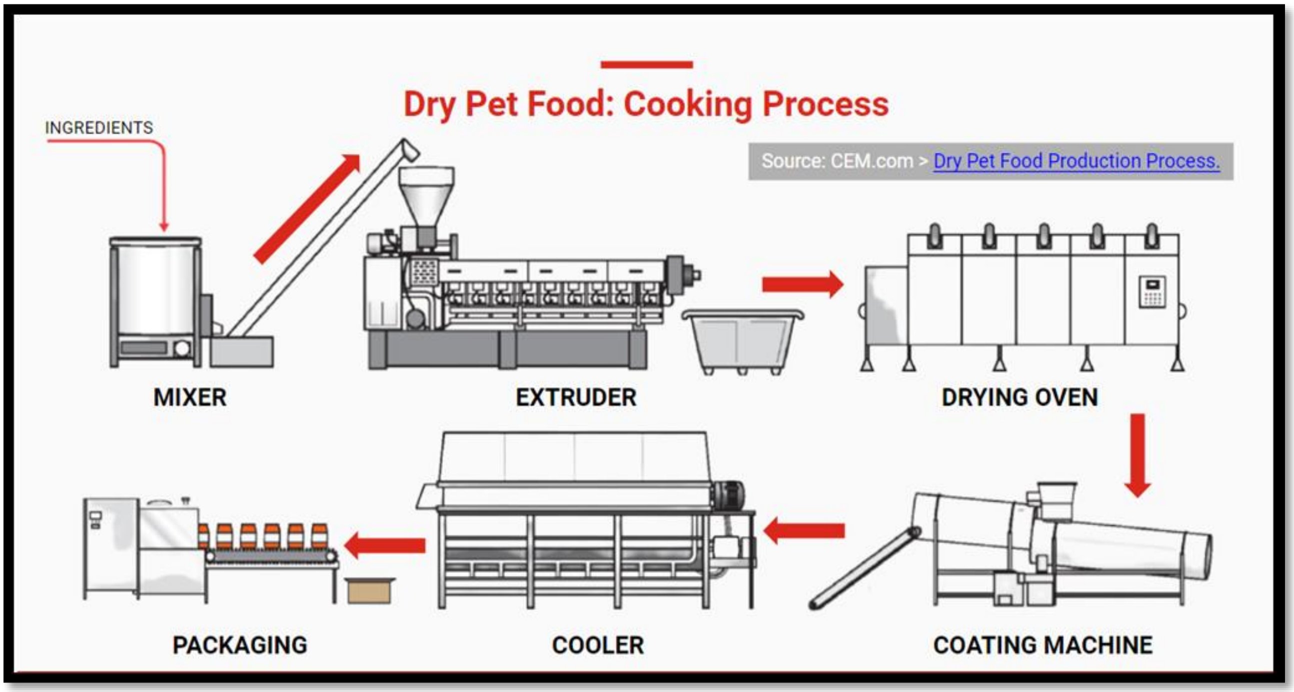
The first objective of this study was to produce formulas utilizing four different chicken protein sources, and to evaluate the effects of those protein sources on palatability in dogs. The second objective was to utilize descriptive sensory analysis to determine aroma and appearance sensory attributes for each product. A consumer sensory study was used to determine acceptability and preference between each product. Lastly, SPME GC-MS was utilized to identify volatile compounds of each dry dog food sample to determine correlations between compounds and sensory aroma attributes.

Table 1.1 Average global warming potential estimates of chicken ingredients with application in US pet foods

Ingredient	LCA Study Location	Carbon Footprint (kg CO_2 Eq/kg Functional Unit	Reference
Chicken	Sweden	1.9	(Cederberg, 2009)
Chicken	Switzerland	2.4	(Katajajuuri, 2007)
Chicken	Canada	1.00	(Vergé et al., 2009)
Chicken	Portugal	1.62	(González-García et al., 2014)
Chicken	USA	1.99	(Putman et al., 2017)
Poultry by-product meal	Portugal	0.73	(Campos et al., 2020)
Poultry fat	Portugal	0.67	(Campos et al., 2020)
Hydrolyzed feather meal	Portugal	0.6	(Campos et al., 2020)

Functional unit = 1 kg live weight

Figure 1.1 Dry pet food production process



(*Dry Pet Food Production Process*, n.d.) https://cem.com/en/dry-pet-food-production-process

Chapter 2 - Effects of different chicken protein sources on palatability in dry dog food

ABSTRACT

As the demand for dog food production continues to rise, along with the number of dogs per household in the US, sustainable ingredients must be considered for the pet food industry. Consumers have a negative perspective when it comes to animal by-products in their pet's food. However, animal by-products contribute a quality source of dietary protein, vitamins, and minerals which contribute to nutritionally complete pet foods. Along with being nutritionally complete, foods must also appeal to the dog while eating. To better understand canine preference for different chicken proteins, the objective of this study was to produce formulas utilizing four different chicken protein sources, and to evaluate the effects of those protein sources on palatability in dogs. Five treatments were produced, through extrusion, using fresh chicken at two different inclusion levels: (FC14 and FC25), dried chicken (DC), chicken meal (CM), and chicken by-product meal (CBPM). Four trained panels of twenty-five adult dogs, made up of majority Beagles, randomly divided by sex, were used for palatability testing using the two-bowl test. Based on the palatability results from this study, the CBPM treatment was preferred ($p<0.05$) over the FC14, FC25, and DC treatment. The CBPM treatment was at parity to the CM treatment ($p>0.05$). It can be concluded that the CBPM treatment was palatable to dogs.

INTRODUCTION

As the number of dogs per household continues to rise, so does the demand for more dog food. Sales are predicted to rise 5% from 2020 to 2021 and are expected to continue to increase for the next five years (APPA, 2021). The COVID-19 pandemic, along with humanization and premiumization trends, are driving this increase in sales (Sprinkle, 2021). Many pet owners consider their pets as members of the family. This humanization trend by consumers has shifted pet owners' preferences for certain food ingredients to be more “human-grade” and less processed (Meineri et al., 2021). Chicken is commonly used within the pet food industry. With the addition of more fresh chicken in pet food formulas, this puts a strain on the global demand for chicken protein sources and may begin to compete with the human food industry (Okin, 2017) (Nijdam et al., 2012). As the demand for dog food increases, raw material supplies are increasingly challenged, and could have an ecological impact on the current food systems.

As pet food production continues to rise, the utilization of more sustainable ingredients must be considered, especially in regard to protein sources. Chicken has the lowest environmental impact when compared to other animal protein sources, and chicken by-products can be used within the pet food industry (Knight, 2020). Unfortunately, the pet owners’ uncertainty regarding by-products tends to lead to a negative perception and are often labeled as poor-quality ingredients (Laflamme et al., 2014). By-products, when rendered properly, provide quality protein and trace minerals to canine foods (Donadelli et al., 2019). Protein quality is determined by measuring how well the amino acid profile and bioavailability of those amino acids match the requirements of the dog’s life stage (Donadelli et al., 2019). By utilizing by-products, the environmental effects of organic waste disposal are reduced (Gooding & Meeker, 2016). While previous studies have measured digestibility between fresh chicken and chicken by-products used in formulas, canine preference between the two ingredients, or other chicken has not been measured (Murray et al., 1997).

Aside from the food product being nutritionally balanced, the product must also be palatable and accepted by the dog. Unlike humans, dogs consume their daily nutritional requirements from a single food. Therefore, it is imperative that the foods are palatable enough for a pet to consume an adequate volume to meet their nutritional requirements. Food consumption is the primary indicator used in the pet food industry to evaluate the palatability of products (Aldrich & Koppel, 2015). A complete profile of finished food products, including texture, flavor, and aroma, are needed to help decipher canine preference. Previous studies have measured canine food preference through odor, taste, and texture: (Griffin, 2003; Houpt et al., 1978; Koppel et al., 2014; Neufield, 2012; Samant et al., 2021; Walker et al., 2006). Food preference of dogs for ingredient effects has been previously researched: soybean inclusion (Félix et al., 2012), fiber inclusion (Koppel et al., 2015), vegetable ingredient-based formulas (Callon et al., 2017), and raw versus cooked meats (Tsai, 2019). Wei-Lun Tsai (2019) measured canine preference for raw meat sources versus cooked meat sources. The limitation with this study is that dogs are typically not fed raw material and the material changes once processed/extruded. Still, a limited number of studies can be found with a focus on food preference of dogs for ingredients themselves. A deeper understanding of the preference of protein sources by dogs may further support the use of by-products in pet foods, and further convince consumers of their benefits. Therefore, it was the objective of this study to produce formulas utilizing four different chicken protein sources, and to evaluate the effects of those protein sources on canine palatability.

MATERIALS & METHODS

Formulation

Five experimental formulas were formulated using the computer software, Concept 5 (Creative Formulation Concepts, LLC Annapolis, MD, USA) with four different chicken protein sources; fresh chicken (FC; BHJ USA Inc. Saint Joseph, MO, USA), dried chicken (DC; Protein Solutions, LLC. Joplin, MO, USA), chicken meal (CM; Tyson Foods. Clarksville, AR, USA), and chicken by-product meal (CBPM; Darling International Inc. Nishnabotna, MO, USA). The CM, DC, and CBPM formulas were formulated at an ingredient inclusion level of 14%. The fresh chicken formulas were formulated at two separate inclusion rates: a 14% fresh chicken (FC14) inclusion rate and a 25% fresh chicken (FC25) inclusion rate. The FC14 formula was formulated to match the volume (14%) of the chicken ingredients in the CM, DC, and CBPM formulas, while the FC25 formula was formulated to have similar crude protein levels as the CM, DC, and CBPM formulas (25%). The remainder of each formula was comprised of brewer's rice, rice protein concentrate, soybean oil, cellulose, beet pulp, vitamins, minerals, and an antioxidant blend **(Table 2.1)**. Lactic acid was added to dry formulas to lower the food's pH to also maximize antimycotic effects (Hand & Lewis, 2010) (Montelongo et al., 2013) The five experimental formulas were formulated to meet AAFCO (2021) canine maintenance nutritional requirements **(Appendix A)**.

Formula Production

Five experimental formulas were produced in the Hill's Experimental Food Lab at the Hill's Pet Nutrition Center (Topeka, KS, USA). All dry ingredients were weighed, and then mixed with a ribbon blender (Wenger Mfg., Sabetha, KS, USA) in the feeder/delivery system.

Ingredients were mixed for five minutes, and then transferred to the hammer mill (Jacobs Corporation, Harlan, IA, USA) to be ground to a consistent particle size (No. 16 mesh sieve) before entering the preconditioner. Once complete, the dry ingredients were placed into a movable bin so the mix could be taken to the preconditioner (Wenger Mfg., Sabetha, KS, USA). Dry ingredients were added to the preconditioner at a feed rate of 14-14.5 lb./min. In the preconditioner, moisture, steam, lactic acid, and choline chloride were added to the dry ingredients and mixed with paddles at 235-240 rpm. Temperature of the mixture reached 185°F at the throat of the preconditioner before exiting to the extruder.

A single screw extruder (Model X-85; Wenger Mfg., Sabetha, KS, USA) with a common medium shear pet food screw profile was used. After extrusion, the product was conveyed to a dual pass dryer (AeroDry; Buhler Aeroglide, Cary, NC, USA) set at 150-155°F to obtain a final moisture of less than 10%. After drying, the product was collected and transferred to a ribbon mixer where soybean oil was added manually and mixed for three minutes to achieve equal distribution. Finally, the finished product was packaged in poly-lined plastic bags, (15-pounds per bag), passed through a metal detector, and stored in ambient conditions until used for scheduled testing.

Kibble Analysis

All five experimental formulas were analyzed for moisture, protein, and fat utilizing near-infrared spectroscopy (NIR; Foss NIRS DS2500, Eden Prairie, MN, USA) in the Hill's Experimental Food Lab. NIR analysis was used to measure moisture, protein, and fat levels during production, therefore when production hit a steady state, the product could then be collected. Bulk density was analyzed at regular intervals during production until the density reached the target measurement of 23 lb./ft^3. A final NIR analysis was conducted after the soybean oil was topically applied. In the Hill's Food Science Lab, twenty kibbles from each experimental formula were measured for diameter, cut length, weight, and analyzed for texture. Digital calipers (Mitutoyo ABSOLUTE A500-171, Aurora, IL, USA) were used to measure the diameter and cut length in millimeters while an analytical scale (Ohaus Explorer Scales, Pleasant Prairie, WI, USA) was used to measure the weight in grams. Quality tests were also performed on the formulas to determine water activity and pH levels. Water activity was measured using a water activity meter (Aqua Lab 4TEV, Pullman, WA, USA) instrument. To increase shelf life, dry dog foods tend to have a water activity below 0.60 (Samant et al., 2021). The pH was measured by mixing 10 g of ground kibbles with 90 mls of water. The mixture was stirred intermittently for 20 minutes, then allowed to settle for 5 minutes before measuring the pH with a probe (VWR Symphony SP70P, Radnor, PA, USA).

Texture analysis was performed to evaluate the kibbles for hardness using a TA.XT plus Texture Analyzer (Texture Technologies Corporation, Hamilton, MA, USA), equipped with 50-kg load cells. The procedure used was a modified version from the method used by Dogan and Kokini (2007) and has been used in other studies (Alvarenga et al., 2018; Manbeck et al., 2017; Smith, 2018). A 25 mm cylindrical probe was used for a compression test with a pre-test speed of 2 mm/s, a test speed of 1 mm/s, a post-test speed of 10 mm/s, and a strain level of 50%. A total of 20 kibbles, from each formula, were measured for hardness. The first peak fracture force (kg) was taken as a measure of hardness for each kibble analyzed.

A final composite sample of each formula was sent to a commercial laboratory, (Eurofins Scientific, Inc., Des Moines, IA, USA) for a full nutritional panel analysis. A combination of methods organized by the Association of Official Agricultural Chemists (AOAC) and Eurofins proprietary methods were used to perform nutritional analyses to Hill's Standards.

Palatability Testing Procedure

This study used the same method as Hall et al., (2018) to measure palatability using the trained canine panel at the Hill's Pet Nutrition Center (Topeka, KS, USA). All canine use was approved by the Institution Animal Care and Use Committee, Hill's Pet Nutrition Center (Topeka, KS, USA) (Hill's IACUC #CP26/permit number: 590). Four trained panels of 25 adult dogs, made up of majority Beagles, randomly divided by sex, were used for palatability testing. Dogs were deemed to be in good health, trained for palatability testing, calibrated annually, and were able to discriminate between foods. Dogs were provided with access to water *ad libitum*.

Palatability was measured for the five formulas (FC14, FC25, DC, CM, and CBPM) using the two-bowl test, fed one meal per day in the morning, over the duration of two days for a total of two meals. For each day of the palatability test, caloric intake was set to meet each dog's daily needs based on their weight, and the feeding time allotment was set for 30 minutes. Bowls were placed on the designated feeding scales and presented simultaneously to each dog. The test was considered complete once each dog reached their daily caloric intake or after 30 minutes had expired. On the second day of testing, the bowls were switched to avoid side bias. Once feeding was complete, the final weight from each bowl was recorded. Intake ratio and first bite were recorded during the palatability test. The first bite was measured for each dog at the beginning of feeding. Load scales under each bowl helped determine which food had the first bite by measuring the change in weight. The change in weight of the bowls was monitored continuously to also assess the consumption rate. At the end of the test, the intake ratio (IR) was calculated and the bowl with the most food consumed was considered the preferred food (Griffin, 2003):

$$Intake\ Ratio = \frac{Food\ A\ Consumed}{(Total\ Consumption\ Food\ A + Total\ Consumption\ Food\ B)}$$

Statistical Analysis

The model statement for kibble measurements contained cut length, diameter, and weight as fixed variables. The model statement for the texture analysis contained hardness. All means were separated using Fisher's Least Significant Difference (LSD) with a significance of (α = 0.05). LSDs are shown with superscript letters to determine ($p<0.05$) differences between samples.

For palatability equivalency testing, food consumption was measured using the IR. A Student's t-test was conducted to classify the results of the overall panel. A p-value < 0.05 expresses that the IR of the test was either greater than or less than 0.50, with a 95% statistical certainty (Vondran 2013). The first bite data was analyzed using a Chi^2 test.

RESULTS & DISCUSSION

Formulation

Experimental formulas were formulated to ensure limited impacts of the other ingredients on palatability **(Table 2.1)**. No palatants or chicken fat were added to keep the base formulas as bland as possible so that the protein is not diluted or masked. To accommodate the differences in the nutritional matrix of the different chicken proteins, minor adjustments to brewer's rice, rice protein concentrate, and soybean oil were made. The use of bland or flavor neutral ingredients, (brewer's rice, rice protein concentrate, and soybean oil), in the formulas should not affect palatability with any minor changes to meet nutritional requirements. The other ingredients, cellulose, beet pulp, lactic acid, choline chloride, vitamins, minerals, and the antioxidant blend were held as constant as possible across all five experimental formulas.

Production

All five formulas were batched and produced on the same day to minimize variability by ensuring raw materials were from the same supplier lot codes. During extrusion, all processing parameters were held constant to limit physical differences to the finished food for palatability testing. All five formulas were successful runs producing usable finished products for palatability, analytical, and other scheduled testing.

While all five formulas were successful and collected, the FC25 formula did not reach the target of 25% inclusion of fresh meat. This was most likely due to the increased levels of water from the high level of fresh chicken being added to the mix in the preconditioner. This resulted in a doughy texture in the extruder that was not able to form an extruded kibble. The maximum level of fresh chicken inclusion reached was 22%. Further changes in the processing parameters may have allowed the 25% level of inclusion but would have added additional variables to the study design.

Kibble Analysis

Image references of the five canine formulas are shown in **Figures 2-1 to 2-5**. NIR results collected post-production are reported in **Table 2.2**. The FC25 formula measured the highest in bulk density (23.44 kg/m^3) and highest in moisture (9.2%). This was most likely due to the elevated levels of water from the high rate of inclusion of fresh chicken. However, the FC14 formula measured the lowest in moisture (6.4%). This could be a result of the drying step. Measured protein was lowest in the FC14 (21.62%) formula because of the lower protein contribution from the fresh chicken ingredient (due to higher moisture dilution) while the CM formula had the highest protein (26.68%) because it had the highest concentration of protein as an ingredient. Fat % was lowest for the FC25 formula due to less soybean oil added compared to the FC14, DC, CM, and CBPM formulas. This may be an indirect result of the higher fat content in the fresh chicken ingredient at that high level of inclusion.

Kibble diameter, cut length, mass, and hardness values are provided in **Table 2.3**. The cut length of the kibbles differed ($p<0.05$) between experimental formulas. The FC25 kibbles measured the largest at 5.44 mm while the DC kibbles measured the smallest at 4.76 mm. The kibble diameter between the CBPM, CM, DC, and FC14 kibbles were not different ($p>0.05$) from each other (ranging between 12.53-12.89mm) but did differ ($p<0.05$) from FC25, which measured the smallest at 12.06 mm. Kibble mass also differed ($p<0.05$) between formulas. Mass was similar between the CM, FC14, and FC25 kibbles (0.30 g) while the CBPM kibbles weighed a little more (0.32 g) and the DC kibbles weighed a little less (0.28 g).

Hardness in this study was defined as the peak force (kg) of each kibble compression. Hardness in pet food is measured as the amount of force that is needed to initially fracture a kibble which simulates a first bite for dogs. According to Dogan & Kokini, (2007), the force needed to initially fracture a kibble is related to the internal structure which includes porosity, size of pores, and cell wall thickness. Texture can influence the final product’s palatability; therefore, it was important that each formula was similar in hardness (Koppel et al., 2015). Each formula was different ($p<0.05$); however, the largest difference was seen between the DC (28.31 kg of force) kibbles and the CM (25.82 kg of force) kibbles. (Dunsford et al., 2002) concluded that smaller kibble cell walls reinforce each other, thus requiring more force to break the kibble. This may be the case for the DC formula in this study since it measured the smallest for cut length. Processing variables have also been shown to influence hardness and texture of formulas (Alvarenga et al., 2018).

Quality check results for all five canine formulas have been provided in **Table 2.4**. After testing water activity (a_w), the FC25 formula measured the highest at 0.58 a_w whereas the FC14 formula measured the lowest at 0.37 a_w. The CM, CBPM, and DC formulas measured around 0.50 a_w. The pH for the FC14 formula measured the lowest at 4.78 while the FC25, DC, CM, and CBPM formulas measured around a pH of 5.

Nutrient analysis results of the five canine formulas have been provided in **Table 2.5**. This analysis provides insight on how the formulas are nutritionally comparable to one another. To ensure all products were similar nutritionally, each formula's predicted (DMB) nutritional requirements were compared before production. Analytical results conclude that nutrients analyzed above the AAFCO minimum requirements for adult dogs at maintenance (**Appendix A**).

By design, all the formulas were very similar with the primary difference being the different chicken sources. Processing parameters were kept as close as possible to maintain focus on the impact of protein sources on palatability. The impact of changing processing parameters could be a topic for future research.

Palatability Results

For each palatability test, the CBPM formula was considered the test food (food A) and the DC, CM, FC14, and FC25 formulas were considered the control foods (food B). The palatability and first consumption results are concluded in **Table 2.6**. The CBPM formula was preferred ($p<0.05$) over the FC14 formula with an IR of 0.735. The CBPM formula was preferred ($p<0.05$) over the FC25 formula with an IR of 0.673. The CBPM formula was preferred ($p<0.05$) over the DC formula with an IR of 0.579. The CBPM formula was a parity ($p = 0.1427$) with the CM formula with an IR of 0.517. When evaluating which first bite of food was taken by each dog, there was a difference ($p<0.05$) between each palatability test: the CBPM versus the CM formula (32 vs. 18), the CBPM vs the DC formula (33 vs. 17), the CBPM vs the FC14 formula (41 vs. 9), and the CBPM vs the FC25 formula (39 vs. 11) over the two-day trial. The majority of the first bites from the dog panels were from the CBPM formula. It is uncertain if these results are related to aroma preference for the CBPM formula as it has been discussed that first consumption/approach responses are very subjective and are not the best indicators of palatability. In addition, these methods' data can often be difficult to measure, and the repeatability of these measures is questionable (Griffin, 2003).

Some of these palatability results were unexpected when compared to previous studies. According to (Li et al., 2017) dogs have a higher taste preference for fat, however in this study DC (12.86%) and FC14 (12.8%) had the highest fat levels but were less preferred compared to CBPM (11.23%). We also know that too much fat can lead to faster oxidation, giving a rancid smell that is unappealing to dogs (Hand et al., 2010).

According to Hand et al. (2010), dogs prefer consistent, larger kibbles. All formulas were similar in diameter size, apart from the FC25 formula that measured different ($p<0.05$). The smallest cut length kibble, the DC formula, may have influenced texture, as it measured the hardest (28.31 kg). The FC25 formula was the next hardest (27.49 kg) in texture, however this may be correlated to having a more "spongey" texture, as seen in Alvarenga et al. (2018), because it measured the highest in moisture (9.2%) and bulk density (23.44kg/m^3). The FC25 formula also had the least amount of starch content which may have led to these results. Results of this study may further indicate that minor differences in the kibble analysis may not have an impact on canine palatability. Previous research suggests that odors might be the primary drivers in a dogs' food choice (Basque et al., 2019; N. J. Hall et al., 2017; Horowitz, 2017; Houpt et al., 1978; Houpt & Smith, 1981). A dog's food selection is highly driven by smell based on the physiology of their highly developed nasal cavities. Houpt et al. (1978) found that when dogs were presented with a bland diet supplemented with a meat odor, they preferred it over a controlled diet with no odor. Manabe et al. (2010) determined that oils and short chain fatty acids are recognized in a special section of the olfactory bulb, and that this strong odor may be a driving factor behind a dog's liking. Determining the sensory characteristics and the aromatic compounds within the five formulas may help better understand the preference from the palatability tests.

CONCLUSION

Testing of the four different chicken proteins presented challenges in keeping processing constant between formulas. Using a high amount of fresh chicken proved to be difficult in forming a finished product at the target moisture and density while maintaining similar extrusion processing. Based on the palatability results from this study, the CBPM formula was preferred over the FC14, FC25, and DC formulas. It was a palatability parity to the CM formula. The definitive palatability results indicate that the minor differences in the kibble analysis may not have had an impact on canine palatability, and that the aroma from the different chicken protein sources did. It can be concluded that the CBPM formula in this study is highly palatable and in addition to being nutritionally beneficial, may provide increased food enjoyment by pets. Future studies should continue to analyze animal by-products as the need for a sustainable pet food industry is necessary.

Table 2.1 Formula Composition: fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), chicken by-product meal (CBPM)

Ingredients, %	**FC14**	**FC25**	**DC**	**CM**	**CBPM**
Fresh Chicken	14	25	-	-	-
Dried Chicken	-	-	14	-	-
Chicken Meal	-	-	-	14	-
Chicken By-Product Meal	-	-	-	-	14
Brewers Rice	50	44.19	53	53	53
Rice Protein Concentrate	16.07	16	12.93	14.25	12.9
Soybean Oil	8	4.52	8	8	8
Cellulose	3	3	3	3	3
Beet Pulp	2.5	2.5	2.5	2.5	2.5
Dicalcium phosphate	2.33	0.87	1.93	1.01	1.95
Calcium carbonate	1	1	1.6	1.2	1.6
Lactic acid	1.2	1.2	1.2	1.2	1.2
Potassium chloride	0.91	0.73	0.86	0.86	0.86
Sodium chloride	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.25	0.25	0.25	0.25	0.25
Vitamin Premix	0.15	0.15	0.15	0.15	0.15
Mineral premix	0.05	0.05	0.05	0.05	0.05
Antioxidant blend	0.04	0.04	0.04	0.04	0.04

Table 2.2 Kibble NIR measurements (As Fed) and density post-production of canine formulas: fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), chicken by-product meal (CBPM)

Item	**FC14**	**FC25**	**DC**	**CM**	**CBPM**
Moisture, %	6.4	9.2	8.3	7.5	8.2
Fat, %	12.8	11.04	12.86	11.44	11.23
Protein, %	21.62	23.99	23.86	26.68	24.81
Density, kg/m3	22.9	23.44	22.4	22.5	22.4

Table 2.3 Kibble measurements and texture analysis: fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), chicken by-product meal (CBPM)

Item	**FC14**	**FC25**	**DC**	**CM**	**CBPM**	**p-value**
Cut Length, mm	5.15^{c}	5.44^{a}	4.76^{d}	5.28^{bc}	5.42^{ab}	<0.0001
Diameter, mm	12.69^{a}	12.06^{b}	12.58^{a}	12.53^{a}	12.89^{a}	0.0005
Mass, g/kibble	0.30^{b}	0.31^{b}	0.28^{c}	0.31^{b}	0.32^{a}	<0.0001
Hardness, kg	26.98^{abc}	27.49^{ab}	28.31^{a}	25.82^{c}	25.84^{bc}	0.013

^{abc} indicates that within a row, unlike letters differ ($p<0.05$)

Table 2.4 Kibble quality analysis: fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), chicken by-product meal (CBPM)

Item	**FC14**	**FC25**	**DC**	**CM**	**CBPM**
a_w	0.35	0.56	0.52	0.46	0.48
pH	4.78	4.92	4.98	5.08	5.01

Table 2.5 Nutrient composition analysis (DMB): fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), chicken by-product meal (CBPM)

Nutrient	FC14		FC25		DC		CM		CBPM	
	Predicted	Analyzed	Predicted	Analyzed	Predicted	Analyzed	Predicted	Analyzed	Predicted	Analyzed
Protein (crude), %	23.36	26.56	26.75	27.58	27.13	26.56	27.81	28.33	26.74	27.05
Fat (crude), %	14.25	13.26	13	11.46	12.71	13.26	12.46	13.13	12.87	13.19
Fiber (crude), %	3.44	2.28	3.8	3.53	3.06	2.28	3.09	2.71	3.13	2.82
Ash, %	6.71	6.21	6.03	5.21	7.17	6.21	7.11	6.64	7.35	6.82
Calcium, %	1.29	1.42	1.18	1.08	1.54	1.42	1.58	1.5	1.54	1.49
Phosphorus, %	0.8	0.78	0.6	0.61	0.8	0.78	0.8	0.81	0.8	0.82
Potassium, %	0.7	0.69	0.65	0.66	0.7	0.69	0.7	0.7	0.7	0.7
Sodium, %	0.29	0.3	0.32	0.32	0.32	0.3	0.32	0.31	0.32	0.32
Chloride, %	0.99	0.9	0.96	0.99	0.91	0.9	0.91	1.03	0.94	0.94
Magnesium, %	0.06	0.08	0.06	0.07	0.07	0.08	0.07	0.08	0.07	0.08
Manganese, ppm	39.93	27.11	36.47	28.68	36.42	27.11	34.05	26.02	39.21	30.37
Zinc, ppm	231.66	185.41	247.04	216.22	200	185.41	210.43	190.83	216.1	194.12
Arginine, %	1.81	0.96	2.05	0.98	2.05	0.96	2.1	1.02	1.99	0.97
Histidine, %	0.55	0.32	0.63	0.31	0.68	0.32	0.64	0.33	0.59	0.3
Isoleucine, %	0.92	0.54	1.06	0.56	1.12	0.54	1.07	0.59	1.06	0.56
Leucine, %	1.81	1.08	2.07	1.05	2.1	1.08	2.06	1.08	2.03	1.04
Lysine, %	0.98	0.72	1.19	0.56	1.5	0.72	1.37	0.71	1.3	0.65
Methionine, %	0.55	0.35	0.63	0.33	0.66	0.35	0.64	0.34	0.59	0.31
Meth-Cys, g	0.94	0.55	1.06	0.56	1.06	0.55	1.03	0.54	1	0.52
Phenylalanine, %	1.17	0.65	1.32	0.67	1.28	0.65	1.27	0.67	1.26	0.64
Phenyl-Tyro, g	2.21	1.07	2.48	1.13	2.37	1.07	2.36	1.11	2.32	1.09
Threonine, %	0.83	0.53	0.97	0.47	1.04	0.53	1	0.5	1.02	0.49
Tryptophan, %	0.29	0.34	0.32	0.34	0.32	0.34	0.32	0.35	0.31	0.33
Valine, %	1.3	0.73	1.47	0.79	1.46	0.73	1.44	0.8	1.45	0.78
Linoleic Acid, %	5.98	4.76	4.53	3.19	5.38	4.76	5.33	4.52	5.43	4.61
Vitamin A, IU/kg	23541	12035	25832	13237	21278	12035	21087	11818	21768	12797
Vitamin D, IU/kg	1397	1139	1541	1478	1247	1139	1243	1225	1241	1334
Vitamin E, IU/kg	165.3	227.69	173.56	163.27	153.95	227.69	149.58	147.46	150.45	151.83
Thiamine, ppm	56.64	58.03	61.61	63.81	51.48	58.03	51.24	52.51	51.14	54.86
Riboflavin, ppm	13.06	11.71	14.41	12.14	12.5	11.71	12.53	10.71	13.8	12.26
Niacin, ppm	213	222	236	254	218	222	210	202	209	218
Pyridoxine, ppm	12.13	11.93	13.39	12.47	11.54	11.93	11.37	9.9	11.08	10.61
Pantothenic Acid, ppm	23.55	22.55	26.43	24.38	23.56	22.55	22.24	18.97	21.79	20.61
Folic Acid, ppm	3.42	2.97	3.73	2.77	3.15	2.97	3.18	3.56	3.72	3.5

B12, ppm	0.13	0.07	0.15	0.08	0.13	0.07	0.13	0.06	0.18	0.07
Biotin, ppm	0.3	0.36	0.34	0.31	0.34	0.36	0.34	0.27	0.48	0.41
Choline, ppm	2280	2667	2544	2427	2240	2667	2107	2721	2247	2592

Table 2.6 The effect of experimental formulas on canine palatability: fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), chicken by-product meal (CBPM)

Diet Comparison (A vs B)	IR of Food A[1]	p-value	First Bite of Food A, n[2]	p-value
CBPM vs CM	0.527	0.1427	32*	0.005
CBPM vs DC	0.579*	0.0001	33*	0.001
CBPM vs FC14	0.735*	0.0001	41*	<0.0001
CBPM vs FC25	0.673*	0.0015	39*	<0.0001

*Comparison differs ($p<0.05$)
[1]IR of Food A = intake (g) of Food A/Total (g) of Food A + Food B
[2]First Bite: number of first bites to bowl A (50 observations)

Figure 2.1 Fresh Chicken 14% (FC14)

Figure 2.2 Fresh Chicken 25% (FC25)

Figure 2.3 Dried Chicken (DC)

Figure 2.4 Chicken Meal (CM)

Figure 2.5 Chicken By-Product Meal (CBPM)

Chapter 3 - Descriptive sensory analysis and consumer acceptance of dry dog food with different chicken proteins

ABSTRACT

Of the animal protein sources used in canine pet food formulations, chicken has the smallest carbon footprint from a sustainability perspective. Chicken by-product meal is considered to be a high-quality dietary protein and provides trace minerals that dogs need from a nutritional standpoint. Due to the negative reputation that all animal by-products have, and the current humanization trends, many pet owners are opting for fewer by-products and more fresh meat in their pets' food. Previous studies have utilized sensory analysis to measure characteristics of dry dog food samples that may impact the pet owner's preconceived bias. However, sensory characteristics of chicken proteins formulated in dry dog foods have yet to be measured. The objectives of this study were to develop a sensory profile for each of the five canine formulas containing different chicken proteins and to assess consumer acceptance of them. Five highly trained descriptive panelists described aroma and appearance attributes for the formulas using a consensus profile method. The descriptive panelists identified twelve aroma sensory attributes and six appearance attributes between the five dog food samples. For consumer acceptance testing, dog owners (n = 99) were recruited to evaluate the five canine formulas for aroma, appearance, and overall liking. Rank orders were also recorded to understand consumers' preference between the dog food samples. Consumers liked the chicken by-product meal formula the most and scored it the highest in overall liking, as well as aroma and appearance liking. Consumers were asked to rank the dog food samples based on their

overall liking, then were provided the chicken protein source used in each sample. Knowing this information, consumers were asked to re-rank the samples. Once consumers were aware of the main protein ingredient, 86% of consumers opinions changed and consumers ranked the fresh chicken and dried chicken samples more preferred than the chicken meal and chicken by-product meal samples. This study indicates that chicken by-products are accepted by consumers, however it is the label that deters them.

INTRODUCTION

The pet food industry has seen a steady increase in overall growth in the United States. A total of $103 billion was spent within the industry in 2020, with consumers spending about $42 billion just on pet food (APPA, 2021). Of the $42 billion spent, dog food accounted for nearly half of the total pet food sales in the US (Mintel, 2021). The humanization trend is driving sales growth within the pet food industry as consumers are willing to spend more on premium products (Nielsen, 2016) (Mintel 2021). Meineri et al. (2021) also highlight that humanization trends have influenced pet owners' preferences, especially for proteins, towards ingredients that are perceived as "human-grade" and less processed. A better understanding of the sensory attributes, and if there is a preference between different chicken protein sources by dogs, or their humans that purchase the food, may further support the use of by-products in dry dog foods.

Scientists utilize sensory analysis in the pet food industry to provide information as to why pets may find certain products palatable or unpalatable. Sensory analysis also provides a better understanding of how changing a characteristic in dog food products may impact the pet owner's perception and acceptance (Koppel, 2014). Both analytical and hedonic sensory analysis techniques, using human panelists, can help determine properties, such as appearance, aroma, and liking, that are important for pet food acceptability (Di Donfrancesco et al., 2014). Previous studies have used sensory analysis in canine foods to find preference through odor, taste, and texture attributes (Chanadang et al., 2016; Di Donfrancesco et al., 2012, 2014; Koppel et al., 2015). Although perception may be different between dogs and humans, sensory analysis can provide an increased understanding of why dogs prefer some products over others.

Product acceptance of pet food is determined by two different participants who look at the product in very different ways. Not only does the food have to be accepted by the pet, but the pet parent must also feel that the product is of sufficient quality and value, underscoring the importance of sensory studies. (Koppel, 2017) breaks the process down into three elements: the pet owner (who buys the food), the pet (who eats the food), and the food (physical characteristics of the food itself). As mentioned in the previous chapter, consumption studies can determine a dog's preference for certain foods. By combining consumption studies with the insights from human panelists, both trained and untrained, scientists can create a greater insight into understanding what makes a highly palatable food. Therefore, the objective of this study was to develop a sensory profile for each of the formulas containing different chicken protein sources, and to assess consumer acceptance of the formulas.

MATERIALS & METHODS

Samples

Five experimental formulas were produced in the Experimental Food Lab (EFL) at the Hill's Pet Nutrition Center in Topeka, KS. The five formulas were formulated to meet American Feed Control Officials (AAFCO 2021) canine maintenance nutritional requirements and utilized four different chicken proteins: chicken meal (CM), dried chicken (DC), chicken by-product meal (CBPM), fresh chicken. The fresh chicken was tested at two levels: at 14% inclusion (FC14) and at 25% inclusion (FC25). The remainder of each formula was composed of brewer's rice, rice protein concentrate, soybean oil, cellulose, beet pulp, vitamins, minerals, and an antioxidant blend **(Table 2.1 - Chapter 2)**.

Sensory Analysis

All human sensory research was approved by the Institutional Review Board for Protection of Human Subjects (IRB #10608) at Kansas State University.

Descriptive Sensory Analysis

Five highly trained panelists from the Sensory Analysis Center, Kansas State University, Manhattan, KS, analyzed the five canine formulas for aroma and appearance characteristics. Each of the panelists had over 1,000 hours of general descriptive sensory analysis training. Each of these panelists also helped with the development of the initial lexicon for dry dog foods (Di Donfrancesco et al., 2012), along with multiple other pet food panels.

For this study, the trained panelists evaluated five canine formulas formulated with different chicken proteins (n = 5: DC, CM, CBPM, FC14, FC25). An initial list of attributes and their definitions, developed from the Di Donfrancesco et al. (2012) lexicon was used to characterize the sensory profiles for each of the samples **(Table 3.1)**. Intensity for each aroma and appearance attribute was measured using a numeric scale of 0–15 with 0.5 increments where 0 = none and 15 = extremely high. The appearance of the kibbles was also measured in terms of uniformity of color, shape, and size. These terms were recorded as "*yes uniformity*" or "*no uniformity*."

Each canine formula evaluation was conducted for two, 1.5-hour sessions. Samples were first evaluated individually by the panelists and then in a group discussion. Each sample was presented in a 12-ounce Styrofoam cup containing 20 grams of kibbles and was analyzed for aroma and appearance. Kibble samples were prepared 30 minutes prior to the testing and were coded with three-digit random numbers. The testing room was quiet, free of any distractions, and was maintained at ambient temperature. Aroma attributes evaluated included: *barnyard*, *brothy, brown aromatics, cardboard, earthy, grain, liver, meaty, musty/dusty, oxidized oil, stale, toasted, vitamin,* and *overall aroma*. Appearance attributes evaluated included: *brown, porous, oily, grainy, fibrous,* and *surface roughness*. After individual evaluations from panelists were completed, the final aroma and appearance profile was determined after a discussion led by the panel's leader. This discussion provided the consensus intensity score for each attribute. This hybrid method (consensus method) has been used in other sensory studies to evaluate dry dog food (Di Donfrancesco et al., 2014; Koppel et al., 2013).

Consumer Study

A Central Location Test (CLT) was conducted in the Sensory Analysis Center at the Kansas State University, Olathe, KS, campus to assess dog owners' acceptance of the five formulas. Consumers, (n = 99) familiar with dry dog food products, were recruited from the Olathe, Kansas, area (19 males & 80 females). Recruitment for the consumers was accomplished through the Sensory Analysis Center database. Consumers were screened for their age (must be > 18 years old but < 65 years old), dog ownership (must own at least 1 dog), and involvement in dry dog food purchasing and feeding (sole person for purchasing or shared responsibility). Consumer demographics are provided in **Table 3.5**.

Quantitative data was collected using a tablet computer and questionnaires were administered through Compusense at hand software (Compusense Inc., Guelph, Ontario, Canada). The questionnaire is provided in **Appendix B**. Approximately 25 consumers attended each session, for a total of 4 sessions. A 9-point hedonic scale was used for the acceptance/liking questions where 1 = *dislike extremely* and 9 = *like extremely*. Consumers were also asked to rank the samples in order from 1 = *most liked* to 5 = *least liked*. The dog food samples were provided monadically and in a randomized order to the consumers. Each dog food sample was presented in a 4-ounce disposable translucent plastic Souffle cup covered with a translucent plastic lid and labeled with a randomized three-digit code. When told to do so, consumers removed the lid and evaluated the aroma first and then the appearance of the kibble samples. Consumers were asked to keep the kibble samples in the order in which they were received to rank each one at the end of the session. Once the initial ranking was complete, consumers were provided with the chicken protein source (FC14, FC25, DC, CM, & CBPM) used in each of the kibble samples. Given this additional information, consumers were then asked to re-rank the samples using the same scale (1 = *most liked* and 5 = *least liked*). Before closing the test, consumers were asked additional questions regarding what sustainability meant to them.

Data Analysis

Descriptive and consumer data was analyzed using XLStat Software (Addinsoft, New York, NY, USA). Principal Component Analysis (PCA) was used to elicit relationships between attributes and kibble samples from the descriptive analysis data. Analysis of variance (ANOVA) was used to analyze liking scores from consumers, with Fisher's Protected Least Significant Difference (LSD) post-hoc mean separation to determine ($p<0.05$) differences between samples.

Friedman analysis of variance was performed to analyze ranking scores by consumers. Partial Least Squares Regression analysis (PLSR) was conducted to better understand how the sensory characteristics of the kibble samples may relate to the consumers' liking.

RESULTS & DISCUSSION

Descriptive Analysis

Sensory Characteristics

Results from descriptive sensory analysis indicated that the intensities for all attributes, excluding overall aroma, were in the low intensity range (0 - 4) **(Table 3.2)**. This low-end range is similar to previous studies that also analyzed sensory characteristics of dry dog food samples: Di Donfrancesco et al. 2014 and Chanadang et al. 2016. Di Donfrancesco et al. (2012) indicates these lower intensity characteristic scores are due to the blended and complex nature of the product category. Aroma attributes including *barnyard, brothy, brown aromatics, cardboard, grain, musty/dusty, oxidized oil, stale, toasted,* and *vitamin* were present in all five samples. *Meaty* and *earthy* were included as possible choices in the attribute list but were not identified in any of the samples. The *meaty* aroma is typically associated with topical palatants that are added to commercial formulas, however no palatants were added to any of the experimental formulas. The *earthy* aroma was not chosen because the panelists felt that *musty/dusty* aroma was the better attribute to use. The aroma attribute, *liver,* was only found in the CBPM sample. The overall aroma intensities of each sample were in the moderate intensity range (5 - 10) with FC14 and FC25 having the strongest *overall aroma*. While still considerably low, the CBPM sample had the highest score for *barnyard* aroma while the FC25 sample had the highest score for *oxidized oil* aroma.

All five samples were characterized similarly in appearance for *brown/tan color, porous, oily, grainy,* and *surface roughness attributes* **(Table 3.3)**. The CBPM sample was characterized as *fibrous,* whereas CM, DC, FC14, and FC25 were not. The FC25 sample measured the highest for *surface roughness* and the FC14 sample measured the lowest for *oily*. The FC14 and FC25 samples measured slightly lower in *brown/tan* color than the DC, CM, and CBPM samples.

Panelists discussed the appearance of each sample in terms of uniformity of the color, shape, and size. The consensus for each is provided in **Table 3.5**. DC was the only sample characterized as *uniform in color* and none of the samples were considered *uniform in shape*. FC14 and CBPM were characterized as *uniform in size* while CM, DC, and FC25 kibble samples were not.

A PCA graph was constructed to better understand the relationship between the five formulas' aroma and appearance characteristics and is shown in **Figure 3.1**. The principal components (PC) 1 and 2 in the biplot describe 65.8% of the variation observed within the samples. PC1 explains 36.71% of the variation of the samples and is positively loaded with *vitamin*, *musty/dusty* and *grain*. and is negatively loaded with *liver, barnyard, fibrous,* and *oily*. The CBPM formula correlated closest to the *liver* aroma and *fibrous* appearance while the CM and FC14 correlated closest to the *grain* aroma. PC2 explains 29.04% of the variation of the samples and is positively loaded with *brown aromatics* and negatively loaded with *oxidized oil* and *overall aroma*. In PC2, the FC25 formula correlated closest to the *oxidized oil* and *overall aroma*. The FC14, DC, and CM samples were not correlated close to any of the appearance attributes. All three of these kibble samples received lower intensity scores for appearance.

In a similar study, Koppel et al. (2015) measured the aroma and appearance attributes using descriptive analysis to determine the sensory characteristics of coated versus uncoated dry dog foods with different fiber inclusions. The base formula, also used as the control food, was formulated using chicken by-product meal as the animal protein source. The descriptive analysis results identified aroma attributes in the control formula such as *barnyard, toasted, grain, stale,* and *cardboard* and similar appearance attributes*: porous, fibrous,* and *grainy* (Koppel et al., 2015). These attributes are similar to the attributes measured for the CBPM, CM, DC, FC14, and FC25 samples. All intensity scores from the CBPM sample in this study, aside from the *porous* appearance attribute, measured in similar ranges as measured in the Koppel et al. (2015) study.

A second study in 2016, Chanadang et al. also used chicken by-product meal as a main animal protein source in one of their dry dog food test formulas. Their objective was to determine the impact of rendered protein meal oxidation levels on shelf-life, sensory characteristics, and the acceptability by consumers. The descriptive panel measured the intensity scores for the attributes: *oxidized oil, stale, cardboard,* and *rancid*. When comparing the descriptive attributes from the Chanadang et al. (2016) dog food samples preserved with antioxidants, to the CBPM sample from this study, the attribute intensity scores for *oxidized oil, stale,* and *cardboard* are similar. Chanadang et al. (2016) used *rancid* as a descriptive attribute while this study used *oxidized oil* and *stale* instead. Lin et al., (1998) also demonstrated this effect when they reported their dry dog foods, formulated with poultry fat, oxidizing faster than foods not formulated with poultry fat. To eliminate any confounding factors aside from the chicken protein sources, palatants and additional fats were not applied to the final products in this study. This approach is not a representation of commercial products, nor was it intended to be. The intent of this study was to focus on the sensory perception of the chicken protein sources used and how those may affect canine palatability and consumer liking.

Table 3.1 Sensory attributes, definitions, and references for aroma and appearance. (Intensity scale 0-15).

Aroma		
Attribute	Definition	Reference & Intensity
Overall	Overall dog food aroma.	Purina Dog Chow = 8.0
Barnyard	Combination of pungent, slightly sour, hay-like aromatics associated with farm animals and the inside of a barn.	White pepper in Swanson Chicken Broth 99% Fat Free (0.90g /300ml) = 6.0
Brothy	The aromatic sensation associated with juices from boiled poultry.	Boxed unsalted Swanson Chicken Broth = 4.0
Brown aromatics	Aromatic impression always characterized as some degree of darkness; generally associated with other attributes (I.e., toasted, nutty, sweet, etc.) and does not have its own individual character.	Kretshner Wheat Germ = 3.0
Cardboard	The aromatics associated with cardboard or paper packaging.	Cardboard = 7.5
Earthy	The slightly musty aromatics associated with raw potatoes and damp humus, slightly musty notes.	Raw button mushrooms = 8.0
Grain	The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice and oats.	Cereal Mix (dry) = 5.0
Liver	Aromatics associated with cooked organ meat/liver.	Grilled beef liver = 6.0
Meaty	A measure of how much a sample is recognized as distinctly animal muscle tissue (poultry, fish, and beef).	Swanson's Beef Broth = 5.0
Musty/Dusty	Dry, dirt-like aromatic associated with dry, brown soil.	Kretshner What Germ = 5.0
Oxidized Oil	The aromatic associated with aged or highly used oil and fat.	Microwave oven heated Wesson Vegetable Oil = 3.0
Stale	The aromatics characterized by lack of freshness.	Mama Mary's Pizza Crust = 4.5
Toasted	A moderately browned/baked aromatic impression	General Mills Cheerios crushed = 7.0
Vitamin	The aromatics associated with a just-opened bottle of vitamin pills (oxidized thiamin)	Nature Made B-Complex capsule = 4.0
Appearance		
Brown	Light to dark evaluation of brown color of product.	Pantone Coated Plus Series 2310CP=4.0 Pantone Coated Plus Series 2311CP =8.0 Pantone Coated Plus Series 2313CP =12.0

Porous	Presence of pores/air bubbles on the surface	Cheerios = 8.0
Oily	The amount of oil perceived on the product surface	Planters cocktail Peanuts = 2.5
Grainy	The perception of small round particles that appear to be relatively harder than the surrounding product	Malt-O-Meal = 12.0
Fibrous	The perception of visible fibers and filaments on the product	Post shredded wheat = 12.0
Surface roughness	Indentations/bumps on surface; smooth to rough	Cheerios = 5.0 Wheaties = 9.0
Color uniformity	A measurement describing uniformity between the kibbles regarding color (Yes/No)	
Shape uniformity	A measurement describing uniformity between the kibbles regarding the shape (Yes/No)	
Size uniformity	A measurement describing uniformity between the kibbles regarding the size (Yes/No)	

*Definitions and references listed were used from the dry dog food lexicon (Di Donfranseco et al., 2012)

Table 3.2 Aroma profile from descriptive analysis (intensity scale 0-15): Fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), and chicken by-product meal (CBPM).

Kibble	**FC14**	**FC25**	**DC**	**CM**	**CBPM**
Attribute					
Overall	7	7	6	5	6
Barnyard	2.5	3	3	2	3.5
Brothy	2	2	2	2	2
Brown aromatics	3	2	2	3	3
Cardboard	3	4	4	4	3
Earthy	0	0	0	0	0
Grain	4	3	3	4	3
Liver	0	0	0	0	2
Meaty	0	0	0	0	0
Musty/Dusty	3	3	3	3	2.5
Oxidized Oil	2	4	3	3	2.5
Stale	2.5	3	3	2	3
Toasted	3	3	3	3	3
Vitamin	2	2	3	2.5	2

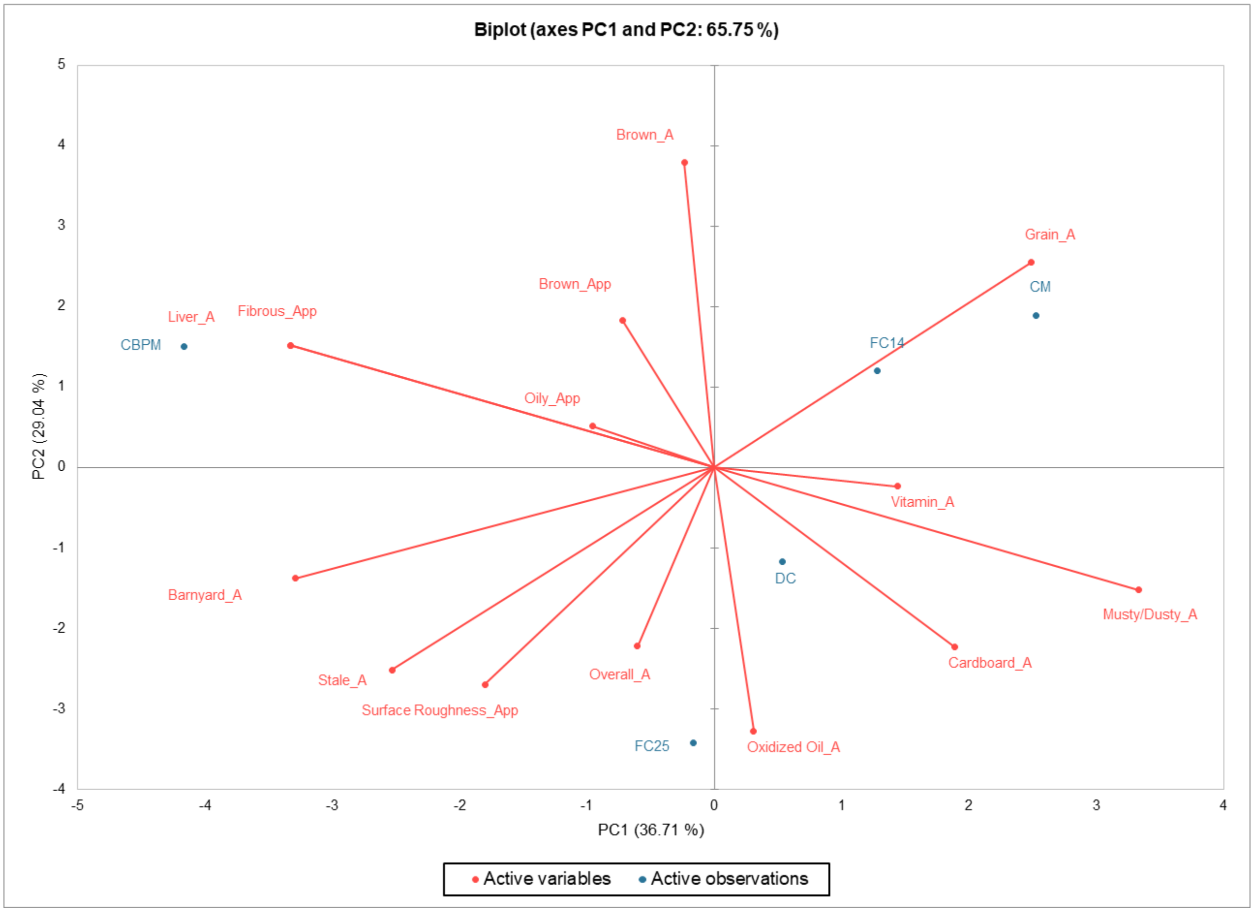
Table 3.3 Appearance profile from descriptive analysis (intensity scale 0-15): Fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), and chicken by-product meal (CBPM).

Kibble	**FC14**	**FC25**	**DC**	**CM**	**CBPM**
Attribute					
Brown/Tan	9	9	10	10	10
Porous	2	2	2	2	2
Oily	2	2.5	3	3	3
Grainy	2.5	2.5	2.5	2.5	2.5
Fibrous	0	0	0	0	2
Surface Roughness	3	4	3	3	3.5

Table 3.4 Uniformity in appearance from descriptive analysis: Fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), and chicken by-product meal (CBPM)

Kibble	**FC14**	**FC25**	**DC**	**CM**	**CBPM**
Attribute					
Color	no	no	yes	no	no
Shape	no	no	no	no	no
Size	yes	no	no	no	yes

Figure 3.1 Principal Components Analysis (PCA) based on descriptive analysis for aroma and appearance



Consumer Study

Acceptance & Aroma Intensity Scores

Results of the consumer acceptance testing indicated that there were differences ($p<0.05$) among the samples for *aroma liking* (5.08 - 5.65 average range), *appearance liking* (5.71 - 6.38 average range), and *overall liking* (5.36 - 6.07 average range) **(Table 3.6)**. Consumers scored the CBPM sample the highest for *aroma*, *appearance*, and *overall liking* while the FC14 sample scored the lowest. The *aroma liking* of the samples was not different ($p>0.05$) between the CBPM, CM and DC samples, but the FC14 sample was different ($p<0.05$) and was least liked. The FC25 sample was not different ($p>0.05$) in *aroma liking* from the DC, CM, CBPM or FC14 samples. The CM and DC samples were not different ($p>0.05$) in *appearance liking* from the CBPM or FC25 samples but were different ($p<0.05$) from the FC14 sample which was liked the least. The CBPM sample was most liked ($p<0.05$) in *appearance liking* than the FC25 and FC14 samples. Similar results were concluded for *overall liking*, with the CM and DC samples not being different ($p>0.05$) in *overall liking* from the CBPM or FC25 samples, but they were different ($p<0.05$) from the FC14 sample which was liked the least. The CBPM sample measured different ($p<0.05$) in *overall liking* from the FC25 and FC14 samples and was liked by the consumers the most. Consumers were also asked how much they thought their dog would like or dislike each sample. Results indicated that their prediction of dog liking follows the majority of the consumers liking. Consumers scored the CBPM sample the highest for their *dog liking* while FC14 scored the lowest for *dog liking.* Di Donfrancesco et al. (2014) also determined that this data could be influenced by consumers overall liking and is not an accurate presentation of actual dog preference. Differences in liking, even if statistically significant, were not extremely large and all the average scores were > 5.00 and < 7.00, indicating that all samples were somewhat liked even if at a different degree.

Once consumers were finished evaluating the *aroma*, *appearance*, and *overall liking* of the samples, they were asked to rank the samples in order of 1 = *most liked* to 5 = *least liked* **(Table 3.7)**. Consumers demonstrated a preference for the CBPM and CM samples versus the FC14 and FC25 samples. The DC sample measured the same ($p>0.05$) against the CBPM, CM, and FC25 samples.

Once the first round of ranking was complete, the consumers were then provided with the chicken protein sources used in each formula sample they were analyzing. They were then asked to re-rank the samples with this additional knowledge of the dog food samples **(Table 3.8)**. The FC14, FC25, and DC samples were more preferred over the CM and CBPM samples in the second round of ranking. Only 14% of consumers did not change their ranking after being provided with the protein sources used. The other 86% of consumers communicated that they changed their ranking because they prefer “healthy, natural, fresh, unprocessed, and no by-products" when making dog food purchasing choices.

Once ranking of the formulas was complete, consumers were asked what factors were most important to them when purchasing a dog food product and what sustainability meant to them (**Appendix B**). The top two purchasing factors consumers chose were nutrition and the ingredient statement. Consumers were least concerned about the marketing, claims, and labeling when purchasing foods for their dogs **(Figure 3.9)**. These results indicate that consumers are most concerned with what ingredients are used in their pet’s foods and how the ingredients contribute to their pet's health versus what they perceive in the kibble itself. In 2021, Just Right, a personalized dog food brand, commissioned the Nutritional Knowledge for Dogs survey to learn more about consumer’s shopping habits (Hardt, 2021). Of the 800 dog owners surveyed, 66% said that quality of ingredients is extremely important and 90% said that their dog's preference is the most important factor when buying food products (Hardt, 2021). These results provide insight that consumers are becoming more observant of what ingredients are used in their dog’s food, however the amount of education that consumers have about the ingredients varies. The Pet Food Consumer Habit Survey asked 3,300 dog owners where they received their information about dog food and their top three answers were veterinarians, online resources, and pet store staff (Phillips-Donaldson, 2021). Only 5% of those dog owners said they received information from the actual pet food manufacturers (Phillips-Donaldson, 2021). This provides an important opportunity for pet food manufacturers to educate consumers and to improve the sustainability of pet foods (Swanson et al., 2013).

In addition to determining purchasing factors, consumers were also asked what sustainability meant to them **(Figure 3.10)**. The top two answers chosen were ‘environmentally friendly’ and ‘maintain over time.’ According to the Environmental Protection Agency (EPA), sustainability is defined as a harmonious and productive system in which humans and nature can exist without jeopardizing the needs and requirements of future generations (Meeker & Meisinger, 2015). Without this definition available, a little over half of the consumers had a good understanding of what sustainability means. According to Beaton (2022), pet food manufacturers are continuously improving their sustainability goals for food products as consumers become more educated on what pet food sustainability means.

Table 3.5 Consumer demographics from Central Location Test (CLT)

Dog Owner Demographics	Categories	Frequency
Gender	Male	19
	Female	80
Age	18-35	14
	36-50	43
	51-65	42
Number of Dogs	1 Dog	53
	2 Dogs	43
	3 Dogs	2
	4 Dogs	1
Age of Dog	Puppy (0-12 Months)	11
	Adult (1-8 years)	74
	Senior (9+ Years)	14
Size of Dog	Less than 20 lbs.	22
	More than 21 lbs.	77
Role in Dog Food Purchasing	Primary Shopper	10
	Share Shopping	89
Feeding Involvement	Feed All the time	36
	Most of the time	62
	Some of the time	1

Table 3.6 Consumer panel (N=99) of dog owner's evaluation of "liking" (hedonic scale 1-9: 1 = dislike extremely to 9 = like extremely). Fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), and chicken by-product meal (CBPM)

Sample Attribute	**FC14**	**FC25**	**DC**	**CM**	**CBPM**	**p-value**
Aroma liking	5.08^{b}	5.18^{ab}	5.59^{a}	5.61^{a}	5.65^{a}	0.047
Appearance liking	5.71^{c}	5.89^{bc}	6.24^{ab}	6.29^{ab}	6.38^{a}	0.008
Overall liking	5.36^{c}	5.59^{bc}	5.93^{ab}	6.00^{ab}	6.07^{a}	0.008
Dog liking*	5.98^{b}	6.20^{ab}	5.93^{ab}	6.41^{ab}	6.58^{a}	0.140

*Predicted by consumers opinion
^{abc} indicates that within a row, unlike letters differ ($p<0.05$)

Table 3.7 Rank order preference pre-ingredient knowledge by consumers (1 = most preferred to 5 = least preferred). Fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), and chicken by-product meal (CBPM)

Sample	**FC14**	**FC25**	**DC**	**CM**	**CBPM**
	3.74^{c}	3.39^{bc}	2.83^{ab}	2.59^{a}	2.46^{a}

^{abc} indicates that within a row, unlike letters differ ($p<0.05$)

Table 3.8 Rank order preference post-ingredient knowledge by consumers (1 = most preferred to 5 = least preferred). Fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), and chicken by-product meal (CBPM)

Sample	**FC14**	**FC25**	**DC**	**CM**	**CBPM**
	2.39^{a}	2.07^{a}	2.53^{a}	3.5^{b}	4.52^{c}

^{abc} indicates that within a row, unlike letters differ ($p<0.05$)

Figure 3.2 Factors most important to consumers when purchasing dog food products asked at the end of the questionnaire

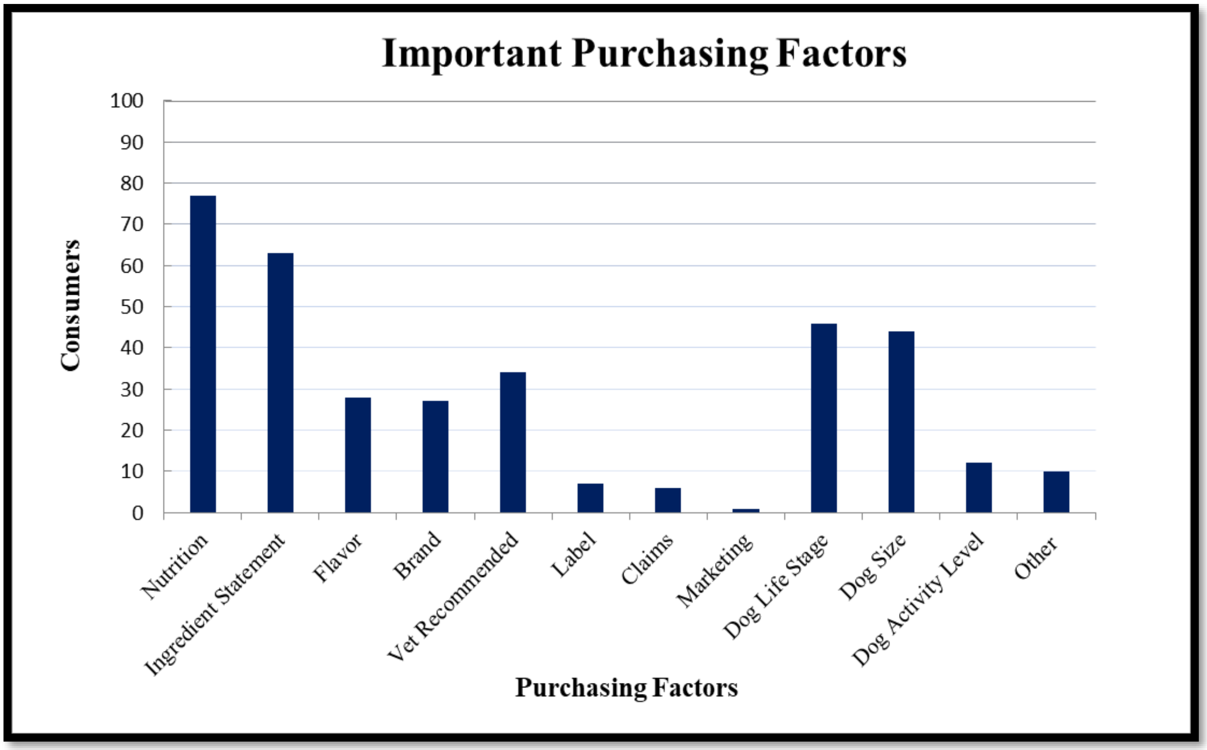
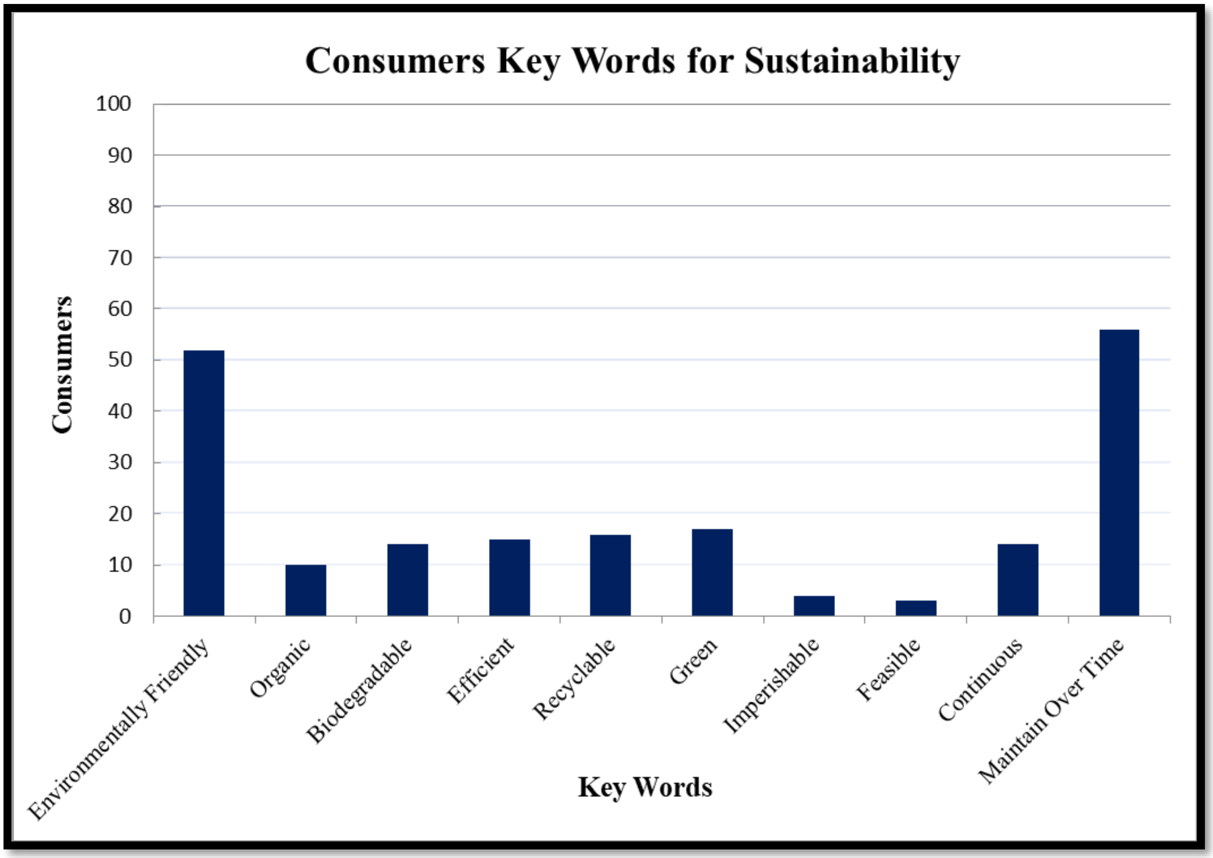


Figure 3.3 Key words describing what sustainability meant to consumers asked at the end of the questionnaire

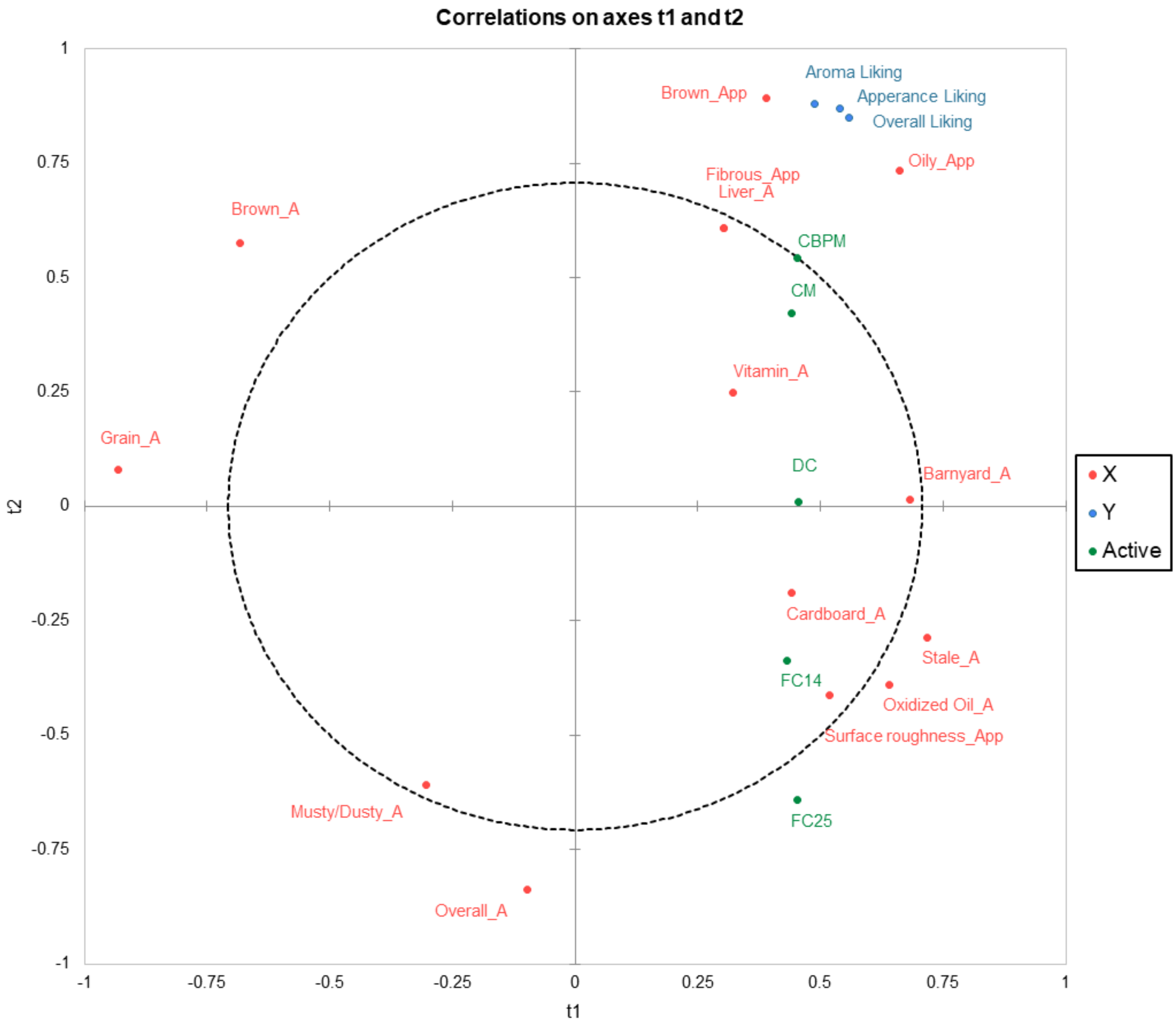


Descriptive and Consumer Data Association

To compare and understand the relationship between consumers' *overall liking* and sensory characteristics of samples, Partial Least Squares regression (PLSR) analysis was performed **(Figure 3.4)**. In the graphic it shows that the CBPM and CM samples were closest to the consumer's *aroma, appearance,* and *overall liking* point. These samples were correlated closest to the *liver* aroma and *fibrous*, *oily,* and *brown* appearance. Di Donfrancesco et al. (2014) measured consumer acceptance of dry dog foods samples and results indicated that the appearance attributes contributed more to overall liking than the aroma attributes. This indicates that the appearance attributes may have driven consumers' liking more than the aroma. The FC14 and FC25 samples were correlated closer to the *cardboard, stale,* and *oxidized oil* aromas and to the *surface roughness* appearance. Di Donfrancesco et al. (2014) also determined that along with appearance, consumers overall liking scores were based more in favor of the mild aromas in the samples. Their sample with the highest *oxidized oil* aroma, based on descriptive analysis, was liked the least by consumers in aroma liking (Di Donfrancesco et al., 2014). Chanadang et al. (2016) used the aroma attributes *cardboard*, *stale,* and *oxidized oil* to describe undesirable aroma characteristics in pet food samples. Consumers from this study scored pet foods with higher intensities in the aroma attributes which decreased their overall liking scores.

Based on palatability results from **Chapter 2**, results suggest that dogs prefer chicken by-products over fresh chicken because by-products are associated with *liver* and *barnyard* aromas. In contrast, the high levels of fresh chicken were associated with *oxidized oil* aromas and are less preferred. As mentioned in Koppel (2014), there are limitations to using human panelists to evaluate canine preference. However sensory analysis methods can be used to better understand the pet, pet owner, and pet owners' selection of food products by providing additional information to pet food manufacturers. Di Donfrancesco et al., (2012) selected, defined, and referenced seventy-two sensory attributes to describe the appearance, aroma, flavor, and texture of dry dog foods. Previous studies, Koppel et al., (2015) and Chanadang et al. (2016) have used sensory analysis methods to determine if any of the sensory attributes that the human panelists could distinguish in the formulas may provide a better understanding of canine preference in palatability tests. These studies, along with future studies, are all useful to the pet food industry to continuously improve dry dog food products.

Figure 3.4 Partial Least Square Regression. Dependent variable (Y): Overall liking score (from consumer panel), Explanatory variable (X): Descriptive sensory analysis (from trained panel). Observations: Kibble samples



CONCLUSION

Descriptive sensory analysis indicated that the intensities for all attributes, excluding *overall aroma,* were in the low intensity range which is similar to previous studies that have also analyzed sensory characteristics of dry dog food samples. This is due to the complex nature of the extruded dog food category as many different ingredients are needed to make an iso-nutrition product. Consumers liked the CBPM sample the most and the FC14 sample the least and also predicted that their dog(s) would like the CBPM sample the most. The correlation between the CBPM sample and *liver* aroma along with *oily, fibrous,* and *brown* appearance could be related to consumer's overall liking. Canine palatability results from the previous chapter **(Chapter 2)** and overall liking results from consumers in this chapter, indicate that chicken by-products should be utilized as ingredients in dry dog foods. Consumers scored the CBPM sample the highest in preference ranking, however, when they learned of the chicken protein sources used, they changed the CBPM sample to the lowest preference ranking. Towards the end of the questionnaire, consumers were asked which purchasing factors they considered when purchasing their dogs' food. The top two terms chosen were 'nutrition' and 'ingredient label' which indicates the consumers are paying more attention to what ingredients are used in their dog's food. Consumers were also asked what sustainability meant to them, and the top two terms chosen were 'environmentally friendly' and 'maintained over time.' As consumers gain more awareness of sustainability practices, biased opinions about animal by-products used in pet foods may be eliminated and seen as a positive move that helps to reduce the environmental impact.

Chapter 4 - Volatile constituents and effect on sensory characteristics of dry dog food formulated with different chicken proteins

ABSTRACT

Qualitative analysis utilizing gas chromatography-mass spectrometry (GC-MS) coupled with the headspace solid-phase microextraction (SPME) method was performed on five dry canine foods containing different chicken protein sources. Separation and tentative identification of thirteen compounds were determined from the highest probability (≥75%) and highest intensity scoring of each compound found in each dry dog food sample. Intensities for each compound were different ($p<0.05$) between the five formulas, but chromatograms were similar due to the ingredients (aside from the chicken proteins) used and the consistency of processing parameters. Intensities and number of compounds were low but reflected the mild aroma of the samples determined by the descriptive analysis. The majority of compounds consisted of carboxylic acids and aldehydes. Partial least squares regression was performed to identify correlations between sensory characteristics and instrumental data. Hexanal, heptanoic acid, 2-heptanone, and octanoic acid seemed to be related to oxidized oil aromatics while acetic acid, propanoic acid, and butanoic acid correlated closest to the liver attribute. Similar volatile compounds with different intensities were measured in this study; however, it is uncertain whether these volatiles were aromatic in the dry dog food samples or if they contributed to canine preference of the different chicken proteins.

INTRODUCTION

Dog food formulas are made with many different ingredients to form an iso-nutritional product. Aside from the food product being nutritionally balanced, the product must also be palatable and accepted by the dog. Food consumption is the primary indicator used in the pet food industry to evaluate the palatability of products, though it provides little insight into the complete profile including texture, flavor, and aroma which are needed to help decipher canine preference (Aldrich & Koppel, 2015). Among all these influencing factors, numerous studies suggest that olfaction has an integral role as dogs make their food choices (N. J. Hall et al., 2017; Pétel et al., 2018; Yin et al., 2020). Koppel et al. (2013) describes pet foods as “interesting objects for aromatic composition studies” because of the complexity of each formula. Along with animal palatability and sensory analysis, instrumental analysis can be used to better understand the diverse and complex array of volatile compounds within dog food samples.

Instrumental analysis, such as gas chromatography (GC), paired with mass spectrometry (MS), is best suited for analysis of molecular compounds and can be useful for analyzing the chemical compounds found in dry dog foods. The method of solid phase microextraction (SPME) paired with GC-MS has been used to determine the aromatic compounds found in the headspace of dog food samples in multiple studies (Koppel et al., 2013; Di Donfrancesco et al., 2017; Yin et al., 2020). Additionally, there are multiple studies available that investigate the volatile aromatic composition of raw ingredients (grains and meats) that are commonly utilized when formulating pet food products (Buśko et al., 2010; M. Chen et al., 2017; Wettasinghe et al., 2000). Dry dog foods are formulated utilizing many different ingredients and understanding the aromatic composition of these products may provide additional insight into compounds driving canine preference (Koppel et al., 2013).

Chicken proteins are commonly used within the pet food industry, including chicken by-products. However, given the current humanization and premiumization trends, pet owners' preferences for fresh chicken over chicken by-products is putting a strain on the global demand for overall chicken protein sources (Okin, 2017) (Nijdam et al., 2012). Along with previous studies evaluating compounds using SPME GC-MS within dog food samples, studies analyzing chicken meat ingredients have been performed (Goodridge et al., 2003; Horvat, 1976; Rivas-Cañedo et al., 2009; Schindler et al., 2010; Wettasinghe et al., 2000, 2001). Wettasinghe et al. (2001) reported that the total volatile flavor concentration of chicken by-product blend was three times higher than that observed for cooked chicken white muscles.

Other than studies measuring the volatile compounds in fresh chicken and chicken by-products separately, there is a lack of published research as to the impact of different chicken protein sources utilized in dry dog foods and their impact on volatile compounds detected by SPME GC-MS. It is uncertain if any of these volatile compounds found in fresh chicken, dried chicken, chicken meal, or chicken by-product meal correlate to canine preference. If there is a correlation between volatile compounds found in chicken by-products to sensory analysis and canine preference, this may further support the use of chicken by-products in pet foods. The objectives of this study were to detect the highest intensity volatile compounds in the five formulas manufactured with the different chicken proteins and to understand the possible relationship between instrumental and descriptive sensory analysis data.

MATERIALS & METHODS

Samples

Five experimental formulas were produced in the Experimental Food Lab (EFL) at the Hill's Pet Nutrition Center in Topeka, KS. The five formulas were formulated to meet American Feed Control Officials (AAFCO 2021) canine maintenance nutritional requirements and utilized four different chicken proteins: chicken meal (CM), dried chicken (DC), chicken by-product meal (CBPM), fresh chicken. The fresh chicken was tested at two levels: at 14% inclusion (FC14) and at 25% inclusion (FC25). The remainder of each formula was composed of brewer's rice, rice protein concentrate, soybean oil, cellulose, beet pulp, vitamins, minerals, and an antioxidant blend **(Table 2.1 - Chapter 2)**.

Extraction of volatile compounds

Headspace-solid phase microextraction (HS-SPME) was used to sample the volatile compounds found in each of the five dry canine formulas containing the different chicken proteins. This similar method has been used in previous studies (Koppel et al., 2013; Di Donfrancesco et al., 2017). Whole kibbles from each formula were weighed out, 2 g, and placed in a 20 mL screw-cap vial (Wheaton, DWK Life Sciences, Millville, NJ, USA) equipped with a polytetrafluorethylene/silicone septum (Wheaton, DWK Life Sciences, Millville, NJ, USA). The internal standard utilized was 50µL of thujone dissolved in methanol (Sigma Aldrich, St. Louis, MO, USA) for a final concentration of 5.007µg/mL aqueous thujone solution. Vials were incubated in an autosampler (MPS Robotic Pro, Gerstel, Linthicum Heights, MD, USA) for 25 minutes at 50 °C and then agitated at 250 rpm. Following incubation, a 2 cm 50/30µm divinylbenzene/carboxen/PDMS fiber (Supelco Analytical, Bellefonte, PA, USA) was utilized.

The fiber was exposed to the sample headspace for 30 minutes at 50 °C. After sampling, analytes were desorbed from the SPME fiber to the injection port of the GC at 250°C for 6 minutes in splitless mode.

Chromatographic Separation and Mass Spectrometer Analysis

Separation and tentative identification of the volatile compounds were performed on a gas chromatograph (7890A Agilent Technologies, Inc., Santa Clara, CA, USA) coupled with a mass spectrometer detector (5977 Agilent Technologies, Inc., Santa Clara, CA, USA). The GC-MS system was equipped with a DB-WAXetr (Agilent J&W polyethylene glycol) column (Agilent Technologies, Inc., Santa Clara, CA, USA; 30 m x 0.25 mm x 0.25 µm film thickness). The initial temperature of the column was 35 °C. The temperature was maintained for 1 minute, and then increased by 6 °C per minute to 230 °C where it was held at this temperature for 5 minutes. All samples were analyzed in triplicates. Tentative identification of compounds was conducted by matching the mass spectra of unknowns in each sample with those in the Agilent MassHunter Qualitative NIST 2017 library. Compounds with the highest probability (>75%) and highest intensity were evaluated. Each compound from each chromatogram was corrected using the internal standard then the average of each corrected intensity was calculated and recorded for each formula.

Descriptive Analysis data for regression

As previously discussed in **Chapter 3**, a sensory panel of five highly trained panelists from the Sensory Analysis Center, Kansas State University, Manhattan, KS, analyzed the aroma characteristics for the five canine formulas. Each of the panelists had over 1,000 hours of general descriptive sensory analysis training and helped develop the initial lexicon for dry dog foods (Di Donfrancesco et al., 2012). Descriptive sensory analysis, using the modified consensus method, was used to determine the aroma attributes. Those aroma attributes were *barnyard, brothy, brown aromatics, cardboard, grain, liver, musty/dusty, oxidized oil, stale, toasted, vitamin,* and *overall aroma.*

Data analysis

Analysis of variance (ANOVA) was used to analyze volatile compound intensities with Fisher's Protected Least Significant Difference (LSD) post-hoc mean separation to determine ($p<0.05$) differences between samples. 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). This approach for measuring correlation has been utilized in other studies. To perform ANOVA and PLSR, XLSTAT software was utilized (Addinsoft, New York, NY, USA).

RESULTS & DISCUSSION

Volatile Composition

Qualitative analysis utilizing HS-SPME GC-MS was used to determine any correlation between canine palatability **(Chapter 2)** and descriptive sensory analysis **(Chapter 3)**. All five formulas yielded the same thirteen compounds that had both a probability ≥75% and high intensity scoring on the chromatograms. Volatile compounds, along with the internal standard corrected intensity/abundance value of each compound, are recorded in **Table 4.1**. Intensities for each compound were different ($p<0.05$) between the five formulas. For this study, the decision was made to focus on the top volatiles to help elucidate different intensities between the samples formulated with the different chicken proteins. Chromatograms were similar due to the ingredients (aside from the chicken proteins) used and the consistency of processing parameters. The number of compounds were lower compared to other dog food instrumental studies possibly due to the method used and the exclusion of additional topicals such as chicken fat, flavors, or palatants. **Figure 4.1** provides an illustration of intensities for thirteen compounds found in all five formulas. The FC25 formula measured the highest intensity for almost all the compounds where the FC14 formula measured the lowest intensity for most of the compounds. Many of the compounds consisted of carboxylic acids.

Maillard Reaction Compounds

The Maillard reaction is a form of non-enzymatic browning that occurs in foods when proteins and/or amino acids chemically react with carbohydrates of reducing sugars. Applying heat during cooking accelerates and continues this process which typically amplifies aromas. Furans are considered the most abundant compounds of the Maillard reaction (Shibamoto, 1989). The FC25 formula's second highest intensity peak compound was 2-pentylfuran. This result could have been from the high amount of fresh chicken used during processing. Molina-Garcia et al. (2017) explained that organic acids, detected in dry dog foods, may be formed by the Maillard reaction. The highest intensity peak measured in the CBPM sample was the carboxylic acid compound, butanoic acid. Previous literature defines butanoic acid as having a rancid aroma (Chen et al., 2019), however, rancid aromatics were not detected by the descriptive sensory panelists in **Chapter 3**. The Maillard reaction may explain the CBPM formula's higher intensity scores for acetic acid, butanoic acid and propanoic acid because of both the rendering and extrusion process. Compounds from the Maillard reaction may correlate with the positive palatability results reported in **Chapter 2**, which may also contribute to drivers of liking. Unique to the CBPM ingredient, versus the other chicken sources, it contains organ meat. This difference may have been associated with the liver attribute the descriptive panelists described in **Chapter 3**, however, previous research has not correlated this aroma attribute to these carboxylic acid compounds**.** Molina-Garcia et al. (2017) explains that organic acids, detected in dry dog foods, may be formed by the Maillard reaction or by secondary decomposition of lipid oxidative products.

Lipid Oxidation Compounds

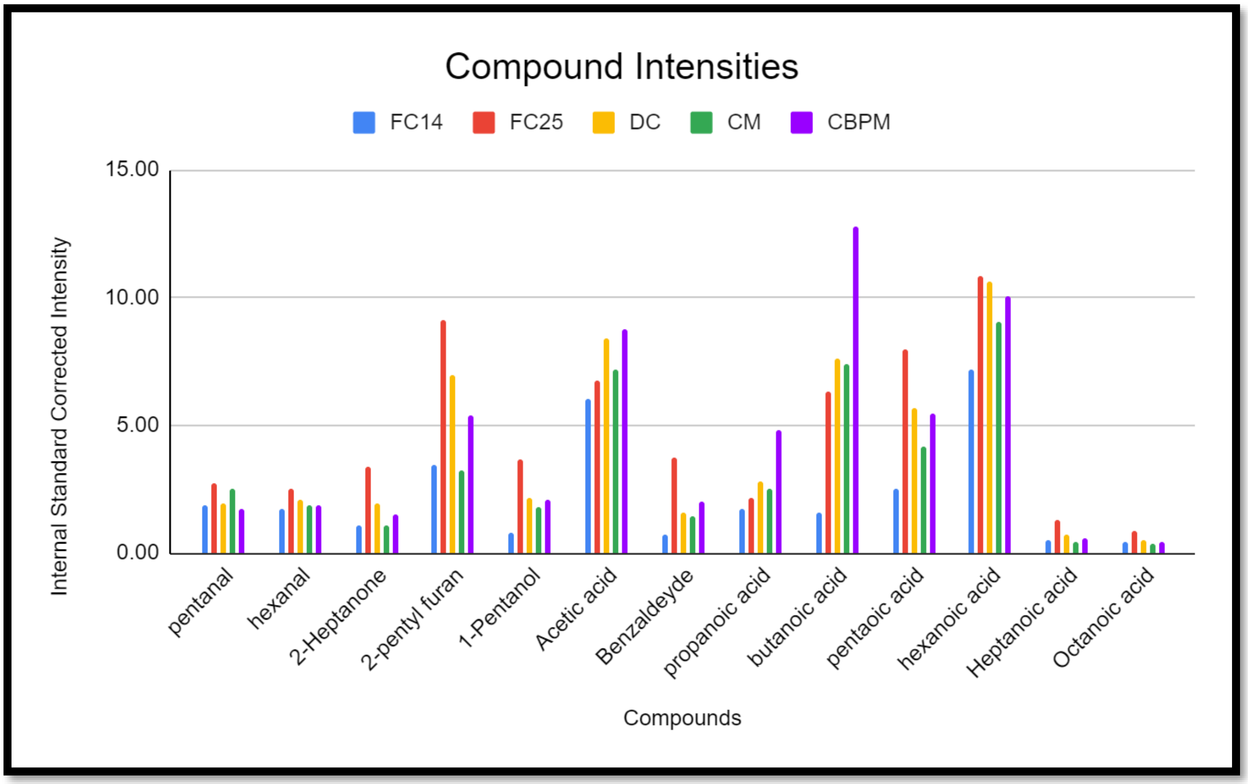
The second highest peak compound measured in four of the five samples was hexanoic acid. In previous studies hexanoic acid has been shown to be a major compound during extensive lipid oxidation (Di Donfrancesco et al., 2017) (Lampi et al., 2015). Hexanal, pentanal, and 2-heptanaone are also compounds produced during lipid oxidation (Elmore et al., 1999). The FC25 formula intensities measured slightly higher in hexanal, pentanal, and 2-heptanaone compared to the other samples. This may be due to the higher contribution of fat from the fresh chicken ingredient. According to Koppel et al. (2014), rancidity in pet foods is recognized by volatile compounds that result from the oxidation of fatty acids (e.g., hexanal, heptanal, octanal). The FC25 formula received the highest intensity score for the attribute oxidized oil. Benzaldehyde was presented at relatively lower levels between all five canine formulas. Di Donfrancesco et al. (2017) also reported lower intensity levels of benzaldehyde in their dog food samples. Benzaldehyde can be a thermal reaction product of hexanal and has been identified in cooked rice and other dry dog food products (Buttery et al., 1988; Donfrancesco & Koppel, 2017). Aside from acetic acid and hexanoic acid, the FC14 formula measured on the lower scale for all compound intensities. The FC14 formula contributed the lowest % of protein and fat from the fresh chicken ingredient and therefore provides overall lower aromatic compounds.

Table 4.1 Internal standard corrected intensity/abundance of compounds found in formulas: Fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), and chicken by-product meal (CBPM)

Sample	**FC14**	**FC25**	**DC**	**CM**	**CBPM**	**p-value**
Compounds						
Alcohols						
1-Pentanol	0.82^{c}	3.65^{a}	2.16^{b}	1.80^{b}	2.09^{b}	<0.0001
Aldehydes						
Pentanal	1.90^{bc}	2.75^{a}	1.94^{bc}	2.49^{ab}	1.70^{c}	0.025
Hexanal	1.74^{b}	2.55^{a}	2.07^{b}	1.89^{b}	1.88^{b}	0.008
Benzaldehyde	0.71^{c}	3.76^{a}	1.57^{b}	1.42^{b}	2.03^{b}	<0.0001
Furans						
2-Pentylfuran	3.45^{d}	9.10^{a}	6.96^{b}	3.21^{d}	5.40^{c}	<0.0001
Ketones						
2-Heptanone	1.10^{c}	3.38^{a}	1.93^{b}	1.07^{c}	1.50^{bc}	<0.0001
Carboxylic Acids						
Acetic acid	6.03^{c}	6.76^{bc}	8.42^{a}	7.18^{b}	8.74^{a}	<0.0001
Propanoic acid	1.69^{d}	2.16^{cd}	2.79^{b}	2.51^{bc}	4.81^{a}	<0.0001
Butanoic acid	1.58^{d}	6.30^{c}	7.61^{b}	7.42^{bc}	12.80^{a}	<0.0001
Pentaoic acid	2.54^{d}	8.00^{a}	5.70^{b}	4.20^{c}	5.45^{b}	<0.0001
Hexanoic acid	7.20^{c}	10.85^{a}	10.59^{a}	9.02^{b}	10.06^{ab}	0.001
Heptanoic acid	0.48^{bc}	1.30^{a}	0.71^{b}	0.46^{c}	0.58^{bc}	<0.0001
Octanoic acid	0.45^{b}	0.87^{a}	0.51^{b}	0.34^{b}	0.45^{b}	0.01

*Least significant differences are shown with superscript letters following the average intensity scores. Letters that are the same for a treatment attribute in a row are not significantly different ($p>0.05$).

Table 4.2 Internal standard corrected intensity/abundance of compounds found in formulas: Fresh chicken 14% (FC14), fresh chicken 25% (FC25), dried chicken (DC), chicken meal (CM), and chicken by-product meal (CBPM)



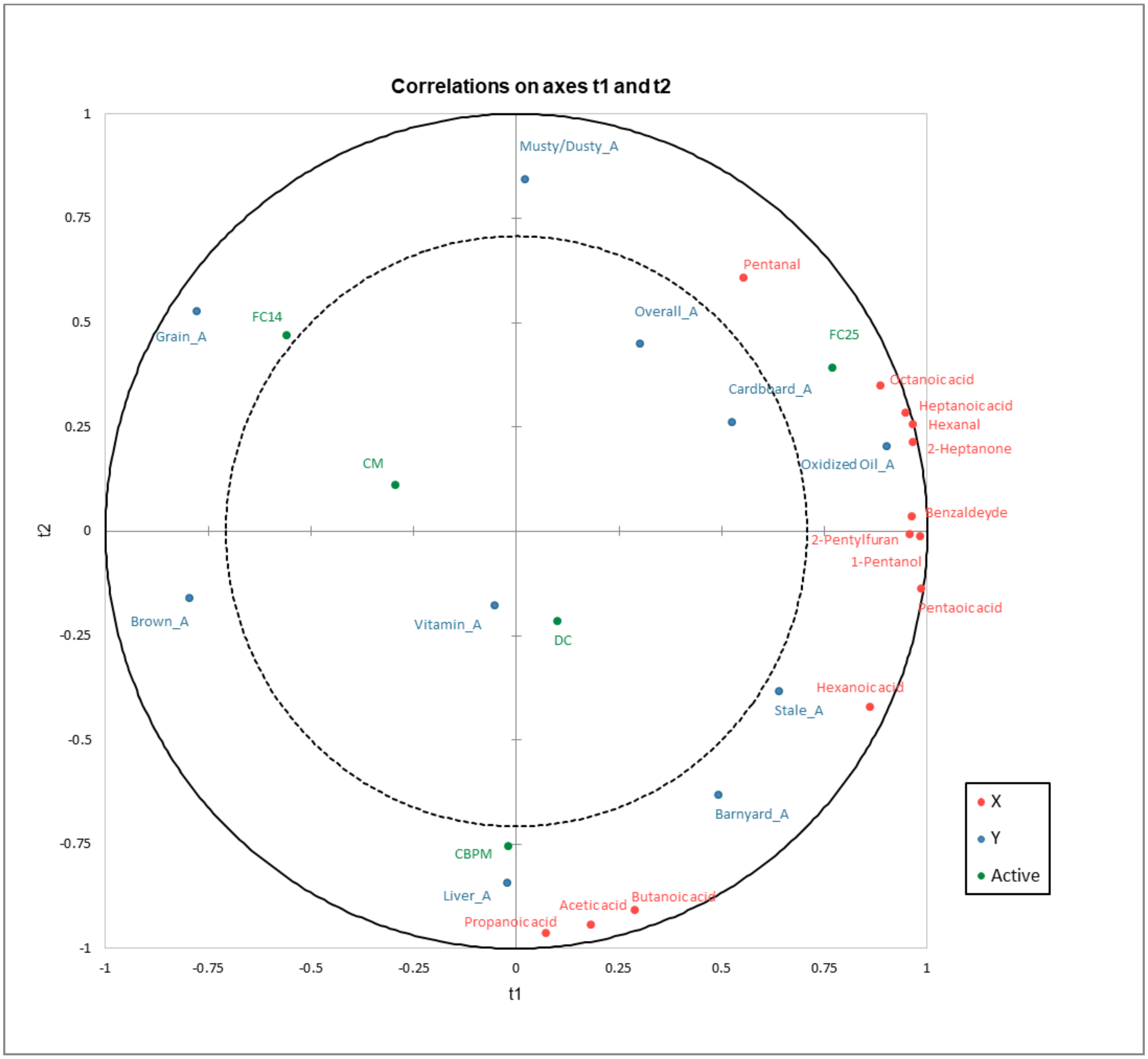
Association of Sensory Attributes and Volatile Compounds

Partial least square regression (PLSR) results with sensory aroma attributes related to instrumental data is shown in **Figure 4.2.** Volatile compound data variation explained 57% of descriptive sensory analysis data variation. PLSR results indicated that hexanal, heptanoic acid, and 2-heptanone correlate to *oxidized oil* aromatics. Previous literature has also reported correlations between hexanal and oxidized oil aromatics in dry dog foods (Koppel et al., 2013; Di Donfrancesco et al., 2017; Yin et al., 2020).

PLSR results also indicated that acetic acid, propanoic acid, and butanoic acid correlated closest to the *liver* attribute. Previous literature does not indicate that these three compounds correlate to this aroma attribute. Although Yin et al. (2020) reported acetic acid and propanoic acid were among the major aroma compounds evaluated and butanoic acid was reported in two of the dog food samples. Descriptive sensory analysis was not conducted in their study to determine the aromas of these dog food samples.

As described by Chambers & Koppel (2013), it can be difficult to associate volatile compounds with sensory characteristics. In this study, canine palatability results indicated a preference for the CBPM formula when compared to the other chicken formulas. The CBPM formula had higher levels of Maillard reaction compounds while the FC25 formula had higher levels of lipid oxidized compounds. The FC25 formula was not preferred when compared to the CBPM formula in canine palatability testing, or in consumer testing, and according to SPME GC-MS correlations this may be because of the intensities of the lipid oxidation compounds.

Figure 4.1 Partial Least Squares Regression Dependent variable (Y): Descriptive sensory data, Explanatory variable (X): Instrumental volatile data, Observations: Kibble samples



CONCLUSION

Thirteen compounds were separated and qualitatively identified in five dry dog food samples manufactured with different chicken protein sources by the highest probability (≥75%) and highest intensity scoring of each compound found. Intensities for each compound were low but different ($p<0.05$) between the five formulas with the FC25 formula measuring the highest intensity for almost all the compounds and the FC14 formula measuring the lowest intensity for most of the compounds. Partial least squares regression was performed to identify correlations between sensory characteristics and instrumental data. Results indicate some associations found between volatile compounds and sensory attributes such as hexanal, heptanoic acid, and 2-heptanone to *oxidized oil* aromatics and acetic acid, propanoic acid, and butanoic acid to *liver* aromatics. The results from this study concluded that SPME GC-MS qualitatively identified volatile compounds from the headspace from the dry dog foods formulated with the different chicken proteins, however it does not indicate if these compounds were actually aromatic. Chambers & Koppel (2013) describe that it is often difficult to associate volatile compounds with specific sensory characteristics and more research to better understand the relationships between the two is needed.

Figure 4.2 Fresh Chicken 14% (FC14) Chromatogram

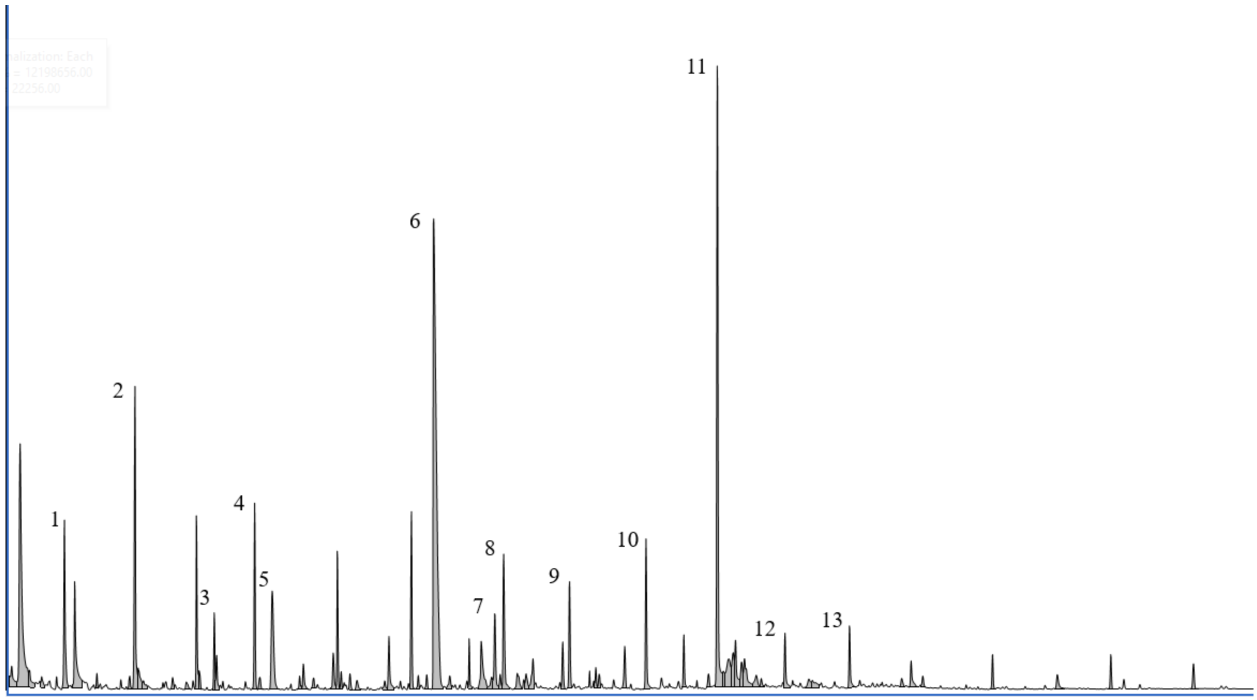


Figure 4.3 Fresh Chicken 25% (FC25) Chromatogram

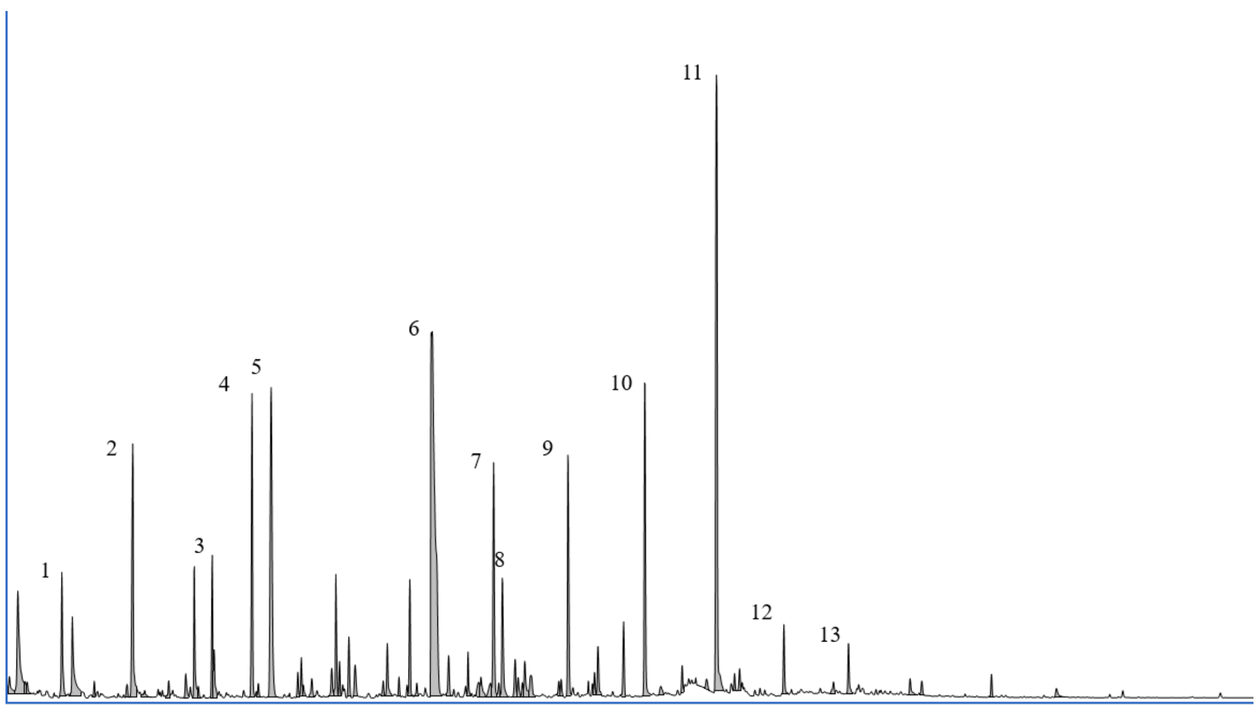


Figure 4.4 Dried Chicken (DC) Chromatogram

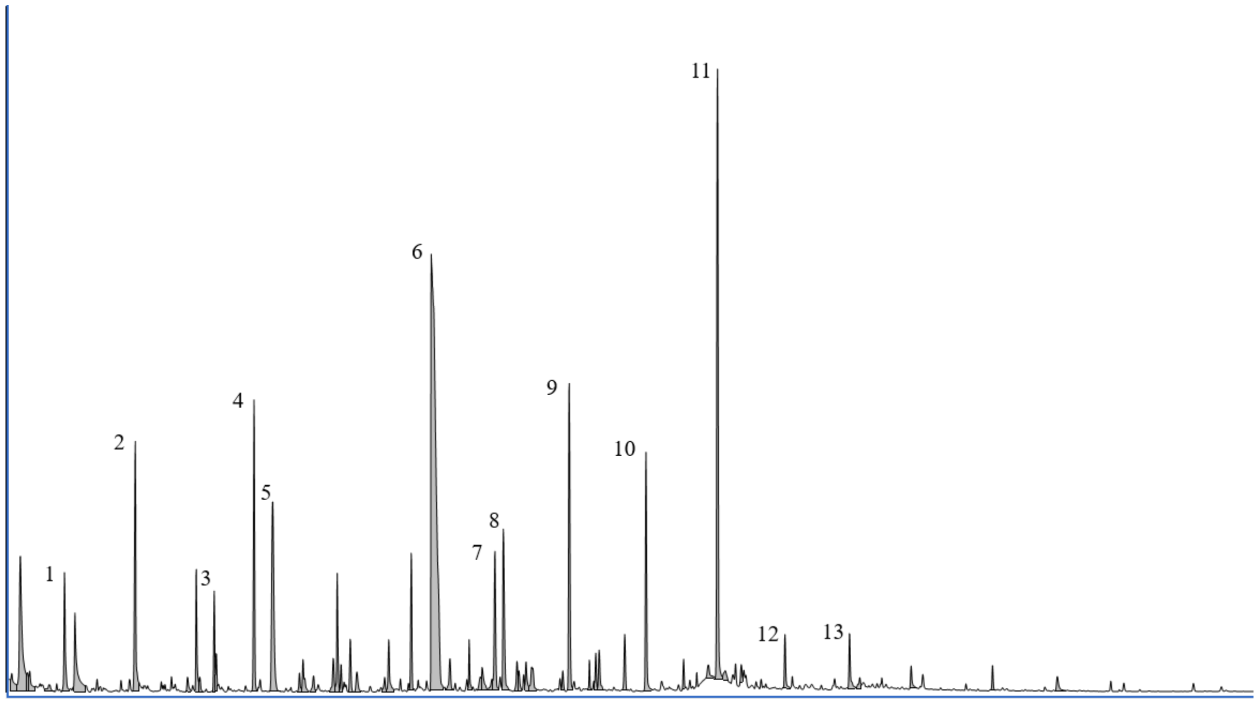


Figure 4.5 Chicken Meal (CM) Chromatogram

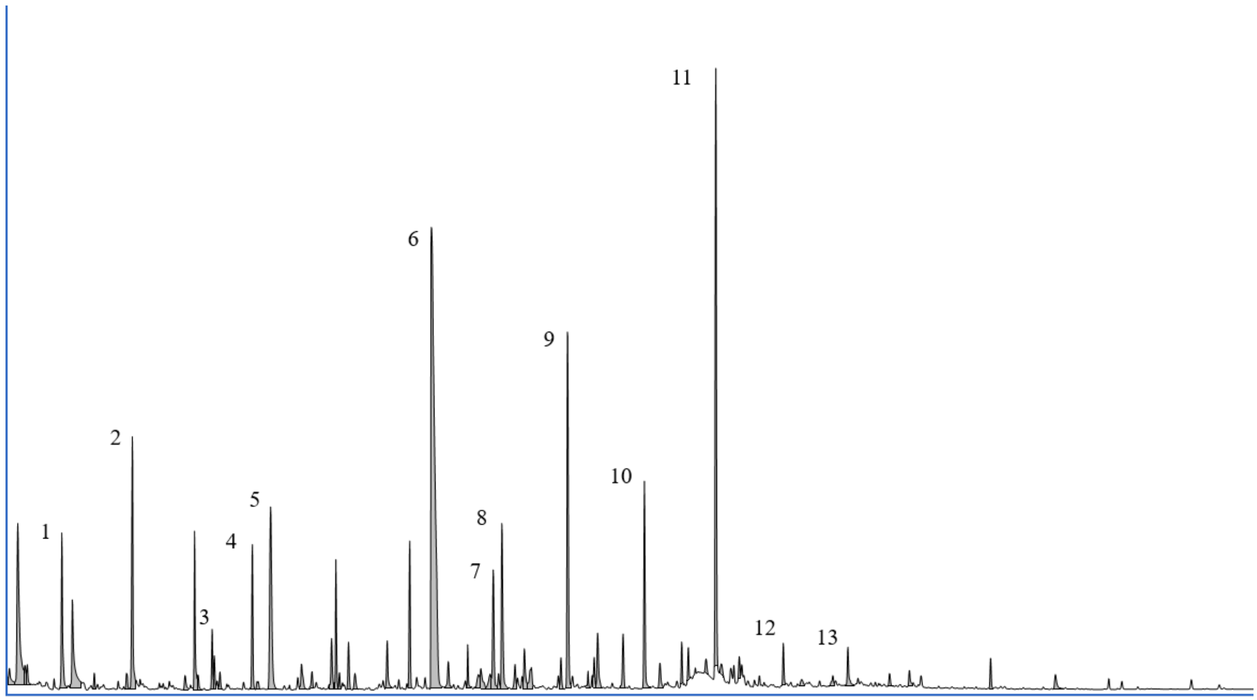
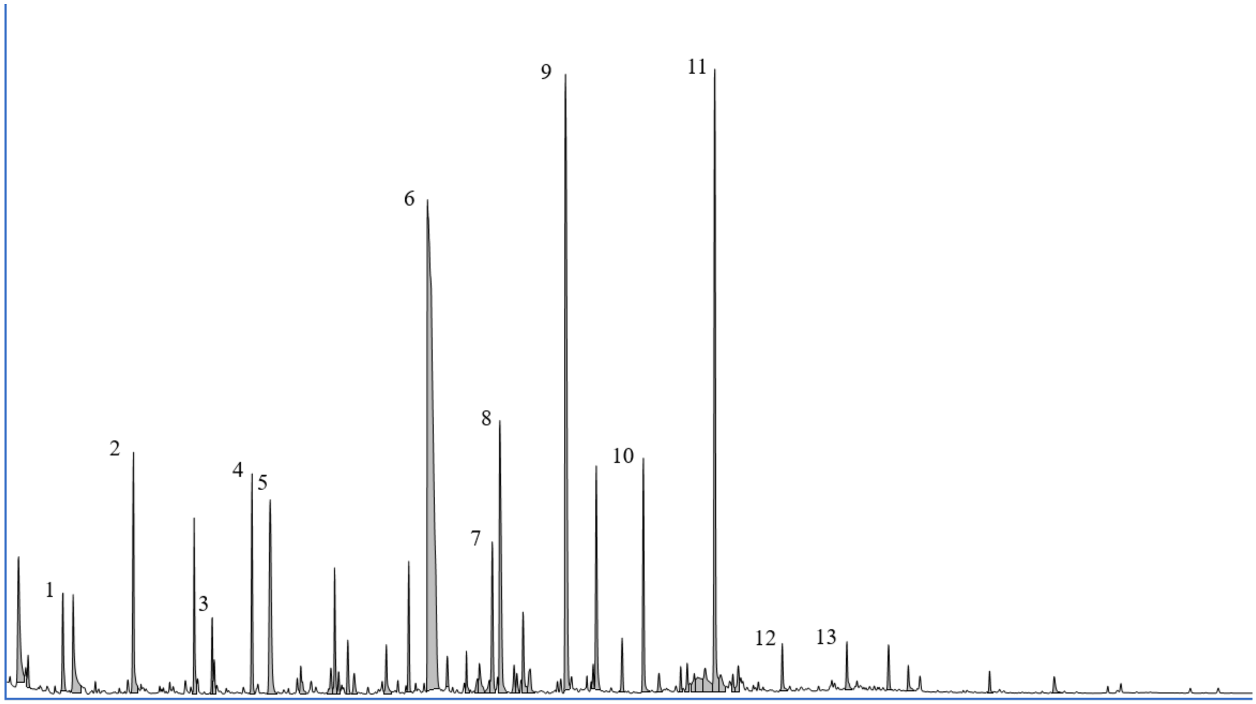


Figure 4.6 Chicken By-Product Meal (CBPM) Chromatogram



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Appendix A - AAFCO canine nutrient profile for adult maintenance requirements (DMB) - Chapter 2

Nutrient	**Minimum**	**Maximum**
Protein, %	18	
Fat, %	5.5	
Calcium, %	0.5	2.5
Phosphorus, %	0.4	1.6
Potassium, %	0.6	
Sodium, %	0.08	
Chloride, %	0.12	
Magnesium, %	0.06	
Iron, mg/kg	40	
Copper, mg/kg	7.3	
Manganese, mg/kg	5	
Zinc, mg/kg	80	
Iodine, mg/kg	1	11
Selenium, mg/kg	0.35	2
Arginine, %	0.51	
Histidine, %	0.19	
Isoleucine, %	0.38	
Leucine, %	0.68	
Lysine, %	0.63	
Methionine, %	0.33	
Methionine-Cystine, %	0.65	
Phenylalanine, %	0.45	
Phenylalanine-Tyrosine, %	0.74	
Threonine, %	0.48	
Tryptophan, %	0.16	

Valine, %	0.49	
Linoleic Acid, %	1.1	
Vitamin A, IU/kg	5,000	250,000
Vitamin D, IU/kg	500	3,000
Vitamin E, IU/kg	50	
Thiamine, mg/kg	2.25	
Riboflavin, mg/kg	5.2	
Niacin, mg/kg	13.6	
Pyridoxine, mg/kg	1.5	
Pantothenic Acid, mg/kg	12	
Folic Acid, mg/kg	0.216	
B12, mg/kg	0.028	
Choline mg/kg	1360	
Ca:P Ratio	1	2

Appendix B - Questionnaire for consumer study - Chapter 3

21509 INCA CLT - Hill's Dog Food (Chelsie)
Test Date; 12/16/21
N=100

Sensory and Consumer Research Center

Welcome Panelist name!

Click the *next* button to begin

1. I agree to participate as a panelist in research conducted by the Sensory & Consumer Research Center.
2. I understand that the purpose of this project is to participate in a **dog food** test.
3. I understand that this is a **confidential test** and I'm not to talk with others about what I evaluated.
4. I will receive participation payment when I complete this study.
5. I understand my performance as an individual will be treated as research data and will in no way be associated with me for anything other than identification purposes, thereby assuring confidentiality of performance and responses.
6. I understand that I do not have to participate in this research and may choose not to participate without penalty.
7. I understand that I may withdraw at any time.
8. If I have any questions concerning this study, I understand that I may contact Martin Talavera at 913-307-7324 at the KSU Olathe Campus.
9. If I have any questions about my rights as a consumer or about the manner in which this research was conducted, I may contact Dr. Rick Scheidt, Chair, Committee on Research Involving Human Subjects, 1 Fairchild Hall (532-2334), Manhattan, KS, 66506.

By typing my name in the space below, I am providing my electronic signature and acknowledging that I understand the above statements.

You will be evaluating 5 dry dog food samples today that provide balanced nutrition for your pet.

You will be evaluating the kibble, one sample at a time, based on their appearance and aroma.

We are interested in what you think about these dog food samples, NOT what your dog might think, unless specified in the question.

Please read all instructions and questions carefully.
Click *next* to continue.

Please remove mask now so that you can smell the kibble samples.

Please wait for your FIRST Kibble sample.

INCA 1/9

DO NOT OPEN CONTAINER!

When you have received sample **BC111**, click *next* to continue.

1:00

Open the container now.

SNIFF the kibble before answering the following question.

Sample: BC111

How much do you like or dislike the **aroma** of this kibble?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample: BC111

How much do you like or dislike the **appearance** of this kibble?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Considering the **aroma** and **appearance**, how much do you like or dislike this kibble sample **overall**?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

INCA 2/9

How much do you think **your dog** would **like or dislike** this kibble?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Using between 1 and 5 single terms or descriptors, please say what you **LIKED** about this product.

Sample: BC111

Using between 1 and 5 single terms or descriptors, please say what you **DISLIKED** about this product.

Sample: BC111

Push your sample aside, making sure to keep them all in the same order as you receive them.

Click Next.

Thinking about the 5 dry dog food kibble samples you just reviewed, please RANK them in order of PREFERENCE.

- Click & drag the code of the MOST PREFERRED sample into the box labeled "1st".
- Click & drag the code of the SECOND MOST PREFERRED sample into the box labeled "2nd".
- Repeat for the THIRD MOST PREFERRED (3rd box) and FOURTH, and FIFTH samples

- Samples are listed from left to right in the order that they were served.

1st | 2nd | 3rd | 4th

5th

BC111 | BC222 | BC333 | BC444 | BC555

All 5 of the samples are foods that **provide the same nutrition to your dog** and are made using the same ingredients, with the **only difference being the protein source used**:

- **229 - Dried Chicken**
- **706 - Chicken Meal**
- **467 - Chicken By-Product Meal**
- **101 - Fresh Chicken at Moderate Inclusion**
- **893 - Fresh Chicken at High Inclusion**

Based on the Protein Ingredient information given above, the Aroma and Appearance, please RANK the 5 kibble samples in order of PREFERENCE.

- Click & drag the code of the MOST PREFERRED sample into the box labeled "1st".
- Click & drag the code of the SECOND MOST PREFERRED sample into the box labeled "2nd".
- Repeat for the THIRD MOST PREFERRED (3rd box) and FOURTH, and FIFTH samples

- Samples are listed from left to right in the order that they were served.

1st	2nd	3rd	4th

5th

Chicken ingredient is listed in a photo under each code.

BC111	BC222	BC333	BC444	BC555
Sample	Sample	Sample	Sample	Sample

Did having the **ingredient information** change how much you **liked or disliked** the different dry dog food kibbles you evaluated?

- ○ Yes
- ○ No

If learning the protein ingredient in the dry dog kibble **changed your kibble ranking**, please explain why.

Please put your mask on now before answering last few questions.

What **age range** does your dog fall? (If you have multiple small dogs please check all that apply)

- ☐ Puppy (0-12 months)
- ☐ Adult (1-8 years)
- ☐ Senior (9 years and older)

When purchasing DRY dog food, which **BRAND** do you prefer? (choose one)

- Science Diet ???
- Eukanuba ???
- Purina Pro Plan ???
- Royal Canin ???
- Pedigree ???
- Blue Buffalo ???
- Wellness ???
- Natural Choice ???
- Merrick Pet Foods ???
- Natural Balance ???
- Alpo ???
- Nature's Recipe ???
- Purina ONE ???
- Beneful ???
- Iams ???
- Other (please specify)
- I don't feed my dog DRY dog food

How often do you change dry dog food brands?

- I never change dog food brands
- Once a month
- Once every 2 to 6 months
- Once a year
- Less than once a year

Which best describes **your role** in **purchasing** pet food, supplements and treats for your household/family?

- I do all of the purchasing
- I do more than half the purchasing
- I do half the purchasing
- I do less than half the purchasing
- I do not do any of the purchasing

What **factors are most important** to you for purchasing a product (check all that apply)

- Nutrition ???
- Ingredient Statement ???
- Flavor ???
- Brand ???
- Vet Recommended ???
- Label ???
- Claims ???
- Marketing ???
- Life Stage ???
- Dog Size ???
- Dog Activity Level ???
- Other (please specify)
- None of the above

What does **sustainability** mean to you?

- Environmentally Friendly ???
- Organic ???
- Biodegradable ???
- Efficient ???
- Recyclable ???
- Green ???
- Imperishable ???
- Feasible ???
- Continuous ???
- Maintain Over Time ???
- Other (please specify)
- None of the above

Sensory and Consumer Research