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Analysis of Acrylamide in Processed Foods Obtained from Korean Markets

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ABSTRACT – The purpose of this study was to determine the level of acrylamide in various processed foods, some of which were chosen because they were known to contain an excessive amount of acrylamide. A total of 190 food products based on steamed rice, cereals, and potato chips were purchased from retail markets and analyzed with the LC-MS/MS method. Acrylamide was found to be widely distributed in all of the foods. The fried potato chips contained the highest levels of acrylamide, at 470–3,572 μg/kg; these were lowered to 38–633 μg/kg by vacuum frying. The median concentration of acrylamide was higher in snacks containing potato (448 μg/kg) than in those with no potato (133 μg/kg). The concentrations of acrylamide were 2–96 μg/kg in Korean staple foods, 48–61 μg/kg in bone-extract soups, and 0–57 μg/kg in Bulgogi sauce. These results suggest that the components of processed foods and the processing methods are important determinants of acrylamide formation.

Key words: acrylamide, Korean foods, processing, potato, vacuum frying, LC-MS/MS

Introduction

On April, 2002, the Swedish National Food Authority (SNFA) announced that researchers from Stockholm University had discovered high levels of acrylamide in the following starch-based foods that had been cooked at temperatures greater than 120°C; potato chips, French fries, cookies, cereals, and breads. Similar results have been subsequently reported for other countries, including The Netherlands, Canada, United Kingdom, Switzerland, Germany, and Japan. For example, preliminary studies conducted in the laboratories of Health Canada confirmed the Swedish results.[1–7)] Acrylamide is a byproduct of the processing of foods at high temperatures, and is usually not present before such processing. Acrylamide formation is particularly likely to occur in carbohydrate-rich foods.[1,7,8)]

Acrylamide is considered as a potential human carcinogen.[9)] However, some of the foods now reported to contain acrylamide have been consumed for many years, and there is no clear evidence that acrylamide in foods causes cancer in humans.[10)] Therefore, the impact of acrylamide in foods requires further investigation.

The prospective risk assessment of acrylamide first requires its levels to be determined in various processed and/or cooked foods.[3)] There are few reports on the distribution and content of acrylamide in representative foods consumed in Korea. The purposes of this study were to determine the occurrence of acrylamide in processed foods (including characteristic Korean foods purchased from markets) and the processing methods that would minimize acrylamide concentrations.

Materials and Methods

Samples

Fried potato chips and snacks were first selected, because the SNFA indicated these as the greatest contributors to acrylamide exposure. Secondly, representative foods in Korea such as steamed rice were selected. For all selected sample groups, the five most popular brands with different production codes were selected. When acrylamide was detected in the selected samples, more brands and production codes of similar food types were analyzed. Sample groups were sorted according to the processing temperature (with a cutoff of 120°C) and

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Table 1. Processed foods in which acrylamide content was determined

Processing temperature	Groups	Processed foods
High (≥ 120°C)	Potato chips	Potato chips, vacuum-fried potato chips
	Snacks	With or without potato
	Cereal products, coffee	Bread, hardtacks, breakfast cereals, coffee
	Sugar products	Candy, chocolate
Low (< 120°C)	Traditional foods	Steamed rice, scorched rice, Bulgogi sauce (liquid), Bone-extract soup
Others	Fats and oils	Palm oil, rapeseed oil, corn oil, soybean oil, sesame oil, olive oil
	Others	Liquid milk, fried egg, infant formula

the characteristics of processed foods. The samples are listed in Table 1.

Samples were stored at under 4°C until analysis, and were homogenized before analysis (those samples with a high water content were analyzed immediately after being purchased from markets).

Reagents

All reagents were of analytical grade unless otherwise stated. Acrylamide and formic acid were purchased from Sigma Chemical (St. Louis, MO, USA). Methanol and acetic acid were purchased from Merck (Darmstadt, Germany). $^{13}C_3$-labelled acrylamide (1,000 μg/mL, an internal standard) and d_5-3-chloropropanediol (a recovery standard) were purchased from Cambridge Isotope Laboratory (Andover, MA, USA). Purified water obtained from an ultra pure water system (Human Science, Seoul, Korea) was used in all aqueous solutions.

Standard solutions

Stock solutions of acrylamide, $^{13}C_3$-labeled acrylamide, and d_5-3-chloropropanediol were prepared in distilled water at concentrations of 1,000 μg/mL. The standards were protected from light and stored in a refrigerator at 4°C.

Internal standard solution – A 1.0-mL aliquot of the $^{13}C_3$-labeled acrylamide stock solution was diluted to 1,000 ng/mL, and 2 mL of the resulting solution was added to each sample before extraction.

Recovery standard solution – A working recovery standard (20 μg/mL) was prepared by dilution of the 1,000 μg/mL standard solution with water. A 2-μL aliquot of this working recovery standard solution was added to 1 mL of each sample and mixed thoroughly prior to purification with a C_{18} cartridge.

Calibration standard solution – Using a microsyringe, 0.1, 5, 25, 100, 200, 300, 400, and 500 μL of acrylamide stock solution were transferred to a series of 10-mL volumetric flasks, and 20 μL of the internal standard and 20 μL of the recovery standard were added to each flask and then diluted to the correct volume with water. All standard solutions were stored at 4°C and then left to stand at room temperature for about 30 min prior to analysis.

LC-MS/MS analysis

An improved liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of acrylamide in processed foods was followed our published methods.[11)]

Sample analysis was conducted with a high-performance liquid chromatography (HPLC) solvent delivery system (Sykam S2100, Sykam, Germany) coupled to MS/MS with an electrospray ionization source (Quattro Micro, Manchester, UK). MassLynx 4.0 software was used to operate the device and perform spectral analysis. The samples were separated in the Aqua C_{18} HPLC column (2×250 mm) packed with 5-μm particles (Phenomeonex, Torrance, CA, USA), using the mobile phase with aqueous 0.2% acetic acid and 1% methanol at a flow rate of 0.2 mL/min for 14 min. The volume of each sample injected was 20 μL. The electrospray positive ionization source was operated at a capillary voltage of 4.2 kV, source temperature of 120°C, desolvation temperature of 240°C, desolvation gas flow rate of 650 L/h with nitrogen, and an argon gas pressure of 2.5 mbar (used as the collision gas). Acrylamide was determined by multiple-reaction monitoring (MRM). MRM was performed by monitoring the 72- to 55-m/z transition for acrylamide, the 75- to 58-m/z transition for $^{13}C_3$-acrylamide, and the 116- to 98-m/z transition for d_5-3-chloropropanediol. For all MRM transitions the dwell time was 1 sec and the inter-scan delay time was 0.2 sec.

Sample preparation

A portion of each sample equal to the manufacturer's recommended serving size was crushed and homogenized. Ten grams of each sample was weighed into a 250-mL beaker to which 2 mL of internal standard and 98 mL of water were added. The acrylamide in samples was extracted into water with stirring by a magnetic stirrer for 20 min. A portion of the aqueous phase was centrifuged at 9,000 rpm for 10 min. A 1-mL aliquot of the aqueous phase was promptly pipetted into a 10-mL polypropylene graduated conical tube with a cap, to which 2 µL of recovery standard solution was added and mixed well. A C_{18} solid-phase extraction cartridge was conditioned with 5 mL of methanol followed by 5 mL of water. An aliquot of the sample was purified with a C_{18} cartridge, and the eluent was discarded. Acrylamide residues were eluted with 2 mL of water, and the collected eluent was passed through a 0.45-µm membrane and collected for LC-MS/MS analysis. The water extracts were directly analyzed by LC-MS/MS.

Results and Discussion

Foods purchased from a Korean market were analyzed in triplicate using HPLC-MS/MS to determine the concentration of acrylamide present. The analytical results of 190 processed foods from 16 food groups are presented in Table 2 and Figs 1–7. In the preparation of samples, it was very important to homogenize them thoroughly and to be careful in the manipulations in order to reduce variability between samples.[12] In this study, these problems were reduced by using both an internal standard ($^{13}C_3$-acrylamide) and a recovery standard (d_5-3-chloropropanediol).

Potato products

Potato chips (including French fries) exhibited relatively high levels of acrylamide, as indicated in Table 2 and Fig. 1 (median, 1,121 µg/kg; range, 470–3,572 µg/kg). These levels are consistent with the values reported previously by other researchers: Ono et al.[2] reported a median and range of 1,270 µg/kg and 439–1,870 µg/kg, respectively, in potato chips from Japanese markets; Nemoto et al.[6] reported a range of 466–3,340 µg/kg; and Takatsuki et al.[3] reported that acrylamide concen-

Table 2. Acrylamide levels in several food groups obtained from retail markets in Korea

Processing temperature	Food group	Acrylamide (µg/kg)		No of samples
		Median	Range	
High (≥ 120°C)	Potato chips	1,121	470–3,572	20
	Vacuum-fried potato chips	117	38–633	20
	Snacks with potato	448	155–1,992	20
	Snacks with no potato	130	21–433	20
	Breads (soft)	48	26–85	7
	Hardtacks	605	379–795	5
	Breakfast cereals	102	56–350	7
	Coffee	246	121–539	7
	Candy	53	11–389	7
	Chocolate	91	25–310	10
Low (< 120°C)	Steamed rice	32	ND[1]–96	8
	Scorched rice	79	55–187	8
	Bulgogi sauce	49	ND–57	5
	Bone-extract soup	51	48–61	6
Others	Palm oil, rapeseed oil, corn oil, soybean oil	ND	ND	12
	Sesame oil	102	58–111	6
	Olive oil	13	7–20	5
	Liquid milk	5	ND–7	6
	Fried egg	ND	ND	5
	Infant formula	90	47–102	6

ND[1] : not detected (<2 µg/kg)

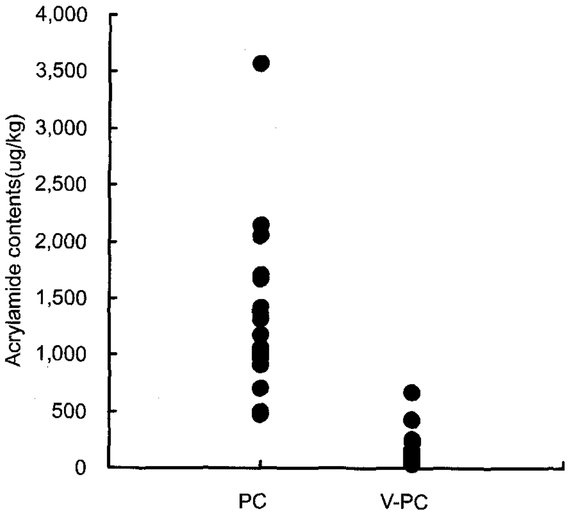

Fig. 1. Levels of acrylamide in potato chips (PC) and vacuum-fried PC (V-PC).

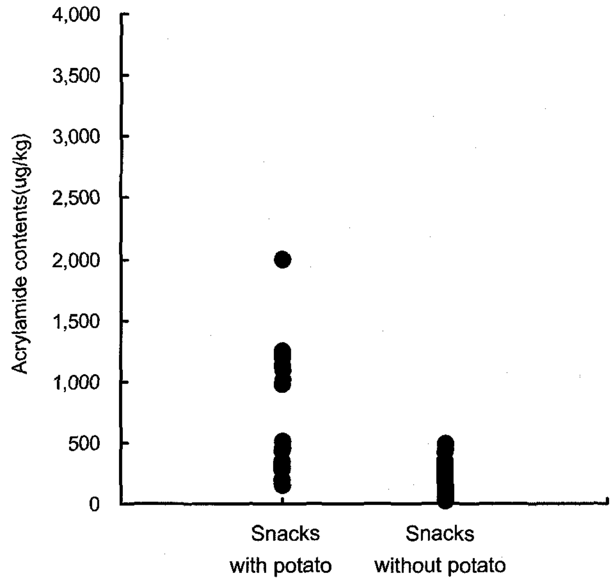

Fig. 2. Levels of acrylamide in snacks.

trations were range of 467–3,544 μg/kg. The wide variations in the levels of acrylamide in potato chips are attributable to variations in the quality of raw materials and processing conditions. In a typical chipping potato, glucose is the most abundant reducing sugar, and asparagine is the most abundant amino acid. Free amino acids and reducing sugars in the potato tuber are important precursors of acrylamide formation, as well as chip flavor components.[13)] There have been recent reports of a Maillard reaction involving, as a first step, a glucose decomposition product and asparagines.[14,15)] Since the asparagine concentration varies widely[16)] between potato varieties, one possible method to reduce acrylamide formation is to use the potato variety with the lowest asparagine contents for the production of potato chips and French fries.[17)] Furthermore, the acrylamide concentrations in potato chips were dependent on the manufacturing heating duration and temperature. The levels of acrylamide were about 10% lower in vacuum-fried potato chips (median, 117 μg/kg; range, 39–633 μg/kg) than in potato chips fried using other methods (Table 2).

Snacks

The levels of acrylamide were approximately three times higher in snacks containing potato (median, 448 μg/kg; range, 115–1,992 μg/kg) than in those with no potato (median, 113 μg/kg; range, 21–433 μg/kg; Table 2 and Fig. 2). These levels are consistent with the values reported previously by other researchers,[4, 5)] and illustrate the importance of food ingredients to acrylamide formation.

Cereal products and coffee

The levels of acrylamide in breakfast cereals are presented in Table 2 and Fig. 3 (median, 102 μg/kg; range,

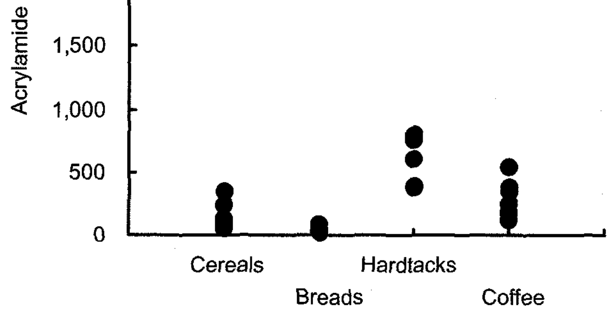

Fig. 3. Levels of acrylamide in cereals, breads, hardtacks, and coffee.

56–350 μg/kg), and are similar to the values (33–213 μg/kg) reported previously by Ono et al.[2] However, the range is smaller than that (<30–1,400 μg/kg) reported by SNFA.[5] The levels of acrylamide in coffee samples are presented in table 2 and figure 3 (median, 246 μg/kg; range, 121–539 μg/kg). Nemoto et al.[6] reported that roasted coffee beans contained acrylamide at 169 μg/kg. The daily intake of acrylamide due to coffee consumption is smaller than that from other foods.

The levels of acrylamide in breads are presented in Table 2 and Fig. 3 (median, 48 μg/kg; range, 26–85 μg/kg), which are consistent with some of the values reported previously by other researchers[4-6,17] but not with others.[18] These results suggest that the formation of acrylamide is dependent on processing conditions such as temperature and water content, and the constituent materials.

The levels of acrylamide in hardtacks are presented in Table 2 and Fig. 3 (median, 605 μg/kg; range, 379–795 μg/kg), which are consistent with some of the values reported previously by other researchers[4,6] but, not with others.[18] The levels of acrylamide were higher in hardtacks than in scorched rice products and breads. Also, the formation of acrylamide was dependent on processing conditions such as temperature and water content.

Sugar products

The levels of acrylamide in chocolate are presented in Table 2 and Fig. 4 (median, 91 μg/kg; range, 25–310 μg/kg), which are consistent with the values reported previously by other researchers.[4-6,17] The levels of acrylamide in candies are presented in Table 2 and Fig. 4 (median, 53 μg/kg; range, 11–389 μg/kg), and varied with the constituent materials added for taste.

Traditional foods produced at low temperature

The levels of acrylamide in steamed and scorched rice samples are presented in Table 2 and Fig. 5. The median values of acrylamide in steamed rice and scorched rice samples were 36 and 109 μg/kg, and the ranges were 2–96 μg/kg and 55–184 μg/kg, respectively (note that the lower detection limit was 2 μg/kg). The levels in processed rice foods did not agree with the results of Park,[18] who did not detect any acrylamide. This indicates that the formation of acrylamide in carbohydrate-rich foods such as rice-based products depend on the heating temperature and storage conditions.

The levels of acrylamide in Bulgogi sauces are presented in Table 2 and Fig. 5 (median, 49 μg/kg; range, 2–57 μg/kg). Although Bulgogi sauces contain many reactants produced at a relatively low temperature, acrylamide could be a byproduct of the Maillard reaction. Bulgogi is one of the most popular Korean traditional foods, and has a characteristic meaty and spicy aroma.

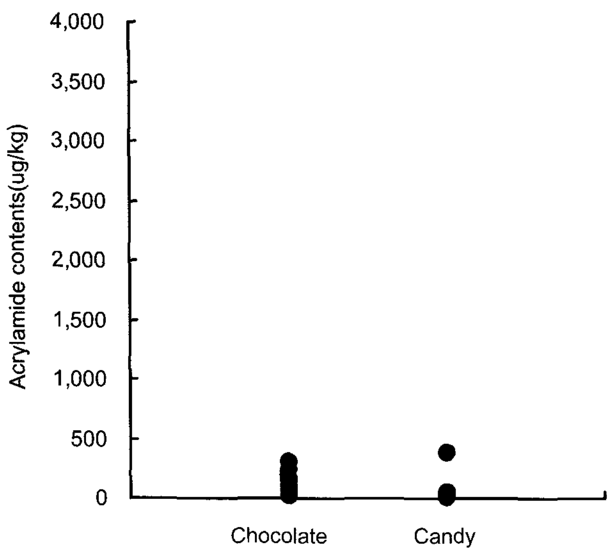

Fig. 4. Levels of acrylamide in chocolate and candy.

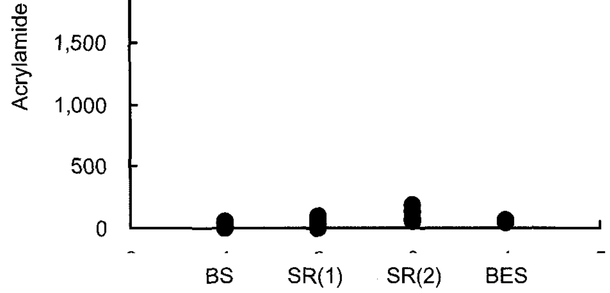

Fig. 5. Levels of acrylamide in Bulgogi sauce (BS), steamed rice (SR(1)), scorched rice (SR(2)), and bone-extract soups (BES).

Meat produces many flavors during cooking due to the complex interaction of precursors derived from fat and lean. Therefore, assessments of the risk of acrylamide in Korean diets should consider Korean traditional roasted and/or grilled foods such as Bulgogi.

Bone-extract soup is one of the most popular Korean traditional soups. Despite the extraction occurring in hot water (with a maximum temperature of 100°C), acrylamide was detected in bone-extract soups. The levels of acrylamide in bone-extract soups are presented in Table 2 and Fig. 5 (median, 51 μg/kg; range, 48–61 μg/kg), which are not consistent with the results of Park[18] for boiled foods. Our results indicate that acrylamide can be formed if foods are boiled for a long time. Many foods that undergo certain heat treatments may produce acrylamide via nonenzymatic browning reactions.[19] It should be noted that browning reactions involve numerous types of chemical reactions, such as oxidation, reduction, hydrolysis, and hydration, as well as reactions involving free radicals.

Edible oils

The levels of acrylamide in commercial edible fats and oils are presented in Table 2 and Fig. 6. Acrylamide was not detected in the oils that are widely used to fry foods in the food industry. However, sesame and olive oils – which are commonly used for condiments in household food preparation for their characteristic flavors – contained acrylamide at 7–111 μg/kg. The median values for sesame and olive oils were 86 and 10 μg/kg, respectively. The higher level in sesame oils could be due to the extraction of oils from the sesame (Sesamum indicum) seeds forming many characteristic components including flavor compounds after roasting.[20] It is hypothesized that glycerol produced from lipids forms acrolein via a dehydration reaction during roasting. Acrolein is oxidized to give acrylic acid, which reacts with ammonia to yield acrylamide.[19] The levels of acrylamide varied between edible oils. These results indicate that the formation of acrylamide in fried foods could be controlled by using different types of oil.

Others

The levels of acrylamide in infant formula are presented in Table 2 and Fig. 7 (median, 90 μg/kg; range, 47–100 μg/kg). Acrylamide was also detected in baby foods. In the worst case, a baby would consume as much acrylamide per kilogram bodyweight as a highly exposed adult. The levels of acrylamide in liquid milk are presented in Table 2 and Fig. 7 (median, 5 μg/kg; range, 2–7 μg/kg). The levels of acrylamide in liquid milk were lower than those of other foods in this study.

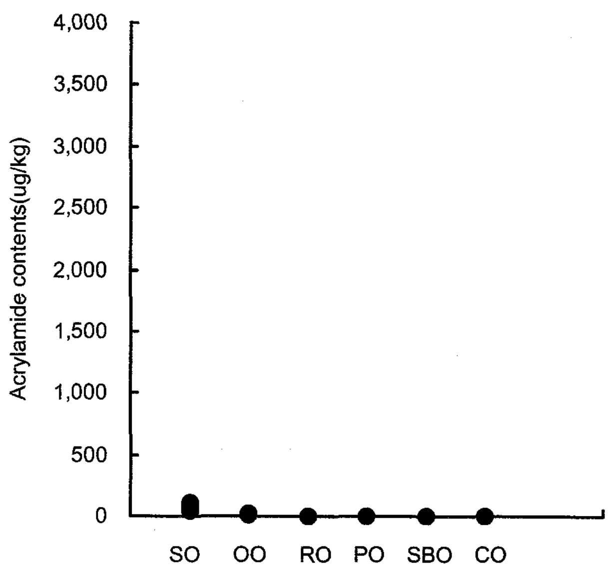

Fig. 6. Levels of acrylamide in edible oils (SO, sesame oil; OO, olive oil; RO, Rapeseed oil; PO, palm oil; SBO, soybean oil; CO, corn oil).

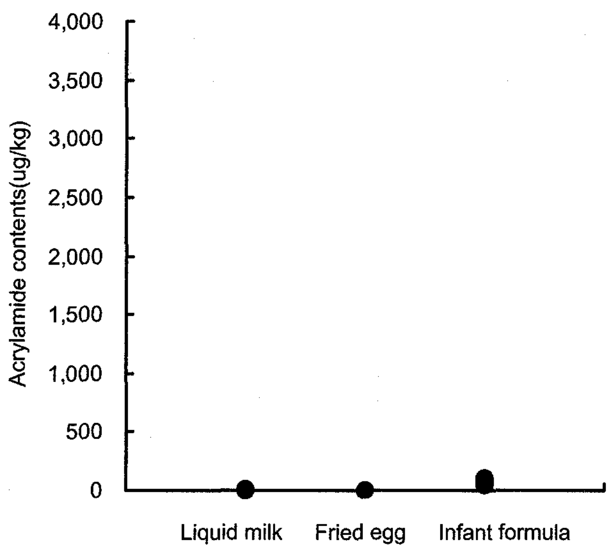

Fig. 7. Levels of acrylamide in liquid milk, fried egg, and infant formula.

국문초록

본 연구는 우리나라 사람이 일상적으로 섭취하는 식품 중에서 아크릴아마이드 잔류량을 조사하여 위해도 평가를 위한 자료로 제공함은 물론 아크릴아마이드가 가장 많이 검출되는 가공식품에서 아크릴아마이드를 기술적으로 저감화 시킬 수 있는 방법을 연구하기 위한 기초 데이터로 활용하고자 수행하였다. 밥, 곡류 가공품, 포테이토칩 등을 포함한 190 가지 시판제품에 잔류하는 아크릴아마이드 함량을 LC-MS/MS 분석법으로 측정하였다. 아크릴아마이드는 여러 가지 가공식품에서 매우 다양한 수준으로 검출되었고 감자칩에서 470~3,572 μg/kg로 가장 많이 검출되었다. 일반적으로 스낵류에서는 감자를 함유한 제품이 감자를 함유하지 않은 제품보다 상대적으로 높게 검출되었으며, 이때 각각의 중간값은 448 과 133 μg/kg이었다. 한국 사람들이 주식으로 섭취하는 밥에서는 불검출~96 μg/kg까지 검출되었다. 끓이는 식품 중의 하나인 곰탕에서도 48~61 μg/kg가 검출되었고, 불고기 양념에서는 불검출~57 μg/kg까지 검출되었다.

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