The effect of high pressure on the formation of volatile products in a model Maillard reaction

Mark Bristow and Neil S. Isaacs

Department of Chemistry, University of Reading, Reading, UK RG6 6AD

Reaction progress in the formation and subsequent decay of several of the volatile products from a model Maillard reaction between lysine and xylose has been followed at pH 7 and 10 and at elevated pressures. At low pH, the buildup and decay of 5-methyl-4-hydroxy-3(2H)-furanone and several minor products were observed. The application of high pressure results in a much diminished maximum concentration of each although the time to the maximum is unaffected. At pH 10, products contain nitrogen heterocycles with 2-methylpyrazine being the principal one which builds up and only slowly decays with time. Again, the yield is greatly reduced by pressure. The results are interpreted in terms of the inhibition by pressure of the formation of the precursor, the Amadori rearrangement product which affects subsequent products. In some instances rates of formation are also found to be slightly inhibited while degradation of these products is accelerated. The corresponding mechanisms are examined in the light of these results.

Introduction

In recent years, the application of high pressures in processing of food materials has been the subject of intense scrutiny.[1–3] Benefits associated with this method of treatment include sterilization from moulds, yeasts and bacteria while retaining fresh flavour, consequences which have an immediate application in the preservation and marketing of fruit-based foods in particular. It has been our aim to elucidate the chemical changes which occur on pressure treatment of foods especially those changes which differ from those which are products of thermal treatment. In this paper, further studies of a model Maillard reaction are discussed.

The Maillard reaction is the name given to the complex sequence of events which occur when protein and carbohydrate are heated together at temperatures in excess of 80 °C,[4,5] Scheme 1. The generation of a multitude of flavour compounds and brown colouration is the basis of the familiar changes which occur during cooking. It would be surprising if the application of high pressure during such a complex sequence of reactions produced no changes in the products and their consequent flavours and colours, information which would be needed before exploitation of this technology could proceed.
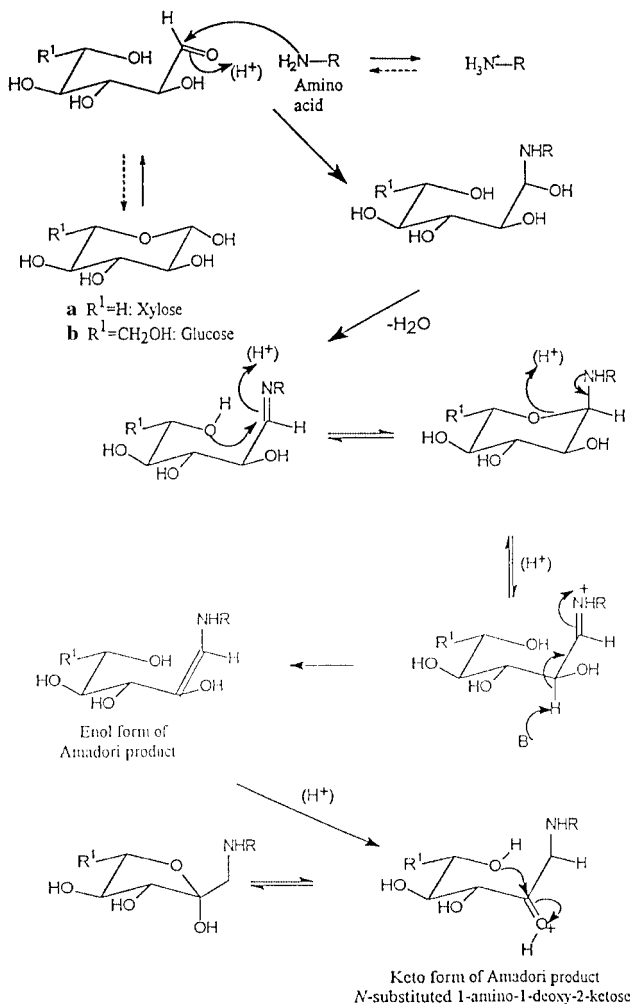
The Maillard reaction occurs in two or three distinct stages. Initially condensation of a carbonyl function from carbohydrate with an amino function in the protein leads to an imine which rearranges to the amino form (Amadori rearrangement), an isolable intermediate, **2**, Scheme 2.[6] This subsequently is transformed into a variety of products, both volatiles and brown polymer ('melanoidin'). We have previously shown[7] that the first stage, the formation of the Amadori rearrangement product (ARP) is accelerated by pressure while the second, its degradation, is retarded.

The ARP therefore is formed more rapidly but decomposes more slowly under high pressure conditions. The present work examines the subsequent reactions which give rise to a large number of volatile products, mainly oxygen and nitrogen heterocycles, the flavour constituents. For this purpose, lysine and xylose were chosen as the model protein and carbohydrate since reactions between these two take place more rapidly and at lower temperatures than those between other analogous ingredients. The effect of pressure on this system has been
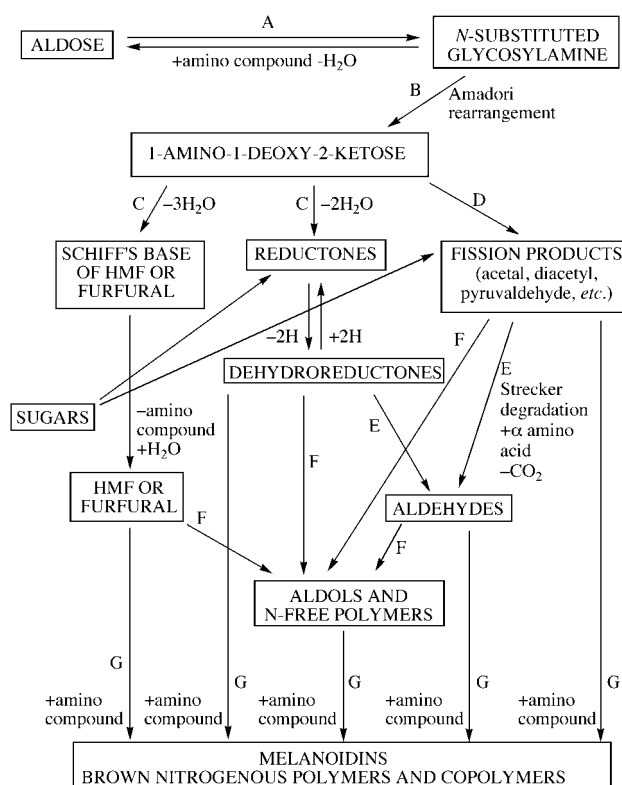
Scheme 1 Stages in the Maillard reaction (after Hodge[4]).

examined by Ames and Apriyantono[8] who identified a range of products at varying pH and by Hayashi[9] who studied the effect of pressure on products. In this work, the yields, both as a function of time and pressure, of six principal products are reported together with their rates of formation and decay and associated volumes of activation. It must be said, however, that systems of this kind are immensely complex and produce a remarkable array of products many of which are transient and evolve with time towards polymeric materials. The products obtained vary with the particular reagents as well as with conditions of pH, temperature and time.

Scheme 2 Formation of the Amadori rearrangement product (ARP, **2**).

a R^1=H: Xylose
b R^1=CH$_2$OH: Glucose

Enol form of
Amadori product

Keto form of Amadori product
N-substituted 1-amino-1-deoxy-2-ketose

Experimental

Buffer solutions

Acetate. Sodium acetate (10 g) was dissolved in water (100 mL) and the pH adjusted to 7.00 by the addition of sodium hydroxide. The solution was degassed by ultrasound and stored over nitrogen.

Bicarbonate. Potassium bicarbonate (0.2 M) was adjusted to pH 10.00 by the addition of sodium hydroxide. The solution was degassed by ultrasound and stored over nitrogen.

Reactions at 1 bar

Xylose (30 g, 0.2 mol) and lysine monohydrochloride (36 g, 0.2 mol) were dissolved in an appropriate buffer solution, either acetate or bicarbonate (200 mL) and the pH further adjusted to 7.00 or 10.00 by the addition of alkali. A nitrogen atmosphere was introduced and the solution stirred magnetically and heated maintaining the temperature at 100 ± 0.5 °C. Aliquots (20 mL) were removed periodically and analysed.

Reactions at high pressure

A sample (approximately 25 mL) of each solution made up as described above was placed in a cylindrical poly(tetrafluoro-ethylene) (PTFE) vessel closed by a piston. This was placed in the high pressure vessel described previously[10] set at 100 °C. The desired pressure was applied and the reaction allowed to proceed for a specified time after which the vessel was depressurized, the sample removed and analysed. Kinetic plots were built up point by point, each being duplicated.
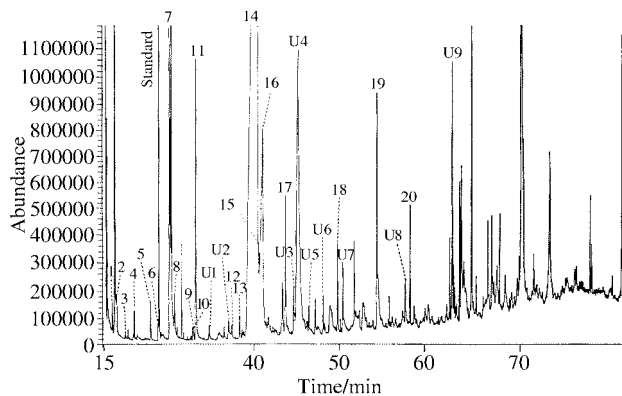
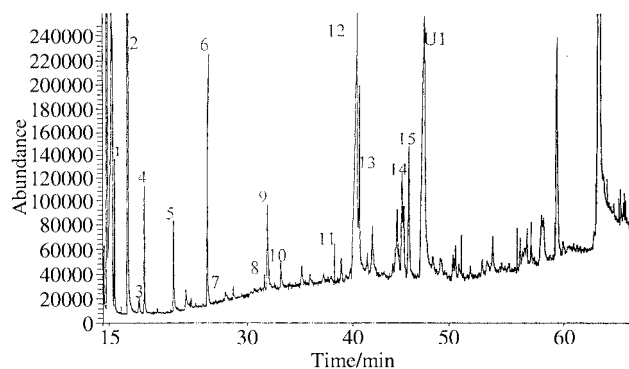**Fig. 1** Liquid chromatogram of products formed at pH 7.

Fig. 2 Liquid chromatogram of products formed at pH 10.

Reactions carried out to a maximum of 2 kbar were conducted in a high pressure sampling cell from which a series of aliquots of the reaction solution could be obtained while maintaining pressure.

Analysis of reaction solutions

Samples (20 mL) were extracted by ether (3 × 100 mL), the combined extracts dried (MgSO$_4$) and carefully rotary evaporated at <30 °C. Final adjustment to 0.1 mL in a graduated vial was made by evaporation in a stream of nitrogen and 5-methyl-hexan-3-one (0.2 μL, 10% in ether) was added as a GLC standard.

Finally the solution was analysed by GLC using a 60 m capillary column (DB-3, J and W Scientific) with the following temperature programme; 30 °C isothermal, 15 min; 30 °C to 225 °C ramped at 4 °C min^{-1}; 225 °C isothermal for 10 min giving a total analysis time of 74 min. Typical chromatograms are shown in Figs. 1 and 2. Area counts for the major peaks relative to the standard were then recorded. The reproducibility of these measurements was about ±5%. For each of the aqueous samples before extraction, the absorbance at 400 nm for a 1 cm pathlength sample was also measured, Fig 3.

Identification of products

The gas chromatograms, Figs. 1, 2 obtained showed more than fifty components, mostly base-line separated. About twenty of these were conclusively identified and others somewhat tentatively, see Tables 1 and 2 for comparison with authentic specimens and for their linear retention indexes[11] (LRIs), defined by eqn. (1), in which LRI$_x$ = LRI for unknown compound x, t_x =

$$LRI_x = 100 [(t_x - t_n)/(t_{n+1} - t_n) + n] \qquad (1)$$

retention time for compound x, t_n = retention time for *n*-alkane of n carbon atoms eluting before x, t_{n+1} = retention time for *n*-alkane of $(n + 1)$ carbon atoms eluting after x, by which comparisons between these values and literature values may be compared.

Table 1 Identification of products in Fig. 1

No.	Name	M^+	No.	Name	M^+
1	pentane-2,3-dione	100	16	2-furoic acid	112
2	ethyl propanoate	102	17	2-methyl-3,5-dihydroxy-4H-pyran-4-one, **5**	142
3	pyrazine	80	18	2,5,5-trimethylcyclohexane-1,3-dione	154
4	3-methylenepentan-2-one	98	19	bifuran, $C_{10}H_{10}O_3$	178
5	butyl acetate	116	20	5-acetyl-7-methyl-2,3-dihydro-1H-pyrrolizine	163
6	methylpyrazine	94	U1	unidentified	124
7	2-furylmethanol	98	U2	unidentified	112
8	1-acetoxypropan-2-one	116	U3	unidentified	140
9	2-acetylfuran	110	U4	unidentified	102
10	2,5-dimethylpyrazine	108	U5	unidentified	152
11	2(3H)-furanone	84	U6	unidentified	135
12	2-propanoylfuran	124	U7	unidentified	126
13	3-methylcyclopentane-1,2-dione	112	U8	unidentified	194
14	5-methyl-4-hydroxy-3(2H)-furanone, **1**	114	U9	unidentified	162
15	2,5-dimethyl-4-hydroxy-3(2H)-furanone	128			

Table 2 Identification of products in Fig. 2

No.	Name	M^+	No.	Name	M^+
1	2-methylpent-1-ene	84	9	2(5H)-furanone	84
2	propan-1-ol-2-one	74	10	N,N-diethylformamide	101
3	propanoic acid	74	11	3-methylcyclopentane-1,2-dione	112
4	butan-3-ol-2-one	88	12	5-methyl-4-hydroxy-3(2H)-furanone	114
5	butan-1-ol-2-one	88	13	2,5-dimethyl-4-hydroxy-3(2H)-furanone	128
6	methylpyrazine	94	14	3-hydroxy-2-methylpyran-4-one	126
7	4-methylpentan-4-ol-2-one	116	15	2,3-dihydro-3,5-dihydroxy-6-methylpyran-4-one	144
8	2,5-dimethylpyrazine	108	U1	unidentified	147
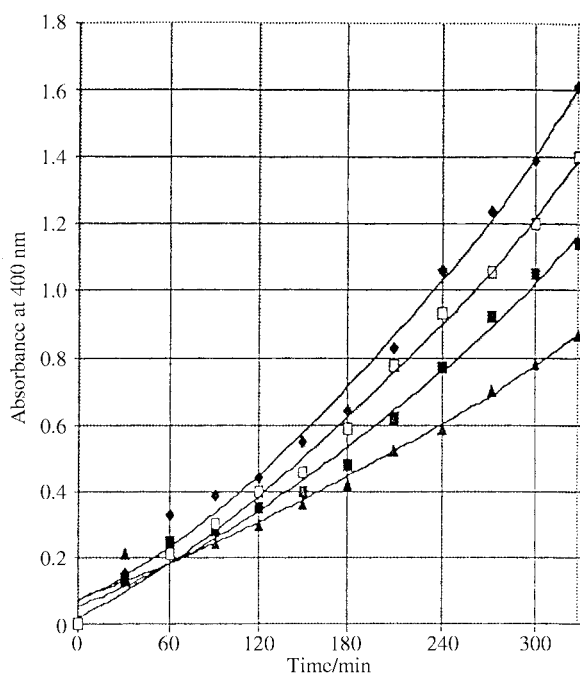
Fig. 3

In addition, identification was assisted by GC/MS analysis. The difference between the two chromatograms emphasises the sensitivity of the product ratios to the pH in which they are formed.

Rate measurements

Where appropriate, data were fitted by computer to a linear, or exponential function in order to obtain relative rate constants. For the analysis of rates from buildup and decay curves such as in Fig. 4, a programme analysing the sequential reaction (2) was

$$A \xrightarrow{k_a} B \xrightarrow{k_b} C \qquad (2)$$
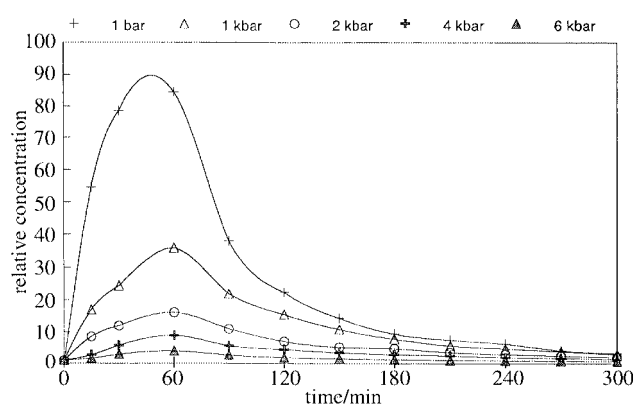
Fig. 4

used for fitting experimental to calculated concentration curves by inspection.

Discussion

Products formed at pH 7

5-Methyl-4-hydroxy-3(2H)-furanone 1. This is the principal volatile constituent from xylose and lysine and imparts a characteristic caramel odour to the product. This compound is not stable under reaction conditions but its concentration rises to a maximum and then decays away completely with time. Under our conditions the maximum occurred at a time of 60 min. The effect of high pressure, however, was mainly on the amount formed. As pressure was increased the time to reach maximum concentration and the rate profile in general were unaltered but the amount of this product diminished until, at 6 kbar, hardly any was seen to form, Fig. 4.

Rates of formation of **1** were obtained from concentration *vs.* time data, Fig. 4, on the assumption of two consecutive reactions. Rates of formation were of first order but rates of decay best fitted a zero order plot, and were most reliably obtained from experiments using authentic **1** as starting material heated

Table 3 Rates of formation and decay of 5-methyl-4-hydroxy-3(2*H*)-furanone as a function of pressure, pH 7, 100 °C

Pressure/bar	A_o	k_a/min^{-1}	k_b/min^{-1}
1	235	0.0297	0.0297
1000	76.5	0.0211	0.0213
2000	38.5	0.0205	0.0205
4000	24.9	0.0140	0.0223
6000	13.3	0.0133	0.0201

in the same buffer solution and under the same conditions as in the Maillard reaction, Table 3.

The rate of the formation step falls slightly with pressure corresponding to an apparent activation volume, $\Delta V^{\ddagger}$, calculated from eqn. (3) as $+4 \pm 1$ cm^3 mol^{-1}. The rate of decay of

$$-\mathrm{d}(\ln k)/\mathrm{d}p = \Delta V^{\ddagger}/RT \qquad (3)$$

the furanone was not reliably obtained from these data, but from separate experiments on the pure compound heated in the same buffer solution at the same temperature. Reactions followed zero-order kinetics suggesting enolization as the slow step followed by rapid scavenging of the enol. Pressure slightly accelerated the decomposition, the volume of activation was found to be -5 ± 1 cm^3 mol^{-1}.

The simulation also reproduces initial quantities of the precursor to the furanone, A_o, whose values decrease markedly with pressure, consistent with the reduced rate of decay of the ARP or whatever other intermediate is controlling this reaction. The mechanism of formation of **1** from the ARP, **2**, is plausibly *via* the deoxyoxopentose **3** as shown in Scheme 3.[12]
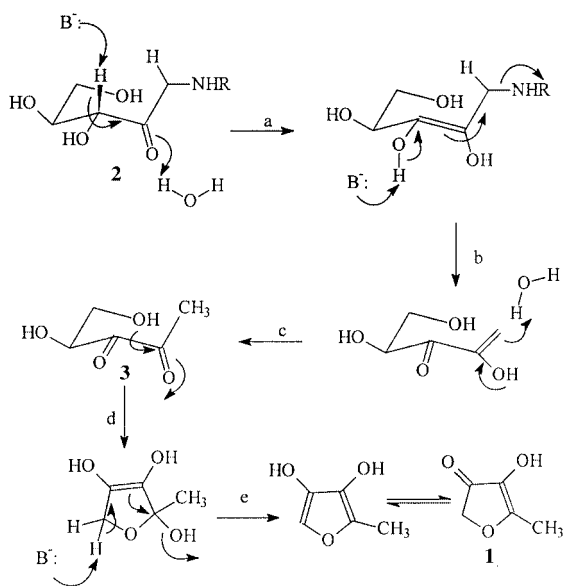
Scheme 3 Formation of 5-methyl-4-hydroxy-3(2*H*)-furanone, **1**.

In such a complex sequence and with incomplete knowledge of the charge states of the intermediates, it is difficult to predict the volume of activation but the observed positive value suggests that step b, the expulsion of the nitrogen residue, a dissociative reaction, is rate-determining. This step is formally similar to the decomposition of the Amadori rearrangement product which has been previously shown also to have a positive volume of activation.[7] Retardation of the rate of decomposition of the ARP in forming melanoidins was further confirmed in the present work although it now appears that this only applies at neutral or acidic pH while at high pH pressure actually accelerates browning.[12] Neutral proton transfers are usually accompanied by no volume change so the various

enolizations are unlikely to be contributing significantly to the volume of activation.

That decomposition of the furanone occurs is evident from its disappearance from the gas chromatogram with time but the product was not identified. When the reaction was followed by NMR spectroscopy carried out in D$_2$O, no spectral changes were apparent. This suggests the product is chemically very similar and a ring-opened form, **4**, is a possible contender, Scheme 4. The mass spectra of both starting material and
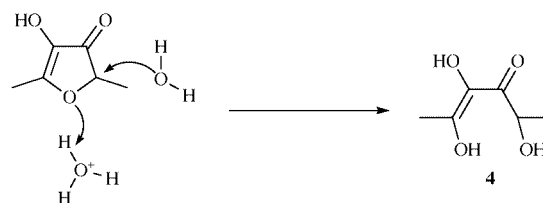
Scheme 4 Possible degradation route of furanone, **1**.

product are identical, $m/z = 114$ being the highest peak in each case corresponding to M$^+$ for **1** and $(M - 18)$ for the assumed product, **4**, but no peaks at higher mass corresponding to self-aldolization products were observed.

The reduction in the peak quantity of furanone produced under high pressure is therefore due to the retardation in its rate of formation together with the acceleration of its decomposition. The decomposition of furanone was also studied in the presence of xylose and was found to be accelerated fourfold. Furthermore, pressure was now found to have a very large rate-accelerating effect, $\Delta V^{\ddagger} = -35$ cm^3 mol^{-1}. NMR of the final product showed a sugar residue to be present. These data are consistent with a condensation reaction between furanone and carbohydrate supplementing the route to disappearance of the furanone, Scheme 5 and must further complicate interpretation of rates measured under conditions of the Maillard reaction.
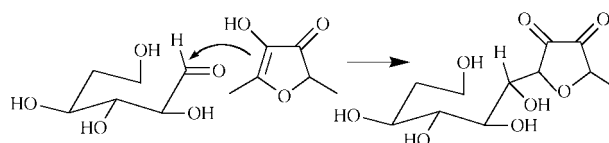
**Scheme 5** Reaction between furanone, **1**, and xylose.

2-Methyl-3,5-dihydroxy-(4*H*)-pyran-4-one, 5. A further major product in the reaction between xylose and lysine is 2-methyl-3,5-dihydroxy-(4*H*)-pyran-4-one (5-hydroxymaltol) **5**. This six-carbon compound requires reaction of xylose with a C1 unit, methanal or an equivalent in its formation, Scheme 6.[14] Methylations are a feature of the reaction as for example in the formation of various methylated pyrazines at higher pH.

The degradation of **5** can lead to acyclic carbonyl compounds[15] and has been shown to be the precursor of 2-acetylfuran, a minor product detected in this study. Other routes could also involve condensations with enolates building up large and eventually coloured molecules. It was noted that, when a pure sample of 5-hydroxymaltol was heated in water, some 50 products were obtained, furans, furanones, pyranones and carboxylic acids. This intermediate is evidently able to act as a second source of some of the other products of the reaction and to create reactive species which later are incorporated in melanoidins.

The rate of formation and the maximum yield of 5-hydroxymaltol diminish with pressure similar to the behaviour of **1**, Table 4; at 6 kbar, it becomes undetectable. Analysis of the kinetic plots gave $\Delta V^{\ddagger}$ for formation $= +5 \pm 1$ cm^3 mol^{-1} although decay rates obtained from the sequential reaction analysis were not of great reliability. It seems that as before,

Table 4 Rates of formation of 5-hydroxymaltol

p/bar	$k/10^{-3}$ min^{-1}
1	58.5
1000	57.3
2000	41.7
4000	31.7
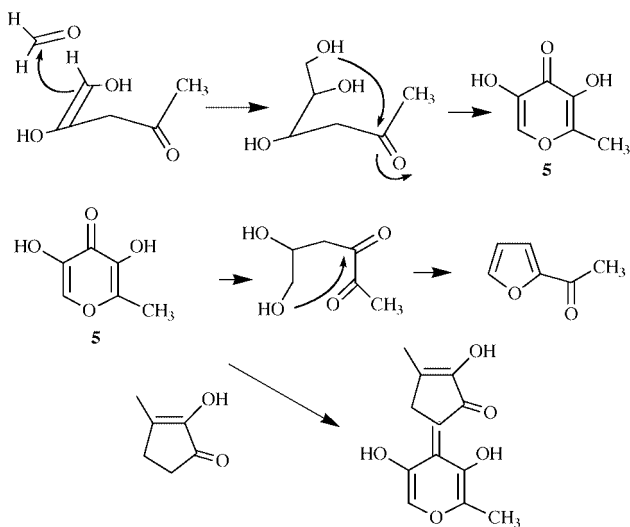
Scheme 6 Formation and decay of hydroxymaltol, **5**.

reduction of the rate of formation due to retardation of the fission of the Amadori product is followed by more rapid decomposition by associative steps.

3(2*H*)-Furanone. This product, formed both at pH 7 and at pH 10 again builds up to a maximum after 60 min and then decays away, the maximum yield being strongly reduced by pressure. 3(2*H*)-Furanone is formed in the retro-aldol fragmentation of xylose which loses a molecule of formaldehyde on treatment with base, Scheme 7. The reaction appears to be
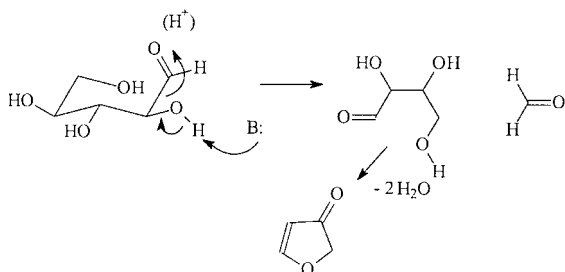
Scheme 7 Formation of 3(2*H*)-furanone.

unrelated to the ARP so it may be coincidence that the reaction profile is similar to those of ARP-derived products.

3-Methylcyclopentane-1,2-dione. Building up to a maximum concentration also after 60 min, the maximum yield of this compound is also suppressed by pressure; at 1 kbar only about 10% of that formed at atmospheric pressure is observed. This product is observed in reactions between glucose and amino-acids[16] but in the present case xylose would need to acquire a further carbon atom so little can be said about the mechanism with any certainty.

Products formed at pH 10

At higher pH, products separated and identified are shown in Fig. 2 and Table 2. There are significant differences between these and the neutral products since at pH 10 nitrogen hetero-
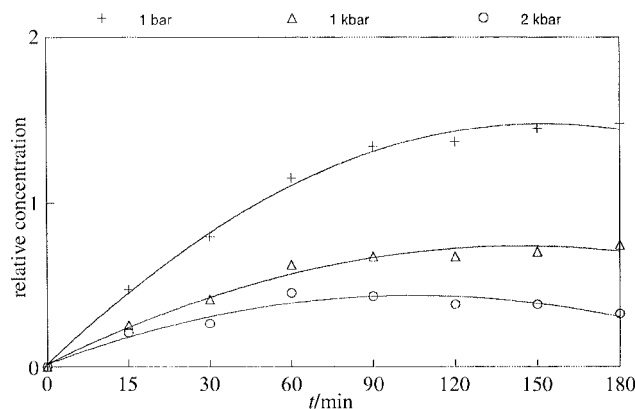
Fig. 5

cycles make their appearance. In the present study, methyl-pyrazine is the one formed in largest quantity and whose formation has been studied. Other workers under similar conditions have observed 2,5-dimethylpyrazine as predominant.[13] Unlike the oxygen heterocycles, this compound is reasonably stable and builds up to a static concentration at 1 bar then diminishes with a half life of about 20 h. However, the rates of formation are retarded quite strongly by pressure, $\Delta V^{\ddagger}_{app} = +12$ cm^3 mol^{-1} and the maximum concentrations are greatly reduced, Fig. 5. At high pressure furthermore, degradation begins to become apparent and is accelerated. However, rates of formation even at the highest pressures are hardly affected by decomposition so that the reduction of the yield must be due to the effect of pressure on the precursor.

Pyrazines can result from the condensation between two α-aminoketone moieties. Methylpyrazine is plausibly formed from the precursors shown in Scheme 8[17] but frequently numerous methylated pyrazines are formed in similar reactions which would each require the requisitely substituted amino ketone. The pattern of methylated pyrazines depends upon the particular amino acids present although the sugar also has a bearing on this.[18] Ammonia has been suggested as being involved[19] and it has been reported that only the nitrogens originate in the amino acids, the remainder of the ring being built up from sugar material.[20] Conceivably the pyrazine which is also formed is then methylated by a formaldehyde equivalent since frequently numerous methylated pyrazines are formed in similar reactions. Aminoketones can arise from an amino acid such as lysine by transamination to a 1,2-dione which in turn arises by retro-aldol cleavage of 1-deoxyosone and 2-methylpyrazine is a major product from heating glycerol with ammonia. The ratio of 2-methyl- to 2,5-dimethylpyrazines appears to be very sensitive to conditions.

It is not clear in this immensely complex system which of or even whether these reactive intermediates are available as such but a ring-forming reaction of this type would be expected to have a large negative volume of activation (*e.g.* -18 cm^3 mol^{-1} for imine formation) so no such reaction appears to be rate-controlling.
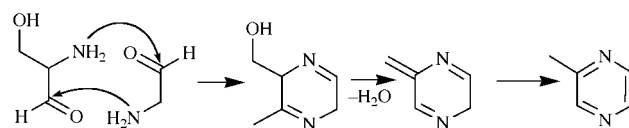
Scheme 8 Formation of methylpyrazine.

In conclusion it appears that the volatile products of the Maillard reaction are generally suppressed by the application of high pressure and that many of them appear to be controlled by a common precursor such as the ARP whose decomposition has a positive volume of activation. If food processing is carried out at high pressure and at temperatures high enough

for Maillard chemistry to occur, then cooked flavours are likely to be diminished.

Acknowledgements

References

1 *High Pressure Processing of Foods*, D. A. Ledward, R. G. Earnshaw, D. E. Johnston and A. P. M. Hastings (eds.), University of Nottingham Press, Loughborough, 1995.

2 *High Pressure Bioscience and Biotechnology*, R. Hayashi and C. Balny (eds.), Elsevier Science BV, Amsterdam, 1996.

3 D. Knorr, V. Heinz, O. Schlüter and M. Zenker, *High Pressure Food Science*, *Bioscience and Chemistry*, N. S. Isaacs, (ed.), Royal Society of Chemistry, 1998, p. 227.

4 J. E. Hodge, *J. Agric. Food Chem.*, 1953, **1**, 928.

5 H. E. Nursten, *Food Chem.*, 1980, **6**, 263.

6 J. M. Ames, *The Maillard Reaction in Biochemistry of food proteins*, B. J. F. Hudson (ed.), Elsevier Applied Science Publishers, London, 1992, p. 99.

7 N. S. Isaacs and M. Coulson, *J. Phys. Org. Chem.*, 1996, **9**, 639.

8 A. Apriyantono and J. M. Ames, *J. Sci. Food Agric.*, 1993, **61**, 477.

9 R. Hayashi, *Agric. Biol. Chem.*, 1991, **55**, 2071.

10 N. S. Isaacs, *High pressure techniques in chemistry and physics*, W. Holzapfel and N. S. Isaacs (eds.), Oxford University Press, 1997, p. 324.

11 From a data base made available by courtesy of D. S. Mottram whose help in identification we acknowledge with gratitude.

12 I. Blank and L. B. Fay, *J. Agric. Food Chem.*, 1996, **44**, 531.

13 V. Hill, PhD Thesis, Reading University, 1998.

14 T. Shono and Y. Matsumura, *Tetrahedron Lett.*, 1976, 1363; S. Torii, H. Tanaka,T. Anoda and Y. Shimizu, *Chem. Lett.*, 1976, 495.

15 M. Kim and W. Baltes, *J. Agric. Food Chem.*, 1996, **44**, 282.

16 G. Brule and P. Dubois, *Ann. Technol. Agri.*, 1971, **20**, 305.

17 T. Shibamoto and R. A. Bernhard, *J. Agric. Food Chem.*, 1977, **25**, 609.

18 A. Arnoldi, C. Arnoldi, O. Baldi and A. Griffini, *J. Agric. Food Chem.*, 1988, **36**, 988.

19 H. I. Hwang, T. G. Hartman, R. T. Rosen and C. T. Ho, *J. Agric. Food Chem.*, 1993, **41**, 2112; *ibid.*, 1994, **42**, 1000.

20 P. E. Koehler, M. E. Mason and J. A. Newell, *J. Agric. Food Chem.*, 1969, **17**, 393.