Acrylamide Content Distribution and Possible Alternative Ingredients for Snack Foods

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ABSTRACT

Acrylamide (AA) contents in 294 snack foods including cereal-based, root- and tuber-based, and seafood-based foods, nuts, dried beans, and dried fruits purchased in Taiwan were determined by gas chromatography–mass spectrometry in this study. The highest levels of average AA content were found in root- and tuber-based snack foods (435 µg/kg), followed by cereal-based snack foods (299 µg/kg). Rice flour–based, seafood-based, and dried fruit snack foods had the lowest average AA content (<50 µg/kg). This is the first large surveillance of AA content in snack foods in Taiwan. The results could provide important data regarding intake information from the snack foods. In addition, the results showed a great diversity of AA content in snack foods prepared from different ingredients. Rice- and seafood-based products had much lower AA than those made from other ingredients. This information could constitute a good reference for consumers to select products for healthy snacking.

Acrylamide (AA) (CH$_2=\text{CHCONH}_2$; CAS no. 79-06-01) is a synthetic monomer used as a precursor in production of polyacrylamide for water treatment, paper processing, and electrophoretic separation in bioscience fields. In April 2002, the presence of AA was first reported in various foods, particularly fried and baked starchy foods, and determined to be the result of high-temperature treatment (31). AA is formed in food via reactions between amino acids (such as asparagine) and reducing sugars (such as glucose), which have been considered to be among Maillard reactions. One of the purposes of cooking processes is to reduce or destroy microorganisms such as pathogens or viruses. Nevertheless, the presence of AA due to high temperature treatment has attracted the attention of consumers and scientific community. AA is recognized as a potential mutagen and reproductive toxicant. The International Agency for Research on Cancer has labeled AA as Group 2A, a probable human carcinogen, since 1994 (14). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) warned that the unintentional contaminant AA in certain foods might be of public health concern since it has been shown to cause cancer in animals. AA is a neurotoxin, while glycidamide is genotoxic and/or carcinogenic (1). The Joint FAO/WHO Expert Committee on Food Additives noted that the morphological changes seen in nerves of rats cannot be excluded for individuals with a high dietary exposure to AA (11). In addition, the committee pointed out that AA could cause reproductive problems and tumors in rats and mice with high dietary exposure to AA (11, 12).

Because diet could be the most important source of human intake of AA, the prevalence of AA in foods (11, 16, 23, 25–28, 32, 37) has been a subject of active studies worldwide for evaluating human exposure to AA. The European Commission Joint Research Centre’s Institute for Reference Materials and Measures established a database of AA levels in food between 2003 and 2006 (38). Furthermore, the European Food Safety Authority describes the 10,366 results from the European AA monitoring project submitted from 23 members of the European Union and Norway in the period from 2007 to 2009 (8, 33–35). Dietary intakes of AA were also determined to estimate the exposure of consumers to AA (2, 5, 6, 13, 15, 19–22, 29). Data from the report of the FAO/WHO in 2002 (10) and the Joint FAO/WHO Expert Committee on Food Additives in 2005 and 2010 (11, 12) show that the dietary intake was between 0.3 to 0.8 and 1 µg/kg of body weight per day for the general population in developing countries. However, more data are needed to conduct risk assessment from food consumption.

Many food companies are dedicated to reducing AA content in foods. The latest version of the Toolbox in 2011 focused on the three main product categories with the highest risk of AA, i.e., potatoes, cereals, and coffee (4). Snack foods are popular food items around the world as well as in Taiwan. Starch, originated from potato, corn, or rice, is a major component in snack foods and results in the formation of AA after frying or baking. The objectives of this study were to investigate the AA contents in snack foods in Taiwan and to offer suggestions for future improvement in developing low-AA-content snack foods.

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TABLE 1. Major ingredients of snack foods

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Major ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal based</td>
<td>Wheat, corn, rice, mixed (wheat/potato or corn/potato) flour</td>
</tr>
<tr>
<td>Root or tuber based</td>
<td>Whole potato, potato starch, taro</td>
</tr>
<tr>
<td>Seafood base</td>
<td>Fish, shrimp, squid</td>
</tr>
<tr>
<td>Nuts</td>
<td>Pistachio, almond, peanut, cashew</td>
</tr>
<tr>
<td>Dried beans</td>
<td>Soybean, green pea, black soybean, mung bean, broad bean</td>
</tr>
<tr>
<td>Dried fruit</td>
<td>Papaya, coconut, other fruit, lemon peel, orange peel</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Food sampling. Snack food samples were purchased from supermarkets in Taipei from 1 June to 5 November 2005. The 294 snack food samples analyzed were categorized as cereal based, root and tuber based, seafood based, nuts, dried beans, and dried fruits. The major ingredients of each snack food sample subgroup are shown in Table 1.

Analysis of AA by GC-MS. The AA contents of snack food samples were measured according to the procedure described in our previous study using a validated gas chromatography–mass spectrometry (GC-MS) method (3) as follows: 1 ml of 1,2,3,13C4-acrylamide obtained from CIL (Andover, MA) (1 mg/ml) was made up to 100 ml with methanol supplied by Sigma (St. Louis, MO) (stock internal standard [ISTD], 10 µg/ml). One milliliter of the stock ISTD was transferred to a 100-ml volumetric flask and made up to volume with water to give a concentration of 1 µg/ml for sample preparation. AA (0.1 mg) from J. T. Baker was weighed into a 100-ml volumetric flask and made up to volume with deionized water. The stock AA solution was diluted with deionized water to 5, 10, 20, 40, 60, and 80 ng/ml. A sample (40 g) was ground in a blender. A subsample (5 g) was homogenized with 30 ml of deionized water in a centrifuge tube for extraction. The mixture was shaken at high speed on a horizontal shaker for 1 h and centrifuged at 3,500 rpm in a Labofuge 400 centrifuge for 15 min at 25°C. The top centrifuged layer was transferred to a 10-ml brown test tube. Oasis HLB cartridges and MCX cartridges were conditioned with 5 and 3 ml of methanol followed by 5 and 3 ml of water, respectively. The centrifuged layer (3 ml) and 0.1 ml of ISTD were passed through an Oasis HLB cartridge connected in tandem to an Oasis MCX cartridge; the first 1-ml volume was discarded, and the remaining portion was collected into a 10-ml brown test tube. The cartridge was further eluted with 4 ml of water into the same tube, and the combined eluates were made up to 6 ml for derivatization. The bromination reagent was prepared from potassium bromide (20 g; Sigma), hydrobromic acid (1 ml; Sigma) and bromine-saturated water (saturated at 0°C, 16 ml) made to a total volume of 100 ml with water. The reagent was stored at 4°C. For derivatization, the eluates (6 ml) were added to 6 ml of bromination reagent and stored at 4°C for 15 h. The excess bromine was decomposed by adding 60 µl of sodium thiosulfate (1 M; Sigma), and the solution was transferred to a 250-ml separation funnel. The solution was extracted with ethyl acetate (Riedel-deHaen, Seelze, Germany) twice by using a vertical shaker at high speed for 20 min. The organic solution was collected to another separation funnel; 4 g of sodium sulfate was added before shaking for 20 min. The dehydrated solution and ethyl acetate used to wash the separatory funnel were transferred to a condensation vessel. The pooled organic fractions were evaporated at 40°C with a rotary evaporator to almost complete dryness, and the fractions were removed to a brown tube. The solvent was removed under a gentle nitrogen gas stream. The residue was dissolved in 40 µl of ethyl acetate, and 10 µl of triethylamine supplied by Sigma was added to convert 2,3-dibromopropionamide (2,3-DBPA) to 2-bromopropionamide (2-BPA). One microliter of the sample was injected for GC-MS analysis. All determinations were made on a Saturn 2200 ion trap GC-MS system (Varian, Walnut Creek, CA) equipped with a tandem mass spectrometer (MS/MS), 3400CX GC, 1078 or 1077 injector, and 8200 autosampler. Samples were separated on a column (length, 30 m; inside diameter, 0.25 mm; HP-5MS; J&W Scientific, Folsom, CA) with a 0.25-µm film thickness. The oven temperature for GC was programmed as follows: 50°C hold for 1 min, increase of 5°C/min to 240°C, and hold for 5 min. The following parameters were used for injection samples into the GC system: injector, 200°C; helium carrier, 1.0 ml/min; 1-µl injection, made in the splitless mode with the split vent closed for 0.75 min. The parameters used for MS were as follows: transfer line temperature, 170°C; manifold temperature, 190°C; scan range, 60 to 1,600 m/z; ionization voltage, 70 eV; ionization mode, EI-Auto. AA contents of food samples were determined by the stable isotope dilution method. The ratio of the peak area response for 2-BPA (m/z, 151) to the peak area response for the ISTD (m/z, 154) was determined for the calibration standards, blanks, and spiked samples. AA was determined from a graph constructed by plotting the peak area ratio against the amount of AA.

Method detection limit and recoveries. The method detection limit was 5 µg/kg. The recoveries of the method were between 102 and 110% when wheat flour dough was spiked with AA at 10 to 50 µg/kg.

RESULTS AND DISCUSSION

Distribution of AA contents in snack foods. The distribution of AA contents in snack foods, taking wheat flour–based snack foods as an example, is shown in Figure 1. It shows a distribution skewed to the right. The left tail is tightly packed together, and the right tail is widely spread apart. The largest value (3,586 µg/kg) was more than 100 times greater than the smallest value (5 µg/kg). The results showed that the distribution of AA content in snack foods was significant.
TABLE 2. Acrylamide contents in snack foods surveyed in Taiwan\textsuperscript{a}

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of samples analyzed</th>
<th>Acrylamide content (μg/kg)</th>
<th>Arithmetic mean</th>
<th>Geometric mean</th>
<th>Median</th>
<th>Min–max</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal based</td>
<td>245</td>
<td></td>
<td>299</td>
<td>123</td>
<td>154</td>
<td>&lt;5–3,586</td>
<td>156</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>203</td>
<td></td>
<td>305</td>
<td>137</td>
<td>178</td>
<td>&lt;5–3,586</td>
<td>145</td>
</tr>
<tr>
<td>Rice flour</td>
<td>12</td>
<td></td>
<td>25</td>
<td>12</td>
<td>5</td>
<td>&lt;5–107</td>
<td>133</td>
</tr>
<tr>
<td>Corn flour</td>
<td>22</td>
<td></td>
<td>271</td>
<td>110</td>
<td>104</td>
<td>&lt;5–403</td>
<td>76</td>
</tr>
<tr>
<td>Mixed flour (wheat/potato)</td>
<td>6</td>
<td></td>
<td>794</td>
<td>390</td>
<td>337</td>
<td>49–2,479</td>
<td>119</td>
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<tr>
<td>Mixed flour (rice/potato)</td>
<td>2</td>
<td></td>
<td>1,290</td>
<td>1,138</td>
<td>1,290</td>
<td>682–1,898</td>
<td>67</td>
</tr>
<tr>
<td>Root or tuber based</td>
<td>21</td>
<td></td>
<td>435</td>
<td>236</td>
<td>384</td>
<td>&lt;5–3,497</td>
<td>173</td>
</tr>
<tr>
<td>Whole potato</td>
<td>9</td>
<td></td>
<td>452</td>
<td>349</td>
<td>419</td>
<td>121–1,138</td>
<td>72</td>
</tr>
<tr>
<td>Potato starch</td>
<td>11</td>
<td></td>
<td>627</td>
<td>187</td>
<td>204</td>
<td>&lt;5–3,497</td>
<td>160</td>
</tr>
<tr>
<td>Taro</td>
<td>1</td>
<td></td>
<td>89</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seafood based</td>
<td>6</td>
<td></td>
<td>11</td>
<td>8</td>
<td>5</td>
<td>&lt;5–25</td>
<td>85</td>
</tr>
<tr>
<td>Fish</td>
<td>4</td>
<td></td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>&lt;5–25</td>
<td>100</td>
</tr>
<tr>
<td>Shrimp</td>
<td>1</td>
<td></td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squid</td>
<td>1</td>
<td></td>
<td>&lt;5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts</td>
<td>4</td>
<td></td>
<td>62</td>
<td>56</td>
<td>67</td>
<td>26–86</td>
<td>41</td>
</tr>
<tr>
<td>Dried fruits</td>
<td>9</td>
<td></td>
<td>9</td>
<td>6</td>
<td>5</td>
<td>&lt;5–44</td>
<td>139</td>
</tr>
<tr>
<td>Dried bean based</td>
<td>9</td>
<td></td>
<td>101</td>
<td>20</td>
<td>19</td>
<td>&lt;5–748</td>
<td>242</td>
</tr>
<tr>
<td>Soybean</td>
<td>4</td>
<td></td>
<td>55</td>
<td>31</td>
<td>59</td>
<td>&lt;5–98</td>
<td>91</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td></td>
<td>169</td>
<td>39</td>
<td>23</td>
<td>&lt;5–748</td>
<td>191</td>
</tr>
<tr>
<td>Total</td>
<td>294</td>
<td></td>
<td>299</td>
<td>105</td>
<td>141</td>
<td>&lt;5–3,586</td>
<td>166</td>
</tr>
</tbody>
</table>

\textsuperscript{a} CV, coefficient of variation; Min–max, minimum to maximum.

foods might be very large, possibly due to different food compositions. Similar findings of high variations in AA contents in foods have been attributed to great differences in processing or storage conditions (27, 29, 30).

AA contents of snack foods. AA contents of snack foods are summarized in Table 2, which is part of an ongoing survey; more data will be accumulated. The arithmetic mean, geometric mean, and median of AA contents of cereal-based snacks were 299, 123, and 154 μg/kg, respectively (range, <5 to 3,586 μg/kg). Among different bases for snack foods, the progression of AA content was as follows: rice flour < corn flour < wheat flour < wheat/potato mixed flour < rice/potato mixed flour (regardless of arithmetic mean, geometric mean, or median). The geometric mean and median were similar and about one-half of the arithmetic mean. The AA contents of rice flour–based snack foods ranged from <5 to 107 μg/kg, which was the lowest among the cereal-based snack foods. Both fried rice crackers and grilled rice crackers were found to have low AA contents (47 and 112 μg/kg, respectively) in Japan (27). In Hong Kong and Turkey, low AA contents were also reported for rice products such as fried rice vermicelli, flattened rice noodles, deep-fried sizzling rice, baked rice, fried rice, or grilled rice (16, 26). However, once rice was mixed with potato, the AA content was dramatically increased to 682 to 1,898 μg/kg. It appeared that rice yielded lower AA in products and could be an alternative ingredient for the industry to develop low-AA snack food products.

There were 21 samples analyzed in the group of root- and tuber-based snack foods. The AA content ranged from 5 to 3,497 μg/kg, in which the arithmetic mean, geometric mean, and median were 435, 236, and 384 μg/kg, respectively. We further categorized samples into whole-potato–based and potato starch–based snack foods and found that the former had lower AA content than the latter in arithmetic mean. Nevertheless, the geometric mean and the median did not exhibit the same pattern. The data illustrated that the geometric mean appeared to be a better choice to represent the AA content in food whenever the distribution variance was relatively large. Ono et al. (27) also reported that the AA concentrations in non–whole-potato-based snacks (which are made from potato starch), wheat flour, or whole milled corn that were shaped, fried, and seasoned were lower than those of potato crisps and whole–potato–based fried snacks. In addition, only one taro-based snack was sampled in this study, and it had low AA content (89 μg/kg). Beyond its use in snack foods, taro can be served as a dessert or as an ingredient in bakery products. The low AA content in taro products also offers an option for formulation of snack foods.

Among the group of seafood-based snack foods made with fish, shrimp, and squid, six samples were studied, and the arithmetic mean, geometric mean, and median of AA content were 11, 8, and 5 μg/kg (range, <5 to 25 μg/kg). The low AA content was probably due to the lack of reducing sugars and asparagines in seafood.

For the nut subgroup, four samples including pistachio, almond, peanut, and cashew were sampled, and the arithmetic mean, geometric mean, and median of AA content were 62, 56, and 67 μg/kg (range, <26 to 86 μg/kg). The National Institute of Health Science (of Japan) (24) also reported that AA contents of roasted peanuts were in the range of 57 to 92 μg/kg, those of roasted cashew nuts were in the range of 9 to 30 μg/kg, and those of roasted almonds and pistachios were...
324 and 34 μg/kg, respectively. Lukac et al. (18) have reported that the AA concentration in roasted almonds can be reduced by controlling the roasting temperature and moisture content of almonds. A recent study showed that the AA level was decreased by about 50.2% after 3 days of storage at 60°C (39).

Among dried fruits, nine samples were analyzed, and their AA contents were less than 10 μg/kg. Although reducing sugars were present, the lack of asparagine was the reason for the low AA. Although high AA content has been reported in carbohydrate-rich products, the results were similar to those in heated protein-rich foods. Boiling appears to be a good method to reduce AA content (17). The National Institute of Health Science also analyzed dried fruit and vegetable samples and reported that apple chips, banana chips, pumpkin chips, and carrot chips contained AA in the range of 9 to 30 μg/kg. A recent report from the United Kingdom Food Standards Agency showed that dried fruit had a low AA content, in the range of 3 to 51 μg/kg (36).

In the group of dried beans, nine samples were analyzed, and the average AA content was 101 μg/kg. In the sample that contained the highest AA, 748 μg/kg, black soybean and five kinds of cereals, including rice and oats, were labeled as major ingredients. The multiple ingredients seemed to offer constituent sources for the formation of AA.

**Ingredients’ influence on AA content.** The comparison of the distributions of AA content in five categories (wheat, rice, corn, potato, and seafood) is shown in Figure 2. For rice-based snack foods, there were more than 50% of the products with AA contents lower than 50 μg/kg, which was the target in French fries (9). Among wheat-, corn-, and potato-based snack foods, more than 60% of the products had AA contents in the range of 51 to 499 μg/kg. Few of them (no more than 20%) had AA contents lower than 50 μg/kg. The AA content in potato-based snack foods was relatively high (500 to 999 μg/kg) among all the samples tested. Wheat-, corn-, and potato-based snack foods all were found to contain AA concentrations of >1,000 μg/kg. None of the seafood-based snack foods in this study showed AA contents greater than 50 μg/kg. The distributions of AA contents showed significant differences based on the major ingredient of various snack foods. The results provide a reference for manufacturers to modify ingredients for developing new low-AA products. There are no statutory maximum AA levels at this stage. Nevertheless, the European Commission Recommendation [C(2010) 9681 final] has specified "indicative values" for AA content based on European Food Safety Authority monitoring data from 2007 to 2008 (7). Taking potato crisps and breakfast cereals as examples, the indicative values are 1,000 and 400 μg/kg, respectively. Some products with high AA contents in this study showed the necessity for snack manufacturers to take action for mitigating their production in the future.

We have established a database of AA contents in snack foods in Taiwan. The results showed a great diversity of AA content in snack foods prepared from different ingredients. Rice- and seafood-based products had much lower AA than those made from other ingredients. The information would be a good reference for consumers to select healthy snacking.

**ACKNOWLEDGMENT**

This study is part of a research project sponsored by the Department of Health of Taiwan, Republic of China (project no. DOH 94-TD-F-113-011). The financial support is greatly appreciated.

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