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Maillard reactions: do the properties of liquid matrices matter?

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The amounts of browning due to the interaction of lysine and xylose occurring when these reactants were in different liquids have been investigated. The reactants were suspended/solvated in water, corn oil, glycerol, different propylene gylcols and mixtures of these liquids. In water the amount of browning was found to equate to the concentration of the reactants to the third power. In glycerol and polypropylene glycol 76° the amount of browning was higher than that achieved for the same amount of reactants in water. In corn oil and polypropylene glycol 1200 no browning was observed. In all samples the addition of water to another liquid caused the level of browning to be increased, until a maximum was achieved. This maximum may have corresponded to the point where all the reactants were soluble in the matrix. Further addition of water decreased the amount of browning. In all cases the amount of browning seemed to relate to the concentration of the reactants if they were calculated as just occurring in the water portion of the matrix. Values calculated in this way were significantly, but constantly a little lower than the experimental results in all cases except for the corn oil, where the values directly corresponded. The predictability of these values was surprising considering that the matrices gave miscible and phase-separated systems. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

It is now well accepted that the rate of the Maillard reaction and the nature of the coloured products are governed by its immediate chemical environment, one important factor being the water activity (Eichner and Karel, 1972; Eichner, 1974; Labuza, 1980). Concepts such as the solubility and concentration of the reacting compounds are often used to explain the 'bell-shaped' curves associated with the relationship between water activity and rate of browning observed in systems with a high solids content (King, 1988; Karel and Buera, 1994). It is postulated that the reactants become soluble in the water layers surrounding the non-soluble material and are therefore sufficiently mobile to interact. Additional water may dilute the concentration of the reactants to the point where the rate of reaction diminishes. The consistency of the other components within the matrix may also affect the mobility of the reactants. Polymers, at high concentration, would limit the rate of diffusion of the reactants. If the polymers composing the matrix are in a glassy state, it is possible that the diffusibility of smaller reactant molecules in the system will be much curtailed. A reduction in the reaction and low colour formation would therefore be expected owing to diffusional limitations. Such a relationship between the glass transition temperature of the matrix and the browning rate has been demonstrated by Karel and Buera (1994) and Karmas and Karel (1994).

Labuza (1980) has also observed that the addition of different humectants to a solid matrix may result in significant differences in the amount of browning occurring at the same water activity (a_w). An explanation for this might be the degree of plasticization (and hence change in the mobility of the matrix) caused by the humectants. In the majority of studies it has been assumed that the humectant does not take part directly in the reaction. However, accelerated chemical reactions have been reported in liquid humectants commonly used in food products and in model systems. According to Seow and Cheah (1985*a*,*b*) and Obanu *et al.* (1977), there can be notable colour formation at elevated temperatures as a result of direct chemical interaction between humectants and protein/amino acids. However, the extent of direct

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*To whom correspondence should be addressed. Fax: 0115 951 6142; e-mail: sandra.hill@nottingham.ac.uk interaction with humectants at low temperatures is insufficient to explain the accelerated chemical changes.

Compared to the numbers of studies on relatively dry systems, there is little reported work on the rates of browning that occur in liquid systems. Yet within a food material it is within the liquid/solid boundaries or in the 'free' water phase that the reaction is expected to take place. This present study has been performed to ascertain the importance of different physico-chemical mechanisms (e.g. solubility, concentration of reactants and products, mobility of the molecules and their ability to diffuse) for the progress of the Maillard reaction in liquid systems.

Liquids of different viscosities and densities were used that show different varying degrees of partitioning with water. Glycerol, corn oil and a range of liquid polyols were used. The influence of these liquids on the rate of the Maillard reaction evoked by the presence of the reactants lysine and xylose is reported.

MATERIALS AND METHODS

Materials

A model system was studied consisting of L-lysine free base, D(+)-xylose, glycerol (MW 92, PG92) and propylene glycol (MW 76, PG 76) which were obtained from the Sigma Chemical Company. Polypropylene glycols (MW 425 (PPG425) and 1200 (PPG1200) were supplied by Fisons Laboratory Reagents. Refined corn oil was purchased from J. Sainsbury. Distilled water was the primary liquid. The glycerol, glycols and corn oil are all referred to as the secondary liquids. All secondary liquids were kept as moisture-free as possible, but it could be reasonably assumed that some water might be associated with the liquids. Measurements on glycerol showed that contamination with water was less than 0.65%.

The reactants, lysine and xylose, were used as supplied except when they were ground together using a mortar and pestle until the average particle length had been reduced to approximately one quarter of the original size (as estimated by light microscopy).

Methods

Sample preparation

The reactants consisted of a 1:1 ratio of xylose and lysine. This mixture was added to distilled water to give a range of lysine concentrations between 8.33 and 33.3%. Liquid/liquid systems consisted of reactants–water–secondary liquid (glycerol, PG76, PPG425, PPG1200 or corn oil). To every 10 g of the water–secondary liquid solution was added 1 g xylose and 1 g lysine. The amount of secondary liquid varied from 0 to 10 g. The samples were stored in screw capped glass bottles at 20°C for a week. The colours were then assessed.

Measurement

Colour measurement The colour development was estimated after storage for a week at the controlled temperature of 20°C. The Hunterlab Color Quest Sphere Spectrocolorimeter Sn C5330 (Virginia, USA) was used to estimate darkness values: a black sample has a darkness of 100% and a white sample has a darkness of 0%. Samples were also assessed for absorbance. Each sample was diluted with sufficient distilled water so that the aqueous phase had an absorbance value of less than 1.0 at 480 nm. Typically, a dilution factor of 100 was used. Initial studies showed that extraction and subsequent dilution gave reproducible values. Absorbances versus concentration of the diluted brown solution gave a linear relationship. Some samples were assessed using both darkness and absorbance methods. There was a good linear relationship ($r^2 = 0.998$) between these two estimates.

Other measurements

(a) *Water activity (aw).* Initial water activities of the samples were measured using a Decagon Water Activity Meter CX-1.
(b) *Solubility measurement.* Measurements were carried out to determine the solubility of lysine. The ninhydrin reaction (Plummer, 1987) was used to measure the amount of amino acid in solution.
(c) *Viscosity measurement.* The viscosities of the samples were measured using a Bohlin CS Rheometer at 25°C with different measuring geometries depending on the samples.
(d) *Moisture absorbed by the secondary liquids.* The liquids were stored for 24 h over distilled water and the percentage increase in weight was calculated.

RESULTS AND DISCUSSION

Colour formation with the reactants

Each liquid and each combination of liquids used as a matrix was mixed with lysine and stored for one week. No apparent colour formation was observed. This was also the case when xylose was mixed with the matrices.

The rate of the Maillard reaction in single liquid matrices

Figure 1 demonstrates that, in some liquids, colour development did not occur, for example in oil, PPG425 and PPG1200. With compounds that forming the matrix, the amount of brown colour development varied. PG76 gave the most colour, followed by glycerol, with water being the least productive. The liquids used to form the matrix provided a range of a_ws. However, this variable cannot be the major influence on colour

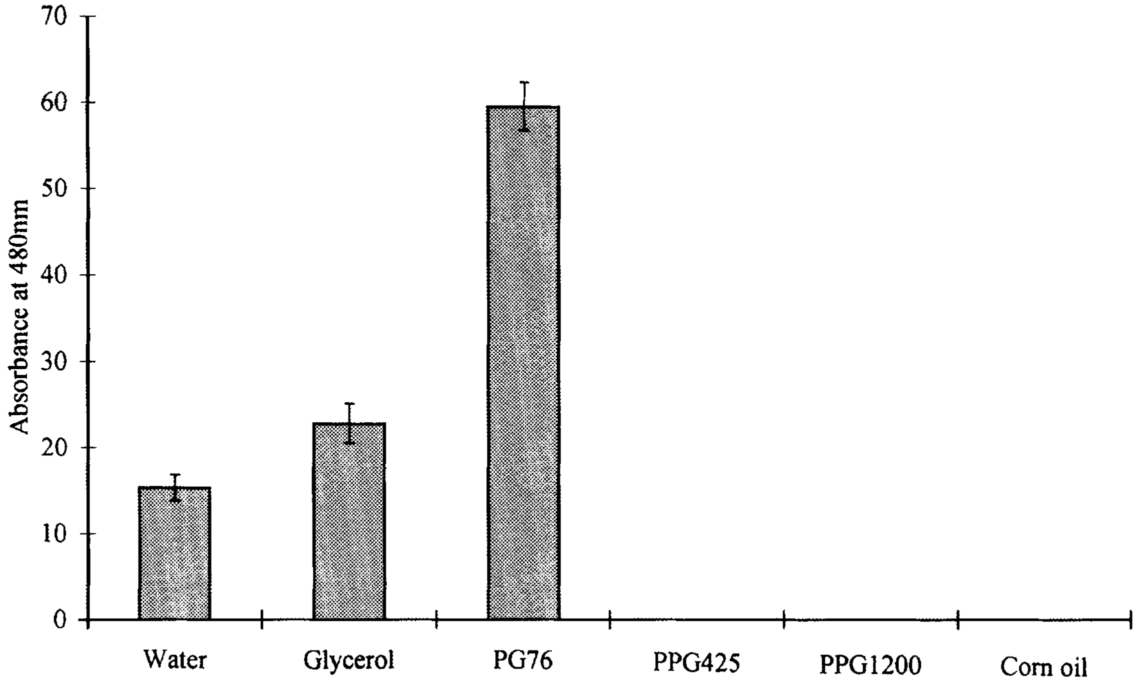

Fig. 1. Rates of browning as indicated by absorbance at 480 nm of liquids (suitably diluted in water) incubated for one week at 20°C in the presence of 8.33% lysine and 8.33% xylose. The liquids (water, glycerol, corn oil and propylene glycol (PPG) solutions were of different molecular weights (76, 425 and 1200). Results were recorded in duplicate.

formation as glycerol gave an intermediate degree of browning at low a_w (0.01) while PG76 gave the darkest coloration at the relatively high a_w of 0.84 (see Table 1).

If a_w did not control the generation of colour, differences in the solubility of the reactants in the various liquids might be the cause of differing amounts of colour. However, the results shown in Table 1 do not wholly support this. Although zero solubility equates to no colour formation, the ranked order of the other samples does not indicate a simple correlation. An alternative and/or additional explanation must be sought, possibly involving a more sophisticated understanding of the roles of diffusion, mobility, solubility and concentration. For the liquid matrices investigated and reported in this paper, features involving the solubility and viscosity of the system have been explored in more detail.

Figure 2 indicates that the amount of colour produced in both glycerol and water depends on the concentration of the reactants added. The dependence is apparently different for the two matrices. In water, colour is dependent on the concentration to at least the third power (darkness is proportional to $[\text{lysine}]^3$). This is clearly demonstrated over a wide range of concentration in Fig. 3. However, for glycerol, over the narrow range used, colour and reactant concentrations are linearly related. At all concentrations used in Fig. 2, the amount of browning in glycerol was higher than in water. At all levels of addition it was observed that the reactants had not fully dissolved in the glycerol at the start of the study with the particulates being seen at the surface of the liquid. Therefore the concentrations quoted in Fig. 2 are an over-estimate of the actual levels. This over-estimate would be greatest at the high concentration values, if it is assumed that the maximum amount of material had already dissolved and the excess is not participating in the reaction. Some particulates were still observable at the end of the week's study. This may explain the differing dependencies on browning levels and lysine concentration for glycerol and water matrices. The low reactant concentration in solution for the glycerol makes the higher browning in this matrix, compared to water, even more marked.

Table 1. The absorbances recorded for liquids containing lysine and xylose incubated for one week at 20°C

Liquid	Absorbance	Water activity	Measured viscosity (Pas)	% Soluble lysine	% Moisture absorbed
Water	15.4 ± 1.5	0.983 ± 0.010	0.002	91.9 ± 0.02	—
PG76	59.9 ± 2.3	0.868 ± 0.009	0.047	74.9 ± 0.02	9.85 ± 0.11
PPG1200	0	0.175 ± 0.014	0.185	1.07 ± 0.01	1.91 ± 0.14
PPG425	0	0.135 ± 0.011	0.072	0.59 ± 0.01	4.31 ± 0.07
Glycerol	23.0 ± 2.8	0.012 ± 0.006	0.969	45.0 ± 0.03	5.54 ± 0.13

Estimates for the water activity, viscosity (at shear rate of $5\,s^{-1}$) and percentage of lysine soluble in the different liquids are shown. The amount of water absorbed in 24 h by each liquid is also indicated. Results are the average of duplicate readings.

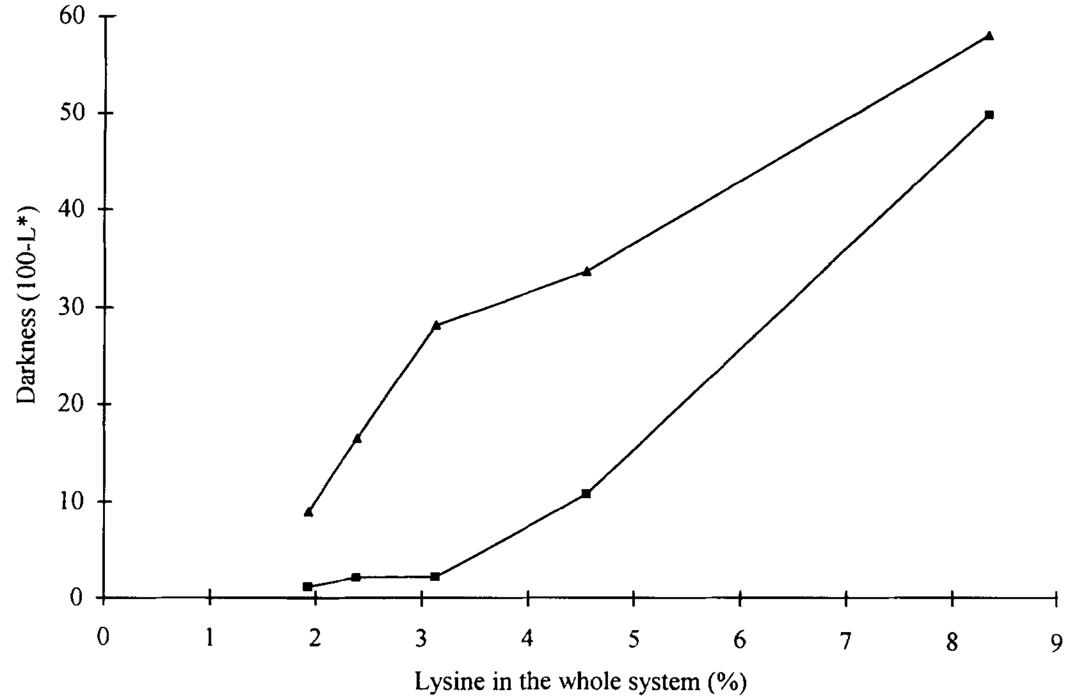

Fig. 2. Darkness values for systems of water (–■–) and glycerol (–▲–) containing different levels of xylose and lysine in a 1:1 ratio. Mixtures were stored for 7 days at 20°C before determination using a Hunter colorimeter.

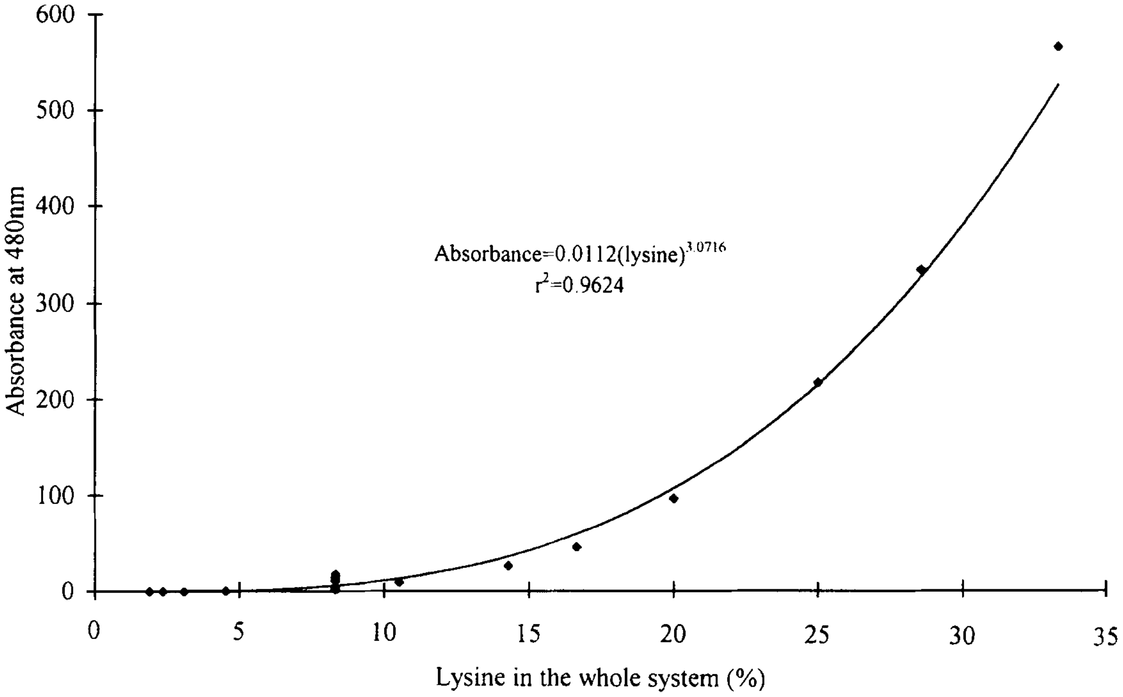

Fig. 3. Absorbance (480 nm) of water samples prepared containing different levels of xylose and lysine in a 1:1 ratio. Mixtures were stored for one week at 20°C and diluted to absorbance (480 nm) values less than 1.0 before measurement.

Constant reactant concentration in solution as the Maillard reaction proceeds

It could be considered that, as the reactants dissolved, there would be a very high concentration in the solution around the particulates. As all the particles are grouped together there could well be high colour generation. This colour would be expected to be more than that generated if the reactants were dispersed equally throughout the matrix if colour generation is proportional to concentration to the third power. Low mobility of dissolving reactants would enhance the effect.

The importance of reactants becoming soluble, taking part in the reaction, and then being replaced by freshly dissolving reactants is a likely scenario for high colour generation.

To test if this was occurring in the glycerol systems, reactants of different particle size were added. Although small particulates gave rise to greater coloration at the start of the assessment, after storage for one week all samples gave the same degree of colour formation (absorbance for small particles = 56.0 ± 0.1, for normal particles = 57.0 ± 0.2). The effects on colour formation if the samples were mixed during the incubation period were also investigated. No differences were observed between the amount of colour formation occurring in the static and mixed samples. It is therefore clear that the high colour formation in samples with low solubility of reactants is not due to a high concentration gradient near the particulates as they dissolve.

Water generated by the reaction

A characteristic feature of the Maillard reaction is that water is liberated in the early stages (Hodge, 1953). Water generation may be relevant to the rate of reaction in the systems being described for at least two reasons. It may drive the reaction forward and can change the concentration of the reactants in solution by:

1. driving the reaction in a certain direction. It is notable that several of the liquids used have a high affinity for water. This is demonstrated in Table 1 where the amount of water absorbed by the liquid is shown. Hygroscopic behaviour of the medium would encourage the Maillard reaction to proceed through the initial stages towards melanoidin production. The higher affinity of PG76 compared to glycerol might explain the differences in browning rate shown in these two liquids.
2. changing the concentration of reactants. Whatever the composition of the matrix, if the Maillard reaction can proceed, water will be generated. This water may mix with the rest of the matrix causing dilution of the soluble reactants and hence lower the browning rate. In cases where solubility is limited, the increase in water may generate sufficient aqueous phase to allow additional solubility of the reactants. If the water generated does not readily diffuse into the total matrix it could be considered that a growing aqueous layer could be generated around the reactants. This would encourage solubility and further colour and water generation.

Viscosity of the matrix

The viscosity of the matrix could be expected to affect the mobility of the reactants and hence the speed of the reactions. The results shown in Table 1 indicate that the browning rates are higher in glycerol and propylene glycol (MW 76) than in water, and the other two polypropylene glycols (MW 425 and MW 1200) and corn oil gave no colour at all. There is no obvious correlation between the viscosity and the browning rates that have been observed in the model systems studied. In other studies it has been demonstrated that the addition of polysaccharides that increase viscosity to a great extent only marginally decreased the amount of brown coloration formed as a result of the Maillard reaction.

The Maillard reaction rate in water/liquid matrices

As discussed, matrices that contain single liquids will become mixtures of water plus the liquid if the Maillard reaction proceeds. In addition, food materials often contain mixtures of substances. Glycerol and propylene glycols are organic compounds often used to lower a_w levels in food. The humectants are used in combination with water and it is these mixed samples that will now be discussed. On combination of liquids, three different systems can occur. These are:

1. fully phase-separated systems where the volume of each phase is the same as the single compounds (little or no interaction between the two liquids), e.g. PPG1200 and corn oil with water
2. a fully miscible matrix with no observable interface between liquids, e.g. glycerol–water and PG76–water
3. a phase-separated system where the observed phases are not equivalent to the volumes of material added, e.g. PPG425–water.

Fully phase-separated systems with little or no interaction between the two liquids

Corn oil is not miscible with water and gave a fully phase-separated system. The browning rate for samples of corn oil mixed with water could therefore be expected to relate to the concentration of the reactants in the water phase alone. The concentration of the reactants in this water phase can be calculated. From the relationship shown in Fig. 3 the corresponding absorbance could be predicted for each lysine concentration in the water phase. These predicted results are shown in Fig. 4 along with the actual absorbances for the corn oil system. Clearly the absorbance values correlate with the concentration of reactants in the water phase. At zero water levels there was no coloration, but there is no solubility.

Fully miscible systems

Glycerol is highly polar and is miscible with water in all proportions. The reactants, lysine and xylose, were added to the homogeneous glycerol–water solutions and the rate of browning followed using the standards methods. If no water was added to the glycerol, high browning occurred although the reactants were not fully solubilised (Fig. 1). On addition of water, the reactants

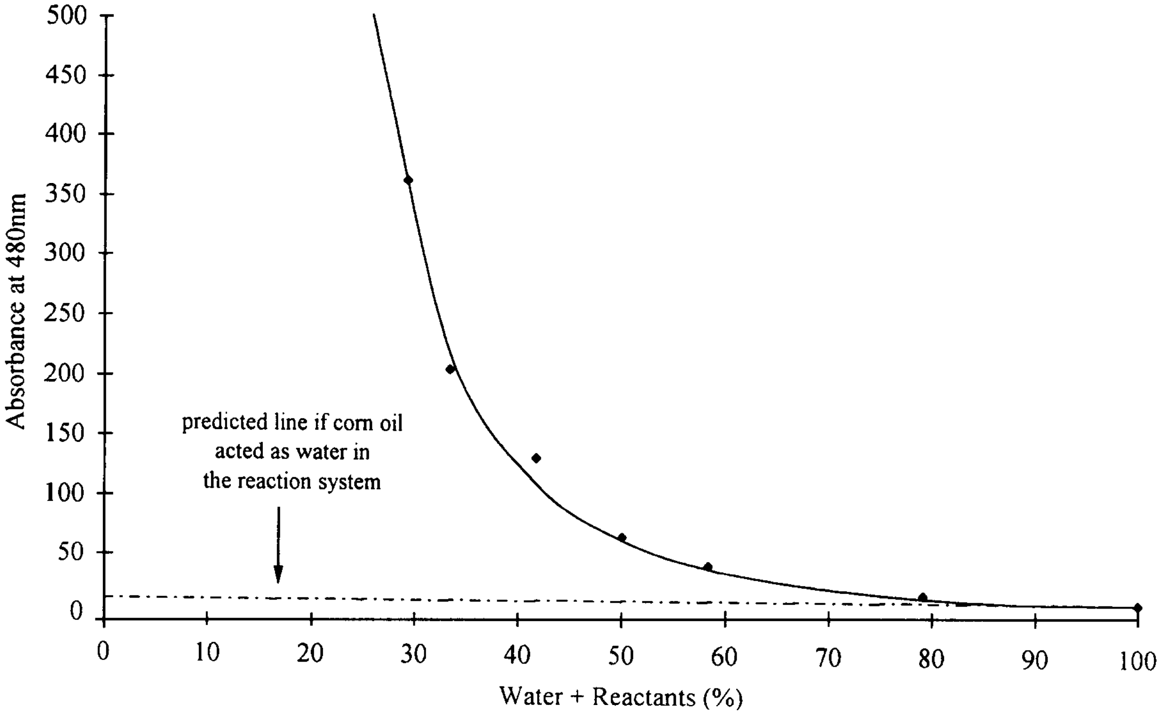

Fig. 4. Absorbance (480 nm) of corn oil samples (◆) containing different amounts of water plus xylose and lysine (1:1 ratio, 2 g per 10 g of liquid). Samples were stored for one week at 20°C. The predicted absorbance levels, assuming all reactants were in the aqueous phase, is also shown (—).

were solubilised but particulate matter was still observed until the matrix was less than 60% glycerol. As water was added to the glycerol, the amount of browning observed became greater as the water and reactants contents increased up to 30–40% (w/w). As the water content increased further, the browning tended to decrease. Figure 5 illustrates the amount of browning in the mixed system.

The action of corn oil (Fig. 4) within the matrix is that of an inert filler (not involved in the reaction nor as a diluent of the reactants). Comparison of the absorbances obtained for the glycerol system do not directly correlate to the browning levels expected for a reaction in the water phase alone (Fig. 5). At low water contents, actual browning levels are lower than predicted. This could be because of the low solubility of the reactants or incomplete partitioning of the components to the water phase. At higher water levels, however, it would seem that higher-than-predicted browning occurs if just the water phase is considered. The cross-over point for the actual browning levels and the predicted levels occurs at approximately the point where the reactants are thought to be soluble in the matrix. Clearly the addition of water to glycerol enhances browning compared to that expected to occur if the matrix were composed of water.

PG76 and water mixtures seem to be as well mixed as the glycerol–water system. There was no phase separation in the PG76–water–xylose–lysine system before or after the Maillard reactions occurred. As the water content increased in mixed PG76–water systems, the browning increased until the water and reactant content was around 30%. At greater water concentrations, browning levels decreased (Fig. 6). In the undiluted PG76, the browning was more than in glycerol (Fig. 1). Obvious differences between PG76 and glycerol are that the former has a lower viscosity and the reactants were more soluble (see Table 1). More browning could also be related to the ability of PG76 to absorb water from the first stage of the Maillard reaction and therefore drive the reaction forward. The variation in the predicted browning (if only occurring in the water phase) and the actual values show the same trend as seen for glycerol.

The basis for the assessment of the rate of the Maillard reaction has been to monitor colour formation. It is appreciated that colour generation is in the latter stages of the Maillard reaction and that the factors governing these stages may not be the same as those relevant at the initial stages. A way of probing the first stages of the Maillard reaction is to monitor loss of the reactants. The reaction mixtures of glycerol–water and PG76–water were assessed for lysine levels at the end of the incubation period. Figure 5 indicates that the low levels of lysine correlate well with the high browning levels recorded in the glycerol–water system. This was also the case for the PG76–water matrix.

Phase-separated system with some interaction between the water and the secondary liquid

The reactants in the pure PPG425 (no added water) were not solubilised and gave no brown coloration. When mixed with water the initial solutions appeared to

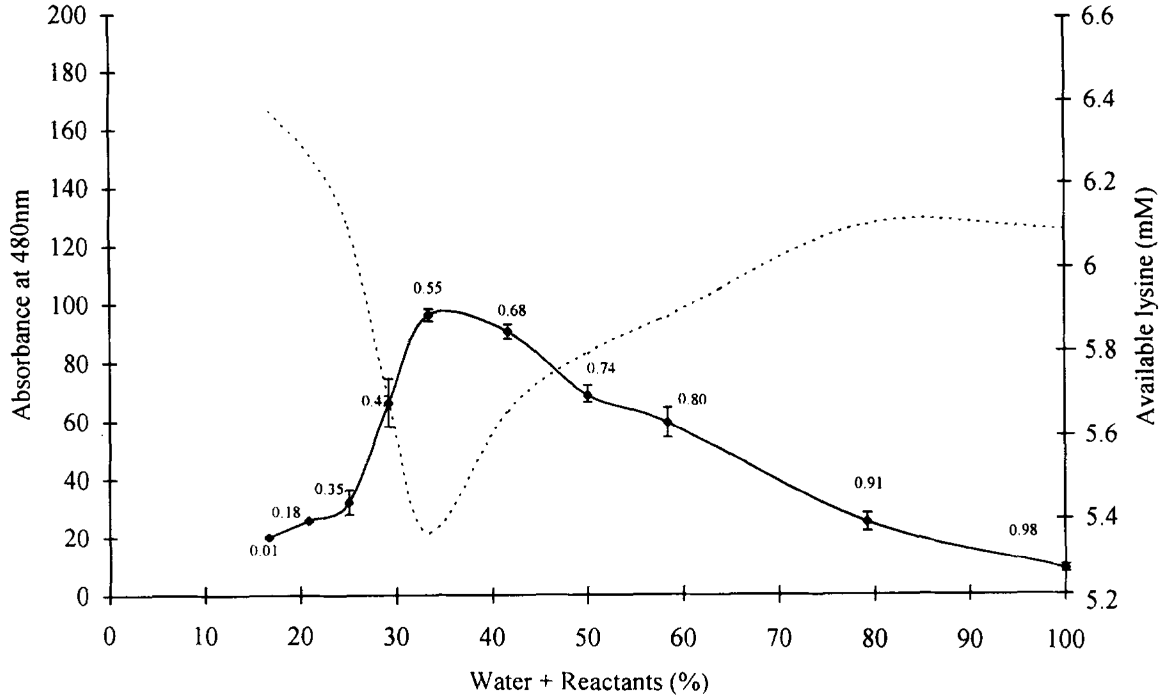

Fig. 5. Absorbance values (480 nm) of glycerol samples (–◆–) containing different amounts of water plus xylose and lysine (1:1 ratio, 2 g per 10 g of liquid). Also indicated are the water activity values for each sample. Other details as in Fig. 4. The levels of lysine found after the storage period are shown (···).

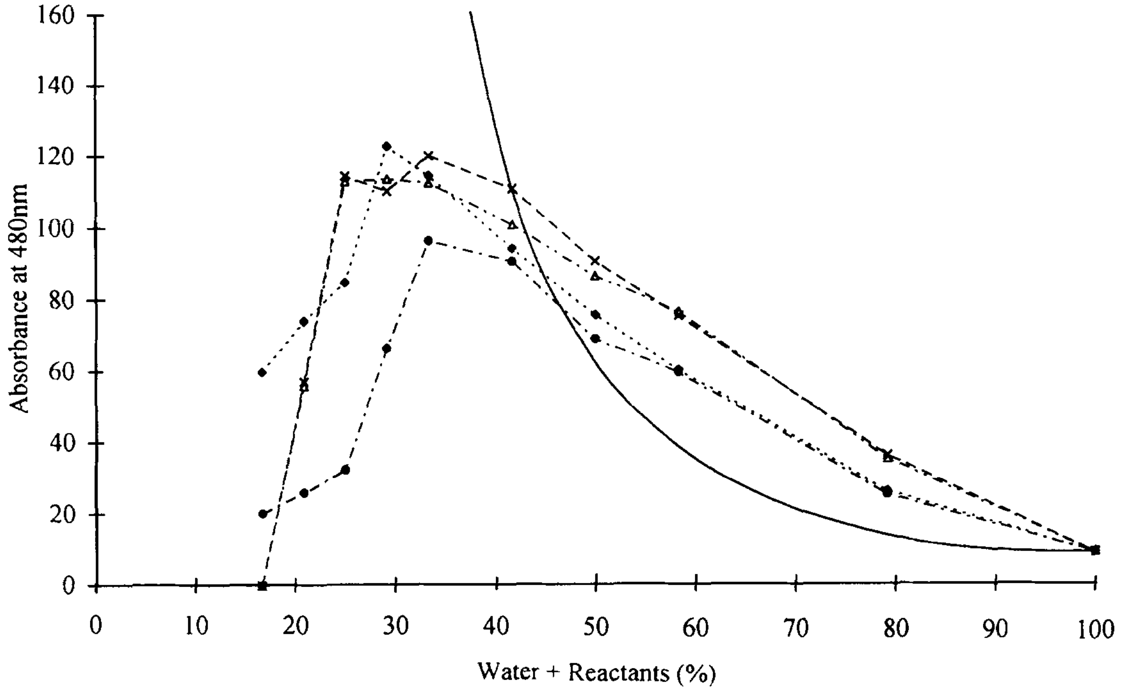

Fig. 6. Absorbance (480 nm) of glycerol samples (–●–), polypropylene glycol 76 (··◆··), 425 (–×–) and 1200 (–·△·–) containing different amounts of water plus xylose and lysine (1:1 ratio, 2 g per 10 g of liquid). Other details as in Fig. 4.

be fully mixed and gave a homogeneous solution, which was unchanged after storage for one week. However, when the reactants were added to the solution, colour development was accompanied by phase separation. The upper layer was colourless but the bottom layer was brown in colour. At lower water contents, the brown product was sticky and difficult to solubilise on addition of excess water. The amount of browning was maximal at lower water contents (20 to 30%) and exceeded that in the equivalent glycerol and PG76 solutions (see

Fig. 6). Whole samples of the PPG425 and water mixtures were diluted and tested for the lysine concentration as for the other mixtures discussed. Low levels of remaining lysine correlated well with high browning levels.

The volume of the bottom phase-separated layer was less at lower water contents, and increased as the water content increased. However, compared to the amount of water added, the volume of the lower layer was less than the water added initially, which suggests that there was some interaction between PPG425 and the water system. Density measurements were carried out on the upper layer of the samples to determine whether water or PPG425 were responsible. The results pointed to PPG425 being the major component in the colourless upper layer. However, the values were not the same for the samples, the density increasing as the water increased.

A comparison of the absorbance results for PPG425–water systems, with the values predicted for browning in pure water, showed similar relationships to those of glycerol and PG76. The excess in browning above that predicted at high water contents observed for PPG425 was more than for glycerol and PG76.

PPG1200 samples showed phase separation. The upper layer was transparent but the bottom layer was cloudy. After the reactants were mixed and left for a week, the lower layer was brown (Maillard product, melanoidin) and the upper layer was colourless. Measurements of the densities of the two layers indicated that the upper layer was the PPG1200. In the pure PPG1200 sample, there was no browning but with water present at levels above 15% w/w there was a sudden increase in browning (Fig. 6). The brown product at low water content was hard and looked like tar. The coloured product was difficult to dissolve and took a long time to be solubilised in the added water (100 ml). However, loss of lysine still correlated well with the browning values. The absorbance values estimated for PPG1200 were very similar to those obtained for PPG425 (Fig. 6).

CONCLUSION

A major observation within this study is the low solubility of the reactants in some of the matrices. The model systems chosen for this study have high levels of reactants (0.57 M Lysine and 0.56 M xylose) compared to many other published studies of the Maillard reaction. Typical reactant starting concentrations range from 0.01 M to 0.05 M (Eichner and Karel, 1972). However, in foods, and in model systems, water contents are often low and the solubility of the reactants would be a major feature.

Colour formation was dependent on the concentration of the reactants to the third power when they are dissolved in water. However, low solubility but high colour formation was observed for the glycerol and PG76 samples, compared to water. This must indicate that phenomena other than, or in addition to, concentration are relevant in predicting the amount of browning.

The use of polyols to form the matrix has been reported by many other workers. Their interest has been to use these compounds to modify the water activity or the mobility of systems (Karel and Buera, 1994; Karmas and Karel, 1994). Within this study, a wide range of water activities has been created and the water activity for maximum browning was very different for each of the matrices. Clearly it was not the water activity that was the dominant factor for browning. All the matrices used in this study were liquid and although their bulk viscosity varied this variation was not sufficient to have had a major influence on the amount of browning observed (Petriella *et al.*, 1988).

Another factor that may explain the increased browning in limited water could be the hygroscopic nature of the matrix. Water is generated in the initial reversible steps occurring in the Maillard reaction. A matrix that abstracts water from the site of production could encourage the reaction forwards to colour formation. Colour production might also be favoured by situations where hydrogen bonding is limited and where charge changes of reactants are easily accomplished (Lu *et al.*, 1995).

In this paper, variations in colour formation have been considered as being the result of physicol-chemical environment. The matrix has been considered inert, not taking part directly in the Maillard reaction and only influencing rates of reactions rather than altering the chemical pathways causing browning. However, there is evidence that the environment in which the Maillard reaction takes place does perturb the chemical pathway. One obvious example is the pH of the system. As high quantities of lysine have been used in this study, pH values in excess of 10 have been recorded at the start of the analysis when water alone was the matrix. However, the actual pH of the system will depend of the levels of lysine in solution and within the phase-separated systems domains of different pH can be imagined. Other studies (Mustapha, 1997) have indicated that the brown products produced by the interaction of lysine and xylose are different, depending on the liquid matrix.

Most real food systems are a combination of materials. The results from this study highlight the difficulties in understanding the Maillard reaction in even relatively easy systems. One major problem is that the system never reaches an equilibrium situation and small changes in the physical or chemical environment can have major effects on the reaction. This present work indicates that prediction of the amount of browning due to the Maillard reaction might be best undertaken using knowledge of the solubility of the likely reactants in the matrix and then calculating the concentration of material in the water fraction present in the system.

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