Preserving nutritional, visual, and functional quality of thermally processed low-acid canned foods for dogs and cats

by

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B.S., Kansas State University, 2016
M.S., University of Illinois at Urbana-Champaign, 2018

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2021

Abstract

Limited published research has addressed the quality of canned pet food. Detailed information regarding these thermally processed foods is needed. Specific concerns include functionality of carbohydrate hydrocolloids, discoloration due to supplemental copper, and thiamine degradation due to thermal processing.

An experiment with the following treatments was conducted to evaluate how carbohydrate hydrocolloids affect heat penetration, color, and texture of canned pet food: 1% dextrose (D), 0.5% guar gum and 0.5% dextrose (DG), and 0.5% guar gum with either 0.5% kappa carrageenan (KCG), 0.5% locust bean gum (LBG), or 0.5% xanthan gum (XGG). The D treatment had the thinnest ($P < 0.05$) batter consistency (23.64 cm/30 sec), the greatest ($P < 0.05$) lethality (20.24 min), and the lowest ($P < 0.05$) finished product toughness (67 N x mm). The DG treatment had thicker ($P < 0.05$) batter consistency (6.6 cm/30 sec), increased ($P < 0.05$) toughness (117 N x mm), and decreased ($P < 0.05$) lethality (18.63 min) and expressible moisture (26.93%) compared to D. The KCG, LBG, and XGG treatments were the thickest (average 2.75 cm/30 sec; $P < 0.05$) treatments pre-thermal processing with lethality comparable ($P > 0.05$) to DG. The KCG treatment was firmer (27.00 N; $P < 0.05$) and tougher (370 N x mm; $P < 0.05$) than LBG and XGG (average 15.59 N and 235 N x mm, respectively), but contained more ($P < 0.05$) expressible moisture (23.59% vs average 16.54%, respectively). The addition of carbohydrate hydrocolloids thickened pre-retort batters and lowered lethality. Further, guar gum with either xanthan gum or locust bean gum created softer textures with greater water holding capacities compared to products with kappa carrageenan and guar gum.

Two experiments were conducted to determine the effect of alternative copper sources on the development of off-colors and black blemishes. In the first experiment, a no added copper

control (NC) was compared to copper-lysine-glutamate at 60 and 300 mg/kg dry matter (DM) (LG60 and LG300, respectively), copper amino acid complex at 60 and 300 mg/kg DM (CA60 and CA300, respectively), and copper sulfate at 60 and 300 mg/kg DM (CS60 and CS300, respectively). Addition of copper darkened (average L* 47.57; $P < 0.05$), decreased ($P < 0.05$) red (average a* 2.07) and yellow hues (average b* 9.20), and lowered ($P < 0.05$) the vitamin E content (average 211 mg/kg DM) compared to NC (55.4, 8.43, 17.20 and 252 mg/kg DM, respectively). Treatments did not affect ($P > 0.05$) vitamins A or B_1 or fatty acid profiles. A second experiment included the following treatments compared to NC: copper-glutamate at 6 and 12 mg/kg DM (CG6 and CG12, respectively), copper amino acid complex at 6 and 12 mg/kg DM (CA6 and CA12, respectively), and copper sulfate at 6 and 12 mg/kg DM (CS6 and CS12, respectively). Addition of copper decreased ($P < 0.05$) red and yellow hues, with the exception of the similar ($P > 0.05$) yellow scores for NC (19.22) and CG6 (18.18). Vitamin E generally decreased ($P < 0.05$) with the addition of copper, except for NC and CS12, which were similar ($P > 0.05$) to each other (average 111.89 mg/kg DM). The number of blemishes was greatest ($P < 0.05$) for CG6 and CG12 (average 4.05 blemishes/slice). Copper amino acid complex may be a suitable alternative to copper sulfate at lower levels (i.e. 6 and 12 mg/kg DM), as these two sources exhibited similar color hues and number of blemishes.

An experiment was conducted to evaluate dried yeasts as sources of thiamine that may survive thermal processing of a canned cat food. There were 2 levels of vitamin premix (with or without) and four sources of yeast: no yeast (NY), Lalmin B-Complex Vitamins (LBV), a spray dried yeast from The Peterson Company (BY), and BGY Advantage (EA). Treatments containing BY (-33.8 mg/kg DM) exhibited similar ($P > 0.05$) processing losses of thiamine compared to those with NY (-31.3 mg/kg DM), while EA (-40.5 mg/kg DM) and LBV (-55.6 mg/kg DM) were

both greater ($P < 0.05$) than NY. All treatments exhibited similar ($P > 0.05$) processing losses of thiamine compared to the treatment containing the vitamin premix at standard commercial levels without any yeast. Thiamine loss for BY was consistent with standard ingredients (including vitamin premix) used in canned cat food. As such, the BY yeast may be an acceptable thiamine source for canned cat foods.

Overall, the quality of canned pet food is influenced by ingredient composition and the thermal process. The results from this dissertation provide insight regarding ingredient and processing interactions on commercial canned pet food.

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Major Professor
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Acknowledgements

I thank my advisor, Dr. Greg Aldrich, for his mentorship and for allowing me to focus my dissertation research on canned pet food. I also thank Dr. Fadi Aramouni, Dr. Kadri Koppel, Dr. Umut Yucel, and Dr. Robert Larson for agreeing to serve on my committee and for pushing me to be a better scientist.

A special thanks to the following people for their contributions to this dissertation:

Dr. Hulya Dogan for helping me with the texture analysis of my samples and for providing constructive feedback on Chapter 2;

Júlia Guazzelli Pezzali for her major contributions to the project in Chapter 3;

The Zinpro Corporation, especially Dr. Dana J. Tomlinson, for supporting the projects in Chapters 3 and 4;

Simmons Pet Food, especially Leah Lambrakis, Dr. Brittany White, and Dr. Markus F. Miller Jr., for supporting the project in Chapter 5;

Dr. Nora M. Bello for consulting on the statistical analysis for the project in Chapter 5.

A huge thank you is in order to all of the current and past graduate students and undergraduate students of the Kansas State University Pet Food Laboratory. Your help during the long days producing canned pet food was invaluable and this dissertation would not have been possible without you. Thank you for also becoming friends and lifting me up when I needed it the most.

Thank you to my family and friends for your unwavering support and for encouraging me to continue when the journey was rough.

Finally, thank you to my cat, Layla, for adapting to every new place we've moved to and for never letting me forget the importance of pet food.

Dedication

To my mom for teaching me the importance of education and for showing me that pursuing a dream is worth the sacrifice.

Chapter 1 - Literature review

Relevance and product basics

The U.S. pet food industry is worth roughly 36.9 billion US dollars [American Pet Products Association (APPA), 2020] and continues to see growth year after year. This growing industry provides many different product forms for pet owners to choose from. Thermally processed low-acid canned pet foods, commonly referred to as wet foods or simply canned foods, are offered to pets in roughly 40.3% of households with a dog and 56.6% of households with a cat (Simmons Research, 2018). This suggests that a large number of pets and pet owners interact regularly with wet food.

Wet pet food comes in two main formats (Kvamme and Phillips, 2003). The first is the loaf or pâté-style. Texture of this food format ranges from very smooth to flakey and depends on marketing targets for the product. Visual inclusions, such as whole peas, carrots, and rice may be added to the formula for visual recognition and appeal for the pet owner. The second main category for wet pet foods is cuts in gravy. Some companies may term this category as chunks in gravy. Nevertheless, the cuts in gravy product consists of reformed meat pieces or shredded meats in a liquid or semi-solid phase (Kvamme and Phillips, 2003; Rokey, 2005). Consistency of the liquid or semi-solid phase ranges from a broth to a thicker gravy. Visual inclusions can also be added to the cuts in gravy format for owner appeal.

At the time of this literature review, wet pet foods were available in three main categories of packaging or container type (Kvamme and Phillips, 2003). The first is a rigid metal can ranging in content weight from 85 to 623 grams. This is considered the most traditional packaging format (Kvamme and Phillips, 2003) and can contain either loaf/pâté style products or cuts in gravy products. The two newer packaging formats are cups/tubs and pouches (National Research Council

(NRC), 2006). Cups and tubs are a semi-rigid type of packaging with a plastic or metallic body and a flexible plastic/metallic lid. Some companies refer to this packaging as a tray (Case et al., 2011a). However, for the sake of this literature review, cups will refer to small (roughly 85-141 grams) semi-rigid containers and tubs will refer to larger (roughly 226-340 grams) semi-rigid containers. This packaging category may also include a reusable plastic lid that can be used for resealing and storing partial containers of food. They are more popular for cuts in gravy wet food formats, though loaf/pâté style products may also be found in the smaller cups. Finally, pouches are a flexible packaging type made of layers of plastic and metal (Kvamme and Phillips, 2003). They are typically single-serving sizes and almost exclusively contain cuts in gravy food formats. The AAFCO definition for "canned" is applied to any animal food that is processed according to the federal regulations described in the following section and is not specific to the traditional can packaging [Association of American Feed Control Officials (AAFCO), 2020]. Therefore, it is still appropriate to refer to pet food processed in semi-rigid and flexible containers as canned pet food.

Terminology, regulations, and calculations for thermal processing

Wet pet foods are produced under a method described as thermal processing. This category must follow federal regulations for processing low-acid foods with an acidic pH greater than 4.6 [U.S. Food and Drug Administration (FDA), 2020]. Food products with pH levels less than 4.6 are separated from a regulation standpoint. Their more acidic pH is a critical factor in controlling bacteria, resulting in lower applications of heat required as compared to foods with less acidic pH levels (Black and Barach, 2015). The exact pH of thermally processed wet pet food has not been well documented. It is reasonable to assume that the pH of wet pet food is close to the pH of meat, the primary ingredient in many wet pet foods (Kvamme and Phillips, 2003). For example, the pH

levels of chicken, pork, and beef liver were documented as 6.5-6.7, 5.8, and 5.8, respectively (Black and Barach, 2015; Briozzo et al., 1987).

The goal of thermal processing is to achieve commercial sterility, which is federally defined in the United States as a condition free of microorganisms that can reproduce at ambient temperatures and microorganisms and spores that are public health concerns through the application of heat [U.S. Food and Drug Administration (FDA), 2020]. Foods that also control for water activity do not need to consider microorganisms and spores that are public health concerns. However, wet pet foods do not control water activity; as such, the first definition is most appropriate. *Clostridium botulinum* is the bacterium of concern in thermal processing of food products for human or animal consumption. It is a spore-forming bacterium, meaning it can produce spores that survive extreme conditions, such as 5-10 hours in boiling water (Black and Barach, 2015), and return to a vegetative state when more favorable conditions are achieved. Cells in the vegetative state can produce neurotoxins that, if consumed repeatedly, lead to food poisoning in mild cases and a disease known as botulism in extreme cases (Maslanka et al., 2015). Outcomes of botulism can include paralysis and death. Thus, wet pet foods must be processed under validated scheduled processes, meaning the selected processes and conditions employed to achieve commercial sterility [U.S. Food and Drug Administration (FDA), 2020]. Commercial sterility does not mean the food product is free of microorganisms, including *Clostridium botulinum*. Instead, foods are typically processed to achieve a 12-log reduction in *Clostridium botulinum* (Morris, 2011). Any food product that does not receive the scheduled processing may contain unsafe levels of *Clostridium botulinum* and should be recalled to prevent possible consumer illness.

Thermal processing of low-acid foods can be achieved through two different approaches. The first technique is called aseptic processing and involves commercially sterilizing a food

product, cooling it, and filling it into pasteurized containers [U.S. Food and Drug Administration (FDA), 2020]. The second method is commonly called retort processing and involves commercially sterilizing food after it has been packaged inside non-sterilized containers. A retort is defined as a closed piece of equipment used for thermal processing of foods [U.S. Food and Drug Administration (FDA), 2020]. Wet pet foods are rarely processed with aseptic processing. Therefore, this literature review will discuss retort processing unless otherwise specified.

There are 4 main types of retorts that can be used for thermal processing. The first is called a still retort. These retorts utilize a basket system to hold containers in place during processing (Black and Barach, 2015). They are commonly used to process wet pet food packaged in metal cans. A sub-category is a still retort with overpressure. Overpressure is an amount of pressure applied by the retort in addition to the pressure from the heating material (Black and Barach, 2015). For example, the pressure inside a still retort using steam at a 121.11 ℃ is 103.4 kPa. The same retort with overpressure may operate with an additional 68.9-137.9 kPa of pressure (Black and Barach, 2015). This specialized retort type is necessary for processing food in semi-rigid and flexible containers. Examples of these packaging types include pouches and cups or tubs with flexible lids, which are growing in popularity in the wet pet food segment. The second category of retorts is the hydrostatic retort. In this system, containers are loaded onto a chain and carried through sections of water, steam, and cooling water to achieve commercial sterility. Some hydrostatic retorts also provide motion to the cans by spinning them or rocking them on the chain (Black and Barach, 2015). This type of retort is predominately used to process rigid cans in the wet pet food industry, but specialized hydrostats that can process flexible containers are available. The final two categories of retorts are continuous agitating retorts and batch agitating retorts. They apply motion to containers during processing through different mechanisms. Continuous agitating

retorts rotate the containers in a shell while moving the containers from one end of the retort to the other. On the other hand, batch agitating retorts utilize the basket system of still retorts but agitate containers by rotating them end-over-end or side-over-side (Black and Barach, 2015). Continuous and batch agitating retorts are rarely employed in the production of wet pet foods. Therefore, all references in this literature review to retort processing will refer to either still retorts or hydrostatic retorts unless otherwise specified.

Thermal processing of food is described by many calculations. The first is the D-value, which is the length of processing time at a set temperature, typically 121.11 °C, to achieve a 1 logarithmic reduction in the bacterial load (Morris, 2011). As was mentioned previously, *Clostridium botulinum* is the bacterium of public health significance for canned foods. Typically, a 12-log reduction is the target for this bacterium (Morris, 2011). The D-value is used to calculate the F_0 value (Equation 1.1; Morris, 2011), or the relative length of processing time at a reference temperature (i.e. 121.11 °C). Researchers must know the *Clostridium botulinum* content (or content of a less-pathogenic microorganism with similar thermal stability) of the food before (N_i) and after (N_f) thermal processing to calculate this value. The equation also utilizes the assumptions that heating throughout the product is uniform, which is likely not true. The typical F_0 value for wet pet foods is reported as 12-14 minutes and the minimum acceptable F_0 may be as low as 8 minutes (Hagen-Plantinga et al., 2017).

Equation 1.1. Calculation of F_0. D is the desired D-value for the process. N_i is the *Clostridium botulinum* content of the food before thermal processing. N_f is the *Clostridium botulinum* content of the food after thermal processing.

$$F_0 = D\left(\log N_i - \log N_f\right)$$

The D-value can also be used to calculate a z-value, which represents the change in temperature of the food product required to achieve a 1 log reduction in the D-value (Morris, 2011). It can also be referred to as the thermal resistance constant (Singh and Heldman, 2014).

This can be done by calculating D-values for different temperatures or by using a standard value. For example, the standard z-value for *Clostridium botulinum* typically used in thermal processing calculations is 10 ℃, which came from experiments with *Clostridium botulinum* 213-B in a pH7 phosphate buffer (Toledo et al., 2018). However, it may not be appropriate to utilize this value in all situations. The relationship between temperature and the logarithmic decrease in the D-value is only linear for a narrow temperature range (Brennan, 2006) and should not be applied to reference temperatures outside that range. Other characteristics that can affect the z-value include the method of heating (i.e. conduction vs. convection; Singh and Heldman, 2014) and well as the specific heat of the container and food product.

The z-value can be used to calculate an F-value when the loads of *Clostridium botulinum* (or any bacterium of interest) are not known. This value looks similar to the F_0, except the subscript is the reference temperature and a superscript of the z-value used may also be included (Singh and Heldman, 2014). For example, the F-value utilizing the reference temperature of 121.11 ℃ and the z-value of 10 ℃ for *Clostridium botulinum* would appear as $F_{121.11}^{10}$. It may also be called the lethality value (Equation 1.2; Singh and Heldman, 2014). Temperature at the coldest point in the canned food, typically the geometric center, is recorded at set intervals ($T_C(t)$ and Δt, respectively) by thermocouples (Black and Barach, 2015). This information is used to approximate the information provided by the *Clostridium botulinum* contents before and after thermal processing. The integral can be solved with the trapezoid rule, wherein the products of each time interval (Δt) and respective $10^{\frac{T_C(t)-121.11℃}{10℃}}$ are summed together (Equation 1.3).

Equation 1.2. Calculation of lethality. $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements**.**

$$Lethality = \int 10^{\frac{T_C(t)-121.11℃}{10℃}} \Delta t$$

Equation 1.3. Calculation of lethality by the trapezoid rule. $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$Lethality = \sum_{0}^{t} 10^{\frac{T_C(t)-121.11℃}{10}} \Delta t$$

Recently, researchers have utilized the reference temperature (100 ℃) and z-value (33 ℃) for thiamine to describe the magnitude of processing and its effect on nutritional quality. This value is called the cook value (C_{100}) and can be calculated similarly to lethality (Equations 1.4 and 1.5). For example, a food product processed to a higher cook value might retain less thiamine and have lower nutritional quality. Researchers have found strong correlations between F-values and C_{100} (Majumdar et al., 2017), so this literature review will mainly discuss lethality as a measure of processing intensity.

Equation 1.4. Calculation of cook value (C_{100}). $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$C_{100} = \int 10^{\frac{T_C(t)-100℃}{33℃}} \Delta t$$

Equation 1.5. Calculation of cook value (C_{100}) by the trapezoid rule. $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$C_{100} = \sum_{0}^{t} 10^{\frac{T_C(t)-100℃}{33}} \Delta t$$

Nutritional and physicochemical effects of thermal processing

Thermal processing can influence the nutritional and physicochemical quality of foods in addition to microbiological safety. Increased thermal processing decreased rat ileal digestibility of most amino acids in a canned cat food (Hendriks et al., 1999) and decreased net protein utilization of a liquid enteral formula by rats (Takeda et al., 2015). This led to an increased level of undigested protein available as substrate for bacteria present in the colon, resulting in greater cecal

concentrations of ammonia. This could be due to the development of Maillard reaction products; wherein, increased browning and decreased α-amino groups have been reported (Benjakul et al., 2018). The Maillard reaction occurs between reducing sugars, such as dextrose and amino groups, such as those found in the structures of lysine and arginine (BeMiller, 2019). The average canned pet food from a survey of 20 commercial products contained 4.5 g of fructoselysine, 36.7 g of carboxymethyllysine, 1.4 g of hydroxymethylfurfural, and 6.9 mg of cross-linked lysinoalanine per kg of diet dry matter (DM; van Rooijen et al., 2014). It is unknown if consumption of these compounds is harmful to dogs and cats. However, they have been identified with wide ranging implications on health when consumed by humans (Zhang et al., 2020) and the reaction may decrease the avilability of involved amino acids. Maillard reaction rates are high for foods processed with high temperatures (BeMiller, 2019), which may overcome the low concentration of reducing sugar reactants present in canned foods. Another possible cross-linkage between proteins includes disulfide bonds with the sulfur-containing amino acids methionine and cysteine, but this would not explain the decrease in digestibility of other amino acids.

Retort processing is known to affect the physicochemical properties of food. For example, processing to greater degrees resulted in less firm sardines in oil (Ali et al., 2005) and rohu balls in curry (Majumdar et al., 2017). Other parameters of texture, such as cohesiveness, springiness, and chewiness as measured with a two-compression texture profile analysis (TPA), also decreased when rohu balls in curry were processed to greater degrees (Majumdar et al., 2017). For example, cohesiveness describes the degree of deformation before the food sample ruptures and lower values indicate that the food product can withstand less force before breaking apart. Springiness quantifies the ability of the food product to recover after the first compression and lower values suggest a less resilient product. Finally, chewiness describes the amount of mastication work required when

consuming the product, and lower chewiness values suggest the food product does not require as much effort to chew. All in all, decreased values for these parameters suggest that textural quality decreased when rohu balls in curry were processed to greater degrees. However, the human sensory panelists preferred the rohu balls with lower textural properties that had been processed to greater intensities. This highlights the importance of sensory and consumer panels when developing products and processes. The scheduled process, or the time and temperature combination employed to achieve a desired F_0 value, can influence texture as well. For example, canned cat foods processed to the same F_0 value but with different combinations of retort time and temperature exhibited differences in firmness, adhesiveness (an indication of stickiness), and chewing work (Hagen-Plantinga et al., 2017). These differences may have influenced feline preference for the wet foods, among other changes brought on by differences in the scheduled processes.

Color has also been influenced by the magnitude of retort processing, likely due to the formation of Maillard reaction products. In general, processing to greater F_0 values led to darker products and increased red and yellow hues (Majumdar et al., 2017; Takeda et al., 2015). However, a human sensory panel recorded similar liking scores of "like very much" to the color of rohu balls processed to different F_0 values (Majumdar et al., 2017). This suggests that increased processing may not influence the liking for the color of food products. Increased processing did not affect liking of appearance but did increase liking of flavors and odors present in the samples (Majumdar et al., 2017). Despite reports with foods for human consumption, these effects have not been documented in wet pet foods.

Thermal processing of foods is intended to lower the risk of contamination from *Clostridium botulinum* to 1 in 1 billion grams of food product (Morris, 2011) and to allow for storage of the food product under non-refrigerated conditions (Singh and Heldman, 2014).

However, this method may lead to other biochemical reactions in the food, such as changes in nutritional quality, texture, and color. These outcomes may negatively influence health and consumer and pet owner liking. Unfortunately, limited research with pet foods in this area has been reported. More controlled evaluations need to be reported to better understand the effects of thermal processing on wet pet foods and the requirements to consistently manufacture quality products. This is necessary to determine the ideal scheduled process to provide commercial sterility and yet not unduly influence nutrition or sensory attributes of the food products.

Thermal processing has wide-ranging effects on canned pet foods. The intention is to destroy vegetative cells and spores of *Clostridium botulinum*, but other consequences occur as well. Generally, processing to greater degrees decreases nutritional quality and affects functional and visual appeal. However, the ingredients selected for canned pet food can influence these effects and improve the overall quality of products. Limited research identified carbohydrate hydrocolloids, supplemental copper forms, and thiamine-containing ingredients as primary concerns in canned pet food. Much of the research available is not with this food format. This fact highlights the importance of further research to address these concerns.

Carbohydrate hydrocolloids

Hydrocolloids are ingredients that form gels or influence viscosity in environments containing water. They can be either non-starch polysaccharides (multiple carbohydrate units bonded together to form one large molecule that could not be hydrolyzed into smaller units if on their own; BeMiller, 2019) or proteins. Non-starch polysaccharide hydrocolloids are more commonly used in wet pet food vs. protein hydrocolloids. Moving forward, the term “carbohydrate hydrocolloids” will be used to describe non-starch polysaccharide hydrocolloids.

The mechanisms of carbohydrate hydrocolloids are driven by their chemical structures. For example, kappa carrageenan is a linear molecule with repeating galactose and 3,6-anhydrogalactose units and sulfur ester substitutions (Phillips and Williams, 2009). This structure allows potassium ions to fit between kappa carrageenan molecule and form junction zones (Phillips et al., 1990), which result in a gel network that sets upon cooling. Another sub-group of hydrocolloids are the galactomannans, which consist of linear 1,4-β-D-mannan chains with D-galactose substitutions (Phillips and Williams, 2009). The distribution of the galactose substitutions is a defining characteristic of locust bean gum (17-26% galactose) and guar gum (33-40% galactose). Their branched structure and abundant hydroxyl groups are the driving forces behind their viscosity development in high-moisture foods (BeMiller, 2019). On the other hand, xanthan gum consists of a linear 1,4-D-glucose backbone with trisaccharide side chain substitutions consisting of an acetylated α-D-mannose, β-D-glucuronic acid, and a β-D-mannose that is acetylated 50% of occurrences in the molecule (Phillips and Williams, 2009). This causes the molecule to form single and double helical regions that form hydrogen bonds to influence viscosity and form gel structures with galactomannans (BeMiller, 2019).

Carbohydrate hydrocolloids are primarily included in wet pet food formulations to improve the structure of a loaf or pâté style product. Examples of these ingredients include guar gum, kappa carrageenan, locust bean gum, and xanthan gum. A colloquial term for this ingredient category is "gums and gelling agents" because they provide viscosity and/or a gel structure. They are good sources of total dietary fiber, primarily soluble dietary fiber (Kienzle et al., 2001; Sunvold et al., 1995a, 1995b), and may also provide health benefits to dogs and cats. However, these ingredients are scrutinized more than previously, with claims of "no carrageenan" becoming more common (Phillips-Donaldson, 2016). This new method for product differentiation has pushed pet food

companies to find replacements for traditional carbohydrate hydrocolloids, but with little knowledge of their functionality to wet pet food products.

The majority of research on carbohydrate hydrocolloids in wet pet foods have evaluated their effects on pet performance and health. For example, inclusion of a gelling agent (guar gum + carrageenan, locust bean meal + carrageenan, or wheat starch) in wet pet foods increased canine apparent ileal digestibility of organic matter, fat, gross energy, and total amino acids but decreased apparent total tract digestibility of dry matter in the same dogs (Karr-Lilienthal et al., 2002). Similarly, ether extract and crude protein apparent total tract digestibility were reduced with the addition of 3.5% guar gum to a meat-based diet fed to dogs (Diez et al., 1997). Increasing the concentration of these types of ingredients in wet pet food diets was reported to decrease apparent total tract digestibility of crude protein and organic matter while not affecting fecal dry matter content or fecal consistency (Zentek et al., 2002). The decrease in apparent total tract digestibility of crude protein might be explained by the fermentability of carbohydrate hydrocolloids. For example, locust bean gum and guar gum exhibit moderately high (> 60%) organic matter disappearance after 24 hours of *in vitro* fermentation with feline (Sunvold et al., 1995a) and canine (Sunvold et al., 1995b) fecal inoculum. Increased fermentation by colonic microbiota could lead to increased excretion of microbial protein. This would artificially increase the crude protein present in feces and thereby decrease the estimate of apparent total tract crude protein digestibility. Another potential explanation is related to the effects of carbohydrate hydrocolloids on viscosity of intestinal digesta. The viscosity of human digesta (*in* vitro) with added guar gum increased during 6 hours of simulated gastric digestion and remained unchanged after up to 15 hours of simulated small intestine digestion (Dikeman et al., 2006). However, dilution with saliva and gastric sections decreased the effect of food viscosity on gastric emptying rate when the model

meal contained up to 1.5% locust bean gum (Marciani et al., 2000). This phenomenon has not been verified in dogs or cats.

Carbohydrate hydrocolloids are rarely included in wet pet foods for a physiological effect. Instead, they are commonly added to improve product and processing functionality. One such benefit is increasing the viscosity of pre-retort batters. For instance, addition of a carbohydrate-based hydrocolloid or a blend of two hydrocolloids increased the apparent viscosity of pre-processed meat batters (Kim et al., 2018). This was likely influenced by increased interactions between different components of the batters, as evidenced by decreased expressible liquids and fat separation when hydrocolloids were added. The concentration of hydrocolloids influenced viscosity with greater proportions thickening samples and increasing their apparent viscosity (Casas et al., 2000). Hydrocolloids may also exhibit synergistic effects on viscosity. In other words, the viscosity may be greater when two hydrocolloids are used in combination with each other than when they are used alone. This was observed with the combination of xanthan gum and guar gum vs. guar gum alone (Casas et al., 2000). However, this has not been confirmed in complex food samples like canned pet food. Viscosity of wet pet food batters is crucial for the container filling process and potentially for thermal processing. Greater viscosity decreased the rate of thermal processing in aseptic processing (Ahmad et al., 1999) and in agitated retort processing (Singh et al., 2016) and masked the effect of pouch residual air during still retort processing (MacNaughton et al., 2018). However, this has not been addressed in wet pet foods processed in still retorts or hydrostatic retorts without container agitation.

Carbohydrate hydrocolloids can influence characteristics of processed products, such as texture. Conflicting results are reported for the effects of these ingredients on finished product texture. Increasing the level of a 30% guar gum 70% xanthan gum blend from 0.13% to 0.32% in

chicken-based sausages decreased firmness, chewiness, and resilience (Andrès et al., 2006), but the addition of carrageenan, guar, locust bean, or xanthan gums to a veal-based meatball increased firmness (Demirci et al., 2014). A sensory panel evaluated beef and pork based sausages and observed lower intensity of firmness and elasticity (i.e. less able to return to shape prior to oral compression) and greater intensity of grainy texture in sausages containing xanthan gum or guar gum compared to sausages containing iota carrageenan or potato starch (Solheim and Ellekjær, 1993). The differing conclusions between the studies are likely due to experimental design (i.e. inclusion of a control, carbohydrate hydrocolloids alone or in combination) and analytical methodology (i.e. texture profile analysis vs. Warner-Bratzler shear-force vs. human sensory panel). Food product formulation likely also plays a large role in the conclusions drawn from texture analysis since carbohydrate hydrocolloids are known to form gel networks that interact with proteins present in the food matrix (Rather et al., 2015). Thus, restructured meat products with differing intrinsic nutritional and functional characteristics may not interact with the same carbohydrate hydrocolloid in the same manner.

Somewhat different conclusions were also drawn about the effect of carbohydrate hydrocolloids on color when multiple types of food products and methodologies were compared. Increased amounts of carbohydrate hydrocolloids resulted in lighter meatballs (Demirci et al., 2014) and sausages (Andrès et al., 2006). However, the level of fat explained more of the variation in lightness (33.6% vs. 26.5%), redness (50.7% vs. 6.6%), and yellowness (6.9% vs. none) than the level of carbohydrate hydrocolloids (Andrès et al., 2006). This would suggest that these ingredients were not driving the changes in the color of these products. Higher inclusions of carbohydrate hydrocolloids increased red and yellow hues (Demirci et al., 2014). However, inclusion of these ingredients did not affect redness and yellowness in restructured hams and

decreased lightness by at most 1.68 units when 1% hydrocolloids were included in the formula (Kim et al., 2018).

To date, there are no published accounts of these effects in wet pet foods. While information gleaned from the literature on restructured meat products intended for human consumption is valuable, carbohydrate hydrocolloids generally serve different functions in these food categories. Many studies report the use of hydrocolloids as fat replacers, while the primary function in wet pet food is for structural integrity. As such, results from human food may not be directly applicable to wet pet food. Similar research is necessary in the wet pet food industry to define the important effects carbohydrate hydrocolloids have on product quality.

Sources of supplemental copper

Copper is a transition metal and an essential micronutrient for companion animals. Complete and balanced pet foods usually contain a supplemental copper source because many ingredients do not contain sufficient levels of copper to meet nutritional needs (Ferreira et al., 2005). Copper oxide was previously used as a supplemental copper source, but its use was discontinued because copper in this form is not biologically available to chickens (Baker et al., 1991) and growing cattle (Kegley and Spears, 1994). Copper sulfate became the predominant supplemental copper source after documentation of higher bioavailability compared to copper oxide. This improved bioavailability was measured by changes in qualitative cecal scores for color and bloating in chicks (Jensen and Maurice, 1978) and increased levels of plasma copper and ruminal soluble copper in calves (Kegley and Spears, 1994). Tribasic copper chloride is another example of an inorganic copper source and may be more biologically available than copper sulfate (Lu et al., 2010; Luo et al., 2005; Miles et al., 1998). Another popular source is chelated copper; these forms consist of a copper ion bound to a single amino acid or to multiple amino acids.

Commercial examples documented in peer-reviewed research include copper proteinate (Correa et al., 2014) and copper-glycine (Ševčiková et al., 2003). Chelated copper sources are marketed as organic forms with increased bioavailability compared to an inorganic source (i.e. copper sulfate).

Copper is involved in many biological oxidation reactions and functions as a cofactor for such enzymes. These enzymes can affect iron utilization, energy metabolism (Gropper et al., 2017), hemicellulose digestibility (Aoyagi and Baker, 1995), and coat color (Aulerich et al., 1982; Liu et al., 2015) among others. These mechanisms have not been directly verified in cats and dogs. Cats appear to be more affected by insufficient levels of dietary copper versus super-fortification levels. Kittens consuming copper deficient diets (< 1 μg copper/g diet) developed lesions on connective tissues and exhibited retarded growth and reduced food intake (Doong et al., 1983). Further, queens consuming diets deficient in copper take longer to become pregnant than queens consuming sufficient or super-fortified diets (Fascetti et al., 2000). In general, the liver is the largest organ storage of copper for cats, followed by the renal cortex and medulla (Paßlack et al., 2014). The consumption of diets deficient in dietary copper consistently reduced liver copper but inconsistently affects plasma copper (Doong et al., 1983; Fascetti et al., 2000). As such, liver copper concentration is often used to assess copper deficiency and body storage in cats. Cat sex and age should be considered as male cats tend to store more copper than females (Fascetti et al., 2002) and older cats store less copper in the renal cortex than younger cats (Paßlack et al., 2014). However, the extent of chronic kidney disease does not appear to affect copper storage (Paßlack et al., 2014). Copper storage may be an issue for dogs with chronic hepatitis (Cedeño et al., 2016) or other health concerns and for specific breeds of dogs with genetic predispositions, such as Labrador retrievers (Fieten et al., 2016) and Bedlington terriers (Forman et al., 2005; Haywood et al., 2016).

Copper is also involved in food-based reactions. Often copper functions as an oxidizing agent, or pro-oxidant, in oxidations reactions. The most stable form of the copper ion (Cu^{2+}) can be reduced and accept a free electron to form Cu^{+}. Free electrons can come from other components of the food product, such as lipids. Oxidation of lipids contributes to rancidity and can be quantified by measuring changes in free fatty acid composition, primary and secondary oxidation products, or reducing agents (anti-oxidants; i.e. vitamin E and ascorbic acid). Copper additions as low as 0.70 ppm to lipid ingredients, such as rapeseed oil, can increase production of the lipid oxidation products like hexanal and 2-hexenal over 35 days of storage in the presence of oxygen (Andersson and Lingnert, 1998). Studies assessing copper-catalyzed lipid oxidation observed greater peroxide values (a measure of primary lipid oxidation) and anisidine values (a measure of secondary lipid oxidation) when copper was added to emulsions containing canola oil (Osborn-Barnes and Akoh, 2003), egg, or soy lecithin (Wang and Wang, 2008).

Reports documenting the effects of copper on lipid oxidation in meat and animal-based products are inconsistent. A survey of salted cod noted a correlation between copper-catalyzed lipid oxidation and yellow-brown discoloration of the meat (Lauritzsen et al., 1999). This relationship was also observed when the addition of 10 mg/kg copper sulfate to mackerel skin and meat increased lipid oxidation (Ke and Ackman, 1976). However, other researchers have not observed this. For example, copper content was not a significant factor in development of rancid odors in raw Atlantic mackerel during storage (Maestre et al., 2011) or in changes to color and fatty acid composition in beef Longissimus thoracis muscle (Kitagawa et al., 2018). Similarly, the addition of 50 ppm copper sulfate along with 2% salt to turkey breast and thigh meat did not alter the oxidation level compared to 2% salt alone (Salih et al., 1989). Differences in meats and copper

supplementation levels could influence the conclusions and lack of consensus across multiple studies.

While copper is often touted as a pro-oxidant of lipids, there is evidence that it can also initiate oxidation of other nutritional components of food. As an example, addition of increasing levels of copper in minced cuttlefish did not decrease thiobarbituric acid reactive substances (TBARS) after multiple freeze-thaw cycles but decreased protein solubility and ATPase activity (Thanonkaew et al., 2006). These changes indicate a decrease in protein functionality, specifically myosin, actin, actin-myosin, and troponin-tropomyosin. The form Cu^{2+} was also associated with decreased redness (measured by a^* values from the CIELAB color space system) in that same study, which is a departure from increased yellowness (measured by b^* values from the CIELAB color space) often associated with lipid oxidation. Additionally, copper promoted the formation of α-amino adipic semialdehyde and γ-glutamic semialdehyde from amino acids in porcine myofibrillar proteins (Villaverde and Estévez, 2013). This further supports the concept that copper can oxidize amino acids and protein components.

Chelation of copper has been proposed as a method to decrease copper-catalyzed oxidation. Oxygen consumption of a system containing ground beef and 10 μM of cupric ion decreased with increasing levels of carnosine (Jun Lee et al., 1999), suggesting that carnosine had a protective effect and could form a complex with copper (Decker et al., 1992). This effect could be due to histidine, an amino acid with a strong affinity for copper ions at either the carboxyl oxygen or the nitrogen in the imidazole ring (Abramenko et al., 2001). Other promising chelating agents to minimize copper-catalyzed oxidation include tannic acid, ellagic acid, and other polyphenols (Ramanathan and Das, 1993).

The U.S. wet pet food industry has acknowledged visible differences due to copper supplementation, primarily “black spots or product graying” (Shields, 1998). Development of these blemishes, especially in light colored products, was linked to increased pet owner complaints. The phenomenon of dark spots has previously been identified on external surfaces of canned meat for human consumption and linked to iron and tin in the can body and products of sulfur amino acids from the canned food. This phenomenon was initiated by the oxidation of sulfur amino acids, such as cysteine and methionine, and the production of hydrosulfide (SH^-) and hydrogen sulfide (SH_2; Dantas et al., 2014). The removal of hydrogen ions (H^+) from both compounds left sulfur ions (S^{2-}) able to reaction with iron (Fe^{2+}) or tin (Sn^{2+}) from the can wall where enamel or lacquer coatings were weak. Brown spots were caused by the reaction between sulfur ions and tin ions, whereas black spots were the result of sulfur ions interacting with iron ions (Dantas et al., 2014; Kontominas et al., 2006). In those situations, improvements to can coating integrity as well as lower food product filling temperatures and higher processing temperatures with shorter processing times were decreased the development of black spots (Kontominas et al., 2006). However, these imperfections were only noted where food came in direct contact with the can wall and were not linked to copper inclusion. Additionally, there is no published research documenting the changes in color and oxidation of wet pet foods when copper sulfate was added to the formula. This needs to be done to fully describe the issue and identify what is reacting with copper to bring about these changes in visual quality. Potential solutions to this challenge include complexing copper with amino acids and other chelating agents or increasing the level of antioxidants in wet pet foods to overcome the oxidative power of copper.

Thiamine

Thiamine is an essential vitamin for mammals. It is comprised of a thiazole and a pyrimidine ring connected to each other by a methylene bridge. This form is called the "free form" and is the structure found in plants. Thiamine found in meats and other animal products is phosphorylated and is mainly in the form thiamine diphosphate. Synthetic forms of thiamine include thiamine mononitrate and thiamine hydrochloride. They are added to processed food products to increase thiamine content.

Thiamine deficiency has been documented in a few human populations, such as the elderly (O'Keeffe et al., 1994; Puxty et al., 1985), alcoholics (Hercberg et al., 1994; Herve et al., 1995), and individuals after gastric vertical sleeve surgery (Tang et al., 2018). Researchers have also proposed that thiamine deficiency is underreported in developing countries where diets are not varied and hospitals do not have the resources needed to accurately diagnose thiamine deficient patients (Adamolekun and Hiffler, 2017). Beriberi is the disease brought on by thiamine deficiency in humans and is observed in three types: dry, wet, and acute. Dry beriberi mainly affects the nervous system while wet beriberi mainly affects the heart and the cardiovascular system (Gropper et al., 2017). Acute beriberi is rare in adults and is mainly documented in nursing infants whose mothers consume thiamine deficient diets. The effects of thiamine deficiency can be seen within 1 month of consuming a thiamine deficient diet.

Thiamine deficiency is also reported in cats. They have a high requirement compared to other mammals due to their carnivorous diet and thiamine's coenzyme role in converting isoleucine, leucine, and valine into utilizable energy (Gropper et al., 2017). Generally, a deficiency of thiamine can lead to decreased appetite, weight loss, and problems with the central nervous system (Case et al., 2011b). Symptoms usually appear within the first month of consuming the

thiamine deficient diet. Visual symptoms that can be observed by an owner or veterinarian include incoordination, holding the tail erect to aid with balance, seizures, dilated pupils, decreased appetite and accompanying weight loss, vomiting, ventroflexion, and tilting of the head (Loew et al., 1970; Marks et al., 2011; Moon et al., 2013; Palus et al., 2010; Steel, 1997). Most case studies report normal serum chemistry profiles and complete blood counts and those with access to magnetic resonance imaging (MRI) report edema and lesions mirrored on both sides of the brain. This type of damage to the brain can lead to learning deficiencies. For example, cats consuming a thiamine deficient diet for 2-3 weeks were less able to learn a new task compared to cats consuming a thiamine sufficient diet for the same duration (Irle and Markowitsch, 1982). Activity of erythrocyte transketolase was previously used as an indirect method for measuring thiamine content in blood to diagnose a deficiency, but this method has been deemed less accurate at quantify thiamine status (Puxty et al., 1985; Talwar et al., 2000). Fecal and urinary thiamine levels have also been used to determine thiamine absorption, but these samples were unreliable and quantification of thiamine was challenging due to the low levels present (Partington et al., 1964). The widely-used method is to diagnose thiamine deficiency with whole blood analysis of thiamine. Some cats described in the previous case studies died due to thiamine deficiency while others survived after supplementation with oral or injected thiamine. This illustrates that thiamine deficiency is a serious and moral concern for cats.

The National Research Council suggests recommended allowances of 5.5 mg thiamine/kg of diet DM for weaned kittens, 5.6 mg thiamine/kg of diet DM for adult cats at maintenance, and 6.3 mg thiamine/kg of diet DM for queens during late gestation and peak lactation [National Research Council (NRC), 2006]. These recommendations are nearly 4 times the recommended allowance for puppies after weaning (1.38 mg thiamine/kg diet DM; NRC, 2006) and nearly 2.5

and 2.8 times the recommendations for adult dogs (2.25 mg thiamine/kg diet DM; NR 2006) and bitches during late gestation and peak lactation (2.25 mg thiamine/kg diet DM; NRC, 2006), respectively. The Association of American Feed Control Officials (AAFCO, 2020) utilizes the NRC recommended allowance of 5.6 mg thiamine/kg of diet DM for adult cats at maintenance as their minimum recommendation for all feline life stages (growth, reproduction, and adult maintenance). Nevertheless, a survey of 90 commercial canned pet foods conducted in late 2012/early 2013 found that 15.6% of foods tested did not meet the 5.6 mg of thiamine/kg of diet DM recommendation (Markovich et al., 2014). All of the foods tested were either formulated to meet AAFCO's feline adult maintenance minimum recommendations or tested with an adult feline feeding study following AAFCO's guidelines. This phenomenon is echoed in the numerous Food and Drug Administration (FDA) recalls that have occurred in recent years. The risk of a cat consuming a thiamine deficient commercial diet is still present even though the problem has been identified and recognized by pet food companies and regulatory agencies.

Thermal processing of canned food to reduce levels of *Clostridium botulinum* damages the thiamine molecule by breaking the methylene bridge between the thiazole and pyrimidine ring (Mauri et al., 2007). This destroys the molecule and makes it unavailable to the dog or cat consuming the food. As sterilization time and temperature are increased, retention of thiamine decreases for many types of foods, such as peas, corn, tomato juice, lima beans (Bendix et al., 1951), soymilk (Kwok et al., 1998), red gram splits (Nisha Rekha et al., 2004). Acidification of foods prior to thermal sterilization can improve thiamine retention (Briozzo et al., 1987; Dwivedi and Arnold, 1972; Nisha Rekha et al., 2004). However, acidification cannot completely prevent thiamine losses and may affect palatability of the processed foods. Other ingredients in the food can enhance the destruction of thiamine during thermal processing. For example, sodium bisulfite

can be used to preserve color of carbohydrate ingredients (i.e. beans and potatoes) after processing (Krokida et al., 2000), but it can also accelerate the destruction of thiamine. As little as 0.05% sodium bisulfite can reduce thiamine retention to 35-40% (Luh et al., 1978). Antioxidants, such as casein hydrolysate and rosemary extract, only slightly improve thiamine retention by 3-4% (Szymandera-Buszka, 2003). Fish-based ingredients appear to have a greater effect on thiamine losses due to a "thiamine destruction factor", commonly referred to as thiaminase. This destruction factor is found in tissue from eviscerated whitefish and can destroy 65% of thiamine in the system (Deutsch and Hasler, 1943). Other implicated fish species include whole freshwater smelt, goldfish, creek chub, fathead minnow, sucker, channel cat, bullhead, and mudminnow and viscera of Menomonee whitefish, carp, white bass, sauger pike, and burbot (Deutsch and Hasler, 1943). Most forms of this enzyme are destroyed during thermal sterilization. However, a heat-stable form was found in North Pacific fish species, including skipjack tuna, green snapper, and black cod (Hilker and Peter, 1966). Many potential solutions have been attempted by pet food companies to prevent thiamine deficiency, such as over-fortification of diets, avoidance of problematic ingredients, and balancing the need for a process to minimize destruction of thiamine while minimizing the risk of illness due to *Clostridium botulinum*. Unfortunately, these efforts have not been able to prevent thiamine deficiency in commercial diets. Thiamine analysis after diet production could identify the issue before food is sent to warehouses and retail locations or shortly after in time for a recall. This solution is costly, time-consuming, and generally not practical for modern industrial food processing practices.

Work addressing processing conditions has not solved the issue of thiamine deficiency in thermally processed canned pet foods. Another avenue is to include a protected source of supplemental thiamine that could survive processing. For instance, many ingredients possess a

"thiamine binding protein" or a method for protecting thiamine during a germination or growth stage (Gołda et al., 2004). Some examples include the yeast *Saccharomyces cerevisiae* (Iwashima et al., 1979), chicken egg white (Muniyappa and Adiga, 1979) and egg yolk (Muniyappa and Adiga, 1981), buckwheat (Mitsunaga et al., 1986), sesame seeds (Shimizu et al., 1995), sunflower seeds (Watanabe et al., 1998), wheat germ (Adachi et al., 2000), legume seeds (Adamek-Świerczyńska and Kozik, 2002), and mung beans (Gunarti et al., 2013).

Yeasts are of particular interest for a few reasons. They are utilized in industrial applications and the food industry for their fermentation of carbohydrates and sugars. This suggests that yeasts have high requirements for thiamine as a cofactor for carbohydrate metabolism. Thiamine binding proteins in yeast strains are linked to intra- and inter-cellular transport and decreases as yeast cells age (Iwashima et al., 1979; Nishimura et al., 1986). Active/raw yeasts can contain 66 μg of thiamine per gram of DM (Varga and Maráz, 2002), but are not sources of biologically available thiamine and may compete with the host organism for thiamine consumed from foods (Kingsley and Parsons, 1947). Inactivation of yeast cells may improve bioavailability and only slightly decrease total thiamine content. Thiamine content in these yeasts ranges from 22.2 μg/g DM to 51 μg/g DM and can be decreased with exposure to lipid peroxides (Parteshko et al., 1975) and storage of 6-12 months (Varga and Maráz, 2002). Despite promising results, inclusion of yeast in thermally processed wet pet food has not been evaluated as a source of protected thiamine. Evaluation of promising ingredients should include the reliability of thiamine content from multiple production lots and the ability of the intrinsic thiamine to withstand retort processing.

Summation and research objectives

Wet pet foods are a common selection for US pet owners and are often produced with retort sterilization. This method creates a reactive environment wherein changes in visual, functional, and nutritional quality are observed. However, the peer-reviewed literature does not reflect this need and importance. Research with products for human consumption suggests that quality can be affected and altered by ingredient selection and may provide insight to their effects in pet food. However, the conclusions drawn from human food research are rarely directly applicable. It is important to understand these effects in wet pet food before trying to innovate, especially given their purpose for commercial product differentiation, pet owner perception, and nutritional quality for dogs and cats.

It is clear that there are pressing challenges in quality of thermally processed wet pet foods that are not fully addressed by the current literature. Therefore, the main objective of this dissertation was to address these concerns. First, the effects of common carbohydrate-based hydrocolloid systems on heat penetration, texture, and color were examined in a model wet pet food. Second, different forms of copper were investigated in two experiments to describe the mechanism behind copper-induced color changes and identify a form that minimizes these changes compared to copper sulfate. Third, dried yeasts were evaluated for their thiamine content and thiamine retention after retort processing in comparison to thiamine mononitrate. The overall goal of this dissertation was to characterize the effects of ingredients currently used in wet pet foods to provide the pet food industry with previously undocumented effects while testing new solutions to these pressing challenges.

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Chapter 2 - The effects of carbohydrate hydrocolloids on heat penetration, color, and texture of pâté-style canned pet foods

Abstract

Carbohydrate hydrocolloids are commonly used in canned pet food. However, their functional effects have never been quantified in this food format. The objective was to determine the effects of select carbohydrate hydrocolloids on the heat penetration, color, and texture of canned pet food. Treatments were added to the formula as 1% dextrose (D) and 0.5% guar gum with 0.5% of either dextrose (DG), kappa carrageenan (KCG), locust bean gum (LBG), or xanthan gum (XGG). Data were analyzed as a 1-way ANOVA with batch as a random factor and separated by Fisher's LSD at $P < 0.05$. Batter consistency (distance traveled in 30 sec) thickened with increasing levels of hydrocolloids (D = 23.64 cm; DX = 6.60 cm; average of KCG, LBG, and XGG 2.75 cm). The D treatment (12.08 min) accumulated greater lethality during the heating cycle compared to all others (average 9.09 min). Redness (a*) and yellowness (b*) were similar for KCG, LBG, and XGG (average 4.41 and 15.39, respectively). The KCG treatment (27.00 N) was the firmest and D and DG (average 8.75 N) the softest with LBG and XGG (average = 15.59 N) intermediate. Toughness exhibited similar relationships except D (67 N x mm) was less tough than DG (117 N x mm). The D treatment showed the greatest expressible moisture (49.91%), LBG and XGG the lowest (average 16.54%), and DG and KCG intermediate (average 25.26%). Carbohydrate hydrocolloids influenced heat penetration of the foods, likely due to differences in batter consistency, and affected product texture.

Keywords: Expressible moisture, gel, gum, thermally processed, wet pet food

Introduction

Wet pet foods are commercially sterilized, low-acid products and come in formats similar to stews and pâtés in appearance. These products primarily consist of meats and water and contain binding/structural ingredient systems similar to restructured meat products for human consumption (BeMiller, 2019; Phillips & Williams, 2009). Carbohydrate hydrocolloids, such as carrageenan and guar, locust bean, and xanthan gums, are common choices. They are able to increase the viscosity of unprocessed meat batters and emulsions (Casas et al., 2000; Kim et al., 2018) and may increase or decrease the firmness of a finished product, depending on the formulation (Andrès et al., 2006; Demirci et al., 2014). Though it is likely that these ingredients are included in commercial pet foods for similar functional benefits, this has not been a primary area of investigation. Instead, research has focused on the nutritional value of carbohydrate hydrocolloids as soluble fibers (Kienzle et al., 2001; Sunvold et al., 1995a; Sunvold et al., 1995b).

The incorporation of carbohydrate hydrocolloids in commercial pet foods has waned as companies wish to differentiate their products from their competitors' offerings (Phillips-Donaldson, 2016). There are no reports of why pet food companies use the inclusion of carbohydrate hydrocolloids or lack thereof to distinguish their formulas from others. Mixed findings suggest that the addition of similar ingredients to canned pet foods causes softer stools in dogs (Zentek et al., 2002) while others reported firmer stools compared to a control diet (Karr-Lilienthal et al., 2002). A small segment of the population believes carbohydrate hydrocolloids to be toxic or carcinogenic to dogs and cats, though this in not confirmed in the literature (Cohen & Ito, 2002; Woodard et al., 1973). Regardless, pet food companies are looking for new ingredients to replace the commonly used carbohydrate hydrocolloids. However, there are no reports quantifying and differentiating the functional effects of these ingredients in canned pet foods. This

limits the ability to identify alternative, label friendly ingredients with similar functionality. Research with sausage, meatballs, and other restructured meat products can provide some insight, but many of these products utilize hydrocolloids for their ability to mimic the mouthfeel of fat (Phillips & Williams, 2009). As such, conclusions from restructured meat products for human consumption may not be directly applicable due to differences in formulation and processing methods.

The objective of this experiment was to determine the effects of guar gum and blends of guar gum with another hydrocolloid on color, texture, and heat penetration of wet pet foods. The hypothesis was that the addition of carbohydrate hydrocolloids would decrease heat penetration and alter the color and texture of processed foods. Additionally, systems containing guar gum with an additional carbohydrate hydrocolloid would have different textures driven by the mechanism of the additional carbohydrate hydrocolloid.

Materials and methods

Experimental treatment design and rationale

Five treatments were designed to show the effects of guar gum, kappa carrageenan, locust bean gum, and xanthan gum on functional properties of canned pet food. Dextrose was chosen as a space-holding control ingredient for its aqueous solubility (Jackson & Silsbee, 1922) and its similar moisture content compared to the carbohydrate hydrocolloids of interest. Controlling the moisture content of the samples was a concern to minimize confounding effects on heat penetration (Li et al., 2015). Guar gum was specifically chosen because of its high thickening power (Phillips & Williams, 2009) and its prevalence in commercial canned pet foods. As such, guar gum was also included in treatments containing either kappa carrageenan, locust bean gum, and xanthan gum an in attempt to mimic commercial pet foods.

These carbohydrate hydrocolloids have different structures that affect their mechanisms and functional properties. Briefly, kappa carrageenan is a linear molecule with repeating galactose units and 3,6-anhydrogalactose units connected with alternating α-1,3 and β-1,4 bonds (Phillips & Williams, 2009). Xanthan gum consists of a 1,4 linked β-D-glucose backbone with a side chain of a glucuronic acid and two mannose units every other glucose unit. Locust bean gum and guar gum are the most similar as they have the same linear 1,4-β-D-mannose backbone with galactose side chains connected with 1,6-α-glycosidic bonds. However, they differ in their galactose content; locust bean gum contains less galactose by weight compares to guar gum (17-26% vs. 33-40%; Phillips & Williams, 2009).

There are limited recommendations for the inclusion level of these ingredients in canned pet food. Locust bean gum is used in pet foods at 0.2-0.5% (Phillips & Williams, 2009) for its binding effects, or the ability to increase interactions between macromolecules themselves and with the solvent (Eliasson, 1996). Additionally, a model lunch meat formula contained 0.6% kappa carrageenan for its gelling effects (Phillips & Williams, 2009). The inclusion level of 0.5% for individual hydrocolloids was chosen based on these recommendations as well as preliminary data wherein higher inclusion levels were firmer than commercial canned pet foods (data not presented).

This led to the creation of five experimental treatments (Table 2.1): 1% dextrose (D), 0.5% dextrose and 0.5% guar gum (DG), 0.5% guar gum and 0.5% kappa carrageenan (KCG), 0.5% guar gum and 0.5% locust bean gum (LBG), and 0.5% guar gum and 0.5% xanthan gum (XGG).

Experimental treatment production and batter analyses

Prior to diet production, frozen blocks of mechanically deboned chicken (CJ Foods, Bern, KS) were ground with a lab-scale meat grinder (Weston Pro Series #32, Southern Pine, NC) fitted

with a die plate with 7 mm diameter holes. Treatments were replicated three times over three days of production, with each treatment made on each day. Water was heated in a stock pot until it reached 40 ℃, at which point the tempered ground chicken was added and the mixture was brought back up to 40 ℃. Brewer's rice (Lortscher Animal Nutrition, Bern, KS), spray-dried egg white (Rembrandt Foods, Okoboji, IA), sunflower oil (Kroger, Manhattan, KS), potassium chloride (Lortscher Animal Nutrition, Bern, KS), vitamin premix (Lortscher Animal Nutrition, Bern, KS), and trace mineral premix (Lortscher Animal Nutrition, Bern, KS) were added to the stock pot and heated to 60 ℃ with continuous stirring. Once the batter reached the target temperature, dextrose (Fairview Mills, Seneca, KS) and/or the respective hydrocolloid ingredient(s) (Danisco, New Century, KS) were added and the batter was stirred continuously for 5 minutes while maintaining temperature. Treatment order was randomized each day to maintain similar initial internal can temperatures, which can influence heat penetration and the required length of processing (Ahmad et al., 1999; Berry & Bush, 1989). After mixing, 21 cans (300 x 407; House of Cans, Lincolnwood, IL) were filled with 405 ± 5 g of batter for each treatment.

Three consistency measurements were taken per treatment replication using a Bostwick consistometer (CSC Scientific Company, Fairfax, VA). Briefly, this analysis utilizes a sloped trough and slide gate to determine how thick or thin a sample of a set volume is. Measurements of distanced in centimeters traveled in thirty seconds are recorded. A sample that travels farther is considered to have thinner consistency and a sample that doesn't travel as far is considered to have thicker consistency. The Bostwick consistometer methodology was chosen because it does not require room temperature samples, which is a concern for viscosity analysis. Generally, viscosity of food samples is greater (i.e. thicker) at cooler temperatures (Casas et al., 2000) and those values would not be relevant for samples collected directly from production. In the present experiment,

all samples were analyzed at the same temperature immediately after the complete batter was mixed for 5 minutes at 60 °C. Additionally, the Bostwick consistometer is widely used by the pet food industry because of its low cost and limited required training (Côté et al., 2019; Mouquet et al., 2006).

Three pH measurements were taken with a pH meter (P/N 54X002608; Oakton Instruments, Vernon Hills, IL) fitted with a pointed pH probe (model #FC240B; Hanna Instruments, Smithfield, RI). Finally, three samples for water activity were collected and stored in covered containers to return to room temperature for measurement with a water activity meter (Decagon CX-2; Meter Group, Pullman, WA).

Heat penetration data collection and calculations

Treatments prepared on the same day were processed in the retort at the same time. Four thermocouples (Ecklund-Harrison Technologies, Fort Meyers, FL) per treatment were placed in cans prior to filling and connected to a data capture system (CALSoft v. 5; TechniCAL LLC, Metairie, LA) to record temperature in the center of the cans during processing. Fill weight and gross headspace were recorded for these cans as well. Specifically, gross headspace was measured as the distance from the top of the can body to the top of the batter inside the container. Once measurements were taken, lids (300 x 407 sanitary lids; House of Cans, Lincolnwood, IL) were sealed onto the cans with a seamer (Dixie Seamer, 91118; Athens, GA). Cans were seamed and randomly loaded into a still retort (Dixie, 00-43; Athens, GA) and processed at 144.79 kPa and 121 °C. Thermocouple-containing cans were randomly distributed among all other cans. Temperature inside the retort was also recorded by the data capture system. The intent was that the data capture system would record temperature inside the retort and inside each can every 15 seconds. However, the data capture system could not consistently record temperature at this rate

during production of replicates 1 and 2. The longest length of time between temperature measurements was 7.75 minutes and the average ± standard deviation excluding the normal time intervals was 1.23 ± 1.30 minutes. The cooling cycle was started once the coldest can among all treatments in the retort containing a thermocouple achieved a minimum lethality, or the relative amount of time at a constant reference temperature of 121.11 ℃ (Sing & Heldman, 2014), of 8 min. This value has been reported as a minimum for commercial canned pet food (Hendriks et al., 1999) and was chosen to remain consistent with pet food industry practices. Cans were cooled in the retort with municipal water (20 ℃) until the last can containing a thermocouple dropped below 50 ℃ before removal from the retort.

Calculations for lethality (Equation 2.1; Singh & Heldman, 2014) and cook value (C_{100}; Equation 2.2) were made using the thermocouple temperature data (Figure A.1). $T_C(t)$ is the internal can temperature at any given time t and Δt represents the length of time between temperature measurements. The reference temperature for the item of interest and the z represents the change in temperature required to see a 1 log reduction in the D value, or the amount of time required to see a 1 log reduction in the amount of the item of interest (Morris, 2011; Singh & Heldman, 2014). These values were 121.11 ℃ and 10 ℃, respectively, for the calculation of lethality. The z value comes from experiments measuring the heat resistance of *Clostridium botulinum* 213-B in a pH7 phosphate buffer (Toledo et al., 2018) and has been used in a preliminary experiment with thermally processed pet food (Molnar et al., 2017). The C_{100} utilized the reference temperature (100 ℃) and z value (33 ℃) were for thiamine, the weakest nutrient. Both equation integrals were solved using the trapezoid rule (Equations 2.3 and 2.4, respectively). These calculations were used to discuss the effect of the treatments on heat penetration and dissipation. For example, higher lethality and C_{100} were indirect indicators of faster rates of heat

penetration. This methodology was used in a preliminary study of the effects of container size and type on lethality values of wet pet food processed for the same amount of time (Molnar et al., 2017).

Equation 2.1. Calculation of lethality. $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$Lethality = \int 10^{\frac{T_C(t)-121.11℃}{10℃}} \Delta t$$

Equation 2.2. Calculation of cook value (C_{100}). $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$C_{100} = \int_0^t 10^{\frac{T_C(t)-100℃}{33℃}} \Delta t$$

Equation 2.3. Calculation of lethality by the trapezoid rule. $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$Lethality = \sum_0^t 10^{\frac{T_C(t)-121.11℃}{10}} \Delta t$$

Equation 2.4. Calculation of cook value (C_{100}). $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$C_{100} = \sum_0^t 10^{\frac{T_C(t)-100℃}{33}} \Delta t$$

Analysis of processed experimental treatments

Four cans per combination of treatment and block were blended (14-speed Osterizer; Sunbeam Products, Boca Raton, FL) and freeze dried (model #HR7000-L; Harvest Right, LLC, Salt Lake City, UT) for analysis of dry matter (AOAC 934.01) in duplicate. Can vacuum was measured on 4 cans per treatment replicate with a glycerin-filled vacuum gauge (#25.300/30; Fisher Scientific, Hampton, NH) fitted with a rubber collar (#10816-11; Wilkens-Anderson Co., Chicago, IL) and metal tip.

Three cans per treatment replicate were analyzed for pH (meter: P/N 54X002608; Oakton Instruments, Vernon Hills, IL; probe: model #FC240B; Hanna Instruments, Smithfield, RI), free liquid, and expressible moisture by centrifugation as an indication of water holding capacity (Jauregui et al., 1981). Briefly, free liquid was quantified as the mass of the liquid phase, if present, upon opening the can. Expressible moisture was determined by weighing approximately 1 g of sample into two Whatman Grade 3 filter papers (GE Healthcare Life Sciences, Piscataway, NJ) and one Whatman Grade 50 filter paper (GE Healthcare Life Sciences, Piscataway, NJ) folded into a thimble shape and centrifuging in a 50 mL polypropylene centrifuge tube (Globe Scientific Inc., Mahwah, NJ) at 2,000 x g (Sorvall® RC 6 Plus; Thermo Electron Corporation, Waltham, MA). As-is and dried filter paper weights were recorded before and after centrifuging to account for residue transfer to the filter papers (Equations 2.5-2.7). Analysis of expressible moisture was conducted in quadruplicate for each can per treatment replicate.

Equation 2.5. Calculation of expressed moisture + residue after centrifugation.

$$Expressed\ residue, g = Dried\ filter\ paper\ after\ centrifugation, g - Dried\ initial\ filter\ paper, g$$

Equation 2.6. Calculation of expressed moisture + residue after centrifugation.

$$Expressed\ residue, g = Dried\ filter\ paper\ after\ centrifugation, g - Dried\ initial\ filter\ paper, g$$

Equation 2.7. Calculation of expressed moisture + residue after centrifugation.

$$Expressible\ moisture, \% = \frac{Equation\ 2.5 - Equation\ 2.6}{As - is\ weight\ of\ sample} * 100$$

Texture was characterized with a modified back extrusion test using a texture analyzer (TA-XT2; Texture Technologies Corp., Hamilton, MA) fitted with a 5.08 cm diameter and 2 cm tall cylindrical probe and a 30 kg load cell. The trigger force was set to 5 g and the test speeds (pre-, test, and post-) were set to 1 mm/s. The probe was pressed into the center of the products in cans to a depth of 2 cm. Firmness was recorded as the largest force measurement observed during the 2 cm compression. Many times this value was similar or not different from the force measurement recorded at the end of the compression. Toughness was calculated as the area under the curve of the compression peak using the trapezoid rule (Figure A.2). Five cans from each treatment replicate were analyzed and values were averaged together to generate composite values. This methodology was selected instead of a texture profile analysis procedure because some of the treatments did not form structures that would remain stable if removed from the can.

Three cans per replicate of treatment were analyzed for color with a CIELAB color-space colorimeter (CR-410 Chroma Meter, Konica Minolta, Chiyoda, Tokyo, J.P) with five measurements taken from four evenly spaced regions of pâté from each can. Sections were created by removing the product from the can and slicing 3 times with a knife. In cases where slices could not be created, color of the top and bottom of the product were measured while inside the can and internal color by separating product into 3 sections of roughly the same size. Color was described in terms of L* (brightness), a* (red to green scale), and b* (yellow to blue scale).

Statistical analysis

Data were analyzed as a randomized complete block design with treatment as the fixed effect and day as the random block using statistical analysis software (SAS 9.4; SAS Institute, Cary, NC). Values were presented as least square means ± the standard error of the mean and differences were calculated using Fisher's LSD in the GLIMMIX procedure. The CORR procedure

was used to calculate r and p-values for Pearson correlations. All tests were considered significant if $P < 0.05$.

Results

Batter pH and batter water activity were not different ($P > 0.05$) among treatments and averaged 5.94 and 0.990, respectively (Table 2.2). Consistency was affected ($P < 0.05$) by treatment. The D treatment was the thinnest (23.64 cm) and often traveled the full 24 cm in less than 30 seconds. Guar gum alone (DG = 6.60 cm) decreased ($P < 0.05$) consistency, resulting in a thicker batter, in comparison to D. The KCG, LBG, and XGG batters exhibited the lowest consistency with no differences ($P > 0.05$) between them (average = 2.75 cm).

No differences ($P > 0.05$) were noted in can fill weight (average = 406.6 grams), gross headspace (average = 13.87 mm), initial internal can temperature (average = 55.67 °C), or can vacuum (average = -12.9 kPa) across treatments (Table 2.3). Data from three thermocouples were removed from statistical analysis due to failure (2) and low fill weight (1).

Heating cycle length and cooling cycle length across the three days averaged 81.25 ± 1.392 min and 79.58 ± 22.735 min, respectively. The complete cycle, heating cycle, and cooling cycle lethality were all affected ($P < 0.05$) by the treatments (Table 2.4). Heating cycle lethality was greater for D (12.08 min) compared to the four other treatments (average = 9.09 min). The same relationship was observed for the complete cycle lethality (D = 20.24 min; average of all others = 18.46 min). On the other hand, D (8.17 min) accumulated lower ($P < 0.05$) cooling cycle lethality than LBG and XGG (average = 9.60 min) with DG and KCG not different ($P > 0.05$; average = 8.97 min) from any of the treatments. The complete cycle C100 were not affected ($P > 0.05$; average = 197.10 min) by the treatments. However, D accumulated more ($P < 0.05$) C100 during the heating cycle (137.01 min) and less ($P < 0.05$) C100 during the cooling cycle (64.90 min)

compared to all other treatments (averages = 117.24 min and 78.66 min, respectively). Total lethality and C100 were very strongly correlated (r = 0.98; P < 0.0001). The same relationship was observed between heating cycle lethality and C100 (r = 1.00; $P < 0.0001$) and between cooling cycle lethality and C100 (r = 0.95; $P < 0.0001$).

Many processed treatment characteristics were affected by the differences in carbohydrate hydrocolloid content (Table 2.5). The only experimental treatment to exhibit two phases was D, with 16.91% ± 1.629% of the product mass as a free liquid phase. The finished product pH was greatest ($P < 0.05$) for KCG (6.38) and lowest for dextrose-containing treatments (D and DG; average = 5.96) with LBG and XGG (average = 6.26) intermediate. Total moisture was greater ($P < 0.05$) for D and DG (average = 79.21%) than for LBG and XGG (average = 77.29%) with KCG (77.83%) intermediate and not different ($P > 0.05$) from any other treatment. Expressible moisture as a percentage of the total sample mass was greatest ($P < 0.05$) for D (49.91%) and lowest for LBG and XGG (average = 16.54%) with DG and KCG intermediate (average = 25.26%). The KCG treatment exhibited the greatest ($P < 0.05$) firmness and toughness (27.00 N and 370 N x mm, respectively) of all experimental treatments, followed by LBG and XGG (average = 15.59 N and 235 N x mm, respectively). The replacement of 0.5% dextrose with guar gum nearly doubled ($P < 0.05$) toughness (D = 67 N x mm; DG = 117 N x mm) but did not affect ($P > 0.05$) firmness (average = 8.75 N). Increasing the level of dextrose darkened ($P < 0.05$) the product. Additionally, LBG was lighter ($P < 0.05$) than DG with KCG and XGG intermediate and not different ($P > 0.05$). No differences ($P > 0.05$) in a* and b* were noted between KCG, LBG, and XGG (averages = 4.41 and 15.39, respectively). However, the inclusion of dextrose in D and DG resulted in redder (average = 8.37) and yellower (average = 22.05) color.

Discussion

The aim of this experiment was to quantify the functional characteristics present in canned pet food containing different carbohydrate hydrocolloids, specifically guar gum, kappa carrageenan, locust bean gum, and xanthan gum. Treatments were designed to show the effects of common carbohydrate hydrocolloid systems and guar gum alone at inclusion levels mimicking commercial canned pet food.

Consistency was affected by the treatments in the present experiment and generally increased when the total carbohydrate hydrocolloid content of the treatment increased. Thickness of a hydrocolloid solution is dependent in the interactions between the hydrocolloid molecules and the solvent or liquid component of the system (Eliasson, 1996). As such, increasing the amount of carbohydrate hydrocolloids increases the number of reactions possible with the solvent. The DG was approximately 3.5 times thicker than D, while KCG, LBG, and XGG were only an average of 2.4 times thicker than DG. Guar gum has a high thickening power compared to many carbohydrate hydrocolloids (Phillips & Williams, 2009) because it contains many hydroxyl groups that can form hydrogen bonds with water.

Consistency was chosen as the metric to describe viscosity, which is known to affect the rate of heat penetration and the time required to reach lethality in food products (Singh et al., 2016). The Bostwick consistometer does not measure viscosity directly and is influenced by other factors including gravitational forces, though it does allow for analysis of samples during can filling. Consistency is also listed in U.S. federal regulations as a potential critical factor for scheduled processes for thermally processed low-acid foods [U.S. Food and Drug Administration (FDA), 2020]. Studies with other food products have found conflicting results regarding the correlation between direct viscosity and Bostwick consistency measurements (Ordiz et al., 2015;

Vercruysse & Steffe, 1989). Similar research should be conducted with pet foods to validate the Bostwick consistometer as a method for apparent viscosity analysis.

Batter pH and batter water activity were not different among the treatments. The lack of difference in batter pH suggested that minimal reactions occurred during the 5 minute mixing after the addition of carbohydrate hydrocolloids. It is also possible that differences may have been detected if the data were analyzed as the concentration of hydrogen ions instead of as pH values (i.e. the negative logarithm of the concentration of pH values). This would have yielded a range of average concentration of hydrogen ions from 1.07 x 10-6 - 1.25 x 10-6 hydrogen ions in the batters. The lack of difference observed in batter water activity was mainly influences by the low concentration of carbohydrate hydrocolloids, which generally do not affect water activity when their inclusion level is less than 2% (BeMiller, 2019).

The intention of this experiment was to begin the cooling cycle after the last can containing a thermocouple reached a lethality value of 8 minutes. However, treatments appear slightly over-processed as the lowest average heating cycle lethality was 8.69 minutes (Table 2.3). Similarly, a previous experiment with processing canned foods to different F_0 values also struggled to hit their targets (Hendriks et al., 1999). Three thermocouples failed during the present experiment, but more than the minimum 10 thermocouples recommended by the Institute for Thermal Processing Specialists (IFTPS, 2014) were successful across the three replicates for each treatment. Nevertheless, thermal processing parameters of initial internal can temperature, fill weight, gross headspace, and vacuum were constant. This indicates that differences in lethality were due to the treatments and not influenced by confounding factors. There are no published reference values for commercial canned pet foods. However, a preliminary study of canned pet food with initial internal can temperatures around 30 ℃ and can volumes of 88.7 mL and 162.7 mL observed post-

processing can vacuums of -0.8 kPa (Molnar et al., 2017). Initial internal can temperatures in that experiment were roughly 50% colder than the present experiment and likely influenced the differences in post-processing can vacuum.

Differences were observed in the heating and cooling of the experimental treatments. Specifically, D obtained greater lethality and C100 during the heating phase and lower values during the cooling phase of retort processing. This could indicate a faster rate of heat penetration and heat dissipation compared to all other treatments. The thickening of pre-retort batters due to the increase in hydrocolloid content likely increased the resistance to heat, leading to lower lethality and C_{100} when the foods were processed under the same time and temperature conditions. Previous research of the effect of viscosity on heat penetration found that increased food thickness decreased the average heating slope and increased the amount of time required to thermally process a food (MacNaughton et al., 2018). This suggests that thinner food consistencies may benefit production facilities by decreasing the amount of time to process a food, which could allow for more products to be made in the same amount of time. It is likely that the heating and cooking lag factors (j_h and j_c, respectively) and the heating and cooling penetration factors (f_h and f_c, respectively) were influenced by the treatments. The lag factors describe how long a food product initially takes to begin heating or cooling while the penetration factors describe the rate of heating or cooling (Toledo et al., 2018). The present experiment did not investigate these parameters, however future experiments should do so to provide more understanding of how carbohydrate hydrocolloids affect thermal processing.

The C_{100} calculation has never been applied in literature to canned pet foods. This metric can describe the detrimental effect of increased thermal processing on quality changes such as texture and nutrients. Thiamine degradation is an important concern for pet foods, especially those

for cats. Consumption of a thiamine deficient diet can be deadly within a few weeks (Davidson, 1992; Loew et al., 1970). Deficient canned pet foods should be recalled to prevent illness and death but recalls are costly to pet food companies. As such, this is a great concern for the pet food industry. However, there are no published reference values for acceptable and unacceptable cook values as it relates to thiamine content or other quality factors. The data presented in this study suggest that canned pet foods processed under commercial conditions have cook values of at least 195.91-201.90 minutes. Future experimentation needs to determine a maximum C_{100} before texture and thiamine are degraded to an unacceptable level.

Color was largely similar between KCG, LBG, and XGG, which was expected. Carbohydrate hydrocolloids are rarely involved in browning reactions. For example, an experiment with chicken sausages found that the level of carbohydrate hydrocolloids only explained 26.5% of the variation in lightness, 6.6% of the variation in redness, and none of the variation in yellowness (Andrès et al., 2006). Instead, other factors, such as fat inclusion level, were more significant. Differences in color were identified in pâté-style wet pet foods containing different soluble proteins at a 2.5% inclusion level (Polo et al., 2009). Companies wishing to alter the color of their products with ingredients at low inclusion levels may have more success changing soluble proteins than carbohydrate hydrocolloids. Regardless, values for the lightness, redness, and yellowness of canned pet foods containing different carbohydrate hydrocolloids have never been published. A pilot study with canned pet foods presented CIELAB color-space values for commercial products, but the ingredient composition of the formulas were not disclosed (Hsu et al., 2020). As such, the values presented for KCG, LBG, and XGG could serve as reference values for chicken-based canned pet foods containing the respective carbohydrate hydrocolloids.

The D and DG treatments appear to have confounding factors influencing the color. First, D was processed to a higher total lethality, which increased the redness and yellowness of thermally processed shrimp in curry (Mallick et al., 2010). The DG treatment was similar to D in redness and yellowness, which suggests that degree of processing is not the only confounding factor. It is highly likely that the dextrose in both treatments participated in a Maillard reaction during thermal processing. This reaction occurs between α-amino groups in proteins and reducing sugars (Benjakul et al., 2018) and is associated with increased redness and yellowness and more acidic pH levels in infant formula (Takeda et al., 2015). This suggests that D and DG do not serve as controls for redness and yellowness in the present experiment. There are no published values for the redness and yellowness of canned pet foods containing dextrose. The data for these two treatments are useful benchmarks for pet food companies who wish to use dextrose to increase the redness and yellowness of their products.

The pH levels of the processed foods were affected by the treatments. The D and DG treatments had more acidic pH, which could be tied to the production of Maillard reaction products mentioned in the previous paragraph. It also appeared that pH became more basic after thermal processing with the degree of change dependent on the carbohydrate hydrocolloids present. This would suggest that thermal processing caused a degree of hydrogen bonding, thus decreasing the amount of free hydrogen ions and explaining the shift to a more neutral pH for treatments LBG and XGG. The KCG treatment shifted even more because the sulfate half-ester groups in kappa carrageenan are negatively charged (BeMiller, 2019) and shift the pH even more. It may also be that the differences in pH are related to the differences in color. Chicken breasts classified by visual color assessment were further differentiated by pH and CIELAB color values (Fletcher, 1999). Specifically, pH was slightly more acidic for lighter chicken breasts and slightly more neutral for

darker chicken breasts. This may be related to denaturation of myoglobin due to processing (Andrés-Bello et al., 2013), however this effect would be small in the present experiment due to the low amounts of myoglobin present in chicken meat (Kranen et al., 1999). Unfortunately the water activity of the processed foods was not measured in the present experiment. Even though it was not anticipated that water activity would be different due to the low inclusion levels of carbohydrate hydrocolloids (BeMiller, 2019), this information would have enhanced the discussion.

Firmness, toughness, and expressible moisture were affected by the experimental treatments. Specifically, firmness was higher when guar gum was included with another hydrocolloid and toughness increased with higher total carbohydrate hydrocolloid inclusions. Increasing the level of hydrocolloids in a product will increase the gel strength (Phillips & Williams, 2009); this is observed in the toughness parameter. Experiments with 0.5-1.5% carbohydrate hydrocolloids in meatballs (Demirci et al., 2014) and restructured hams (Kim et al., 2018) observed this phenomenon as increases in firmness. It may be that 0.5% guar gum, as in DG, in wet pet foods is not enough to influence firmness compared to a sample without a hydrocolloid. The effect of increased carbohydrate hydrocolloid content was also observed in expressible moisture. As was mentioned in the discussion of consistency, hydrocolloids with hydroxyl groups are able to form hydrogen bonds with water (Phillips & Williams, 2009). Increasing the level of those hydrocolloids introduces more hydroxyl groups, resulting in more bonding with water. This would lower the amount of water that could be expressed and has been observed in restructured hams (Kim et al., 2018). The difference between D and DG highlights this well and illustrates the strong power of guar gum to interact with water. It is possible that processing D to a higher total lethality decreased the overall protein functionality (Hendriks et al.,

1999) and confounded the observed lower toughness and higher level of expressible moisture. In future experiments, treatments with significantly different consistencies should be thermally processed separately to ensure that all treatments receive the same level of processing.

The KCG treatment was firmer and tougher with lower levels of expressible moisture compared to LBG and XGG. This is caused by the different gel structures formed with these hydrocolloids. Gels created by the combination of kappa carrageenan and potassium ions (i.e. from potassium chloride) can withstand substantial application of force before fracturing (Phillips et al., 1990; Wang et al., 2018). These gel systems are typically described as "firm" and "brittle" (Phillips & Williams, 2009). On the other hand, guar gum, locust bean gum, and xanthan gum form bonds with the hydrogen atoms in water to form a gel structure (Phillips & Williams, 2009). Specifically, gels containing xanthan gum and a galactomannan are described as "firm" and "rubbery" (Phillips et al., 1990). These different gelation mechanisms are defining features in this analysis. For example, a difference in expressible moisture between DG and KCG was not observed or expected because kappa carrageenan does not participate in many hydrogen bonds with water.

Firmness and toughness of canned pet food is rarely reported and expressible moisture has never been documented. As such, the values presented in this manuscript can serve as references for commercial product development and improvement. These metrics may be important to pet owner acceptability and pet palatability and food preference. Reports indicate that cats prefer a softer food requiring less work to chew in the first 7 days of consuming a canned food (Hagen-Plantinga et al., 2017). The softer textures for LBG and XGG vs. KCG may be preferred by cats, but this was not a focus of the present study. Future work should expand upon this study and utilize palatability testing with dogs and cats as well as consumer testing with pet owners to determine which textures are preferred and why they are preferred.

This work highlighted multiple areas for future research. First, the effect the carbohydrate hydrocolloid concentration has on batter consistency, heat penetration, and finished product texture and expressible moisture should be investigated. As learned from this research, dextrose is not a good control ingredient and should be replaced with ground meat as a space-holding ingredient to avoid the changes in pH and color that were observed in the present experiment. The use of the primary meat as the control ingredient is standard practice in testing the effects of carbohydrate hydrocolloids in restructured meat products for human consumption. Another alternative control ingredient could be cellulose, which is a carbohydrate ingredient but has no effect on viscosity [National Research Council (NRC), 2006]. Second, the Bostwick consistometer should be validated against direct apparent viscosity methods. This could be done simultaneously to other work in an experiment evaluating carbohydrate hydrocolloids. Findings from such an experiment may confirm that Bostwick consistency is an appropriate methodology or suggest that a different method should be the standard. Finally, the changes due to dextrose inclusion at low levels were unexpected. The effect of inclusion level on pH, color, and Maillard reaction products should be explored in the event that dextrose is essential for future experiments or for commercial products.

Conclusions

Carbohydrate hydrocolloid inclusions affected heating, texture, and expressible moisture of wet pet foods. Thickening batter consistency to 6.60 cm traveled in thirty seconds or thicker likely decreased the rate of heat penetration and resulted in lower accumulation of lethality and C_{100}. Addition of at least 0.5% guar gum toughened wet pet foods and decreased expressible moisture, but at least 1% hydrocolloid content was needed to observe differences in firmness. Dextrose inclusion at either 0.5% or 1% decreased product pH and increased the red and yellow

hues. Replacement of guar gum alone may need to focus on increased consistency prior to thermal processing. On the other hand, researchers should address the greater firmness and toughness and lower expressible moisture observed when kappa carrageenan and guar gum were used in combination compared to guar gum with either xanthan gum or locust bean gum. The differences observed in the present experiment illustrated the importance of carbohydrate hydrocolloids to wet pet foods. These distinctions may influence pet palatability and pet owner preference.

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Tables

Table 2.1. Ingredient composition of thermally processed wet pet foods[1] containing different hydrocolloid ingredients.

Ingredient, %	D	DG	KCG	LBG	XGG
Mechanically separated chicken	56.00	56.00	56.00	56.00	56.00
Water	38.35	38.35	38.35	38.35	38.35
Brewer's rice	3.00	3.00	3.00	3.00	3.00
Potassium chloride	0.50	0.50	0.50	0.50	0.50
Spray-dried egg white	0.50	0.50	0.50	0.50	0.50
Sunflower oil	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10
Trace mineral premix[3]	0.05	0.05	0.05	0.05	0.05
Dextrose	1.00	0.50	-	-	-
Guar gum	-	0.50	0.50	0.50	0.50
Kappa carrageenan	-	-	0.50	-	-
Locust bean gum	-	-	-	0.50	-
Xanthan gum	-	-	-	-	0.50

[1] D = 1% dextrose; DG = 0.5% guar gum and 0.5% dextrose; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum.
[2] One kg of vitamin premix supplies 17,163,000 IU vitamin A, 920,000 IU vitamin D_3, 79,887 IU vitamin E, 22.0 mg vitamin B_{12} (cobalamin), 4,719 mg vitamin B_2 (riboflavin), 12,186 mg vitamin B_5 (d-pantothenic acid), 14,252 mg vitamin B_1 (thiamine), 64,730 mg vitamin B_3 (niacin), 5,537 mg vitamin B_6 (pyridoxine), 720 mg vitamin B_9 (folic acid), and 70.0 mg vitamin B_7 (biotin).
[3] One kg of trace mineral premix supplies 88,000 mg zinc sulfate, 38,910 mg ferrous sulfate, 11,234 mg copper sulfate, 5,842 mg manganous oxide, 310 mg sodium selenite, and 1,584 mg calcium iodate.

Table 2.2. Batter characteristics of thermally processed wet pet foods[1] containing different hydrocolloid ingredients.

Measurement	D	DG	KCG	LBG	XGG	SEM	P-value
Batter consistency, cm/30 sec	23.64[a]	6.60[b]	1.69[c]	3.63[c]	2.94[c]	0.719	< 0.0001
Batter pH	5.90	5.93	5.95	5.94	5.97	0.081	0.7411
Batter water activity	0.984	0.996	0.992	0.987	0.992	0.0067	0.2528

[abc] Treatment means with unlike superscripts are different ($P < 0.05$).

[1] D = 1% dextrose; DG = 0.5% guar gum and 0.5% dextrose; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum.

Table 2.3. Processing controls of thermally processed wet pet foods[1] containing different hydrocolloid ingredients.

Measurement	D	DG	KCG	LBG	XGG	SEM	P-value
Number of thermocouples	12	12	11	11	11	-	-
Initial internal can temperature, ℃	55.32	56.13	58.51	55.01	53.40	1.768	0.2038
Can fill weight, g	404.6	404.9	405.1	404.3	404.0	0.54	0.3900
Gross headspace, mm	14.83	14.53	13.29	13.17	13.53	0.350	0.4248
Can vacuum, kPa	-12.4	-11.9	-16.0	-12.7	-11.4	1.76	0.4605

[1] D = 1% dextrose; DG = 0.5% guar gum and 0.5% dextrose; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum.

Table 2.4. Lethality and cook value (C_{100}) for thermally processed wet pet foods[1] containing different hydrocolloid ingredients.

Measurement	D	DG	KCG	LBG	XGG	SEM	P-value
Total lethality, min	20.24[a]	18.63[b]	18.27[b]	18.33[b]	18.26[b]	0.470	0.0121
Heating lethality, min	12.08[a]	9.71[b]	9.25[b]	8.74[b]	8.66[b]	0.566	0.0177
Cooling lethality, min	8.17[b]	8.92[ab]	9.01[ab]	9.59[a]	9.61[a]	1.714	0.0428
Total C_{100}, min	201.90	196.41	195.19	196.42	195.56	2.923	0.2307
Heating C_{100}, min	137.01[a]	120.67[b]	118.84[b]	115.38[b]	114.06[b]	3.870	0.0196
Cooling C_{100}, min	64.90[b]	75.74[a]	76.36[a]	81.04[a]	81.50[a]	9.233	0.0099

[ab] Treatment means with unlike superscripts are different ($P < 0.05$).

[1] D = 1% dextrose; DG = 0.5% guar gum and 0.5% dextrose; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum.

Table 2.5. Finished product characteristics of thermally processed wet pet foods[1] containing different hydrocolloid ingredients.

Measurement	D	DG	KCG	LBG	XGG	SEM	P-value
pH	5.95^{c}	5.97^{c}	6.38^{a}	6.27^{b}	6.24^{b}	0.080	< 0.0001
Total moisture, %	79.37^{a}	79.04^{a}	77.83^{ab}	77.30^{b}	77.28^{b}	0.798	0.0418
EM[2], % of sample	49.91^{a}	26.93^{b}	23.59^{b}	15.92^{c}	17.16^{c}	1.905	< 0.0001
Firmness, N	9.03^{c}	8.47^{c}	27.00^{a}	16.30^{b}	14.87^{b}	2.673	< 0.0001
Toughness, N x mm	67^{d}	117^{c}	370^{a}	245^{b}	225^{b}	32.5	< 0.0001
L*	53.61^{c}	56.88^{b}	57.59^{ab}	59.09^{a}	58.65^{ab}	1.044	0.0023
a*	8.18^{a}	8.56^{a}	4.03^{b}	4.68^{b}	4.51^{b}	1.244	0.0108
b*	21.40^{a}	22.69^{a}	14.64^{b}	15.93^{b}	15.59^{b}	1.511	< 0.0001

^{abcd} Treatment means with unlike superscripts are different ($P < 0.05$).

[1] D = 1% dextrose; DG = 0.5% guar gum and 0.5% dextrose; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum.

[2] EM = expressible moisture

Chapter 3 - Effects of copper course and supplementation level on degradation products, color, and fatty acid profile in canned pet food

Abstract

Discolored thermally processed canned pet foods may be perceived by pet owners as moldy or adulterated. Interactions between trace minerals, especially copper, and fatty acids or carbohydrates may occur during canning. Today, copper is supplemented primarily as highly reactive inorganic copper sulfate. Chelated copper sources may protect the metal ion and prevent formation of undesirable/visually unfavorable reactions. Therefore, the objective of this work was to determine the effects of 3 mineral premixes at 2 levels of inclusion in a canned pet food diet on color, degradation products, and fatty acid profile. In a 2 x 3+1 augmented factorial arrangement of treatments, canned pet food diets were as follows: no mineral premix (NC), 60 mg/kg dry matter (DM) from copper sulfate (CS60), copper-lysine-glutamate (LG60), and copper amino acid complex (CA60), or 300 mg/kg DM from the same copper sources (CS300, LG300, and CA300, respectively). Diets were produced in three 20-can batches over 3 days (replicates) and cooked in a still retort (Dixie; Athens, GA). All treatments were analyzed for copper, iron, zinc, manganese, thiamine, vitamin A, and vitamin E. Fatty acids were quantified for NC, CS60, LG60, and CA60. Color was analyzed by slicing the full canned product into 4 segments and measuring each portion with a colorimeter (CIELAB color system). Data were analyzed as a linear mixed model (SAS; version 9.4, SAS Institute, Cary, NC) with significance set at $P < 0.05$. Fatty acids were not affected ($P > 0.05$) by source, nor were thiamine or vitamin A by concentration or source. Vitamin E concentration of NC was greater ($P < 0.05$) than all other treatments. The control (NC), as a

reference, was lighter, redder, and more yellow ($P < 0.05$) than all other treatments. Yellowness was not different ($P > 0.05$) between copper-supplemented treatments and LG300 was darker ($P < 0.05$) than all treatments but did not differ ($P > 0.05$) from CS60 or CA60. This research indicated that all copper sources tested led to color change of canned pet food when over-supplemented. Further research is needed to determine if copper at lower levels might decrease discoloration to undetectable levels.

Keywords: Cat, color change, copper, dog, oxidation, thermally processed food

Abbreviations: DM = dry matter; CS = copper sulfate; LG = copper-lysine-glutamate; CA = copper amino acid complex; NC = negative control treatment with no copper supplementation; CS60 = treatment containing 60 mg copper/kg dry matter from copper sulfate; CS300 = treatment containing 300 mg copper/kg dry matter from copper sulfate; LG60 = treatment containing 60 mg copper/kg dry matter from copper-lysine-glutamate; LG300 = treatment containing 300 mg copper/kg dry matter from copper-lysine-glutamate; CA60 = treatment containing 60 mg copper/kg dry matter from copper amino acid complex; CA300 = treatment containing 60 mg copper/kg dry matter from copper amino acid complex; FAME = fatty acid methyl esters;

Introduction

Thermally processed wet pet food, commonly referred to as canned pet food, is increasing in popularity. In 2018, 40.3% of households with dogs and 56.6% of households with cats fed a wet pet food, a jump from 29.8% and 54.4 %, respectively, in 2010 (Simmons Research, 2018). Copper is a trace mineral that plays multiple roles in metabolism, including oxidation of iron and as a component of superoxide dismutase, cytochrome c oxidase, and lysyl oxidase (Gropper et al., 2017). Dogs and cats must consume it in their diet to meet their nutritional requirements. Few ingredients used in commercial pet foods contain sufficient amounts of copper, so manufacturers supplement this mineral to ensure the nutrient profile set by the Association of American Feed Control Officials (AAFCO) is met. Copper sulfate is the most common inorganic copper supplement used in pet food after the discontinuation of copper oxide due to its low bioavailability (Baker et al., 1991; Kegley and Spears, 1994).

Light-colored pet foods (predominately chicken-based) receive greater complaints from pet owners due to gray discoloration and dark blemishes. Pet food companies have identified copper as a reactant in this color change (Shields, 1998). Color change related to copper is not unique to wet pet food products. A yellow/brown discoloration of commercially-salted cod has been linked to lipid oxidation, which in turn was correlated with increasing copper levels (Lauritzsen et al., 1999). Copper is involved in oxidation of food products that can be quantified through analysis of reactants and products of the reaction. As an example, rapeseed oil with increasing concentrations of copper had greater concentrations of hexanal and 2-hexanal, which are secondary products of lipid oxidation, after 35 days of storage (Andersson and Lingnert, 1998). Other secondary markers of lipid oxidation include decreases in vitamin A (Nieva-Echevarría et al., 2017), thiamine (Perez-Ruiz et al., 1992; Stepuro et al., 1997), and vitamin E (Yoshida et al.,

1994). Total fatty acid content and the proportions of saturated and unsaturated fatty acids can also indicate the degree of oxidation (Gómez-Estaca et al., 2017). Chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA; Yoshida et al., 1993) or peptides isolated from chickpeas with high quantities of histidine (Torres-Fuentes et al., 2014) have been reported to retard or inhibit lipid oxidation in model food systems. Thus, organically-chelated copper may function in a similar manner and prevent or minimize the discoloration of a complex food matrix such as canned pet food.

However, there is no available research addressing the potential link between copper sulfate and product discoloration in thermally processed wet pet food. Nor has the use of organic copper sources been evaluated to supply copper without altering product color. Therefore, the objective of this experiment was to evaluate the effects of three different supplemental copper sources on color and indirect and direct indicators of oxidation in thermally processed pet food. The indirect indictors included vitamin A, thiamine, and vitamin E. The direct indicator was the fatty acid profile of the foods. The hypothesis was that using a copper complex (i.e. copper amino acid complex) or chelated copper (i.e. copper-lysine-glutamate) would minimize changes in color, vitamins, and fatty acid contents relative to an inorganic source such as copper sulfate.

Materials and methods

Dietary treatments were arranged in a 2 x 3+1 augmented factorial, with a negative control lacking copper supplementation (NC), two copper supplementation levels [60 or 300 mg copper/kg dry matter (DM)], and three copper sources (copper sulfate, copper-lysine-glutamate, and copper amino acid complex; Zinpro Corporation, Eden Prairie, MN). The high levels of copper supplementation were selected to ensure that differences between treatments could be identified. The 300 mg/kg DM supplementation of copper is greater than the 250 mg/kg DM maximum level

recommended for dogs [Association of American Feed Control Officials (AAFCO), 2017]. However, these treatments were not intended for consumption by dogs or cats. This resulted in seven total treatments including: no copper (NC), 60 mg/kg DM supplementation with copper sulfate (CS60), 300 mg/kg DM supplementation with copper sulfate (CS300), 60 mg/kg DM supplementation with copper-lysine-glutamate (LG60), 300 mg/kg DM supplementation with copper-lysine-glutamate (LG300), 60 mg/kg DM supplementation with copper amino acid complex (CA60), and 300 mg/kg DM supplementation with copper amino acid complex (CA300). The NC treatment was used as a base formula for all others and level of chicken was decreased to allow or inclusion of copper supplements (Table 3.1).

Three treatment replicates were produced over three days with each treatment made once daily. One-half of the required water and chicken (CJ Foods, Bern, KS) were heated in a stock pot to 40 ℃. White rice (Kroger, Manhattan, KS), spray-dried egg white (Rembrandt Foods, Okoboji, IA), soybean oil (Kroger, Manhattan, KS), potassium chloride (Bill Bar and Company, Overland Park, KS), vitamin premix (DSM, Heerlen, N.L.), and copper source (Zinpro Corporation, Eden Prairie, MN) were added to the pot and continuously stirred while heating to 60 ℃. Once this temperature was reached, the remaining water, guar gum (Dupont, New Century, KS), and carrageenan (Dupont, New Century, KS) were added and mixed. When the temperature returned to 60 ℃, aliquots were loaded into cans (size 300 x 407; Freund Container & Supply, Lisle, IL) and lids (300; Freund Container & Supply Lisle, IL) were seamed onto the body (Dixie Seamer, 91118; Athens, GA). Cans for all treatments were placed inside a retort (Dixie, 00-43; Athens, GA) and cooked at 121 ℃ and 144.79 kPa for 1 hour. At the end of the cycle, steam was turned off and the retort was filled with municipal water to cool the cans before removal.

Three cans from each batch and treatment were composited and subsampled for determination of moisture (AOAC 930.15), fat by acid hydrolysis (AOAC 954.02), crude protein (AOAC 990.03), crude fiber (AOCS Ba 6a-05), and ash (AOAC 942.05; Midwest Laboratories, Omaha, NE). Mineral analysis (copper, iron, magnesium, and zinc) and vitamin analysis (thiamine, vitamin A, and vitamin E by alpha-tocopherol acetate; NP Analytical Laboratories, St. Louis, MO) were completed on all treatment replicates as well.

Three cans from each batch and treatment were analyzed for color using a colorimeter (CR-410 Chroma Meter, Konica Minolta, Chiyoda, Tokyo, J.P.) standardized with a white plate. Products were sliced into 4 sections for analysis to quantify color throughout the loaf. The L*, a*, and b* values were recorded from the colorimeter, with L* representing lightness/darkness (the greater the value, the lighter the product), a* the red/green factor (positive values representing red and negative values representing green), and b* the yellow/blue factor (positive value representing yellow and negative values representing blue).

After color analysis, three cans from each batch of the NC, LG60, CA60, and CS60 treatments were homogenized for analysis of fatty acid methyl esters (FAME) by gas chromatography (6890N, Agilent Technologies, Santa Clara, CA) with a flame ionization detector (FID) and coupled with a DB-23 capillary column (column length = 60 m, internal diameter = 250 μm, film thickness = 0.25 μm). The 60 mg/kg DM copper supplementation treatments were selected for fatty acid analysis because their copper contents were more practical than the 300 mg/kg DM copper supplementation treatments. Helium carrier gas flow rate was 1.5 mL/min and the back inlet pressure and temperature were 36.01 psi and 250 ℃, respectively. One microliter samples were collected using an Agilent 7683 autosampler in the splitless mode. Gas chromatography oven temperature was increased in gradients and followed the outlined sequence

for a 23.75 minute total run time: 150 ℃ for 1 minute, increase 25 ℃/min to 175 ℃, increase at 4 ℃/min to 230 ℃, hold at 230 ℃ for 8 minutes. The FID detector was operated at 260 ℃ with 30 mL/min hydrogen flow to the detector and 400 mL/min air flow. Sampling rate of the FID was 20 Hz and data were processed using interpretation software (Agilent Chemstation; Santa Clara, CA).

The experiment was organized in a 2 x 3+1 augmented factorial arrangement of treatments. Data for all analyses were analyzed as a one-way ANOVA using the mixed model procedure with day as a random effect (GLIMMIX, SAS, version 9.4; SAS Institute, Cary, NC). The fixed effect of treatment for comparison of fatty acid contents included the NC, LG60, CA60, and CS60 experimental treatments. Statistical analysis of all other dependent variables included all seven experimental treatments from the 2 x 3+1 factorial arrangement. Effect of treatment was determined using the ANOVA F-test and means for each treatment were separated by Fishers LSD (Marini, 2003). *P*-values less than 0.05 were considered different.

Results

Nutrient composition of the treatments were similar, except for ash, which was numerically lower for NC, compared to all other treatments (Table 3.2). Copper concentration was successfully altered ($P < 0.0001$) by changing the inclusion level (Table 3.3). The NC treatment contained 3.50 mg/kg DM copper, the 60 mg/kg DM treatments contained 62.23 mg/kg DM copper, and the 300 mg/kg DM treatments contained 303.51 mg/kg DM copper. Vitamin A (average = 30,569.57 IU/kg DM) and thiamine (average = 27.85 mg/kg DM) were not different ($P > 0.05$) among treatments. Vitamin E for NC (252.38 mg/kg DM) was 15.35% greater ($P < 0.0001$) than all other treatments (average = 211.38 mg/kg DM). However, no differences for vitamin E were noted between the 60 and 300 mg/kg DM copper treatment levels, and no difference between copper sources was observed.

All color components were affected by treatments (Table 3.4). Adding a copper supplement darkened ($P < 0.0001$) canned food, with a tendency for 60 mg/kg DM copper-supplemented pet food to be lighter than its 300 mg/kg DM copper-supplemented counterparts. No difference in lightness was observed for copper source comparisons LG vs. CS or CA vs. CS. Redness of canned food was affected by copper supplementation ($P < 0.0001$). Treatments with added copper yielded products that were less red than NC and increasing the level of copper supplementation from 60 mg/kg DM to 300 mg/kg DM further reduced red hues in the product. For yellow/blue coloring, adding copper resulted in less yellow products ($P < 0.0001$), but no differences were identified between supplementation levels or copper sources.

Fatty acid concentrations were not affected ($P > 0.05$) by adding 60 mg/kg DM of copper from any copper source (Table 3.5). Palmitic acid (16:0), cis oleic acid (18:1 cis), and cis linoleic acid (18:2 cis) were the predominant fatty acids, averaging 238.5 g/kg, 312.4 g/kg, and 273.5 g/kg, respectively.

Discussion

The copper levels tested in this experiment were higher than the minimum AAFCO recommendations for dog and cat food to ensure that any nutrient or color alterations could be identified. It was expected that nutrient composition would be similar among the treatments, except for copper. All 60 and 300 mg/kg DM treatments were close to the targeted supplementation levels. Even though the copper content in CA300 was lower than in CS300 and LG300, it was still greater than the 60 mg/kg DM treatments and therefore considered a viable treatment for this experiment. The average 300 mg/kg DM treatment contained 4.73 times more copper than the average 60 mg/kg DM treatment, suggesting a successful formulation and overall production.

Vitamin A, thiamine, and vitamin E were measured to assess their stability in the presence of different copper sources and levels. Variation in vitamin A analysis was high and may be due to sampling or analytical techniques. Previous research found that thiamine can be oxidized in the presence of copper (Perez-Ruiz et al., 1992; Stepuro et al., 1997). However, those experiments were conducted in the presence of oxygen, whereas thermally processed canned pet foods are nearly anaerobic systems. Thus, thiamine oxidation due to copper supplementation may not occur in canned pet food applications. Unfortunately the initial vitamin E contents of the formulas before retort processing was not analyzed. This information would have aided in understanding the effects of the copper sources on oxidation separately from the effect of processing. The primary source of vitamin E in the formulas was likely the soybean oil. Reports of vitamin E content in soybean oil ranged from 1.2 mg total tocopherols/kg as-is (DellaPenna, 2005) to 122.0-165.4 mg α-tocopherol/kg as-is for fortified oils (Hemery et al., 2015). While it was expected that NC food would have high levels of vitamin E, the lack of difference observed between 60 and 300 mg/kg DM treatments and between the copper sources was not expected. Greater inclusion of copper decreased vitamin E levels and could contribute to radical formation and impact lipid oxidation (Yoshida et al., 1994). However, research with copper sulfate and tribasic copper chloride in broiler chick feed only revealed differences in vitamin E after 10 days of storage (Lu et al., 2010; Luo et al., 2005). It is possible that differences were not noted in this study because storage was not a factor. Another possibility is that the four types of tocopherols (α, β, γ, and δ) were affected by copper source differently. The analysis in this experiment only measured d,l-α-tocopherol and did not capture changes to the other tocopherols.

Color was impacted by adding different copper sources and concentrations to the formulation. Higher L*, a*, and b* values indicate a lighter, redder, and more yellow product and

lower L*, a*, and b* values indicate a darker, greener, and bluer product, respectively. Simply, adding copper darkened the loaf with less red and yellow hues. While the LG300 product was darker and less red than that of LG60, the differences were small (1.36 and 0.74, respectively) and there are no reports of the minimum difference in CIELAB color values needed for differentiation by pet owners. As such, it is unknown if pet parents would be able to recognize these differences or if these differences would affect their impression of the food. Reports of copper-correlated discoloration in chicken-based products are sparse. However, discoloration has also been observed in salted cod, though characterized as a yellow-brown color change (Lauritzsen et al., 1999). Researchers indicated that discolored flesh was more yellow and oxidized than white flesh, and the differences were correlated with copper content of the flesh. However, these research findings differ from results with cuttlefish subjected to freeze-thaw cycles and supplemented with copper. In that instance, increased a* values (red hues) were reported while increased b* values (yellowness) were linked to iron supplementation (Thanonkaew et al., 2006).

If differences in color were due to copper-induced oxidation, it might be detected as a change in concentrations of fatty acids. Increased concentrations of saturated fatty acids and decreased concentrations of unsaturated fatty acids are associated with thermal oxidation of lipid sources (Kim et al., 2013). However, no differences in fatty acid composition between samples were observed. This would indicate that oxidation of thermally processed foods for pets may not be the mechanism behind the observed color change. Similar research suggested that the addition of copper to rapeseed oil (Andersson and Lingnert, 1998) or a lipid-based emulsion (Osborn-Barnes and Akoh, 2003) did not affect oxidation on day 0 of a storage study, but did increase undesirable lipid oxidation products, such as hexanal, 2-hexanal, or peroxide or anisidine values during prolonged storage. In a chick-feeding study, in which diets were fortified with a copper-

glycine chelate or copper sulfate, there was a difference in pentadecanoic acid (C 15:0) in breast meat (Winiarska-Mieczan and Kwiecień, 2015). Yet these experiments were conducted with aerobic and respiratory systems, which differ from the anaerobic environment of food inside a can.

It is possible that the different lipid sources in the present experiment (mechanically deboned chicken and soybean oil) influenced the results. Mechanically deboned chicken is a source of saturated and unsaturated fatty acids at roughly 297-318 g/kg and 682-701 g/kg of total fatty acids, respectively (Püssa et al., 2009; Trindale et al., 2004). Soybean oil is slightly more unsaturated (821 g/kg of total fatty acids) with oleic (211.9 g/kg of total fatty acids) and linolenic (525.5 g/kg of total fatty acids) as the predominant unsaturated fatty acids (Aharoni et al., 2005).Thermal oxidation of lard and soybean oil resulted in similar directions of change (i.e. increases or decreases) in fatty acid contents with the exception of oleic acid (Kim et al., 2013). Oleic acid content, a predominant fatty acid in the present experiment, increased in soybean oil but decreased in lard after thermal oxidation. Therefore, the effect of copper-induced oxidation on oleic acid content may have been masked by the presence of the two lipid sources. Understanding the fatty acid content of raw diet batters prior to retort processing may have aided in detecting these changes or understanding why differences were not observed. Additionally, other analyses may measure lipid oxidation more accurately. These measures include hexanal, a by-product of linoleic acid oxidation, malondialdehyde by the thiobarbituric acid reactive substance assay, primary oxidation products by the peroxide value, and secondary oxidation products by the p-anisidine value (Nielsen, 2010). They may be more valid analyses due to the relatively low total fatty acid content in the formulas compared to a pure lipid source. Nevertheless, the present study did not observe differences in fatty acids when copper was supplemented. This may suggest a different mechanism causing changes in coloration, which does not involve lipid oxidation.

The work described in this paper demonstrates that copper affects vitamin E content and color of chicken-based thermally processed pet food. However, it does not appear that lipids, vitamin A, or thiamine were altered by copper addition. It does indicate that lower LG concentrations may minimize differences in product redness compared to higher concentrations. While the intent was to produce treatments with over-fortified copper levels to ensure measurable differences, the amount used may have been too high to detect differences in vitamin E and color components a* and b* between sources. Future work could determine if more practical copper supplementation levels cause measurable differences in the parameters examined in this paper. Another avenue would be to identify product components interacting with copper and resulting in discoloration. Perhaps the color compounds are the result of an interaction of copper with carbohydrates or proteins, for example. Copper inclusion in cuttlefish muscle decreased a* values (Thanonkaew et al., 2006), which was also observed in the present experiment. This resulted in protein degradation associated with decreased protein solubility. One proposed mechanism is that copper bound to protein can undergo redox cycling and produce radicals that lead to oxidative damage of the protein (Ramirez et al., 2005). This mechanism was discovered with human serum albumin in an aerobic system, so the results may not be applicable to the anaerobic environment inside a can. Another possibility is that oxymyoglobin is oxidized when copper is reduced and binds to histidine (Moiseeva and Postnikova, 2001). Low levels of myoglobin have been measured in chicken muscle (Kranen et al., 1999) and a reduction in oxymyoglobin may explain the reduced redness observed in the present experiment. However, discoloration due to copper-catalyzed oxidation has not been tested with chicken oxymyoglobin. This hypothesis could be tested by measuring levels of oxymyoglobin and the contribution of oxymyoglobin to red hues (Zhu and Brewer, 1999) in diets before and after retort processing.

Conclusions

The work described herein shows that adding supplemental copper to canned pet food decreased lightness, redness, and yellowness of the product regardless of its source. Vitamin E concentration was reduced, suggesting that an oxidation reaction occurred and may have caused the color change. However, fatty acid concentrations were not affected, suggesting that the oxidation reaction with copper does not involve lipids. Future work should focus on identifying which compounds are serving as the reactants to cause the color change as well as determining if these changes can be observed at lower levels of supplemental copper. Additionally, chelating copper with lysine-glutamate for use at lower levels may lessen the reduction in redness compared to copper sulfate and copper amino acid complexes. Similar chelated copper sources should be evaluated in future work as well.

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Tables

Table 3.1. Ingredient composition of canned pet food[1] containing different copper sources at different copper supplementation levels.

Ingredient, g/kg	NC	LG60	LG300	CA60	CA300	CS60	CS300
Mechanically separated chicken	550.5	550.0	548.0	550.0	548.0	550.0	548.0
Water	383.5	383.5	383.5	383.5	383.5	383.5	383.5
White rice	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Carrageenan	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Guar gum	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Potassium chloride	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Spray dried egg	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Soybean oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Copper source	-	0.5	2.5	0.5	2.5	0.5	2.5

[1] NC = no supplemental copper; LG60 = 60 mg/kg dry matter (DM) copper supplementation from copper-lysine-glutamate; LG300 = 300 mg/kg DM copper supplementation from copper-lysine-glutamate; CA60 = 60 mg/kg DM copper supplementation from copper amino acid complex; CA300 = 300 mg/kg DM copper supplementation from copper amino acid complex; CS60 = 60 mg/kg DM copper supplementation from copper sulfate; CS300 = 300 mg/kg DM copper supplementation from copper sulfate.

Table 3.2. Nutrient composition of canned pet food[1] containing different copper sources at different copper supplementation levels.

Nutrient	NC	LG60	LG300	CA60	CA300	CS60	CS300
Moisture, g/kg	776	778	774	772	768	776	774
		---------- Dry matter basis ----------					
Crude protein, g/kg	384	394	385	377	391	392	397
Crude fat, g/kg	333	354	341	369	334	350	334
Crude fiber, g/kg	6.3	7.5	5.7	8.4	7.7	5.2	1.4
Ash, g/kg	67.0	84.2	80.1	82.0	83.6	87.0	77.0
Iron, mg/kg	76.61	68.02	75.00	80.57	83.53	75.04	74.82
Manganese, mg/kg	3.08	2.39	2.52	2.98	4.31	2.63	2.70
Zinc, mg/kg	28.66	27.07	33.76	31.93	36.16	27.32	30.04

[1] NC = no supplemental copper; LG60 = 60 mg/kg dry matter (DM) copper supplementation from copper-lysine-glutamate; LG300 = 300 mg/kg DM copper supplementation from copper-lysine-glutamate; CA60 = 60 mg/kg DM copper supplementation from copper amino acid complex; CA300 = 300 mg/kg DM copper supplementation from copper amino acid complex; CS60 = 60 mg/kg DM copper supplementation from copper sulfate; CS300 = 300 mg/kg DM copper supplementation from copper sulfate.

Table 3.3. Copper and vitamin contents of canned pet food[1] containing different copper sources at different copper supplementation levels.

Nutrient, dry matter basis	NC	LG60	LG300	CA60	CA300	CS60	CS300	SEM[2]	*P*-value
Copper, mg/kg	3.50[d]	58.6[c]	309[a]	68.2[c]	285[b]	65.9[c]	317[a]	4.9	< 0.0001
Vitamin A, IU x 10^3/kg	37.24	31.55	27.93	27.24	30.73	29.59	29.72	2.698	0.2248
Thiamine, mg/kg	26.7	28.3	30.8	26.5	28.2	27.7	26.8	2.13	0.5460
Vitamin E, mg/kg	252[a]	204[b]	217[b]	219[b]	212[b]	214[b]	202[b]	8.6	0.0186

[abcd] Means within the same row that do not share a superscript are different ($P < 0.05$).

[1] NC = no copper supplementation; LG60 = 60 mg/kg dry matter (DM) copper supplementation from copper-lysine-glutamate; LG300 = 300 mg/kg DM copper supplementation from copper-lysine-glutamate; CA60 = 60 mg/kg DM copper supplementation from copper amino acid complex; CA300 = 300 mg/kg DM copper supplementation from copper amino acid complex; CS60 = 60 mg/kg DM copper supplementation from copper sulfate; CS300 = 300 mg/kg DM copper supplementation from copper sulfate.

[2] SEM = pooled standard error of the mean.

Table 3.4. CIELAB color values of canned pet food[1] containing different copper sources at different copper supplementation levels.

Color component	NC	LG60	LG300	CA60	CA300	CS60	CS300	SEM[2]	*P*-value
L*	55.44[a]	48.02[b]	46.66[c]	48.15[b]	47.62[bc]	47.12[bc]	47.82[b]	0.400	< 0.0001
a*	8.43[a]	2.44[b]	1.70[d]	2.25[bc]	2.05[bcd]	2.10[bcd]	1.88[cd]	0.157	< 0.0001
b*	17.20[a]	9.17[b]	9.36[b]	9.01[b]	9.24[b]	9.04[b]	9.36[b]	0.319	< 0.0001

[abcd] Means within the same row that do not share a superscript are different ($P < 0.05$).
[1] NC = no copper supplementation; LG60 = 60 mg/kg dry matter (DM) copper supplementation from copper-lysine-glutamate; LG300 = 300 mg/kg DM copper supplementation from copper-lysine-glutamate; CA60 = 60 mg/kg DM copper supplementation from copper amino acid complex; CA300 = 300 mg/kg DM copper supplementation from copper amino acid complex; CS60 = 60 mg/kg DM copper supplementation from copper sulfate; CS300 = 300 mg/kg DM copper supplementation from copper sulfate.
[2] SEM = pooled standard error of the mean.

Table 3.5. Fatty acid composition of canned pet food[1] containing different sources of copper at a 60 mg/kg DM[2] copper supplementation level.

Fatty acid, g/kg of total identified fatty acids	NC	LG60	CA60	CS60	SEM[3]	*P*-value
12:0	0.341	0.327	0.322	0.469	0.0737	0.4237
14:0	5.83	5.80	5.82	5.75	0.096	0.9203
14:1	1.56	1.53	1.55	1.50	0.043	0.8193
16:0	238	239	239	239	1.5	0.5402
16:1 isomer	4.59	4.60	4.62	4.63	0.064	0.9820
9-16:1	47.9	47.5	47.9	47.3	0.70	0.9098
16:2	2.13	2.11	2.11	2.08	0.050	0.8775
17:0	1.47	1.48	1.52	1.48	0.027	0.5215
18:0	59.9	60.5	59.8	59.6	1.21	0.5633
18:1 cis	313	311	312	314	1.7	0.4209
18:1 isomer	15.9	15.9	15.9	15.7	0.077	0.1654
18:2 cis	273	274	272	274	1.6	0.8320
18:3n6	2.39	2.41	2.30	2.38	0.043	0.2599
18:3n3	17.1	15.0	17.0	14.9	1.87	0.5156
20:0	0.802	0.835	0.842	0.874	0.0623	0.6830
20:1	2.28	2.38	2.33	2.42	0.064	0.2370
20:2	1.54	1.26	1.94	1.96	0.210	0.0974
20:3n6	2.62	3.12	3.33	2.89	0.226	0.1770
20:4	10.1	11.1	9.71	8.84	0.849	0.2159

[1] NC = no copper supplementation; LG60 = 60 mg/kg DM copper supplementation from copper-lysine-glutamate; CA60 = 60 mg/kg DM copper supplementation from copper amino acid complex; CS60 = 60 mg/kg DM copper supplementation from copper sulfate.
[2] DM = dry matter
[3] SEM = pooled standard error of the mean.

Chapter 4 - Effects of copper course at minimum levels on off-colors, blemishes, and vitamin E content in canned pet food

Abstract

In previous research, supplementation of wet pet food with super-fortified levels of copper decreased vitamin E content and caused product darkening, which prevented the observation and analysis of black blemishes reported in commercial products. The objective of this study was to determine the effect of different sources of copper included at minimum recommended levels on color, vitamin E, and blemishes. Treatments were arranged in a 2 x 3+1 factorial, with 2 levels of copper supplementation (6 and 12 mg/kg dry matter), 3 copper sources (CG = copper-glutamate, CA = copper amino acid complex, and CS = copper sulfate), and a negative control with no added copper (NC). Color was quantified with a colorimeter using CIELAB color space values and blemishes were enumerated with Image J software. Data were analyzed as a general linear mixed model. Adding copper decreased ($P < 0.05$) vitamin E, a*, and b* values, with higher a* values ($P < 0.05$) for CG vs. CS. However, CG treatments contained more ($P < 0.05$) blemishes than CS treatments. Minimal levels of supplemental copper from CG may enhance preservation of overall color, but could increase the occurrence and size of blemishes. No disadvantage was observed for CA vs. CS, indicating that CA could be a suitable alternative to CS.

Keywords: blemish, color change, copper, thermal process, vitamin E.

Introduction

Copper must be supplemented in commercial diets for pets as it is an essential nutrient for dogs and cats and other ingredients in the formulation may not provide enough to meet an animal's requirements [1]. Traditionally, copper oxide was used as the copper source in pet foods, but it was found to be biologically unavailable in chickens [2] and growing cattle [3]. After this discovery, copper sulfate became the most commonly used form of supplemental copper in companion animal diets. Shortly after this change, pet food companies noticed discoloration in thermally processed low-acid pet food, commonly referred to as wet pet food. This discoloration was characterized as an overall darkening and appearance of small black spots particularly in light-colored meat-based foods (i.e. chicken, turkey, etc.) [4]. These differences in color could be perceived as adulterated or spoiled product by the pet owner, leading to eroded consumer confidence.

Copper is known to function as an oxidizing agent in food reactions and is reduced from Cu^{2+} to Cu^{+}. The electron donor for this reaction can come from many different ingredients, such as lipids or proteins. Copper-catalyzed lipid oxidation has been observed in simplistic models, such as emulsions containing canola oil [5] or soy lecithin [6] and during storage of rapeseed oil [7], but results are not conclusive in meat and animal-based products. Higher levels of copper were correlated to lipid oxidation and yellow-brown discoloration in salted cod [8] but did not result in development of rancid odors in Atlantic mackerel [9] or increased oxidation levels in turkey breast [10]. Limited research regarding the effects of copper on protein oxidation has demonstrated decreased protein functionality and increased red hues in minced cuttlefish [11]. However, none of these mechanisms have been determined to cause the discoloration observed in wet pet foods.

One previous study addressed the inclusion of different copper sources in wet pet foods and found that treatments supplemented with copper were darker, less red and yellow, and exhibited decreased vitamin E levels [12]. Organic/chelated copper sources were included in the study to determine if complexed copper protected the mineral from reacting with other ingredients. In that work copper was supplemented at high levels [60 and 300 mg/kg dry matter (DM)] and the foods were too dark for detection of black blemishes. Other differences, specifically lightness (L*), redness (a*), and vitamin E content were not distinguishable between the three copper sources tested. Therefore, the objective of this experiment was to determine the effects of organic and inorganic copper sources at minimally-supplemented levels [13] on concentrations of vitamin E, CIELAB color space values, and blemishes in a thermally processed wet pet food. The hypothesis was that an organic copper source would influence vitamin E content, color, and blemish count less than an inorganic copper source.

Materials and methods

Treatments were arranged in a 2 x 3+1 augmented factorial, with two copper premix levels (6 mg/kg DM or 12 mg/kg DM), three sources of copper [copper-glutamate (CG), copper amino acid complex (CA), or copper sulfate(CS)], and a negative control with no copper premix. This yielded 7 treatments: no copper supplementation (NC), copper-glutamate supplementation at 6 mg/kg DM (CG6), copper-glutamate supplementation at 12 mg/kg DM (CG12), copper amino acid complex supplementation at 6 mg/kg DM (CA6), copper amino acid complex supplementation at 12 mg/kg DM (CA12), copper sulfate supplementation at 6 mg/kg DM (CS6), and copper sulfate supplementation at 12 mg/kg DM (CS12). These levels were chosen to meet the minimum recommended copper allowances for dogs (7.3 mg/kg DM) and cats (5 mg/kg DM) [13].

Copper premixes were made in advance of diet production and were formulated to provide 6 mg/kg DM at a 0.25% inclusion and 12 mg/kg DM at a 0.50% inclusion (Table 4.1). Sources of copper (Zinpro Corporation, Eden Prairie, MN) were ground using a mortar and pestle before mixing with corn starch (Fairview Mills, Seneca, KS) in a table-top planetary mixer (KitchenAid Artisan, St. Joseph, MI) for 5 minutes. The copper premixes were exchanged for whole white rice flour. Mechanically deboned chicken meat (CJ Foods, Inc., Bern, KS) was ground through a 7 mm circular die plate (Weston Pro Series #32, Southern Pine, NC) before diet production.

Experimental batches (n=3) were produced over 3 days with each treatment made once daily. Water was heated in a pot to 40 °C, at which point chicken, white rice (Kroger, Manhattan, KS), spray-dried egg white (Rembrandt Foods, Okoboji, IA), soybean oil (Kroger, Manhattan, KS), potassium chloride (Bill Bar and Company, Overland Park, KS), vitamin premix (DSM, Heerlen, N.L.), and a copper premix (per protocol) were added. Guar gum (Danisco, Copenhagen, D.E.) and carrageenan (Danisco, Copenhagen, D.E.) were added once the mixture reached 60 °C. Cans (300 x 407; Freund Container & Supply, Lisle, IL) were filled with 400 g of each diet and lids (300; Freund Container & Supply, Lisle, IL) were seamed (Dixie Seamer, 91118; Athens, GA). Cans from all treatments prepared on the same day were placed into a retort (Dixie, 00-43; Athens, GA) and processed together at 121 °C and 144.79 kPa until a lethality of 8 minutes was reached. The first can of each treatment contained a thermocouple (Ecklund-Harrison Technologies, Fort Myers, FL) connected to a data logger (TechniCAL, LLC, Metairie, LA) to record internal product temperature. These collected data were recorded every 15 seconds (0.25 minutes) with software (CALSoft 5; TechniCAL LLC, Metairie, LA) for lethality calculations utilizing standard values for *Clostridium botulinum* (Equation 4.1) [14]. Briefly, the software recognized $T_C(t)$ as the internal can temperature at time t and Δt as the length of time between

temperature recordings. Processing was deemed "complete" when the last can containing a thermocouple reached a lethality value of 8 minutes. After processing was complete, the retort was drained and filled with municipal water for 20 minutes, twice, before cans were removed from the retort.

Equation 4.1. Calculation of lethality. $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$Lethality = \int 10^{\frac{T_C(t)-121.11℃}{10℃}} \Delta t$$

Three cans from each day and treatment were composited and subsampled for analysis of moisture (AOAC 930.15), crude protein (AOAC 990.03), acid-hydrolyzed fat (AOAC 954.02), crude fiber (AOCS Ba 6a-05), ash (AOAC 942.05), iron (AOAC 985.01), manganese (AOAC 985.01), zinc (985.01), and copper (AOAC 985.01; Midwest Laboratories, Omaha, NE). Vitamin E was determined as alpha tocopherol acetate (NP Analytical Laboratories, St. Louis, MO). Three cans from each day and treatment were sliced into 4 sections and each slice surface was analyzed with a colorimeter (CR-410 Chroma Meter, Konica Minolta, Chiyoda, Tokyo, J.P.) for color components. Color components were brightness (L*) and measurement on the red/green (a*) and yellow/blue (b*) scales. Pictures were taken of slices with a manual digital camera (Nikon D3100; Melville, NY) for blemish enumeration and characterization (ImageJ, Rockville, MD). Blemishes were defined as small darkened areas visible to the human eye with those ≥ 1 mm^2 in area being the most easily recognizable.

Results were analyzed as a one-way analysis of variance (ANOVA) with 7 treatments [15]. Data were analyzed as a generalized linear mixed model (SAS 9.4; SAS Institute, Cary, NC). Fishers LSD tests were used to separate treatment means with differences at $P < 0.05$. Contrasts between supplementation levels and copper sources were conducted to elaborate the results.

Probability-values less than 0.05 were considered different and between 0.05 and 0.10 were considered a trend.

Results

Average nutrient content of treatment pet foods was 75.49% moisture, 24.51% dry matter (DM), 34.52% DM crude protein, 41.05% DM acid-hydrolyzed fat, 0.61% DM fiber, and 5.66% DM ash (Table 4.2). Iron and zinc were expected to be similar across treatments, as iron and zinc-containing ingredients were not altered. Manganese was also not supplemented and fell below the detection limit of 1 mg/kg as-is basis for all diets.

Copper targets were successfully met and were different ($P < 0.0001$) between the two supplementation levels (Table 4.3). Average copper content across copper sources for the 6 mg/kg added copper treatments was 7.59 mg/kg and for the 12 mg/kg added copper treatments was 14.19 mg/kg DM. The level of copper in NC was below the detection limit of 1 mg/kg as-is basis; however, the average DM copper content can be estimated by subtraction of target supplemented copper as 1.89 mg/kg. Vitamin E content was altered ($P = 0.0104$) by the addition of copper. The NC treatment contained more ($P < 0.05$) vitamin E than the copper-containing treatments except for CS12, which was not different. No benefit ($P > 0.05$) was observed for using CG vs. CS premixes for retaining vitamin E, though more ($P < 0.05$) vitamin E was retained when CS was used vs. CA.

Differences across treatments were noted in a* ($P < 0.0001$) and b* ($P = 0.0001$) color values, but not L* ($P = 0.1744$; Table 4.4). Compared to NC, the copper-containing treatments were less ($P < 0.05$) red and yellow. Treatments targeting 12 mg/kg of copper were less red and yellow, in addition to darker, than those supplemented with 6 mg/kg of copper. No differences ($P > 0.05$) were noted for a chelated vs. inorganic copper sources in terms of L*, a*, and b* values.

However, there does appear to be a benefit for using CG instead of CS, with higher ($P < 0.05$) a* values and a trend for higher ($P < 0.10$) b* values for CG. Treatments containing CG at the lower supplementation level were closer in redness to NC than the same level of CS. On the other hand, no differences in L* and b* values were noted between the two copper sources nor between a* values at higher copper supplementation.

Supplementing copper with CG at both levels increased ($P = 0.0332$) the total number of blemishes per slice of product (Table 4.5). Including CS or CA at either level did not change the total number of blemishes compared to NC. When considering only blemishes > 1 mm^2 in area were considered, targeting a supplementation of 6 or 12 mg/kg DM copper with CA or CS did not alter the number of larger spots compared to NC. Supplementing with CG resulted in more total blemishes and more blemishes > 1 mm^2 than any other copper source, with no differences noted between the two supplementation levels. The majority of spots for each treatment were smaller than 1 mm^2 with most around 0.1-0.5 mm^2 (Figure 4.1). Most blemishes between 1 and 16 mm^2 were between 1 and 2 mm^2 and numbers decreased as blemish area increased (Figure 4.2). Area of the largest blemishes observed in a treatment ranged from 1.73 mm^2 (NC) to 74.79 mm^2 (CA12). The average blemish size ranged from 1.20 mm^2 (CS6) to 2.41 mm^2 (CG12 and CA12; Table 4.5).

Discussion

In this experiment, 3 copper sources were included at 2 inclusion levels to determine if a chelated copper source would induce fewer visual changes in a chicken-based formula compared to copper sulfate, the standard copper source used in wet pet food. The hypothesis was that copper chelation would make the copper less reactive, ultimately causing less change in product color and the development of black blemishes. Copper-glutamate was selected because copper-lysine-glutamate showed promise when supplemented at a 60 mg/kg level in a previous experiment [12].

Copper amino acid complex was included to illustrate the differences when copper is chelated with specific amino acids vs. non-specific amino acids. Both products are chelated at a one-to-one metal to amino acid ratio, but the specific vs. non-specific amino acid complex may have affected processing differences.

Copper content was successfully altered by the addition of custom copper premixes. Levels used in this experiment were lower than previously examined in wet pet foods [12] and more accurately represented the copper concentration found in commercial pet foods. The vitamin E results were surprising. It was anticipated that the higher level of copper supplementation would result in a greater decrease in vitamin E content. Instead, an increased concentration of vitamin E was observed for the CA and CS sources. The lack of difference in vitamin E between NC and CS12 was also unexpected. This may have occurred due to the low level of copper supplementation; a previous study observed an average 15.36% reduction in vitamin E by adding a copper supplement targeting 60 or 300 mg/kg DM to a thermally processed wet pet food [12]. Reduction of vitamin E due to copper supplementation in broiler chick feed was only observed after 10 days of storage [16,17]. It is possible that copper's effect on vitamin E is only evident at super-fortified levels or after prolonged storage. Further, differences noted in the present experiment may simply be due to sampling or analytical variation. For example, vitamin E content was numerically 15.77 mg/kg DM higher with the higher inclusion of CA vs. the lower level. The standard deviation for vitamin E content of CA6 (± 20.25 mg/kg DM) was greater than for CA12 (± 6.49 mg/kg DM) and prevented differentiation between the treatments. Additionally, it would have aided in understanding the effects of copper source and supplementation level on vitamin E if the pre-retort processing vitamin E level was known. It is assumed that the primary source of vitamin E was the soybean oil, however levels of tocopherols can vary depending on processing

and fortification. This experiment measured dl-α-tocopherol to determine vitamin E content. However, unfortified soybean oils are low in α-tocopherols [18] and differences may have been more detectable if all eight forms of vitamin E were analyzed.

The present study showed that food darkening was not affected by level of copper supplementation, but increased copper concentration resulted in decreased redness and yellowness of product. This agrees with the reduction in a* values with higher levels of copper supplementation and supports that higher copper levels often result in darkened wet pet foods [12]. In the previous study, differences in yellow color changes were not quantifiable at higher copper concentrations; the current study supports the notion that the previous over-fortification of copper masked differences in a* and b* values between supplementation levels and sources. These color shifts were small, especially the yellow-blue shift, but products could be differentiated by the human eye with proper training. Other researchers have linked copper to increased yellow hues and lipid oxidation [8] and to increased red hues and protein oxidation [11]. The increase in red hues observed in the present experiment suggest a protein oxidation reaction with copper. Analyses to identify protein oxidation could include functionality tests, such as protein solubility, or analysis of dispensable and indispensable amino acids.

This is the first study to quantify and characterize blemishes in thermally processed wet pet foods. Theoretically, incidence of blemishes would indicate that the added copper serves as a reactive agent and may result in blemish formation. Increasing the level of supplemental copper did not increase the number of blemishes observed, though this could be due to the variation observed. The data suggest that CG provides a more reactive copper species than CA and CS at both supplementation levels. Wet pet foods are not the only food products to exhibit black spots. Potatoes develop black spots and discoloration when physically damaged, but this has not been

linked to copper content [19]. Blemishes described as black stippling [20] and russeting (development of brown areas) [21] were documented in citrus and apples, respectively, after the application of copper fungicides to control a disease called black spot. These blemishes were dependent on the amount and type of copper fungicide used, with increased application resulting in more blemishes [20]. Use of a fungicide containing copper oxychloride resulted in more russeting compared to no fungicide application, while no difference was observed between a copper hydroxide fungicide and no fungicide [21]. Similar results were observed with the application of fungicides to prevent black spot in Navel oranges. Application of fungicides with either copper ammonium acetate, copper oxychloride, copper hydroxide and ferric chloride, or cuprous oxide all reduced the severity and incidence of black spot. Additionally, the severity of damage to the rind was greater for copper oxychloride fungicide than all other copper-containing fungicides [22]. These results agree with the present experiment, which found that copper form affected the number of black blemishes observed in wet pet food.

Another mechanism to consider is the development of sulfide black and brown in canned meat. In that instance, the oxidation of sulfur-containing amino acids due to thermal processing produced hydrosulfide and hydrogen sulfide that interacted with iron and tin from the can wall to form iron sulfide and tin sulfide [23,24]. These compounds created visible black and brown spots, respectively. However, these spots are only found on parts of canned meat that directly touch the can wall and were not associated with copper. It is possible that thermal processing caused copper ions to dissociate from the respective complex and form copper sulfide, resulting in a dark spot. If this were the case, filling cans at lower temperatures and/or thermal processing at higher temperatures for shorter periods of time may help minimize this spotting [24].

Future work should address pet owner acceptability and perception of thermally processed pet foods with different copper sources at different levels. There is limited published research addressing whether or not consumers can detect color differences in meat products and what they prefer. However, consumers most liked the color of cooked broiler chicken breast meat with higher L* and lower b* values [25]. This suggests that pet owners may also prefer lighter and less yellow products, but this has not been confirmed with wet pet foods. Even though smaller shifts were seen here compared to previous literature, supplementation levels should remain at practical values to simulate commercial production of thermally processed wet pet foods. A panel of pet owners should identify blemishes and determine if there is an acceptable blemish size and/or number of blemishes in a product.

Another direction is to identify the mechanism behind this discoloration, which was not achieved in this work. Much of the literature suggests a reaction between copper and protein. Amounts of myoglobin, oxymyoglobin, and metmyoglobin could be quantified in wet pet foods containing different copper sources. While myoglobin concentrations are greater in heart muscles from broiler chickens, low levels have been detected in *adductor*, *pectineus*, and *sartorius* muscles [26] and could be oxidized to metmyoglobin, resulting in a color change. Identification of the mechanism of discoloration would aid in the creation of new copper sources that do not react as copper sulfate or the tested chelated copper sources but are biologically available to dogs and cats.

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Tables and figures

Table 4.1. Ingredient composition of thermally processed wet pet food[1] containing different copper sources at minimum levels to meet AAFCO[2] recommendations.

Ingredient, %	NC	CG6	CG12	CA6	CA12	CS6	CS12
Mechanically separated chicken	55.10	55.10	55.10	55.10	55.10	55.10	55.10
Water	38.35	38.35	38.35	38.35	38.35	38.35	38.35
White rice	3.00	2.75	2.50	2.75	2.50	2.75	2.50
Carrageenan	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Guar gum	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Potassium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Spray-dried egg	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Soybean oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CG copper premix[4]	-	0.25	0.50	-	-	-	-
CP copper premix[5]	-	-	-	0.25	0.50	-	-
CS copper premix[6]	-	-	-	-	-	0.25	0.50

[1] NC = no added copper source; CG6 = 6 mg/kg DM of added copper from copper glutamate; CG12 = 12 mg/kg DM of added copper from copper glutamate; CA6 = 6 mg/kg DM of added copper from copper amino acid complex; CA12 = 12 mg/kg DM of added copper from copper amino acid complex; CS6 = 6 mg/kg DM of added copper from copper sulfate; CS12 = 12 mg/kg DM of added copper from copper sulfate.
[2] AAFCO = Association of American Feed Control Officials
[3] One kg of vitamin premix supplies 17,163,000 IU vitamin A, 920,000 IU vitamin D_3, 79,887 IU vitamin E, 22.0 mg vitamin B_{12} (cobalamin), 4,719 mg vitamin B_2 (riboflavin), 12,186 mg vitamin B_5 (d-pantothenic acid), 14,252 mg vitamin B_1 (thiamine), 64,730 mg vitamin B_3 (niacin), 5,537 mg vitamin B_6 (pyridoxine), 720 mg vitamin B_9 (folic acid), and 70.0 mg vitamin B_7 (biotin).
[4] 500 g of CG copper premix contains 1.0292 g of copper-glutamate and 498.9708 g of cornstarch.
[5] 500 g of CA copper premix contains 2.5631 g of copper amino acid complex and 497.4369 g of cornstarch.
[6] 500 g of CS copper premix contains 1.0373 g of copper sulfate and 498.9627 g of cornstarch.

Table 4.2. Average nutrient content of thermally processed wet pet food[1] containing different copper sources at minimum levels to meet AAFCO[2] recommendations.

Nutrient	NC	CG6	CG12	CA6	CA12	CS6	CS12
Moisture, %	75.17	75.37	74.73	75.07	76.17	75.67	76.27
Dry matter, %	24.83	24.63	25.27	24.93	23.83	24.33	23.73
	---------- Dry matter basis ----------						
Crude protein, %	34.03	33.25	34.17	35.14	35.92	34.68	34.47
Acid-hydrolyzed fat, %	40.05	41.93	40.87	40.61	40.62	42.74	40.51
Crude fiber, %	0.71	0.44	0.66	0.58	0.64	0.63	0.57
Ash, %	5.20	4.18	6.09	5.82	6.35	6.05	5.95
Iron, mg/kg	64.14	65.52	69.02	66.03	63.60	63.85	69.46
Manganese, mg/kg	ND[3]	ND[3]	ND[3]	ND[3]	ND[3]	ND[3]	ND[3]
Zinc, mg/kg	33.81	32.21	36.52	33.11	33.56	33.42	33.30

[1] NC = no added copper source; CG6 = 6 mg/kg DM of added copper from copper glutamate; CG12 = 12 mg/kg DM of added copper from copper glutamate; CA6 = 6 mg/kg DM of added copper from copper amino acid complex; CA12 = 12 mg/kg DM of added copper from copper amino acid complex; CS6 = 6 mg/kg DM of added copper from copper sulfate; CS12 = 12 mg/kg DM of added copper from copper sulfate.

[2] AAFCO = Association of American Feed Control Officials.

[3] ND = below detection limits.

Table 4.3. Copper and vitamin E contents of thermally processed wet pet food[1] containing different copper sources at minimum levels to meet AAFCO[2] recommendations.

Nutrient, dry matter basis	NC	CG6	CG12	CA6	CA12	CS6	CS12	SEM[3]	P-value
Copper, mg/kg	ND[4]	7.58[b]	14.11[a]	7.65[b]	14.12[a]	7.53[b]	14.33[a]	0.519[5]	< 0.0001[5]
Vitamin E, mg/kg	112.72[a]	94.47[b]	92.67[b]	76.07[c]	91.84[bc]	92.95[b]	111.06[a]	5.347	0.0104

[abc] Least square means within the same row that do not share a common superscript are different ($P < 0.05$).

[1] NC = no added copper source; CG6 = 6 mg/kg DM of added copper from copper glutamate; CG12 = 12 mg/kg DM of added copper from copper glutamate; CA6 = 6 mg/kg DM of added copper from copper amino acid complex; CA12 = 12 mg/kg DM of added copper from copper amino acid complex; CS6 = 6 mg/kg DM of added copper from copper sulfate; CS12 = 12 mg/kg DM of added copper from copper sulfate.

[2] AAFCO = Association of American Feed Control Officials.

[3] SEM = pooled standard error of the mean.

[4] ND = below detection limits.

[5] NC treatment was not included in the statistical analysis.

Table 4.4. CIELAB color space values of thermally processed wet pet food[1] containing different copper sources at minimum levels to meet AAFCO[2] recommendations.

Color component	NC	CG6	CG12	CA6	CA12	CS6	CS12	SEM[3]	P-value
L*	64.92	64.91	61.50	63.70	63.10	64.30	63.17	0.926	0.1744
a*	10.58^{a}	9.55^{b}	8.12^{cd}	8.32^{c}	6.97^{e}	8.67^{c}	7.45^{de}	0.328	< 0.0001
b*	19.22^{a}	18.18^{ab}	16.14^{cde}	17.08^{bcd}	16.04^{de}	17.19^{bc}	15.38^{e}	0.362	0.0002

^{abcde} Least square means within the same row that do not share a common superscript are different ($P < 0.05$).

[1] NC = no added copper source; CG6 = 6 mg/kg DM of added copper from copper glutamate; CG12 = 12 mg/kg DM of added copper from copper glutamate; CA6 = 6 mg/kg DM of added copper from copper amino acid complex; CA12 = 12 mg/kg DM of added copper from copper amino acid complex; CS6 = 6 mg/kg DM of added copper from copper sulfate; CS12 = 12 mg/kg DM of added copper from copper sulfate.

[2] AAFCO = Association of American Feed Control Officials.

[3] SEM = pooled standard error of the mean.

Table 4.5. Characterization of blemishes found in thermally processed wet pet food[1] containing different copper sources at minimum levels to meet AAFCO[2] recommendations.

Characterization	NC	CG6	CG12	CA6	CA12	CS6	CS12	SEM[3]	P-value
Number of blemishes/slice	0.06^{b}	4.00^{a}	4.09^{a}	0.95^{b}	1.89^{ab}	0.72^{b}	1.33^{b}	0.861	0.0337
Number of blemishes/slice ≥ 1 mm^2	0.06^{c}	1.72^{ab}	2.05^{a}	0.42^{c}	0.78^{bc}	0.20^{c}	0.69^{bc}	0.356	0.0142
Largest spot area, mm^2	1.73	13.88	15.18	7.88	74.79	4.75	6.30	-	-
Average spot area, mm^2	1.63	1.86	2.41	1.80	2.41	1.20	1.35	-	-

[abc] Least square means within the same row that do not share a common superscript are different ($P < 0.05$).
[1] NC = no added copper source; CG6 = 6 mg/kg DM of added copper from copper glutamate; CG12 = 12 mg/kg DM of added copper from copper glutamate; CA6 = 6 mg/kg DM of added copper from copper amino acid complex; CA12 = 12 mg/kg DM of added copper from copper amino acid complex; CS6 = 6 mg/kg DM of added copper from copper sulfate; CS12 = 12 mg/kg DM of added copper from copper sulfate.
[2] AAFCO = Association of American Feed Control Officials.
[3] SEM = pooled standard error of the mean.

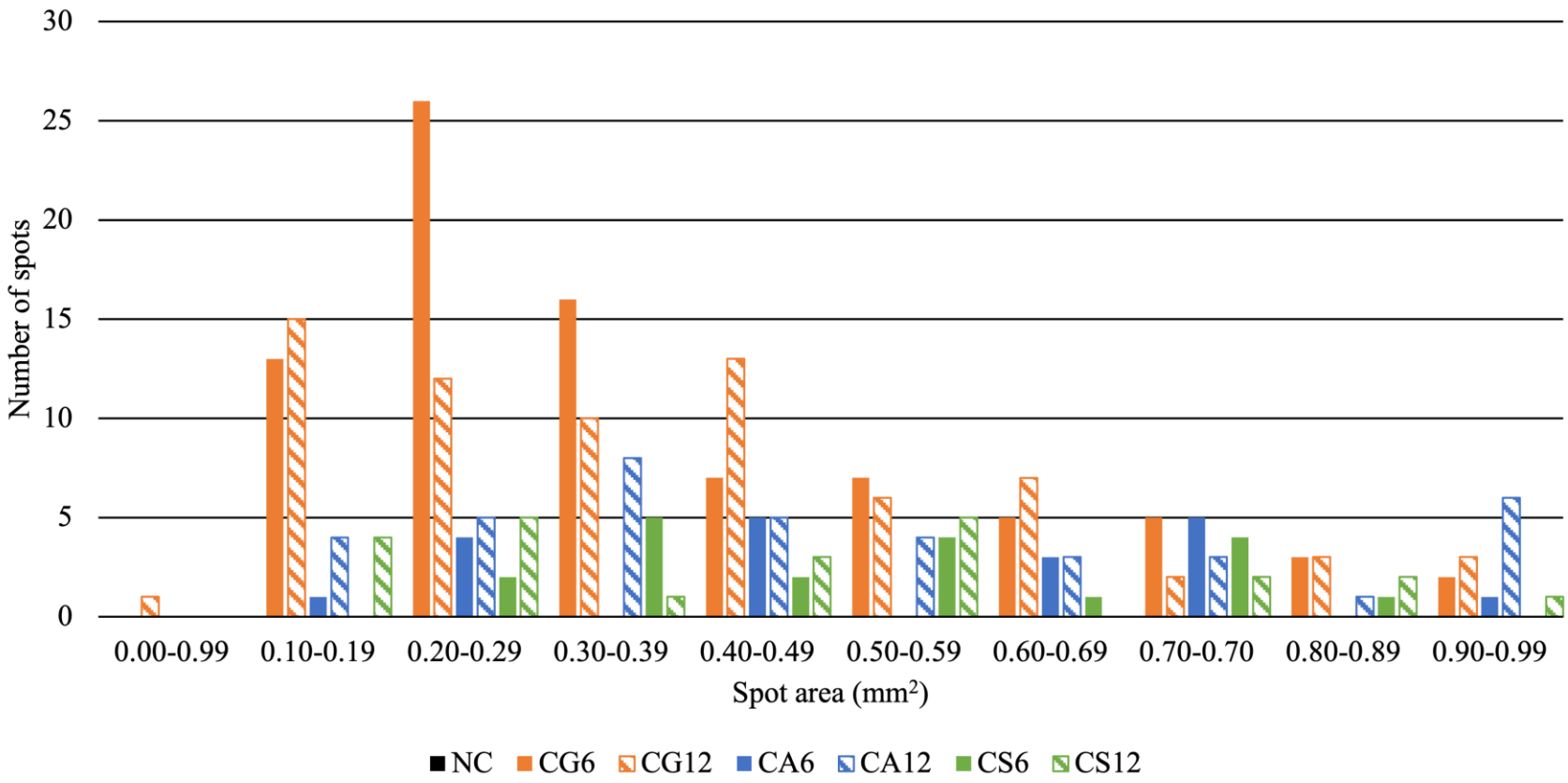

Figure 4.1. Distribution of spots < 1 mm² in area found in thermally processed wet pet food[1] containing different copper sources at minimum levels to meet AAFCO[2] recommendations.

[1] NC = no added copper source; CG6 = 6 mg/kg DM of added copper from copper glutamate; CG12 = 12 mg/kg DM of added copper from copper glutamate; CA6 = 6 mg/kg DM of added copper from copper amino acid complex; CA12 = 12 mg/kg DM of added copper from copper amino acid complex; CS6 = 6 mg/kg DM of added copper from copper sulfate; CS12 = 12 mg/kg DM of added copper from copper sulfate.
[2] AAFCO = Association of American Feed Control Officials.

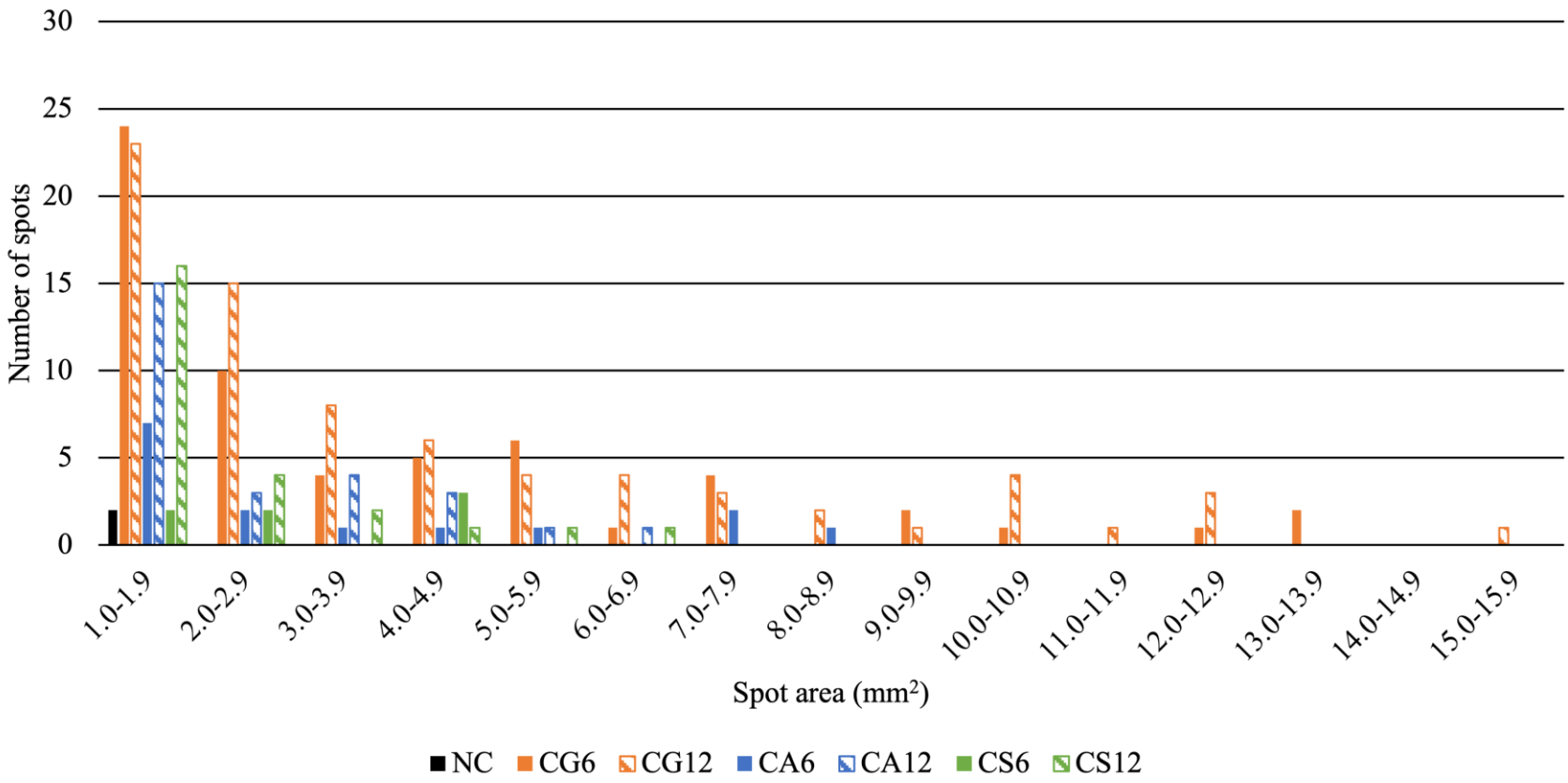

Figure 4.2. Distribution of spots ≥ 1 mm² and < 16 mm² in area found in thermally processed wet pet food[1] containing different copper sources at minimum levels to meet AAFCO[2] recommendations.

[1] NC = no added copper source; CG6 = 6 mg/kg DM of added copper from copper glutamate; CG12 = 12 mg/kg DM of added copper from copper glutamate; CA6 = 6 mg/kg DM of added copper from copper amino acid complex; CA12 = 12 mg/kg DM of added copper from copper amino acid complex; CS6 = 6 mg/kg DM of added copper from copper sulfate; CS12 = 12 mg/kg DM of added copper from copper sulfate.

[2] AAFCO = Association of American Feed Control Officials.

Chapter 5 - Inclusion of dried yeasts but not vitamin premix influences thiamine loss after thermal processing in canned cat food

Abstract

Significant improvement in thiamine retention of canned cat food has not been achieved by altering processing conditions. Some ingredients, such as yeasts, may provide a protected form of thiamine able to withstand retort processing. Therefore, the objective was to evaluate dried yeasts as thiamine sources in canned cat food . Samples of commercially available dried yeasts were analyzed for thiamine, yielding three ingredients with acceptable contents: LBV, BY, and EA (4283.8, 36.8, and 12.0 mg/kg dry matter (DM) thiamine, respectively). Experimental treatments were arranged as a 2 x 4 factorial with 2 levels of vitamin premix (with or without) and 4 levels of yeast (NY = none, LBV, BY, or EA). The inclusion of LBV was chosen to match the thiamine contribution from the vitamin premix (0.08%) and inclusions of BY and EA were capped at 5%. Replicates (n = 3) were processed in a horizontal still retort to an average total F0 of 79.23 minutes. Change in DM thiamine content was calculated as the difference between pre- and post-retort thiamine contents. Data were analyzed in a randomized complete block design with pre-retort thiamine content as a covariate and production day as a random effect. Main effects of vitamin premix and yeast and their interaction were considered significant at *P*-values less than 0.05. The Fisher's LSD post hoc comparison test was used to determine if means were different. Thiamine content of LBV and BY were lower and EA was higher than the initial screening lots (1271.7, 30.1, and 24.9 mg/kg DM, respectively). On average, experimental formulas retained 33.75% thiamine. The main effect of yeast (P = 0.0232) and the interaction between vitamin premix and yeast (P = 0.0002) were significant while the main effect of vitamin premix (average -42.9 mg/kg DM) was not significant (P = 0.0670). Thiamine loss between NY (-31.3 mg/kg DM)

and BY (-33.8 mg/kg DM) were similar whereas EA (-40.5 mg/kg DM) and LBV (-55.6 mg/kg DM) lost more thiamine than NY. The experimental formula of EA with vitamin premix (-70.3 mg/kg DM) lost more thiamine than no yeast, BY, or EA without vitamin premix (average -17.4 mg/kg DM) and all others (average -57.3 mg/kg DM) were intermediate. Inclusion of vitamin premix with a yeast source did not minimize thiamine loss (average = 33.75% retention) and BY exhibited similar thiamine retention to intrinsic thiamine in standard ingredients.

Keywords: Degradation, retention, retort, thiamine, vitamin B_1, wet pet food

Introduction

Cats and other carnivores have a high requirement for thiamine (National Research Council (NRC), 2006) and consumption of a deficient diet can result in paralysis and death within a few weeks (Loew et al., 1970; Davidson, 1992). Other observed symptoms of deficiency include impaired learning in young cats (Irle and Markowitsch, 1982), low blood levels of transketolase (Baggs et al., 1978), and brain lesions visible with magnetic resonance imaging (Palus et al., 2010; Chang et al., 2017). Reports in the literature indicate this is a widespread and common problem; a survey of 90 commercially available canned cat foods found that 15.6% of tested products contained less than 5.6 mg/kg thiamine on a dry matter basis (DMB) and did not meet the NRC's recommended allowance (Markovich et al., 2014). Recalls due to insufficient thiamine have affected prominent companies in the pet food industry. Thus, this is a real and pervasive problem and not simply a function of naive or haphazard production practices.

Thiamine molecules (chemical formula: $C_{12}H_{17}N_4OS^+$) consist of a thiazole and a pyrimidine connected with a methylene bridge. The methylene bridge between the two portions of the thiamine molecule is relatively weak and can be destroyed during food processing (Mauri et al., 2007). Increased temperature and greater processing time decreased retention of thiamine in foods for human consumption (Bendix et al., 1951; Kwok et al., 1998; Nisha Rekha et al., 2004). These conditions are utilized to achieve commercial sterility, which is required by federal regulations in the United States. Commercial sterility is defined as the condition achieved by the application of heat wherein microorganisms able to reproduce when food is stored in ambient conditions and microorganisms and spores with a public health concern are not present in the food [U.S. Food and Drug Administration (FDA), 2020]. Foods that control for water activity in addition to the application of heat do not need to consider microorganisms and spores with a public

health concern. Most canned pet foods do not control for water activity, so the first definition is considered most appropriate. Therefore, altering processing conditions to minimize thiamine degradation to a significant and meaningful level is unlikely. This leaves the ingredient composition of canned cat food as another avenue. Thiamine is typically supplied as the form thiamine mononitrate in a vitamin premix and/or as a separate ingredient. However, other ingredients may provide thiamine in a different form. One example is *Saccharomyces cerevisiae*, a common yeast already used in commercial pet food. Its use as a protein source and as a palatability enhancer in extruded food for companion animals is documented (Martins et al., 2014; Reilly et al., 2020). In addition, yeast cells have a mechanism to bind thiamine for transport. Researchers isolated binding proteins from young *Saccharomyces cerevisiae* cells that were able to bind 63.4 picomoles of thiamine per milligram of protein at pH 5.5 (Iwashima et al., 1979). Additionally, genetic analysis of *Saccharomyces cerevisiae* strains used for production of ethanol has identified amplified genes responsible for biosynthesis of thiamine (Stambuk et al., 2009). However, this ingredient category has never been evaluated as a source of thiamine that might provide fortification through the retort process.

The objectives of this experiment were to identify yeast-based ingredients which might function as thiamine sources and to evaluate their effect on the loss of thiamine in canned cat food after thermal processing. The hypothesis was that yeast-based sources of thiamine would retain more thiamine after thermal processing compared to thiamine mononitrate, the standard thiamine source included in commercial vitamin premixes.

Materials and methods

Yeast ingredient screening

Six commercial dried yeasts were sourced from companies supplying ingredients to the pet food industry. They could be classified as two brewer's yeasts, one ingredient including an active yeast and a brewer's yeast, one combination of a yeast extract and a brewer's yeast, one Saccharomyces cerevisiae fermentation product (FP; Diamond V, Cedar Rapids, IA), and one fortified inactive yeast (LBV, Lalmin B-Complex Vitamins; Lallemand Bio-Ingredients, a division of Lallemand Inc., Montréal, QC, CA). The two brewer's yeasts (BY and EA, respectively) are product #1064B (The Peterson Company, Kalamazoo, MI) and BGYADVANTAGE (The F.L. Emmert Company, Cincinnati, OH). The ingredient including an active yeast and a brewer's yeast (NS; NUCLEO-SACC) and the ingredient combining a yeast extract and a brewer's yeast (YS; YEA-SACC1026 OA) were both sourced from the same company (Alltech Inc., Nicholasville, KY). Five lots of each, with the exception of LBV (n = 4), were collected for all ingredients. Each lot was analyzed for chemical composition in duplicate. Three dried yeasts were selected from these six for evaluation in a canned cat food. Thiamine content was the primary selection criterion, with higher thiamine contents preferred and more likely to meet the minimum recommended amount of 5.6 mg thiamine per dry matter kg of processed canned cat food [Association of American Feed Control Officials (AAFCO), 2020].

Experimental treatment production

Eight formulas were created to test the retention of a standard vitamin premix containing thiamine mononitrate as the main thiamine source separate from and in addition to the yeast ingredients LBV, BY, and EA (Table 5.1). The experimental treatments were arranged as a 2 x 4 factorial with 2 categorical levels of vitamin premix (without vitamin premix or with vitamin

premix) and 4 categorical levels of dried yeast (no yeast, LBV, BY, or EA). The formulas with vitamin premix included a vitamin premix at 0.08% of the total mass. This vitamin premix contained thiamine mononitrate as the thiamine source. The 0.08% inclusion was chosen to emulate a commercial canned cat food providing roughly 2,343% of the AAFCO minimum recommended level for 5.6 mg thiamine per kg of diet dry matter [Association of American Feed Control Officials (AAFCO), 2020]. The level of LBV (0.65%) was chosen to match this value based on the average dry matter thiamine content from the initial ingredient screening. The levels for BY and EA were capped at 5% even though this level was not expected to provide the AAFCO minimum recommended level for thiamine after thermal processing. This maximum level was chosen to ensure that all experimental treatments were practical from a processing and ingredient formulation perspective.

Formulas were produced once per replicate day of processing (n = 3). Production of a 1,361 kg batch of basal batter began by grinding (Scansteel Foodtech, Denmark) frozen mechanically deboned low ash chicken (Simmons Animal Nutrition, Siloam Springs, AR), ground chicken (Simmons Animal Nutrition, Siloam Springs, AR), and pork liver (BHJ USA, LLC., Omaha, NE) through a die plate with 6.35 mm openings. Next, the ground meats were mixed (MTB-40-100P; MTC, Temple, TX) with a gravy prepared prior with a triblender (F3218; Alfa Laval Inc., Richmond, VA). This gravy contained the water, guar gum (Tilley Chemical Company Inc., Baltimore, MD), potassium chloride (Bill Barr & Company, Overland Park, KS), mineral mix (Trouw Nutrition USA LLC, Highland, IL), kappa carrageenan (Mannasol Products Ltd., Cheshire, United Kingdom), taurine (Avid Organics, Gujarat, India), salt (Compass Minerals America, Overland Park, KS), and 50% vitamin E (DSM Nutritional Products, Heerlen, NL) for the formula. Steam was added while mixing to increase moisture content and to raise the

temperature of the batter to 50 °C. The batter was processed through a three-plate emulsifier (Comvair-149.14 kW; Reiser, Canton, MA) and 91 kg of batter was sub-sampled to create the eight canned pet foods. Each formula was produced by adding the ground brewer's rice (Les Aliments Dainty Foods, Windson, ON, Canada), vitamin premix (Nutra Blend LLC, Neosho, MO), and/or yeast (depending on the treatment) directly to 7.6 kg of basal batter. Batter temperature was maintained at a minimum of 50 °C with a hot plate. The addition was done while mixing the batter with a power drill (#DR560; Black + Decker, Towson, MD) equipped with a paint mixing attachment for 1 minute to minimize clumping and to ensure a complete distribution of the added dry ingredients. A sample of each batter was collected and frozen for chemical analysis before cans (size 307 x 109; Crown Holdings, Philadelphia, PA) were filled with 156 g of batter and seamed (Pneumatic Scale Angelus, Stow, OH) with an easy-open lid.

A minimum of 1 can per formula replicate contained a thermocouple (Ecklund-Harrison Technologies Inc., Fort Myers, FL) for thermal process validation and cook value calculations. Lethality and cook value (C_{100}) were calculated using Equation 5.1 (Singh and Heldman, 2014) and Equation 5.2 respectively, wherein $T_C(t)$ is the temperature recorded by the thermocouple at time t and Δt represents the length of time between temperature measurements (15 seconds or 0.25 minutes). The temperature of 121.11 °C and z-value of 10 °C for the calculation of lethality are reference values for *Clostridium botulinum*. This hardy bacterium poses a public health concern for thermally processed low-acid foods (Black and Barach, 2015). Reference values for C_{100} (temperature = 100 °C; z-value = 10 °C) were derived from experiments with thiamine because the nutrient is highly sensitive to thermal processing. The units for lethality and C_{100} are relative time (minutes) the food product could have been processed for at the respective reference temperature. Both integral equations were solved using the trapezoid rule (Equations 5.3 and 5.4,

respectively). All cans for each production day were processed in a horizontal steam batch retort (Versatort Multimode 1520; Allpax, Covington, LA) under a process schedule designed to mimic a worst-case scenario in production with a 10 minute come-up cycle, a 63 minute cooking cycle (target temperature = 123 ℃), and a 27 minute cooling cycle. Afterwards, cans were removed from the retort and cooled to room temperature before analysis.

Equation 5.1. Calculation of lethality. $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$Lethality = \int 10^{\frac{T_C(t)-121.11℃}{10℃}} \Delta t$$

Equation 5.2. Calculation of cook value (C_{100}). $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$C_{100} = \int 10^{\frac{T_C(t)-100℃}{33℃}} \Delta t$$

Equation 5.3. Calculation of lethality by the trapezoid rule. $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$Lethality = \sum_{0}^{t} 10^{\frac{T_C(t)-121.11℃}{10}} \Delta t$$

Equation 5.4. Calculation of cook value (C_{100}). $T_C(t)$ is the internal can temperature at any given time, t. Δt is the time interval between temperature measurements.

$$C_{100} = \sum_{0}^{t} 10^{\frac{T_C(t)-100℃}{33}} \Delta t$$

Chemical analyses

All samples were analyzed by a commercial laboratory (Midwest Laboratories; Omaha, NE) for moisture (minimum detection level = 0.01%; AOAC 930.15), thiamine (minimum

detection level = 0.01 mg/kg as-is basis; AOAC 942.23), crude protein (minimum detection level = 0.2% as-is basis; AOAC 99003), crude fat (minimum detection level = 0.1% as-is basis; AOAC 2003.05 for dry samples, AOAC 954.02 for wet samples), crude fiber (minimum detection level = 0.2% as-is basis; AOCS Ba 6a-05), ash (minimum detection level = 0.1% as-is basis; AOAC 942.05), sulfur (minimum detection level = 0.01% as-is basis; AOAC 985.01), phosphorus (minimum detection level = 0.05% as-is basis; AOAC 985.01), potassium (minimum detection level = 0.05% as-is basis; AOAC 985.01), magnesium (minimum detection level = 0.05% as-is basis for premixes, 0.01% as-is basis for all other samples; AOAC 985.01), calcium (minimum detection level = 0.05% as-is basis for premixes, 0.01% as-is basis for all other samples; AOAC 985.01), sodium (minimum detection level = 0.05% as-is basis for premixes, 0.01% for all other samples; AOAC 985.01), iron (minimum detection level = 50 mg/kg as-is basis for premixes, 5 mg/kg as-is basis for all other samples; AOAC 985.01), manganese (minimum detection level = 20 mg/kg as-is basis for premixes, 1 mg/kg as-is basis for all other samples; AOAC 985.01), copper (minimum detection level = 20 mg/kg as-is basis for premixes, 1 mg/kg as-is basis for all other samples; AOAC 985.01), and zinc (minimum detection level = 20 mg/kg as-is basis for premixes, 1 mg/kg as-is basis for all other samples; AOAC 985.01). Results below the minimum detection level were treated as zeros. Frozen mechanically deboned low ash chicken, frozen pork liver, and frozen ground chicken were not analyzed for crude fiber content. Samples of the post-retort diets were generated by compositing three cans from the individual replicates. All analyses were conducted in duplicate. Nitrogen free extract (NFE) was calculated by subtracting the DMB contents of crude protein, crude fat, crude fiber, and ash from 100%.

Statistical analysis

Data from the initial screening of dried yeasts were presented as mean values across analyzed lots with their corresponding standard deviation.

Initial internal can temperature, lethality, and C_{100} were analyzed as a 2-way analysis of variance (ANOVA) with vitamin premix and yeast as fixed effects and production day as a random effect. The procedure GLIMMIX was used for this analysis (SAS v. 9.4; SAS Institute, Cary, NC). Nutrient contents of pre-retort batters and processed diets were presented as mean values ± standard deviation. Thiamine content of individual formulas was compared to 5.6 mg/kg DMB, the minimum recommended value [Association of American Feed Control Officials (AAFCO), 2020], using a single-sample lower-tail t test. Dietary thiamine content was considered less than the minimum recommended value if the *P*-value was less than 0.05.

Change in thiamine content was calculated by subtracting pre-retort batter thiamine content from processed diet thiamine content. Data were transformed (wherein "x" represents a data point: $(x * -1)^{1/3}$) to meet the model assumptions of equal variance and normality for statistical analysis. Significance of the main effects for vitamin premix and yeast and their interaction were determined with a 2-way analysis of covariance (ANCOVA). Pre-retort batter thiamine content was included as a covariate and production day as a random blocking factor. Retention was analyzed with this methodology instead of as a percent loss or percent retention to maximize the stability of the response variable and to account for the variation due to initial thiamine content (Bowley, 2015). Denominator degrees of freedom were corrected using the Kenward-Roger adjustment. Individual means were separated by Fisher's LSD and significance was set at $\alpha = 0.05$. The procedure GLIMMIX was employed in this analysis (SAS v. 9.4; SAS Institute, Cary, NC). Data were presented as means with a 95% confidence interval (CI) in pre-transformation units.

Results

Differences in nutrient composition were observed in the initial screening of dried yeast ingredients (Table 5.2). Thiamine content was highest for LBV (4283.8 mg/kg DMB) with all other ingredients similar to each other (9.9-36.8 mg/kg DMB). Ranges for crude protein (from 11.8% DMB in FP to 57.3% DMB in LBV), crude fiber (from 0.35% DMB in NS to 38.52% DMB in FP), and ash (from 5.87% DMB in NS to 41.25% DMB in YS) were also wide. Crude fat content was less varied with the highest contents measured in LBV and YS (average = 2.62% DMB), lowest in NS, FP and BY (average = 0.48% DMB), and intermediate in EA (1.68% DMB). Notable findings for the mineral concentrations included higher levels of calcium, iron, and manganese in YS (14.640% DMB, 1627.0 mg/kg DMB, and 146.70 mg/kg DMB, respectively) and a higher level of zinc in BY (207.5 mg/kg DMB).

Nutrient composition varied among ingredients used to create the basal batter (Table 5.3) and among the ingredients added by hand (Table 5.4). Ingredients in the basal batter contributed low levels of thiamine (average = 4.3 mg/kg DMB). The ingredient with the highest thiamine content was the vitamin premix at 17933.3 mg/kg DMB. Brewer's rice contained low levels (1.6 mg/kg DMB) consistent with the ingredients included in the basal batter. On average, the three yeasts selected contained 515% more crude protein, 84.8% less crude fat, 95.0% less crude fiber, 78.5% less ash, and 55.4% more NFE than the vitamin premix. The BY and EA yeasts contained similar levels of thiamine, averaging 27.5 mg/kg DMB. While LBV contained more thiamine than BY and EA, it still only contained 7.09% of the thiamine provided in the vitamin premix.

At least 1 thermocouple was successful for all formula replicates, except for the formula containing BY and no vitamin premix on production day 3. No main effects of vitamin premix or yeast or their interaction were detected ($P > 0.05$) for thermal processing data. Initial internal can

temperature averaged 30.01 ℃ and total lethality and C_{100} averaged 79.23 and 280.76 minutes, respectively.

Change in thiamine content was affected ($P < 0.05$) by the main effect of yeast and the interaction between yeast and vitamin premix (Figure 5.1). The greatest loss of thiamine was observed for the LBV yeast (average = -55.6 mg/kg DMB; 95% CI = -69.9 mg/kg DMB, -43.3 mg/kg DMB). More thiamine was lost with EA (average = -40.5 mg/kg DMB; 95% CI = -46.4 mg/kg DMB, -35.1 mg/kg DMB) vs. NY (average = -31.3 mg/kg DMB; 95% CI = -39.0 mg/kg DMB, -24.7 mg/kg DMB) with BY (average = -33.8 mg/kg DMB; 95% CI = -39.2 mg/kg DMB, -28.9 mg/kg DMB) not different from either. The formula including the vitamin premix and EA (average = -70.3 mg/kg DMB) exhibited a greater loss in thiamine content compared to formulas without the vitamin premix and either EA, BY, or NY (average = -17.4 mg/kg DMB). Formulas containing LBV with or without the vitamin premix and EA or NY with the vitamin premix (average = -57.3 mg/kg DMB) were intermediate and not different from the others. Inclusion of the vitamin premix (average = -62.8 mg/kg DMB; 95% CI= -97.4 mg/kg DMB, -37.6 mg/kg DMB) did not affect ($P > 0.05$) the change in thiamine content compared to formulas without the vitamin premix (-23.0 mg/kg DMB; 95% CI = -41.7 mg/kg DMB, -10.9 mg/kg DMB). This represented an average 33.75% retention of thiamine after thermal processing regardless of vitamin premix inclusion.

Discussion

The aim of this work was to identify yeast ingredients as potential thiamine sources and to evaluate the stability of their intrinsic thiamine during thermal processing in canned food for cats. The initial screening of commercially available yeasts yielded three ingredients for inclusion in canned cat foods for thermal processing. These yeasts were LBV, BY, and EA and their average

DMB thiamine contents were 4283.8, 36.8, and 12.0 mg/kg, respectively, across multiple production lots. Dried yeasts have not been extensively evaluated for thiamine content. Limited reports suggested thiamine content of 5.1 mg/kg as-is basis in a brewer's yeast (Viñas et al., 2003), 22.2 mg/kg DMB in a *Candida tropicalis* yeast biomass (Parteshko et al., 1975), and 6.6 mg/kg as-is basis in a torula brewer's yeast [National Research Council (NRC), 2006]. The wide range in thiamine contents observed suggests that one value cannot accurately describe all dried yeasts. Thiamine content should be analyzed when evaluating a new ingredient and source. Nevertheless, the dried yeasts selected for thermal processing contained at least 100% more thiamine compared to published values for brewer's yeasts.

Analysis of the yeasts used for formula production found that LBV used in the formulas contained 70.3% less thiamine than the average screening lot. On the other hand, EA used in formula production contained 107.5% more thiamine. The BY ingredient was the most consistent and only contained 18.2% less thiamine. Storage can affect thiamine content in yeasts, with reported losses of 13-44% within 6 months of storage and 55-68% within 12 months (Varga and Maráz, 2002). As such, it is possible that LBV and BY supplied for experimental formula production were older than the lots included in the initial screening. The higher thiamine content in EA used to produce formulas suggested that thiamine content in the ingredient was more variable than suggested by the screening. It is also possible that the differences in thiamine content between samples was influenced by the inherent variability in thiamine analysis. Nevertheless, these differences led to lower than expected batter thiamine contents for those containing BY or LBV and higher than expected values for batters containing EA. Other nutrients were more consistent between the screening and production yeast samples. Supply chain management and

accurate analytical data for these ingredients is critical if their intended purpose is to supply thiamine for commercial canned cat foods.

Thiamine content of ingredients used to produce commercial canned cat food is scarce in the literature. Data herein suggested that thiamine content of these ingredients did not contribute to the overall thiamine content of the diet in a meaningful way. This information was still valuable for understanding the background thiamine content of the experimental formulas. Ground brewer's rice was chosen as the space-filling ingredient for its low thiamine content and similar dry matter content compared to the vitamin premix and selected yeasts. This led to similar moisture contents across all 8 formulas. Randomization of formula production order resulted in similar initial internal can temperatures as well. Differences in these thermal processing parameters affect the kinetics of heat penetration (Hallman and Stevens, 1932; Li et al., 2015). This could influence the degree of cook and thiamine losses observed after thermal processing. These findings suggested that differences observed in thiamine loss in this experiment were due to ingredient inclusions and not influenced differently by processing conditions. The effect of thiaminase, an enzyme capable of destroying thiamine, was not considered because the meats were held constant and the enzyme is found in fish (Deutsch and Hasler, 1943; Hilker and Peter, 1966), which were not included in the treatments.

It is important to note that scheduled process employed in the present experiment resulted in greater lethality than is normal for canned pet food. The typical lethality for commercial products was reported between 12 and 14 minutes (Hendriks et al., 1999). This would result in a lower degree of thermal processing and less thiamine loss would likely be observed. Another experiment with a less harsh scheduled process would be necessary to determine the effect of the test ingredients on thiamine loss in a more typical production setting.

The intention of the 2 x 4 factorial design was to determine if thiamine loss was affected by the inclusion of a standard vitamin premix containing thiamine mononitrate and/or dried yeasts. Average thiamine retention across all formulas was lower than reported in a preliminary study at roughly 69% retention (Molnar et al., 2017). However, that experiment processed wet cat foods to a lethality of 8 minutes, which was less thermal processing than applied in this experiment and likely influenced the degree of thiamine retention. The 33.75% thiamine retention observed herein was more in-line with approximately 30% thiamine retention for thermally processed beef liver (Briozzo et al., 1987) and 23.8-27.8% retained thiamine for pressure-cooked red-gram splits (Nisha Rekha et al., 2004).

Thiamine loss was not minimized by including the vitamin premix in the formula. This was not expected and suggested that thiamine mononitrate and forms of thiamine intrinsic to standard canned cat food formulas have similar retention due to thermal processing. However, the different yeasts tested did not exhibit similar thiamine losses. The NY level of the yeast fixed factor represented no yeast inclusion in the formula and quantified changes in thiamine present in standard canned cat food ingredients. The only yeast level to exhibit similar changes in thiamine loss was BY. Even though LBV contained more thiamine than BY and EA, it exhibited the lowest retention. It is important to note that experimental treatments containing LBV met minimum recommended nutrient allowances [Association of American Feed Control Officials (AAFCO), 2020] and were practical formulations. Thiamine loss was not minimized by the combination of the vitamin premix and yeast. Instead, most formulations were similar and loss increased when vitamin premix was included with EA. All formulas were comparable to the formula containing the vitamin premix and no yeast, which was considered most similar to what is found in the marketplace, in terms of thiamine loss due to thermal processing.

This study did not address the effect of storage on thiamine loss in raw ingredients and canned cat food. However, storage losses are suggested by the differences in thiamine in screening lots of LBV and BY and the lots used in formula production. Differences may also be observed during the storage of the thermally processed foods containing a yeast ingredient. Future research should address the effect of storage on thiamine content of these ingredients in comparison to a vitamin premix containing thiamine mononitrate. Storage and thiamine analysis of formulas similar to those presented herein would be necessary to determine if thiamine content decreases below 5.6 mg/kg DMB at any point during the intended shelf life.

It appeared that BY had the most favorable thiamine survival of the three yeasts tested, pending storage testing. The ingredient contained more DMB thiamine than standard canned cat food ingredients (with the exception of vitamin premix), yet its thiamine loss was similar. Additional testing with this ingredient should determine the ideal amount of BY when batter viscosity, processed formula texture, and feline thiamine bioavailability are considered in addition to thiamine loss. The scheduled process employed in such an experiment should more closely resemble standard processing conditions instead of mimicking a worst-case scenario. While the intention of this experiment was to validate yeast ingredients based on thiamine loss due to retort processing, it is clear that LBV had the highest thiamine content of any yeast examined. Therefore, it is reasonable to conduct further research with the ingredient even though it exhibited poor retention in the present experiment.

Conclusions

The results from this experiment suggested that thiamine loss was different when three commercially available yeasts or no yeast were included in the diet formulation and the inclusion of a standard vitamin premix did not improve thiamine retention as expected. However,

formulations with LBV or BY alone and LBV, BY, or EA with the vitamin premix did result in products that met or exceeded nutrient allowances. They may be suitable sources of thiamine due to their high thiamine content relative to standard ingredients in commercial canned cat foods alone. The results discussed suggest that BY was the most favorable yeast in terms of thiamine loss due to thermal processing. Future research is necessary to fine-tune canned cat food formulas with this ingredient to meet all requirements for food safety, pet health, and pet owner acceptance.

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Tables and figures

Table 5.1: Ingredient composition of canned cat foods containing different levels of a vitamin premix and/or a dried yeast ingredient[1].

	No vitamin premix				Contains vitamin premix			
Ingredient, % as-is	NY	LBV	BY	EA	NY	LBV	BY	EA
Basal batter[2]	94.92	94.92	94.92	94.92	94.92	94.92	94.92	94.92
Ground brewer's rice	5.08	4.43	0.08	0.08	5.00	4.35	-	-
Vitamin premix	-	-	-	-	0.08	0.08	0.08	0.08
Lallemand yeast	-	0.65	-	-	-	0.65	-	-
Peterson yeast	-	-	5.00	-	-	-	5.00	-
Emmert yeast	-	-	-	5.00	-	-	-	5.00

[1] NY = no yeast; LBV = Lalmin B-Complex Vitamins; BY = spray-dried brewer's yeast #1064B; EA = BGYADVANTAGE.

[2] 1 kg of basal batter contains 283.48 g mechanically deboned low ash chicken, 230.33 g frozen pork liver, 223.59 g water, 148.83 g ground chicken, 106.30 g steam, 3.19 g guar gum, 1.35 g potassium chloride, 1.06 g mineral premix for cats, 0.74 g kappa carrageenan, 0.57 g taurine, 0.53 g salt, and 0.02g 50% vitamin E.

Table 5.2: Nutritional composition (mean ± standard deviation) of commercial yeast ingredients[1] selected as potential sources of protected thiamine for canned cat food.

Nutrient	NS	YS	FP	EA	LBV	BY
n	5	5	5	5	4	5
Moisture, %	6.9 ± 0.21	4.5 ± 0.12	8.3 ± 0.15	4.3 ± 0.28	5.0 ± 0.41	7.4 ± 0.81
Dry matter, %	93.1 ± 0.21	95.5 ± 0.12	91.7 ± 0.15	95.7 ± 0.28	95.0 ± 0.41	92.6 ± 0.81
	---------- Dry matter basis ----------					
Thiamine, mg/kg	13.9 ± 2.58	10.6 ± 2.07	9.9 ± 0.85	12.0 ± 1.59	4283.8 ± 176.18	36.8 ± 5.97
Crude protein, %	45.6 ± 0.34	23.4 ± 1.04	11.8 ± 0.66	54.5 ± 1.09	57.3 ± 1.29	48.6 ± 4.34
Crude fat, %	0.41 ± 0.390	2.74 ± 0.292	0.69 ± 0.251	1.68 ± 0.256	2.50 ± 0.286	0.34 ± 0.207
Crude fiber, %	0.35 ± 0.130	4.59 ± 1.775	38.52 ± 2.261	3.65 ± 0.683	0.38 ± 0.150	0.50 ± 0.556
Ash, %	5.87 ± 0.132	41.25 ± 0.776	7.90 ± 0.465	7.08 ± 0.221	6.34 ± 0.202	6.35 ± 0.945
NFE[2], %	47.8 ± 0.47	28.0 ± 2.13	41.1 ± 1.30	33.1 ± 0.60	33.5 ± 1.10	44.2 ± 5.35
S, %	0.43 ± 0.013	0.25 ± 0.005	0.31 ± 0.034	0.41 ± 0.011	0.54 ± 0.044	0.39 ± 0.040
P, %	1.480 ± 0.0387	0.952 ± 0.0313	0.324 ± 0.0422	0.782 ± 0.0271	1.093 ± 0.1007	1.434 ± 0.1747
K, %	1.710 ± 0.0615	0.430 ± 0.0247	1.865 ± 0.0925	2.337 ± 0.0553	2.546 ± 0.0759	1.459 ± 0.3053
Mg, %	0.196 ± 0.1962	1.629 ± 0.0722	0.427 ± 0.0507	0.308 ± 0.0076	0.118 ± 0.0089	0.195 ± 0.0202
Ca, %	0.24 ± 0.044	14.64 ± 0.497	0.64 ± 0.0.056	0.49 ± 0.029	0.23 ± 0.013	0.33 ± 0.028
Na, %	0.020 ± 0.0000	0.020 ± 0.0000	0.767 ± 0.1178	0.017 ± 0.0067	0.095 ± 0.0173	0.101 ± 0.1422
Fe, mg/kg	68 ± 6.3	1627 ± 64.7	156 ± 23.6	112 ± 8.1	47 ± 3.1	175 ± 64.2
Mn, mg/kg	9.32 ±1.563	146.70 ± 8.808	20.40 ± 4.518	38.12 ± 1.943	10.25 ± 0.612	9.24 ± 2.477
Cu, mg/kg	29.49 ± 1.906	8.65 ± 0.788	4.01 ± 0.558	16.96 ± 1.067	5.50 ± 0.265	6.01 ± 2.479
Zn, mg/kg	82 ± 3.3	105 ± 6.1	68 ± 15.4	60 ± 7.0	88 ± 9.2	208 ± 103.3

[1] NS = NUCLEO-SACC; YS = YEA-SACC; FP = *Saccharomyces cerevisiae* fermentation product; EA = BGYADVANTAGE; LBV = Lalmin B-Complex Vitamins; BY = spray-dried brewer's yeast #1064B.

[2] NFE = nitrogen free extract, calculated (Dry matter basis contents of crude protein, crude fat, crude fiber, and ash subtracted from 100).

Table 5.3: Nutritional composition of ingredients[1] in basal batter used to produce canned cat food containing different sources of thiamine.

Nutrient	MDC	PL	GC	GG	PC	MP	KC	TA	ST	VE
Moisture, %	67.3	75.2	71.7	7.9	1.0	5.8	8.9	0.4	0.7	1.3
Dry matter, %	32.8	24.8	28.3	92.1	99.0	94.2	91.1	99.6	99.3	98.7
	---------- Dry matter basis ----------									
Thiamine, mg/kg	1.2	9.2	1.8	3.1	5.5	1.6	1.4	2.1	2.3	15.2
Crude protein, %	41.4	67.7	46.2	4.8	0.7	11.5	3.0	71.7	0.6	1.4
Crude fat, %	50.2	24.5	43.7	0.2	0.0	1.3	0.7	0.0	0.0	53.2
Crude fiber, %	-	-	-	2.53	0.03	0.37	4.18	0.33	0.00	0.57
Ash, %	7.04	4.78	11.06	0.86	100.67	56.97	42.70	0.65	100.50	43.87
NFE[2], %	-	-	-	91.6	0.0	29.9	49.4	27.3	0.00	1.0
S, %	0.39	0.73	0.44	0.06	0.12	4.21	3.97	25.02	0.00	0.13
P, %	1.663	1.213	2.332	0.05	0.000	0.157	0.030	0.000	0.000	0.000
K, %	0.478	0.828	0.523	0.177	48.717	29.967	17.717	0.037	0.010	0.000
Mg, %	0.096	0.068	0.120	0.032	0.075	0.158	0.668	0.000	0.000	0.057
Ca, %	2.69	0.03	4.05	0.07	0.08	0.83	0.95	0.01	0.06	0.26
Na, %	0.233	0.470	0.298	0.033	0.458	0.350	0.942	0.035	37.083	0.372
Fe, mg/kg	51	544	60	21	0	36733	650	0	22	138
Mn, mg/kg	0.00	7.85	0.00	2.20	0.00	3661.67	34.00	0.00	0.00	0.00
Cu, mg/kg	0.00	97.78	0.00	2.15	0.00	3553.33	0.00	0.00	0.00	0.00
Zn, mg/kg	56	201	73	7	0	31250	0	0	12	0

[1] MDC = frozen mechanically deboned low ash chicken; PL = frozen pork liver; CM = frozen ground chicken; GG = guar gum; PC = potassium chloride; MP = mineral premix; KC = kappa carrageenan; TA = taurine; ST = salt; VE = vitamin E 50%.
[2] NFE = nitrogen free extract, calculated (Dry matter basis contents of crude protein, crude fat, crude fiber, and ash subtracted from 100).

Table 5.4: Nutritional composition of hand-add ingredients[1] used to produce canned cat food containing different sources of thiamine.

Nutrient	BR	VP	LBV	BY	EA
Moisture, %	12.5	7.2	5.4	7.7	4.7
Dry matter, %	87.5	92.8	94.6	92.3	95.3
	---------- Dry matter basis ----------				
Thiamine, mg/kg	1.6	17933.3	1271.7	30.1	24.9
Crude protein, %	9.2	8.7	56.4	51.6	52.4
Crude fat, %	0.8	7.9	0.8	0.5	2.3
Crude fiber, %	0.42	29.97	0.08	0.70	3.73
Ash, %	0.70	29.17	6.19	5.65	6.37
NFE[2], %	88.9	24.3	36.5	41.6	35.2
Sulfur, %	0.12	0.40	0.44	0.48	0.41
Phosphorus, %	0.202	0.000	1.145	1.507	0.832
Potassium, %	0.158	0.237	2.415	1.592	2.335
Magnesium, %	0.056	0.143	0.115	0.182	0.327
Calcium, %	0.02	7.90	0.15	0.24	0.56
Sodium, %	0.000	0.072	0.023	0.010	0.020
Iron, mg/kg	14	513	51	63	120
Manganese, mg/kg	19.40	188.50	11.27	7.47	42.05
Copper, mg/kg	3.52	8.42	5.02	22.88	17.63
Zinc, mg/kg	21	84	122	144	54

[1] BR = ground brewer's rice; VP = vitamin premix; LBV = Lalmin B Complex Vitamins; BY = spray dried brewer's yeast #1064B; EA = BGY Advantage.
[2] NFE = nitrogen free extract, calculated (Dry matter basis contents of crude protein, crude fat, crude fiber, and ash subtracted from 100).

Table 5.5: Nutritional composition (mean ± standard deviation) of pre-retort canned cat food batter containing different levels of a vitamin premix and/or a dried yeast ingredient[1].

	No vitamin premix				Contains vitamin premix			
Nutrient	NY	LBV	BY	EA	NY	LBV	BY	EA
Moisture, %	82.7 ± 0.34	79.7 ± 4.29	82.3 ± 0.25	81.7 ± 1.79	81.0 ± 0.88	81.3 ± 0.93	81.1 ± 0.60	82.7 ± 1.13
Dry matter, %	17.3 ± 0.34	20.4 ± 4.29	17.7 ± 0.25	18.3 ± 1.79	19.1 ± 0.88	18.7 ± 0.93	18.9 ± 0.60	17.3 ± 1.13
	---------- Dry matter basis ----------							
Thiamine, mg/kg	4.1 ± 1.38	54.1 ± 14.07	10.7 ± 1.40	8.5 ± 1.55	137.3 ± 15.95	191.5 ± 7.26	161.3 ± 21.23	169.2 ± 26.50
Crude protein, %	34.6 ± 3.48	37.4 ± 4.99	45.8 ± 4.36	48.9 ± 1.56	35.3 ± 3.83	34.4 ± 4.95	47.0 ± 4.25	47.5 ± 1.90
Crude fat, %	28.1 ± 5.73	30.9 ± 6.65	26.8 ± 5.45	28.3 ± 6.94	28.3 ± 4.21	24.5 ± 3.40	29.3 ± 2.50	29.8 ± 4.30
Crude fiber, %	0.46 ± 0.014	0.52 ± 0.023	0.85 ± 0.162	1.11 ± 0.139	0.37 ± 0.337	0.58 ± 0.188	1.01 ± 0.236	1.15 ± 0.093
Ash, %	6.67 ± 1.918	6.56 ± 1.863	8.43 ± 2.439	7.91 ± 1.691	6.14 ± 1.146	7.58 ± 2.136	7.32 ± 1.171	7.97 ± 1.706
NFE[2], %	30.3 ± 0.34	24.6 ± 7.75	18.1 ± 0.74	13.8 ± 4.07	29.9 ± 5.98	32.9 ± 7.89	18.1 ± 4.00	15.4 ± 3.31
Sulfur, %	0.41 ± 0.051	0.42 ± 0.071	0.51 ± 0.060	0.52 ± 0.066	0.42 ± 0.069	0.42 ± 0.075	0.51 ± 0.040	0.50 ± 0.055
Phosphorus, %	0.895 ± 0.2275	0.831 ± 0.0973	1.177 ± 0.1080	1.045 ± 0.1628	0.846 ± 0.1461	0.838 ± 0.2043	1.150 ± 0.1331	1.071 ± 0.0073
Potassium, %	0.815 ± 0.1824	0.770 ± 0.3486	1.144 ± 0.1782	1.352 ± 0.2929	0.721 ± 0.2023	0.834 ± 0.2207	1.030 ± 0.1901	1.420 ± 0.2257
Magnesium, %	0.071 ± 0.0110	0.065 ± 0.0108	0.104 ± 0,0084	0.141 ± 0.0154	0.066 ± 0.100	0.068 ± 0.0121	0.098 ± 0.0060	0.146 ± 0.0040
Calcium, %	1.12 ± 0.442	1.00 ± 0.113	1.10 ± 0.173	1.24 ± 0.285	1.06 ± 0.206	0.98 ± 0.323	1.15 ± 0.258	1.26 ± 0.044

Sodium, %	0.323 ± 0.0421	0.319 ± 0.1067	0.324 ± 0.0383	0.317 ± 0.0677	0.329 ± 0.0536	0.318 ± 0.0595	0.334 ± 0.0501	0.311 ± 0.0488
Iron, mg/kg	278 ± 77.1	268 ± 101.5	255 ± 81.9	295 ± 92.1	274 ± 91.1	269 ± 91.6	277 ± 88.2	294 ± 86.4
Manganese, mg/kg	20.07 ± 6.297	19.17 ± 6.315	14.03 ± 6.388	26.63 ± 7.060	19.82 ± 6.824	20.48 ± 7.476	18.65 ± 7.447	27.17 ± 6.385
Copper, mg/kg	32.38 ± 11.120	29.23 ± 16.042	36.83 ± 11.188	35.37 ± 12.648	31.28 ± 11.274	31.45 ± 14.080	35.62 ± 10.690	36.28 ± 11.303
Zinc, mg/kg	155 ± 51.8	161 ± 57.5	189 ± 55.0	171 ± 56.5	168 ± 59.8	158 ± 63.6	196 ± 59.2	171 ± 57.0

[1] NY = no yeast; LBV = Lalmin B-Complex Vitamins; BY = spray-dried brewer's yeast #1064B; EA = BGYADVANTAGE.
[2] NFE = nitrogen free extract, calculated (Dry matter basis contents of crude protein, crude fat, crude fiber, and ash subtracted from 100).

Table 5.6: Nutritional composition (mean ± standard deviation) of processed canned cat food containing different levels of a vitamin premix and/or a dried yeast ingredient[1].

	No vitamin premix				Contains vitamin premix			
Nutrient	NY	LBV	BY	EA	NY	LBV	BY	EA
Moisture, %	82.9 ± 0.98	82.6 ± 1.24	83.1 ± 0.74	81.9 ± 1.09	82.7 ± 1.13	82.5 ± 1.32	82.7 ± 0.74	82.0 ± 1.08
Dry matter, %	17.1 ± 0.98	17.4 ± 1.24	17.0 ± 0.74	18.1 ± 1.09	17.3 ± 1.13	17.5 ± 1.32	17.3 ± 0.74	18.1 ± 1.08
	---------- Dry matter basis ----------							
Thiamine, mg/kg	0.7* ± 1.02	19.9 ± 4.29	5.8 ± 0.54	2.2* ± 0.37	54.7 ± 18.64	59.8 ± 7.29	60.7 ± 11.04	46.5 ± 5.78
Crude protein, %	38.7 ± 1.63	40.8 ± 2.06	50.7 ± 0.38	49.9 ± 2.62	43.5 ± 7.73	40.2 ± 2.21	47.3 ± 5.76	49.6 ± 2.28
Crude fat, %	28.6 ± 8.48	28.3 ± 8.63	28.6 ± 4.01	30.0 ± 6.22	31.9 ± 12.50	27.6 ± 8.12	27.0 ± 1.83	30.3 ± 3.85
Crude fiber, %	0.46 ± 0.285	0.72 ± 0.514	0.86 ± 0.287	1.56 ± 0.267	0.85 ± 0.605	0.94 ± 0.268	0.79 ± 0.222	1.93 ± 0.225
Ash, %	6.14 ± 1.184	6.43 ± 0.720	7.12 ± 0.909	8.31 ± 1.715	6.51 ± 1.421	6.21 ± 1.065	6.84 ± 1.010	7.98 ± 1.449
NFE[2], %	26.1 ± 5.74	23.7 ± 5.38	12.7 ± 2.61	10.3 ± 1.71	17.2 ± 8.13	25.1 ± 5.16	18.1 ± 5.93	10.0 ± 0.83
Sulfur, %	0.47 ± 0.061	0.46 ± 0.051	0.54 ± 0.066	0.53 ± 0.046	0.48 ± 0.093	0.46 ± 0.069	0.53 ± 0.058	0.52 ± 0.038
Phosphorus, %	0.975 ± 0.1348	0.958 ± 0.0660	1.138 ± 0.2293	1.092 ± 0.1393	1.080 ± 0.2244	0.955 ± 0.1131	1.132 ± 0.1821	1.134 ± 0.1146
Potassium, %	0.810 ± 0.2040	0.875 ± 0.2017	1.186 ± 0.1969	1.377 ± 0.2237	0.956 ± 0.3560	0.856 ± 0.2072	1.101 ± 0.1816	1.338 ± 0.1146
Magnesium, %	0.076 ± 0.0073	0.076 ± 0.0048	0.104 ± 0.0121	0.150 ± 0.0117	0.092 ± 0.0221	0.077 ± 0.0064	0.098 ± 0.0158	0.150 ± 0.0090
Calcium, %	1.22 ± 0.199	1.14 ± 0.072	0.95 ± 0.433	1.30 ± 0.233	1.26 ± 0.489	1.19 ± 0.217	1.12 ± 0.366	1.42 ± 0.225

Sodium, %	0.323 ± 0.0498	0.319 ± 0.0491	0.324 ± 0.0502	0.317 ± 0.0529	0.329 ± 0.0529	0.318 ± 0.0566	0.334 ± 0.0488	0.311 ± 0.0430
Iron, mg/kg	278 ± 90.0	268 ± 83.1	255 ± 104.0	295 ± 95.2	274 ± 100.5	269 ± 94.6	277 ± 103.6	294 ± 88.7
Manganese, mg/kg	20.07 ± 7.624	19.17 ± 7.255	14.03 ± 9.938	26.63 ± 8.240	19.82 ± 7.907	20.48 ± 7.955	18.65 ± 9.353	27.17 ± 7.558
Copper, mg/kg	33.65 ± 11.707	33.37 ± 11.098	38.92 ± 10.864	37.50 ± 10.596	36.38 ± 10.570	33.42 ± 11.288	38.63 ± 13.543	35.85 ± 10.078
Zinc, mg/kg	176 ± 61.3	176 ± 55.9	179 ± 79.3	180 ± 61.4	181 ± 69.0	176 ± 59.6	188 ± 72.6	181 ± 58.1

[*] Least square mean thiamine content is less than 5.6 mg/kg dry matter basis (AAFCO, 2020; $P < 0.05$).

[1] NY = no yeast; LBV = Lalmin B-Complex Vitamins; BY = spray-dried brewer's yeast #1064B; EA = BGYADVANTAGE.

[2] NFE = nitrogen free extract, calculated (Dry matter basis contents of crude protein, crude fat, crude fiber, and ash subtracted from 100).

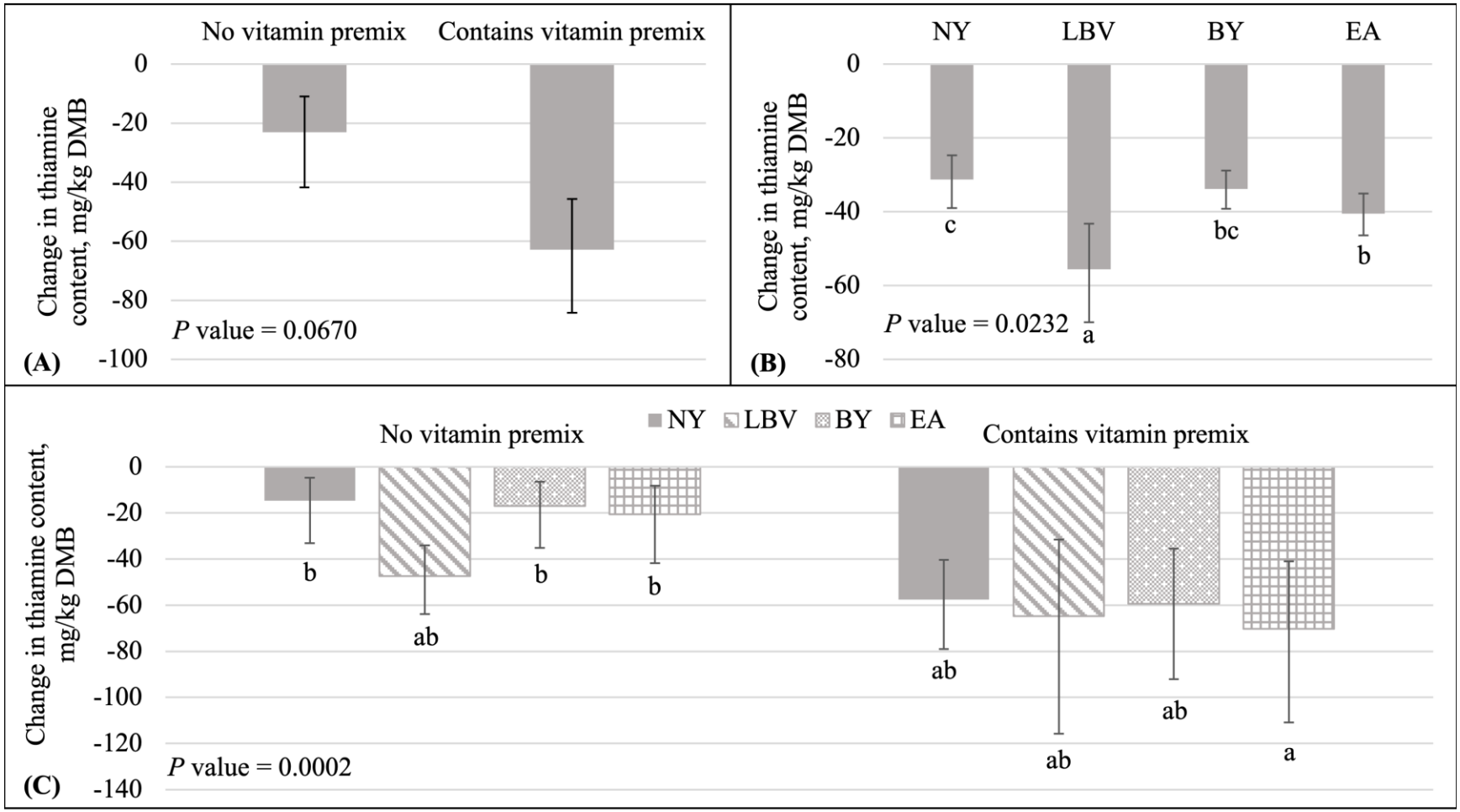

Figure 5.1: Change in dry matter basis (DMB) thiamine content (mean values with 95% confidence interval) of canned cat food: (A) = Main effect of vitamin premix; (B) = Main effect of yeast[1]; (C) = Interaction of vitamin premix and yeast.

[abc] Means within the same chart that do not share a superscript are different ($P < 0.05$).

[1] NY = no yeast; LBV = Lalmin B-Complex Vitamins; BY = spray-dried brewer's yeast #1064B; EA = BGYADVANTAGE.

Appendix A - Supplementary data for Chapter 2

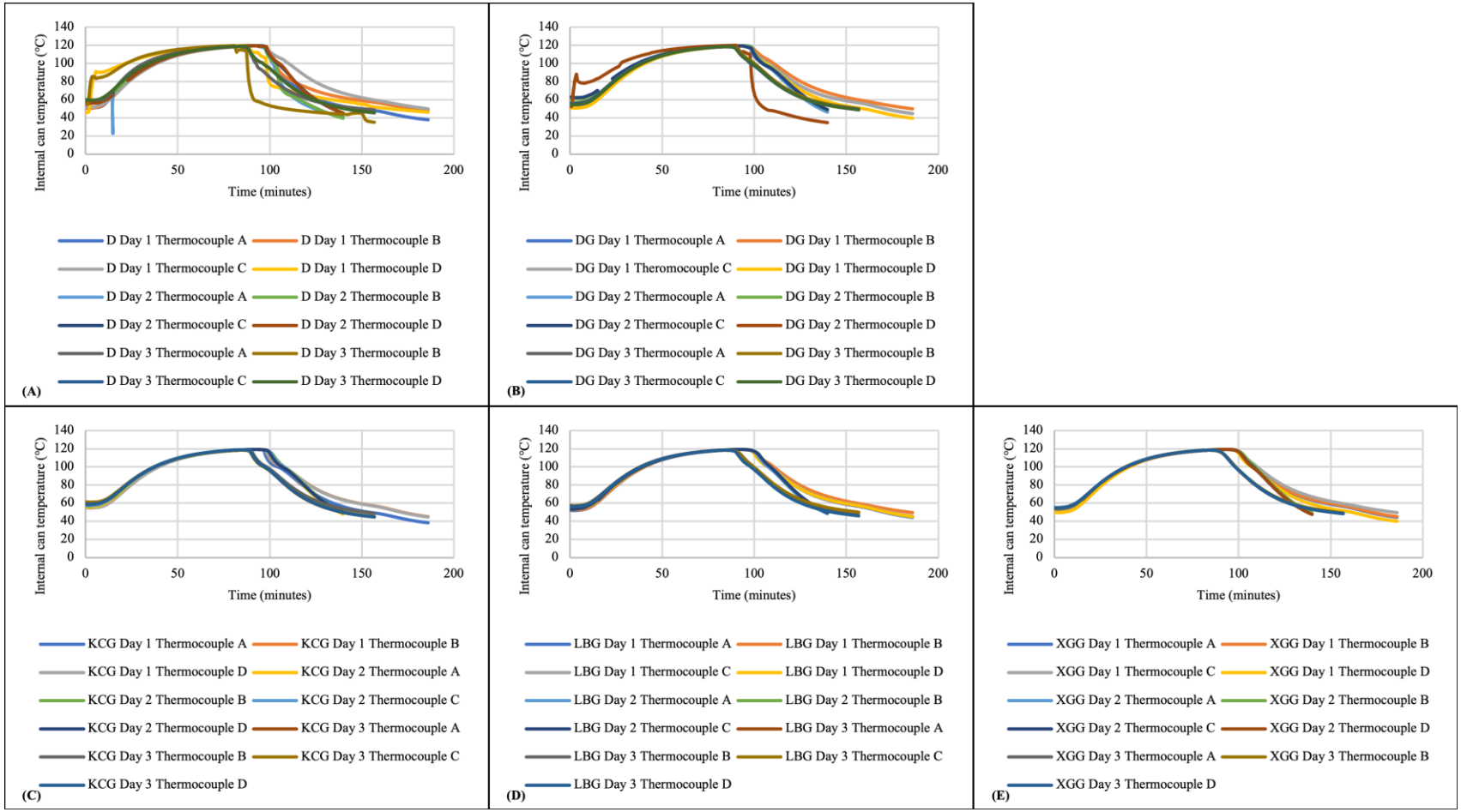

Figure A.1: Internal can temperatures for thermally processed wet pet foods containing different carbohydrate hydrocolloid ingredients[1].

[1] D = 1% dextrose; DG = 0.5% guar gum and 0.5% dextrose; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum.

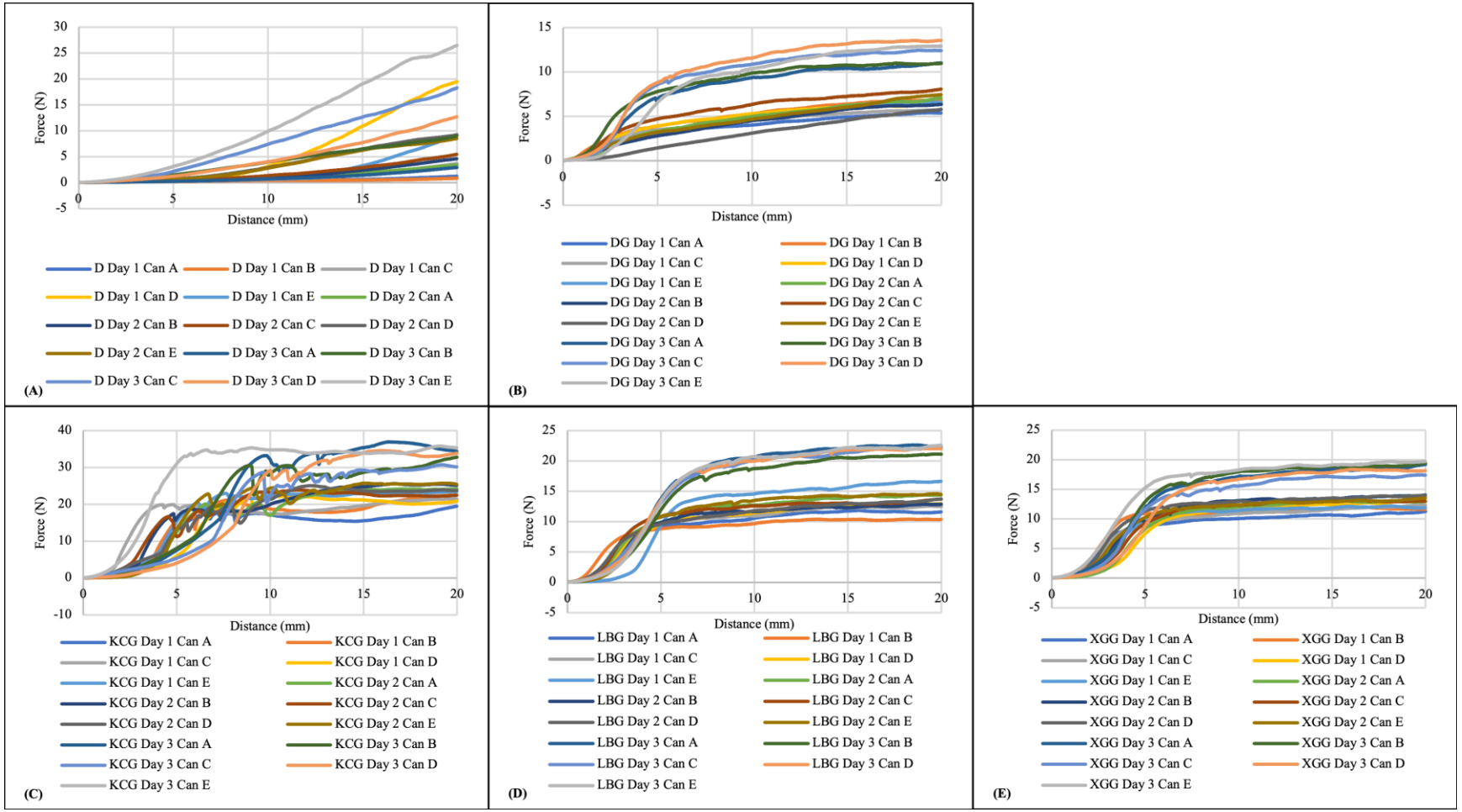

Figure A.2: Force deformation curves from modified back extrusion procedure applied to thermally processed wet pet foods containing different carbohydrate hydrocolloid ingredients[1]

[1] D = 1 % dextrose; DG = 0.5% guar gum and 0.5% dextrose; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum.