

Real-Time Monitoring of Thermal Flavor Generation in Skim Milk Powder Using Atmospheric Pressure Chemical Ionization Mass Spectrometry

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The feasibility of monitoring volatile flavor compounds formed by thermal treatment of skimmed milk powder in real time by atmospheric pressure chemical ionization mass spectrometry (APCIMS) was established. Skim milk powder samples were heated isothermally (70 to 120 °C) at different moisture contents (2.2 and 12.7 g water/100 g dry solids). Headspace was sampled and analyzed continuously in full scan mode (30–180 amu) by APCIMS. The identity of the volatile compounds monitored by APCIMS was confirmed by coupled GC–EI–APCIMS. The concentration measured by the APCIMS was the net effect of three processes, namely formation of the compound, partition from the skim milk powder into the gas phase, and dilution due to the headspace sampling method used. Preliminary experiments established that the technique could follow the effects of heating temperature and moisture content on the formation of selected compounds from skim milk powder.

KEYWORDS: On-line; Maillard; APCIMS; isothermal; moisture content

INTRODUCTION

Many food flavors are formed through thermal reaction pathways such as the Maillard reaction or caramelization. Most studies have focused on the chemistry of the pathways, usually in aqueous systems, as they provide a convenient, homogeneous phase for reaction. However, thermal flavor generation in many food products occurs either as the food matrix becomes dehydrated (e.g., bread crust) or in low water systems (e.g., caramel production where the matrix consists chiefly of fat, dairy proteins, and sugars). There is, therefore, much interest in understanding the effects of time, temperature, and water content on the reactions that create flavors in low moisture (<40%) foods. The system is dynamic and the factors are inter-related, which calls for appropriate experimental design as well as appropriate analytical methods.

Analytical methods used to study the rate and extent of the Maillard reaction include following the formation of colored compounds (1) or the depletion of starting materials (2). An on-line colorimetric method was described (3) for following the Maillard reaction in a continuous flow liquid system. Gogus et al. (4) measured the extent of Maillard reaction using ¹⁴C labeled precursors. Another approach has been to investigate intermediate formation in the Maillard reaction. Early intermediates such as the Amadori compound and the deoxy sugars have been studied (5), as have dicarbonyl compounds (6) and the tasteless intermediate 5,6-dihydro-3-hydroxypyridone (DHHP) (7).

With the exception of the on-line colorimetric method, all the methods involve a series of experiments under isothermal

conditions, followed by extraction and GC–MS to obtain the data necessary for an Arrhenius plot (e.g., 3, 8–10). There are several disadvantages with this approach. First, data generation is time-consuming as many analyses have to be carried out. Second, it is difficult to adapt the kinetics obtained under isothermal conditions to the changing time–temperature conditions found in real food processes. Third, few of the methods follow flavor compounds. Fourth, most of the methods are better-suited to high-water systems rather than low-water systems. If a real time analysis of flavor formation could be developed, many of the disadvantages listed above could be overcome.

Some reports of real-time analysis, applicable to thermal flavor generation in low-water systems, have appeared. Electronic noses have the potential for real-time measurements of many volatiles, and the formation of volatiles from Blockmilk during heating has been monitored (11). Because the electronic nose could not specifically identify the volatiles, the results indicated the overall course of the reaction, but were qualitative rather than quantitative measures of flavor formation. The use of direct mass spectrometric (MS) techniques for real-time monitoring of volatile organic compounds from macerated fruits (12, 13) and from food during eating has been achieved (14–16). Both proton transfer reaction (PTR) MS and atmospheric pressure chemical ionization (APCI) MS are capable of monitoring between 10 and 30 volatiles simultaneously with sensitivity in the ppb region. PTRMS has already been applied to the release of volatiles from roasting coffee (17).

The purpose of this paper is to test the feasibility of using direct APCIMS to continuously monitor volatiles in the headspace above heated skim milk powder (SMP). The issues of

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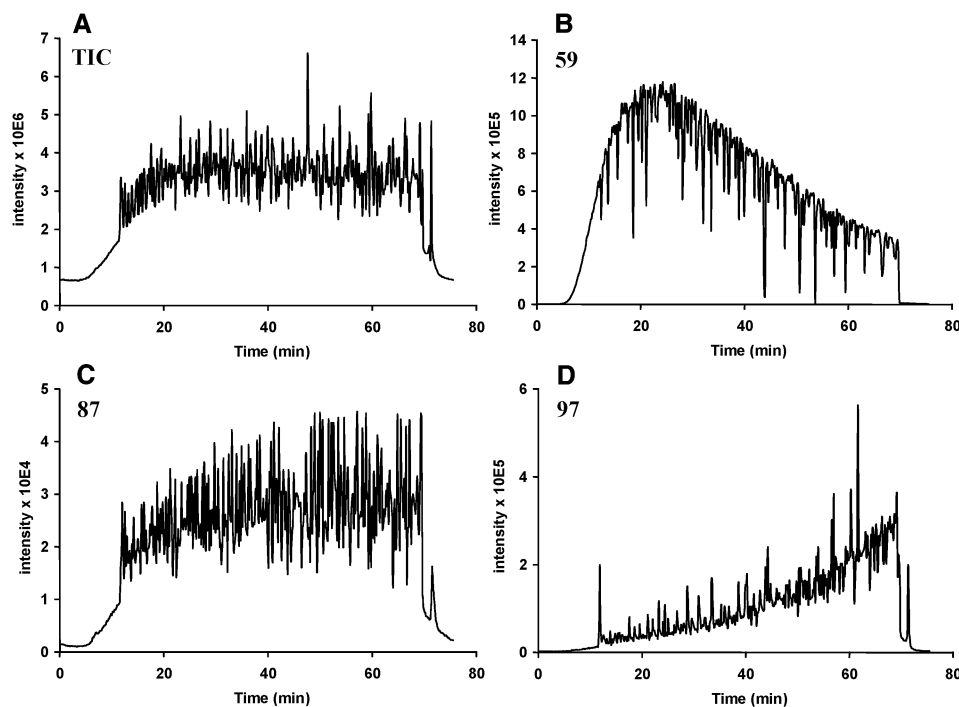


Figure 1. APCIMS time–intensity profiles for SMP heated at 90 °C with moisture content of 4.6 g/100 g dry solids. A, TIC (Total ion chromatogram); B, 59 (ion signal at m/z 59); C, 87 (ion signal at m/z 87); D, 97 (ion signal at m/z 97).

interfacing to a high-temperature vessel, sensitivity, and identification of the volatile flavors formed are addressed. Preliminary experiments with samples heated at different temperatures and different initial moisture contents show the potential of the technique.

MATERIALS AND METHODS

Sample Preparation. Skim Milk Powder (SMP; Sainsbury's, U.K.) was ground using a pestle and mortar before use. Moisture content was varied by storage of the dry material above saturated salt solutions (18) or by desiccation over phosphorus pentoxide, and was measured by vacuum oven drying at 70 °C to constant mass. To study the effect of temperature on the formation of selected compounds (shown later in Figures 4 and 5), samples were heated for 60 min between 70 and 120 °C at a moisture content of 3.0 g/100 g dry solids. To study the effect of moisture content (shown later in Figures 6 and 7), the initial moisture content of SMP was varied between 0 and 12.7 g/100 g dry solids (dsb) and all samples were heated at 90 °C for 60 min.

Sample Heating. SMP (6 g) was placed in a 100-mL Schott glass bottle fitted with a screw-top lid containing a loose-fitting 4-mm-diameter tube so that the contents remained at atmospheric pressure throughout the heating/cooling period. Samples were heated isothermally in an oven at temperatures between 70 and 120 °C for 60 min. Headspace (5 mL/min) was sampled from the 4-mm tube in the Schott bottle lid into the APCI ionization source through a heated (150 °C) transfer line. APCIMS (MS-NOSE, Micromass, Altrincham, UK) was operated in the gas phase using positive-ion, full-scan mode for the mass range 30–180 amu (Corona pin voltage 4 kV, source temperature 50 °C, scan time 10 s, and inter-scan delay 0.02 s). Data were collected using MassLynx software.

Identification of Ions Monitored on APCIMS by Coupled GC–EI–APIMS. A sample of SMP was heated for 6 h at 100 °C at a moisture content of 8.1 g/100 g dry solids in a GC oven with the lid loosely sealed. After the sample was cooled to 40 °C, headspace was collected onto a Tenax trap (SGE, Milton Keynes, U.K.) by flushing with nitrogen at 30 mL/min for 15 min. The Tenax trap was thermally desorbed (SGE) onto a BP-1 column (SGE; 30 m × 0.25 mm; film thickness 1 μm), and volatile compounds were cryotrapped by placing a section of the column in liquid nitrogen. Helium was the carrier gas with a head pressure of 20 psi. Temperature program was as follows:

30 °C for 2 min, 5 °C/min to 100 °C, 10 °C/min to 200 °C, and hold for 2 min. The exit of the column was split using a Y piece (SGE) and equal lengths of deactivated fused silica (SGE) led to the EI–MS and the APCIMS sources. The EI–MS was operated in full-scan mode, monitoring ions over a mass range of 25–200. Gas-phase APCIMS was operated in positive-ion, full-scan mode for the mass range 30–200 (scan time 0.4 s; inter-scan delay 0.02 s). Compounds were identified by using retention indices where authentic standards were available, or mass spectral matching (NIST library).

RESULTS AND DISCUSSION

Feasibility of On-Line Monitoring. Initially, the sensitivity of APCIMS was tested to ensure that it was adequate to monitor volatile flavor compounds as they were generated. There was also concern that the sampling of the headspace at high temperatures might lead to nonuniform flow and therefore erratic changes in headspace concentration. A simple system was set up using a headspace volume of 100 mL and a low sampling rate (5 mL/min) to address the sensitivity and flow problems mentioned above. The system was designed to be open to the atmosphere to avoid pressure changes, which would further complicate the situation, but this design also allowed loss of moisture from the sample during heating. It is worth pointing out that many low-water food systems are processed under these conditions, so the model system does represent the commercial process. Skim milk powder (SMP) was chosen as the material as it is easily obtainable and is a well-defined, real food system. It was heated at 90 °C and an initial moisture content of 4.6 g/100 g dry solids for 70 min.

The trace from APCIMS monitoring of the headspace above heated SMP is shown in Figure 1. Although the trace is “noisy”, there are clear trends in the trace, rather than random noise, indicating that sampling of headspace at elevated temperature is feasible. As it was not known which ion masses would be of interest, the headspace was monitored in full-scan mode (30–180 amu) which produced the total ion chromatogram (TIC, Figure 1A). The TIC represents all volatiles in the headspace and, after an initial increase, it remained fairly

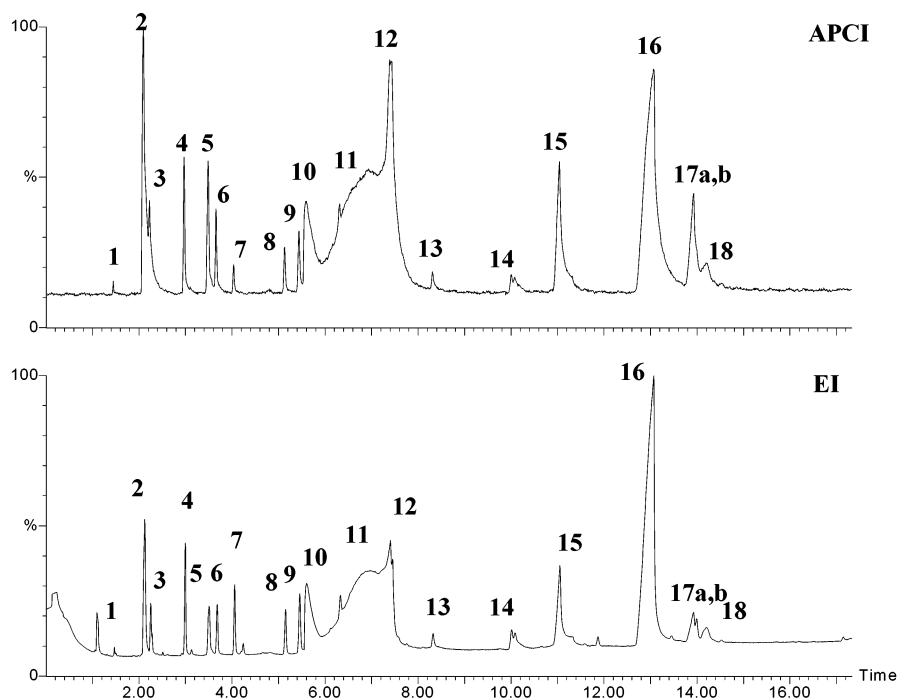


Figure 2. Chromatograms from GC analysis of headspace collected from a sample of skim milk powder (SMP; moisture content 8.1 g/100 g dry solids, heated 6 h at 100 °C) with simultaneous APCI (top) and EI (bottom) detectors. Numbers 1–18 = peaks identified in Table 1.

Table 1. Identification of GC, APCIMS, and EIMS Peaks

peak no.	API mass(es)	MW	identification	confirmation ^a
1	45	44	acetaldehyde	***
2	59	58	acetone	**
3	68	68	furan	***
4	73	72	2-methyl propanal	***
5	87	86	diacetyl	***
6	73 (102)	72	2-butanone	***
7	83	82	2-methyl furan	***
8	87 (85, 69)	86	3-methyl butanal	***
9	87 (85)	86	2-methyl butanal	***
10	47	60	oxybis methane	*
11	61	60	acetic acid	***
12	75	74	1-hydroxy-2-propanone	***
13	89	88	acetoin	***
14	101	100	2-methyl dihydrofuranone	*
15	97	96	furfural	***
16	98 (81)	98	furfuryl alcohol	***
17a	87 (85, 114)	?	unknown	
17b	126	126	furfuryl formate (overlapping peaks)	***
18	111	110	2-acetyl furan	***

^a Symbols are as follows: * = identification by library mass spectrum; ** = confirmation by mass spectrum of authentic standard; *** = confirmation by mass spectrum and LRI of authentic standard.

constant over the 60-min heating period. The TIC was then analyzed to extract individual ion profiles and study the pattern of production over the heating period. **Figure 1B, 1C, and 1D** shows the results for three ions (m/z 59, 87, and 97) which showed very different behaviors: one being produced early during heating, one being produced throughout heating, and the other produced only toward the end of the heating period. At this stage, the precise identities of the compounds relating to m/z values 59, 87, and 97 were not known, but they were established later.

Because heating was isothermal, these differences in time profiles may reflect the different stages of the Maillard reaction at which each compound is produced, but interpretation of the data needs care. The headspace concentration measured is the net result of generation of the volatile compounds, their partition

between the SMP and the gas phase, and dilution of the gas phase by removal of 5 mL/min to the APCIMS. Under isothermal conditions, partition would be constant during the experiment, but changes in the water content of the matrix might cause some change in partition (probably minor at 4.6 g water/100 g dry solids). Dilution by itself should be the same for all compounds, but the mechanism here is more “dynamic headspace dilution” which can create significant differences between the release of compounds in aqueous solution (19). However, the fact that the data in **Figure 1A, B, C, and D** show clear trends (and trends which seem to be compound-specific) suggests that these differences are real and that the data can be interpreted with some degree of certainty, at least under isothermal conditions.

The conclusions of the preliminary experiments, therefore, were that sensitivity was adequate to follow a range of volatile compounds (only three compounds are shown here for clarity, but it was possible to follow about 12 compounds), consistent sampling could be achieved (although smoothing the raw traces would simplify their analysis), and that there were observable differences in the time course of volatile production. The one remaining problem was the identity of the compounds, as the APCIMS could give only information on the ion mass of the compounds which did not allow unequivocal identification.

Identification of Volatile Compounds. To identify compounds, a sample of SMP was heated to 90 °C, then cooled to 40 °C, and headspace was collected on a Tenax trap (SGE). This was then desorbed onto a GC column connected to both EI-MS and APCIMS detectors. **Figure 2** shows the chromatograms obtained from the APCIMS and EI-MS detectors, both operating in full-scan mode. The traces were very similar qualitatively, indicating similar sensitivity to compounds between the two systems. The identities of 18 compounds were obtained by comparing the mass spectra from the EI detector with library matches, and/or the mass spectra and linear retention indices of authentic compounds (**Table 1**). In general, the APCI ion mass is equal to the molecular weight (MW) plus one, because the technique produces the protonated molecular ion

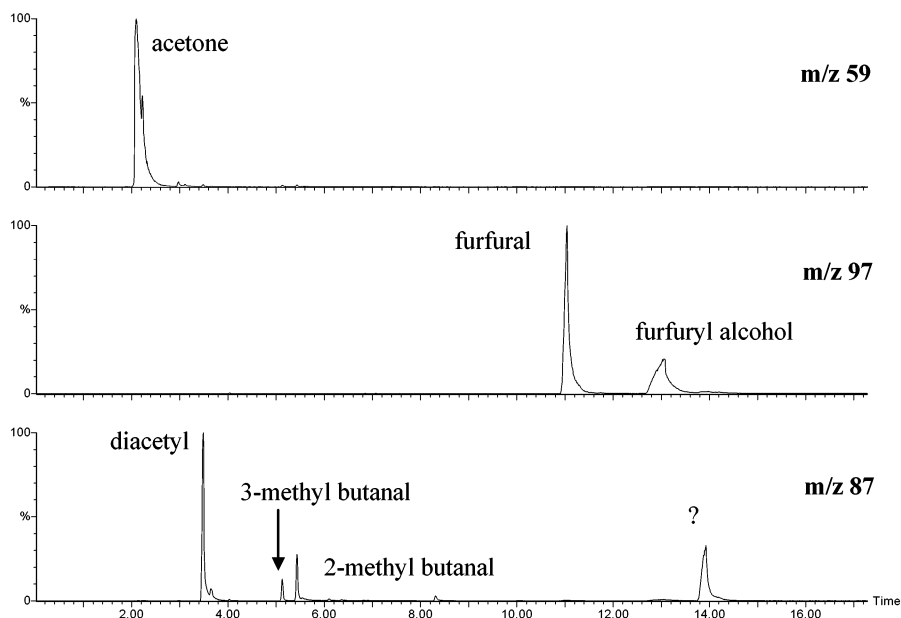


Figure 3. Extracted ion profiles from the APCI chromatogram shown in Figure 2.

(MH^+). Furan, furfuryl alcohol, and furfuryl formate are exceptions that do not add a proton, and this may be connected with delocalization of charge in the furan ring. Alcohols and, to some extent, aldehydes can undergo dehydration during APCIMS giving a $(M - H_2O + H)^+$ ion, and this can be seen in Table 1 for furfuryl alcohol and 3-methylbutanal. 2-Butanone appears to form a minor adduct giving an ion 29 mass units greater than the protonated molecular ion, while oxybis methane apparently fragments, forming an ion at m/z 47.

To determine whether the APCI ion mass was due to a single compound, extracted ion profiles were obtained from the APCI full scan data. Figure 3 shows that for ion mass 59, the only compound contributing was acetone; the trace contains no other peak at this ion mass. For ion mass 97, however, the main contribution was from furfural with some additional contribution from furfuryl alcohol. For ion mass 87, there were three known compounds and several unknown compounds (one of which coeluted with furfuryl formate) contributing throughout the trace. From these experiments, it was possible to assign compounds to APCI ion masses and determine the limits on data interpretation; for instance, the EI-MS trace contained some compounds not visible on the APCIMS trace.

The compounds identified were all of low molecular weight, and were known products of thermal flavor reactions such as the Strecker degradation (20). Larger, more complex Maillard products, such as maltol or furaneol, which may be of interest because they contribute to the flavor of a cooked product, are formed later in the course of the reaction (20, 21). As these are less volatile, they are less likely to be observed in the headspace and therefore less suitable for analysis using the headspace APCI technique.

Effect of Temperature. Having established the identity of the compounds monitored by APCIMS, further experiments were carried out to study the effect of different isothermal treatments (70 to 120 °C) on SMP at a moisture content of 3 g/100 g dry solids. The APCI profiles of the heated SMP generally increased in intensity with temperature. Smoothing the plots by averaging adjacent data points, as in Figure 4, enabled the effect of temperature on the production of acetone and furfural/furfuryl alcohol to be seen more clearly. Figure 4 also emphasizes the different time-courses of production for the

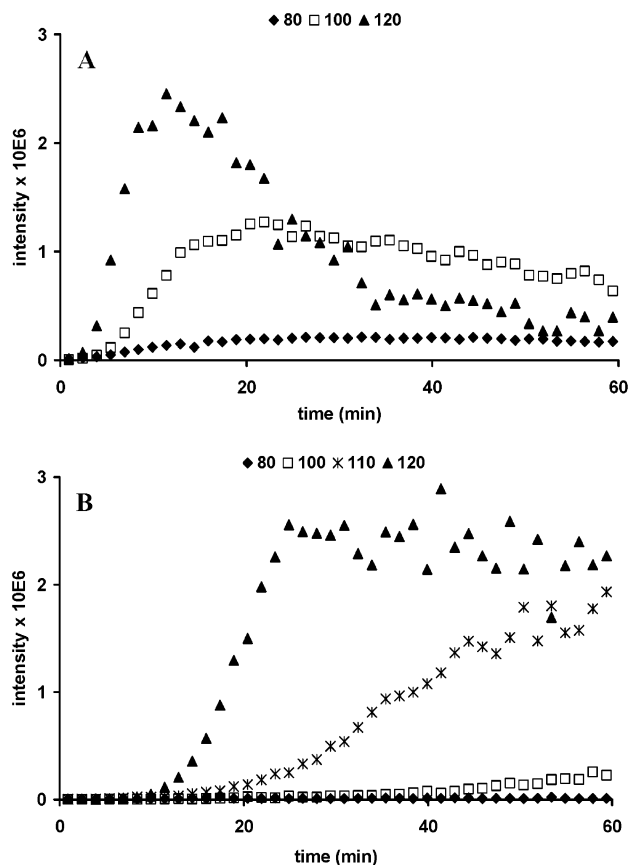


Figure 4. Time-intensity plots (smoothed and averaged every 1.5 min) showing production of acetone (A) and furfural/furfuryl alcohol (B) from SMP (3 g water/100 g dry solids) heated isothermally at different temperatures. Legend is temperature (°C). Plots A and B were obtained from the extracted ion chromatograms of m/z 59 and 97, respectively, and intensity refers to the signal for these ions.

two compounds. Increasing the temperature increased not only the maximum amount of a compound, but also meant that it reached its maximum earlier in the heating period. The general shape of the time profile is the same at each temperature, indicating the same processes are taking place.

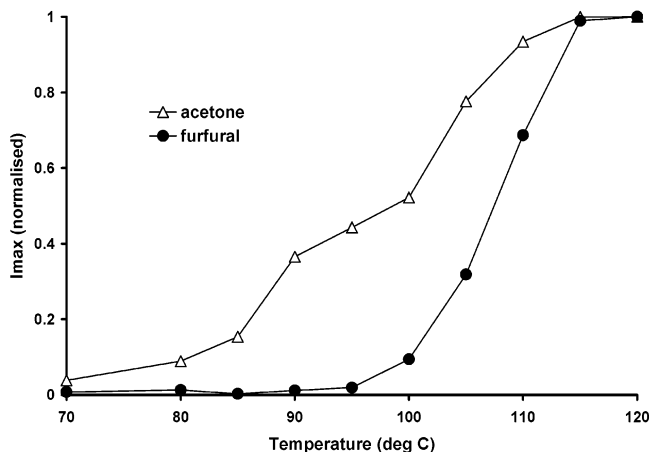


Figure 5. Effect of heating temperature on production of acetone and furfural/furfuryl alcohol from SMP at 3 g water/100 g dry solids. Amounts of acetone and furfural/furfuryl alcohol are expressed as the maximum amount formed, normalized to show the relative changes.

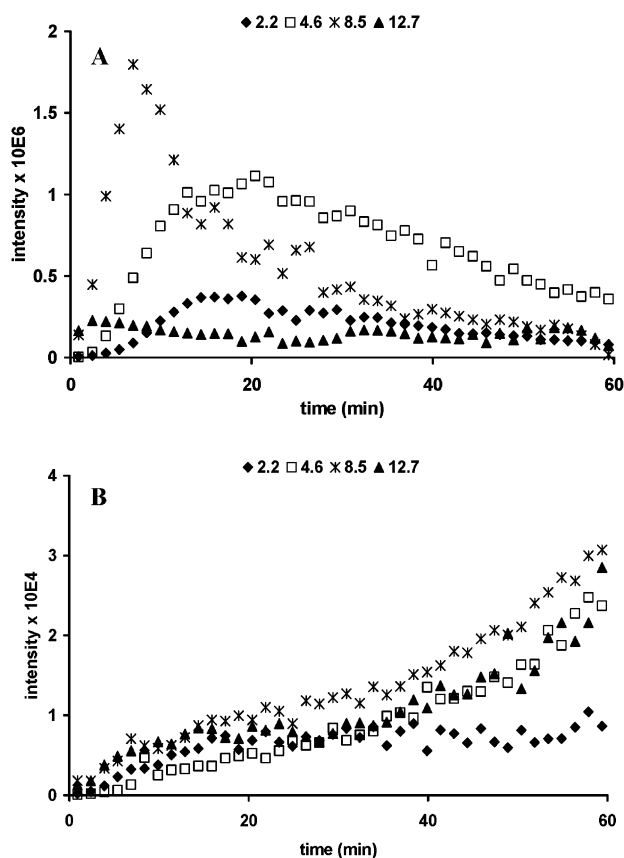


Figure 6. Time-intensity plots (smoothed and averaged every 1.5 min) showing production of acetone (A, m/z 59) and furfural/furfuryl alcohol (B, m/z 87) at four moisture contents (all heated at 90 °C). Legend is moisture content expressed as g pf water per 100 g of dry solids, and intensity refers to the signal obtained for the m/z 59 and m/z 97 ions.

The maximum intensity (I_{max}) reached for each compound at each temperature was calculated, and the data were normalized against the highest value for each compound to enable easy comparison (**Figure 5**). I_{max} values increased with temperature for each compound, but at different rates. For example, the amount of acetone increased over the whole temperature range, but furfural/furfuryl alcohol started to increase only above 95 °C. The differences in **Figure 5** may reflect different activation energies for the different compounds. The data in **Figures 4**

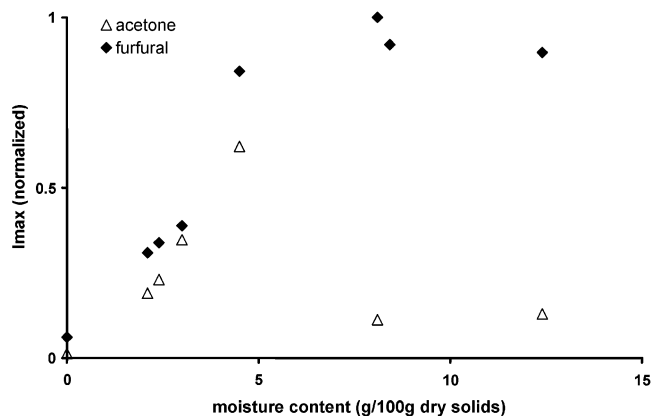


Figure 7. Effect of moisture content on production of acetone and furfural from SMP heated at 90 °C. The concentrations of acetone and furfural are expressed as normalized values.

and **5** are further evidence that the headspace-APCIMS monitoring is consistent and can produce valid, real-time data for the volatile compounds monitored here.

Effect of Moisture Content. Because the rate of thermal flavor generation is dependent on temperature and moisture content, experiments were carried out with SMP hydrated to different starting moisture contents. For acetone (m/z 59) there was an increase in production up to a moisture content of 8.5 g/100 g dry solids but a decrease at 12.7 g/100 g dry solids (**Figure 6A**). This may represent the interplay between reactant mobility (increases with increasing moisture) and the rate of dehydration reactions (decreases with increasing moisture content) (22). For ion mass 97 (furfural and furfuryl alcohol) little difference was seen in reaction at any of the moisture contents (**Figure 6B**). Furfural production was slow because of the relatively low heating temperature of 90 °C (compared with **Figures 4** and **5**). However, when the data from **Figure 6** were expressed as maximum volatile intensity plotted against moisture content, clear trends could be seen (**Figure 7**).

CONCLUSIONS

The production of some selected volatile compounds from heated SMP was successfully monitored in real time using APCIMS. This method enabled time profiles of different compounds to be compared and has the potential to provide kinetic data. Different conditions could be distinguished, both in their effect on the rate of production and the overall amount generated. Changes in temperature or moisture content resulted in changes in reaction.

Many of the volatile compounds monitored by the APCIMS method were identified using GC with simultaneous EIMS and APCIMS detection. A limitation of the APCI method was that some ion masses corresponded to several compounds, whose individual time-profiles could not, therefore, be measured.

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