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A study of different indicators of Maillard reaction with whey proteins and different carbohydrates under adverse storage conditions

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ABSTRACT

This study examined different indicators of each stage of Maillard reaction under adverse storage conditions in a system with whey proteins and lactose or glucose. The analysis of lysine loss by the *o*-phthaldialdehyde method can be considered a good indicator of the early stage, showing considerable differences in reactivity when systems with mono and disaccharides were analyzed. Capillary electrophoresis proved to be a sensitive method for evaluating the extent of glycosylation of the native proteins, providing valuable information when the loss of lysine was not significant. The estimation of the Amadori compound from the determination of total 5-hydroxymethyl-2-furfuraldehyde would have correlate well with reactive lysine content if the advanced stages of the reaction had not been reached. For assessing the occurrence of the intermediate and final stages, the measurement of free 5-hydroxymethyl-2-furfuraldehyde and color, proved not to be suitable for storage conditions.

1. Introduction

Whey proteins are widely acknowledged for their high nutritional value due to their excellent functional properties (i.e. emulsification, gelation, foaming and water-binding capacity) (Tunick, 2008). They are extensively used as ingredients for a variety of applications in the food industry due to their excellent functional properties. The nutritional and the functional attributes of these proteins are closely related to their structure, but chemical changes occurring during industrial processes can alter both characteristics (Martínez-Alvarenga et al., 2014; Oliver, Melton, & Stanley, 2006). The Maillard reaction is one of the most frequent reactions that take place in technological processes where heat treatments are involved. This reaction is also known as non-enzymatic browning and it can occur during storage as well (Guyomarc'h, Warin, Muir, & Leaver, 2000). It begins with the condensation of a reducing sugar with aminoacids or the ε-amino group of lysyl residues of proteins. Three stages in the Maillard reaction are usually considered: the early, the intermediate and the final stage. In the first stage, the reaction of amino acids with reducing sugars forms glycosylamines, which rearrange into Amadori compounds and results in the loss of lysine availability, lessening their nutritional value due to the blockage of its free ε-amino group (Hurrell, 1990). At the intermediate and the final stages, sensory changes take place as a result of the formation of volatile compounds and pigments. In the final stage, color changes become noticeable, associated with the formation of brown pigments, which are a complex series of polymers and co-polymers called melanoidins. These compounds have significant effects on food quality, and therefore color changes become a key factor for the acceptance of the product by the consumers.

In order to optimize processes and storage conditions, chemical markers have proved to be useful tools for assessing the food deterioration and for managing the changes that take place in the products. With regard to the development of the Maillard reaction, both the formation of certain products and the disappearance of reagents have often been used as indicators of the different stages of the reaction (Rufián-Henares, García-Villanova, & Guerra-Hernández, 2002; Rufián-Henares, Guerra-Hernández, & García-Villanova, 2002). Different markers might be more suitable to use at each stage. To analyze the first stage, the loss of reactants or the formation of Amadori compound can be studied (Schwietzke, Malinowski, Zerge, & Henle, 2011). At an intermediate stage the formation of volatile products or fluorescent compounds can be determined and in the final stage, the development of color is commonly studied (Damjanovic Desic & Birlouez-Aragon, 2011; Morales & van Boekel, 1998).

A number of factors influence the development of Maillard reaction, including temperature, time, initial pH, water activity (a_w),

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physical state of the matrix, reactant concentration and type of carbohydrate (Labuza & Baisier, 1992). The carbohydrates involved in the reaction are monosaccharides and reducing disaccharides. The nonreducing disaccharides like sucrose and trehalose cannot take part unless they are hydrolyzed first (Schebor, Burin, Buera, & Chirife, 1999). Although all studies indicate that the reaction rate of the monosaccharides is greater than that the disaccharides, the difference between them may vary as a reflection of environmental conditions (Ledesma-Osuna, Ramos-Clamont, & Vázquez-Moreno, 2008; Naranjo, Malec, & Vigo, 1998).

The purpose of the present research was to evaluate different parameters to follow the evolution of the Maillard reaction in a system with whey proteins and lactose or glucose and to compare their applicability as indicators of each stage of this reaction under adverse storage conditions. The first stage was analyzed by the loss of reactive lysine, the extent of glycosylation of the native proteins by capillary electrophoresis and the estimation of the Amadori compound by the determination of total HMF. The intermediate stage was studied by the free HMF measurement and color analysis was employed to evaluate the final stage. In addition, the sensitivity of each method to detect differences between the reactivity of mono and disaccharide was compared.

2. Materials and methods

2.1. Chemicals

Acetonitrile, acetic acid glacial, sodium acetate anhydrous, sodium borate, trichloracetic acid, glucose and lactose were purchased from J.T.Baker (Xalostoc, Mexico). N-acetyl-L-cysteine, OPA reagent, 1-fluoro-2,4-dinitrobenzene (FDNB), standards of HMF, β-lactoglobulin and α-lactalbumin were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Oxalic acid dihydrate was obtained from Fluka (Buchs, Switzerland). Whey protein isolate was obtained from BIPRO® Davisco Foods International Inc. (Eden Prairie, MN, USA); lactose content was <0.1%, determined by Schaffer-Somogyi method (AOAC 959.11, 1990) and protein content was 97%, dry basis (Kjeldahl method, AOAC 920.105, 1990). Deionized water was purified through a Simplicity® Water Purification Systems (Millipore, Bedford, MA, USA). All other reagents were of analytical grade or better.

2.2. Sample storage procedure

Two model systems containing whey proteins and glucose or lactose were prepared by mixing the components. The ingredients of the systems were weighed according to the molar ratio sugar:reactive lysine 9:1, which was the same as in milk. The components corresponding to each model system were dissolved in a phosphate buffer solution pH 6.5 (20% w/v). The systems were freeze-dried for 48 h at a temperature of −55 °C and a chamber pressure of 0.04 mbar in a freeze-drier ALPHA 1-4 LD2 Martin Christ Gefriertrocknungsanlagen GMBH (Osterode am Harz, Germany). Lyophilized samples were placed in desiccators containing a saturated solution of Mg $(NO_3)_2{\cdot}6H_2O$ at 4 °C. When the a_w nearly reached 0.52, the desiccators were moved to 25 °C and samples were equilibrated until weight was constant, at which point a_w was measured in an AquaLab Series 3TE, Decagon Devices Inc. (Pullman, WA, USA). Portions of 1.5 g of equilibrated samples were sealed in hermetic flasks and stored in oven at 37 °C. Two flasks were analyzed periodically. Control samples, after equilibration without thermal treatment, were evaluated in triplicate and designated as time zero.

2.3. Methods

2.3.1. Determination of reactive lysine

Spectrophotometric method of *o*-Phthaldialdehyde/N-acetyl-L-cysteine was used for determination of reactive lysine (Medina Hernández & García Alvarez-Coque, 1992). The samples were dissolved in 2% (w/v) sodium dodecylsulphate solution. Triplicate determinations of each duplicate sample were carried out, then the average of six measurements is reported. Absorbance was measured at 336 nm with a Hewlett Packard spectrophotometer HP 8453 (Hewlett Packard, Palo Alto, CA, USA). To calculate the reactive lysine content, a calibration curve was constructed by plotting the absorbance of standard solutions of whey proteins versus their lysine concentration. The reactive lysine of the proteins was previously determined by the FDNB method (Booth, 1971) and the value obtained was 9.50 g lysine/16 g Nitrogen. In order to determine the probable interference of the free amino groups of amino acids, small peptides and amines, the samples were dissolved in pH 9.0 sodium borate buffer solution and the proteins were precipitated with 10% trichloroacetic acid solution (Goodno, Swaisgood, & Catignani, 1981). The supernatant was evaluated and free amino acids were always negligible.

Kinetic rate constants for loss of lysine (k) and their standard error were calculated by exponential regression analysis of $L_t = L_0\ e^{-kt}$, where L_t = reactive lysine concentration at time t and L_0 = reactive lysine concentration at t = 0. The confidence intervals were estimated for a significance level of 95% by means of the Student *t*-test. Analysis of variance (ANOVA) was employed to ensure that the constants were different from zero.

Total nitrogen content was determined in triplicate by the Kjeldahl method (AOAC 920.105, 1990) using a digestor Bloc Digest 6 P-Selecta (J.P.Selecta S.A., Barcelona Spain) and a steam distillation system Pro-Nitro M P-Selecta (J.P.Selecta S.A., Barcelona, Spain).

2.3.2. Capillary electrophoresis

Capillary electrophoresis was carried out with a Beckman P/ACE 5010 (Beckman Coulter Inc., Fullerton, CA, USA) equipped with UV detection system and controlled by a P/ACE Station 1.21 (Beckman, Fullerton, CA, USA) software system. The separations were performed using a fused-silica capillary (Alltech Associates Inc., Deerfield, IL, USA) of 57 cm × 50 μm ID. The distance between detection window and outlet was 7 cm, resulting in an effective length of 50 cm. During sample analysis, a constant voltage at 20 kV was applied and the temperature of the separation was kept at 25 °C with circulating coolant surrounding the capillary placed in a cartridge. Detection was performed on the capillary at 214 nm. An electrophoresis buffer consisting of 50 mM sodium borate (pH 9.25) was prepared, which was used for electrophoretic separation and for dissolving samples. The capillary was rinsed by flushing with 0.1 N NaOH, water and electrophoresis buffer during 2 min each to any electrophoretic separation. Prior analysis all model systems were dialyzed against ultrapure water to remove any excess of sugars and salts, and then freeze-dried. Samples were dissolved in electrophoresis buffer at 2 mg/mL and injected hydrodynamically (20 s at 0.5 psi) at the anode. Buffers and samples were filtered through a 0.20 μm membrane filter and then deaerated ultrasonically prior to use. Each sample was analyzed in quadruplicate and the first electropherogram in a series was always discarded.

The electropherogram of the whey protein isolate was compared with those of the standards of β-lactoglobulin and α-lactalbumin and no differences were observed. Hence, it was considered that the proteins were not initially damaged. In addition, proteins stored under the same a_w and temperature conditions, but in the absence of carbohydrates, were analyzed by capillary