DEVELOPMENT AND EVALUATION OF A DOG BISCUIT WITH CAROTENOID AS AN ANTIOXIDANT

By

STACEY J KOWALSKI

Bachelor of Science in Animal Science

Oklahoma State University

Stillwater, OK

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AS AN ANTIOXIDANT

Thesis Approved:

Dr. Timothy Bowser

Thesis Adviser

Dr. Stephen Damron

Dr. William McGlynn

Dr. A. Gordon Emslie

Dean of the Graduate College

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INTRODUCTION

In 2002, consumers spent $46 billion on pet food and pet care supplies in the United States (Crossley, 2003). It is estimated that by 2012 pet treats and supplements sales alone will reach $1.7 billion, according to an article by New Report (2008). That is a 39% increase in sales from 2007. Nutraceutical treats are increasing in demand as well as other specialized products such as products for aging or obese dogs. This increase in demand for new types and safer foods for dogs is an important reason that more research is being done on antioxidants in the industry.

This project was completed to create healthier dog treats. Testing was completed to find out if antioxidants that are known to improve an animal's health can also improve shelf life of a dog treat when compared to one that did not have any antioxidants added. Antioxidants that are used should improve shelf-stability and increase shelf-life along with the added health benefits for the animal. The treat itself is well liked by the dog, small in size, does not contain artificial ingredients, and it should be marketable. It contains no artificial preservatives or dyes.

Three different carotenoids were used as the antioxidants. They are lycopene, lutein with 5% zeaxanthin, and natural carotene, which contains both α-carotene and β-carotene. Though lycopene, lutein, and carotene are similar in structure, they do come

from different sources. Some of these health benefits include improving eye health, cardiovascular health, and preventing some forms of cancers. The purpose of this study is to develop a shelf-stable, nutritious, and healthy dog treat that is unique, provides some benefit to the dog, and is highly marketable.

CHAPTER II

REVIEW OF LITERATURE

Pet Food

There are many different kinds of pet foods available today. Pet foods can be found as dried treats and foods and canned, moist, or semi-moist foods and treats. Within these types of foods, an owner also gets a choice of flavors and varieties. Food can be bought according to the animal's age, size, type, or other health issues. The food that is chosen can greatly affect a pet's overall health and quality of life.

According to Philips (2004), people are more willing to spend extra money to provide healthy food for their pet than they are for themselves. The pet food industry is considered by many to be "recession proof" (Philips, 2004). This is very important to the pet food industry in today's economy. Many of the larger companies such as Procter & Gamble, Mars, and Nestlé all have a pet food division. These divisions may help bring in revenue for the companies when other divisions are not showing a profit during bad economic times.

Owners tend to buy food based on what their pet will eat and what is good for them, not on price (Philips, 2004). There is an increasing trend in today's market that people tend to think if a food is good and safe for them, that it should also be good and safe for their pet (Philips, 2004). Things such as the addition of probiotics in foods and

omega-3 fatty acids that were first made popular in human foods are now being seen in pet foods (Philips, 2004). Because of this, it is safe to assume that the next breakthrough in pet food nutrition could be the addition of antioxidants.

Antioxidants

An antioxidant is a substance that is used to preserve food by slowing rancidity and discoloration due to oxidation according to the US Food and Drug Administration (Nanditha et al., 2009). An antioxidant was defined by Halliwell (1991) as a substance that can significantly delay or prevent oxidation even when it is present at a lower concentration in comparison to those of an oxidizable substrate.

Antioxidants have become a widely used food additive because of their ability to improve shelf life of foods without damaging sensory or nutritional qualities (Nanditha et al., 2009). Antioxidants include tocopherols, carotenoids, BHA (Butylated Hydroxy Anisole), BHT (Butylated Hydroxy Toluene), Tertiary-butyl hydroquinone (TBHQ), gallates, rosmarinic acid, and synthetic antioxidants (Nanditha et al., 2009).

Antioxidants occur naturally in many foods. The amount of antioxidant is especially important for preserving foods that contain fat to reduce the amount of off-odors and flavors due to rancidity that might appear and to reduce or prevent the creation of decomposed products that could be harmful (Sims et al., 1977). They have also been found to be helpful in preventing the growth of microorganisms in baked goods (Nanditha et al., 2009). Shelf life can also be improved and the organoleptic and nutritional qualities of bakery products can be preserved by using antioxidants (Nanditha et al., 2009). Food antioxidants can be grouped depending on their function (Figure 1) (Madhavi et al., 1996). There are both primary and secondary antioxidants. Primary and

secondary antioxidants are further divided into groups. The food antioxidants that will be mainly focused on in this paper are the secondary antioxidants.

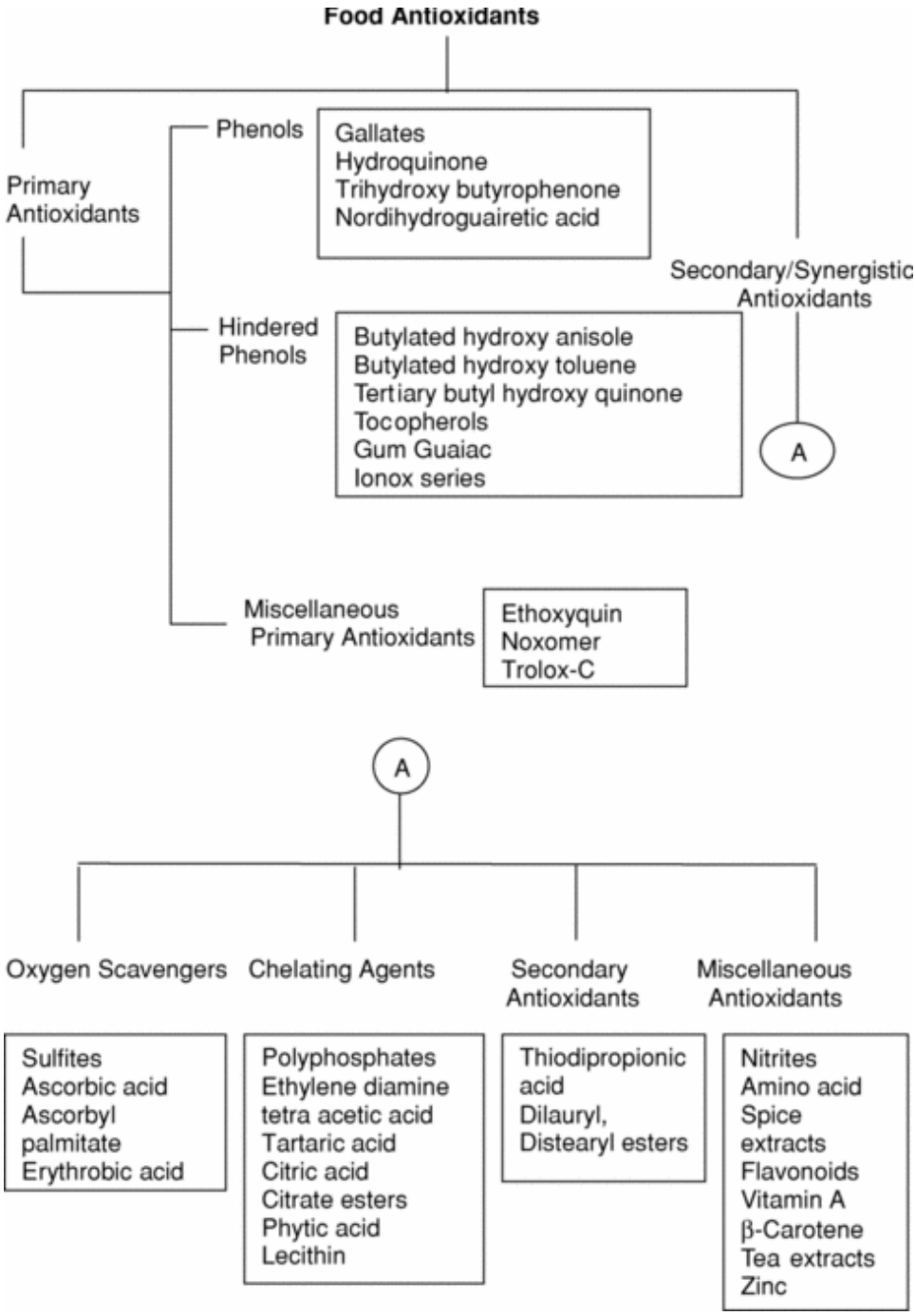


Figure 1: Classification of food antioxidants (Madhavi et al. 1996)

Primary antioxidants stop the free-radical chain reaction by giving an electron to free radical, converting them to a more stable product (Nanditha et al., 2009). They are effective at low levels and may become pro-oxidants at high levels (Nanditha et al., 2009). They are able to delay or prevent the process because they can prevent the propagation step by reacting with the peroxy or alkoxy radicals or by reacting with a fat free radical (Nanditha et al., 2009).

$$AH + R\bullet \rightarrow A\bullet + RH$$

$$AH + ROO\bullet \rightarrow A\bullet + ROOH$$

$$AH + RO\bullet \rightarrow A\bullet + ROH$$

The antioxidant free radical can react with the chain-propagation reactions which will cause the formation of peroxy antioxidant compounds (Nanditha et al., 2009).

$$A\bullet + ROO\bullet \rightarrow ROOH$$

$$A\bullet + RO\bullet \rightarrow ROA$$

Phenolic groups, at this point, may have substituted alkyl or electron-releasing groups to the para or ortho positions, decreasing the reactivity of the –OH group (Nanditha et al., 2009). Using an inductive effect, the electron-donating groups can increase the electron density on the –OH group and raise the amount of reactivity with lipid radicals (Nanditha et al., 2009). Using butyl or ethyl groups as a substitution at the paraposition improves the activity and reduces the amount of propagation reactions involving antioxidant free radicals (Nanditha et al., 2009).

$$AOO\bullet + RH\bullet \rightarrow AOOH + R\bullet$$

$$A\bullet + RH\bullet \rightarrow AH + R\bullet$$

$$A\bullet + O\bullet 2 \rightarrow AOO\bullet$$

Antioxidants can also be categorized as either natural or synthetic. When deciding which one to use, it is important to determine who is receiving the product and its intended use. Table 1 lists the advantages and disadvantages of each (Nanditha et al., 2009).

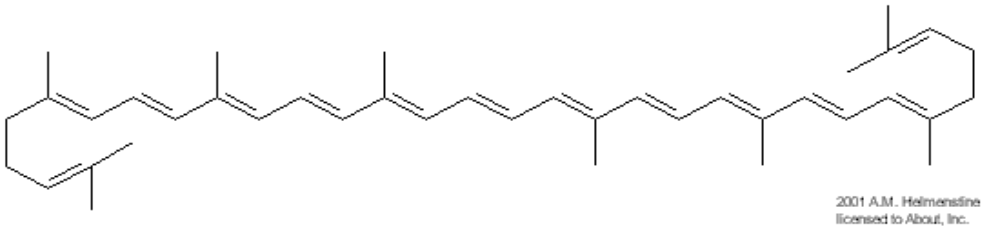
Table 1: Comparison of natural antioxidants with synthetic antioxidants (Nanditha et al., 2009)

Synthetic Antioxidants	
Advantages	**Disadvantages**
Mechanism of action and their use is well established.	Showed positive results for some toxicological studies.
More efficient.	May impart color, aftertaste, and flavor.
Comparatively cheaper.	Not readily accepted by consumer, as they are synthetic compounds.
Stable at processing conditions of temperature and time.	Their use is regulated by PFA act.
Easy purification steps.	
Natural Antioxidants	
Readily accepted by the consumer as considered to be safe.	Usually more expensive if purified.
Considered as safe for consumption and are under "generally recognized as safe" (GRAS).	Properties of different preparations vary if not purified.
No positive results for toxicological studies as on now.	Mechanism of action is not well established.
Along with antioxidant property also serves other purposes such as coloring agent in case of ß-carotene.	Not stable under high temperature and time combinations of processing.

Carotenoids of Interest for Pet Treats

Lycopene:

Lycopene is popularly associated with tomatoes and eye health. Tomatoes are a good source of lycopene, but so are watermelons, red grapefruits, and radishes. Lycopene is found in bright colored vegetables that are usually red. While lycopene does aide in eye health, it also provides many other health benefits. These health benefits are thought to be possible due to lycopene's antioxidative effects. Figure 2 shows the chemical structure of lycopene according to Helminstine (2001).



Lycopene
Molecular Weight: 536.89
Molecular Formula: $C_{40}H_{56}$

Figure 2: Lycopene (Helminstine, 2001)

Several studies have been completed that show humans who consume tomatoes are less likely to develop some cancers, heart disease, and other chronic diseases (Rao, 1999; Agarwal, 2000). Other studies have shown an inverse relationship between consuming tomatoes and the risk of heart disease and cancer (Giovannucci et al., 1995; Kristenson et al., 1997; Dorgan et al., 1998).

The carotenoid that is considered the most powerful singlet oxygen quencher is lycopene (Devaraj et al., 2008). It is very hydrophobic and is found in cell membranes and other lipoprotein components (Rao et al., 1999). It is also believed to be able to inactivate hydrogen peroxide and nitrogen dioxide (Bohm et al., 1995; Lou et al., 1995). Lycopene is a 40-carbon atom that contains an open chain hydrocarbon that has double bonds that are arranged in a linear array, 11 of which are conjugated and 2 are non-conjugated (Rao et al., 1999; Devaraj et al., 2008). These bonds have the ability to undergo isomerization from trans- to mono- or to poly-cis isomers by thermoenergy, light, or during chemical reactions (Rao et al., 1999). Usually between 79 and 91% of lycopene is found as all-trans lycopene (Clinton et al., 2003).

Lycopene may be the most potent scavenger reactive oxygen species (ROS) when compared to the other major dietary carotenoids (DiMascio et al., 1989; Mortenson et al.,

1997). Lycopene has been found to be twice as active as β-carotene in keeping lymphocytes from cell death or having NO_2 radical induced membrane damage (Tinkler et al., 1994; Bohm et al., 1995).

It has been found that in raw foods lycopene can be converted from *trans* to *cis* isomer when the food is cooked, processed, or stored (Tonucci et al., 1995). Loss of lycopene during processing or cooking appears to be minimal because it is fairly heat resistant (Ong et al., 1992; Mangels et al., 1993; Scott et al., 1995; Tonucci et al., 1995).

Effective levels of lycopene supplementation have not been determined. Different studies show different results in what is required to achieve the antioxidative effect. In a study completed by Devaraj et al. (2008), it was found that 30 mg of lycopene was enough to reduce DNA damage by approximately 9%. Other studies found up to 50% lymphocyte DNA protection by using a tomato puree supplementation that provided between 7 and 16 mg of lycopene each day (Riso et al., 1999; Porrini et al., 2000).

A study completed by Devaraj et al. (2008) found that the supplementation of 30 mg/day of lycopene reduces lymphocyte DNA's ability to be oxidized and reduces urinary 8-OHdG, which has been found at increased levels in target tissues of some animal cancer models.

Another factor to consider when using lycopene is deciding whether to use a synthetic form of lycopene or a tomato-based lycopene. This is important because there could be large differences in manufacturing costs between using the natural vs. synthetic. Hoppe et al. (2003) performed a study to determine if there is a difference between synthetic and tomato-based lycopene. In the study they compared LycoVit 10%, which

contains synthetic lycopene, against Lyc-O-Mato^{TM} Beads 5%, which contains natural tomato lycopene. It was found that neither form of lycopene affected other circulating carotenoids. Further, synthetic lycopene and natural tomato-based lycopene resulted in the same trans- and total lycopene response indicating that both sources have the same bioavailability.

Many factors have the ability to affect the bioavailability of lycopene. Isomerization from all trans- to cis- conformation caused by heat, presence of dietary lipids, and lycopene being freed from the food matrix by processing increases the bioavailability of dietary lycopene (Stahl et al., 1992).

Lycopene can be used as a preservative very easily in baked goods. It is not harmed by heat or light, which is important when it is used in a bakery, shelf-stable product. It may have the potential to provide many health benefits to humans when consumed. There is currently not a lot of research on the affects of lycopene on dogs. It is thought that they should get the same benefits from the carotenoid as humans do.

Lutein and Zeaxanthin:

Lutein is a well known antioxidant. When people think of lutein and where it comes from, they usually think of kale or spinach or other dark green leafy vegetables. While lutein is found in these vegetables, it is also quite abundant in marigolds and many other variously colored fruits. Lutein is very important in fighting against age-related macular degeneration (Mozaffarieh et al., 2003). Figure 3 shows the chemical structure of lutein and Figure 4 shows the chemical structure of zeaxanthin according to Chrysantis (2009).

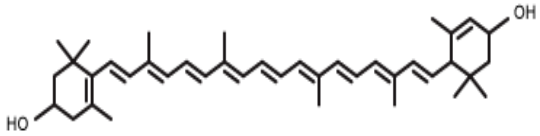


Figure 3: Lutein (Chrysantis, 2009)

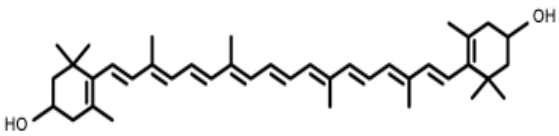


Figure 4: Zeaxanthin (Chrysanti, 2009)

Lutein and zeaxanthin are very similar to lycopene in there mode of operation as an antioxidant. They both help keep products from oxidizing. They are also stable under baking conditions. There is no synthetic form of lutein on the market yet, so the natural form must be used. This may increase the cost for the manufacturer and the consumer.

Lutein and zeaxanthin are the two major carotenoids in the macula and retina (Bone et al., 1988; Handelman et al., 1988). Lutein is usually found in the perifoveal region and zeaxanthin is found in the foveal reagion (Bones et al., 1988; Snodderly et al., 1991). Studies are still being done to learn exactly what part these carotenoids play in macular health. One function is the limitation of the harmful photo-oxidative effects of blue light through its absorption (Dichtburn, 1973; Kirschfeld,1982; Bone et al., 1984). They are also thought to be able to protect against harmful effects of photochemical reactions (Foote et al., 1970; Snodderly et al., 1991; Snodderly, 1995). They can also reduce the effects of chromatic aberration and light scatter on visual performance (Reading et al., 1974; Nussbaum et al., 1981).

To be able to obtain these benefits and delay age-related macular degeneration from lutein and zeaxanthin, diet supplementation is needed in humans (Snodderly, 1995; Jaques, 1999; Pratt, 1999; Moeller et al., 2000). Studies have shown that diets supplemented with foods rich in lutein and zeaxanthin have been able to increase macular pigment density in most human subjects (Hammond et al., 1997; Landrum et al., 1997; Berendschot et al., 2000).

Though there are many studies that indicate that lutein and zeaxanthin play a major part in macular health, the proof of a beneficial effect is still missing (Mozaffarieh et al., 2003). It is also unclear what real benefit these carotenoids can give to a patient that already has age-related macular degeneration (Mozaffarieh et al., 2003).

A study completed by Bone et al. (2001) relied on post mortem analysis and clinical records showed that donors that had a history of age-related macular degeneration had lower amounts of macular carotenoids when compared to donors that had no history of the disease. Another study that focused on a person's history and risk factors for the disease found that if a person already had the disease in one eye or had the risk factors, such as age, usually had lower levels or an absence of macular pigments (Beatty et al., 2001).

While it is still unknown what role lutein and zeaxanthin play in macular health, it is clear that they are acceptable carotenoids to use to help improve the shelf life of a product. With the research is still limited in regards to the effects that these carotenoids play on human health, it is understandable that there is virtually no research available on its affects on dogs. Like lycopene, it is assumed that the effect on humans is the same for dogs.

It may be slightly more expensive to use lutein and zeaxanthin in the product, so it will be up to the manufacturer to determine if the extra cost is worthwhile compared to other carotenoids.

Natural Carotene:

β-Carotene is one of the most well known antioxidants. Many vegetables and fruits contain this antioxidant. Carrots are probably the most well known β-carotene

containing vegetable. Other vegetables that contain β-carotene are sweet potatoes, spinach, broccoli, and cantaloupe. β-carotene is thought to improve immune health, eye health, and help guard against some cancers. Figure 5 shows the chemical structure of β-carotene according to Nanditha (2009).

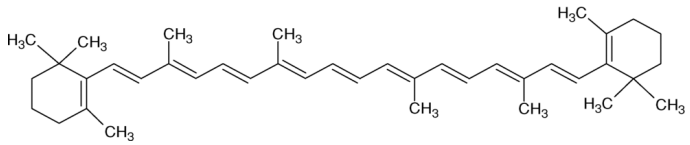


Figure 5: Beta-carotene (Nanditha, 2009)

β-Carotene is the most studied carotenoid and is known to play a role in the immune system and promoting health for many species (Massimino et al., 2003). It is a well known fact that as humans and animal's age, their immune systems weaken (Miller, 1989; Miller 1994). This decrease in immune response is thought to be related to a decrease in the responsiveness of the thymus-derived T-cells (Massimino et al., 2003).

Like humans, recent studies indicate that dogs immune function also declines with age as is shown by the decline in chemotaxis, mitogen stimulation, and phagocytosis (Greeley et al., 1996; Hayek, 1998; Meydani et al., 1998). A study by Strasser et al. (1989) shows that a dog also has an increase in mature neutrophils and immunoglobulin G and a decrease in white blood cell and immature neutrophils.

In the study competed by Massimino et al. (2003), it was found that feeding an aging dog β-carotene can positively effect immunocompetence. Chew et al. (2000) completed a similar study finding that dogs can absorb dietary β-carotene which is taken up by blood lymphocytes and neutrophils. The blood lymphocytes help distribute β-carotene to all of the subcellular organelles (Chew et al., 2000). This may help improve a dog's immunological health by improving the health of the cell.

Like lycopene and lutein, β-carotene should be able to improve the shelf-life of baked goods. It is stable under baking temperatures for a short time and is able to help protect the food from the effects of oxidation. It is stable under freezing temperatures as well, making this one a little more versatile than lutein or lycopene. It just seems to be a little more durable.

There is a synthetic form of β-carotene available. This form is what is usually found in supplements. Using the synthetic form might make production a little cheaper for the manufacturer.

CHAPTER III

ABSTRACT

The pet food industry is rapidly growing. The number of pets that people have and the amount of money people are willing to spend on their pet is increasing. Research is expanding in pet food that will improve dog treat palatability, shelf stability, and provide some benefit for the animal. If antioxidants are added to a dog treat, some of these concerns might be met. The purpose of this study was to develop a shelf-stable, nutritious, and healthy dog treat that is unique, profitable, provides some benefit to the dog, and is highly marketable.

Carotenoids are known to improve shelf stability by preventing the treat from being oxidized. This antioxidant effect is what also provides benefit to the animal by preventing some of the animal's cells from oxidizing which is one of the causes of the physical effects of aging. For this study, three different carotenoids were added individually to the same dog treat recipe. The carotenoids used were lycopene, lutein with 5% zeaxanthin, and natural carotene which contains both α-carotene and β-carotene. Each dog biscuit weighed 5 g and had 6 mg of carotenoid in it. The amount of carotenoid used was the largest recommended dose to use for lycopene according to the American Macular Degeneration Foundation (AMDF). An accelerated 6-week shelf-life study was completed by placing 20 dog treats into a sealed poly bag that was stored at 35C.

Proximate analysis was completed to calculate moisture, lipid, protein, and ash

content on week 0 only. Color analysis, water activity, and antioxidant concentration were measured on weeks 0, 1, 2, 3, 4, 5, and 6. An in-home pet preference test was completed by testing the three biscuits that have the added antioxidants against a similar product without carotenoids that was already on the market. The results from water activity show that the biscuits with antioxidants had less variability over time. Color scores varied more for the top of the biscuits than for the bottoms. No biscuit was preferred more than the other statistically.

INTRODUCTION

Billions of dollars are spent every year on the care and well-being of pets in the United States. The amount that is spent on pets continues to increase every year. There is an increased demand for products that may improve the pet's quality of life. An ingredient that could potentially improve a pet's health and also provided some benefit for the producer would be best.

Antioxidants are known to aid the health of animals by preventing the oxidation of cells. The prevention of oxidation also helps extend the shelf-life of some products in much the same way. Carotenoids are antioxidants that are becoming popular and thus have appeal to consumers.

Three of the most popular carotenoids are lycopene, lutein, and carotene. The objective of this study is to create a new dog biscuit, add the carotenoids one at a time to the biscuit to see which one is better at extending shelf-life, and which one is preferred most by dogs. The objectives that are to be addressed in this study are as follows:

OBJECTIVES

1. Develop a unique dog biscuit.
2. Three different antioxidants will be added, one at a time, to the dog biscuit recipe.
3. Make process flow chart for biscuit processing and list CCPs. Discuss product and process safety.
4. Propose a conceptual package and trademark suitable for the products.
5. Perform consumer/pet preference tests (in home) for the products against a leading national competitor's product.
6. Perform shelf life study for products.

METHODOLOGY

This section discusses the materials and methods used for the development and evaluation of the dog biscuits. All variables that were considered important and controllable were controlled; including but not limited to variability in the raw ingredients and always using the same scale throughout the experiments. There were precautions to prevent cross contamination such as always changing latex gloves between handling the different types of samples.

Dog Treat

The dog treats were made using ingredients from local stores and were brought at the same time, so all ingredients were from the same lot. The dog treat was made of the following ingredients:

1. 27.72% peanut butter
2. 19.14% molasses
3. 18.48% whole oat flour
4. 17.82% honey
5. 15.84% rolled oats
6. 1% antioxidant

The antioxidants that were used were all carotenoids. They were lycopene, lutein, and natural carotene. They were all provided by Chr. Hansen (Horsholm, Denmark) and were in a liquid form. The lycopene was NutriPhy Lycopene 100 (product number 601841). The carotene was Natural Carotene 103 and was made from a mixture and α- and β-carotene (product number 615144). The lutein was NutriPhy Lutein 100 which also contain 5% zeaxanthin (product number 612688). The control biscuit was formulated

from the same ingredients but without the antioxidant. The control biscuit was made from the following ingredients:

1. 28% peanut butter
2. 19.33% molasses
3. 18.66% whole oat flour
4. 18% honey
5. 16% rolled oats

The different samples of the dog treats were all evaluated the same way despite the different carotenoid that was used as an antioxidant because the carotenoids are used were all structurally similar and to ensure the result can be compared between the test groups.

Creation of Product

The molasses and half of the oat flour were combined and heated in a microwave (Panasonic Household Microwave Oven, Secaucus, NJ, model number NN-L77278A) to gelatinize. They were heated until the mixture had an internal temperature of 65C. The mixture was heated in 10 second intervals, stirred and the temperature was taken. This gelatinization was done so the dough would be easier to mix and the biscuits would be softer because of the re-ordering of the amylase molecules in the starch. The honey and peanut butter were added to the warm mixture. The molasses and oat flour being warm helped soften the peanut butter and aided in mixing. The rest of the oat flour was added and combined with the other ingredients, followed by the rolled oats. The mixture was thoroughly stirred which helped combine the ingredients and to cool it down. Once the batter was 20C, the antioxidant was added. The batter was rolled out on a cookie sheet

using a rolling pin to a thickness of about ¼ of an inch. It was then cut using an apple corer. An apple corer was used because it can create small, uniform biscuits. The biscuits were weighed to be sure that they weighed 5g ±0.05g. The treats were baked at 177C (350F) for 7 minutes in a gas oven (Maytag Self Cleaning Gas Oven, Newton, IA, CRG760). After that, they were removed from the oven, taken off of the cookie sheet and allowed to cool in ambient temperature on a flat tray for 20 minutes.

Each batch made 350 biscuits of each type. That was enough biscuits for all tests including the preference tests. All biscuits were made on the same day. For week 0 of the shelf life study, there were 30 biscuits of each type because additional biscuits were needed for proximate analysis. For weeks 1-6 of the shelf life study, 20 were made of each type. The remainder of the biscuits was used for the preference tests.

Packaging and Storage

Once cool, the biscuits were then placed into Cryovac pouches (Duncan, SC, 5x9, oxygen transfer rate of 2.5 cc m^2/day, model number PP730b). The different types of biscuits were packaged and stored separately. Latex gloves were used throughout the handling process and were changed whenever a different type of dog biscuit was handled to prevent cross-contamination. The dog biscuits were packaged in groups of twenty. There were seven sets of dog biscuits made, one for each week of the trial period. The bags were then sealed using a heat sealer. They were not vacuum sealed because the packaging used was not suitable for vacuum sealing. The biscuits were not flushed with any gases because we wanted to find out how well the biscuits would do without the added gases. All of the bags were then moved to an oven (Cole-Parmer Instrument Company, Chicago, IL, model number 52000-55) for the shelf life study except for week

0. The biscuits were stored at 35C. Week 0 was tested immediately. All other settings on the oven were set to the manufacturers settings.

There were 16 spare biscuits from each group. The biscuits were divided evenly into two groups, eight biscuits were refrigerated (White-Westinghouse Refrigerator, White Consolidated IND., Cleveland, OH, model number MRT21GNEW1) at 3C and eight were stored in a freezer (Biofreezer, Forma Scientific Inc, Marretta, OH, model number 8517) at -75C. These samples were vacuum sealed in Prime Source vacuum pouches (Carroltton, TX, 8x10, 3 mil standard barrier, model number 75001829) before storage. All subsequent spare week's samples were handled the same way. Figure 6 in the appendix is a flowchart of the process for creating the dog treat. The flowchart also shows potentials critical control points for manufacturing.

Proximate Analysis

Proximate analysis was completed on biscuits from Week 0. Proximate analysis was completed to determine the percent of moisture, percent of fat, and percent of ash by modifying the AOAC method. Analysis was completed by taking a 2g finely ground sample of each of the treats that were created. To get the 2g sample, 5 dog biscuits of each type were ground using a mortar and pestle. It was put it into a pre-weighed Waltman 41 15cm ashless filter paper and he ends of the paper were folded toward the center and were held in place using a pre-weighed paper clip.

The percent of protein was also determined by sending a sample Dr. Guadalupe Davila-El Rassi's lab FAPC 316 and the test was completed by EE Chin Ng. Percent of crude protein was found by the AOAC method for Leco® Combustion. The percent of

carbohydrates was found by adding the percent protein, percent moisture, percent fat, and percent ash and subtracting from 100.

Percent Moisture

The percent moisture was determined by using AOAC method number 950.46. The method was modified by using the Waltman 41 15cm ashless filter paper instead of the aluminum pans. The sample was prepared as stated above. The samples were placed on a tray and then put in a drying oven at 102C for 24 hours. Once dry, the samples were placed into a desiccator (Pyrex Sleeve Top Desiccators, Fisher Scientific, Pittsburg, PA, model number 08-631B) for 30 minutes to cool and then reweighed and the weight recorded.

Percent Fat

The percent of crude fat was found next. The procedure used was modified from AOAC method number 960.39. The modification was using the ashless filter paper instead of a thimble and sand. The percent of crude fat was found by placing the same samples in a soxhlet containing petroleum ether for 18 hours in a fume hood. The samples were then removed and allowed to air dry for 30 minutes, placed in the drying oven for 30 minutes at 104C for 30 minutes to be sure all the petroleum ether had evaporated, and then placed in the vacuum desiccator for 30 minutes to cool. The samples were reweighed and the weight recorded.

Percent Ash

The percent of ash was found by using AOAC method 920.153. The same samples that were used for the previous tests were placed in ceramic crucibles. The ceramic crucibles were weighed before the sample was placed inside. They were then

put in an ash oven (Isotemp® Oven, Fisher Scientific, Pittsburg, PA, model number 655F) at 550C for 24 hours. They were removed once the test was completed and set into a vacuum desiccator for one hour to finish cooling off. The samples were reweighed and the weight recorded.

Shelf life Study

The shelf-life study was completed as an accelerated 6-week shelf-life study. Each week was equivalent to one month of a regular shelf life study. The method for the shelf life study was taken from Man et al., (2000). According to their method, holding a product at 35C to 40C can bring about a 4-fold increase in aging compared to ambient temperature (Man et al., 2000). This method is only suitable if the product is sensitive to higher temperatures and is the product is meant to be shelf stable (Man et al., 2000). Other methods and testing parameters are required for other types of products (Man et al., 2000).

The dog treats were placed into separate, heat sealed vacuum pouches (the same type of Prime Source vacuum pouches that were mentioned before), 20 per bag. There was a separate bag for each type of biscuit and for each week of the shelf life study. The bags were placed in a programmable oven, (Cole-Parmer Instrument Company, Chicago, IL, model number 52000-55) at 35C for the length of time required for that specific set of samples, 1-6 weeks. The only time the programmable oven was opened was to remove the set of samples for that particular week.

Water Activity

Water activity was determined for each week including an initial measurement at week 0. It was important to measure water activity to help determine shelf stability

because water activity measures the amount of free water that is in the product. This free water can increase microbial growth and the rate of rancidity. As long as the water activity is kept below 0.80, the product is considered shelf stable. An Aqua Lab Series 3 (Decagon Devices, Inc., Pullman, WA) was used for each week of the shelf-life study. For this test, two dog biscuits were ground using a mortar and pestle. Enough of the sample was put into the sample cups to cover the bottom of the cup and fill it no more than half full. The sample was put into the machine and tested in duplicate. All results were recorded and transferred to an Excel® spreadsheet for analysis.

Colorimeter

A color score was taken on the dog biscuits for weeks 0, 1, 2, 3, 4, 5, and 6. This test was completed to find out if the color of the dog biscuit would change over time. The test was completed as quickly as possible after the treats were removed from the programmable oven. For this test a Minolta (Color Space Conversion) Spectrophotometer CM-3500d (Minolta Co., Ltd, Ramsey, NJ) was used. The machine was properly calibrated before each testing session. The medium size orifice plate was used and the treats were placed on a plastic Petri dish for testing. The dish was wiped clean with a Kimwipe® between each sample. The treats were placed on the testing apparatus one at a time. The "blank" for the sample was calculated from the control sample for the week being tested. The top and bottom of the dog biscuits were tested separately. There could be color changes that occurred because of the bottom touching the pan and having more direct contact with the heat. Both the top and the bottom side of the biscuits that were tested was done so using the same dog biscuits and were tested in the same order for both sides. So, not only were the tests always completed in the order

of control, lutein, lycopene, and carotene, but also each sample per category was done in the same order. Each sample was tested in triplicate.

Preference Test:

The preference test was completed to determine if the biscuits that were created in this study were preferred over a major brand. The method for this test was established from ISO 5495:2005. The major brand used was Blue Dog Bakery Peanut Butter and Molasses Flavored Dog Treats (Blue Dog Bakery, Seattle, WA, lot number 01/02/11Z). For the test, twelve pet dogs and their owners participated in an in-home test of the products. All three of the study's formulas (A, B, and C) were tested to compare them to each other and to the Blue Dog Bakery Peanut Butter and Molasses Flavored Dog Treats (X). Biscuit A was lycopene, biscuit B was lutein, and biscuit C was carotene. The test was completed as a two-sided paired test. Dogs and owners were selected based on an agreed upon criteria. They were then separated into two groups, as shown below. Each set of treats were presented six times to each dog in a random order to determine preference. This gives 72 observations per pair

Group I, 6 dogs	A/B, A/C, B/C, A/X, B/X, and C/X
Group II, 6 dogs	B/X, C/X, A/C, A/B, B/C, and A/X

Number of dogs participating:

To get at least 95% certainty of which dog treat was preferred, α was fixed at 0.05 and p_d at 40% (the % of dogs that are expected to be able to distinguish a difference between samples). The β value was set at 0.10. At least 65 observations were required. For this trial, 12 dogs participated and 6 observations were completed per dog for a total of 72 observations per pair (a safety margin of 7 observations per pair).

Conducting the Test:

Participants were given four different treats. Each treat was approximately one teaspoon of product and were stored at room temperature. When samples were compared, they were presented two at a time either on separate sample dishes or in separate hands. The individual observations were randomized in each test. A sample of the score sheet is shown in Figure 6 in the appendix.

Lycopene Assay

The lycopene assay was completed to determine the amount of the antioxidant that was present in the sample for a given test period. The lycopene assay was completed in triplicate. For this test, 10g of sample was ground finely using a mortar and pestle. Only 0.25g of the sample was used for the extraction.

Extraction

The extraction was completed to remove only the carotenoid from the dog treat. This method was modified from the method established by Sadler et al., (1990). The extraction that was completed for this study was done by placing 0.25g of the sample into a 250mL Erlenmeyer flask that was covered with aluminum foil. Then 25mL ethanol (Ethanol-190 proof 95% alcohol, Pharmco Products Inc., Brookfield, CT, product number E190), 25mL acetone (Fisher Scientific, Pittsburg, PA, product number A949-1), and 50mL hexane (EM Science, Cherry Hill, NJ, product number 110-54-3) was added. The flask was agitated for 10 minutes in a shaker. The agitation was paused and 15mL of de-ionized water was added. The flask was agitated for an additional 5 minutes. The top was then covered with aluminum foil and allowed to sit in a fume hood for 15 minutes. Once the layers had separated and the bottom layer was completely clear, approximately

40mL of the top layer (hexane) was removed. It was placed in a brown bottle and sealed with a screw top and parafilm. It was refrigerated until needed. The other layers were disposed of by placing them in a brown sealed container labeled as the "A can" and disposed of according to Environmental Health and Safety guidelines.

Conducting the Test

This test was completed based on a method created by Davis et al., 2002. A Beckman DU 520 General Purpose UV/Vis Spectrophotomer (Beckman Coulter Inc., Fullerton, CA) was turned on and allowed to warm up for 30 minutes at 503nm. While the machine was warming up, the samples were prepared pipetting 3mL of the sample into a crystal cuvette. All samples were completed in triplicate. A blank was also created by pipetting 3mL of hexane into a crystal cuvette. The program used for this test was 314 FIX λ Lycopene. This program was setup by Darren Scott at Oklahoma State University and was based on the method by Davis et al., in 2002. All results were recorded by hand and transferred to an Excel® spreadsheet.

CHAPTER IV

RESULTS AND DISCUSSION

Most of the data is also available in tables and figures in the appendices. There were similar results among the dog biscuits in regards to proximate analysis and palatability. The biscuits that had the antioxidant added did have better results for water activity. There was variation among all the dog biscuits for the lycopene assay. This chapter breaks the information down into the same sections that were used before and better explains the results of the tests that were completed.

Dog Treat

In developing a dog treat that could potentially be made by a company, first conceptual ideas were discussed. Dr. Timothy Bowser (OSU) and Dr. William McGlynn (OSU) were consulted to help decide which recipe would be used. The dog biscuit that was to be made needed to be shelf stable, easy to produce, the ingredients easily attainable, and it should be marketable.

A chicken, vegetable, and potato flour biscuit was made originally. The biscuit was difficult to make and it was hard to achieve an acceptable water activity. Rolling the dough into a biscuit shape proved to be nearly impossible. The raw ingredients also required refrigeration, which would require more equipment. Though it could be done, this biscuit was not chosen because of the problems that were mentioned.

Another product that was tried though it was not a biscuit was a reformed beef jerky-type product. The product had problems from the beginning because it was hard to get the antioxidant to attach properly to the meat. It was also hard to keep the pH of the meat in an acceptable range. Many natural antioxidants lower the pH of the food that they are added to. Once the meat's pH was below the isoelectric point, it was difficult to form the jerky into strips.

The biscuit that was created was an oat flour based biscuit. This biscuit was both easy to produce and had a low water activity. The ingredients chosen were oat flour, rolled oats, honey, molasses, peanut butter, and the needed antioxidant. The oat flour and rolled oats were used because they are easily attainable year around, reasonably priced, can be mixed well with other ingredients. When in a commercial setting, it is important to sift the oat flour for foreign contaminants such as metals or plastics that may harm the consumer or their pet. The honey acted as a sweetener, which dogs like, and as a humectant to improve shelf life and water activity. Molasses also acts as a humectant. It does not increase the amount of sugar in the biscuit as much as honey does, which is important for canine health. Molasses also has a flavor that dogs seem to really like. Peanut butter was used because it is a high-quality source of protein and other vitamins, has a low water activity, can be combined with other ingredients easily, and is a well-known favorite of dogs. The antioxidants that were added to the dog biscuit were done so because of their availability, the amount of information currently available on their health benefits for humans, and their benefits in foods.

Control Points for Manufacturing

The flour should be sifted before using to help with mixing and to ensure there are

no foreign contaminates in the flour. If there are letters of guarantee from the supplier, this step is only considered a control point and not a critical control point.

The baking temperature and time were decided based on how easily it would work in a commercial setting while being able to reach an internal temperature suitable for killing any microorganisms. This is very important, especially since peanut butter is the main ingredient in the recipe. Peanut butter is known to be easily contaminated with *E. coli*. This step is only considered a control point, not a critical control point. The biscuits are small and being heated for a significant amount of time at a temperature that will destroy pathogens.

After the product is baked and has reached the proper internal temperature, it is important to check the biscuits for metal. This is usually done with a wand metal detector. Any biscuits that contain metal must be removed and discarded properly. This step is considered a critical control point and is very important to ensure the safety of the product. It is a critical control point because metal pieces from the mixers or other machines could break off in the batter and be baked into the biscuit.

Packaging

The packaging that was used for these tests was used because of price and availability. They were free and were shipped directly to the lab. If any packaging could have been used, it would have been one that did not allow light in. Light breaks down antioxidants. If the dog biscuits are kept out of the light, then theoretically the dog biscuit will benefit from the antioxidant that was added longer. The packages should be heat sealed to prevent oxygen from entering or exiting the package. They should not be vacuum sealed because vacuum sealing tends to cause some of the antioxidant to come

out of the dog biscuit and stains the packaging. Also, the biscuits tend to have a wet feeling if they have been vacuum sealed. The way the biscuits were packaged for the shelf-life study worked well. Other than making the packaging more opaque or making it out of an aluminum type material, they should be packaged in a similar way for maximum freshness and desirability.

Labeling

The label for the package should contain the company name and the name of the product. The label on the back of the package should contain a list of ingredients in the order of abundance in the product. It should also show a proximate analysis of the product including moisture, fat, protein, and carbohydrate content. The place the product was manufactured, lot number, and contact information for the manufacturer should also be on the label. An example of the label are listed in the appendix as Figure 8 and Figure 9.

Proximate Analysis

The results for the proximate analysis are shown in Table 2 in the appendix. The levels of moisture, fat, ash, protein, and carbohydrates are the same for all treatment groups. This was expected because the only variable between the types of biscuits was the antioxidant added. The addition of an antioxidant did not have any effect on the proximate analysis.

Water Activity

The results for the water activity are shown in Table 3 in the appendix. Table 4 shows the results after statistical analysis. Duncan's Multiple Range Test was used in SAS. In this table, means with different letters in the same row are significantly different ($P \leq 0.05$). Weeks 0 and 1 both show variability throughout all the treatment groups.

After that period of time, the water activity was relatively constant for the treatment groups that contained antioxidants. The control group however, had a lot more variability than the other treatment groups. This indicates that the addition of an antioxidant may help stabilize water activity.

Colorimeter

The results for the colorimeter (color score) are listed as an L*a*b* numbers. Duncan's Multiple Range Test was used in SAS. The L* value represents black (0) to white (100). The a* value measures red to green and the b* value measures yellow to blue. The data was collected and transferred to an Excel file. Tables 5-10 show the results after statistical analysis. Table 5 shows the L* value for the tops, Table 6 shows the a* value for the tops, Table 7 shows the b* value for the tops, Table 8 shows the L* value for the bottoms, Table 9 shows the a* value for the bottoms, and Table 10 shows the b* value for the bottoms. In these tables, means with different letters in the same row are significantly different ($P \leq 0.05$). The higher variability among the bottom values may be caused by the increase heat that the bottoms would have experienced in the oven that the tops would not have undergone.

Preference Test

This test was completed to determine a difference between the palatability of the biscuits with the antioxidants and the biscuit by Blue Dog Bakery. The results however, do not support this. While there is a difference between the dog biscuits that were created and the biscuit by Blue Dog Bakery, there is not much difference between the three sample dog biscuits. The results can be found in Table 11 in the appendix. A summary of the results can be found in Table 12 in the appendix. Some of the data that

was collected was not used do to participant error. In planning this experiment human error was taken into consideration. Enough tests were completed correctly so the results were not compromised.

There was not a large enough difference between many of the trials to be able to determine a definite preferred dog biscuit. This caused the hypothesis of the dog biscuits all being different to be rejected. The results show that biscuit "B" was chosen more often when compared to the other biscuits. The order of preference was B>A>C>X or lutein>lycopene>carotene> national brand. While the antioxidant containing dog biscuits tested similar toward each other, they were chosen more often by the pet than the Blue Dog Bakery dog biscuit. Unfortunately, there was no biscuit chosen more often than the one it was compared to. When analyzed at an $\alpha=0.05$, there is no difference statistically among the test groups.

Lycopene Assay

The lycopene assay was one of the easier tests to complete. The results for this test can be found in Table 13 and illustrated in Figure 10. A statistical analysis of the data is available in Table 14. Duncan's Multiple Range Test was used in SAS to complete the analysis. In Table 14, means with different letters in the same row are significantly different ($P \leq 0.05$). The results show that statistically the control and lutein are more similar in concentration to each other than to lycopene or carotene. Likewise, lycopene and carotene are more similar in concentration than to lutein or to the control. Because the lutein biscuits have a lower concentration when compared to the other biscuits with antioxidants added may indicate a need for an assay that is set to find the

concentration of lutein. The lutein may have been present at a higher concentration, but the machine may have been able to determine the amount at a wavelength of 503nm.

CHAPTER V

CONCLUSION

The results show that while the dog biscuits with the carotenoid added may not have been preferred more than the national brand, the carotenoid did help stabilize water activity. All biscuits had similar results for proximate analysis and the preference tests. There was some variability among the treatment groups and overtime in regards to color stability. The odor of the biscuits did not seem to change over time. All biscuits for all treatment groups had similar odor intensities. It would be up to the manufacturer whether the benefits of adding the carotenoid are worth the cost. Increased levels of the carotenoid may show better results. Other carotenoids or antioxidants may provide the desired result at a cheaper price.

Another thing that occurred that was unexpected was that the lycopene and carotene dyed the package they were in. Lycopene only did this if the package was vacuum sealed. Carotene dyed the packages regardless of package type or sealing method. Some of the sensory panelists said they would not buy the product because they worried that it would dye their furniture or turned their hands an orange color. The objectives that were to be addressed and their conclusions are listed below.

OBJECTIVES

1. Develop a unique dog biscuit.

a. The biscuit was created.

2. Three different antioxidants will be added one at a time to the dog biscuit recipe.
    a. Lycopene, lutein, and natural carotene were added to the biscuits.
3. Make process flow chart and list CCPs. Discuss product and process safety.
    a. The flow chart for the process can be found in Figure 7.
    b. Product and process safety was in the Results and Discussion chapter.
4. Propose a conceptual package and trademark suitable for the products.
    a. Conceptual packaging was discussed in the results and discussion chapter and a company name and product name were created. A search of the government website www.uspto.gov was used to determine if the name had been trademarked.
        i. The company name that is available to be trademarked is Flying Norb, which will be the name of my pet food line.
        ii. The product name will be Norb's Natural PeaNutty Nuggets.
5. Perform consumer/pet preference tests (in home) for the products against a leading national competitor's product.
    a. Testing was completed and no difference was found among the test groups.
6. Perform shelf life study for products.
    a. The shelf life study and evaluation of the product was completed.
    b. The testing was completed, the biscuits with an antioxidant present showed less variability with the water activity over time than the control.

The biscuits with carotene and lycopene added had higher concentrations of antioxidant present according to the lycopene assay.

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APPENDICES

PREFERENCE TEST DATA SHEET
Completed forms due: _____

DATE:_____ TIME: _____

OWNER: _____ PET: _____

Preference test data collection table

trial #	left side	right side	choice	eat? (yes or no)
1.	B	A		
2.	B	A		
3.	A	B		
4.	B	A		
5.	A	B		
6.	A	B		

Anything unusual (please describe):

Comments:

Purpose of the test: to determine the pet's preference for one product over the other.

Explanation: The products are "chips" or "wafers" cut from the chew "bones" that were presented in the previous test. Six trials are used to determine repeatability of the test. Product locations (left or right) are changed at random in case the pet has a preference for eating food presented in a particular manner.

Instructions:

1. Select a time for the test when the pet is not distracted.
2. Present the product "chips" to the pet as shown in the table. ["Left side" means that the product is presented to the pet in a clean dish on YOUR left. "Right side" means that the product is presented to the pet in a clean dish on YOUR right.]
3. Record the pet's choice (the chip that he/she was the most interested in) in the "CHOICE" column in the table.
4. Record if the pet ate the product of choice in the last column "EAT?" in the table (enter YES or NO). If the pet partially ate the chip, enter a YES.
5. Try to complete the entire table in one session, but if there are distractions, then complete when possible. Call Jodi when forms are complete to arrange for pickup (269-9796).

Figure 6: Score card provided for pet owners. Pet owners used this score card to record their results from the preference test. A separate sheet was used for each test session (Bowser, 2009)

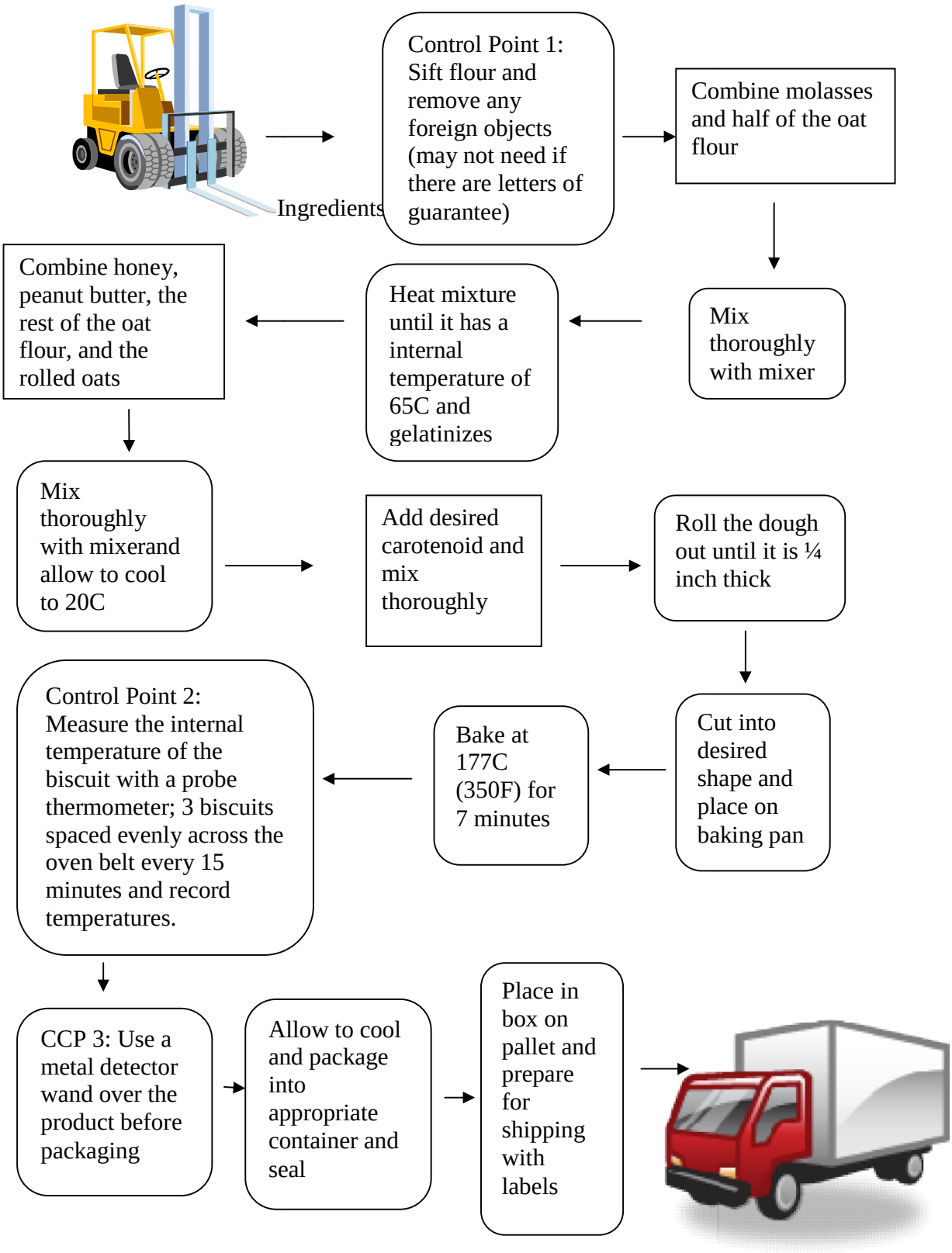

Figure 7: Flowchart of the process for creating the dog biscuit

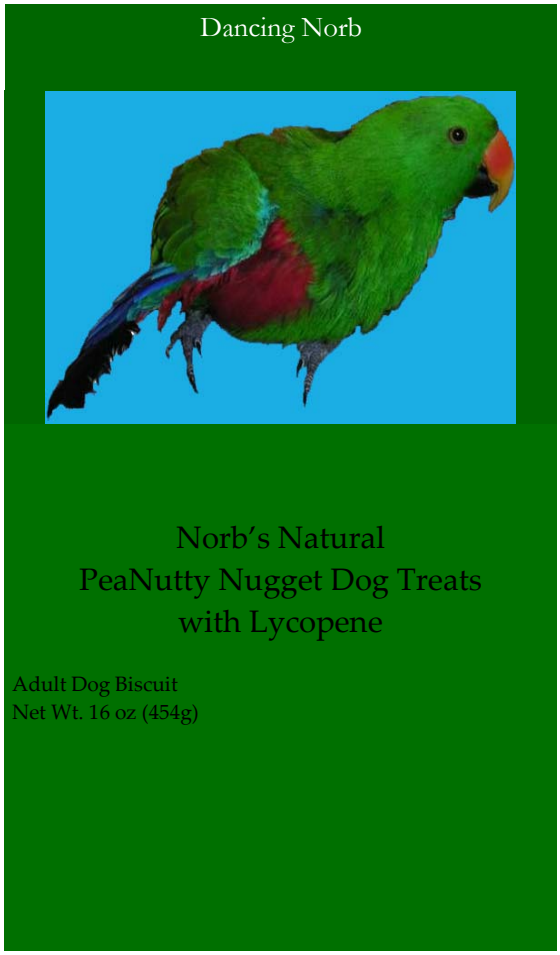

Figure 8: Front label for dog food package.

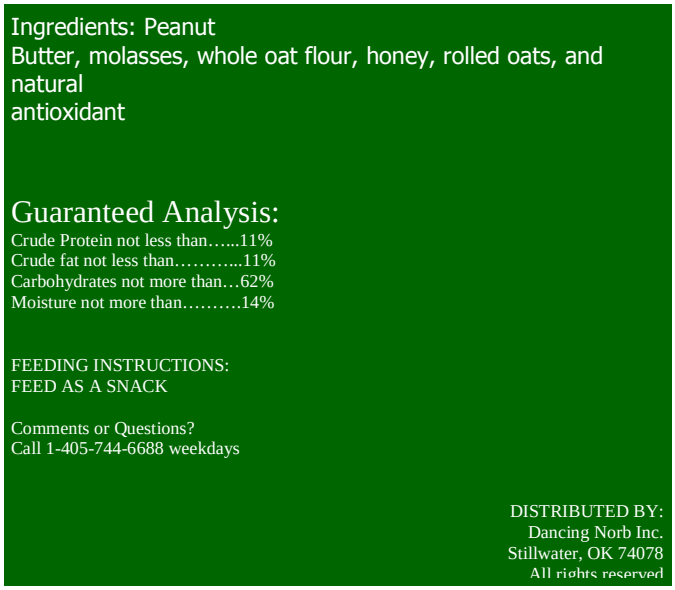

Figure 9: Back label for dog food package.

Table 2: Results for proximate analysis for week 0

TRT	% Moisture	% Fat	% Ash	% Protein	% CHO
Control	12.89505	11.67614	1.976692	11.96	61.49212
Lycopene	12.90325	12.36186	2.069446	11.8	60.86544
Lutein	12.81426	12.56128	2.098801	11.72	60.80566
Carotene	13.36963	11.72608	2.139209	12.19	60.57508

Table 3: Summary of results for water activity for all treatment groups

WEEK	CONTROL	LYCOPENE	LUTEIN	CAROTENE
0	0.546	0.394	0.506	0.438
1	0.514	0.539	0.570	0.554
2	0.525	0.546	0.552	0.538
3	0.514	0.524	0.534	0.526
4	0.518	0.535	0.542	0.542
5	0.517	0.542	0.539	0.552
6	0.513	0.533	0.538	0.535

Table 4: Statistical analysis for the water activity results for all treatment groups and for all testing periods.

	CONTROL	LUTEIN	LYCOPENE	CAROTENE
WEEK 0	0.546^{a}	0.506^{b}	0.394^{d}	0.438^{c}
WEEK 1	0.514^{d}	0.57^{a}	0.539^{c}	0.554^{b}
WEEK 2	0.525^{b}	0.552^{a}	0.546^{a}	$.538^{ab}$
WEEK 3	0.514	0.534	0.524	0.526
WEEK 4	$.518^{a}$	0.542^{b}	$.535^{b}$	$.542^{b}$
WEEK 5	$.517^{a}$	$.539^{b}$	$.542^{b}$	$.552^{c}$
WEEK 6	$.513^{a}$	$.538^{b}$	$.533^{c}$	$.535^{bc}$

Means with different letters in the same row are significantly different ($P \leq 0.05$)
n=4

Table 5: L* Values for the Tops of the Biscuits

	CONTROL	LUTEIN	LYCOPENE	CAROTENE
WEEK 0	27.039[a]	28.817[a]	32.303[b]	34.696[b]
WEEK 1	29.111	29.084	28.269	26.433
WEEK 2	25.272	24.446	23.791	22.797
WEEK 3	26.257[a]	26.118[a]	21.297[b]	23.203[b]
WEEK 4	23.705	23.419	20.497	22.639
WEEK 5	26.876	24.288	25.808	24.246
WEEK 6	28.000	26.291	25.193	25.349

L*=0 (black) - 100 (white).
Means with different letters in the same row are significantly different ($P \leq 0.05$)
n=4

Table 6: a* Values for the Tops of the Biscuits

	CONTROL	LUTEIN	LYCOPENE	CAROTENE
WEEK 0	5.870[a]	6.546[a]	12.775[b]	14.013[b]
WEEK 1	4.759[a]	5.775[ab]	7.057[ab]	8.757[b]
WEEK 2	3.9732[a]	5.467[ab]	6.478[b]	8.201[c]
WEEK 3	5.563[ab]	4.388[a]	6.899[b]	9.273[c]
WEEK 4	5.449[a]	4.913[a]	8.156[b]	7.566[b]
WEEK 5	5.780[ab]	5.066[a]	8.658[b]	7.182[ab]
WEEK 6	4.776	4.665	7.117	6.440

a*=red to green
Means with different letters in the same row are significantly different ($P \leq 0.05$)
n=4

Table 7: b* Values for the Tops of the Biscuits

	CONTROL	LUTEIN	LYCOPENE	CAROTENE
WEEK 0	6.886[a]	9.618[a]	14.044[b]	18.680[c]
WEEK 1	9.120	10.230	10.703	7.652
WEEK 2	8.008[a]	9.308[ab]	7.735[a]	11.729[b]
WEEK 3	10.273[ab]	7.725[a]	7.670[a]	13.677[b]
WEEK 4	8.341[a]	6.955[a]	9.339[ab]	14.202[b]
WEEK 5	10.488	6.729	9.011	6.917
WEEK 6	7.122	5.654	6.776	6.243

b*=yellow to blue
Means with different letters in the same row are significantly different ($P \leq 0.05$)
n=4

Table 8: L* Values for the Bottoms of the Biscuits

	CONTROL	LUTEIN	LYCOPENE	CAROTENE
WEEK 0	29.872[a]	31.377[a]	35.489[b]	36.482[b]
WEEK 1	27.006	26.524	26.845	27.361
WEEK 2	26.819[a]	24.721[ab]	23.771[ab]	22.754[b]
WEEK 3	27.004[a]	25.000[ab]	21.968[b]	21.941[b]
WEEK 4	25.376[a]	23.112[ab]	24.353[ab]	22.235[b]
WEEK 5	33.332[a]	28.114b	28.400[b]	24.136[c]
WEEK 6	31.564[a]	26.177[b]	25.862[b]	29.251[a]

L*=0 (black) - 100 (white).
Means with different letters in the same row are significantly different ($P \leq 0.05$)
n=4

Table 9: a* Values for the Bottoms of the Biscuits

	CONTROL	LUTEIN	LYCOPENE	CAROTENE
WEEK 0	6.776[a]	7.521[a]	17.300[b]	16.841[b]
WEEK 1	7.399[a]	6.751[a]	9.183[ab]	11.081[b]
WEEK 2	8.344	7.205	7.597	7.015
WEEK 3	7.537	7.584	9.399	7.895
WEEK 4	6.681[a]	6.005[a]	9.251[b]	8.044[ab]
WEEK 5	11.882[a]	8.967[b]	13.942[a]	7.733[b]
WEEK 6	10.686[ac]	5.701[b]	9.265[c]	11.777[a]

a*=red to green
Means with different letters in the same row are significantly different ($P \leq 0.05$)
n=4

Table 10: b* Values for the Bottoms of the Biscuits

	CONTROL	LUTEIN	LYCOPENE	CAROTENE
WEEK 0	9.427[a]	11.911[a]	22.388[b]	24.748[b]
WEEK 1	9.947[ab]	10.525[ab]	9.543[a]	13.649[b]
WEEK 2	16.160[a]	12.729[b]	11.230[b]	11.411[b]
WEEK 3	11.579	11.817	10.151	8.606
WEEK 4	9.210	8.367	11.395	12.096
WEEK 5	21.690[a]	13.888[b]	17.107[ab]	7.089[c]
WEEK 6	16.077[a]	8.107[b]	9.778[b]	14.927[a]

b*=yellow to blue
Means with different letters in the same row are significantly different ($P \leq 0.05$)
n=4

Table 11: Final results of the paired comparisons test for the preference test.

				Test 1		Test 2		Test 3		Test 4		Test 5		Test 6		
	Owner	Pet	No.	A	B	A	C	B	C	A	X	B	X	C	X	SUM
PANEL A	Bekah S.	Koa	1	4	2	4	2	4	2	2	4	4	2	2	4	36
	Suzanne H.	Dixie	2	4	2	5	1	3	3	3	3	6	0	4	2	36
	Richelle S.	Tiki	3	2	4	4	2	3	3	3	3	4	2	3	3	36
	Kyle F.	Lyla	4	3	3	3	3	4	2	/	/	6	0	6	0	30
	Tim B.	Buster	5	3	3	3	3	3	3	3	3	2	4	2	4	36
	Smith	Roxy	6	2	4	3	3	4	2	3	3	2	4	3	3	36
	Cheyanne C.	Dixie	7	3	3	3	3	3	3	4	2	5	1	2	4	36
PANEL B	Alisha P.	Fergie	8	4	2	3	3	5	1	4	2	4	2	4	2	36
	Remington R.	Dally	9	2	4	5	1	3	3	4	2	5	1	5	1	36
	Lacey G	Kozmo	10	1	5	4	2	3	3	4	2	2	4	6	0	36
	Kate H.	Cody	11	1	5	1	5	4	2	2	4	2	4	2	4	36
	Peter M.	Toby	12	3	3	3	3	3	3	2	4	4	2	2	4	36
		SUM		32	40	41	31	42	30	34	32	46	26	41	31	426

Table 12: Summary of final results from the sensory panel

Paired	Preferred	Responses for Preferred Samples (Out of 72 Possible)	α-level
A/B	B	40	<0.05
A/C	A	41	<0.05
B/C	B	42	<0.05
A/X	A	34	<0.05
B/X	B	46	<0.05
C/X	C	41	<0.05

Table 13: Summary of results from lycopene assay for all treatment groups and weeks.

DAY	CONTROL	LYCOPENE	LUTEIN	CAROTENE
0	0.019	0.602	0.081	0.436
1	0.015	0.355	0.010	0.388
2	0.002	0.357	0.056	0.328
3	0.010	0.386	0.075	0.449
4	0.010	0.338	0.102	0.403
5	0.010	0.361	0.037	0.424
6	0.074	0.374	0.106	0.432

All numbers reported as mg/g of fresh tissue.

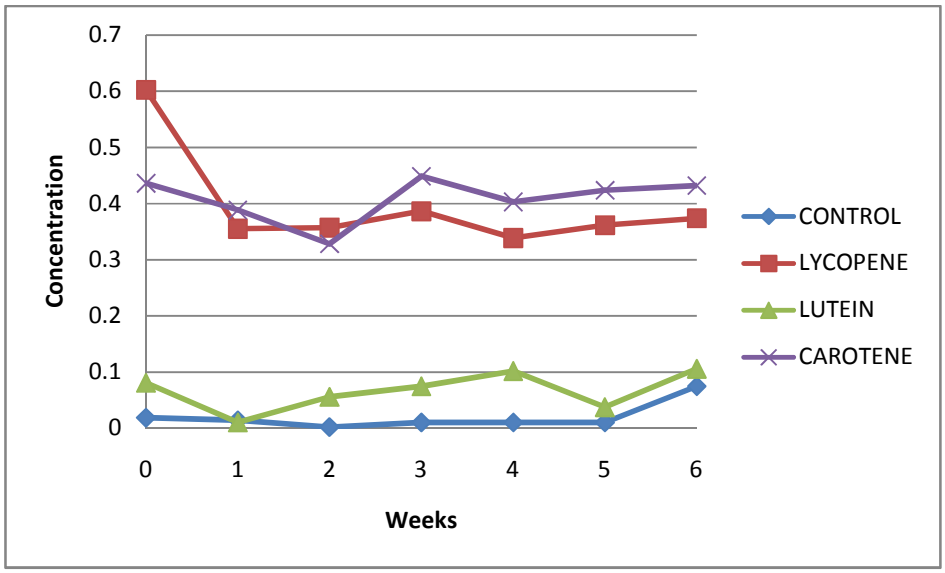

Figure 10: The results for the lycopene assay for all treatment groups and weeks

Table 14: Statistical analysis for the lycopene assay results for all treatment groups for all testing periods

	CONTROL	LUTEIN	LYCOPENE	CAROTENE
WEEK 0	0.019^{d}	0.081^{c}	0.602^{a}	0.436^{b}
WEEK 1	0.015^{a}	0.010^{a}	0.355^{b}	0.388^{b}
WEEK 2	0.002^{c}	0.561^{b}	0.357^{a}	$.328^{a}$
WEEK 3	0.010^{d}	0.075^{c}	0.386^{b}	0.449^{a}
WEEK 4	$.010^{d}$	0.102^{c}	$.339^{b}$	$.403^{a}$
WEEK 5	$.010^{c}$	$.037^{c}$	$.361^{b}$	$.424^{a}$
WEEK 6	$.075^{c}$	$.106^{c}$	$.374^{b}$	$.432^{a}$

Means with different letters in the same row are significantly different ($P \leq 0.05$)
n=4

VITA

Stacey Joann Kowalski

Candidate for the Degree of

Master of Science

Thesis: DEVELOPMENT AND EVALUATION OF A DOG BISCUIT WITH CAROTENOID AS AN ANTIOXIDANT

Major Field: Food Science

Biographical:

Personal Data: Born In Fort Smith, Arkansas, August 20, 1983, the daughter of Meveline and Mark Kowalski.

Education: Graduated from Spiro High School with honors, Spiro, Oklahoma, in May, 2001; received Associate of Art degree in Liberal Arts with honors from University of Arkansas, Fort Smith, Arkansas in May, 2003; received Bachelor of Science degree in Animal Science from Oklahoma State University, Stillwater, Oklahoma in May, 2007; completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in July, 2009.

Experience: Raised in Spiro, Oklahoma; employed by Oklahoma State University as a laboratory assistant, January, 2005 to December, 2007; employed by Oklahoma State University as a small animal caretaker, June, 2005 to May, 2008, acted as weekend supervisor, August, 2007 to May, 2008; employed by Oklahoma State University as a research assistant and acted as laboratory manager, July, 2007 to July, 2009; employed by PetSci LLC as an independent consultant, January, 2008 to July, 2009.

ADVISER'S APPROVAL: Dr. Timothy Bowser

Name: Stacey Joann Kowalski Date of Degree: July, 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: DEVELOPMENT AND EVALUATION OF A DOG BISCUIT WITH CAROTENOID AS AN ANTIOXIDANT

Pages in Study: 52 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: The purpose of this study was to develop a shelf-stable, nutritious, and healthy dog treat that is unique, profitable, provides some benefit to the dog, and is highly marketable. For this study, three different carotenoids were added individually to the same dog treat recipe. The carotenoids used were lycopene, lutein with 5% zeaxanthin, and natural carotene which contains both α-carotene and β-carotene. An accelerated 6-week shelf-life study was completed by placing 20 dog treats into a sealed poly bag that was stored at 35C. Proximate analysis was completed to calculate moisture, lipid, protein, and ash content on week 0 only. Color analysis, water activity, and antioxidant concentration were measured on weeks 0, 1, 2, 3, 4, 5, and 6. An in-home pet preference test was completed by testing the three biscuits that have the added antioxidants against a similar product without carotenoids that was already on the market.

Findings and Conclusions: The results from water activity show that the biscuits with antioxidants had less variability over time. Color scores varied more for the top of the biscuits than for the bottoms. The preference test showed that no biscuit was preferred more than the other statistically. The carotene and lycopene biscuits had similar levels of antioxidant present and the control and lutein biscuits had similar levels of antioxidant present during the lycopene assay. In conclusion, though the biscuit that was created was well liked by the pets and their owners and the antioxidant did help with water activity, it may not be worth the added costs to include these carotenoids at these levels in a dog biscuit.

ADVISER'S APPROVAL: Dr. Timothy Bowser