Acrylamide formation in carbohydrate-rich food powders consumed in Korea

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Abstract

Acrylamide formation in carbohydrate-rich botanical powders consumed in Korea was investigated in this study. Free asparagine and monosaccharide contents were measured as the chief precursors to acrylamide formation. The highest levels of acrylamide were detected in heated lotus roots, followed by potatoes, Jerusalem artichokes, and yams. Tubers and rhizomes contained more asparagine than sugar and had a high ratio of free asparagine to free amino acids. Acrylamide was formed preferably when sugars were the limiting factor, rather than when the same amount of asparagine was limited. This study demonstrated the potential for several botanical powders in the Korean diet to be contaminated by acrylamide.

Keywords: acrylamide; asparagine; Maillard reaction; monosaccharides

Introduction

Acrylamide is classified as: *probably carcinogenic to humans* (Group 2A) (IARC, 1994), and its contamination in food was first reported in starch-rich foods by Swedish scientists (Tareke *et al*., 2002). Analysis of a wide range of foods revealed that it is primarily formed in carbohydrate-rich foods cooked at high temperatures, including potato chips and fries, biscuits, crackers, and toast (EC, 2002). Given that carbohydrate-rich ingredients constitute the main part of Korean diet, evaluating the acrylamide-forming potential of these carbohydrate-rich foods is essential. Jeong *et al*. analysed the acrylamide contents in Korean commercial foods and found that a brewed tea product made from Jerusalem artichoke (a tuber receiving attention from people with diabetes due to its high inulin contents) contained a higher level of acrylamide than potato-based foods (Jeong *et al*., 2020). However, given that processed foods are heated under different conditions with a variety of ingredients, determining the acrylamide-forming potential of individual food ingredients has been challenging.

The key pathway by which acrylamide formation occurs in foods is the reaction between asparagine and free monosaccharides, typically represented by fructose and glucose (Becalski *et al*., 2003). Asparagine, an amino acid present in high contents in potatoes and cereals, is a crucial component in the production of acrylamide via the Maillard reaction at temperatures above 100°C (Friedman, 2003). Stadler *et al*. proved that the sugar–asparagine adduct, N-glycosylasparagine, is a direct precursor of acrylamide, indicating the involvement of the Maillard reaction (Stadler *et al*., 2002). Clear relationships between asparagine content in food systems and acrylamide formation have also been demonstrated in wheat and rye bread model systems, whereas no dependence on the glucose or fructose content has been observed (Bråthen and Knutsen, 2005; Elmore *et al*., 2005). Contrarily, a stronger correlation has been observed between acrylamide content and glucose and fructose concentrations than between acrylamide and asparagine contents, after frying in potatoes (De Wilde *et al*., 2005). However, most studies on the relationship between these precursors and acrylamide formation

have been restricted to certain food matrices, especially wheat, rye, or potatoes; thus, the contributions of the precursors in various food systems, specifically for tubers and rhizomes, are poorly understood.

Acrylamide formation is strongly dependent on heating conditions. Previous studies have demonstrated that the amount of acrylamide increased with increasing frying and baking temperatures (Bråthen and Knutsen, 2005), and that the acrylamide content reaches a maximum at approximately 190–210°C. Moreover, while the acrylamide content increased with heating time, prolonged heating tended to reduce the acrylamide content (Becalski *et al*., 2003; Corradini & Peleg, 2006). Nevertheless, correlations between heating temperatures or time and acrylamide formation varied in dry and aqueous systems. Therefore, considering the contributions of the heating temperature, time, and moisture content is vital for determining the heating conditions. By identifying the optimum conditions for acrylamide formation, it would be possible to compare the acrylamide-forming potentials of individual ingredients.

The aim of this study was to evaluate the acrylamide-forming potentials of carbohydrate-rich botanical powders consumed in Korea, in terms of the contributions of asparagine, glucose, and fructose contents as acrylamide precursors. Concentrations of each precursor were measured in the raw powders, subsequently being treated with an optimised heating condition established by several experiments using potato powder. Then, the quantitative relationships of the precursors and acrylamide formation were investigated.

Materials and Methods

Chemicals

The acrylamide analytical standard and the $^{13}C_3$-acrylamide internal standard were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (HPLC Reagent, 99.9%), formic acid (glacial, 99.0%), and acetic acid (glacial, 99.7%) were purchased from Samchun Pure Chemicals (Gyeonggi, Korea). To prepare the acrylamide standard solutions of required concentrations, an aqueous stock solution (0.02 mg/mL) of acrylamide was prepared and diluted with water. A stock solution of $^{13}C_3$-acrylamide (1 mg/mL) was prepared in methanol and diluted with 0.1% aqueous formic acid for use as the internal standard (200 ng/mL). All the stock solutions were stored at 4°C prior to use. D-(–)-fructose (HPLC grade, ≥99.0%) and D-(+)-glucose (GC grade, 99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA), acetonitrile (HPLC reagent, 99.9%) was purchased from Samchun Pure Chemicals (Gyeonggi, Korea), and acetone (HPLC reagent, ≥99.5%) was purchased from Fisher Chemical (Waltham, MA, USA). Distilled deionised water was used in all the experiments, and the in-house deionised water was re-purified using a water purification system (Aqua MAX-Ultra System, Younglin Instrument, Gyeonggi, Korea).

L-aspartic acid, L-asparagine (HPLC grade, ≥98%), 2-mercaptoethanol (GC grade, ≥99.0%), and phthaldialdehyde (HPLC grade, ≥97%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Monosodium phosphate (ACS grade, ≥98%) was purchased from Fisher Chemical (Waltham, MA, USA). Boric acid (≥99.5%) was purchased from Wako Pure Chemical (Osaka, Japan). Sodium hydroxide (≥98%) was purchased from Samchun Pure Chemicals (Gyeonggi, Korea). 40 mM sodium phosphate buffer (pH 7.8) and 0.4 M boric acid buffer (pH 10.2) solutions were filtered using polyvinylidene difluoride (PVDF) membrane filters (0.22 μm GV, Merck Millipore, MA, USA).

Sample preparation

A total of 25 samples of 15 types of powders were purchased from the best-selling brands for each type of powder on commercial Korean websites. The samples include the powders frequently consumed in Korea, such as rice, starchy rice, and lotus root. For most of the powder samples, only one brand was used; however, for powders with high acrylamide contents, e.g., potato, lotus root, Jerusalem artichoke, and yam, three brands were analysed and each sample was presented with different numbers. Since wheat flour is the most used carbohydrate-rich powder, three wheat flour brands were analysed. For tapioca starch and potato starch, three different brands were mixed and analysed. All the products other than tapioca starch originated from South Korea. Furthermore, all the samples were free of additives and were not subjected to heating processes for drying (steaming or stir-frying).

Optimisation of heating conditions

Dough preparation and heating method

The powders were freeze-dried; thereafter, water was added to attain specific moisture contents. The initial moisture content of the dough was presented as the ratio of the added water (g) to the total weight (g) of the mixed dough (%, w/w). The examination of the various recipes employing botanical powders with 50, 55, 60, 65, and 70% (w/w) moisture contents indicated that their textures were distinguishable and moulded readily. The mixed dough (5.5 g) was spread evenly in a round shape with a thickness of 1 mm. Thereafter, the dough samples were heated on a preheated copper mould to the

required temperatures. The copper mould, produced by Banseok Tech (Gyeonggi, Korea), had a thickness and diameter of 1 mm and 7 cm, respectively; its surface was maintained at an intended temperature using a hot plate (Daihan Scientific, Gangwon, Korea), and the temperature was measured using a surface thermometer (Testo, Lenzkirch, Germany). As in previous studies (Bråthen and Knutsen, 20052005; Corradini and Peleg, 2006), the heating temperature ranged from 160 to 200°C, and the heating time ranged from 2 to 18 min.

Upon heating the dough completely, it was cooled down immediately in a deep freezer (–80°C) for 2 min. The dough was then kept in a desiccator until its weight became constant (weight change below 0.3%), followed by lyophilization.

Establishing optimum heating conditions

To study the acrylamide-forming-potential of each powder type, heating conditions were established. For the optimisation of the heating temperature, time, and initial moisture content, potato powder was used. Since potato forms high levels of acrylamide, it was considered the best matrix for readily distinguishing between the experimental conditions.

First, the acrylamide content was measured at 2, 5, 7, 10, 12, 15, and 18 min after initiation of heating, and optimum heating time was set at the point where the acrylamide content showed no further increase. To determine whether the moisture and temperature affect the heating time significantly, doughs with different moisture contents (50 and 65%) were heated at two temperatures (160 and 200°C). With the heating time fixed at the time determined in the first experiment, the temperature required for realising the highest acrylamide content was determined. Doughs with 50 and 65% moisture contents were heated separately at 160, 170, 180, 190, and 200°C. Finally, the initial moisture contents were optimised by the analysis of the doughs with 50, 55, 60, 65, and 70% moisture contents, using the heating time and temperature obtained in the previous experiments. The set heating conditions were applied in the analysis of the acrylamide-forming potentials of the powders.

Analytical method for acrylamide

The analysis of the acrylamide was done following the method reported by Jeong *et al*. (2020) and outlined briefly below.

Sample treatment

The sample was homogenised, and the internal standard, $^{13}C_3$-acrylamide (2 mL, 200 ng/mL), and water (18 mL) were added to 2 g of the sample. Acrylamide was extracted using a shaker (SI-600R, Lab companion, Jeiotech, Seoul, Korea) and centrifuged. The supernatant was then filtered through a 0.2 μm PVDF syringe filter (25 mm PVDF, Chrom4 GmbH, Hauptstraβe, Germany). Oasis HLB (6 mL, 200 m, Waters Corporation, MA, USA) and a Bond Elut AccuCAT cartridge (3 mL, 200 mg, Agilent, CA, USA) were employed sequentially for the clean-up. The final eluate was used to quantify the acrylamide content.

Acrylamide quantification using HPLC-MS/MS

For acrylamide quantification, a Nexera X2 system (Shimadzu, Kyoto, Japan) equipped with LCMS-8050 (Shimadzu, Kyoto, Japan) was used. Isocratic elution with an aqueous solution containing 0.2% acetic acid and 0.5% methanol (flow rate = 0.2 mL/min) was performed on a C18 HPLC column (INNO Column C18, 5 μm, 120 Å; 2.0 mm × 250 mm, YoungJin Biochrom, Gyeonggi, Korea). A 20 μL aliquot of the sample was injected and the oven temperature was maintained at 35°C. Electrospray in positive ionization mode was used for detection.

Analysis of free asparagine and free monosaccharide (fructose and glucose) contents

Sample treatment

The dough made for the heating process was freeze-dried and homogenised. The sample (1 g) was mixed with water (18 mL) in a 50 mL conical tube, and the mixture was sonicated for 15 min in a water bath at 60°C. The resulting mixture was further shaken for 20 min at 37°C using a shaker (SI-600R, Lab companion, Jeiotech, Seoul, Korea), and centrifuged at 2,898 g for 20 min. The supernatant was filtered through a 0.22 μm PVDF syringe filter, and 3 mL of the filtrate was used for the analysis of free asparagine. Another 3 mL was diluted with 3 mL of acetonitrile for the analysis of fructose and glucose.

Quantification of free asparagine using HPLC-FLD

The method established by Kang (2007) was adjusted for the analysis of asparagine in the dough extract, using a high-performance liquid chromatography–fluorescence detector (HPLC-FLD). For the derivatization, 10 μL of 0.5 M sodium hydroxide and 10 μL of 0.4 M boric acid buffer, pH 10.2, were added to 20 μL of the dough extract. A few seconds later, 20 μL of o-phthaldialdehyde solution (50 mg/mL in 2-mercaptoethanol) was added, and the solution was maintained for 2 min. An aliquot of the resulting reaction mixture (20 μL) was injected into the HPLC system. A Dionex Ultimate 3000 (ThermoFisher, MA, USA) system connected to a ZORBAX Eclipse AAA column (4.6 mm × 150 mm, 3.5 μm, Agilent, CA, USA) was used for the analysis. The detailed elution condition using eluents A (40 mM sodium phosphate buffer,

Table 1. Gradient elution condition of HPLC-FLD for asparagine analysis.

Time (min)	A (%)	B (%)	Flow rate (mL/min)
0	100	0	2
3.9	100	0	2
5	90	10	2
10	90	10	2
11	50	50	1.5
18	50	50	1.5
19	100	0	2
25	100	0	2

pH 7.8) and B (acetonitrile: methanol: water = 45:45:10) was listed in Table 1. Free asparagine was quantified using the Dionex Ultimate 3000 Fluorescence detector (ThermoFisher, MA, USA). The excitation and emission wavelengths were 345 and 460 nm, respectively.

Quantification of free monosaccharides using HPLC-ELSD

The method established by Montesano *et al*. (2016) was adjusted for the analysis of free monosaccharides, using high-performance liquid chromatography–evaporative light scattering detection (HPLC-ELSD). A Dionex Ultimate 3000 (ThermoFisher, MA, USA) system comprising a carbohydrate analysis column (aminopropylmethylsilyl-bonded amorphous silica, 10 µm, 125 Å; 3.9 mm × 300 mm, Waters, MA, USA) was used. The isocratic elution with a mixture of acetone (20%), acetonitrile (60%), and water (20%) was employed. The flow rate was 1 mL/min, and 20 µL of the sample was injected. PL-ELS2100 (Varian, CA, USA) was used as the detector, and the evaporator and nebuliser temperatures were 35 and 80°C, respectively. The nitrogen gas flow rate was 1.6 SLM.

Statistical analysis

IBM SPSS Statistics (version 25.0, SPSS Inc., Chicago, IL, USA) was used for statistical analyses. One-way analysis of variance (ANOVA) and paired *t*-tests were used to test the effects of temperature and moisture content on the acrylamide content. Post-hoc comparison of means (Tukey test) was performed to determine significant differences in acrylamide contents among different levels within each heating factor group. The term "significant" is used to indicate differences for which $p < 0.05$. In addition, a simple linear regression between the acrylamide contents and the concentrations of its precursors was performed to determine the correlations between asparagine or sugar contents and acrylamide contents. The most appropriate regression model was determined based on the Pearson correlation coefficient, r.

Results and Discussion

Optimisation of the heating conditions

Heating time

The acrylamide contents of potato doughs with moisture contents of 50% and 65% were measured after heating at 160 or 200°C for 2, 5, 7, 10, 12, 15, and 18 min.

As the acrylamide contents of the powders increased with increased heating time, the plateau of the acrylamide content was observed between 15 and 18 min. Since this tendency appeared constant when moisture contents of 50 or 65% were used, it was concluded that heating for 18 min would result in the stabilization of the acrylamide content of the doughs even under the different experimental conditions employed. Thus, the 18 min heating time was selected as the heating time condition. The acrylamide contents in heated potato doughs were presented by heating times in Figure 1.

Heating temperature

The potato doughs with two moisture contents (50 or 65%) were heated at 160, 170, 180, 190, and 200°C for 18 min to ensure consistency in the trend between the temperature and acrylamide content at various moisture content settings. For both moisture contents, the acrylamide content tended to increase with increasing heating temperature and was stabilised at temperatures in the range 190–200°C and 170–200°C in the doughs with moisture contents of 50 and 65%, respectively (Table 2).

In the 160–180°C range, the acrylamide content was significantly higher for the doughs with 65% moisture content than the doughs with 50% moisture content. This indicates that heat transfer was accelerated in the interior portion of the dough with the higher moisture content. In addition, the higher moisture content possibly enhanced the mobility of asparagine and the sugars (Michalak *et al*., 2016). Thus, the moisture in the dough stimulated heat transfer and reactant mobility, probably causing the generation of higher levels of acrylamide. At 190 and 200°C, there was no significant difference between the acrylamide in two moisture content doughs.

Doughs with 65% moisture content showed no significant difference in acrylamide contents at temperatures in the range 170–200°C (Table 2), indicating that temperature has less significant effects on acrylamide formation at higher moisture contents. In other words, the reactants could generate a considerable amount of acrylamide at relatively lower temperatures as a result of improved heat transfer and reactant mobility.

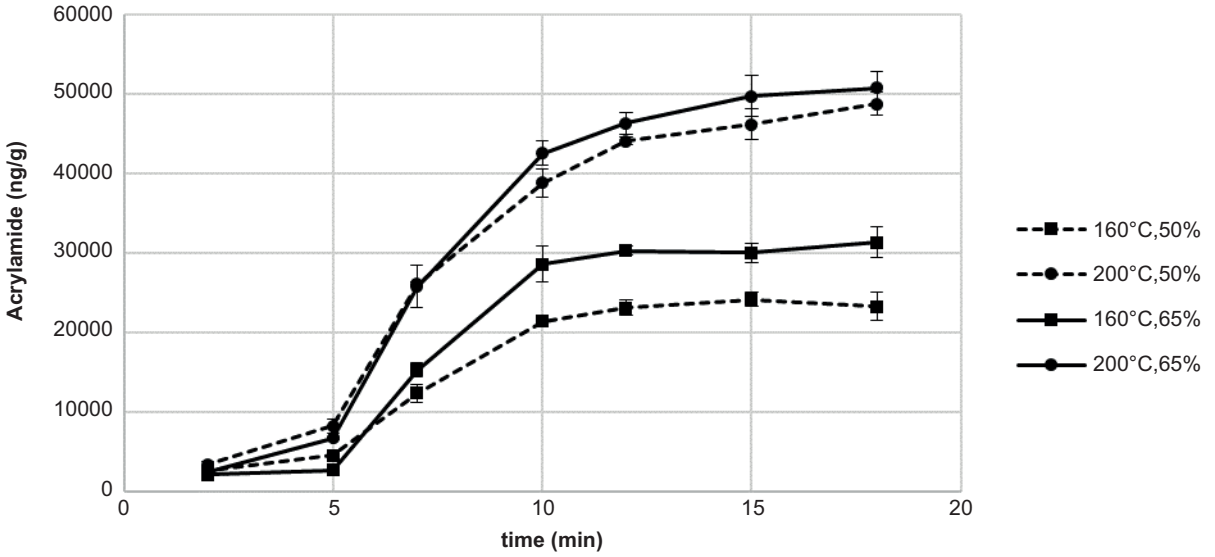

Figure 1. Acrylamide contents in potato doughs heated for various times, with multiple temperature (°C) and moisture content (%) conditions compared.

Table 2. Acrylamide content of potato dough heated for 18 min at various temperatures.

Temperature (°C)	Acrylamide content (ng/g)	
	Moisture content 50% Mean ± SD	Moisture content 65% Mean ± SD
160*	23154.1[a] ± 549.61	34694.8[a] ± 206.13
170*	26673.6[a] ± 359.44	48361.7[b] ± 4614.0
180*	40629.0[b] ± 335.02	53488.1[b] ± 502.31
190	47551.9[bc] ± 4514.0	53775.7[b] ± 516.02
200	51096.3[c] ± 1484.6	54875.2[b] ± 2005.1

[a–c]Values with the same letters indicate no significant differences between the acrylamide contents in the same moisture content with varying temperatures (one-way ANOVA, $p < 0.05$).
*Significantly different between the acrylamide contents of the two moisture contents in the same temperature (*t*-test, $p < 0.05$).

Table 3. Acrylamide content of potato dough heated for 18 minutes at various moisture contents.

Moisture content (%)	Acrylamide content (ng/g)	
	Temperature 170°C Mean ± SD	Temperature 200°C Mean ± SD
50*	26026.3[a] ± 1036.8	51096.3[a] ± 1484.6
55*	31456.0[a] ± 686.93	51511.7[a] ± 1141.4
60*	42687.4[b] ± 1562.4	53936.6[a] ± 1715.5
65	48793.9[c] ± 2849.9	52655.5[a] ± 2150.2
70*	45906.2[bc] ± 2077.4	53217.6[a] ± 769.84

[a–c]Values with the same letters indicate no significant differences between the acrylamide contents in the same temperature with varying moisture contents (one-way ANOVA, $p < 0.05$).
*Significantly different between the acrylamide contents of the two temperatures in the same moisture content (*t*-test, $p < 0.05$).

Initial moisture content

As presented in Table 2, acrylamide contents in two different moisture content doughs were not significantly different at 200°C. In contrast, acrylamide contents were distinguishable by moisture content in potato doughs heated at 170°C and 180°C. Besides, in moisture content 65%, there were no significant differences with the acrylamide content in doughs heated at 200°C, which is the maximum acrylamide level observed in the experiment. Thus, while these two temperatures could determine the moisture content where the maximum acrylamide content is measured, the lower temperature, 170°C, was adopted for experimental convenience. Therefore, an additional temperature, i.e., 170°C, was examined to determine the effect of moisture content on acrylamide formation. The acrylamide contents after heating at 170 and 200°C were compared, while varying the initial moisture content from 50 to 70% (Table 3).

No significant differences in acrylamide content were observed for different initial moisture contents at 200°C. However, at 170°C, the acrylamide level increased with increasing moisture content, which is in line with the observations in previous studies on the influence of moisture content on acrylamide formation (Ciesarova *et al.*, 2006; Rydberg *et al.*, 2005). However, this tendency was suppressed at moisture contents of 65%, with the acrylamide contents resulting from moisture contents of 65 and 70% showing no significant difference. This might be due to the reactant mobility competing with the dilution effect. It was reported that an optimum Maillard reaction rate was available at an intermediate

water activity (a_w) because the reactants were diluted at higher a_w, whereas at lower a_w, the mobility of reactants was limited (Lingnert *et al*., 2002). It is expected that 70% of moisture content was above that required for reactant dissolution, and thus facilitates reactant dilution (Bell, 2020).

Consequently, it appears that the acrylamide content was stabilised at a moisture content of 65% at various temperatures. Since the acrylamide contents observed at temperatures between 170 and 200°C in the 65% moisture content dough were not significantly different, 170°C was chosen as the experimental temperature for further study. The final heating conditions were determined to be 65% moisture, 170°C, and 18 min.

Acrylamide and its precursors in various carbohydrate-rich powders

Free asparagine, fructose, glucose, and acrylamide contents

After the heating process at the determined heating conditions (65%, 170°C, and 18 min), the acrylamide contents of the powders were quantified. The free asparagine content and the contents of two different free monosaccharides (fructose and glucose) in the freeze-dried dough mixture were also analysed before cooking and the results are shown in Table 4. The method detection limits (MDL) and the method quantification limits (MQL) were measured for asparagine (0.01, 0.02 μmol/g), free monosaccharides (0.04, 0.1 μmol/g), and acrylamide (0.05, 0.2 nmol/g).

Powders of tubers or rhizomes, lotus root, potato, Jerusalem artichoke, and yam tended to show high acrylamide contents after being heated, with a sample of heated lotus root powder showing the highest acrylamide content. Most of the heated cereal grain powders, including wheat and rice powders, showed relatively low acrylamide contents, with oat powder showing the highest acrylamide content among heated grain powders.

The acrylamide content of the heated wheat-1 and buckwheat powders were 5.5 and 5.0 nmol/g, respectively. The wheat-2 and wheat-3 powders had acrylamide contents of 0.7 and 1.0 nmol/g, respectively, when they were heated, which were much lower than that of the wheat-1 powder (5.5 nmol/g).

Low acrylamide contents were found in heated soybean (0.6 nmol/g), rice (1.7 nmol/g), and starchy rice (2.7 nmol/g) powders. The corn powder showed an acrylamide content of 11 g nmol/g after heating. Cereal grain powders, such as rice, starchy rice, wheat, whole wheat, and corn powders, had relatively low asparagine contents and generally showed low acrylamide contents upon heating. The acorn powder contained 15.4 μmol/g of asparagine and 12.8 μmol/g of free monosaccharides. Its acrylamide content was below the MQL when it was heated.

Acrylamide contents were in the range of 176.6–1291.1 nmol/g in the three lotus root powders after heating. One of those heated samples had the highest acrylamide content (1291.1 nmol/g). In addition, the acrylamide contents of heated potato and Jerusalem artichoke powders were in the ranges of 604.7–713.3 and 214.0–549.6 nmol/g, respectively (range of acrylamide content of the three samples in each powder). As previously mentioned, a higher amount of acrylamide was detected in tea bags made with Jerusalem artichoke than in potato-based processed foods (Jeong *et al*., 2020). However, the acrylamide content was higher in potato powders than in Jerusalem artichoke powders under the same heating conditions. Furthermore, heated Jerusalem artichoke powders had the third-highest acrylamide content after lotus root and potato powders. Heated yam powders had an acrylamide content in the range of 67.4–129.6 nmol/g.

Asparagine contents were also high in lotus root (82.8–232.6 μmol/g), potato (182.1–247.9 μmol/g), Jerusalem artichoke (236.9–472.6 μmol/g), and yam (46.1–93.7 μmol/g) powders, all of which were tubers or rhizomes. Both plant parts have a role in nutrient storage. Given that asparagine has high nitrogen to carbon ratio and low reactivity, it can be stored as a good nitrogen source and is thus concentrated in plants (Lea *et al*., 2007). Tapioca starch and potato starch powders showed acrylamide contents below the MQL after heating.

Effect of free asparagine and free monosaccharides on acrylamide formation

Free asparagine and free monosaccharide (glucose and fructose) contents were analysed as the precursors of acrylamide. The acrylamide contents of each heated powder were compared as a function of the limiting factors, either asparagine or the sugars.

For powders with more asparagine than sugar (potato-1, potato-3, buckwheat, JA-1, JA-2, JA-3, oat, soybean, lotus root-2, lotus root-3, yam-1, and yam-2), their acrylamide contents were presented as a function of sugars. For powders containing more sugar than asparagine (potato-2, rice, starchy rice, wheat-1, whole wheat, corn, lotus root-1, and yam-3), their acrylamide contents were compared as a function of asparagine content. Powders of wheat-2, wheat-3, tapioca starch, and potato starch were excluded because their sugar contents were below the MQL. Acorn powder was also excluded because its acrylamide content was below the MQL upon heating.

Being proportional to the limiting factors in raw powders, the acrylamide formation corresponded to the

Table 4. Free asparagine and free monosaccharide (fructose and glucose) contents of the raw powder before heating; acrylamide content in the heated dough of the powder.

Type of powder	Acrylamide (nmol/g) Mean ± SD	Asparagine (µmol/g) Mean ± SD	Free monosaccharides (µmol/g) Mean ± SD
Potato1[a]	604.7 ± 38.8	247.9 ± 15.3	45.5 ± 1.2
Potato2	623.5 ± 24.8	182.1 ± 10.0	235.8 ± 7.1
Potato3	713.3 ± 21.9	189.3 ± 15.4	43.5 ± 1.1
Lotus root1	176.6 ± 4.4	82.8 ± 5.6	106.4 ± 2.2
Lotus root2	1291.1 ± 38.2	228.8 ± 8.2	50.9 ± 0.7
Lotus root3	820.7 ± 34.5	232.6 ± 5.0	36.5 ± 0.3
JA1[b]	549.6 ± 16.9	236.9 ± 18.0	38.3 ± 1.6
JA2	255.5 ± 2.3	472.6 ± 34.9	33.1 ± 0.5
JA3	214.0 ± 15.7	340.1 ± 17.5	31.1 ± 0.9
Yam1	129.6 ± 6.7	93.7 ± 7.5	24.7 ± 0.5
Yam2	67.4 ± 2.4	78.4 ± 4.8	28.4 ± 0.3
Yam3	68.3 ± 1.7	46.1 ± 3.6	79.3 ± 2.0
Oat	18.4 ± 0.2	52.5 ± 3.4	19.3 ± 0.2
Corn	11.6 ± 0.1	11.6 ± 0.8	35.3 ± 0.6
Whole wheat	10.8 ± 0.2	13.0 ± 0.6	29.7 ± 0.3
Buckwheat	5.0 ± 0.1	50.3 ± 2.8	22.8 ± 0.1
Wheat1	5.5 ± 0.2	9.4 ± 0.3	23.3 ± 0.3
Wheat2	0.7 ± 0.01	20.6 ± 1.2	n.d.[c]
Wheat3	1.0 ± 0.03	6.5 ± 0.04	n.d.
Rice	1.7 ± 0.2	7.8 ± 0.1	22.0 ± 0.4
Starchy rice	2.7 ± 0.02	9.0 ± 0.5	11.7 ± 0.1
Acorn	0.1 ± 0.01	15.4 ± 0.6	12.8 ± 0.1
Soy bean	0.6 ± 0.03	92.0 ± 8.4	13.5 ± 0.1
Tapioca starch[d]	0.1 ± 0.001	8.4 ± 0.1	n.d.
Potato starch[d]	0.1 ± 0.003	8.8 ± 0.1	n.d.

[a]Each number indicates different products from three brands.
[b]JA: Jerusalem artichoke.
[c]n.d: not detected (below the MDL value).
[d]Three products were mixed and analysed.

asparagine or sugar content of the powders, which is consistent with the results of reported studies that were conducted with several food matrices (potato, wheat, or rye). In previous studies, it was concluded that sugar contents were the main determinants of acrylamide formation in potato (Elmore *et al*., 2005; Halford *et al*., 2012), while in wheat and rye, the acrylamide content depended on asparagine content (Curtis *et al*., 2010; Granvogl *et al*., 2007). Acrylamide formations based on asparagine and sugar contents as the limiting factors are compared in Figure 2.

Figure 2 illustrates that acrylamide contents inclined to be magnified steeper in heated powders as free monosaccharide contents increased, rather than when free asparagine contents did, which implied that the same amounts of limiting factors produced different levels of acrylamide. This observation was contrary to the expectation that the same amount of limiting factors, either asparagine or sugar, would produce the same amount of acrylamide following a one-to-one molecular reaction. Multi-response kinetic models, related to the effect of asparagine, free amino acids, and sugar contents on acrylamide formation, were suggested by Balagiannis *et al*. (2019) and Gökmen *et al*. (2012) (Figure 2).

Figure 3, illustrating the various pathways for acrylamide formation suggests that acrylamide could be generated via both the "specific amino acid pathway (SP)" and the "generic amino acid pathway (GP)." In the SP, asparagine is exhausted by the reaction with sugars, directly forming a decarboxylated Schiff base, which in turn generates acrylamide. In the GP, asparagine reacts with a variety of carbonyl intermediates, which are produced by the Maillard reaction via the interaction between the amino acids (including asparagine) and sugars in food.

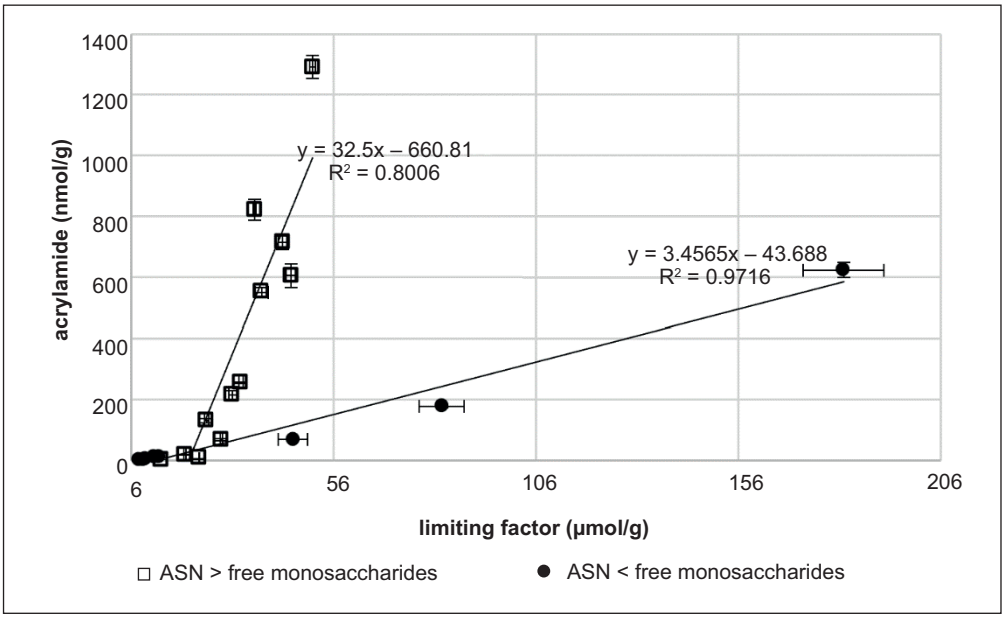

Figure 2. Acrylamide content as a function of its limiting factors, either asparagine or free monosaccharide contents, depending on the food system.

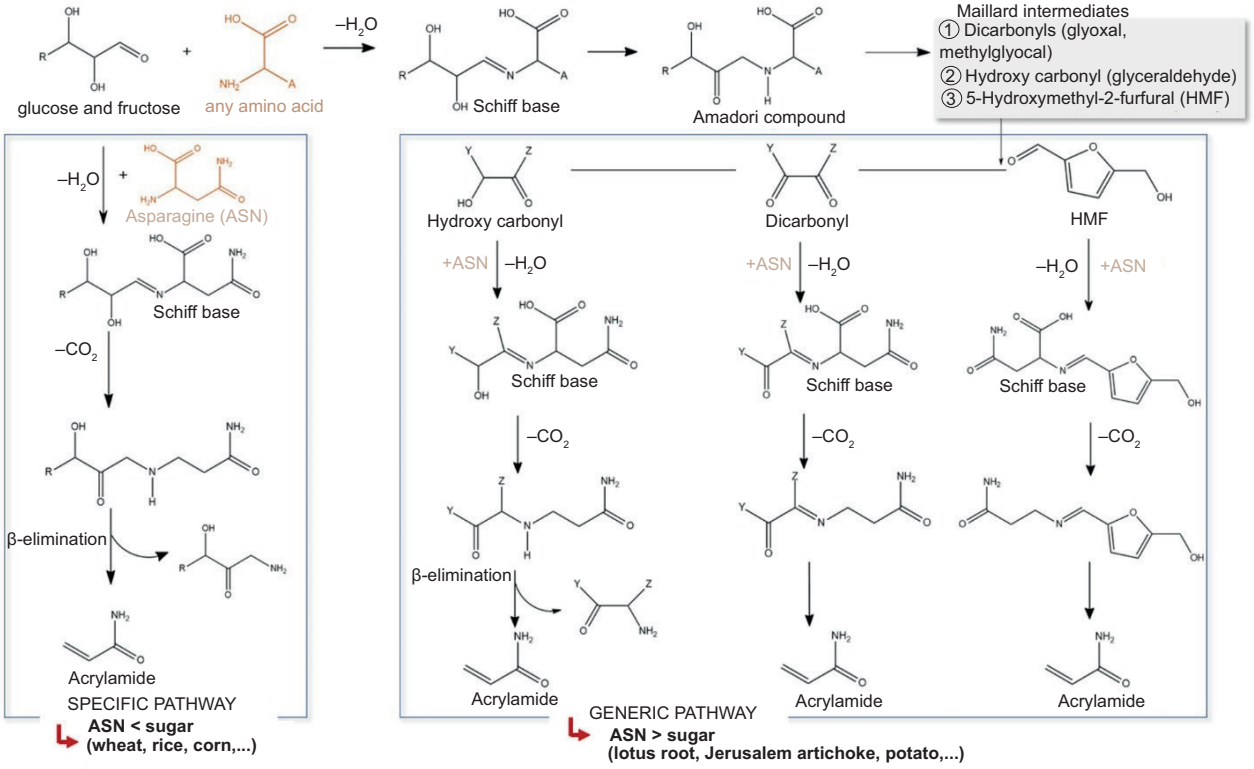

Figure 3. Acrylamide formation pathways based on reactions between monosaccharides and amino acids. The overall reactions were adapted from Balagiannis *et al.* (2019), with the HMF pathway added from Gökmen *et al.* (2012).

Under asparagine-limited conditions, asparagine is exhausted by the SP, making the absolute amount of potential acrylamide more limited. Besides, in the abundance of asparagine with the same level of limited sugars, asparagine still generates acrylamide via GP using Maillard intermediates produced by the SP. Consequently, there would be higher chances for acrylamide formation when the sugars are the limiting factors than when asparagine is limited. This phenomenon occurs mainly due to the asparagine being the essential substance for the acrylamide formation, while sugars could be substituted by the Maillard intermediates.

Table 4 shows that the asparagine-to-sugar ratios in the powders containing more asparagine than sugar ranged from approximately 2:1 to 14:1. Excessive asparagine would increase the possibility for the generic pathway to occur, allowing more asparagine to react with the carbonyl intermediates. In addition, in this study, the heating process was prolonged to 18 min, possibly resulting in the accumulation of more Maillard intermediates than was obtained in the study conducted by Balagiannis *et al.* (2019).

The contributions of each pathway to acrylamide formation were dependent on the food composition. Equimolar solutions of fructose and asparagine formed acrylamide predominantly via the SP (Knol *et al*., 2010). Likewise, in a wheat biscuit model with excessive sugar, the SP was dominant (Nguyen *et al*., 2016). In contrast, in a French fry model with sugar levels lower than asparagine levels (ratio of asparagine/sugar 1.5–10), the GP was dominant in acrylamide formation owing to the accumulation of reactive carbonyl intermediates (Balagiannis *et al*., 2019). The study demonstrated that acrylamides produced via the GP was approximately five-fold higher than that produced via the SP (the ratio of asparagine/sugar 1.5, heated at 165°C for 5 min).

These results indicate that when the asparagine level in a food system is limited, most of the acrylamide is formed via the direct reaction between asparagine and sugars. However, in the presence of higher levels of asparagine, Maillard intermediates are primarily responsible for acrylamide formation. As shown in Figure 2, samples with asparagine exceeding sugars formed approximately nine-folds more acrylamide than the other samples. The GP is deemed to account for this phenomenon, which is promoted by the Maillard reaction in food systems with high asparagine contents.

Given that the Maillard reaction is involved in the pathway, the presence of free amino acids (FAA) could control the acrylamide formation by competing with asparagine in the reaction to the carbonyl intermediates comprising a part of the GP. The competitive effect was observed in a previous study on acrylamide formation in model systems containing asparagine, glucose, and various amino acids (Koutsidis *et al*., 2009). When asparagine (25 mmol/kg), glucose (50 mmol/kg), and amino acids (25 or 75 mmol/kg) were reacted at 160°C for 20 min, acrylamide formation decreased when the additional amino acid content was 75 mmol/kg. The extent of the decrease in acrylamide formation depended on the type of the added amino acid (12 and 81% reductions in the presence of valine and tryptophan, respectively).

Although FAA other than asparagine were not quantified in this study, the amount of FAA in the tubers, lotus root, and several grains could be estimated based on the results of previous studies. Reported FAA data were utilised to calculate the ratio of free asparagine to the free amino acids pool (Table 5). The concentration of the FAA pool was compared with the mean concentrations of free monosaccharides, which were measured in this study to provide plausible evidence for the occurrence of the Maillard reaction in the powders.

The FAA concentrations exceeded the contents of the free monosaccharides in most of the powders by approximately 2 to 46-folds, excluding the corn powder. Consequently, these amino acids would competitively participate in the Maillard reaction; consuming monosaccharides, in turn, could lead to the carbonyl products, not only acrylamide.

Asparagine constituted 20–50% of the FAA pool in tubers and rhizomes, which showed high levels of acrylamide. In oats, which has the largest acrylamide content among the cereal grains, free asparagine constituted 40% of the FAA. Wheat, rice, and corn, with low acrylamide contents, had relatively smaller free asparagine contents (5–16% of the FAA). The powders with higher asparagine contents than sugars tended to have a larger portion of asparagine to the FAA than in the other powders. It is presumed that asparagine in cereal grains, such as wheat or rice, was inhibited from participating in the Maillard reaction by the other amino acids. Thus, in addition to the GP, the competition between amino acids might as well affect the substantial difference of acrylamide formation as a function of the limiting factors as shown in Figure 2.

Conclusions

A variety of carbohydrate-rich powders that are consumed in Korea were examined for their acrylamide-forming potential. Among the powders, tubers, and rhizomes, which are used by plants for nutrient storage, were the most potent contributors to acrylamide formation. Under the same heating conditions, the highest acrylamide content was detected in lotus root, followed by potato, Jerusalem

Table 5. Mean concentrations of free amino acids (FAA), mean ratios of free asparagine to FAA (ASN/FAA) (data from published studies[a]), and mean concentrations of free monosaccharides in powders (data from the present study[b]).

	Free monosaccharides (μmol/g)	FAA (ASN/FAA) (μmol/g)	Reference
Lotus root	64.6	1080.6 (0.5)	Edo *et al*. (2016)
Jerusalem Artichoke	34.2	1566.6 (0.3)	Jung and Shin (2016); Bobrivnyk *et al*. (2017)
Yam	44.1	159 (0.2)	Duan *et al*. (2016); Kim and Park (2014)
Potato	108.3	250.4 (0.3)	Halford *et al*. (2012); Davies (1977)
Oats	19.3	134.5 (0.4)	Mustafa *et al*. (2007); Jun *et al*. (2017)
Wheat	23.3	66.85 (0.16)	Mustafa *et al*. (2007)
Rice	22.0	199.0 (0.05)	Kamara *et al*. (2010)
Corn	35.3	23.3 (0.1)	Harrigan *et al*. (2007a, 2007b)

[a]Contents of 20 amino acids (Gly, Ala, Ser, Thr, Cys, Val, Leu, Ile, Met, Pro, Phe, Tyr, Trp, Asp, Glu, Asn, Gln, His, Lys, and Arg) were collected from analytical results of given references. The ND was considered as zero.
[b]Asparagine and monosaccharide contents are from the results of this study.

artichoke, and yam. Asparagine and monosaccharide levels, represented by fructose and glucose contents, were also measured as the limiting factors of acrylamide formation in the powders. The same amounts of the limiting factors, i.e., either asparagine or sugar, produced different levels of acrylamide. In the acrylamide-forming pathway, asparagine could be consumed to form acrylamide or carbonyl intermediates, via the Maillard reaction. Thus, it was expected that with higher levels of asparagine in food, there would be a greater chance of forming acrylamide because these carbonyl Maillard intermediates may react again with asparagine. The competition between asparagine and other amino acids was identified as another factor that possibly contributes to higher acrylamide formation in the food in which asparagine dominated the free amino acid composition.

The results of this study proved that several food ingredients consumed in Korea, such as lotus root and Jerusalem artichoke, are highly potent to be contaminated by acrylamide. In addition, the present study demonstrated that the reaction pathways in real food systems alter depending on the quantitative relationships of the precursors. Investigations on the Maillard intermediates in food matrices with different asparagine/sugar ratios are necessary to control acrylamide formation in food. It is worth conducting a further study on the relative contributions of asparagine and the other amino acids producing Maillard intermediates, with which acrylamide could be formed through the generic pathway.

References

Balagiannis, D.P., Mottram, D.S., Higley, J., Smith, G., Wedzicha, B.L. and Parker, J.K., 2019. Kinetic modelling of acrylamide formation during the finish-frying of french fries with variable maltose content. Food Chemistry. 284: 236–244. https://doi.org/10.1016/j.foodchem.2019.01.075

Becalski, A., Lau, B.P.Y., Lewis, D. and Seaman, S.W., 2003. Acrylamide in foods: occurrence, sources, and modelling. Journal of Agricultural and Food Chemistry. 51(3): 802–808. https://doi.org/10.1021/jf020889y

Bell, L.N., 2020. Moisture effects on food's chemical stability. In: Barbosa-Cánovas, G.V., Fontana, A.J., Jr, Schmidt, S.J. and Labuza, T.P. (eds.) Water activity in foods: fundamentals and applications. John Wiley & Sons, Hoboken, NJ, pp. 227–253.

Bobrivnyk, L., Remeslo, N., Stepanets, L. and Fedorenchenko, L., 2017. Chemické složení hlíz vybraných odrůd topinamburu. Listy Cukrovarnicke a Reparske. 133(3): 104–107.

Bråthen, E. and Knutsen, S.H., 2005. Effect of temperature and time on the formation of acrylamide in starch-based and cereal model systems, flat breads and bread. Food Chemistry. 92(4): 693–700. https://doi.org/10.1016/j.foodchem.2004.08.030

Ciesarova, Z., Kiss, E. and Kolek, E., 2006. Study of factors affecting acrylamide levels in model systems. Czech Journal of Food Sciences. 24(3): 133. https://doi.org/10.17221/3308-CJFS

Corradini, M.G. and Peleg, M., 2006. Linear and non-linear kinetics in the synthesis and degradation of acrylamide in foods and model systems. Critical Reviews in Food Science and Nutrition. 46(6): 489–517. https://doi.org/10.1080/10408390600758280

Curtis, T.Y., Powers, S.J., Balagiannis, D., Elmore, J.S., Mottram, D.S., Parry, M.A. and Halford, N.G., 2010. Free amino acids and sugars in rye grain: implications for acrylamide formation. Journal of Agricultural and Food Chemistry. 58(3): 1959–1969. https://doi.org/10.1021/jf903577b

Davies, A., 1977. The free amino acids of tubers of potato varieties grown in England and Ireland. Potato Research. 20(1): 9–21. https://doi.org/10.1007/BF02362297

De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W. and Calus, A., 2005. Influence of

storage practices on acrylamide formation during potato frying. Journal of Agricultural and Food Chemistry. 53(16): 6550–6557. https://doi.org/10.1021/jf050650s

Duan, Y., Kim, G., Joung, S. and Kim, H., 2016. Nutritional evaluation of Korean yam (Dioscorea batatas DECNE.). Journal of Korean Applied Science and Technology. 33(2): 391–400. https://doi.org/10.12925/jkocs.2016.33.2.391

Edo, K., Hashimoto, T., Nishio, S., Sawada, E. and Nakagawa, H., 2016. Free amino acid contents of roots of the lotus cultivars "Bicchu" and "Lotus" grown in Tokushima prefecture. Journal for the Integrated Study of Dietary Habits. 27: 221–224. https://doi.org/10.2740/jisdh.27.3_221

Elmore, J.S., Koutsidis, G., Dodson, A.T., Mottram, D.S. and Wedzicha, B.L., 2005. Measurement of acrylamide and its precursors in potato, wheat, and rye model systems. Journal of Agricultural and Food Chemistry. 53(4): 1286–1293. https://doi.org/10.1021/jf048557b

European Commission (EC), 2002. Opinion of the Scientific Committee on Food (SCF) on new findings regarding the presence of acrylamide in food. EC, Brussel.

Friedman, M. 2003. Chemistry, biochemistry, and safety of acrylamide. A review. Journal of AgricUltural and Food Chemistry. 51(16): 4504–4526. https://doi.org/10.1021/jf030204+

Gökmen, V., Kocadağlı, T., Göncüoğlu, N. and Mogol, B.A., 2012. Model studies on the role of 5-hydroxymethyl-2-furfural in acrylamide formation from asparagine. Food Chemistry. 132(1): 168–174. https://doi.org/10.1016/j.foodchem.2011.10.048

Granvogl, M., Wieser, H., Koehler, P., Von Tucher, S. and Schieberle, P. 2007. Influence of sulfur fertilization on the amounts of free amino acids in wheat. Correlation with baking properties as well as with 3-aminopropionamide and acrylamide generation during baking. Journal of Agricultural and Food Chemistry. 55(10): 4271–4277. https://doi.org/10.1021/jf070262l

Halford, N.G., Muttucumaru, N., Powers, S.J., Gillatt, P.N., Hartley, L., Elmore, J.S. and Mottram, D.S., 2012. Concentrations of free amino acids and sugars in nine potato varieties: effects of storage and relationship with acrylamide formation. Journal of Agricultural and Food Chemistry. 60(48): 12044–12055. https://doi.org/10.1021/jf3037566

Harrigan, G.G., Stork, L.G., Riordan, S.G., Ridley, W.P., MacIsaac, S., Halls, S.C. and Wen, L., 2007a. Metabolite analyses of grain from maize hybrids grown in the United States under drought and watered conditions during the 2002 field season. Journal of Agricultural and Food Chemistry. 55(15): 6169–6176. https://doi.org/10.1021/jf070493s

Harrigan, G.G., Stork, L.G., Riordan, S.G., Reynolds, T.L., Ridley, W.P., Masucci, J.D. and Smith, R.G., 2007b. Impact of genetics and environment on nutritional and metabolite components of maize grain. Journal of Agricultural and Food Chemistry. 55(15): 6177–6185. https://doi.org/10.1021/jf070494k

International Agency for Research on Cancer (IARC), 1994. Acrylamide. IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 60. IARC, Lyon.

Jeong, H., Hwang, S. and Kwon, H., 2020. Survey for acrylamide in processed foods from Korean market and individual exposure estimation using a non-parametric probabilistic model. Food Additives and Contaminants: Part A. 37(6): 916–930.. https://doi.org/10.1080/19440049.2020.1746410

Jun, H., Yoo, S., Song, G. and Kim, Y., 2017. Effect of particle size of naked oat flours on physicochemical and antioxidant property. Korean Journal of Food Preservation. 24(7): 965–974. https://doi.org/10.11002/kjfp.2017.24.7.965

Jung, B. and Shin, T., 2016. Food components and antioxidant activities of dried Jerusalem artichoke with white and purple colors. Journal of Korean Society of Food Science and Nutrition. 45(8): 1114–1121. https://doi.org/10.3746/jkfn.2016.45.8.1114

Kamara, J.S., Konishi, S., Sasanuma, T. and Abe, T., 2010. Variation in free amino acid profile among some rice (Oryza sativa L.) cultivars. Breeding Science. 60(1): 46–54. https://doi.org/10.1270/jsbbs.60.46

Kang, O., 2007. Comparison of free amino acids in soybean paste (Doenjang) by different extraction solvents and analytical methods. Korean Journal of Food Cookery Science. 23(1): 150–155.

Kim, H. and Park, E., 2014. Change of amino acids contents of Gastrodia elata Blume with harvest times and seed tuber. Journal of Plant Biotechnology. 41(4): 229–235. https://doi.org/10.5010/JPB.2014.41.4.229

Knol, J.J., Linssen, J.P. and Van Boekel, M.A., 2010. Unravelling the kinetics of the formation of acrylamide in the Maillard reaction of fructose and asparagine by multi-response modelling. Food Chemistry. 120(4): 1047–1057. https://doi.org/10.1016/j.foodchem.2009.11.049

Koutsidis, G., Simons, S.P., Thong, Y.H., Haldoupis, Y., Lazaro, J.M., Wedzicha, B.L. and Mottram, D.S., 2009. Investigations on the effect of amino acids on acrylamide, pyrazines, and Michael addition products in model systems. Journal of Agricultural and Food Chemistry. 57(19): 9011–9015. https://doi.org/10.1021/jf9014763

Lea, P.J., Sodek, L., Parry, M.A., Shewry, P.R. and Halford, N.G., 2007. Asparagine in plants. Annals of Applied Biology. 150(1): 1–26. https://doi.org/10.1111/j.1744-7348.2006.00104.x

Lingnert, H., Grivas, S., Jägerstad, M., Skog, K., Törnqvist, M. and Åman, P., 2002. Acrylamide in food: mechanisms of formation and influencing factors during heating of foods. Scandinavian Journal of Nutrition. 46(4): 159–172. https://doi.org/10.1080/110264802762225273

Michalak, J., Gujska, E., Czarnowska, M., Klepacka, J. and Nowak, F., 2016. Effect of storage on acrylamide and 5-hydroxymethylfurfural contents in selected processed plant products with long shelf-life. Plant Foods for Human Nutrition. 71(1): 115–122. https://doi.org/10.1007/s11130-015-0523-4

Montesano, D., Cossignani, L., Giua, L., Urbani, E., Simonetti, M.S. and Blasi, F., 2016. A simple HPLC-ELSD method for sugar analysis in Goji berry. Journal of Chemistry. 2016: 1–5. https://doi.org/10.1155/2016/6271808

Mustafa, A., Åman, P., Andersson, R. and Kamal-Eldin, A., 2007. Analysis of free amino acids in cereal products. Food Chemistry. 105(1): 317–324. https://doi.org/10.1016/j.foodchem.2006.11.044

Nguyen, H.T., Peters, R.J. and Van Boekel, M.A., 2016. Acrylamide and 5-hydroxymethylfurfural formation during baking of

biscuits: Part I: Effects of sugar type. Food Chemistry. 192: 575–585. https://doi.org/10.1016/j.foodchem.2015.07.016

Rydberg, P., Eriksson, S., Tareke, E., Karlsson, P., Ehrenberg, L. and Törnqvist, M., 2005. Factors that influence the acrylamide content of heated foods. In: Friedman, M. and Mottram, D. (eds.) Chemistry and safety of acrylamide in food, Vol. 561. Springer, Boston, MA, pp. 317–328.

Stadler, R.H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P.A. and Riediker, S., 2002. Acrylamide from Maillard reaction products. Nature. 419(6906): 449–450. https://doi.org/10.1038/419449a

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S. and Törnqvist, M., 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. Journal of Agricultural and Food Chemistry. 50(17): 4998–5006. https://doi.org/10.1021/jf020302f