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Applications of the Maillard reaction in the food industry

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This paper summarises some recent work concerned with the development of colour and flavour via the Maillard reaction in both aqueous and restricted moisture model systems. High performance liquid chromatography (HPLC) and capillary electrophoresis (CE), both with diode array detection, are discussed for their ability to separate reaction products. The use of the diode array data to classify reaction products is presented. The coloured reaction products identified from aqueous sugar–amino acid systems are summarised, and their contribution to the colour of total model systems is considered. The effects of temperature/time, pH and high pressure on the development of colour and flavour in Maillard model systems are presented. Colour measurement data and quantitative descriptive analysis (QDA) data are given for a starch–glucose–lysine model system extruded at different feed pH values. The use of a laboratory reaction cell to mimic most of the conditions encountered in the extruder is discussed. Its use to obtain information for the successful prediction of colour development in the extruder is presented. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The Maillard reaction has been used to produce foods that look and taste attractive for thousands of years; for as long as food has been cooked. The modern food industry relies on the application of the Maillard reaction to produce many foods, e.g. coffee and bakery products, that possess the colour and flavour demanded by the consumer.

The chemistry underlying the Maillard reaction is extremely complex (Ledl and Schleicher, 1990; Ames, 1992). In 1953, John Hodge published his consolidated scheme (Fig. 1) which summarised the chemical reactions which were understood to comprise the Maillard reaction at that time (Hodge, 1953). The Hodge scheme remains widely used today. In essence, it states that a reducing sugar, such as glucose, condenses with a compound possessing a free amino group, such as an amino acid, to give a condensation product. Subsequently, a range of reactions takes place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations, which ultimately lead to the formation of brown nitrogenous polymers and co-polymers, known as melanoidins.

Although the Hodge scheme is very useful, it has some drawbacks. First, the scheme is simply a summary of the reactions that take place. Secondly, in the intervening years, a vast amount of research on the Maillard reaction has been undertaken (e.g. Eriksson, 1981; Waller and Feather, 1983; Fujimaki *et al.*, 1986; Finot *et al.*, 1990; Labuza *et al.*, 1994; Ikan, 1996; O'Brien *et al.*, in press). More recent work has established that other important pathways, not accounted for by the Hodge scheme, also exist. For example, Namiki (1988) has shown that a free radical route operates, especially at high pH. In both the food and medical fields, the central importance of the Amadori rearrangement product (ARP), which is implied by the Hodge scheme, has been questioned (Edwards and Wedzicha, 1992; Fu *et al.*, 1994, Wedzicha *et al.*, 1994).

Nursten (1986) gave a list of 12 symptoms of the Maillard reaction. Probably, the most important, as far as the food manufacturer is concerned, are the formation of colour and discoloration, the formation of flavours or off-flavours, the production of compounds with antioxidant activity, the reduction in nutritional value, and the formation of compounds with potentially toxic properties.

Various factors influence the Maillard reaction (Ames, 1990) and they can be considered as food processing and storage variables. They include the nature of the reactants (the composition of the raw material), the temperature–time combination used during heating and storage, the pH and water activity of the food,

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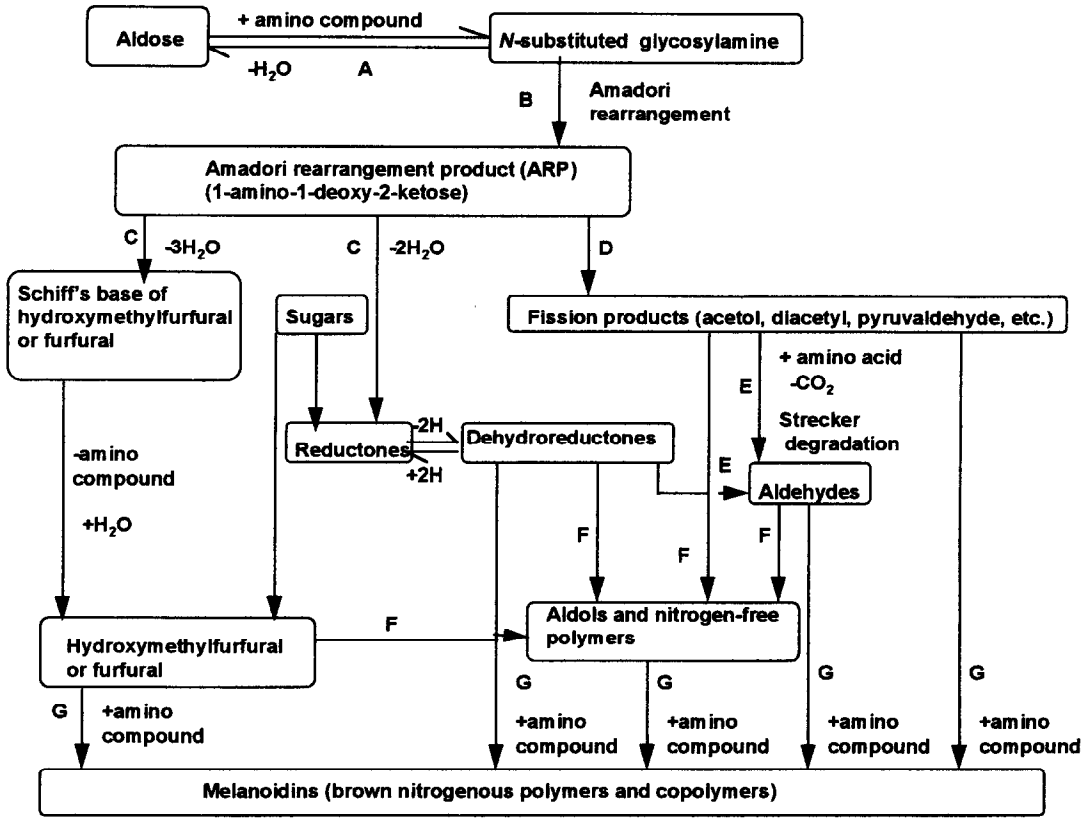

Fig. 1. Hodge's outline of the Maillard reaction (based on Hodge, 1953).

the presence of oxygen and metals, and the presence of any reaction inhibitors, such as sulfur dioxide. By manipulating these variables, the balance of the various chemical pathways making up the Maillard reaction changes. This results in a modification to the profile of reaction products and, in turn, an affect on quality attributes, such as flavour and colour.

This paper discusses some recent studies designed to lead to an increased understanding of (a) the development of colour and flavour due to the Maillard reaction, (b) the effect of various processing variables on the Maillard reaction and, (c) how quality, associated with the Maillard reaction, may be predicted in food systems.

UNDERSTANDING THE MAILLARD REACTION

Developing separation methods

Once the cascade of reactions that comprise the Maillard reaction has begun, it is very difficult to stop. Therefore, when the profile of reaction products that exists at any one point in time is to be studied, it is essential that the analytical procedures applied do not themselves distort the results, e.g. by promoting further reactions, including the formation of artifacts. For this reason, attention should be given to the use of gentle methods which are rapid and simple, such as high performance liquid chromatography (HPLC) or capillary electrophoresis (CE). The aim of the work described in this section was to separate, classify and identify coloured and colour-related Maillard reaction products.

Bailey *et al.* (1996*a*) studied coloured and colour-related Maillard reaction intermediates and products by refluxing aqueous sugar (xylose or glucose)–amino acid (glycine or lysine) solutions with control of the pH at 5 throughout heating. One molal concentrations of each reagent were used. Direct injection of the total reaction products on to an HPLC column, with monitoring at 254, 280, 360 and 460 nm, showed that the complexity of the chromatograms obtained depended on the reactant sugar and amino acid (Bailey *et al.*, 1996*a*), xylose–lysine giving the most complex, and glucose–glycine the simplest, profiles (Fig. 2). Four types of chromatographic behaviour were observed: a tailing broad band (observed most clearly at 360 nm) a convex broad band (observed most clearly at 460 nm), unretained peaks and resolved peaks. Some unretained peaks and some resolved peaks were observed at each monitored wavelength. Analysis of the aqueous sugar–glycine systems by CE as well as by HPLC demonstrated that CE gave superior resolution of the reaction products (Royle *et al.*, in press).

Most foods are not aqueous solutions and the study of a system that is closer to food is of more interest to industry. Mixtures of starch, glucose and lysine (96:3:1, m:m:m) were extrusion cooked, using a twin screw machine, at a total moisture content of 15% and a die

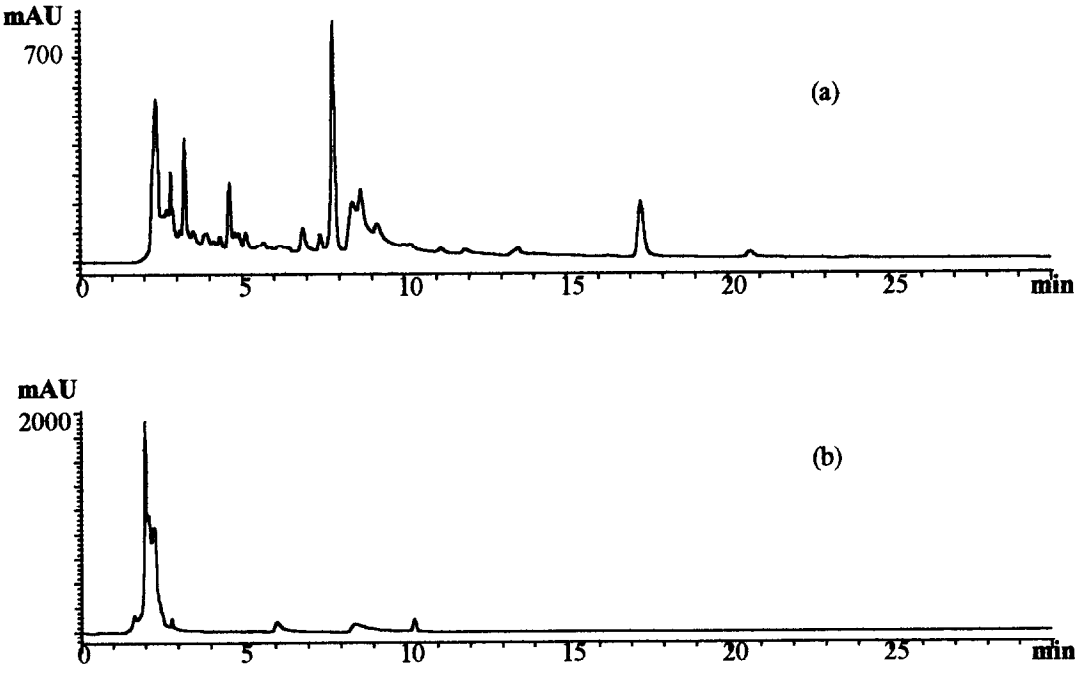

Fig. 2. HPLC analysis of (a) the aqueous xylose–lysine system refluxed for 15 min, and (b) the aqueous glucose–glycine system refluxed for 2 h. pH controlled at 5 during heating. Detection wavelength 280 nm. Spherisorb ODS2, linear water/methanol gradient, 5–45% methanol over 30 min.

temperature of 150°C (Bates *et al.*, 1994). When the methanol extracts of the starch–glucose–lysine extrudates were separated by HPLC and CE, again, CE gave better resolution (Ames *et al.*, 1997). However, when the ultimate aim is to isolate selected reaction products, prior to structural analysis by NMR and MS, HPLC is preferred over CE because it is easier to scale-up to the semi-preparative mode and larger samples can be injected.

The presence of ARPs is sometimes used as a indicator of the Maillard reaction in foods. The separation of mixtures of ARPs is usually achieved either by HPLC, with post-column derivatisation, or by GC of the silylated oxime derivatives (Eichner *et al.*, 1990), and separation takes about 35 min in each case. However, Yaylayan and Forage (1991) separated the ARP of glucose and tryptophan from its thermal degradation products without derivatisation using reversed phase HPLC and a mobile phase with the pH decreased to 2.3 with phosphate buffer. Underivatised ARPs of glucose and phenylalanine and of glucose and proline have recently been separated by CE using the method described by Royle *et al.* (in press). Reproducible migration times of between 4 and 6 min were obtained for those two ARPs. This suggests that CE could potentially provide a simple and rapid technique for the analysis of mixtures of ARPs.

Classifying Maillard reaction products

Volatile Maillard reaction products contribute to food flavour and complex mixtures of them may be separated and identified by routine gas chromatography–mass spectrometry (GC–MS). The task of assigning structures to non-volatile Maillard reaction products is less straightforward. Due to the similarities between the structures of many of the compounds concerned, it is often necessary to analyse isolated reaction products by nmR (both ^{1}H and ^{13}C) and MS in order to completely elucidate their chemical structures. Since the isolation step is a time-consuming procedure, it is very useful to have an on-line technique capable of providing sufficient data to allow classification of the reaction products. Data obtained from an HPLC or CE diode array detector may be used in this way.

Diode array spectra have been obtained for standard compounds and compared to those for the resolved peaks of the aqueous model systems using the same HPLC conditions (Bailey *et al.*, 1996*a*). Spectra of standard pyrroles, furanones and pyrazines were virtually identical, within each chemical class. Where the diode array spectra (but not necessarily the retention times) of the resolved peaks of the model system matched those of the standards, they were described as, e.g. furanone-like or pyrrole-like. The peaks from the glucose–lysine model system refluxed for 2 h at pH 5 that possessed pyrrole-like and furanone-like spectra are indicated in Fig. 3, together with the diode array spectra for the standard 4-hydroxy-5-methyl-3(2*H*)-furanone and 2-pyrrolealdehyde.

Identifying Maillard reaction products

In spite of the difficulties encountered in obtaining structures for coloured Maillard reaction products,

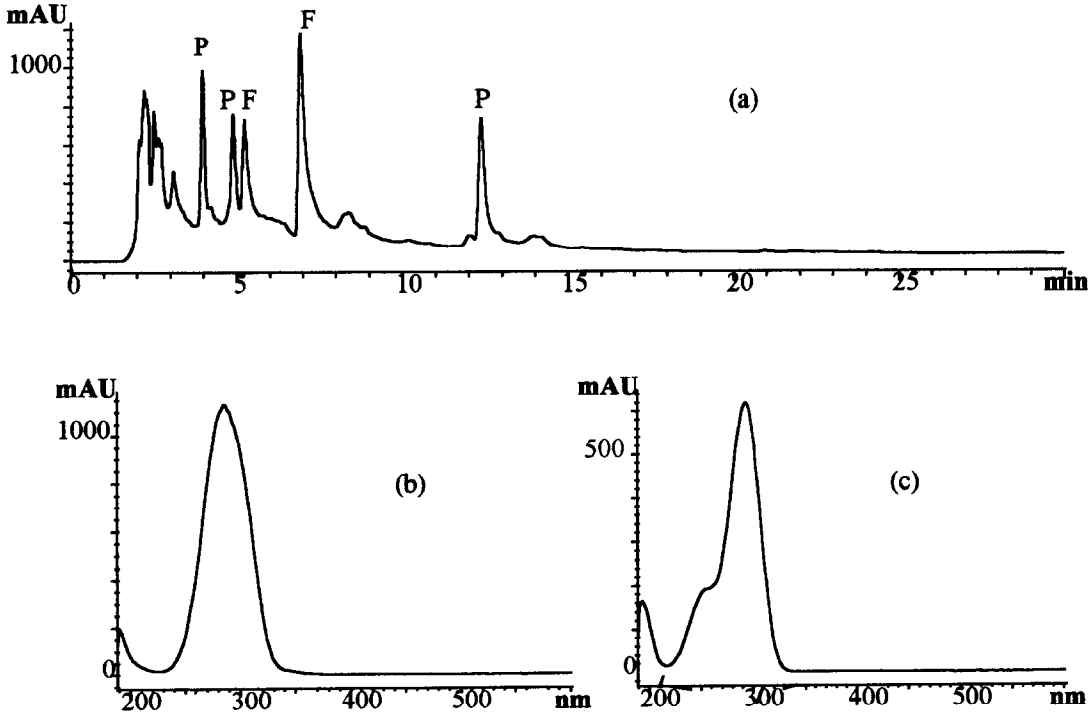

Fig. 3. HPLC analysis of (a) the aqueous glucose–lysine system refluxed for 2 h with the pH controlled at 5 during heating, detection wavelength 280 nm, P: peak with a pyrrole-like spectrum; F: peak with a furanone-like spectrum. Spherisorb ODS2, linear water/methanol gradient, 5–45% methanol over 30 min. (b) diode array spectrum of 4-hydroxy-5-methyl-3(2*H*)-furanone, (c) diode array spectrum of 2-pyrrolealdehyde.

several have been reported from model systems (Ledl and Schleicher, 1990; Ames, 1992), including five that have been reported from aqueous sugar/amino acid solutions (Ames *et al.*, 1993). Recently, Arnoldi *et al.* (1997) reported the first structure of a three-ring reaction product formed in an aqueous xylose–lysine solution (see Fig. 4). The presence of furan rings, a cyclopentenone ring, carbonyl groups, a hydroxymethyl group and an interannular carbon–carbon bond are features that this compound possesses in common with some of the other previously reported structures (Ames *et al.*, 1993). The formation of this compound is unclear. It seems likely that the two furan rings derive directly from the carbon skelton of xylose and it is probable that aldol condensation of xylose fragments are also involved in its formation (Arnoldi *et al.*, 1997). The contribution to the total colour of Maillard model systems made by these low molecular weight compounds is likely to be small, since macromolecular reaction products appear to be responsible for the majority of colour in such systems. However, the structure of the macromolecular material in sugar–amino acid model systems is unclear. It is possible that it comprises a relatively colourless backbone with low molecular weight substructures (such as those discussed here) attached at intervals.

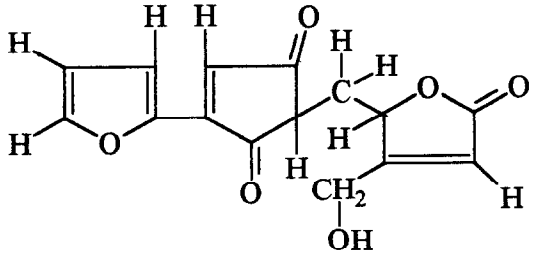

Fig. 4. Structure of a novel three-ring compounds identified from a xylose–lysine system (Arnoldi *et al.*, 1997).

EFFECT OF VARYING THE PROCESSING CONDITIONS ON THE MAILLARD REACTION

Once the methods have been developed for separating, classifying and identifying Maillard reaction products, they may be applied (with modifications where appropriate) to the study of the effect of varying the processing conditions on the reaction products.

Effect of temperature and time

When an aqueous xylose–lysine solution, which had been refluxed for 15 min with pH control, was subjected to (a) further refluxing so the total heating time was 60 min, or (b) storage at 40°C for up to 3 weeks (Ames *et al.*, 1996), very different chromatograms were obtained (Fig. 5). One resolved peak (HP) developed when the sample was refluxed for 60 min but it was absent from the stored sample, while a second resolved peak became prominant only on the chromatogram of

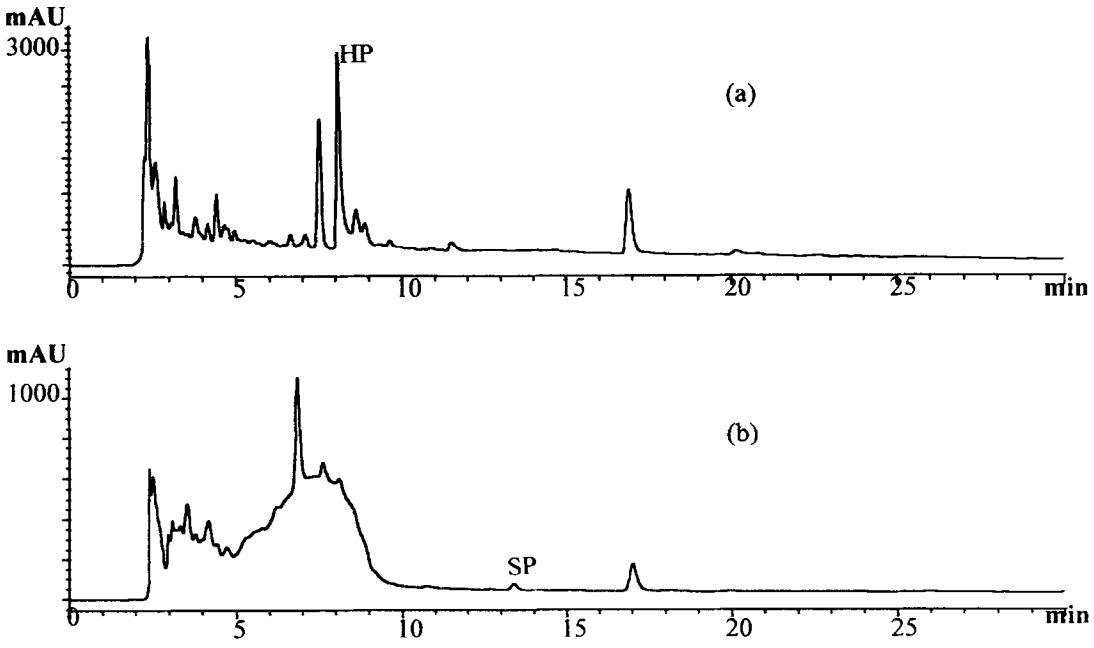

Fig. 5. HPLC analysis of an aqueous xylose–lysine system refluxed without pH control for (a) 60 min, and (b) after storage of the 15 min refluxed system at 40°C for 3 weeks. Detection wavelength 280 nm. Spherisorb ODS2, linear water/methanol gradient, 5–45% methanol over 30 min. See Fig. 2a for the chromatogram after refluxing for 15 min.

the stored sample (SP). However, the most noticeable difference between the chromatograms of the sample refluxed for 60 min and the stored sample, was the development of a band of poorly resolved material in the stored sample and a general loss of resolution of sample components.

Differences between the profiles of reaction products isolated from foods could be used to identify marker compounds which might indicate the history of the sample.

Effect of high pressure

Considerable interest is currently being shown in the use of high pressure (often in the region 100–800 MPa) as an aid to preserving food or as a means of making products with different quality attributes (Ledward, 1995). Aqueous glucose–lysine solutions incubated at 50°C over the pH range 6.5–10.1, at either atmospheric pressure or at 600 MPa, showed a rate of colour development (measured by absorbance at 420 nm) that increased with pH. However, the effect of pressure varied according to the pH of the system (Table 1). At pH 6.5, browning was faster at atmospheric pressure, while, at pH 8.0 and 10.1, high pressure increased the rate of colour development (Hill *et al.*, 1996). The effect of pressure seemed to be reversed in the region pH 7–7.5.

Table 1. Pseudo zero-order rate constants (absorbance units at 420 nm/h) for aqueous glucose–lysine solutions of initial pH 6.5, 8.0 and 10.1, incubated at 50°C at atmospheric pressure and 600 MPa (Hill *et al.*, 1996)

pH	Atmospheric pressure	600 MPa
6.5	0.02	0.006
8.0	0.1	0.5
10.1	3	23

At lower pH, such as pH 6.5, the system will be buffered mainly by the carboxylic acid group of the amino acid. It has been suggested that high pressure favours the ionic form of this group and the resulting pressure-induced pH decrease, about 0.2 of a pH unit for every 100 MPa pressure applied (Heremans, 1995), would be expected to reduce the rate of browning, since colour development in Maillard systems is known to be retarded by decreasing the pH (Ames, 1990). At higher pH, including pH 8.0 and 10.1, buffering will be by the amino groups and dissociation of these groups is reported to be largely independent of pressure (Heremans, 1995). Therefore, in the system described, at high pH, the pH of both the atmospheric and pressurised systems would be the same, implying that pressure may enhance the rate of colour development at high pH. At low pH, pressure may also enhance colour development, but the effect may be overriden by the pressure-induced pH decrease in the pressurised system resulting in reduced browning. In all the systems, pressure may also modify the chemical reactions taking place, thus influencing the rate of colour development.

Yields of the major volatile reaction products from the glucose–lysine system (initial pH 10.1) incubated at 60°C to the same degree of browning (5 h at 600 MPa or 8 h at atmospheric pressure) are shown in Table 2. A dramatic decrease in yields of compounds was obtained at high pressure, with no compound being reported at more than 30% of the level at which it was present in

Table 2. Yields (μg/0.1 mol glucose) of volatiles from the pH 10.1 aqueous glucose–lysine system incubated at atmospheric pressure (AP) and at 600 MPa (Hill *et al.*, 1997)

Identity	AP	600 MPa	Relative % yield at 600 MPa
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	108	17	16
Methylpyrazine	257	3	1
2,5-and/or 2,6-Dimethylpyrazine	3951	110	3
Trimethylpyrazine	758	20	3
3-Methyl-1,2-cyclopentanedione	25	4	16
2-Acetyl-1,4,5,6-tetrahydropyridine	30	9	30
2-Acetylpyrrole	38	ND	NA
2,5-Dimethyl-2,5-cyclohexadiene-1,4-dione	7	ND	NA
2,3-Dihydro-3,5-dihydroxy-(4*H*)-pyran-4-one	442	5	1
7-Acetyl-5,6-dimethyl-2,3-dihydro-(1*H*)-pyrrolizine	78	12	15

the atmospheric system (Hill *et al.*, 1997). High pressure will increase the rates of reactions that possess negative volumes of activation, such as those where the numbers of molecules decrease, e.g. condensation reactions (Matsumoto *et al.*, 1985). Therefore, it seems that 'condensation-like' reactions, leading ultimately to melanoidins, are favoured by high pressure but that yields of small (volatile) molecules are reduced. This suggests that high pressure may be used to produce food ingredients, such as caramel colours, with different properties from those manufactured by conventional means.

Effect of pH

Aqueous systems

pH has a major influence on the importance of many pathways followed during the Maillard reaction, and it, therefore, strongly influences the profile of reaction products. When the diode array spectra were compared of the resolved HPLC peaks of aqueous xylose–lysine solutions that had been refluxed for 15 min, either with the pH maintained at 5 during heating, or by allowing the pH to fall (final pH 4.0), some peaks were common to both systems while others were detected on only one of the chromatograms and they are given in Table 3 (Bailey *et al.*, 1996*b*). Such data may indicate possible common reaction pathways under different sets of processing conditions, and also compounds that may be used as markers to reveal the processing history of the sample.

Extruded systems

Starch–glucose–lysine extrudates were prepared from feedstocks adjusted to pH 3.4 or 7.7 by the addition of citric acid or sodium bicarbonate (Bates *et al.*, 1994). Reverse phase HPLC of the methanol extracts showed one major resolved peak in each (Ames *et al.*, 1997). They were identified as 5-hydroxymethylfurfural (HMF) (pH 3.4) and 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone (pH 7.7). Surprisingly, HMF was also identified in small amounts in the pH 7.7 system. The formation of this compound is favoured by low pH in aqueous Maillard model systems and its identification from the pH 7.7 model extrudate is perhaps surprising. However, HMF is found at low levels in liquid heat-treated milk (Meissner and Erbersdobler, 1996), a system with a pH of about 6.5.

Using the method described by Bates *et al.* (1994), colour measurement data were also obtained for the ground extrudates prepared from feedstocks with the pH adjusted to values in the range 3.4–7.7 (Table 4). As the pH increased, the L* value decreased (indicating increased darkness of the extrudate), the a* value increased (indicating increased redness) and the hue angle decreased (indicating a shift from yellow–brown to orange–brown). Analysis of variance (ANOVA) of the data showed that pH had a significant effect on the L*, a* and hue angle values (Bates, 1996). The effects of pH on the b* value (yellowness) and C* value (colourfullness relative to the surroundings) were less clear and not significant. The quantitative descriptive analysis (QDA) data for the unground extrudates were also

Table 3. HPLC diode array data for the major resolved peaks detected in the aqueous xylose–lysine model systems refluxed for 15 min with control of the pH at 5 and without pH control (final pH 4.0) (Bailey *et al.*, 1996*b*)

Common peaks[a]			Unique peaks[b]		
Rel. t_R[c] (min)	λmax (nm)[d]	Spectral family	Rel. t_R (min)	λmax (nm)	Spectral family
0.48	271sh, 297	A	0.62*	271sh, 299	A
0.66	269sh, 302	A	1.08#	269sh, 299	A
1.13	267sh, 297	A	0.60#	317	C
1.00	269, 333	B	1.01*	317	C
1.33	269, 333	B	1.98*	229, 277	D
1.62	269, 333	B	2.74#	237, 351	F
2.58	261sh, 293	E			

[a] Peaks detected in both systems.
[b] Peaks detected in only one of the systems. *Detected only in the system heated with pH control. #Detected only in the system heated without pH control.
[c] Retention time (t_R) relative to the retention time of the first member of Spectral Family B.
[d] sh, shoulder.

Table 4. Colour measurement data for the ground starch–glucose–lysine extrudates prepared from feedstocks of different pH and a feed moisture content of 15% (Bates, 1996)

pH of feedstock	L*	a*	b*	Hue angle	C*
3.4	70.97	3.44	17.73	79.31	18.09
4.0	67.14	5.14	19.25	75.05	19.92
5.0	66.88	5.77	20.00	73.95	20.83
7.4	59.55	8.81	19.85	65.94	21.73
7.7	56.70	8.18	17.42	64.80	19.24

obtained, using a trained sensory panel, and are displayed in Fig. 6. The scores for darkness, colour intensity, brown, orange and red all increased with pH, while higher scores for yellowness and puffiness were obtained as the pH decreased. The mean scores of all these terms differed significantly between the samples.

The colour measurement and sensory data show that Maillard browning increased with pH, as expected. The use of methanol to extract the coloured material from the extrudates was inefficient and most of the coloured material remained bound to the starch matrix. Covalent bonds could be involved. Further work, including enzymic digestion of the starch, has recently been carried out (Ames *et al.*, 1998).

PREDICTION OF QUALITY ASSOCIATED WITH THE MAILLARD REACTION IN FOOD SYSTEMS

It is useful to be able to predict the quality of a food product, given that the properties of the starting materials and the processing conditions are known. There is also interest in being able to define the processing conditions required to produce a product with a stated specification, when the properties of the raw materials have changed. In both cases, the first step is to understand how the properties of the product vary with the composition of the raw materials and the processing conditions.

Many food-processing operations require large amounts of material for trial runs. Therefore, there is a need for laboratory-scale equipment that can be used to obtain meaningful data when the effect of varying the raw material or the processing conditions is studied. Bates *et al.* (1994) designed a laboratory reaction cell that could mimic most of the conditions encountered in an extruder (rapid heating and cooling, short residence time, temperature and pressure). It allowed data to be obtained over a wide range of processing conditions and using very small (20 g) samples. It could not mimic shear and the products were not expanded. The reaction cell was used to obtain information that could be used to predict the colour development of the starch–glucose–lysine mixture in the extruder.

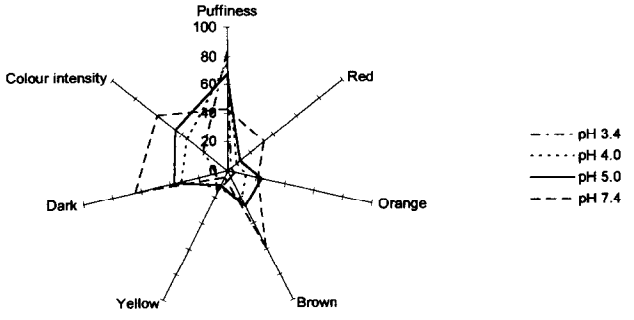

Fig. 6. Spider plot of mean attribute scores for the unground starch–glucose–lysine extrudates prepared from feedstocks of different pH and a feed moisture content of 15% (Bates, 1996).

Linear regression of the colour measurement data obtained at pH values from 3.4 to 7.7 in the reaction cell and the extruder are shown in Table 5. For the b* and C* values, the R^2 values are very low for the extruded samples and the gradients for the reaction cell and extruded samples are rather different. The gradients for the L*, a* and hue angle values were very similar for the reaction cell and extruded samples and so linear regression equations were obtained for these colour measurements, using the reaction cell data (eqns 1–3).

$$L^* = -2.6(\text{pH}) + K1 \quad (1)$$

$$a^* = 0.9(\text{pH}) + K2 \quad (2)$$

$$\text{Hue angle} = -3.6(\text{pH}) + K3 \quad (3)$$

where K1–3 are the intercepts.

By assuming the same gradients for the extrusion data and the mean L*, a* and hue angle values, the K values were calculated to be 79.88, 1.27 and 91.95, respectively. The regression equations were used to predict these values for the extrudate prepared at pH 6.5. The predicted values (with the experimental values in brackets) were, L*, 63.0 (63.3); a*, 7.1 (7.3); hue angle, 68.5 (69.0).

Future work could involve developing a model that would better fit all the experimental colour measurement data and which could be used to accurately predict the effects of pH, moisture content and temperature and time of heating on colour development. The application of such a model to a wider range of model food

Table 5. Linear regression data obtained for the starch–glucose–lysine extrudates on plotting colour responses against feedstock pH (Bates, 1996)

Measurement	Reaction cell			Extruder		
	R^2	Intercept	Gradient	R^2	Intercept	Gradient
L*	0.80	95.9	−2.6	0.63	81.3	−3.0
a*	0.82	−4.0	0.9	0.74	−0.2	1.2
b*	0.95	−6.6	3.0	0.01	18.4	0.1
Hue angle	0.93	106.4	−3.6	0.77	90.0	−3.2
C*	0.94	−7.0	3.1	0.15	17.6	0.5

systems as well as foods themselves, also needs to be undertaken.

CONCLUSION

The ability to predict the properties of food products is of great importance to the food industry because it gives the manufacturer control over product quality. In order to predict the quality of a food, the effects of variations in the raw material and processing parameters, e.g. temperature and time of heating, pH and moisture content, are required. The study of the effects of processing variables and raw material quality requires appropriate analytical methods and a knowledge and understanding of the chemistry taking place. Nevertheless, this sequential approach of understanding the chemistry underlying the Maillard reaction, studying the processing variables that influence its course, and developing methods for quality attributes dependent on it, represents a promising approach to help industry to maximise the acceptability and nutritional value of food and to minimise the levels of compounds with potentially toxic properties.

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