IMPACT OF MINERAL ELEMENTS (Ca, Mg AND Fe) ON MAILLARD REACTION IN MODEL SYSTEMS WITH CASEIN

Danute Terese Ramonaityte

Kaunas University of Technology,
Kaunas, LT-50524, Lithuania, e-mail: danute.ramonaityte@ktu.lt

Abstract

Non-enzymatic browning, known as Maillard reactions (MR), occurs in many foods, including dairy products. The initial reactants are the amino compounds and reducing sugars. However, the MR in the real food systems proceeds in presence of other macro- and micro-components. The task of the work was to determine the influence of Ca, Mg and Fe on the browning characteristics of the MR model systems consisting glycine or casein and lactose or glucose, and to estimate the interaction of the elements with the high molecular weight (HMW) products formed during MR. The extent of browning in MR solutions was evaluated by measuring absorbance at 420 and 550 nm. The HMW melanoidins were separated by means of dialysis.

It was determined that Ca^{2+}, Mg^{2+} promote, and Fe^{2+} slows down the formation of colored MR compounds in the lactose–casein as well as in glucose–casein model systems by thermal treatment to 12 h at boiling temperature. The amount of Ca in the non-dialyzable part of the systems increased over a longer time of interaction from 28–38 to 62–71 %, while the amount of non-dialyzable Mg stays fixed about 26%. The peak of non-dialyzable Fe (~50%) was found in the first hour of interaction.

The results demonstrate the possible involvement of Ca, Mg and Fe in the thermal changes of dairy products and the impact of Maillard reactions on the solubility and supposed bioavailability of the elements.

Key words: casein, Maillard reaction, mineral elements, influence

Introduction

Non enzymatic browning, known as the Maillard reactions (MR), occurs in many foods, including dairy products. The initial reactants in the MR are the amino compounds (mainly free amino acids or proteins) and reducing sugars (as glucose, lactose and others). In the case of milk products, casein and lactose are particularly involved. I the early step, the free amino groups of the proteins (such as amino groups of lysine residues in casein) react non-enzymatelly with reducing sugars. In the advanced and final steps of the reaction proteins are modified into colored, fluorescent and cross-linked molecules, which form later the brown polymers – melanoidins. This reactions is of importance in many heated, dried and stored foods because the formation of flavoring ingredients, browning products, the loss of nutritive value (Ames, 1998, Van Boekel, 1998).

MR in the real food systems proceeds in presence of many others macro- and microcomponents. It is determined that the development of non-enzymatic browning in foods and the nature of compounds formed in different stages of MR depend on the presence of such minor substances as oxidizing or reducing agents (Bobbio *et al*., 1985; Swales, Wedzicha, 1992; Zidertman *et al.*, 1989), formaldehyde (Vasiliauskait8 and Wedzicha, 1997), urea (Braekman et al., 2001) and mineral elements, especially transition metals (Birglouez-Arragon, 1997; Cheng, Kowakishi, 1993; Hayase *et al*., 1996; Randleman and Inglett, 1990).

Diverse studies have suggested that melanoidins behave as anionic hydrophilic polymers, which can form stable complexes with metal cations. According to several authors, Ca, Mg, Fe, Cu and Zn are bound to some degree by soluble and insoluble melanoidins derived from different amino acids-sugar model systems (Rendleman, 1987; O'Brien and Morrissey, 1997). However, heated methionine-sugar mixtures had a little effect on Ca and Mg solubility (Delgrado-Andrade *et al.*, 2004). It was predicated, that the effects of browning products generated during food processing should be taken into account, particularly in trace element solubility and bioavailability (Morales, 2005). Heat treatment of reducing sugar-casein model system also affected the calcium bioavailability by in vitro and in vivo assay (Seiquer *et.al.*, 2001).

The results that appear in the literature give not sufficient information about the Maillard reactions in food systems near to realistic for milk products, where some metal present in the reaction media. The aim of this study is to define the influence of mineral elements Ca, Mg

and Fe on the browning characteristics of the model systems from glycine or casein and lactose or glucose solutions, and to evaluate the interaction of the elements with high molecular weight products formed during Maillard reactions.

Materials and Methods

Sample preparation. Glucose and lactose (Merk, Darmstadt, Germany), glycine (Sigma Chemical Co, St. Louis, MO, USA) and dried casein were used to prepare the samples. Mineral elements (Ca, Mg and Fe) were added as water solutions of the salts: anhydrous $CaCl_2$, $MgCl_2 \times H_2O$ and $FeSO_4 \times 7H_2O$.

The concentration of reactants in 100 ml model solutions was as followed: lactose or glucose – 0.25 mol; glycine – 0.25 mol; casein – 0.5 g; calcium – 120 or 240 mg, magnesium – 60 or 120 mg and iron – 20 mg. The model mixtures with casein were prepared in Na_2SO_3 solution (0.7 %). The concentrations of Ca and Mg were selected to simulate usual content of minerals in cow's milk or dairy products, and the concentration of Fe used similar as in fortified milk formulas.

Thermal treatment and dialysis of model samples. Prepared model mixtures were heated at boiling temperature in a flask under reflux for 6 hours and samples were taken after 1, 2, 3, 4, 5 and 6 hours to monitor the course of the reaction.

Maillard reaction products formed in each stage of heating were separated by means of dialysis into two fractions: (1) high molecular weight (HMW) compounds (>12 000 Da), and (2) dialyzable compounds. 10 ml of each solution was dialyzed for 72 hours at temperature 4–6 °C against 1000 ml bidistilled water, which was changed for first time after 8 h, and later for each 12 h.

Spectrometric analysis. Browning of samples was evaluated spectrophotometrically as absorbance at 420 nm (A_{420}) and 550 nm (A_{550}) using Spectrophotometer Varian Carry 1/3, USA. Browning index (BI) was calculated as: $BI=A_{420}-A_{550}$. For browning measurements, due to the insoluble nature of the brown pigments in proteins, the model samples (5.0 ml) were digested with 1 ml pancreatin solution (50 mg/ml) at 45 °C for 2 h. Trichloracetic acid solution (50% w/v) was added to stop the enzymatic reaction and the samples were centrifuged (8000 g for 10 min) and filtered trough filter paper (Davies *et al.*, 1998). For comparison, the direct measurement of absorbance carried out in the non-digested samples.

The results of measurements were calculated as average of triplicates.

Determination of mineral elements. The content of Ca, Mg and Fe in non-dialyzable fractions was determined by flame AAS method after dry ashing (550±25 °C) of non-dialysable parts of the model systems.

Results and Discussion

Calcium added in concentration corresponded its content in cow's milk (120 mg or 3.0 mmol/100 ml) stimulated the browning interaction in heated lactose-glycine system. The increase of absorbance at 420 nm was more rapid at longer heating time. When a higher content of calcium was added to the system (240 mg or 6.0 mmol/100 ml) the stimulating effect of Ca^{2+} on the accumulation of browning compounds was lower (Fig. 1*a*). This suggested that effect of Ca ions on the MR in lactose-glycine system become slight limiting.

Supposedly, in the presence of Ca ions in an appropriate amount the interaction of some charged advanced MR compounds eased due the formation of Ca bridges among reacting groups, and the rate of accumulation of brown melanoidins expand. However, at the abundance of Ca^{2+} ions in the system the blocking effect on the development of MR was observed. On the other hand, by higher content of calcium lactose-casein system (Fig. 1*b*) can lead to form of more HMW complexes, including protein-bound melanoidins, which availability to enzymatic digestion was badly, and a part of melanoidins can to be precipitated by adding of trichloracetic acid. Unfortunately, browning status of precipitates was evaluated in this work only visual, and a higher darkness of the precipitates of the samples with higher

Ca content was observed in this way. Both additives of Ca shortened an incubation time, after which measurable absorption values were obtained in the samples.

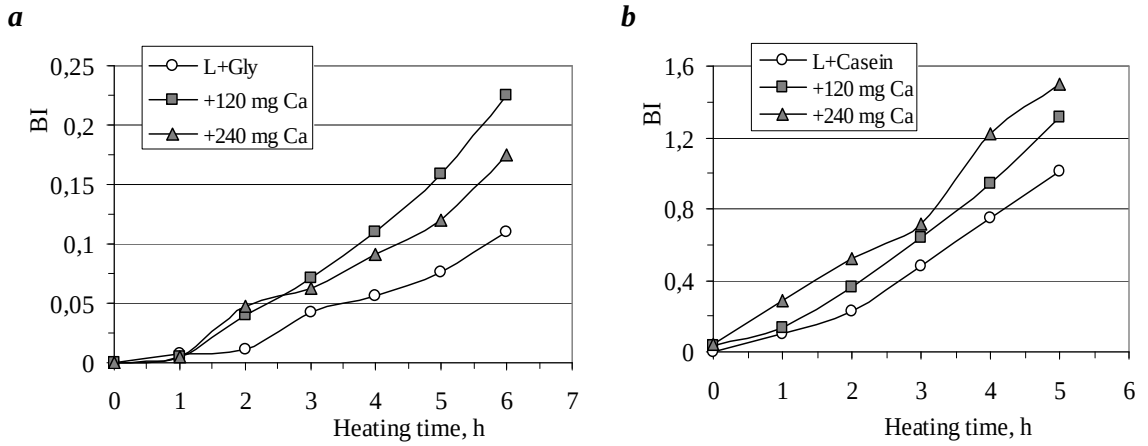

Figure 1. Change of browning index (BI) value in MR model systems by heating with added Ca: *a*) lactose-glycine (L+Gly) **and *b*) lactose-casein** (L+Casein)

The browning of test solutions with added Mg, was measured at 2 different means – without use of enzyme and after enzyme digestion. The absorbance A_{420} in the non-digested samples was directly proportional to the concentration of Mg added and reflected rather opalescence than absorption. However, the dependence of the accumulations of color compounds on the level of Mg in the digested samples (60 mg or 2.5 mmol/100 ml and 120 mg or 5.0 mmol/100 ml) was similar as in case of Ca addition (Fig. 2). Moreover, the browning rate in glucose-casein model systems was higher than in lactose-casein systems, but the effect of Mg^{2+} ions was similar, as it can see by comparison the data given in Fig. 1*a* -1*b*, and 2*a* - 2*b*

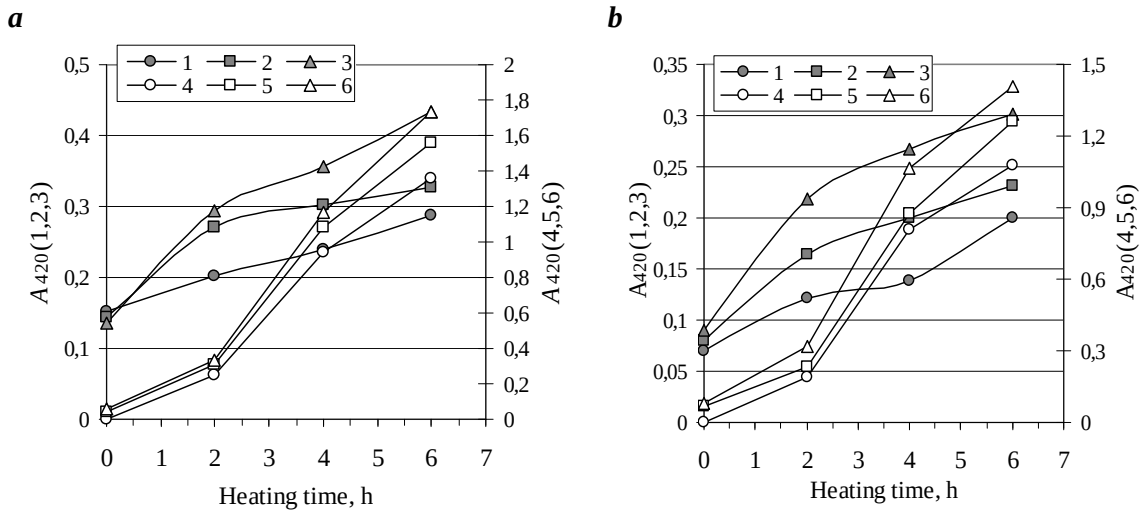

Figure 2. Change of absorbance in glucose-casein (*a*) and lactose-casein (*b*) model systems by heating: 1 and 4–no Mg added; 2 and 5–60 mg; 3 and 6–120 mg Mg. Filled and addle points show data without and after enzyme digestion, respectively.

The change of absorbance A_{420} and A_{550} in

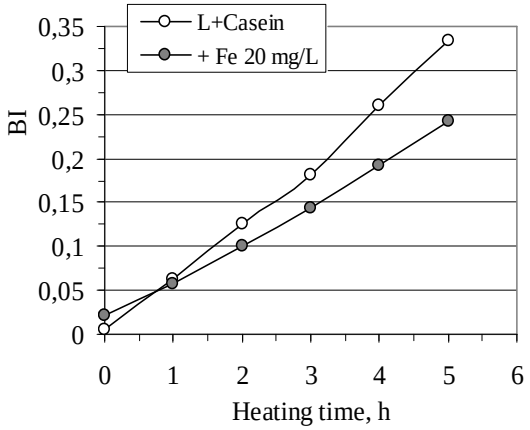

Figure 3. Influence of Fe^{2+} on the change of browning index (BI) in lactose-casein system (L+Casein) **by heating**

time, expressed as increase of browning index, showed that Fe added in concentration of 20 mg or 0.36 mmol/L inhibited the formation of melanoidins in the lactose-casein system (Fig. 3). It was previous determined (Cheng *et.al.*, 1991, Hayase, 1996) that iron involves in MR not only as complexing but also as oxidizing agent. On this reason, the oxidative effect on the brown chromophores can be proposed in this experiment.

According to the data obtained by analysis distribution of added Ca, Mg and Fe between dialyzable and non-dialyzable fractions of the heated model systems, a part of the elements is associated with HMW melanoidins. By progress of MR in sugar- casein solutions the relative amount of calcium incorporated into structure of non-dialyzable compounds, increased from 28–36% to 63–71% (Fig. 4). The binding properties of HMW compounds derived in the lactose containing model systems trended to be was similar as in glucose systems (the separate data is not given). As it was reported in the studies, Ca^{2+} ions are able to form complexes with melanoidins (Rendleman, 1987, Seiquer, 2001). The results of our experiments are in agreement with these findings and indicate that bioavailability of Ca in milk products trends to decrease by thermal treatment.

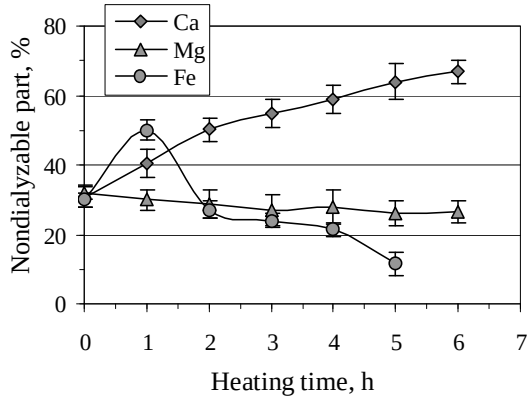

Figure 4. Percentage of non-dialyzable elements in both glucose-casein and lactose-casein model systems by heating (present as average of percentage values)

The percentage of Mg in non-dialyzable fractions of tested model systems remained near to constant during all heating time.

The complexation of Fe by non-dialyzable compounds was other than Ca and Mg. In the first hour of lactose-casein interaction, when the products from early and advanced stages of MR begin to form and when the concentration of protein-bound melanoidins is low, the percentage of Fe added, that remains in non-dialyzable fraction, increased approximately from 30% to 50%. As the reactions proceed and the products of final stage of MR accumulate, the amount of Fe^{2+} bound to HMW fraction suddenly decreased to 13.5%. It is proposed that metal binding ability of melanoidins formed in sugar-casein system decreased due the oxidative effect of Fe^{2+} theirself, incorporated into the structure of casein-bound melanoidins.

Conclusion

The results of the study demonstrate the possible participation of Ca, Mg and Fe in the thermal changes of foods containing casein and reducing sugars and the impact of Maillard reactions on the solubility and supposed bioavailability of the elements.

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