Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 87 (2004) 329–337
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of heated glucose–lysine and glucose–methionine model-systems on mineral solubility

Cristina Delgado-Andrade, Isabel Seiquer *, Rosa Nieto, María Pilar Navarro

Unidad de Nutrición, Estación Experimental del Zaidín, Camino del Jueves, CSIC, Armilla, 18100 Granada, Spain

Received 1 August 2003; received in revised form 1 December 2003; accepted 1 December 2003

Abstract

Equimolar mixtures of glucose–lysine or glucose–methionine, 40% moisture, were heated at 150 °C for 30, 60, or 90 min. Samples were characterized by means of pH measurement, browning development and free amino acid content. Solubility assays were performed to study the influence of obtained MRPs on Ca, Mg, Fe, Cu and Zn solubility, under intestinal conditions of pH and ionic strength. Results showed that heated mixtures of glucose–lysine and glucose–methionine had little effect on Ca and Mg solubility. However, early compounds formed in both model systems seemed to favour trace element solubility, maximum after 30 min of heating in the glucose–lysine system, and after 60 min in the glucose–methionine one. Browning development and solubility results reflected the slowing down of the Maillard reaction when methionine was one of the reactants. The effects of browning products generated during food processing should be taken into account, particularly in trace element availability.

Keywords: Maillard reaction products; Lysine; Methionine; Calcium; Magnesium; Copper; Iron; Zinc

1. Introduction

Various factors may influence mineral availability, but especially interesting are those that play a role at the digestive level, since the mineral species resulting from the digestion process will be important determinants of subsequent cell mucosal uptake and absorption. Solubility in the intestinal lumen is not synonymous with absorbable minerals; however, major minerals and trace elements must be soluble in the gastro-intestinal tract prior to absorption, hence the importance of knowing their solubility stage.

During digestion, several chemical and physical reactions take place, and the mineral elements may interact with other components of foods or the gastrointestinal content. As a result of these interactions, the solubility stage of mineral elements could be altered and, consequently, their availability modified. Dietary factors are of capital importance in this context and, to the original components of raw food must be added those newly formed during industrial or domestic processes, by means of which the food environment may be altered and new external factors may be introduced.

Thermal treatments are frequently used to preserve foods and make them edible. One of the most common reactions to occur during processes and storage is the Maillard reaction (MR), which involves the condensation of the carbonyl group of reducing sugars with the amino group of amino acids and proteins, generating a series of products (Schiff's bases, premelanoidins and melanoidins), widely known as Maillard reaction products (MRPs), the characteristics and molecular weights of which depend both on the source of reactants and the reaction conditions (Cammerer & Kroh, 1995). The early MRPs are simple, but the advanced MRPs become more complicated with different molecular weights.

Diverse studies have suggested that MRPs are capable of behaving as anionic polymers and of complexing metal ions, producing soluble and insoluble complexes. According to several authors (Rendleman, 1987; Wijewickeme & Kitts, 1997) calcium and copper are bound by soluble and insoluble melanoidins derived from different amino acid–sugar model systems. MRPs,

* Corresponding author. Tel.: +34-58-572757; fax: + 34-58-572753.
E-mail address: iseiquer@eez.csic.es (I. Seiquer).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.12.002

prepared from monosodium glutamate and glucose, are capable of fixing Ca, Zn, Cu and Mg (O'Brien & Morrissey, 1997). Other Maillard browned compounds, from proline, leucine, glycine, glutamic acid or lysine, autoclaved with glucose, bind zinc and decrease its apparent availability, and lysine products present the greatest binding capacity (Whitelaw & Weaver, 1988). After the addition of a heated casein–glucose–fructose sample to a calcium solution, a higher percentage of metal appears insoluble in comparison with the raw sample, while the rest remains soluble but, partially, in non-ionic form (Aspe, Vaquero, & Navarro, 1993). Homma and his coworkers (Homma & Murata, 2001; Homma, Aida, & Fujimaki, 1986) also demonstrated the existence of metal chelating compounds in browned pigments of coffee with different affinities for iron, copper and zinc. Finally, some browned products of toasted bread have little calcium binding capability, but when bread containing lactose is toasted, it gives rise to pigments which insolubilize calcium ions (Rendleman, 1987).

On the other hand, MRPs from lysine are frequently present in food, as this amino acid is very sensitive to thermal treatment and has often been reported to be highly reactive in the MR (Ashoor & Zent, 1984). In this respect, lysine availability also decreases more than the other amino acids (VanBarneveld, Batterham, Skingle, & Norton, 1995). Thus, derivatives from the glucose–lysine model system are frequently used to study diverse aspects of MRPs, such as browning development, complex formation (Bailey, Ames, & Monti, 1996), effects on protein digestibility (Friedman, 1996), transport and metabolism studies (Erbersdobler, Brandt, Scharrer, & Von Wagenheim, 1981).

Although less reactive than lysine, methionine is also degraded by the MR during food processing (Nielsen, De Weck, Finot, Liardon, & Hurrell, 1985) generating volatile compounds (Tsai, Hiserodt, Ho, Hartman, & Rosen, 1998) and losing availability (Hurrell & Carpenter, 1981). In nutritional terms, these losses could be of considerable importance because the sulphur amino acids, together with lysine, are normally the first limiting amino acid in many food proteins.

Given the abundance of MRPs in the food we eat, and their resistance to the digestive process, during this process they have many opportunities to interact with dietetic minerals and form more or less soluble complexes which thus affect their absorption. Therefore, the study of some of these products and their influence on mineral solubility in the pH and osmolarity conditions normally found in the intestine, is considered a first step in determining their possible effects on mineral availability.

The goals of the present study were to perform a comparative analysis to determine the point at which the presence of MRPs from glucose–lysine or glucose–methionine model systems is capable of modifying the degree of solubility of various minerals, under the above-described conditions.

2. Materials and methods

2.1. Sample preparation

Glucose (Merck, Darmstadt, Germany), lysine and methionine (Sigma Chemical Co., St. Louis, MO; USA) were used to prepare the samples. Equimolar mixtures of glucose–lysine monohydrate (GL0) or glucose–dl-methionine (GM0), 40% moisture and in unbuffered systems, were heated in an oven (Selecta 2000210, Barcelona, Spain) at 150 °C for 30 min (GL30 and GM30), 60 min (GL60 and GM60) or 90 min (GL90 and GM90), using open recipients. Heating times and humidity contents of samples were selected to simulate usual cooking times and moisture contents of different foods, which, moreover, promote the development of the MR (O'Brien & Morrissey, 1997). After heating, the reaction was stopped and the mixtures were lyophilized (FTS System, Inc., TDS-3, New York, USA) as described by Delgado-Andrade, Seiquer, and Navarro (2002).

Samples were weighed before and after the heating and lyophilizing processes, in order to determine the percentage of lost samples.

2.2. Determination of pH in water solution

One hundred milligram of each sample/10 ml demineralized and neutralized water (Milli-Q Ultrapure Water System, Millipore Corp., Bedford, MA, USA) were stirred for an hour in a water bath at room temperature. The pH was measured in the water sample solutions or in the corresponding supernatants (PHM250 ion analyzer MeterLab, Radiometer Copenhagen, Denmark).

2.3. Development of brown colour and free amino acid content

One hundred milligram of each sample (GL, GL30, GL60, GL90, GM, GM30, GM60 or GM90) were suspended in a final volume of 10 ml of demineralized and neutralized water. Samples were then stirred for 1 h in a water bath at room temperature (P Selecta, J.P. Selecta S.A., Unitronic 320 OR) and centrifuged at 5000 rpm for 45 min (Heraeus Christ GMBH, Osterode/Harz 03400). Absorbance was measured at 420 nm in solutions or corresponding supernatants (Milton Roy Spectronic 1201).

Free amino acid contents of samples were determined using the analytical procedure of Fernández-Fígares,

Pérez, Nieto, Aguilera, and Prieto (1995), omitting the acid hydrolysis step.

2.4. Solubility assays

Solubility assays were performed to study the influence of obtained MRPs on the solubilities of Ca, Mg, Fe, Cu and Zn in a conjoint solution. One hundred milligram of each sample were individually suspended in a final volume of 10 ml of a 3.75 mM Ca, Mg, Fe, Cu and Zn solution. To simulate intestinal conditions, pH and ionic strength were adjusted in solutions containing samples to 6.8–7 and 0.165, respectively, using 4 N NaOH and 1.65 M KCl (Rendleman, 1987). The solutions were then stirred in a water bath at room temperature for 2 h and centrifuged at 8000 rpm. (Hettinch Zentrifugen, Universal 30RF D-78532 Tuttligen) for 2 h. After centrifugation, the Ca, Mg, Fe, Cu and Zn contents was measured in the obtained fractions. The percentages of soluble and insoluble minerals were calculated from the initial mineral concentrations.

2.5. Analytical techniques

Total fractions of precipitate and supernatant from the solubility assays were completely digested by the addition of concentrated HNO_3, $HClO_4$ and by heating at high temperatures in a sand beaker (J.R. Selecta, S.A., COMBIPLAC, num. 0373930). After appropriate dilution with milli-Q water, mineral analysis in each fraction was performed with flame AAS in a Perkin–Elmer Analyst 700 Spectrophotometer (Norwalk, CO, USA). Standard solutions were prepared from stock Tritisol solutions of Ca ($CaCl_2$ in 6.5% HCl, 1000 mg Ca, Merck), Mg ($MgCl_2$ in 6% HCl, 1000 mg Mg, Merck), Fe ($FeCl_3$ in 15% HCl, 1000 mg Fe, Merck), Cu ($CuCl_2$ in H_2O, 1000 mg Cu, Merck) and Zn ($ZnCl_2$ in 0.06% HCl, 1000 mg Zn, Merck).

The interassay coefficients of variation for the different minerals were as follows: Ca 2.07%, Mg 5.16%, Fe 3.21%, Cu 6.99%, Zn 1.93%. Milk powder standard (certified reference material BCR No. 63, Community Bureau of Reference, Brussels, Belgium) was used to check calcium and magnesium recoveries (mean ± SD of 10 determinations): Ca measured value 13.48 ± 0.04 mg/g, Ca certified value 13.49 ± 0.10 mg/g; Mg measured value 1.29 ± 0.02 mg/g, Mg certified value 1.26 ± 0.02 mg/g. Bovine liver standard (certified reference material BCR No. 185, Community Bureau of Reference, Brussels, Belgium) was simultaneously used to check iron, copper and zinc recoveries (mean ± SD of 10 determinations): Fe measured value 215 ± 5 μg/g, Fe certified value 214 ± 5 μg/g; Cu measured value 192 ± 5 μg/g, Cu certified value 189 ± 4 μg/g; Zn measured value 146 ± 2, Zn certified value 142 ± 3.

All glassware and polyethylene sample bottles were washed with 10 N nitric acid, and milli-Q water was used throughout. All analyses were performed in triplicate.

2.6. Statistical treatment

The results for the weight losses of the sampling process, pH determinations, absorbance measures and solubility assays were tested statistically by one-way analysis of variance (ANOVA), followed by Duncan's test to compare means with significant variation ($p < 0.05$). Correlations were established between pH, absorbance and free residual amino acids.

3. Results and discussion

3.1. Characterization of the MRPs obtained

After heating, weight loss in the glucose–lysine and glucose–methionine mixtures was observed, increasing as heating time increased. However, the loss pattern was not identical for the two systems: in the first 30 min of heating, the glucose–lysine system decreased by 22% of its initial weight (Table 1), mainly water losses with a small fraction of dry matter, as suggested by the weight after lyophilization. The mixture already showed marked browning due to MR development, and volatile

Table 1
Weight changes in the process for obtaining MRPs from 50 g initial weight of samples

Heating time (min)	Sample					
	Glucose–lysine			Glucose–methionine		
	After heating		Weight after freeze-drying (g)	After heating		Weight after freeze-drying (g)
	Weight (g)	Weight loss (%)		Weight (g)	Weight loss (%)	
30	39.03 ± 0.36a	21.94 ± 0.48a	25.14 ± 0.29a	43.08 ± 0.16a	13.84 ± 0.21a	29.50 ± 0.37a
60	25.65 ± 0.57b	48.70 ± 0.71b	22.64 ± 0.03b	28.99 ± 0.09b	42.02 ± 0.12b	24.21 ± 0.34b
90	22.36 ± 0.01c	55.28 ± 0.10c	21.62 ± 0.27b	22.76 ± 0.41c	54.48 ± 0.53c	21.93 ± 0.79b

Different letters in the same column mean significant differences ($p < 0.05$, ANOVA one-way and Duncan test). Values are mean ± SD of six determinations.

compounds are assumed to have formed (Friedman, 1996). The formation of these volatile MRPs generally increases with time and heating temperature (Chan & Reineccius, 1994); between 30 and 60 min of heating time higher weight losses were observed. The last 30 min of heating, especially, produced a reduction of the water fraction, since the weight after freeze-drying did not vary between the GL60 and GL90 samples (Table 1).

In the glucose–methionine mixture, however, 30 min of heating resulted only in a decrease of the water fraction as, after lyophilization, the weight sample was nearly identical to the initial weight (29.5 vs. 30 g). As described for the glucose–lysine mixture, the highest reduction of water and dry matter took place between 30 and 60 min of heating treatment and, after 90 min, the weight of the dry sample did not vary significantly.

Thus, although the final weights of the samples after 90 min of heating were very similar, the glucose–lysine system seemed to follow a faster development of the reaction than the glucose–methionine system, a result that was expected due to the higher reactivity of lysine in the MR (Hurrell & Carpenter, 1981; Malec, Gonzales, Naranjo, & Vigo, 2002).

The pH of the GL0 sample was 9.9, due to the use of lysine monohydrate, while that of the GM0 sample was 6.4 (Table 2). It is well documented that an increase in pH causes an increase in the rate of Maillard browning. Moreover, a higher pH favours the initial condensation step of the MR and influences many subsequent pathways as the most chromogenic routes, yielding reductones and fission products (Bates, Ames, MacDougall, & Taylor, 1998). Therefore, the high pH of the GL0 sample could contribute to the faster reaction development described for this model system.

3.2. pH changes and browning

The MR itself has a strong influence on pH and, in unbuffered systems, pH falls through the reaction due to the disappearance of basic amino groups at the early stages (Hill, Ledward, & Ames, 1996). Thus, in our glucose–lysine and glucose–methionine model systems, pH decreased with heating time, as has been described in several model systems (Monti, Bailey, & Ames, 1998; Renn & Sathe, 1997). The rate of pH decline was more pronounced for the glucose–lysine system than for the glucose–methionine one, especially during the first 30 min of treatment, in which the glucose–lysine system underwent a rapid and marked decrease (3.15 units of pH). However, the greatest pH decline in the glucose–methionine system was only of 1.9 units, and took place between 30 and 60 min of heating. Such differences should be considered in the context of the diverse natures of the amino acids involved since, as mentioned above, lysine is one of the most highly affected. Moreover, in unbuffered systems, the amino compounds may well influence the rate of browning through their effect on pH.

pH has an important effect on the extent of MR and strongly influences the profile of the reaction products obtained; in most cases, accordingly, reaction rates have been found to rise with increasing pH (Ames, 1998). In this sense, Ashoor and Zent (1984) demonstrated maximum colour development at around pH 10 for various glucose–amino acid model systems. In accordance with the results of the present experiment, Hill et al. (1996) observed, in a xylose–lysine model system at initial pH values of 8 or 6.3, that the rate of pH decline during 1 h of incubation at 50 °C was higher at basic pH (0.57 vs. 0.12).

On the other hand, under alkaline conditions, the Schiff base and the Amadori compound undergo chain fragmentation, and the fragments quickly react to form melanoidins (Rizzi, 1994).

The time-course of colour formation in the glucose–lysine model system was more accelerated than in the glucose–methionine one, and the browning degree reached after each heating time was also higher in samples containing lysine. Similarly, after the heat treatment of various amino acid–sugar combinations, Ashoor and Zent (1984) showed that the glucose–lysine system produced a higher browning rate at 420 nm. It is generally accepted that reactants and reaction conditions strongly affect the browning and structure of final products (Manzocco & Maltini, 1999).

Table 2
pH and absorbance at 420 nm of water sample solutions or corresponding supernatants

Heating time	Sample					
	Glucose–lysine			Glucose–methionine		
	pH	Precipitate	Absorbance	pH	Precipitate	Absorbance
0	9.93 ± 0.02a	–	0.001 ± 0.000a	6.39 ± 0.03a	–	0.007 ± 0.001a
30	6.78 ± 0.01b	–	0.573 ± 0.001b	6.06 ± 0.01b	–	0.011 ± 0.001a
60	5.85 ± 0.01c	–	0.989 ± 0.001c	4.12 ± 0.01c	+	0.126 ± 0.002b
90	5.35 ± 0.01d	+	0.348 ± 0.001d	4.35 ± 0.01d	++	0.219 ± 0.002c

Different letters in the same column mean significant differences ($p < 0.05$, ANOVA one-way and Duncan test). Values are mean ± SD of six determinations.

According to Bates et al. (1998), the dissociation constants of the two amino groups of lysine favour the initial step of MR, as well as the more chromogenic routes of the reaction. In the present glucose–lysine system, the absorbance value at 420 nm was maximum after an hour of heating time (Table 2), probably due to the high concentration of soluble brown pigments. In the last 30 min of treatment, insoluble melanoidins were formed, shown by the appearance of a dark pellet. Thus, the spectrophotometric measure of absorbance, performed on the soluble fraction, decreased in the GL90 sample. In this sense, Fogliano, Monti, Musella, Randazzo, and Ritieni (1999), in a gluten-glucose system heated to 150 °C, also observed an important increase of browning until 45 min of heating, followed by a decrease at longer heating times. Monti et al. (1998), using different amino acid–sugar mixtures refluxed for up to 120 min, also reported that, in the glucose–lysine system, the highest absorbance values appeared after 1 h of heating, to subsequently reach a plateau.

Water activity (a_w) is an important factor for MR, which is favoured within an interval of moisture content (Reineccius, 1990). Thus, the MR activation energies increase with decreasing water proportion (Cremer & Eichner, 2000). In the present experiment the moisture content of the assayed samples drastically decreased after 60 min of heating, and thus, the lower a_w could be another limiting factor in the browning rate. Fogliano et al. (1999) pointed out that samples from the gluten-glucose system, heated under wet conditions, produced more colour at all temperatures studied, compared to those heated under dry conditions.

In both model systems, glucose–lysine and glucose–methionine, the decline in the reaction rate pH tended to follow the same general trend as the browning rate, as Renn and Sathe (1997) described for the glucose–leucine model system, without finding a correlation between the two parameters. However, in the present assay, a correlation was found between pH and absorbance: $r = -0.9773$ and -0.9942 for glucose–lysine and glucose–methionine, respectively, until 60 min of heating ($p < 0.001$), $r = -0.7027$ and -0.8880 for glucose–lysine and glucose–methionine, respectively, including all heating times ($p < 0.01$).

As the heating time was increased, free residual methionine, and especially, lysine decreased. Also recorded were decreased pH and an enhanced browning rate. However, statistical analysis only found a correlation between pH and lysine decrease ($r = 0.9886$, $p = 0.01$). The amino acid losses followed a similar trend to the browning rate, but there was no correlation between the two occurrences, as has been described in other amino acid–sugar model systems (Renn & Sathe, 1997).

The highest proportions of lysine and methionine were lost during the first 30 min of heating, when the glucose–methionine samples had only taken on a slight yellow colour, while those of glucose–lysine had already attained a brown colouring, and absorbance at 420 nm had increased. In this regard, Renn and Sathe (1997) suggest that MR occurs in three stages, of different duration depending on the reaction conditions. During the first stage, amino acids and sugars mostly react exclusively with each other, producing early compounds that are uncoloured but capable of reducing the nutritive value (Malec et al., 2002). This is in agreement with the loss of free methionine and the absence of browning in the GM30 sample (Table 2).

3.3. Influence of the glucose–lysine samples on mineral solubility

The presence of GL0 in 3.75 mM Ca, Mg, Fe, Cu and Zn solutions did not lead to significant changes in Ca solubility and increased the Mg soluble fraction (Table 3), as previous assays carried out under the same conditions but without samples showed percentages of soluble mineral of $93.6 \pm 6.50\%$ and $80.6 \pm 6.91\%$ for Ca and Mg, respectively. Heating the glucose–lysine mixture (GL30, GL60 and GL90 samples) caused only slight decreases in Ca and Mg solubility (Table 3, Fig. 1), with the element remaining almost soluble. In this sense, O'Brien and Morrissey (1997) studied the

Table 3
Soluble and insoluble minerals in the presence of the glucose–lysine samples

Mineral	Soluble mineral (μmol)				Insoluble mineral (μmol)			
	Sample[a]				Sample[a]			
	GL0	GL30	GL60	GL90	GL0	GL30	GL60	GL90
Ca	37 ± 1.1Aa	35 ± 1.0Ba	35 ± 1.0Ba	35 ± 1.0Ba	0.8 ± 0.1Aa	1.6 ± 0.2Ba	1.5 ± 0.2Ba	1.4 ± 0.1Ba
Mg	37 ± 1.0Aa	34 ± 1.0Bab	35 ± 1.1Aba	33 ± 1.0Ba	1.1 ± 0.1Aa	1.9 ± 0.4ABab	2.1 ± 0.4ABa	2.2 ± 0.2Ba
Cu	36 ± 1.0Aa	29 ± 2.0Bc	18 ± 1.0Cb	13 ± 1.0Db	0.9 ± 0.1Aa	7.5 ± 0.8Bc	19 ± 1.5Cb	24.5 ± 0.5Db
Fe	1.3 ± 0.1Ab	32 ± 2.0Bb	28 ± 1.0Cc	12 ± 1.0Dc	34 ± 1.0Ab	6.4 ± 1.5Bc	9.7 ± 0.1Cc	26.5 ± 0.5Dc
Zn	16 ± 1.0Ac	34 ± 1.0Bab	22 ± 2.0Cd	14 ± 2.0Ad	21 ± 1.0Ac	3.6 ± 1.4Bb	12 ± 1.0Cd	21 ± 1.1Ad

[a] GL0, unheated glucose–lysine mixture; GL30, GL60 and GL90, glucose–lysine mixtures heated at 150 °C for 30, 60 or 90 minutes, respectively. Values are mean ± SD of six determinations. Different capital letters in the same file mean significant differences, for either soluble or precipitated minerals ($p < 0.05$, ANOVA one-way and Duncan test). Different small letters in the same column mean significant differences ($p < 0.05$, ANOVA one-way and Duncan test).

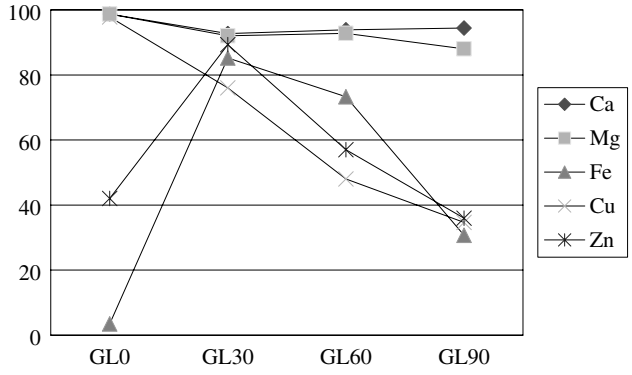

Fig. 1. Effects of the heat treatment of glucose–lysine samples on mineral solubility (%). GL0, unheated glucose–lysine mixture; GL30, GL60 and GL90, glucose–lysine mixtures heated at 150 °C for 30, 60 or 90 min, respectively.

affinities of MRPs-minerals and suggested that Ca should be the one with least affinity, followed by Mg. In solubility assays carried out in our laboratory with heated casein–glucose–fructose mixtures, it was shown that all the soluble calcium was ionic calcium (Seiquer, Aspe, Vaquero, & Navarro, 2001). Thus, it could be hypothesized that, in the presence of glucose–lysine heated mixtures, most of the Ca remains in ionic form. However, it could also be, to a slight extent, bound to the soluble melanoidin fraction; Rendleman (1987) reported considerable soluble complex formation between Ca and brown pigments in coffee and only small amounts of insoluble Ca complexes. Under our experimental conditions, small quantities of insoluble compounds were also formed.

In the absence of sample under the initial conditions, almost all the Fe was precipitated ($91.5 \pm 2.36\%$). The presence of the GL0 sample in the mineral solution did not modify iron solubility, probably due to the basic pH of the GL0 sample. Most iron absorption takes place in the initial portions of the small intestine since, the higher the pH, the lower the iron solubility (Charlton & Bothwell, 1983). A significant increase in Fe solubility was observed with the GL30 sample (Table 3). This could be due, in part, to the lower initial pH of this sample (Table 2). Moreover, free lysine, still 37.2% present in the GL30 sample, could complex Fe, as the ability of lysine to chelate this mineral and improve its absorption has been shown (Van Campen, 1973). Another factor, which could contribute to enhancing Fe solubility, is the capacity of soluble melanoidins to complex Fe (Homma & Murata, 1994).

Iron solubility significantly decreased in the presence of the most heated samples, GL60 and GL90 (Table 3). This might be explained by the progressive destruction of free lysine (34.1% and 21.5% of free lysine in GL60 and GL90, respectively) and the formation of more advanced MRPs, which were partially insoluble in GL90 (Table 2). Using a glucose–glycine model system Yoshimura, Iijima, Watanabe, and Nakazawa (1997) indicated that the high molecular weight fraction possesses greater iron-chelating power than the low molecular weight fraction, and the binding of certain non dialyzable melanoidins with iron has also been described (Hashiba, 1986). More recently, Homma and Murata (1994) showed (in coffee) the presence of ligands with molecular weights from 36 to 50 kDa, capable of insolubilizing Fe.

The GL0 sample solubilized practically all the copper (from $1.08 \pm 0.52\%$ of soluble Cu without sample to $97.8 \pm 0.89\%$, Fig. 1). Several amino acids, including lysine, may form soluble complexes with Cu, and in biological media only a little Cu remains as free ion (Berthon, Hacht, Blais, & May, 1986). Cu solubility is directly related to intestinal Cu absorption; for this reason, Cu–Lys complexes have been used in animal nutrition to improve copper absorption (Nockels, DeBonis, & Torrent, 1993). The presence of glucose–lysine heated mixtures led to a significant reduction of the soluble copper fraction, which progressively diminished with the longer heating time (Table 3). The loss of free lysine could have contributed to this reduction, but the ability of MRPs to insolubilize Cu should also be taken into account. O'Brien and Morrissey (1997) suggest that the affinity between MRPs and Cu is even higher than with the amino acid involved. It has been pointed out that the copper-chelating affinity of MRPs is implicated in the antioxidant activity attributed to some of these compounds (Wijewickeme & Kitts, 1997). Moreover, with increased heating time, the Cu-chelating ability of the final products is higher (Moon, Murata, & Homma, 1994), which, in accordance with the present results, favours Cu insolubilization. Therefore, the soluble percentage of Cu in the presence of heated mixtures was lower than the other minerals, except in GL90, where no significant differences were found with respect to the other trace elements.

Initial in vitro Zn solubility in intestinal conditions was reduced in the GL0 solution (from 75.0 ± 6.58 % without sample to 42.0 ± 1.58 %). The behaviour of Zn in the presence of the glucose–lysine heated samples was similar to that of iron, except that, with GL0, higher values of the soluble mineral were recorded (Table 3). Several assays, carried out with the amino acid–sugar model system, have shown the ability of these products to chelate Zn (O'Brien & Morrissey, 1997). The Zn-chelating activity of browning pigments in foods has been studied in corn flakes (Johnson, 1991) and in coffee (Homma, Nakamura, Asakura, Sekiguchi, & Murata, 1990), where the most active Zn-binding fraction contains low molecular weight compounds. These authors later demonstrated that the high molecular weight fractions are also able to chelate Zn (Homma & Murata, 1994). It would be reasonable to assume that, in the

GL30 solution, the soluble compounds formed had a high Zn-binding ability; with increased heating time, the products are assumed to be of higher molecular weight, and they should complex Zn, producing its precipitation. This hypothesis is in accordance with the reasoning of Whitelaw and Weaver (1988), who observed a decrease in Zn bioavailability with the advance of the browning process.

Our results show that MRPs from glucose–lysine do not lead to major modifications in Ca and Mg solubility. Like Friedman (1996), we found trace elements to be the most reactive, the affinity order being Fe<Cu>Zn. The stronger binding of Cu compared to Zn is consistent with the findings of other investigators (Homma et al., 1986; O'Brien & Morrissey, 1997).

3.4. Influence of the glucose–methionine samples on mineral solubility

Effects on Ca and Mg solubility due to the presence of glucose–methionine mixtures were quantitatively minor, both in comparison with the no-sample solution and between the different samples (Table 4, Fig. 2). A slight but significant decrease in the soluble Mg fraction was observed in the presence of the GM90 sample, which could be related, as has been described, to the ability of advanced MRPs to chelate minerals (Rendleman, 1987). In spite of the proven implication of methionine in the MR, there is a lack of information about the influence of MRPs from this amino acid on mineral speciation. The few biological assays, concerning the effects of MRPs from methionine on Mg availability, show disparate results, reporting both no effects (Delgado-Andrade, 2002) and increased Mg absorption (Andrieux & Saquet, 1984).

As has been described for the glucose–lysine model system, MRPs from glucose–methionine do not seem to have major effects on Ca solubility. However, when protein is used as a reactant, rather than amino acids, there is a decrease in Ca solubility, which has been shown with heated mixtures of casein–glucose–fructose

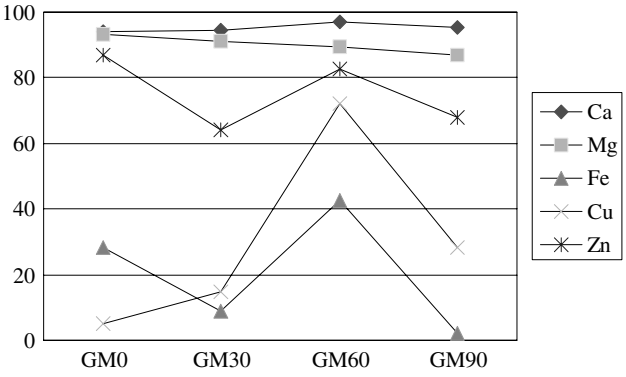

Fig. 2. Effects of the heat treatment of glucose–methionine samples on mineral solubility (%). GM0, unheated glucose–methionine mixture; GM30, GM60 and GM90, glucose–methionine mixtures heated at 150 °C for 30, 60 or 90 min, respectively.

(Seiquer et al., 2001). The different effects on Ca speciation confirm the importance of the reactants and the reaction conditions for the behaviour of the final products.

The presence of the GM0 sample did not modify Cu solubility with respect to the no-sample solution, with nearly all the Cu remaining precipitated, contrary to what was observed with the GL0 sample (Table 4). Thus, as expected, soluble complexes with Cu do not seem to have been formed under our experimental conditions, since methionine is not cited among the amino acids with ability to complex Cu (Berthon et al., 1986). However, Aoygi and Baker (1994) demonstrated that certain Cu–methionine products may counteract the inhibitory effect of some substances on Cu absorption.

Heat treatment of the glucose–methionine samples improved Cu solubility, more markedly after 60 min (Fig. 2). It can be hypothesized that the higher Cu solubility may be ascribed to the ability of premelanoidins and soluble melanoidins to chelate Cu. With 90 min of heat treatment, levels of insoluble melanoidins were raised, and, as a consequence, so were those of the precipitated Cu fraction (Table 4).

Table 4
Soluble and insoluble minerals in the presence of the glucose–methionine samples

Mineral	Soluble mineral (μmol)				Insoluble mineral (μmol)			
	Sample[a]				Sample[a]			
	GM0	GM30	GM60	GM90	GM0	GM30	GM60	GM90
Ca	35 ± 1.0a	36 ± 1.0a	36 ± 1.1a	36 ± 1.0a	0.7 ± 0.1Aa	0.6 ± 0.1Aa	0.7 ± 0.1Aa	1.1 ± 0.1Ba
Mg	35 ± 1.1Aa	34 ± 1.0ABa	34 ± 1.0ABb	33 ± 1.0Bb	1.9 ± 0.4ABa	2 ± 0.1ABa	1.7 ± 0.1Aa	2.3 ± 0.2Ba
Cu	2 ± 0.5Ab	5.6 ± 0.6Bb	27 ± 1.0Cc	11 ± 1.0Dc	35 ± 1.0Ab	28 ± 2.0Bb	9 ± 0.4Cc	24 ± 0.3Db
Fe	12 ± 1.0Ac	3.3 ± 0.4Bc	16 ± 1.0Cd	0.8 ± 0.2Bd	24 ± 0.1Ac	35 ± 1.0Bc	20 ± 1.0Cd	36 ± 1.0Bc
Zn	33 ± 1.0Ad	24 ± 1.1Bd	31 ± 1.0Ae	26 ± 1.0Be	4.3 ± 0.3Ad	10 ± 0.4Bd	4.9 ± 0.4Ae	10 ± 0.5Bd

[a] GM0, unheated glucose–methionine mixture; GM30, GM60 and GM90, glucose–methionine mixtures heated at 150 °C for 30, 60 or 90 min, respectively. Values are mean ± SD of six determinations. Different capital letters in the same file mean significant differences, for either soluble or precipitated minerals ($p < 0.05$, ANOVA one-way and Duncan test). Different small letters in the same column mean significant differences ($p < 0.05$, ANOVA one-way and Duncan test).

Iron, initially insoluble at neutral pH, was partially solubilized in the presence of GM0 (Table 4). It should be taken into account that the more acid pH of the sample (6.39) could have contributed to the soluble complexes formation of iron–methionine. In this sense, Glidewell and Glidewell (1993) reported the complexing of iron with several amino acids containing $–SCH_3$, such as methionine. Moreover, the decrease in –SH groups during food processing (Castrillón, Navarro, & Alvarez-Pontes, 1997) has been related to a decrease in Fe absorption (Taylor, Martinez-Torres, Romano, & Layrisse, 1986). Of several amino acids, only methionine and cysteine are able to mimic the positive effect of the "meat factor" in iron absorption (Layrisse, Martínez-Torres, Leets, Taylor, & Ramírez, 1984). A positive effect of methionine on Zn absorption has also been described (Lonnerdal, 2000), which could be related to the higher Zn solubility ($86.9 \pm 1.15\%$) observed in the presence of the GM0 sample with respect to the no-sample solution ($75.0 \pm 0.53\%$).

Glucose–methionine complexes, formed after 30 min of heating, did not favour Fe and Zn solubility, contrary to what was described for Cu. The stronger binding of soluble MRPs for Cu than for Fe and Zn (O'Brien & Morrissey, 1997) could explain the positive influence on Cu solubility. Due to the slow reaction development in the glucose–methionine system, after 60 min of heat treatment, soluble MRPs are assumed to have increased, which would have allowed an increase in the soluble Fe and Zn fractions with respect to the GM30 sample. However, during the last 30 min of heating, with the browning development, insoluble melanoidins increased (Table 2) and the Fe and Zn soluble fractions were significantly reduced, more markedly in the Fe fraction. MRPs formed in the presence of GM90 seemed to have a higher affinity for Cu and Fe than for Zn and this may contribute to the lower Zn precipitation compared with other minerals. In this respect, assays carried out using heated casein–glucose–fructose mixtures, digested alone or included in a diet, show that Zn precipitation is minor when the browning products form part of a diet, due in part to the presence of other minerals that compete with Zn for binding sites on chelating compounds (Navarro, Aspe, & Seiquer, 2000).

In summary, current findings show that heating mixtures of glucose–lysine and glucose–methionine has little or no effect on Ca and Mg solubility. With respect to the trace elements, early compounds formed in both model systems seem to favour mineral solubility, maximum after 30 min of heating in the glucose–lysine system, and after 60 min in the glucose–methionine one, supporting the slowing down of the MR when methionine is one of the reactants, and this is also reflected in the browning development. It is therefore concluded that the effects of browning products generated during food processing should be taken into account, especially regarding trace element availability, as in vitro solubility may be considered as a prior indication of in vivo mineral utilization.

References

Ames, J. M. (1998). Applications of the Maillard reaction in the food industry. *Food Chemistry, 62*, 431–439.

Andrieux, C., & Saquet, E. (1984). Effects of Maillard' s reaction products on apparent mineral absorption in different parts of the digestive tract. The role of microflora. *Reproduction and Nutrition Development, 23*, 379–386.

Aoygi, S., & Baker, D. H. (1994). Copper–amino acid complexes are partially protected against inhibitory effects of L-cysteine and L-ascorbic acid on copper absorption in chicks. *Journal of Nutrition, 124*, 388–395.

Ashoor, S. H., & Zent, J. B. (1984). Maillard browning of common amino acids and sugars. *Journal of Food Science, 49*, 1206–1207.

Aspe, T., Vaquero, M.P. & Navarro, M.P. (1993). Changes in the speciation and utilization of calcium in rats by the presence of untreated or heated casein, separately or in combination with glucose and fructose. In *Bioavailability'93-nutritional, chemical and food processing implications of nutrient bioavailability*. (pp. 68–72). Nutrient-Bioavailability Symposium (Part II), University of Antwerp, Belgium.

Bailey, J. R., Ames, J. M., & Monti, S. M. (1996). An analysis of the non-volatile reaction products of aqueous Maillard model system at pH 5, using reversed-phase HPLC with diode-array detection. *Journal of the Science of Food and Agriculture, 72*, 97–103.

Bates, L., Ames, J. M., MacDougall, D., & Taylor, P. C. (1998). Laboratory reaction cell to model Maillard color development in a starch-glucose–lysine system. *Journal of Food Science, 63*, 991–996.

Berthon, G., Hacht, B., Blais, M. J., & May, P. M. (1986). Copper-histidine ternary complex equilibrium with glutamine asparagine and serine. The implications for computer-simulated distributions of copper (II) in blood plasma. *Inorganic Chimica Acta, 125*, 219.

Cammerer, B., & Kroh, L. W. (1995). Investigation on the influence of reaction conditions on the elementary composition of melanoidin. *Food Chemistry, 53*, 55–59.

Castrillón, A. M., Navarro, M. P., & Alvarez-Pontes, E. (1997). Changes in chemical composition and nutritional quality of fried sardine (*Clupea pilchardus*) produced by frozen storage and microwave reheating. *Journal of the Science of Food and Agriculture, 75*, 125–132.

Chan, F., & Reineccius, A. (1994). The reaction kinetics for the formation of isovaleraldehyde, 2-acetyl-1-pyrrolone, di(H)di(OH)-6-methylpyranone, phenylacetaldeh yde, 5-methyl-2-phenyl-2-hexenal. and 2-acetylfuran in model systems. In T. P. Labuza, G. A. Reineccius, V. Monnier, J. O'Brien, & J. Baynes (Eds.), *Maillard reactions in chemistry, food and health* (pp. 131–139). Cambridge: The Royal Society of Chemistry.

Charlton, R. W., & Bothwell, T. H. (1983). Iron absorption. *Annual Review of Medicine, 34*, 55–68.

Cremer, D. R., & Eichner, K. (2000). The reaction kinetics for the formation of strecker aldehydes in low moisture model systems and in plant powders. *Food Chemistry, 71*, 37–43.

Delgado-Andrade. 2002. Reacción de Maillard: Influencia sobre la biodisponibilidad mineral. Doctoral Thesis. Granada University. Delgado-Andrade, Granada, Spain.

Delgado-Andrade, C., Seiquer, I., & Navarro, M. P. (2002). Copper metabolism in rats fed diets containing Maillard reaction products. *Journal of Food Science, 67*, 855–860.

Erbersdobler, H. F., Brandt, A., Scharrer, E., & Von Wagenheim, B. (1981). Transport and metabolism studies with fructose amino acids. *Progress in Food and Nutrition Science, 5*, 217.

Fernández-Fígares, I., Pérez, L., Nieto, R., Aguilera, J. F., & Prieto, C. (1995). The effect of heat treatment on ileal amino acid digestibility of growing broilers vetch and bitter vetch meals. *Animal Science, 60*, 493–497.

Fogliano, V., Monti, S. M., Musella, T., Randazzo, G., & Ritieni, A. (1999). Formation of coloured Maillard reaction products in a gluten-glucose model system. *Food Chemistry, 66*, 293–299.

Friedman, M. (1996). Food browning and its prevention: An overview. *Journal of Agriculture and Food Chemistry, 44*(3), 631–653.

Glidewell, C., & Glidewell, S. M. (1993). The fate of nitrite in food processing: isolation of dinuclear and tetranuclear iron-sulphur nitrosyl complexes from cysteine and methionine sources. *Food Chemistry, 46*, 225–230.

Hashiba, H. (1986). Oxidative browning of Amadori compounds-color formation by iron with Maillard reaction products. In M. Fujimaki, M. Nakmiki, & H. Kato (Eds.), *Amino-carbonyl reactions in food and biology systems* (pp. 155–164). Amsterdam: Elsevier Science.

Hill, V. M., Ledward, D. A., & Ames, J. M. (1996). Influence of high hydrostatic pressure and pH on the rate of Maillard browning in a glucose–lysine system. *Journal of Agriculture and Food Chemistry, 44*, 594–598.

Homma, S., & Murata, M. (1994). Characterization of metal chelating compounds in soluble coffee. In T. P. Labuza, G. A. Reineccius, V. Monnier, J. O'Brien, & J. Baynes (Eds.), *Maillard reactions in chemistry, food and health* (p. 413). Cambridge: The Royal Society of Chemistry.

Homma, S., & Murata, M. (2001). Metal chelating compounds in instant coffee. *Annals of Nutrition and Metabolism, 45*(S), 394.

Homma, S., Aida, K., & Fujimaki, M. (1986). Chelation of metal with brown pigments in coffee. In M. Fujimaki, M. Nakmiki, & H. Kato (Eds.), *Amino-carbonyl reactions in food and biology systems* (pp. 165–172). Amsterdam: Elsevier Science.

Homma, S., Nakamura, Y., Asakura, T., Sekiguchi, N. Y, & Murata, M. (1990). Separation and characterization of metal chelating compounds in coffee. In P. A. Finot, H. U. Aeschbacher, R. F. Hurrell, & R. Liardon (Eds.), *The Maillard reaction in food processing, human nutrition and physiology* (pp. 279–284). Basel: Birkhaüser.

Hurrell, R. E., & Carpenter, K. J. (1981). The estimation of avilable lysine in foodstuffs after Maillard reaction. In C. Eriksson (Ed.), *Progress in food and nutrition science. Maillard reaction in foods* (vol. 5, pp. 78–85). Pergamon Press: Oxford.

Johnson, P. E. (1991). Effect of food processing and preparation on mineral utilization. In M. Friedman (Ed.), *Nutritional and toxicological consequences of food processing* (pp. 483–498). New York: Plenum Press.

Layrisse, M., Martínez-Torres, C., Leets, I., Taylor, P., & Ramírez, J. (1984). Effects of histidine, cysteine, glutathione or beef on iron absorption in humans. *Journal of Nutrition, 114*, 217–223.

Lonnerdal, B. (2000). Dietary factors influencing zinc absorption. *Journal of Nutrition, 130*(5S Suppl), 1378S–1383S.

Malec, L. S., Gonzales, A. S., Naranjo, G. B., & Vigo, M. S. (2002). Pereyra Influence of water activity and storage temperature on lysine availability of a milk like system. *Food Research International, 35*, 849–853.

Manzocco, L., & Maltini, E. (1999). Physical changes induced by the Maillard reaction in a glucose–glycine solution. *Food Research International, 32*, 299–304.

Monti, S. M., Bailey, R. G., & Ames, J. M. (1998). The influence of pH on the non-volatile reaction products of aqueous Maillard model systems by HPLC with diode array detection. *Food Chemistry, 62*, 369–375.

Moon, G., Murata, M., & Homma, S. (1994). Cu (II) chelating activity of glucose–lysine model melanoidin. In T. P. Labuza, G. A. Reineccius, V. Monnier, J. O'Brien, & J. Baynes (Eds.), *Maillard reactions in chemistry, food and health* (p. 417). Cambridge: The Royal Society of Chemistry.

Navarro, P., Aspe, T., & Seiquer, I. (2000). Zinc transport in Caco-2 cells and Zinc in rats: Influence of the heat treatment of a casein-glucose–fructose mixture. *Journal of Agricultural and Food Chemistry, 48*, 3589–3596.

Nielsen, H. J., De Weck, D., Finot, P. A., Liardon, R., & Hurrell, R. F. (1985). Stability of tryptophan during food processing and storage. 1. Comparative losses of tryptophan, lysine and methionine in different model systems. *British Journal of Nutrition, 53*, 281–292.

Nockels, C. F., DeBonis, J., & Torrent, J. (1993). Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. *Journal of Animal Science, 71*, 2539–2545.

O'Brien, J., & Morrissey, P. A. (1997). Metal ion complexation by products of the Maillard reaction. *Food Chemistry, 58*, 17–27.

Reineccius, G. A. (1990). The influence of Maillard reaction on the sensory properties of foods. In P. A. Finot, H. U. Aeschbacher, R. F. Hurrell, & R. Liardon (Eds.), *The Maillard reaction in food processing, human nutrition and physiology* (pp. 157–170). Basel: Birkhaüser.

Rendleman, J. A. (1987). Complexation of calcium by the melanoidin and its role in determining bioavailability. *Journal of Food Science, 6*(52), 1699–1705.

Renn, P. T., & Sathe, S. K. (1997). Effects of pH, temperature, and reactant molar ratio on L-leucine and D-glucose Maillard browning reaction in an aqueous system. *Journal of Agriculture and Food Chemistry, 45*, 3782–3787.

Rizzi, G. P. (1994). The Maillard reaction in foods. In T. P. Labuza, G. A. Reineccius, V. Monnier, J. O' Brien, & J. Baynes (Eds.), *Maillard reactions in chemistry, food and health* (pp. 11–19). Cambridge: The Royal Society of Chemistry.

Seiquer, I., Aspe, T., Vaquero, P., & Navarro, P. (2001). Effects of heat treatment of casein in the presence of reducing sugars on calcium biavailability: in vitro and in vivo assays. *Journal of Agricultural and Food Chemistry, 49*(2), 1049–1055.

Taylor, P. G, Martinez-Torres, C., Romano, E. L, & Layrisse, M. (1986). The effect of cysteine-containing peptides released during meat digestion on iron absorption in humans. *American Journal of Clinical Nutrition, 43*, 68–71.

Tsai, J. H., Hiserodt, R. D., Ho, C., Hartman, T. G., & Rosen, R. T. (1998). Determination of volatile organic selenium compounds from the Maillard reaction in a selenomethionine–glucose model system. *Journal of Agricultural and Food Chemistry, 46*, 2541–2545.

VanBarneveld, J. R., Batterham, E. S., Skingle, D. C., & Norton, B. W. (1995). The effect of heat on amino acids for growing pigs. 4. Nitrogen balance and urine, serum and plasma composition of growing pigs fed on raw or heated-treated field peas (*Pisum sativum*). *British Journal of Nutrition, 73*, 259–273.

Van Campen, D. (1973). Enhancement of iron absorption from ligated segments of rat intestine by histidine, cysteine and lysine: effects of removing ionizing groups and of stereoisomerism. *Journal of Nutrition, 103*, 139–142.

Whitelaw, M. L., & Weaver, M. L. (1988). Maillard browning effects on in vivo availability of zinc. *Journal of Food Science, 53*, 1508–1510.

Wijewickeme, A. N., & Kitts, D. (1997). Influence of reaction conditions on the oxidative behaviour of model Maillard reaction products. *Journal of Agricultural and Food Chemistry, 45*, 4571–4576.

Yoshimura, Y., Iijima, T., Watanabe, T., & Nakazawa, H. (1997). Antioxidative effect of Maillard reaction products using glucose–glycine model system. *Journal of Agricultural and Food Chemistry, 45*, 4106–4109.