The Effect of Processing and Ingredient Interactions on Thiamine Degradation in Canned Cat Food: A Modern Nutrition-Health Dilemma

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Abstract

Water soluble vitamins play an integral role in 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 the cat, like other carnivores, has 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.

Keywords: Thiamine, Nutrition, Cat, Canning, Retort

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. [1] reported that thiamine degrades due to heat processing, and alkaline conditions in canned meat products, and during storage. Since 2008, there have been numerous thiamine related recalls [2] affecting companies and products such as Iams Proactive Health Cat and Kitten Food (FDA, 2011), Nestle Well Pet LLC
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 [16]. Water soluble B vitamins also aid in the production of metabolic energy through the TCA cycle [14-17]. Thiamine acts as a coenzyme known as 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 [18]. Thiamine is important for carbohydrate metabolism, and plays a key role in brain development, function, maintenance, and communication processes [19,20]. In its cationic form, thiamine plays a role in the generation of acetylcholine, which stimulates cerebellum activity, and aids in nerve tissue repair [17].

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 [21]. 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 [3]. However, it was not correctly identified and published until 1936 [18]. Thiamine is produced naturally by organisms including plants, fungi, and bacteria [22]. There are several common pet food ingredients that contain thiamine in meaningful quantities and include yeast, peas, beans, wheat germ, oats, some meats (including pork and beef), and soy [14,23]. 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 [7]. Thiamine exists physiologically in various thiamine esters including thiamine monophosphate, thiamine diphosphate, and thiamine tri phosphate [24]. 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 food are thiamine hydrochloride and thiamine mononitrate. Thiamine mononitrate is used more often [15]. Thiamine hydrochloride is a sulfur-containing vitamin composed of a thiazole ring and a pyrimidine group joined by a methylene (Figure 1) [17,25,26]. Thiamine is
Thiamine requirements for cats and dogs were established by the National Research Council based on the limited amount of research in the target species [3]. Like most carnivores, cats have a relatively high thiamine requirement (5.60 mg/kg diet DM on a 4,000 Kcal diet); whereas, dogs as more omnivorous species require a lower level (1 mg/kg diet DM on a 4,000 Kcal diet) [30]. Cats in late gestation have a 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 [31-35]. 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 [32,33]. “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 [34]. Canned products are hermetically sealed to prevent unwanted growth of microorganisms with high heat resistance and it also prevents entry of pathogens that might produce toxins during storage [33].

For 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 [33,36]. 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 [37,38].

A heat penetration study is completed to collect data for calculating commercial sterility [37]. 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 [39]. The minimal F-value for pet food is 6 [37].

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. [32], reported that digestibility of amino acids in a rat model decreased as heat processing time increased in an autoclave. 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

Within the parameters of the regulations there are several processing conditions to consider when exploring mechanisms involved with thiamine degradation in a canned pet food application. Ariahu, et al. [40] measured the retention of thiamine in brine, sauce, and soup formulated with periwikle (Tympánostomus fusca tus) in a low acidic condition and processed at four different temperatures (0 to 40 minutes) and found that thiamine retention followed first order kinetics [40]. This means that the thiamine decay in the study was directly proportional to the time and temperature. This was confirmed by Durance et al. and Rehka et al. [36]. But does the degradation for thiamine occur simultaneous to commercial sterility? Perhaps not, as Ariahu et al. [40] 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 then thiamine degraded. But this is a very limited sampling and more research would help 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 [41-43]. Thiamine exists in several ester forms, but depending on the food matrix, it can be degraded into several different products. For example, Dwivedi, Wantanabe and Asahi [44] 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 thiocrome (a carbine 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, Rehka, et al. 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 food (pH 6.5, 15 minutes, and 120°C) [41]. In a study with canned salmon Durance, et al. [36] showed that thiamine loss was lower when the processing time decreased fifteen percent. The pH may also influence the results as thiamine stability was greatest between pH 2.0 and 4.0 chromatography to isolate the degradation of the molecule [45]. 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 byproducts. These include hydrogen sulfite, elemental sulfur, 4-methyl-5-(β-hydroxyethyl) thiazole, and other minor products [34]. 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 [46,47] 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-35 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 casein ate, 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 [46] believed that the fat containing systems protect the thiamine. Processing conditions prior to retort preparation of a canned cat food was evaluated by Trible [42] and it was determined that batter moisture affected thiamine content at 65%. As the retort time increased, thiamine retention decreased.

Storage

Commercial pet food is stored in a warehouse, on the store shelf, and in the pet owner’s home for an extended period of time - for a canned pet food this can exceed two years. As the product ages, thiamine and other labile nutrients degrade. This happens starting at the very moment of harvest. For example, Poel, et al. [48] 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 as 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 tetra phosphate [48]. Peñas, et al. [49] stored four different dehydrated vegetables (dehydrated garlic, onions, potatoes, and carrots) in conditions comparable to a
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. [56]. Not to be confused with sodium bisulfate which is an acidifier and was shown to not directly affect thiamine concentrations any different than hydrochloric acid in a canned cat food application [57].

**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 food, meat and fish play a prominent role in the recipe. One type of protein in particular that affects thiamine retention in pet food is fish. Fish and Shellfish contain a thiamine degrading enzyme, thiaminase. Hilker, et al. [8] 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 [58]. High concentrations of thiaminase were also found in the liver and intestines of Ukrainian fish [58]. The thiaminase enzyme degrades thiamine by a base-exchange reaction of the methylene group and pyridine moiety [58] (Figure 2). Thiaminase I comes from fish, shellfish, ferns, and some bacteria, and thiaminase II is found in some bacteria. Not only are these thiamine degrading enzymes derived from different sources, they 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 [59]. Fortunately, the thiaminase activity (because it is a protein) can be reduced with denaturing heat treatment [58]. In an extreme situation, the destruction of dietary thiamine could result from eating fish if the quantity of thiaminase was sufficient [58]. The processing of fish could reduce thiaminase activity in the final product [60]. Rendered fish meal used in pet food is extensively heated which should effectively inactivate thiaminase [53]. Thiaminase I can also be found in some bacteria such as P. thiaminolyticus and C. sporogenes [61].

**Figure 2: Chemical Base Substitution of Thiamine interacting with Thiaminase I (ACD/ChemSketch).**


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Thiaminase is typically measured either by spectro fluoro metric thiochrome assay [62] or by radiochemical method with 14C-thiazole [63]. The radiochemical method uses thiamine hydrochloride in the 12C-thiamine form and thiazole-2-14C-thiamine hydrochloride (14C-thiamine) to measure the 14C-thiazole activity [58].

Other Factors

Athar, et al. [64] 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 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 [64]. Athar, et al. concluded from the experiment 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. [23] 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. [23] 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 [65] 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 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. In order for thiamine to be measured 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 [66]. 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. 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 [66]. The HPLC is a rapid method that measures compounds using emission and excitation of wavelength [66]. 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 on 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. Thiamine is carried in the erythrocytes or is bound to free plasma [67,68].

Thiamine can also be measured by fluorescence with a fluorimeter [69]. 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, [17] 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 [17].

Summary

Even though thiamine deficiencies are a well-known problem in the pet food industry, most of the survival 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. This shortcoming for information in canned pet food products should be corrected.

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 interactions among the thiamine in pet food products.

Research has shown that using ingredients with low-oxidized fats and using preservatives like rosemary amines. 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.

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