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Identification of 5,5′-oxy-dimethylene-*bis*(2-furaldehyde) by thermal decomposition of 5-hydroxymethyl-2-furfuraldehyde

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The thermal degradation of 5-hydroxymethyl-2-furfuraldehyde (5-HMF) was performed over a temperature range of 100–220°C in a stove during exposure periods of 15 min. The chromatographic analysis of the reaction mixtures was achieved by high performance liquid chromatography (HPLC)/diode array detection using a reversed-phase column Spherisorb ODS2, and the chromatograms recorded at 280 nm. Under the assayed conditions, apart from the peak of residual 5-HMF (RT 8.1 min), three major peaks corresponding to RT 14.5 min (compound 2), 20.9 (compound 3), 23.0 (compound 4) and a minor peak corresponding to 23.1 min (compound 5) were observed. Compound 2 was isolated by semipreparative HPLC and identified as 5,5′-oxy-dimethylene-*bis*(2-furaldehyde), a symmetric ether of 5-HMF (M^+ 234; $C_{12}H_{10}O_5$), by UV, ^{1}H-NMR and GC–MS spectral analyses.

INTRODUCTION

On heating carbohydrate solutions, the presence of many products has been demonstrated (Popoff and Theander, 1976; Hodge and Osman, 1976; Olsson *et al.*, 1977; Olsson *et al.*, 1978; Nilsson-Thorel *et al.*, 1993). Nevertheless, the main breakdown product seems to be 5-hydroxymethyl-2-furfuraldehyde (5-HMF), which justifies its selection for quality control purposes (Lee and Nagy, 1988); its concentrations are helpful and frequently used as an indicator of time of storage and/or to measure the degree of thermal abuse in carbohydrate foodstuffs. Several authors have successfully determined this compound in several products of caramel (Alfonso *et al.*, 1980), tomato paste (Allen and Chin, 1980; Porretta and Sandei, 1991), fruit juices (Mijares *et al.*, 1986; Fuleki and Pelayo, 1993), wines (Williams *et al.*, 1983), plant extracts (Kiridena *et al.*, 1994), pharmaceutical syrups and infusion fluids (Cook *et al.*, 1989), honey (Salinas *et al.*, 1991; Viñas *et al.*, 1992), milk (Morales *et al.*, 1992), khoa (Sahaia *et al.*, 1992), brandies (Mir *et al.*, 1992), baby cereals (Guerra-Hernández *et al.*, 1992) and coffee (Chambel *et al.*, 1997).

However, the well-known combinations of 5-HMF with some food components, and the chemical instability of the compound, may diminish its usefulness as a chemical marker of quality of food, particularly when strong thermal or prolonged heating treatments of food are employed. Durham *et al.* (1982) have reported the formation of 5-hydroxymethylfuroic acid and furan-2,5-dicarboxylic acid by 5-HMF decomposition under autoclaving conditions. More recently, Schrödter (1992) and Kroh (1994) also admitted the formation of 2,5-furandialdehyde, 5-methylfurfural and furfural by oxidation, reduction and decarboxylation of 5-HMF, respectively, under certain conditions of thermal decomposition. Additionally, formaldehyde and formic acid could be formed. Furthermore, *in vivo* trials they have reported the presence of *N*-(5-hydroxymethyl-2-furoyl)glycine and 5-hydroxymethyl-2-furoic acid when 5-HMF was administered *per os* or intravenously to rats (Germond *et al.*, 1987).

Thus, under certain heating conditions, namely when roasting is carried out, as with cocoa and coffee beans, the concentrations of 5-HMF found in foodstuffs may

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not correspond either to the real levels of heating practised or to the initial production levels of 5-HMF. The question of whether or not 5-HMF is a suitable indicator of heating has not yet been satisfactorily answered (Mannermaa *et al.*, 1992). Therefore, we performed this thermal degradation study within a range of temperatures, from 100 to 220°C, during 15 min, in order to gain insight of the degradation mechanisms of 5-HMF and in an attempt to find a more stable chemical marker as a heat processing indicator.

A very accurate and precise method of high performance liquid chromatography (HPLC)/UV diode array detection was used to follow and quantify the remaining 5-HMF and the products arising from its thermal decomposition.

MATERIALS AND METHODS

Chemicals

5-HMF (analytical grade, 99%) was obtained from Sigma Chemical Co. All other chemicals (methanol and acetic acid) used were of analytical grade (Merck Darmstadt) and deionized water was applied throughout the study.

Thermal assay conditions

Nine equal amounts of 5-HMF (50 mg) were spread out on the bottom of nine identical porcelain capsules and heated in a stove under controlled conditions at $T = 100$, 120, 140, 160, 180, 200, 210, 215 and 220°C for 15 min. Subsequently, each sample was quantitatively transferred to a 5 ml flask with methanol and immediately diluted to the volume mark. The mixture was filtered and 20 μl were injected into a HPLC/diode array detector.

HPLC analysis

To chemically identify and quantify the 5-HMF degradation products, an analytical HPLC unit (Gilson), with a reversed-phase Spherisorb ODS2 (5 μm, particle size; 25.0 cm × 4.6 mm) column was used. The solvent system employed was a gradient of water plus acetic acid (0.2%) (A) and methanol (B). The gradient was as follows: 0 min 7.5% B, 7 min 40% B, 18 min 60% B, 24 min 80% B, 29 min 90% B. Elution was performed at a solvent flow rate of 1 ml min^{-1}. Detection was achieved with a Gilson diode array detector. Spectral data from all peaks were accumulated in the range 200–600 nm, and chromatograms were recorded at 280 nm.

Isolation of compound 2 (RT 14.5 min)

From the assays at $T = 200$ and 210°C, compound 2, eluted at RT 14.5 min, was isolated by a semipreparative HPLC using a Spherisorb ODS2 (10 μm, particle size; 25.0 cm × 10.0 mm) column and a 2 ml loop. The gradient employed was as cited above and a flow rate of 2 ml min^{-1} was used.

The eluted compound 2 was concentrated in vacuum below 40°C and was subsequently used for GC–MS and ^{1}H-NMR analyses. Purity of compound was previously detected by analytical HPLC with diode array detection, where a single peak at RT 14.5 min with a characteristic shape could be seen.

GC–MS analysis

MS spectra were achieved by electron impact mode (70 eV) in a HP 5980/5970 MSD GC-MS system, equipped with a DB-5 MS capillary column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness (J&W Scientific), coupled directly to the mass detector. The sample (1.5 μl) dissolved in methanol was injected in the splitless mode at 220°C. The initial column temperature was 70°C, maintained for 1.0 min and then programmed to increase at 25°C min^{-1} to 280°C. Data acquisition was performed in the full-scan mode (35:600) after optimization of the MS under auto-tuning conditions with perfluortributylamine.

^{1}H-NMR analysis

The ^{1}H-NMR spectra were recorded at 300 MHz using $CDCl_3$ as the solvent and tetramethylsilane as the internal standard in a Bruker ARX 500 instrument. Shifts were measured in δ values.

RESULTS AND DISCUSSION

Thermal assays: formation and degradation of compounds

Under the thermal conditions of the experimental design when 5-HMF was heated, apart from the residual 5-HMF at RT 8.1 min (peak 1), the most prominent peaks appeared at RT 14.5 min, 20.9 min and 23.0 min, corresponding to compounds 2, 3 and 4, respectively. At 23.1 min, a very insignificant peak could also be seen (compound 5). Chromatograms of 5-HMF and its degradation products obtained at several treatment temperatures are shown in Fig. 1A–J. Diode array detection indicated a clear build-up, with increasing temperatures, of new compounds with similar UV shapes to the parent compound, suggesting that furfuryl chromophores were generally present in the major compounds formed.

An examination of the profiles shows a UV-absorbing compound at about RT 20.9 min (compound 3), which appeared first at 100°C (Fig. 1B) in a low concentration, reaching its highest concentration at about 160°C (Fig. 1E); thereafter, it exhibited a gradual decline and

was almost undetectable at about 210°C (Fig. 1H).

At 160°C, two other peaks (compounds 2 and 4) were formed as is clear from inspection of the HPLC chromatograms (Fig. 1E). The degradation behaviours of the compounds were quite different. Compound 4 reached its highest concentration at 180°C (Fig. 1F), whereas compound 2 was maximum at 200°C (Fig. 1G) and was almost the only compound detectable at 210°C (Fig. 1H), the temperature at which the former sharply decreased.

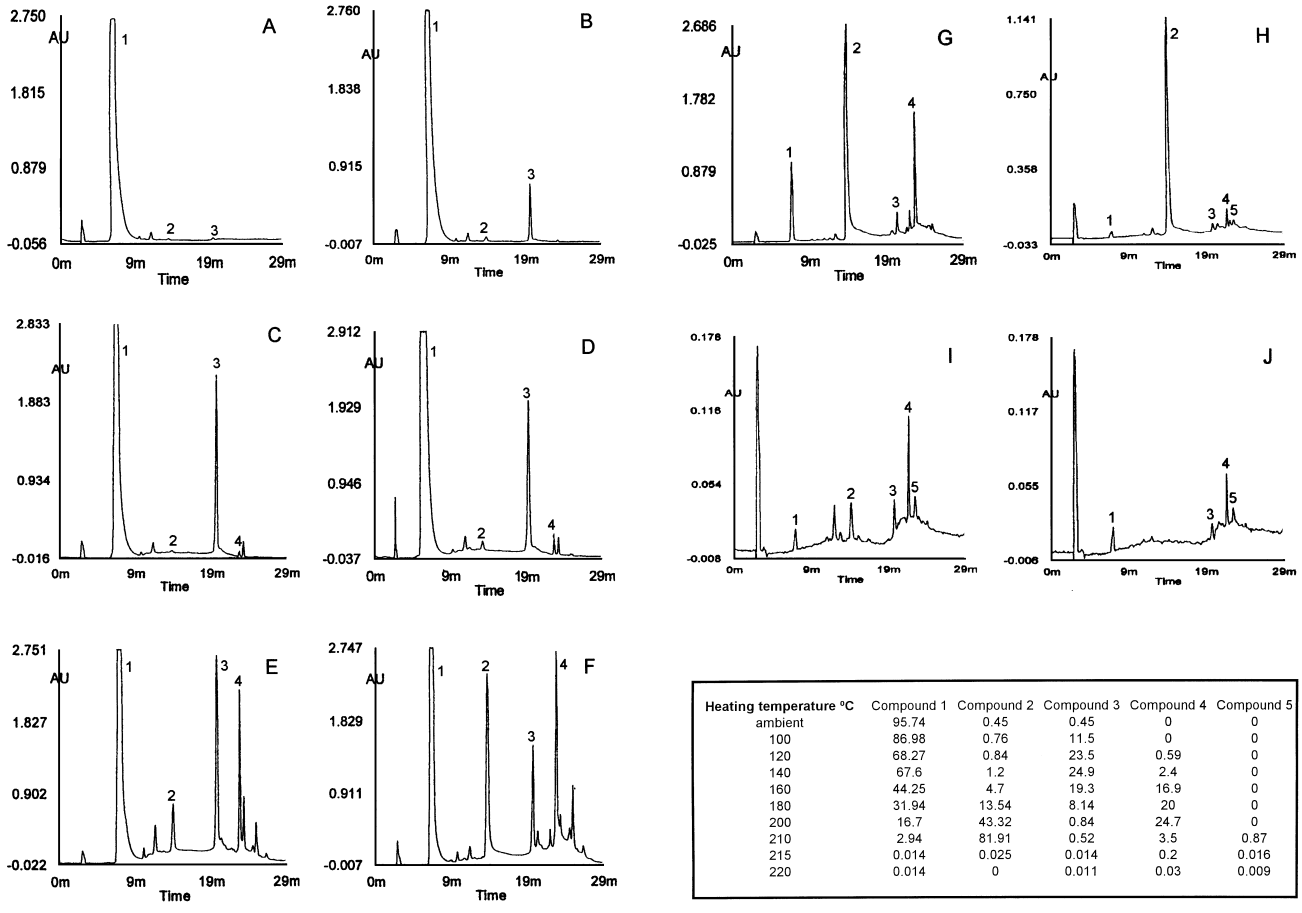
Figure 2 shows the global evolution of all five compounds plotted as the relative percentage of areas vs thermal assay temperature and confirms the above-mentioned individual observations of each compound; the gradual decrease of 5-HMF from 100 to 210°C, at which it is almost completely decomposed, is also clearly observed.

Identification of compound 2

After isolation, compound 2 was identified on the basis of its spectral UV and ^{1}H-NMR characteristics, and on the basis of its MS fragmentation data which were as follows: UV spectrum data (obtained in diode array detector): λ_{max} at 281.4 nm and a shoulder at 234.2 nm. When comparing the UV spectrum of compound 2 with that of 5-HMF, both showed the same shape, which indicated similarity to the electronic spectrum of furanic aldehyde compounds. ^{1}H-NMR data (300 MHz, $CDCl_3$, TMS as internal standard): δ 9.64 (s, 2H, 2 × CHO), 7.22 (d, J = 3.5 Hz, 2H, 2 × C3–H), 6.54 (d, J = 3.6 Hz, 2H, 2 × C4–H), 4.64 (s, 4H, 2 × CH_2). GC-MS data (% relative intensity): M^+ 234 ($C_{12}H_{10}O_5$) (1%), 206 (7%), 177 (1%), 138 (3%), 125 (7%), 123 (8%), 110 (43%), 109 (100%), 96 (2%), 95 (18%), 82 (30%), 81 (99%), 79 (7%), 69 (13%), 68 (5%), 53 (94%), 52 (36%), 51 (31%), 50 (16%), 41 (30%), 39 (59%), 38 (25%).

All data were consistent with the molecular formula $C_{12}H_{10}O_5$ and with the chemical structure of 5,5′-oxy-dimethylene-*bis*(2-furaldehyde), a compound already reported by Popoff and Theander (1976).

From a theoretical point of view, the formation of compound 2 can easily be explained from two 5-HMF molecules with intermolecular water elimination. Schrödter (1992) reported the formation of 2,5-furandialdehyde, among other compounds, as a result of the decomposition of glucose under Curie-Point-Pyrolysis conditions at 300°C which may not be in contradiction to our results, bearing in mind that a direct comparison is not possible because, in the thermal



Heating temperature °C	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5
ambient	95.74	0.45	0.45	0	0
100	86.98	0.76	11.5	0	0
120	68.27	0.84	23.5	0.59	0
140	67.6	1.2	24.9	2.4	0
160	44.25	4.7	19.3	16.9	0
180	31.94	13.54	8.14	20	0
200	16.7	43.32	0.84	24.7	0
210	2.94	81.91	0.52	3.5	0.87
215	0.014	0.025	0.014	0.2	0.016
220	0.014	0	0.011	0.03	0.009

Fig. 1. High performance liquid chromatography (HPLC) chromatograms showing the effect of over heating of 5-hydroxymethyl-2-furfuraldehyde (5-HMF) at different temperatures and corresponding to: (A) 5-HMF standard; (B) at 100°C; (C) at 120°C; (D) at 140°C; (E) at 160°C; (F) at 180°C; (G) at 200°C; (H) at 210°C; (I) at 215°C and (J) at 220°C.

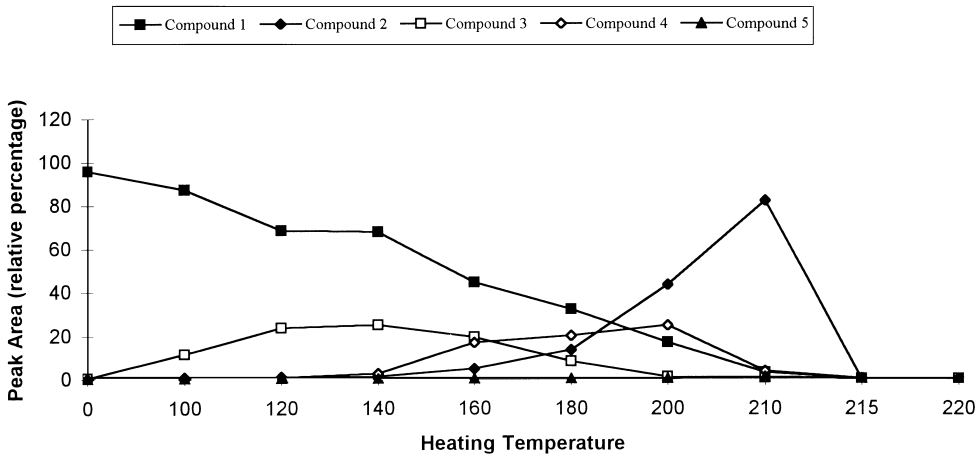


Fig. 2. Plots of the relative percentage of areas of peaks vs temperatures of thermal decomposition of 5-hydroxymethyl-2-furfuraldehyde (5-HMF).

degradation assays, both starting compounds (glucose vs 5-HMF) and the experimental conditions (300°C vs 100–220°C) were considerably diversified. Nevertheless, the formation of some of those compounds is not excluded in our study. In addition, the formation of several other compounds, involving a sequence of oxidation reactions of 5-HMF followed by decarboxylations, or eventually an intermolecular esterification, could be foreseen. However, this hypothesis needs experimental confirmation which awaits separation of all the observed compounds and their subsequent identification.

CONCLUSIONS

The 5-HMF was thermochemically decomposed when exposed for 15 min to temperatures as low as 100°C, and was almost completely decomposed at 210°C. Under experimental conditions, several other unstable compounds were also formed. Compound 2, a symmetric ether, and dimer of the parent compound, was chemically identified as 5,5′-oxy-dimethylene-*bis*(2-furaldehyde) (M^+ 234; $C_{12}H_{10}O_5$). This compound might be a better marker of thermotreatment of foods up to 200–210°C than 5-HMF itself.

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