ACRYLAMIDE IN JAPANESE PROCESSED FOODS AND FACTORS AFFECTING ACRYLAMIDE LEVEL IN POTATO CHIPS AND TEA

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Abstract: Acrylamide concentrations in processed foods sold in Japanese markets were analyzed by LC-MS/MS and GC-MS methods. Most potato chips and whole potato-based fried snacks showed acrylamide concentration higher than 1000 μg/kg. The concentrations in non-whole potato based Japanese snacks, including rice crackers and candied sweet potatoes, were less than 350 μg/kg. Those in instant precooked noodles were less than 100 μg/kg with only one exception. The effect of storage condition of potato tubers on acrylamide concentration in potato chips after frying was also investigated. Sugar content in the tubers increased during cold storage, and the acrylamide concentration increased accordingly. The concentrations of asparagine and other amino acids, however, did not change during the cold storage. High correlations were observed between the acrylamide content in the chips and glucose and fructose contents in the tubers. This fact indicated that the limiting factor for acrylamide formation in potato chips is reducing sugar, not asparagine content in the tubers. Effects of roasting time and temperature on acrylamide concentration in roasted green tea are also described.

Key words: Acrylamide; LC-MS/MS; GC-MS, Japanese food; potato; cold storage; glucose; fructose; asparagine; roasting condition; tea

1. ACRYLAMIDE ANALYSIS OF PROCESSED FOODS IN JAPANESE MARKET

After the press release on the presence of acrylamide in common processed foods by University of Stockholm and National Food Administration of Sweden in April 2002, we started analysis of acrylamide in processed foods in Japanese market (National Food Research Institute, 2002; Ono et al., 2003).

Commercial processed foods were purchased at supermarkets in Tsukuba, Japan from 31 May to 5 June 2002. Our analytical sample preparation procedure is shown in Fig. 1. Food samples to be analyzed were pulverized by a food processor. After addition of the internal standard acrylamide-d_3 solution, the sample was shaken, and then water was added. The mixture was homogenized and centrifuged. The supernatant was subjected to a mixed-mode SPE cartridge, which had been conditioned with methanol and water, and eluted with water.

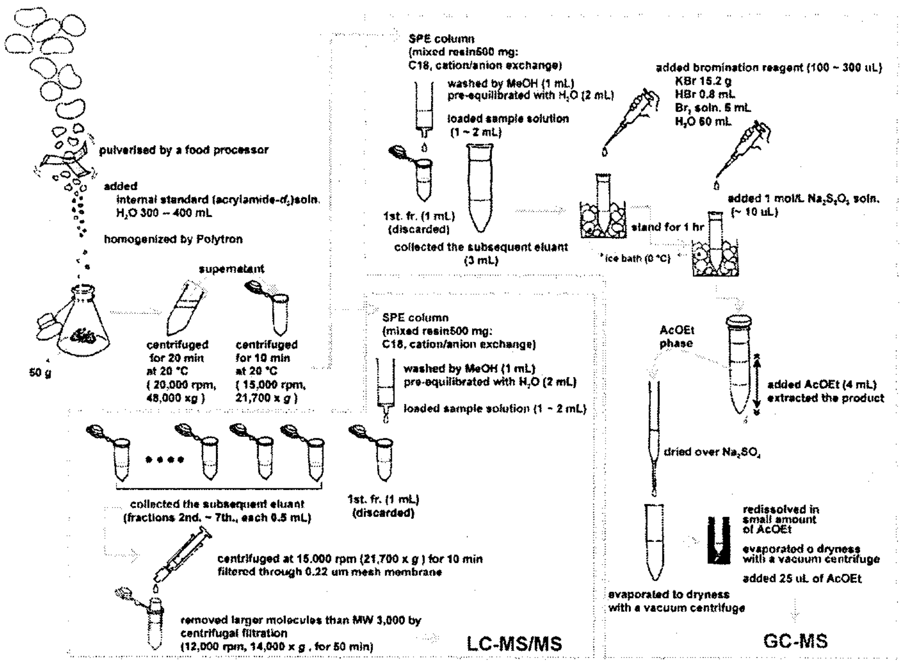

Figure 1. Sample preparation procedure for LC-MS/MS and GC-MS analyses.

For LC-MS/MS analysis, an aliquot of the eluate was centrifuged and passed through a syringe filter with a hydrophilic membrane. The filtrate was ultrafiltered and kept at -20°C under dark until LC-MS/MS analysis on an API 3000 triple quadrupole instrument equipped with a Turbo Ion Spray source (Applied Biosystems) coupled to a Nanospace SI-2 HPLC system (Shiseido). The sample (2 μl) was injected into the HPLC with an Atlantis dC18 column (2.1×150 mm, 3 μm, Waters), a C18 column for polar molecules, and eluted by 10 % methanol in water isocratically at a flow rate of 0.1 ml/min at 40°C. The eluate from 4.8 min to 10.2 min after the sample injection was introduced to the mass spectrometer operating in the positive-ion mode. Acrylamide was eluted at 5.4 min, and detected as a transition ion at *m/z* 72>55 by selected reaction monitoring. The internal standard was detected at *m/z* 75>58.

For GC-MS analysis, the SPE eluate was mixed with a bromination reagent (Castle et al., 1991) in an ice bath until the reddish color of bromine remained. The reaction mixture was stood for 1 hr, and then an aqueous sodium thiosulfate solution was added to destroy excess bromine until the solution became colorless. The brominated acrylamide was extracted with ethyl acetate and dried over anhydrous sodium sulfate. After most of the solvent was removed by centrifugal evaporation under reduced pressure at 30°C, the residue was transferred to a micro grass tube to be kept at 4°C till GC-MS analysis. The sample (1 μl) was introduced into the GC-MS-QP2010 system (Shimadzu) with a CP-Sil 24 CB Lowbleed/MS column (50% phenyl, 50% dimethylpolysiloxane (equivalent to OV-17), 0.25 mm i.d. ×30 m, 0.25 μm film thickness, Varian) by splitless injection method. Injector temperature was 120°C, and the temperature program used was as follows: isothermal for 1 min at 85°C, increased at a rate of 25°C/min to 175°C, isothermal for 6 min, increased at a rate of 40°C/min to 250°C, and isothermal for 7.52 min. Fragment ion peaks of 2,3-dibromopropionamide derived from acrylamide at *m/z* 150 $[C_3H_5NO^{79}Br]^+$ and 152 $[C_3H_5NO^{81}Br]^+$, and those from acrylamide-d_3 at *m/z* 153 $[C_3H_2D_3NO^{79}Br]^+$ and 155 $[C_3H_2D_3NO^{81}Br]^+$ were detected by selected ion monitoring.

The limit of detection and limit of quantification of acrylamide were 0.2 ng/ml (6 fmol) and 0.8 ng/ml (22 fmol), respectively, on the LC-MS/MS, and those of 2,3-dibromopropionamide derived from acrylamide were 12 ng/ml (52 fmol) and 40 ng/ml (170 fmol), respectively, on the GC-MS. Repeatability given as RSD was <5% and <15% for the LC-MS/MS and the GC-MS methods, respectively. High correlation (r^2=0.946, n=74) was observed between values obtained by the two methods.

Fig. 2 shows the results of our analysis of Japanese processed foods (National Food Research Institute, 2002; Ono et al., 2003). The ranges of acrylamide concentration in potato chips, cookies, and breakfast cereals observed in this survey were within those reported from Western countries in the FAO/WHO consultation meeting (FAO/WHO, 2002). Most potato chips and whole potato based fried snacks showed acrylamide concentration

>1000 µg/kg. Rice crackers, which are processed by grilling at 200-300°C for several minutes or frying at 160-260°C for about a few minutes, contained 20 to 300 µg/kg acrylamide. Non-whole potato based snacks, made from potato starch, wheat flour, or corn whole meal, shaped, fried, and seasoned, contained similar level of acrylamide as did rice crackers'. Candied sweet potatoes, which are cooked by frying at around 160°C for several minutes followed by syrup coating, also showed lower acrylamide concentrations than did potato chips. Instant noodles and won-tons contained about 10 to 60 µg/kg acrylamide, with one exception with a concentration >500 µg/kg. Roasted barley grains used as a tea substitute or herb tea blend contained 200-600 µg/kg acrylamide, comparable to the concentration in coffee powder (FAO/WHO, 2002). Other Japanese and Asian type foods measured here contained around 100 µg/kg acrylamide or less.

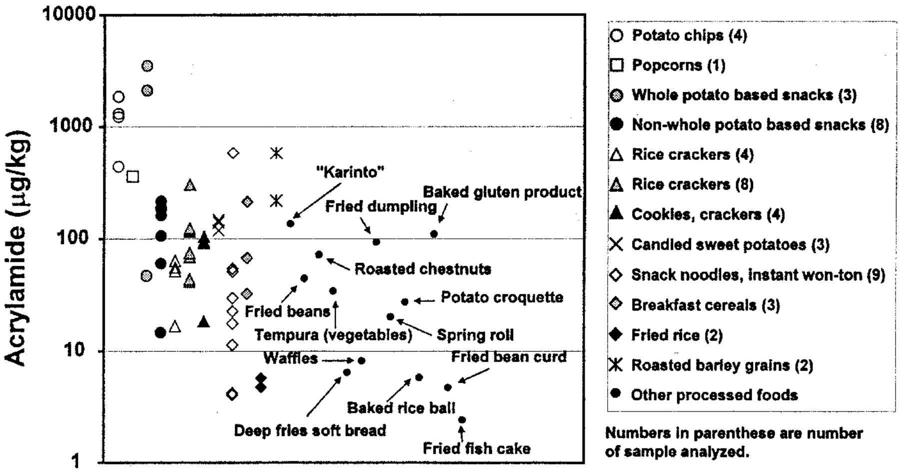

Figure 2. Acrylamide concentration in processed foods in Japanese market.

2. EFFECT OF STORAGE CONDITIONS OF POTATO TUBERS ON ACRYLAMIDE FORMATION DURING FRYING

A large variation has been found in acrylamide concentration in potato based foods. This cannot be accounted for only by difference of processing conditions. It is known that sugar content in potato tuber increases during cold storage by starch degradation, which is known as "low temperature sweetening." This sugar level change during the storage should affect acrylamide formation during cooking because reaction of reducing sugars with asparagine is thought to be a major rout for acrylamide formation in starchy foods. We then investigated the change of sugar and amino acid contents during storage of potato tubers and its effect on acrylamide concentration after frying (Chuda et al., 2003).

Potato tubers (cultivar Toyoshiro) with similar weight and starch values were selected for the storage experiment. Toyoshiro is the most popular cultivar for potato chips in Japan. Three tubers stored under each condition were cut in half longitudinally, with a half used for chipping and the other for amino acid and sugar analyses by HPLC. For chipping, 4 slices 1.3 mm thick were prepared from each of 3 tubers (total 12 slices). Slices were washed for 100 sec in water and fried at 180°C for 90 sec in cottonseed oil. The acrylamide content of the chips was determined by the GC-MS method.

The concentrations of asparagine and other amino acids did not change during the cold storage. On the other hand, sugars increased during cold storage (Fig. 3). Acrylamide concentration after frying increased accordingly (Fig. 4). The acrylamide content in chips showed a high correlation with glucose content in the tubers (r^2=0.884, n=20). A similar correlation was observed for fructose (r^2=0.884, n=20). No correlation was observed with asparagine content (r^2=0.056, n=20). This fact indicated that the limiting factor for acrylamide formation in fried potato-based foods is the reducing sugar and not the asparagine content in the tubers.

We investigated the sugar contents of tubers stored at various temperatures for 2 weeks. When they were stored at 6°C or below, the sugar content was higher than when stored at 8°C or above. Acrylamide concentration in the chips also increased when the tubers were stored at 6°C or below. Therefore, refrigeration of potatoes for chips and French fries at 6°C or below should be avoided to suppress the acrylamide formation. Avoiding the cold storage also suppresses browning during frying, probably by inhibition of accumulation of reducing sugars in the tubers.

There should be cultivar difference in sugar content and sugar accumulation pattern during the cold storage. Thus we are now investigating difference in accumulation of acrylamide by frying into potato chips among cultivars and breeding lines for selection of material with low potential of acrylamide formation even after storage.

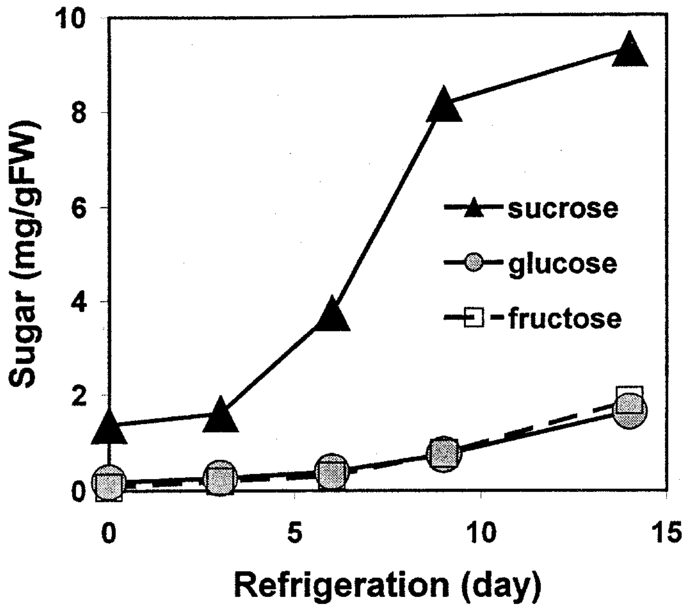

Figure 3. Changes of sugar contents in potato tubers during refrigeration at 2°C.

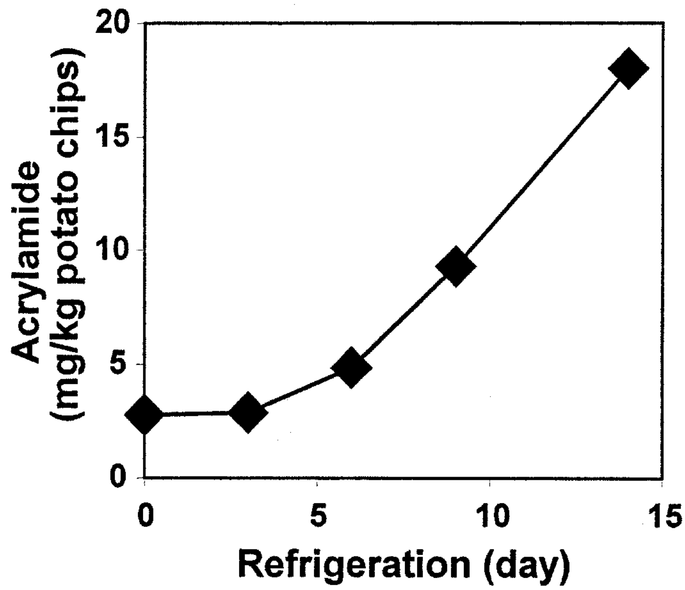

Figure 4. Effect of refrigeration period of tubers on acrylamide content in potato chips.

3. ACRYLAMIDE ANALYSIS OF ROASTED GREEN TEA LEAVES

Various types of tea (Fig. 5) are consumed in Asian countries. In Japan, non-fermented green teas are popular, and among green teas, Sencha is consumed most. Hojicha, roasted green tea, is often served in Japanese restaurants. Recently Chinese type semi-fermented Oolong tea has become popular. Bottled Oolong tea is now sold in stores.

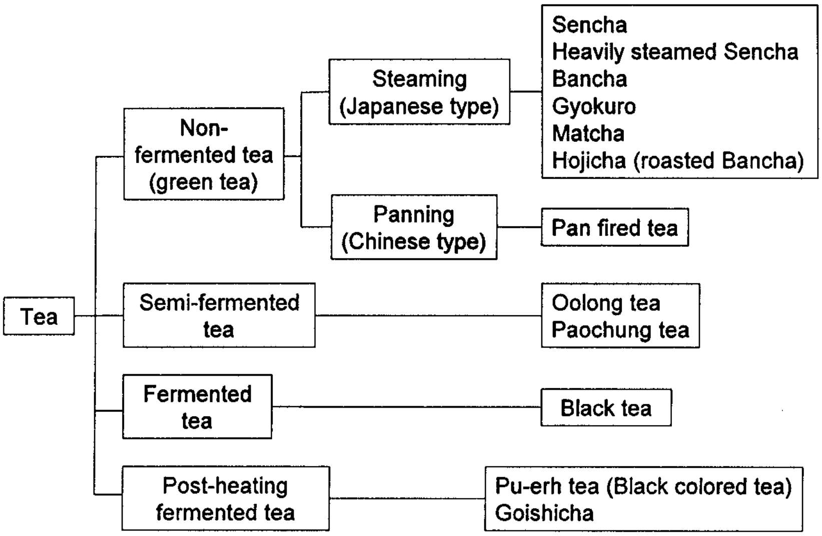

Figure 5. Types of tea.

Results of analyses of tea and tea substitutes reported by the Ministry of Agriculture, Forestry and Fisheries of Japan (2003) (Table 1) show that the acrylamide level in green tea is not as high as in roasted products such as Houjicha and roasted cereal grains used as tea substitutes or in herb teas. Based on the analytical data, it appears that steeping in boiling or hot water resulted in extraction of most of the acrylamide in the infusions. We also investigated the effect of roasting conditions on acrylamide accumulation in the tea products. In the course of the analysis, it was found that epigallocatechin in the tea samples inhibited the formation of the brominated derivative. We then fractionated the SPE eluate and used only a 1.5 ml portion after discarding 1 ml early eluate used for bromination to avoid contamination of epigallocatechin eluted in the later fractions.

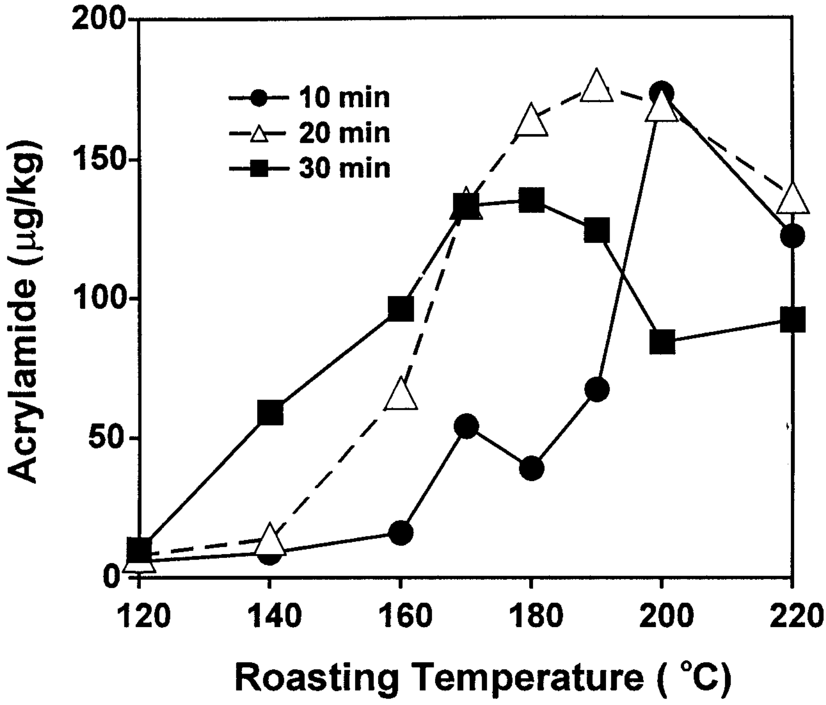

Figure 6. Effect of roasting temperature on acrylamide content in tealeaves.

Green tea samples prepared by the conventional method were roasted in a laboratory oven for 10, 20, or 30 min, and the acrylamide content measured by the GC-MS method was plotted against roasting temperature (Fig. 6). For each roasting time, a maximum of acrylamide content was observed. The maximum shifted at lower temperatures with increased roasting times. In this graph, the maximum acrylamide concentration is about 180 μg/kg, and this seems to be the maximum potential of acrylamide accumulation of this tea sample. But some roasted green tea samples showed values above 500 μg/kg (Table 1). Therefore, not only roasting time and temperature but also components of tealeaves should affect the acrylamide level in tea products. Effect of the leaf components on the acrylamide formation is the subject to be studied next.

Table 1. Acrylamide in tea* (The Ministry of Agriculture, Forestry and Fisheries of Japan, 2003)

	Sample No.	Acrylamide (ng/g)		Condition of infusion
		Product	Infusion	
Green tea	1	nd	nd	10 g / 90°C 430 ml
(Sencha)	2	20	nd	1 min
	3	30	nd	
Green tea	1	70	nd	10 g / 90°C 430 ml
(Pan fired tea)	2	50	nd	1 min
	3	100	3	
Hojicha	1	520	10	15 g / 90°C 650 ml
(roasted green tea)	2	260	4	0.5 min
	3	570	11	
	4	190	4	
Black tea	1	20	nd	5 g / boiling water 360 ml
	2	20	nd	1.5 - 4 min
	3	nd	nd	
Oolong tea	1	60	nd	15 g / 90°C 650 ml
	2	90	2	0.5 min
Roasted barley grains	1	320	14	50 g / hot water 1500 ml
(Mugicha)	2	290	11	5 min standing after boil up
	3	180	5	
Roasted pearl barley	1	130	nd	30 g / hot water 1000 ml
grains (Hatomugicha)	2	130	nd	15 min boiling,
	3	120	7	5 min standing

*Limit of detection: 20 ng/g (product), 2 ng/g (infusion).

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