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Measurement of Acrylamide and Its Precursors in Potato, Wheat, and Rye Model Systems

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The relationship between acrylamide and its precursors, namely, free asparagine and reducing sugars, was studied in cakes made from potato flake, wholemeal wheat, and wholemeal rye, cooked at 180 °C, from 5 to 60 min. Between 5 and 20 min, major losses of asparagine, water, and total reducing sugars were accompanied by large increases in acrylamide, which maximized in all three products between 25 and 30 min, followed by a slow linear reduction. Acrylamide formation did not occur to a large degree until the moisture contents of the cakes fell below 5%. Linear relationships were observed for acrylamide formation with the residual levels of asparagine and reducing sugars for all three food materials.

KEYWORDS: Acrylamide; asparagine; reducing sugars; free amino acids; rye; wheat; potato

INTRODUCTION

The formation of significant levels of the suspected carcinogen acrylamide in heated foods high in carbohydrate, from the reaction between free asparagine and intermediates of the Maillard reaction, has been widely reported (*1*, *2*). The formation of such intermediates is determined by the concentration and types of sugars and amino acids present. These intermediates also react with other amino acids to form colored products (melanoidins) and flavor compounds. Thus, the formation of acrylamide from asparagine is one of a number of competing processes. For this reason it is postulated that the yield of acrylamide should be sensitive to the free amino acid and sugar compositions of the food substrate and to conditions which are known to promote the Maillard reaction, such as temperature and moisture level. Because the desired consequences of the Maillard reaction (color and flavor) share intermediates with acrylamide formation, the most important question is whether the processes can be controlled independently, for example, through temperature control.

Studies have measured high levels of acrylamide (>200 μg/kg) in cooked potato, wheat, and rye products (*3*), foods that are widely used within the food-manufacturing industry.

The aim of this work is to understand the effect of the heating process on acrylamide formation and associated changes in the concentrations of free amino acids and sugar precursors in simple food systems comprising potato, rye, or wheat.

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EXPERIMENTAL PROCEDURES

Materials. Amino acids [alanine (Ala), β-alanine (β-Ala), α-aminobutyric acid (Aaba), γ-aminobutyric acid (Gaba), asparagine (Asn), aspartic acid (Asp), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Try), tyrosine (Tyr), and valine (Val); all 99+% purity], sugars (fructose, glucose, maltose, sucrose, and trehalose; all 99+% purity), and ethyl acetate (Pestanal grade) were purchased from the Sigma-Aldrich Co. Ltd. (Poole, U.K.). Sodium thiosulfate pentahydrate, hydrobromic acid, hydrochloric acid, potassium bromide (all Analar grade), and bromine (99.8%) were purchased from Fisher Scientific Ltd. (Loughborough, U.K.). A solution of [1,2,3-$^{13}C_3$]-acrylamide (1 mg/mL) in methanol (99% ^{13}C) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Methanol (Analar grade) was purchased from Merck Ltd. (Poole, U.K.).

Drum-dried potato flake (*4*), wholemeal wheat flour, and wholemeal rye flour were sourced from United Kingdom food producers.

Preparation and Cooking of Cakes Made from Potato, Wheat, and Rye. Water was added to the potato flake and flours to give the following compositions: potato/water (1:1.3), wholemeal rye/water (2.2:1), and wholemeal wheat/water (2.5:1). Approximately 500 g of dough was prepared for each batch of experiments. Cakes were prepared using a mechanical dough maker (Crypto-Peerless, Peerless Ltd., Halifax, U.K.) and a mechanical dough roller. The cakes were cut using a pastry cutter and pricked to minimize rising during cooking. Uncooked cakes weighed ~18 g; they were 3 mm thick and 73 mm in diameter. Cakes were cooked in an electric moving band impingement oven (Impinger II, Lincoln Foodservice Products Inc., Fort Wayne, IN) at 180 °C for from 5 to 60 min (**Table 1**); at each cooking time three cakes were cooked. Potato cakes had a higher initial moisture content than the cereal-based cakes and hence took a longer time to reach a state where they could be milled for analysis. This is why there are more data points for the cereal-based cakes at lower cooking times.

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Table 1. Cooking Times and Moisture Contents of Potato, Rye, and Wheat Cakes

cooking time (min)	moisture content (%)		
	rye	potato	wheat
0	41.60	59.73	39.60
5	24.46	nd[a]	22.76
10	3.86	24.25	10.33
12.5	2.52	nd	4.09
15	1.88	3.82	2.77
17.5	0.86	2.46	0.55
20	0.66	1.64	0.25
22.5	nd	1.26	nd
25	0.73	1.17	0
27.5	nd	0.81	nd
30	0.64	0.84	0
35	0.65	0.73	0
40	0.50	1.02	0
50	0.70	0.75	0
60	0.42	0.62	0

[a] Not determined.

Measurement of Water Content. Oven-drying was used to determine the moisture content of the wheat and rye flours and potato flake. Samples (~2 g) were weighed accurately into predried and weighed metal oven-drying dishes with lids. The weight losses after heating at 120 °C for 1 h in a fan oven were recorded. The samples were then heated for a further hour at 120 °C, allowed to cool, and reweighed. Three replicates were performed for each sample. Moisture losses in cooked cakes were measured by weighing the cooked cakes directly before and immediately after cooking. Cakes were allowed to cool and then weighed again, before they were ground for analysis. The moisture content of the cooked cakes were used to calculate the concentrations of acrylamide and its precursors in the cakes on a dry weight basis. Hence, changes in these concentrations with cooking time were not influenced by changes in moisture content.

Measurement of Acrylamide. The cakes were ground, and 5 g was taken for analysis. Acrylamide was analyzed as the dibromo derivative by gas chromatography−mass spectrometry (GC-MS) using the method of Castle et al. (*5*), with the following modifications. The extracting medium was methanol (30 mL), rather than water, because addition of water to the ground rye cakes caused gelatinization, rendering extraction difficult. The solubility of acrylamide in methanol is high (*6*), and starch gelatinization in the rye did not occur when methanol was used as the solvent. After refluxing, [1,2,3-$^{13}C_3$]acrylamide internal standard (500 ng), rather than methacrylamide, was added to the extract, along with 15 mL of brominating reagent. The bromination was allowed to proceed overnight at room temperature, rather than in a refrigerator.

The brominated extract (2 μL) was injected onto a Clarus 500 GC-MS system (Perkin-Elmer Life and Analytical Sciences, Inc., Boston, MA) in splitless mode at 250 °C, the split opening after 0.5 min. Pulsed injection was used; helium carrier gas flow rate was 5 mL/min for 0.5 min, followed by a decrease to 1 mL/min over 0.5 min. Flow rate was maintained at 1 mL/min for 10 min and then increased over 0.5 min to 5 mL/min, until the end of the run. A DB-17 MS capillary column was used (30 m × 0.25 mm i.d., 0.15 μm film thickness; Agilent, Palo Alto, CA). The oven temperature was 85 °C for 1 min, rising at 8 °C/min to 200 °C and then at 30 °C/min to 280 °C for 10 min. The transfer line was held at 280 °C and the ion source at 180 °C.

Electron impact mass spectra were obtained at 70 eV. The mass spectrometer was operated in selected ion monitoring mode. Four ions were used to characterize brominated [1,2,3-$^{13}C_3$]acrylamide (*m*/*z* 108, 110, 153, and 155), and another four ions were used to characterize brominated acrylamide (*m*/*z* 106, 108, 150, and 152). The ion *m*/*z* 155 was used to quantify brominated [1,2,3-$^{13}C_3$]acrylamide, and the ion *m*/*z* 152 was used to quantify brominated acrylamide.

Determination of Free Amino Acids. The free amino acid content of the three foods was measured using the EZ-Faast amino acid derivatization technique for GC-MS (Phenomenex, Torrance, CA) (*7*). Arginine and citrulline are two amino acids that could not be analyzed using this technique. Preliminary work showed that cysteine and cystine were not present in any of the samples at levels high enough for quantification. The amino acid compositions of cakes cooked for 50 and 60 min were not analyzed.

The sample was measured into a 7 mL vial. Preliminary work showed that levels of amino acids were relatively high in potato flake, but lower in rye flour and lowest in wheat flour. Therefore, the sample size used for wheat flour and cakes cooked from it was 1.0 g, for whole rye 0.50 g samples were used, and for potato the samples were 0.20 g. Hydrochloric acid (0.01 M) was added (5 mL) to the vial, and the sample was stirred for 15 min at room temperature. After stirring, the sample was allowed to settle for 45 min. An aliquot of supernatant (2 mL) was then centrifuged at 7200*g* for 30 min. One hundred microliters of the centrifuged supernatant was then derivatized.

The EZ-Faast amino acid analysis kit was used to prepare derivatized amino acids for analysis by GC-MS. The preparation of a sample for GC-MS began with the addition of 20 nmol of norvaline internal standard, followed by a solid-phase extraction and then a two-step derivatization at room temperature. The derivatized amino acids were extracted into isooctane/chloroform (100 μL) and analyzed in electron impact mode at 70 eV using the Clarus 500 GC-MS system. An aliquot of the derivatized amino acid solution (2 μL) was injected at 250 °C in split mode (5:1) onto a 10 m × 0.25 mm Zebron ZB-AAA capillary column. The oven temperature was 110 °C for 1 min, then increased at 30 °C/min to 320 °C, and held at 320 °C for 2 min. The transfer line was held at 320 °C, and the carrier gas flow rate was kept constant throughout the run at 1.1 mL/min. The ion source was maintained at 220 °C. Samples and standards were analyzed in triplicate.

Standards of 19 nonbasic amino acids (Ala, β-Ala, Aaba, Gaba, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Val) in 0.1 M hydrochloric acid and 3 basic amino acids (Asn, Gln, and Try) in water were prepared. A calibration curve was plotted for each amino acid, and the gradient of this curve was used to calculate the amount of each amino acid in the three foods and the cakes prepared from them. A specific mass spectral fragment ion was chosen for quantification of each amino acid. The area of this ion in the peak of each amino acid was measured relative to the area of the *m*/*z* 158 ion of norvaline.

Analysis of Sugars by Ion Chromatography. Finely ground cakes (0.5 g) were extracted using 10 mL of water for 30 min with occasional shaking. Trehalose was used as an internal standard (300 μg). Color was removed by the addition of 0.1 g of activated carbon. The extract was then shaken and left to stand for 30 min. The carbon was removed by centrifugation at 5000 rpm for 10 min. The extract was further purified using Sep-Pak Plus C_{18} cartridges (Waters Corp., Milford, MA) and filtered through a 0.2 μm filter disk (Whatman Inc., Clifton, NJ). Doughs and flours were extracted using 10 mL of 50% methanol. The methanol was evaporated, and the extract was then treated in the same way as for the cakes.

Analysis was performed using an 8220i Dionex ion chromatography system (Dionex Corp., Sunnyvale, CA). Twenty microliters of sample was injected onto a Carbopac PA10 column (Dionex) at room temperature using an autosampler. A gradient program was set up using 200 mM NaOH (solvent A) and water at a flow rate of 1 mL/min, 50% solvent A, held for 10 min, and increased to 100% at 35 min. The column was then washed for 10 min with 550 mM sodium acetate in 100 mM NaOH and re-equilibrated with 50% solvent A for 10 min. A pulsed amperometric detector was used with the following settings: 420 ms at 0.05 V, 180 ms at 0.75 V, and 420 ms at −0.15 V, while the sensitivity was set at 3K. Chromatographic analysis of peaks was performed using Turbochrom (Perkin-Elmer Life and Analytical Sciences, Inc.). Standards of glucose, maltose, fructose, and sucrose were used for quantification.

RESULTS AND DISCUSSION

The units used for amino acid data in this paper are micromoles per kilogram, to allow direct comparison among the different amino acids, in their role as precursors. Sugar data are also presented in a similar way. Most acrylamide data published to date have been presented as micrograms per kilogram, and these units are used in this paper, except where its formation from its precursors is discussed.

Table 2. Acrylamide Contents of Potato, Rye, and Wheat Cakes

cooking time (min)	acrylamide content[a] (μg/kg)		
	rye	potato	wheat
5	59 (28)	nd[b]	48 (17)
10	758 (210)	410 (76)	132 (7)
12.5	1004 (128)	nd	116 (15)
15	2627 (115)	2242 (138)	358 (53)
17.5	2194 (219)	4334 (152)	708 (49)
20	3104 (464)	5113 (313)	913 (121)
22.5	nd	5544 (2239)	nd
25	3166 (723)	6404 (1104)	1058 (15)
27.5	nd	5786 (243)	nd
30	2451 (212)	6805 (287)	1089 (100)
35	nd	6436 (285)	1014 (8)
40	2200 (112)	6402 (104)	868 (188)
50	1786 (59)	5299 (213)	769 (189)
60	1586 (152)	4512 (55)	690 (86)

[a] On a dry weight basis. Values are means of three replicates with standard deviations in parentheses. [b] Not determined.

Water Content. The mean moisture contents of wholemeal flour, rye flour, and potato flake were 14.8, 14.0, and 6.7%, respectively. The moisture contents of the cooked cakes directly after cooking are shown in **Table 1**. The appearance and aroma of the cakes cooked for >20 min indicated that they were overcooked and bore little relationship to commercial products.

Little work has been carried out on the effect of moisture content on acrylamide formation. Leung et al. (*8*) measured water loss in a fritter made from wheat flour, which was deep-fried at 170, 190, or 210 °C. The original moisture content of the fritter was ~48% and fell to ~10% after 15 min at 170 °C; that is, over one-fifth of the original water in the fritter remained, at which time the acrylamide content was ~200 μg/kg. Our results show that moisture loss was greater from the wheat cakes analyzed in this experiment at 15 min (>90%), possibly because the oil surrounding the fritter provided a barrier to diffusion of moisture from the fritter's surface. Leung et al. (*8*) showed that, for the fritters, moisture content was inversely proportional to acrylamide content, at all three temperatures.

Acrylamide. The concentrations of acrylamide in the potato, wheat, and rye cakes are shown in **Table 2**, and the three curves, showing acrylamide formation with time for each of the products at 180 °C, are compared in **Figure 1**. All three curves are similar, with a rapid increase in acrylamide formation between 10 and 20 min, reaching a maximum value between 20 and 35 min, followed by a slow linear decrease. Maximum concentrations for acrylamide in potato, rye, and whole wheat cakes were 6800, 3200, and 1100 μg/kg, respectively. Because the cakes were cooked for a relatively long time, compared to commercial products, these values were relatively high. Acrylamide formation in potato strips cooked at 200 °C (*9*) gave a similar maximum value in a slightly shorter time. A similarly shaped curve was obtained by Pollien et al. (*10*), using proton-transfer reaction mass spectrometry to measure acrylamide formation in the headspace above a dried potato slice weighing 0.5 g at 170 °C.

Acrylamide formation was relatively low when the moisture levels in the cakes were >5%. Below 5% moisture, a linear relationship was observed, between moisture and acrylamide, up to and including the maximum amount formed (**Figure 2**). By altering the initial moisture content of gingerbread doughs and measuring the acrylamide formed, Amrein et al. suggested that this relationship was coincidental (*11*). Once maximum formation had been reached, in all three foods there was a relatively slow linear decrease of acrylamide with time, and the rate of this decrease was similar for all three foods. This decrease could be due to secondary reactions between acrylamide and other food components, or evaporation of acrylamide from the surface of the cakes could have occurred. Taubert et al. (*12*) showed that loss of acrylamide occurred in fried potato below its polymerization temperature of 175 °C. As no acrylamide was detected in the frying oil, they suggested that acrylamide had degraded and had not migrated from the surface of the potato.

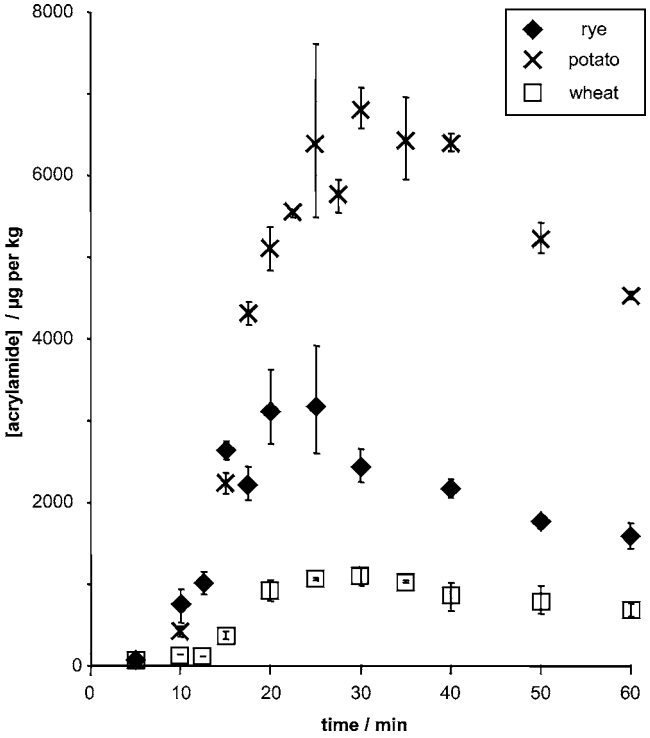

Figure 1. Effect of cooking time at 180 °C on acrylamide concentrations in rye, potato and wheat cakes (Error bars represent standard deviations of three replicates.).

Free Amino Acids. The potato data presented in this paper are from drum-dried potato flakes (Maris Piper/Pentland Dell 1:1). Losses of free amino acids, when Netted Gem potatoes were drum-dried, were less than the losses of free amino acids when the same potatoes were made into chips but greater than when they were made into French fries (*13*). Another study (*14*) showed that >80% of both glycine and methionine were lost from potatoes, as a result of drum-drying, and apart from isoleucine and serine, losses for all free amino acids measured were ≥40%. Asparagine and aspartic acid were measured as one peak, with a loss when drum-dried of 67%. When dehydrated potato granules were prepared using the add-back process, losses of asparagine were ~20% (*15*).

Noti et al. (*16*) reported levels of 0.15−0.4 g/kg of asparagine in 10 samples of wheat flour. Surdyk et al. (*17*) measured asparagine levels of 0.17 g/kg in a white wheat flour. Few data are available on the free amino acid compositions of rye. Springer et al. (*18*) showed that levels of free asparagine varied across the rye grain, with the lowest level in the endosperm and the highest level in the bran. Acrylamide levels in wafers made from rye flours reflected their free asparagine content, with levels of acrylamide in bran being greater than in cooked wholemeal rye, which were greater than in cooked rye flour without bran.

The individual free amino acid contents of cakes made from rye, potato, and wheat are shown in **Tables 3**, **4**, and **5**,

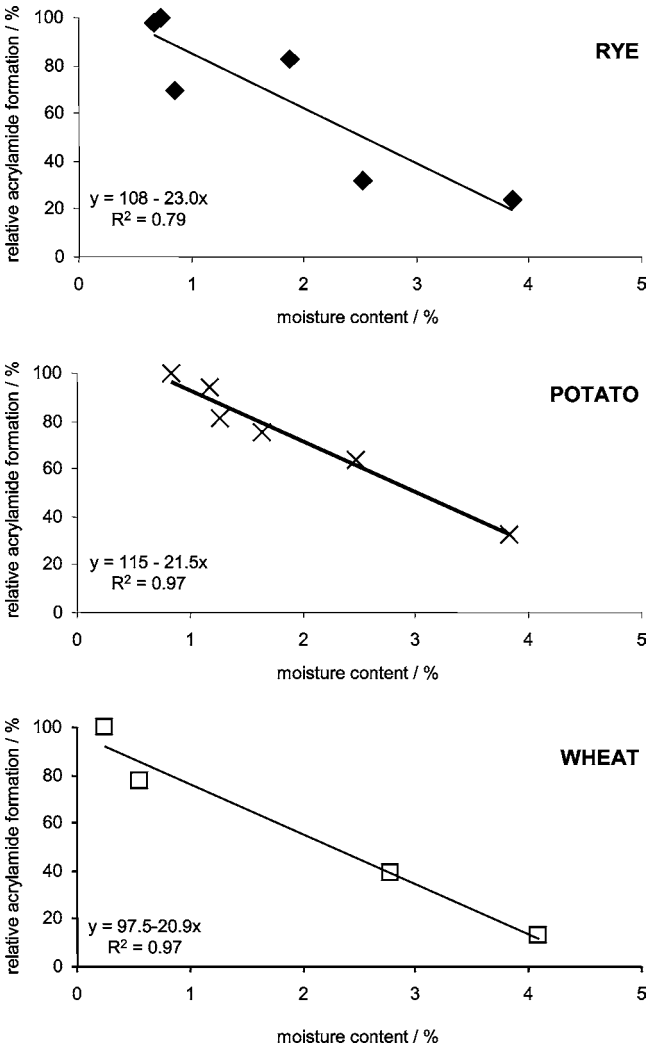

Figure 2. Variation of relative acrylamide formation with moisture content at 180 °C in rye, potato and wheat cakes, at moisture levels below 5%.

respectively. All values are reported on a dry weight basis, and those values obtained at 0 min are for unheated rye flour, potato flake, and wheat flour. Analysis on a dry weight basis allows for any changes in amino acid values, due to concentration effects caused by loss of water from the cakes during cooking. Data for arginine are not presented, as the analytical technique used is not suitable for arginine. However, using capillary electrophoresis, we have measured arginine concentrations in potato flake, rye flour, and wheat flour at 5400, 1100, and 720 μmol/kg, respectively. No arginine data were obtained for the cooked cakes.

Uncooked potato flake contained the highest concentrations of both asparagine and total free amino acids of the three foods, followed by rye flour and then wheat flour (**Table 7**). In addition, asparagine comprised 38% of the total free amino acids in potato flake, higher than that in rye flour (26%) and wheat flour (16%). Asparagine was the free amino acid present at the highest amounts in both potato flake and rye flour, whereas aspartic acid was present at slightly higher levels than asparagine in wheat flour. The asparagine concentration in the uncooked potato flake was 3500 mg/kg, equivalent to ~700 mg/kg in fresh potato, assuming a moisture content of 80% (*19*). When compared to other literature values for asparagine in fresh potatoes, this value is low, suggesting that losses had occurred during the drum-drying process.

The concentrations of all of the amino acids in all three foods were much reduced after 40 min of heating, although some amino acids increased in concentration in some of the products, early in the cooking process. At 10 min in potato, the earliest time at which a heated potato cake was analyzed, there was an increase in all amino acids, apart from glutamine, when compared to the uncooked flake. At 15 min all amino acids had fallen to below their original concentrations. Some of the free amino acids in rye increased after 5 min, in particular γ-aminobutyric acid, which doubled in concentration and did not fall below its original concentration until 15 min. In wheat, glutamic acid showed a 3-fold increase upon heating and decreased only after 15 min of heating. Asparagine, aspartic acid, and alanine showed no losses until 15 min. **Figure 3** shows the decomposition curves for asparagine in potato, wheat, and rye.

The average coefficient of variance for the amino acid data was 12%, with histidine giving the greatest value of 24%. Glycine had the lowest coefficient of variance of 7% and that of asparagine was 9%.

No authors have previously reported the effect of cooking time on free amino acid composition in potatoes, wheat flour, or rye flour, but other relevant systems have been studied. Talley and Eppley (*20*) investigated heated amino acid losses in model potato chips by heating an amino acid and a reducing sugar together on filter paper disks in fat at 103 °C. The decomposition curves for each of 22 amino acids with glucose were plotted against log(time). Asparagine decomposed more quickly when it was heated with fructose than with glucose, as did γ-aminobutyric acid, lysine, and histidine, whereas leucine decomposed to the same degree with both sugars.

Kaminski et al. prepared malt extracts from rye, wheat, and barley (*21*). They studied the effect of cooking temperature (80−160 °C for 180 min) and pH (2.5−8.5; 180 min at 120 °C) on free amino acid and sugar levels in all three extracts and the

Table 3. Free Amino Acid Composition of Rye Flour and Rye Cakes Cooked at 180 °C

cooking time (min)	free amino acid concn[a] (μmol/kg)																					
	Ala	Gly	Aaba	Val	β-Ala	Leu	Ile	Thr	Ser	Gaba	Pro	Asn	Asp	Met	Glu	Phe	Gln	Orn	Lys	His	Tyr	Try
0	2721	851	16	602	76	480	227	264	328	456	1173	4793	3123	57	1071	207	329	17	167	75	103	123
5	3364	938	22	556	71	242	196	315	476	854	1156	7717	4252	28	1844	111	298	49	218	80	104	149
10	2149	666	14	400	59	193	149	220	301	911	889	3517	2832	19	642	81	116	13	107	52	58	90
12.5	2125	571	15	355	56	134	129	184	290	478	876	4202	3203	14	1098	66	53	32	114	49	66	97
15	463	187	tr	98	32	37	37	47	64	218	274	589	811	tr	96	18	18	6	31	8	16	25
17.5	638	223	7	129	40	44	50	54	99	154	358	995	1206	tr	166	25	17	18	44	13	27	33
20	289	160	tr	61	16	21	23	23	34	95	150	348	442	–	48	9	12	tr	18	tr	7	9
25	135	107	tr	30	9	9	9	12	21	47	53	188	181	–	20	5	12	tr	19	tr	5	5
30	145	109	tr	23	11	8	7	9	18	54	32	153	97	–	9	tr	6	tr	11	tr	tr	tr
40	132	108	tr	21	10	7	7	10	16	33	34	149	121	–	11	tr	7	tr	15	tr	tr	tr

[a] On a dry weight basis. Values are means of three replicates (tr, <5 μmol/kg; –, <1 μmol/kg).

Table 4. Free Amino Acid Composition of Spray-Dried Potato Flake and Potato Cakes Cooked at 180 °C

cooking time (min)	free amino acid concn[a] (μmol/kg)																					
	Ala	Gly	Aaba	Val	β-Ala	Leu	Ile	Thr	Ser	Gaba	Pro	Asn	Asp	Met	Glu	Phe	Gln	Orn	Lys	His	Tyr	Try
0	2910	554	95	3110	145	1050	1370	1036	2370	6460	995	27000	4290	651	2760	1130	4150	242	2380	371	1480	328
10	3378	645	110	3630	146	1255	1551	1288	2630	6790	1180	31339	5496	818	3275	1317	3261	257	2615	466	1814	383
15	2598	545	74	2714	122	806	1163	915	1992	5131	898	20427	4498	531	2221	935	930	166	1746	303	1413	301
17.5	2067	436	60	2006	125	612	888	619	1199	3603	735	11463	3860	348	1161	675	110	86	1068	112	1030	215
20	2073	451	59	1793	129	479	790	519	1169	3442	653	8315	3411	295	740	564	79	82	830	77	828	167
22.5	1734	355	53	1510	120	336	616	283	598	1797	534	3314	2312	164	331	364	55	67	527	48	604	129
25	1386	281	42	1148	98	280	516	240	471	1315	438	2377	2045	134	204	325	39	56	424	34	516	109
30	882	217	38	840	83	214	409	179	300	810	360	1279	1706	113	156	274	45	49	338	11	474	87
35	736	205	32	723	81	167	317	128	228	638	260	874	1333	78	92	219	42	36	257	–	359	61
40	510	173	26	609	69	161	306	136	212	482	210	760	1217	83	109	235	61	33	280	–	403	70

[a] On a dry weight basis. Values are means of three replicates (tr, <5 μmol/kg; –, <1 μmol/kg).

Table 5. Free Amino Acid Composition of Whole Wheat Flour and Whole Wheat Cakes Cooked at 180 °C

cooking time (min)	free amino acid concn[a] (μmol /kg)																					
	Ala	Gly	Aaba	Val	β-Ala	Leu	Ile	Thr	Ser	Gaba	Pro	Asn	Asp	Met	Glu	Phe	Gln	Orn	Lys	His	Tyr	Try
0	748	403	7	219	13	316	99	95	147	1154	332	1327	1399	41	180	107	65	9	136	31	71	946
5	713	384	7	161	13	129	68	71	117	783	302	1319	1405	14	543	50	43	10	87	29	37	852
10	698	358	7	150	14	105	59	55	108	539	292	1077	1263	12	423	41	28	8	64	24	31	748
12.5	784	371	9	139	14	110	60	79	166	620	288	1542	1777	12	688	46	27	16	106	29	55	898
15	515	249	5	116	14	80	49	41	73	491	257	725	916	8	267	35	20	5	42	16	28	529
17.5	259	130	tr	46	14	34	21	27	57	125	126	272	562	tr	112	17	11	9	29	tr	22	354
20	115	72	tr	30	13	15	12	8	18	80	83	102	214	tr	39	8	7	tr	11	tr	6	135
25	51	51	–	11	6	7	tr	tr	11	34	28	44	68	–	9	tr	tr	tr	7	tr	tr	48
30	40	46	–	9	5	tr	tr	tr	5	23	19	33	53	–	5	tr	tr	tr	6	–	tr	30
40	29	38	–	tr	tr	tr	tr	tr	tr	10	8	26	30	–	tr	tr	tr	tr	6	–	–	6

[a] On a dry weight basis. Values are means of three replicates (tr, <5 μmol/kg; –, <1 μmol/kg).

Table 6. Sugar Composition of Rye Flour, Potato Flake, and Wheat Flour and Cakes Cooked from Them at 180 °C

cooking time (min)	sugar content[a] (μmol/kg)										
	rye				potato			wheat			
	glucose	fructose	sucrose	maltose	glucose	fructose	sucrose	glucose	fructose	sucrose	maltose
0	11900	13400	33700	32400	28600	24600	14000	3020	2360	28500	43900
5	7530	7380	37900	29000				4090	2670	27800	40900
10	9620	10800	37100	19200	24000	22500	14400	3290	2640	26800	27900
12.5	4790	6010	41000	12200				2830	2400	28300	28800
15	5950	7730	36700	6580	7380	14074	11400	3930	2800	25800	24100
17.5	3760	3380	37400	4140	1720	5558	11600	3920	2130	27400	14400
20	3560	3510	32300	3410	817	3179	11700	3970	2500	28800	10500
22.5					tr	1582	10700				
25	3480	3470	26400	1300	tr	1235	10700	4370	2870	23000	6490
27.5					tr	1396	10200				
30	3360	3020	25500	tr	–	1096	8720	3870	2820	21800	5420
35	2920	2690	23000	–	–	tr	8210	3640	2700	18900	3500
40	2390	2180	24100	–	–	1166	7860	3330	2480	16600	3010

[a] On a dry weight basis. Values are means of three replicates (tr, <800 μmol/kg; –, <400 μmol/kg).

effect of cooking time at 120 °C (up to 180 min) in wheat extract. Generally, in all three malt extracts, glutamine and glutamic acid, which were measured as one peak, and histidine decomposed substantially more readily than the other amino acids. At ≥140 °C, cooking for 180 min resulted in the loss of >95% of total free amino acids, and only alanine in the wheat extract was present at >10% of its original level. In our work, alanine was one of the most stable amino acids in both rye and wheat flour, although less stable than glycine.

Sugars. Several authors have measured individual sugar contents in potato with regard to acrylamide formation (*9*, *22*–*26*). Sugar content varies greatly among potato varieties. For example, in different potato cultivars, Amrein (*22*) measured glucose concentrations from 100 to 2500 mg/kg and fructose concentrations between 30 and 1500 mg/kg of fresh weight.

Table 7. Chemical Composition of Rye Flour, Whole Wheat Flour, and Potato Flake

	rye	potato	wheat
asparagine (μmol/kg)	4800	27000	1300
total free amino acids (μmol/kg)	18400	70300	8500
asparagine relative to total free amino acids (%)	26	38	16
other major amino acids (%)	Asp, 17	Gaba, 9	Asp, 16
	Ala, 15	Arg, 8	Gaba, 15
	Pro, 6	Asp, 6	Try, 11
total reducing sugars (μmol/kg)	57700	53200	49300

The sugar content of wheat flour has been widely reported, although few data have been published in the past 10 years (*17*). Seppi (*27*) found that levels of fructose, glucose, maltose, and

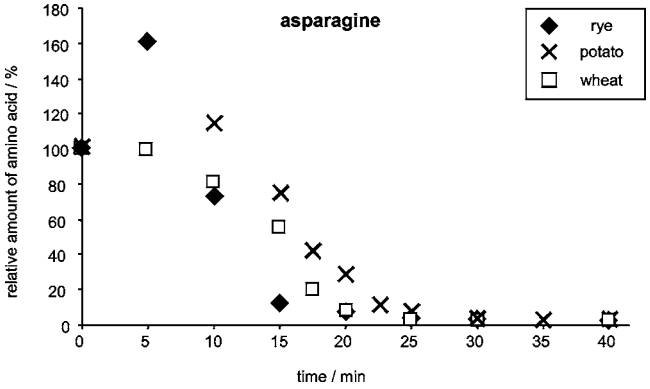

Figure 3. Decomposition of asparagine with time at 180 °C in rye, potato and wheat cakes.

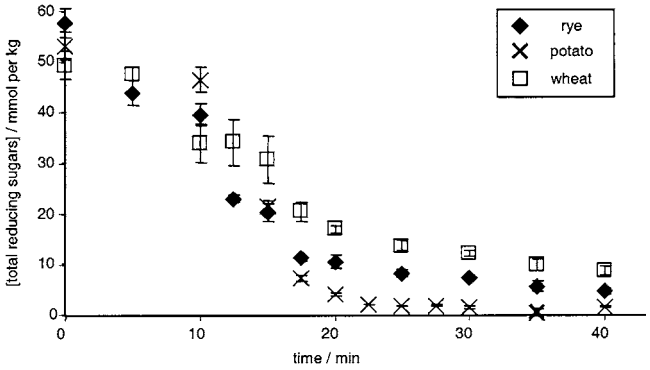

Figure 4. Decomposition of total reducing sugars with time at 180 °C in rye, potato and wheat cakes (Error bars represent standard deviations of three replicates.).

sucrose were higher in wholemeal flour than in white flour. He also showed that large increases in maltose occurred when dough was rested, even without yeast present. The sugar contents of wholemeal flours from wheat and rye were measured by Salomonsson et al. (*28*). The composition and concentrations of sugars in both cereals were similar. Sucrose was measured at the highest concentration in both cereals, ~5 times greater by weight than the combined concentrations of fructose and glucose. Raffinose was also measured in both cereals, but maltose was not measured.

The sugar contents of potato flake, rye flour, and wheat flour are shown in **Table 6**. Again, all values are reported on a dry weight basis, and those values obtained at 0 min are for unheated potato flake, rye flour, and wheat flour. The major difference between the cereals and potato was that maltose was not detected in potato, whereas in rye, maltose comprised 56% and in wheat, 89%, of the total reducing sugars. Total reducing sugars, that is, glucose, fructose, and maltose, were similar in all three foods at between 49 and 58 μmol/kg and for all three foods, glucose levels were similar to fructose levels. Sucrose, a nonreducing sugar, showed little or no loss in cereals until after 20 min. It decomposed linearly with heating time for potato, with >80% remaining after 20 min, suggesting that sucrose is relatively unimportant as a precursor of acrylamide. Leszkowiat et al. (*29*) showed that sucrose, as part of a fried potato model system, could participate in the Maillard reaction. They suggested that partial hydrolysis of sucrose to fructose and glucose had occurred.

Pollien et al. (*10*) showed that, in model systems, fructose generated more acrylamide than glucose when heated with asparagine at 150 °C. Talley and Eppley (*20*) reported that heated asparagine decomposed more quickly at 103 °C in the presence of fructose than in the presence of glucose. This effect was also observed by Biedermann et al. (*23*) and Rydberg et al. (*9*), when both sugars were separately added to potato samples, which were then heated at 150 and 180 °C, respectively. Maillard systems heated at 120 °C and pH 6.8, which contained fructose, exhibited 3 times greater mutagenicity toward a strain of *Salmonella typhimurium* than those containing glucose, although in relative terms the mutagenicities of all of the systems were low (*30*).

In the present study the decomposition of maltose in the two cereals was rapid, relative to that of glucose and fructose. In rye, levels of fructose and glucose decreased slowly upon heating, and in wheat, levels did not change. Hollnagel and Kroh (*31*) showed that in Maillard reaction model systems containing glycine at 100 °C, degradations of both glucose and maltose occurred at similar rates. They also showed that at least 10% molar conversion of maltose to glucose occurred, which would explain why glucose levels were maintained in the cakes made from cereal. In addition, Theander and Westerlund (*32*) reported an increase in fructose when wheat flour was drum-dried and suggested it may arise from the breakdown of fructose-containing carbohydrates, such as raffinose and fructans, which were not measured in the present work. In potato, glucose and fructose decomposed readily, so plots of total reducing sugars against cooking time were similar for all three foods (**Figure 4**). Within each food the decomposition curves for fructose and glucose were similar. After 20 min, 34% of total reducing sugars remained in wheat, 18% in rye, and 7.5% in potato.

Relationships among Acrylamide, Asparagine, and Reducing Sugars. In the potato flake, the molar concentration of total free amino acids was slightly greater than the molar concentration of total reducing sugars (**Table 7**), with a ratio of amino acids to reducing sugars of 1.2. In both cereals, the molar concentrations of total reducing sugars were greater than the molar concentrations of total free amino acids (amino acids/reducing sugars = 0.30 for rye and 0.16 for wheat). Hence, in rye and wheat, there was a large excess of reducing sugar, and so relative losses of reducing sugars in the two cereals were small, due to the small pool of free amino acids. In potato, the slight excess of free amino acids means that reducing sugar loss was greater, when compared with the cereals, and amino acid loss was correspondingly less. These observations can be related to the formation of acrylamide in all three foods. For data points obtained before maximum acrylamide formation had occurred, a plot of asparagine loss against acrylamide formation gave a straight line, the gradient of which equated to the conversion rate of asparagine to acrylamide (**Figure 5**). In potatoes only 0.29% of asparagine molecules were converted to acrylamide; in rye the value was 0.80%, and in wheat it was 0.98%. These data show a very important fact, that is, that acrylamide is lost when all of the asparagine has disappeared, implying that during cooking as acrylamide is formed it is also being lost in other reactions.

There were also strong linear correlations between the decomposition of most of the other free amino acids and acrylamide formation. Glutamine correlated relatively poorly with acrylamide, compared to the other amino acids. It was the only amino acid in all three foods that had a correlation with acrylamide with an R^2 value of <0.9. There were also strong correlations between asparagine and many of the other amino acids. The amino acids that correlate poorly with asparagine were those which decomposed relatively quickly during the first 5 min of cooking and were mainly found in the two cereals. These include valine, leucine, isoleucine, phenylalanine, and

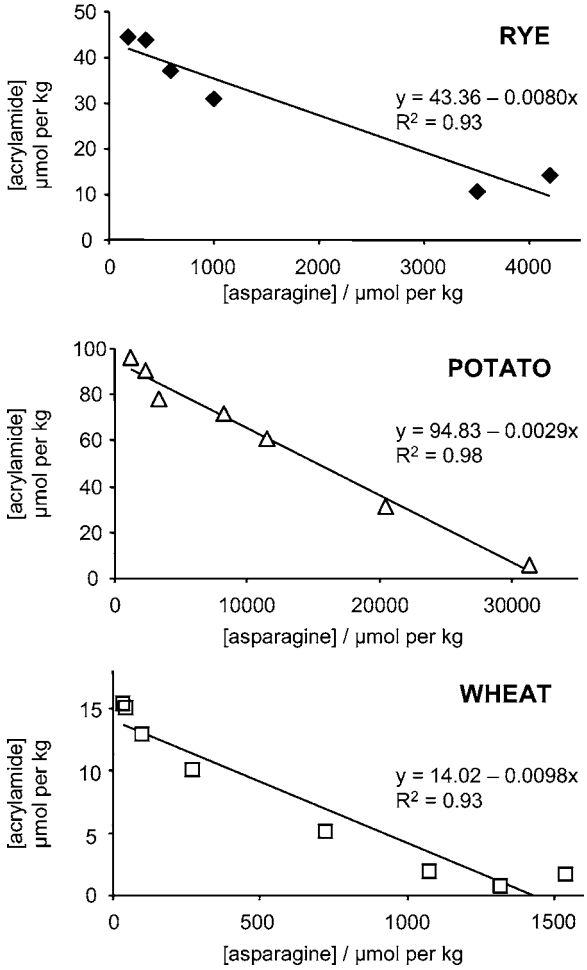

Figure 5. Variation of acrylamide formation with free asparagine loss at 180 °C in rye, potato and wheat cakes.

γ-aminobutyric acid. Glutamine was poorly correlated with asparagine in all three samples.

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