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Effect of Maillard reaction conditions on the degree of glycation and functional properties of whey protein isolate – Maltodextrin conjugates

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ABSTRACT

The Maillard reaction has been used as a natural alternative to improve protein functionality by covalent coupling with saccharides. However, if reaction conditions are not properly selected, glycation can lead to a loss in functional properties. The objective of our research was to study the effect of temperature, time, water activity and reactants molar ratio on the degree of glycation and color development in whey protein isolate conjugated with maltodextrins. Three different levels of glycation (low, medium and high) were selected to investigate functional properties. The extent of glycation was assessed by quantifying the loss of amino groups using the *o*-phthaldialdehyde technique. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to evaluate the molecular weight of the glycoproteins. Color changes were determined using a Minolta colorimeter and calculating the browning index. Functional properties evaluated were solubility, rheological behavior, foam overrun and foam stability. Temperature and water activity were the most influential factors determining the degree of glycation and color change. The correlation coefficient between blocked amino groups and color was of 0.743. Whey protein isolate exhibited lower solubility at pH 5 and conjugates at pH 4. Consistency index and foaming properties improved according to the level of glycation achieved.

1. Introduction

Milk proteins consist mainly of caseins and whey proteins, 80% and 20% respectively (Walstra, Wouters, & Geurts, 2006, chap. 21). Whey proteins are widely used as raw materials in the food industry due to their nutritional value and functional properties. Modern processing techniques have allowed the separation and fractionation of whey proteins to create whey protein powders with different protein contents (Tunick, 2008). Whey protein isolate (WPI) contains at least 90% protein, with negligible amounts of fat, sodium, and lactose and is an excellent source of essential amino acids. Despite the good functional properties of proteins from different sources, increasing consumer demand for functional foods has prompted food scientists to improve protein functionality.

The Maillard reaction (MR) is a complex series of chemical reactions that occur naturally between the amino group of an amino acid, peptide or protein and the carbonyl group of a reducing sugar or an end-product of lipid peroxidation. It is classified in 3 stages (Hodge, 1953): initial, intermediate, and final. There is now extensive scientific evidence proving that protein functionality can be significantly improved by covalent coupling with saccharides through the MR without the use of any chemical reagents (Akhtar & Dickinson, 2007; Chevalier, Chobert, Popineau, Nicolas, & Haertlé, 2001; Liu et al., 2012; Nakamura, Kato, & Kobayashi, 1992; Oliver, Melton, & Stanley, 2006a; Sun, Hayakawa, & Izumori, 2004; Zeng, Zhang, Guan, & Sun, 2011). This strategy has been regarded by many scientists as the safest and most promising alternative for the food industry (Kato, 2002; Mine & Yang, 2010; Oliver, Melton, & Stanley, 2006b). Conjugation is based on the Amadori rearrangement of the MR (Shepherd, Robertson, & Ofman, 2000). Different factors such as temperature, time, relative humidity (RH), pH, and reactants molar ratio affect the rate and extent of the MR, hence, the nature of the products formed and their functional properties (Pan & Melton, 2007; Scaman, Nakai, & Aminlari, 2006). The majority of research studies report glycation of proteins with saccharides at high temperatures or for an extended period of time which could lead to browning, odor formation, and irreversible loss of the

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protein structural integrity (Akhtar & Dickinson, 2007; Broersen, Voragen, Hamer, & Jongh, 2004; Hiller & Lorenzen, 2010; Jiang & Brodkorb, 2012; Li et al., 2009; Lillard, Clare, & Daubert, 2009; Mesa, Silván, Olza, Gil, & Castillo, 2008). On the other hand, there is scarce information on the effect of mild MR conditions on the degree of glycation and functional properties of proteins with different levels of glycation produced by this method. This is a relevant study topic since it has been reported that under such conditions (≤60 °C), the Amadori product formed in the initial stage is rather stable (Malec, Pereyra Gonzales, Naranjo, & Vigo, 2002). The present study was therefore aimed at evaluating the effect of temperature, time, relative humidity (RH), and reactants' molar ratio on the degree of glycation between WPI and maltodextrin (MD) in order to obtain mixtures with different levels of glycation which were then used to evaluate their functional properties.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) (92.7% protein content), BiPRO® Lot no. JE 214-9-420, from Davisco Foods International (Eden Prairie, MN, USA) was used in the preparation of protein solutions and emulsions. It was not denatured, lactose-free, and comprised mainly of β-lactoglobulin and α-lactalbumin, including as well small amounts of bovine serum albumin, immunoglobulins, glycomacropeptide, lactoferrin, lactoperoxidase and proteose peptones. Maltodextrin with an average molecular weight of 1672 Da, *o*-phthaldialdehyde (OPA, 79760), L-lysine, sodium bromide, ammonium sulfate, sodium tetraborate, sodium dodecyl sulfate (SDS), ethanol, and β-mercaptoethanol were purchased from Sigma–Aldrich Co. (Toluca, Edo de Mex, Mexico). All chemicals used in this study were of analytical grade.

2.2. Description of the study

The present investigation was divided in two parts. Initially, the effect of temperature (50, 60 °C), time (24, 48 h), RH (50, 80%), and amino/carbonyl ratio (1:1, 1:2) on the degree of glycation between WPI and MD was evaluated employing a 2^4 full factorial design, measuring the percentage of blocked lysine in each treatment as response variable with the OPA method. These conditions have shown to cause minimal changes to the protein structure (Chevalier, Chobert, Dalgalarrondo, Choiset, & Haertlé, 2002; Gauthier, Bouhallab, & Renault, 2001; Morgan, Leonil, Mollé, & Bouhallab, 1999; Morgan et al., 1999; Wooster & Augustin, 2007a). Color was also evaluated as an indirect measurement of the different stages of the MR. In the second part of the study, 3 glycoproteins (GP) with different levels of glycation (low, medium, and high) were selected. The solubility, foaming properties, and viscosity of the conjugates were evaluated and compared with their respective controls with the aim of knowing how many saccharide molecules need to be attached per molecule of protein in order to obtain improved functionality.

2.3. Preparation of Maillard conjugates

Appropriate amounts of WPI and MD at 1:1 (30 g of protein and 45 g of polysaccharide) and 1:2 (18.75 g of protein and 56.25 g of polysaccharide) amino:carbonyl ratio were dissolved in Milli-Q water and stirred for approximately 1 h at room temperature. Mixtures were then lyophilized, ground and the resulting powders were kept at 50 and 60 °C in desiccators previously equilibrated at 50 and 80% RH for 24 and 48 h. As controls, WPI and MD were treated separately under the same conditions as previously described. Lyophilized mixtures of the biopolymers without any treatments (Mixture 1:1, and Mixture 1:2) were evaluated as well.

2.3.1. Determination of degree of glycation

The degree of glycation was defined as the average number of lysine residues conjugated with MD moieties (Wooster & Augustin, 2007a) and was determined using the OPA colorimetric assay based on the method of Church, Swaisgood, Porter, and Catignani (1983). The OPA solution was prepared by dissolving 200 mg of OPA in 5 mL of pure ethanol and mixing this solution with 125 mL of 0.1 M sodium tetraborate buffer (pH 9.75), 0.5 mL of β-mercaptoethanol and 12.5 mL of 10% (w/v) SDS solution. Afterwards, the solution was diluted to a final volume of 250 mL with milli-Q water. The assay consisted of briefly mixing by inversion in a quartz cuvette 3 mL of OPA solution with 50 μL of sample solution (1 mg/mL) and measuring the absorbance at 340 nm after 1 min of incubation at room temperature. A standard curve was constructed using L-lysine by preparing a stock solution (1 mg/mL) and then diluting to concentrations ranging from 0.02 mg/mL up to 0.1 mg/mL with milli-Q water. The moisture content of each conjugate was taken into account when calculating the percentage of blocked lysine (Equation (1))

$$\text{Available amino groups} = \frac{\frac{x}{L_{mw}} * 2}{\frac{1}{P_{mw}}} \quad (1)$$

where x is the value obtained in the equation of the standard curve, L_{mw}, the Lysine molecular weight, and P_{mw}, the protein molecular weight.

2.3.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Polyacrylamide gel electrophoresis was performed under denaturing conditions following the method of Laemmli (1970), in a Bio-Rad Mini-Protean III apparatus, using a stacking gel of 10% and a running gel of 4% (w/v). Samples of 20 μg of protein were mixed (1:1) and denatured in 2X sample buffer (62.5 mM Tris–HCl, pH 6.8; 25% glycerol; 2% SDS; 0.01% Bromophenol Blue; 10% β-mercaptoethanol) at 95 °C for 3 min. Electrophoresis was carried out in tris-glycine-SDS buffer (pH 8.35) at room temperature for 30 min at 80 V, and for 2 h at 100 V. Subsequently, a fixing solution (40% methanol, 10% acetic acid, and 50% distilled water) was applied to gels, which were manually shaken for 15 min. Gels were rinsed with distilled water and stained with a Coomassie brilliant blue (CBB G-250) solution for 30 min. Finally, staining solution was removed with an acetic acid solution (10%) thrice within 24 h.

2.3.3. Color development

Color changes were determined using a Konica Minolta Chroma Meter CR-300 series (Konica Minolta Sensing, Inc., USA) with diffuse illumination/0° to obtain the CIE L^* a^* b^* values and then calculate the browning index (Equation (3)). The instrument was calibrated with a standard white tile ($Y = 88.2$, $x = 0.309$, $y = 0.316$) before measurements.

$$x = \frac{a + 1.75(L)}{5.645(L) + a - 3.012(b)} \quad (2)$$

$$\text{BI} = \frac{100(x - 0.31)}{0.172} \quad (3)$$

where L, a, and b are the values obtained in the colorimeter, BI is the browning index, and x is the value obtained in equation (2).