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Correlations between the Amounts of Free Asparagine and Saccharides Present in Commercial Cereal Flours in the United Kingdom and the Generation of Acrylamide during Cooking

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A range of commercially available cereals (mainly rye and wheat) used to manufacture U.K. bakery products were obtained, and the levels of free amino acids and sugars were measured. Selected samples were cooked as flours and doughs to generate acrylamide and the data compared with those obtained from a model system using dough samples that had been additionally fortified with asparagine (Asn) and sugars (glucose, fructose, maltose, and sucrose). In cooked flours and doughs, Asn was the key determinant of acrylamide generation. A significant finding for biscuit and rye flours was that levels of Asn were correlated with fructose and glucose. The results suggest that for these commercial cereals, selection based on low fructose and glucose contents, and hence low asparagine, could be beneficial in reducing acrylamide in products (e.g., crackers and crispbreads) that have no added sugars.

KEYWORDS: Acrylamide; asparagine; cereals; flours; milling; rye; sugars; wheat

INTRODUCTION

Studies by Swedish scientists correlating concentrations of acrylamide−hemoglobin adducts in laboratory animals with the intake of fried feed (*1*) have led to intense investigations of acrylamide in foods worldwide. Initial studies showed that the highest concentrations of acrylamide were formed when carbohydrate-rich foods such as cereals and potatoes were heated, whereas only moderate amounts were produced in protein-rich foods (*2–4*). The presence of acrylamide in foods is of concern because dietary intake of acrylamide could be associated with cancer formation in humans (*5–7*).

It is now generally accepted that acrylamide is formed in foods from the amino acid Asn (*8, 9*) in a thermal reaction that requires the presence of a reducing sugar (*10*). Consequently, interest in crop Asn and sugars has increased dramatically in recent years.

Asparagine accumulates in all plants during normal physiological processes such as seed germination and nitrogen transport and in response to external stresses caused by, for example, drought, mineral deficiencies, or pathogen attack (*11*). Cereals use Asn (and Gln) to store nitrogen in (storage) proteins. Hence, during ripening the concentration of free Asn does not increase because it is incorporated into proteins, whereas during sprouting, increased enzyme activity may lead to increased amounts of free Asn (and sugars) (*12*). For some wheat varieties grown under certain conditions, it has been shown that free Asn was correlated with protein (*13, 14*), and concentrations of the former increased markedly at higher protein contents resulting from elevated nitrogen fertilization (*12, 13*). Consequently, concentrations of free Asn might be expected to vary in commercial cereals due to changes in agronomic and environmental conditions, although there is still not enough information available to determine a clear trend. For example, free Asn concentrations in 31 different European varieties of wheat ranged from 74 to 664 mg kg^{-1} within a single crop year, with at least one variety showing a 2-fold variation (*15*). In a recent study, nitrogen fertilization correlated with an approximate 4-fold increase in free Asn for a single wheat variety in each of two crop years (*13*). However, some of the most dramatic increases in free Asn have been reported for wheat grown in sulfate-deficient soils (*12, 16, 17*). For example, increases in free Asn concentrations of up to 30-fold in wheat compared to samples receiving a normal amount of fertilizer have been reported (*16*). Interestingly, acrylamide amounts generated by heating these samples did not rise in proportion to the measured increases in Asn (*16*). The authors suggested that this could be due to the reaction becoming limiting in sugars at the relatively high Asn concentrations found in sulfur-deprived wheat. In rye, free Asn concentrations have been reported to range from 319 to 791 mg kg^{-1} (*18*).

There is less information concerning amounts of sugars in cereals, how these vary between varieties, and changes resulting from agronomic practices. A recent study has demonstrated that reducing sugar concentrations may, however, be less susceptible

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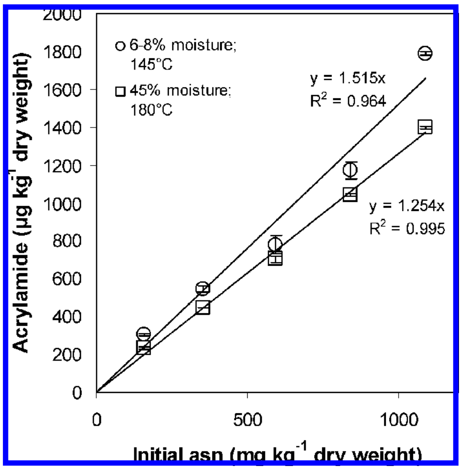

Figure 1. Correlation between added Asn and acrylamide generated in cooked wheat dough at different moisture contents. The cooking times were 20 min.

to the impact of fertilization regimens compared to Asn (*17*). Furthermore, the relative reactivity of individual sugars in cereal products is unclear. Taemans et al. (*15*) demonstrated that acrylamide could be formed from either fructose, glucose, or sucrose in a flour matrix, whereas Mustafa et al. (*19*) and Surdyk et al. (*20*) found that added fructose had little effect on acrylamide in rye crispbreads and yeast-leavened wheat bread, respectively. When considering the effect of saccharides on acrylamide generation, it is important to consider both reducing and nonreducing sugars. This is because nonreducing disaccharides such as sucrose can undergo thermally induced hydrolysis to reducing monosaccharides (*15*).

Under the conditions of heating, for example, baking, Asn and sugars are consumed in Maillard reactions with the simultaneous formation of acrylamide (*21–23*). Under normal growing conditions, the concentration of Asn in cereals is very much lower than that of the sugars (e.g., fructose, glucose, maltose, sucrose), and this has the effect of limiting acrylamide formation in these products (*21*). For this reason, the effect of changes in sugars, for example, from recipe additions, is often ignored or perhaps confused. Classical chemistry tells us that the rate of formation of acrylamide will be dependent on the concentrations of both precursors, that is, Asn and sugars. However, because sugars may also be precursors of intermediates (via Maillard reactions) that then go on to react with Asn (*23*), the relationship between the parent carbohydrates and acrylamide generated is likely to be complex.

Hence, the objectives of this study were three-fold:

1. Assess the potential impact of changes in free Asn and sugars on acrylamide generated during cooking in a model system using wheat dough that had been additionally fortified with precursors.

2. Assess the correlations between free Asn, sugars, and acrylamide generated during cooking in commercial bread wheat samples.

3. Assess the variability of free Asn and simple sugars in other commercial cereals used to manufacture U.K. bakery products.

The overall aim was to further understand the correlation between free Asn, simple sugars. and acrylamide generated from commercial cereal raw materials used to manufacture U.K. bakery products. Data on additional variables that have been shown to affect acrylamide formation, such as other amino acids (*24*) and divalent metal ions (*25, 26*), were also collected. The latter information can be found in the Supporting Information.

MATERIALS AND METHODS

Chemicals. All reagents and chemicals were purchased from suppliers in the United Kingdom. Amino acids (Asn, Asp, Ala, Arg, Gln, Glu, Gly, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, Val), sugars (arabinose, fructose, glucose, maltose), acrylamide, and [$^{13}C_3$]acrylamide (>98 atom %) were all of high purity (>99%); all other chemicals were of analytical grade.

Bakery Raw Materials. Flours, grain, and salt were obtained from commercial mills and bakery manufacturers and suppliers in the United Kingdom: bread wheat and cake flours were from the 2004 harvest; biscuit and rye samples were from the 2006 harvest. Mill fractions of bran, germ, and white and wholemeal flour were obtained from a single breadmaking grist (2004 harvest).

Preparation of Samples. Wheat grains were reduced to fine flour using a type DLFU laboratory grinder (Buhler-Miag, Switzerland). Experimental dough samples, comprising flour (1000 g), salt (20 g), and water (600 g for white; 700 g for wholemeal), were vacuum-mixed to a work input of 36 kJ kg^{-1} using a rapid mechanical dough development procedure (*27, 28*). Asparagine and sugars were added as required via the dough water. Low-moisture samples were prepared by freeze-drying the dough to a target moisture content of 7% using a Supermodulyo 12K freeze-dryer (Edwards, Crawley, U.K.). The dried sample was reduced to a fine homogeneous powder in a BL 300 domestic blender (Kenwood, Havant, U.K.).

Production and Analysis of Acrylamide. Flours and experimental dough samples were cooked in a closed-cell apparatus that has been used to simulate the conditions of baking (*21*). Briefly, samples were contained in stainless steel HPLC tubes of 75 mm length with an internal diameter (i.d.) of 7.75 mm, and acrylamide was generated by cooking the samples for 20 min in the oven of a Carlo Erba Mega series (Milan, Italy) gas chromatograph (GC): flours were heated at 160 °C; low- (6−8%) and high-moisture (45%) dough samples were heated at 145 and 180 °C, respectively. Acrylamide was extracted from the cooked samples using water and converted to 2-bromopropenamide prior to analysis using GC-MS/MS according to the procedure of Hamlet et al. (*29*). The limit of detection was 0.5 μg kg^{-1}.

Moisture Determination. The moisture content of samples was determined gravimetrically following heating overnight at 102 ± 1 °C.

Free Amino Acids. The procedures of Benedito de Barber et al. (*30*), Kim et al. (*31*), and Bartok et al. (*32*) were adapted for the extraction, cleanup, and analysis, respectively.

Stock standard solutions of all amino acids were prepared at 1 mg mL^{-1} in deionized water (Tyr was prepared at 0.5 mg mL^{-1} in 0.01 M HCl). Mixed standards were prepared by blending and serially diluting the stock standards with water to give calibration standards in the range from 0.5 to 20 μg mL^{-1}. Derivatization reagent was prepared by dissolving 10 mg of *o*-phthaldialdehyde (OPA) in 100 μL of methanol, making up to 1 mL with borate buffer (0.4 M, pH 10.2) and then adding 20 μL of 3-mercaptopropionic acid. The reagent was allowed to stand for ≤90 min prior to use and remained stable at 4 °C for ≤1 week.

Samples (2.5 g) were extracted with acetic acid (0.01 M, 20 mL) using a macerator prior to centrifuging for 20 min at 23000g_{av} and 5 °C. The supernatant was decanted, the maceration and centrifugation process was repeated, and the supernatants were combined and adjusted to 100 mL with 0.01 M acetic acid.

An aliquot of the extract (3 mL) was mixed with 0.01 M HCl (1:1 v/v) and 4 mL of the solution applied to an Isolute SCX-2 (Kinesis, Cambridge, U.K.) solid phase extraction cartridge (preconditioned with 3 mL each of methanol and 0.01 M HCl). The cartridge was washed with 2 × 2.5 mL of 0.01 M HCl and eluted with 0.10 M K_3PO_4 (2 × 2 mL), and the eluate was passed through a 0.2 μm syringe filter into an autosampler vial for analysis using HPLC.

The HPLC system consisted of a P4000 pump and an AS3000 autosampler with an automated precolumn derivatization unit (Thermo Separation Products). The automated derivatization sequence was as follows: sample extract solution (20 μL) and borate buffer (150 μL) were combined and vortex mixed (0.5 min); 20 μL of OPA reagent was added and vortex mixed (0.5 min); 1200 μL of water was added and vortex mixed (0.5 min). Derivatized samples were separated on a 150 × 4.6 mm, 5 μm, ZORBAX Eclipse-AAA (Agilent) column prior

Table 1. Effect of Sugars, Added to Model Dough at 2−10 Times Natural Levels, on Acrylamide Production (Mean and Standard Error from Three Replicates) after 20 min at 180 °C (All Data Are Expressed on a Wet Weight Basis)

	sugars (g 100 g^{-1} of dough)				acrylamide (μg kg^{-1})	
dough sample[a]	sucrose	glucose	maltose	fructose	dough	relative increase[b]
no addition	0.226	0.053	1.053	0.031	158 (4.2)	
3 times added sucrose	0.736	0.053	1.053	0.031	160 (1.3)	2.93 (sucrose)
10 times added glucose	0.226	0.501	1.053	0.031	164 (0.6)	12.69 (glucose)
2 times added maltose	0.226	0.053	2.211	0.031	170 (0.4)	10.08 (maltose)
3 times added maltose	0.226	0.053	3.342	0.031	188 (0.4)	13.07 (maltose)
4 times added maltose	0.226	0.053	4.446	0.031	201 (3.6)	12.28 (maltose)
5 times added fructose	0.226	0.053	1.053	0.162	172 (4.3)	103.98 (fructose)

[a] Flour (1000 g) + salt (20 g) + water (600 g). The free Asn and total free amino acids compositions were 110 and 740 mg kg^{-1} of dough, respectively [b] Expressed as the increase in acrylamide (μg kg^{-1}) per g 100 g^{-1} sugar added.

Table 2. Distribution of Asparagine and Sugars in U.K. Commercial Mill Fractions Obtained from a Single Bread Wheat Grist (2004 Harvest; Protein Content 14% As-Is)

(**A**) Asparagine (All Data Expressed on an As-Is Moisture Basis)

			Asn[a]		
mill fraction	composition (%)	nitrogen (%)	mg kg^{-1} of fraction	% total amino acids[c]	contribution[b] (%)
whole grain	100	14	374 (30)	16	
white flour	82	11	141 (11)	21	38.9
bran	15	27	911 (65)	24	45.9
germ	3	2	1506 (83)	26	15.2

(**B**) Sugars (All Data Expressed on an As-Is Moisture Basis)

	sugars[a] (g 100 g^{-1})			
mill fraction	fructose	glucose	maltose	sucrose
whole grain	0.107 (0.007)	0.163 (0.011)	0.594 (0.038)	0.516 (0.033)
white flour	0.040 (0.003)	0.060 (0.006)	0.890 (0.014)	0.415 (0.025)
bran	0.806 (0.028)	0.776 (0.045)	0.048 (0.001)	0.945 (0.075)
germ	0.481 (0.020)	0.411 (0.037)	0.505 (0.045)	8.572 (0.495)

[a] Data are means of duplicate measurements with standard deviation in parentheses. [b] Contribution to Asn in wholemeal flour. [c] Sum of measured Asn, Asp, Ala, Arg, Gln, Glu, Gly, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, and Val.

to detection using an LC-240 fluorescence detector (Perkin-Elmer) operating at excitation and emission wavelengths of 340 and 450 nm, respectively. The injection volume was 20 μL. A gradient program was set up using 0.02 M phosphate buffer at pH 7.80 (solvent A) and 45:45:10 (v/v) acetonitrile/methanol/water (solvent B) at 1.5 mL min^{-1}: 57% solvent B increased to 100% over 1.8 min, held for 18 min, then decreased to 0% over 5 min.

Amino acids were quantified by an external standard method, and each sample was analyzed in duplicate. Method performance for asparagine in flour (typical) was as follows: limit of detection, 4.1 mg kg^{-1}; accuracy, 97 ± 7% (spiked flour, 95% confidence limit); precision (RSD%), 9.2% (flour containing 141.8 mg kg^{-1} asparagine); uncertainty, ±15.2% at 141.8 mg kg^{-1}.

Sugars. Benzoic acid solution (50% saturated) was prepared by dissolving 2.0 g of benzoic acid in 1 L of water. Carrez A was prepared by dissolving zinc acetate (43.8 ± 0.1 g) in approximately 800 mL of water. Glacial acetic acid was added (6 mL) and the solution made to 1 L with water. Carrez B was prepared by dissolving potassium ferrocyanide (21.2 ± 0.1 g) in approximately 800 mL of water prior to dilution to 1 L. Individual stock solutions of fructose, glucose, maltose, and sucrose were prepared at 0.1 g 100 mL^{-1} in 50% saturated benzoic acid. Internal standard solution (arabinose) was prepared at 1 g 100 mL^{-1} in 50% saturated benzoic acid. Mixed calibration standards ranging from 4 to 640 μg mL^{-1} were prepared in acetonitrile/water (60:40 v/v), each with an internal standard concentration of 400 μg mL^{-1}.

Samples (0.2 g for dough, 1 g for flours) were weighed accurately into 50 mL centrifuge tubes. Internal standard solution (1.00 mL), Carrez A and B solutions (5 mL each), and water (4 mL) were added, and the tube was capped, mixed, and shaken for 20 min. Acetonitrile (10 mL) was added, and the contents were mixed prior to centrifugation at 2000g_{av} for 10 min. The supernatant was passed through a 0.2 μm syringe filter prior to analysis by HPLC.

The HPLC system consisted of a GP40 gradient pump and an ED40 electrochemical detector coupled with an amperometric cell and an AS3500 autosampler (Dionex Corp., Sunnyvale, CA). The HPLC column was a 250 × 4 mm, 5 μm, CarboPac PA-1, and the mobile phase was 200 mM sodium hydroxide solution (isocratic elution, 15 min).

Sugars were quantified by an internal standard method using the sugar/arabinose responses and the slope and intercept of a least-squares fit to the calibration data set. Each sample was analyzed in duplicate. The limits of detection ranged from 0.3 to 1.0 mg kg^{-1} depending on the sugar.

RESULTS AND DISCUSSION

Model System. Before any of the cereal samples were processed, a simple addition experiment was performed to confirm that acrylamide levels in cooked wheat doughs (45% moisture and 180 °C; 6−8% moisture and 145 °C) did relate to flour asparagine levels. As can be seen from **Figure 1** there was a linear relationship between added Asn and acrylamide generated, at both high and low dough moistures, consistent with data reported elsewhere (*33*).

In a matching experiment, simple sugars (fructose, glucose, maltose, and sucrose) were added in amounts up to 10 times the normal concentrations found in dough. The contributions to acrylamide from each of the sugars were calculated by

Table 3. Amino Acid and Sugar Variation in U.K. Commercial Bread Wheat Varieties (2004 Harvest)

(**A**) Asparagine and Total Amino Acids (All Data Expressed on an As-Is Moisture Basis)

		Asn			total amino acids[a]		
flour description	*n*	mean (mg kg^{-1})	range (mg kg^{-1})	relative range	mean (mg kg^{-1})	range (mg kg^{-1})	Asn range (%)
French	3	216	207−226	1.1	1859	1722−1974	11.0−12.5
Einstein	4	291	242−393	1.6	1722	1541−1941	14.7−20.3
Claire	3	393	258−517	2.0	2391	2136−2841	12.1−18.4
Robigus	4	306	250−375	1.5	1937	1535−2253	11.1−18.4
Canadian	2	328	321−336	1.0	2179	2024−2335	14.4−15.9
German E	3	368	320−441	1.4	1666	1406−2089	21.1−22.9
Malacca	3	447	315−540	1.7	2765	2576−2940	11.3−18.8
Solstice	9	448	315−593	1.9	2068	1473−2653	19.8−23.9

(**B**) Sugars (Grams per 100 g), Mean and Range (All Data Expressed on an As-Is Moisture Basis)

		fructose		glucose		maltose		sucrose		total sugars	
flour description	*n*	mean	range	mean	range	mean	range	mean	range	mean	range
French	1	0.029		0.056		0.595		1.086		1.766	
Einstein	4	0.053	0.049−0.059	0.079	0.074−0.084	0.501	0.389−0.568	1.301	1.199−1.390	1.934	1.889−2.027
Claire	2	0.055	0.043−0.067	0.075	0.067−0.083	0.411	0.300−0.522	1.022	0.992−1.053	1.563	1.503−1.624
Robigus	2	0.078	0.074−0.083	0.090	0.082−0.098	0.463	0.301−0.625	1.130	1.075−1.186	1.762	1.642−1.882
Canadian	1	0.020		0.047		0.524		1.001		1.591	
German E	1	0.041		0.068		0.602		1.025		1.735	
Malacca	2	0.050	0.045−0.055	0.070	0.067−0.073	0.543	0.510−0.576	1.007	0.940−1.075	1.670	1.628−1.712
Solstice	3	0.060	0.051−0.066	0.088	0.083−0.092	0.436	0.384−0.531	1.267	1.179−1.388	1.850	1.721−1.926

[a] Sum of measured Asn, Asp, Ala, Arg, Gln, Glu, Gly, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, and Val.

Table 4. Composition of Individual U.K. Bread Wheat Flours and Doughs and the Amounts of Acrylamide Generated by Heating (All Data Are Expressed on an As-Is Moisture Basis)

(**A**) Flour Samples

		sugars (g 100 g^{-1})[a]						
whole wheat flour	Asn[a] (mg kg^{-1})	fructose	glucose	maltose	sucrose	moisture (g 100 g^{-1})	protein (%)	acrylamide generated[b] (μg/kg)
French	212 (10)	0.036 (0.006)	0.072 (0.015)	0.595 (0.014)	0.922 (0.143)	11.8	11.1	447 (85)
Canadian	295 (10)	0.019 (0.002)	0.061 (0.012)	0.583 (0.051)	0.901 (0.093)	11.3	16.3	470
Robigus	330 (49)	0.074	0.082	0.301	1.186	13.7	10.7	934 (53)
German E	346 (64)	0.041	0.068	0.602	1.025	13.8	13.5	570 (30)
Solstice	375 (45)	0.049 (0.001)	0.105 (0.014)	0.501 (0.028)	1.093 (0.122)	13.5	13.2	667
Solstice	413 (26)	0.066	0.092	0.384	1.179	13.4	12.3	734 (35)
Claire	457 (80)	0.060 (0.006)	0.097 (0.012)	0.351 (0.044)	0.973 (0.080)	14.3	11.7	671 (39)
Malacca	512 (40)	0.052 (0.003)	0.089 (0.015)	0.454 (0.073)	0.954 (0.107)	13.9	12.9	737 (110)
Solstice	538 (19)	0.060 (0.003)	0.093 (0.010)	0.354 (0.032)	1.134 (0.241)	14.3	12.9	1067 (11)

(**B**) Dough Samples

		sugars (g 100 g^{-1})[a]				
whole wheat dough	Asn[a] (mg kg^{-1})	fructose	glucose	maltose	sucrose	acrylamide generated[b] (μg/kg)
French	121 (6)	0.0790 (0.0058)	0.1649 (0.0049)	0.8368 (0.0148)	0.3757 (0.0078)	186 (9)
Canadian	189 (16)	0.0966 (0.0028)	0.2097 (0.0049)	0.5940 (0.0098)	0.3592 (0.0136)	183 (10)
Robigus	205 (9)	0.1017 (0.0055)	0.1956 (0.0054)	1.3207 (0.0523)	0.5021 (0.0218)	344 (8)
German E	233 (18)	0.1290 (0.0060)	0.2959 (0.0009)	0.6732 (0.0062)	0.3030 (0.0060)	302 (16)
Solstice	216 (13)	0.1489 (0.0032)	0.2679 (0.0022)	0.7304 (0.0129)	0.3540 (0.0010)	332 (10)
Solstice	233 (22)	0.1145 (0.0091)	0.2220 (0.0038)	0.7214 (0.0010)	0.3853 (0.0002)	346 (1)
Claire	233 (34)	0.1176 (0.0009)	0.2279 (0.0008)	0.6311 (0.0004)	0.4153 (0.0081)	320 (22)
Malacca	325 (33)	0.1174 (0.0042)	0.2506 (0.0074)	0.7511 (0.0111)	0.3717 (0.0107)	383 (21)
Solstice	282 (34)	0.1080 (0.0083)	0.2146 (0.0192)	0.6919 (0.0042)	0.4830 (0.0168)	436 (13)

[a] Samples were heated samples at 160 °C for 20 min (data are mean of ≤3 replicates with standard deviations in parentheses). [b] Data are mean of ≤2 replicates with standard deviations in parentheses.

dividing the change in acrylamide resulting from the sugar addition by the addition factor (e.g., 5×) and the acrylamide concentration in the unfortified dough.

Under the conditions of cooking in dough, that is, 20 min at 180 °C (45% moisture), the order of reactivity was fructose ≫ glucose/maltose > sucrose (see **Table 1**). These findings were similar to those reported elsewhere for fortified wheat (*33*) and model Maillard systems (*34*). It has been suggested that the formation of a key intermediate from sugars which goes on to react with Asn occurs via a single step for fructose and via multiple steps for glucose (*23*). This may account for the observed differences in reactivity of these sugars with respect

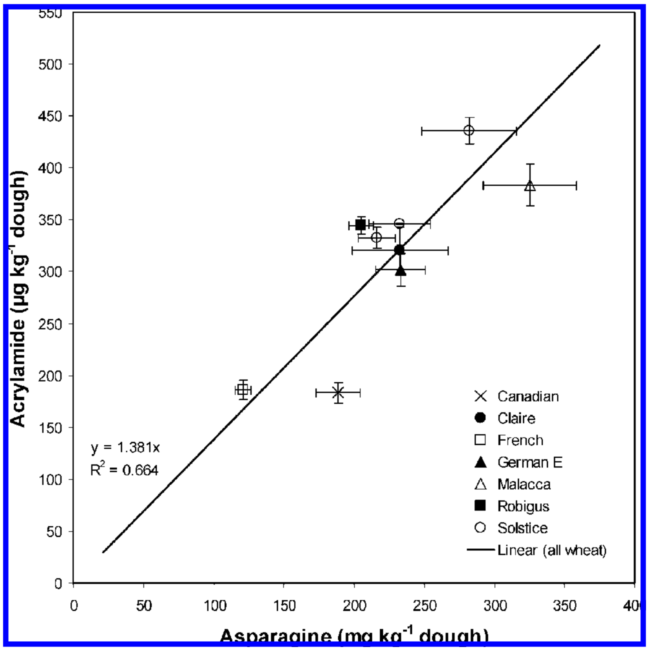

Figure 2. Correlation between free Asn in wheat dough (45% moisture) and acrylamide formed after heating for 20 min at 180 °C. Dough samples were prepared from different commercial wheat varieties exhibiting a range of Asn concentrations (data are mean and standard deviation).

to acrylamide generation. However, because fructose concentrations in the wheat flour used were relatively low, it could only account for about 2% of the acrylamide formed. The sum of the contributions to acrylamide formed in cooked dough from all of the flour sugars analyzed was 11%. These results suggest that natural variations in sugar concentrations in, for example, wheat are unlikely to have a significant effect on acrylamide generated during cooking. However, changes in sugar concentrations resulting from dough processing, such as the addition of yeast (*35*), and from recipe additions, for example, some short dough biscuits (*36*), would be expected to affect acrylamide formation.

Correlations between Free Amino Acids, Sugars, and Acrylamide in Bread Wheat Flours. *Distribution of Amino Acids and Sugars in the Wheat Berry.* A U.K. bread wheat grist (11% protein) was milled on a commercial scale into wholemeal, bran, germ, and white flour (starchy endosperm), and the distribution of free amino acids and sugars was measured in each of the mill fractions (see **Table 2**).

The highest levels of free amino acids and sugars were found in the germ and the bran. Milled germ contained the highest amount of Asn (1506 mg kg^{-1}) and white flour the least (141 mg kg^{-1}), and these data were consistent with those reported elsewhere (*15*). However, because the bran (Asn = 911 mg kg^{-1}) was more abundant than the germ, this fraction made the greatest contribution to the overall Asn content of the wholemeal flour (see **Table 2A**). Hence, for a given grist, high-extraction wheat flours would be expected to contain higher levels of Asn (*12*).

Free Amino Acids and Sugars in Bread Wheat Flours. To assess the potential variation between and within the commercial wheat flours, 31 samples of grain representing 8 wheat varieties were obtained directly from 9 mills across the United Kingdom. These were all breadmaking grists from the 2004 harvest year for use in U.K. bread products with protein contents ranging from 10.3 to 16.3% (mean = 12.3%). The grain was milled to a whole meal and analyzed immediately. The variation of free Asn and total amino acid concentrations in each sample of wheat is shown in **Table 3A**. Free Asn varied from 207 to 593 mg kg^{-1} (mean = 366 mg kg^{-1}) across all varieties, that is, a range of approximately 3:1. In contrast to data reported elsewhere (*13, 14*), no correlations were obtained between free Asn and wheat protein and total free amino acids. Similarly, there was no correlation between free Asn and the reducing sugars fructose and glucose. Free Asn amounts varied widely, both within and between varieties, and considerable overlap between cultivars was also apparent. These results suggest that variety selection is unlikely to be a simple option for reducing acrylamide.

Compared to free Asn in wheat, sugar concentrations showed very little variation, both within and between varieties (**Table 3B**), suggesting that they are less affected by agronomical changes, for example, due to fertilization (*17*).

Acrylamide Generation in Bread Wheat Flours and Doughs. To confirm that measured Asn in wheat flour did correlate with acrylamide in cooked cereal products, selected samples representative of the measured range of free Asn concentrations were cooked as flours and doughs using a closed cell reactor, and the levels of acrylamide were measured.

The composition of the bread wheat flours and the amount of acrylamide generated are given in **Table 4A**. As expected, free Asn content was a reasonable indicator of acrylamide potential in the cooked wheat flours (*13*). Although the amounts of acrylamide formed were consistent with the addition experiments (see **Figure 1**) and results reported elsewhere (*33*), the correlation between free Asn and acrylamide ($R^2 = 0.41$) was not as good as might be expected. To normalize potential inconsistencies due to milling (particle size) and moisture (*21*) that could affect acrylamide formed, the flours were prepared as doughs and cooked to generate acrylamide. **Figure 2** shows that acrylamide generated in the dough samples also followed free Asn amounts with a linear relationship, but with an improved correlation consistent with the addition experiments (see **Figure 1**). Given that amylases, gylcosidases, and starch damage are additional variables in dough, the relationship was reasonable ($R^2 = 0.66$). However, the relatively strong correlation between acrylamide generated in flours and that generated in the doughs ($R^2 = 0.73$) suggests that other, as yet unknown, factors may also be important in determining acrylamide. Comparison of the data given in sections **A** and **B** of **Table 4** shows that the amount of acrylamide formed in the flours was also approximately twice that formed in doughs ($y = 0.444x$ for acrylamide in dough versus acrylamide in flours; $R^2 = 0.7313$), and this could be explained by the different moisture contents (*21*), which were ca. 13 and 45%, respectively.

Variation of Amino Acids and Sugars in Other Commercial Cereals. A wide range of grains and flours, representative of those used in U.K. products such as biscuits, cakes, confectionery, and crispbreads, were obtained from commercial sources. The concentrations of free Asn, total free amino acids, and sugars were measured, and the links within these and to other variables, such as protein, were explored.

Biscuit and Confectionery Flours. This category represents a wide range of cereals used in biscuits, crackers, and confectionery wafers and, potentially a wide range of free amino acid and sugar concentrations. A total of 41 cereal flours from the 2006 harvest year were obtained directly from product manufacturers and commercial suppliers. These were mostly soft wheat varieties grown in the United Kingdom (e.g., white and wholemeal wheat with protein concentrations ranging from 8 to 11%) together with some barley, maize, oats, rice, and rye.

The variation of free Asn concentrations in all flours is shown in **Table 5A**. As expected, Asn concentrations varied widely

Table 5. Amino Acid and Sugar Variation in U.K. Commercial Biscuit and Confectionery Flours (2006 Harvest)

(**A**) Asparagine and Total Amino Acids (All Data Are Expressed on an As-Is Moisture Basis)

		Asn			total amino acids[a]		
flour description	*n*	mean (mg kg^{-1})	range (mg kg^{-1})	relative range	mean (mg kg^{-1})	range (mg kg^{-1})	Asn range (%)
rice	3	61	51−79	1.5	357	284−431	11.8−22.2
wheat (white)	25	115	48−155	3.2	1034	410−1394	8.6−17.5
barley	1	199			1105		18.0
wheat (wholemeal)	7	255	106−346	3.3	1644	909−2304	11.6−21.6
maize	1	288			1275		22.6
oats	2	370	350−391	1.1	1130	1076−1183	32.5−33.0
rye	1	396			1944		20.4

(**B**) Sugars (Grams per 100 g), Mean and Range (All Data Are Expressed on an As-Is Moisture Basis)

		fructose		glucose		maltose		sucrose		total sugars	
flour description	*n*	mean	range	mean	range	mean	range	mean	range	mean	range
rice	3	0.019	0.007−0.041	0.146	0.061−0.199	0.016	0.000[b]-0.033	0.319	0.192−0.493	0.499	0.340−0.765
wheat (white)	25	0.057	0.033−0.088	0.117	0.028−0.154	0.923	0.288−1.272	0.492	0.273−0.666	1.589	0.805−1.934
barley	1	0.117		0.125		0.527		0.914		1.684	
wheat (wholemeal)	7	0.120	0.084−0.159	0.237	0.199−0.300	0.604	0.329−0.922	0.773	0.703−0.879	1.733	1.419−2.085
maize	1	0.052		0.172		0.152		1.032		1.408	
oats	2	0.019	0.017−0.020	0.018	0.017−0.020	0.194	0.155−0.234	1.097	1.012−1.182	1.328	1.286−1.371
rye	1	0.213		0.396		0.467		0.753		1.830	

[a] Sum of measured Asn, Asp, Ala, Arg, Gln, Glu, Gly, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, and Val. [b] Less than 1 mg kg^{-1}.

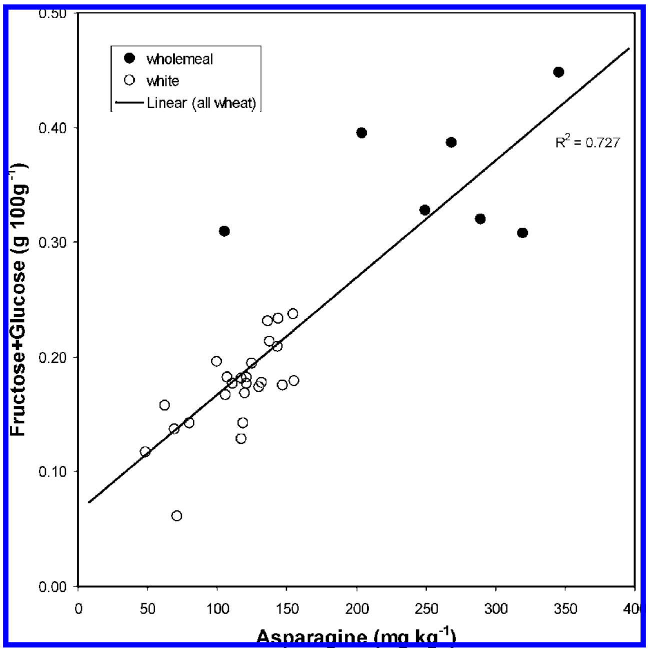

Figure 3. Correlation between free Asn and reducing sugars (fructose and glucose) in U.K. commercial biscuit wheat flours.

between the cereal types, with rye having the highest amount (396 mg kg^{-1}) and rice the lowest (61 mg kg^{-1}). For the wheat samples, the relative range of Asn contents was similar to the bread wheat flours, that is, approximately 3:1 for both white (48−155 mg kg^{-1}) and wholemeal (106−346 mg kg^{-1}) flours. Compared to the wholemeal bread flours (mean Asn = 366 mg kg^{-1}), the mean free Asn concentration in the wholemeal wheat flours (255 mg kg^{-1}) was lower, and this was probably due, on average, to the lower protein content of the wheats. However, in keeping with the bread wheat flours, free Asn amounts did not correlate with wheat protein.

Similarly, there was a large range of sugar concentrations across all cereal types (see **Table 5B**) with rye, wholemeal wheat, and barley having the highest amounts of fructose and glucose. In keeping with the data from the bread wheat samples, the sugar concentrations in the wheat flours showed very little variation.

Inspection of data from the wheat flours revealed that amounts of free Asn were correlated with both fructose ($R^2 = 0.685$) and glucose ($R^2 = 0.696$), whereas weak or little correlation was found for the disaccharides maltose and glucose. **Figure 3** shows the correlation between free Asn and the sum of fructose and glucose ($R^2 = 0.727$). This tandem variation of Asn and reducing sugars may offer some opportunity for grist selection, particularly in products that do not use added sugars. These data were in contrast to those obtained for the wholemeal bread flours, for which free Asn was not correlated with the reducing sugars fructose and glucose ($R^2 = 0.070$). One possible explanation for the latter result could be due to the selection criteria for bread wheat, which is based on protein quality/quantity, and this may bias the correlation in some way.

Cake Flours. In the United Kingdom, the modification of cake flour using chlorine treatment to improve baking performance is no longer permitted. Selective modification of the quality properties of cake flour can be achieved by heat treatment using temperatures that can reach up to 140 °C. To assess the effects of heat treatment, 15 U.K. cake flours representing a wide range of grists and heat treatments were obtained and the concentrations of free amino aids measured. These were mainly group three (soft) wheats with 10−40% and group four (hard) wheats (protein contents 6−7%). The variation in sugars was not assessed because cake products utilize sugar at levels well in excess of the natural variation in flour.

The concentrations of free Asn and total amino acids in the cake flours are shown in **Figure 4**. The samples are ordered by increasing levels of heat treatment, and despite the wide range of temperatures used (0−140 °C), there was no apparent impact on flour Asn or total amino acid levels. Cake flours have a lower level of gluten and a higher proportion of the starchy endosperm than, for example, bread flours, and this is reflected in the lower mean free Asn concentration of 68 mg kg^{-1} (range = 22−95 mg kg^{-1}). With the exception of one sample, the cake flours

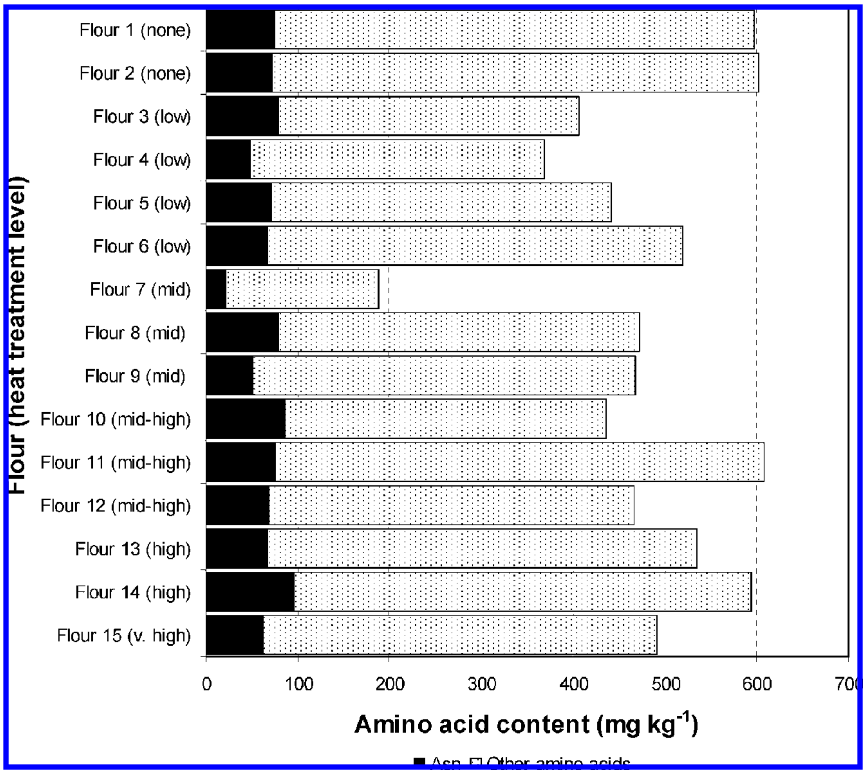

Figure 4. Free amino acid concentrations in U.K. commercial cake flours: flours 1–15 represent different grists and heat treatments (ordered by increasing heat treatment levels). Other amino acids = sum of Asp, Ala, Arg, Gln, Glu, Gly, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, and Val.

Table 6. Amino Acid and Sugar Variation in U.K. Commercial Crispbread Rye Flours (2006 Harvest)

(**A**) Asparagine and Total Amino Acids (All Data Are Expressed on an As-Is Moisture Basis)

		Asn			total amino acids[a]		
flour description	*n*	mean (mg kg^{-1})	range (mg kg^{-1})	relative range	mean (mg kg^{-1})	range (mg kg^{-1})	Asn range (%)
Ursus	12	816	660–979	1.5	3883	3239–4713	19.4–22.8
mixed	1	826			4382		18.8
Picasso	2	841	827–856	1.0	4194	3872–4515	18.3–22.1
Hacada	2	915	914–916	1.0	4528	4468–4588	19.9–20.5

(**B**) Sugars (Grams per 100 g), Mean and Range (All Data Are Expressed on an As-Is Moisture Basis)

		fructose		glucose		maltose		sucrose		total sugars	
flour description	*n*	mean	range	mean	range	mean	range	mean	range	mean	range
Ursus	12	0.096	0.055–0.117	0.195	0.164–0.247	0.422	0.321–0.585	0.943	0.870–1.115	1.655	1.484–1.952
mixed	1	0.211		0.222		0.256		0.803		1.492	
Picasso	2	0.082	0.082–0.129	0.205	0.172–0.237	0.406	0.386–0.427	1.036	0.921–1.150	1.752	1.561–1.943
Hacada	2	0.092	0.091–0.093	0.190	0.188–0.191	0.353	0.339–0.367	0.938	0.919–0.956	1.572	1.537–1.608

[a] Sum of measured Asn, Asp, Glu, Ser, Gln, Gly, Thr, Arg, Ala, Tyr, Val, Met, Trp, Phe, Ile, Leu, and Lys.

also showed much less variation in free Asn levels than, for example, bread flours, and this is probably because the flour is more refined.

Crispbread Flours. A total of 16 rye grain samples representing 3 varieties and 1 mixed rye flour used to manufacture U.K. crispbreads were obtained from commercial sources. Grains were milled into wholemeal flour, the concentrations of free amino acids and sugars were measured, and links between precursors were explored.

Table 6A shows the variation in free Asn and total amino acids in each of the rye flours. Asn concentrations in rye ranged from 660 to 979 mg kg^{-1} (mean = 831 mg kg^{-1}) and were approximately 2–3 times higher than those found in the bread wholemeal wheats (see **Table 4A**).

Although the total sugar concentrations were fairly constant (see **Table 6B**), fructose amounts showed much greater variation both within and between varieties, and the overall range was approximately 4:1 (0.055–0.211 g 100 g^{-1}). The mean concentrations of both reducing sugars (mean glucose = 0.197 g 100 g^{-1}; mean fructose = 0.103 g 100 g^{-1}) were approximately twice that of the wholemeal wheat flours, indicating that the contribution to acrylamide generated by cooking these cereals could be significant. However, this remains to be determined because previous studies have shown that Asn in rye is the key determinant of acrylamide formed during cooking and not added fructose (*19*).

Figure 5 shows that for some varieties, at least, free Asn was correlated with the reducing sugars in a similar way to that

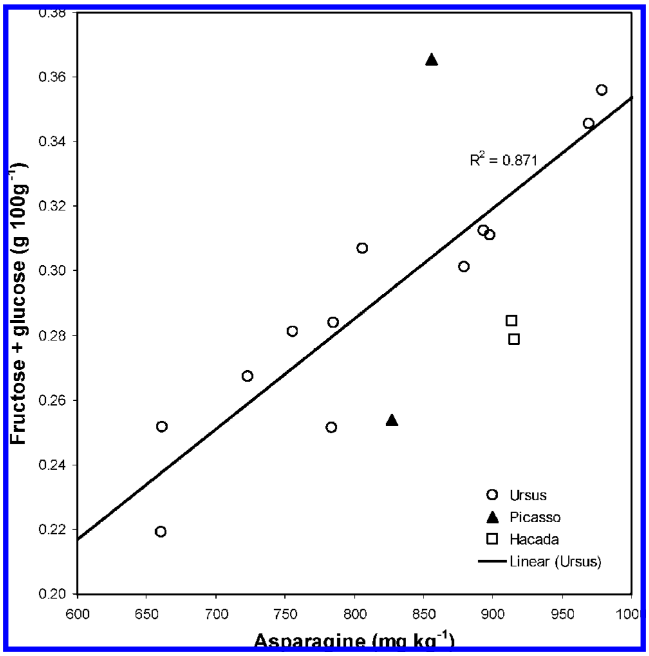

Figure 5. Correlation between Asn and reducing sugars (fructose and glucose) in U.K. commercial crispbread rye flours.

of the biscuit and confectionery wheat flours. This tandem relationship between Asn and the reducing sugars may also offer some opportunities for selective gristing, particularly in products such as crispbreads that do not use added sugars in their recipe.

Implications for Cereal Manufacturers. In summary, it is evident that free Asn amounts in commercial flours vary widely both within and between cereal types, even within a single growing season. Asparagine levels varied 10-fold across all cereal types and were highest in rye followed by oats, wholemeal wheat, maize, barley, wheat flours, and finally rice. Concentrations of fructose and glucose in wholemeal rye were also twice those seen in wholemeal wheat.

This natural variation means that reducing asparagine by selecting a single variety of grain is not an easy option. However, free Asn and reducing sugars do appear to vary in tandem in soft wheat and in rye flours, so selective gristing may be worthwhile for products in which there is no added fructose/glucose in the recipe. No such correlation was found with bread flours, possibly because they are selected on the basis of criteria based on the type and quantity of functional protein present.

Supporting Information Available: Distribution of all measured amino acids (Asn, Asp, Ala, Arg, Gln, Glu, Gly, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, Val), sugars, and divalent metals in cereals used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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