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Data Report

Acrylamide in dog food

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ABSTRACT — This study reported acrylamide, a carcinogenic substance produced by the Maillard reaction, in dog food, as a part of understand the mechanism of canine carcinogenesis. These results indicated that the average concentrations of acrylamide in dry, retort, and canned dog food were 39.6, 11.0, and 10.7 ng/g, respectively. Among the three, dry dog food exhibited significantly higher concentration. The daily intake of acrylamide by dogs was calculated to be 590 ng/kg/day, which is approximately four times higher than that of humans.

Key words: Acrylamide, Dog food, LC–MS/MS, Maillard reaction, QuEChERS

INTRODUCTION

Although the number of dogs has decreased in recent years (Anicom, 2017), more than 80% dogs have been confined indoors. Moreover, the significance of their existence has changed from being mere pets to being family members, and a shift has occurred in canine diet, that is, from human food to dog food, which considers nutritional balance. Thus, a variety of dog food has become available based on breeds, age, and even therapeutic diets. To ensure the safety of dog food, the "Law Concerning the Safety of Feed for Pet Animals" (Ministry of the Environment, 2008) was enacted in 2010. This law was passed in response to the deaths of numerous dogs and cats caused by melamine-contaminated pet food in North America (Oshima, 2009). The law currently regulates not only melamine but also two additives, five pesticides, and ten contaminants. However, previous studies have also reported other harmful ingredients, such as aflatoxin, arsenic, and cadmium, present in dog food (Bischoff and Rumbeiha, 2012; Kim *et al*., 2018). Although scholars have reported tumors as the most common cause of canine mortality among dogs in Japan (Anicom, 2017; Inoue *et al*., 2015), the impact of environmental factors, such as diet, remains unclear.

By contrast, environmental factors have significantly contributed to cancer development among humans, (Lichtenstein *et al*., 2000) smoking and diet have exhibited significant effects (Cancer Causes and Control, 1996). Several studies have reported on the formation of heterocyclic amines and acrylamide in human food upon heating (Aaslyng *et al*., 2013; Puangsombat *et al*., 2012). Among them, Mottram *et al*. (2002) and Stadler *et al*. (1994) have found that acrylamide is formed by the Maillard reaction between asparagine and reducing sugars in raw materials when heated at high temperatures (> 120°C) through processes, such as frying, baking, and roasting. In Japan, Yoshida *et al*. (2005) detected acrylamide in potato chips and became a major social problem that a carcinogen was contained in a snack that many people eat on a daily basis. In particular, Johnson *et al*. (1986) provided evidence that acrylamide induc-

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es testicular mesothelioma and other tumors in rats after oral administration, which the International Agency for Research on Cancer (IARC) of the World Health Organization classified as Group 2A or probable carcinogens (World Health Organization, 1994).

In manufacturing dry dog food, the production process includes heating various powdered feed ingredients which are mixed with water to induce homogenization. The samples are then mixed and sterilized under high-temperature and high-pressure conditions in the extrusion process. Dog food are extracted from holes, cut into small pieces, and then heated and dehydrated under hot air to remove excess water. In the same manner, retort and canned food are subject to high-temperature and high-pressure processing for sterilization before sealing (Ministry of Agriculture, Forestry and Fisheries, 2015). Despite varying conditions, both processes involve heat treatments. Given the use of raw materials, the study infers that acrylamide formation during processing is possible.

In this regard, the study aimed to detect and measure carcinogen-related substances in dog food to elucidate the mechanism of carcinogenesis in animals. Previous studies have argued that acrylamide formation occurs during heat treatment (Mottram *et al*., 2002; Stadler *et al*., 1994). In other words, we measured the content of acrylamide in dog food, which has been reported to be produced by heat treatment, and compared acrylamide in dry type, retort type, and canned food.

MATERIALS AND METHODS

30 dry, 13 retort, and 8 canned dog food variants from 10, 6, and 2 companies were purchased as samples, respectively. A total of 51 samples were purchased from a nearby market in 2019-2020.

The dry and wet variants of retort and canned food were cut and ground by using a mill mixer (AS ONE CORPORATION, Osaka, Japan) to prepare samples for extraction. The extraction of acrylamide from samples was performed as previous study (Mastovska and Lehotay, 2006). In particular, 10 g of cut and ground samples was collected in a 50-mL centrifuge tube. Then, 50 μL (1,000 ng) of 20 μg/mL D_3-acrylamide (2,3,3-D_3, Cambridge Isotope Laboratories, Inc., Andover and Tewksbury, MA, USA) standard solution, 5 mL of hexane (special grade reagent, Fujifilm Wako Pure Chemicals, Osaka, Japan), and 10 mL of distilled water (Fujifilm Wako Pure Chemicals) were added to the samples and stirred in a lab mixer. Further, 10 mL of acetonitrile (for liquid chromatography analysis, Fujifilm Wako Pure Chemicals) was added, and the mixture was stirred. The centrifuge tube was then placed horizontally on a shaker (NTS-4000B, TOKYO RIKAKIKAI CO, LTD, Tokyo, Japan) and shaken at 160 rpm for 30 min. A homogenizer (T25 digital ULTRA TURRAX, IKA®-Werke GmbH & CO. KG, Staufen, Germany) was used to homogenize the sample for 1 min. A packet of QuEChERS Extract Tubes for Acrylamides (Agilent Technologies, Santa Clara, CA, USA) was added, mixed vigorously for 1 min, and centrifuged at 5,000 rpm for 5 min. From the acetonitrile layer (middle layer), 1 mL was transferred to a vial of Bond Elut QuEChERS AOAC Dispersive SPE (Agilent Technologies), mixed using the lab mixer for 30 sec, and centrifuged at 5,000 rpm for 1 min. A sample from the supernatant was obtained for LC-MS/MS (LC800, GL Science Co., Ltd., Tokyo, Japan and 3200 QTRAP, AB Sciex, Framingham, MA, USA), measurement. The operation was performed independently from each sample to prepare two samples for measurement for each sample.

LC-MS/MS with electrospray ionization in a multiple reaction monitoring mode was used to measure acrylamide content. The set mass numbers (mass-to-charge ratio) were 72.087 > 55.100 and 75.085 > 58.100 m/z for acrylamide and D_3-acrylamide, respectively. The mobile phases were 2.5% methanol solution containing 0.1% formic acid (solution A) and methanol containing 0.1% formic acid (solution B). Linear gradient analysis was performed with A:B = 100:0 at 0 min and A:B = 0:100 at 5 min. The flow rate was set to 200 μL/min at a column temperature of 40°C.

A Tukey-Cramer test was used to determine significant differences among three mean values of acrylamide concentrations.

RESULTS AND DISCUSSION

In the manufacturing process for dry food, the product was subjected to heat during the extrusion and drying processes (Ministry of Agriculture, Forestry and Fisheries, 2015). During extrusion, the product was subjected to high pressure and temperature for sterilization and dried under hot air to remove excess moisture. Furthermore, acrylamide production is expected during these processes because the raw materials include meat and grains. Therefore, acrylamide was detected in 51 samples of dry, retort, and canned dog foods (Fig. 1), particularly in all dry samples (lower limit of detection: 5 ng/g) with the mean and median values of 39.6 and 38.3 ng/g, respectively. A significant difference was observed

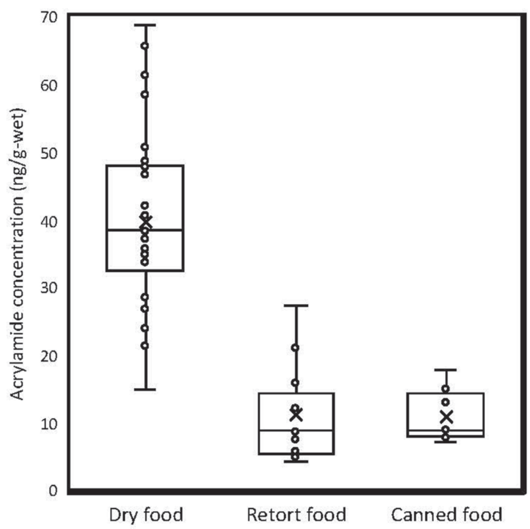

Fig. 1. Comparison of acrylamide concentrations between dog food types. ○: each date. Top of the error bar was maximum value of acrylamide in dog foods. Bottom of the error bar was minimum value of acrylamide in dog foods. ×: average value of acrylamide in dog foods. Top line of box was showed 75% of data. Middle line of box was showed median of data. Bottom line of box was showed 25% of data.

between dry and retort food and between dry and canned food ($p < 0.01$). However, no significant difference was observed between retort-type and canned food. The heating method used for these two types of food (retort-type and canned food) were boiling, which less likely produces acrylamide.

In a previous study, although the number of samples was small (i.e., four samples), the level of acrylamide detected in dry food was 106-358 ng/g (Veselá and Šucman, 2013). In the present study, acrylamide concentrations ranged 14.7-68.6 ng/g in dry food, which was lower than that in the previous report. This difference lacks a clear definition because of variations in the analytical method, the survey year, country, number of surveys, and brands. However, the current study infers that such difference may have been caused by an improvement in heating conditions in various manufacturing processes because the survey was conducted approximately 10 years ago. To understand the trend of acrylamide concentration, the regular and continuous monitoring of acrylamide levels in dog food is necessary.

Various studies have examined acrylamide levels in human diet. WHO had surveyed acrylamide concentrations in food products from Norway, Sweden, Switzerland, the United Kingdom, and the United States and found that acrylamide was detected in most foods at levels ranging from several tens to several thousand ng/g (World Health Organization, 2002). In Japan, the National Institute of Health Sciences reported that acrylamide levels in Japanese human food products ranged from less than the detection limit (solid sample: < 9 ng/g, liquid sample: < 3 ng/mL) to several thousand ng/g. Furthermore, acrylamide concentrations in Japanese human food products were at the same level as those in other countries, particularly in baked and fried foods (National Institute of Health Sciences, 2002). Acrylamide concentration in dog food in the present study was at the same level. And, acrylamide in dogfood may have been also generated during heat treatment in the manufacturing process. For humans, a balanced diet with various food types is recommended from the perspective of cancer prevention, and the average intake of acrylamide is estimated to be 154-166 ng/kg/day (National Institute for Environmental Studies, 2016). But, in for dogs, dog food is given as a comprehensive nutritional diet, and in contrast to humans, dogs are recommended to feed only on dog food. For example, if a 20-kg adult dog eats 300 g of dry food per day, then the daily acrylamide intake would be 11.9 µg/day (590 ng/day/kg), which is four times higher than that of humans. It was considered that this difference can be attributed to low body weight and a diet with approximately similar acrylamide levels. Feeding boiled food, such as retort food and canned, in addition to dry food may be effective in reducing the risk for carcinogenesis by acrylamide in dogs. Although the IARC classified acrylamide under group2, acrylamide intake and the risk of carcinogenesis in humans have recently been re-evaluated and reported to be less likely to cause cancer (Pelucchi *et al*., 2015). The results of carcinogenicity studies of acrylamide in dogs have not been reported, acrylamide induction of benign tumors of the thyroid and mammary glands and mesothelioma of the testes has been reported in rats (Friedman *et al*., 1995). Therefore, future studies should elucidate whether daily acrylamide intake increases the risk of carcinogenesis among dogs.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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