

Evaluating sensory characteristics, consumer acceptance and
volatile compounds in freeze-dried cat treats

by

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Abstract

The freeze-dried pet food category is steadily growing along with growth in pet ownership and the importance of pet humanization trend. However, freeze-dried pet food, treats, or related freeze-dried meat products has not been studied relative to their sensory characteristics, in which descriptive sensory analysis or comparison to volatile compounds analysis has been conducted. The objectives of these studies were to 1) develop a lexicon to describe the sensory characteristics of freeze-dried cat treats products, 2) determine consumer perception of freeze-dried cat treats and emotional responses of both cats and cat owners, and compare consumer acceptance of the products between a Central Location Test (CLT) and a Home Use Test (HUT), 3) determine the volatile aromatic compounds that generate perceivable aromas from these treats and 4) examine the relationship between aromatic sensory attributes, consumer acceptance and volatile components. Thirty-two products, available in the US market, were used to develop the sensory lexicon. The products represented a range of characteristics within the product category, such as cost, meat type, and ingredient composition. Five highly trained descriptive sensory evaluators identified, defined and referenced twenty-seven appearance, aroma, and texture sensory attributes. Six of the samples were evaluated in CLT and HUT consumer tests and volatile analysis. Volatile compounds from the products were analyzed using headspace solid-phase microextraction (SPME), gas-chromatography – mass spectrometry (GC-MS). A total of 9 appearance, 5 texture/handfeel, 13 aroma attributes, and more than 60 volatiles were identified to describe the samples. Principal component analysis (PCA) was used to map the scores obtained during the validation phase of the lexicon terminology. Overall fish and cardboard were common aromatics, while the samples were differentiated by overall beef, poultry, and decaying animal attributes. The more abundant volatiles included hydrocarbons,

aldehydes, and ketones. There was a significant difference in overall liking scores on some product samples from the owners and cats perspectives when comparing the CLT and HUT. Both cats and their owners seemed to prefer the single ingredient treats the most. The consumers were segmented into five clusters. The emotions of the owners and the cats were similar between the samples. The primary emotions noted for the owners included happy, interested, curious, relaxed and comfortable; while, the cats were happy, curious, excited, content/satisfied and comfortable. Combining the sensory aromatic attributes with volatile compounds helps understand sensory properties of these and similar healthy pet products. Chicken treat samples with single ingredients and fish treats with mixed ingredients were higher in oxidized oil aroma, which was associated with hexanal. This research will help the pet food industry identify the characteristics of freeze-dried cat treats, how this relates to consumer acceptance and the potential white spaces (gaps) in the product category.

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Chapter 1 - Literature Review

Sensory Analysis

Sensory evaluation is a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (Stone and Sidel, 2004). The sensory evaluation method has been a useful tool for providing the sensory characteristics for products in various areas. For example, these include product development, food science, research and development, quality assurance, and quality control in both food and non-food fields (Lawless & Heymann, 2010).

There are three main classes of sensory testing. Two are classified as analytic tests and include discrimination testing and descriptive testing, and the third is the affective testing which is categorized as a hedonic type of test (Lawless & Heymann, 2010). Each of these classes is used for a different purpose.

Discrimination tests examine if there is a detectible difference between products. For example, a respondent is asked to select the odd item out from three samples in a triangle test, in which two items are the same and third is different (Lawless & Heymann, 2010).

Descriptive tests aim to describe the sensory characteristics of the products, identify the attributes and quantify the intensities of those product characteristics. In product development, this test assists in identifying sensory profiles of the products which help detecting how close those characteristics are to the target. For quality assurance, this test is helpful for troubleshooting the product characteristics and defects. Moreover, with statistical analysis techniques, combining the descriptive information with instrumental or consumer acceptance data can help to attain better understanding of the products. The more common descriptive

analysis techniques are flavor profile, quantitative descriptive analysis, texture profile, and spectrum.

Affective or hedonic tests investigate how much the consumers like or dislike a product. This test provides opportunities for researchers to probe the reasons for liking and disliking certain products. Moreover, with statistical analysis, the test would also provide information on potential consumer segmentation. Preference testing, also an affective test, investigates whether the consumers prefer one product over another while acceptance testing is to quantify degree of liking or disliking of a product. The most common scale used to quantify acceptability is the 9-point hedonic scale. This scale has been shown to be useful in the hedonic assessment of foods, beverages, and non-food products for decades. However, there are potential problems associated with category scales such as the categories are not quite equally spaced, the neutral (“neither like nor dislike”) category makes the scale less efficient and consumers tend to avoid the extreme categories (Lawless & Heymann, 2010). Peryam and Pilgrim (1957) mentioned that the hedonic scores can be changed as a result of differences in environment conditions. However, the relative changes among the samples would be similar.

Based on locations, consumer study is classified into to Central Location Test (CLT) and Home Use Test (HUT). The Central Location Test is the most popular type. It is conducted in the facilities provided by the agency, which could be in malls or a mobile testing laboratory, for example. These settings would assist in controlling proper product preparation, product presentation and evaluation environment. However, the consumers might be limited in product exposure. In the Home Use Test (HUT) the participants are asked to use the products under normal circumstances. This test would provide more realistic information, but the cost would be more expensive and time consuming (Lawless & Heymann, 2010). To explore and develop

new product concepts, qualitative consumer studies such as interviews or observations are the most applicable techniques (Lawless & Heymann, 2010). The methods are flexible and less structured which allow the flow of investigation to change depending on the situation.

Conducting focus groups, one-on-one interviews and observational method would assist probing and extracting in-depth information from the consumers. In addition, these approaches would be useful for explaining behavior.

Lexicon Development

Sensory lexicons are standardized vocabularies that objectively describe the sensory properties of consumer products which facilitates communication across diverse audiences. It has been used as a tool for communication with panelists, product developers, marketing professionals and suppliers, and the need for lexicons has been increased (Lawless and Civille, 2013).

Sensory lexicons have been developed and used for describing sensory characteristics of products in various areas. For example, food and beverages (Suwonsichon et al., 2020; Belisle, 2017; Chambers et al., 2016; Pujchakarn et al., 2016; Bett-Garber et al., 2013; Lee et al., 2013), personal care products (Dooley et al., 2009; Sun et al., 2014) and fragrances (Verrielle et al., 2012) have created sensory lexicons. In meat products, the standardized lexicon of meat known as warmed-over flavor was developed using beef patties in which varying reheating methods have been applied (Johnson and Civille, 1986). Cooked lean beef and cooked fatty beef aroma intensity decreased for the reheated samples when compared to the control. On the other hand, cardboard and oxidized/rancid/painty aroma attributes were scored higher in the reheated patties. The terms generated are applicable to determining off-flavor in meats and helpful in the area of

meat product development, quality control and shelf-life stability. This has been beneficial to these industries as their sensory terminologies are standardized and used across multiple panels, companies and countries.

Sensory Analysis and Pet Food

Pets are unable to describe the reasons why they like one product over another.

Palatability testing with animals provides limited information on the reasons for liking or preference. Tests with animals could be expensive, require large amounts of material, and are time consuming (Booth, 1976). Although perception systems - the process of recognizing and interpreting sensory stimuli - are different between *Felis catus* and *Homo sapiens* in terms of taste and olfactory cues (Chaudhari and Roper, 2010; Thorne, 1992; Neufeld, 2012), the pet owner would likely be the ones who determine whether the food is acceptable for their pets (Koppel, 2014). Pet food manufacturers need to satisfy pets' palatability and also the pet owners' expectation towards their pets' food (Delime et al., 2018). Thus, using human sensory panels to assess cat food sensory properties could be a way to link the pet and pet owner perception of food with a product concept to address sustained consumption by the pet. Appearance and smell are important factors that contribute to acceptance by the pet owner (Delime et al., 2018). Delime et al. (2020) conducted cross-cultural research to describe the link between pet food odor and the pet owners' emotional response for consumers from three different countries, Kansas (USA), Brittany (France), and the Island of Reunion. It was reported that pet owners' perception of pet food odor, as well as emotional experience could be affected by culture. This supports the idea that developing pet food needs to satisfy pet owners' expectations, appearance and smell, but also the origin of the consumers matter.

In the field of pet food studies, Di Donfrancesco, Koppel, and Chambers (2012) developed an initial lexicon for sensory properties of dry dog food using the products available in the US market. There have been some studies on development of sensory evaluation method using human taste panels to optimize dried cat food products by (Pickering, 2009a) and canned cat food by (Pickering, 2009a), and development of an aroma attributes lexicon for cat foods, for retorted cat foods (Koppel and Koppel, 2018).

In pet food consumer studies, Di Donfrancesco et al. (2014) and Di Donfrancesco, Koppel and Aldrich (2018) conducted a Central Location Test (CLT) with dog owners to assess preference. Di Donfrancesco, Koppel and Aldrich (2018) also conducted a Home Use Test (HUT) for palatability study with dogs. Even though it was harder to control the testing environment, conducting a Home Use Test would be an appropriate method to evaluate a more natural feeding environment - particularly with cats (Tobie et al, 2015). Conducting CLT in comparison to HUT with cat treats has not previously been reported.

Associations of Sensory Analysis with Instrumental Methods

Sensory descriptive analysis is a method which utilizes highly trained panelists as measurement instruments to identify attributes and quantify intensities of product sensory attributes (Lawless and Civille, 2013). This approach can provide sensitive information that analytical methods may not (Hootma, 1992). In these cases, when the association between sensory and instrumental analysis has been established, using analytical methods could be more cost and time effective versus a human panel (Lawless & Heymann, 2010). Combining instrumental measurements with sensory analysis techniques and investigating relationship between sets of data could be helpful for such things as the relationship between aroma attributes

and chemical compounds (Chambers & Koppel, 2013). Evaluating aromatic composition with descriptive analysis could increase the understanding of product attributes (Lawless & Heymann, 2010; Yang, 2020; Lu et al., 2017; Velásquez et al., 2019; Lee et al., 2018). Headspace analysis is one example of instrumental determination procedures for characterizing volatile components in food (Chambers & Koppel, 2013).

Sensory attributes have been associated with volatile compounds in various kinds of food products. For example, the technique has been applied to such products as coffee (Velásquez et al., 2019), Sichuan peppers (Yang, 2020), green tea (Lee et al., 2018), and wines (Torrens et al., 2010; Lee et al., 2006; Nui, 2011). Some studies have investigated the association between aromatic sensory attributes and volatile compounds using headspace solid-phase microextraction (SPME), gas-chromatography – mass spectrometry (GC-MS) in pet foods (Koppel, Adhikari & Di Donfrancesco, 2013; Di Donfrancesco & Koppel, 2017). However, the academic literature regarding cat treats and their volatiles has not previously been published in the academic literature.

Cats and Nutritional Requirements

According to their nature and nutritional requirement, cats require high protein concentration in their diets (AAFCO, 2014) and specific amino acids, such as methionine, cysteine, taurine and arginine that can only be met by consumption of animal tissue (Zoran, 2002). Cats are considered hypercarnivores, obligate meat eaters, solitary hunters and usually take live prey with a body mass less than their own (Holiday and Steppan, 2004). Consumption of vertebrate prey are reflected in their nutritional requirements, dental anatomy, digestive physiology, and their drivers of intake (Watson, 2011). Hewson-Hughes et al. (2011) reported

that cats have a limitation to their carbohydrate intake. A high-carbohydrate intake and limited intake of calories from protein and fat lead to a deficiency in those key macronutrients. To balance their macronutrient intake, domestic cats will voluntarily select low-carbohydrate foods (Verbrugghe & Hesta, 2017).

The Association for Pet Obesity Prevention (APOP) reported that 59.5% of cats and 55.8% of dogs in the United State were classified as obese (APOP, 2019). Obesity is a common nutritional disorder and could be associated with the initiation of certain diseases (German, 2006). Even though some diseases, medications, and genetic defects can lead to obesity, the imbalance between energy intake and expenditure remains a predominant cause. Houpt & Smith (1981) suggested that obesity is mostly related to owner-induced variables. For treatment of obesity total energy restriction or starvation would effectively lead to weight reduction but could cause protein/body mass loss (German, 2006). Moreover, starvation could also cause feline hepatic lipidosis (feline fatty liver syndrome), polio encephalomalacia of the caudal colliculus (neurological disease) and congestive heart failure (Anholt, Himsworth, & Britton, 2016). Hence, it is more suitable for the cats to be supplemented in protein and micronutrients, and controlled in fat and energy intake (German, 2006).

Butterwick and Hawthorne (1998) evaluated weight loss in cats at different levels of energy restriction at adult maintenance nutrient levels to achieve a target body weight. Increasing the energy restriction resulted in higher average weight loss in cats compared to moderate energy restriction. However, this higher rate of weight loss may not be a desirable result. The higher energy restriction level could cause an increase in the proportion of weight loss from lean body mass and a decrease in the proportion of weight loss from body fat (Butterwick and Hawthorne, 1998). Another study relating food intake and body weight control by Wei et al. (2011)

presented that two identical nutrient profiles on a dry-matter basis with different water content affected food intake and body weight in cats. Energy intake and body weight in cats decreased when feeding canned diets compared to a freeze-dried version of the same diet (Wei et al., 2011). Thus, to keep the cats healthy and in shape, adjusting their food intake based on nutritional requirements should be considered, especially when it comes to adding extra calories, such as treats, to their normal daily diet.

Palatability of Cat Foods

Cats are discriminative in food selection and they are sensitive to flavor differences in food (Pickering, 2009b). Food selection by the cat is based on sensory properties, smell, taste, mouth-feel, previous experiences and genetic variation. Another key driver of palatability is nutritional content as cats adapt intake of key nutrients to meet specific targets (Watson, 2011). Food preference in domestic pets depends on individual animal variation and previous diet or experience (Rofe and Anderson, 1970; Bradshaw, 2006). Rofe and Anderson (1970) reported that domestic animals, either on the farm or in the home, have limited freedom in food choice selection and mostly depend on their owners. However, Bradshaw et al. (2000) stated that food preferences of house cats and farms cats were different. Among cats from different farms distinct differences in food preferences were observed as well (Bradshaw et al., 2000).

There are studies related to cats choices of preference. Bradshaw et al. (1996) and Xia, (2006) revealed that carnivores have taste buds that are highly responsive to amino acids and unresponsive to many mono- and disaccharides. Houpt & Smith (1981) found that cats prefer salmon and commercial cat food to that of rats. They prefer chicken flavored commercial cat food over liver flavored commercial cat food (Mugford, 1977). Adult cat acceptability of

different diets was investigated by Kane, Morris and Rogers (2013) and among fat sources and fat levels, their results showed that the diets made with bleached tallow were preferred over diets made with butter or chicken fat. Among different levels of fat, the 25% fat diet was liked more than those with 10% or 50% fat. Hegsted et al. (1956) founded that domestic cats tend to select a new food rather than ones in which they are familiar.

Single-bowl and two-bowl tests are examples of consumption tests commonly used in the industry (Aldrich & Koppel, 2015; Koppel, 2014; Li et al., 2017). The single-bowl test is best for acceptability measurement of a food sample. The consumption amount and intake ratio are important factors indicating acceptance (Griffin, 2003). The set-up for a single-bowl test is suitable for testing in a home environment (Koppel, 2014). Two-bowl tests are for preference evaluation of one food over another (Griffin, 2003). The sample which is first sniffed or tasted, the total amount of each food consumed, and the ratio of food consumed relative to the total amount of both foods consumed are important factors to ascertain a preferred food choice. The two-bowl test is typically conducted using trained dogs/cats (Koppel, 2014). For more options on preference testing, Li et al. (2017) proposed a preference ranking procedure. Using trained dogs to extract treats from five puzzle toys, the first sample extracted was counted as the most preferred. The results demonstrated that dogs were able to rank some samples preferred in a deliberated order. This method was verified by Tsai (2019).

Freeze-Dried Pet Foods

Freeze-drying is a preservation method which combines the benefits of both frozen and dehydrated food processing methods. During the process of freeze-drying or lyophilization the water in the product is crystalized at low temperature, followed by a vacuum treatment which

converts the solid phase of water into a vapor directly (sublimation) resulting in a dry product that retained its shape and flavor (Rey et al., 1975; Tsinontides, 2004; Liu et al., 2008; Ciurzynska and Lenart, 2011; Dincer, 2017).

The fundamental freeze-drying steps are freezing, vacuum, heat, and condensation. After the product is frozen to prepare its condition for low temperature drying, the product is placed under vacuum. This is the sublimation process which enables the frozen solvent in the product to vaporize into a gas without passing through the liquid phase. The acceleration of sublimation phase is done by incremental heat application to the frozen product. To complete the separation process, low-temperature condenser plates (low temperature) remove the vaporized solvent from the vacuum chamber by converting it back to a solid in the condensation phase (Dincer, 2017).

Low temperature dehydration helps retain freshness of flavor, color and aroma as well as nutritional value similar to the frozen food and provides shelf stable convenience of canned or dehydrated food without significant degradation of flavor, texture, and nutritional content (Dincer, 2017). Freeze-dried products are light in weight as 98% of water is removed from the product (Dincer, 2017). Water activity (a_w) has an impact on freeze-dried food stability during storage (Sun et al., 2002). The growth of microorganisms and chemical reactions can be inhibited by reducing water activity. However, deteriorative reactions such as lipid oxidation and enzymatic reactions still occur at relatively low a_w values (Martinez and Labuza 1968). Furthermore, the low moisture content and porous property in freeze-dried products may increase the sensitivity to lipid oxidation. Moreover, Srinivasan et al. (1996) stated that lipid oxidation leads to acceleration of protein oxidation (Sun et al., 2002). Oxidation reactions can cause deterioration of flavor, color, and texture (Kanner 1994).

Application of freeze-drying is common with food and also with pharmaceutical and biological products. Manufacturing freeze-dried products translates into higher investment, energy consumption, and maintenance costs (Dincer, 2017 and Yalcin, 2016). Because of high energy consumption and high costs of both operation and maintenance, freeze-drying has always been the most expensive method for producing dehydrated products (Ciurzynska and Lenart, 2011). Compared to conventional air-drying, there is no significant affect to color deterioration of foods from freeze drying (Shishegarha and Ratti, 1999). As an example, the volume reduction of berries was in the range of 5-16 % after freeze-drying process; whereas, after air-drying it was approximately 80% (Jankovie, 1993). Texture of the freeze-drying preservation method was much crisper, when compared to a hot air-dried banana product (Pan et al, 2008). The freeze-dried products can be reconstituted by adding water (Rey et al., 1975). Rehydration potential is impacted by porosity (Farkas and Singh, 1991). Rehydration of freeze-dried food is 4-6 times greater than of air-dried foods because of higher porosity of the product. This would suggest it is a good process for ready-to-eat and instant food products (Ratti, 2001). However, freeze-dried pet food, treats, or related freeze-dried meat products has not been a studied relative to sensory characteristics in which descriptive sensory analysis or comparison to volatile compounds analysis has been conducted.

Research Objectives

The market for freeze-dried pet food is growing and attracting more consumers. With the nutritional content that matches the cats' dietary requirements, an increase in pet humanization, and the demand for healthy food, freeze dried cat treats may be an ideal treat to reward and feed cats.

The first objective of this research was to develop a sensory lexicon for freeze-dried cat treats. The lexicon should describe sensory characteristics of freeze-dried cat treat products, such as appearance, texture and aroma, and may be useful to researchers and professionals in the pet food industry and related fields to improve new product development and aid quality control.

The second objective was to compare consumers' acceptance of products relating a Central Location Test (CLT) and a Home Use Test (HUT) and gain insight regarding consumer perception of freeze-dried cat treats and their emotional responses from both cats and cat owners. Even though the pet parents are an important factor in making pet food purchasing decisions, preference by the animal is also an essential element that motivates pet owners to make a purchase decision.

The last objective was to characterize volatile compound profiles of the freeze-dried cat treat samples and explore the relationships between volatiles in the samples relative to aromatic sensory characteristics. Moreover, combining this information with consumer data from the previous study (Chapter 3) will assist understanding drivers of liking for freeze-dried cat treats.

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Chapter 2 - Lexicon Development for Freeze Dried Cat Treats

Abstract

The objective of this study was to develop a lexicon to describe the sensory characteristics of freeze-dried cat treats products. A total of thirty-two product samples sold in the US market were selected by researchers and used for creating this lexicon. Five highly trained panelists from the Sensory Analysis Center (Manhattan, Kansas) assessed the freeze-dried cat treats samples using descriptive analysis techniques. An initial lexicon was developed using a subset of 10 samples. The lexicon was validated by evaluating 30 commercial freeze-dried cat treats samples. The panelists identified, defined, and referenced twenty-seven appearance, aroma, and texture sensory attributes. A total of 9 appearance, 5 texture/hand feel, and 13 aroma attributes were identified to describe the samples. The appearance of the samples varied widely in color, size and shape, as well as surface characteristics such as fibrousness. Principal component analysis (PCA) was used to map the scores obtained during the validation phase of the lexicon terminology. Overall fish and cardboard were common aromatic descriptors, while the samples were differentiated by overall beef, poultry, and decaying animal attributes. These results may help the pet food industry characterize freeze-dried cat treats and potentially identify gaps in the product category.

Introduction

According to Association of American Feed Control Officials, treat products are a subset of pet food that are not intended as a source of complete and balanced nutrition, but are for intermittent feeding ostensibly as a reward for pets (AAFCO, 2020). Cat owners commonly use treats to bond with their cats. Treats also provide additional calories to the cats' daily intake. Thus, to avoid obesity treats need to be offered judiciously. The cat as an obligate carnivore requires high levels of protein in their diet to support their (National Research Council, 2006). There are many cat treat choices available in the market. Freeze-dried cat treats are one of the options in this category that provides cats a meat-based treat option.

Freeze-dried food is a dried food product which combines the benefits of both freezing and dehydration to process foods. The product is frozen at low temperatures to freeze the water. Then, the product is placed under vacuum so to sublimate the solid (frozen water) state directly to the vapor (gaseous) phase (Rey et al., 1975; Liu et al., 2008; Dincer, 2017). It retains freshness of flavor, color and aroma as well as nutritional value similar frozen food, and provides consumers a convenient shelf-stable option (Dincer, 2017).

The freeze-dried pet food market is estimated to grow from 2020 – 2026 (Global Market Insights, 2020). The freeze-dried pet food category is growing within the natural pet food segment. Even though the cost of freeze-dried products is relatively higher than regular cat treats, the concerns regarding nutrition and health have intensified which has led to an increase in market share for freeze-dried pet foods. Freeze-dried pet products typically contain single meats and (or) ingredients; however, the trend for blended meats has also become more popular in recent years (Global Market Insights, 2020).

Sensory lexicons have been developed for different product categories. Standardized vocabularies facilitate communication across diverse audiences describing sensory properties of products by using panels as instruments (Lawless and Civille, 2013). Although the perception systems are different between *Felis catus* and *Homo sapiens* in terms of taste and olfactory (Chaudhari and Roper, 2010; Thorne, 1992; Neufeld, 2012), the pet owners would be the ones who determine whether the food is acceptable for their pets (Koppel, 2014). Thus, using a human sensory panel to assess cat food could be a way to link the pet and owner's perception of food into a product concept and into sustained consumption by the pet.

There have been some studies regarding development of sensory evaluation methods using human taste panel for optimization of dried cat food products (Pickering 2009a) and canned cat foods (Pickering 2009b), and development of an aroma attributes lexicon for retorted cat foods (Koppel and Koppel, 2018). The terms generated are applicable in determining off-flavor in meats and helpful in the area of meat product development, quality control and shelf-life stability. However, none of the analysis on freeze-dried cat treats sensory characteristics has been reported. The objective of this study was to develop a lexicon to describe the sensory characteristics of freeze-dried cat treats products, and to evaluate 30 commercial samples using this lexicon.

Materials and Methods

Samples

From an initial list of more than three hundred commercial freeze-dried cat treat products available in the US market, total of thirty-two samples were selected for this study (Di Donfrancesco, Koppel, and Chambers, 2012). The products were selected by the researchers based on discussions targeting products that represented a wide range of characteristics in the product category, such as size, shape, color, cost, meat type, brand, manufacturer, and ingredients. Ten of the product samples were randomly selected from the list to use in terminology development and description. For sample evaluation, a new set of twenty- three product samples were used (Table 2.1), except for one product sample #152 which had been presented in the terminology development and description phase. The samples used in the study were within “best by date” and stored according to the instructions on the package. Each sample was contained in different size packages. Approximately 120 grams of each sample was acquired and stored at room temperature until evaluation.

Table 2.1.

Freeze-dried Cat Treat Used in Lexicon Development and Evaluation.

Sample code	Ingredients	Manufacturing country
113	Duck, Orange	USA
197	Wild cod	Canada
242	Minnows	USA
277*	Goat, venison, wild boar, duck, lamb, lamb liver, mackerel, wild boar liver, goat liver, venison liver, goat kidney, goat tripe, venison kidney, venison tripe, wild boar kidney, mixed tocopherols (preservative)	USA
326	Salmon	USA
341	Salmon, Mixed tocopherol	USA
373*	Turkey	USA
480	Pork liver	USA

503	Quail, Natural flavor, Taurine, Organic blueberry, Organic barley grass, Chia seeds, Broccoli sprouts, Carrots, Plums, Whey, Sea salt, Kelp, Yeast extract, Citric acid, Mixed tocopherols, Rosemary extract, Yeast culture, Minerals (Potassium Chloride, Zinc Protein, Zinc Sulfate, Ferrous sulfate, Iron protein, Copper sulfate, copper protein, manganese sulfate, manganese protein, sodium selenite, calcium iodate, Calcium carbonate, Vitamins (Ascorbic acid, Vitamin E supplement, Niacin supplement, Calcium carbonate, Vitamin A supplement, Thiamine Mononitrate, Pyridoxine hydrochloride, riboflavin supplement, Vitamin D3 supplement, Biotin, Vitamin B12 supplement, Folic acid), Dried Bacillus licheniformis Fermentation Product, Dried Aspergillus oryza fermentation product, Dried Aspergillus niger fermentation product, Dried Enterococcus faecium fermentation product, Dried Lactobacillus casei fermentation product, Dried Lactobacillus acidophilus fermentation product, Dried Bacillus subtilis fermentation product, Dried Lactobacillus plantarum fermentation product, Dried lactobacillus lactis fermentation product.	USA
527	Freeze-Dried white fish	USA
562*	Salmon	USA
611*	Minnows	USA
627	Turkey, Turkey heart, Turkey liver, Turkey gizzard	USA
647	Salmon	USA
698	Freeze dried sirloin beef, cheddar cheese (pasteurized milk, cheese cultures, less than 2% salt, enzymes) butterfat, nonfat dry milk, yeast extract, natural flavors.	USA
721	Salmon, tuna, Shrimp	USA
801	Kangaroo meat	USA
823	Chicken Heart, Chicken Liver, Chicken Gizzard	USA
825*	Shrimp	USA
834	Rabbit	USA
853*	Tuna	USA
874	Chicken, Mixed tocopherol	USA
W129*	Beef liver, lamb liver, wild boar liver, goat, wild boar, lamb, goat liver, beef, goat kidney, goat tripe, beef kidney, beef tripe, lamb kidney, lamb tripe, mixed tocopherols (preservative)	USA
W145*	Chicken, turkey, chicken liver, chicken heart, turkey liver, turkey heart, monkfish, chicken gizzard, turkey gizzard, mixed tocopherols (preservative)	USA
W946*	Chicken breast	USA
215, W541	Whole Atlantic mackerel, flounder, monkfish, whole Atlantic herring, Acadian redfish, silver hake, mixed tocopherols (preservative)	USA

229, W137	Lamb meat, lamb heart, lamb tongue, lamb tail, lamb kidney, lamb spleen	USA
325*, 152, W408	Whitefish	USA
376, W604	Beef	USA
532, W303	Shrimp	USA
738, W866	Chicken breast	USA
818, W106	Ground wild boar with bone, wild boar liver, wild boar heart, wild boar kidney, wild boar blood, wild boar trachea	USA

Notes: * = The samples used in initial lexicon development

W = The samples were served in wet form

Sample Preparation

The samples were served in medium size snifter glasses and each was covered with a watch glass for evaluation of appearance and aroma, and in 96 ml plastic cups (Dart, Mason, Michigan, USA) covered with lids for texture evaluation. Samples were weighed (3 grams) into each container and coded with 3-digit random numbers. All dry form samples were served without further manipulation. Six grams of purified water was added to the wet form samples, representing the products served in reconstituted form. Samples were prepared 30 minutes prior to the testing (water was added to the samples 4 min before evaluation).

Panelists

Five highly trained panelists from the Center of Sensory Analysis and Consumer Behavior, Kansas State University (Manhattan, KS, USA) participated in this study. The panelists had received 120 h of general descriptive analysis training on a variety of food products. This training included acuity tests for basic tastes, odors, texture, mouthfeel and descriptive capabilities such as techniques and practice in attribute identification, terminology development, and intensity scoring. The panelists had extensive experiences in descriptive

analysis testing for more than 1,000 h with a variety of food products and had participated in studies using the consensus method like this study. For this project the panelists received further orientation to freeze-dried cat treats using samples that may or may not be included in the study.

The research was approved by the Institutional Review Board for Protection of Human Subjects (IRB #10055) at Kansas State University.

Terminology Development and Description

The initial descriptive terminology was developed using ten of the freeze-dried cat treat product samples (Table 2.1). Development of descriptive terminologies was conducted using a consensus approach. The panelists conducted group discussions to select attribute terms, definitions and references for product description. Three 1.5 h orientation sessions were held to establish the initial attributes and descriptive references for freeze-dried cat treats. Attributes, definitions, and references were proposed by the panelists and were based on previous work and experience. The use of similar attribute determination and description procedures are documented from previous studies for food items such as fruit juice (Koppel and Chambers, 2010), snacks (Kumar and Chambers, 2019) and pet food (Di Donfrancesco, Koppel, and Chambers 2012; Koppel and Koppel 2018).

The panel was aware that additional descriptive terminology and references could be added during the testing phase by agreement among the panel. This consensus profile method is particularly useful in lexicon development studies as new attributes can easily be added, defined, and referenced when they appear in products the panelists are seeing for the first time.

Sample Evaluation and Lexicon Validation

Twenty-three of the freeze-dried cat treat products were used for evaluation. Seven of them were evaluated in both dry and wet form for a total of 30 samples. The samples were randomized, and panelists were served three to four samples per session. Twelve 1.5 h sessions were held for evaluation. Samples were evaluated for appearance, followed by aroma and texture (hand feel).

A modified flavor profile method (Caul, 1978) using a scale from 0 to 15 with 0.5 increments was used for intensity quantification, where 0 represents none and 15 represents extremely high. Some of the attributes were evaluated using percentages of presence and/or absence of the characteristics. Each panelist individually scored intensities for attributes present in the sample according to the appearance, aroma, and texture references included in the lexicon. Then, the panel leader led a discussion to determine the consensus scores for each attribute. When a new descriptive attribute was needed, the panel would discuss the term and find appropriate reference(s) and definition(s) for addition to the attribute list using consensus method. If agreed upon the term was added to the lexicon. The samples were presented to the panel three times, during orientation, evaluation and side-by-side after the profiles had been collected. First, they were individually evaluated. For confirmation, after all the thirty samples were evaluated, each was served side-by-side to fine-tune the intensity score and recheck the presence of each attribute. The testing room was maintained at 21 ± 1 °C and $55 \pm 5\%$ relative humidity. Steamed towels were used to aid cleansing of the olfactory pathways and to wipe panelists hands.

Data Analysis

Excel spreadsheets (Microsoft Office, Redmond, WA, USA) were used for data collection. The consensus data generated from the descriptive analysis was analyzed by principle component analysis (PCA), correlation matrix, using XLStat version 2019.4.2.12345 (Addinsoft, New York, NY). To visualize and understand the sample profiles, PCA was conducted to analyze aroma attributes. Because overall beef and poultry aroma attributes were not present in the dry samples, they were excluded from the analysis. Agglomerative hierarchical clustering (AHC) analysis was conducted for grouping the samples using Ward's method (Bouguettaya et al., 2015).

Results and Discussion

Lexicon Development

The initial lexicon included 20 attributes for appearance, texture/hand feel, and aroma characteristics (Table 2.2). Eight attributes were associated with appearance: white, yellow and brown color, uniformity of size, fibrous, surface roughness, residual particles and powdery residual. Three attributes described texture: powdery, surface roughness and moistness of mass. Nine attributes described aroma: overall fish, shellfish, heated oil, oxidized oil, cardboard, brown, musty/dusty, poultry, and decaying animal.

The panelists discussed and changed some of the terms, definitions and references based on their previous experiences. For example, oxidized oil was used instead of rancid attributes. Di Donfrancesco, Koppel, and Chambers (2012) also noted that the rancid description was redundant with oxidized oil and based on the panelists discussion, oxidized oil was the attribute that would more appropriately describe the samples. The terms big and/or small in size, bits,

nuggets, chunks and kibbles shape characteristics were first proposed for appearance description terms for the samples. Instead, uniformity of size was used as the attribute for sample description, where ‘0’ is ‘completely different’ and ‘100’ is ‘completely similar’ on the scale of 0 to 100 percent. The panelists mentioned that the two attributes “broken pieces of the chunks” and “residual flakes” were different and should be defined accordingly. Then, they proposed residual particles and powdery residuals to describe those characteristics of the products.

During discussion, the panelists reviewed terminologies, definitions, and descriptive references. Based on their experience, the panelists proposed reference materials that could be used as a standard to compare samples. The references were tested and matched to the samples. The panelists had further discussions and re-examinations to confirm that the references described each of the attribute characteristics intended. However, different kinds of products, especially when commercial products are used as references, may have unique characteristics which go beyond specific sensory attribute. For example, soy sauce was used as a reference for the brown attribute. The soy sauce lexicon developed by Cherdchu, Chambers, and Suwonsichon (2013) identified that different brands of soy sauces have unique aromas and flavors. Several brands of soy sauces were presented to the panelists, and aroma characteristic of the two brands used in this study (Table 2.2) were comparable to the product samples brown aroma. For poultry aroma attribute, two of the different brands of canned chicken broth were proposed as a reference. One of the products included ingredients that created an aroma profile with poultry, and vegetable notes. The selected broth was mentioned to be a better representative of the poultry aroma characteristic, as the poultry aroma could be better distinguished.

Lexicon Validation

The panelists started sample evaluations using a lexicon that included 20 attributes (Table 2.2). During evaluation, using consensus method, some attributes were added to the lexicon. These included yellow color for appearance, oily hand feel, greasy hand feel for texture, liver, overall dairy, overall grain and beef aroma attributes.

Although some attributes were scored specifically in a given sample, each was included in the developed lexicon to ensure all sensory attributes are included. This resulted in a final lexicon of 27 attributes (Table 2.2).

Table 2.2.

List of Appearance, Texture and Aroma Attributes, Definitions and References of the Freeze-Dried Cat Treat Products.

Attribute	Definition	Reference
<u>Appearance</u>		
White color*	Light to dark evaluation of white color of product.	Pantone Coated Plus Series 7527 CP = 7.0
Yellow color	Light to dark evaluation of yellow color of product.	Pantone Coated Plus Series 7527 C = 10.0 Pantone Coated Plus Series 7401 CP = 3.0 Pantone Coated Plus Series 7401 C = 6.0
Orange color*	Light to dark evaluation of orange color of product.	Pantone Coated Plus Series 7403 C = 8.0 Pantone Coated Plus Series 7411 C = 5.0
Brown color*	Light to dark evaluation of brown color of product.	Pantone Coated Plus Series 7413 C = 8.0 Pantone Uncoated Plus Series 7562 UP = 3.0
Uniformity of size*	A measurement describing uniformity of product regarding size (%).	Pantone Uncoated Plus Series 7562 U = 5.0 Define by percentage: 0% = completely different
Fibrous*	The perception of visible fibers and filaments or strands of muscle like tissue on the	100% = totally similar Celery Stem = 4.5

	product.	Preparation: Cut celery stem into half inch, and serve 4 pieces in 3.25 oz. cup
		Chicken Breast = 13.0
		Preparation: Cook Chicken Breast in a microwave oven until the inner temperature of the chicken reaches 165F in a covered 3 Quart Pyrex dish. Allow to cool and then cut into 1/2" cubes. Serve 5 pieces in 3.25 oz. cup
		Post Shredded Wheat = 14.0
		Preparation: Serve 4 pieces of Post Shredded Wheat in 3.25 oz. cup
Surface Roughness*	Visual evaluation of indentations/bumps on surface; smooth to rough.	Marshmallows = 2.0
		Preparation: Serve 1 piece of Kraft Jet-Puffed Marshmallows in 3.25 oz cups
		Cheerios = 5.0
		Preparation: Serve 1 Tablespoon in 3.25 oz cups
		Wheaties = 9.0
		Preparation: Serve 1 Tablespoon in 3.25 oz cups
Residual Particles*	Presence of broken pieces from product sample.	Define by percentage: 0% = No broken pieces
		100% = All broken pieces
Powdery Residual*	Powdery residuals visible on the bottom container.	Post Shredded Wheat + ¼ tsp crumb = 5.0

		Preparation: In medium snifter, put 4 pieces of Post Shredded Wheat and add ¼ teaspoon of grinded Post Shredded Wheat.
		Post Shredded Wheat + 1 tsp crumb = 10.0
		Preparation: In medium snifter, put 4 pieces of Post Shredded Wheat and add 1 teaspoon of grinded Post Shredded Wheat.
<u>Texture/Feel</u>		
Powdery*	Perception of a powdery substance/ coating on the product by using fingers to feel the product.	<p>Fritos Corn Chips Original = 3.0</p> <p>Preparation: Serve 4-5 pieces in 3.25 oz. cup</p> <p>Cheetos Cheese Puff = 6.0</p> <p>Preparation: Serve 2 pieces in 3.25 oz. cup</p> <p>Skinny Pop Popcorn White Cheddar = 8.0</p>
Surface Roughness*	The amount of indentions/bumps and surface abrasions, which can be perceived by gently manipulating one piece between thumb and forefinger	<p>Preparation: Serve 6-7 pieces in 3.25 oz. cup</p> <p>General Mills Cheerios = 3.0</p> <p>Preparation: Serve 1 Tablespoon in 3.25 oz cups</p> <p>General Mills Wheaties = 9.0</p> <p>Preparation: Serve 1 Tablespoon in 3.25 oz cups</p> <p>Post Shredded Wheat = 12.0</p>
Moistness of Mass*	The amount of moisture perceived in the product after gently compressing between thumb and forefinger	<p>Preparation: Serve 4 pieces of Post Shredded Wheat in 3.25 oz. cup</p> <p>Kroger Frozen Lima Beans = 3.0</p> <p>Preparation: Serve thawed Lima beans in 3.25 oz. cup</p> <p>Kroger Canned Lima Beans = 5.0</p> <p>Preparation: Rinse Lima beans. Serve in 3.25 oz. cup</p>

Oily Hand Feel	The amount of oil coating appearing on the product which can be perceived by gently manipulating one piece between thumb and forefinger	Fritos corn chips = 5.0 Preparation: Serve 4-5 pieces in 3.25 oz. cup Lays potato chips = 8.0
Greasy Hand Feel	Degree of oil/grease layer perceived on top of sample presented which can be perceived by gently manipulating one piece between thumb and forefinger	Preparation: Serve 3-4 pieces in 3.25 oz. cup Johnsonville Summer Sausage Original = 7.5 Preparation: Serve a 0.5 cm thick sliced Summer Sausage in 3.25 oz. cup
<u>Aroma</u>		
Overall Fish*	An overall impression of fishy aromatics and processed flavor associated with fish such as salmon and tuna.	Nature Made Fish Oil 1200 mg softgels = 7.0 Preparation: Cut 1 pill into half and pour the fish oil into a medium snifter.
Shellfish*	The aromatics that could be associated with shellfish such as clams, shrimp, oysters, and crab.	Reese All-Natural Clam Juice = 3.0 Preparation: Mix 1 part clam juice with 1 part water, serve 1 Tablespoon in medium covered snifter.
Heated oil*	The aromatics commonly associated with heated oil.	Kroger fake crab meat = 6.0 Preparation: Cut into small pieces. Place 5g in a medium snifter. Wesson Vegetable Oil = 7.0 Preparation: 1/3 cup oil heated for 2 min on high power in the microwave oven. Served 1/3 cup oil in medium individual snifters covered with a watch glass.
Oxidized oil*	The aromatics associated with aged oil and fat. May also be defined as rancid or painty at higher levels.	Microwave Oven Heated Vegetable Oil = 6.0 Preparation: Add 300ml of oil from a newly purchased and opened bottle of Wesson Vegetable Oil to a 1000ml glass beaker. Heat in the microwave oven on high power for 3 minutes. Remove from the microwave and let sit at room temperature to cool for approximately 25minutes. Then heat another three minutes, let cool another 25 minutes, and heat for one additional 3 minutes interval. Let beaker sit on counter uncovered overnight. Serve 1 tablespoon in

Cardboard*	The aromatic associated with cardboard or paper packaging. The intensity rating is only for the 'cardboardy' character within the reference.	<p>medium sniffers, covered. Cardboard = 7.5</p> <p>Preparation: 2" cardboard (cereal boxes) square in 1/2 cup of water. Serve in a medium snifter.</p>
Brown*	Woody, brown, slightly sour fermented aroma	<p>Kikkoman soy sauce (1:6) = 4.0</p> <p>Preparation: Dilute 1 part of soy sauce with 6 parts of deionized water. Serve 1 tablespoon in medium sniffers</p> <p>La Choy soy sauce (1:8) = 6.0</p> <p>Preparation: Dilute 1 part of soy sauce with 8 parts of deionized water. Serve 1 tablespoon in medium sniffers</p>
Musty/Dusty*	A dry aromatic, dirt-like, associated with stored dry grain, brown soil.	<p>Kretschmer Wheat Germ = 5.0</p> <p>Preparation: Serve 1 tablespoon wheat germ in medium sniffers.</p>
Poultry*	An aromatic impression that is associated with poultry.	<p>Canned Campbell's Chicken Broth = 6.0</p> <p>Preparation: Place 1 tablespoon in a medium snifter</p> <p>Swanson Canned White Chicken Breast = 9.0</p> <p>Preparation: Drain the water out of canned chicken. Serve 10 g of the chicken in medium snifter.</p>
Decaying Animal*	The aromatics reminiscent of decaying animal material.	<p>Dimethyl Disulfide = 12.0</p> <p>Preparation: Dilute Dimethyl Disulfide 10,000 ppm in Propylene Glycol. Dip a smelling strip into the prepared solution approximately 1 cm from the tip. Put in a test tube and cover with cap</p>
Liver	Aromatic associated with cooked organ meat/liver.	<p>Grill beef liver = 7.5</p> <p>Preparation: Thaw Skylark sliced frozen beef liver. Pan-fry beef liver until an internal temperature of 160F. Chop into 0.5 cm cube and serve 1 Tablespoon in medium snifter.</p>

Overall Dairy	Aromatics associated with products made from milk such as cream, milk, sour cream or buttermilk.	Great Value Non-Fat Dry Milk = 2.5
Overall Grain	Light brown, dusty, musty, sweet aromatics associated with grains.	Preparation: Mix 1 teaspoon of NFDM and 1 teaspoon of water in medium snifter. Cereal Mix (dry) = 5.0 Preparation: Mix ½ cup of each General Mills Rice Chex, General Mills Wheaties and Quaker Quick Oats. Put in a blender and “pulse” blend into small particles. Place 1 Tbsp in a medium snifter.
Overall Beef	Brown, roasted, serummy aromatics commonly associated with cooked beef.	Dillon's 85%Lean Ground Beef = 7.5 Preparation: Brown 0.2 kg. 85% lean ground beef until an internal temperature of 165F is reached. Serve 1 Tablespoon in medium snifter

*= 20 attributes listed in the initial lexicon

The most common aroma attributes for freeze-dried cat treats were overall fish and cardboard. The overall fish aroma attribute was defined as an impression of fishy aromatics and processed flavor associated with fish such as salmon and tuna. The samples containing salmon, whitefish, shrimp, cod and minnows as ingredients were scored relatively high in the overall fish aroma – ranging from 5 to 12 in intensity. The highest intensity of overall fish attribute was 12 in sample W303 which was made from shrimp. The lowest intensity was 5 in samples 341 and 527 which were made of salmon and white fish, respectively. However, samples 480, 113, 834, 229, W137, 801, 823, 503 and 627 were made with pork liver, duck, rabbit, lamb, kangaroo, chicken, quail, and turkey, respectively, and had a low overall fish aroma (intensity scores ranged from 2 to 5). This could possibly be because meat products, other than fish, might also contain volatile compounds causing a fishy aroma. For example, Trimethylamine was recognized as the primary component of fishy odor (Herath et al., 2019). The compound was also detected in chicken juice (Bota and Harrington, 2006). All the other attributes from the initial lexicon were scored for

sample evaluation except the decaying animal attribute. The decaying animal aroma attribute was detected in one of the samples presented during initial lexicon development sessions. To affirm that all the key attributes were covered, the samples from the orientation (sample 152) was brought back for evaluation as the panelists were able to detect the decaying animal attribute from this sample. The intensity for decaying animal was scored at 2.5 in sample 152 which was served without added water. The sample W408 served in wet form had a higher intensity for decaying animal aroma (scored at 10). Although sample 152 and 527 were made from the same kind of meat (whitefish) according to the ingredient label, their aroma characteristics were slightly different. This could be because they were from different manufacturers and processed differently. Sample 527 was weaker in overall fish and shellfish aroma. However, the decaying animal aroma was not evident in sample 527; although the heated oil attribute intensity was a 3.

The heated oil aroma attribute was highest in sample 229 which was made from lamb meat, lamb heart, lamb tongue, lamb tail, lamb kidney, and lamb spleen. The intensity of heated oil was scored at 5.5 while the oxidized oil aroma was not present in the sample. On the other hand, sample 215, made of mixture of six different kinds of fish, was scored the highest intensity in the oxidized oil aroma among all the samples. The intensity of oxidized oil attribute was scored at 8 while the heated oil aroma characteristic was not present in this sample. Lipid oxidation can occur in the stored triglycerides or the tissue phospholipids in meat and meat products. Lipid oxidation occurring in both cooked and refrigerated/frozen meats causes deterioration in the quality of meat and meat products (Love and Pearson, 1971). The reaction is the major chemical degradative reaction in meat products (Schindler et al., 2010) and formation of off-flavor compounds (Kiritsakis, 1998). In stored freeze-dried meat, this reaction may cause protein denaturation and cross-linking (Love and Pearson, 1971). Kiritsakis (1998) reported that

several major aldehydes are formed in oxidized olive oil which were pentanal, hexanal, octanal, and nonanal. However, it was reported that 2-pentenal and 2-heptenal were the main compounds responsible for off-flavors (Kiritsakis, 1998). In Chapter 4, 2-pentenal was also detected in sample 215. This could support the oxidized oil aroma intensity in the sample. In addition, nonanal, 2,3-octanedione, pentanal, 3-hydroxy-2-butanone, 2-pentyl furan, 1-octen-3-ol, butanoic acid, pentanal and hexanoic acid were the compounds associated with lipid oxidation in beef muscle (Stetzer et al., 2008).

Overall beef, poultry, musty/dusty, brown and decaying animal attributes were unique for certain products. Only sample 866, pure chicken breast – served with added water, had a poultry aroma. The same product served without water did not have the aroma, just as the other poultry products. Sample W604 and 376 were the same product made of 100% beef and evaluated in different serving methods, with and without water, respectively. Similar results occurred with sample W604 which was served with added water and was the only sample scored with the overall beef attribute. Suggesting that water and not flavor was the only factor removed from the freeze-dried product. Exposing the product to water reconstituted the product's original property (Dincer, 2017), which may have allowed the aroma release from the sample. Boutboul et al. (2000) studied aroma compounds in corn starch using invert gas chromatography. They compared measurements between dry and humid conditions and reported the retention volumes of volatiles were higher in humid conditions. Confirming that water could possibly be the carrier for those water-soluble compound release from the food matrix.

Overall dairy aroma was detected in samples 698, 376, W604 and W137. It is possible that dairy aromatics could be present in sample 698 because of non-fat dried milk contained in the ingredient. There was no other aroma attribute presented in 698 but overall dairy. However,

the overall dairy aroma was also detected in samples 376 and W604 which were single ingredient samples made from beef. In the lexicon development of beef flavor (Adhikari et al. 2011), dairy flavor attribute was also present. It was described as the aromatics associated with products made from cow's milk such as cream, milk, sour cream or buttermilk. Milk (2% fat) was used as the reference for flavor evaluation. In the current study non-fat dry milk was used as the aroma reference. This would suggest that beef itself has a dairy aroma characteristic. Moreover, the overall dairy note was also found in the sample made from lamb, W137. Not all lamb-based samples had the overall dairy attribute though; it was not observed in sample, 229 which was served without water.

Liver aroma was detected in samples 480, 229 and W106 at 5.5, 2, and 5 intensity, respectively. This aroma was most likely from the ingredient itself that contained parts of liver. Sample 480 was made of pork liver while 229 was made from lamb and W106 was made from wild boar with addition of their liver. However, the liver aroma was also scored in sample 834, which was rabbit meat.

Samples W106 and W408 had the two highest intensity scores (score = 9) for the cardboard aroma attribute while sample 627 was the highest score for musty/dusty (score=6). Whitson et al. (2010) reported that cardboard flavor was derived from a combination of compounds, specifically pentanal, heptanal, nonanal, 1-octen-3-one, and dimethyl trisulfide. It could be that the samples contained these compounds. Brown aromatic was identified in a range from 1.5 to 4.5 in samples 834, 229, 801, 341, 823, 503, 818, and 197. Sample 215 had the highest intensity in brown aroma.

The oily hand feel, and greasy hand feel attributes were scored differently. Oily hand feel was described as the amount of oil coating appearing on the product which can be perceived by

gently manipulating one piece between thumb and forefinger while greasy hand feel was described as degree of oil/grease layer perceived on top of sample presented which can be perceived by gently manipulating one piece between thumb and forefinger. It appeared that the samples that were scored for the oily hand feel attribute were not scored for the greasy hand feel and vice versa. Oily hand feel was a more common characteristic than the greasy hand feel. Only samples 818, W137, W106 and W604 had the greasy hand feel characteristic. The intensity ranged from 3.5 to 6 with W106 the highest and W604 was the lowest.

Moistness of mass characteristic was present only in samples served with added water. The intensity was between 3 to 7. Powdery and surface roughness texture were highest at 11 (sample 341) and 13 (sample 647) intensity scores, respectively. The texture appeared to be different depending upon the type of meat in the product. Manufacturing process seems to have an impact on the product texture. For example, both sample 326 and 647 were made of 100% salmon; however, the powdery and surface roughness texture scores were different. Sample 326 seemed to be blended and pressed before being freeze-dried while 647 seemed to be cuts of flesh that were freeze-dried. Sample 326 was scored lower in both powdery and surface roughness.

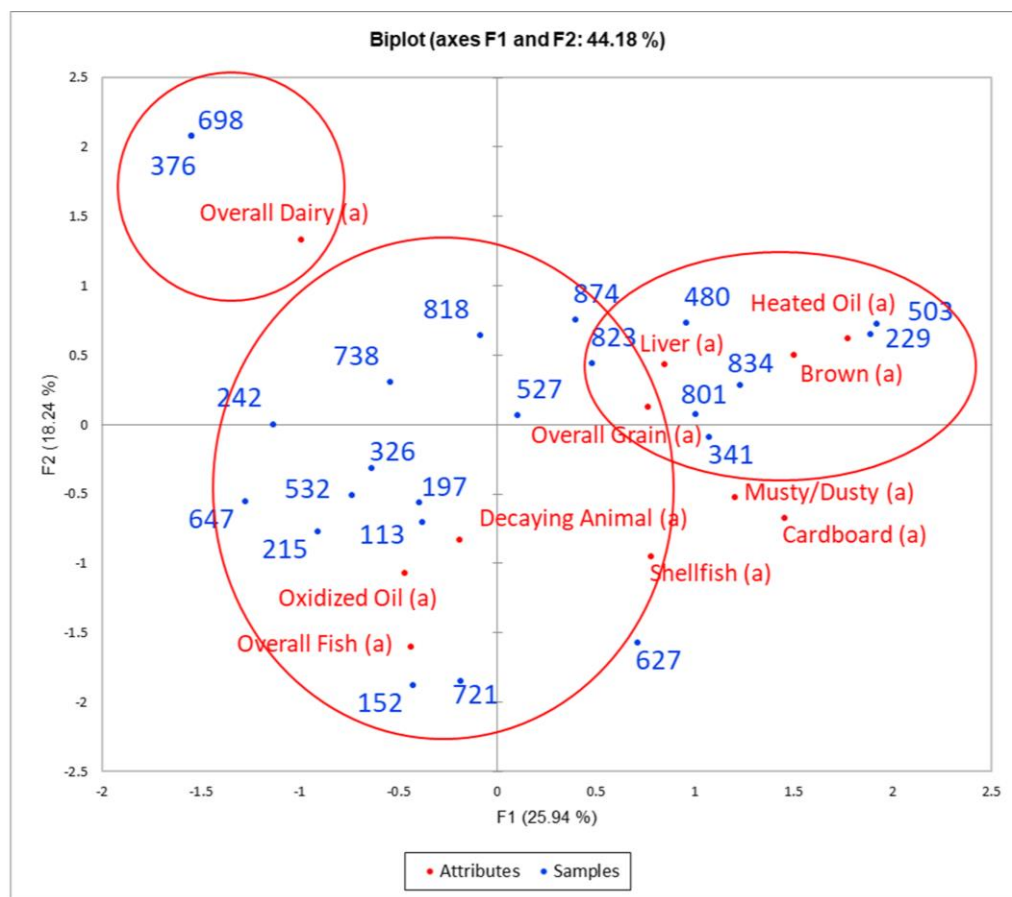
Principal Component Analysis (PCA)

Aromatic profiles of the samples were further analyzed using principle component analysis for better understanding and visualization. The results illustrated that the samples were spaced broadly across the map (Figure 2.1). This confirmed that the attributes for the Lexicon were successful at providing product diversity descriptions. All the dry samples were included in the principle component analysis.

The PC1 coordinate explained 25.94% of the variation and separated samples according to the higher versus lower presence of heated oil, cardboard and brown aromatic attributes. The PC2 coordinate explained 18.24% of the variability and was positively populated with overall dairy and negatively with overall fish and shellfish. PC3, explaining 16.52% of variability, is positively loaded with oxidized oil and musty/dusty and negatively loaded with decaying animal. PC4, explaining 9.94% of variability, separated samples according to the higher versus lower presence of liver attribute. The first 4 PCs were necessary to effectively explain the variance (eigenvalue > 1) and accounted for 70.63% of the variability. The first two PCs were shown on Figure 2.1. Based on similarity of aroma characteristics, Agglomerative Hierarchical Clustering (AHC) analysis grouped the samples three main groups: (1) samples 480, 834, 229, 801, 341, 823 and 503 were stronger in overall grain, liver, heated oil and brown aroma attributes, (2) samples that were stronger in overall fish, oxidized oil, shell fish and decaying animal aroma characteristics such as samples 113, 215, 326, 627, 721, 818, 738, 242, 532, 197, 527, 874, 647, and 152 and (3) samples 376 and 698 were dominant in overall dairy.

Figure 2.1.

Principle Component Analysis of Aroma Attributes of Evaluated Freeze-Dried Cat Treats, Showing Principle Components 1 and 2.



Based on the white (open) spaces on the map (Figure 2.1), additional product development opportunities might be available in order to fill the sensory spaces with differentiated products. On the map's upper right quadrants, there could be space for products that were low in overall fish and oxidized oil and high in overall dairy aroma characteristics. In this study, the overall dairy attribute was present in products made from beef, although the overall beef aroma characteristic was not present in any of the freeze-dried product samples served in dry form. The study by Koppel and Koppel (2018) also noted that products from a white space analysis with retorted cat foods had aroma attributes which could possibly have pronounced beef or fish aromatics. This could support the idea that these aroma attributes could be candidates to fill the void. Furthermore, based on the results from this study, the poultry and beef aroma attributes were not present in the freeze-dried poultry and beef samples, as well as

lamb aroma characteristic in lamb meat samples. These could be a potential opportunity for development of freeze-dried cat treats consisting of these aromas. Although some characteristics may not be successful in the market, there is a potential to fill a niche in the market (Koppel and Koppel, 2018).

Limitations

Due to the large variety of meat types and ingredients of the freeze-dried cat treat products available in the market, a greater variety of samples could have provided more attributes. Further, a study of individual types of meats should be conducted to provide broader detail. Moreover, the freeze-dried treat samples with water added should be evaluated in comparison to the dry samples. The samples in this study were randomly selected by researchers. The commercial products were from various manufacturers, processing, raw materials, and production dates. It is possible that some appearance and aroma attributes were not captured by this study. Producing the freeze-dried cat treat products in the lab scale would have assisted in controlling these variations. Additional information of these products should be collected by evaluating the products using laboratory instrumental measurements for physical and chemical compositions of the products.

Although the perception systems are different between *Felis catus* and *Homo sapiens* in terms of taste and olfactory cues (Chaudhari and Roper, 2010; Thorne, 1992; Neufeld, 2012), using humans to communicate flavor profiles of the samples would benefit from a comparison to palatability tests with cats. Future research should focus on understanding the volatile characteristics and consumer acceptance, as well as cat acceptance of these products.

Conclusion

The development of a freeze-dried cat treat lexicon generated 27 sensory attributes describing appearance, texture and aroma characteristics. A highly trained panel selected, defined and provided references for each attribute. Intensity scores were anchored on a 0-15 scale for evaluation. Common aroma attributes among freeze-dried cat treat products were overall fish and cardboard. Unique aromatics presented in certain products were overall beef, poultry, and decaying animal attributes. This Lexicon could assist researchers and sensory professionals working in the pet food industry to describe characteristics of freeze-dried cat treat products for their appearance, aroma, and texture, and provide an understanding of the consumer preferences from drivers of liking and for utilization in product quality control. Future research should correlate the data from this lexicon development and product evaluation to volatile compound analysis and consumer liking. Combining the information together may be useful for future new product development.

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Chapter 3 - Consumer Acceptance of Freeze-Dried Cat Treats

Abstract

The natural food trend continues to grow fueled by expanding pet humanization in the pet industry. A majority of pet owners now consider their pet as a family member. Although pet food purchase depends on the owner, palatability rests with the pet. The objectives of this study were to 1) compare consumer acceptance of the products between a Central Location Test (CLT) and a Home Use Test (HUT), and 2) to understand consumer perception of freeze-dried cat treats and the emotional responses of both cats and their owners. A CLT (n=104) and HUT (n=57) were performed using chicken and fish-based products made with single meats (SI), single meats with preservative (SP) and from mixed ingredients (MI). The single meat samples of both chicken and fish were liked the most. The average overall liking scores for most of the products when comparing the CLT and HUT were not different. The perceived naturalness of the products and purchase intent were increased ($P<0.05$) after owners were shown the ingredient statements. The single ingredients were scored the highest among the products in naturalness. There was no difference among the samples on liking scores and intake of the cats between the first and the second day ($p>0.05$). The ten most frequently selected terms describing owner's emotions were Caring, Comfortable, Content/Satisfied, Curious, Friendly, Happy, Interested, Loved/Loving, Nurturing, and Relaxed. The ten most frequently selected terms describing cat's emotions were Calm, Comfortable, Content/Satisfied, Curious, Engaged, Excited, Focus, Happy, Loved/Loving, and Relaxed. This research could benefit the pet food industry and product developers regarding consumer preference and better understanding of the relationship between owners and their pets.

Introduction

Most pet owners consider their pets as family members. There has been a greater tendency for pet parents to express interest in pet foods which are similar to human foods and the desire that their pets to be fed human-like products. This pet humanization has become more common in pet industry (Jander, 2019; Wall, 2018). The natural food trend also continues to grow as an attractive attribute for pet owners in parallel to human food trends (Phillips-Donaldson, 2011; Sprinkle, 2018).

The Nielsen Global Health and Wellness Survey (2015) stated that the three most desirable attributes rated by the respondents were foods that are fresh, natural and minimally processed. Although the definition for “Natural” has not been clearly identified by FDA, the review by Roman et al (2017) described that food naturalness is important for consumers. In pet food, the AAFCO definition of natural is “A feed or ingredient derived solely from plant, animal or mined sources, either in its unprocessed state or having been subject to physical processing, heat processing, rendering, purification, extraction, hydrolysis, enzymolysis or fermentation, but not having been produced by or subject to a chemically synthetic process and not containing any additives or processing aids that are chemically synthetic except in amounts as might occur unavoidably in good manufacturing processes” (AAFCO, 2017). Freeze-drying could fit well into one of these categories, as an alternative processing method for the natural trend that alters products the least from their original stages. Murley (2019) evaluated how ingredient statements affect perceptions of naturalness of whole foods. They reported that the ingredient statement was more influential than the product description on packaging for naturalness perceptions. By reviewing ingredient statements, it was observed that familiarity with an ingredient and chemical

sounding names of ingredients have influence on whether the food is considered to be natural to consumers (Murley & Chambers, 2019; Chambers et al., 2017).

Even though the food trends are changing, it remains important to focus on nutritional requirements of the pet. Cats are obligate carnivores – they need a high level of protein in their diet and other nutrients that are found exclusively from animal sources (Holiday and Steppan, 2004). Providing treats is a way for owners to reward and build relationships with their cats. Freeze-dried cat treats, which are primarily made with various meats, may be a good choice for adding extra protein to the diet. In respect to quality, freeze-drying may provide better retention of nutrients when compared to conventional drying (Ratti, 2001).

Although pet owners choose what to feed their pets, the acceptability by the pet is first and foremost (Koppel, 2014). Conducting pet food consumer studies with human subjects via hedonic analysis and with pets via acceptance or preference tests could help guide producers developing products to meet consumer needs. Few studies have been conducted which focus exclusively on cats. For example, Delime et al., (2020) conducted CLT with dog and cat owners to evaluate odor perception of kibbles. Di Donfrancesco et al. (2014) and Di Donfrancesco, Koppel and Aldrich (2018) conducted a CLT with dog owners to assess their preference dry dog foods manufactured with sorghum and sorghum fractions. Di Donfrancesco, Koppel and Aldrich (2018) also conducted a HUT for dry dog foods manufactured with sorghum palatability with dogs. Even though it is harder to control the testing environment, conducting HUT would be an appropriate approach in a more natural feeding practice, particularly with cats (Tobie et al, 2015).

Animals are unable to verbally communicate their preferences. However, they have established special relationships with their humans to communicate nonverbally through

behavior (Arañuri et al., 2017). There are a variety of approaches to measure animal emotions. For example, by measuring behavioral and physiological changes with a coding system they were able to identify relationships between behavioral and facial expressions relative to how they reacted to emotional stimuli (Bennett, Gourkow, & Mills, 2017). Another group investigated cats' vocal characteristics and usage of vocalization (Yeon et al., 2011). One of the most common approaches to investigate pet emotions is through their owner perceptions (Paul, Harding, & Mendl, 2005). This method aided better understanding regarding owners' emotions to their pets and the relationship between them (Morris, et al. 2007; Marten, et al. 2016; Su, et al. 2018; Arañuri et al. 2017; Tsai et al. 2020).

Measuring pet liking of food and owner perception of animal liking of food in home condition is lacking in the field (Koppel, 2014). Conducting CLT and HUT might assist in understanding consumer perceptions. The objectives of this study were to 1) compare consumer acceptance of products between the CLT and HUT, and 2) understand consumer perceptions of freeze-dried cat treats and the impact of ingredient statements and the emotional responses of both cats and cat owners.

Materials and Methods

Samples

Six commercial freeze-dried cat treat products were selected for the consumer study from the list on Chapter 2. The samples were selected by researchers based on discussions aiming for ingredient statement variations. These were purchased online via Chewy.com and Amazon.com. All samples were within the "best by" date on the package. The products were stored at room temperature according to package directions. The two flavors of the freeze-dried cat treat


samples used in this study were selected by researchers based on the most relevant flavors available in the US market which were fish and chicken (Rowlands, 2020; Fracchia, 2020; Chewy.com; Petco.com). The samples were divided into two sets according to the flavors. Each set had three samples based on the ingredients, single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI). The sample list with ingredient statements are reported in Table 3.1.






For the CLT, the samples were served in 96 ml disposable polystyrene translucent plastic Souffle cups (Dart, Mason, Michigan, USA) covered with clear polyethylene terephthalate lids (Dart, Mason, Michigan, USA) and labeled with a three-digit code. Approximately three grams of the sample was placed into each cup.

For the HUT, the samples were prepared in 29.5 ml disposable polystyrene translucent plastic Souffle cups (Dart, Mason, Michigan, USA) covered with clear polyethylene terephthalate lids (Dart, Mason, Michigan, USA) and labeled with a three-digit code. Each cup was filled with 3-4 pieces of the sample (approximately 1.5-2 grams).

Table 3.1.

Freeze-dried Cat Treat Samples and Ingredients Used in the Central Location Test and Home Use Test.

Samples	Ingredients	Photo
Fish single ingredient (FSI),	Salmon	

Fish single meat with preservative (FSP)	Salmon (preserved with mixed Tocopherol)	
Fish mixed ingredients (FMI)	Whole Atlantic mackerel, flounder, monkfish, whole Atlantic herring, Acadian redfish, silver hake, mixed tocopherols (preservative)	
Chicken single ingredient (CSI),	Chicken breast	
Chicken single meat with preservative (CSP)	Chicken (preserved with mixed Tocopherol)	
Chicken mixed ingredients (CMI)	Cage Free Chicken: Heart, Liver, Gizzard	

Subjects

Participants were recruited from the Kansas City area via email through the database of Sensory and Consumer Research Center, Kansas State University (Olathe, Kansas, USA). They were asked to complete the online screening through Compusense *at hand* software (Compusense Inc., Guelph, Ontario, Canada). The participants had to meet the criteria that they were cat owners, over 18 years of age, not working in pet care/pet food industry or in marketing research, did not participate in any consumer study and market research about pet food in the past three months, had cats in their house, were required to feed their cats with cat treats and had to be personally responsible for at least 50% of cat food purchases.

A total of 54 men and 50 women, participated in the CLT. A sub-group of 60 participants from the CLT were asked to participate in the HUT as well.

Central Location Test

A CLT was conducted at the Sensory and Consumer Research Center, Kansas State University (Olathe, Kansas, USA.) Demographic characteristics of the consumers are reported in Table 3.2.

Table 3.2.

Consumers Demographic from Central Location Test.

Cat owner characteristics	Categories	Frequency	%
Gender	Male	54	52%
	Female	50	48%
Age	Under 18 years old	0	0%
	18 - 20 years old	1	1%
	21 - 30 years old	10	10%
	31 - 40 years old	21	20%
	41 - 50 years old	35	34%
	51 - 60 years old	18	17%
	61 years or older	19	18%

Income	Less than \$20,000	1	1%
	\$20,000 - \$39,999	4	4%
	\$40,000 - \$59,999	8	8%
	\$60,000 - \$79,999	10	10%
	\$80,000 - \$99,999	22	21%
	\$100,000 or more	59	57%
Education	Some high school or less	0	0%
	Completed high school/GED	2	2%
	Some college/technical school	11	11%
	Completed college/technical school	61	59%
	Some post-graduate study or more	30	29%
Number of Pets	One	29	28%
	Two	32	31%
	Three	23	22%
	Four	10	10%
	Five or more	10	10%
Kind of Pets	Cat	104	59%
	Dog	56	32%
	Lizard	2	1%
	Bird	1	1%
	Fish	6	3%
	Horse	1	1%
	Rabbit	1	1%
	Hamster	1	1%
	Guinea pig	1	1%
	Other (please list): Chickens, Alpaca, Goat, Snake, Turtle	4	2%
Number of Cats	One	62	60%
	Two	31	30%
	Three	5	5%
	Four or more	6	6%
Role in Cat Food Purchasing	I do all the cat food purchasing	59	57%
	I do more than half the purchasing	32	31%
	I do half the purchasing	12	12%
	I do less than half the purchasing	1	1%

Questionnaires were administered by Compusense *at hand* software (Compusense Inc., Guelph, Ontario, Canada). A tablet computer was provided to each participant for evaluation.

A total of 12 testing sessions were conducted in one day. The number of participants varied from 7 to 11 people per session. Fifty percent of the participants were served with the set of fish samples first while the other fifty percent were served with the set of chicken samples. The samples were served monadically and the three samples within each set were randomized. All samples were served to all participants during a 45 minutes session. Each participant was compensated (\$35) after the completion of the session.

Home Use Test

Sixty cat owners participated in the Central Location Test were included for the study, in which 57 owners completed the testing. The demographics of the participants shown in Table 3.3.

Table 3.3.
Consumers Demographic from Home Use Test.

Cat owner characteristics	Categories	Frequency	%
Gender	Male	28	49%
	Female	29	51%
Age	Under 18 years old	0	0%
	18 - 20 years old	1	2%
	21 - 30 years old	1	2%
	31 - 40 years old	10	18%
	41 - 50 years old	20	36%
	51 - 60 years old	8	15%
	61 years or older	15	27%
Income	Less than \$20,000	1	2%
	\$20,000 - \$39,999	1	2%
	\$40,000 - \$59,999	4	7%
	\$60,000 - \$79,999	5	9%
	\$80,000 - \$99,999	32	58%
	\$100,000 or more	12	22%
Education	Some high school or less	0	0%
	Completed high school/GED	0	0%

Some college/technical school	5	9%
Completed college/technical school	31	56%
Some post-graduate study or more	19	35%

Thirty of the participants received the fish samples and the other thirty received the chicken samples. Each participant received a packet of six three-digit coded cups of sample (three samples in duplicate), a short questionnaire sheet and an owner instruction on the testing procedures and the timeline of the study. Each participant was compensated (\$25) after the completion of the 8-day testing and the questionnaires.

The participants were informed that the HUT study would require eight days to complete. During this time, they were required to feed their own cat with the provided samples. While performing the test with the selected cat for the study, other cats in the home were to be separated in a multi-cat household. On each evaluation day, participants were asked to log in to the survey and complete the questionnaire on Compusense *at hand* software (Compusense Inc., Guelph, Ontario, Canada). One sample code was served for two consecutive days with one day in between each sample code.

The research was approved by the Institutional Review Board for Protection of Human Subjects (IRB #10055) and the Institutional Animal Care and Use Committee (IACUC #4388) for Kansas State University.

Questionnaire

Central Location Test

Participants were asked to open the sample cup and rate their overall liking on a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely. Also, the participants were asked to indicate how much they thought their cats would like the sample.

A 5-point Just-About-Right (JAR) scale, where 1 indicated “too weak”, 3 “just about right”, and 5 “too strong” was used for color intensity of pieces, overall shape among pieces, size of pieces, aroma and hardness evaluation of each sample. Comparing between before and after seeing the ingredients statement of each sample, the participants were first asked to rate naturalness of the products on a 9-point scale, where 1 = not natural at all to 9 = extremely natural and purchase intent on a 9-point scale, where 1 = extremely unlikely to purchase and 9 = extremely likely to purchase. Then, on the same scale, the participants were asked to read the ingredient label and answer the following questions; based on the ingredient statement listed above, how natural do you think this cat treat is and based on the ingredient statement listed above, how likely are you to purchase this cat treat.

Furthermore, the participants were asked to Check-All-That-Apply (CATA) for the opinions about the ingredient statement, similar to Murley (2019). In addition, participants predicted estimated cost, where 1 = extremely not expensive and 9 = extremely expensive. Moreover, participants were also asked to describe their likes and dislikes for each sample.

Home Use Test

To evaluate each sample at different time points, before, during and after feeding the treat samples to the cat, the participants were asked to report emotion responses using check-that-all-apply (CATA) with the listed emotion terms for the owner and the cat.

Based on the emotion terms for owners and cats developed in the study by Tsai et al. (2020), the 62 and 55 terms for owners and cats were used in this study. The list was shown in Table 3.4 (owner's) and Table 3.5 (cat's). Even though the cats might not eat the treat sample, the owners needed to rate their liking as well as their cat liking on a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely. Furthermore, they had to report emotion responses of themselves and their cats before feeding the treat samples. In addition, open-ended questions were included for the owners to provide more information about their opinions on what they and their cats like and/or dislike about the product.

Table 3.4.
Owner's Emotion Terms.

Positive emotions		Negative emotions	
amazed	friendly	afraid/fearful	reluctant
amused	generous	alone	sad
appreciative	giddy	angry	scared
calm/harmony	goofy	anxious/nervous	sorry
careful	happy	cautious	stressed
caring	humorous	confused	sympathetic
close	important	discouraged	uncomfortable
comfortable	interested	doubtful	upset
companioned	intrigued	embarrassed	worried
complete	loved/loving	empathetic	
connected	nurturing	frustrated	
content/satisfied	mindful	guilty	
curious	playful	hopeless	
energetic	quiet	hurt/painful	
excited	refreshed	indifferent	
fun	relaxed	jealous	
free	safe	mean	
focused	warm	regretful	

Table 3.5.

Cat's Emotion Terms.

Positive emotions		Negative emotions	
calm	fascinated	alert	hurt/painful
comfortable	focused	angry	impatient
companioned	free-spirited	anxious/nervous	panicked
competitive	fun	bored	resistant/reluctant
confident	happy	cold/indifferent	sad
content/satisfied	loved/loving	combative	shamed
crazy	loyal	confused	sick
curious	peaceful	defensive	stressed
dependent	playful	desperate	unaware
determined	proud	distrustful	uncomfortable
energetic	relaxed	exhausted	unnerved
engaged	safe/secure	fearful/scared	upset
entertained	warm	homesick	worried
excited		hostile	

Data Analysis

A spreadsheet application (Excel, Microsoft Office Pro ver. 2013) was used to calculate means and percentages. An application addon XLSTAT (Addinsoft, New York, NY, USA) was used for Analysis of Variance using Tukey's Honestly Significant Difference, Penalty Analysis and Check All That Apply Data Analysis for CATA data. For Just-About-Right data, scores 1-2 were grouped "too low" and scores 4-5 were grouped as "too high". Scores of 3 were considered as "just about right". Penalty Analysis or Mean Drop Analysis was conducted on "Just-About-Right" data to determine a mean drop on liking score if respondents do not rate a particular attribute on Just About Right (Schraidt, 2009). Clusters among consumers were grouped according to their overall liking based on results from CLT by Agglomerative Hierarchical Clustering (AHC). Data from the incomplete surveys were analyzed by ignoring the missing data.

Results and Discussion

Central Location Test

A total of 22% of cat owners fed their cats treats two times a day or more, 42% feed one time every day, 26% feed more than two times a week, 4% feed one time a week, 3% feed 2-3 times a month, and 2% feed less than one time a month.

A total of 22% of the participants typically give treats regularly, 19% feed if they feel like it, 16% feed if the cat behaves well, 14% feed the cat at special occasions, 8% feed when they want to spend time with their cat, 6% feed when they want the cat to behave (going to the vet, for instance), 3% feed each morning/night and none feed the cat when they are sad.

A total of 36% of the participants feed soft and chewy foods/treats, 27% feed crunchy, 17% feed catnip, 5% was lick able/liquid, freeze-dried and jerky were 4% each, Veterinary diets and dehydrated were 3% each and 1% fed other kinds (cooked meat and crunchy with filling).

Frequency of the owners reading the ingredient statement when purchasing cat food products were 2% Never, 24% Rarely, 38% Occasionally, 33% Most of the time and 3% Always.

The Importance for the owners reading the ingredient statement when purchasing cat food products were 4% not at all important, 13% slightly important, 46% moderately important, 34% very important and 3% extremely important.

The percentage of the owners' responses who agreed on the statements about them and feeding their cats were shown in Table 3.6.

Table 3.6.

Cat Owners' Opinion on Statements about Feeding Their Cats.

Cat Owners Opinion	%
I always follow a healthy and balanced diet for my cats.	15%
I always look for natural ingredients in the foods that I feed my cats.	14%
I am very particular about the healthiness of my cats' food.	13%
If I do not understand the name of an ingredient or if the name is unfamiliar, I do not buy the food product.	7%
The healthiness of cat treats makes no difference to my choice.	6%
The healthiness of food has little impact on my cats' food choices.	5%
I do not read ingredient statements and do not worry about natural ingredients in my cats' foods.	5%
I feed my cats what I would like to feed them and I do not worry about the healthiness of the food.	4%
I do not avoid feeding my cats any foods, even if they may cause weight gain.	4%
I do not care about natural ingredients in the foods that I feed my cats.	3%
In my opinion, additives in foods are not harmful for my cats' health.	3%
I do not care about additives in my cats' diet.	2%

Owner and cat overall liking average scores on the single ingredient sample were higher ($p < 0.05$) than single meat with preservative (SP) and mixed ingredients (MI) for both chicken and fish sample sets (Table 3.7).

Table 3.7.

Average Overall Liking Scores from CLT Consumer Test (N=104) of Cat Owner's Evaluation of Owner's Liking and Cat's Liking (1-9 Hedonic Score; 1 = Dislike To 9 = Likes Extremely).

	Sample	Owner Overall Liking	Cat Overall Liking
Fish	SI	6.14 a	7.11 a
	SP	4.64 b	5.52 b
	MI	4.36 b	5.47 b
Chicken	SI	6.13 a	6.70 a
	SP	5.30 b	5.66 b
	MI	5.13 b	5.95 b

*Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$). The data was analyzed to compare within the same type of meat.

**Sample variations were single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI)

The average liking scores for most liked samples were above 6 where 5 = neither like nor dislike and 9 = extremely like. In the fish sample set, both the owner's liking and expected cat's liking showed that the fish mixed ingredients (FMI) sample was scored the lowest in the sample group. However, it was not significantly different from the fish single meat with preservative (FSP) (average liking scores were approximately 4 for owner and 5 for cat). Based on the average score, the FMI and FSP were slightly disliked. In the group of chicken samples, both owner's and cat's liking score for chicken single meat with preservative (CSP) was the lowest but not significantly different from the chicken mixed ingredients (CMI) ($p > 0.05$). The prediction of cat liking followed owner's liking and was always estimated a bit higher than the owners' liking. The factor leading to this result was possibly because of the owners' interpretation of their cats. Di Donfrancesco (2014) performed Central Location Test to compare consumer acceptance on eight dry dog food variations using commercial products available in

the US market. Similar results were presented in the study that the prediction of pet's liking on most of the samples followed owner's liking.

The experimental design was organized to compare among the sample with the same kind of meat. Thus, the scores should not be compared across the groups (chicken and fish). Although the study on taste preference in cats by Houpt and Smith (1981) reported that cats prefer fish and commercial food to rats, cats preferred salmon over fish, liver, chicken or beef flavor commercial cat food (Adamec, 1976) and the chicken flavored commercial cat food over the liver flavored (Mugford, 1977).

The FSI product was liked the most among the three fish samples. Eighty-nine percent of the consumers rated the color as JAR (Table 3.8).

Table 3.8.

Percentage of Consumers Responded to Color, Shape, Size, Aroma and Hardness on Just-About-Right Scale.

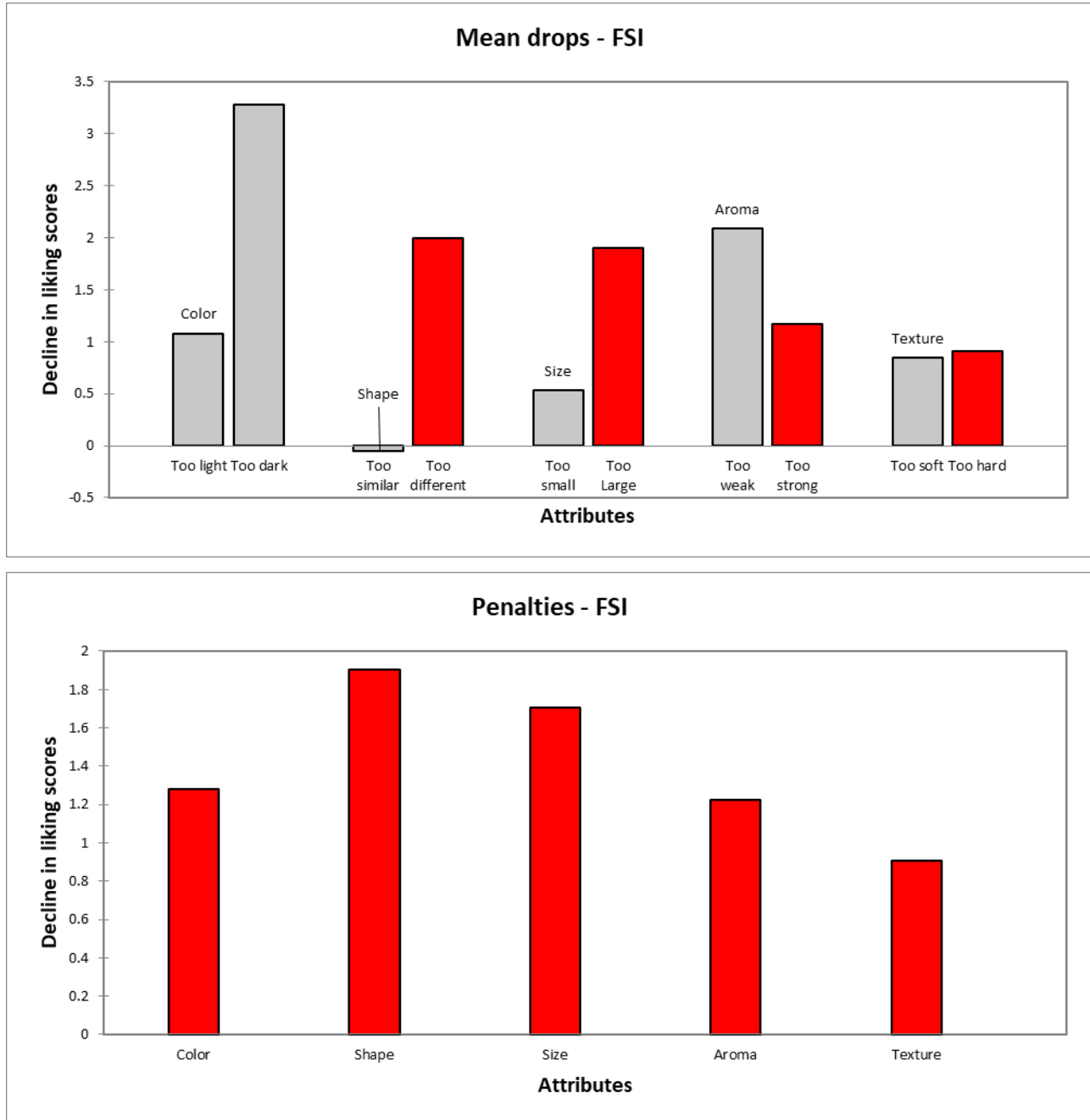
		Level	Color	Shape	Size	Aroma	Hardness	*Sa
Chicken	SI	Too little	31%	2%	5%	58%	10%	mpl e
		JAR	69%	68%	48%	41%	60%	
		Too much	0%	30%	47%	1%	31%	
	SP	Too little	38%	17%	4%	35%	7%	vari atio
		JAR	59%	71%	70%	63%	52%	
		Too much	3%	12%	26%	3%	41%	
	MI	Too little	7%	0%	17%	12%	1%	ns wer
		JAR	60%	53%	53%	70%	42%	
		Too much	34%	47%	30%	18%	57%	
Fish	SI	Too little	10%	2%	5%	2%	4%	sing le
		JAR	89%	58%	65%	63%	50%	
		Too much	1%	40%	30%	35%	46%	
	SP	Too little	19%	17%	0%	11%	35%	ingr edie
		JAR	60%	75%	34%	60%	49%	
		Too much	21%	8%	66%	30%	16%	
	MI	Too little	49%	20%	6%	8%	19%	
		JAR	50%	45%	48%	40%	56%	
		Too much	1%	35%	46%	52%	25%	

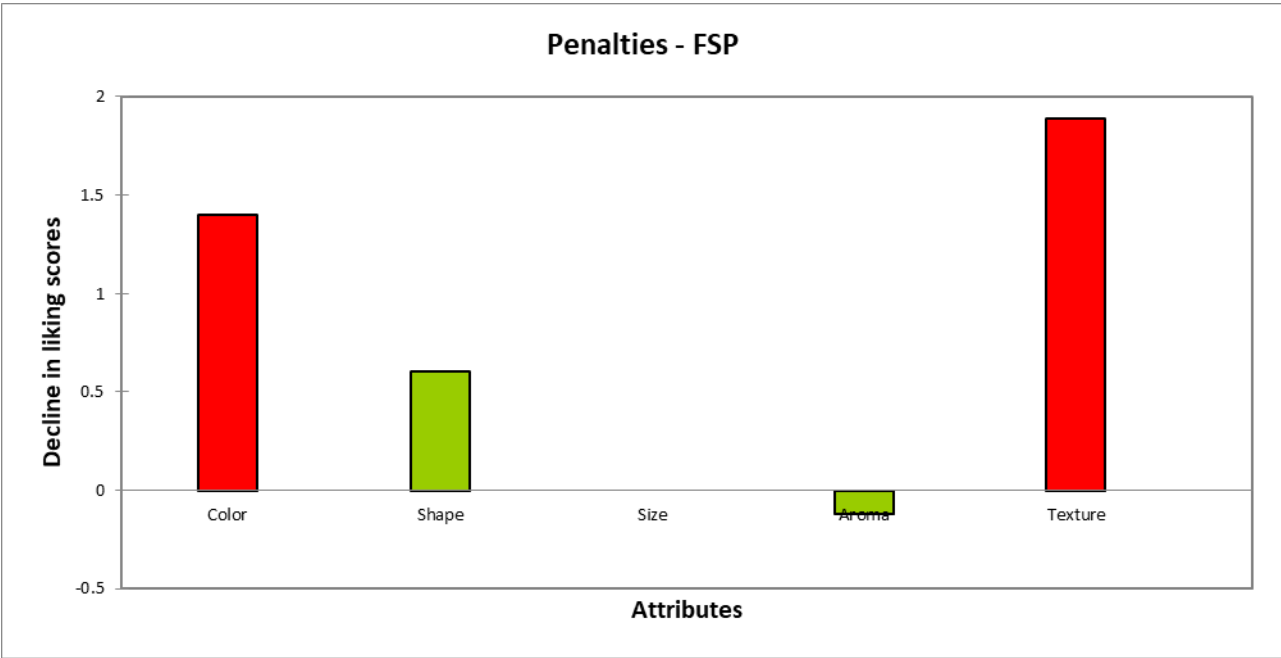
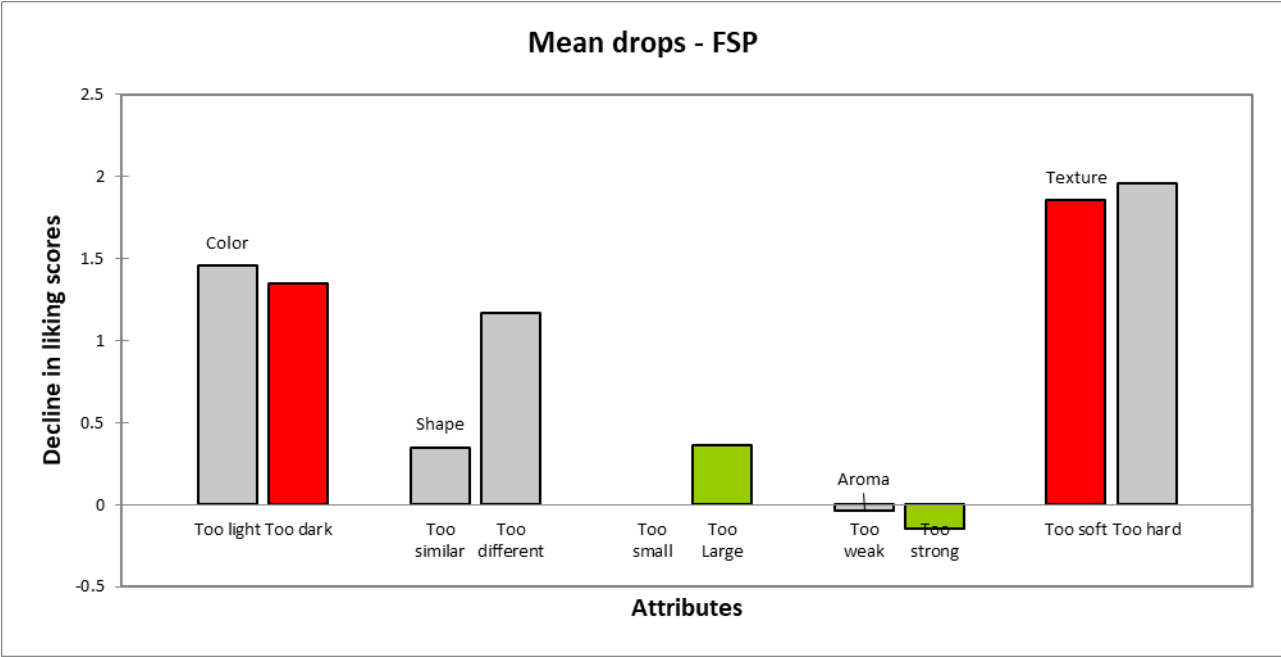
nt (SI), single meat with preservative (SP) and mixed ingredients (MI)

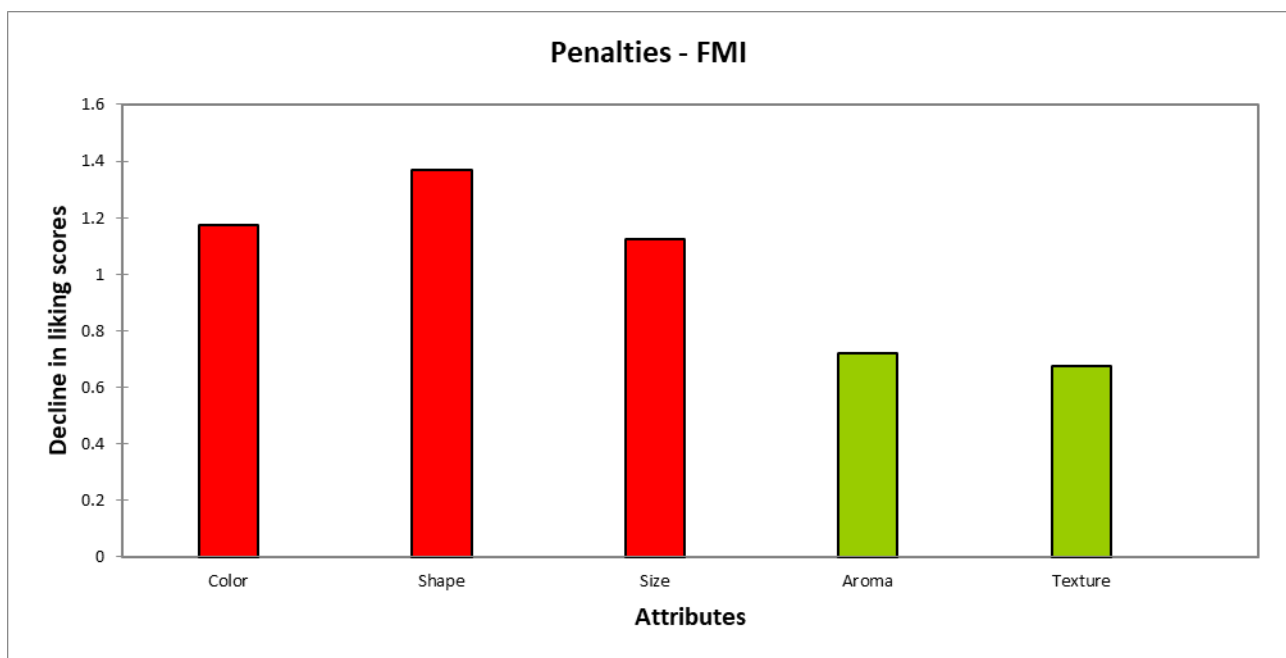
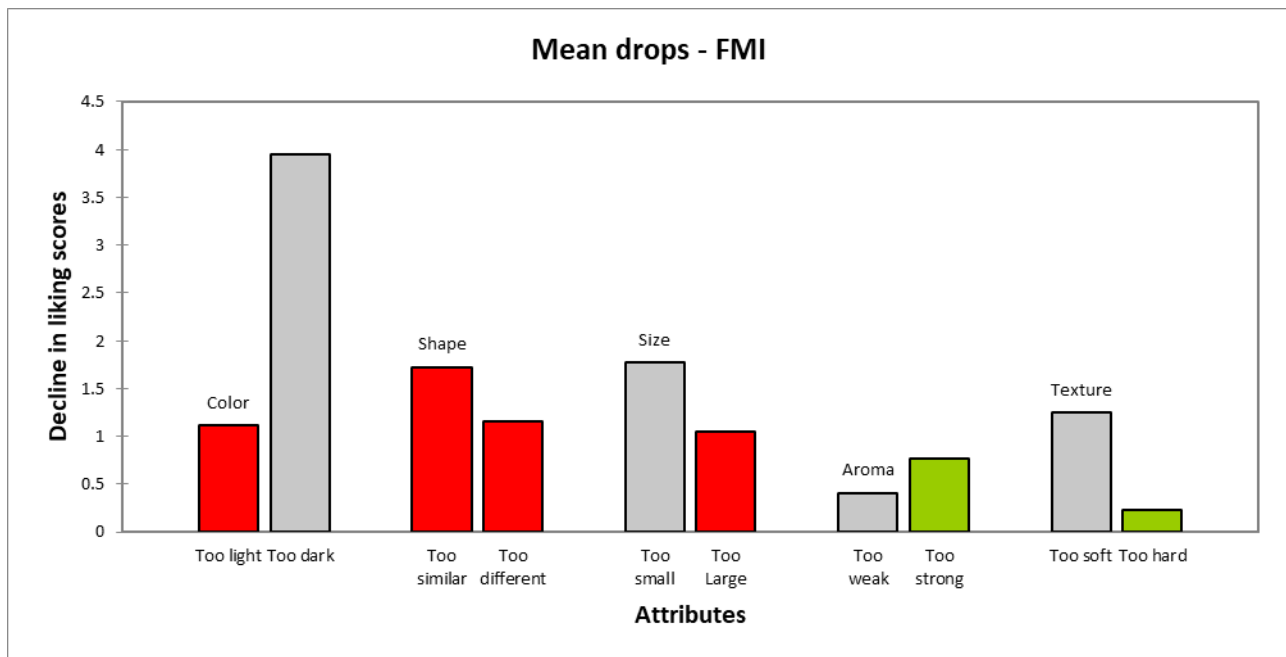
The penalty analysis results showed that the consumers strongly penalized the FSI product as they considered it too different in shape, too large in size, too strong in aroma and too hard in texture. The consumers strongly penalized the FSP product as it was too dark in color, and too soft in texture. The FMI product was penalized when the consumers considered it too light in color, too similar in shape and too large in size (Figure 3.1). CSI product was scored the highest in overall liking among the three chicken product samples. The product was highly penalized when it was too different in shape, too large in size and too hard in texture. The consumers strongly penalized the CMI product as too dark in color, too large in size, too strong in aroma and too hard in texture. Even though the mean drop from the penalty analysis could not be computed whether the CMI product was too weak or too strong in aroma, the overall penalty was significant which showed that the aroma does matter for the consumers. Similarly, the mean drop test could not be computed as to whether the CSP product was too similar or too different in shape. This suggests that the shape does matter for the consumers as the overall penalty was significant. The CSP was strongly penalized when considered too light in color and if too hard in texture (Figure 3.2).

Figure 3.1.

Penalty Analysis Graphs for Color, Shape, Size, Aroma and Texture from Central Location Test for Fish Samples.





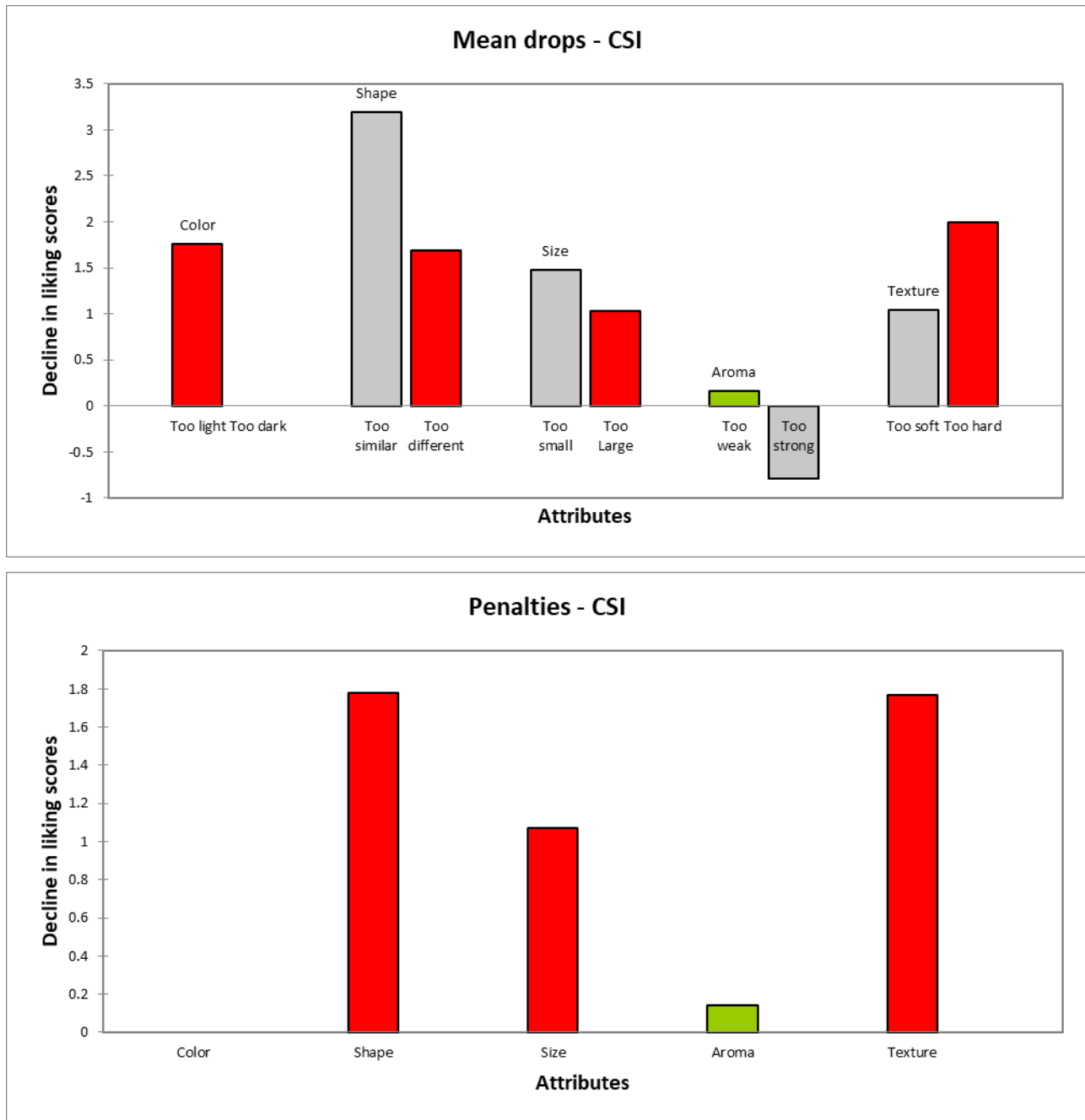


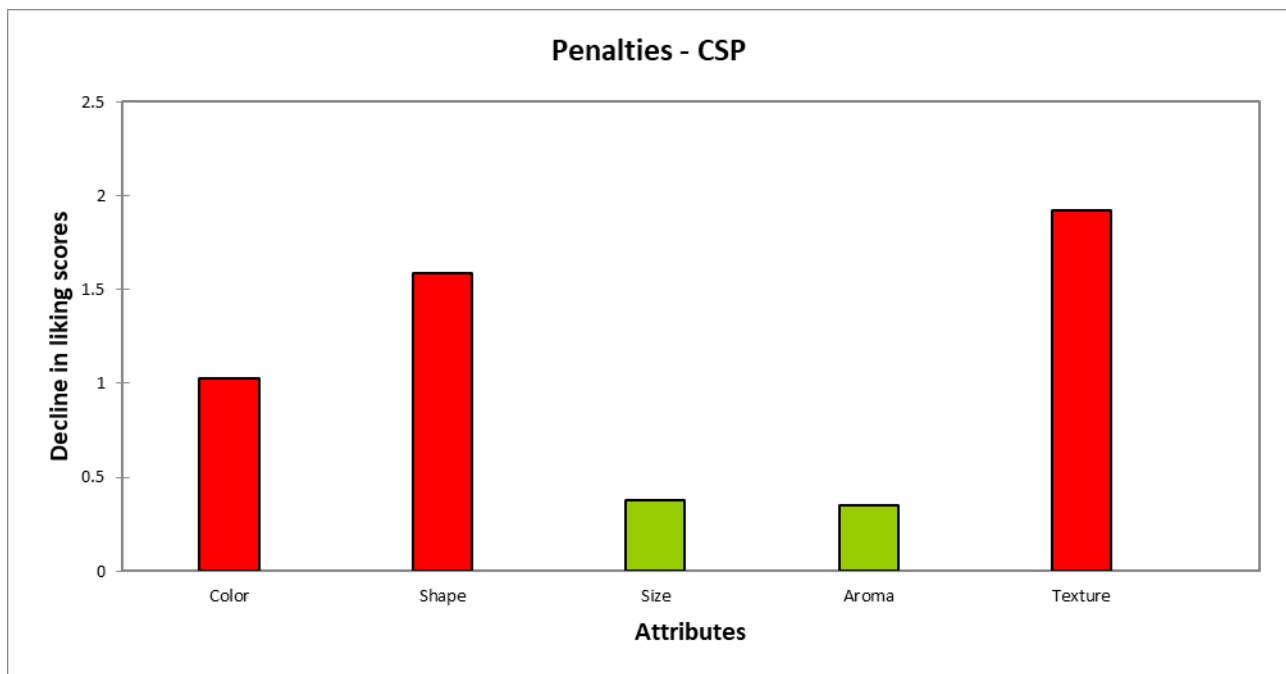
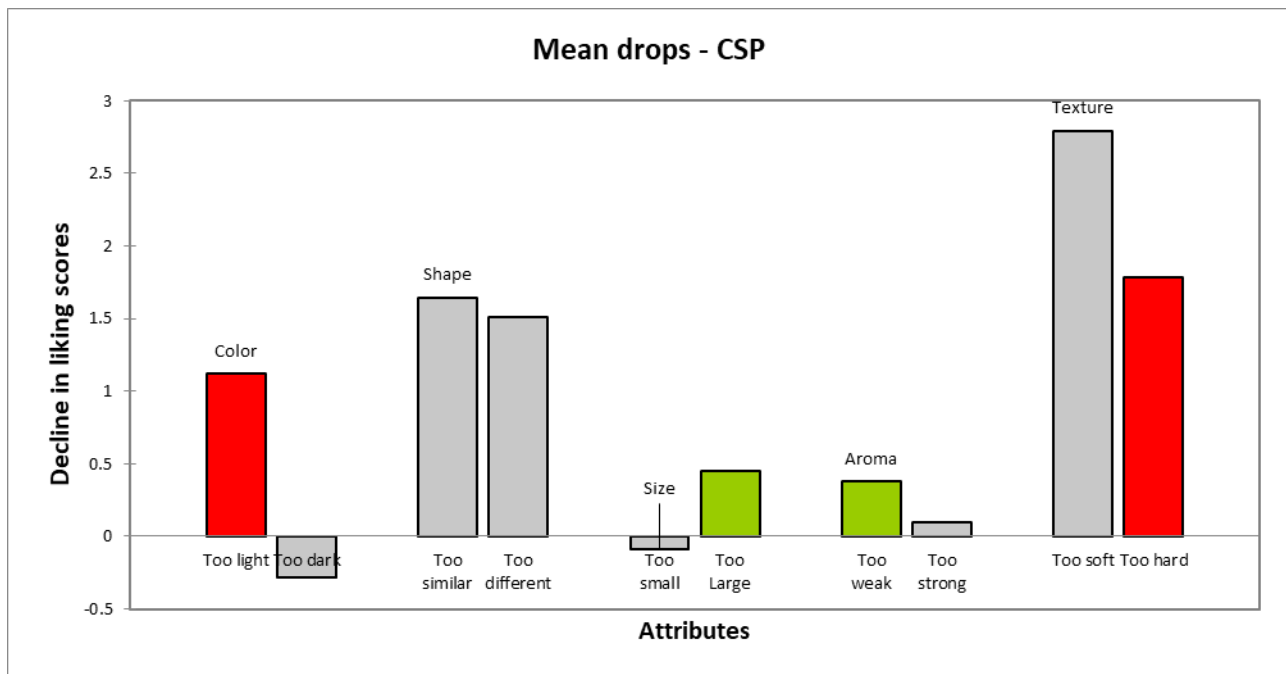
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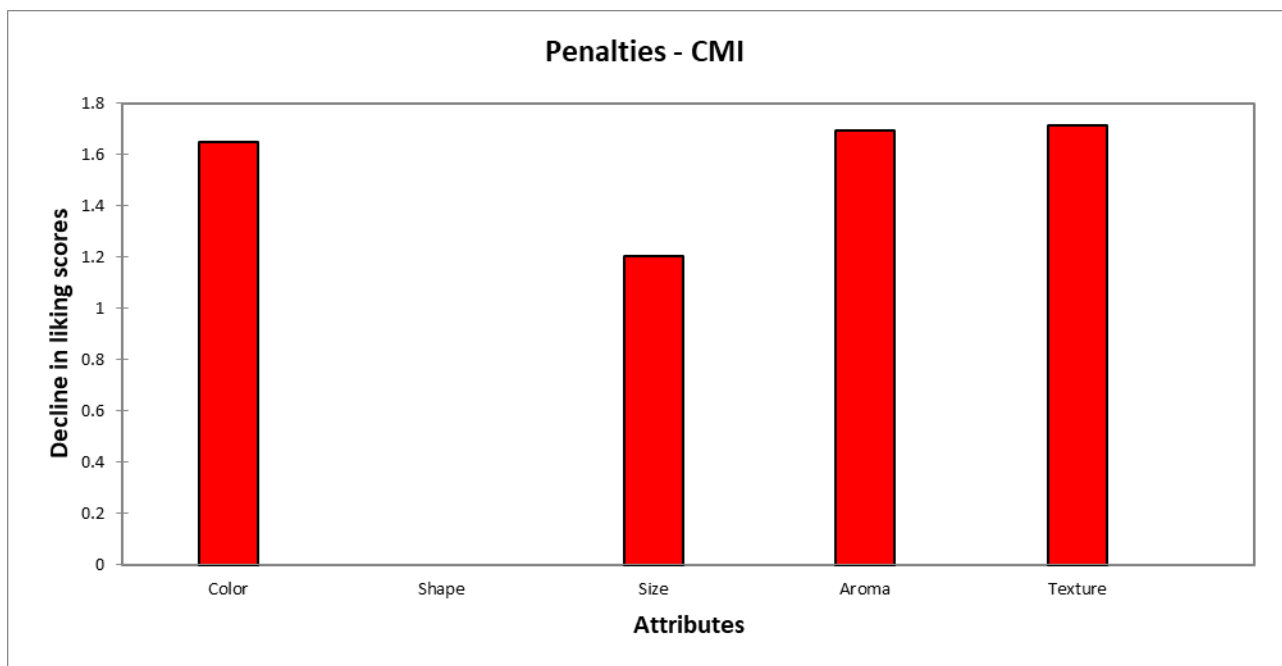
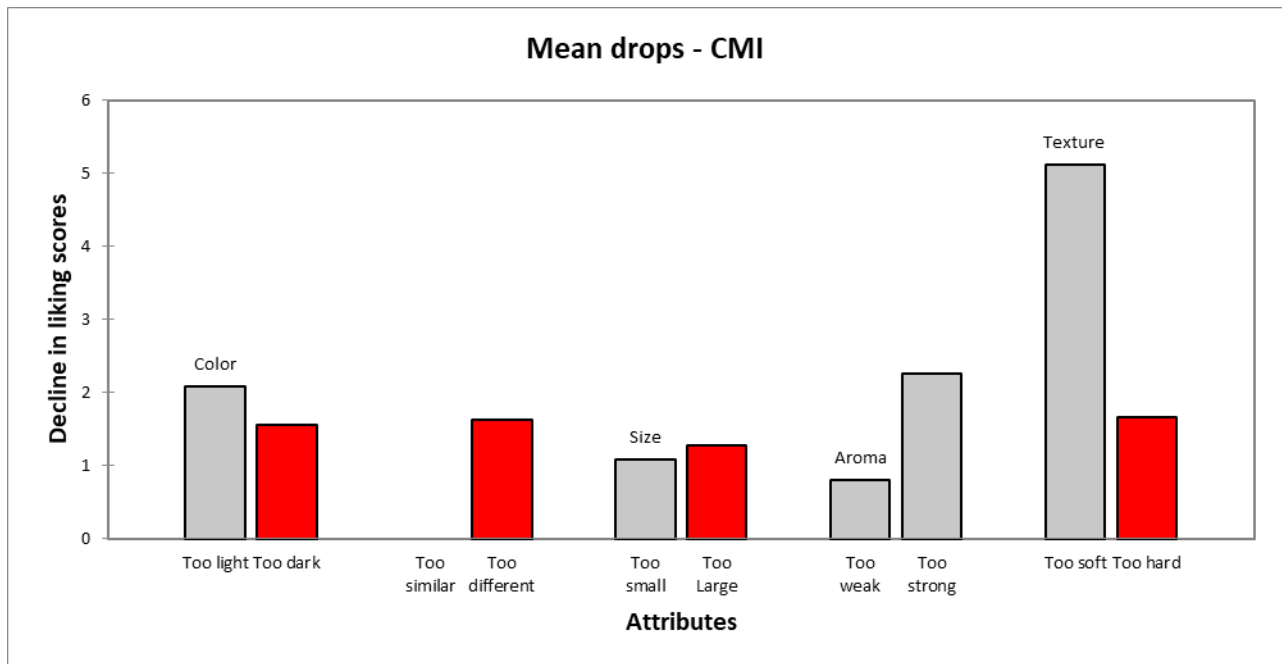
*Fish cat treats with single ingredient (FSI), Fish cat treats single meat with preservative (FSP) and Fish cat treats with mixed ingredients (FMI)

Figure 3.2.

Penalty Analysis Graphs for Color, Shape, Size, Aroma and Texture from Central Location Test for Chicken Samples.







Note:

*Chicken cat treats with single ingredient (CSI), Chicken cat treats single meat with preservative (CSP) and Chicken cat treats with mixed ingredients (CMI)

The comparison between before and after seeing the ingredient statements for the purchase intent scores were rated significantly higher ($P < 0.05$) after seeing the ingredient statement for all the samples except for the chicken single meat with preservative (Table 3.9). After seeing the ingredient statement, the consumers were more likely ($P < 0.05$) to purchase both the chicken and the fish single ingredient products and less likely ($P < 0.05$) to purchase the single meat with preservative products.

Table 3.9.

Average Score from CLT Consumer Test (N=104) of Cat Owner's Evaluation of "Purchase Intent" for Before and After Seeing the Ingredient Statement (1-9 Point Scale; 1 = Extremely Unlikely to Purchase to 9 = Extremely Likely to Purchase) and "Cost Estimation" (1-9 Point Scale; 1 = Extremely Expensive To 9 = Extremely Inexpensive).

	Sample	Purchase Intent Before	Purchase Intent After	Cost Estimate
Fish	SI	6.45 Ba	7.55 Aa	3.12 b
	SP	4.40 Bb	5.02 Ac	4.71 a
	MI	4.37 Bb	5.73 Ab	4.53 a
Chicken	SI	6.24 Ba	7.57 Aa	4.24 b
	SP	4.92 Ab	5.11 Ac	5.71 a
	MI	5.39 Bb	6.83 Ab	4.04 b

* Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$). The data was analyzed to compare within the same type of meat.

** Lower case shows significant difference between the three samples (column). Upper case shows significant difference within the sample, before vs after (row)

*** Sample variations were single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI)

The average score for cost estimation is reported in Table 3.8. Within the chicken sample set, the single meat with preservative (CSP) was estimated to be the least expensive relative to the other two variations ($p < 0.05$). Similarly, the fish single meat with preservative (FSP) was also estimated to be less expensive than the fish single ingredient (FSI) ($p < 0.05$). However, it was not different from the fish mix ingredient (FMI) ($p > 0.05$).

The results from a pet food consumer study reported by Di Donfrancesco et al. (2014) indicated that the sample perceived as having the lowest cost was one of the samples that earned the lowest score for overall liking for dog food. In this study, the FSP, FMI and CSP samples which were scored lower in overall liking tended to be perceived as lower cost as well.

Naturalness Perception

The single ingredient samples for both chicken and fish (CSI and FSI) were perceived to be significantly more natural than the other two variations. Chicken single meat with preservative (CSP) was rated lower ($P < 0.05$) in naturalness than the other two. Similarly, fish single meat with preservative was perceived to be the least natural but not different from the fish mixed ingredients (Table 3.10).

Table 3.10.

Average Score from CLT Consumer Test (N=104) of Cat Owner's Evaluation of "Naturalness Perception" for Before and After Seeing the Ingredient Statement (1-9 Point Scale; 1 = Extremely Unnatural to 9 = Extremely Natural).

	Sample	Naturalness Perception	Naturalness Perception
		Before	After
Fish	SI	7.50 Ba	8.40 Aa
	SP	4.52 Bb	5.41 Ac
	MI	4.83 Bb	6.47 Ab
Chicken	SI	7.34 Ba	8.29 Aa
	SP	4.75 Ac	5.22 Ab
	MI	6.53 Bb	8.09 Aa

* Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$). The data was analyzed to compare within the same type of meat.

** Lower case shows significant difference between the three samples (column). Upper case shows significant difference within the sample, before vs after (row)

*** Sample variations were single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI)

After seeing the ingredient statements, a consumers' perception of product naturalness was slightly changed. The results among all the samples were perceived to be more natural after seeing the ingredient statement with the exception of the chicken single meat with preservative which was not rated higher in naturalness. The perception of naturalness of the single ingredient samples was still rated the highest and the single meat with preservative samples was the lowest for both chicken and fish. However, there was no difference between the single ingredient and mixed ingredients in the chicken sample set.

Chambers & Castro (2018) reported that consumers are likely to use different definitions for what constitutes "natural." Ingredient familiarity and chemical sounding names influence how people perceive naturalness of the product. Murley & Chambers (2019) also noted that consumers primarily look at the whole product to make decisions about naturalness, but one of the factors such as ingredient familiarity could influence their decisions. Also, Murley (2019) reported that product identity and ingredients are the two cues that consumers use to make decisions about the naturalness of food product. People rely more on the ingredient statement than product identity when making decisions about naturalness on products.

Table 3.11.

Frequency of Consumer's Selection of Check-All-That-Apply on Opinion About Ingredient Statement.

Statements	Fish			Chicken		
	SI	SP	MI	SI	SP	MI
Too long	3 (a)	2 (a)	22 (b)	0 (a)	2 (a)	1 (a)
Too short	4 (a)	3 (a)	0 (a)	6 (a)	8 (a)	1 (a)
Has chemical names	2 (a)	61 (c)	47 (b)	0 (a)	62 (b)	0 (a)
Food sounds good for cats	96 (c)	37 (a)	65 (b)	85 (b)	40 (a)	84 (b)
Food sounds tasty for cats	80 (c)	47 (a)	63 (b)	79 (b)	48 (a)	78 (b)
Contains unnatural ingredients	3 (a)	46 (c)	32 (b)	2 (a)	43 (b)	2 (a)
Ingredients come from nature	89 (c)	44 (a)	67 (b)	82 (b)	35 (a)	88 (b)
Ingredients made in a lab	1 (a)	33 (c)	20 (b)	0 (a)	42 (b)	1 (a)
Has unhealthy ingredients	2 (a)	18 (b)	14 (b)	1 (a)	30 (b)	4 (a)
Ingredients cause cancer	0 (a)	7 (a)	6 (a)	0 (a)	9 (b)	0 (a)
Has healthy ingredients	92 (b)	53 (a)	62 (a)	99 (c)	46 (a)	85 (b)
Not appropriate for cats	0 (a)	5 (a)	3 (a)	0 (a)	6 (a)	3 (a)
Don't recognize ingredients	1 (a)	49 (c)	26 (b)	0 (a)	45 (b)	0 (a)
Extra flavor added	6 (a)	11 (a)	6 (a)	2 (a)	9 (a)	2 (a)

* Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$). The data was analyzed to compare within the same type of meat.

*** Sample variations were single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI)

Based on the similar ingredient statement structure of the single ingredient and the single meat with preservative samples, the consumers selected the following opinion statements more frequently for FSI and CSI ingredient statements: “food sounds good for cats”, “food sounds tasty for cats”, “ingredients come from nature and had healthy ingredients”. For CSP and FSP, consumers’ opinions included: “the ingredients contained chemical names”, “contains unnatural ingredients”, “ingredients made in lab”, “had unhealthy ingredients”, “ingredients cause cancer” and “don’t recognize ingredients”. The ingredient statement structure of the mix ingredients for chicken and fish samples were slightly different. The consumers thought FMI ingredient statement was too long and had unhealthy ingredients while CMI ingredient statement was “food

sounds good for cats”, “food sounds tasty for cats” and “ingredients come from nature” (Table 3.11).

Consumer Segmentation

Based on owners’ overall liking scores, the consumers were grouped into five clusters (Figure 3.3). Consumers in cluster 3 (n=38) seemed to moderately like most of the products (average score > 6) except for the FMI (significantly lower liking score than the other two variations). On the other hand, Cluster 1 (n=15) contained consumers that disliked majority of the products (average score range = 3.4 – 4.7). Among the chicken samples, CMI was liked the most in cluster 1. In cluster 2 (n=17), consumers disliked most of the products (score < 5) except for CSI that was neither liked nor disliked and CSP that was liked the most by the consumers in this cluster (score > 6.5). In cluster 4 (n=20), the consumers liked the single ingredient products made from both chicken and fish (CSI and FSI) (score > 6.5). In cluster 5, consumers moderately liked most of the products (score > 5.5) except for FSP (scored the lowest among the three variations in the sample with same kind of meat) (Table 3.12).

Figure 3.3.

Dendrogram from Agglomerative Hierarchical Clustering Analysis Grouping of the Consumers Based on Overall Liking Scores.

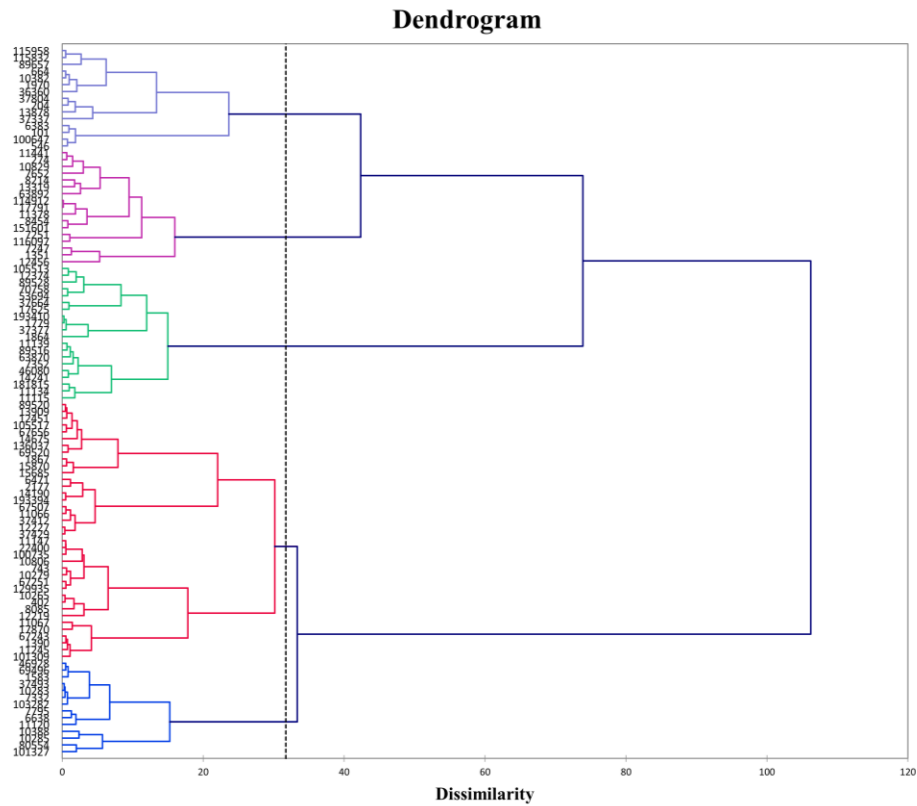


Table 3.12.

Average Owner Liking Scores of Each Sample for Consumer Clusters.

Class	FSI	Fish			Chicken	
		FSP	FMI	CSI	CSP	CMI
1 (N=15)	4.67 a	3.47 a	3.60 a	3.40 a	4.07 a	5.53 b
2 (N=17)	3.53 a	4.71 a	3.65 a	5.35 b	6.76 c	2.88 a
3 (N=38)	7.24 c	6.13 b	4.92 a	6.32 a	6.03 a	6.32 a
4 (N=20)	6.85 b	3.95 a	3.25 a	7.50 b	3.25 a	3.65 a
5 (N=14)	6.93 b	2.79 a	6.07 b	7.50 b	5.79 a	6.29 a

* Means with the same letter in each row are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$). The data was analyzed to compare within the same type of meat.

** Sample variations were fish (F) and chicken (C) - single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI).

***1-9 hedonic score; 1 = dislike to 9 = likes extremely.

Home Use Test

A sample was fed over 2 days during the HUT and the cats intake acceptance on the treats were not different between day1 and day2 ($P > 0.05$). This contrasts with Hegsted et al. (1956) that reported domestic cats tend to select a new food rather than the ones they are familiar with.

The cats overall liking scores tended to be somewhat higher than their owners overall liking scores (Table 3.13). A similar trend was observed in the CLT.

Among the three variations of chicken and fish samples, cat liking scores were not different (Table 3.13). However, the owner overall liking score for the chicken samples presented that the chicken single ingredient (CSI) was rated higher than the chicken mix ingredient (CMI) ($P < 0.05$). Meanwhile, the owner liking score for chicken single meat with preservative (CSP) was not different from the CSI nor CMI ($P > 0.05$).

Table 3.13.

Average Score from HUT Consumer Test (N=57) Of Cat Owner's Evaluation of "Owner's Liking and Cat's Liking" (1-9 Hedonic Score; 1 = Dislike to 9 = Likes Extremely).

	Sample	Owner Overall Liking	Cat Overall Liking
Fish	SI	6.00 a	6.04 a
	SP	4.57 b	5.07 a
	MI	5.43 ab	5.66 a
Chicken	SI	6.54 a	6.92 a
	SP	6.09 ab	6.41 a
	MI	5.75 b	5.82 a

*Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$). The data was analyzed to compare within the same type of meat.

** Sample variations were single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI)

The single ingredient of the fish (FSI) sample was rated higher than the single meat with preservative (FSP) ($P < 0.05$). Whereas, the owners liking score for fish mix ingredient (FMI) was not different from the FSI nor FSP ($P > 0.05$).

Emotions while preparing to feed, during the offering and feeding process and after feeding the treats for both owners and cats was not difference ($P > 0.05$).

The results were that the ten most frequently identified emotions before, during and after feeding their cats were Caring, Comfortable, Content/Satisfied, Curious, Friendly, Happy, Interested, Loved/Loving, Nurturing, and Relaxed (Figure 3.4). These were all positive emotions. Meanwhile the terms that were not selected were Alone, Jealous, Mean, and Scared which were categorized into negative terms. For cats' emotions – as interpreted by their owners – Calm, Comfortable, Content/Satisfied, Curious, Engaged, Excited, Focus, Happy, Loved/Loving, and Relaxed were the top ten most frequently identified (Figure 3.5); while Angry, Combative, Hostile, and Hurt/Painful were not selected at all. Most of the selected emotion terms from this study, based on feeding situations, were positive emotions. On the other hand, negative emotions frequently mentioned by the cat owners were mostly related to the experience of going to the veterinarian (Tsai et al., 2020). For example, Sad, Stressed and Frustrated for cat owners; and Anxious and Scared for cats.

Figure 3.4.

Frequency of the 10 Most Common Emotions for Cat Owners' Emotion.

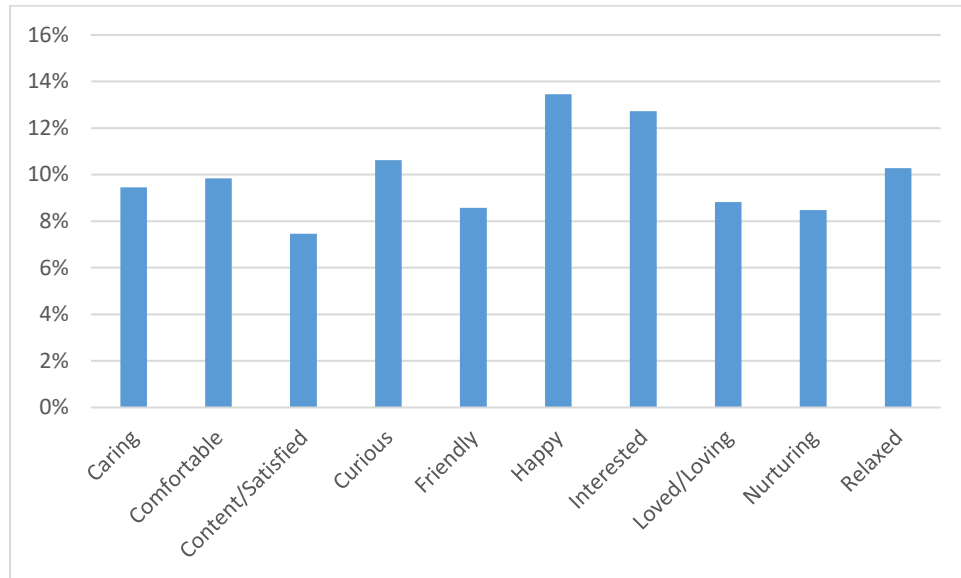
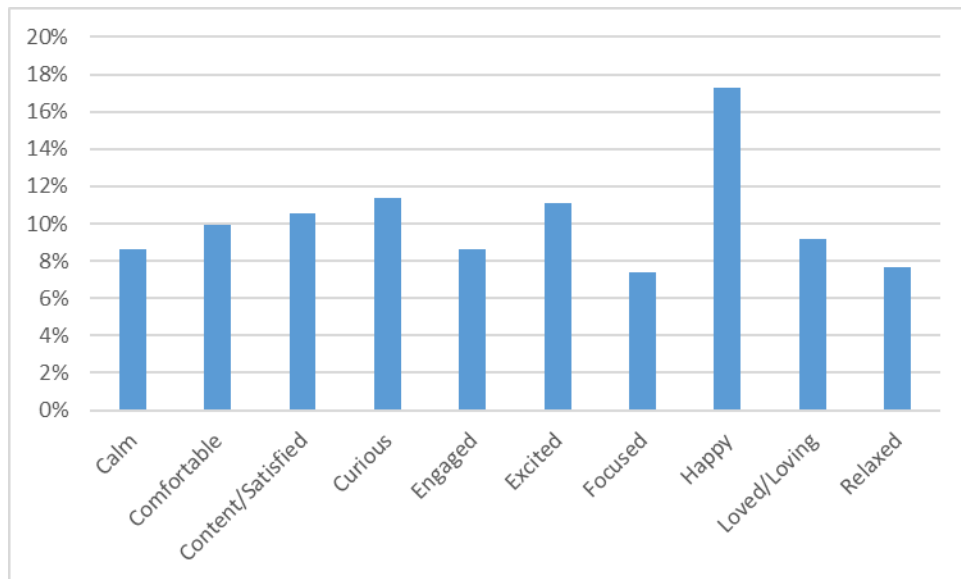


Figure 3.5.

Cats' emotions (interpreted by their owners) of the 10 most common emotions.



Comparison of CLT vs HUT Liking Score

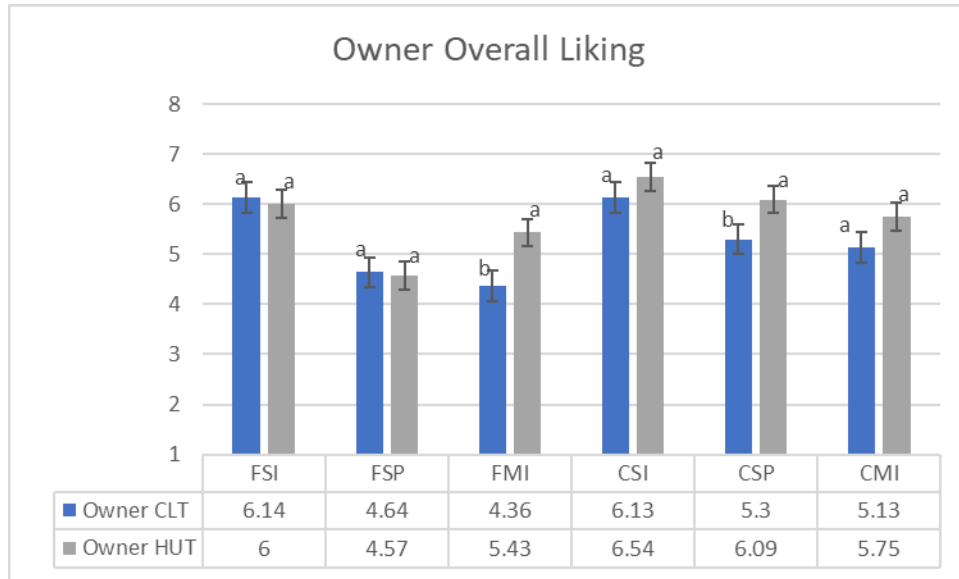
Overall liking scores of the owners and the cats from the Central Location Test and the Home Use Test were compared. The liking scores for CSI, CMI and FSP samples were not significantly different between the CLT and HUT ($P > 0.05$) (Figure 3.6 and 3.7). Also, the owner liking score for FSI and cat liking score for FMI were not different when the average liking scores were approximately 6 and 5, respectively. However, the owner liking score from HUT for samples CSP and FMI were higher than CLT ($P < 0.05$). Also, the cat liking score for sample CSP from HUT was higher than CLT. On the other hand, the cat liking score from HUT was significantly lower for sample FSI which the average liking score was 6.04 from HUT and 7.11 from CLT. The overall liking of both owners and cats for most of the samples were slightly higher conducting home use test. Moreover, cat's liking scores tended to follow the owner's based on the owner's interpretation. For more cost effective and less time consuming, conducting CLT seemed provide sufficient information.

Even though the owners and the cats liking score tended to be higher in HUT for some samples, the results from this study did not reveal a pattern for either increasing or decreasing the liking scores in the comparison between the CLT and HUT. Peryam and Pilgrim (1957) suggested that the hedonic rating can be affected by changes in environmental conditions. As the HUT participants also participated in the CLT, the liking scores affected by the carry over effect since they were familiar with the samples and had seen the information of the samples. Although a HUT is more time consuming and more expensive (Lawless & Heymann, 2010), it may be worth more at providing realistic information from the test. According to the results, as the liking scores were different between CLT and HUT depending on the samples, feeding the treats to the cats in the actual home environment and observing their body movements, facial expression and

vocalization might provide a more accurate interpretation regarding the cat's acceptance of the treat.

Figure 3.6.

Bar Graph Comparing Overall Liking of the Owners Between Central-Location-Test and Home-Use-Test.



*Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$). The data was analyzed to compare within the same type of meat.

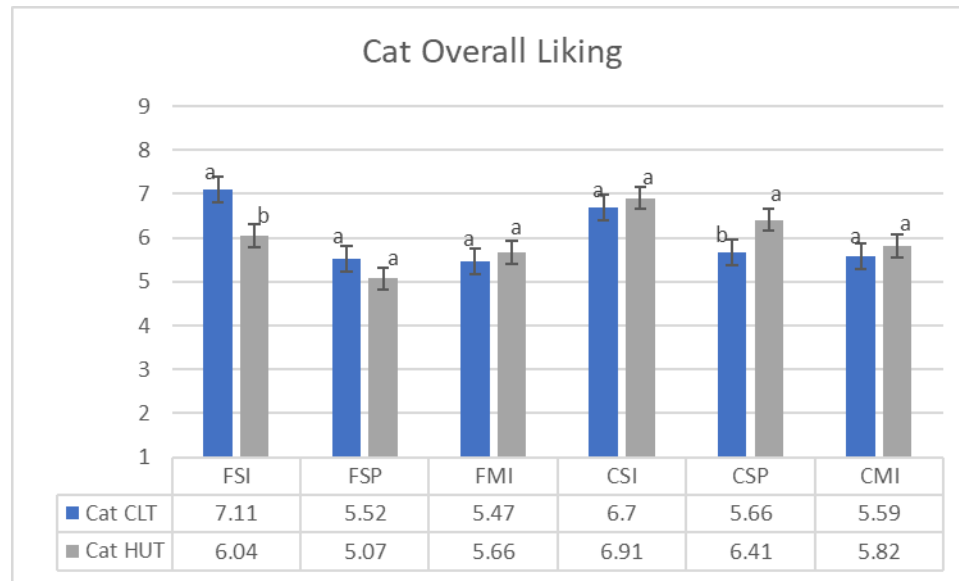
** Sample variations were single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI)

***The overall liking scores were based on a 9-point scale -1 (extremely dislike) to 9 (extremely like).

****CLT: n=104 / HUT: n=28(fish samples - F) & n=29(chicken samples - C)

Figure 3.7.

Bar graph comparing overall liking of the cats between Central-Location-Test and Home-Use-Test.



*Means with the same letter are not significantly different ($P \leq 0.05$). Scores not sharing the same letter were significantly different ($p \leq 0.05$). The data was analyzed to compare within the same type of meat.

** Sample variations were single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI)

***The overall liking scores were based on a 9-point scale -1 (extremely dislike) to 9 (extremely like).

****CLT: n=104 / HUT: n=28(fish samples - F) & n=29(chicken samples - C)

Consumer comments from open-ended questions

The consumers liked about the FSI product saying that it looked like real fish, contained all-natural ingredients and had a good appearance. However, hardness in texture and sharpness of the pieces were some of the concerns which were expressed.

Smell of FSP product was not strong. The product was made of salmon. They liked the texture and uniformity of shape and size. However, they did not like that the FSP product contained preservatives that they did not recognize. Also, the product was crumbly and would be messy to feed as a treat to their cats.

FMI had a fishy smell that the cats may like and contained different kinds of fish in the ingredient statement. Nevertheless, the appearance did not remind them (owners) of natural products as they said it looked too processed. Furthermore, the strong smell, preservatives added and the mixture of many kinds of fish caused it to be perceived as it was made from leftover fish parts were the reasons the consumers did not like.

Although the consumers disliked the CSP product because of its foamy/spongy texture, too processed appearance – not natural and preservative on the ingredient, it did have a good size and shape and uniformity of size that the consumers liked. Moreover, it was mentioned that the aroma would be a “cat’s liking” smell.

What the consumers liked about the CSI product was that it seemed like an unprocessed chicken – real meat - with no preservative and looked just like natural dried meat. Along with the simple one ingredient kind of treat, the product looked like quality food. Nonetheless, the consumers did not like the lack of aroma and the too dry texture of the product.

The consumers liked the color and aroma of the CMI product. It also had a good appearance, with all natural, healthy and simple ingredients with no preservative. Yet, the hardness of the texture was not liked.

Limitations

There were some limitations to this study. To focus more on the effects of ingredient statements, less flavor variations were used in the study. Only six samples and two flavor variations, were selected for testing from a huge variety of the product category. The CLT and HUT were conducted based on the population only in a specific geographic location (Kansas). Moreover, the number of participants in HUT was small compared to CLT (CLT: n=104 and

HUT: n=28(fish samples) and n=29(chicken samples). Furthermore, the HUT participants were the same group as the CLT participants. The carry over effect could have an impact on the HUT results as the participants had already seen the products and the products' information during the CLT study. Moreover, the instruction for HUT stating: "Please serve the treat sample as you regularly would during a treat offering process" could also had impact on the evaluation variations as the freeze-dried products can be served with and without water. Demographic information of the cats could have been collected. This could have provided wider point of view from the data. Also, as mentioned by Koppel (2014), importance of odor and appearance of cat food to cat owner should have been added to fulfill the research information in the field.

Conclusion

Six freeze-dried cat treat products were evaluated, comparing between the Central Location Test (CLT) and Home Use Test (HUT). The liking scores for most of the products between the two tests (CLT and HUT) were not different, but several consumer clusters were identified. The results did not show any trend as to whether the products were liked more in the HUT or vice versa. The results from Home Use Test resulted in no difference among the samples on cat's overall liking scores and feeding intake of the cats between the first and the second day ($p>0.05$). However, prediction of a cats' preference on food followed their owners' liking. The single ingredient for both chicken and fish sample groups were liked the most and scored the highest in naturalness among the products. Most of the samples were penalized in owner's overall liking score because aroma was too strong. Even though the cost estimate for the single ingredient products were higher than the others, the purchase intent for those products were also higher. Naturalness perception of the products and purchase intent significantly increased after

the seeing the ingredient statements. Emotions of both the owner and their cat of before, during and after feeding process were not significantly different. Not all the terms describing owners' and cats' emotions gathered from the focus group by Tsai et al. (2020) were selected by the consumers in this study. Alone, Jealous, Mean and Scared terms describing owners emotions and Angry, Combative, Hostile, and Hurt/Painful terms describing cats emotions, categorized into negative terms, were not used by the consumers in this study. In comparison between CLT and HUT tests, conducting CLT tended to be less time consuming and more cost effective than HUT. However, the results from HUT seemed to provide a more accurate interpretation on the cat's acceptance as the owner was able to evaluate from the actual feeding environment. With uncertain pattern on the liking scores, more testing should be investigated in the future study. With the trend of pet humanization and natural food trend, this could help the pet food industry and related field to better understanding of consumers. Conducting the test in various area would capture a wider population of the consumers.

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Chapter 4 - Association of Sensory Characteristics with Volatile Compounds and Consumer Acceptance in Freeze-Dried Cat Treats

Abstract

Association of aromatic sensory attributes and volatile compounds has been studied in pet food products, but not in freeze-dried cat treats. Further, freeze-dried cat treats volatile profiles have not been reported in current literature. The objectives of this study were to 1) characterize the volatile compounds in freeze-dried cat treat products, 2) relate the descriptive sensory aromatic attributes to the aromatic volatile compounds and 3) combine the sensory characteristics and the volatile components information with both owner and pet liking. Volatile compounds from the six freeze-dried cat treat products (three chicken and three fish samples) were analyzed using headspace solid-phase microextraction (SPME), gas-chromatography – mass spectrometry (GC-MS). More than 60 volatiles were identified to describe the samples. To correlate the instrumental aromatic data with the descriptive sensory aromatic data, Partial Least Squares regression was performed, also for the descriptive aroma data with the consumer liking. The relationship of the information could help understand sensory properties of these and similar healthy pet products and drivers of liking, which would be beneficial for pet food and other industries.

Introduction

Compared to the more conventional dry kibble and wet pet food format, freeze-dried pet food is one of the non-traditional kinds that is growing rapidly in the pet food market (Phillips-Donaldson, 2020). Considering additional caloric intake during the day, freeze-dried cat treat products are an option may complement the cats' nutritional needs. This kind of treat is primarily made from various kinds of meat and is high in protein.

The term that describes an animal's response to food is palatability (Watson, 2011) and is a key attribute of pet foods for describing acceptability of food to the palate or taste and a measurement of its attractiveness and consumption. This is as important to manufacturers as it is to pets and their owners. Food selection by cats is based on sensory properties such as smell, taste, and mouthfeel (Watson, 2011) and is subject to individual animal variation and previous diet or experience (Rofe and Anderson, 1970; Bradshaw, 2006). Another key driver of palatability is nutritional content; cats can adapt their intake for key nutrients in order to meet specific targets for calories and essential nutrients like amino acids (Watson, 2011).

Studying aromatic composition together with descriptive studies could help to better understand specific details about products (Lawless & Heymann, 2010; Yang, 2020; Lu et al., 2017; Velásquez et al., 2019; Lee et al. 2018). Headspace analysis may be used to characterize the volatile components in food (Chambers & Koppel, 2013). Some studies exploring the association of aromatic sensory attributes and volatile compounds have used headspace solid-phase microextraction (SPME), gas-chromatography – mass spectrometry (GC-MS) to determine the aromatic compounds present in pet foods (Koppel, Adhikari & Di Donfrancesco, 2013; Di Donfrancesco & Koppel, 2017). However, academic literature regarding cat treats and their volatiles is non-existent.

The aims of this study was to 1) characterize the volatile compounds in freeze-dried cat treat products, 2) relate the descriptive sensory aromatic attributes of six freeze-dried cat treat products to the aromatic volatile compounds and 3) compare the sensory characteristics and the volatile component information with both owner and pet liking. To associate aromatic sensory attributes, volatile components and consumer liking, descriptive data from Chapter 2 and consumer study data from Chapter 3 were related among the same product samples.






Materials and Methods

Samples

Six commercial grade freeze-dried cat treat product samples used in the lexicon development (Chapter 2) and the consumer study (Chapter 3) were evaluated for volatile analysis. The six product samples were described in Table 4.1. They were purchased online via Chewy.com and Amazon.com. All samples were within the “best by” date on the package. The products were stored at room temperature according to package directions. The two flavors of the freeze-dried cat treat samples used in this study were selected by researchers based on the most popular flavors available in the US market which were fish and chicken. The samples were divided into two sets according to the flavors. Each set has three samples based on the ingredients, single ingredient (SI), single meat with preservative (SP) and mixed ingredients (MI).

Table 4.1.

Freeze-dried Cat Treat Samples Used in Volatile Compounds Analysis.

Samples	Ingredients	Photo
Fish single ingredient (FSI),	Salmon	 A photograph showing several irregular, light brown, freeze-dried pieces of salmon. Below the pieces is a ruler with markings from 7 to 20 cm.
Fish single meat with preservative (FSP)	Salmon (preserved with mixed Tocopherol)	 A photograph showing four irregular, light brown, freeze-dried pieces of salmon. Below the pieces is a ruler with markings from 8 to 19 cm.
Fish mixed ingredients (FMI)	Whole atlantic mackerel, flounder, monkfish, whole atlantic herring, acadian redfish, silver hake, mixed tocopherols (preservative)	 A photograph showing numerous small, irregular, light brown, freeze-dried pieces of various fish. Below the pieces is a ruler with markings from 7 to 20 cm.
Chicken single ingredient (CSI),	Chicken breast	 A photograph showing several irregular, light brown, freeze-dried pieces of chicken breast. Below the pieces is a ruler with markings from 7 to 19 cm.
Chicken single meat with preservative (CSP)	Chicken (preserved with mixed Tocopherol)	 A photograph showing several irregular, light brown, freeze-dried pieces of chicken. Below the pieces is a ruler with markings from 7 to 20 cm.

Chicken mixed
ingredients (CMI)

Cage Free Chicken: Heart, Liver,
Gizzard



Extraction Methods of Volatile Chemicals by SPME

To characterize aroma profiles of freeze-dried cat treat products, the volatile compounds were extracted using headspace solid phase micro extraction (HS-SPME). This similar method has been used in several studies (Koppel et al., 2013; Di Donfrancesco et al., 2017; Yang, 2020).

The samples were broken into small pieces. A 10 mL screwcap vial (Supelco Analytical, Bellefonte, PA, USA) equipped with a polytetrafluoroethylene/silicone septum (Supelco Analytical, Bellefonte, PA, USA) was used as a container for the analysis. About 0.4 gram of sample was weighed in each vial. Each of the vials was added with 10 mL 100 ppm 1,3-dichlorobenzene dissolved in methanol (Sigma Aldrich, St. Louis, MO, USA), which was used as the internal standard.

The SPME fiber 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco Analytical, Bellefonte, PA, USA) was inserted into the vial and exposed to the sample headspace for 1 min at 40 °C for volatiles extraction. The fiber was inserted in the auxiliary injection port at 150 °C for 5 min for cleaning before each evaluation. The sample was stirred at 250 rpm and incubated at 50 °C for 1 min using the autosampler. After sampling, the fiber was removed from the vial. The volatile compounds were desorbed into the gas-chromatographic system injection port, using a splitless injection for 2 minutes at 240 °C. Ultra-high purity helium gas was used as the carrier.

Chromatographic Analysis

A gas chromatograph-mass spectrometer (Shimadzu GCMS – QP2020, Shimadzu Corporation, Kyoto, Japan) was used for isolation, tentative identification, and semi-quantification of the volatile compounds. The compounds were separated on an SH-Rxi-5Sil MS Crossbond column (Shimadzu, Tokyo, Japan; 30 m × 0.25 mm × 0.25 µm film thickness) (Restek, State College, PA, USA).

The column was ramped from the initial temperature of 40 °C to 220 °C with 7 °C min⁻¹ rate. Mass spectrometry was performed using electron-impact ionization at 70 eV (200 °C). The 25.71 min run time was recorded in full scan mode (scanned for masses between 35–350 m/z mass range). Volatile compounds were identified using NIST library version 14. All samples were analyzed in 3 replicates. The semi-quantification of volatile compounds was manifested by the ratio of peak area, which was calculated by the GC peak area divided by the peak area of internal standard.

Descriptive Analysis Method for Sensory Characteristics

Five highly trained panelists evaluated the appearance, texture and aroma of the products, using the modified flavor profile consensus method. All panelists, from the Sensory Analysis Center and Consumer Behavior, Kansas State University (Manhattan, KS, USA), had been trained with at least 1,000 h of experience in evaluating a variety of food products in descriptive sensory analysis panels. Three grams of samples were served in a sniffing glass covered with a watch glass for aroma and appearance evaluation. For texture, three grams of the samples were served in 96.12 ml plastic cups, covered with lids.

A total of eight aroma sensory characteristics were detected from the six samples. The aroma characteristics terms included: overall fish, shellfish, heated oil, oxidized oil, cardboard, brown, musty/dusty, and overall grain. A modified flavor profile method using a scale from 0 to 15 with 0.5 increments was used for intensity quantification, where 0 represents none and 15 extremely high (Di Donfrancesco and Koppel, 2017; Yang, 2020). In between the sample evaluation, the panelists used moist washcloths to help eliminate aromas from their nostrils and wipe down their fingers.

Consumer Acceptance

Central Location Test (n=104) and Home Use Test (n=57) were performed (Chapter 4). The participants were asked to open the sample cup and answered the questionnaires, rating their overall liking on a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely. Also, the participants were asked to indicate how much they thought their cats would have liked the sample. Then, they were asked to take the samples home to do Home Use Test, feeding their cats in normal home condition and evaluating their overall liking on a 9-point hedonic scale.

Statistical Analysis

Partial least square regression (PLSR) was performed using statistical software, version 2019.4.2.12345 (Addinsoft, MS Excel, NY, USA). The multivariate statistical technique has been used in several research studies to identify associations between chromatographic analysis data (X-matrix) and aromatic sensory characteristics of food (Y-matrix) (Koppel et al., 2013; Di Donfrancesco et al., 2017; Lee et al., 2018; Velásquez et al., 2019; Yang, 2020). This statistical analysis technique was also applied to determine correlations between descriptive sensory

analysis data (X-matrix) and consumer acceptance data (Y-matrix) (Adhikari et al., 2010; Tenenhaus et al., 2005).

In this study, the variables (volatile compounds) were selected according to Variable Importance in the Projection (VIP) value (Velásque et al., 2019). The variables that presented values <1 were excluded, except for those chemicals that were present in all the samples. Total 116 volatiles were removed from the list. The remaining 70 volatile compounds (X-matrix) and eight aromatic sensory attributes (Y-matrix) were associated using PLS regression.

All the sensory characteristics (X-matrix) presented in the six samples were correlated to consumer acceptance data (Y-matrix) to determine drivers of liking. The attributes included: five appearance attributes (orange color, brown color, fibrous, surface roughness, and powdery residual), three texture attributes (powdery, surface roughness and oily hand feel) and eight aroma characteristics (overall fish, shellfish, heated oil, oxidized oil, cardboard, brown, musty/dusty, and overall grain).

Results and Discussion

Volatile compounds

A total of 186 compounds were identified in six freeze-dried cat treats, mainly made of two kinds of meat (chicken and fish) (Table 4.2). These compounds were grouped as: alcohols (22 compounds), aldehydes (20 compounds), alkanes (58 compound), alkenes (12 compounds), alkynes (one compound), amines (two compounds), amides (three compound), benzene derivatives (five compounds), carboxylic acids (three compounds), esters (23 compounds), furans (three compounds), ketones (24 compounds), pyrazines (four compounds), terpenes (two compounds), ethers (three compounds), and nitrile (one compound).

More volatiles were present in FSI, FMI and CMI, with detection of 65, 65 and 64 compounds, respectively. On the other hand, the least were present in CSI product sample, where 44 compounds were detected.

Total volatile concentration among the products ranged from 5.68 mg/kg (chicken cat treats with single ingredient - CSI) to 68.28 mg/kg (fish cat treats single meat with preservative - FSP). Overall concentration of volatiles was higher in fish cat treats than in the chicken cat treats. Among the chicken treat samples, total volatile concentration was higher in the chicken cat treats with mixed ingredients (CMI), which contained chicken heart, liver and gizzard, than the chicken cat treats with single ingredient (CSI) product. In contrast, the fish with mixed ingredients (FMI) contained lower volatile concentration than the fish cat treats with single ingredient (FSI) product. Similar results were observed in study of volatile flavor composition for cooked by-product blends of chicken, beef and pork investigated using gas chromatography–mass spectrometry (Wettasinghe et al., 2001). It was reported that the total volatile flavor concentration of chicken by-product blends (CB) was three times higher than that observed for cooked chicken white muscles (CM). In contrast, the total volatile concentration in beef and pork blends was lower than that in their muscle samples (Wettasinghe et al., 2001).

The volatile compounds consisted largely of hydrocarbons. Among alkane, alkene and alkyne groups, the most abundant compound was alkanes ranging from 1.32 to 10.02 mg/kg. However, the group that presented the highest concentration was amines, specifically Trimethylamine (compound #114 on Table 4.1). Trimethylamine was recognized as the primary component of fishy odor (Herath et al., 2019), that is also present in fish and seafood products (Miyasaki, Hamaguchi & Yokoyama, 2011; Emmanuel et al., 2011). Based on the result, more

Trimethylamine was found in fish cat treats (highest in FSP – 44.89 mg/kg) than in the chicken cat treats (lowest in CSI – 0.63 mg/kg).

The second largest group were ketones, then esters, alcohols and aldehydes. Among the six samples, in each group of volatiles, ketones, alcohols and aldehydes were present the most in FSI (9.07, 9.01, and 7.33 mg/kg for ketones, alcohols and aldehydes, respectively). On the other hand, ketones, alcohols and aldehydes presented in CSI the least (0.6, 0.43 and 0.95 mg/kg, respectively). Benzaldehyde, known as almond oil (Koppel et al., 2013), was reported in both fish and chicken meat in the literature (Rivas-Cañedo et al., 2009; Schindler et al., 2010; Varlet et al., 2006; Methven et al., 2007; Leduc et al., 2012). In this study, the compound was present in almost every samples but CSI. However, benzaldehyde could possibly migrate from plastic packaging materials (Vera et al., 2012).

Esters were present in CMI the most and CSI the least, with 19.81 and 0.73 mg/kg, respectively. In CMI, the concentration content of the ester compound was mainly from Acetic acid, methyl ester or Methyl acetate (10.86 mg/kg). The compound presented in the literature (Nonaka et al., 1967) Also, this compound has been found in many food products such as fig, apple, papaya, and fruits (Pubchem).

2-Methyl-2-butenal, and 2-Methyl-2-pentenal presented in all the three fish cat treats but none in the chicken cat treats. 2-Methyl-2-butenal, was also present in fish meat from a previous study (Methven et al., 2007). None of the ethers and amides compounds were detected in any of the chicken treats. Benzene and terpene compounds were not present in both fish and chicken single ingredient treat samples. Nitrile was not detected in fish cat treats.

1-Octen-3-ol, 3-Methyl-butanal, Hexanal, Dodecane, Trimethylamine, Acetic acid, Acetic acid, methyl ester, 2-Butanone and 2-Heptanone were the common compounds found in

all the six samples in this study. However, 3-Methyl-butanal, Dodecane and Trimethylamine were identified in previous studies on fish and seafood volatiles (Joffraud, 2001; Wierda et al., 2006; Methven et al., 2007; Leduc et al., 2012; Miyasaki, Hamaguchi & Yokoyama, 2011; Emmanuel et al., 2011). Hexanal was one of the volatiles present in all of the six samples. Belitz et al. (2001) mentioned that it was the main product of oxidation of linoleic acid. Oxidation reaction of unsaturated fatty acids are exposed to oxygen and form new molecules (Chipault and Hawkins, 1971; Ismail et al., 2016). All lipids containing unsaturated fatty acids oxidize over time (Ismail et al., 2016). Lipid oxidation generates a number of volatile compound products, including heptanal, octanal, octanol, pentanal and hexanal which causes rancid off-flavors and odors (Ross and Smith, 2006). Hexanal and heptanal (off-flavour compounds) were used as compounds to monitor quality of products due to lipid oxidation in food samples (Thongwong et al., 1999; Du et al., 2000; Chitsamphandhvej et al., 2008). In this study, heptanal was also present in most of the products, except for sample FSP.

Table 4.2. Volatile Compounds Content (mg/kg) in the 6 Different Freeze-dried Cat Treat Samples, AVE: Average, SD: Standard Deviation, n.d.: Not Detected, Rt – Retention Time, PLS: the Code Used on PLS Regression Analysis.

					FSI		FSP		FMI		CSI		CSP		CMI	
PLS	#	Compounds	Rt- min	Rt- max	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
Alcohols																
	1	1-Methoxy-2-propanol	4.11	4.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16	0.01	n.d.	n.d.
	2	1-Penten-3-ol	4.16	4.19	6.48	0.76	2.67	0.17	1.92	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	3	1-Pentanol	5.29	5.31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06	0.00	0.09	0.01	0.11	0.02
C1	4	2-Penten-1-ol, (Z)-	5.34	5.35	0.86	0.12	n.d.	n.d.	0.49	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C2	5	1-Hexen-3-ol	5.51	5.52	0.07	0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C3	6	2,3-Butanediol	5.57	5.73	n.d.	n.d.	0.09	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.08	0.05
C4	7	1-Propoxy-2-propanol	6.63	6.64	0.05	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	8	n-Tridecan-1-ol	8.56	8.57	n.d.	n.d.	n.d.	n.d.	0.03	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C5	9	1-Butoxy-2-Propanol	8.64	8.65	0.23	0.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C6	10	1-Heptanol	9.25	9.25	0.35	0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C7	11	1-Octen-3-ol	9.46	9.49	0.47	0.12	0.19	0.02	0.26	0.00	0.11	0.01	0.37	0.02	0.41	0.12
	12	3-Tetradecyn-1-ol	10.07	10.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.25	0.02	n.d.	n.d.
C8	13	4-Methyl-2-propyl-1-pentanol	10.60	10.60	0.34	0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	14	3,5-Octadien-2-ol	10.75	10.75	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06	0.00	n.d.	n.d.	n.d.	n.d.
	15	4-Octyne-3,6-diol	11.43	11.43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.44	0.02	n.d.	n.d.
	16	2-Ethyl-1-dodecanol	11.76	11.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05	0.00	n.d.	n.d.	n.d.	n.d.
	17	2-Butyl-1-octanol	11.91	11.91	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11	0.02	n.d.	n.d.	n.d.	n.d.
	18	2-Propyl-1-heptanol	12.19	12.19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.42	0.02	n.d.	n.d.
	19	2,6-dimethyl-cyclohexanol	12.40	12.41	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.75	0.22
C9	20	1-Norbornanemethanol, acetate	15.72	15.72	0.12	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	21	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	17.20	17.20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04	0.01	n.d.	n.d.	n.d.	n.d.

Table 4.2. Cont.

PLS	#	Compounds	Rt- min	Rt- max	FSI		FSP		FMI		CSI		CSP		CMI	
					AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
	22	1-Dodecanol	19.34	19.37	0.13	0.06	0.23	0.26	0.10	0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Total Alcohols			9.10		3.19		2.81		0.43		1.73		1.34	
		Aldehydes														
	23	2-Methyl-propanal	3.22	3.24	0.40	0.37	n.d.	n.d.	0.92	0.17	n.d.	n.d.	1.18	0.09	1.05	0.84
C10	24	3-Methyl-butanal	3.90	3.93	0.20	0.09	0.47	0.03	0.53	0.05	0.03	0.00	0.41	0.03	1.09	0.58
	25	Pentanal	4.34	4.35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.12	0.01	0.18	0.02	0.25	0.14
C11	26	2-Methyl-2-butenal	4.94	4.97	1.30	0.24	0.18	0.03	0.14	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	27	2-Pentenal, (E)-	5.15	5.15	n.d.	n.d.	n.d.	n.d.	0.06	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C12	28	Hexanal	5.84	5.87	0.43	0.06	0.19	0.04	0.37	0.03	0.56	0.05	0.49	0.05	0.62	0.25
	29	2-Ethyl-2-butenal	6.17	6.21	0.12	0.01	0.10	0.03	0.06	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C13	30	2-Methyl-2-pentenal	6.41	6.44	2.42	0.43	0.15	0.03	0.14	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31	4-Heptenal, (Z)-	7.75	7.78	n.d.	n.d.	0.14	0.06	0.10	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	32	4,4-Dimethylpent-2-enal	7.25	7.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04	0.02
C14	33	Heptanal	7.80	7.83	0.34	0.11	n.d.	n.d.	0.10	0.01	0.03	0.00	0.08	0.01	0.11	0.05
C15	34	2-Ethyl-2-pentenal	8.41	8.41	0.27	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C16	35	Benzaldehyde	9.17	9.20	0.34	0.07	0.48	0.02	0.18	0.00	n.d.	n.d.	0.25	0.04	0.34	0.13
	36	2,4-Heptadienal, (E,E)-	9.88	9.88	n.d.	n.d.	n.d.	n.d.	0.11	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	37	Octanal	9.97	10.01	0.18	0.03	0.11	0.01	0.08	0.00	0.02	0.00	n.d.	n.d.	0.07	0.04
	38	2-Ethyl-2-hexenal	10.06	10.07	0.22	0.06	n.d.	n.d.	n.d.	n.d.	0.05	0.00	n.d.	n.d.	0.54	0.19
	39	Nonanal	12.17	12.20	0.68	0.29	0.27	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C17	40	2-Propyl-2-heptenal	13.69	13.71	0.27	0.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18	41	9-Octadecenal, (Z)-	16.29	16.30	0.11	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C19	42	2-Butyl-2-octenal	17.53	17.54	0.06	0.01	n.d.	n.d.	n.d.	n.d.	0.14	0.01	n.d.	n.d.	n.d.	n.d.
		Total Aldehydes			7.33		2.10		2.79		0.95		2.61		4.12	

Table 4.2. Cont.

					FSI		FSP		FMI		CSI		CSP		CMI	
PLS	#	Compounds	Rt- min	Rt- max	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
Alkane																
	43	2,2-Dimethoxybutane	5.17	5.34	0.07	0.02	0.24	0.28	0.06	0.01	0.02	0.00	n.d.	n.d.	n.d.	n.d.
	44	1,2-Dimethyl-cis-cyclohexane	7.99	8.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.15	0.05
C20	45	Bicyclo[3.3.1]nonane	8.74	8.74	0.04	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	46	2,2,6-Trimethyl-octane	9.08	9.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.24	0.03	n.d.	n.d.
	47	2,2,3,5-Tetramethyl-heptane	9.54	9.54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14	0.02	n.d.	n.d.
C21	48	2,2,4,6,6,-Pentamethyl-heptane	9.75	9.76	n.d.	n.d.	0.79	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	49	2,2,4,6,6-Pentamethyl-heptane	9.77	9.79	n.d.	n.d.	n.d.	n.d.	1.09	0.05	n.d.	n.d.	0.40	0.02	n.d.	n.d.
	50	Undecane	9.92	9.95	n.d.	n.d.	n.d.	n.d.	0.36	0.01	0.04	0.00	n.d.	n.d.	0.18	0.07
	51	3-Ethyl-3-methylheptane	10.10	10.10	n.d.	n.d.	n.d.	n.d.	0.03	0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22	52	2,2-Dimethyl-heptane	10.41	10.41	n.d.	n.d.	0.80	0.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C23	53	2,2-Dimethyl-decane	10.41	10.42	0.30	0.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	54	2,2,11,11-tetramethyl-dodecane	10.42	10.60	n.d.	n.d.	n.d.	n.d.	0.26	0.01	0.03	0.03	1.33	0.16	n.d.	n.d.
	55	2,2,4,4-Tetramethyloctane	10.61	10.61	n.d.	n.d.	n.d.	n.d.	1.03	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	56	3-Methyl-5-propyl-nonane	10.70	10.73	0.50	0.23	0.93	0.06	0.63	0.02	n.d.	n.d.	1.33	0.05	n.d.	n.d.
C24	57	2,5-Dimethyl-undecane	10.74	10.75	n.d.	n.d.	0.64	0.06	n.d.	n.d.	n.d.	n.d.	0.82	0.02	n.d.	n.d.
	58	3,6-Dimethyl-undecane	10.77	11.33	n.d.	n.d.	0.70	0.05	1.79	0.09	0.10	0.00	n.d.	n.d.	0.22	0.07
	59	5,6-Dimethyl-decane	10.86	10.86	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09	0.01	n.d.	n.d.
C25	60	2,6,11-Trimethyl-dodecane	10.97	10.99	n.d.	n.d.	0.12	0.00	n.d.	n.d.	n.d.	n.d.	0.23	0.01	n.d.	n.d.

Table 4.2. Cont.

PLS	#	Compounds	Rt- min	Rt- max	FSI		FSP		FMI		CSI		CSP		CMI	
					AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
	61	3,5-Dimethyl-heptane	11.04	11.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.48	0.02	n.d.	n.d.
C26	62	2,9-Dimethyl-undecane	11.06	11.07	n.d.	n.d.	0.33	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	63	3-Ethyl-2,2-dimethyl-pentane	11.11	11.13	0.49	0.28	0.70	0.05	n.d.	n.d.	0.07	0.00	n.d.	n.d.	0.14	0.05
	64	5-Ethyl-2,2,3-trimethyl-heptane	11.15	11.17	n.d.	n.d.	n.d.	n.d.	0.80	0.05	n.d.	n.d.	n.d.	n.d.	0.09	0.04
	65	3,3,4-Trimethyl-decane	11.29	11.33	n.d.	n.d.	n.d.	n.d.	0.25	0.02	n.d.	n.d.	1.40	0.02	n.d.	n.d.
	66	2,5-Dimethyl-dodecane	11.51	11.92	n.d.	n.d.	n.d.	n.d.	0.30	0.05	n.d.	n.d.	n.d.	n.d.	0.05	0.02
C27	67	2,8,8-Trimethyl-decane	11.58	11.59	n.d.	n.d.	0.48	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.08	0.04
	68	3,3-Dimethyl-undecane	11.59	11.59	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.89	0.03	n.d.	n.d.
	69	2-Methyl-5-propyl-nonane,	11.80	11.81	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.15	0.03	n.d.	n.d.
	70	5-Butyl-nonane	11.82	13.43	n.d.	n.d.	n.d.	n.d.	0.92	0.07	n.d.	n.d.	n.d.	n.d.	0.05	0.02
	71	Hexadecane	12.08	12.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.13	0.05
C28	72	4-Methyl-dodecane	12.08	12.09	0.50	0.51	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C29	73	4-Ethyl-2,2,6,6-tetramethyl-heptane	12.25	12.26	n.d.	n.d.	0.12	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	74	Undecane, 5-methyl-	12.33	12.36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.20	0.02	0.52	0.03	n.d.	n.d.
	75	4,6-Dimethyl-dodecane	12.33	12.33	n.d.	n.d.	n.d.	n.d.	0.33	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C30	76	4,5-Dipropyl-octane	12.42	12.88	n.d.	n.d.	n.d.	n.d.	0.92	0.02	0.03	0.00	n.d.	n.d.	n.d.	n.d.
	77	2,6,11,15-Tetramethyl-hexadecane	12.44	12.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.35	0.02	n.d.	n.d.
C31	78	3,3,6-Trimethyl-decane	12.55	12.55	n.d.	n.d.	0.21	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 4.2. Cont.

PLS	#	Compounds	Rt- min	Rt- max	FSI		FSP		FMI		CSI		CSP		CMI	
					AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
	79	3,3,8-Trimethyl-decane	12.87	12.88	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.08	0.01	n.d.	n.d.
C32	80	4,4-Dimethyl-undecane	13.08	13.20	0.27	0.40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09	0.01	n.d.	n.d.
	81	2,3,5,8-Tetramethyl-decane	13.24	13.25	n.d.	n.d.	n.d.	n.d.	0.26	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	82	5-(2-Methylpropyl)-nonane	13.33	13.43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.17	0.04	n.d.	n.d.	n.d.	n.d.
	83	3-Methyl-undecane	13.51	13.59	n.d.	n.d.	n.d.	n.d.	0.18	0.00	n.d.	n.d.	0.26	0.01	0.07	0.03
	84	Cyclododecane	14.02	14.02	n.d.	n.d.	n.d.	n.d.	0.23	0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C33	85	Dodecane	14.16	14.19	0.99	0.77	0.19	0.02	0.31	0.03	0.19	0.00	0.12	0.02	0.21	0.08
	86	2-Chloro-octane	14.38	14.38	n.d.	n.d.	n.d.	n.d.	0.06	0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	87	5-Methyl-5-propyl-nonane,	14.42	14.42	n.d.	n.d.	n.d.	n.d.	0.08	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	88	Nonadecane	14.60	14.60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.12	0.00	n.d.	n.d.	n.d.	n.d.
	89	Heptadecane	14.73	14.74	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.13	0.00	n.d.	n.d.	n.d.	n.d.
	90	1-Iodo-dodecane	14.98	14.99	n.d.	n.d.	n.d.	n.d.	0.05	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	91	4,4-Dimethyl octane	15.04	15.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02	0.00	n.d.	n.d.	n.d.	n.d.
	92	Hexylcyclohexane	15.08	15.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.07	0.00	n.d.	n.d.
	93	2-Methyl-dodecane	15.34	15.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.07	0.00	n.d.	n.d.	n.d.	n.d.
	94	2,3-Dimethyldodecane	15.58	15.58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05	0.00	n.d.	n.d.	n.d.	n.d.
C34	95	Tridecane	16.15	16.16	0.07	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C35	96	1-(1-Hydroxy-1-heptyl)-2-methylene-3-pentyl-cyclopropane	17.78	17.79	0.09	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C36	97	Tetradecane	17.89	18.06	0.12	0.04	n.d.	n.d.	0.05	0.00	0.06	0.00	n.d.	n.d.	0.05	0.02
C37	98	1,1,3,4-Tetrachloro-1,2,2,3,4,4-hexafluorobutane	22.48	22.50	n.d.	n.d.	n.d.	n.d.	0.04	0.00	0.02	0.00	n.d.	n.d.	n.d.	n.d.

Table 4.2. Cont.

PLS	#	Compounds	Rt- min	Rt- max	FSI		FSP		FMI		CSI		CSP		CMI	
					AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
	99	1,1,2,3,4,5-Hexachloro- 1,2,3,4,5,5-hexafluoro- pentane	22.47	22.48	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05	0.02
C38	100	2,6,10,14-Tetramethyl- pentadecane	23.15	23.16	0.21	0.03	1.83	0.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Total Alkane			3.66		8.09		10.02		1.32		9.98		1.47	
		Alkene														
C39	101	E,Z-4- Ethylidenecyclohexene	7.36	7.39	n.d.	n.d.	0.20	0.08	0.07	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	102	3,5,5-Trimethyl-2- hexene	9.34	9.37	0.71	0.16	0.77	0.06	0.92	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C40	103	1-Docosene	11.76	11.76	0.50	0.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	104	5-Undecene	11.90	11.90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.15	0.08
	105	9-Methyl-5-undecene, (Z)-	12.40	12.40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.43	0.01	n.d.	n.d.
	106	4-Methyl-1-undecene	13.35	13.35	n.d.	n.d.	n.d.	n.d.	0.18	0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	107	Decahydro-1,6- dimethyl-naphthalene	13.78	13.79	n.d.	n.d.	n.d.	n.d.	0.05	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	108	3-Methyl-2-undecene, (Z)-	13.88	13.88	n.d.	n.d.	n.d.	n.d.	0.11	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	109	1-Tridecene	14.00	14.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.28	0.00	n.d.	n.d.	n.d.	n.d.
C41	110	2,6,6-Trimethyl-1- cyclohexene-1- carboxaldehyde	14.05	14.05	n.d.	n.d.	0.09	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	111	Decahydronaphthalene	15.82	15.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.12	0.00	n.d.	n.d.	n.d.	n.d.
	112	5-Hexyl-3,3-dimethyl- cyclopentene	17.39	17.39	n.d.	n.d.	n.d.	n.d.	0.07	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Total Alkene			1.21		1.06		1.40		0.40		0.43		0.15	

Table 4.2. Cont.

					FSI		FSP		FMI		CSI		CSP		CMI	
PLS	#	Compounds	Rt- min	Rt- max	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
		Alkyne														
	113	3-Dodecyne	15.55	15.56	n.d.	n.d.	n.d.	n.d.	0.14	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Amine														
C42	114	Trimethylamine	2.81	2.87	15.33	4.96	44.89	3.98	13.29	2.47	0.63	0.26	3.44	0.39	5.14	1.95
	115	2-Propen-1-amine	11.63	11.63	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.83	0.06	n.d.	n.d.
		Total Amine			15.33		44.89		13.29		0.63		4.27		5.14	
		Amide														
	116	N,N-Dimethylformamide	5.55	5.60	0.43	0.22	0.57	0.11	0.11	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C43	117	N,N-Dimethylacetamide	7.26	7.28	0.22	0.08	n.d.	n.d.	0.06	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	118	N,N-Dimethylpropanamide	8.82	8.86	0.19	0.07	0.17	0.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Total Amide			0.83		0.74		0.17		0.00		0.00		0.00	
		Benzene compounds														
C44	119	p-Xylene	7.22	7.24	n.d.	n.d.	0.56	0.07	n.d.	n.d.	n.d.	n.d.	0.24	0.02	n.d.	n.d.
C45	120	o-Xylene	7.70	7.71	n.d.	n.d.	0.25	0.03	n.d.	n.d.	n.d.	n.d.	0.28	0.03	n.d.	n.d.
C46	121	1,2,3-Trimethylbenzene	9.87	9.87	n.d.	n.d.	0.17	0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	122	Mesitylene	9.88	9.88	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.07	0.01	n.d.	n.d.
	123	Toluene	5.35	5.36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.17	0.02	0.12	0.06
		Total Benzene compounds			0.00		0.99		0.00		0.00		0.76		0.12	

Table 4.2. Cont.

					FSI		FSP		FMI		CSI		CSP		CMI	
PLS	#	Compounds	Rt- min	Rt- max	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
Carboxilic acid																
C47	124	Acetic acid	3.28	3.38	0.81	0.30	1.08	0.11	0.79	0.09	0.29	0.01	1.66	0.24	1.11	0.92
C48	125	4-Hydroxy-butanoic acid	8.01	8.02	n.d.	n.d.	0.07	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	126	Oleic Acid	9.25	9.26	n.d.	n.d.	n.d.	n.d.	0.14	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Total Carboxilic acid			0.81		1.16		0.92		0.29		1.66		1.11	
Esters																
C49	127	Acetic acid, methyl ester	3.07	3.10	0.51	0.69	0.24	0.04	1.49	0.17	0.42	0.02	1.70	0.06	10.86	5.57
	128	Methyl propionate	3.66	3.69	0.18	0.12	n.d.	n.d.	0.05	0.01	n.d.	n.d.	0.14	0.03	0.62	0.07
	129	Methyl isobutyrate	4.17	4.18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.33	0.02
	130	Butanoic acid, methyl ester	4.63	4.66	0.10	0.01	n.d.	n.d.	0.05	0.02	0.02	0.00	n.d.	n.d.	0.42	0.15
	131	2-Hydroxy-propanoic acid-methyl ester, (.+/- .)-	4.83	4.86	0.07	0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.13	0.03	1.39	0.36
C50	132	2-Methyl-2-propenoic acid-2-hydroxypropyl ester	4.84	4.84	n.d.	n.d.	0.27	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	133	Methyl isovalerate	5.44	5.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.20	0.11
	134	Methyl valerate	6.25	6.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.36	0.09
	135	3-Hydroxy-butanoic acid methyl ester	6.90	6.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.13	0.05
	136	Hexanoic acid, methyl ester	8.24	8.26	0.09	0.01	n.d.	n.d.	n.d.	n.d.	0.12	0.02	0.07	0.00	3.32	0.89
	137	3,5,5-Trimethylhexyl acetate	9.32	9.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.13	0.00	n.d.	n.d.
C51	138	Carbonic acid, prop-1-en-2-yl undecyl ester	9.92	9.92	n.d.	n.d.	0.43	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	139	Oxalic acid, 2-ethylhexyl hexyl ester	9.94	9.94	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.26	0.01	n.d.	n.d.

Table 4.2. Cont.

PLS	#	Compounds	Rt- min	Rt- max	FSI		FSP		FMI		CSI		CSP		CMI	
					AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
	140	Heptanoic acid, methyl ester	10.38	10.39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.52	0.08
	141	Methyl 6,6,8,8,10,10-hexamethyl-3-oxo-2,5,7,9,11-pentaoxa-6,8,10-trisilatridecan-13-oate	10.65	10.66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03	0.00	n.d.	n.d.	0.05	0.02
	142	Octanoic acid, methyl ester	12.53	12.53	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.00	0.29
	143	Sulfurous acid, isohexyl 2-pentyl ester	13.60	13.60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11	0.01	n.d.	n.d.
C52	144	2-Bromo-propanoic acid butyl ester	11.62	11.63	n.d.	n.d.	0.51	0.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	145	Nonanoic acid, methyl ester	14.59	14.59	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.28	0.02
	146	Decanoic acid, methyl ester	16.56	16.56	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.24	0.08
C53	147	2-Methyl-propanoic acid-3-hydroxy-2,2,4-trimethylpentyl ester	17.60	17.60	0.07	0.01	n.d.	n.d.	n.d.	n.d.	0.08	0.03	n.d.	n.d.	n.d.	n.d.
	148	Dodecanoic acid, methyl ester	20.18	20.18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09	0.03
	149	(E)-Dodec-5-en-4-olide	20.96	20.96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04	0.00	n.d.	n.d.	n.d.	n.d.
	Total Esters				1.03		1.45		1.59		0.73		2.54		19.81	
	Furans															
C54	150	2-Ethyl-furan	4.36	4.37	0.71	0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C55	151	2,4-Dimethylfuran	4.54	4.55	0.12	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	152	2-pentyl-furan	9.72	9.73	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.30	0.01	0.37	0.02	0.54	0.30
	Total Furans				0.83		0.00		0.00		0.30		0.37		0.54	

Table 4.2. Cont.

					FSI		FSP		FMI		CSI		CSP		CMI	
PLS	#	Compounds	Rt- min	Rt- max	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
Ketones																
	153	Acetone	2.95	2.96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.23	0.64
C56	154	2-Butanone	3.41	3.44	1.25	0.38	1.63	0.12	2.63	0.24	0.42	0.01	1.72	0.03	1.24	0.83
	155	2,3-Pentanedione	4.31	4.32	n.d.	n.d.	n.d.	n.d.	0.16	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C57	156	3-Penten-2-one, (E)-	4.89	4.90	0.03	0.00	n.d.	n.d.	0.13	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C58	157	2-Heptanone	7.54	7.57	0.15	0.04	0.28	0.04	0.04	0.00	0.13	0.02	0.30	0.03	0.47	0.15
	158	Butyrolactone	8.01	8.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.17	0.01	n.d.	n.d.
	159	Dimethyl sulfone	8.08	8.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06	0.01	n.d.	n.d.
C59	160	6-Methyl-2-heptanone	8.90	8.90	0.16	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	161	2,5-Octanedione	9.53	9.53	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09	0.05
	162	2-Methyl-1-hepten-6-one	9.55	9.57	0.47	0.12	0.41	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.10	0.02
	163	2-Octanone	9.60	9.68	n.d.	n.d.	n.d.	n.d.	0.41	0.01	n.d.	n.d.	n.d.	n.d.	0.17	0.05
C60	164	2,3-Dimethyl-2-cyclopenten-1-one	9.88	9.88	0.27	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C61	165	1,4-Cyclohexanedione	10.55	10.55	1.35	0.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	166	1-Methyl-2-pyrrolidinone,	10.85	10.85	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.10	0.03
	167	5-Ethyldihydro-2(3H)-furanone	11.06	11.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16	0.05
	168	2-Nonanone	11.85	11.87	2.95	0.69	0.55	0.03	n.d.	n.d.	n.d.	n.d.	0.22	0.03	0.21	0.08
C62	169	3,5-Octadien-2-one	11.93	11.96	1.72	0.38	0.65	0.04	0.92	0.03	0.05	0.01	0.34	0.03	n.d.	n.d.
	170	5-t-Butyl-hexa-3,5-dien-2-one	13.25	13.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.17	0.07
	171	2-Methyl-cyclopentanone	13.66	13.66	n.d.	n.d.	n.d.	n.d.	0.11	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 4.2. Cont.

PLS	#	Compounds	Rt- min	Rt- max	FSI		FSP		FMI		CSI		CSP		CMI	
					AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
	172	7a-Methyl-3-methylenehexahydrobenzofuran-2-one	13.80	13.80	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16	0.07
C63	173	2-Decanone	13.97	13.99	n.d.	n.d.	0.25	0.02	n.d.	n.d.	n.d.	n.d.	0.29	0.02	0.20	0.07
	174	4-(1-Acetylcyclopentyl)-but-3-en-2-one	15.81	15.81	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16	0.06
C64	175	2-Undecanone	16.00	16.00	0.65	0.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C65	176	2-(1-Methyl-2-oxopropyl)cyclohexanone	17.03	17.04	0.05	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total Ketones					9.07		3.77		4.39		0.60		3.10		5.46	
Pyrazines																
C66	177	Methyl pyrazine	6.35	6.40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11	0.02	0.22	0.03
C67	178	2,5-Dimethyl pyrazine	8.08	8.17	0.07	0.02	0.25	0.02	n.d.	n.d.	n.d.	n.d.	0.34	0.02	0.56	0.14
C68	179	Trimethyl pyrazine	10.02	10.03	n.d.	n.d.	0.19	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	180	2-ethyl-3,5-dimethylpyrazine	11.62	11.62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.15	0.07
Total Pyrazines					0.07		0.45		0.00		0.00		0.45		0.93	
Terpenes																
	181	.alpha.-Pinene	8.60	8.63	n.d.	n.d.	0.13	0.01	0.09	0.01	n.d.	n.d.	0.07	0.01	n.d.	n.d.
C69	182	D-Limonene	10.65	10.65	n.d.	n.d.	0.28	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total Terpenes					0.00		0.41		0.09		0.00		0.07		0.00	

Table 4.2. Cont.

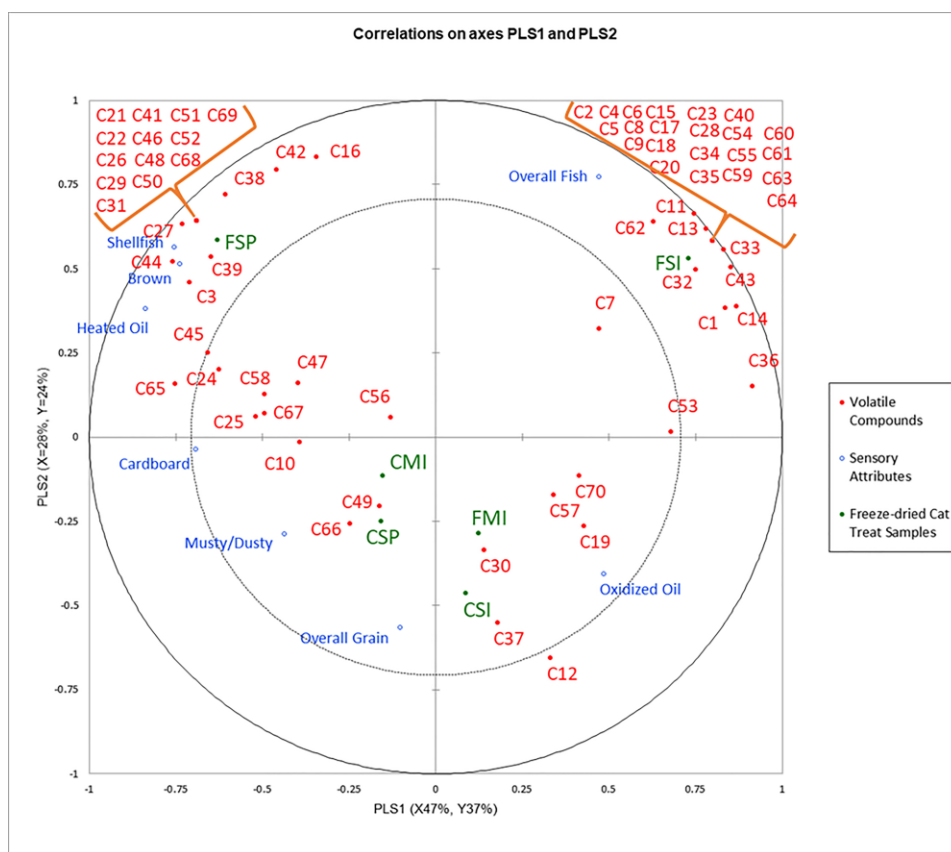
					FSI		FSP		FMI		CSI		CSP		CMI	
PLS	#	Compounds	Rt- min	Rt- max	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD	AVE	SD
Ethers																
C70	183	propylene oxide	2.96	2.98	0.78	0.92	n.d.	n.d.	2.27	0.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	184	4-Ethyl-2-methoxy-phenol	10.93	10.93	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09	0.04
	185	Decyl heptyl ether	12.29	12.29	n.d.	n.d.	n.d.	n.d.	0.19	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total Ethers				0.78		0.00		2.46		0.00		0.00		0.09	
Nitriles																
	186	Hexane nitrile	7.30	7.31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05	0.00	n.d.	n.d.	0.05	0.01
Total Volatiles Concentration					50.04		68.28		40.06		5.68		27.97		40.33	

Association of Volatile compounds and descriptive sensory attributes

The correlation between descriptive sensory data on aromatic attributes (Y-matrix) and the instrumental aromatic profile (X-matrix) showed that there were two main groups of association (Figure 4.1).

Figure 4.1.

Partial Least Squares Regression Factors 1 (x = 47%, y = 37%) and 2 (x = 28%, y = 24%). X-matrix: Chromatographic Analysis Data and Y-matrix: Descriptive Sensory Data.



Note: Red dots (C): chemicals from the chromatographic analysis; Blue dots: aroma sensory attributes from the descriptive sensory data. Green dots: Freeze-dried cat treat samples. (Fish cat treats with single ingredient (FSI), Fish cat treats single meat with preservative (FSP), Fish cat treats with mixed ingredients (FMI), Chicken cat treats with single ingredient (CSI), Chicken cat treats single meat with preservative (CSP) and Chicken cat treats with mixed ingredients (CMI))

Compounds 2,3-Butanediol (C3), 2,2,4,6,6-Pentamethyl-heptane (C21), 2,2-Dimethyl-heptane (C22), 2,9-Dimethyl-undecane (C26), 2,8,8-Trimethyl-decane (C27), 4-Ethyl-2,2,6,6-tetramethyl-heptane (C29), 3,3,6-Trimethyl-decane(C31), E,Z-4-Ethylidenecyclohexene (C39), 2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde (C41), Trimethylamine (C42), p-Xylene (C44), o-Xylene (C45), 1,2,3-Trimethyl-benzene (C46), 4-Hydroxy-butanoic acid (C48), 2-Methyl-2-propenoic acid-2-hydroxypropyl ester (C50), Carbonic acid, prop-1-en-2-yl undecyl ester (C51), 2-Bromo-propanoic acid butyl ester (C52), 2-(1-Methyl-2-oxopropyl)-cyclohexanone (C65), Trimethyl pyrazine (C68) and D-Limonene (C69) were highly correlated to shellfish, heated oil and brown sensory aromatic attributes, upper left corner on Figure 4.1. Trimethylamine (C42) had higher association to shellfish attribute ($r > 0.7$) than overall fish ($r > 0.5$). Upper right quadrant on Figure 4.1 presented another group of compounds were related to overall fish attribute, which included 2-Penten-1-ol, (Z)- (C1), 1-Hexen-3-ol (C2), 1-Propoxy-2-propanol (C4), 1-Butoxy-2-Propanol (C5), 1-Heptanol (C6), 4-Methyl-2-propyl-1-pentanol (C8), 1-Norbornanemethanol, acetate (C9), 2-Methyl-2-butenal (C11), 2-Methyl-2-pentenal (C13), Heptanal (C14), 2-Ethyl-2-pentenal (C15), 2-Propyl-2-heptenal (C17), 9-Octadecenal, (Z)- (C18), Bicyclo[3.3.1]nonane (C20), 2,2-Dimethyl-decane (C23), 4-Methyl-dodecane (C28), 4,4-Dimethyl-undecane (C32), Dodecane (C33), Tridecane (C34), 1-(1-Hydroxy-1-heptyl)-2-methylene-3-pentyl-cyclopropane (C35), Tetradecane (C36), 1-Docosene (C40), N,N-Dimethylacetamide (C43), 2-Ethyl-furan (C54), 2,4-Dimethylfuran (C55), 6-Methyl-2-heptanone (C59), 2,3-Dimethyl-2-cyclopenten-1-one (C60), 1,4-Cyclohexanedione (C61), 3,5-Octadien-2-one (C62), 2-Decanone (C63) and 2-Undecanone (C64) ($r > 0.75$). Hexanal is an off flavor (Chitsamphandhvej et al., 2008). Chitsamphandhvej et al. (2008) used hexanal and heptanal contents in samples to determine lipid oxidation resulting in deterioration of food

quality. Sources of omega-3 fatty acids, EPA and DHA are primary from seafood products such as fish and fish-oil (Saito et al., 2008). In degradation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the taste or odor was linked to fishy attribute (Ismail et al., 2016). It was shown that heptanal (C14) was more correlated to the overall fish attribute ($r > 0.6$) than other attributes. The fish sample also had higher correlation to heptanal and overall fish attribute.

The study by Di Donfrancesco and Koppel (2017) identified that hexanal is related to oxidized oil sensory attribute, also Koppel, Adhikari, and Di Donfrancesco (2013). In this study, although hexanal seemed to be related to the oxidized oil aromatic attribute, 4,5-Dipropyl-octane (C30), 1,1,3,4-Tetrachloro-1,2,2,3,4,4-hexafluoro-butane (C37), 3-Penten-2-one, (E)- (C57) and propylene oxide (C70) were higher correlated to the oxidized oil attribute ($r > 0.8$) than the hexanal ($r = -0.01$). Hexanal was even closer to overall grain than the oxidized oil.

On Figure 4.1, the first two partial least squares factors explained 75 % of the X-matrix (volatile data) variability and 61% of the Y-matrix (sensory descriptive data) variability.

Di Donfrancesco and Koppel (2017) reported that 1-nonen-3-ol and (E,E)-3,5-octadien-2-one was correlated to musty aroma. Musty notes can be described differently and the references used can be varied conducting descriptive sensory analysis (Vazquez-Araujo et al., 2011) In this study, musty/dusty was related to 2,5-Dimethyl-undecane (C24), 2,6,11-Trimethyl-dodecane (C25) and o-Xylene (C45) ($r > 0.6$).

3-Methyl-butanal (C10), Acetic acid (C47), 2-Butanone (C56), 2-(1-Methyl-2-oxopropyl)-cyclohexanone (C65) and 2,5-Dimethyl pyrazine (C67) were close to the cardboard aroma from this study ($r > 0.6$). However, in whey protein study by Whitson et al. (2010), it was reported that cardboard flavor was from a combination of pentanal, heptanal, nonanal, 1-octen-3-one, dimethyl trisulfide, not from just one compound. In addition, Czerny (2009) investigated

odor-active compounds in cardboard. Vanillin, (E)-non-2-enal, (R/S)- γ -nonalactone, 2-methoxyphenol, (R/S)- δ -decalactone, p-anisaldehyde, 3-propylphenol were detected as the highest odor intensities.

As chicken and fish, especially salmon, were the two main kinds of meat used as ingredients in the six samples of freeze-dried cat treat products in this study, the list of volatile compounds presented in this study were compared to previous studies based on the raw ingredients - in chicken (Du et al., 2001; Rivas-Cañedo et al., 2009; Jayasena et al., 2013; Nonaka et al., 1967; Horvat et al., 1976; Goodridge et al., 2003; Minor et al., 1965; Siegmund & Pfannhauser, 1999; Schindler et al., 2010), fish and seafood (Varlet et al., 2006; Joffraud, 2001; Jónsdóttir et al., 2008; Wierda et al., 2006; Oliveira et al., 2005; Methven et al., 2007; Leduc et al., 2012; Miyasaki, Hamaguchi & Yokoyama, 2011; Emmanuel et al., 2011).

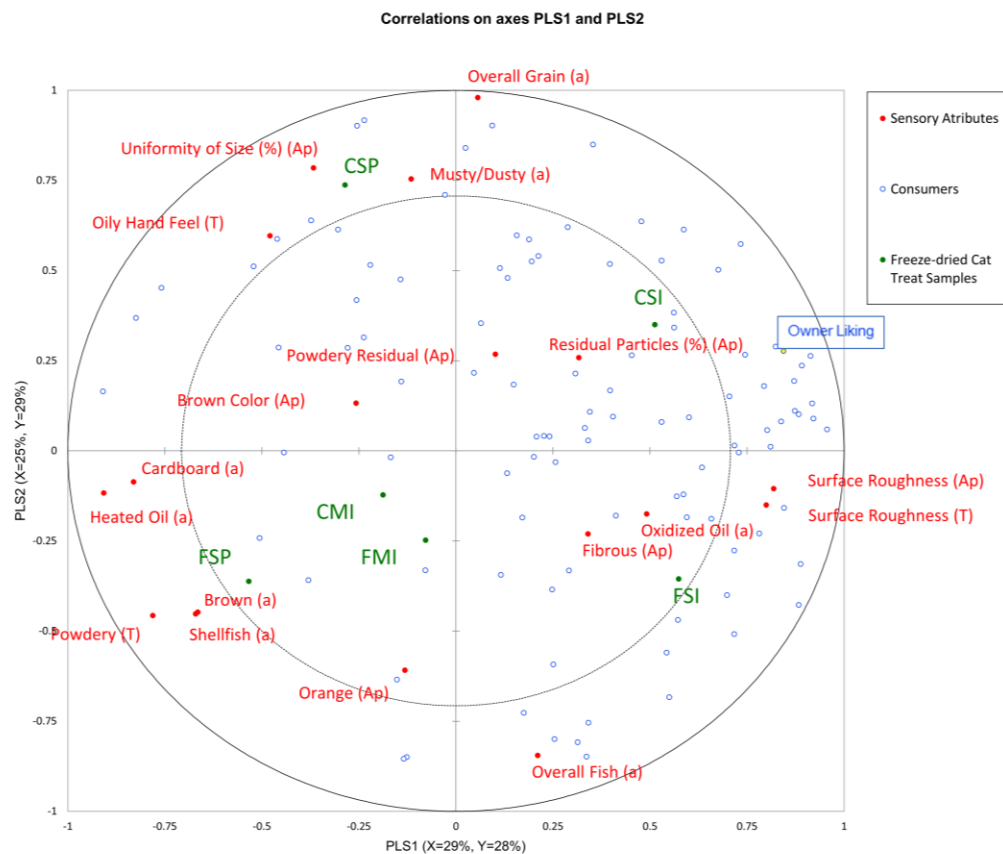
2,3-Butanediol (C3), 2,2,4,6,6-Pentamethyl-heptane (C21), 2,2-Dimethyl-heptane (C22), 2,9-Dimethyl-undecane (C26), 2,8,8-Trimethyl-decane (C27), 4-Ethyl-2,2,6,6-tetramethyl-heptane (C29), 3,3,6-Trimethyl-decane (C31), 2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde (C41), 1,2,3-Trimethyl-benzene (C46), 4-Hydroxy-butanoic acid (C48), 2-Methyl-2-propenoic acid-2-hydroxypropyl ester (C50), Carbonic acid, prop-1-en-2-yl undecyl ester (C51), 2-Bromopropanoic acid butyl ester (C52), Trimethyl pyrazine (C68) and D-Limonene (C69) were closely related to brown aroma attribute. Soy sauce was used as a reference for brown aroma attribute. 2,3-Butanediol (C3) was one of the volatiles presented in soy sauce (Sun et al., 2010).

Descriptive sensory attributes and consumer acceptance

Presented on the map (Figure 4.2), most of the consumers liked the CSI and FSI more in which had higher correlation with surface roughness texture and appearance. These attributes could possibly be the drivers of liking of these products.

Figure 4.2.

Partial Least Squares Regression Factors 1 ($x = 29\%$, $y = 28\%$) and 2 ($x = 25\%$, $y = 19\%$). X-matrix: Descriptive Sensory Data and Y-matrix: Consumer Overall Liking Data.



Note: Red dots: aroma sensory attributes from the descriptive sensory data; Blue dots: consumer acceptance data, “owner liking” in the square represents the average score of the consumer; Green dots: Freeze-dried cat treat samples. (Fish cat treats with single ingredient (FSI), Fish cat treats single meat with preservative (FSP), Fish cat treats with mixed ingredients (FMI), Chicken cat treats with single ingredient (CSI), Chicken cat treats single meat with preservative (CSP) and Chicken cat treats with mixed ingredients (CMI))

The first two partial least squares dimensions explained 57 % of the descriptive sensory data (X-matrix) variability and 48% of the consumer acceptance (Y-matrix) variability.

CSI and FSI were negatively correlated to FSP, CMI and FMI. FSP, CMI and FMI were characterized by cardboard, heated oil, brown and shellfish aromas. Less participants liked these samples compared to FSI and CSI. The consumers tended to be more concentrated on the right side of the map where FSI and CSI is located. These two samples were negatively correlated cardboard, heated oil, brown and shellfish aroma attributes. This could possibly be that the FSI and CSI had low intensities in cardboard, heated oil, brown and shellfish aroma attributes. Di Donfrancesco et al. (2014) indicated that appearance had more influence driving overall liking of consumers more than aroma of the samples while Delime et al. (2018) mentioned that appearance and aroma are both important factors that would contribute to the pet owners acceptance. In this study, the average of the owner liking also showed higher correlation with surface roughness texture and appearance ($r > 0.75$) than the other attributes. However, more of the appearance attributes appeared to have higher correlation to the average owner liking than the aroma attributes.

Limitations

The samples used in this study were limited to just two main different kind of meat. It would provide another perspective comparing among various kinds of meats. Variation of the matrix that the compound is in and concentration of the compound could possibly be the cause of difficulties to associate volatile compounds with a specific sensory characteristic (Chambers & Koppel, 2013). Thus, in the future study, Gas Chromatography Analysis with Olfactometric Detection would be helpful for the relationship identification.

Conclusion

Total of 186 volatile compounds were detected from the six freeze-dried cat treat samples. Majority of the compounds were hydrocarbons. The total concentration of volatiles was the highest in the FSI sample and the lowest in CSI sample. Trimethylamine was relatively high in the fish cat treat samples. Combining the sensory aromatic attributes with volatile compounds helped in better understanding sensory properties of these products. The use of sensory attributes from the lexicon development (Chapter 2) associated with consumer acceptance helped to understand drivers of liking of the freeze-dried cat treat products. This could potentially be beneficial in quality control purpose, product development for pet food and other industries.

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Appendix A - Screener and Questionnaire Used in Consumer Study

(Chapter 2)

Screener

Q1: What is your gender? [Quota 50:50]

- ☐ Male
- ☐ Female

Q2: Which of the following categories best describes your current age?

- ☐ Under 18 years old **(DQ)**
- ☐ 18 - 20 years old
- ☐ 21 - 30 years old
- ☐ 31 - 40 years old
- ☐ 41 - 50 years old
- ☐ 51 years old or older

Q3: Do you or does any member of your immediate family, work for any of the following types of companies? (Check all that apply)

- ☐ Advertising or public relations **(DQ)**
- ☐ Market research **(DQ)**
- ☐ Broadcast or print media **(DQ)**
- ☐ Pet food manufacturer or distributor **(DQ)**
- ☐ Grocery store or store that sells pet food or supplies **(DQ)**
- ☐ Auto manufacturing/sales
- ☐ Humane Society **(DQ)**
- ☐ Dog or cat breeding, training, or grooming facility **(DQ)**
- ☐ Pet store **(DQ)**
- ☐ Veterinarian **(DQ)**
- ☐ Pet daycare or kennel **(DQ)**
- ☐ Credit card company or bank
- ☐ Cosmetics company
- ☐ None of the above

Q4: When was the last time, if ever, you participated in any type of consumer or market research about pet food?

- ☐ In the last month **(DQ)**
- ☐ In the last two months **(DQ)**
- ☐ In the last three months **(DQ)**
- ☐ In the last 4-6 months
- ☐ More than 6 months ago
- ☐ I have never participated in a consumer research study about a food or beverage.

Q5: Do you have any cats in your home?

- ☐ Yes
- ☐ No **(DQ)**

Q6: What percent of the cat food decisions do you personally make for your household?

- ☐ Less than 50% **(DQ)**
- ☐ 50%
- ☐ More than 50%

Q7: Do you feed any of these types of foods to your cat(s)? (Check all that apply)

- ☐ Canned Cat Food
- ☐ Dry Cat Food
- ☐ Cat Treats **(Required)**
- ☐ Cat Food Toppers
- ☐ Raw Food
- ☐ Leftovers

Q8: How would you describe the lifestyle of your cat(s)?

- ☐ Indoor - cat never goes outside
- ☐ Outdoor - cat does not come into my house **(DQ)**
- ☐ Indoor & Outdoor - cat comes into my home AT NIGHT
- ☐ Indoor & Outdoor - cat does NOT come into my home AT NIGHT **(DQ)**
- ☐ None of the above **(DQ)**

Q9: Would you be willing to take treats home to your cat and fill out a questionnaire for an additional incentive of a \$25 Amazon gift card?

- ☐ Yes
- ☐ No

Demographic questions

Q1: What is your gender?

- ☐ Male
- ☐ Female

Q2: Which of the following categories best describes your current age?

- ☐ Under 18 years old
- ☐ 18 - 20 years old
- ☐ 21 - 30 years old
- ☐ 31 - 40 years old
- ☐ 41 - 50 years old
- ☐ 51 - 60 years old
- ☐ 61 years or older

Q3: Which of the following best describes your annual household income before taxes?

- ☐ Less than \$20,000
- ☐ \$20,000 - \$39,999
- ☐ \$40,000 - \$59,999
- ☐ \$60,000 - \$79,999
- ☐ \$80,000 - \$99,999
- ☐ \$100,000 or more

Q4: What is the last level of education that you completed?

- ☐ Some high school or less
- ☐ Completed high school/GED
- ☐ Some college/technical school
- ☐ Completed college/technical school
- ☐ Some post-graduate study or more

Q5: How many pets do you currently have in your household?

- ☐ One
- ☐ Two
- ☐ Three
- ☐ Four
- ☐ Five or more

Q6: Please indicate what kind of pets you have in your household? (Check all that apply)

- ☐ Cat
- ☐ Dog
- ☐ Lizard
- ☐ Bird
- ☐ Fish
- ☐ Horse
- ☐ Rabbit
- ☐ Hamster
- ☐ Guinea pig
- ☐ Other (please list)

Q7: How many cats do you currently have in your home?

- ☐ One
- ☐ Two
- ☐ Three
- ☐ Four or more

Q8: Which statement best describes your role in cat food purchasing for your household/family?

- ☐ I do all the cat food purchasing
- ☐ I do more than half the purchasing
- ☐ I do half the purchasing
- ☐ I do less than half the purchasing

Q9: "I know my cat well enough that I can tell what he or she likes/dislikes." (If you have more than one cat, please refer to the one that will participate in the test.)

- ☐ Strongly disagree
- ☐ Disagree
- ☐ Neither agree nor disagree
- ☐ Agree
- ☐ Strongly agree

Q10: How often do you usually give your cat treats?

- ☐ 2 times a day or more
- ☐ 1 time every day
- ☐ More than 2 times a week
- ☐ 1 time a week
- ☐ 2 to 3 times a month
- ☐ 1 time a month
- ☐ Less than 1 time a month

Q11: When do you give your cat treats? (Check all that apply)

- ☐ If the cat behaved well
- ☐ If I feel like it
- ☐ If the cat asks for it
- ☐ I typically give treats regularly
- ☐ When I'm sad
- ☐ When I want to spend time with my cat
- ☐ When I want the cat to behave (eg: go to vet)
- ☐ At special occasions
- ☐ Other: (Specify)

Q12: Which of the following types of cat treats do you usually give to your cats? (Check all that apply)

- ☐ Crunchy
- ☐ Soft and Chewy
- ☐ Freeze-Dried
- ☐ Catnip
- ☐ Veterinary Diet
- ☐ Jerky
- ☐ Dehydrated
- ☐ Lickable/Liquid
- ☐ Other: (Specify)

Q13: How often do you look at the ingredient statement when purchasing cat food products?

- ☐ Never
- ☐ Rarely
- ☐ Occasionally
- ☐ Most of the time
- ☐ Always

Q14: When you read an ingredient label, how important are the ingredients in your cat food purchase?

- ☐ Not at all Important
- ☐ Slightly Important
- ☐ Moderately Important
- ☐ Very Important
- ☐ Extremely Important

Q15: How much do you agree with the following statements?

Q15.1: I would be really upset if my cat disappears

- ☐ Strongly Disagree
- ☐ Disagree
- ☐ Neither Agree nor Disagree
- ☐ Agree
- ☐ Strongly Agree

Q15.2: Being with my cat brings me a lot of happiness and pleasure

- ☐ Strongly Disagree
- ☐ Disagree
- ☐ Neither Agree nor Disagree
- ☐ Agree
- ☐ Strongly Agree

Q15.3: I find comfort in the presence of my cat

- ☐ Strongly Disagree
- ☐ Disagree
- ☐ Neither Agree nor Disagree
- ☐ Agree
- ☐ Strongly Agree

Q15.4: I am very close to my cat

- ☐ Strongly Disagree
- ☐ Disagree
- ☐ Neither Agree nor Disagree
- ☐ Agree
- ☐ Strongly Agree

Q16: Which of the following statements do you think apply to yourself? (Check all that apply)

- ☐ I am very particular about the healthiness my cats' food.
- ☐ I always follow a healthy and balanced diet for my cats.
- ☐ I feed my cats what I would like to feed them and I do not worry about the healthiness of food.
- ☐ I do not avoid feeding my cats any foods, even if they may cause weight gain.
- ☐ The healthiness of food has little impact on my cats' food choices.
- ☐ The healthiness of cat treats makes no difference to my choice.
- ☐ I do not care about additives in my cats' diet.

- I always look for natural ingredients in the foods that I feed my cats.
- If I do not understand the name of an ingredient or if the name is unfamiliar, I do not buy the food product.
- I do not care about natural ingredients in the foods that I feed my cats.
- I do not read ingredient statements and do not worry about natural ingredients in my cats' foods.
- In my opinion, additives in foods are not harmful for my cats' health.
- I try to feed my cats with foods that do not contain additives.

Central Location Test Questionnaire

Q1: Overall, please indicate how much you like or dislike this cat treat.

- Extremely dislike
- Moderately dislike
- Dislike
- Slightly dislike
- Neither like nor dislike
- Slightly like
- Like
- Moderately like
- Extremely like

Q2: Please indicate what best describes the INTENSITY/STRENGTH of:

Q2.1: Color intensity of pieces

- Much too light
- Slightly too light
- Just-about-right
- Slightly too dark
- Much too dark

Q2.2: Overall shape among pieces

- Much too similar
- Slightly too similar
- Just-about-right
- Slightly too different
- Much too different

Q2.3: Size of pieces

- Much too small
- Slightly too small
- Just-about-right
- Slightly too large
- Much too large

Q2.4: Aroma

- ☐ Much too weak
- ☐ Slightly too weak
- ☐ Just-about-right
- ☐ Slightly too strong
- ☐ Much too strong

Q2.5: Hardness

- ☐ Much too soft
- ☐ Slightly too soft
- ☐ Just-about-right
- ☐ Slightly too hard
- ☐ Much too hard

Q3: How natural do you think cat treat \${SAMPLEBC} is?

- ☐ Extremely unnatural
- ☐ Moderately unnatural
- ☐ Unnatural
- ☐ Slightly unnatural
- ☐ Neither natural nor unnatural
- ☐ Slightly natural
- ☐ Natural
- ☐ Moderately natural
- ☐ Extremely natural

Why did you say this sample was \${WILDCARD1}?

Q4: Please indicate the statement that best describes how much you think your cat would like or dislike this sample. (Please refer to the cat that would be participating in the home use test (if you are selected).)

- ☐ Extremely dislike
- ☐ Moderately dislike
- ☐ Dislike
- ☐ Slightly dislike
- ☐ Neither like nor dislike
- ☐ Slightly like
- ☐ Like
- ☐ Moderately like
- ☐ Extremely like

Q5: How likely would you be to purchase this product if it was available at a reasonable price where you normally shop?

- ☐ Extremely unlikely to purchase
- ☐ Moderately unlikely to purchase
- ☐ Unlikely to purchase
- ☐ Slightly unlikely to purchase
- ☐ Neither likely nor unlikely to purchase
- ☐ Slightly likely to purchase
- ☐ Likely to purchase
- ☐ Moderately likely to purchase
- ☐ Extremely likely to purchase

Q6: Please read the ingredient label and answer the following questions.

Q6.1: Based on the ingredient statement listed above, how likely are you to purchase this cat treat?

- ☐ Extremely unlikely
- ☐ Moderately unlikely
- ☐ Unlikely
- ☐ Slightly unlikely
- ☐ Neither likely nor unlikely
- ☐ Slightly likely
- ☐ Likely
- ☐ Moderately likely
- ☐ Extremely likely

Q6.2: Based on the ingredient statement listed above, how natural do you think this cat treat is?

- ☐ Extremely unnatural
- ☐ Moderately unnatural
- ☐ Unnatural
- ☐ Slightly unnatural
- ☐ Neither natural nor unnatural
- ☐ Slightly natural
- ☐ Natural
- ☐ Moderately natural
- ☐ Extremely natural

Q6.3: Choose all of the following statements that you think apply to this ingredient list? (Choose all that apply)

- ☐ Too long
- ☐ Too short
- ☐ Has chemical names
- ☐ Food sounds good for cats
- ☐ Food sounds tasty for cats

- Contains unnatural ingredients
- Ingredients come from nature
- Ingredients made in a lab
- Has unhealthy ingredients
- Ingredients cause cancer
- Has healthy ingredients
- Not appropriate for cats
- Don't recognize ingredients
- Extra flavor added
- None of the above

Q7: Please indicate what you think the estimated cost of this cat treat would be.

- Extremely expensive
- Moderately expensive
- Expensive
- Slightly expensive
- Neither expensive or unexpensive
- Slightly unexpensive
- Unexpensive
- Moderately unexpensive
- Extremely unexpensive

Q8: What, if anything, did YOU like about this cat treat? Please be as specific as possible.

Q9: What, if anything, did YOU dislike about this cat treat sample? Please be as specific as possible.

Home Use Test Questionnaire

Q1: Please check the statement that applies to the test today

- My cat ATE the treat today
- My cat DID NOT eat the treat today

Q2: Overall, please indicate how much you like or dislike this cat treat.

- Extremely dislike
- Moderately dislike
- Dislike
- Slightly dislike
- Neither like nor dislike
- Slightly like
- Like
- Moderately like
- Extremely like

Q3: Overall, how much do you think your cat liked or disliked this cat treat?

- ☐ Extremely dislike
- ☐ Moderately dislike
- ☐ Dislike
- ☐ Slightly dislike
- ☐ Neither like nor dislike
- ☐ Slightly like
- ☐ Like
- ☐ Moderately like
- ☐ Extremely like

Please include any comments or notes about the treat & feeding experience you may have here. For example, any usual or unusual behavior from your cat during/after eating the treat.

Q4: Please include any comments or notes about the treat & feeding experience you may have here. For example, any usual or unusual behavior from your cat during/after eating the treat.

Q4.1: When getting ready to feed this treat to my cat, I felt (Check all that apply)

amazed	friendly	afraid/fearful	reluctant
amused	generous	alone	sad
appreciative	giddy	angry	scared
calm/harmony	goofy	anxious/nervous	sorry
careful	happy	cautious	stressed
caring	humorous	confused	sympathetic
close	important	discouraged	uncomfortable
comfortable	interested	doubtful	upset
companioned	intrigued	embarrassed	worried
complete	loved/loving	empathetic	
connected	nurturing	frustrated	
content/satisfied	mindful	guilty	
curious	playful	hopeless	
energetic	quiet	hurt/painful	
excited	refreshed	indifferent	
fun	relaxed	jealous	
free	safe	mean	
focused	warm	regretful	

Q4.2: When he/she saw me getting ready to feed this treat, I think my cat was (Check all that apply)

calm	fascinated	alert	hurt/painful
comfortable	focused	angry	impatient
companioned	free-spirited	anxious/nervous	panicked
competitive	fun	bored	resistant/reluctant
confident	happy	cold/indifferent	sad
content/satisfied	loved/loving	combative	shamed

crazy	loyal	confused	sick
curious	peaceful	defensive	stressed
dependent	playful	desperate	unaware
determined	proud	distrustful	uncomfortable
energetic	relaxed	exhausted	unnerved
engaged	safe/secure	fearful/scared	upset
entertained	warm	homesick	worried
excited		hostile	

Q4.3: During the offering and feeding process, I felt (Check all that apply)

amazed	friendly	afraid/fearful	reluctant
amused	generous	alone	sad
appreciative	giddy	angry	scared
calm/harmony	goofy	anxious/nervous	sorry
careful	happy	cautious	stressed
caring	humorous	confused	sympathetic
close	important	discouraged	uncomfortable
comfortable	interested	doubtful	upset
companioned	intrigued	embarrassed	worried
complete	loved/loving	empathetic	
connected	nurturing	frustrated	
content/satisfied	mindful	guilty	
curious	playful	hopeless	
energetic	quiet	hurt/painful	
excited	refreshed	indifferent	
fun	relaxed	jealous	
free	safe	mean	
focused	warm	regretful	

Q4.4: During the offering and eating process, I think my cat was: (Check all that apply)

calm	fascinated	alert	hurt/painful
comfortable	focused	angry	impatient
companioned	free-spirited	anxious/nervous	panicked
competitive	fun	bored	resistant/reluctant
confident	happy	cold/indifferent	sad
content/satisfied	loved/loving	combative	shamed
crazy	loyal	confused	sick
curious	peaceful	defensive	stressed
dependent	playful	desperate	unaware
determined	proud	distrustful	uncomfortable
energetic	relaxed	exhausted	unnerved
engaged	safe/secure	fearful/scared	upset
entertained	warm	homesick	worried
excited		hostile	

Q4.5: After my cat ate this treat, I felt (Check all that apply)

amazed	friendly	afraid/fearful	reluctant
amused	generous	alone	sad
appreciative	giddy	angry	scared
calm/harmony	goofy	anxious/nervous	sorry
careful	happy	cautious	stressed
caring	humorous	confused	sympathetic
close	important	discouraged	uncomfortable
comfortable	interested	doubtful	upset
companioned	intrigued	embarrassed	worried
complete	loved/loving	empathetic	
connected	nurturing	frustrated	
content/satisfied	mindful	guilty	
curious	playful	hopeless	
energetic	quiet	hurt/painful	
excited	refreshed	indifferent	
fun	relaxed	jealous	
free	safe	mean	
focused	warm	regretful	

Q4.6: After he/she ate this treat, I think my cat felt (Check all that apply)

calm	fascinated	alert	hurt/painful
comfortable	focused	angry	impatient
companioned	free-spirited	anxious/nervous	panicked
competitive	fun	bored	resistant/reluctant
confident	happy	cold/indifferent	sad
content/satisfied	loved/loving	combative	shamed
crazy	loyal	confused	sick
curious	peaceful	defensive	stressed
dependent	playful	desperate	unaware
determined	proud	distrustful	uncomfortable
energetic	relaxed	exhausted	unnerved
engaged	safe/secure	fearful/scared	upset
entertained	warm	homesick	worried
excited		hostile	

Q5: What, if anything, did you and your cat LIKE about this cat treat? Please be as specific as possible.

Q6: What, if anything, did you and your cat DISLIKE about this cat treat sample? Please be as specific as possible.

Appendix B - Example of In-Home Use Test Instruction

Check List

In this bag, you have the following:

- Question List (2 pages, double sided)
- 3 different samples, 2 packages for each sample – 6 packages total

Study Overview

- This In-Home Use Test study will take 8 days to complete.
- The samples are a commercial grade freeze-dried cat treat product.
- One sample code should be served for 2 consecutive days with a break-day in between each sample code.
- You, as the cat owner, will be feeding your own cat. (If you have more than one cat, please refer to the one that will participate in the test and separate the others while performing the test.)
- On BOTH days of the evaluation, you must log in to the survey and complete the brief questionnaire. The question list shows you the questions that will be asked for each sample. Feel free to take notes on those pages, if needed, for reference when completing the online questionnaire.
- For each treat, the surveys must be completed by MIDNIGHT on Day 1 & 2.
 - **Even if your cat didn't eat the treat – you must log in and complete the survey.**
- All surveys must be completed in order for you to receive your incentive payment.
- If you have any questions or concerns, please email the Sensory & Consumer Research Center (consumerresearch@ksu.edu).

Testing Instructions

1. Please follow the sample serving order for each testing day as indicated in the table.

May 13	May 14	May 15	May 16	May 17	May 18	May 19	May 20
Sample 1 (code 485)	Sample 1 (code 485)	Break	Sample 2 (code 943)	Sample 2 (code 943)	Break	Sample 3 (code 721)	Sample 3 (code 721)

2. There are a couple pieces of the treats in each container. Please serve the treat sample as you regularly would during a treat offering process.
3. Please observe the cat eating the samples. Refer to the question list and take any notes needed for your reference.
4. Complete the online surveys each test day before midnight. There are no surveys on the break-days between samples.
5. Log in to the survey: **kstate.at-hand.net/edu**
6. Use your username to access the survey. Your sample set is number: