Analysis of Thermally Produced Compounds in Foods by Thermospray Liquid Chromatography–Mass Spectrometry and Gas Chromatography–Mass Spectrometry

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The feasibility of using thermospray liquid chromatography–mass spectrometry using water as chemical ionization (CI) reagent in discharge mode for molecular weight determination was demonstrated with two known thermally produced compounds. Subsequently, the method was used to determine the molecular weight of a third compound thermally produced in meats. The same molecular weight was obtained using pure water as CI reagent with discharge and a 0.1 M ammonium acetate solution without discharge. The molecular weight of 114 was confirmed by gas chromatography–mass spectrometry (GC–MS). An electron impact mass spectrum obtained by GC–MS matched a previously published spectrum for 4-hydroxy-5-methyl-3(2*H*)-furanone.

Keywords: *Chemical markers; thermospray LC–MS; GC–MS*

INTRODUCTION

We have been investigating the feasibility of using thermally produced compounds in foods as indicators of time–temperature exposure, particularly at the center of food particles undergoing a continuous aseptic processing at high temperatures (125–135 °C). Since direct temperature measurement at the center of a flowing particle is very difficult, it is expected that the yield of such marker compounds could be used to estimate the lethality delivered to the cold spot of the particulate food using calibration established in the laboratory by chemical and microbiological measurements (Ramaswamy et al., 1994).

Numerous compounds are thermally produced in foods, and many were identified by gas chromatography–mass spectrometry (GC–MS) (MacLeod and Ames, 1986). Many of these thermally produced compounds might have a potential to be used as time–temperature integrators. However, the ease of quantitative determination from a complex food is one of the key requirements. We have selected three compounds as potentially useful markers, because they are easily determined by HPLC and formed from precursors commonly present in foods (Kim and Taub, 1993). Two compounds, M1 and M3, were identified as 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one and 5-(hydroxymethyl)furfural, respectively, by GC–MS. This paper reports a positive identification of the third marker, M2, by thermospray liquid chromatography–mass spectrometry (TSP-LC-MS) and GC–MS. We are particularly interested in M2, because it is formed in meats in addition to M1 and their concurrent determination offers several advantages over determination of one marker (Kim, 1993; Ross, 1993).

Thermospray LC–MS is a useful technique for determination of molecular weight of nonvolatile compounds (Blakley and Vestal, 1983; Vestal, 1984). Recently, TSP-LC-MS was used for a variety of compounds including glycosides (Iida and Murata, 1991), phenylurea herbicides (Bagheri et al., 1992), cyclosporins (Abian et al., 1992), sugar oligomers (van der Hoeven et al., 1992), and 25-hydroxyvitamin D_3 (Vicchio et al., 1993).

In thermospray vaporization, a supersonic jet of aerosol with high solute concentration is created as the liquid stream flows through a heated capillary tube. In direct thermospray ionization LC–MS, 0.1 M ammonium acetate is often used as the chemical ionization reagent (Garteiz and Vestal, 1985; Arpino, 1990, 1992). It is believed that clustered ammonium ions are formed as primary thermospray products and the analyte molecules are ionized in the gas phase by protonation from these ammonium ions (Alexander and Kebarle, 1986). In the external ionization mode, a solvent ion plasma is produced by an electron beam ("filament-on" mode) or an electrical discharge ("discharge" mode). These solvent ions are used to ionize the analyte by chemical ionization (CI) reactions. Pure methanol and acetonitrile without ammonium acetate have been shown to carry out filament-on or discharge ionization (Arpino, 1990).

We have been using anion exclusion chromatography (AEC) with UV detection using 10 mM sulfuric acid as eluant for determination of these intrinsic chemical markers (ICM). It was not desirable to introduce sulfuric acid into the thermospray system while attempting to see if AEC and TSP-LC-MS could be used in tandem for molecular weight determination of M2. We found that a similar UV chromatogram was obtained using 100% water. The question was whether

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pure water could be used to generate solute ions in TSP-LC-MS.

In limited cases thermospray mass spectra were obtained without ammonium acetate in filament-on or discharge mode. For example, insecticides were analyzed by TSP-LC-MS using water/methanol gradient (Yang and Vestal, 1987), and triglycerides were analyzed using acetonitrile/methylene chloride gradient (Sanders and Charpentier, 1987). Steroids were analyzed with 100% methanol (Das et al., 1988). However, there has been no report of using pure water in TSP-LC-MS to our knowledge. Therefore, it was of interest to find out whether molecular weight information could be obtained using pure water as eluant in TSP-LC-MS.

We were also interested in using TSP-LC-MS as a mass selective detector for the markers from a complex mixture derived from thermally processed foods. In this paper, we demonstrate the capability of combined TSP-LC-MS and GC–MS for determination of the chemical identity using a partially purified compound as well as for selectively determining thermally produced compounds in a complex mixture.

EXPERIMENTAL PROCEDURES

Sample Preparation. A sample containing M1 and M3 was prepared by heating 10% D-fructose solution in a sealed glass ampule for 30 min in a 131 °C oil bath (Fisher Scientific, Pittsburgh, PA). To make sample containing M2, 10 g of beef was homogenized with 10 mL of water using a Polytron (Brinkmann Instruments, Westbury, NY), and the homogenate was centrifuged. A 5 mL aliquot of the supernatant was heated in a sealed ampule (Wheaton, Millville, NJ) for 30 min in a 131 °C oil bath, centrifuged, and filtered using a 0.45 μm Nylon 66 membrane filter (Alltech, Deerfield, IL). The filtrate was freeze-dried and reconstituted in a smaller volume of water to achieve 5-fold concentration. The concentrated material was loaded on a 3 × 25 cm gel filtration (Bio-Gel P-2, Bio-Rad, Hercules, CA) column and eluted with deionized water. Collected fractions (1.6 mL/fraction) were analyzed for M2 by AEC with photodiode array (PDA) (Model 990, Waters, Milford, MA) detection (Kim and Taub, 1993). Fraction 51 (hereafter called M2-A), which contained the highest concentration of M2, was kept at 4 °C until analysis by LC–MS or GC–MS. A 100 μL aliquot of the concentrated material was also injected into the AEC-PDA system and the eluting M2 was collected (called M2-B).

GC–MS. GC–MS analysis was performed using a Hewlett-Packard Model 5890 Series II gas chromatograph coupled to a Series 5971 mass selective detector. An HP Ultra 2 capillary column (5% cross-linked phenylmethyl silicone, 25 m × 0.2 mm, film thickness 0.33 μm) was used with helium as the carrier gas and a flow rate of 0.7 mL/min. Oven temperature was 115 °C throughout the analysis. Injector temperature and detector temperature were 200 and 280 °C, respectively. A 1 μL volume of the aqueous sample was injected directly.

TSP-LC-MS. Alltech anion exclusion column (cross-linked polystyrene/divinylbenzene, 7.8 × 150 mm) was used for LC separation. The MS system included a Vestec (Houston, TX) thermospray interface and a Hewlett-Packard 5970A mass spectrometer. The sample injection volume was 10–50 μL; 100% deionized water was used as eluant at a 1 mL/min flow rate most of the time. The variable-wavelength UV detector was used at 285 nm. The thermospray interface–mass spectrometer system was operated in both positive and negative ion mode with discharge ionization. A 0.1 M ammonium acetate solution was used for direct thermospray ionization to confirm results by discharge ionization.

RESULTS AND DISCUSSION

TSP-LC-MS of M1 and M3. When fructose is heated, both M1 and M3 are formed along with other compounds via loss of 2 and 3 mol of water, respectively (Shaw et al., 1967, 1971; Kim and Taub, 1993). The M1 and M3 in the heated fructose were used to see whether thermospray mass spectra could be obtained using 100% water as the CI reagent.

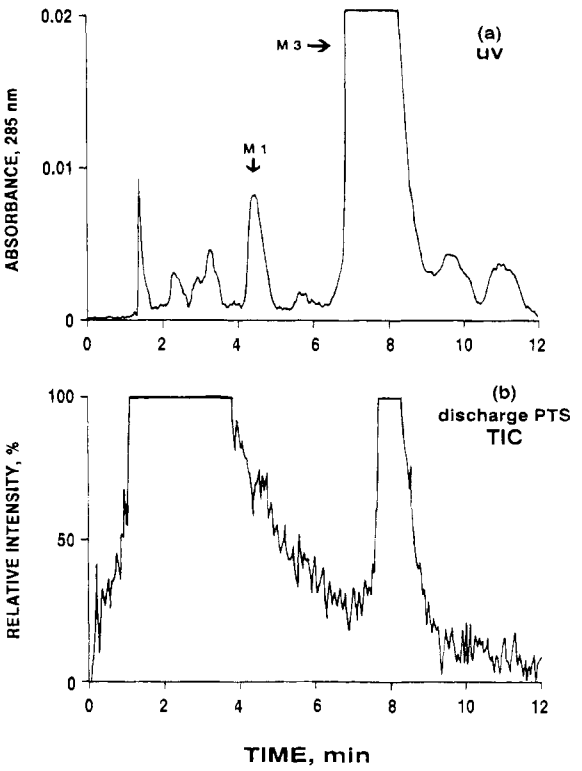

Figure 1. (a) Chromatogram of compounds in heated fructose separated by ion exclusion chromatography using water as eluant and detected by UV at 285 nm; (b) TIC mass chromatogram from the same separation obtained using PTS with discharge.

Figure 1 shows the UV chromatogram at 285 nm (a) for the heated fructose sample and the total ion current (TIC) mass chromatogram (b) obtained in tandem by positive ion thermospray with discharge using 100% water as eluant. The UV chromatogram shows a minor peak at 4.6 min corresponding to M1 and a major peak at 8 min for M3. Unheated fructose does not absorb at 285 nm. The signal intensity in the TIC chromatogram was concentrated around 2.5 min corresponding to unreacted fructose and other degradation products. The 2.5 min retention time for fructose was confirmed by injecting a solution of fructose and detecting it at 210 nm. The TIC chromatogram also showed a strong peak corresponding to M3 at 8 min. However, the signal due to M1 at 4.6 min was hardly discernible from the background noise in the TIC chromatogram. Overall, the TIC chromatogram resembled a UV chromatogram obtained at a less selective wavelength of 210 nm instead of 285 nm.

The TIC mass chromatogram was scanned between 4.4 and 5.0 min in the *m/z* 105–250 range. As shown in Figure 2a, *m/z* 145 was the predominant signal corresponding to $(M1 + H)^+$ as observed before for 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (MW 144) (R. T. Rosen, Center for Advanced Food Technology, Rutgers University, personal communication, 1991). A smaller signal at *m/z* 163 corresponding to $(M1 + H_2O + H)^+$ was also observed. In a separate negative ion thermospray (NTS) experiment, *m/z* 144 was observed as a predominant signal corresponding to electron-capture negative chemical ionization.

The 7.5–8.5 min scan of the TIC shows *m/z* 127 for $(M3 + H)^+$, corresponding to 5-(hydroxymethyl)furfural

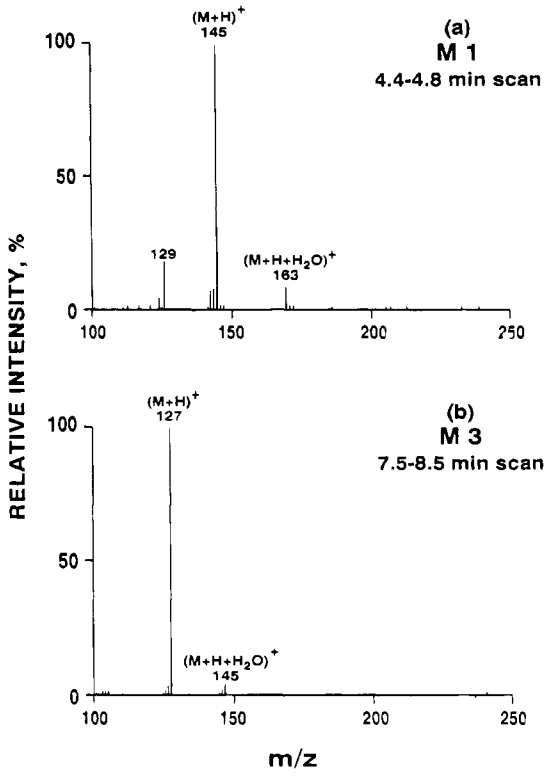

Figure 2. (a) PTS mass spectrum of M1 obtained by 4.4–4.8 min scan of the above TIC mass chromatogram; (b) PTS mass spectrum of M3 obtained by 7.5–8.5 min scan.

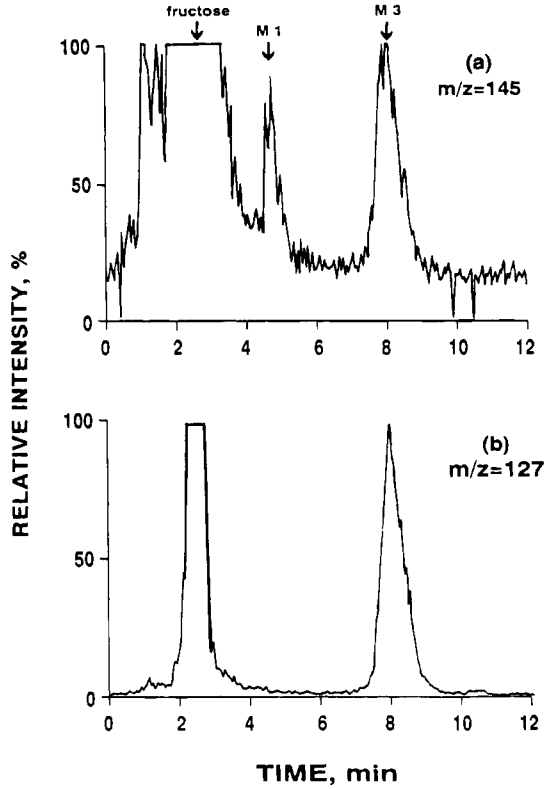

Figure 3. (a) Selective mass spectrum (m/z = 145) obtained from the TIC mass chromatogram in Figure 1b showing M1; (b) selective mass spectrum (m/z = 127) showing M3 at 8 min.

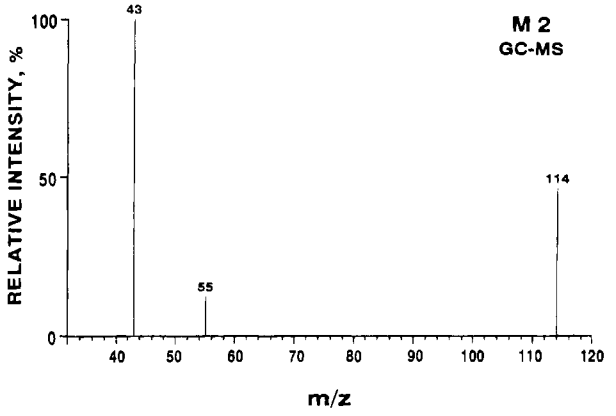

Figure 4. EI spectrum of M2 obtained by GC–MS analysis.

(MW 126) (Figure 2b). A signal at m/z 145 was observed at a lower intensity for $(M3 + H_2O + H)^+$. These results indicate that, in discharge mode, 100% water is a good source of protons and can be used as the reagent gas to carry out a less energetic positive chemical ionization for direct molecular weight information. In a separate experiment in the negative thermospray mode, m/z 125 was observed corresponding to $(M3 - H)^-$. In general, positive thermospray results were less noisy than negative thermospray results and more suitable for direct molecular weight information. As a matter of fact, with all three markers $(M + 1)^+$ was always the predominant peak, which makes molecular weight determination quite straightforward. When ammonium acetate was used, both $(M + 18)^+$ and $(M + 1)^+$ peaks were observed.

The TIC chromatogram in Figure 1 was scanned for m/z 145 to generate a selective mass chromatogram for M1 (Figure 3a). The M1 signal, which was not discernible in the TIC chromatogram, was observed at 4.6 min. The m/z 145 peak at 8 min is due to $(M3 + H_2O + H)^+$. The ratio of M3/M1 signal in Figure 3a is smaller than that in Figure 1a, because the $(M3 + H_2O + H)^+$ signal is much smaller than $(M3 + H)^+$ as shown in Figure 2b. The large peak at 2.5 min is believed to be due to $(\text{fructose} - 2H_2O + H)^+$. The mass chromatogram for m/z 127 in Figure 3b shows $(M3 + H)^+$ at 8 min and a larger peak at 2.5 min due to $(\text{fructose} - 3H_2O + H)^+$. These results show that TSP-LC-MS can be used for mass selective detection of the markers. However, there were no real advantages over the simpler UV detection, because there are no coeluting compounds that absorb at 285 nm when anion exclusion column is used.

GC–MS of M2. When the M2 sample purified by gel filtration (M2-A) was analyzed by GC–MS, a predominant TIC signal was observed at 3.6 min. When the TIC chromatogram was scanned at 3.6 min, three signals corresponding to m/z 114, 55, and 43 were obtained (relative intensity 47:12:100, Figure 4). Unfortunately, we could not find an EI spectrum from a computer library that matched the observed spectrum. Therefore, we could not be certain whether m/z 114 corresponds to the parent molecular ion or not. Also, the EI spectrum was too simple to allow construction of possible structures.

TSP-LC-MS of M2. We turned to TSP-LC-MS for definitive molecular weight determination for M2. M2 was partially purified by both gel filtration (M2-A) and anion exclusion chromatography (M2-B). When M2-A was analyzed by anion exclusion column with a UV detector and the thermospray mass spectrometer connected in tandem, results in Figure 5 were obtained. The UV chromatogram at 285 nm (Figure 5a) showed a major peak at 5.8 min corresponding to M2. The sample was also analyzed using a PDA detector. The compound with 5.8 min retention time showed an absorption maximun at 285 nm.

Figure 5b shows a positive thermospray TIC chromatogram obtained from M2-A in discharge mode. A small peak was observed at 5.8 min in addition to larger

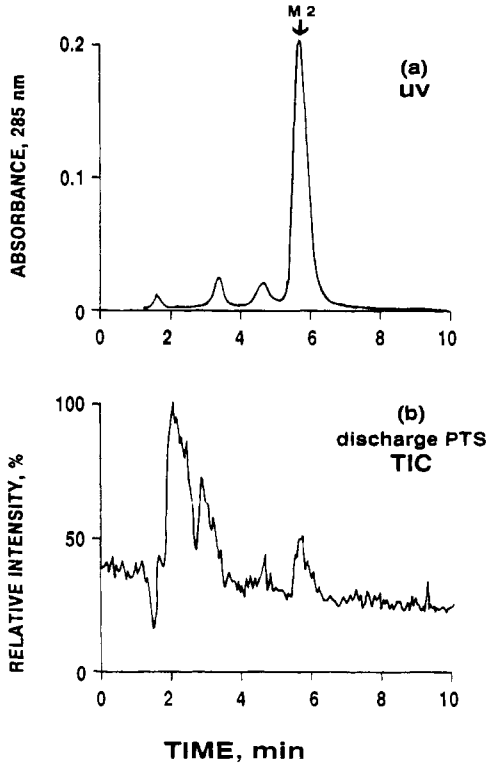

Figure 5. (a) UV chromatogram of partially purified M2; (b) TIC mass chromatogram of the same sample obtained by PTS with discharge.

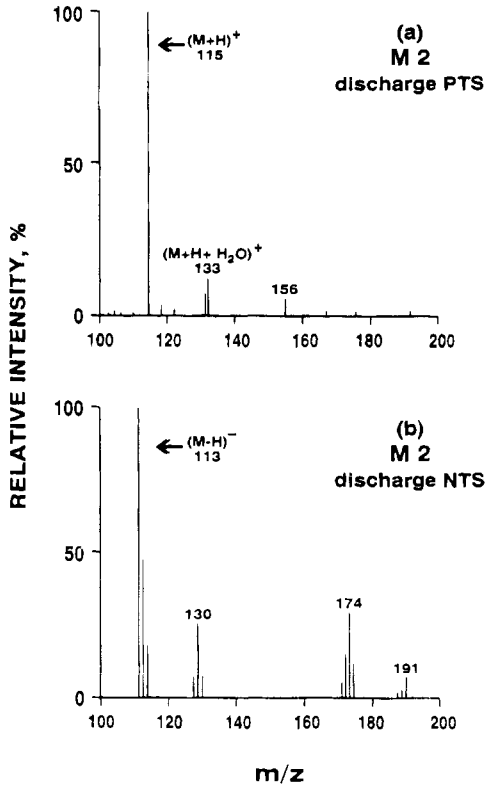

Figure 6. (a) PTS mass spectrum of M2 obtained by 5.6–6.0 min scan of the TIC mass chromatogram in Figure 5b; (b) NTS mass spectrum of M2 obtained similarly from the NTS-TIC mass chromatogram.

peaks at 2–3.5 min due to impurities which do not absorb strongly at 285 nm. When the TIC chromatogram was scanned at 5.6–6.0 min, a signal with *m/z* 115 possibly corresponding to $(M + H)^+$ was observed as a major peak (Figure 6a). A smaller peak was also observed at *m/z* 133 possibly corresponding to $(M + H_2O + H)^+$. A similar scan from a negative thermospray chromatogram showed a major peak at *m/z* 113 possibly corresponding to $(M - H)^-$ (Figure 6b). These PTS-LC–MS results suggest that the molecular weight of M2 is 114 as observed by GC–MS.

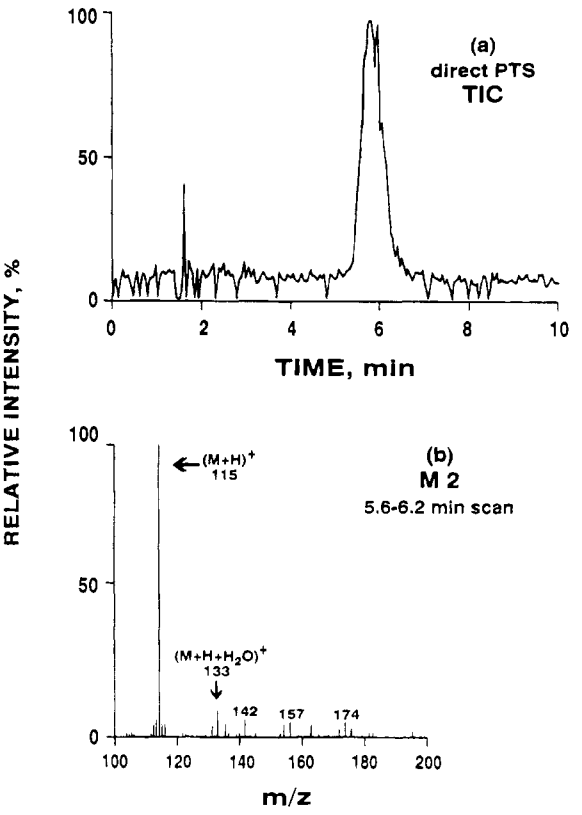

Figure 7. (a) TIC mass chromatogram of purified M2 obtained by direct PTS using 0.1 M ammonium acetate eluant; (b) PTS mass spectrum of M2 obtained by 5.6–6.2 min scan of the TIC mass chromatogram.

The M2 sample collected from the anion exclusion column (M2-B) was analyzed by direct PTS without discharge using a 0.1 M ammonium acetate solution as eluant. The M2 sample was also analyzed by discharge PTS using pure water. Figure 7a shows a single major peak at 5.8 min. A similar chromatogram was obtained by UV detection at 285 nm, suggesting that this sample contains M2 at a very high purity. A predominant peak with *m/z* 115 was observed by scanning the TIC chromatogram at 5.6–6.0 min (Figure 7b). A small peak with *m/z* 133 was also observed as in Figure 6a. Same results were obtained from M2-B sample by discharge PTS. Thus the molecular weight of M2 was determined as 114 from two different preparations by three different methods, i.e., GC–MS, TSP-LC-MS in discharge mode using water, and direct thermospray LC–MS using ammonium acetate.

Identification of M2. Once convinced that the molecular weight of M2 is 114, we reexamined the EI spectrum obtained by GC–MS. The unique feature of the EI spectrum was a strong signal with *m/z* 43. There are three classes of volatile compounds in food that show *m/z* 43 as the strongest signal (ten Noever de Brauw et al., 1979): (1) compounds containing an acetyl group, such as diacetyl, 2,3-pentanedione, 2-acetylthiazole, and 2-acetyl-3-methylpyrazine; (2) compounds containing a propyl or isopropyl group; and (3) 5-methylfurans, such as 2-acetyl-5-methylfuran and 5-methylfuranones. Both classes 1 and 3 yield a fragment corresponding to $CH_3-C{\equiv}O^+$ with *m/z* 43.

The high intensity of the parent ion at 114 (Figure 4) suggests that M2 is a cyclic compound (ten Noever de

Brauw et al., 1979). The UV absorption maximum of 285 nm also suggests that M2 is a cyclic compound with conjugated double bonds. M1, which belongs to class 3, has an absorption maximum at 298 nm. Moreover, M1 and M2 have a similar chromatographic behavior on the anion exclusion column, suggesting that M1 and M2 are structurally similar.

On the basis of these considerations, we could come up with four possible (hydroxymethyl)furanone structures consistent with a molecular weight of 114. A compound, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, which is related to one of the (hydroxymethyl)furanone structures by having a methyl group instead of a hydrogen atom (MW = 128), was reported (Rodin et al., 1965). This compound has an absorption maximum at 289 nm and an EI spectrum with *m/z* 43 as the strongest peak like M2. It is responsible for burnt pineapple flavor. When an aqueous extract from canned pineapple was analyzed by anion exclusion chromatography, a compound was observed with the same retention time as M2 and a UV absorption maximum at 289 nm. The same retention time indicated that M2 is closely related to 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone.

A literature search on (hydroxymethyl)furanones yielded a reference for 4-hydroxy-5-methyl-3(2*H*)-furanone, which showed an EI spectrum identical to that in Figure 4 and an absorption maximum at 287 nm (Tonsbeek et al., 1968). What was most striking to us was that this compound was produced from beef broth, which was consistent with our first observation of M2 in heated beef. Tonsbeek et al. (1969) also identified ribose 5-phosphate as a natural precursor of 4-hydroxy-5-methyl-3(2*H*)-furanone. Indeed, we observed that when beef was heated with either ribose or ribose 5-phosphate, the yield of M2 was greatly enhanced. From these observations, we positively identified M2 as 4-hydroxy-5-methyl-3(2*H*)-furanone.

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LITERATURE CITED

Abian, J.; Stone, A.; Morrow, M. G.; Creer, M. H., Fink, L. M.; Lay, J. O., Jr. Thermospray High-Performance Liquid Chromatography/Mass Spectrometric Determination of Cyclosporins. *Rapid Commun. Mass. Spectrom.* **1992**, *6*, 684.

Alexander, A. J.; Kebarle, P. Thermospray Mass Spectrometry. Use of Gas-Phase Ion/Molecule Reactions to Explain Features of Thermospray Mass Spectra. *Anal. Chem.* **1986**, *58*, 471.

Arpino, P. Combined Liquid Chromatography Mass Spectrometry. Part II. Techniques and Mechanisms of Thermospray. *Mass Spectrom. Rev.* **1990**, *9*, 631.

Arpino, P. Combined Liquid Chromatography Mass Spectrometry. Part III. Applications of Thermospray. *Mass Spectrom. Rev.* **1992**, *11*, 3.

Bagheri, H.; Brouwer, E. R.; Ghijsen, R. T.; Brinkman, U. A. Th. Trace-Level Enrichment of Phenylurea Herbicides from Surface and Drinking Water Coupled On-Line with Column Liquid Chromatography-Thermospray Mass Spectrometry. *Analusis* **1992**, *20*, 475.

Blakley, C. R.; Vestal, M. L. Thermospray Interface for Liquid Chromatography/Mass Spectrometry. *Anal. Chem.* **1983**, *55*, 750.

Das, P. R.; Pramanik, B. N.; Malchow, R. D.; Ng, K. J. Comparison of Methanol Thermospray (filament on) and Direct Chemical Ionization Mass Spectrometry to the study of Biologically Important Steroids. *Biomed. Environ. Mass Spectrom.* **1988**, *15* (5), 253.

Garteiz, D. A.; Vestal, M. L. Thermospray LC/MS Interface: Principles and Applications. *LC Mag.* **1985**, *3*, 334.

Iida, J.; Murata, T. Application of Thermospray Liquid Chromatography/Mass Spectrometry to Analysis of Glycosides. *Anal. Sci.* **1991**, *7*, 963.

Kim, H.-J. Two Marker Approach for Validating Aseptic Processing of Particulate Foods. *Act. Rep. R&D Assoc.* **1993**, *45*, 28.

Kim, H.-J.; Taub, I. A. Intrinsic Chemical Markers for Aseptic Processing of Particulate Foods. *Food Technol.* **1993**, *47* (1), 91.

MacLeod, G.; Ames, J. M. Capillary Gas Chromatography-Mass Spectrometric Analysis of Cooked Beef Aroma. *J. Food Sci.* **1986**, *51*, 1427.

Ramaswamy, H. S.; Awuah, G.; Kim, H.-J.; Choi, Y.-M. Evaluation of a Chemical and a Microbiological Technique for Process Lethality Measurement in an Aseptic Processing Holding Tube Simulator. Presented at the R&D Associates Fall meeting, Boston, MA, Oct 1994.

Rodin, J. O.; Himel, C. M.; Silverstein, R. M. Volatile Flavor and Aroma Components of Pineapple. I. Isolation and Tentative Identification of 2,5-Dimethyl-4-Hydroxy-3(2H)-Furanone. *J. Food Sci.* **1965**, *30*, 280.

Ross, E. W. Relation of Bacterial Destruction to Chemical Marker Formation during Processing by Thermal Pulses. *J. Food Process Eng.* **1993**, *16*, 247.

Sanders, R. A.; Charpentier, B. A. Thermospray LC/MS of Triglycerides. Presented at the 35th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, CO, May 24–29, 1987.

Shaw, P. E.; Tatum, J. H.; Berry, R. E. Acid-catalyzed degradation of D-fructose. *Carbohydr. Res.* **1967**, *5*, 266.

Shaw, P. E.; Tatum, J. H.; Berry, R. E. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, a Degradation Product of a Hexose. *Carbohydr. Res.* **1971**, *16*, 207.

ten Noever de Brauw, M. C.; Bouwman, J.; Tas, A. C.; La Vos, G. F. *Compilation of Mass Spectra of Volatile Compounds in Food*; Central Institute for Nutrition and Food Research: Zeist, Netherlands, 1979.

Tonsbeek, C. H. T.; Plancken, A. J.; Weerdhof, T.v.d. Components Contributing to Beef Flavor. Isolation of 4-Hydroxy-5-methyl-3(2H)-furanone and its 2,5-Dimethyl Homolog from Beef Broth. *J. Agric. Food Chem.* **1968**, *16*, 1016.

Tonsbeek, C. H. T.; Koenders, E. B.; van der Zijden, A. S. M.; Losekoot, J. A. Components Contributing to Beef Flavor. Natural Precursors of 4-Hydroxy-5-methyl-3(2H)-furanone in Beef Broth. *J. Agric. Food Chem.* **1969**, *17*, 397.

van der Hoeven, R. A. M.; Niessen, W. M. A.; Schols, H. A.; Bruggink, C.; Voragen, A. G. J.; van der Greef, J. Characterization of Sugar Oligomers by On-Line High-Performance Anion-Exchange Chromatography-Thermospray Mass Spectrometry. *J. Chromatogr.* **1992**, *627*, 63.

Vestal, M. L. High-Performance Liquid Chromatography-Mass Spectrometry. *Science* **1984**, *226*, 275.

Vicchio, D.; Yergey, A.; O'Brien, K.; Allen, L.; Ray, R.; Holick, M. Quantification and Kinetics of 25-Hydroxyvitamin D_3 by Isotope Dilution Liquid Chromatography/Thermospray Mass Spectrometry. *Biol. Mass Spectrom.* **1993**, *22*, 53.

Yang, L.; Vestal, C. H. Analysis of Insecticides by Thermospray Liquid Chromatography-Mass Spectrometry. Presented at the 35th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, CO, May 24–29, 1987.

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