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The kinetics of Maillard reaction in lactose-hydrolysed milk powder and related systems containing carbohydrate mixtures

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ABSTRACT

The kinetics of Maillard reaction in lactose-hydrolysed skim milk powder and related systems containing carbohydrate mixtures were analysed. The effect of the increase of water activity and temperature during storage of the commercial product was also evaluated. In systems with two and three carbohydrates, a marked decrease of the reaction rate was observed when monosaccharides were partially replaced by lactose, notwithstanding the fact that the former still remained in a higher proportion than lysine. The rate of available lysine loss in lactose-hydrolysed milk was mostly affected by the presence of galactose. The reaction rate constants at a_w 0.52 and at 37 and 50 °C were higher than at a_w 0.33. However, no significant differences were observed at 60 °C. Temperature is the most important factor to be controlled in order to minimise nutritional deterioration during storage.

1. Introduction

Nutritional and functional damage of proteins during processing and storage of foods may occur, mainly due to the Maillard reaction with reducing sugars. Under mild conditions, the nutritional deterioration is mainly attributed to the reduction of lysine availability, the most reactive amino acid due to its free ε-amino group (Arnoldi, 2002).

The Maillard reaction is influenced by many factors, including temperature, time, initial pH, water activity (a_w), physical state of the matrix, reactant concentration and type of carbohydrate involved (Labuza & Baisier, 1992). The effect of glass transition on the kinetics of diffusioncontrolled chemical reactions such as the Maillard reaction has been reported by many researchers. Below the glass transition temperature (T_g), the reaction rate markedly decreases as a result of the high viscosity of the amorphous matrix, which limits molecular mobility (Karmas, Buera, & Karel, 1992; Lievonen, Laaksonen, & Roos, 1998). The reactivity of reducing sugars is related to the proportion of the open chain, which is the active form. Hence, the percentage of the acyclic carbonyl is a relevant factor when comparing the reaction rates of the different reducing sugars in the Maillard reaction (Yaylayan, Ismail, & Mandeville, 1993). Those with the most stable acyclic forms would be more reactive. In general, the order of reactivity of reducing sugars was reported to decrease as follows: pentoses > hexoses > disaccharides > maltodextrins (Chevalier, Chobert, Popineau, Nicolas, & Haertlé, 2001; Rufián-Henares, García-Villanova, & Guerra-Hernández, 2004).

Dairy products are very sensitive to the Maillard reaction, due to their high content of reducing sugars and lysine-rich proteins. Particularly, lactose-hydrolysed milk is very prone to protein deterioration, as lactose is partially replaced by glucose and galactose, which react with lysine at a higher rate than the disaccharide.

Most of the research on nutritional damage in lactose-hydrolysed milk by Maillard reaction was performed in liquid milk (Evangelisti, Calcagno, Nardi, & Zunin, 1999; Messia, Candigliota, & Marconi, 2007; Tossavainen & Kallioinen, 2008). The a_w of skim lactose-hydrolysed milk powder is very low, but it must be noted that the recommended shelf life of this product amounts to almost one year, and that fluctuations of temperature and the possible increase of a_w during this extended period of time, would enhance nutritional damage.

The Maillard reaction rate is not easy to analyse in this product, because it is a complex system which contains more than one sugar. Some studies have addressed the progress of this reaction in systems containing two or more sugars (Carulli, Calvano, & Palmisano, 2011; Chávez-Servín, Romeu-Nadal, Castellote, & López-Sabater, 2006; García-Baños, del Castillo, Sanz, Olano, & Corzo, 2005; Messia et al., 2007; Rutherfurd & Moughan, 2008). Nevertheless, in those where the lessening of the different sugars was evaluated, results were not easy to compare (Chávez-Servín et al., 2006; García-Baños et al., 2005).

The aim of this work was to analyse the kinetics of Maillard reaction in systems containing mixtures of carbohydrates, such

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as lactose-hydrolysed skim milk powder. For this purpose, model systems containing casein and either glucose, galactose, or lactose, or mixtures of these sugars were studied. The initial stage was analysed through the loss of available lysine and the decrease of reducing sugars. In order to analyse the changes in the rate constants when another sugar was added, the decrease of each reactive in model systems with one sugar and then in systems combining two or three of them were compared. The effect of the increase of water activity and temperature during storage of lactose-hydrolysed skim milk powder was also evaluated.

2. Materials and methods

2.1. Materials

Casein, glucose, galactose, lactose, O-phthaldialdehyde (OPA), *N*-acetyl-L-cysteine (NAC), $MgCl_2$, phenyl-β-D-glucoside and N-trimethylsilylimidazole (TMSI) were obtained from Sigma–Aldrich. (St. Louis, MO) and sodium dodecylsulfate was purchased from Mallinckrodt (Hazelwood, MO). All other chemicals were of analytical grade. The commercial lactose-hydrolysed skim milk powder contained 32.4% protein, 1% fat and 52% carbohydrate (21% glucose, 21% galactose and 10% lactose). The analysis started two weeks after its manufacturing date.

2.2. Preparation of samples

Six model systems containing casein and either glucose, galactose, or lactose, or binary mixtures of these carbohydrates were prepared. Afterwards, another system with casein and the three above mentioned sugars was also analysed. The carbohydrates and protein employed in model systems are present in lactose-hydrolysed milk, although the ratio between the two reactants is lower. The initial total of reducing sugars:available lysine molar ratio in all systems was 3:1. The proportion selected was high enough to avoid affecting the reaction rate, due to the differences between carbohydrates:lysine molar ratios of lactose-hydrolysed milk and the model systems, but low enough to allow the measurement of sugar loss.

In the systems with two sugars, the initial molar ratio between them was 1:1 and in the system with three sugars, the initial molar ratio between them was 1:1:1, e.g., in the system containing glucose and lactose, the initial glucose:lactose:available lysine molar ratio was 3:3:2, and in the system with glucose, galactose and lactose, the initial glucose:galactose:lactose:available lysine molar ratio was 1:1:1:1.

Model systems were prepared by freeze-drying a dispersion of dry ingredients (20% w/w) in 0.1 M phosphate buffer solution (pH 6.5). A Stokes freeze-dryer model 21 (Philadelphia, PA) was used, which operated at a −40 °C condenser plate temperature and a chamber pressure of less than 100 mm Hg during 48 h was used. The freeze-dried samples were then equilibrated at 25 °C in vacuum desiccators over saturated solution of $MgCl_2{\cdot}6H_2O$ at a_w 0.33 until weight was constant.

Lactose-hydrolysed skim milk powder was equilibrated at a_w 0.33 and 0.52 using a desiccator containing saturated salt solutions of $MgCl_2{\cdot}6H_2O$ and $Mg(NO_3)_2{\cdot}6H_2O$, respectively until weight was constant.

2.3. Measurement of water activity

To measure the a_w of the samples, an AquaLab Water Activity metre Series 3TE with internal temperature control (Decagon Devices, Inc., Pullman, WA) was used.

2.4. Determination of available lysine

Available lysine was measured using the *o*-phthaldialdehyde/*N*-acetyl-L-cysteine spectrophotometric method reported by Medina Hernández and García Alvarez-Coque (1992). For analysis, samples containing approximately 0.2 mg lysine were dissolved in 2% w/v sodium dodecylsulfate solution. Absorbance was measured at 335 nm with a Hewlett Packard HP 8453 spectrophotometer (Palo Alto, CA, USA). Three replicates were analysed for each sample. To calculate the available lysine content, a standard curve with casein was plotted as previously reported by Malec, Pereyra Gonzales, Naranjo, and Vigo (2002).

Total nitrogen content was determined in duplicate by the Kjeldahl method using a Büchi digestor Model 430 and a nitrogen distillation apparatus Model 320 (Büchi Laboratoriums-Technik AG, Flawil, Switzerland).

2.5. Determination of carbohydrates

Carbohydrates were determined by gas chromatography of trimethylsilylated derivatives according to the method described by Troyano, Olano, Fernández-Díaz, Sanz, and Martínez-Castro (1991) with modifications. Samples (200–300 mg) were gently mixed with approximately 10 ml of deionised water in a 10 ml volumetric flask and filled to volume by adding extra water. The mixtures were held for 1 h at 25 °C and then centrifuged at 1500 rpm for 15 min. Two millilitres of supernatant were added with 1 ml 0.5% phenyl-β-D-glucoside in 80% methanol, as internal standard, and diluted to 10 ml with methanol. After 1 h at room temperature, the mixture was filtered and 0.5 ml of the filtrate were evaporated under vacuum at room temperature. The dry residue was derivatised and immediately injected. Standard solutions containing different proportions of glucose, galactose, lactose and phenyl-β-D-glucoside were prepared, to study the response factor (RF) of carbohydrates relative to the internal standard.

Gas chromatographic analyses were carried out on a Shimadzu 17A gas chromatograph (Kyoto, Japan) equipped with a split injector and a flame ionisation detector (FID). The temperature of the injector and detector was 300 °C. Carrier gas was nitrogen set at a flow rate of 0.8 ml min^{-1}; the split ratio was 1:10. The column was a DB 1701, 30 m × 0.25 mm ID; 0.25 μm film thickness (J & W, Folsom, CA,). Oven initial temperature was 200 °C for 8 min, later increased at a rate of 30 °C min^{-1} until reaching 210 °C, remaining constant for 11 min. Three replicates were analysed for each sample.

2.6. Differential scanning calorimetry (DSC)

The glass transition temperatures for the casein–glucose, casein–galactose and casein–lactose systems at a_w 0.33 were determined using a Mettler Toledo DSC 822e (Schwerzenbach, Switzerland). The instrument was calibrated using indium (156.6 °C), lead (327.5 °C) and zinc (419.6 °C). The samples were heated from −50 to 100 °C at a heating rate of 10 °C min^{-1}, using hermetically sealed aluminium pans of 40 μl capacity; an empty pan was used as a reference. Thermograms were evaluated employing the Mettler Stare program. An average value of two replicate samples was reported. The T_g values were taken as the onset temperature of the glass transition temperature range.

2.7. Kinetic study

Several 500 mg samples of the equilibrated model systems were weighed, sealed immediately into a glass flask to avoid adsorption of water from the environment, and stored at 37 °C. Similarly, samples of lactose-hydrolysed skim milk powder equilibrated at both