JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification and Monitoring of Intermediates and Products in the Acrylamide Pathway Using Online Analysis

GUY A. CHANNELL, FLORIAN WULFERT, AND ANDREW J. TAYLOR*

Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

Acrylamide formation under controlled processing conditions was studied in a starch matrix by analyzing volatile compounds in the gas phase using online mass spectrometry. Compounds were identified using mass spectral analysis, authentic standards, and the labeling patterns from isotopically labeled asparagine and sugars. Acrylamide, 3-aminopropanamide, methylpyrazine, 3-oxopropanamide, and aminopropan-2-one were assigned to the ions at *m*/*z* 72, 89, 95, 88, and 74, respectively. Ion *m*/*z* 60 was proposed as the transamination product of glyoxal, but labeling experiments did not support this assignment. Temporal formation of acrylamide and related compounds was studied in 51 samples containing asparagine and selected sugars or carbonyls. Data from the experiments were analyzed to investigate correlations between the amounts of acrylamide, intermediates, and pyrazines formed. A strong correlation between 3-aminopropanamide and acrylamide was found in all samples, whereas other correlations were reactant specific. Preliminary multiway analysis of the data identified temporal similarities in the ion profiles and showed that dynamic monitoring can follow the production and utilization of intermediates leading to acrylamide.

KEYWORDS: Ion trap; chemical pathway; APCI-MS; PARAFAC

INTRODUCTION

After the discovery of acrylamide in foods, initial research established the chemical origins and pathways leading to acrylamide (*1*–*5*). This was followed by attempts to manipulate the Maillard reaction to decrease the amount of acrylamide formed through the Maillard reaction while maintaining the desirable color and flavor (*6*–*8*). It is clear from these studies that a better understanding of some key branch points in the Maillard pathways is needed to understand why certain reactants and processing conditions encourage the pathways to take one route over another.

Previous studies using conventional extraction and analysis have provided information on the formation of intermediates and end products during the course of the reaction, but the experiments are time-consuming, limiting more detailed investigation on the temporal sequence of events and on the effects of processing conditions on the branch points. Some online monitoring of acrylamide formation has been reported (*9, 10*), and suitable reactors and systems have been developed to control temperature and humidity conditions during the reaction (*11*).

The drawback with online monitoring is assigning compounds of interest to the ions monitored by the mass spectrometer (MS) systems using the limited information available from the mass spectra (often restricted to the *m*/*z* value of the protonated molecular ion). In Maillard systems, isobaric compounds are often present, and fragmentation of some compounds during ionization can also make assignment difficult. Gas chromatography (GC) of volatiles using online and conventional MS detectors has been used to assign some compounds unequivocally (*12*), but GC conditions may not be suitable for the analysis of the reactive intermediates involved in acrylamide formation. Techniques such as MS2 (controlled fragmentation of an ion) can provide extra information for some compounds (*13*). The approach investigated in this paper was to combine MS2 with the use of authentic compounds (when available) and with the use of specifically labeled reactants to assign ions to known compounds in the established acrylamide pathways. With assignments in place, the results from the online system were compared with published data (obtained using conventional means) to ensure agreement between the techniques and to identify potential benefits of the online system.

To better relate the experiments to food, acrylamide formation was measured in a low-moisture waxy maize starch matrix. The effect of different sugars on acrylamide formation was then studied using an experimental design that also considered the effect of different reactant ratios (and amounts) on the formation of acrylamide and related compounds.

To understand the effect of reactants on acrylamide and intermediate formation, different data analyses were applied to the large, multidimensional data sets produced from online monitoring. Initial, simple inspection of the data was followed by correlation analyses and then by multiway methods (*14*) to

* Corresponding author (e-mail andy.taylor@nott.ac.uk).

10.1021/jf7037423 CCC: $40.75
Published on Web 07/15/2008

understand the relationship between the intermediates and the formation of acrylamide and other end products, such as pyrazines, which result from branch points in the pathways.

MATERIALS AND METHODS

Authentic Compounds. 3-Aminopropanamide (3-APA) was obtained as the hydrochloride salt (alaninamide hydrochloride) from TCI America Inc. (Portland, OR), and acrylamide was a product of Sigma-Aldrich. Aminopropan-2-one hydrochloride was a kind gift from Dr. Mark Fitzsimons, Petroleum and Environmental Geochemistry Group, University of Plymouth, U.K. The authentic standards were introduced as aqueous solutions (acrylamide 125 μM, 3-APA 125 μM, and aminopropan-2-one 2.5 mM) at different flow rates (0.05, 0.2, 1.0, 5.0, 20.0 μL/min) through the humidifier port of the matrix reactor system with no starch plug present but with all other conditions as described below.

Sample Preparation. A waxy maize starch slurry (Roquette U.K. Ltd., WAXILYS 200, 50 g/L) was mechanically mixed with aqueous solutions of the reactants and internal standard (2-isobutyl-3-methoxypyrazine; 5 nmol/g dry starch), then heated at 70 °C to form a weak gel, poured into 96-well plates, and freeze-dried. The resulting cylinders (plugs) of porous starch (5 mm diameter × 10 mm; water content = 1−2 g/100 g of dry weight) were then adjusted to $a_w = 0.84$ over saturated KCl solution at 25 °C. For identification of key compounds, both asparagine and sugar reactants were incorporated into the starch matrix, each at 100 μmol/g of dry starch. Reactants were asparagine (asn), fructose (fru), glucose (glu), asparagine with 98% ^{15}N label in the amide nitrogen (asnN15) or in the amide and α nitrogens (98%; asnN15×2), glucose labeled with 99% ^{13}C in the C1 and C2 positions (gluC13×2), and fructose labeled with 99% ^{13}C at C1 (fruC13), all obtained from Cambridge Isotopes Laboratories Inc., Andover, MA. The combinations used in the starch matrix were asn + glu, asn + fru, asnN15 + glu, asnN15 + fru, asnN15×2 + glu, asnN15×2 + fru, and asn + gluC13×2.

To study the effects of sugars and carbonyls on the formation of acrylamide and its intermediates, a D-optimal designed experiment was set up with three factors: carbonyl type (lactose, glucose, methyl α-D-glucopyranoside, fructose, ascorbic acid, and pyruvic acid, all obtained from Sigma-Aldrich, Poole, U.K.; and 3-deoxyglucosone obtained from Toronto Chemicals, Ontario, Canada), amounts of reactants (50, 100, or 200 μmol of reactant/g of dry starch), and ratios of carbonyl to asparagine (1:4, 2:1, 1:1, 1:2, and 4:1, based on the reactant concentrations above). In all, 51 samples were produced.

Thermal Processing and MS Conditions. Plugs (triplicates) were heated in a matrix reactor consisting of a Sulfinert stainless steel tube (7 × 70 mm) held at 170 °C. Heating time was 11 min with a humidifying gas flow through the reactor ([5 μL/min water vaporized into 50 mL/min N_2 (*11*)]. The reactor was linked to a custom-built APCI source (*13*) through seven, parallel 30 cm × 530 μm fused silica capillaries (to provide laminar flow without excessive back pressure) in a heated (200 °C) transfer line to prevent condensation of volatile compounds formed in the reactor at 170 °C. The 50 mL/min flow from the reactor was mixed with 1.35 L/min of makeup gas (dry N_2) and connected to the source of an ion trap (IT) MS (ThermoFinnigan Deca XP). Assuming optimal gas flow through the system, residence times in the reactor and transfer line were calculated as 3.2 and 0.5 s, respectively. The IT-MS monitored ions from *m/z* 50 to 280 in the MS1 mode, and acrylamide was measured using MS2 of the parent ion *m/z* 72 (protonated molecular ion for acrylamide). MS2 conditions were as follows: *m/z* range, 50−75; isolation width = 1.2; collision energy = 27%; activation $Q = 0.55$; activation time = 30 ms; maximum injection time = 500 ms. Raw data were averaged over 12 microscans to give a data point for each ion, every 5 s. To assign compounds to ions, MS2 was used on other selected ions using the same conditions but with the appropriate *m/z* range. Overall percentage coefficient of variation in the system (from plug preparation to MS analysis) was typically 10%.

Product Formation. To measure the total amount of products formed during the reaction, the areas under selected ion traces were calculated, once the matrix reactor had achieved the set temperature of 170 °C. Acrylamide amounts were expressed as the area under the MS2 ion at *m/z* 55. The amounts of acrylamide in the gas phase and in the solid matrix are linearly related (*15*), and this was reconfirmed in this study, although only 0.1% of acrylamide was released into the gas phase (data not shown).

MS Interpretation. Mass Frontier software (HighChem Ltd., Bratislava, Slovakia) was used to predict fragmentation of compounds during ionization or under MS-MS conditions to provide supporting information for tentative identifications.

Correlation Analyses. Ion areas were correlated using the Pearson correlation function (Matlab, The MathWorks, Natick, MA) to determine the correlations between the amounts formed during heating. Partial least-squares analysis was applied to determine the ions best correlated to predict acrylamide formation using a Matlab module. The temporal data were analyzed using the PARAFAC algorithm (Eigen-Vector, Wenatchee, WA) in a Matlab environment. Data from the 51 samples were assembled in a matrix and integrated using the 3D-PCA functionality of PARAFAC to identify common trends in the form of discrete ion profiles.

RESULTS AND DISCUSSION

Assignment of Compounds to Ions. The first step was to identify and select compounds that were important in the acrylamide pathway. Acrylamide formation from a typical carbonyl (in this case an α-dicarbonyl, pyruvaldehyde) and asparagine is illustrated in **Figure 1**. The potential pathway from tautomer **2** to acrylamide suggested by Stadler (*5*) is included, and the hypothesis is tested later in this paper. It is clear that the decarboxylated Schiff base represents a branch point which can lead either to the transaminated form of the sugar/carbonyl or to 3-aminopropanamide (3-APA). Because these compounds are, respectively, the precursors of pyrazines or acrylamide, they are key intermediates. Because pyruvaldehyde and glyoxal have been identified as important carbonyls in Maillard systems (*1, 2*), the related transaminated compounds (aminopropan-2-one and aminoethanal) were targets for online monitoring. Methylpyrazine, formed by combination of transaminated glyoxal and transaminated pyruvaldehyde, was also a key compound, along with 3-APA and acrylamide itself.

Because the online system monitors compounds solely on the basis of *m/z* values, the potential formation of isobaric compounds was studied. Initially, a theoretical approach was adopted by considering carbonyl compounds that could arise from sugar degradation and substituting them into the reaction pathways in **Figure 1** to calculate the molecular weights of the intermediates. These values were then converted into predicted *m/z* values for the intermediates, assuming ionization by protonation of the intact molecule (**Table 1**). The values show some common ions formed from asparagine (e.g., acrylamide and 3-APA), but the other intermediates have *m/z* values that are different and depend on the nature of the sugar or carbonyl source. The values in **Table 1** indicate that if ionization produces the $[M + H]^+$ ion and if the general chemical pathways shown in **Figure 1** are responsible for acrylamide production, then interference through isobaric compounds will be minimal. There is the possibility that some $[M + H]^+$ ions may fragment and create ions with *m/z* values that may interfere. Rather than approach this issue theoretically (e.g., by using fragmentation software to predict ion fragments from the ions in **Table 1**), an experimental approach using labeled compounds, authentic compounds (when available), and mass spectral analysis was adopted. The labeling patterns and the extent of label incorporation were studied and interpreted as follows. If an ion mass was completely shifted by the presence of an isotopic label, if the ion mass agreed with the predicted labeling pattern, and if the ion intensities of the unlabeled and labeled ions were the

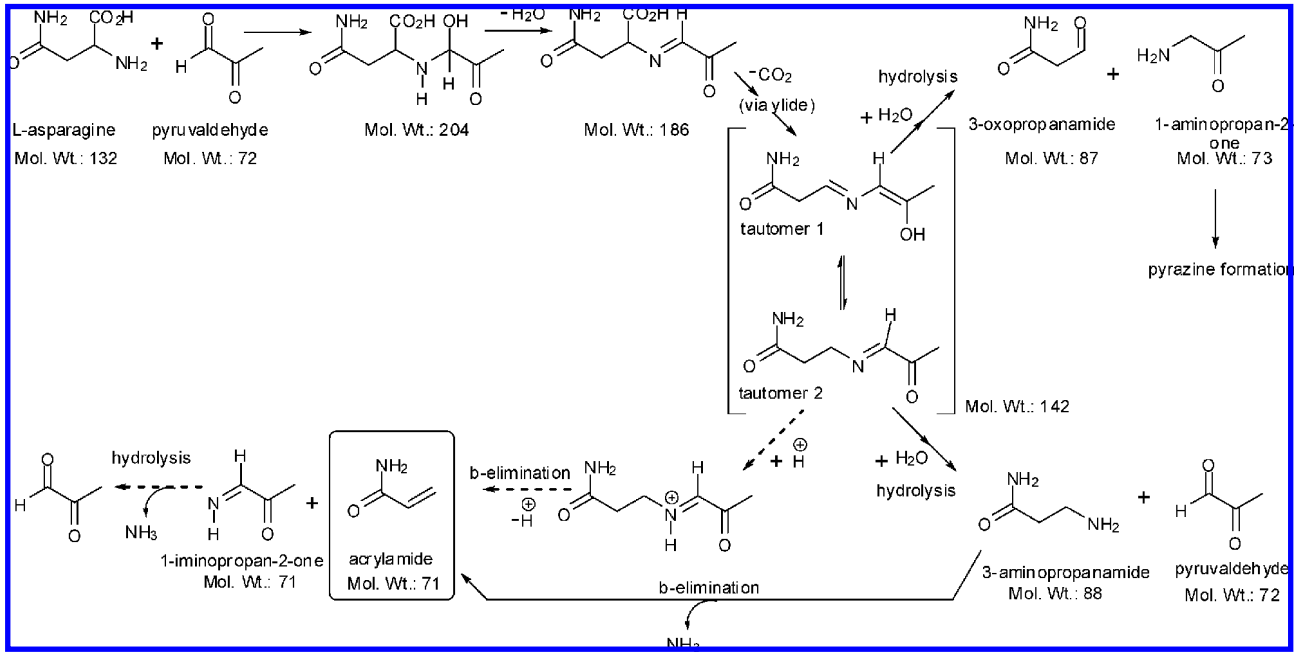

Figure 1. Proposed chemical pathways to acrylamide from asparagine and a dicarbonyl compound, pyruvaldehyde. The dotted lines from tautomer **2** to acrylamide indicate a potential, alternative mechanism (*5*).

Table 1. Carbonyl Reactants and Calculated *m/z* Values of $[M + H]^+$ Ions for the Different Classes of Intermediates and Products Predicted from Reactions with Asparagine through the Pathway in **Figure 1**[a]

carbonyl	carbonyl reagent	Schiff base	decarboxylated Schiff base	3-oxopropanamide	transaminated carbonyl	3-APA	imine	acrylamide
glyoxal	59	173	129	**88**	60	**89**	58	**72**
glycol aldehyde	61	175	131	**88**	62	**89**	60	**72**
pyruvaldehyde	73	187	143	**88**	74	**89**	72	**72**
diacetyl	87	201	157	**88**	88	**89**	86	**72**
3-hydroxy-2-oxopropanal	89	203	159	**88**	90	**89**	88	**72**
1-hydroxy-2,3,-butandione	103	217	173	**88**	104	**89**	102	**72**
4-hydroxypentan-2,3-dione	117	231	187	**88**	118	**89**	116	**72**
3,4,5-trihydroxypentanal	135	249	205	**88**	136	**89**	134	**72**
3-deoxyglucosone	163	277	233	**88**	164	**89**	162	**72**

[a] Boldface entries indicate common asparagine-derived ions. The column headed "imine" relates to the potential formation of acrylamide through the dotted line shown in **Figure 1**.

same, the interpretation was that the ion was wholly due to the proposed compound. If other interfering ions or ion fragments were contributing to the ion intensity, some residual intensity should be observed in the presence of label.

Samples were prepared in the waxy maize starch matrix using labeled reactants and heat processed as described previously. Asparagine labeled with ^{15}N in the amide position and in the α-amino and amide nitrogens was used along with ^{13}C-labeled glucose (C1 and C2 positions) and labeled fructose (C1 position). The aim was to determine the contribution of asparagine and glucose/fructose to the reaction products. Authentic compounds and MS2 were also used to provide information that would aid assignment of compounds to ions.

Acrylamide. The ion at *m/z* 72 corresponds to the $[M + H]^+$ ion of acrylamide. The presence of a MS2 sibling ion at *m/z* 55 supported the tentative identification, and fragmentation software also predicted this MS2 ion. Further confidence in the identification was provided through the use of authentic compounds. Authentic acrylamide was introduced into the reactor system as an aqueous solution (125 μm) through the evaporative humidifier port of the matrix reactor (170 °C) with no sample plug present. The ion intensities at *m/z* 72 and the MS2 ion at *m/z* 55 were measured for the calibration standards as well as for acrylamide formed from the unlabeled glucose and fructose samples. Statistical analysis of the intensities of the ions at *m/z* 72 and 55 from both the calibration and experimental samples produced a linear relationship with a correlation coefficient of 0.9992, strongly suggesting that MS-MS of the ion at *m/z* 72 to form an ion at *m/z* 55 was due to one compound only. If there were other ion contributions at *m/z* 55 (e.g., from fragmentation of other compounds), then a lower correlation coefficient would be expected.

Further confirmation was provided by studying the incorporation of label into acrylamide from isotopically labeled reactants and comparing predicted incorporation (using the general chemical pathway in **Figure 1**) with experimental observations. Asparagine with a ^{15}N amide label caused the peak at *m/z* 72 to decrease to baseline levels while the ion intensity at *m/z* 73 showed the same intensity as *m/z* 72 with unlabeled reactants (**Figure 2**). Asparagine with both α and amide nitrogen atoms labeled with ^{15}N gave the same pattern as the amide-labeled reactant, indicating that there was no contribution to *m/z* 72 from the α nitrogen. Reactions using glucose with a ^{13}C label at the C1 and C2 positions gave the same pattern as the unlabeled glucose, indicating that the ion at *m/z* 72 contained no C1 or C2 carbons from glucose (**Figure 2**). Fructose was then used as the carbonyl source with unlabeled and labeled asparagine. The underlying patterns of labeling were the same as those observed with labeled glucose except that fructose formed an ion at *m/z* 74 (proposed as aminopropan-2-one),

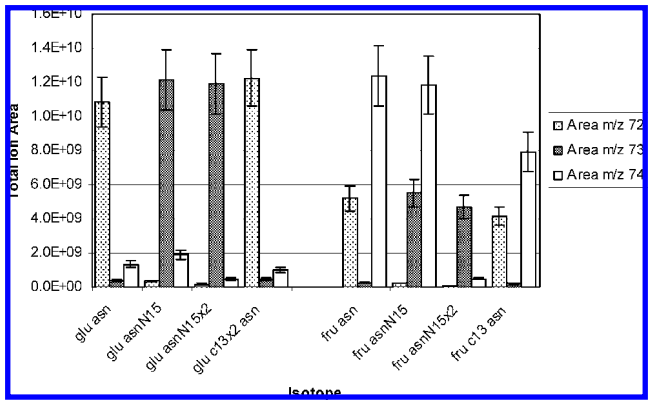

Figure 2. Incorporation of label into *m/z* 72 from glucose/asparagine and fructose/asparagine mixtures in the waxy maize starch matrix heated under standard conditions. glu, glucose; asn, asparagine; fru, fructose; asnN15, asparagine with ^{15}N label in amide position; asnN15×2, asparagine with ^{15}N label in amide and α nitrogens; glu C13×2, glucose with ^{13}C label in C1 and C2; fru C13, fructose with ^{13}C label in C1.

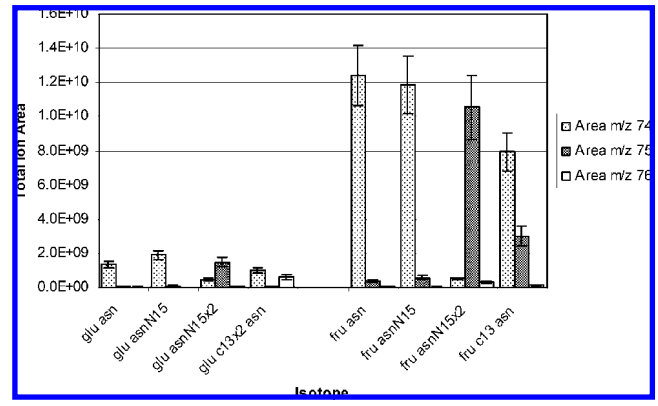

Figure 3. Incorporation of label into *m/z* 74 from glucose/asparagine and fructose/asparagine mixtures in the waxy maize starch matrix heated under standard conditions. glu, glucose; asn, asparagine; fru, fructose; asnN15, asparagine with ^{15}N label in amide position; asnN15×2, asparagine with ^{15}N label in amide and α nitrogens; glu C13×2, glucose with ^{13}C label in C1 and C2; fru C13, fructose with ^{13}C label in C1.

which would have masked any double label incorporation. However, the changes in *m/z* 72 and 73 with fructose/asparagine showed the pattern predicted by the chemical pathway and agreed with the glucose labeling pattern (**Figure 2**). The sum of these experimental data is compelling evidence that the ions at *m/z* 72 and 55 can be wholly assigned to acrylamide in this system and that acrylamide quantification based on the MS2 ion at *m/z* 55 is justified. The results from the labeling experiments were scrutinized for signs of acrylamide formation through the alternative mechanism (cleavage of tautomer **2** to form acrylamide and iminopropan-2-one, both potentially *m/z* 72), but there was no evidence from the labeling patterns for this pathway. We conclude that acrylamide production in these samples occurs entirely through the 3-APA pathway.

Ion *m/z* 74. The ion at *m/z* 74 has been linked to the transamination compound (aminopropan-2-one) formed from pyruvaldehyde. This compound is seen in other Strecker reactions (e.g., formation of 3-methylbutanal from glucose and leucine), and evidence for tentatively assigning aminopropan-2-one to *m/z* 74 has been presented previously (*11*). MS2 of the authentic compound produced a spectrum containing *m/z* 74 (10%, parent ion) and *m/z* 56 (85%). Fragmentation software predicted the formation of *m/z* 56 by protonation of aminopropan-2-one at the carbonyl oxygen. MS2 of *m/z* 74 produced from an asparagine−glucose reaction gave the same two ions in the same ratio. As discussed previously for acrylamide identification, the fact that the parent and sibling ions relate to the proposed structure provides some evidence for identification, and the fact that both the authentic compound and the ion in the thermal reaction give the same parent/sibling ion ratio strongly suggests that any contributions to the ion intensity at *m/z* 74 from other compounds are minimal.

Further evidence was obtained from labeling experiments using the same labeled reactants as for acrylamide identification (**Figure 3**). From the proposed chemical pathways, the nitrogen in aminopropan-2-one should be derived solely from the α amino group of asparagine. Experiments with amide-labeled and double-labeled ^{15}N asparagine showed that the ion mass at *m/z* 74 shifted to 75 when the α nitrogen of asparagine was labeled, but no change in ion mass was observed with amide label (**Figure 3**). With labeled glucose, an increase of 2 amu was observed, whereas labeled fructose showed an increase of 1 amu, results that are in agreement with the proposed pathways to aminopropan-2-one. These results are the best evidence that can currently be obtained to show that the ion at *m/z* 74 is entirely due to the presence of aminopropan-2-one.

Ion *m/z* 60. From the predictions in **Table 1**, *m/z* 60 was tentatively assigned to the transamination compound of glyoxal (aminoethanal). Authentic compound was not available, so labeling patterns were studied. Aminoethanal was expected to incorporate the α amino nitrogen from asparagine, but the labeling experiments showed that the amide nitrogen was quantitatively incorporated to give an ion at *m/z* 61 with the same intensity as ion *m/z* 60 from the unlabeled mixture (data not shown). The double-labeled asparagine gave the same labeling pattern as the single-labeled asparagine, as did label from glucose labeled with ^{13}C at the C1 and C2 positions. With fructose, the amounts of *m/z* 60 were higher, but the labeling patterns were identical to those found in the glucose experiments. The identity of *m/z* 60 is therefore unknown, and the hypothesis that it is a marker for glyoxal production is in doubt. The fact that *m/z* 60 is found in all samples, irrespective of carbonyl type, suggests it is an asparagine-derived compound, with acetamide a potential candidate.

Ion *m/z* 89. An authentic sample of 3-APA (hydrochloride form) was introduced as an aqueous solution (125 μM) through the evaporative humidifier port of the matrix reactor (170 °C), and the resulting mass spectrum was monitored. The rationale was that the hydrochloride form of the compound would break down to liberate 3-APA when the solution was vaporized at high temperature. The temperature and operating conditions were the same as the reaction conditions used to form acrylamide from the reactants, and therefore the behavior of 3-APA was measured under identical conditions, whether injected as the authentic compound or measured as a reaction product. The spectra showed that 3-APA hydrochloride underwent spontaneous breakdown to acrylamide (*m/z* 72) at low flow rates and produced both *m/z* 89 and 72 at higher flow rates (data not shown). Therefore, a comparison of authentic compound with the thermally produced ion at *m/z* 89 yielded limited information, although the sibling ion from MS2 of the authentic and experimental samples was the same (*m/z* 71). Because of the degradation, labeling experiments were used to predict which products would be labeled and to confirm the identity of ion 89 in this system.

According to the proposed chemical pathways, 3-APA derived from asparagine should incorporate nitrogens from both the

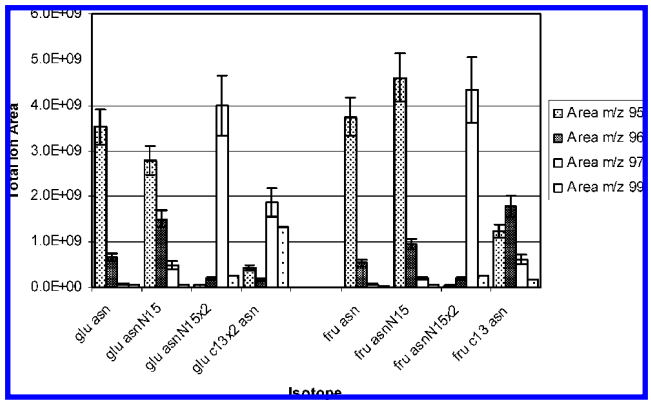

Figure 4. Incorporation of label into *m/z* 95 from glucose/asparagine and fructose/asparagine mixtures in the waxy maize starch matrix heated under standard conditions. glu, glucose; asn, asparagine; fru, fructose; asnN15, asparagine with ^{15}N label in amide position; asnN15×2, asparagine with ^{15}N label in amide and α nitrogens; glu C13×2, glucose with ^{13}C label in C1 and C2; fru C13, fructose with ^{13}C label in C1.

amide and α amino groups. In the presence of the amide-labeled asparagine, the mass of 3-APA should increase by 1 amu to 90 and, in the presence of the double-labeled asparagine, *m/z* 91 was expected. Results (not shown) proved that unlabeled glucose and asparagine produced *m/z* 89 with very minor amounts of *m/z* 90 and 91. In the presence of amide-labeled asparagine, *m/z* 89 increased significantly due to incorporation of label by another compound, 3-oxopropanamide (see **Figure 1**), but *m/z* 90 increased from baseline levels to a level roughly equal to *m/z* 89 with unlabeled reactants, suggesting significant incorporation of amide label into 3-APA. With the double-labeled asparagine, *m/z* 89 was shifted to *m/z* 91. With labeled glucose, the ion profile was very similar to that obtained from the unlabeled reactants, showing no incorporation of ^{13}C label into the ions at *m/z* 89, 90, or 91. Again, all of the evidence supports the proposal that the ion at *m/z* 89 can be assigned to 3-APA.

Ion *m/z* 95. If methylpyrazine formation occurs through the condensation of compounds derived from transamination of pyruvaldehyde and glyoxal (*16*), then each compound will incorporate ^{15}N label from the α-amino group of asparagine and the pyrazine should show an increase of 2 amu in the presence of α-labeled asparagine. If the dicarbonyl has been formed from glucose or fructose, then ^{13}C label from position C1 should also be present in the pyrazine. Inspection of **Figure 4** shows no significant incorporation of amide nitrogen from asparagine but significant incorporation from α-amino nitrogen with both glucose and fructose. Significant ^{13}C incorporation was also observed from glucose and fructose (see shift from *m/z* 95 to 97/99 for glucose and from *m/z* 95 to 96/97 for the single-labeled fructose). The labeling patterns are consistent with assigning methylpyrazine to *m/z* 95. Further inspection of the data from the glucose and fructose samples showed that both carbonyls produced about the same amount of methylpyrazine, and this was potentially due to pyruvaldehyde being limiting in the glucose systems, whereas the fructose system was limited by the availability of glyoxal. Support for this hypothesis came from an examination of the amounts of pyrazine and dimethylpyrazine in the glucose and fructose systems, where pyrazine formation was greater in the glucose system (pyrazine formation involves condensation of two molecules derived from glyoxal), whereas dimethylpyrazine (condensation of two pyruvaldehyde-derived molecules) levels were higher in the fructose system.

The approaches described above are intended to provide objective data to support the assignments and to comply with the accepted principles of compound identification in scientific publications. With conventional GC-MS analysis, there are established parameters such as retention time, mass spectral match, and authentic compound comparison, but online monitoring needs to develop its own parameters, and we propose the approach above, as well as the previously reported practice of using GC with EI and online detectors (*12, 17*).

Application of Online Monitoring to Study Acrylamide Formation. The assignments described above were used to study the relative amounts of acrylamide (and related products) generated from samples containing different reactants. A statistically designed experiment was set up with three factors: different "sugar" types (glucose, fructose, lactose, ascorbic acid, pyruvic acid, 3-deoxyglucosone, methyl α-D-gluopyranoside), different amounts of reactants, and different ratios of reactants as these are reported to affect acrylamide formation (*6, 18*). Sugar types were restricted to nonvolatile compounds to avoid interference with the online MS analysis. Fifty-one samples were prepared and analyzed in 4 days. Data from the online analysis were expressed as total amount of each compound formed during the 11 min heating period. Because the amount of each compound monitored in the gas phase is a result of partition from the starch matrix, it is not valid to compare the amounts of different compounds in the same sample, but it is valid to compare the amounts of the same compound in different samples because the matrix and processing conditions were identical. A linear relationship between acrylamide in the gas and solid phases has been shown previously (*15*).

Inspection of the ion spectra evaluated the formation of pyruvaldehyde and glyoxal in the heated samples, using the ions at *m/z* 74 and 95 as markers. *m/z* 74 is the transamination product of pyruvaldehyde and can combine with the transamination product of glyoxal to yield methyl pyrazine (*m/z* 95). Samples containing glucose or fructose showed the highest levels of *m/z* 95 (same levels for both sugars), but the other sugar-related compounds showed lower levels (10 times less; data not shown). The hypothesis that glucose and asparagine formed pyruvaldehyde and glyoxal was further supported by the presence of ions corresponding to the decarboxylated Schiff base (*m/z* 143) and dimethylpyrazine (*m/z* 109; the condensation of two pyruvaldehyde-derived molecules) plus pyrazine (*m/z* 81; condensation of two glyoxal-derived molecules). Fructose samples showed the highest levels of *m/z* 74, and the ion corresponding to dimethylpyrazine was also present at the highest level of all samples. This simple data analysis showed that online monitoring could provide rapid, qualitative evaluation of the systems and that the results were in agreement with the established chemical pathways leading to acrylamide.

To extract more information from the data, the amounts of each compound formed in all 51 samples were correlated (Pearson correlation). The hypothesis was that strong correlations would show common pathways in the 51 samples, whereas pathways specific to a particular carbonyl or sugar should show lower correlations. **Table 2** gives the values between ions that showed the best correlations. The strongest correlation (0.9823) was between the ions at *m/z* 89 and 55, which confirms the role of 3-APA as a precursor of acrylamide (*2, 19*) in all of the formulations tested. **Figure 5** shows a plot of the correlations between ions 55 and 89 to illustrate the lack of outlying data points, which reinforces the hypothesis that this correlation indicates a common reaction step in all 51 samples. The next best correlation (0.8959) was with *m/z* 143, which is believed to correspond to the decarboxylated Schiff base formed from

Table 2. Pearson Correlation of Ion Areas in All 51 Samples[a]

area	ion 55	ion 60	ion 74	ion 88	ion 89	ion 95	ion 143
ion 55	1						
ion 60	0.5710	1					
ion 74	0.2446	0.7726	1				
ion 88	0.2030	0.1742	−0.0281	1			
ion 89	0.9823	0.5411	0.1526	0.3380	1		
ion 95	0.7585	0.6873	0.7026	0.0812	0.6750	1	
ion 143	0.8959	0.4652	0.3274	0.1654	0.8386	0.8121	1

[a] Ion area is proportional to the overall amounts of Maillard products formed during heating at 170 °C for 11 min. Samples contained asparagine with different carbonyl compounds at different concentrations (50−200 μmol/g of dry starch) and with different ratios of carbonyl to asparagine (1:4 to 4:1). See text for full details.

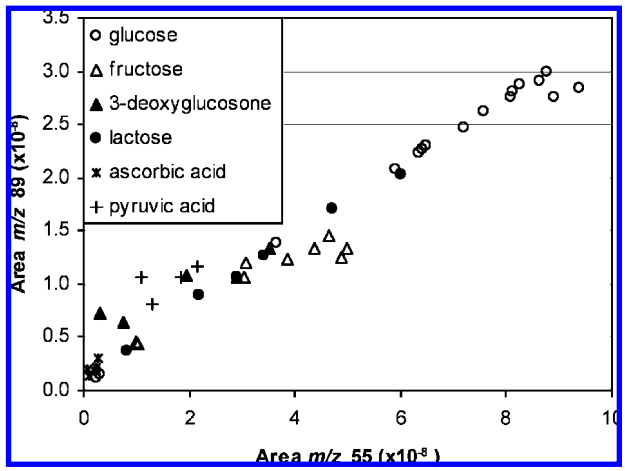

Figure 5. Relationship between ion *m*/*z* 55 (acrylamide) and ion *m*/*z* 89 (proposed as 3-aminopropanamide) for the different sugar-related reactants.

asparagine and pyruvaldehyde, and there was a complementary correlation (0.8386) between ions 89 and 143. Interestingly, there was no significant correlation between acrylamide and the glyoxal-derived decarboxylated Schiff base (predicted *m*/*z* 129), although there are signs of glyoxal-derived compounds, for example, pyrazine and methylpyrazine. Ion *m*/*z* 88 is potentially 3-oxopropanamide.

It is difficult to interpret these data further as they consider overall compound formation in a wide range of samples and because it seems that some important compounds are transient (or below detection limits) and are not observed in the mass spectra. The other limitation is to recognize that the amount of a compound measured in the gas phase is the net result of formation and further reaction and, therefore, the amounts of some compounds measured may be highly dependent on reactant composition. Further data analysis therefore considered the formation of ions from specific sugars or carbonyls.

Table 3 gives the significant correlations between ions for the different sugar and sugar-related reactants. For example, a strong correlation between *m*/*z* 55 and 74 was seen for the 3-deoxyglucosone samples, and the relationships between these ions for the other reactants were then plotted. **Figure 6** shows two trends, the upper trend correlated with samples containing fructose or 3-deoxyglucosone and the lower trace representing the samples containing the other carbonyl reactants. This suggests that pyruvaldehyde is generated through reaction of fructose and 3-deoxyglucosone with asparagine in the first cycle of the Maillard reaction and then reacts with another molecule of asparagine to form acrylamide. The production of pyruvaldehyde from 3-deoxyglucosone is already established (*20*), and there was a strong correlation between *m*/*z* 55 and 74 for the 3-deoxyglucosone samples (**Table 3**). According to **Figure 6**, glucose produces lower levels of *m*/*z* 74, whereas the amounts formed from ascorbic acid and pyruvic acid were very low. *m*/*z* 74 was well correlated with the ion at *m*/*z* 95 for lactose samples but was negatively correlated with *m*/*z* 88 in 3-deoxyglucosone samples (**Table 3**).

Table 3. Pearson Correlation of Ion Areas from 51 Samples Containing Asparagine plus Different Sugar or Carbonyl Reactants in Different Amounts and Ratios[a]

ion area correlation	*m*/*z* 55	*m*/*z* 60	*m*/*z* 74	*m*/*z* 88	*m*/*z* 89	*m*/*z* 95	*m*/*z* 143
m/*z* 55	1						
m/*z* 60	0.999^{DG}	1					
m/*z* 74	0.985^{DG}	0.988^{L}	1				
m/*z* 88	0.906^{G}	-0.853^{DG}	-0.941^{DG}	1			
m/*z* 89	0.998^{L}	0.979^{DG}	0.921^{DG}	0.921^{G}	1		
m/*z* 95	0.958^{G}	0.986^{L}	0.993^{L}	0.794^{G}	0.947^{DG}	1	
m/*z* 143	0.961^{DG}	0.995^{A}	0.970^{DG}	0.791^{L}	0.963^{MG}	0.997^{P}	1

[a] Ion areas represent the amounts of Maillard products formed during heating at 170 °C for 11 min. Each value represents the correlation coefficient between the two ions and the superscript represents the type of carbonyl for which the correlation occurred (DG, 3-deoxyglucosone; G, glucose; L, lactose; A, ascorbic acid; P, pyruvic acid; MG, methyl-α-D-glucopyranoside).

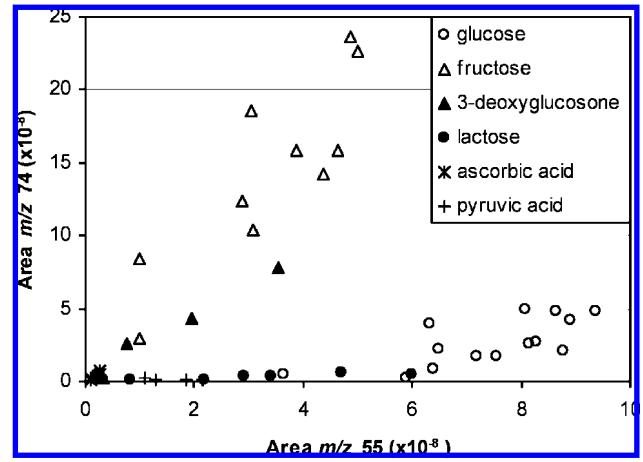

Figure 6. Correlation of the overall amounts of ion *m*/*z* 55 (acrylamide) and ion *m*/*z* 74 (proposed as aminopropan-2-one) from selected carbonyl sources reacted with asparagine at different concentrations and in different ratios of carbonyl to asparagine (see Materials and Methods for details).

Following the two-way correlations described above, all of the data were analyzed to determine the ion combinations that could best predict acrylamide formation within this set of samples. Partial least-squares analysis was used to identify the key ions that correlated with a target (in this case acrylamide formation). The mathematical relationship between the target and the contributing variables was then calculated. This led to eq 1, which shows that acrylamide formation was positively correlated with the unknown ion at *m*/*z* 60, pyrazine (*m*/*z* 81), methylpyrazine (*m*/*z* 95), and dimethylpyrazine (*m*/*z* 109) and negatively correlated with aminopropan-2-one (*m*/*z* 74).

$$\text{ion } 55 = 0.179 \times \text{ion } 60 - 0.394 \times \text{ion } 74 + 1.025 \times \text{ion } 81 + 0.608 \times \text{ion } 95 + 0.838 \times \text{ion } 109 \quad (1)$$

The fact that aminopropan-2-one is a precursor of methyl- and dimethylpyrazine, yet the precursor and the product show negative and positive correlations, is difficult to explain. It may indicate that the complex relationships between the intermediates in the chemical pathways cannot be represented with the linear correlations inherent in PLS analysis or that other pathways which produce/consume *m/z* 74 need to be considered in the data interpretation.

Dynamic Temporal Analysis. The data analysis presented above has focused on the overall amounts of products formed but ignored the temporal formation of compounds. In a preliminary analysis, PARAFAC scanned all of the ion data and computed discrete temporal profiles. The ions associated with the profiles were then examined to ascertain if they were consistent with the experimental systems used.

Figure 7 shows the six most significant ion profiles and the major ions associated with them. Profile 1 is associated with ions *m/z* 167 and 137; both ions were derived from the internal standard added to the waxy maize starch matrix during manufacture. The release profile of this compound shows matrix retention is of the order of 100 s and that the initial release is rapid. Clearance time under ideal conditions was calculated (from gas flows and system volumes) as 3.5 s in the absence of a matrix. The difference in clearance times (100 and 3.5 s) is presumably due to a change in gas flow rate with matrix present as well as the potential partition between the matrix and the gas phase.

Profile 2 is linked to ion *m/z* 88, which did not feature strongly in the earlier correlation studies, maybe because it reaches maximum intensity early in the reaction and then decays away. The origin of the other ion in profile 2 (*m/z* 279) is not known. Aminopropan-2-one (*m/z* 74) and the ion at *m/z* 60 were associated with profile 3 and showed a fairly constant level throughout the heating period. Profile 4 is associated with a single ion at *m/z* 212, an ion that was found in only the methyl D-glucopyranoside samples. Acrylamide (*m/z* 55) was the ion associated with profile 5, and this too showed a fairly steady level throughout the reaction. The ions at *m/z* 144 and 127 were associated with profile 6, and these are proposed as sugar breakdown products, for example, hydroxymethylfurfural and derivatives.

With the ability to assign compounds to ions, the next step is to improve the data processing to make better use of the information-rich outputs from online monitoring. The ability to process multiple samples in a time efficient way and to investigate samples with compositions and structures close to real foods provides new opportunities to screen Maillard reactions and give an overall picture of the effects of composition and processing. Coupled with conventional GC-MS studies, it could assist in our understanding of the dynamics of these reactions.

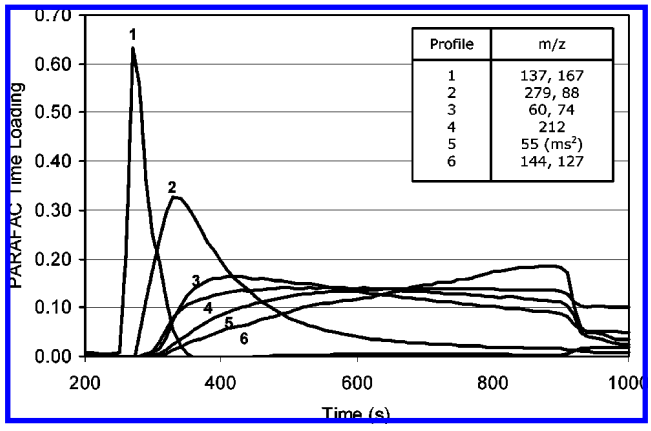

Figure 7. Time profile factors identified in 51 samples with different reactant compositions using PARAFAC data analysis.

LITERATURE CITED

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

(2) 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.

(3) Blank, I.; Robert, F.; Goldmann, T.; Pollien, P.; Varga, N.; Devaud, S.; Saucy, F.; Huynh-Ba, T.; Stadler, R. H. Mechanisms of acrylamide formation—Maillard-induced transformation of asparagine. *Chem. Saf. Acrylamide Food* **2005**, *561*, 171–189.

(4) Granvogl, M.; Schieberle, P. Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. *J. Agric. Food Chem.* **2006**, *54*, 5933–5938.

(5) Stadler, R. H.; Robert, F.; Riediker, S.; Varga, N.; Davidek, T.; Devaud, S.; Goldmann, T.; Hau, J.; Blank, I. In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *J. Agric. Food Chem.* **2004**, *52*, 5550–5558.

(6) Rydberg, P.; Eriksson, S.; Tareke, E.; Karlsson, P.; Ehrenberg, L.; Tornqvist, M. Investigations of factors that influence the acrylamide content of heated foodstuffs. *J. Agric. Food Chem.* **2003**, *51*, 7012–7018.

(7) Amrein, T. M.; Limacher, A.; Conde-Petit, B.; Amado, R.; Escher, F. Influence of thermal processing conditions on acrylamide generation and browning in a potato model system. *J. Agric. Food Chem.* **2006**, *54*, 5910–5916.

(8) Stadler, R. H. Acrylamide formation in different foods and potential strategies for reduction. *Chem. Saf. Acrylamide Food* **2005**, *561*, 157–169.

(9) Cook, D. J.; Taylor, A. J. On-line MS/MS monitoring of acrylamide generation in potato- and cereal-based systems. *J. Agric. Food Chem.* **2005**, *53*, 8926–8933.

(10) Pollien, P.; Lindinger, C.; Yeretzian, C.; Blank, I. Proton transfer reaction mass spectrometry, a tool for on-line monitoring of acrylamide formation in the headspace of Maillard reaction systems and processed food. *Anal. Chem.* **2003**, *75*, 5488–5494.

(11) Channell, G. A.; Taylor, A. J. On line monitoring of the Maillard reaction using a film reactor coupled to ion trap mass spectrometry In *Process and Reaction Flavors*; Weerasinghe, D. K., Sucan, M. K., Eds.; American Chemical Society: Washington, DC, 2005; Vol. 905, pp 181–191.

(12) Wright, J.; Wulfert, F.; Hort, J.; Taylor, A. J. Effect of preparation conditions on release of selected volatiles in tea headspace. *J. Agric. Food Chem.* **2007**, *55*, 1445–1453.

(13) Jublot, L.; Linforth, R. S. T.; Taylor, A. J. Direct atmospheric pressure chemical ionisation ion trap mass spectrometry for aroma analysis: speed, sensitivity and resolution of isobaric compounds. *Int. J. Mass Spectrom.* **2005**, *243*, 269–277.

(14) Bro, R. Review on multiway analysis in chemistry—2000–2005. *Crit. Rev. Anal. Chem.* **2006**, *36*, 279–293.

(15) Cook, D. J.; Channell, G. A.; Taylor, A. J. On line monitoring of acrylamide formation In *Chemistry and Safety of Acrylamide in Food*; Friedman, M., Mottram, D. S., Eds.; Springer: New York, 2005; Vol. 561, pp 303–316.

(16) Hofmann, T.; Bors, W.; Stettmaier, K. Studies on radical intermediates in the early stage of the nonenzymatic browning reaction of carbohydrates and amino acids. *J. Agric. Food Chem.* **1999**, *47*, 379–390.

(17) Lindinger, C.; Pollien, P.; Ali, S.; Yeretzian, C.; Blank, I.; Mark, T. Unambiguous identification of volatile organic compounds by proton-transfer reaction mass spectrometry coupled with GC/MS. *Anal. Chem.* **2005**, *77*, 4117–4124.

(18) Bagdonaite, K.; Viklund, G.; Skog, K.; Murkovic, M. Analysis of 3-aminopropionamide: a potential precursor of acrylamide. *J. Biochem. Biophys. Methods* **2006**, *69*, 215–221.

(19) Schieberle, P.; Kohler, P.; Granvogl, M. New aspects on the formation and analysis of acrylamide. *Chem. Saf. Acrylamide Food* **2005**, *561*, 205–222.

(20) Yaylayan, V. A.; Keyhani, A. Origin of 2,3-pentanedione and 2,3-butanedione in D-glucose/L-alanine Maillard model systems. *J. Agric. Food Chem.* **1999**, *47*, 3290–3294.

Received for review December 21, 2007. Revised manuscript received March 16, 2008. Accepted May 18, 2008. The U.K. Food Standards Agency supported this research (Grant C03047).

JF7037423