*Food Chemistry* **43** (1992) 359–367

# Melanoidins from glucose and glycine: composition, characteristics and reactivity towards sulphite ion

**B. L. Wedzicha & M. T. Kaputo**

*Procter Department of Food Science, University of Leeds, Leeds LS2 9JT, UK*

(Received 3 December 1990; accepted 8 March 1991)

Melanoidins with $M_r$ > 12000 were prepared by reaction of glucose with glycine (molar ratios 1:9 to 9:1) at 55° and 90°C, initial pH 5·5. Extinction coefficients ($E^{1\%}$) at 450 nm were 36·9 ± 6·9 and 40·5 ± 2·9 at the two temperatures, respectively, and do not depend on reaction time or the molar ratio of glucose to glycine used to prepare the polymer. All the melanoidins had similar degrees of dehydration (c. 3 mol $H_2O$/mol glucose) and stoichiometry with respect to glucose and glycine, but the degree of decarboxylation of glycine varied in the range 0–38% of residues incorporated into the polymer. The melanoidin prepared when [glucose] > [glycine] was more reactive towards sulphite species than when [glucose] < [glycine], and the extent of bleaching of melanoidin at 450 nm was proportional to the number of sulphur atoms incorporated into the polymer. These observations are related to the nature of the chromophores in melanoidins and the mechanism of polymerisation.

## INTRODUCTION

In a previous paper (Wedzicha & Kaputo, 1987) we considered the stoichiometry of the reaction of sulphur(IV) oxospecies (S(IV)) with the high molecular weight coloured products (melanoidins) of Maillard browning of glucose and glycine. Melanoidins with $M_r$ > 12000 were prepared by reaction of 1·25 mol glucose with 1 mol glycine when 1·0 M glucose was heated with 0·5 M glycine (pH 5·5, 90°C, 22 h). It was demonstrated that this product combined with S(IV) to the extent of one atom of sulphur becoming incorporated for every two or three molecules of glucose used to form the polymer. The degree of unsaturation of the melanoidin was estimated by bromination of the double bonds and was found to equal the number of sites reactive towards S(IV).

A wide range of procedures for the preparation of melanoidins has been used by previous workers and, to illustrate the diversity of systems tried, the compositions of some model browning reactions and the purpose for which they were studied are given in Table 1. There seems to be no consensus as to what constitutes the best model Maillard reaction. We see that, in general, the hexose– or pentose–glycine systems are the most widely studied, the particular advantage of glycine being that it demonstrates the reactivity of the α-amino moiety without complications of reactive side chains. There are few investigations of the effects of reactant concentration on the composition and properties of melanoidins and this must, surely, provide important information regarding the mechanism of the build up of the polymers.

It may be speculated that melanoidins are formed as a result of the random polymerisation of carbohydrate degradation products (e.g. 3-deoxyosuloses) or adducts of these with amino compounds. The regularity of the polymers with respect to nitrogen-containing and nitrogen-free subunits, and whether the frequency of the subunits is affected by the composition of the reaction mixture with respect to aldose and amino acid, are not known. For this reason we decided to initiate a systematic investigation of the structure of melanoidins by considering the relationship between composition of reaction mixtures and certain properties of the high molecular weight products. In the first instance we consider the yield, absorbance and reactivity towards S(IV) of high molecular weight melanoidins prepared from glucose and glycine.

*Food Chemistry* 0308-8146/92/$05.00 

**Table 1. Model Maillard browning systems reported in the literature**

| System | [Aldose] (M) [Amine] (M) | pH or buffer | T (°C) | Purpose | Reference |
|---|---|---|---|---|---|
| Glucose–glycine | 0·1–1<br>0·1–0·5 | 2–8 | 50–90 | Kinetics of browning | Song *et al.*, 1966; Nam & Kim, 1984 |
| | Equimolar 0·4, 1·0, 2·0 | 6·8 | 95 | Characterisation of melanoidins | Hayase *et al.*, 1984; Kim *et al.*, 1985; Kim & Park, 1986; Kato *et al.*, 1986, 1987 |
| | 1·67<br>1·0 | | 100–125 | Products and composition | Maillard, 1912, 1916 |
| | 2·0<br>2·0 | 6·8 | Reflux | Physiological effects of melanoidins | Fujimaki *et al.*, 1979 |
| | Variable | | 50–110 | Rate of formation of melanoidins | Obretenov *et al.*, 1986 |
| | 0·5–2·0<br>0·25–1·0 | 5·5 | 55 | Kinetics of inhibition of browning | McWeeny *et al.*, 1969; Wedzicha & Vakalis, 1988 |
| Glucose–glycine or alanine | 1·5<br>1·5 | | 90 | Kinetics of browning and characterisation of melanoidins | Taguchi & Sampei, 1986 |
| Glucose–glycine or methionine | 0·2, 1·0<br>0·2, 1·0 equimolar | 3·5 (phthalate) | Reflux | Characterisation of melanoidins | Feather & Nelson, 1984; Feather & Huang, 1985 |
| Glucose–lysine | 0·33<br>0·054 | 4–8 (methanol) | 90–110 | Kinetics of browning | Lee *et al.*, 1984 |
| | 0·6<br>0·9 | 7·9 | 100 | Physiological effects of melanoidins | Takeuchi *et al.*, 1987 |
| Glucose–arginine or histidine | 1·0<br>2·0 | 3–11 | 80–110 | Antibacterial compounds | Einarsson, 1987*a*,*b* |
| Glucose or fructose–glycine | 0·5<br>0·5 | 5·5 | 100 | Kinetics and mechanism of browning | Kato *et al.*, 1969 |
| Glucose or fructose–glycine | Ratio 2·5:1·0 | 5·0 | 50 | Inhibition of browning | Burton *et al.*, 1962 |
| Glucose, fructose or HMF–glycine | 0·2<br>0·2 | 3·5 (phthalate) | Reflux | Characterisation of melanoidins | Feather & Nelson, 1984 |
| Glucose or xylose–butylamine, glycine or ammonia | | 5·2–6·5 (water or methanol) | 100 | Characterisation of melanoidins | Kato & Tsuchida, 1981 |
| Glucose or xylose–butylamine, glycine or ammonia | 2·0<br>2·0 | 5·2–6·5 (water or methanol) | 100 | Reductone content of melanoidins | Kato *et al.*, 1968 |
| Xylose–glycine | 0·2<br>0·2 | 5·0 (acetate) | 100 | Separation and characterisation of melanoidins | Motai, 1974<br>Motai & Inoue, 1974 |
| | 2·0<br>2·0 | 6·5 | 90 | Reductone content of melanoidins | Gomyo *et al.*, 1972 |
| | 0·8<br>0·8 | Acetate | 90–100 | Antioxidant activity of products | Kirigaya *et al.*, 1968 |
| | 1·0<br>1·0 | 8·2 (phosphate) | Reflux | Isolation of coloured product | Nursten & O'Reilly, 1986 |
| Xylose or arabinose–glycine, alanine or urea | 1·0<br>1·0 | | 22, 68<br>100 | Characterisation of melanoidins | Benzing-Purdie *et al.*, 1983; Benzing-Purdie & Ratcliffe, 1986 |
| Various | 1·0–1·25<br>0·69 | 3–9 | 50 | Rate of browning | Spark, 1969 |
| Various | Variable | 6–7·2 | 65, 100 | Rate of browning | Wolfrom *et al.*, 1974 |
| Various | Variable | Alkaline | Hot | Characterisation of melanoidins | Rubinsztain, *et al.*, 1984 |

## MATERIALS AND METHODS

All the reagents were of AnalaR grade and were obtained from BDH Chemicals, Poole, UK. Melanoidins were prepared by heating glucose (0·1–1·0 M) and glycine (0·1–1·0 M), initial pH 5·5, at 55° and 90°C for varying lengths of time. Nondialysable melanoidins were obtained as described by Wedzicha & Kaputo (1987) and were tested for freedom from specific low molecular weight carbonyl compounds by TLC of 2,4-dinitrophenylhydrazones, and by the radiochemical method described for freedom from other low molecular weight components. The reactivity of melanoidins towards S(IV) was measured as described previously (Wedzicha & Kaputo, 1987), except that S(IV)–melanoidin reaction mixtures were, in general, contained in flasks with air in the headspace.

Non-dialysable melanoidins with $M_r$ > 12000 were further fractionated by ultrafiltration, using an Amicon TRC 10A apparatus fitted with PM30 or XM100A membranes with a cut-off of $M_r$ = 30000 and 100000, respectively. The apparatus was operated at a pressure of 1·75 to 2·9 kg/cm$^2$. Possible adsorption of melanoidins on to the membrane was checked by comparing absorbances of filtrates and retentates with that of the melanoidin solution initially taken for ultrafiltration. No significant adsorption was identified.

The error in composition of melanoidins, determined by microanalysis, was as reported previously, i.e. coefficients of variation for C, H and N were 2·5, 3·1 and 2·9%, respectively, from 10 preparations.

## RESULTS AND DISCUSSION

### Preliminary studies

Dialysis is perhaps the most straightforward method of separating high molecular weight products from reactants and precursors and was used to prepare melanoidins with relative molecular mass $M_r$ > 12000. Figure 1 shows the development of absorbance at 450 nm in the system glucose–glycine and in the retentate after the reaction mixture is dialysed against water. These data show the usual 'induction' period in colour formation and it is seen that in the early stages of browning, the contribution of melanoidins with $M_r$ > 12000 to the absorbance is, as expected, small but this increases with time. Similar trends in behaviour are observed when data for reactions at 55°C are compared with those for reactions at 90°C, but the time scale is much shorter at 90°C, the apparent $Q_{10}$ value for the increase in absorbance being *c.* 10 to 11. At both temperatures the absorbances due to the reaction mixtures and the nondialysable melanoidins, and the yields of nondialysable melanoidins, depend on

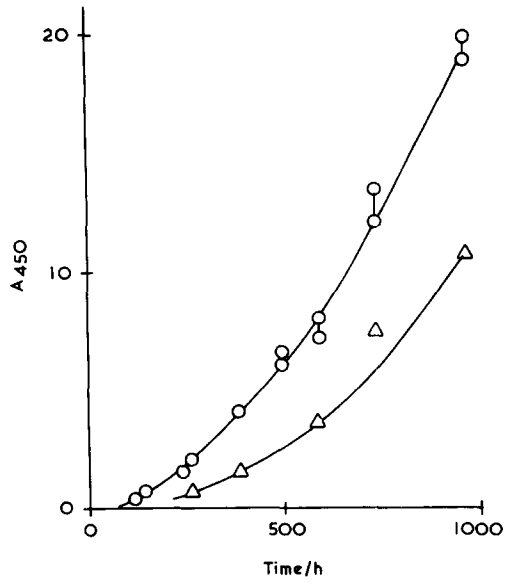



**Fig. 1.** Absorbance–time behaviour of the glucose–glycine reaction and of the nondialysable melanoidins formed. Initial reaction conditions: [glucose] = 1·0 M; [glycine] = 0·5 M; pH 5·5; 55°C. ○, reaction mixture; △, nondialysable melanoidins.

(time)$^2$ and, therefore, conform to the kinetics of browning suggested by Haugaard *et al.* (1951) and Kato *et al.* (1969) at constant reaction mixture composition.

It is interesting to compare the absorbances of nondialysable melanoidins prepared at 55° and 90°C, given in Table 2. The extinction coefficients ($E^{1\%}$) are seen to give no discernible trend with reaction time and may be summarised as 36·9 ± 6·9 and 40·5 ± 2·9 for melanoidins prepared at 55° and 90°C, respectively. This suggests that the chromophores produced in melanoidins with $M_r$ > 12000 obtained in the early stages of heating are similar to those at later stages and, within the sensitivity of the measurements, are independent of heating temperature. These variables in melanoidin preparation are, therefore, relatively unimportant.

**Table 2. Extinction coefficients (450 nm, 1 cm cells) of a 1% (w/w) solution of nondialysable melanoidins ($E^{1\%}$) (prepared by heating glucose (1 M) with glycine (0·5 M), initial pH 5·5, 55°C and 90°C for different lengths of time. The pH values of reaction mixtures at the end of the heating period were 3·69 and 3·71 at 55° and 90°C, respectively)**

| Temperature: 55°C | | Temperature: 90°C | |
|---|---|---|---|
| Time (h) | $E^{1\%}$ | Time (h) | $E^{1\%}$ |
| 120 | 33·3 | 3 | 40·0 |
| 240 | 46·7 | 6 | 36·0 |
| 480 | 47·6 | 9 | 41·5 |
| 600 | 43·0 | 12 | 44·0 |
| 720 | 33·5 | 15 | 42·9 |
| 840 | 33·7 | 18 | 38·7 |

**Table 3. Yields and extinction coefficients (450 nm, 1 cm cells) of a 1% (w/w) solution of nondialysable melanoidins ($E^{1\%}$) (prepared by heating glucose and glycine at various concentrations at 90°C, initial pH 5·5, for 22 h. The final pH values of reaction mixtures were, respectively, 4·88, 4·39, 4·12, 4·19 and 4·41. Yields are the weights of dry product obtained from 30 ml reaction mixture)**

| [Glucose] (M) | [Glycine] (M) | Yield (mg) | $E^{1\%}$ |
|---|---|---|---|
| 0·1 | 0·9 | 4 | 39·5 |
| 0·5 | 1·0 | 59 | 35·4 |
| 1·0 | 1·0 | 58 | 39·9 |
| 1·0 | 0·5 | 57 | 31·1 |
| 0·9 | 0·1 | 2 | 33·7 |

Melanoidins may also be prepared from different molar ratios of glucose to glycine. Table 3 shows the absorbance behaviour of such mixtures, which span an 81-fold change in molar ratio of reactant concentrations, heated at 90°C for 22 h. It is evident that there is, again, no apparent trend in $E^{1\%}$. The yield of melanoidin is also relatively unaffected by composition for molar ratios of 0·5:1·0 to 1·0:0·5 (glucose:glycine). The initial reaction between glucose and glycine, leading to the formation of early intermediates in browning, is known to be of first order with respect to glucose and glycine (Labuza *et al.*, 1977; Wedzicha & Vakalis, 1988), which means that the rate when [glucose] = [glycine] = 1·0 M will be twice as great as that when, say, [glucose] = 1·0 M and [glycine] = 0·5 M. Thus, when the concentrations of glucose and glycine are comparable, the rate-determining step in the formation of nondialysable melanoidins does not appear to be sensitive to initial reactant concentration. Also, this result does not conform to the kinetic equation for browning proposed by Haugaard *et al.* (1951) and Kato *et al.* (1969), in which the dependence on glucose and glycine concentrations was given as first and second order, respectively. On the other hand, the yield of melanoidin falls markedly when the molar concentration of one of the reactants is small; the rate of the initiating reaction when [glucose] = 0·1 M and [glycine] = 0·9 M will be 9% of that when [glucose] = [glycine] = 1·0 M and the corresponding yield of melanoidin would be *c.* 5 mg. We see, therefore, that although the actual yields are lower than this amount, they are at least of the correct order of magnitude.

A more detailed consideration of the yield of melanoidin is possible for the reaction of 1·0 M glucose + 0·5 M glycine at 55°C. The turnover of glucose may be estimated from the kinetics of the S(IV)-inhibited Maillard reaction (Wedzicha, 1984*a*; Wedzicha & Vakalis, 1988; Wedzicha & Garner, 1991). The mechanism of the S(IV)-inhibited Maillard reaction may be summarised by the scheme in Fig. 2, which shows two consecutive rate-determining processes, i.e. formation of intermediates I and II. In the presence of S(IV),

$$\text{Glucose} + \text{glycine} \xrightarrow[k_1]{\text{slow}} \text{I} \xrightarrow[k_2]{\text{slow}} \text{II} \xrightarrow[\text{S(IV)}]{\text{fast}} \text{DSH}$$

**Fig. 2.** Kinetic model for the S(IV)-inhibited Maillard reaction of glucose and glycine when the concentrations of glucose and glycine are much greater than that of S(IV) (Wedzicha, 1984*a*). DSH represents 3,4-dideoxy-4-sulphohexosulose, I and II are intermediates, and $k_1$ and $k_2$ are rate constants.

intermediate II is converted to 3,4-dideoxy-4-sulphohexosulose (DSH). When the concentrations of glucose and glycine are large compared to that of S(IV), the concentration of DSH at any time $t$ is related to rate constants $k_1$ and $k_2$ by

$$[\text{DSH}] = k_1 t - (k_1/k_2)\{1 - \exp(-k_2 t)\}$$

It has been shown that intermediate I is 3-deoxyhexosulose (DH) and that S(IV) interrupts the pathway to formation of colour by reacting quickly with an important intermediate such as 3,4-dideoxyhexosulos-3-ene (DDH). This is formed by dehydration of DH, and could well be intermediate II in the mechanism (Fig. 2). If the extent of formation of DSH is indicative of the amount of glucose, which would, in the absence of S(IV), have been converted to an intermediate in colour formation, this might also be indicative of the possible extent of melanoidin formation. Such an idea assumes, of course, that the intermediate in question does not, for instance, react with its precursors in subsequent reactions in the absence of S(IV).

The rate constant $k_1$ is dependent on [S(IV)], and its value in the absence of the additive must be used. This is $1{\cdot}6 \times 10^{-5}$ M/h (Wedzicha & Vakalis, 1988) for [glucose] = 1 M and [glycine] = 0·5 M, and $k_2 = 5{\cdot}5 \times 10^{-3}$/h at the same concentration for a reaction at initial pH 5·5 and 55°C. The yield of melanoidin was calculated from the knowledge that the nondialysable product consists of an average of 5 mol glucose combining with 4 mol glycine with the elimination of 3 mol $H_2O$ and 0·17 mol $CO_2$ (Wedzicha & Kaputo, 1987). Thus, the relative molecular mass of the subunit of the polymer is 180·4, i.e. the conversion of 1 mol glucose to intermediate gives rise to 180·4 g polymer if all that intermediate is converted to melanoidin.

The concentration of glucose which has undergone reaction at various times, together with the theoretical yield of melanoidin in a 30 ml reaction mixture, should all the glucose reacted have been converted to the polymer, is shown in Table 4, together with actual yields of nondialysable melanoidins. It is evident that the actual and theoretical yields are of similar magnitude, indicating that a high conversion of intermediates to polymers must be taking place. For times <600 h the data show the yields of nondialysable melanoidins to be smaller than predicted and in keeping with the expectation that only a proportion of the melanoidin formed will have $M_r > 12000$. However, at times of 720

**Table 4. Extent of conversion of glucose to intermediates in browning calculated from the kinetics of the S(IV)-inhibited Maillard reaction of glucose (1 M) and glycine (0·5 M), initial pH 5·5, 55°C. (The yield of melanoidin is the amount formed in 30 ml reaction mixture. Theoretical values are based on the assumption that all glucose-derived intermediate is used to make the polymer by combining with glycine in the ratio 5:4 (glucose–glycine) with the loss of 3 mol $H_2O$/mol glucose and 0·17 mol $CO_2$/mol glycine. Measured values are the weights of nondialysable melanoidins obtained from reaction mixtures at given times)**

| Time (h) | [Glucose] converted (mM) | Theoretical yield (mg) | Measured yield (mg) |
|---|---|---|---|
| 240 | 0·94 | 5·1 | 4·6 |
| 480 | 4·8 | 26 | 16 |
| 600 | 6·7 | 36 | 30 |
| 720 | 8·6 | 47 | 51 |
| 840 | 10·5 | 57 | 82 |

and 840 h, yields higher than the theoretical are observed. This is indicative of a faster rate of polymerisation than predicted by the formation of an intermediate in the S(IV)-inhibited Maillard reaction. No kinetic data for the S(IV)-inhibited reaction at 90°C are available.

We conclude from these preliminary experiments that the conditions for melanoidin production are not critical as far as the extinction coefficients of the products are concerned. If one accepts that the degree of polymerisation of melanoidins increases with reaction time, the fact that the extinction coefficient does not seem to change with time implies that it does not depend on the degree of polymerisation. This would be consistent with our suggestion (Wedzicha & Kaputo, 1987) that the absorbance is due to isolated chromophores rather than an extended chromophore. If we imagine two possibilities for formation of colour as being either a gradually increasing degree of conjugation of polymer with accompanying bathochromic shift or the increase in concentration of relatively small chromophores, the data presented here are consistent with the latter.

### Microanalysis data

If the overall reaction for the formation of melanoidin is the combination of $a$ molecules of aldose consisting of $l$, $m$ and $n$ atoms of C, H and O, respectively, and $b$ molecules of amino acid consisting of $p$, $q$ and $r$ atoms of C, H and O, respectively, and 1 atom of N, then the formula of the melanoidin is (Wedzicha, 1984*b*):

$$C_{la+pb-x}H_{ma+qb-2y}O_{na+rb-2x-y}N_b$$

where, respectively, $x$ and $y$ molecules of $CO_2$ and $H_2O$ are formed in the reaction. For the glucose–glycine reaction, $l = 6$, $m = 12$, $n = 6$, $p = 2$, $q = 5$ and $r = 2$. The unknowns $a$, $x$ and $y$ may be found by solving the following simultaneous equations:

$$C - 2 = 6a - x$$
$$H - 5 = 12a - 2y$$
$$O - 2 = 6a - 2x - y$$

where C, H and O represent the number of the corresponding atoms in the empirical formula expressed with N = 1, i.e. $b = 1$. This assumes that the aldehyde product formed as a result of Strecker degradation (loss of $CO_2$) is incorporated completely into the polymer and represents one of the limiting situations. The situation when none of the aldehyde product is incorporated into the melanoidin will be considered after the initial assumption is discussed.

Empirical formulae with calculated stoichiometries are given in Table 5. Different workers have treated the data for the extent of dehydration differently. For example, Kato & Tsuchida (1981) expressed the loss of water as mol $H_2O$/mol sugar after allowing for the elimination of 1 mol water when the amine condenses with the sugar. On the other hand, Feather & Nelson (1984) consider $y/a$ to be a better indicator of dehydration per mole of sugar. The only system which may be compared to the data obtained here is that of Feather & Nelson (1984) who found $y/a = 3$. The data in Table 3 give, respectively, the following values of $y/a$: 3·1, 2·9, 2·8, 3·0, 2·6, 2·8, 2·8 and 3·1. These results are comparable to those of Feather & Nelson (1984) with many of the values close to 3. It is, of course, inappropriate to calculate the mean and standard deviation of this set of values since it is unlikely that all the results will belong to one population.

Whereas dehydration is thought to arise from degradation of the aldose, it is likely that one important contributor to $CO_2$ formation is Strecker degradation of the amino acid. The yield of $CO_2$, expressed as mol $CO_2$/mol amino acid, is much more variable than the degree of dehydration. The lowest values of $x$ were obtained when the amount of one reactant far exceeded

**Table 5. Empirical formulae and calculated number of moles of glucose $a$ incorporated per nitrogen atom into nondialysable melanoidins, with the liberation of $x$ mol $CO_2$ and $y$ mol $H_2O$. (Reaction conditions: initial pH 5·5, 90°C, 22 h. Measurements carried out on whole nondialysable melanoidin fraction except for [a] 12 000 < $M_r$ < 30 000, [b] 30 000 < $M_r$ < 100 000 and [c] $M_r$ > 100 000.**

| [Glucose] (M) | [Glycine] (M) | Empirical formula (N = 1) | | | $a$ (mol) | $x$ (mol) | $y$ (mol) |
|---|---|---|---|---|---|---|---|
| | | C | H | O | | | |
| 0·1 | 0·9 | 6·6 | 9·5 | 4·1 | 0·78 | 0·08 | 2·43 |
| 0·5 | 1·0 | 8·1 | 11·7 | 4·6 | 1·08 | 0·38 | 3·13 |
| 1·0 | 1·0 | 8·1 | 11·8 | 4·9 | 1·06 | 0·25 | 2·96 |
| 1·0 | 0·5 | 9·3 | 12·5 | 5·4 | 1·25 | 0·18 | 3·75 |
| 0·9 | 0·1 | 8·3 | 12·1 | 5·7 | 1·05 | 0 | 2·75 |
| 1·0[a] | 0·5 | 8·8 | 12·5 | 5·4 | 1·16 | 0·18 | 3·21 |
| 1·0[b] | 0·5 | 8·8 | 12·4 | 5·5 | 1·15 | 0·10 | 3·20 |
| 1·0[c] | 0·5 | 9·3 | 12·5 | 5·1 | 1·27 | 0·33 | 3·87 |

that of the other. Indeed, when [glucose] = 0·9 M and [glycine] = 0·1 M, no evidence for the formation of $CO_2$ could be obtained. If, as found by Feather & Huang (1985), $CO_2$ is derived almost completely from the Strecker degradation of amino acid, the extent of decarboxylation could be as great as 38% of the amount of amino acid incorporated into the melanoidin. A simple, but elegant, experiment carried out by Feather & Huang (1986) involved measurement of the incorporation of $^{14}C$ when glycine, labelled selectively in positions 1 and 2, was used to form the melanoidins. Some 60 to 70% of carboxyl groups appeared to be lost from glycine molecules when incorporated into melanoidins as a result of reaction with glucose under unspecified conditions. NMR studies of the polymer prepared using glucose and $^{13}C$-enriched alanine reveals that intact amino acid is also incorporated.

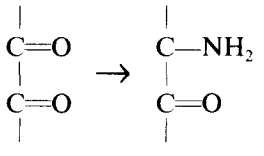
In Strecker degradation of glycine, each mole of $CO_2$ formed is accompanied by 1 mol formaldehyde. Thus, if $x$ mol HCHO are liberated and the product is not incorporated into the melanoidin, the empirical formula of the melanoidin becomes

$$C_{la+pb-2x}H_{ma+qb-2y-2x}O_{na+rb-3x-y}N_b$$

and the stoichiometry of the reaction leading to formation of melanoidin may be found similarly to that described above, using empirical formulae given in Table 5. Calculation shows all values of $x$ and $y$ to be the same as those given in Table 5, but differences were seen in some values of $a$. The greatest difference is when $x$ = 0·38 mol and the value of $a$ is 0·06 mol greater than the value shown in Table 5. For the other values of $a$ the difference was in proportion to the value of $x$. Any uncertainty regarding the fate of the formaldehyde is not significant with respect to the calculation of molar ratios of reactants taking part in the polymerisation. This is one of the consequences of using glycine as the amino acid because the aldehyde formed has a low relative molecular mass. The choice of an amino acid with a substantial side chain could allow a more detailed evaluation of the fate of the aldehyde, but, of course, the reactivity of such an aldehyde might differ considerably from that of formaldehyde. In some melanoidin preparations the yield of formaldehyde is relatively high and the significance of this in melanoidin structure has yet to be explored.

With the exception of the experiment in which the concentration of glycine far exceeded that of glucose, the stoichiometry of our polymers is in the range of four to five molecules of glucose for every four amino acid molecules used to make up the polymer and seems insensitive to the degree of decarboxylation of the amino acid. A simple, though perhaps naive, view of the polymer is one of alternating glucose- and amino acid-derived units with the occasional occurrence of pairs of glucose-derived units. Alternatively, the backbone of the polymer could well be a string of glucose-derived units with the amino acid-derived entities as side groups; the slight excess of glucose-derived units over glycine-derived units suggests that there is occasionally a side group missing. The fact that polymer composition is not affected by the degree of decarboxylation merely reflects the noninvolvement of the carboxyl group in polymer formation. In their $^{13}C$-NMR investigation, Feather & Huang (1986) identified the carboxyl group of alanine in the polymer produced from glucose and alanine, but were unable to distinguish whether or not it was substituted in the polymer; we see no reason why the carboxyl group should not be free. Also, the degree of decarboxylation of amino acid does not appear to affect the extinction coefficient of the melanoidin.

The decarboxylation is accompanied by conversion of an $\alpha$-dicarbonyl group to an amino ketone, i.e.



and the only part of the amino acid which is incorporated into any polymer derived from the Strecker degradation product is the nitrogen atom. In considering any polymerisation mechanism we need to imagine at least two types of subunit: a carbohydrate degradation product–glycine unit and an amino ketone derived from the Strecker reaction.

These data also show that the polymerisation reaction might be more specific than is perhaps appreciated; i.e. it occurs between a small number of intermediates in a fairly regular manner. This is implied from the way in which the composition of melanoidins is insensitive to the composition of the reaction mixture. Interestingly, the three molecular weight fractions ($12\,000 < M_r < 30\,000$, $30\,000 < M_r < 100\,000$ and $M_r > 100\,000$) show similar stoichiometries of glucose- to glycine-derived subunits, suggesting that the nondialysable melanoidin fraction is reasonably homogeneous.

## Reaction with sulphur(IV) oxospecies

In our previous paper (Wedzicha & Kaputo, 1987) we described how the extent of reaction of S(IV) towards melanoidins is equal to the number of C=C bonds which may be brominated. It is, therefore, of interest to compare melanoidins of different composition for their reactivity towards S(IV) as another parameter related to structure and composition.

The ratios of atoms of sulphur to atoms of carbon (S/C) in the product when nondialysable melanoidins are allowed to react with S(IV) are given in Table 6, together with the composition of the polymer in terms of its subunits, i.e. the number of glucose subunits per

**Table 6. Effect of composition of reaction mixtures used to prepare nondialysable melanoidins on the composition of products formed when melanoidins are allowed to react with S(IV). (Composition of products given as S/C ratio and the numbers of subunits of the melanoidin containing either one atom of nitrogen per atom of sulphur ($N_N$) or one molecule of glucose per atom of sulphur ($N_G$). Reaction conditions for preparation of melanoidins: initial pH 5·5, 90°C, 22 h. Reaction conditions for S(IV)–melanoidin reactions: [melanoidin] = 5·2 g/litre except for runs marked *a*, *b* and *c* for which [melanoidin] = 2·2 g/litre, [S(IV)] = 38 mM, pH 5·5, 40°C, 22 days. Measurements carried out on whole nondialysable melanoidin fraction except for [a] $12000 < M_r < 30000$, [b] $30000 < M_r < 100000$ and [c] $M_r > 100000$.**

| [Glucose] (M) | [Glycine] (M) | $10^3$ S/C | $N_N$ | $N_G$ |
|---|---|---|---|---|
| 0·1 | 0·9 | 41·7 | 3·6 | 4·7 |
| 0·5 | 1·0 | 29·3 | 4·3 | 3·9 |
| 1·0 | 1·0 | 23·0 | 4·9 | 4·2 |
| 1·0 | 0·5 | 34·7 | 3·3 | 2·9 |
| 0·9 | 0·1 | 42·6 | 2·8 | 2·7 |
| 1·0[a] | 0·5 | 33·0 | 3·5 | 3·0 |
| 1·0[b] | 0·5 | 34·0 | 3·4 | 3·0 |
| 1·0[c] | 0·5 | 30·0 | 3·6 | 2·8 |

atom of sulphur, or nitrogen atoms per atom of sulphur. We see that melanoidins prepared with [glucose] > [glycine] tend to be more reactive towards S(IV), but this reactivity does not appear to be correlated to the composition of the melanoidin with respect to glucose and glycine units, or the degree of dehydration (Table 5). It is, on the other hand, interesting to see that the most reactive melanoidins (per glucose unit) are also the least decarboxylated.

On the basis of these results we suggest that there exist subtle differences in the subunits which make up the melanoidin if glucose or glycine are in excess; these differences are not evident from the spectrophotometric behaviour of the melanoidins at 450 nm. It is also clear from Table 6 that they are not related to the molecular weight distribution of the nondialysable melanoidins.

### Bleaching of melanoidins

It is, of course, important to attempt to relate changes in composition to the bleaching effect of S(IV). The S/C ratio in the products of S(IV)–melanoidin reactions may be varied by stopping the reaction at given times, and it is found that the absorbance of such mixtures at 450 nm is related to S/C as shown in Fig. 3. This relationship is encouraging because it is consistent with S(IV) removing an amount of chromophore proportional to the amount of sulphur incorporated into the melanoidin. This agrees with the idea of isolated chromophores which react independently with S(IV).

An investigation of the effect of composition of glucose–glycine mixtures used to prepare melanoidins on the progress of the bleaching shows that the final

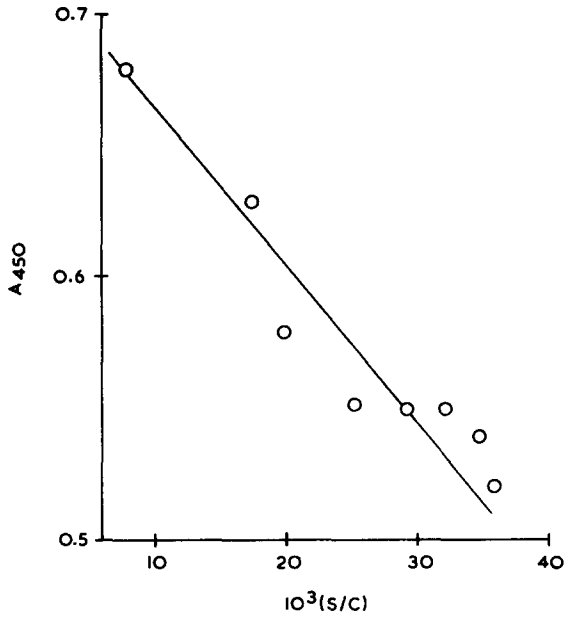



**Fig. 3.** Relationship between absorbance at 450 nm and the S/C ratio when nondialysable melanoidins react with S(IV). Melanoidins prepared by heating glucose (1·0 M) and glycine (0·5 M) (initial pH 5·5) at 90°C for 22 h. Initial conditions for reaction of melanoidins with S(IV): [melanoidin] = 5·43 g/litre, [S(IV)] = 34 mM, pH 5·5 (acetate buffer), 40°C.

extent of bleaching is similar (34 to 38% reduction in absorbance at 450 nm) for all the melanoidins listed in Table 5 with the exception of those from [glucose] = 0·9 M and [glycine] = 0·1 M for which the reduction in absorbance was 50%. Thus, we see that while, for a given melanoidin, the extent of bleaching depends on the amount of S(IV) reacted with the polymer, S/C is not the only variable which determines bleaching when melanoidins from different glucose–glycine mixtures are compared.

The fact that bleaching is only partial implies that the chromophore which is reacting towards S(IV) is not the only chromophore present or that, after reaction with S(IV), the modified chromophore still gives rise to some absorbance at 450 nm. It is, of course, easy to criticise the use of single wavelength measurement as an indicator of browning for two reasons. First, perceived colour is the integral of the transmittance (or reflectance) of the sample multiplied by the spectral response of the eye and the output of the light source. Secondly, melanoidins could be regarded as a mixture of chromophores with different absorbance maxima, and the absorbance at a given wavelength would be a complex function of the extent to which polymerisation has proceeded. The data presented here are encouraging because they imply that there may indeed be some value in the single wavelength measurement to determine the concentration of melanoidin formed, although this provides no indication of the intensity of the perceived colour.

## GENERAL REMARKS

Melanoidins with $M_r$ > 12000 are expected to consist of at least 60 subunits (average $M_r$ of subunit when [glucose] = 1 M, [glycine] = 0·5 M is 180·4) and because of their length, such polymers may appear homogeneous even if the distribution of different types of subunit is random. However, the tendency for glucose and glycine to become incorporated into the melanoidin in similar amounts, despite attempts to force the stoichiometry to change by varying the composition of reaction mixtures, e.g. a high proportion of glucose : glycine, suggests that there might not be any significant run of nitrogen-free subunits.

The polymerisation appears to be directed towards a certain composition and the mechanism is, perhaps, more specific than once imagined. The fact that any changes in composition, and particularly the degree of decarboxylation, are not reflected in the extinction coefficient suggests that the chromophore is isolated from the amino acid-derived part of the polymer. It is not specifically associated with subunits containing intact glycine moieties or amino ketone groups. Also, the chromophores appear to be isolated from one another and seem to react independently with S(IV). The independent and possibly additive behaviour of the chromophores suggests that absorbance measurements at a single wavelength might be satisfactory as a method for following melanoidin formation; this need not be the case for low molecular weight products, which were not investigated here.

It may be relevant that Motai (1974) found that the extinction coefficient $E^{1\%}$ of melanoidins from xylose–glycine is related to relative molecular mass according to

$$E^{1\%} = 2{\cdot}75M_r^{0{\cdot}29}$$

when polymers with $M_r$ = 2140, 3550, 5600 and 14200 were fitted to the equation. There is, however, no reason why xylose–glycine melanoidins should behave exactly as glucose–glycine melanoidins, and the molecular weight range studied by Motai (1974) is mostly below the minimum relative molecular mass of the melanoidins investigated in this paper.

Since it is unlikely that Strecker degradation occurs after polymerisation, we see evidence that the two types of subunit may be incorporated into the polymer in varying amounts (the extent of Strecker degradation is 0 to 38% of glycine residues incorporated) and suggest that the inclusion of these is nonspecific and one or the other may add to a growing polymer at random.

For the browning reaction considered in more detail ([glucose] = 1 M, [glycine] = 0·5 M, 55°C) the yield of melanoidin at long reaction times is greater than expected from the turnover of glucose in the S(IV)-inhibited Maillard reaction of glucose and glycine. In the presence of S(IV), the reaction product, DSH, is relatively unreactive while excess of S(IV) is present (Wedzicha & Garner, 1991). It is possible that, in the absence of S(IV), intermediates and products of browning may interact with precursors of the intermediates which are scavenged by S(IV). The data presented here suggest that this may be so. Thus, it might be wrong to imagine melanoidins as composed of, say, DDH–glycine reaction products, despite DDH being one of the substances known to be most reactive towards colour formation (Burton *et al.*, 1963). One should, therefore, consider the extent to which DH and even *its* precursors are directly involved in polymer formation.

## REFERENCES


Benzing-Purdie, L. M. & Ratcliffe, C. I. (1986). A study of the Maillard reaction by $^{13}C$ and $^{15}N$ CP–MAS NMR: Influence of time, temperature and reactants on major products. In *Amino-Carbonyl Reactions in Food and Biological Systems*, ed. M. Fujimaki, M. Namiki & H. Kato. Elsevier Science Publishers, Amsterdam, pp. 193–205.

Benzing-Purdie, L. M. Ripmeester, J. A. & Preston, C. M. (1983). Elucidation of the nitrogen forms in melanoidins and humic acid by nitrogen-15–cross polarisation–magic angle spinning nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem.*, **31**, 913–15.

Burton, H. S., McWeeny, D. J. & Biltcliffe, D. O. (1962). Sulphites and aldose–amino reactions. *Chem. Ind. (London)*, 219–21.

Burton, H. S., McWeeny, D. J. & Biltcliffe, D. O. (1963). Non-enzymic browning: the role of unsaturated carbonyl compounds as intermediates and of $SO_2$ as an inhibitor of browning. *J. Sci. Food Agric.*, **14**, 911–20.

Einarsson, H. (1987*a*). The effect of time, temperature, pH and reactants on the formation of antibacterial compounds in the Maillard reaction. *Lebensm. Wiss. u. Technol.*, **20**, 51–5.

Einarsson, H. (1987*b*). The effect of pH and temperature on the antibacterial effect of Maillard reaction products. *Lebensm. Wiss. u. Technol.*, **20**, 56–8.

Feather, M. S. & Huang, R. D. (1985). Maillard polymers derived from D-glucose-1- and -6-$^{14}C$, glycine-1- and -2-$^{14}C$, and *C*-1- and *S*-methyl-$^{14}C$-methionine. *J. Carbohydr. Chem.*, **4**, 363–8.

Feather, M. S. & Huang, R. D. (1986). Some studies on a Maillard polymer derived from L-alanine and D-glucose. In *Amino-Carbonyl Reactions in Food and Biological Systems*, ed. M. Fujimaki, M. Namiki & H. Kato. Elsevier Science Publishers, Amsterdam, pp. 183–92.

Feather, M. S. & Nelson, D. (1984). Maillard polymers derived from D-glucose, D-fructose, 5-(hydroxymethyl)-2-furaldehyde, and glycine and methionine. *J. Agric. Food Chem.*, **32**, 1428–32.

Fujimaki, M., Homma, S., Arakawa, N. & Inagaki, C. (1979). Growth response of rats fed with a diet containing non-dialysable melanoidins. *Agric. Biol. Chem.*, **43**, 497–503.

Gomyo, T., Kato, H., Udaka, K., Horikoshi, M. & Fujimaki, M. (1972). Chemical studies on melanoidins. Part III. Effects of heating on chemical properties of melanoidin prepared from glycine–xylose system. *Agric. Biol. Chem.*, **36**, 125–32.

Haugaard, G., Tumerman, L. & Silvestri, H. (1951). A study of the reaction of aldoses with amino acids. *J. Am. Chem. Soc.*, **73**, 4594–600.

Hayase, F., Kim, S. B. & Kato, H. (1984). Decolourisation and degradation products of the melanoidins by hydrogen peroxide. *Agric. Biol. Chem.*, **48**, 2711–17.

Kato, H. & Tsuchida, H. (1981). Estimation of melanoidin structure by pyrolysis and oxidation. *Prog. Food Nutr. Sci.*, **5**, 147–56.

Kato, H., Noguchi, G. & Fujimaki, M. (1968). Reductone contents of non-dialysable melanoidins. *Agric. Biol. Chem.*, **32**, 916–19.

Kato, H., Yamamoto, M. & Fujimaki, M. (1969). Mechanisms of browning degradation of D-fructose in special comparison with D-glucose–glycine reaction. *Agric. Biol. Chem.*, **33**, 939–48.

Kato, H., Kim, S. B. & Hayase, F. (1986). Estimation of the partial chemical structures of melanoidins by oxidative degradation and $^{13}C$ CP–MAS NMR. In *Amino-Carbonyl Reactions in Food and Biological Systems*, ed. M. Fujimaki, M. Namiki & H. Kato. Elsevier Science Publishers, Amsterdam, pp. 215–23.

Kato, H., Lee, I. E., Chuyen, N. V., Kim, S. B. & Hayase, F. (1987). Inhibition of nitrosamine formation by non-dialysable melanoidins. *Agric. Biol. Chem.*, **51**, 1333–8.

Kim, S. B. & Park, Y. K. (1986). Decolourisation and degradation products of melanoidin on ozonolysis by active oxygen. II. Decolourisation and degradation products of melanoidin on ozonolysis. *Bull. Korean Fish Soc.*, **19**, 36–44.

Kim, S. B., Hayase, F. & Kato, H. (1985). Decolourisation and degradation products of melanoidins on ozonolysis. *Agric. Biol. Chem.*, **49**, 785–92.

Kirigaya, N., Kato, H. & Fujimaki, M. (1968). Studies on antioxidant activity of non-enzymic browning reaction products. Part 1. Relations of colour intensity and reductones with antioxidant activity of browning reaction products. *Agric. Biol. Chem.*, **32**, 287–90.

Labuza, T. P., Warren, R. M. & Warmbier, H. C. (1977). The physical aspects with respect to water and non-enzymatic browning. *Adv. Exp. Biol.*, **86B**, 379–418.

Lee, C. M., Sherr, B. & Koh, Y. N. (1984). Evaluation of kinetic parameters of a glucose–lysine Maillard reaction. *J. Agric. Food Chem.*, **32**, 379–82.

Maillard, L. C. (1912). Action of amino acids on sugars. Formation of melanoidins in a methodical way. *Compt. Rend.*, **154**, 66–8.

Maillard, L. C. (1916). Synthesis of humus-like substances by the interaction of amino acids and reducing sugars. *Ann. Chim. (Rome)*, **5**, 258–317.

McWeeny, D. J., Biltcliffe. D. O., Powell, R. C. T. & Spark, A. A. (1969). The Maillard reaction and its inhibition by sulphite. *J. Food Sci.*, **34**, 641–3.

Motai, H. (1974). Relationship between the molecular weight and colour intensity of colour components of melanoidin from glycine–xylose systems. *Agric. Biol. Chem.*, **38**, 2299–304.

Motai, H. & Inoue, S. (1974). Conversion of colour components of melanoidin produced from the glycine–xylose system. *Agric. Biol. Chem.*, **38**, 233–9.

Nam, S. & Kim, M. L. (1984). Browning pattern and pigment of glucose/glycine model systems. *Korean J. Food Sci. Technol.*, **16**, 218–22.

Nursten, H. E. & O'Reilly, R. (1986). Coloured compounds formed by the interaction of glycine and xylose. *Food Chem.*, **20**, 45–60.

Obretenov, T. D., Kuntcheva, M. J. & Panchev, I. N. (1986). Influence of reaction conditions on forming non-dialysable melanoidins from glucose and glycine. *Food Process. Preserv.*, **10**, 251–8.

Rubinsztain, Y., Ioselis, P., Ikan, R. & Aizenshtat, Z. (1984). Investigations on the structural units of melanoidins. *Org. Geochem.*, **6**, 791–804.

Song, P. S., Chichester, C. O. & Stadtman, F. H. (1966). Kinetic behaviour and mechanism of inhibition in the Maillard reaction. I. Kinetic behaviour of the reaction between D-glucose and glycine. *J. Food Sci.*, **31**, 906–13.

Spark, A. A. (1969). Role of amino acids in non-enzymic browning. *J. Sci. Food Agric.*, **20**, 308–16.

Taguchi, K. & Sampei, Y. (1986). The formation and clay mineral and $CaCO_3$ association reactions of melanoidins. *Org. Geochem.*, **10**, 1081–9.

Takeuchi, H., Nishioka, Y., Fujishiro, M. and Muramatsu, K. (1987). Physiological effects of non-dialysable melanoidin in rats. *Agric. Biol. Chem.*, **51**, 969–76.

Wedzicha, B. L. (1984*a*). A kinetic model for the sulphite-inhibited Maillard reaction. *Food Chem.*, **14**, 173–87.

Wedzicha, B. L. (1984*b*). *Chemistry of Sulphur Dioxide in Foods.* Elsevier Applied Science Publishers, London, p. 193.

Wedzicha, B. L. & Garner, D. N. (1991). The formation and reactivity of osuloses in the sulphite-inhibited Maillard reaction of glucose and glycine. *Food Chem.*, **39**, 73–86.

Wedzicha, B. L. & Kaputo, M. T. (1987). Reaction of melanoidins with sulphur dioxide: stoichiometry of the reaction. *Int. J. Food Sci. Technol.*, **22**, 643–51.

Wedzicha, B. L. & Vakalis, N. (1988). Kinetics of the sulphite-inhibited Maillard reaction: the effect of sulphite ion. *Food Chem.*, **27**, 259–71.

Wolfrom, M. L., Kashimura, N. & Horton, D. (1974). Factors affecting the Maillard browning reaction between sugars and amino acids. Studies on the non-enzymic browning of dehydrated orange juice. *J. Agric. Food Chem.*, **22**, 796–800.