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# On-Line MS/MS Monitoring of Acrylamide Generation in Potato- and Cereal-Based Systems

DAVID J. COOK* AND ANDREW J. TAYLOR

Samworth Flavor Laboratory, Division of Food Sciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, United Kingdom

An on-line MS/MS technique was used to study the generation of acrylamide in rye-, wheat-, and potato-based systems during cooking. Acrylamide release to the gas phase was monitored continuously and was correlated with the acrylamide content of samples using a calibration based upon the partition of [1,2,3-$^{13}C_3$]acrylamide. On-line results at 180 °C were compared with data relating to the same systems obtained through GC−MS analysis. Agreement between the two techniques was notable, both in terms of the temporal profiles of acrylamide generation and when comparing the relative magnitudes of results for potato, wheat, and rye determined by each method. The effects of pH (citric acid) and added amino acids (soy protein hydrolysate) on the generation of acrylamide in hydrated potato flake were modeled at 180 °C. It was concluded that a combined treatment of low levels of each additive could result in significant reductions in acrylamide, although the effects of such treatments on sensory properties such as color and flavor remain to be evaluated.

**KEYWORDS: Acrylamide; cooking; Maillard reaction; on-line analysis; APCI-MS; potato, rye; wheat**

## INTRODUCTION

The worldwide research effort stimulated by the discovery that acrylamide is widespread in the diet at $\mu$g/kg to mg/kg levels (*1*) continues apace. Many of the early questions relating to its occurrence and mechanism of formation in foods have now been answered in some depth, and progress to date has been the subject of two comprehensive reviews (*2*, *3*), to which the reader is referred. Regulatory authorities and the food industry must now attempt to apply this knowledge in circumstances where the risk presented by acrylamide intake from the diet remains hard to evaluate. In the absence of a full understanding of the risk to human health posed by dietary exposure to acrylamide, the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) recently concluded that there may be a public health concern relating to acrylamide in the diet. They recommended that the position regarding dietary intake of acrylamide should be reevaluated in 2−3 years when results of ongoing toxicological studies become available. On the basis of this evaluation, FAO and WHO recommend that efforts to reduce acrylamide levels in foodstuffs should continue and there is hence an ongoing need for both fundamental and applied research in this area.

To achieve the above goal requires the development of sensitive and rapid experimental protocols for the extraction and analysis of acrylamide from food matrixes. The majority of protocols in current use are either GC− or LC−MS-based (*4*) and in either case involve time-intensive sample extraction and purification steps that vary in rigor depending upon the sample matrix concerned. Many techniques require derivatization as an additional stage of sample workup. For a simple time course experiment, samples must be prepared and these procedures replicated at every time point. The development of techniques enabling acrylamide generation to be followed on-line (in "real-time") is an attractive alternative to such time-intensive analyses. On-line monitoring of acrylamide using mass spectrometry has the potential to generate data-rich profiles of acrylamide generation and thus to improve understanding of the kinetic and physical processes involved.

The first report of the application of on-line monitoring to acrylamide analysis was published by Pollien et al. in 2003 (*5*). This paper demonstrated the use of proton-transfer reaction mass spectrometry (PTR-MS) to monitor acrylamide in the gas phase as it was generated during the thermal treatment of potato as well as in Maillard model systems (asparagine plus reducing sugars). Acrylamide was monitored at *m*/*z* 72, and data were reported in the form of ion count versus time. One of the inherent difficulties in the development of this technique to wider application is the nature of the relationship between the gas phase acrylamide signal (which is monitored) and the corresponding concentration of acrylamide in the product (which one would ideally measure). This requires some form of calibration for the partition behavior of acrylamide from a specified sample matrix at each process temperature. Hence, while the on-line system has excellent credentials for rapid comparative studies in the gas phase (e.g. to investigate the influence of additives on acrylamide production during a specific process), it is more complex to express results in terms of the absolute acrylamide content of samples. It is also likely that

* Corresponding author. Phone: +44-(0)115-9516153. Fax: +44-(0)-115−9516142. E-mail: david.cook@nottingham.ac.uk.

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the partition behavior of acrylamide changes as a foodstuff dries out during cooking, and this effect is difficult to quantify.

An on-line MS/MS analysis for acrylamide, based on atmospheric pressure chemical ionization mass spectrometry (APCI-MS), has also been developed and its use to monitor acrylamide generation in a hydrated potato flake system reported (*6*). Using MS/MS analysis (ion transition *m/z* 72→55) enhances the specificity of the technique. A protocol was developed to correlate on-line signal intensities with the acrylamide contents of samples (*6*). The procedure involved placing samples of known acrylamide content in the on-line cell and measuring acrylamide release to the gas phase. Calibration graphs were constructed to allow for differences in partition over the temperature range 150−200 °C. This method applied a correction for the effects of temperature on partition, but it was not able to account for differences due to the sample matrix (e.g. whether potato or cereal based). The calibration also involved assumptions concerning the differences in moisture content and effective surface area for partition between the standards and experimental samples, which were unlikely to hold throughout the time course of heating.

In this paper, we report the use of $^{13}$C-labeled acrylamide as a marker to calibrate the partition of acrylamide during on-line analysis. This allows raw on-line data, measured in the gas phase, to be corrected for the effects upon partition of both sample matrix and oven temperature. Furthermore, this study was designed to enable a comparison of on-line data with corresponding results for samples prepared from the same batch of materials (*7*, *8*) that were analyzed for acrylamide using conventional analytical methods (repetitive time point sampling and GC−MS analysis of the dibromo derivative (*9*)).

## MATERIALS AND METHODS

**Materials.** [1,2,3-$^{13}C_3$]acrylamide was purchased as a 1 mg/mL solution in methanol (Cambridge Isotope Laboratories Inc., Andover, MA). Citric acid and soy protein acid hydrolysate (Amisoy S-1674) were supplied by Sigma-Aldrich (Poole, UK).

Drum-dried potato flake (50% Pentland Dell/50% Maris Piper), wholemeal wheat flour, and wholemeal rye flour were sourced from United Kingdom food manufacturers.

**Sample Preparation.** Water was added to potato/cereal samples in predetermined ratios to give a dough of a consistency that could be rolled, cut, or extruded. The product formulations were potato flake:water (1:1.3), wholemeal rye flour:water (2.2:1), and wholemeal wheat flour:water (2.5:1). The materials and formulations were as described in a parallel study (*7*). The weight of each product used for on-line analysis was varied to give the same weight of dry matter in each instance, based upon their moisture contents as reported previously (*8*). Potato (455 mg), rye (315 mg), and wheat (305 mg) were extruded through a 5-mL disposable syringe to produce a bead of material (about 1.5 mm diameter) that was weighed into the on-line sample holder. This procedure was followed in an attempt to standardize the shape of the samples, thus minimizing variation in the surface area available for partition of acrylamide into the gas phase.

**On-Line Apparatus.** Apparatus (**Figure 1**) consisted of a sample cell (stainless steel HPLC column, 55 mm × 10 mm i.d.; Phenomenex, UK) within a GC oven (Sigma 3B, Perkin-Elmer, UK). The cell was constantly purged with a flow of humidified nitrogen gas heated to 5 °C above operating oven temperature by passage through a 3 m heated transfer line (Hillesheim, Germany). In these experiments, a constant level of humidification was applied by pumping preheated water (0.8 mL/min delivered by HPLC pump, Suprex-Anachem) into a 2 L/min flow of nitrogen gas at a point 2 m along the heated transfer line toward the nitrogen supply. The resulting nitrogen/vapor mixture had a humidity ratio of 340 g of steam/kg of dry nitrogen. A series of needle valves was used to split the flow between exhaust (the majority of the flow) and the sample cell (9 mL/min). The APCI-MS sampling rate

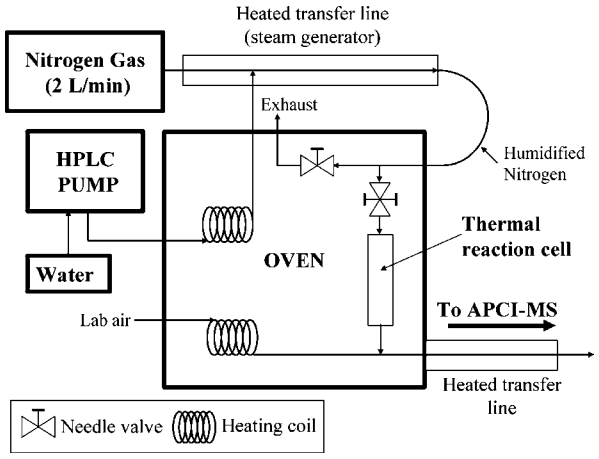


**Figure 1.** Apparatus for on-line monitoring of acrylamide generation under conditions of controlled temperature and humidity.

was adjusted to around 20 mL/min, so that the entire output of the cell was analyzed (to enhance sensitivity). "Makeup" flow was laboratory air, which had first passed through a heating coil inside the GC oven, so that it did not condense volatiles in the sample cell effluent.

**APCI-MS Analysis.** A Thermo Finnigan LCQ Deca Xp ion trap mass spectrometer (ITMS) was fitted with a custom-built APCI gas-phase interface (*10*). The source was designed to form predominantly protonated molecular ions (positive ion mode) with only minor fragmentation.

Out flow from the on-line cell was sampled directly into the APCI source via a 0.53 mm i.d. deactivated fused silica capillary tube within a heated transfer line (180 °C). For the analysis of acrylamide, the ITMS was set up to work in MS/MS mode in the low mass range, from *m/z* 40 to 75. The parent ion *m/z* 72 (acrylamide) fragmented to yield predominantly *m/z* 55 (isolation width, 1.2; collision energy, 25%; activation *Q*, 0.35; activation time: 30 ms; max inject time, 200 ms). Raw data were averaged over 10 microscans. Acrylamide concentrations in the headspace were calculated relative to the signal (*m/z* 55) for a calibrant of known concentration (*11*). Typically, a solution of 3.16 μg/mL acrylamide in $CH_2Cl_2$ was injected into the APCI source at a rate of 1.5 μL/min. This was equivalent to sampling a 20 nL/L headspace of acrylamide into the source at 75 mL/min. Instrument response was linear within the range 3−3000 nL/L acrylamide in the gas phase (*6*).

**Developing a Calibration for the On-Line Analysis: Partition Behavior of [1,2,3-$^{13}C_3$]Acrylamide from Each Food System at 160, 180, and 200 °C.** A solution of [1,2,3-$^{13}C_3$]acrylamide in water (17.5 μg/mL) was used to prepare samples of potato (1:1.3), rye (2.2:1), and wheat (2.5:1) containing 5 mg/kg wb of the label. This concentration gave the desired product composition without further dilution of the wheat product; appropriate dilution with water was applied when mixing rye and potato samples. Sample preparation for on-line analysis was as described previously. The release of [1,2,3-$^{13}C_3$]acrylamide from samples was monitored using the MS/MS transition *m/z* 75→58. Sample blanks were run for each food system to check that there was no significant background signal arising from other food components when monitoring this transition. Three replicate measurements were recorded (about 20 min duration) for each food system at each oven temperature (160, 180, and 200 °C). Data points were sampled at 380-ms intervals. Raw data was smoothed (50 point moving average) and values extracted at 0.5-min intervals. These values were averaged across three replicates and used to plot mean release curves for each sample/temperature.

**On-Line Monitoring of Acrylamide Generation in Model Systems at 160, 180, and 200 °C.** This experiment used nonlabeled samples, but all other aspects of sample preparation and of data analysis were as described in the labeling experiment. Generation of acrylamide was monitored for a 1 h period in potato, wheat, and rye model systems at each oven temperature (three replicates).

**Comparative Study: Effects of Citric Acid and Soy Protein Hydrolysate.** The effects of added citric acid and soy protein hydrolysate on acrylamide generation in the potato model system at

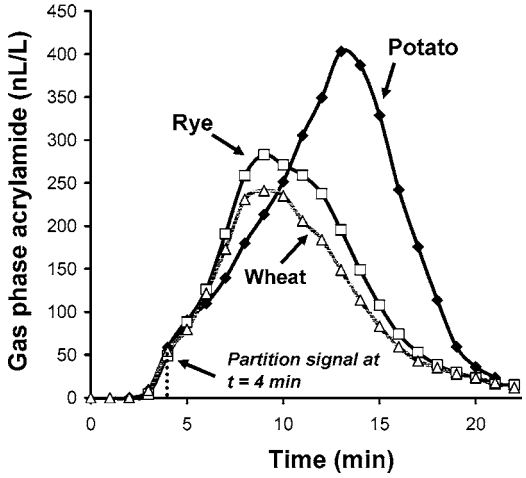


**Figure 2.** Release of 5 mg/kg [1,2,3-$^{13}C_3$]acrylamide from each food system, initially at room temperature, when placed in the on-line cell at an oven temperature of 160 °C. Data are the mean of three replicate measurements.

**Table 1.** Mean Gas Phase Acrylamide Concentrations Recorded 4 min after the Introduction of Samples Spiked with 5 mg/kg $^{13}$C-Labeled Acrylamide into the On-Line Cell

| | gas-phase acrylamide (nL/L),[a] $t = 4$ min | | |
|---|---|---|---|
| sample | 160 °C | 180 °C | 200 °C |
| potato (1:1.3) | 58.3 | 62.2 | 105 |
| rye (2.2:1) | 48.8 | 93.8 | 152 |
| wheat (2.5:1) | 54.1 | 98.9 | 127 |

[a] Mean of three replicate measurements.

180 °C were studied using a 22-point response surface design. Citric acid (1.54 g/100 mL) or soy hydrolysate (1.88 g/100 mL) was added in solution when hydrating the potato flake (1:1.3), along with an appropriate dilution with water. The resulting concentrations of the additives in the product were 0−0.87 g/100 g (wb) citric acid and 0−1.06 g/100 g (wb) soy protein hydrolysate. Preliminary trials had shown that either factor could be effective in reducing acrylamide at the higher of these concentrations, so the experimental design was weighted toward samples with lower additive concentrations, to investigate whether less severe modification of the system could result in significant reductions. Treatments were compared by calibrating the area under each on-line release curve during the first 20 min of heating at 180 °C. Results, expressed as nanograms of acrylamide released to the gas phase between 12 and 20 min, were modeled using Design Expert software version 6.02 (Statease, Minneapolis, MN).

## RESULTS AND DISCUSSION

**Developing a Calibration for the On-Line Analysis: Partition Behavior of [1,2,3-$^{13}C_3$]Acrylamide from Each Food System at 160, 180, and 200 °C.** Calibration by this method involved adding 5 mg/kg of $^{13}$C-labeled acrylamide to a sample matrix, placing it in the on-line cell at a specified temperature, and measuring the release of labeled acrylamide into the gas phase (**Figure 2**). This procedure was replicated for each combination of sample type and oven temperature. Partition factors were derived from the release curves, which were subsequently used to express on-line gas-phase concentrations (nL/L) in terms of the acrylamide content of samples, with due correction for acrylamide partition behavior (**Table 1**). By way of example, the acrylamide content of a potato sample treated at 180 °C was estimated from the gas-phase concentration (nL/L) divided by 62.2 (**Table 1**) and multiplied by 5 mg/kg.

The release of $^{13}$C-labeled acrylamide from samples had an initial lag phase of 3−4 min (while the surface heated to a temperature sufficient for acrylamide to partition into the gas phase) followed by a gradual increase to maximum and subsequent decay (**Figure 2**). The increase in surface acrylamide concentration (after the lag phase) occurred as heat penetrated further into the sample and water and acrylamide from the interior migrated to the surface. Evaporation of water from the sample helps to bring acrylamide to the product surface, as although acrylamide is not particularly volatile (boiling point 125 °C at 25 mmHg, (*12*)), it is highly water soluble. The parameter of the release curves used to estimate the partition of acrylamide for a particular system was the gas phase acrylamide concentration 4 min after the cell was placed on-line (**Table 1**). This parameter was considered the most appropriate because it was reproducible and represented an "instantaneous" measure of the gas phase acrylamide concentration resulting from a concentration of 5 mg/kg acrylamide at the product surface. Beyond this time point, the concentration of acrylamide at the surface increases and is no longer known. Furthermore, the rate of increase in surface acrylamide concentration is a function of the heat transfer properties of the sample and how rapidly it dries out, neither of which influence the partition of acrylamide as it is generated (principally at the sample surface) during an on-line experiment.

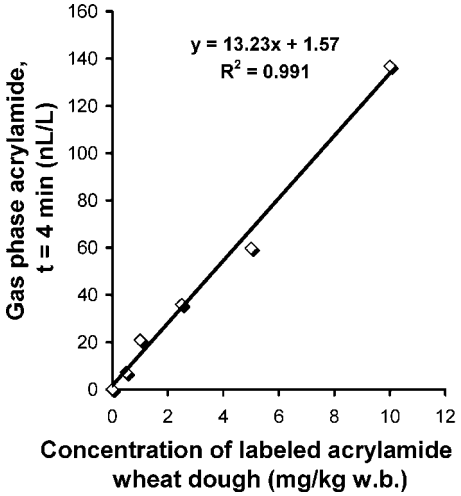


**Figure 3.** Calibration graph demonstrating linearity between the on-line signal at $t = 4$ min and the concentration of [1,2,3-$^{13}C_3$]acrylamide spiked into rye dough (2.2:1). Oven temperature was 160 °C.

To further validate this procedure, the relationship between the gas-phase signal after 4 min and the concentration of $^{13}$C-labeled acrylamide in the product (0.5−10 mg/kg wb) was investigated and showed a linear correlation (e.g. see **Figure 3**, $R^2 = 0.991$).

Calibration of the on-line technique based upon the partition of $^{13}$C-labeled acrylamide for each experimental condition means that the intensities of acrylamide generated can be compared meaningfully across treatments. However in terms of the absolute acrylamide concentration of samples, the calibrated on-line technique represents at best an estimate, which is based upon certain assumptions and involves sources of error. For example, to record the gas-phase signal at 4 min, as opposed to 3.9 or 4.1 min, was an arbitrary decision and the precise time point used modifies the calibration. It is likewise possible that the partition behavior of acrylamide varies with sample moisture content, and of course, this changes during processing. However, since the bulk of moisture loss occurs prior to acrylamide generation, such effects are minimized in this instance.

**Acrylamide Generation in Model Systems at 160, 180, and 200 °C.** *On-Line Analysis.* After raw data had been corrected

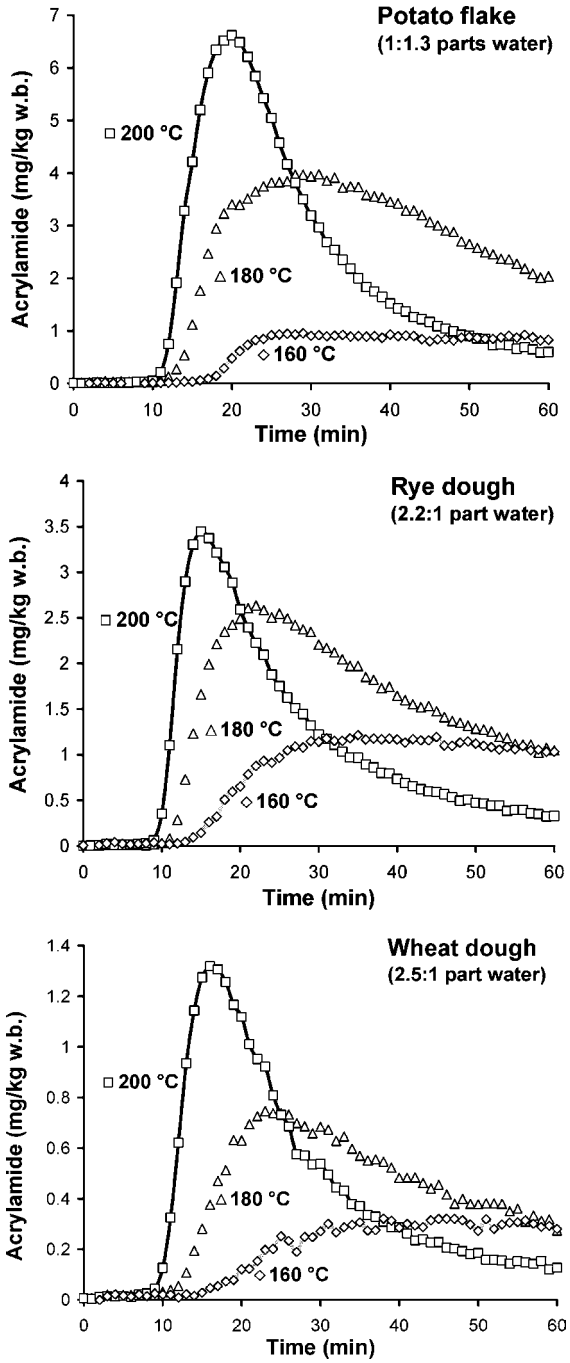


**Figure 4.** On-line acrylamide generation curves for each food system at three oven temperatures. Raw on-line data for gas-phase acrylamide have been expressed in terms of acrylamide concentrations in the products using a calibration based on the partition behavior of [1,2,3-$^{13}C_3$]acrylamide (**Table 1**). Curves are the mean of three replicate measurements.

for partition in accordance with the calibration described in the preceding section, mean acrylamide generation curves were plotted for each sample at each oven temperature (**Figure 4**). Asparagine is acknowledged to be the primary precursor of acrylamide in food systems (*13*−*16*), and the relative amounts of acrylamide produced in the three food systems at each temperature (**Figure 4**) followed the trend in their asparagine contents (i.e. potato > rye > wheat; **Table 2**).

On-line acrylamide generation profiles (**Figure 4**) featured an initial time lag (as the sample dried out and its temperature increased) followed by an increase to a maximum and a subsequent decay. Relevant parameters of the release curves

**Table 2.** Selected Compositional Data for the Food Systems[a]

| | potato flake | wholemeal rye flour | wholemeal wheat flour |
|---|---|---|---|
| asparagine content (mmol/kg db) | 27 | 4.8 | 1.3 |
| asparagine content as percentage of total amino acids (%) | 42 | 28 | 17 |
| total reducing sugars (mmol/kg db) | 53.2 | 57.7 | 49.3 |
| molar ratio of total reducing sugars: total amino acids | 0.82 | 3.34 | 6.32 |
| fructose content (mmol/kg db) | 24.6 | 13.4 | 2.36 |
| maltose content (mmol/kg db) | | 32.4 | 43.9 |

[a] Based upon previously published data relating to the same food materials, as analyzed by Elmore et al. (7).

**Table 3.** Features of the Acrylamide vs Time Profiles (Extracted from the Data of **Figure 4**)

| | | maximum acrylamide concn (mg/kg wb) | time to maximum (min) | maximum rate of acrylamide production (mg/kg wb min$^{-1}$) |
|---|---|---|---|---|
| 200 °C | potato flake (1:1.3) | 6.62 | 20 | 1.27 |
| | wholemeal rye flour (2.2:1) | 3.45 | 15 | 0.90 |
| | wholemeal wheat flour (2.5:1) | 1.32 | 16 | 0.31 |
| 180 °C | potato flake (1:1.3) | 3.97 | 31 | 0.68 |
| | wholemeal rye flour (2.2:1) | 2.64 | 22 | 0.47 |
| | wholemeal wheat flour (2.5:1) | 0.75 | 23 | 0.09 |
| 160 °C | potato flake (1:1.3) | 0.96 | 29 | 0.17 |
| | wholemeal rye flour (2.2:1) | 1.22 | 35 | 0.12 |
| | wholemeal wheat flour (2.5:1) | 0.32 | 46 | 0.03 |

(maximum rate of increase, maximum intensity, time to reach maximum) were extracted to facilitate their comparison (**Table 3**). The maximum rates of acrylamide production in each system were in the ratios 1:(3−5):(4−7.5) (wheat:rye:potato), dependent upon oven temperature. In each product the maximum intensity of acrylamide monitored by the on-line technique increased with oven temperature over the range investigated (**Table 3**). The potato system showed a somewhat higher temperature dependence (a 7-fold increase in maximum between 160 and 200 °C) than was observed for rye and wheat (3−4-fold increase over the same range).

The decay component of the acrylamide generation profile is thought to occur both due to depletion of the precursors of acrylamide (primarily asparagine and reducing sugars) and because of further processes that remove free acrylamide from the system, e.g. Michael addition reactions with amino acids or proteins (*3*, *17*) or thermal degradation (*18*). Elmore et al. (*8*) plotted the time course of both total reducing sugars and asparagine in the model systems at 180 °C. While there is a small time offset to consider when comparing their data with the on-line results (see also following section), the depletion of precursors of acrylamide to between 5 and 15% of starting concentrations within 25 min at 180 °C was clearly demonstrated and coincides with the period when acrylamide release reached a maximum value (**Figure 4**). It is likely that the reactions that remove free acrylamide from the system are also operative during the up-slope of acrylamide production, but at that stage they are outweighed by the rate at which acrylamide is being produced. Hence, the resultant profile is a balance between generation and depletion throughout the time course. Acrylamide production was monitored for a prolonged period (1 h) to investigate the process of depletion after generation had slowed or stopped (e.g. see **Figure 5**). However, in terms of the industrial relevance of the results, it must be borne in mind that most food products undergo time/temperature processes that fall within the first 20 min of the graphs in **Figure 4** and hence lie

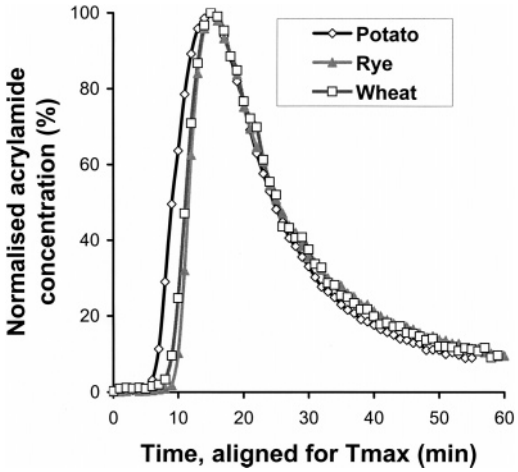


**Figure 5.** Comparison of acrylamide vs time profiles for each product at 200 °C. Concentration data have been normalized and aligned at the time of maximum intensity.

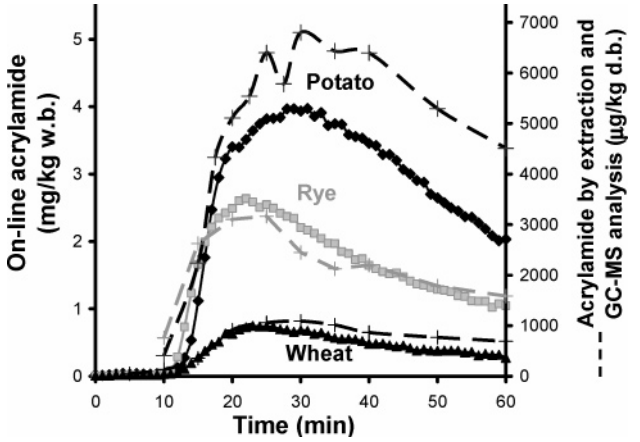


**Figure 6.** Comparison of the temporal profiles of acrylamide generation (180 °C) measured using the on-line system (filled symbols) and by GC–MS analysis of similar products (dashed curves (*8*)). Note: the food materials and sample compositions used in the two studies were the same. However the studies of necessity employed different ovens and sample weights/dimensions. Samples for GC–MS analysis were taken from homogenized whole cakes which were 3 mm thick disks of 73 mm diameter. On-line samples approximated a cylindrical shape with a diameter of 1.5 mm.

on the up-slope of acrylamide production (e.g. see ref *8*). One implication of this is that small changes in process time or temperature within this region might have a significant impact upon the acrylamide content of such foods.

It is pertinent to consider the current on-line results in relation to a kinetic model of acrylamide generation developed by Wedzicha et al. (*17*) and based upon composition vs time data for amino acids, reducing sugars, and acrylamide measured at 180 °C in the same systems utilized in the current research. The kinetic model featured a key intermediate species (Int), which formed a crossover point between acrylamide production (reaction of Int with asparagine) and desirable Maillard products (reaction of Int with other amino acids). The proportion of acrylamide formed from Int was thought to depend on the molar percentage of the total unreacted amino acids represented by asparagine. The molar percentages of asparagine in the model food systems prior to cooking were in the ratio 1:1.6:2.5 (wheat:rye:potato, **Table 2**), whereas the actual molar amounts of asparagine were in the ratio 1:3.7:20. This suggests that more acrylamide was formed in the potato system both because more asparagine was present and because it represented a higher molar proportion of the total amino acids. Since carbonyl sources are required for the generation of acrylamide from asparagine (*19*), it follows that the reducing sugar composition of samples had a bearing on acrylamide generation. It has been reported that fructose gives enhanced molar yields of acrylamide, in comparison to glucose, when reacted with asparagine (*5*, *20*), and it can be noted in this context that the potato sample contained substantially more fructose than the cereal samples (**Table 2**).

The time profile of acrylamide production in the samples was also influenced by their initial moisture contents. Elmore et al. (*8*) demonstrated (for the same food systems) that acrylamide was not produced in significant quantities until the moisture content fell below 5%, and it took longer to reach this moisture content during cooking of the initially wetter potato samples. However, when on-line data for each food system at a particular temperature were normalized and aligned according to the time of maximum release, the similarity of the acrylamide vs time profiles in each product was evident (e.g. see **Figure 5**). The two cereal samples behaved virtually identically, while there was a marginally longer increase to maximum for the potato system. The similarity of these profiles suggests that the same chemical processes are responsible for acrylamide generation and decay in each foodstuff. Thermal decomposition of acrylamide at process temperatures is one mechanism involved in acrylamide loss. Taubert et al. (*18*) reported that when acrylamide was heated in a sealed tube at 180 °C for 10 min, in the presence of a polymerization inhibitor, there was a 40−50% loss due to degradation. In our systems, treated at a comparable temperature, the rate of decay was less rapid (**Figure 4**), which may mean that the presence of the food matrix conferred a degree of stability with respect to degradation of acrylamide. The net rate of decay observed in our experiments was also lower because acrylamide was still being generated after the maximum had passed, albeit at a reduced rate.

*Comparison of On-Line Results with Those Obtained by a Validated Analytical Technique.* Results from the on-line system (180 °C) were compared with published data relating to the same materials that were obtained by the conventional approach of time-point sampling followed by solvent extraction and GC−MS analysis (*8*). When results from the two techniques were plotted on a common time axis (**Figure 6**), there were clear similarities between the resultant acrylamide profiles. When viewing **Figure 6**, it should be noted that the two sets of data have been plotted on separate *y*-axes and hence do not represent the same absolute values of acrylamide content. Indeed, it would be surprising if this had been the case, due to the use of different ovens in the respective trials and implicit differences in sample size and dimensions (see legend to **Figure 6**). What is notable, however, is the agreement between the techniques, both in terms of the temporal profiles of acrylamide generation and when comparing the relative magnitudes of results for potato, wheat, and rye determined by each method. The GC−MS curves (dashed lines in **Figure 6**) typically preceded those from on-line analyses by 2−3 min. The predominant cause for the time lag in the on-line data is thought to be humidification of the purge gas, which slowed the drying rate (*6*) and thus prolonged the induction period before acrylamide generation. Humidification of the purge gas was used as a standard operating condition because prior studies had found that it resulted in both a better signal-to-noise ratio (superior partitioning of acrylamide into humidified nitrogen) and a steadier signal response.

Comparing current results with those from GC−MS analysis supports the validity of data from the calibrated on-line MS/MS technique and demonstrates that it monitored changes in

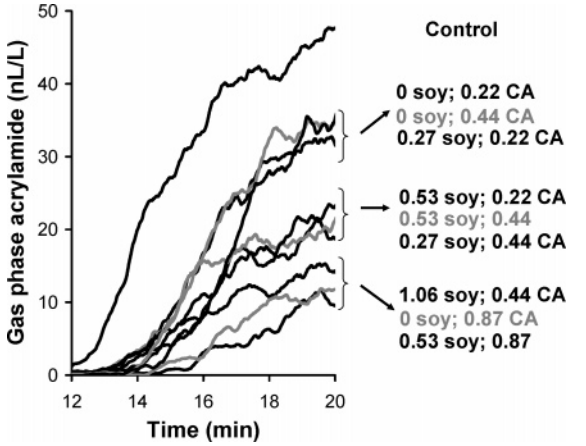


**Figure 7.** Typical on-line acrylamide release curves (180 °C) from hydrated potato flake (1:1.3) treated with citric acid (CA) and soy protein hydrolysate (soy). The legend lists samples in order of decreasing acrylamide intensity at $t = 20$ min. Additive quantities are in g/100 g wb.

acrylamide concentration over time which were meaningfully related to those obtained by a more established (and time-intensive) technique (**Figure 6**). One on-line curve was generated from three replicate measurements, involving a total analysis time of approximately 3.5 h. This represents a substantial time-saving over established analytical techniques, although it is clear that a conventional approach, employing time-point sampling, offers certain options that are not available using on-line monitoring (e.g. full compositional analysis of samples at each data point).

**Effects of Citric Acid and Soy Protein Hydrolysate on Acrylamide Generation in the Potato System.** Several studies have reported that pH modification (*20*−*23*) and the addition of competing amino acids (*17*, *20*, *22*) are potential ways in which to reduce acrylamide formation in food and model systems. These findings were rapidly confirmed using on-line monitoring of hydrated potato flake samples treated with citric acid or added amino acids (data not shown). However, modifying both the pH and amino acid profile of foods can have a profound effect on the course of the Maillard reaction and, when modified to the extent suggested by some studies, would markedly change the color and flavor of food products. The idea behind this study was to investigate the effectiveness of combined treatments of a low level of each factor in reducing acrylamide formation in the potato flake model system. Because the trial was comparative in nature, the results are presented in terms of the amount of acrylamide monitored in the gas phase. Representative on-line traces from the trial (10 of the 22 data points in the model) are shown in **Figure 7**.

The total acrylamide released to the gas phase during the first 20 min of treatment at an oven temperature of 180 °C was modeled within the design space bounded by citric acid concentration [0−0.87 g/100 g (wb)] and soy protein hydrolysate concentration [0−1.06 g/100 g (wb)]. A two-factor interaction model ($R^2 = 0.89$) described acrylamide production in terms of the additive concentrations:

$$\text{total release to 20 min (ng)} = 4.16 - (4.40 \times \text{CA}) - (4.23 \times \text{soy}) + (5.34 \times \text{CA} \times \text{soy}) \quad (1)$$

where CA = added citric acid, g/100 g wb, and soy = added soy protein hydrolysate, g/100 g wb.

The physical meaning of this model is readily visualized from a contour plot showing how acrylamide production varied with the additive concentrations (**Figure 8**). At high levels of each

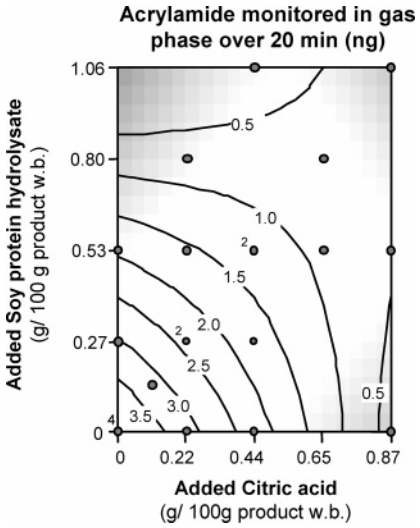


**Figure 8.** Contour plot showing the effects of citric acid and soy protein hydrolysate on acrylamide production monitored by the on-line MS/MS technique. Dark circles show the experimental design points. Numbers adjacent to design points show number of replicate measurements where applicable. Model $R^2 = 0.89$.

**Table 4.** Effect of Citric Acid and Soy Protein Hydrolysate on the pH of Potato Cake (1:1.3)[a]

| citric acid (g/100 g of product wb) | soy protein hydrolysate level (g/100 g of product wb) | pH |
|---|---|---|
| – | – | 5.53 |
| 0.22 | – | 4.48 |
| 0.44 | – | 3.93 |
| 0.87 | – | 3.36 |
| – | 0.27 | 5.56 |
| – | 0.53 | 5.54 |
| – | 1.06 | 5.46 |
| 0.22 | 0.27 | 4.51 |
| 0.44 | 0.53 | 3.95 |

[a] Data are the mean of two replicates, measured at 20 °C.

treatment, acrylamide generation was low, irrespective of whether treatments were applied individually or in tandem. However, at lower levels, there was an additive effect of the two treatments. For example, the model predicts that a combined treatment of 0.22 g/100 g citric acid and 0.27 g/100 g soy hydrolysate would result in 2.38 ng of acrylamide release, but when applied individually, more acrylamide is produced (3.01 ng for soy hydrolysate alone, 3.23 ng for citric acid alone). Acrylamide production for the control was modeled to be 4.16 ng. Hence, the combined treatment of 0.22 g/100 g CA and 0.27 g/100 g soy hydrolysate reduced acrylamide production by 43% (product pH 4.51; **Table 4**), while doubling the amount of each additive caused a reduction of 70% (product pH 3.95; **Table 4**).

Lowering product pH is thought to reduce acrylamide generation by protonating the α-amino group of asparagine, which subsequently cannot engage in nucleophilic addition reactions with carbonyl sources (*23*). The effect of citric acid addition alone was a 23.5% reduction in acrylamide at pH 4.48 (product pH lowered by 1.05 units) and 47% reduction at pH 3.93 (product pH lowered by 1.6 units). These reductions were less in percentage terms than those reported by Jung et al. (*23*) when modifying the pH of corn grits (also with citric acid) prior to frying. A reduction of 1.2 pH units reduced acrylamide generation during baking or frying of the grits by 49−58%;

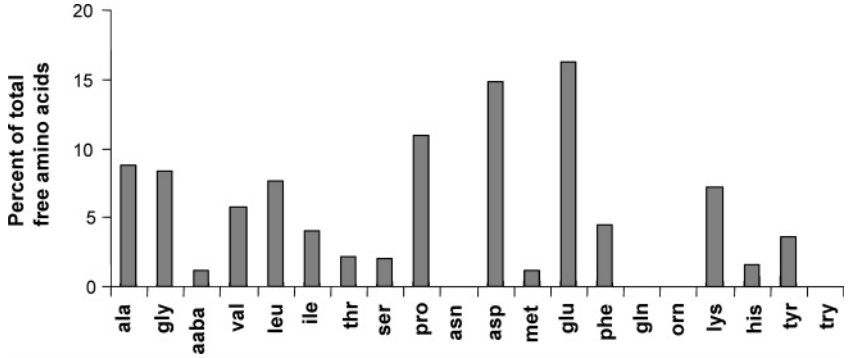


**Figure 9.** Amino acid profile of the soy protein hydrolysate sample used in the trial.

when product pH was lowered by 1.5 units, the reduction was 72−82%. The relationship between pH drop and reductions in acrylamide might vary between products, due to compositional factors, or could reflect different starting pH values of the products. The potato flake system was more acidic than either system investigated by Jung et al. (pH 5.5 as compared with 5.7 or 6.2). Jung et al. reported that the amount of acrylamide formed in buffered solutions of asparagine and glucose over the range pH 4−8 showed a dramatic reduction between pH 6 and pH 5 (*23*). Hence, there may have been less scope for reducing acrylamide by pH modification in the potato flake due to a lower starting pH. Furthermore, it is somewhat simplistic to consider the inactivation of asparagine as a nucleophile in isolation, without also considering the effects upon other amino acids and the course of the Maillard reaction as a whole.

Soy protein hydrolysate is believed to reduce acrylamide by introducing additional amino acids to compete with asparagine for key reaction intermediates (*17*). Since analysis of the amino acid profile of the hydrolysate sample (**Figure 9**) indicated that it contained almost no asparagine (120 mg/kg of the hydrolysate, or 0.012 wt %), it was eminently suited to this purpose. This finding is consistent with previously published data on the amino acid composition of soy protein (*24*, *25*). It is further possible that certain amino acids (e.g. lysine) added in soy protein hydrolysate might reduce free acrylamide via Michael addition reactions.

These findings suggest that combined treatments of citric acid and soy protein hydrolysate at lower rates of addition might be worthy of evaluation for their potential to reduce acrylamide in real food processing systems. However, the impact upon desirable Maillard products such as flavor and color remains to be evaluated. The efficacy of such treatments might further depend on the ratio of reducing sugars:total amino acids in a particular product, and this also requires investigation. In the current potato flake sample, amino acids were present in excess on a molar basis (**Table 2**), which favored a competitive strategy based upon lowering the molar percentage of amino acids represented by asparagine.

## ABBREVIATIONS USED

db, dry weight basis; wb, wet weight basis.

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