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# Melanoidins extinction coefficient in the glucose/glycine Maillard reaction

Sara I.F.S. Martins, Martinus A.J.S. van Boekel*

*Department of Agrotechnology and Food Science, Product Design and Quality Management Group, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands*

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**Abstract**

Melanoidins (brown, nitrogenous polymers and co-polymers) are the final products of the Maillard reaction. The glucose/glycine melanoidins extinction coefficient was determined using $^{14}$C-labelled glucose at three different reaction conditions. The absorbance was measured at different wavelengths (420, 450, 470 and 490 nm) and the extinction coefficient determined for each. The value of the extinction coefficient can be used to recalculate browning, measured as absorbance units, into melanoidins concentration in terms of sugar molecules incorporated. The amount of $^{14}$C-labelled sugar molecules was estimated in melanoidins separated via dialysis with a cut-off value of 3500 Da. These melanoidins only represented ≈12% of the total colour formed. The extinction coefficient of the melanoidins remained constant during the observation period. At 470 nm, values of 0.65 (±0.02) l mmol$^{-1}$ cm$^{-1}$; 0.66 (±0.02) l mmol$^{-1}$ cm$^{-1}$ and 0.62 (±0.05) l mmol$^{-1}$ cm$^{-1}$, were obtained at 120 °C pH 6.8, 100 °C pH 6.8 and 100 °C pH 5.5, respectively. The difference is not significant. The extinction coefficient appeared to not to vary within the pH and temperature range studied. From the elemental analysis, the nondialysable melanoidins elementary composition seemed to be influenced by the reaction conditions, which was supposed to be related to the presence of side-chains on the melanoidin backbone. A trend was observed in the melanoidins C/N ratio: it decreased with increasing reaction pH as well as it changed to a lower level, of about 8, as the extent of browning increased.


*Keywords:* Melanoidins; Extinction coefficient; D-[U-$^{14}$C]-Glucose; Glycine; Maillard reaction

## 1. Introduction

In the Maillard reaction, melanoidins (brown nitrogenous polymers and co-polymers) are known as the main end product of the reaction. These brown polymers have significant effect on the quality of food, since colour is an important food attribute and a key factor in consumer acceptance. Up till now, browning is usually measured spectrophotometrically and expressed in absorbance units, which gives qualitative information in terms of colour formation but cannot be related in quantitative terms to molecular concentration.

Studies on colour formation have been summarized in different review articles (Feather, 1985; Friedman, 1996; Namiki, 1988). Hashiba (1982) concluded that browning was directly proportional to the reducing power of the sugar and to the amounts of glycine consumed, by comparing different sugars with one single amino acid. Rizzi (1997) on the other hand stated that many coloured products appear to be (retro)aldolization/dehydration products of sugars which may or may not be attached to proteins or other sources of amino nitrogen. Up till now three main proposals for the structure of melanoidins have been put forward. Hofmann (1998a) detected low-molecular-weight (LMW) coloured substances, which were able to cross-link proteins via ε-amino groups of lysine or arginine to produce high-molecular-weight (HMW) coloured melanoidins. However one should keep in mind that with proteins there is always high-molecular-weight melanoidins, since proteins are by themselves HMW compounds. On the other hand, Tressl, Wondrak, Garbe, Krüger, and Rewicki (1998) postulated a polymer consisting of repeating units of furans and/or pyrroles, formed during

* Corresponding author. Tel.: +31-317-484281; fax: +31-317-483669.

*E-mail address:* tiny.vanboekel@wur.nl (M.A.J.S. van Boekel).

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the advanced stages of the Maillard reaction and linked by polycondensation reactions. However, in a recent study (Cämmerer, Jalyschko, and Kroh, 2002) intact carbohydrate structures have been identified from acid hydrolysis of melanoidins indicating that sugars are not inevitably degraded to heterocycles such as furans and pyrroles. In a third structural proposal, the melanoidin skeleton is mainly built up from sugars degradation products, formed in the early stages of the Maillard reaction, polymerized through aldol-type condensation and linked by amino compounds, such as amino acids (Cämmerer & Kroh, 1995; Kato & Tsuchida, 1981; Yaylayan & Kamisky, 1998).

The mechanism of the formation of brown colour is not fully understood and the structure of melanoidins is largely unknown, which makes it difficult to quantify these compounds. However, this quantification is necessary when trying to predict or optimize browning in processed foods from a known molecular composition.

According to the Lambert–Beer equation ($A = \varepsilon * c * l$), there is a direct linear relation between absorbance ($A$) and concentration ($c$), through the extinction coefficient (ε), if the factor $l$, the length of the cuvette, is constant. Previous studies, not only in a sugar/amino acid system (Leong, 1999; Wedzicha & Kaputo, 1992) but also in a sugar/protein system (Brands, Wedzicha, & Van Boekel, 2002), have shown that it is possible to relate absorbance caused by nondialysable melanoidins to the number of sugar molecules incorporated in those melanoidins, by heating radioactive glucose (U-$^{14}$C glucose) with an amino acid and/or protein. Experiments in a glucose/glycine system at 55 °C and 90 °C, pH 5.5 (Wedzicha & Kaputo, 1992) suggested that the chromophores in melanoidins with $M_r > 12\,000$ Da formed in the early stages of heating are similar to those at later stages. However, under these conditions the amount of material with $M_r > 12\,000$ Da is believed to be very small (Hofmann, 1998b). In a similar study (55 °C, pH 5.5) but with the melanoidins cut off at 3500 Da it was also reported that the extinction coefficient remained constant throughout the heating time in glucose/amino acid systems (Leong, 1999). Values of ε at 470 nm were estimated to range from 0.34 l mmol$^{-1}$ cm$^{-1}$ for alanine to 0.94 l mmol$^{-1}$ cm$^{-1}$ for glycine. On the other hand in sugar/casein systems (120 °C, pH 6.8), where ε also remained constant throughout the heating period, independently of the sugar, glucose or fructose, a constant value of 0.3 l mmol$^{-1}$ cm$^{-1}$ was obtained (recalculated to ε at 470 nm) (Brands, et al. 2002).

It may be speculated that melanoidins are formed as a result of random polymerization of carbohydrate degradation products or adducts of those with amino compounds. The regularity of the polymers with respect to nitrogen-containing and nitrogen-free subunits is still not clear. However, the starting materials, as well as reaction conditions have a strong influence on the elemental composition of melanoidins (Cämmerer & Kroh, 1995; Wedzicha & Kaputo, 1992). The reaction conditions can determine the type of products that are formed during the Maillard reaction and as a consequence the followed pathways in the melanoidins formation (Tressl, Nittka, & Kersten, 1995).

The aim of the present study was to elucidate if and how the reaction conditions, pH and temperature, can influence the glucose/glycine melanoidins extinction coefficient. Melanoidins were, rather arbitrarily, defined as high-molecular-weight by a lower limit of 3500 Da, which was the nominal cut-off value in the dialysis system used. The average extinction coefficient of melanoidins was determined for three systems (A: pH 6.8, temperature 120 °C; B: pH 6.8, temperature 100 °C; C: pH 5.5, temperature 100 °C) following the method of Leong (1999). A benefit of this approach is that the molar extinction coefficient can be expressed in terms of the concentration of glucose molecules converted into melanoidins, even though the molecular weights of melanoidins are expected to span a very wide range of values. The C/N ratio of the high molecular weight products was also determined for the studied systems.

## 2. Materials and methods

### 2.1. Materials

All chemicals were of analytical grade and were supplied by Sigma chemicals (United Kingdom). D-[U-$^{14}$C]-Glucose (Specific Activity 111 MBq/mmol and 11.4 GBq/mmol) was obtained from Amersham Life Science Ltd (United Kingdom).

### 2.2. Samples preparation

Reaction mixtures (100 ml) of glucose and glycine containing an equimolar final concentration of 0.2 M were prepared in phosphate buffer 0.1 M, pH 6.8 or 5.5. Before making up to the final volume, 1 MBq of D-[U-$^{14}$C]-glucose was spiked into the solution (the concentration of added labelled sugar was negligible). The reaction mixture was then distributed over glass, screw-capped, Schott tubes (16×160 mm), each containing a minimum of 10 ml. Samples were heated in at least duplicate at 120 and 100 °C (pH 6.8) and at 100 °C (pH 5.5). The heating was carried out in an oil bath and the proper safety measures taken. At timed intervals, the samples were withdrawn, immediately cooled in ice and then dialysed.

### 2.3. Dialysis

Approximately 2 ml of the reaction mixture were injected into dialysis cassettes ($M_r > 3500$) (Slide-A-Lyzer Dialysis Cassette, 3.5 K MWCo, Pierce, USA)

and dialyzed against distilled water. The optimum dialysis time was established by carrying out a test, where the retentate of the same sample was counted after different dialysis days. Each day corresponds to two water changes, 2 l each. The $^{14}C$-activity reached equilibrium after 7 days (14 water replacements) (Fig. 1). Only then the contents were removed and the volume adjusted to 10 ml with distilled water.

### 2.4. Scintillation counting

An aliquot (1 ml) of the diluted dialysed fraction was pipetted into 10 ml of scintillation liquid (Emulsifier Scintillator Plus, Packard) in a plastic vial (Liquid Scintillation Vials, Wheaton Scientific), shaken thoroughly and counted immediately in a Liquid Scintillator Analyzer, 1600TR, Packard for 10 min. The count due to $^{14}C$ was corrected for quenching. Quenching is a phenomenon where the observed pulse height is lower than the actual, and there is a shift of the energy spectrum of the isotope to lower energies. Chemical quenching is the result of impurities present in the solution that interfere with the energy transfer process, while coloured solutions act as an optical filter causing colour quenching. Quench correction was done by the internal standard method (Price, 1973). After counting the sample was spiked with an accurately known amount of isotope in an unquenched form and recounted. The specific activity of $^{14}C$-glucose in the reaction mixture was calculated from the counts obtained from 1 ml of a 100-fold diluted unheated reaction mixture and was expressed as number of disintegration per minute (dpm) per mol of glucose. Once the quench-corrected number of counts for a certain sample was known the concentration of U-$^{14}C$-sugar incorporated into the high molecular weight fraction could be calculated by dividing the number of counts per minute by the specific activity of the sugar.

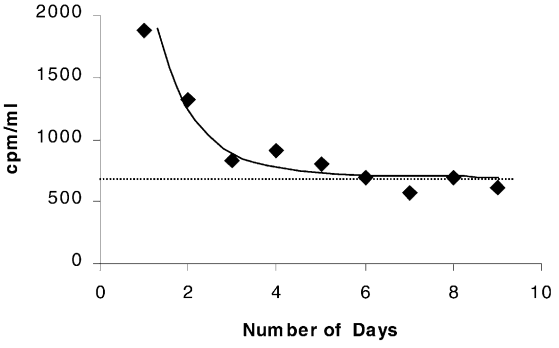


Fig. 1. Change in $^{14}C$-activity in the retentate as a function of dialysis time (two water changes per day) at room temperature. Counts per minute (cpm).

### 2.5. Spectrometric analysis

Browning was measured spectrophotometrically as the absorbance at 420, 450, 470 and 490 nm. Optical pathlength of the cuvette 1 cm. Spectrophotometer Cary 50 Bio, Varian.

### 2.6. Microanalysis

The reaction mixtures (without addition of radio labelled sugar) were dialyzed in the same way as described above and the retentates freeze-dried. Microanalysis was carried out using a CE Instruments Element Analyzer, Type EA 1110 CHN.

## 3. Results and discussion

### 3.1. Extinction coefficient

Browning measured, as absorbance at 470 nm was determined for each studied system before and after dialysis (Fig. 2). Considerable browning was observed before dialysis with the smallest induction period at 120 °C (pH 6.8). At the same pH but lower temperature (100 °C), a longer heating time was required to achieve the same absorbance and at 100 °C and pH 5.5, browning showed the longest induction period. An absorbance of three units was reached after 0.5, 1.8 and 8 h for systems A (120 °C, pH 6.8), B (100 °C, pH 6.8) and C (100°, pH 5.5), respectively. After dialysis the absorbance results show that independently of the reaction conditions, more than 80% of browning, measured spectrophotometrically at 470 nm, passed into the dialysate, namely 88, 88 and 82% in system A, B and C, respectively. The majority of coloured compounds were thus not retained in the HMW fraction ($M_r > 3500$) but below it. This result is in line with literature describing browning in sugar-amino acid systems. Leong (1999) observed that the HMW fraction (>3500 Da) contributed only up to 10% of the absorbance of the glucose/glycine reaction mixture heated in acetate buffer at 55 °C and pH 5.5. Also Hofmann (1998b) in both glucose/glycine and glucose/alanine systems heated in phosphate buffer for 4 h at 95 °C, pH 7, reported that only trace amounts of compounds with molecular weights greater than 3000 Da were formed. These results are in contrast with sugar/protein reactions. A much higher percentage of colour was detected in the HMW fraction ($\geqslant 70\%$), which is as expected since the melanoidins are attached to the protein that is HMW by itself (Brands et al., 2002; Hofmann, 1998b). Colour in the glucose/amino acid reaction mixtures is almost exclusively due to the LMW fraction. The fact that independently of the studied reaction conditions, the same % of high molecular weight fraction was obtained,

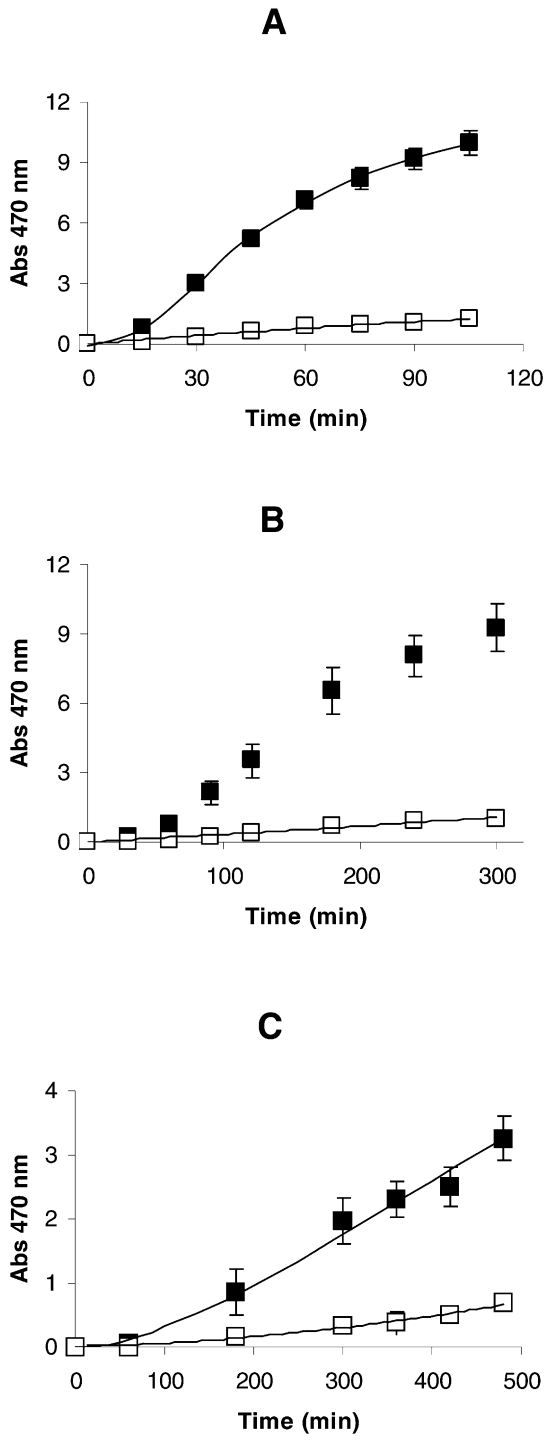


Fig. 2. Browning [measured as absorbance (Abs) at 470 nm] before (closed markers) and after (open markers) dialysis. A—Temperature 120 °C pH 6.8; B—Temperature 100 °C pH 6.8; C—Temperature 100 °C pH 5.5. The error bars represent the standard deviation for each observation.

suggests that the formation of nondialysable (HMW) melanoidins does not appear to be sensitive to temperature and pH.

As mentioned before, the extinction coefficient was calculated based on the Lambert–Beer equation ($A = \varepsilon * c * l$). Since the factor $l$ is constant, there is a direct linear relation between absorbance ($A$) and concentration ($c$), through the extinction coefficient (ε). The concentration of nondialysable melanoidins was expressed as the concentration of U-$^{14}$C glucose incorporated. The extinction coefficient was then the slope of the plot of the absorbance of the retentate after dialysis versus the concentration of radiolabelled glucose incorporated into those melanoidins. In Fig. 3 the results show that over the observation period the extinction coefficient of the nondialysable melanoidins remained constant, in the three systems studied. The extinction coefficient was calculated taking the average of the repetitions carried out under the same conditions. In Table 1 the values of ε are presented for different wavelengths. The chosen wavelengths result from the fact that in literature reports concerning brown colour, usually one of these four wavelengths are used, since there is no maximum in the visible spectrum (400–500 nm approximately) for brown colour (Martins, 1997). The differences in the extinction coefficient according to the used wavelength stress the importance of having standard procedures. The finding that the regression line not always passed through the origin was ascribed to the background measured in the system. Radioactive glucose, not incorporated into the melanoidins was possibly still retained inside of the retentate to some extent. A possible reason is the fouling of the membrane by the melanoidins, slowing down the diffusion, or a complex formation of the melanoidins with the sugar, which would also delay the diffusion.

The evidence is strong that the chromophores produced in the nondialysable melanoidins in the early stages of the reaction are similar to those at later stages, independently of the reaction conditions. In the present study the molar extinction coefficient averaged over the three systems, for glucose/glycine HMW melanoidins was calculated to be $0.64 \pm 0.03$ l mmol$^{-1}$ cm$^{-1}$ at 470 nm. Leong (1999) showed that at 470 nm the extinction coefficient for HMW and LMW glucose/glycine melanoidins was the same. However, the extinction coefficient that they reported was considerably higher for glucose/glycine systems, namely 0.94 l mmol$^{-1}$ cm$^{-1}$ at 470 nm and it varied according to the type of amino acid. Using the kinetic modelling approach in the glucose/glycine system at pH 5.5 and 55 °C, Leong and Wedzicha (2000) estimated ε as a parameter to be 1.0 l mmol$^{-1}$ cm$^{-1}$ from absorbance measurements without radiolabelling. On the other hand, Wedzicha and Kaputo (1992) by applying the same radiolabelled technique showed that ε was not affected by composition molar ratios of glucose and glycine, as well as by the heating temperature. Values of 0.41 l mmol$^{-1}$ cm$^{-1}$ at 90 °C and 0.37 l mmol$^{-1}$/cm$^{-1}$ at 55 °C were obtained, at 450 nm. Also, in a recent study Brands et al. (2002) reported that the melanoidins extinction coefficient

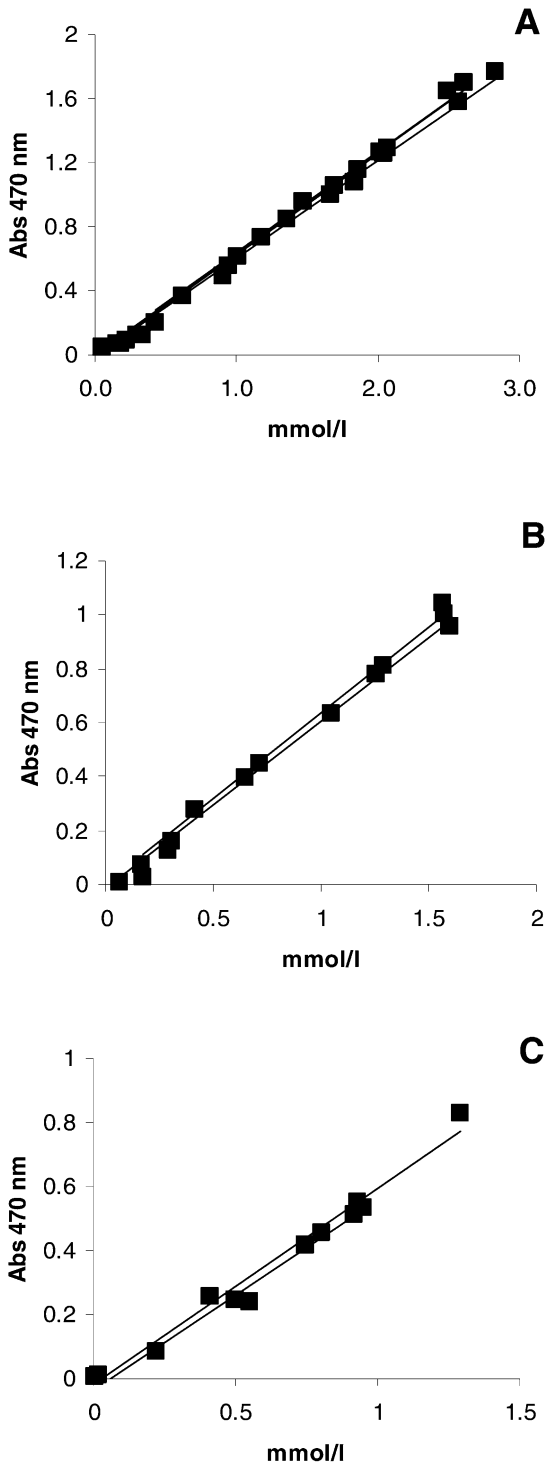


Fig. 3. Browning [measured as absorbance (Abs) at 470 nm] as function of the melanoidin concentration (measured as incorporated sugar); A—Temperature 120 °C pH 6.8; B—Temperature 100 °C pH 6.8; C—Temperature 100 °C pH 5.5. Each line corresponds to a repetition of the experiment. The slope of the lines determines the extinction coefficient value.

remained constant in a sugar/casein system, during the observation period (90 min at 120 °C, pH 6.8) independently of the sugar. They found values of 0.48 and 0.53 l mmol$^{-1}$ cm$^{-1}$ for the glucose/casein and fructose/casein systems, respectively, at 420 nm. In the present study, under the same conditions the obtained extinction coefficient was 1.00±0.03 l mmol$^{-1}$ cm$^{-1}$ at 420 nm, about twice as high than the ε of sugar/casein melanoidins. This means that in sugar/amino acid reaction mixtures, less glucose molecules (or glucose fragments) have to be incorporated into the melanoidins than in the sugar/casein systems, to increase the absorbance by one unit.

Table 1
Extinction coefficient (ε) of glucose/glycine nondialysable melanoidins measured for different wavelengths under different reaction conditions

| | Wavelength (nm) | | | |
|---|---|---|---|---|
| | 420 | 450 | 470 | 490 |
| *120 °C, pH 6.8* | | | | |
| *ε (l mmol$^{-1}$ cm$^{-1}$)* | *1.00±0.03* | *0.77±0.02* | *0.65±0.02* | *0.53±0.01* |
| Intcp.[a] Lower 95% | −0.23 | −0.18 | −0.15 | −0.12 |
| Intcp.[a] Upper 95% | −0.02 | −0.002 | −0.01 | −0.002 |
| *100 °C, pH 6.8* | | | | |
| *ε (l mmol$^{-1}$ cm$^{-1}$)* | *1.01±0.02* | *0.79±0.02* | *0.66±0.02* | *0.53±0.01* |
| Intcp.[a] Lower 95% | −0.12 | −0.09 | −0.09 | −0.057 |
| Intcp.[a] Upper 95% | −0.02 | −0.01 | −0.004 | 0.01 |
| *100 °C, pH 5.5* | | | | |
| *ε (l mmol$^{-1}$ cm$^{-1}$)* | *0.97±0.07* | *0.71±0.05* | *0.62±0.05* | *0.50±0.04* |
| Intcp.[a] Lower 95% | −0.20 | −0.16 | −0.14 | −0.11 |
| Intcp.[a] Upper 95% | 0.07 | 0.05 | 0.05 | 0.05 |

[a] Intercept with the origin (95% confidence interval).

If the degree of polymerization of melanoidins increases with the 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 polymerization. Looking at each system individually, we observe that the ratio of absorbance due to LMW and HMW is constant in each system (Fig. 4). Also, the yield of nondialysable melanoidins was proportional to the total colour formation. Leong (1999) in a similar study, at 55 °C and pH 5.5, reported that the ratio of HMW to LMW showed an initial lag phase, till 0.1 absorbance units of the HMW fraction, increasing afterwards proportionally with time. These results suggest that nondialysable melanoidins formation results from the built-up of LMW components into HMW structures. The chromophore formation can be explained, either by combination of isolated, low molecular weight, coloured chromophores only or together with low molecular weight non-coloured compounds. This combination, though, does not change the chromophore extensively (Fig. 5). Moreover, the result that HMW colourants with molecular weight up to several thousand daltons could not be observed (Hofmann, 1998b) suggests that the built up of LMW into HMW structures only reaches the oligomer size, approximately 13 molecules of glucose and glycine. Once the melanoidin chromophore is formed its concentration increases proportionally with time.

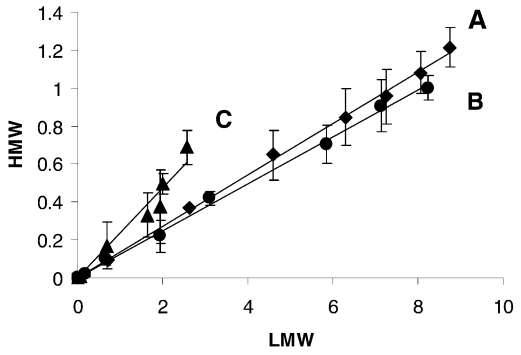


Fig. 4. The absorbance at 470 nm of high molecular weight (HMW) fraction plotted against the low molecular weight (LMW) fraction. A—Temperature 120 °C pH 6.8 (HMW = 0.12×LMW; $R^2 = 0.99$); B—Temperature 100 °C pH 6.8 (HMW = 0.14×LMW; $R^2 = 0.99$); C—Temperature 100 °C pH 5.5 (HMW = 0.24×LMW; $R^2 = 0.96$).

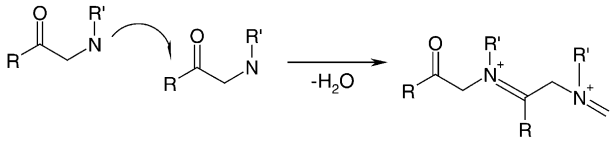


Fig. 5. Formation of high molecular weight components through the combination of coloured low molecular weight subunits (adapted from Leong, 1999). R and R′ may carry a chromophore which does not change extensively when combined with the high molecular weight molecule.

Moreover, other studies reported that changing the reaction conditions influence the elementary composition of the melanoidins (Cämmerer & Kroh, 1995; Wedzicha & Kaputo, 1992). The observed differences were attributed to the possible presence of side chains that do not affect the chromophore extensively. In agreement with this a different slope was observed for the LMW and HMW ratio, in our three studied systems where the extinction coefficient remained constant throughout the heating period. We will come back to the melanoidins elementary composition in the following section.

### 3.2. Microanalysis

From microanalysis results reported in literature (Table 2) it seems that the elementary composition of the melanoidins is influenced by the reaction conditions. For the evaluation of the microanalysis data of the melanoidins in the present study, a stoichiometric reaction model was fitted to the results, as used before (Wedzicha & Kaputo, 1992). The overall reaction for the formation of melanoidin is a combination of $a$ molecules of sugar consisting of $l$, $m$ and $n$ atoms of C, H and O, respectively and $b$ molecules of amino acid consisting of $p$, $q$, $r$ and $s$ atoms of C, H, N and O, to give a melanoidin formula, where $y$ is the number of water molecules: $C_{la+pb}\,H_{ma+qb-2y}\,N_{rb}\,O_{na+sb-y}$. In the glucose/glycine system $l = 6$, $m = 12$, $n = 6$, $p = 2$, $q = 5$, $r = 1$ and $s = 2$. Assuming $b = 1$, the unknowns $a$ and $y$ can be found by solving the following equations: $C = 6a + 2$ and $H = 12a + 5 - 2y$.

The number of carbon dioxide molecules was not calculated. The microanalysis data of nondialysable melanoidins derived at 120 °C and pH 6.8 are shown in Table 3. The results show that throughout the heating time the number of incorporated mol of sugar (or its corresponding degradation product) per amino acid remains constant, around 1.2. This is consistent with the fact that almost 80% of glycine was recovered after the reaction heating period. The same result was found for glucose/glycine heated at 100 °C, pH 7 for 10 h (Cämmerer & Kroh, 1995). Nevertheless, different reports have shown that the reaction conditions have influence on the number of sugar molecules incorporated into the polymer in glucose/glycine systems. In a $H_2O$/Methanol solution (Yaylayan & Kaminsky, 1998) at 65 °C, heated for 7 h a value of 0.91 was reported, while in a solvent-free system at 170 °C for 20 min a value of 2.19 was estimated (Cämmerer & Kroh, 1995). On the other hand, the number of molecules of water eliminated per mol of sugar or corresponding degradation product incorporated seemed to be constant independently of the reaction conditions. In the present study the estimated number was 3. Also Cämmerer & Kroh (1995) independently of the temperature, pH, solvent-free or water content came to the same conclusion, as well as Wedzicha and Kaputo (1992). Also, Feather and Nelson (1984) reported the same value for polymers derived from D-glucose and D-fructose with glycine. According to the proposed structure by Yaylayan and Kaminsky (1998) Amadori products can polymerize through nucleophilic addition reactions of amino groups to the carbonyl moieties of a second molecule, followed by dehydration to form the zwitterionic polymer. Also, Cämmerer and Kroh (1995) suggested that the melanoidin skeleton was mainly built up of sugar degradation products, formed in the early stages of the Maillard reaction. The hypothetical structure proposed was based on the reactions of dicarbonyl compounds (dehydrated, sugar-derived intermediates) that can react among themselves (aldol reaction or nucleophilic addition) as well as have substitution reactions with amino compounds. In these two last studies the position and the type of characteristic IR absorptions found for melanoidins was determined and it was not affected by the different reaction conditions, which means that the main structure of the melanoidin chromophore was not influenced by the reaction conditions. The observed differences in the elementary analysis were attributed to the possible presence of side-chains that do not affect the chromophore. These results are well in line with the fact of the extinction coefficient remaining constant throughout the heating period, independently of the pH and temperature, as well as of the molar composition,

Table 2
Microanalysis results reported in literature under different reaction conditions

| Reference | Reaction conditions | C/N |
|---|---|---|
| Cämmerer and Kroh (1995) | $H_2O$; 60 °C; 160 h; pH*5*; [gly] = [glu] = 0.1 M | 7 |
| | $H_2O$; 100 °C; 10 h; pH*5*; [gly] = [glu] = 0.1 M | 9 |
| Wedzicha and Kaputo (1992) | Acetate Buffer 0.2 M; 90 °C; 22 h; pH *5.5* | 8 |
| | [gly] = 1.0 M; [glu] = 1.0 M | |
| Leong (1999) | Acetate Buffer 0.2 M; 55 °C; 90 h; pH *5.5* | 8 |
| | [gly] = 0.5 M; [glu] = 1.0 M | |
| Feather and Nelson (1984) | 100 °C; 8 h; pH *3.5* | 10 |
| | [gly] = 1.0 M; [glu] = 0.2 M | |
| Bobbio et al (1981) | Citrate Buffer 0.05 M | |
| | [gly] = 0.66 M; [glu] = 1.25 M | |
| | 70 °C; 415 h; pH *3.0* | 12 |
| | 70 °C; 415 h; pH *6.0* | 11 |
| | 80 °C; 80 h; pH *3.0* | 13 |
| | 80 °C; 80 h; pH *6.0* | 10 |
| Hayashi and Namiki (1986) | [ala] = [glu] = 2 M | |
| | 100 °C; 190 min; pH *2.3* | 12 |
| | 100 °C; 53 min; pH *6.5* | 9 |
| | 100 °C; 25 min; pH *9.2* | 7.5 |
| Olsson, Pernemalm, and Theander (1978) | $H_20$; 100 °C; 120 h; pH *5* | |
| | [gly] = 0.5 M; [glu] = 0.75 M | 12 |

the type of amino acid and type of sugar as observed in previous studies (Brands et al. 2002; Leong, 1999; Wedzicha & Kaputo, 1992).

When we compare the C/N ratio values of system A (120 °C, pH 6.8) (Table 3) with the ones of system B (100 °C, pH 6.8) and C (100 °C, pH 5.5) (Table 4) we observed that at higher pH and temperature, in a way the most favourable reaction conditions for formation of melanoidins, the C/N ratio remained constant throughout the heating period. If we decreased the temperature and/or the pH, we observed in the initial period of the reaction a higher value for C/N followed by a decrease till a constant value was reached. It seems that the ratio is indeed lower at higher pH and therefore seems to depend on the reaction conditions. These results are consistent with the fact that at lower pH around 90% of glycine was recovered, in contrast with the 80% at higher pH, independently of the temperature. However, we should be careful with interpretation because elemental analysis could be sensitive to experimental errors. Consequently, the C/N values reported in literature are not consistent, either with pH or temperature (Table 2). However, there is a trend that the C/N ratio decreases with increasing pH (Bobbio, Imasato, & De Andrade Leite, 1981; Hayashi & Namiki, 1986) as well as it changes to a lower level, of about 8, as the extent of browning increases.

Table 3
Microanalysis results from system A[a]

| Time (min) | 120 °C pH 6.8 | | | |
|---|---|---|---|---|
| | C/N | *a*/*b* | *y*/*a* | Abs 470 nm |
| 15 | 11 | 1.2 | 1.8 | 3.6 |
| 30 | 11 | 1.2 | 2.6 | 6.3 |
| 45 | 11 | 1.2 | 3.1 | 8.1 |
| 60 | 11 | 1.2 | 3.2 | 9.2 |

[a] Calculated number of molecules of sugar (*a*) per molecule of amino acid (*b*) and calculated number of molecules of water (*y*) per molecule of sugar (*a*).

Table 4
Microanalysis results from systems B and C

| Time (min) | 100 °C pH 6.8 | | 100 °C pH 5.5 | |
|---|---|---|---|---|
| | C/N | Abs 470 nm | C/N | Abs 470 nm |
| 30 | 15 | 0.5 | | |
| 60 | 14 | 2.0 | 19 | 0.1 |
| 120 | 11 | 4.6 | | |
| 180 | 11 | 6.3 | 16 | 0.6 |
| 300 | 11 | 10.0 | 12 | 1.3 |
| 420 | | | 12 | 2.2 |
| 480 | | | 12 | 2.6 |

## 4. Conclusion

The results of the extinction coefficient are well in line with earlier investigations. There is strong evidence that chromophores formed in the nondialysable melanoidins in the early stages of the reaction are similar to those at the later stages and their formation does not appear to be sensitive to the reaction conditions. Alternative pathways in the formation of the same oligomer can be a possible explanation, as suggested by Yaylayan and Kaminsky (1998). The microanalysis results showed

that the melanoidins for which the extinction coefficient was determined are in line with those reported in literature. With this technique it was possible to determine the extinction coefficient of melanoidins formed in glucose/glycine systems at different reaction conditions. Through these results we are now able to translate the spectrophotometric data into concentrations of reacted sugars and to take browning into account in a kinetic model. This will allow a better prediction for browning in model systems as well as in foods.

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