Concentrations of Free Amino Acids and Sugars in Nine Potato Varieties: Effects of Storage and Relationship with Acrylamide Formation

Nigel G. Halford,*,[†] Nira Muttucumaru,[†] Stephen J. Powers,[‡] Peter N. Gillatt,[§] Lee Hartley,[§] J. Stephen Elmore,[⊗] and Donald S. Mottram[⊗]

[†]Plant Biology and Crop Science Department and [‡]Computational and Systems Biology Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom

[§]Kettle Foods Ltd., Barnard Road, Bowthorpe, Norwich NR5 9JP, United Kingdom

[⊗]Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading RG6 6AP, United Kingdom

Ⓢ Supporting Information

ABSTRACT: Acrylamide forms during cooking and processing predominately from the reaction of free asparagine and reducing sugars in the Maillard reaction. The identification of low free asparagine and reducing sugar varieties of crops is therefore an important target. In this study, nine varieties of potato (French fry varieties Maris Piper (from two suppliers), Pentland Dell, King Edward, Daisy, and Markies; and chipping varieties Lady Claire, Lady Rosetta, Saturna, and Hermes) grown in the United Kingdom in 2009 were analyzed at monthly intervals through storage from November 2009 to July 2010. Acrylamide formation was measured in heated flour and chips fried in oil. Analysis of variance revealed significant interactions between varieties nested within type (French fry and chipping) and storage time for most free amino acids, glucose, fructose, and acrylamide formation. Acrylamide formed in chips correlated significantly with acrylamide formed in flour and with chip color. There were significant correlations between glucose or total reducing sugar concentration and acrylamide formation in both variety types, but with fructose the correlation was much stronger for chipping than for French fry varieties. Conversely, there were significant correlations with acrylamide formation for both total free amino acid and free asparagine concentration in the French fry but not chipping varieties. The study showed the potential of variety selection for preventing unacceptable levels of acrylamide formation in potato products and the variety-dependent effect of long-term storage on acrylamide risk. It also highlighted the complex relationship between precursor concentration and acrylamide risk in potatoes.

KEYWORDS: acrylamide, asparagine, free amino acids, potato, Solanum tuberosum, sugars, color

■ INTRODUCTION

Acrylamide was discovered in a range of mainly plant-derived popular foods in 2002.[1] It is classified by the World Health Organisation and the International Agency for Research on Cancer as "probably carcinogenic to humans", on the basis of its carcinogenic action in rodents; it also has neurological and reproductive effects.[2] Human dietary exposure to acrylamide is at levels that are orders of magnitude lower than those used in rodent toxicology studies, and research is ongoing to try to establish how much risk, if any, it actually represents. Nevertheless, the FAO/WHO Expert Committee on Food Additives has recommended that dietary exposure should be reduced and the European Food Safety Authority (EFSA) issued "indicative" levels for acrylamide in food in early 2011 (http://ec.europa.eu/food/food/chemicalsafety/contaminants/recommendation_10012011_acrylamide_food_en.pdf). These levels are not regulatory limits, nor are they a safety standard, although they have already been misused as such by the popular press in the United Kingdom. Nevertheless, the food industry is under pressure to ensure that the concentrations of acrylamide in its products are below the indicative levels. Fried potato products are major contributors

to dietary exposure to acrylamide (http://www.efsa.europa.eu/en/press/news/datex110420.htm).

The major route for acrylamide formation in food is through the Maillard reaction, a complex series of nonenzymic reactions between amino groups and reducing sugars. The reaction takes place only at high temperatures, such as those that occur during frying, baking, and roasting; boiled products are not affected. The Maillard reaction gives rise to a plethora of products, many of which impart color, aroma, and flavor;[3−5] acrylamide forms only when the amino acid that participates in the final stages of the reaction is asparagine.[6−8] There are other possible routes for acrylamide formation, for example, from 3-aminopropionamide[9] or in wheat grain from gluten.[10] Nevertheless, free asparagine and reducing sugars can be regarded as the most important precursors for acrylamide formation.

The food industry has devised many strategies for reducing acrylamide formation by modifying food processing, and there are anecdotal and published reports of significant reductions

being made as a result.[11,12] These acrylamide reduction strategies have been compiled in a "Toolbox" produced by Food Drink Europe (http://ec.europa.eu/food/food/chemicalsafety/contaminants/ciaa_acrylamide_toolbox09.pdf); they include modification of time/temperature conditions during processing, lowering pH by the addition of citric acid, presoaking in water, addition of antioxidants, and addition of divalent cations, such as calcium chloride. The addition of asparaginase to reduce asparagine concentration prior to cooking has been successful in some products but is not applicable to all foods.

Strategies involving changes to processing are expensive to implement, and there may be a limit to the improvements that can be achieved without adversely affecting the quality and defining characteristics of the product. They may also have little relevance to home cooking. A complementary approach is to reduce the concentrations of the precursors for acrylamide formation in the raw material from the crop, initially by variety selection and then through the identification of existing low acrylamide risk genotypes that could be brought into cultivation, and, in the long term, through plant breeding programs aimed at producing new varieties with lower concentrations of free asparagine and/or reducing sugars.[13] Raw materials derived from such varieties would generate less acrylamide in any form of cooking, industrial or domestic, and would reduce the need to adapt processes.

The relationship between asparagine and sugar concentrations in potatoes and acrylamide formation during processing is surprisingly complicated. Asparagine is the most abundant free amino compound in potato tubers, typically accounting for approximately one-third of the total free amino acid pool.[14−16] This led to sugar concentrations being proposed as the limiting factor for acrylamide-forming potential, and the results of some studies have been consistent with this.[15,17] However, Shepherd et al. showed that asparagine and sugar concentrations contributed approximately equally to the variation in acrylamide-forming potential in a segregating potato breeding population,[18] whereas Elmore et al. found that asparagine as a proportion of the total free amino acid pool was the determining factor in a study of three different potato varieties grown under glass,[19] suggesting that there was competition between different free amino acids for participation in the Maillard reaction. Further evidence of competition between different free amino acids when sugars are limiting was obtained from the analysis of tubers before and after a period of cold storage.[20] When sugar levels were relatively high, acrylamide and aroma compound formation during heating was proportional to sugar concentration, whereas when sugar levels were low, acrylamide and aroma compound formation was proportional to the concentrations of their precursor amino acids expressed as a percentage of total free amino acids.

This complex relationship between precursor concentration and acrylamide formation in potato contrasts with that found in wheat and rye grain, in which there is a clear relationship between acrylamide-forming potential and the concentration of free asparagine.[21−24] Currently, the best advice for potato is that the concentrations of reducing sugars, free asparagine, and other free amino acids must all be considered in variety selection.[13] Here we describe the analysis of nine varieties of potatoes grown in the South East of the United Kingdom in 2009, showing unexpected differences in the relationship between acrylamide formation and precursor concentration

between French fry and chipping varieties, and the effect of long-term storage on acrylamide risk.

■ MATERIALS AND METHODS

Potato Samples. Ten sets of tubers from potatoes (*Solanum tuberosum*) grown commercially at sites in the South East of the United Kingdom (Table 1) in 2009 were analyzed in the study. The

Table 1. Locations of Farms in the United Kingdom That Produced the Potatoes Analyzed in the Study and the Soil Type on Which the Potatoes Were Grown

variety	location	soil type
Lady Claire	Stokesby, East Norfolk	loamy sandy clay
Lady Rosetta	Gresham, North Norfolk	sandy clay loam
Hermes	Caister, East Norfolk	sandy clay loam
Saturna	Caister, East Norfolk	sandy clay loam
Markies	Melton Constable, North Norfolk	sandy loam
Daisy	Melton Constable, North Norfolk	sandy silt loam
Morris Maris Piper	Stonea/March, Cambridgeshire	silt
Thomsett Maris Piper	between Soham/Isleham Cambridgeshire	sandy black
Pentland Dell	Wisbech, Cambridgeshire	silt
King Edward	Great Bentley, Essex	medium sandy loam

potatoes were planted in April and harvested between late September and early November 2009. One tonne of each of the 10 consignments of tubers was held in boxes under carefully controlled storage conditions in two different stores and sampled monthly from November 2009 to July 2010. The French fry varieties (Maris Piper, Pentland Dell, King Edward, Daisy, and Markies) were held at 8.5 °C, whereas the chipping varieties (Lady Claire, Lady Rosetta, Saturna, and Hermes) were held at 9.5 °C. This is in line with normal practice, although commercial storers might change conditions slightly for some varieties and batches. Sprouting was prevented by treatment with the antisprouting agent chlorpropham (CIPC), at the discretion of the store manager. During storage, the potatoes were monitored for disease, defects, chitting, and general visual appearance.

The stability of the tubers during storage was checked by measuring glucose and sucrose concentrations using a YSI 2700 SELECT Biochemistry Analyzer (YSI (UK) Ltd., Fleet, UK). This analyzer uses a biosensor incorporating an immobilized enzyme or enzymes, depending on the substrate to be measured. The substrate is oxidized to produce hydrogen peroxide, which is itself oxidized at a platinum electrode. The current produced is proportional to the concentration of the substrate. D-Glucose is oxidized in the presence of glucose oxidase, producing hydrogen peroxide and glucono-lactone. The sucrose sensor has three enzymes, made up of invertase to convert sucrose to glucose and fructose, mutarotase to convert α-D-glucose to β-D-glucose, and glucose oxidase. Ten tubers of each variety were selected, washed in water, and sliced. The juice from potato slices (ca. 200 g) was collected, made up to 500 mL with water, and stored at 4 °C for 1 h. The instrument was calibrated against sucrose and glucose standard solutions (each 0.5 g/L). The supernatant from the potato solution was aspirated into the instrument, and the sucrose and glucose concentrations were read automatically. After every 10 readings, the instrument self-calibrated, and if a discrepancy was found, it was recalibrated manually against the sucrose and glucose standards. A single sample for each variety by month combination was analyzed.

The objective of this protocol was to ensure that the potatoes were treated in a way that reflected standard industrial practice and that any changes in potato composition or acrylamide formation during processing resulted from the length of storage or variety.

Preparation of Potato Tubers for Acrylamide and Precursor Analysis. The tubers were weighed, and four potato sticks of 0.5 cm cross section were prepared from each, using a French fry cutter. The

ends of the potato sticks were sliced off to remove all peel, and the four were weighed, placed in a foil tray, and blast frozen for 15 min. The frozen samples were placed in a freeze-dryer and lyophilized for 72 h. They were then weighed again before milling in a coffee grinder, and the freeze-dried potato flour samples were stored at −18 °C until analysis.

Determination of Free Amino Acids. A tuber flour sample (0.100 ± 0.005 g) was weighed into a 14 mL screw-top bottle. Hydrochloric acid (10 mL, 0.01 mol/L) was added to the vial, and the sample was stirred for 15 min at room temperature. An aliquot of supernatant (2 mL) was centrifuged at 10000g for 10 min. The amino acids in 100 μL of the centrifuged supernatant were then derivatized using the EZ-Faast amino acid derivatization technique for GC-MS (Phenomenex, Torrance, CA, USA). Arginine could not be measured using this technique. GC-MS analysis of the derivatized sample was carried out using an Agilent 5975 system (Agilent, Santa Clara, CA, USA) in electron impact mode as described previously.[19] One analysis was performed for each sample, but there were five replicate samples per variety for each month. Amino acids were quantified using external calibration curves, prepared using derivatized amino acid standards, and are reported on a dry weight basis.

Analysis of Sugars by Ion Chromatography. Sugar concentrations were measured in tuber flour using the method described previously.[19] Aqueous methanol (50%) containing 100 mg/L trehalose as internal standard was used to prepare an extract of each flour sample (0.100 ± 0.005 g). The supernatant was centrifuged at 10000g for 10 min, and aliquots were diluted 10-fold in water and filtered through a 0.2 μm syringe filter. The extracts were analyzed using a Dionex ion chromatography system with a 250 × 4 mm Carbopac PA1 column (Dionex Corp., Sunnyvale, CA, USA). Each extract was analyzed in triplicate (technical replicates), and there were five biological replicate samples of each variety at each time point. Data are reported on a dry weight basis.

Acrylamide Analysis on Potato Flour. Tuber flour samples (0.500 ± 0.005 g) were weighed into unsealed, glass ampules (1 mL capacity), which were heated, six at a time, for 20 min at 160 °C. These conditions have been used previously[19] and give high levels of acrylamide formation, providing a good, consistent indication of acrylamide-forming potential in different raw materials. One replicate was prepared for each flour sample, with three biological replicate samples for each variety by month combination. The heated potato flour (∼0.4 g, accurately weighed) was extracted with water (40 mL, containing 50 μg/L $^{13}C_3$-acrylamide internal standard) at room temperature in a 50 mL centrifuge tube. After 20 min of shaking, the tube and contents were centrifuged for 15 min at 15 °C and 9000 rpm. This temperature was not low enough for starch gelatinization to occur, which meant that 2 mL of aqueous extract removed from the centrifuge tube could be readily passed through a 0.2 μm syringe filter into a 2 mL vial.

Samples were analyzed by liquid chromatography−mass spectrometry/mass spectrometry (LC-MS/MS) using an Agilent 1200 HPLC system with a 6410 triple-quadrupole mass spectrometer with electrospray ion source in positive ion mode. An isocratic separation was carried out at room temperature using a 100 × 3.0 mm Hypercarb column with a 10 × 3.0 mm Hypercarb precolumn (both 5 μm particle size; Thermo Fisher, Waltham, MA). The mobile phase was 0.1% aqueous formic acid at a flow rate of 0.3 mL/min. The injection volume was 25 μL. The eluant from the column was run to waste from 0 to 4.5 min, and data were collected from 4.5 to 8 min. Acrylamide eluted at around 6 min. A run time of 20 min allowed the cleanup of the column for the following sample. The transitions m/z 72 → 55 and 72 → 27 were measured for acrylamide, and the transition m/z 75 → 58 was measured for $^{13}C_3$-acrylamide. Peaks were symmetrical with no interference from impurities.

The limit of quantitation for acrylamide in the flour was 20 μg/kg, equivalent to a concentration in the extract of 0.25 μg/L. No suppression of acrylamide by the sample matrix was observed. This could be concluded from the fact that the peak areas of the $^{13}C_3$-acrylamide internal standard were similar in both the aqueous standard and the sample (50 μg/kg).

Fried Chips. Potatoes from each variety for each storage point were used to prepare chips. Potatoes (ca. 10 kg) were washed in water using a Haith potato rumbler (Haith Engineering, Doncaster, UK). Solids content was determined hydrogravimetrically, using a gravitator (Blake and Boughton, Thetford, Norfolk, UK). The tubers were sliced using a portable slicer (model CC, Urschel, Valparaiso, IN, USA) with the head set to slice at $^{72}/_{1000}$ of an inch (1.8 mm), which is a typical thickness for hand-cooked chips. Potato slices (1 kg) were fried in sunflower oil (FFA 0.5%) in an electrically powered 12 kW fryer (Blue Seal, Birmingham, UK) at 140 °C with stirring for 7.5 min (again typical for hand-cooked chips). The temperature profile during frying was recorded and, typically, at the end of frying, the temperature was 140 ± 3 °C. Chip oil and moisture contents were determined using an InfraLab instrument (NDC Infrared Engineering Ltd., Maldon, Essex, UK), calibrated using external standards. Chip color was determined on the Hunter Lab scale using a D25LT colorimeter (Hunter Associates Laboratory, Reston, VA, USA) after the chips had been macerated to a diameter of ca. 10−20 mm.

Measurement of acrylamide in the chips was undertaken by an external laboratory (Premier Foods, High Wycombe, UK). The sample was macerated, and acrylamide was extracted into water, converted to 2-bromopropenamide by selective bromination, and analyzed using gas chromatography−tandem mass spectrometry (GC-MS/MS).[25] Quantification was by a stable isotope internal standard method using ^{13}C-labeled acrylamide. Single replicate samples were used for each variety by month combination in this assay.

Statistical Analyses. Analysis of variance (ANOVA) was used for each measured variable presenting biological replication, to assess the overall significance (F tests) of main effects and interactions between varieties nested within type (French fry or chipping) and storage time (months). Means of interest were then compared using the least significant difference (LSD) at 5% based on the residual degrees of freedom (df) from the ANOVA. A natural log (to base e) transformation was used for acrylamide, amino acids, and sugars to account for some heterogeneity of variance, residuals then conforming to the assumptions of ANOVA. Pearson's correlation coefficient (r) was calculated for variables of interest, for French fry and chipping varieties separately and together. Correlations were tested for statistical significance using the F test.

The full data set, averaging over replicates when necessary to give one profile per variety (keeping the two Maris Piper samples separate) by month combination, was also analyzed using principal coordinates (PCO) analysis (see, for example, Krzanowski[26]). For this a 90 by 90 (varieties by months) similarity matrix was derived using the Euclidean distance measure between all profiles, having transformed the variables mentioned above to the $\log_e$ scale as appropriate. Given this matrix and invoking metric scaling, the PCO analysis found points in 89-dimensional space such that distances between the points representing the 90 variety by month combinations were preserved. Finally, dimensionality reduction was applied to the 90 by 89 matrix of coordinates thus formed, to give principal axes for a simplified representation. The first few principal axes (PCos) were retained as accounting for the greatest amount of variation in the set of points. Plotting in these new dimensions revealed the separation between the 90 combinations. Regression analysis was then used to relate the PCos (coordinates) back to the original data to see which variables were most highly correlated with the coordinates, in terms of those with greatest F statistics, and so most likely to be related to the observed separation of the combinations.

RESULTS AND DISCUSSION

Potatoes were supplied by commercial growers in the United Kingdom in 2009 (Table 1). Maris Piper was obtained from two different suppliers, and these two samples will be referred to as Thompsett Maris Piper and Morris Maris Piper. Pentland Dell, King Edward, Daisy, Markies, and Maris Piper are traditionally used for the production of French fries, whereas Lady Claire, Lady Rosetta, Saturna, and Hermes are used for
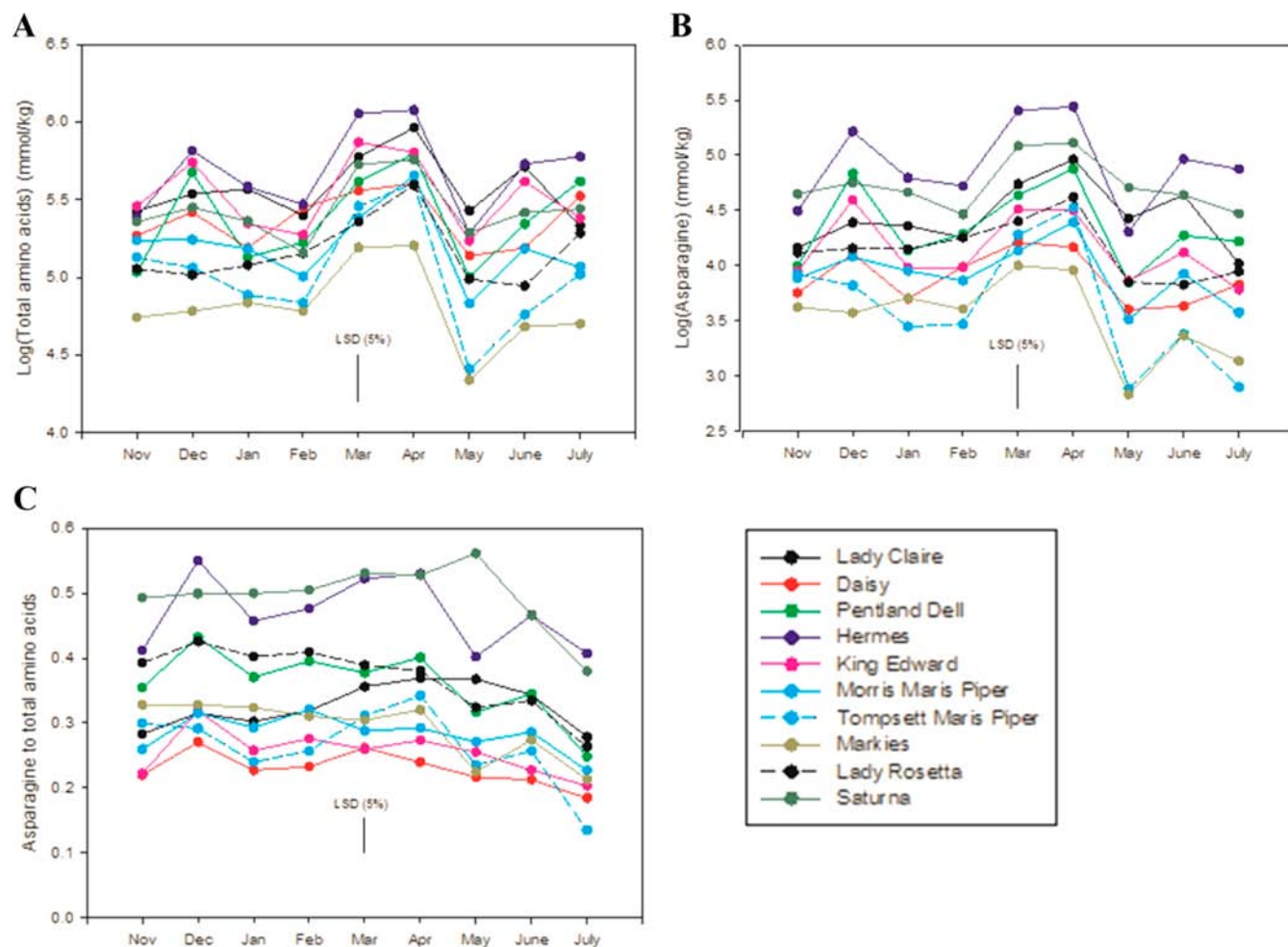
Figure 1. Free amino acid concentrations (mmol/kg on the $\log_e$ scale) in 10 samples of potato tubers produced by commercial growers in the United Kingdom in 2009, placed in long-term storage and sampled monthly from November 2009 to July 2010: (A) total free amino acids; (B) free asparagine; (C) ratio of free asparagine to total free amino acids. The least significant difference (LSD) at 5% is indicated ($n = 5$, df = 359).

chips (called crisps in the United Kingdom). Maris Piper and King Edward are also popular retail varieties.

Free Amino Acids. Total free amino acid and free asparagine concentrations and the ratio of free asparagine to total free amino acids are shown graphically in Figure 1. The data were analyzed using analysis of variance (ANOVA) to generate the least significant difference (LSD) at 5% for comparison of means (shown as a bar in Figure 1). The full data set is given in the Supporting Information. Statistical comparisons had to be made on the $\log_e$ scale for valid application of ANOVA, and so the means of the logged data are presented. This enables the LSD (5%) values to be used to make comparisons on the correct scale.

ANOVA of the free asparagine data showed a significant ($p = 0.004$) interaction between varieties nested within type (French fry and chipping) and month (effectively storage time), indicating that the varieties performed differently within type and across months. Similarly for the total free amino acids, the ANOVA showed a significant ($p = 0.019$) interaction between varieties nested within type and storage time. Asparagine was the most abundant free amino acid in most varieties, followed by glutamine and aspartic acid. The spread in both total free amino acid and free asparagine concentrations between varieties was marked. In November, for example, the average free asparagine concentration for Markies was 39.0 mmol/kg,

whereas for Hermes it was 91.1 mmol/kg. By July, the concentration in Markies had fallen to 23.7 mmol/kg, but for Hermes it had risen to 133.8 mmol/kg, resulting in a >5-fold difference between the two varieties.

Markies was also relatively low in total free amino acids (Figure 1A), whereas Hermes was relatively high. Despite this, Hermes had a relatively high ratio of free asparagine to total free amino acids (Figure 1C). Saturna, Pentland Dell, and Lady Rosetta also had relatively high ratios of free asparagine to total free amino acids (Figure 1C). King Edward and Daisy, on the other hand, were relatively high in total free amino acids but contained more free glutamine than free asparagine, resulting in a relatively low ratio of free asparagine to total free amino acids (Figure 1C). This is consistent with a previous study on King Edward potatoes grown under glass,[19] in which glutamine was shown to be the most abundant free amino acid.

Free asparagine and total free amino acids peaked for most of the varieties in March and April and then declined to May. The relatively high concentrations of free asparagine compared with other free amino acids in potato tubers and cereal grain may reflect asparagine's role as a nitrogen store,[13,27] and the decline in concentrations during late storage could be indicative of the tubers mobilizing resources at this time.

Graphs for the other free amino acids are shown in the Supporting Information, apart from arginine, which cannot be
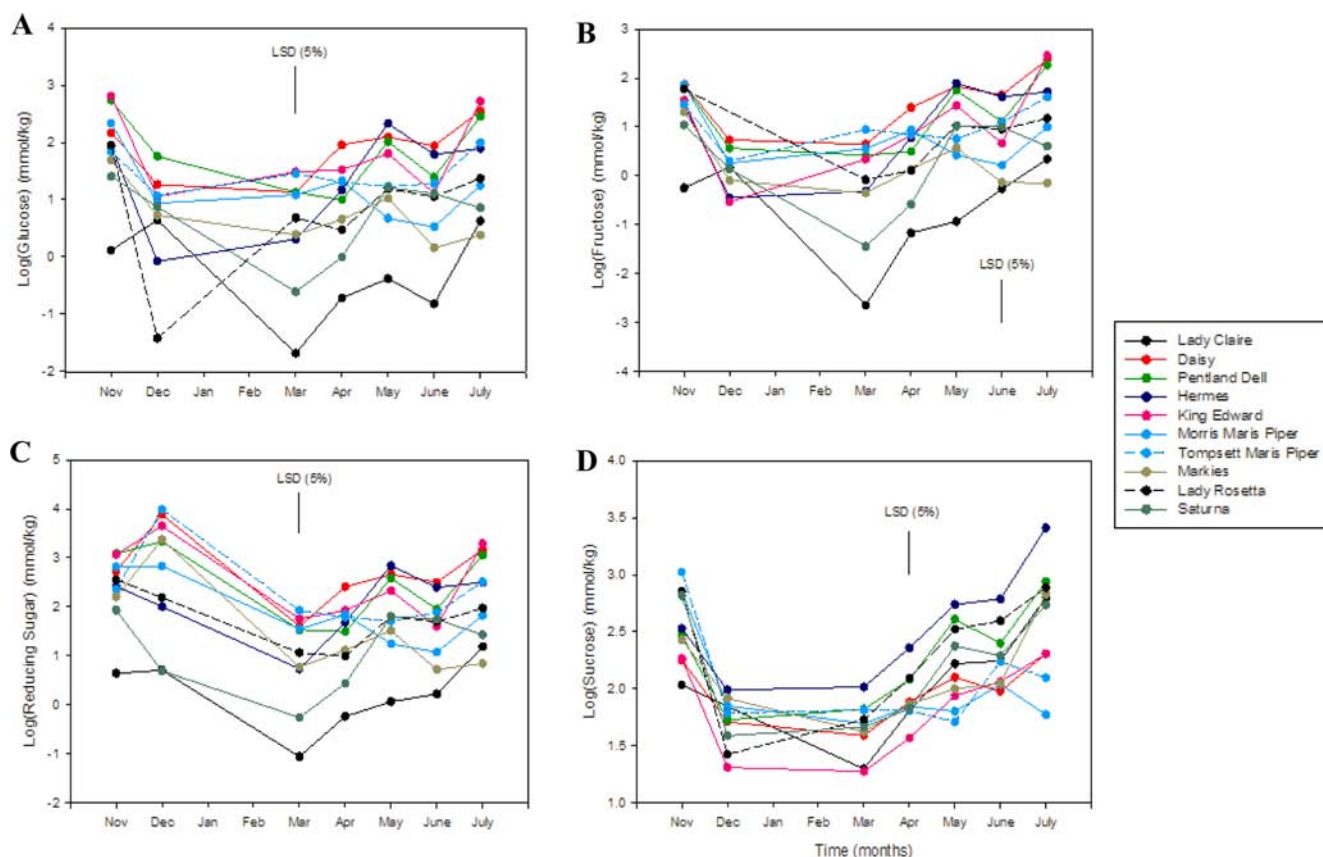
Figure 2. Sugar concentrations (mmol/kg on the $\log_e$ scale) in 10 samples of potato tubers produced by commercial growers in the United Kingdom in 2009, placed in long-term storage and sampled from November 2009 to July 2010: (A) glucose; (B) fructose; (C) total reducing sugars; (D) sucrose. The least significant difference (LSD) at 5% is indicated ($n = 5$, df = 276).

measured by the method used, and cysteine, which was below the concentration for accurate measurement. The interaction between variety nested within type and month was significant ($p < 0.05$) for all except valine ($p = 0.195$), isoleucine ($p = 0.078$), and proline ($p = 0.133$) (plots shown in the Supporting Information). As with asparagine, there were peaks in concentration in March and April, followed by a decline, for aspartic acid, glutamine, ornithine, lysine, and histidine. In contrast, there was a general increasing trend for leucine, threonine, and proline, and a weak one for phenylalanine, but with Maris Piper (from both suppliers) showing a great fall in phenylalanine in May. Serine peaked in December and showed an increasing trend from February onward, whereas glutamic acid showed a general decreasing trend throughout. There were no strong trends for alanine, glycine, valine, or isoleucine. Comparison of the varieties revealed that Markies was notably low in isoleucine, whereas Daisy was high in methionine.

These free amino acids may have differing effects on acrylamide formation. In model systems containing asparagine and glucose, amino acids added to the reaction at equimolar concentrations to asparagine increased the rate of acrylamide formation, probably by promoting the formation of Maillard reaction intermediates, such as highly reactive deoxyosuloses, dicarbonyls, and hydroxycarbonyls, which readily react with asparagine.[28] However, in other circumstances amino acids may act to reduce acrylamide formation from asparagine. At higher concentrations, the secondary amine-containing amino acids, proline and tryptophan, had a strong inhibiting effect on acrylamide formation in the model systems, reducing it by 80%

after 60 min of heating at 160 °C; cysteine and glycine also reduced acrylamide formation, by 55 and 45%, respectively.[28] Two previous studies have also shown the ratio of free asparagine to total free amino acids to be important,[19,20] suggesting that competition between free asparagine and other amino acids for participation in the final stages of the Maillard reaction could be an important factor under some circumstances.

Sugars. Glucose, fructose, total reducing sugar, and sucrose concentrations in tuber flour are shown graphically on the $\log_e$ scale in Figure 2. The entire data set is given in the Supporting Information. Spoilage of samples between removal from the store and analysis of sugars in January and February led to missing data for these months; however, in-store monitoring of glucose and sucrose concentrations did not show any great change during this period (data not shown). Significant ($p < 0.05$) interactions between varieties nested within type and month were found for all sugar variables.

There were considerable variations in the sugar levels across the data set, but some clear trends emerged. Sucrose was the most abundant sugar, whereas glucose was the most abundant reducing sugar, typically 1.5−2 times the concentration of fructose. As with asparagine and total free amino acids, the varieties showed large differences in reducing sugar concentrations. The mean glucose concentration in Lady Claire in November, for example, was 1.17 mmol/kg, whereas in King Edward it was 17.38 mmol/kg. Lady Claire had the lowest concentrations of reducing sugars throughout, apart from the last storage point in July. Saturna was also shown to be

relatively low in reducing sugars, whereas King Edward, Maris Piper, Pentland Dell, Lady Rosetta, Daisy, and Hermes were relatively high. Overall, the French fry varieties were 2.3-fold higher in glucose (4.31 vs 1.85 mmol/kg) and 1.8-fold higher in fructose (2.55 vs 1.39 mmol/kg) than chipping varieties, but 1.3-fold lower in sucrose (7.68 vs 9.65 mmol/kg). There was a significant correlation between glucose and fructose concentrations in both types, but it was stronger for the chipping varieties ($r = 0.838$, $p < 0.001$) than the French fry varieties ($r = 0.666$, $p < 0.001$). There was also a significant correlation between sucrose and glucose ($r = 0.900$, $p < 0.001$) and between sucrose and total reducing sugar concentration ($r = 0.921$, $p < 0.001$).

Amrein et al. also reported a wide range of glucose, fructose, and sucrose concentrations (0.5−14.2, 0.2−2.1, and 2.4−8.9 mmol/kg, respectively, on a fresh weight basis) in 17 varieties of potato grown in Switzerland in 2002,[15] whereas Elmore et al. reported 1.2−7.8, 0.5−2.1, and 27.4−37.4 mmol/kg, respectively, on a dry weight basis for three varieties (King Edward, Maris Piper, and Prairie) grown under glass.[19]

In most of the varieties, sugar concentrations fell between November and December and rose steadily from March to July as dormancy began to break. The exception was Markies, which was very stable in late storage and by July had the lowest reducing sugar concentration. It was notable that concentrations of sucrose as well as reducing sugars rose in the other varieties from March to July, indicating that if the increase in glucose and fructose concentrations arose from the cleavage of sucrose by invertase, sucrose must have been replenished, presumably from the breakdown of starch. Invertase activity is a well-known problem in potato storage, and RNA interference of invertase gene expression has been used successfully to reduce glucose and fructose concentrations in stored potatoes of North American varieties Ranger Russet[29] and Katahdin.[30] Inhibition of starch breakdown through reduced expression of starch-associated R1 and phosphorylase-L (PhL) genes has also been shown to be effective.[31] Again, there was one variety that behaved differently: Maris Piper samples had the highest concentrations of sucrose in November but had the lowest by the end of the storage period in July, suggesting that, in this variety, as sucrose was being cleaved to reducing sugars it was not being replenished from starch breakdown.

There were other interesting differences in the relationship between sucrose and reducing sugars. King Edward, for example, had the highest reducing sugar concentrations, but for most months had the lowest sucrose concentrations, whereas Hermes, Lady Rosetta, and Pentland Dell, in contrast, had relatively high concentrations of all three sugars and Lady Claire had relatively low concentrations of all three sugars. Differences in activities of invertase, the other sucrose cleavage enzyme, sucrose synthase, and the sucrose synthesis enzymes sucrose phosphate synthase and sucrose phosphate phosphatase are the likely explanation for these differences.

Acrylamide Analysis in Heated Potato Flour. Acrylamide formation in heated potato flour has been used previously as an indicator of acrylamide-forming potential[19,20] (the term "acrylamide-forming potential" is used because how much actually forms depends on processing methods). There are many ways of processing potatoes to induce acrylamide formation and, although not a commercial process, heating flour has merit as a standard because it is relatively easy to control and gives high levels of acrylamide formation, providing a good, consistent indication of acrylamide-forming potential in

different raw materials. Tuber flour samples were heated for 20 min at 160 °C, and acrylamide was extracted and analyzed by LC-MS/MS. The data are shown graphically on the $\log_e$ scale in Figure 3 (for the full data set see the Supporting Information).
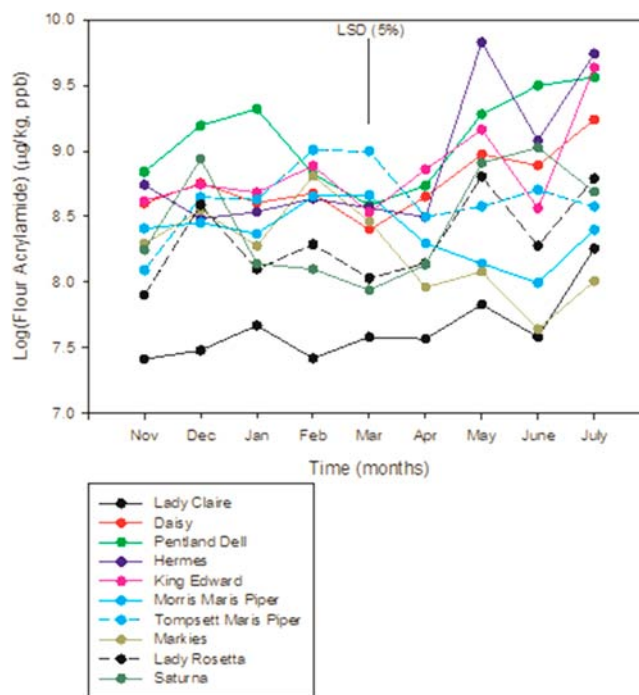
Figure 3. Acrylamide formation (μg/kg on the $\log_e$ scale) in heated potato flour from 10 samples of potato tubers that were produced by commercial growers in the United Kingdom in 2009, placed in long-term storage and sampled monthly from November 2009 to July 2010. The least significant difference (LSD) at 5% is indicated ($n = 3$, df = 180).

There was a significant ($p = 0.039$) interaction between month, type, and variety factors. Lady Claire consistently showed the lowest acrylamide formation until the last storage point in July, when Markies showed the lowest. The Maris Piper and Lady Rosetta samples also gave relatively low acrylamide in late storage. Pentland Dell, Daisy, King Edward, and Hermes, on the other hand, showed consistently high acrylamide compared with the other varieties, and Hermes gave the highest levels in late storage. Acrylamide formation in flour from all of the varieties increased from March to July, reflecting the increase in reducing sugars during this period. The exceptions were Markies and Maris Piper, in which the potential for acrylamide formation was flat or, in the case of Markies, declined during late storage. Again, this reflected the reducing sugar concentrations.

As with the precursor concentrations, the range for acrylamide formation between the different varieties was substantial and statistically significant (see LSD at 5% shown as a bar in Figure 3). In November, for example, acrylamide formed in heated flour from Lady Claire tubers was 1660 μg/kg, whereas in flour from Pentland Dell tubers it reached 7110 μg/kg, a difference of >4-fold. Note that even the figure for Lady Claire is high compared with acrylamide formation in commercial food products, highlighting the importance of controlling acrylamide levels, even with varieties with the lowest acrylamide-forming potential, through the development of optimum processing methods.
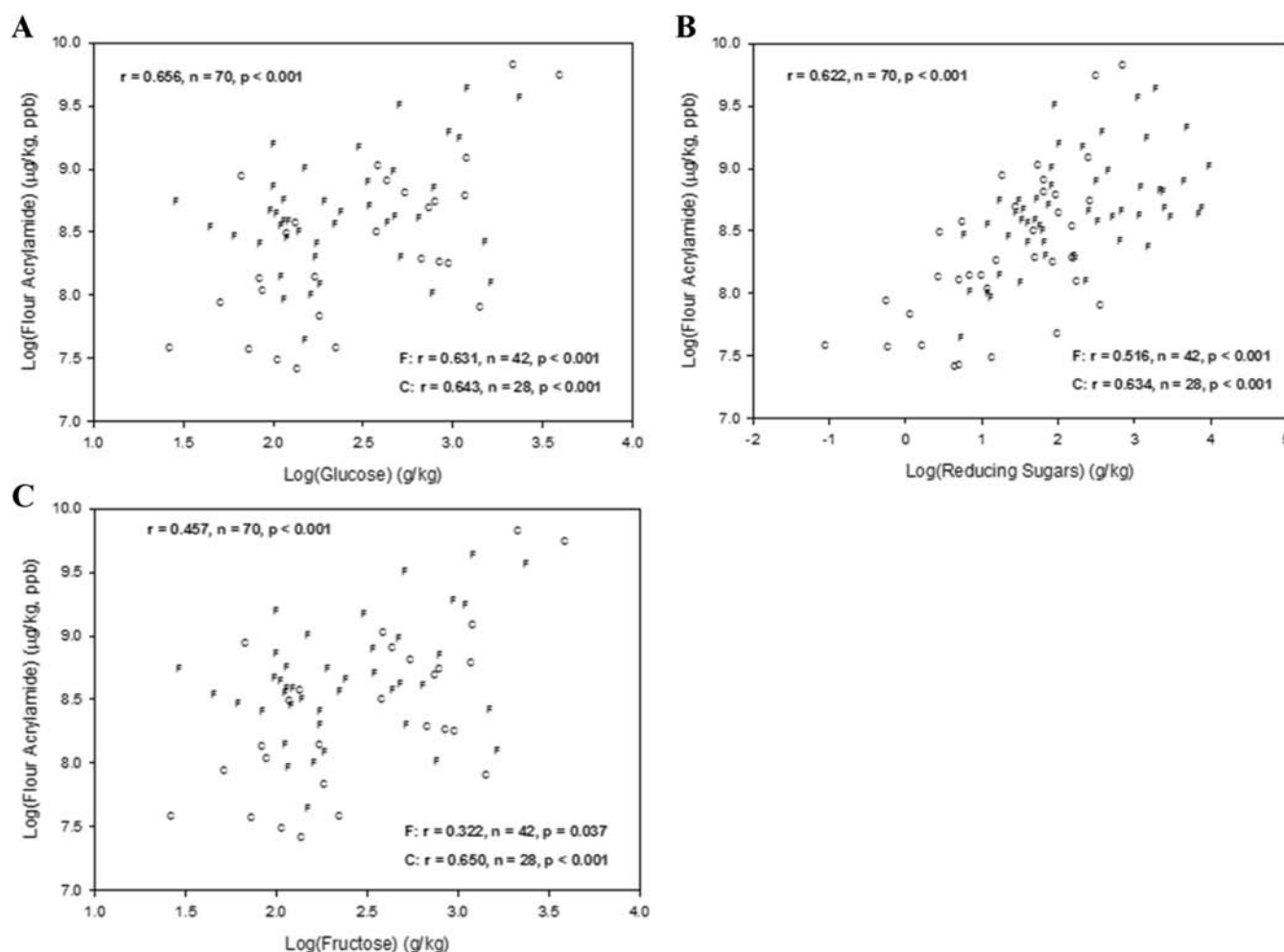
Figure 4. Graphs showing correlations between reducing sugar concentration and acrylamide formation in heated flour from 10 samples of potato tubers that were produced by commercial growers in the United Kingdom in 2009, placed in long-term storage and sampled monthly from November 2009 to July 2010: (A) glucose concentration and acrylamide formation; (B) total reducing sugar concentration and acrylamide formation; (C) fructose concentration and acrylamide formation. Points on the graphs from French fry varieties are denoted F, whereas those for chipping varieties are denoted C, the results for correlation (r) being given for both types overall and then for types separately.
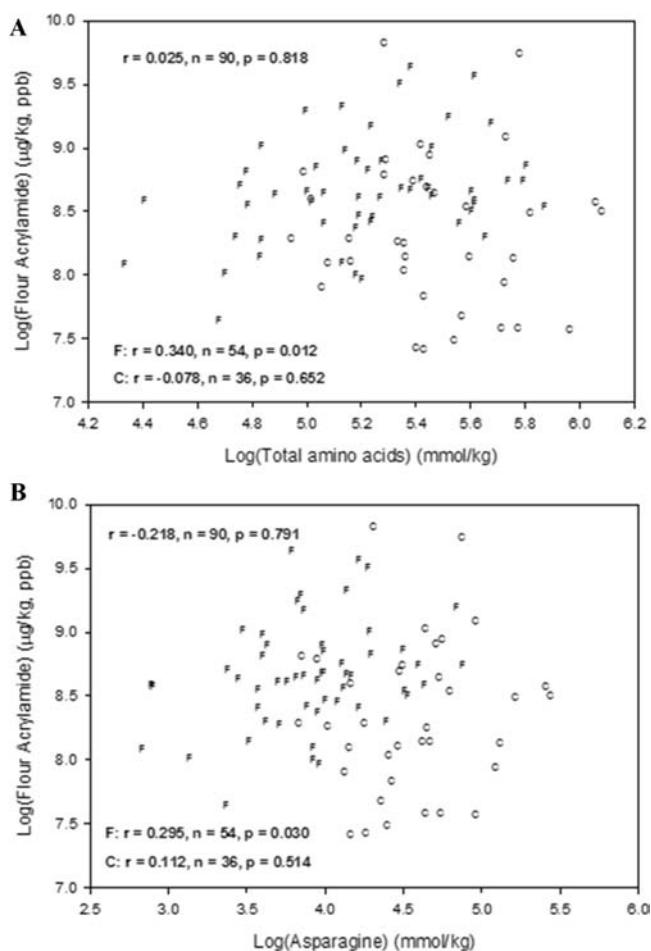
Relationships between Acrylamide Formation and the Concentrations of Free Amino Acids and Sugars. Correlations between acrylamide in the heated flour and the concentrations of free amino acids and sugars were investigated for all of the varieties together and separately for French fry and chipping varieties and revealed some unexpected differences. There were significant correlations between glucose concentration and acrylamide ($r = 0.656$, $p < 0.001$) (Figure 4A) with no difference between chipping and French fry varieties. For total reducing sugars (Figure 4B), overall there was a significant correlation with acrylamide ($r = 0.622$, $p < 0.001$), but the correlation was stronger for chipping varieties ($r = 0.634$, $p < 0.001$) than for French fry varieties ($r = 0.516$, $p < 0.001$). The difference between French fry and chipping varieties was even more apparent when acrylamide was correlated with fructose concentration (Figure 4C). Overall there was a weak but significant correlation with acrylamide ($r = 0.457$, $p < 0.001$). However, the correlation was much stronger for the chipping varieties ($r = 0.650$, $p < 0.001$) than for the French fry varieties ($r = 0.322$, $p = 0.037$). The French fry varieties generally contained higher concentrations of reducing sugars than the chipping varieties, but it is not possible to say whether this was

the explanation for these contrasting correlations without more detailed kinetic studies of acrylamide formation.

Sucrose concentration also correlated with acrylamide formation ($r = 0.601$, $p < 0.001$ overall; $r = 0.513$, $p < 0.001$ for French fry varieties; $r = 0.638$, $p < 0.001$ for chipping varieties). However, as stated previously, sucrose concentration correlated closely with reducing sugar concentration, so this may not necessarily reflect a direct relationship. Sucrose has been shown to contribute to acrylamide formation but only if it is first hydrolyzed through enzymic, thermal, or acid-catalyzed reaction.[32]

The influence of free amino acids also differed between the French fry and chipping varieties. There was a positive correlation between total free amino acid concentration and acrylamide formation in the French fry varieties ($r = 0.340$, $p = 0.012$), but no significant correlation ($p > 0.05$) for the chipping varieties (Figure 5A). Free asparagine concentration also showed a significant positive correlation with acrylamide formation for the French fry varieties ($r = 0.295$, $p = 0.030$) but not for the chipping varieties (Figure 5B). This result justifies inclusion of the consideration of free asparagine concentration in potato variety selection and breeding in the acrylamide "Toolbox" compiled by Food Drink Europe (http://ec.europa.

Figure 5. Graphs showing correlations between free amino acid concentration and acrylamide formation in heated flour from 10 samples of potato tubers that were produced by commercial growers in the United Kingdom in 2009, placed in long-term storage and sampled monthly from November 2009 to July 2010: (A) total free amino acid concentration and acrylamide formation; (B) free asparagine concentration and acrylamide formation. Points on the graphs from French fry varieties are denoted F, whereas those for chipping varieties are denoted C, the results for correlation (r) being given for both types overall and then for types separately.

eu/food/food/chemicalsafety/contaminants/ciaa_acrylamide_toolbox09.pdf). Phenylalanine showed a positive correlation with acrylamide formation in French fry varieties ($r = 0.507$, $p < 0.001$) but not for chipping varieties (plot not shown). Phenylalanine was not a particularly abundant amino acid (see the Supporting Information), and its correlation with acrylamide formation may not necessarily reflect a direct relationship. Another less abundant free amino acid, proline, showed a positive correlation with acrylamide formation but only in the chipping varieties ($r = 0.515$, $p = 0.001$; plot not shown). Proline has been shown to inhibit acrylamide formation rather than increase it,[28] but that would be unlikely to occur when proline is present at much lower concentrations than asparagine, as is the case here (see the Supporting Information). As with phenylalanine, the observed correlation may not reflect a direct relationship.

Two more abundant free amino acids, the acidic amino acids glutamic acid and aspartic acid, also showed interesting correlations with acrylamide formation. Glutamic acid showed a negative correlation with acrylamide formation in the

chipping varieties ($r = -0.748$, $p < 0.001$), but no significant correlation ($p > 0.05$) in the French fry varieties (plot not shown). Aspartic acid showed a similarly negative correlation with acrylamide formation in the chipping varieties ($r = -0.625$, $p < 0.001$), but the correlation with acrylamide in the French fry varieties was positive, although of borderline significance ($r = 0.269$, $p = 0.051$) (plot not shown). It should be noted that there is no known chemical mechanism by which glutamic and aspartic acid could affect acrylamide formation in this way.

Potato Chips. Although the acrylamide data obtained from the heated flour samples provide information about the acrylamide potential of the potatoes from different varieties over storage and allow correlation with precursors, the food industry also needs to know how this relates to acrylamide formation in commercial potato products such as chips. Potato slices from each variety and each storage time were therefore fried in a pilot-scale commercial fryer, and acrylamide and color were analyzed as well as solids, oil, and moisture contents. The data are shown graphically on log_e scales in Figure 6 (for full data set see the Supporting Information). Note that the commercial L value range for hand-cooked chips based on consumer acceptance is typically between 55 and 62. However, the chips in this study were produced from unpeeled potatoes, and the peel would influence the overall chip color, giving a lower measured L value. Most processors would adjust processing parameters to moderate the final chip color; they would also employ color sorting to remove darker chips outside their specification. In contrast, whereas the controlled conditions used to prepare the chips for this study were chosen to be representative of commercial batch-frying conditions, they were kept constant for all potato types, rather than using a subjective optimization process, and all of the chips produced were included, with no defects removed.

Acrylamide formation in the chips was lower than in the heated flour, reflecting the lower temperature and the shorter cooking time. Nevertheless, the acrylamide formed in the chips correlated well with that formed in flour for both French fry and chipping varieties (Figure 7A; $r = 0.756$, $p < 0.001$). Lady Claire was again the best of the varieties in terms of acrylamide risk, with consistently low acrylamide formation. Saturna also performed well; indeed, Saturna and Lady Claire were the only two varieties for which acrylamide levels were consistently below the European Commission's indicator level for potato chips of 1000 μg/kg. Markies dropped below the 1000 μg/kg level as its reducing sugar concentration fell in late storage, confirming its potential as a useful long-term storage variety. Lady Rosetta, on the other hand, was under the indicator level until late storage. Daisy, Pentland Dell, King Edward, and Maris Piper were consistently over the 1000 μg/kg level, despite Maris Piper being relatively stable in late storage. Hermes fluctuated the most, with levels rising rapidly from April onward, as reducing sugars began to accumulate.

Acrylamide formation in chips showed a significant correlation with color on the Hunter L ($r = -0.610$, $p < 0.001$) and Hunter a scales ($r = 0.813$, $p < 0.001$) (Figure 7B,C), again for both French fry and chipping varieties, indicating that the darker the chips were, the more acrylamide they contained. Correlations between the acrylamide concentrations in the chips and the concentrations of free amino acids and sugars in the flour samples were very similar to those for the acrylamide from the heated flour, confirming that the two methods used for acrylamide production are comparable for the investigation of acrylamide-forming potential. The contrasting
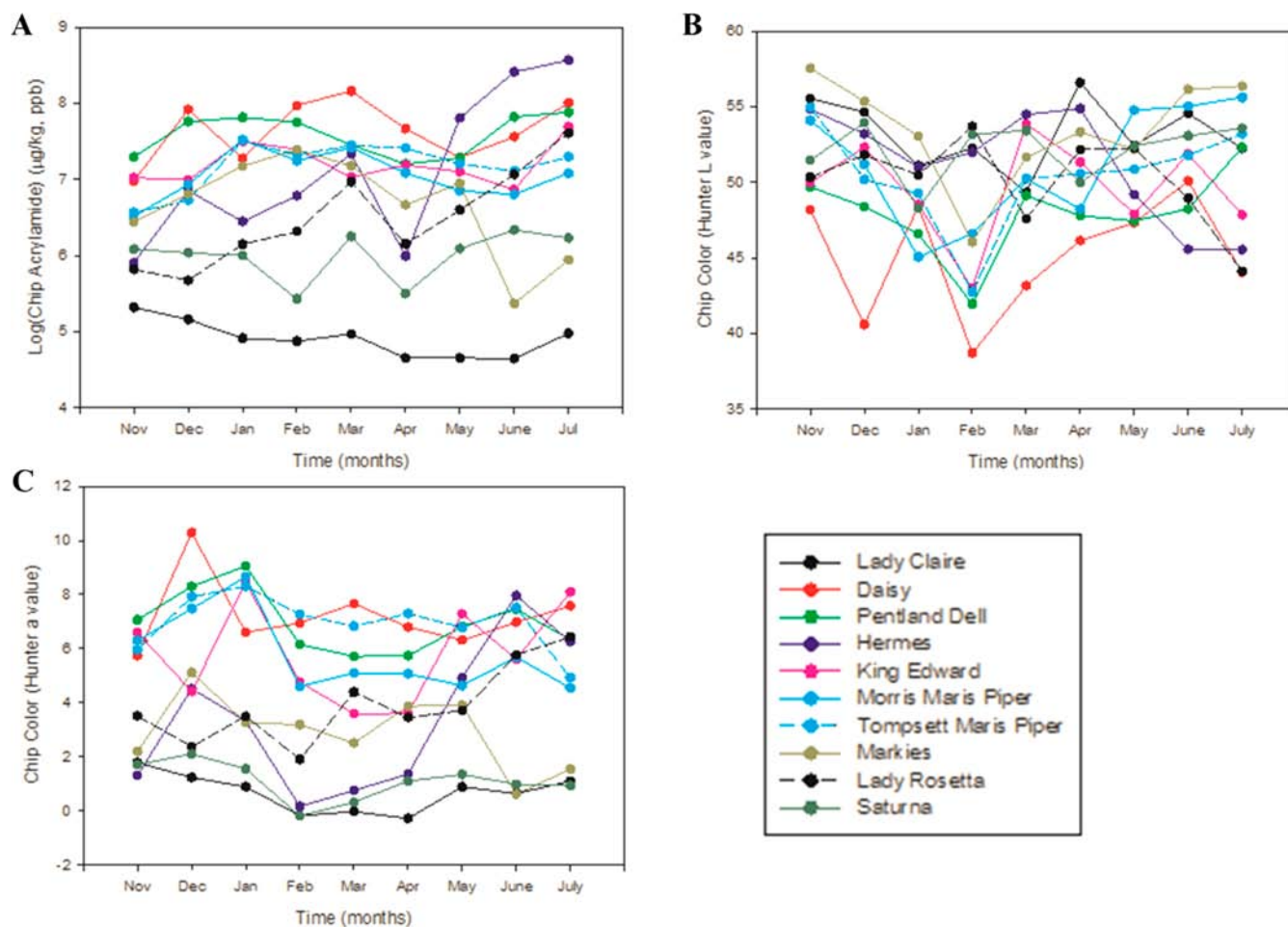
Figure 6. Analysis of chips produced from 10 samples of potato tubers that were produced by commercial growers in the United Kingdom in 2009, placed in long-term storage and sampled monthly from November 2009 to July 2010: (A) acrylamide formation (μg/kg); (B) chip color (Hunter L); (C) chip color (Hunter a).

relationship of aspartic acid with acrylamide formation in the French fry and chipping varieties was even more marked in the chips than in flour ($r = -0.628$, $p < 0.001$ for the chipping varieties; $r = 0.465$, $p < 0.001$ for the French fry varieties).

There was no significant correlation ($p > 0.05$) between acrylamide formation and chip solids, moisture, or oil contents. There were trends for a gradual decrease in chip oil and an increase in chip moisture content over storage time, but with extensive variation for individual varieties.

Principal Coordinates (PCO) Analysis. PCO analysis was performed on the means of amino acids, acrylamide (chip and flour), sugars (glucose, fructose, and sucrose), solids, color (Hunter L and Hunter a), chip oil, and chip moisture data for November–July, to give a global picture of the variety by month combinations and to try to expose the main variables separating them. The first three principal coordinate axes (PCos) accounted for 60.20% of the variation in the distances (between variety by months) data. These were retained and the data visualized on the new coordinate axes (Figure 8). The plot shows that together any two of these first three dimensions can separate the French fry from the chipping varieties, so the differences between the types observed in the univariate analyses of measurements described above are also seen clearly in the PCo analysis, using all the data.

Notably, relating the coordinates to the measured variables via regression, glucose ($F = 71.7$) and asparagine ($F = 64.5$),

followed by chip acrylamide ($F = 60.5$) and flour acrylamide ($F = 57.5$), came out as the top variables for discrimination in the PCo2 direction. Lady Claire, with low glucose and acrylamide, was most different in that direction, confirming the relationship between low glucose and low acrylamide. Of the French fry varieties, Markies stood out most from the others in all months, in the PCo1 direction. Valine ($F = 471.5$), isoleucine ($F = 406.0$), leucine ($F = 254.8$), and phenylalanine ($F = 218.4$) were the most important discriminatory variables in this direction, and Markies had the lowest concentrations in all of these amino acids (see the Supporting Information). Aspartic acid ($F = 65.6$), tryptophan ($F = 61.7$), and γ-aminobutyric acid (GABA) ($F = 61.4$) were most discriminatory in the PCo3 direction, and Pentland Dell appeared to be separated to some extent in this direction. It had fairly high aspartic acid and low but fluctuating tryptophan from November until March and was the only variety with consistent GABA from November until January (see the Supporting Information).

Implications for the Potato Industry. The study was conducted on potatoes from one season, so conclusions about the different varieties should be qualified. Furthermore, acrylamide formation was measured in chips and flour as standard methods, whereas some of the varieties studied would not normally be used for chip production, and no commercial process involves heating potato flour. Nevertheless, the study showed that varietal selection could be a powerful tool in
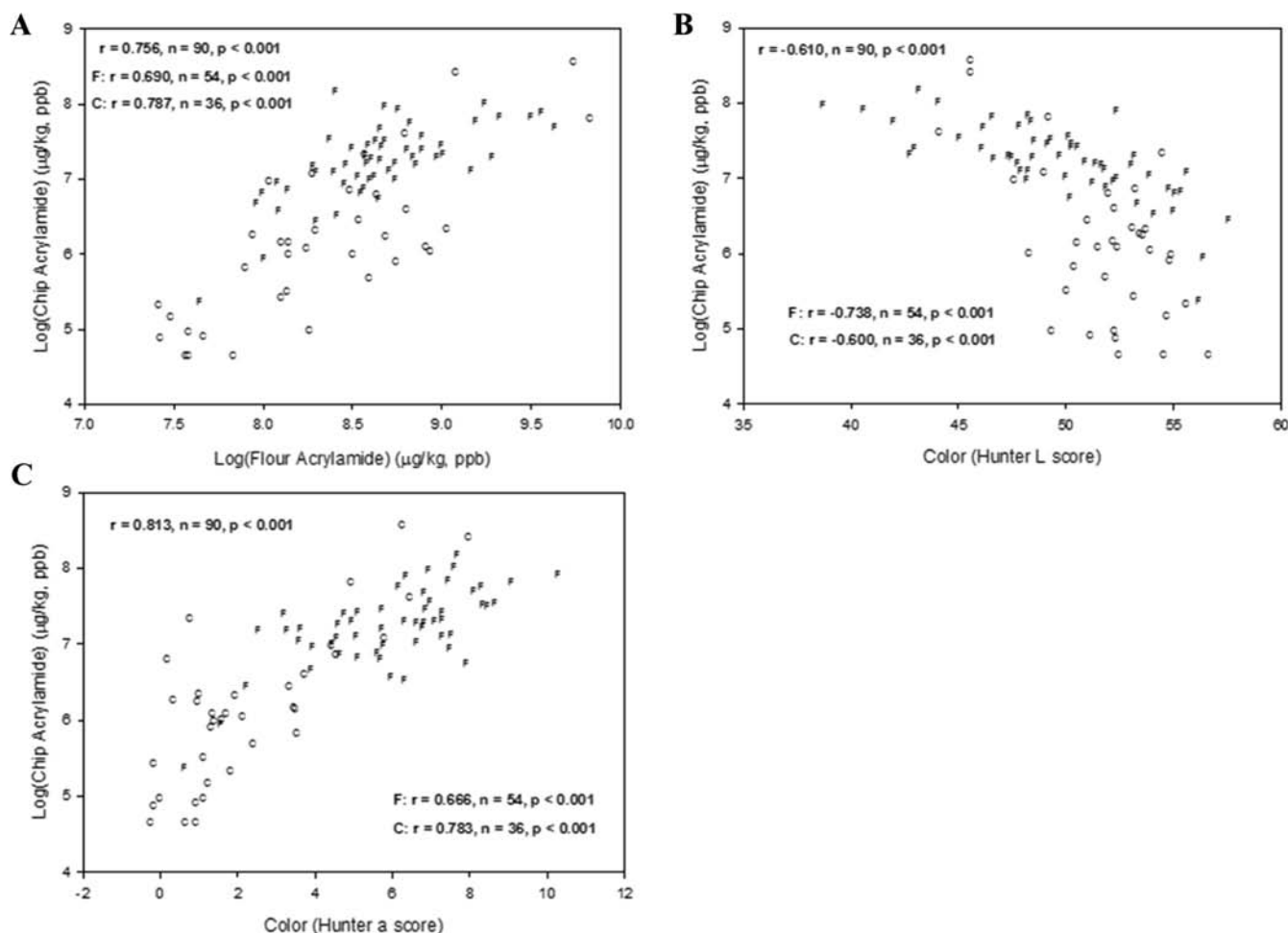
Figure 7. Correlation between (A) acrylamide formed in chips and acrylamide formed in flour, (B) acrylamide formation in chips and color (Hunter *L*), and (C) acrylamide formation in chips and color (Hunter *a*). Points on the graphs from French fry varieties are denoted F, whereas those for chipping varieties are denoted C, the results for correlation (*r*) being given for both types overall and then for types separately.

ensuring that potato products comply with the indicator levels set by the European Commission and other regulatory authorities. Chips produced from Lady Claire and Saturna were consistently below the 1000 μg/kg acrylamide level, as were chips from Lady Rosetta during early storage and from Markies in late storage. However, acrylamide formation also depends on processing methods, so products made with the other varieties may well comply with indicator values in some cases. Acrylamide formation in heated flour showed that the acrylamide-forming potential of all the varieties was considerably higher than 1000 μg/kg; however, it correlated well with acrylamide formation in chips. The flour method is relatively easy to control and gives high levels of acrylamide formation, providing a good, consistent indication of acrylamide-forming potential in different raw materials.

The study highlighted the effect of long-term storage on acrylamide risk and showed it to be variety-dependent, with some varieties being much more prone to fluctuations in acrylamide precursors than others. Concentrations of reducing sugars rose in most varieties in late storage, highlighting the importance of not using potatoes outside their prescribed storage window, but were stable in Maris Piper and actually fell in Markies, potentially making this a valuable variety for chip production from late storage, even though it is usually considered to be a French fry variety. Storage is an important

issue for the potato industry in Europe, North America, and other temperate regions because potato tubers have to be stored for long periods to enable a year-round supply.

Two Maris Piper samples were analyzed in the study. Both came from Cambridgeshire in the East of England, but from farms with differing soil types (silt and sandy black; Table 1). The differences in sugar and free amino acid concentrations between these two samples were relatively small compared with the overall intervarietal differences, indicating that in this case the environmental factor of soil type was less important than the genetic factor of variety.

The study adds considerably to information about the relationship between precursor concentration and acrylamide formation in potato and reinforces the conclusion that the relationship is a complex one. Glucose concentration was the most important factor, and there was also a significant correlation between total reducing sugars and acrylamide formation (an important point because measurement of total reducing sugar is relatively simple using the Benedict or Fehling test). Fructose concentration on its own, however, showed a significant correlation with acrylamide formation only in the chipping varieties, which generally had lower concentrations of both glucose and fructose than the French fry varieties. On the other hand, both total free amino acid concentration and free asparagine concentration correlated with acrylamide formation
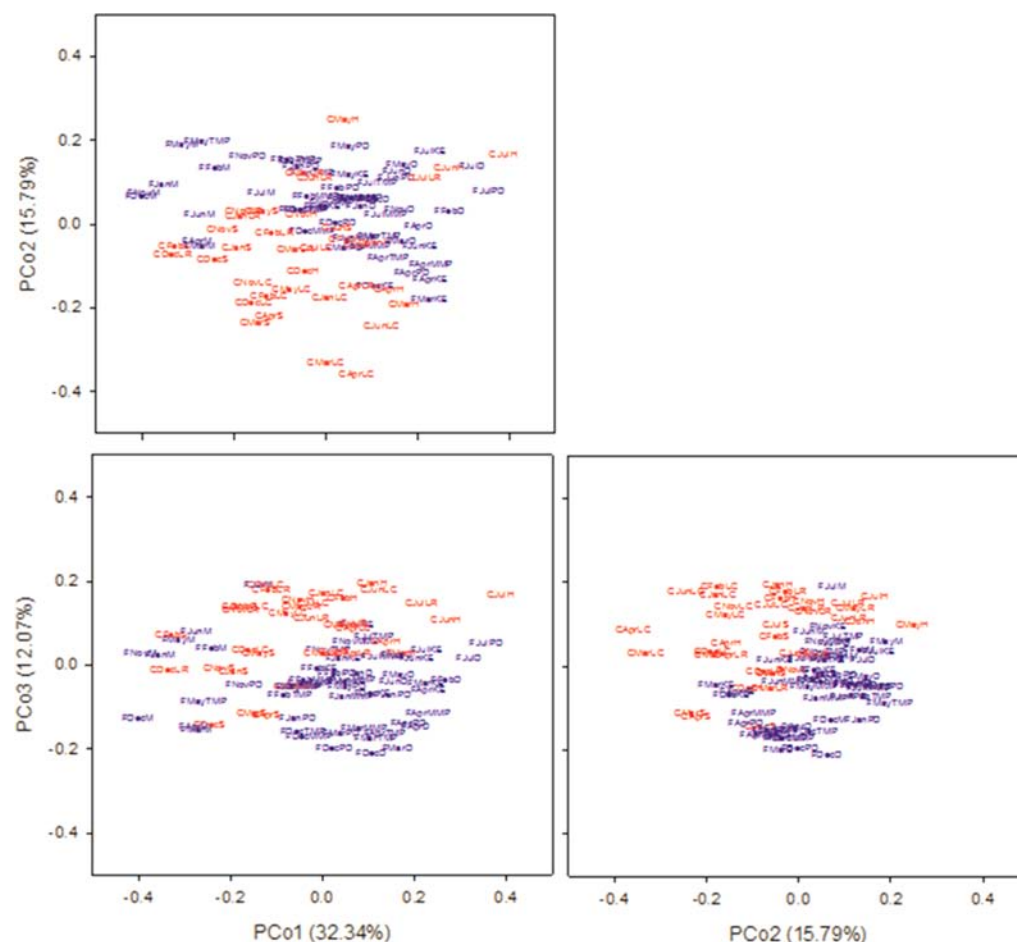
Figure 8. Principal coordinates (PCO) analysis plot. The analysis was performed on the means of amino acids, acrylamide (chip and flour), sugars (glucose, fructose, and sucrose), solids, color (Hunter *L* and Hunter *a*), chip oil, and chip moisture data for November–July. For the plotted points, the first letter in the code denotes a French fry (F) variety (shown in blue) or chip (C) variety (shown in red), the next three letters denote the month, and the final letters denote the variety: Daisy (D), Markies (M), Thomsett Maris Piper (TMP), Morris Maris Piper (MMP), Pentland Dell (PD), King Edward (KE), Lady Claire (LC), Lady Rosetta (LR), Saturna (S), and Hermes (H). The first three PCos were retained, accounting for percentage variance as shown on the axes labels.

in the French fry varieties but not the chipping varieties. This complex relationship between the concentrations of reducing sugars and free amino acids and the formation of acrylamide contrasts starkly with the situation in cereals, in which free asparagine concentration is the over-riding factor.[21−24]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Data showing free amino acid and sugar concentrations and acrylamide formed in heated flour for tubers from nine varieties of potato produced by commercial growers in the United Kingdom in 2009, placed in long-term storage and sampled monthly from November 2009 to July 2010, as well as data for acrylamide, chip color (Hunter *L* and *a*), solids, fat, and moisture content of chips produced from these potatoes, and graphical representations of free amino acid concentrations (mmol per kg on the log$_e$ scale). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: nigel.halford@rothamsted.ac.uk. Fax: +44 1582 763010.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M.. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998−5006.

(2) Friedman, M. Chemistry, biochemistry and safety of acrylamide. A review. *J. Agric. Food Chem.* **2003**, *51*, 4504−4526.

(3) Nursten, H. E. *The Maillard Reaction*; Royal Society of Chemistry: Cambridge, UK, 2005.

(4) Mottram, D. S. The Maillard reaction: source of flavor in thermally processed foods. In *Flavors and Fragrances: Chemistry, Bioprocessing and Sustainability*; Berger, R. G., Ed.; Springer-Verlag: Berlin, Germany, 2007; pp 269−284.

(5) Halford, N. G.; Curtis, T. Y.; Muttucumaru, N.; Postles, J.; Mottram, D. S. Sugars in crop plants. *Ann. Appl. Biol.* **2011**, *158*, 1−25.

12054

dx.doi.org/10.1021/jf3037566 | *J. Agric. Food Chem.* 2012, 60, 12044−12055

(6) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448−449.

(7) Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M.-C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449−450.

(8) Zyzak, D. V.; Sanders, R. A.; Stojanovic, M.; Tallmadge, D. H.; Eberhart, B. L.; Ewald, D. K.; Gruber, D. C.; Morsch, T. R.; Strothers, M. A.; Rizzi, G. P.; Villagran, M. D. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* **2003**, *51*, 4782−4787.

(9) Granvogl, M.; Jezussek, M.; Koehler, P.; Schieberle, P. Quantitation of 3-aminopropionamide in potatoes - a minor but potent precursor in acrylamide formation. *J. Agric. Food Chem.* **2004**, *52*, 4751−4757.

(10) Claus, A.; Weisz, G. M.; Schieber, A.; Carle, R. Pyrolytic acrylamide formation from purified wheat gluten and gluten-supplemented wheat bread rolls. *Mol. Nutr. Food Res.* **2006**, *50*, 87−93.

(11) Claus, A.; Schreiter, P.; Weber, A.; Graeff, S.; Herrmann, W.; Claupein, W.; Schieber, A.; Carle, R. Influence of agronomic factors and extraction rate on the acrylamide contents in yeast-leavened breads. *J. Agric. Food Chem.* **2006**, *54*, 8968−8976.

(12) Mustafa, A.; Aman, P.; Andersson, R.; Kamal-Eldin, A. Analysis of free amino acids in cereal products. *Food Chem.* **2007**, *105*, 317−324.

(13) Halford, N. G.; Curtis, T. Y.; Muttucumaru, N.; Postles, J.; Elmore, J. S.; Mottram, D. S. The acrylamide problem: a plant and agronomic science issue. *J. Exp. Bot.* **2012**, *63*, 2841−2851.

(14) Eppendorfer, W. H.; Bille, S. W. Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower and potatoes as influenced by nitrogen fertilisation and phosphorus and potassium deficiency. *J. Sci. Food Agric.* **1996**, *71*, 449−458.

(15) Amrein, T. M.; Bachmann, S.; Noti, A.; Biedermann, M.; Barbosa, M. F.; Biedermann-Brem, S.; Grob, K.; Keiser, A.; Realini, P.; Escher, F.; Amadò, R. Potential of acrylamide formation, sugars, and free asparagine in potatoes: a comparison of cultivars and farming systems. *J. Agric. Food Chem.* **2003**, *51*, 5556−5560.

(16) Oruna-Concha, M. J.; Duckham, S. C.; Ames, J. M. Comparison of volatile compounds isolated from the skin and flesh of four potato cultivars after baking. *J. Agric. Food Chem.* **2001**, *49*, 2414−2421.

(17) Becalski, A.; Lau, B. P.-Y.; Lewis, D.; Seaman, S. W.; Hayward, S.; Sahagian, M.; Ramesh, M.; Leclerc, Y. Acrylamide in French fries: influence of free amino acids and sugars. *J. Agric. Food Chem.* **2004**, *52*, 3801−3806.

(18) Shepherd, L. V. T.; Bradshaw, J. E.; Dale, M. F. B.; McNicol, J. W.; Pont, S. D. A.; Mottram, D. S.; Davies, H. V. Variation in acrylamide producing potential in potato: segregation of the trait in a breeding population. *Food Chem.* **2010**, *123*, 568−573.

(19) Elmore, J. S.; Mottram, D. S.; Muttucumaru, N.; Dodson, A. T.; Parry, M. A.; Halford, N. G. Changes in free amino acids and sugars in potatoes due to sulfate fertilization, and the effect on acrylamide formation. *J. Agric. Food Chem.* **2007**, *55*, 5363−5366.

(20) Elmore, J. S.; Dodson, A. T.; Briddon, A.; Halford, N. G.; Mottram, D. S. The effects of storage on the formation of aroma and acrylamide in heated potato. In *Controlling Maillard Pathways to Generate Flavors*; Mottram, D. S., Taylor, A. J., Eds.; American Chemical Society: Washington, DC, 2010; pp 95−109.

(21) Muttucumaru, N.; Halford, N. G.; Elmore, J. S.; Dodson, A. T.; Parry, M. A. J.; Shewry, P. R.; Mottram, D. S. The formation of high levels of acrylamide during the processing of flour derived from sulfate-deprived wheat. *J. Agric. Food Chem.* **2006**, *54*, 8951−8955.

(22) Curtis, T. Y.; Muttucumaru, N.; Shewry, P. R.; Parry, M. A.; Powers, S. J.; Elmore, J. S.; Mottram, D. S.; Hook, S.; Halford, N. G. Evidence for genetic and environmental effects on free amino acid levels in wheat grain: implications for acrylamide formation during processing. *J. Agric. Food Chem.* **2009**, *57*, 1013−1021.

(23) Curtis, T. Y.; Powers, S. J.; Balagiannis, D.; Elmore, J. S.; Mottram, D. S.; Parry, M. A. J.; Raksegi, M.; Bedő, Z.; Shewry, P. R.; Halford, N. G. Free amino acids and sugars in rye grain: implications for acrylamide formation. *J. Agric. Food Chem.* **2010**, *58*, 1959−1969.

(24) Granvogl, M.; Wieser, H.; Koehler, P.; Von Tucher, S.; Schieberle, P. 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. *J. Agric. Food Chem.* **2007**, *55*, 4271−4277.

(25) Hamlet, C. G.; Sadd, P. A.; Liang, L. 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. *J. Agric. Food Chem.* **2008**, *56*, 6145−6153.

(26) Krzanowski, W. J. *Principles of Multivariate Analysis: A User's Perspective*, revised ed.; Oxford University Press: Oxford, UK, 2000.

(27) Lea, P. J.; Sodek, L.; Parry, M. A.; Shewry, P. R.; Halford, N. G. Asparagine in plants. *Ann. Appl. Biol.* **2007**, *150*, 1−26.

(28) Koutsidis, G.; Simons, S. P. J.; Thong, Y. H.; Haldoupis, Y.; Mojica-Lazaro, J.; Wedzicha, B. L.; Mottram, D. S. Investigations on the effect of amino acids on acrylamide, pyrazines, and Michael addition products in model systems. *J. Agric. Food Chem.* **2009**, *57*, 9011−9015.

(29) Ye, J.; Shakya, R.; Shrestha, P.; Rommens, C. M. Tuber-specific silencing of the acid invertase gene substantially lowers the acrylamide-forming potential of potato. *J. Agric. Food Chem.* **2010**, *58*, 12162−12167.

(30) Bhaskar, P. B.; Wu, L.; Bethke, P. C.; Vaillancourt, B.; Jansky, S. H.; Busse, J. S.; Buell, R.; Hamernik, A. J.; Jiang, J. Suppression of the vacuolar invertase gene prevents cold-induced sweetening in potato. *Plant Physiol.* **2010**, *154*, 939−948.

(31) Rommens, C. M.; Ye, J.; Richael, C.; Swords, K. Improving potato storage and processing characteristics through all-native DNA transformation. *J. Agric. Food Chem.* **2006**, *54*, 9882−9887.

(32) De Vleeschouwer, K.; Van der Plancken, I.; Van Loey, A.; Henndrickx, M. E. Role of precursors on the kinetics of acrylamide formation and elimination under low moisture conditions using a multiresponse approach — Part I: Effect of the type of sugar. *Food Chem.* **2009**, *114*, 116−126.