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Analysis of the non-volatile Maillard reaction products formed in an extrusion-cooked model food system

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An enzymic method was developed to extract the total non-volatile Maillard reaction products from a model starch–glucose–lysine extrudate. This method, coupled with HPLC using a diode array detector, was used to compare the profiles of Maillard reaction products in extrudates prepared from feedstocks with added citric acid (CA) or sodium hydrogen carbonate (SHC). The chromatograms revealed the presence in both samples of material that was unretained by the HPLC column as well as resolved peaks, but the chromatograms of the SHC sample were more complex. Diode array spectra were obtained for three and seven resolved peaks, respectively, from the CA and SHC extrudates. All three peaks from the CA sample were also present in the SHC sample and one was 5-hydroxymethylfurfural (HMF). © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Although the Maillard reaction is primarily responsible for much of the colour that develops when most foods are heated, the majority of the coloured compounds remain uncharacterised (Ames, 1992). Ames and Nursten (1989) classified coloured Maillard reaction products into two groups: the melanoidins, which have been described as polymeric materials, and the low molecular weight coloured compounds, which typically comprise two to four linked rings. The structural elucidation of Maillard reaction products isolated from food-like systems is still in its infancy, with most studies being confined to aqueous solutions of a single sugar and a single amino acid (Ledl and Schleicher, 1990; Ames *et al.*, 1993). Few studies have dealt with intermediate or low moisture systems, although these are of more relevance to many food items (Tomlinson *et al.*, 1993, 1994).

We have recently reported the extraction of some coloured material from an extrusion-cooked starch–glucose–lysine system using methanol (Ames *et al.*, 1997*a*). Only a small proportion of the total colour was extracted and the need for a method capable of dealing with the total coloured material was indicated. The starch may chemically or physically bind the coloured material produced from glucose–lysine interactions and it may also react directly with lysine to give coloured compounds. Kramhoeller *et al.*, (1993) have elucidated the structures of four compounds formed on heating dextrin or starch with propylamine, and enzymic hydrolysis was required to release one of them from the starch matrix.

The aim of this study was to develop a method for the analysis of the total non-volatile reaction products in a model extrusion cooked system and to use the method to compare the profiles of coloured compounds prepared from feedstocks that, when used to prepare aqueous slurries, differed in pH.

MATERIALS AND METHODS

Materials

Wheat starch type A was obtained from ABR Foods, Corby, UK. D-(+)-glucose, L-lysine monohydrochloride, citric acid (CA), sodium hydrogen carbonate (SHC) and calcium chloride were obtained from BDH, Poole, UK. The lysine was of biochemical grade, the calcium chloride was general purpose reagent grade and the other chemicals were of analytical grade. HPLC grade methanol was obtained from Rathburn Chemicals Ltd,

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Walkerburn, UK. HPLC grade water was prepared in the laboratory using a Purite Labwater RO50 unit (Purite Ltd, High Wycombe, UK). Ultrafiltration membranes (type YM30), with a nominal molecular weight cut-off of 30 000 daltons, were supplied by Amicon (Stonehouse, UK). Enzymes were supplied by Novo Nordisk Bioindustries UK Ltd (Farnham, UK).

Preparation of extruded model system

Wheat starch (type A), D-(+)-glucose and L-lysine monohydrochloride were mixed (0.96 : 0.03 : 0.01, m:m:m) to give a homogeneous extruder feed. The pH of a slurry of the feed was adjusted to 4.0 or 7.7 by adding CA or SHC to the dry mix. The feed was cooked in a twin screw corotating extruder, model MPF50 (APV Baker Ltd, Peterborough, UK) to give an expanded product. Starch, without addition of sugar and amino acid and without addition of CA or SHC (pH of an aqueous slurry: 5.0), was also extruded. Significant extrusion cooking conditions were as follows: residence time, 32 s; moisture content of feed, 18%; die temperature, 151 ± 1°C. Full experimental details have been reported previously (Bates *et al.*, 1994).

Preparation of extracts of non-volatile compounds

Ground extrudate (10 g) was suspended in 100 ml of 0.02% calcium chloride solution. The enzyme, Termamyl (0.2 ml), was added and the pH was adjusted to 5.6 using 1 M HCl or 1 M NaOH. The mixture was kept at 40°C in a shaking waterbath for 1 h prior to ultrafiltration through a membrane with a nominal molecular weight cut-off of 30 000 Da. A blank was also prepared, without any extrudate. Extracts were prepared in triplicate. Absorbance measurements, at 360 and 460 nm, were taken before ultrafiltration.

High-performance liquid chromatography (HPLC)

The HPLC system consisted of an HP 1050 quaternary pump and diode array detector (Hewlett-Packard, Bracknell, UK). Data analysis was carried out using HP Chemstation software. Separations were performed using a Spherisorb ODS2 column, particle size 5 μm, (25 cm × 0.49 cm i.d.) fitted with a 5 μm Spherisorb ODS2 guard cartridge (Hichrom Ltd, Theale, Reading, UK) and a linear water/methanol gradient, 5–85% methanol over 60 min. The injection volume was 20 μl. Chromatograms were obtained at 280, 360 and 460 nm and raw data was collected from 190 to 600 nm. Extracts were analysed in duplicate.

RESULTS AND DISCUSSION

The extrudates prepared with CA were pale yellow, while those prepared with SHC were dark brown. Starch extrudates were off-white. Detailed colour measurement data for them have been reported previously (Bates *et al.*, 1994).

A preliminary study compared the ability of continuous solvent extraction using a range of solvents (supercritical carbon dioxide, methanol, 2% dimethyl formamide in methanol, ethyl acetate and diethyl ether) to extract the colour from the extrudates (Bates, 1996). Methanol extracted more colour than the other solvents and the separation of the methanol-extractable components by isoelectric focusing, capillary electrophoresis and HPLC has been reported (Bates, 1996; Ames *et al.*, 1997*a*). However, most colour remained in the starch matrix and, therefore, attention was turned to enzyme extraction.

A study was performed to assess the ability of six different carbohydrate enzymes to hydrolyse soluble starch, using the incubation conditions outlined in the experimental section. Termamyl gave the greatest rate of release of reducing groups and the highest yield of reducing groups, as assessed by the 3,5-dinitrosalicylic acid method (Chaplin, 1994). Termamyl contains an endoamylase which will hydrolyse 1,4-α-glucosidic linkages in amylose and amylopectin. It breaks down starch rapidly to soluble dextrins and oligosaccharides (Novo Nordisk, 1995).

The enzyme extraction procedure resulted in solubilisation of the extrudates and the enzyme was removed by the 30 000 Da membrane. Absorbance measurements for the extracts prepared from the extrudates are given in Table 1. Values for the CA sample were 1.5- and 3-fold lower at 360 and 460 nm, respectively, than those for the SHC sample. Values for the starch extracts were about 10-times lower than those for the CA sample.

HPLC chromatograms of the extract of extruded starch and the blank extract showed only a few small peaks. In contrast, several prominent peaks were observed for the starch–glucose–lysine mixtures of both pH values. These peaks could be divided into two groups. The first group comprised the peaks that were unretained or poorly retained by the column. Well resolved peaks with retention times of more than 4 min comprised the second group. They were observed at 280 nm for both samples and at 360 nm for the SHC extract (Fig. 1).

The λ_{max} values of the largest resolved peaks for both

Table 1. Absorbance values of the extracts of the extrudates[a]

Sample	Wavelength (nm)	
	360	460
Starch	0.151 ± 0.015	0.043 ± 0.009
CA	1.892 ± 0.007	0.448 ± 0.006
SHC	2.816 ± 0.020	1.313 ± 0.035

[a]Values are means of triplicate readings taken with reference to water.

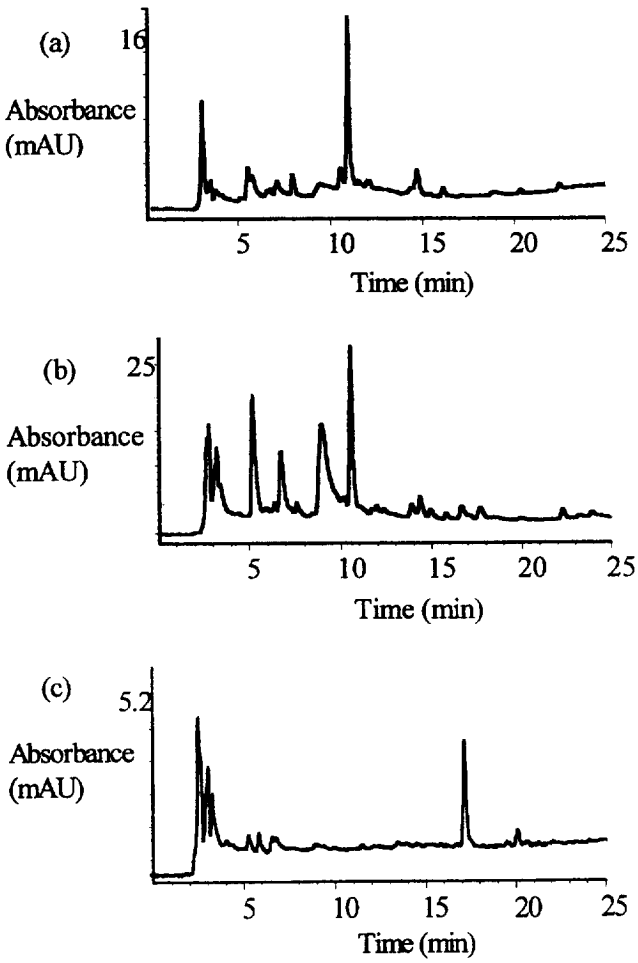

Fig. 1. HPLC chromatograms of the starch–glucose–lysine extrudates: (a) CA, 280 nm; (b) SHC, 280 nm; (c) SHC, 360 nm.

samples are given in Table 2. One peak from both samples had a retention time (10.62 min) and diode array spectrum (λ_{max} 275 nm with a subsidiary maximum at 225 nm) that corresponded exactly with that of authentic HMF analysed using the same HPLC conditions. It represented 37.3 and 18.3%, respectively, of the total peak area at 280 nm, for the CA and SHC samples. No data were obtained that would allow a comparison of the absolute amounts of HMF formed at the two pH values. However, since HMF is a major low molecular weight compound formed when hexose sugars take part in the Maillard reaction under conditions of low pH (Ames, 1992), its presence at lower relative amounts in the higher pH sample was expected in this study. Based on retention time and spectral matches, two other resolved peaks from the SHC extrudate (those at 5.23 and 14.35 min) were also present in the CA sample. The five resolved peaks detected at 360 nm from the SHC sample are particularly interesting since they are coloured. A spectrum was obtained for the peak at 17.16 min and the λ_{max} value is included in Table 2. None of these peaks at 360 nm was detected in the CA sample.

Table 2. λ_{max} values of the largest resolved peaks observed on the chromatograms of the enzyme digests of the CA and SHC extrudates

CA extrudate		SHC extrudate	
Retention time (min)	λ_{max} (nm)	Retention time (min)	λ_{max} (nm)
5.20[a]	295, sh. 230	5.23[a]	295
10.62#[b]	285, 225	6.75	290
14.38[c]	300, sh. 265	9.03	298
		10.62#[b]	285, 225
		14.35[c]	300, sh. 260
		16.76	270, sh. 300
		17.16*	362

sh, Shoulder; #, HMF. *Detected only on the 360 nm chromatogram. All other peaks were detected only at 280 nm.
[a,b,c]Peaks with the same superscript contain the same component(s).

It has been reported previously that the spectra of furans, furanones, pyrroles and pyrazines give UV-visible diode array spectra that are characteristic of each chemical class (Bailey *et al.*, 1996). By comparing the spectra of the 5.23, 6.75, 9.03 and 14.35 min peaks of the SHC extrudate (see Table 2) with those of standard reference compounds, all these sample spectra are tentatively described as furanone-like. Some of the standard reference compounds used by Bailey *et al.*, (1996) have been identified among the volatile components of these starch–glucose–lysine extrudates (Ames *et al.*, 1997*b*). They include furfural in both extrudates and 2-acetylfuran in the CA sample. They were probably not detected in this study of the total reaction products due to their presence at lower levels than in the extracts of the volatiles.

In another study in this laboratory (Bailey *et al.*, 1996), an aqueous solution of glucose and lysine was refluxed for 120 min without pH control and the mixture of reaction products was analysed using the same HPLC conditions as those used in this study. The spectrum of the 17.16 min peak of the SHC extrudate (see Table 2) was very similar to the spectra of three peaks of the aqueous sample. Owing to the different retention times, none of the peaks from the aqueous sample is due to the compound isolated from the extrudate, although some common structural features are likely.

Attempts were made to compare the data presented in Table 2 with equivalent data for the methanol-extractable components of these extrudates (Ames *et al.*, 1997*a*) and to look for components that were common to the two extracts prepared from the same extrudate. However, since different HPLC stationary and mobile phases were used for the two studies, this proved to be very difficult. Nevertheless, HMF was identified in both extrudates from the methanol extracts and from the enzyme extracts prepared in the current study.

Unretained or poorly retained peaks eluted within 4 min of sample injection, i.e. within the void volume of the column for both samples. They were poorly resolved and were observed at all three monitored wavelengths,

indicating that at least some of the material responsible for them was coloured. Spectra of these peaks had λ_{max} values at about 315 nm and showed tailing into the visible region.

It is interesting to note that the 460 nm chromatogram of the SHC extrudate showed no broad band of unresolved material. Such a band was observed on the 420 nm chromatogram of the methanol extract of this extrudate (Ames *et al.*, 1997*a*) and on the 460 nm trace of an aqueous (pH 5) glucose–lysine system refluxed for 2 h (Bailey *et al.*, 1996), and was attributed to high molecular weight melanoidin in both cases. The most likely explanation for its absence from the enzyme extract is that the sample was too dilute for this band to be observed. An alternative explanation is that the enzyme cleaved the unresolved material to smaller molecules that did not form a broad band.

CONCLUSION

A method has been developed for the extraction of the total non-volatile Maillard reaction products of a model extrudate prepared from starch, glucose and lysine. Application of the method to extrudates prepared from feedstocks giving aqueous slurries of different pH followed by HPLC has illustrated differences and similarities between the major resolved reaction products. This method is likely to be appropriate for the analysis of similar compounds from a range of carbohydrate-based foodstuffs.

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