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Study of nonenzymic browning in α-amino acid and γ-aminobutyric acid/sugar model systems

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ABSTRACT

The reactivities, in nonenzymic browning, of γ-aminobutyric acid (GABA), a non-protein amino acid with wide natural occurrence and potential health benefits as it occurs in foods, and of the α-L-amino acids arginine, glutamic acid, glutamine, leucine, lysine, and phenylalanine were investigated by heating equimolar mixtures of glucose and the cited amino acids at 110 °C at pH 6.0 for different times (0–4 h). Linear regression analysis indicated that the colour development in a GABA/glucose mixture was slower than that of a lysine/glucose mixture and comparable to that of a phenylalanine/glucose mixture. High-performance anion-exchange chromatography (HPAEC) with integrated pulsed amperometric detection (IPAD) showed that the decrease in GABA levels (*ca.* 10% after heating for 4 h) as a function of heating time was smaller than that of glucose (*ca.* 30% after heating for 4 h). At the same time, glucose to fructose isomerisation took place. After 20 min of heating at pH 6.0, all mixtures showed a fructose peak, the area of which increased with heating time. However, after correcting for fructose isomerisation, glucose losses were still higher than amino acid losses. In contrast to its precursor glutamic acid, GABA was stable during heating of a solution containing it alone. Heating of GABA-containing D-sugar solutions (xylose, fructose, glucose, maltose and sucrose) showed that the relative order of colour yield was pentose > hexose > disaccharides. As well as glucose to fructose isomerisation, HPAEC–IPAD allowed monitoring of the different isomerisation reactions occurring, and also disaccharide hydrolysis in the different GABA/sugar mixtures.

1. Introduction

The Maillard reaction (MR) is a complex set of reactions, initially between carbonyl groups of reducing sugars and amino groups from amino acids, peptides, or proteins. At early and intermediate stages of the MR, UV-absorbing colourless compounds are formed, whereas dark-brown polymeric components, i.e. melanoidins, are formed at later stages. To study MR kinetics, model systems are frequently used. The rate of the MR and the type of products formed depend on temperature, pH, and water activity conditions (Ajandouz & Puigserver, 1999; Kwak & Lim, 2004; Martins & van Boekel, 2005; van Boekel, 2001).

The type of reducing sugar largely affects Maillard browning (Villamiel, del Castillo, & Corzo, 2006). Pentoses react more readily than do hexoses, which, in turn, are more reactive than are disaccharides. Furthermore, Maillard browning depends on the type of amino acid and the reaction conditions used. Under some conditions, the basic amino acids histidine, arginine and lysine, as well as the hydroxyl group-containing amino acids, serine and threonine, have the highest reactivity with α-dicarbonyl compounds, while the non-polar and acidic amino acids show the lowest reactivity (Piloty & Baltes, 1979). Using other conditions, amino acids can be divided into three groups, depending on the absorbance measured at 420 nm (Ashoor & Zent, 1984). Lysine, glycine, tryptophan and tyrosine result in the most intense Maillard browning. Intermediate browning-producing amino acids include alanine, valine, leucine, isoleucine, phenylalanine, proline, methionine, asparagine and glutamine. The two basic amino acids, histidine and arginine, the two hydroxyl group-containing amino acids (serine and threonine), the two acidic amino acids (aspartic acid and glutamic acid), and the thiol-containing amino acid, cysteine, belong to the group of the least reactive amino acids. Depending on the experimental conditions, sugar and amino acid losses can be described by simple reaction kinetics (zero-, first- or second-order) or by applying more complex multiresponse modelling (van Boekel, 2001).

The non-protein amino acid γ-aminobutyric acid (GABA, NH_2–$(CH_2)_3$–COOH) is enzymically formed by decarboxylation of glutamic acid. GABA is present in vegetables, e.g. spinach, potato and broccoli, fruits, e.g. apple, and cereals, e.g. rice, barley, maize (Oh, Moon, & Oh, 2003). The role of GABA in plants is still unclear. It may play roles in pH regulation, nitrogen storage, plant development, and plant defence (Shelp, Bown, & McLean, 1999). From a

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food technology point of view, it is important that GABA functions as an inhibitory neurotransmitter in animals and humans, and that it is involved in the regulation of cardiovascular functions, such as blood pressure (Hayakawa et al., 2004). Inoue et al. (2003) studied the effect of a fermented milk product containing GABA on the blood pressure of patients with mild hypertension. This study consisted of a 12-week period of daily intake of 12 mg GABA or placebo, followed by 2 weeks of no intake. Blood pressure decreased significantly after 4 weeks of consuming the fermented milk product. It remained decreased throughout the intake period. Two weeks after intake, the blood pressure increased again but remained lower than the original level. No significant changes in blood pressure occurred in the placebo group. GABA also has a role in the recovery from and prevention of chronic alcohol-related diseases (Oh, Soh, & Cha, 2003), inhibits leukaemia cell proliferation, and stimulates cancer cell apoptosis (Oh & Oh, 2004). Evidently, loss of GABA in MRs has already been demonstrated in hydrothermally-treated foods. The levels of 2-furoylmethyl-GABA in hydrolysates of commercial processed tomato products (Sanz, del Castillo, Corzo, & Olano, 2000), orange juice (del Castillo, Villamiel, Olano, & Corzo, 2000) and cereal-based products (Rada-Mendoza, Garcia-Baños, Villamiel, & Olano, 2004) indicated GABA losses in Maillard reactions. Tressl, Kersten, and Rewicki (1993a,b) investigated the formation of specific MR products by heating GABA and reducing sugars at 160 °C for 1.5 h in slightly acidic aqueous media. However, the relative reactivity of GABA, when compared to that of protein α-amino acids, has, to the best of our knowledge, never been investigated. Additionally, amino acid and sugar losses in MRs are usually determined by two separate analyses with time-consuming sample preparation steps for amino acid analysis, e.g. pre- or post-derivatisation (Ajandouz & Puigserver, 1999; Martins, Marcelis, & van Boekel, 2003), while high performance anion-exchange chromatography (HPAEC) with integrated pulsed amperometric detection (IPAD) allows determination of amino acids, together with sugars, without sample derivatisation (Ding, Yu, & Mou, 2002; Yu, Ding, Mou, Jandik, & Cheng, 2002).

Against this background, the present study was designed to study the reactivity of GABA in MRs. Thus, pigment formation in a GABA/glucose model system was compared to that of α-amino acid/glucose model systems. Secondly, the destruction of the amino acids and glucose was simultaneously determined by HPAEC–IPAD. Thirdly, the contribution of sugar to the overall nonenzymic browning was investigated. Therefore, pigment formation, sugar losses in sugar solutions heated at 110 °C, and GABA and sugar losses in GABA/sugar model systems heated at 110 °C were studied.

2. Materials and methods

2.1. Materials

The L-amino acids (arginine, glutamic acid, glutamine, leucine, lysine, phenylalanine), GABA, and the D-sugars (xylose, fructose, glucose, mannose, maltose and sucrose) were obtained from Sigma-Aldrich (Bornem, Belgium). L-norleucine was from Fluka (Bornem, Belgium).

2.2. Model systems

Equimolar mixtures (2.5 ml of 30 mM solution in 50 mM sodium phosphate buffer at pH 6.0) of (i) glucose and each of the amino acids (arginine, glutamic acid, glutamine, leucine, lysine, phenylalanine and GABA), and of (ii) GABA and each of the sugars (xylose, fructose, glucose, maltose and sucrose) were heated in an oil bath (110 °C) in screw-sealed tubes for different times (10–240 min). The sugar solutions alone and the amino acid solutions containing GABA or glutamic acid were also heated under the same conditions. After a heating period of *ca.* 2 min, the solution reached 110 °C. The pH value of the different solutions was 6.0, except for the glucose/glutamic acid mixture and the glutamic acid solution (pH 3.9). To exclude the impact of pH on nonenzymic browning, glucose and the amino acids GABA and glutamic acid, and glucose and glutamic acid alone were also heated at pH 6.0 by using a more concentrated sodium phosphate buffer (200 mM, pH 6.0) for 5–150 min. pH values of the mixtures slightly changed (0.2) during heating. After heating, the tubes were immediately cooled in ice. Analyses were performed in duplicate.

2.3. Measurement of colour change

The colour change of the model systems was determined as the absorbance at $\lambda = 420$ nm against the unheated mixture. The difference between two measurements did not exceed 0.010 extinction units for the different solutions.

2.4. Changes of amino acid and sugar levels

Residual amino acids and sugars were determined by HPAEC–IPAD. The reaction mixtures were diluted 800-fold by adding 200 μl of the reaction mixture to 3.8 ml norleucine solution (1.5 mM) and diluting this solution (100 μl) in deionised water (3.9 ml). A Dionex BioLC system (Sunnyvale, CA, USA), consisting of a GS50 gradient pump with an online degasser, an AS50 autosampler with a thermal compartment and an ED50 electrochemical detector containing a gold working electrode and a pH reference electrode, was used. Separation was performed using an AminoPac PA10 guard column (Dionex, 50 × 2 mm) and analytical column (Dionex, 250 × 2 mm) at a flow rate of 0.25 ml/min at 30 °C. The sample injection volume was 15 μl. To prepare the gradient, mobile phases of 18 MΩ water (mobile phase A), sodium hydroxide (0.250 M, mobile phase B; Baker, Deventer, The Netherlands) and sodium acetate solutions (1.0 M, mobile phase C; Dionex Benelux, Amsterdam, The Netherlands) were used. All three mobile phases were degassed and kept under slight helium overpressure to prevent accumulation of atmospheric carbon dioxide. The chromatographic system control, data acquisition and data analysis were performed using Chromeleon version 6.70 software (Dionex). The levels of amino acids and sugars were simultaneously determined in the diluted reaction mixtures using the following gradient conditions: 0.0–2.0 min, 24% B; 2.0–8.0 min, 24–36% B; 8.0–11.0 min, 36% B; 11.0–18.0 min, 36–20% B and 0–40% C; 18–21 min, 20–16% B and 40% C; 21–23 min, 16% B and 40–70% C; 23–42 min, 16% B and 70% C; 42–42.1 min, 16–80% B and 70–0% C; 42.1–44.1 min, 80% B; 44.1–44.2 min, 80–24% B; 44.2–74 min, 24% B. For the determination of maltose levels (in the GABA/maltose mixture and the maltose solution), slightly modified gradient conditions were used. The holding time for 36% B was 19 min instead of 3 min. The detection waveform for all determinations was borrowed from Ding et al. (2002). The difference between two measurements did not exceed 2.0% for the different solutions.

2.5. Statistical analysis and kinetic modelling

Regression analyses were performed to describe colour changes, and amino acid and sugar losses. The browning rate, corresponding to the slope of the linear regression curve, was estimated (with 95% confidence intervals).

The data of the amino acid/sugar–time curves were fitted to three different kinetic models according to the following equations (van Boekel, 2001): the zero-order reaction, $c = c_0 - kt$, the first-order reaction, $c = c_0 \exp(-kt)$, and the second-order reaction, $\frac{1}{c} = \frac{1}{c_0} + kt$ with c_0 the initial concentration of the reactant, k the

reaction rate constant, and *t* the time. The least sum of squares was used as criterion for model discrimination. Data were analysed using GraphPad Prism 3.0 (Graphpad, San Diego, CA, USA).

3. Results and discussion

3.1. Pigment formation in amino acid/glucose model systems

Fig. 1a and b show the pigment formation as a function of heating time at 110 °C of the amino acid/glucose mixtures and of the glucose solution heated in 50 and 200 mM sodium phosphate buffers, respectively. Absorbance measurements (420 nm) indicated that pigment formation of a glucose mixture heated in 50 mM sodium phosphate buffer at 110 °C increased in the presence of all the amino acids, except for glutamic acid that, in contrast to the other amino acids, caused a sharp decrease in pH value (Fig. 1a). When heated in 200 mM sodium phosphate buffer at 110 °C, browning increased for both the GABA/glucose and the glutamic acid/glucose mixtures (Fig. 1b). While pigment formation was favoured in 200 mM sodium phosphate buffer, browning of the glutamic acid/glucose mixture remained low. Pigment formation in all mixtures (50 mM sodium phosphate buffer) showed an induction period (*ca.* 30 min), after which it linearly increased with heating time (except for lysine). Linear regression analysis of the colour development indicated that the correlation coefficient (*r*) was 0.99 (except for glutamic acid/glucose). The absorbance values of the lysine/glucose mixture increased linearly between 0 and 150 min and were followed by a slower pigment formation. Based on the pigment formation, the amino acids fell into three distinct categories. Increased pigment formation is related to an increased linear slope. Lysine was the highest pigment-producing amino acid (slope 0.012). Phenylalanine, GABA, arginine, glutamine and leucine showed intermediate pigment formations (slopes 0.0066, 0.0062, 0.0038, 0.0033, 0.0038, respectively). The glutamic acid/glucose mixture showed the lowest pigment formation (slope 0).

The induction period for pigment formation was attributed to the production of colourless intermediates in the earlier stages of the MR (Ajandouz & Puigserver, 1999; Baisier & Labuza, 1992). Later, melanoidins were formed. Their levels increased linearly with heating time. Enhanced browning, due to the use of more concentrated phosphate buffers, was in line with Rizzi (2004). He reported on the catalytic role of phosphate and carboxylate ions in Maillard browning. Ajandouz and Puigserver (1999) demonstrated that most amino acids favour nonenzymic browning of glucose solutions at pH values below neutrality and ascribed the high reactivity of lysine to its having two amino groups. As mentioned earlier, nonenzymic browning depends on experimental conditions, since arginine here was an intermediate pigment-producing amino acid, while, under conditions described elsewhere (Ashoor & Zent, 1984; Piloty & Baltes, 1979), different relative reactivities were noted. However, the high absorbance of lysine/glucose mixtures and the intermediate pigment formation of GABA and arginine/glucose mixtures may indicate that, under the experimental conditions used here, basic side chains increased nonenzymic browning.

3.2. Glucose and amino acid losses in amino acid/glucose model systems

Fig. 2a and b show the glucose losses as a function of heating time at 110 °C in amino acid/glucose mixtures and in the control glucose solution heated in 50 and 200 mM sodium phosphate respectively. Fig. 2a indicates that the mixture with the highest pigment formation (lysine) had increased glucose degradation (*ca.* 40% after heating for 4 h). Glucose losses were intermediate (20% < glucose losses < 40% after heating for 4 h) for the intermediate pigment-producing amino acids (phenylalanine, GABA,

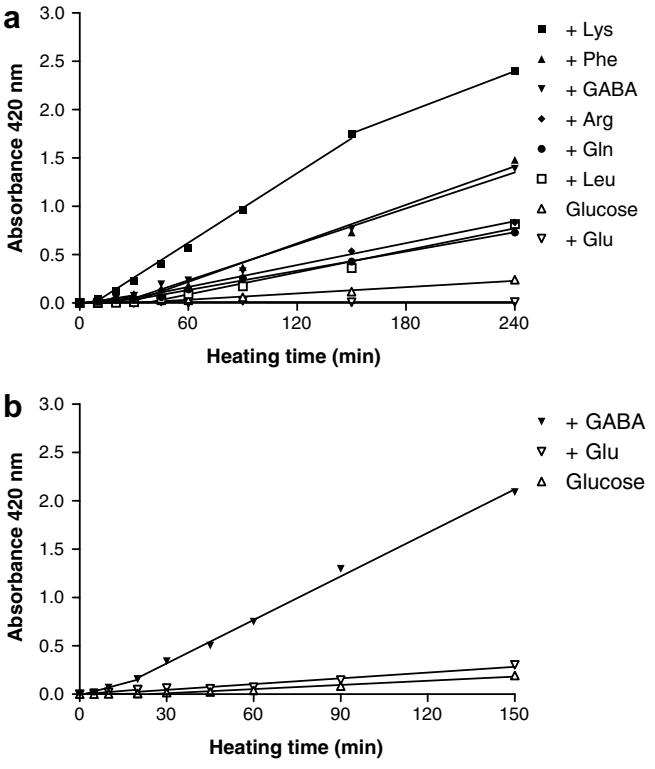

Fig. 1. Colour development in amino acid/glucose mixtures and glucose solutions heated in 50 (a) and 200 mM (b) sodium phosphate buffers (pH 6.0) at 110 °C as a function of heating time.

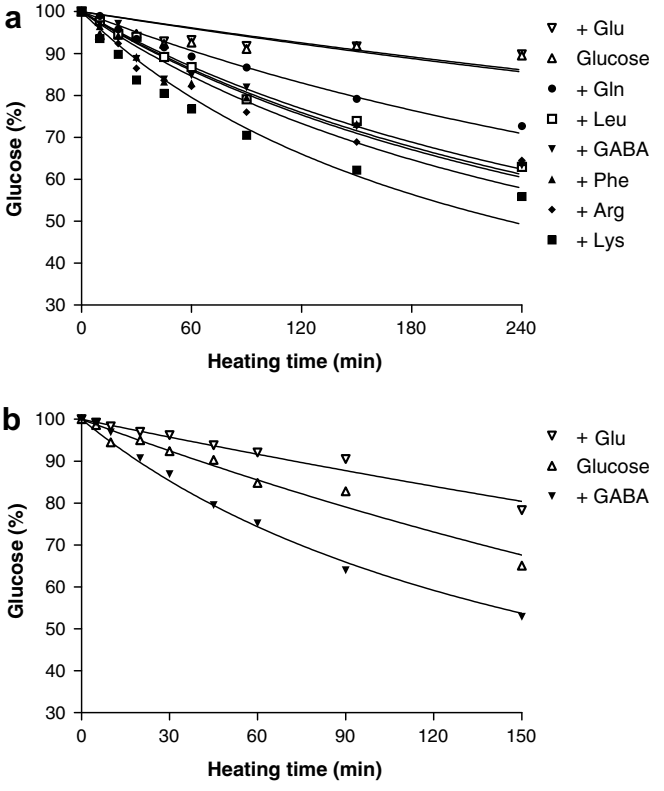

Fig. 2. Glucose losses in solutions containing glucose alone or in the presence of amino acids when heated in 50 (a) and 200 mM (b) sodium phosphate buffers (pH 6.0) at 110 °C as a function of heating time.

arginine, glutamine and leucine). At pH 3.9 (glutamic acid/glucose mixture in 50 mM sodium phosphate buffer), glucose losses were comparable to those of the glucose solution (<20% after heating for 4 h). The increased browning of the mixtures heated in 200 mM sodium phosphate buffer was related to increased glucose losses (Fig. 2b).

Fig. 3 shows the amino acid losses as a function of heating time at 110 °C in amino acid/glucose mixtures heated in 50 mM sodium phosphate buffers. The decreases in amino acid levels were comparable for lysine, phenylalanine, GABA, arginine and leucine. Their losses were lower than the corresponding glucose losses (<20% after heating for 4 h). Neither glucose nor amino acid levels of the different model systems decreased linearly with heating time. Glucose losses in the different mixtures were best described by second-order reaction kinetics, except for the glucose solution and the glutamic acid/glucose mixture heated in 200 mM sodium phosphate buffer (first-order reaction). Glutamine and glutamic acid losses were fitted by first-order reaction kinetics, whereas amino acid losses in all other mixtures were described by second-order reaction kinetics. In contrast to lysine, phenylalanine, GABA, arginine and leucine, the glutamic acid/glucose and glutamine/glucose mixtures heated in 50 mM sodium phosphate buffer showed a greater decrease in amino acid than in glucose levels. Similar to glucose degradation, GABA losses increased during heating of GABA/glucose mixtures in 200 mM sodium phosphate buffer (results not shown).

The higher molar loss of glucose than of the amino acid has been reported in previous studies using equimolar ratios of the two (Ajandouz & Puigserver, 1999; Martins & van Boekel, 2005). The difference in glucose and amino acid degradation can be explained by the amino acid regeneration from the initial condensation products as well as by the parallel reaction of the sugar into (mainly) its isomer fructose. In the present study, a fructose peak was observed in the different amino acid/glucose mixtures at pH 6.0 after heating for 20 min. Fig. 4 illustrates the isomerisation of glucose into fructose for the GABA/glucose mixture. However, after correcting for fructose isomerisation, glucose losses still exceeded amino acid losses (results not shown). These observations indicated that, besides isomerisation reactions, glucose is more strongly involved in the MR than are amino acids, as already suggested by Martins and van Boekel (2005).

The larger decrease of glutamic acid than of glucose concentrations at pH 3.9 (50 mM sodium phosphate buffer), indicated loss of the former in side reactions. Meltretter, Seeber, Humeny, Becker, and Pischetsrieder (2007) recently studied the formation of Maillard oxidation and condensation products from whey proteins during reaction with lactose at pH 6.8. They observed the formation of the pyrrolidone pyroglutamic acid following reaction of the free amino group of glutamic acid with its side-chain carboxyl residue.

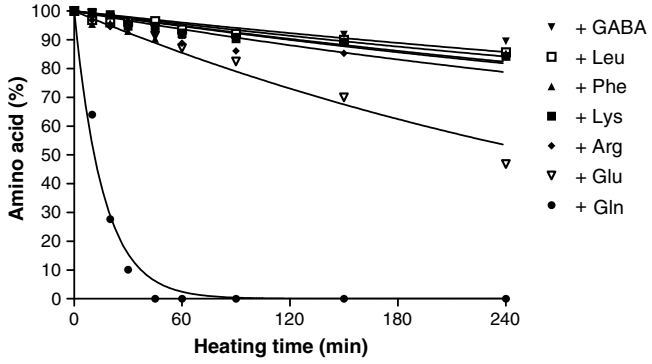

Fig. 3. Amino acids losses in amino acid/glucose solutions heated in 50 mM sodium phosphate buffers (pH 6.0) at 110 °C as a function of heating time.

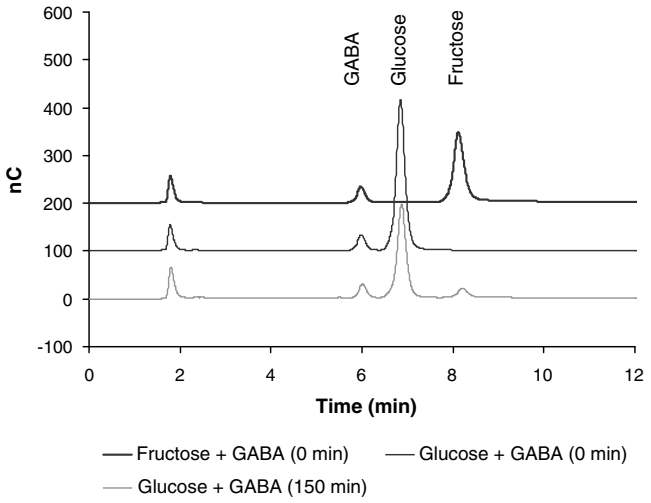

Fig. 4. High performance anion-exchange chromatogram of non-heated (0 min) GABA/fructose and GABA/glucose mixtures, and of GABA/glucose mixtures heated at 110 °C at pH 6.0 for 150 min.

They also showed that the pyrrolidone is formed in the absence of lactose as well. Hence, glutamic acid condensation into pyroglutamic acid may explain the high glutamic acid losses during heating of the glutamic acid/glucose mixture. The reduced glutamic acid losses at pH 6.0 (200 mM sodium phosphate buffer) may indicate that side reactions were favoured in acidic conditions. It is further of note that, in the absence of glucose, heating (for up to 4 h) of glutamic acid solutions (30 mM) in the 50 and 200 mM sodium phosphate buffers at 110 °C resulted in a colourless reaction mixture and in glutamic acid losses (results not shown).

Glutamine is an unstable amino acid. Snowden, Baxter, Bergana, and Pound (2002), and Niquet and Tessier (2007) showed that its degradation results *inter alia* in pyroglutamic acid and ammonia, and in minor levels of glutamic acid. Under certain experimental conditions, the yield of ammonia formation from glutamine reaches *ca.* 100% (Niquet & Tessier, 2007). Hence, the large glutamine losses can be explained by its loss in MR on the one hand, and by its reaction to pyroglutamic acid during heating, on the other.

In contrast to what is known about glutamic acid and glutamine, no thermal degradation of the other amino acids studied here has, to the best of our knowledge, been described. Although one could easily envisage the formation of a pyrrolidone from GABA, heating of the GABA solution alone at 110 °C and pH 6.0 did not result in destruction of GABA (results not shown).

3.3. Pigment formation in sugar and GABA/sugar model systems

Fig. 5a and b show the pigment formation as a function of heating time at 110 °C of the sugar solutions alone and of the GABA/sugar mixtures, respectively. Heating of the sugar solutions indicated that fructose (slope 0.0035) and xylose (slope 0.0038) produced pigments more swiftly than did glucose (slope 0.0011; Fig. 5a). Almost no colour development occurred in the solutions of the disaccharides maltose and sucrose (slope 0.0003 and 0.0004, respectively). As also observed for the amino acid/glucose mixtures, browning of the different sugar solutions showed a lag phase. After the induction period (*ca.* 30 min), pigment formation linearly increased with heating time (except for xylose). In the different GABA-containing sugar solutions, pigment levels were higher than in those containing the sugar alone (Fig. 5b). Under the experimental conditions used, the GABA/xylose mixture showed the highest pigment formation (slope 0.025). The colour develop-

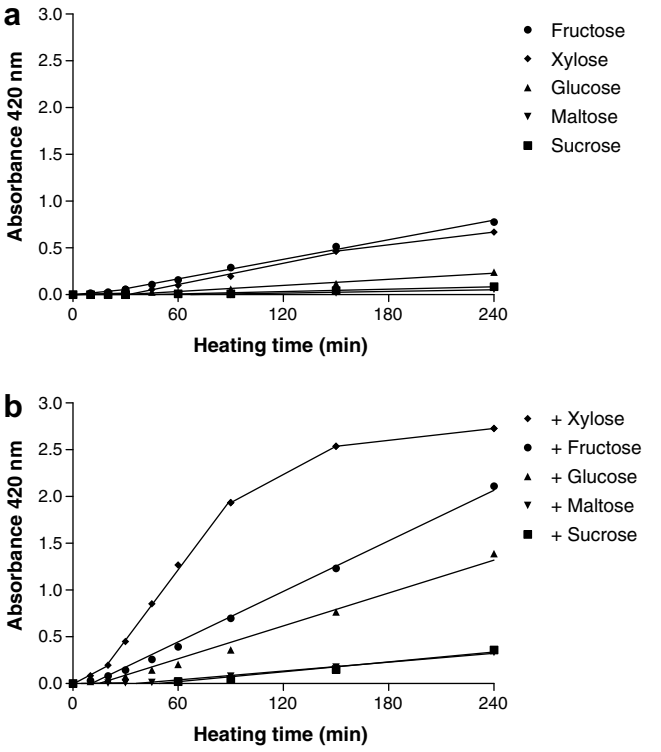

Fig. 5. Colour development in sugar solutions alone (a) and GABA-containing sugar solutions (b) heated at 110 °C at pH 6.0 as a function of heating time.

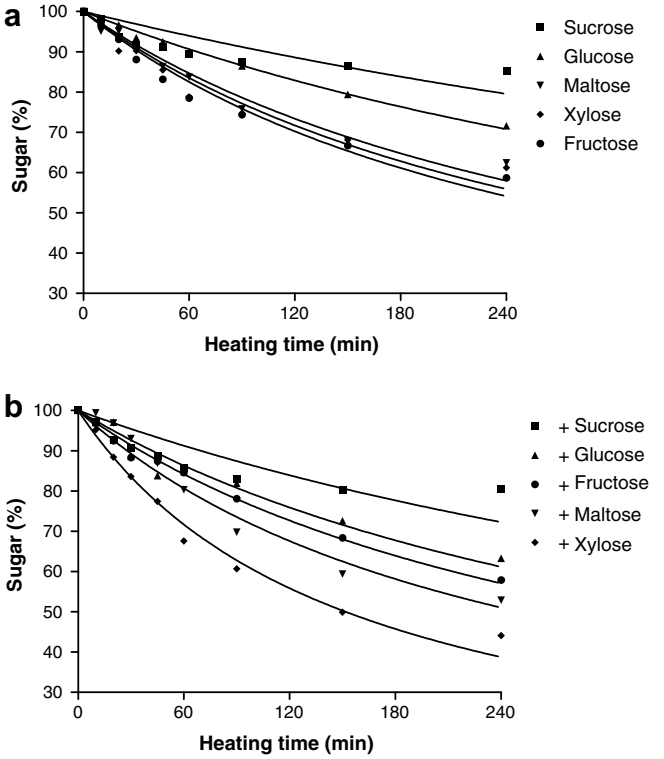

Fig. 6. Sugar losses in solutions containing the sugar alone (a) or in the presence of GABA (b) when heated at 110 °C at pH 6.0 as a function of heating time.

ment was intermediate for the GABA-containing fructose and glucose solutions (slope 0.0090 and 0.0059, respectively), and lowest for the GABA/maltose and GABA/sucrose mixtures (slope 0.0016 and 0.0018, respectively). The linear increase of pigment formation in GABA/fructose and GABA/glucose solutions with heating time indicated that browning can be described by a zero-order reaction kinetic ($r = 0.99$). The colour development of the GABA/disaccharide mixtures showed an induction period of *ca.* 30 min, after which the pigment formation linearly increased with heating time ($r = 0.99$).

The pigment formation in the sugar solutions was attributed to caramelisation reactions. The differences in colour development for the different sugar solutions were in line with del Pilar Buera, Chirife, Resnik, and Lozano (1987). They found that, during heating at pH 6.0, the activation energies for monosaccharide solutions were lower than for disaccharide solutions. The high rate of colour development with fructose was explained by the lower stability of its ring conformation than those of xylose and glucose. Furthermore, the faster pigment formation in xylose than in glucose solutions was explained by the higher level of open-chain sugar in xylose than in glucose solutions. The colour development of the solution of the non-reducing sugar, sucrose, was due to partial thermal hydrolysis (at 110 °C) into glucose and fructose (as discussed further) and their degradation to coloured products.

The increase in melanoidin levels in GABA-containing sugar solutions indicated that the non-protein amino acid behaves like most α-amino acids. Furthermore, our data illustrate that the relative order of nonenzymic colour development in amino acid/sugar mixtures is pentose > hexose > disaccharides, as reported earlier (Villamiel et al., 2006).

3.4. Sugar and GABA losses in sugar and GABA/sugar model systems

Fig. 6a and b show sugar and GABA losses as a function of heating time at 110 °C in sugar solutions and GABA/sugar mixtures, respectively. Sugar levels decreased with heating time (Fig. 6a). Fructose, xylose and maltose solutions showed the highest sugar degradation (*ca.* 35% after heating for 4 h), followed by glucose (*ca.* 20% after heating for 4 h). The low pigment-producing sugar, sucrose, showed the lowest sugar losses (*ca.* 10% after heating for 4 h). Heating of GABA-containing sugar mixtures resulted in increased sugar degradation (Fig. 6b). Sugar losses ranged from *ca.* 20% for sucrose to *ca.* 50% for xylose. Furthermore, higher melanoidin levels in the heated GABA/sugar mixtures were related to higher sugar losses (except for maltose).

As earlier observed, the amino acid (GABA) losses were lower than the sugar losses (Fig. 7). The GABA/xylose mixtures showed the greatest GABA decrease (*ca.* 20% after heating for 4 h), while it was reduced to similar levels for all other GABA/sugar mixtures (*ca.* 10% after heating for 4 h). As observed for the amino acid/glucose mixtures, the decrease in sugar and GABA levels as a function of heating time was not linear. Both sugar and GABA losses were fitted by second-order reaction kinetics. However, the curves indicated that reaction kinetics other than those here used would probably better fit the GABA losses.

The decrease in the sugar levels in the heated solutions was attributed to their degradation in caramelisation reactions. During caramelisation, slow enolisation occurred. After dehydration and several further reaction steps, furan derivatives were obtained. Dehydration of pentoses (e.g. xylose) resulted in furfural, while, from hexoses (e.g. glucose and fructose), 5-hydroxymethyl-2-furaldehyde (HMF) was obtained. Furthermore, in contrast to what is the case for aldoses (glucose), both 1,2- and 2,3-enediols are formed from ketoses (fructose). Therefore, fructose degradation results in a wider product spectrum (Belitz et al., 2004). Hollnagel and Kroh (1998) identified the α-dicarbonyl compounds, glyoxal, methylglyoxal and diacetyl, as degradation products from nonenzymic browning of glucose, fructose and maltose. Haghighat Khajavi, Kimura, Oomori, Matsuno, and Adachi (2005) investigated

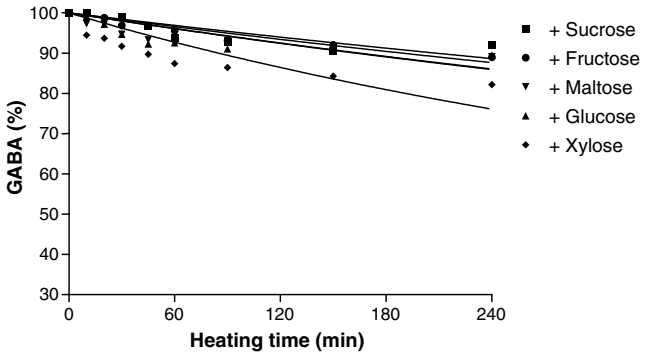

Fig. 7. GABA losses in GABA-containing sugar solutions heated at 110 °C at pH 6.0 as a function of heating time.

sucrose degradation at different temperatures. They indicated that sucrose is hydrolysed into equivalent levels of glucose and fructose, which reacts further to form HMF and acidic compounds (e.g. formic acid). Quintas, Guimaraes, Baylina, Brandao, and Silva (2007) recently proposed a mechanistic model describing the sucrose caramelisation reaction. Our HPAEC–IPAD chromatograms indicated that, besides glucose isomerisation (*cfr. supra*), xylose isomerised into lyxose and fructose into mainly glucose and some smaller levels of mannose. Additionally, the chromatograms allowed monitoring of both sugar hydrolysis (sucrose into fructose and glucose, and maltose into glucose) and isomerisation (maltose into sucrose) reactions during heating of disaccharide solutions and GABA/disaccharide mixtures.

The larger sugar losses in GABA/sugar mixtures than in sugar solutions alone indicated that GABA, as do most of the α-amino acids (*cfr. supra*), increases nonenzymic browning reactions. Tressl, Kersten, and Rewicki (1993a,b) studied the MR products in GABA/reducing sugar (xylose, glucose, rhamnose, fructose and arabinose) model systems. They used gas chromatography and mass spectrometry to determine the origin, the reactive intermediates, and the formation pathways of pyrroles, pyrrolidones, and pyridone structures. They observed that the GABA-specific MR products were comparable to those of peptide-bound lysine.

4. Conclusions

Heating of GABA/glucose and α-amino acid/glucose mixtures at 110 °C in slightly acidic conditions indicated that the GABA/glucose mixture showed intermediate pigment formation, while the GABA precursor glutamic acid was a low pigment-producing amino acid. Determination of glucose and amino acid losses by HPAEC–IPAD indicated that almost all model systems showed greater glucose degradation than amino acid losses at pH 6.0 (except for glutamine). Furthermore, in contrast to the GABA precursor, glutamic acid, GABA was not destroyed during heating of a GABA solution alone. Comparison of colour development of sugar solutions alone (xylose, glucose, fructose, sucrose and maltose), and of GABA-containing sugar solutions, indicated that GABA increased pigment formation. The increased pigment formation went hand in hand with increased sugar losses and small levels of GABA degradation. Finally, the chromatograms obtained by HPAEC–IPAD analyses of the different mixtures showed that xylose–lyxose, glucose–fructose–mannose and maltose–sucrose isomerisation reactions and hydrolysis of disaccharides (sucrose and maltose) in monosaccharides (glucose and fructose, and glucose, respectively) occurred during heating of sugar solutions alone and amino acid/sugar mixtures.

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