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A Study on Disappearance of Nitrite in Cat Food

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ABSTRACT

The disappearance of nitrite in cat foods was examined. Nitrite is an additive that plays the role of a colorant. In the previous study, dog foods used for the same measurement showed that the concentration of nitrite did not change during storage for samples of the same type and lot, but decreased for cat foods. Based on experiments and discussions, it was speculated that nitrite was reduced by bacterial contamination, pH fluctuations, and other additives.

INTRODUCTION

Nitrite (precisely, nitrite root; nitrite ions themselves or species that dissolve in water to produce nitrite ions) are conventionally permitted for use as food additives (coloring agents) in processed meat products. However, since the toxicity of nitrite has been recognized[1, 2], a standard has been established for the residual concentration[3]. On the other hand, the regulation of nitrite concentration in pet food was initiated in 2015. One of the reasons for this is that pets being treated like family members, it is necessary to consider their health as in the case of humans[4]. In Japan, the number of children under the age of 15 is almost the same as the number of dogs and cats raised [5]. Furthermore, the number of dogs and cats kept as pets is much larger than that of other animals. Therefore, the establishment of standards for additives containing nitrite in pet food for dogs and cats, which was rare until now, is expected to become standard practice.

We have long been focusing on nitrite and ensuring whether its addition to pet food is in compliance with the regulations [the amount contained in marketed products [4)5)], and improvement of the official method (measurement method)][6] is under consideration. Previously, nitrous acid content in pet food for dogs and cats was measured using several tens of samples. At that time, it was thought that dry samples with low moisture content had non-uniform color and shapes, and the measurement data were likely to vary. Hence, the samples in the same package were divided among multiple measurements for analysis. Pet food is usually stored at room temperature and has an expiration date of more than one year. For this reason, it was thought that the concentration of the ingredients was stable during storage. However, semi-moist- and wet-type pet foods with high water content when opened once showed a decrease in water content, in addition to mold generation and color fading. Therefore, these foods are stored frozen at -30 ^{0}C until use for measurements. Strangely, the nitrite concentration showed a decrease in cat foods even during frozen storage, in contrast to the case of dog foods. Therefore, present study focused to reveal the reason for this difference based on the experimental results presented in this paper.

Overview of nitrite

Nitrite compounds (mainly sodium nitrite) are accepted as food coloring agents in Japan. The coloring mechanism involves combination of the nitrite part (NO_2) with myoglobin to impart red color and improve the appearance of the food[7]. However, high nitrite concentration may lead to methemoglobinemia[2,4] and gastric cancer[1] and hence, the residual amount (concentration remaining when ingested as food, but not at the time of manufacture) is regulated. The residual amount is reported to be about 1/6 to 1/8 depending on the storage conditions[4]. The official method for nitrite content measurement is the diazo-coupling method (described in "Method"). Unfortunately, there are variations in the calibration curve and extraction of nitrite from the food is time-consuming. It is better to perform all measurements at once, but in certain scenarios, this process is time-consuming and difficult. In Japan, the usage of nitrite is permitted in processed meat products such as sausages and ham, as well as fish eggs such as salmon roe and casserole, and the standard value (maximum allowable concentration) for the residual amount is 70 and 5 mg/kg, respectively. Regarding pet foods, a standard of 100 mg/kg has been tentatively established in Japan under the Pet Food Safety Law, but the Association of American Feed Control Officials (AAFCO) prescribes a standard of 20 mg/kg. We believe that stricter standards are necessary because pets continue to consume more the same food than humans, and the latter standard is more appropriate. Nitrite is a very unstable substance that is converted into nitrate when chemically oxidized and into ammonium when reduced.

MATERIALS AND METHODS

Apparatus

The following instruments were used in the experiments: a PA64C precision balance (Ohaus, Tokyo); a thermostatic bath (Thermominder SDminiN, Taitec, Saitama, Japan); a micro cooled centrifuge, model 3780 (Kubota, Tokyo); a U-5100 spectrophotometer (Hitachi, Ibaraki, Japan); and a Simplicity water purifier (Merck Millipore, Billerica, MA, USA).

Reagents

Sulfanilamide solution was prepared by dissolving 0.5 g of sulfanilamide (special grade, Wako Pure Chemical Industries, Osaka, Japan) in 100 ml of hydrochloric acid (special grade, Wako Pure Chemical Industries). A naphthyl ethylenediamine solution was prepared by dissolving 0.12 g of *N*-(1-naphthyl)ethylenediamine hydrochloride (special grade, Wako Pure Chemical Industries) in 100 ml of water and stored in the dark. The nitrite standard stock solution was prepared by dissolving 0.023 g of sodium nitrite (special grade, Wako Pure Chemical Industries) in 50 ml of water, and this was further diluted 500-fold with water to obtain the nitrite standard solution (0.6 μg/ml nitrite). To prepare an ammonium acetate solution, 10 g of ammonium acetate (special grade, Wako Pure Chemical Industries) was added to 100 ml of water. A sodium hydroxide solution was prepared by adding 2 g of sodium hydroxide (special grade, Nacalai Tesque, Kyoto, Japan) to 100 ml of water. A zinc sulfate solution was prepared by adding 21.4 g of zinc sulfate heptahydrate (special grade, Nacalai Tesque) to 100 mL of water. Ultrapure water (18 MΩ・cm) was used in all experiments.

Samples

Commercially available cat foods listed in Table 1 were used as samples. There were 8 kinds of wet food (water content: 75% or more) that could be purchased in different lots in April and October 2013. The purchased pet foods were promptly measured for the first time, and the remainder was divided into small plastic bags of about 10 g each and stored frozen at −30°C until use. When a frozen sample was used for measurements, it was thawed in a refrigerator at 4°C just before use, and then, the sample weight was measured using a precision balance.

Pretreatment of cat food samples

We used the Standard Methods of Analysis for Hygienic Chemistry, 2015 edition[3], with the pretreatment scale set to 1/4. The treatments were carried out more than twice for each sample and measured thereafter. Approximately 2.5 g of the sample was weighed, added to a small amount of warm water (80^{0}C), homogenized using a mortar and pestle, and then transferred to a 50 ml tube. The equipment used for homogenizing was rinsed lightly with warm water, and the liquid was added to the tube to obtain a volume of 30 ml. Next, 2.5 ml each of the sodium hydroxide solution and zinc sulfate solution were added, and the tube was shaken well and heated in a water bath set to 80^{0}C for 20 min. The tube was then cooled to room temperature, and 5 ml of ammonium acetate solution were added, followed by enough water for a final volume of 50 ml. After centrifugation at 3000 rpm for 5 min at 4^{0}C, the supernatant was filtered through filter paper (Advantec Toyo, Tokyo) to obtain a transparent test solution.

Measurement of nitrite concentrations

Measurements were conducted with samples 1/10 of volume as compared with that in the original method[3]. About 0.1 ml of sulfanilamide solution and 0.1 ml of naphthyl ethylenediamine solution were added to a 2 ml aliquot of each test solution. After allowing the solution to stand at room temperature for 20 min, the absorbance (A1) was measured at 540 nm. To determine the turbidity of the filtrate, we measured the absorbance of the filtrate mixed with water (A0). We used the absorbance obtained from the calculation (A1–A0) to obtain the nitrite concentration from the calibration curve. The calibration curve was prepared by diluting the nitrite standard solution with water and analyzing 2 ml each solution corresponding to 0, 0.12, 0.24, 0.36, 0.48, and 0.6 μg/ml nitrite in the same manner as that for the test solution. Results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Causes of decreased nitrite concentration

As shown in Figure no. 1, the concentration of nitrite decreased with extension of the storage period, but interestingly the concentration of nitrite in some foods increased. Although not discussed in this paper, the nitrite concentration in dog foods did not change due to freezing, indicating almost no degradation during thawing. The formation of nitrite and its decomposition (to ammonia) may influence the variation in nitrite content, depending on temperature changes and enzymatic reactions in bacteria. It is considered that nitrate is converted to nitrite by the reductase in the contaminating bacteria. This enzyme or the bacteria provide a pH level suitable for reaction or growth, and the optimum pH for many kinds of bacteria is near neutral. Moreover, in the case of human foods, a buffer called a pH adjuster is often contained[9], and this suppresses the growth of bacteria by lowering the pH to about 4. It has not been confirmed whether this pH adjuster is included in the pet foods used this time (it is only known that they contain phosphorus). On the other hand, the main factor for degradation is a decrease in pH. As can be seen from the measurement method, nitrite is relatively stable at alkaline pH but easily decomposes under acidic conditions[10]. More fundamentally, the difference is that dogs are omnivorous, but cats are carnivorous (favor carcass). Although it is difficult to examine the meat content of the sample used in this study, it is highly possible that the ratio of meat or fish is high and the corroded one is used. When fish and meat are corroded, the histidine contained in may be changed to histamine[11,12] and alkalized[13]. The possibility that a small amount of vegetables and a pH adjuster was included to compensate for this cannot be denied[9]. At present, there is no provision for the amount of the additive, so it is likely that the manufacturer has added an additive to reduce the pH gradient (to return to acidity) in their own recipe. As a result, it may be accompanied by decomposition of nitrous acid. For canned foods in humans, it is common to place them in cans and sterilize them at high temperatures. However, it is unlikely that pet food is always made with such a process and cost. For this reason, there may be an environment where live bacteria are likely to enter. It is only a hypothesis, but it is thought that there are many vegetables in dog foods. Even if it rots by bacteria, histamine is generated from meat and fish and alkalized, and the sugar contained in vegetables is decomposed to acidify (sour). So the pH is balanced[13]. Therefore, there is no need to add a pH adjuster, and decomposition may not occur easily.

Changes in nitrite concentration due to lot-to-lot differences

Table no. 2, shows the difference in nitrite concentration depending on the purchase date (lot). It is unlikely that the manufacturing method is changed depending on the season of manufacture. Therefore, this result is considered to reflect the effect of temperature at the time of transportation and storage at the store before the consumer purchases, or the type and amount of impurities at the time of packing. In terms of human food, imports from overseas are mainly transported by ship. This takes more than a few months for the transport. The goods for humans that need to be refrigerated should be refrigerated and transported during that time, but pet foods are likely not transported at room temperature. The samples purchased this time are available in April and October. The samples obtained in April can be manufactured and transported a few months ago, and those obtained in October can be considered as well. Hence, it is the October portion that is likely to have been exposed to higher temperatures. As indicated earlier, nitrite is unstable and may be oxidized by air, and the effects of microbial decomposition are expected to be more affected in the summer when the temperature rises to around 40°C. It can be interpreted that those results were reflected.

CONCLUSION

In pet foods, there are few standards for the types and amounts of additives even if only those distributed in Japan are considered. Due to the gradual display obligations on sales products, it is difficult to purchase products that consumers may be able to identify. All additives are to be labeled[14], but no concentration or amount is required. If the display area is small, it can be omitted. First of all, urgent countermeasures will be necessary in this regard. Because smartphones are widely used in Japan, getting information about detailed ingredients in mobile phone apps may be an improvement. Whether a pet should be treated as an animal close to a person or a thing is interpreted differently by law. At least the buyer should be willing to buy a better pet food for their pets, so there should be a strategy for that.

In this study, many factors were also considered based on hypotheses. Actually, we did not measure the pH of pet food and if we were considering transportation, we should have compared foods manufactured in Japan. These are future research challenges.

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Table No. 1: Pet foods used in this study

Sample No.	Manufacturer	Country of origin	Content (meat, seafood, and vegetables)*1	Package form*2	Nitrite addition*3
1	A	America	chicken, turkey, pork, spinach, rice	can	
2	A	America	chicken, pork, seafood, carrot, tomato, spinach	can	
3	B	Thailand	seafood	pouch	
4	B	Thailand	seafood	pouch	
5	B	Thailand	chicken, seafood	pouch	
6	B	Thailand	seafood	pouch	
7	C	Thailand	chicken, beef, seafood	pouch	○
8	C	Thailand	chicken, beef, seafood	pouch	○

*1: The contents displayed on the container were transcribed. More details are unknown.

*2: The pet food used is classified as a wet food (water content: 75% or more), stored at room temperature until opening, and the expiration date is 1-2 years.

*3: Cat foods labeled as containing nitrites.

Table No. 2: Change in nitrite concentration due to differences in purchase month

Sample No.	Purchase month	Nitrite (mg/kg)
1	April	0.637 ± 0.197
	October	0.414 ± 0.028
2	April	0.410 ± 0.167
	October	0.360 ± 0.101
3	April	0.389 ± 0.088
	October	0.036 ± 0.001
4	April	0.421 ± 0.002
	October	0.040 ± 0.004
5	April	0.604 ± 0.300
	October	0.138 ± 0.003
6	April	0.580 ± 0.105
	October	0.124 ± 0.038
7	April	1.186 ± 0.151
	October	1.020 ± 0.080
8	April	1.192 ± 0.010
	October	0.564 ± 0.019

The sample was purchased in 2013, and the first measurement was performed immediately after purchase. The measurement was performed twice at the time of purchase and one week after, and the average value ± standard deviation was shown.

The sample number is the same as in Table 1.

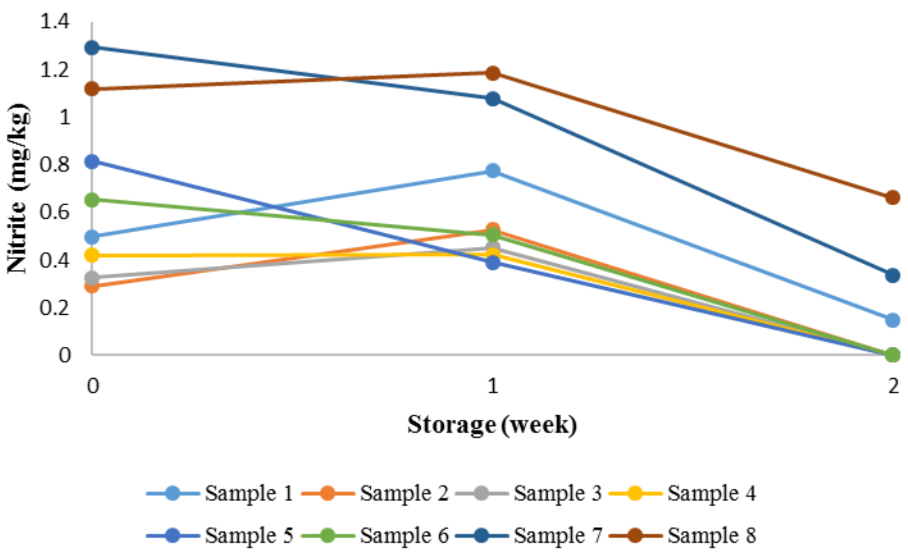

Figure No. 1: Changes in nitrite concentration by storage

The storage period of 0 weeks indicates data measured immediately after purchase.

The sample number is the same as that in Table 1.