

Thiamine in a wet pet food application

by

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## Abstract

Since 2010, there have been seven recalls related to thiamine deficiency in cat food products (FDA, 2017; FSA, 2017). Cats have a high requirement of thiamine (5.6 mg/kg), and deficiencies can lead to death within a month if not treated (AAFCO, 2017). A few studies have been published regarding the impact of retort processing on thiamine loss in canned pet food but no work has been reported on heat penetration in other containers (pouches and trays). Therefore, our objectives were to determine the effect of container size and type on thiamine retention during processing of cat food. Our hypothesis was that thiamine retention would be impacted by container size and type. To address this, a 2x3 factorial arrangement of treatments in which two container sizes (small: 89-104 mL vs medium: 163-207 mL) and three container types (can, pouch, and tray) were evaluated for B-vitamin losses and thermal process lethality of a wet pet food. A model wet cat loaf type formula was produced for all six experimental treatments and each was processed in duplicate over six-days. All ingredients including the vitamin premix (10x level) were thoroughly mixed, heated to 43°C, and containers were manually filled. The filled and sealed containers were cooked in a retort (cans: SJ Reid Retort, Bellingham, WA; trays and pouches: FMC retort, Madera, CA) with thermocouples attached to the center of representative containers (n=14) in each batch. Software (Calsoft Systems, v. 5.0.5) was used to record the internal temperatures. The retort time was targeted to meet an  $F_0=8$  at 121°C and 21 PSI. Treatment sample were analyzed for included pH, moisture, crude protein, crude fat, ash, and B-vitamins. Results were analyzed using the GLM procedure in SAS (v. 9.4; Cary, NC) with means and interactions separated using Fisher LSD method by significant F and an  $\alpha$  of 5%. The proximate composition and pH were similar ( $P > 0.10$ ) among treatments. There was an interaction ( $P < 0.05$ ) between container size and type for time to reach the  $F_0=8$ ; wherein, the

medium can and tray had the longest time (45.5 and 46.3 min, respectively); the small can and tray, and medium pouch were intermediate (35.4, 36.0, and 32.0 min, respectively); and the small pouch had the shortest time (36.0 min). There was no difference for either main effect of container type or size on heating lethality values (each main effect average  $F_0=10.3$ ) and total lethality ranged from 12.7-16.7 min. Thiamine retention was lowest (70%) among the B-vitamins, and there was minimal loss throughout the process. The excess heating beyond  $F_0=8$  may account for the dramatic impact on the retention of heat labile nutrients like thiamine. This may be more difficult to control in the newer packaging systems like pouches and trays.

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# **Chapter 1 - The effect of processing and ingredient interactions on thiamine degradation in canned cat food: A modern nutrition-health dilemma**

## **Abstract**

Water soluble vitamins play an integral role in the normal metabolic function in cats. Thiamine deficiencies are a common issue in the pet food industry because thiamine degrades easily during processing. Specifically, when cats are fed a diet low in thiamine they may develop life threatening health issues including anorexia, ventroflexion, neurological impairment, and possibly death within a few weeks if not treated. However, little research has been published using a pet food matrix regarding what specific factors in pet food processing result in the most losses and whether these can be controlled. Thiamine can be degraded in a canned food due to heat, moisture, long-term storage, sulfites, pH, and thiaminase enzyme activity. Thermal processes used to produce wet pet foods sold in cans, pouches, and trays are required to be heat treated for extended periods of time. This is detrimental to thiamine retention. Because cats, like other carnivores have a very high metabolic requirement for thiamine, they are susceptible to these losses. For this reason, supplementation is often a logical step. However, survival of more than 10% of the thiamine may not be assured. This review summarizes the prevailing literature on the topic with application to pet food. Further, suggestions regarding potential investigations to remedy the issue are discussed. Finding an optimal time x temperature x pH x ingredient combination is a real possibility that has the potential to save many cats in the future.

## **Thiamine and Pet Food**

Vitamins are essential nutrients for companion animal diets that must be provided in small amounts in order to perform biochemical and physiological functions. This is because essential vitamins are either not made by the animal or not produced in sufficient quantities to support vital needs. Thiamine, or Vitamin B1, as it is often described, is one of these essential vitamins. Previous research has concluded that thiamine is susceptible to losses during canning. Jansen et al. (1926) reported that thiamine degrades due to heat processing, alkaline conditions due to additives used in canned meat products, and during storage. Since 2008, there have been numerous thiamine related recalls (Rumbeiha, 2011; Bischoff, 2012) affecting companies and products such as Iams Proactive Health Cat and Kitten Food (FDA, 2011), WellPet LLC (FDA, 2011), Nestle Purina (FDA, 2012), and JM Smucker Company (FDA, 2017). This is not an issue of negligence. Companies struggle to identify the proper level of supplementation for thiamine to compensate for the processing losses that can exceed 90% (NRC, 2006). Carnivores such as the domestic cat exhibit rather acute thiamine deficiency signs within a few weeks (Dreyfus et al., 1961).

In their work Moon et al. (2013) fed male cats a cooked meat diets and female cats stale food diets. The male cats exhibited signs of thiamine deficiency within two weeks and female cats three months. Clinical signs of thiamine deficiency are anorexia, changes in the central nervous system including seizures, head twitching, muscle weakness, incoordination, tachycardia, bradycardia, weight loss, and death (Davidson, 1992; Hilker et al. 1996; Chang et al., 2016). Other common clinical signs of thiamine deficiency include ocular issues like acute blindness, gastrointestinal issues such as vomiting, weight loss, and constipation (Singh et al. 2005). Ventroflexion, a flexion of the spine with movement of the head towards the chest, has been reported as a result of thiamine deficiency, and cats with the deficiency have been reported to

somersault when they jumped from a table to the floor (Loew et al., 1970). It has also been reported that cats manifesting thiamine deficiency had more difficulty learning to complete tasks (Irle and Markowitsch, 1982). Thiamine deficiency also has been reported to cause an overproduction of pyruvate leading to ischemic cells in the brain (Davidson, 1992). The biochemical lesions damage an animal's carbohydrate metabolism and the animal can eventually die within four weeks if not treated with thiamine supplements (Davidson, 1992). Once a thiamine deficiency has been diagnosed, typically, a vitamin B complex injection is administered for three to five days, followed by oral dose for two to four weeks (Plumb, 2011). Thiamine deficiency is a health issue for cats and a potential economic problem for manufacturers of cat food because of its constant need by the animal and devastating result if left unaddressed.

Because thiamine is water soluble, it can only be stored in small limited amounts and is mostly lost through urine (Garosi et al., 2003). Therefore, nutrients such as thiamine must be provided and (or) supplemented in the food on a continuous basis. Cats are obligate carnivores that require high levels of protein and fatty acids (Anholt et al., 2016). They need thiamine in order to utilize enzymes in several metabolic pathways including the urea cycle to adjust to nitrogen levels in the body (Anholt et al., 2016). Cats also need thiamine for synthesis of fatty acids, amino acids, and carbohydrates (Palus et al., 2010). If the diet is insufficient in thiamine, a deficiency can develop into neurological issues and can result in death if supplementation is not provided to overcome the deficiency (Palus et al., 2010).

Thiamine, (2-{3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methylthiazol-5-yl}ethanol), like other water-soluble "B vitamins," are coenzymes that support nutrient utilization (Fattal-Valevski, 2011). Water soluble B vitamins also aid in the production of metabolic energy through the TCA cycle (Gregory, 1997; Bubber et al. 2004; Fattal-Valevski,

2011; Manzetti et al., 2014). Thiamine acts as a coenzyme, known as thiamine pyrophosphate, that becomes di-phosphorylated into thiamine diphosphate (TPP; Analytical Methods Committee, 1999). Once thiamine has been dephosphorylated, it receives electrons in an oxidation-reduction process, and then catalyzes the conversion of pyruvate to acetyl-CoA (Chabrière et al. 1999). Thiamine is important for carbohydrate metabolism, and plays a key role in brain development, function, maintenance, and communication processes (Butterworth 2006; Gibson et al. 2007). In its cationic form, thiamine plays a role in the generation of acetylcholine, which stimulates cerebellum activity, and aids in nerve tissue repair (Manzetti et al., 2014).

Thiamine was the first water-soluble B vitamin to be discovered. Characterization began in 1890 with the recognition of beriberi disorder in humans related to the lack of an essential food element. This was later determined to be thiamine deficiency (Ellefson, 1985). Thiamine was isolated in 1911, and found naturally in grain products such as rice and wheat germ, as well as yeast, legumes, and meat organs, particularly liver, heart, and kidney (NRC, 2006). However, it was not correctly identified and published until 1936 (Williams, 1936). Thiamine is produced naturally by organisms including plants, fungi, and bacteria (Manzetti et al., 2014). There are several common pet food ingredients that contain thiamine and include yeast, peas, beans, wheat germ, oats, some meats (including pork and beef), and soy (Szymandera-Buszka et al., 2014; Fattal-Valeski, 2011). However, these ingredients either lack sufficient thiamine to fully support the diet or are limited in the diet due to other considerations. Thus, supplementation is generally required.

There are several phosphorylated forms of thiamine in the body including the active form, thiamine pyrophosphate. Thiamine pyrophosphate is the coenzyme used in energy production (Davidson, 1992). Thiamine exists physiologically in various thiamine esters

including thiamine monophosphate, thiamine diphosphate, and thiamine triphosphate (Jun. 2008). But, tissue-based thiamine from meat and plant sources in the basal dietary ingredients are not typically included in commercial cat foods in an amount sufficient to support their needs. The two main supplemental forms of thiamine added to pet foods are thiamine hydrochloride and thiamine mononitrate. Thiamine mononitrate is used more often in pet food because of its higher stability compared to thiamine hydrochloride and is non-hygroscopic (Gregory, 1997). Thiamine hydrochloride is a sulfur-containing vitamin composed of a thiazole ring and a pyrimidine group joined by a methylene bridge (Kraut et al., 1962; Davis et al.; 1983, Manzetti, 2014; Figure 1). Thiamine is manufactured synthetically by oxidizing thiothiamine to thiamine sulfate in the presence of hydrogen peroxide and then salts are produced under ion-exchange (Matsukawa et al. 1970; EFSA, 2011). Thiamine mononitrate is produced by oxidizing thiamine sulfate into an ethanol solution (EFSA, 2011), then the final product is precipitated with several filters and dried under vacuum where thiamine hydrochloride is produced by oxidizing thiamine sulfate using ion exchange resin. Then, the thiamine product is decolorized, distilled, precipitated, isolated through centrifugation, and dried under vacuum (EFSA, 2011). The structure of thiamine helps to understand thiamine's instability through the canning process. The methylene bridge connecting the pyrimidine and thiazole moiety can easily be broken down by ingredients such as sulfites (Farrer, 1945). The thiazole moiety is less stable than the pyrimidine structure, and is easily cleaved by hydrolysis. The methylene bridge and sulfur ion within thiamine contribute to its instability during processing. Thiamine is susceptible to degradation during canning due to its ability to dissolve in water. Thiamine is most likely dissolved into water because of the bonding of the hydrogen atoms to the hydroxyl groups and amine tertiary structures. Therefore, the pH of

canned pet food, exposure to high heat for extended periods of time and water solubility are why thiamine is degraded during the canning process.

Thiamine requirements for cats and dogs was established by the National Research Council based on the limited amount of research in the target species (NRC, 2006). Like most carnivores, cats have a relatively high thiamine requirement (5.60 mg/kg diet DM on a 4,000 Kcal diet); whereas, dogs have a more omnivorous species requiring a lower level (1 mg/kg diet DM on a 4,000 Kcal diet; AAFCO, 2015). Cats in late gestation have an even higher thiamine requirement (6.3 mg/kg diet DM on a 4,000 Kcal diet). This difference in the amount of thiamine required, is in part, why thiamine deficiency in thermally processed canned foods may be more prevalent in foods produced for cats than dogs.

## **Thermal Processing**

Thermal processing is critical to the food industry because it helps increase the safety of commercial products while transforming the food matrix into a convenient form for nutrient delivery to the animal (Berry et al., 1993; Hendriks et al. 1999; Awuah et al., 2007; Balsa-Canto et al., 2007). Canned food is produced through a cooking process (commercial sterilization) in which high temperature treatment is intended to destroy microorganisms and prevent growth of clostridium botulinum spores (Hendriks et al., 1999; Awuah et al., 2006). “Commercial sterilization” is defined as thermal processing that will render the food free of microorganisms capable of reproducing in the food in normal non-refrigerated conditions during storage and distribution (21 CFR 113.3), whereas full sterilization is defined as using a chemical or physical process that eliminates microorganisms completely (Zajko and Klimant, 2013). Canned products are hermetically sealed to prevent unwanted growth of microorganisms with high heat resistance.

Hermetically sealed containers also prevent entry of pathogens that might produce toxins during storage (Awuah et al., 2006). For FDA regulatory compliance (21CFR113), all canned foods must have a kinetic study on file reporting time vs temperature necessary to achieve bacterial lethality and verified by the process authority as validation that a product is commercially sterile (Durance et al. 1997; Awuah et al., 2006). The process authority will conduct a lethality studying measuring the f-values of canned products. The f-value measures the amount time at a constant temperature rate to kill a specific number of organisms such as *C. botulinum* using a 12 log cycle reduction (Fellows, 2009; Goff, 2013). A heat penetration study is completed to collect data for calculating commercial sterility (Chen, 2007; Fellows, 2009). Several factors that affect heat penetration of a canned product include retort temperature, processing time, initial temperature, size of container, shape of container, type of retort (still, agitating, etc.), product formula, container type, and vacuum on the container (Pflug, 1980). The minimal F-value for pet food is 6 according to previous thermal processing studies (Fellows, 2009).

Even though thermal processing is required during the canning process, heat can degrade certain nutrients in the product and erode product quality. For example, Hendrik's et al. (1999), reported that digestibility of amino acids in a rat model decreased as heat processing time increased. This research group stated "to maintain optimal amino acid digestibility, the amount of time for thermal processing should be limited." This balance between safety and nutrient destruction could be true for vitamins like thiamine as well.

### **Processing Conditions and Their Effect on Thiamine Stability**

Within the parameters of the regulations there are several processing conditions to consider when exploring mechanisms involved with thiamine degradation in canned pet foods. Ariahu et. al. (2000) measured the retention of thiamine in a brine, sauce, and soup formulated with

periwinkle (*Tympanostomus fuscatus*) in a low acidic condition and processed at four different temperatures (0 to 40 minutes) and found that thiamine retention followed first order kinetics (Ariahu et. al., 2000). This means that the thiamine decay in the study was directly proportional to the time and temperature. This was confirmed by Durance et al. (1997), and Rehka et. al. (2004). But does the degradation for thiamine occur simultaneous to commercial sterility? Perhaps not, as Ariahu et. al., (2000) observed that all of the z-values for clostridium *sporogenes* occurred at lower temperatures than thiamine destruction. Their data suggests that the microbiological safety might be attained at a tenfold lower temperature than thiamine degraded. More research is required to understand if another (nutritional) safety variable might benefit our current model for commercial sterility.

Other factors that influence thiamine degradation during processing include blanching, changes in pH, batter moisture, retort time, and retort temperature (Tribble, 2016; Słupski, 2012; Rekha et al., 2004). Thiamine exists in several ester forms, but depending on the food matrix, it can be degraded into several different products. For example, Dwivedi et al., (1973) performed an experiment with thiamine under alkaline conditions using paper chromatography to isolate the degradation of the molecule. They observed that thiamine was converted into thiochrome (a carbene form of thiamine), thiamine disulfide, and two derivatives of pyrimidine. In the same paper the researchers speculated that the activation of energy for the degradation of the thiazole ring occurs at a lower pH than the cleavage of the methylene bridge. The more acidic the food matrix, the more rapidly the thiazole group on the thiamine molecule will be cleaved and the methylene bridge destroyed. Differences also exist in thiamine levels retained at given pH treatments due to changes in time and temperature. For example, Rekha et al. (2004) evaluated pH, cooking time, and temperature to model the kinetics of thiamine degradation using a split red gram (*Cajanus*

*cajan* L.) model that showed the greatest losses occurred at processing conditions most similar to those used to produce commercial canned cat foods (pH 6.5, 15 minutes, and 120°C). In a study with canned salmon Durance (1997) showed that thiamine loss was lower when the processing time was decreased by fifteen percent. The pH may also influence the results as thiamine stability was greatest between pH 2.0 and 4.0 (Trang, 2013). This suggests that as pH increases to a more neutral or alkaline pH, thiamine will slowly become unstable and may degrade more easily in a pet food matrix.

Once thiamine has been broken down, there are several end byproducts. These include hydrogen sulfite, elemental sulfur, 4-methyl-5-( $\beta$ -hydroxyethyl) thiazole, and other minor products (Dwivedi et al. 1973). Thiamine is degraded by breaking the CH bond separating the pyrimidine and thiazole moieties leading to the destruction of the thiazole ring resulting in hydrogen sulfide production (1973). Morfee and Liska (1971, 1972) conducted a study by incorporating thiamine in milk at 121°C for 50 minutes and observed sulfur as a major end product in more basic conditions. The thiamine-<sup>35</sup>S (sulfur containing degraded thiamine) interacted with the protein in a milk model system and in evaporated milk. When the thiamine interacted with a combination of protein sources like lactose and sodium caseinate, it had the highest level of thiamine degradation as the pH shifted from 7.4 to 6.5. Thiamine broke down the least (12.7 and 16.7%, respectively) in model systems with high milk fat levels. Morfee and Liska (1971) believed that the fat containing systems protected the thiamine. Processing conditions prior to retort preparation of a canned cat food was evaluated by Tribble (2016) and it was determined that target moisture for the batter mixture was 65%. As the retort time increased, thiamine retention decreased.

## **Storage**

Commercial canned pet food is stored in a warehouse, on the store shelf, and in the pet owner's home for an extended period of time, sometimes exceeding two years. As the product ages, thiamine and other labile nutrients degrade. Nutrient degradation begins starting at the very moment of harvest. For example, Poel et. al. (2009) assessed the thiamine concentration in skeletal pork muscles during the harvesting process and showed that the concentration of thiamine decreased immediately upon harvest. This suggests that thiamine degradation occurs very quickly. But they also noted that total thiamine concentration increased in samples 96 and 216 hours after harvest; perhaps this is a result of thiamine phosphate ester production from the pig skeletal muscle during rigor. These thiamine phosphate esters include thiamine monophosphate, thiamine diphosphate, thiamine triphosphate, and thiamine tetraphosphate (Poel et. al., 2009). Peñas et. al. (2013) stored four different dehydrated vegetables (dehydrated garlic, onions, potatoes, and carrots) in conditions comparable to a product sitting on a store shelf at three-month intervals for one year, and analyzed them for the concentration of various vitamins. In general, thiamine decreased in all four vegetables over time degrading more the longer it was stored (Peñas et. al., 2013). The same situation may occur in pet food which has been stored for long periods, but supporting research is lacking.

## **Food Preservatives**

Recent research has shown that various forms of sulfur dioxide, which were once used as a preservative to reduce odor of the meat in pet food, have a negative impact on thiamine retention (Malik and Sibraa, 2006). Sulfites were used to delay the reduction of myoglobin by inhibiting bacterial growth in meat (Clydesdale et al; 1991, Singh, 2005; and Markovich et al., 2013). By slowing the conversion of myoglobin into metmyoglobin, odor in meat containing food is reduced

and the red color preserved longer (Singh, 2005). Thus, sulfur dioxide can increase the shelf life and lengthen the palatability of cooked meat. Several countries including the United States have banned sulfites in meats. Researchers investigated the kinetics of the thiamine degradation in order to minimize thiamine loss and (or) at least better predict and compensate for losses. It was determined that sulfites cleave the methylene bridge between the pyrimidine and thiazole moiety of the thiamine molecule. Both the sulfur ion and methylene bridge lead to thiamine instability during thermal processing.

Other ingredients in the food matrix may contain preservatives like sulfites and sulfur dioxide. These are inorganic bases found in dehydrated potatoes, potato flakes, wine, dried apples, corn gluten meal, and flavorings. These may unwittingly be added to pet food and cause negative effects on thiamine in the can. Sulfur dioxide can also be used with fruits and vegetables to improve color retention (Dwivedi and Arnold, 1972). Sulfur dioxide destroys thiamine by converting it into a thiazole base and pyridyl methanol sulfuric acid (Dwivedi 1972; Steel, 1997; Singh et al. 2005). Even if a food contains an adequate amount of thiamine, it can be inactivated after coming into contact with sulfite treated meat because the sulfites change the structure of thiamine. Of course, the destruction of thiamine in food depends on the quantity of sulfur dioxide in the product (Steel, 1997). For example, thiamine level decreased by 55% with 400 mg sulfur dioxide and 95% with 1,000 mg/kg sulfur dioxide in cat food (Studdert and Labuc, 1991 and Steel, 1997). This type of reduction is dose-dependent and predictable.

Another sulfur containing preservative commonly encountered in food systems is sodium bisulfite. In a simplified model, sodium bisulfite (0-1% levels) added to rice during the soaking step, reduced thiamine concentration (0.08g/100g) of the finished product to undetectable levels (<0.01 mg/100g; Vanier et. al., 2015). This should not be confused with sodium bisulfate, which

is an acidifier and was shown to not directly affect thiamine concentrations any differently than hydrochloric acid in a canned cat food application (Trible, 2016).

### **Ingredients and their Relationship with Thiamine**

Canned pet foods are produced with many types of ingredients, including: water, meats, fats, starches gums, gels, vitamins, and minerals. Each of these may interact with thiamine differently and contribute to its degradation. In canned foods, meat and fish play a prominent role in the recipe. One type of animal protein source in particular that affects thiamine retention in pet food is fish. Fish and shellfish contain a thiamine degrading enzyme, thiaminase. Hilker and Peter (1966) observed that thiaminase tended to be higher in freshwater fish than oceanic fish. Thiaminase is commonly found in high concentration in the kidneys of carp, striped mullet, and alewives (Zajicek et al. 2005). High concentrations of thiaminase were also found in the liver and intestines of Ukrainian fish (Zajicek et al., 2005). The thiaminase enzyme degrades thiamine by a base-exchange reaction of the methylene group and pyridine moiety (Zajicek et al., 2005; Figure 3). There are two types of thiaminase, thiaminase I comes from fish, shellfish, ferns, and some bacteria, and thiaminase II which is found in some bacteria. Not only are these thiamine degrading enzymes derived from different sources, they also hydrolyze the thiamine by different mechanisms. For example, thiaminase I requires a co-substrate in order to act as a transferase; whereas, thiaminase II hydrolyzes thiamine directly (Edwin, 1979). Fortunately, the thiaminase activity (because it is a protein) can be reduced with denaturing heat treatment (Zajicek et al., 2005). In an extreme situation, the destruction of dietary thiamine could result from eating fish if the quantity of thiaminase was sufficient (Zajicek et al., 2005). The processing of fish could reduce thiaminase activity in the final product (Sivasankar, 2004). Rendered fish meal used in pet food is extensively heated which should effectively inactivate thiaminase (Markovich et al., 2013).

Thiaminase I can also be found in some bacteria such as *P. thiaminolyticus* and *C. sporogenes* (Kraft et al., 2014).

Thiaminase is typically measured either by spectrofluorometric thiochrome assay (Fujita, 1954) or by radiochemical method with  $^{14}\text{C}$ -thiazole (Edwin and Jackman, 1974). The radiochemical method uses thiamine hydrochloride in the  $^{12}\text{C}$ -thiamine form and thiazole-2- $^{14}\text{C}$ -thiamine hydrochloride ( $^{14}\text{C}$ -thiamine) to measure the  $^{14}\text{C}$ -thiazole activity (Zajicek et al., 2005).

### **Other factors:**

Athar et. al. (2006) evaluated vitamin retention in three different cereal grain mixtures that have native sources of b-vitamins (oats, maize, and a maize and pea mixture) and found thiamine retention differed such that the maize and pea mixture retained more thiamine than maize and (or) oats alone. The authors speculated that the oats should have had a greater retention because of the high oil content in the oats compared to the other grain ingredients. The authors had hypothesized that the higher oil content would alter the processing (Athar et. al., 2006). Athar et al. (2006) concluded that thiamine is more heat labile during extrusion compared to thiamine in the maize sample. This different response would suggest the possibility that we could select ingredients that promote greater thiamine retention and thereby minimize processing losses to some degree.

Szymandera-Buszka et. al. (2014) evaluated other preservatives like rosemary extract using minced chicken as the model system in a thermal process. When added to minced chicken, rosemary extract extended the half-life of thiamine longer than casein hydrolysate. After the shelf life study, Szymandera-Buszka et. al. (2014) concluded that the low oxidized fat and minced chicken with rosemary extract may have limited thiamine losses during storage. The authors also observed that samples with low oxidized fat and rosemary extract also had a longer shelf life. Rosemary may be used as a possible antioxidant source that could protect thiamine in canned pet

food but more research would need to be conducted by incorporating meat into a pet food matrix as the model system. Then the rosemary and thiamine infused pet food matrix would need to be thermally processed in a retort at 121°C, 21 PSI for 1 hour to represent typical canning conditions in the pet food industry.

One study evaluated the relationship between storage of pet food and nutrient retention over time. Mooney (2016) conducted a shelf life study with extruded pet food comparing ambient storage (20°C and 50% RH) conditions to pet food storage with high temperature and high humidity (50°C and 75% RH). Results from this study concluded that pet food stored in high humidity and high temperatures had a 24% thiamine loss after two weeks and a 35% thiamine loss after six weeks. When looking at the data from ambient storage, thiamine was relatively stable for the first three months but dropped to 65% of starting values by six months.

## **Analytical Methods**

There are many methods to determine thiamine retention in food. However, two common techniques are based on fluorescence and high performance liquid chromatography (HPLC). In order to measure thiamine in a given food sample, it must be extracted from the food matrix first. This is typically done using acid hydrolysis with low strength hydrochloric acid followed by enzyme digestion of starch using clara-diastase (Analytical Methods Committee, 2000). Potassium ferricyanide is a derivatizing agent that transforms thiamine into a thiochrome. Potassium ferricyanide oxidizes thiamine and its phosphate esters into thiochrome under alkaline conditions (Lu et al., 2008 and Wehling). Once thiamine has been converted to thiochrome, the sample can be refined through a gradient elution column and measured using fluorescence detection on the HPLC (Analytical Methods Committee, 2000). The HPLC with fluorescence detection is a rapid method that measures compounds using emission and excitation of

wavelength (Analytical Methods Committee, 2000). Due to the sensitivity of thiamine to light, samples must be prepared in amber glassware to prevent any possible degradation before analysis.

Not only can the HPLC be used to detect thiamine in pet food, it can also be used to measure thiamine in the blood and urine of animals. Typically, thiamine and its phosphate esters are measured in the erythrocytes (Jun, 2008). Thiamine is carried in the erythrocytes or is bound to free plasma (Marks et al., 2011 and Talwar et al., 2000).

Thiamine can also be measured by fluorescence with a fluorimeter (AOAC 942.23). In this procedure, free thiamine is extracted from a 10g sample in dilute acid after autoclaving. The solution is then incubated with buffered enzymes to release bound thiamine. After thiamine is oxidized to thiochrome (fluorophore) (Gregory, 1997), the fluorometer measures the fluorescence of the standard solution with an input filter at 365 nm and output filter at 435nm. Then the standard solution fluorescence is compared it to the fluorescence of the thiamine extracted sample. Wherein, the intensity of the fluorescence increases with increasing concentration of the thiamine in the sample. This method is sensitive and has specific quantification (Gregory, 1997).

## **Summary**

Even though thiamine deficiencies are a well-known problem in the pet food industry, most of the thiamine retention research has been conducted using human food matrices. Pet food differs from human “foods” as they are complete diets containing all the nutrients the animal requires. This creates interactions among the various elements and the deficiencies are more serious as no other food item will offset the insufficiency. A time and temperature relationship affects the concentration of thiamine during thermal processing as thiamine is heat labile. However, more research needs to be conducted on the thermal kinetic relationship of time and

temperature during thermal processing of canned pet foods containing thiamine. Furthermore, in-depth research also needs to be conducted to consider other processing factors, such as pH and ingredient impact of thiamine in pet food products.

Research has shown that using ingredients with low-oxidized fats and using preservatives like rosemary extract may limit thiamine losses during thermal processing (Szymandera-Buszka et al., 2014). Sulfites that are used in food as preservatives also have a negative impact on thiamine and should be used as little as possible in pet food products (Dwivedi, 1973). Enzymes such as thiaminase will inactivate thiamine in canned pet food. When developing pet foods, the industry should be conscious when determining what ingredients are added to the food matrix in order to reduce thiamine loss. Overall, time, temperature, and ingredient interactions reduce thiamine concentration during thermal processing of pet food.

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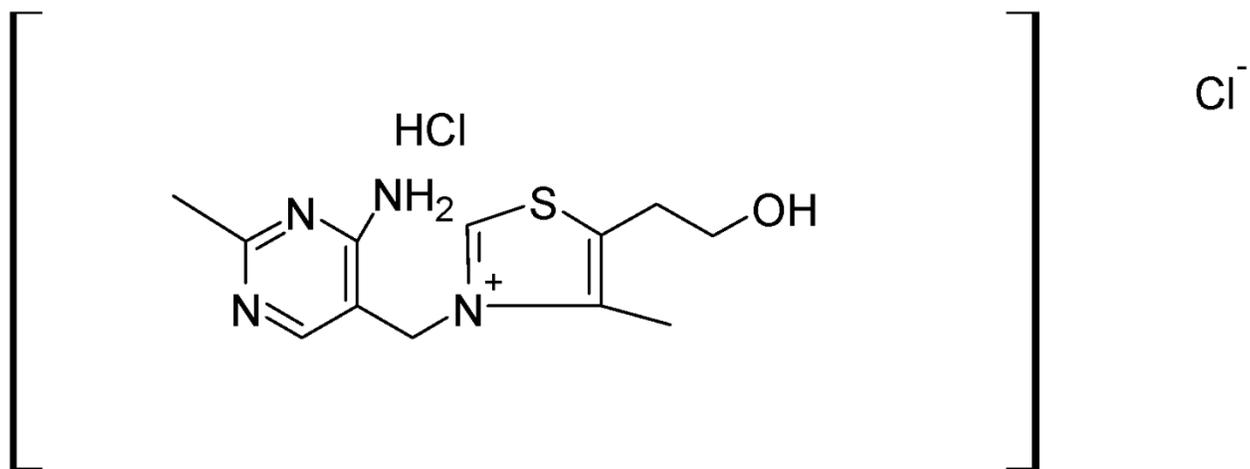
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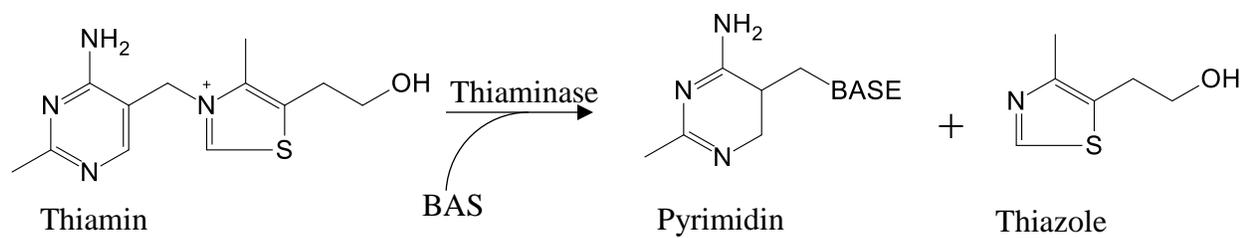
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## Figures



**Figure 1.** Chemical Structure of Thiamine Hydrochloride (ACD/ChemSketch)



**Figure 2.** Chemical Base Substitution of thiamine interacting with thiaminase I (ACD/ChemSketch)

## **Chapter 2 - Retention of thiamine and other water soluble vitamins in a wet pet food application**

### **Abstract**

In 2017, there have already been several recalls related to thiamine deficiency in pet food products (FDA, 2017; FSA 2017). Cats have a high requirement of thiamine (5.6 mg/kg,) and deficiencies can lead to death within a month if not treated (AAFCO, 2017). Few studies have been published regarding the impact of processing in a retort on thiamine loss in pet food. Therefore, it was our objective to determine the effect of container size and type on thiamine retention during processing of cat food. A model wet cat loaf product was used for all six treatments: two sizes (small and medium) and three types of packaging (cans, trays, and pouches). All ingredients including the vitamin premix (10x level) were hand added to a mixer and then containers were manually filled. Within each replicate (50 containers per replicate), thermocouple probes were inserted into separate containers. Once filled, each container was hermetically sealed and cans with thermocouples were attached to the retort data collection system. The retort time was determined by thermocouple heat penetration to meet the  $F_{0=8}$  at 121°C and 21 PSI. Sample analysis included pH, moisture, crude protein, crude fat, ash, and thiamine. Results were analyzed using the GLM procedure in SAS (v. 9.4) with means and interactions separated using Fisher LSD method by significant F values and an  $\alpha$  of 5%. The proximate composition and pH were similar ( $P>0.10$ ) among treatments. Thiamine retention was lowest (70%) and the other B-vitamins had negligible vitamin loss during the canning process.

**Keywords:** thiamine, thiamine deficiency, thermocouples, retort.

## **Introduction**

Cats and other carnivores have a high requirement for thiamine relative to omnivores like the dog (NRC, 2006). Thiamine must be provided in the diet because cats cannot synthesize it adequately to support their needs. At the extreme, thiamine deficiency can result in issues of the central nervous system, paralysis, and death (Bendix et al., 1951; Loew et al., 1970; Wilson et al., 1975; Davidson, 1992; NRC, 2006; Penderis et al., 2007). Reports in the literature indicate this is a widespread and common problem (Rumbeiha, 2011; Markovich, 2014). Recalls have affected prominent companies due to thiamine deficiency (FDA, 2017). This is not due to negligence, but because thiamine can be destroyed by food processing. Riaz et al. (2009) summarized that thiamine losses were 50% retention in pelleting and extrusion, and 70% during canning. Beetner et al. (1974) reported 54% thiamine retention during extrusion, 33% thiamine retention with extrusion at higher temperatures, and only 15% thiamine retention at higher screw speeds. High temperature, alkaline pH changes, sulfites, preservatives and enzymes may have negative effect (Clarke and Gurin, 1935; Reddy and Love, 1999; Palus et al., 2010; Markovich et al. 2013). Today pet foods are produced in many different types and sizes of containers. But information regarding whether the container and volume affect thiamine retention in pet formulas is scarce.

Therefore, the objectives were to determine the effect of container size and type on thiamine retention during thermal processing of a wet cat food.

## **1. Materials and Methods**

### **1.1 Treatments**

The experiment was conducted as a 2x3 factorial arrangement of treatments with two sizes of containers (small and medium) and three containers types (aluminum can, laminate pouch, and plastic tray). Experimental treatments were produced in duplicate over six days in an industrial research and development center. The small containers evaluated were a 3 ounce (88.7 mL) two piece can (209.5 x 107 aluminum can with modified vinyl coating), a 3.5 ounce (88.7 mL) PET foil closure tray-thermoforming tub with 1.2 mm polypropylene/EVOH 10%/Polypropylene base sheet, and a 3 ounce (88.7 mL) pouch made of 12 $\mu$ PET/adhesive/Al Foil 8 $\mu$ /adhesive/Polypropylene 70 $\mu$  with 95mm width x 138 mm height with 25mm gusset. The medium containers were a 5.5 ounce (162.7 mL) two piece (307 x 109.3) aluminum can with modified vinyl coating, a 7 ounce (207 mL) thermoforming tub with base sheet of 1.4 mm polypropylene/EVOH10%/Polypropylene, and a 6 ounce (177.4 mL) pouch made of polyester 12 $\mu$ /adhesive/Al Foil 7 $\mu$ /adhesive/Nylon 15 $\mu$ /adhesive/polypropylene 60 $\mu$  (140mm wide, 180mm high, with 25 mm gusset).

### **1.2 Model Canned Cat Formula**

A model canned cat diet was formulated to mimic a commercial cat food. The same formula was produced for all six treatments (Table 1). All of the ingredients were sourced from commercial suppliers and the same supplier lot code for each ingredient was used throughout the experiment. This included frozen mechanically separated chicken blocks (Protein Inc/BHJ, St Joseph, MO), soy bean oil (preserved with mixed tocopherols, Columbus Foods, Des Plaines, IL), whole brown rice (Gulf Pacific Rice, Houston, TX), kappa carrageenan gum (Marcel Trading, Quezon City, Philippines), medium coarse guar gum (Intercolloid, Wembley,

Middlesex, UK), dried egg product (Rose Acre Farms, Seymour, IN), salt (Cargill, Hutchison, KS), choline chloride (SEM Minerals, Quincy, IL), mineral premix (Prince Agri, Quincy, IL) and taurine (Prinova, Carol Stream, IL). The vitamins were supplied as a standard premix including all vitamins except choline (DSM Nutritional Products, LLC, Parsipanny, NJ) and were added into the batter during production at 10x normal levels allowing the vitamins to be measured more easily and to be able to determine a difference in vitamin retention. All of the raw ingredients were sampled and submitted for background vitamin analysis.

### **1.3 Batter Production**

The frozen blocks of mechanically separated chicken were ground through 9.5 mm openings in a pilot plant extractor and grinder prior to batching. The ground mechanically separated meat was weighed into a horizontal jacketed mixer (Rietz, Evansville, IN). Each ingredient was pre-weighed and added into the mixer and blended for five minutes until uniform while being heated. Batter (68 kg) sufficient to fill 50 containers of product daily (two batches per day) was produced. Once the batter mixture was brought up to a drop temperature of 43.3°C with direct steam injection, the moisture was measured by rapid methods (CEM, Mathews, NC) and recorded. Water was added to adjust the moisture to 78% for all batches. The vitamin premix was then added to the meat mixture then mixed for another 10 minutes. The complete batter was then homogenized (Karl Schnell Emulsifier, Winterbach, Germany) to pass a three and six mm diameter plate.

### **1.4 Filling and Seaming**

The hot emulsified batter was weighed into each replicated container size and type. Once filled and attached to the data collection wiring, the rest of the cans were sealed with a Ferrum Seamer (Schafisheim, Switzerland), small trays were sealed with the Shinwa sealer (Shinwa,

Japan), medium trays were sealed with Raque Sealer (Raque Food Systems, Louisville, KY) and pouches were sealed with a PMP mini vacuum seamer (PakSource Global, LLC., Sarasota, FL)

### **1.5 Retort Process**

Time and temperature data for each batch of cat food heated in the retort (JBT, Madera, CA- trays and pouches; Reid Boiler Works Inc. Bellingham, WA-cans) were monitored with type-T thermocouples (Ecklund-Harrison Technologies Inc., Ft. Meyers, FL). Thermocouple probes (n=14) were inserted into separate containers identified numerically. Once filled, each container was hermetically sealed and cans with thermocouples were attached to the retort data collection system.

Before samples could be placed into the retort, a random sampling of containers were vacuum tested (cans). In addition, for the trays and pouches, five samples per replicate were filled with water and sealed. After the containers were sealed they were subjected to a “burst test” or compression test to determine how much pressure each container could withstand.

### **1.6 Retort Cooking Cycle**

Within each batch, containers were sealed and placed into a retort basket filled with water before the internal batter temperature dropped below 43°C. Ballast containers were placed into two trays above and below the test product to represent a normal mass in a production process. The retort time was determined by thermocouple heat penetration to meet the  $F_0=8$  at 121°C and 21 PSI. Computer software (Calsoft Systems, Schaumburg, IL) was used to track the F values of each container with thermocouples until the target internal temperature reached a steady state. Once a product reached  $F_0=8$ , the retort was cooled with water to monitor the F values during cooling phase to determine additional accumulation of heat for lethality. Temperatures were recorded for each treatment.

## **1.7 Sample Collection**

Chicken, egg and vitamin premix samples were collected during the batching process. The chicken was stored frozen (4°C) and the dry ingredients were stored at room temperature. Samples were collected from: 1) cat loaf batter without vitamins, 2) cat loaf batter with vitamins, and 3) post retort food. Both batters (without vitamins and batter with vitamins) were stored in the refrigerator (4°C) until the processing was completed, then each representative loaf samples with and without vitamins were transferred to the freezer (-20°C). Batter samples 1 and 2 were stored frozen until analysis and finished product was stored at room temperature.

## **Sample Analysis**

After retort, the samples were allowed to cool over night before analysis. Four post-retort samples from each treatment were composited into a single homogenous blend and subsampled for proximate analyses, including moisture (AOAC Official Method 934.01), crude protein (AOAC Official method 990.03), crude fat (AOAC Official method 920.39), ash (AOAC Official Method 942.05), pH (691 pH-Meter, Metrohm, Herisau, Switzerland), and thiamine concentration by the procedure of EN 14122:2003; (K. Schafer and B. Kessler, 2010, at a commercial laboratory (Nestle Purina Analytical Laboratory, St. Louis, MO).

Composited samples of the meat mixture prior to vitamin addition, and after vitamin addition, were transported frozen and on dry ice. These samples were analyzed for thiamine concentration (Nestle Purina Analytical Laboratory, St. Louis, MO). Riboflavin (AOAC 944.33), Niacin (AOAC 944.13), and Pyridoxine (AOAC 961.15) were analyzed in duplicate by the vitamin supplier laboratory (DSM Nutritional Products, LLC; Parsippany, NJ). Pantothenic acid (AOAC 945.74), Biotin (Scheiner, and De Ritter, 1975; modified procedure of Wright, and

Skeggs, 1944), Folic Acid (AOAC 992.05), and Cobalamin (AOAC 952.20) were analyzed in single analysis (Covance Laboratory, Madison, WI). The proportion of B-vitamin retention was determined as final concentration divided by initial concentration and reported as percent retention.

### **Statistical Analysis**

Experimental treatments were arranged in a 2x3 factorial arrangement consisting of two container sizes (small and medium) and three types (cans, pouches, and trays). Results were evaluated by analysis of variance with main effect means and interactions separated using Fisher LSD and significant F using the GLM procedure of SAS (v 9.4; SAS Institute, Inc., Cary, NC).

### **Results and Discussion**

The pH and target moisture, crude protein, crude fat, and ash, did not differ within container size or type (Table 2). For thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid, and cobalamin, the vitamin targets were 3, 209, 90.9, 909.1, 136.4, 272.7, 1.60, 22.2, and 0.6 mg/kg, respectively (Table 3). The thiamine, riboflavin, pantothenic acid levels before retort were greater than the target, while the initial levels of niacin, pyridoxine, biotin, folic acid and cobalamin were slightly less than targeted but both were within the intended range. After processing, the overall average thiamine, riboflavin, and niacin were less ( $P<0.05$ ) than initial, and the pantothenic acid, pyridoxine, biotin, and folic acid were greater ( $P<0.05$ ) than starting values, with cobalamin similar between start and finish.

The main effect mean thiamine concentration was greater ( $P<0.05$ ) for small vs medium containers (2,359 vs 2,540 mg/kg) and the main effect means for pouches was greater ( $P<0.05$ ) for cans or trays (2,540 vs 2,359 and 2,274 mg/kg, respectively). However, when expressed as % retention, they did not differ for either main effect (overall average retention 69%). There

seems to be a wide variation in thiamine retention reported in the literature. The NRC (2006) suggests a retention of only 10%.; whereas, in the Tribble (2016) work using similar processing parameters, retention was lower than the current study at only 48% Cover et al. (1949) reported thiamine retention between 11-39% retention in canned beef and veal, Al-Khalifa and Dawood (1993) observed a 39.9% thiamine retention for roasted chicken, 63.6% for braised chicken, 46.7% for fried chicken, and 58% for poultry that was cooked in the microwave. Clearly, the process conditions and raw material has a great influence. The viscosity of the material and process method can have an influence as well. For example, Sharma and Lal (1998) reported variation in thiamine concentration when heating cow milk with different thermal processing methods ( $371.89 \pm 3.28 \mu\text{g/l}$ ,  $328.47 \pm 3.14 \mu\text{g/l}$ ,  $314.10 \pm 3.79 \mu\text{g/l}$ ,  $298.85 \pm 2.95 \mu\text{g/l}$ ,  $234.73 \pm 3.00 \mu\text{g/l}$  for raw, LTLT pasteurization, microwave boiling, conventional boiling, and in bottle sterilization, respectively;  $P < 0.05$ ). Markovich et al., (2014) analyzed commercial canned cat food products for thiamine levels sold out on the market and discovered 12 of the 90 cat foods analyzed had thiamine levels below Association of American Feed Control Officials (AAFCO) thiamine profiles. The range of thiamine levels from their study ranged from 0.45 to 352 mg/kg (Markovich et al., 2014). The wide range of vitamin degradation among various research projects would suggest that the decay of this vitamin is heavily influenced by processing parameters. Tight controls will help minimize sacrifice and decrease the likelihood of overt deficiencies. Our work would suggest that the differences in concentration due to container size and type, while interesting, disappeared when factored against starting amounts. Such that achieving the desired thermal process  $F_0=8$  for commercial sterility, and no more, is key to preserving thiamine in this canned food matrix. The longer a product is thermally processed in the retort using thermocouples to track a minimal lethality, the more nutrient degradation can occur. All

containers with thermocouples have to reach the target lethality. However, this causes over processing because half of the thermocouples in the containers will be at the target lethality and the other half will be above the target lethality causing over processing of some of the containers in the retort.

Riboflavin concentration and retention were not different for container size (average 86.9 mg/kg) or type (average 86.9 mg/kg). Cover et al. (1949) reported a similar riboflavin retention in their study (87-100%). Al-Khalifa and Dawood (1993) processed poultry and reported 90.4% riboflavin retention for roasting, 83.7% retention for braising, 70.1% for frying, and 116% for microwaving. These studies collectively showed that 15-30% of riboflavin is lost during thermal processing. Tribble (2016) found that riboflavin concentrations were higher in liver than chicken or fish in canned pet food. Rather than heat, riboflavin is sensitive to light exposure, processing, and storage conditions (Rickman et al., 2007). The riboflavin data from this study agrees with previous research that at under a controlled F-value of 8, the type and size of container does not impact the overall riboflavin retention.

The main effect mean for niacin concentration was higher ( $P < 0.05$ ) for small containers than medium containers (860.93 vs 810.39 mg/kg, respectively), but did not differ due to container size or type (overall average = 835.7 mg/kg). There was also no difference in relative niacin retention for either main effect (average = 97.4%, Tribble (2016) also observed no difference in niacin retention under similar processing parameters to this study. Cover et al. (1949) reported a high niacin retention in canned beef and veal (average=94%). This would suggest that in general there is minimal loss of niacin during the canning process. It seems to be relatively stable across most food processes. For example, pelleting, extrusion, and canning are

5-10%, 10-30%, and 5% losses, respectively (Riaz et al., 2009). Adding to this, it appears that container size and type do not impact niacin retention during thermal processing either.

The main effect mean of pyridoxine concentration was not different for either container size or container type (average = 179), nor was the proportions of pyridoxine retained different for container size (average=112%). However, pyridoxine retention was higher ( $P<0.05$ ) for trays than cans and pouches (124 vs 104.3 and 108.9%, respectively). Even though the pyridoxine concentrations differed, the overall pyridoxine retention exceeded 100 percent which would suggest that the raw ingredients contributed a significant proportion of the overall pyridoxine content and that the vitamin is otherwise relatively stable. At least in meat-based foods like a cat formula. However, Ives et al. (1945) reported only 63-72% pyridoxine retention in canned vegetables. While our study had a “gain” in pyridoxine, it is likely due to sampling or analytical variance, a release of bound vitamins during canning, or unaccounted background levels from meats.

The main effect mean for pantothenic acid concentration was higher ( $P<0.05$ ) in small vs medium containers (323.7 vs 309.0 mg/kg) but there was no difference in container type (average = 316 mg/kg). The pantothenic acid relative retention was over 100% for both container size and container type (average = 110%). Tribble (2016) also reported no difference in pantothenic acid concentration when increasing the amount of time cat food was processed in the retort (average = 55 min). However, they observed an increase in pantothenic acid concentration when the batter moisture was at 85%. But, they stated that this was most probably due to batching issues rather than any thermal process variable. Results from our study would tend to confirm that pantothenic acid is relatively stable in canned food process and not influenced by either container size or type.

Biotin was also not affected by container size or type (average = 1.6 mg/kg) and retention exceeded 100% (average = 107%). This is consistent with previous work in this lab. Ives et al. (1945) analyzed canned products with Biotin levels ranging from 0.2 ug/100g to 9.8 ug/100g in a variety of foods including green beans, carrots, corn, salmon, and spinach and biotin made up 68-99% of total solids in canned foods. The authors stated it is was the least water soluble of the B-vitamins. The overall percentages of biotin were high in canned food products. Nollet (2004) reported that biotin is stable in heat, light, and acidic conditions. Results from our study confirm only that biotin is stable to processing, as well as adds to the body of knowledge by demonstrating that container size and type do not influence the overall biotin retention during thermal processing in a retort.

The main effect means for folic acid concentration were higher for small than medium containers ( $P < 0.05$ ; 14.8 vs 13.5 mg/kg). However, there was no difference in folic acid concentration between cans, pouches, and trays (average = 14 mg/kg). The relative folic acid retention differed between small and medium containers ( $P < 0.05$ ), but the relative retention was over 100% for all container sizes. Further, there was no difference for the main effect means for container type. Research completed by Tribble (2016) reported no difference for folic acid retention when increasing the amount of time the canned cat food was cooked in the retort (1.04, 0.96 and 0.92 mg/kg for 45, 60, and 90 minutes, respectively) or when there was an increase in batter moisture (0.91, 0.85, and 1.16 mg/kg for 65%, 75%, and 85% batter moisture, respectively). The folic acid concentration in milk was stable by processing sterilization (Sharma and Lal, 1998). Given the very small concentration and sampling variation that can occur with nutrients like folic acid in the parts per billion, there may be some analytical variance contributing to the retention exceeding 100%. The current study tends to confirm there was no

impact on folic acid concentration or retention for cat food thermally processed in different container sizes or types.

The main effect of cobalamin concentration did not differ between container size or container type (average = 0.4 mg/kg), nor was there a difference in cobalamin retention for container size or type (average = 98%). This agreed with Tribble (2016) who found no difference due to increasing time for canned cat foods produced from a similar formula or process. However, Tribble (2016) did report that batter moisture might have had an impact, though they suggested this could have been due to batter production methodology and (or) sampling variation. Based on our work, cobalamin appears to be quite stable and not impacted by canned pet food processing parameters.

## **Conclusion**

Among the B-vitamins, thiamine appears to be the most dramatically impacted by processing. However, the results from this work had a greater retention (average=69%) than previous researchers had observed. In our work, smaller containers had greater retention for thiamine, niacin, pantothenic acid, and folic acid concentrations. This work suggests that heat penetration may have varying impact on each of the B-vitamin retentions with thiamine most affected. Careful control over thermal process parameters may be able to diminish a significant proportion of the losses observed for thiamine in the cat formulas sold in hermetically sealed thermal processed forms and that container size or type at a given heat penetration should not have an effect.

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## Tables

Table 1: Model wet cat formula with ingredient components to emulate commercial wet cat products out on the market

Ingredient	%
Water	To 100
Chicken	55.33
Rice, Brown	3.000
Egg Product, Dried	0.500
Carrageenan	0.050
Guar	0.350
Soybean oil	0.500
Potassium Chloride	0.500
Choline Chloride	0.092
Vitamin Premix	0.200
Mineral Premix	0.250
Taurine	0.041
Salt	0.035

Table 2: Loaf pH and proximate analysis of a model wet (cat) food thermally processed in two sizes (small and medium) and three container types (can, pouch, and tray)

Proximate Analysis	Container Size					Container Type				
	Target	Small	Medium	SEM	P-Value	Can	Pouch	Tray	SEM	P-Value
pH	6.50	6.49	6.45	0.02	0.2263	6.47	6.46	6.48	0.03	0.7904
Moisture (%)	78.0	78.05	78.10	0.13	0.7938	78.21	77.88	78.23	0.16	0.1649
Crude Protein (%)	35.0	31.58	32.32	0.23	0.0626	32.10	31.53	32.22	0.28	0.2520
Crude Fat (%)	44.3	47.42	47.98	0.24	0.1472	47.40	47.83	47.88	0.29	0.4811
Ash (%)	8.0	9.54	9.40	0.07	0.1873	9.48	9.41	9.53	0.09	0.6324

N=12

Table 3: Comparison of B-vitamin concentrations (mg/kg) of the wet (cat) food before and after retort to the target levels in the model wet cat formula (Dry Matter Basis; n=12)

Vitamin	Target	Before	After	P-value	SEM
Thiamine	3,209.0	3,451.8 <sup>a</sup>	2,391.5 <sup>b</sup>	<0.0001	51.44
Riboflavin	90.9	105.2 <sup>a</sup>	86.9 <sup>b</sup>	<0.0001	1.60
Niacin	909.1	862.7	835.7	0.2370	15.70
Pantothenic acid	136.4	286.5 <sup>b</sup>	316.4 <sup>a</sup>	0.0006	5.29
Pyridoxine	272.7	159.8 <sup>b</sup>	179.3 <sup>a</sup>	<0.0001	3.22
Biotin	1.60	1.51 <sup>b</sup>	1.62 <sup>a</sup>	0.0057	0.03
Folic Acid	22.2	11.5 <sup>b</sup>	14.2 <sup>a</sup>	<0.0001	0.31
Cobalamin	0.60	0.42	0.41	0.4836	0.01

a,b means within a main effect with unlike superscripts differ (P<0.05)

Table 4: Vitamin B concentrations (mg/kg) on DMB and vitamin retention (%) following processing.

	Container Size					Container Type				
	Target	Small	Medium	SEM	P-Value	Can	Pouch	Tray	SEM	P-Value
Thiamine	3,209	2,513 <sup>a</sup>	2,270 <sup>b</sup>	45.15	0.0090	2359 <sup>ab</sup>	2540 <sup>a</sup>	2274 <sup>b</sup>	78.21	0.0369
Retention		71.82	67.00	1.97	0.1343	67.00	74.82	66.40	3.41	0.0859
Riboflavin	90.90	88.12	85.69	1.70	0.3508	86.69	87.00	87.03	2.08	0.9918
Retention		81.35	84.62	3.38	0.5188	84.41	84.84	79.72	4.14	0.6452
Niacin	909.10	860.93 <sup>a</sup>	810.39 <sup>b</sup>	14.27	0.0463	832.90	849.78	824.31	17.48	0.6038
Retention		98.13	96.69	3.60	0.7870	94.09	104.89	93.26	4.40	0.1953
Pyridoxine	136.40	179.40	179.10	5.84	0.9724	172.08	176.22	189.46	7.15	0.2758
Retention		114.44	110.62	1.97	0.4752	104.31	108.96	124.33	4.34	0.0393
Pantothenic	272.70	323.71 <sup>a</sup>	309.04 <sup>b</sup>	4.01	0.0413	311.54	323.43	314.15	4.91	0.2734
Retention		109.78	112.04	2.64	0.5655	107.09	109.53	116.11	3.23	0.2051
Biotin	1.60	1.65	1.59	0.0406	0.3361	1.59	1.62	1.64	0.0497	0.7472
Retention		110.50	103.99	4.65	0.3602	103.95	109.95	108.78	4.14	0.6707
Folic Acid	22.20	14.83 <sup>a</sup>	13.50 <sup>b</sup>	0.37	0.0445	14.71	14.02	13.77	0.46	0.3844
Retention		133.99 <sup>a</sup>	115.65 <sup>b</sup>	3.37	0.0084	128.63	124.97	120.86	4.12	0.4583
Cobalamin	0.60	0.41	0.41	0.0136	0.7415	0.43	0.41	0.39	0.0167	0.3453
Retention		96.91	99.74	4.12	0.6451	104.52	96.38	94.07	5.05	0.3697

a, b means within main effect with unlike superscripts differ (P<0.05)

N=12

## **Chapter 3 - The effect of container size and type on lethality (F-values) during production of thermally processed wet pet foods.**

### **Abstract**

Most studies report heat penetration data in cans but lack information on other containers such as pouches and trays. Therefore, the objective of this work was to determine if heat penetration in various container types and sizes of pet food is similar. The experiment was conducted as a 2x3 factorial arrangement of treatments consisting of two sizes (small: 89-104 mL vs medium: 163 - 207 mL) and three container types (can, pouch, and tray). A model cat loaf formula was produced for all six experimental treatments in duplicate over a six-day period. The containers were commercially sterilized in a retort. with thermocouples attached to the center of representative containers (n=14) in each batch. Software was used to record the internal temperatures and lethality during processing. Data were analyzed using the GLM procedure of SAS (v. 9.4), and main effect means and interactions were separated by significant F test. There was an interaction ( $P < 0.05$ ) between container size and type for time to reach the  $F_0=8$ ; wherein, the medium can and tray had the longest time (45.5 and 46.3 min, respectively), the small can and tray, and medium pouch were intermediate (35.4, 36.0, and 32.0 min, respectively), and the small pouch the shortest time (36.0 min). There was no difference for either main effect of container type or size on heating lethality values (each main effect average  $F_0=10.3$ ). These heating differences may have an impact on the retention of heat labile nutrients like thiamine.

**Keywords:** Thermal processing, lethality, heat penetration, cat food

## Introduction

Conventional thermal processing of cans, pouches, and trays are common in pet food (Chen and Ramaswamy, 2006; Bohrer, 2011). However, there is limited information in pet food for processing of hermetically sealed low acid heat penetration studies on pouches and trays. Not only is there little information on the processing of canned pet products, but there is also little information on the relationship between processing and its effects on nutrient degradation. The FDA only has guidelines on how to make sure that food is commercially sterile (21 CFR 113) in order to inactivate spoilage organisms, enzymes, and any other harmful microorganisms (Anderson and Walker, 2011). Pet food companies focus on making pet food commercially sterile by conducting heat penetration studies but have issues retaining thiamine concentration in their products because there is little literature on the effects of thermal processing on thiamine retention. Thiamine retention issues have caused many recalls in the pet food industry due to thiamine degradation during thermal processing (FDA, 2017; FSA, 2017). Markovich et al. (2013) has confirmed this issue by randomly analyzing commercial pet food for thiamine content and found that many canned cat food products in the market have thiamine concentrations below the animals' requirements (Markovich et al., 2013). Ostensibly due to processing losses.

Therefore, it was our objective to determine if heat penetration thermal death curves are similar among the various container types and sizes by observing the thermal processing lethality ( $F_0$ ) in pet food processed in different container types and sizes.

## **Materials and Methods**

### **Treatments**

The experiment was conducted as a 2x3 factorial arrangement of treatments with two sizes (small and medium) and three container types (cans, pouches, and trays). All six experimental treatments were produced in duplicate over a six-day time frame in an industrial research and development center. The containers evaluated were a 3 ounce (88.7 mL) two piece can (209.5 x107 aluminum can with modified vinyl coating), a 3.5 ounce (88.7 mL) PET foil closure tray-thermoforming tub with 1.2mm polypropylene/EVOH 10%/ Polypropylene base sheet, and a 3 ounce (88.7mL) pouch made of 12 $\mu$ PET/adhesive/Al Foil 8 $\mu$ /adhesive/Polypropylene 70 $\mu$  with 95mm width x 138 mm height with 25 mm gusset. The medium containers were a 5.5 ounce (162.7 mL) two piece (307 x 109.3) aluminum can with modified vinyl coating, a 7 ounce (207 mL) thermoforming tub with base sheet of 1.4mm polypropylene/EVOH10%/Polypropylene, and a 6 ounce (177.4 mL) pouch made of polyester 12 $\mu$ /adhesive/Al Foil 7 $\mu$ /adhesive/Nylon 15 $\mu$ /adhesive/polypropylene 60 $\mu$  (140mm wide, 180mm high, with 25 mm gusset).

### **Model Canned Cat Formula**

A model cat loaf type formula was used for all six treatments in the study. All ingredients were sourced from commercial suppliers and the same lot code for each ingredient was used throughout the experiment. The model cat diet consisted of frozen mechanically separated chicken in frozen blocks (Protein Inc/BHJ, St. Joseph, MO), soy bean oil (preserved with mixed tocopherols, Columbus Foods, Des Plaines, IL), whole brown rice (Gulf Pacific Rice, Houston, TX), kappa carrageenan gum (Marcel Trading, Quezon City, Philippines), medium coarse guar gum (Intercolloid, Wembley, Middlesex, UK), dried egg product (Rose Acre Farms, Seymour,

IN), salt (Cargill, Hutchison, KS), choline chloride (SEM Minerals, Quincy, IL), mineral premix (Prince Agri, Quincy, IL) and taurine (Prinova, Carol Stream, IL). The vitamins were supplied as a standard premix including all vitamins except choline (DSM Nutritional Products, LLC, Parsipanny, NJ) and added into the batter during production at 10x normal levels.

### **Batter Production**

Frozen blocks of mechanically separated chicken were ground in a plant extractor with nine mm openings. After the mechanically separated chicken blocks had been broken down into smaller blocks, it was ground with a meat grinder and weighed into batches. Then a batch of the pre-weighed mechanically separated chicken and pre-weighed ingredients were placed into a horizontal jacketed mixer (Rietz, Evansville, IN). All ingredients were blended for five minutes until uniform while being heated. Sufficient amounts of batter (68 kg) were produced to fill 50 containers of the cat loaf product daily (2 batches per day). Batter moisture was analyzed with rapid methods ((CEM, Mathews, NC) after the batter was brought up to drop temperature (43.3°C). Water was added to adjust the moisture content of the batter. Once the batter had a moisture of 78%, the vitamin premix was added and the meat mixture was mixed for another 10 minutes. The complete meat mixture was homogenized (Karl Schnell Emulsifier, Winterbach, Germany) to pass a three and six mm diameter plate.

### **Equipment**

A horizontal jacketed mixer was used to mix and heat all ingredients into a liquid mixture (Rietz, Evansville, IN). The cans were sealed with a Ferrum Seamer (Schafisheim, Switzerland) and the pouches were sealed with a PMP mini vacuum seamer (PakSource Global, LLC., Sarasota, FL). The small trays were sealed with the Shinwa sealer (Shinwa, Japan) and medium trays were sealed with Raque Sealer (Raque Food Systems, Louisville, KY). Once the cans were

sealed with the seamer, the seams were inspected for seam thickness, width, height, body hook, cover hook, and tightness. The target thickness was 0.046-0.054  $\mu\text{m}$  width and height was 0.106-0.124 $\mu\text{m}$ , body hook was 0.072-0.092 $\mu\text{m}$ , cover hook was 0.065-0.085 $\mu\text{m}$ , and tightness was 80%. All small cans met the required target measures (Table 5). Compression tests were completed on trays and pouches and recorded before containers were placed into the retort.

### **Retort Process**

A horizontal spray FMC retort (JBT, Madera, CA) was used for the trays and pouches while the SJ Reid retort (Reid Boiler Works Inc. Bellingham, WA) was used for the cans. Time-temperature data for each batch of cat food processed in the retort was monitored with data software (Calsoft Systems, v. 5. 0. 5). Thermocouple probes (n=14) were inserted into separate containers identified numerically into the center of the container. Etzel (2014) discussed that thermocouples should be placed into the geometric center of the container because this is the area that takes the longest to reach the internal temperature of 121°C to meet commercial sterility. Once filled, each container was hermetically sealed and cans with thermocouples were attached to the retort data collection system. The other containers with the model cat loaf batter were placed in the retort simultaneous to the thermocouple probed containers.

Within each batch, containers were sealed, and placed into a retort basket before the batter temperature dropped below 43°C. Ballast containers filled with water were placed into trays. There were two trays of ballast containers above and below the test product to represent a normal mass in a production process. The amount of time each product was processed in the retort was dependent upon when all thermocouples reached the  $F_0 = 8$  at 121°C and 21 PSI. The software (v. 5.0.5., Calsoft Systems, Schaumburg, IL) was used to track the F-values (lethality) of each container with thermocouples until the target internal temperature reached a steady state.

Once all thermocouples placed in each product reached  $F_0 = 8$ , the retort was cooled with water and the thermocouples were used to monitor the F-values during the cooling phase to determine additional accumulation of heat for lethality. Time and temperature were recorded for each treatment.

### **Data handling**

Time and temperature data were plotted at thirty second intervals for each thermocouple during the thermal processing for each batch. Previous studies completed by a thermal process authority determined the thermal process conditions for the software including initial product temperature, heating temperature, type of retort safety, quality of final processed food, and packaging types (Awuah et al., 2007; Chen and Ramaswamy, 2007). The Arrhenius equation was used to calculate lethality (F-values) for the heating and cooling portions of the curve using first order kinetics. Goff (2017) defines lethality as the process in minutes required to kill a known population of microorganisms in a food matrix under a specific set of conditions. The equation used was  $L = 10^{\frac{T-TREF}{z}}$ . T stood for temperature at any given time. For the reference temperature (TREF), we used the reference temperature for our target spore forming organism (*Clostridium sporogenes*). Our reference temperature used in the study was 121.1°C (250°F) because we were analyzing a low acid food. A z-value is important for calculating thermal death time calculations and is defined as a temperature change that results in a tenfold reduction in a D-value (Awuah et al., 2007). For our z-value (temperature sensitivity of the microorganism) in the equation stated above, we used 10°C. Our sterilization value was a F-value of 8.0 minutes ( $z = 10^\circ\text{C}$ ) for *Clostridium sporogenes*.

## Statistics

The treatments were arranged in a 2x3 factorial arrangement with two sizes (small and medium) and three container types (cans, pouches, and trays). Results for the processing data were presented as means and standard deviations for the thermocouples in each treatment batch. Data were analyzed by analysis of variance with the main effect means and interactions separated using the GLM procedure of SAS (v. 9.4; SAS Institute, Inc., Cary, NC), means were considered different with  $P < 0.05$ .

## Results and Discussion

Seam integrity was evaluated for all cans (Table 5) and in each case they were within target range. This assures that all containers had a proper seam integrity to sustain the seal throughout the thermal process. All containers (cans, pouches, and trays) had vacuum or burst tests completed before being placed into the retort to assure the containers would not explode during thermal processing (Table 6). Burst tests were also completed post retort on trays and pouches. The seal was measured visually on cans before being placed into the retort to assure none of the cans were leaking (Table 6). Cans had a satisfactory vacuum, and the compression test for trays and pouches was within normal tolerance prior to, and after retort.

Berry and Pflug (1993) stated that both temperatures of the heating and cooling phase during thermal processing must be tracked to determine destruction of microorganisms such as *Clostridium botulinum* and *Clostridium sporogenes*. Studies conducted in industrial facilities use *Clostridium sporogenes* as their model organism. *Clostridium sporogenes* have a D-value of 0.7-1.5 minutes for meats (Fellows, 2009). Awuah et al. (2007) defines D-value as the amount of heating time to reduce 90% of the microorganisms in a population. For this study, we used the assumption of a 5D log reduction with a D-value of 1.6 minutes ( $5 \times 1.6 = 8$  for an F-value of 8).

Fellows (2009) reported a minimum lethality value of 6 for 153 mm diameter x178 mm height cans or lethality of 12 for 83 mm diameter x114 mm height sized cans. Awuah et al. (2007) stated that minimal lethality for low acid foods was  $F_0 = 3$  minutes (12D reduction for *Clostridium botulinum*). Of course, the initial bacterial level, physical parameters of the food matrix, container type, processing system, and conditions must be considered when determining the lethality for processing (Berry and Pflug, 1980). When conducting heat penetration studies, the type of thermocouple or sensor must be accounted for when calculating lethality. Awuah et al. (2007) conducted a study comparing remote sensors to thermocouples and found that container head space, retort rpm, sensor configuration, and sensor-mounting fixture geometry can affect the heat penetration data. In this case, all things were equal with the exception of container size and type.

All thermocouples were placed in the center of each container based on the assumption that the cold spot of a container in a stationary vessel was at the geometric center for conduction heating products (Potter and Hotchkiss, 1998). When running each treatment in the retort, temperature and time were recorded at 30 second intervals using data capture software (CALsoft 5.0.5; TechniCAL Inc., Metairie, LA) for each batch of the model canned cat food. The goal was to process everything for a total lethality of 5, 10, 15 or 25 minutes ( $Z=10^\circ\text{C}$ ) with time ranging from 80-120 minutes but Hendrik's results from the study were 5.3, 8.6, 17.2 and 24.2 with the lethality of 8.6 being under processed and 17.2 lethality value allowing for over processing of the product. However, this can be illusive. Hendriks et al. (1999) wrote that the minimum lethality for pet food should be 8 but anything under 8 increases the risk for spoilage issues from microorganisms and that most pet foods are normally heat processed to a lethality value range of 12-14. The main effect means in our study had heating F-values (lethality) ranging from 9.4 to

12.1 minutes; while the heating F-values (lethality) during thermal processing in the retort were similar (average  $F_0 = 10.1$ ). Stowe et al. (2016) observed no difference for the average time to lethality among tray shapes (oval, triangle, round and rectangle: 15.92, 13.53, 14.38, and 13.64, respectively) during the thermal processing in an agitating retort at 11 RPM rotational speed. However, Stowe et al. (2016) discovered that at 6 RPM rotational speed in an agitating retort the triangular shaped trays heated more slowly than the rectangular and round shaped containers. Stowe et al. (2016) suggested that the data from their experiment indicated that geometry of the container in the retort at higher rotational speeds had an impact on the rate to reach full heat penetration in a product. In our case, the cooking time for each treatment was dependent upon when all thermocouples in a batch reached the lethality of 8 minutes; thus, some of the containers could be exposed to a higher lethality value. From a process perspective, not only were the model canned cat foods cooking while coming up to temperature ( $121^{\circ}\text{C}$ ), but the product in the containers was also still cooking during the cooling process. The F-values (lethality) during the cooling time added an additional 3-6 minutes. During the study, there was over processing when the goal was to achieve a  $F_0=8$  but the total F-value (lethality) was actually 12 for the full duration of the retort process. While longer thermal processing times may provide added assurance for pathogen lethality, it can have a negative effect on heat labile nutrients. For example, the work of Hendriks et al. (1999) demonstrated that the protein quality of the food was negatively affected at the higher F-value. It stands to reason that more heat labile nutrients like the B-vitamin thiamine would be affected negatively as well.

When comparing the total processing time for each container type, cans and trays took more time ( $P < 0.05$ ) to reach the target lethality of  $F_0 = 8$  min compared to the pouches (40.5 and 41.2 vs 30.7 min, respectively). Medium sized containers took more time ( $P < 0.05$ ) to reach the

target lethality than small sized containers (41.3 minutes vs 33.6 min, respectively). An interaction between size and type had different total thermal process times in order to reach the target lethality of eight minutes ( $P < 0.05$ ). Medium sized cans and pouches took longer ( $P < 0.05$ ) to achieve the target time  $F_o = 8$  min than small cans and trays (45.5, 46.3, 35.4, and 36.0 minutes, respectively). Chen and Ramaswamy (2007) summarized in their paper that thermal conductivity will have more resistance on plastic containers than metallic containers. Perhaps this was a factor in our work.

When comparing cooling lethality for the treatments there was no difference for container size (average lethality = 4.6). However, cans had lower ( $P < 0.05$ ) cooling lethality compared to the pouches and trays ( $F_o = 3.3, 4.5,$  and  $5.9,$  respectively). But when summed together, total lethality values were similar for container size and type (average  $F_o = 14$ ).

When comparing the internal temperatures of the six treatments, it took ten minutes during the heating process to see a difference in internal temperature ( $P < 0.05$ ). Within ten minutes of thermal processing, the lowest internal temperature was  $58^\circ\text{C}$  in the 7 ounce tray. Whereas, the 3 ounce can and 85 gram pouch had the highest internal temperature ( $77^\circ\text{C}$ ) at 10 minutes of thermal processing. Again, when comparing at the 45-minute process timeline, where all of the treatments should begin the cooling process, both the medium can and tray had the highest internal temperature ( $120.5^\circ\text{C}$  and  $120.0^\circ\text{C}$ , respectively). At 45 minutes in the retort, the small pouch had the lowest internal temperature of  $87.5^\circ\text{C}$ . Comparing the six treatments after 75 minutes there remained a difference in internal temperatures ( $P < 0.05$ ). Considering the time and temperature relationship, the medium tray took the longest to heat up to temperature ( $121^\circ\text{C}$ ) and the longest to cool down, and the small pouch took the least amount of time to reach  $121^\circ\text{C}$ .

Research published by the NRC (2006) summarized that thermal processing effects nutrient stability in pet food. As noted previously, Hendriks et al. (1999) compared lethality values ranging from 0 to 24.2 and observed a decrease in digestibility of amino acids when the canned cat food as the lethality value increased. Tribble (2016) reported that the longer a can was in the retort, the lower the thiamine retention. This total lethality time may have a negative effect on thiamine retention for the containers that take longer processing times to reach commercial sterilization. This goes past just nutrient availability as Awuah et al. (2007) reported that canning reduced nutritional value and sensory attributes of foods. The form of the food stuff has an effect too. For example, Batifoulier et al. (2005) reported thiamine concentration decreased during the kneading process (3.0 ug/g) and then increased during the second fermentation (5.0 ug/g) and baking the bread (5.5 ug/g). Kwok et al. (1998) reported thiamine retention ( $75 \pm 6$ ) decreased over time when heating soymilk with experimental results of 12%, 32%, and 3% for thiamine processed 90°C, for 60 minutes, 120°C for 15 min, and 140°C for 30 sec, respectively. The first order kinetic parameters used in Kwok et al's, (1998) study for thiamine had a z-value of 30°C,  $k_0$  of  $1.13 \times 10^{11} \text{ min}^{-1}$  and an activation energy of  $97 \text{ kJ mol}^{-1}$  with results confirming thiamine sensitivity during processing. Of course, there is variability in thiamine concentration and retention during processing of different food matrices (Ryley and Kajda, 1992).

## **Conclusion**

The medium can and medium tray took the longest time to cook in the retort while the small pouch took the least amount of time to reach a lethality value ( $F_0$ ) of 8. Container size had no impact on lethality during cooking cycle of thermal processing, lethality during the cooling process, and total lethality. However, the types of containers had no effect on heating lethality or total lethality. Lastly, the both the small and medium sized tray took the longest to cool down. To

that end, both the pouch and tray had the largest cooling lethality values. The longer a container takes to heat during thermal processing, the more nutrient degradation a product will have. Container type had an impact on cooling lethality. The heating differences may impact availability of heat labile nutrients, like amino acids and vitamins.

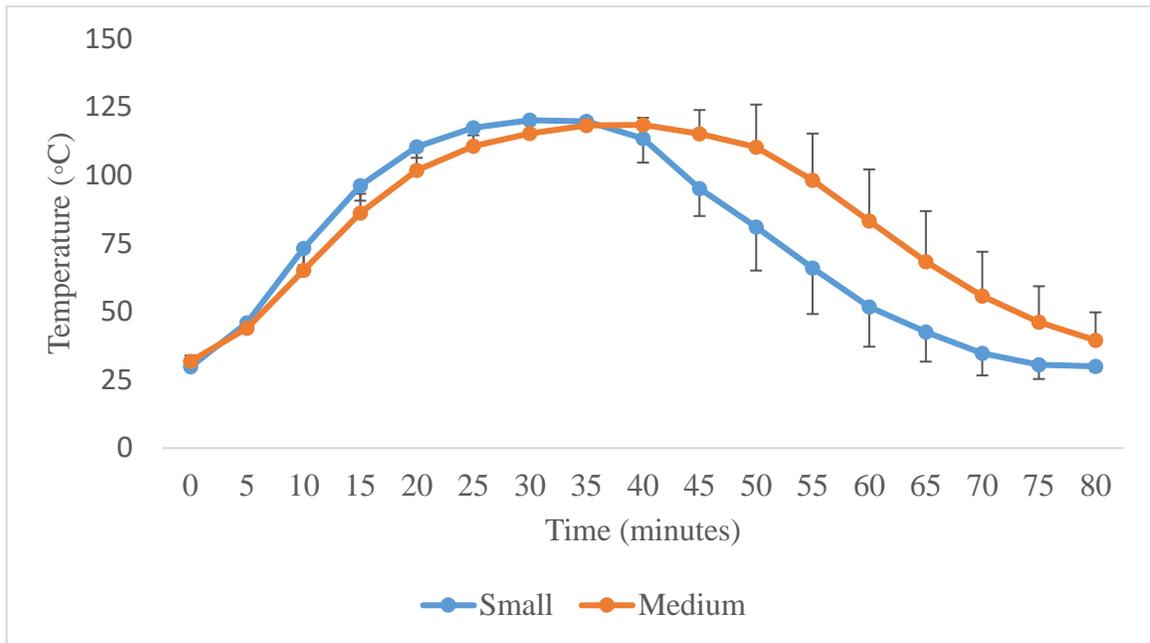
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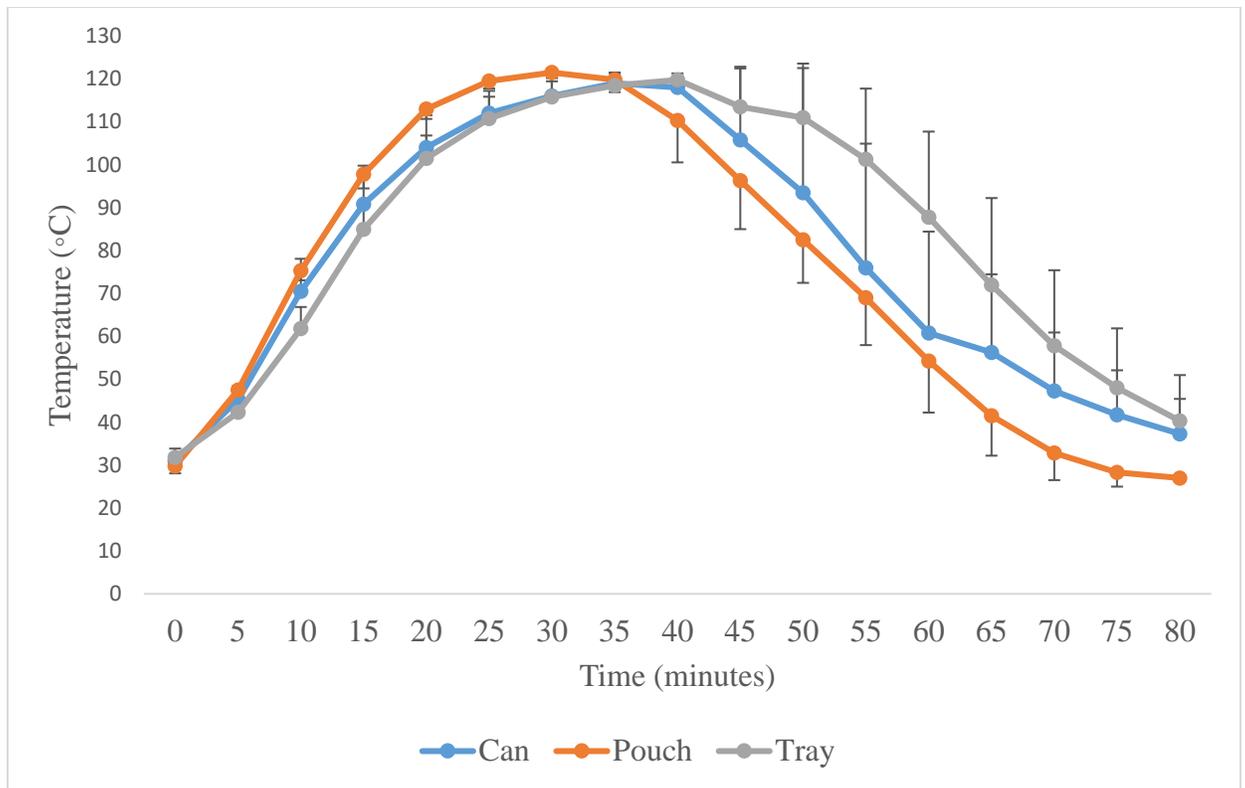
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## Tables and Figures



**Figure 3.** An average of thermocouple temperatures measuring the internal temperature of the model wet loaf product in small and medium sized containers (n = 14) over time (5 minute intervals) (mean  $\pm$  sd)



**Figure 4.** An average of thermocouple temperatures measuring the internal temperatures of the model wet cat loaf (n = 14) over time (five minute intervals) for container types (mean ± sd)

**Table 5.** Quality control measures to validate the small cans were hermetically sealed

	Target	3 oz can (mean $\pm$ std)	3 oz can (mean $\pm$ std)
Thickness ( $\mu\text{m}$ )	0.046-0.054	0.05 $\pm$ 0.00086	0.05 $\pm$ 0.00067
Width and height ( $\mu\text{m}$ )	0.106-0.124	0.116 $\pm$ 0.00083	0.116 $\pm$ 0.00204
Body hook( $\mu\text{m}$ )	0.072-0.092	0.08 $\pm$ 0.00117	0.078 $\pm$ 0.00213
Cover hook ( $\mu\text{m}$ )	0.065-0.085	0.071 $\pm$ 0.00078	0.075 $\pm$ 0.00336
Tightness (%)	80	90	90

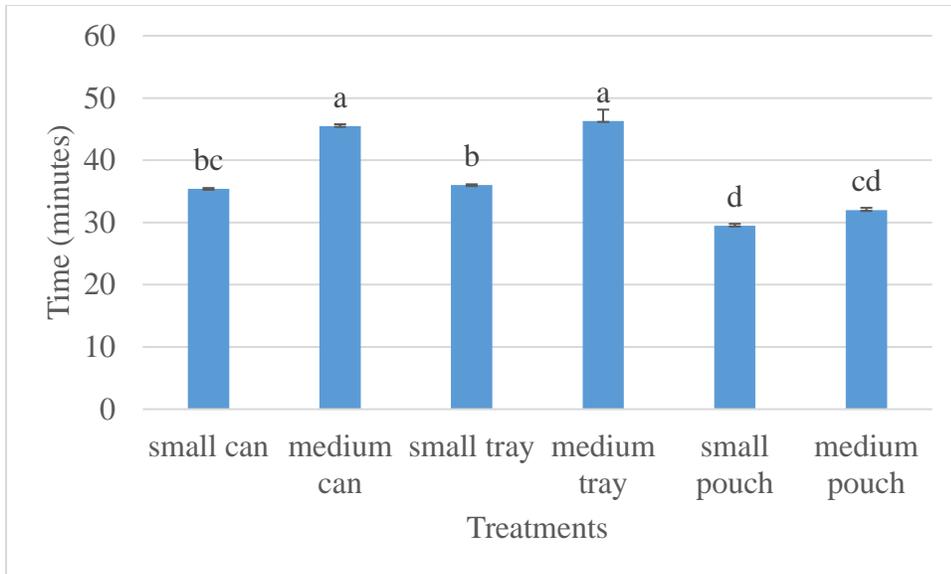
**Table 6.** Time (min) to reach target lethality ( $F_0 = 8$ ), lethality values during heating process, and lethality values during the cooling process

	Container size				Container type				
	small	medium	SEM	P-value	can	pouch	tray	SEM	P-value
Time (min)	33.6 <sup>b</sup>	41.3 <sup>a</sup>	0.9549	0.0005	40.5 <sup>a</sup>	30.7 <sup>b</sup>	41.2 <sup>a</sup>	1.1694	0.0004
Heating lethality (Fo)	9.5	11.1	0.6957	0.1458	9.5	12.1	9.4	0.8520	0.0868
Cooling lethality (Fo)	4.7	4.4	0.1196	0.1192	3.3 <sup>b</sup>	4.5 <sup>a</sup>	5.9 <sup>a</sup>	0.1464	<0.0001
Total lethality (Fo)	14.2	15.5	0.122	0.2640	12.7	16.7	15.3	0.150	0.0569

a, b means within a main effect with unlike superscripts differ ( $P < 0.05$ )

Table 7. Vacuum and Compression test on containers of wet cat food for quality control purposes

Analysis	Small Size Containers			Medium Size Containers		
	can	tray	pouch	can	tray	pouch
Vacuum (mm Hg)	6	-	-	6	-	-
Compression pre-retort (PSI)	-	10.9	21.2	-	7.3	13.6
Burst test post-retort (PSI)	-	10.2	21.2	-	7.4	13.1



**Figure 5.** Interaction of container size and container type for the amount of time (minutes) to reach the target lethality ( $F_0 = 8$ ; mean  $\pm$  sd)  
 a, b means within a main effect with unlike superscripts differ ( $P < 0.05$ )