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Assessment of interfacial and foaming properties of bovine sodium caseinate glycated with galactose

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ABSTRACT

The impact of the initial and advanced stages of glycation of sodium caseinate (SC) with galactose on the interfacial and foaming properties has been investigated at pH 7 and 5. At pH 7, the most remarkable result was the higher stabilizing foam capacity of glycoconjugates as compared to native and heat treated SC, as a result of the higher elastic character and cohesion of the interfacial film formed by glycated SC. At pH 5, native and control heated SC underwent a significant loss of solubility, resulting in a worse dynamic of adsorption at the interface of such systems and the formation of fluid and poorly resistant films. However, solubility of glycated SC remained relatively high, so that, at this pH, only SC glycoconjugates showed interfacial characteristics suitable to stabilize the foam during its formation as well as against mechanisms of foam destabilization at long term. This behavior was attributed to the higher adsorption efficiency and degree of interfacial interaction exhibited by the SC glycoconjugates. These findings highlight the beneficial effect of glycation on the foaming properties of SC which could contribute to broadening the applicability of SC as a foaming agent, mainly in acid foods.

1. Introduction

The interfacial behavior of proteins is important in determining quality attributes of many foods such as milk, meat, mayonnaise, spreads, ice cream, frozen desserts, cakes, breads, and whipped toppings, since their structure depends upon the formation and stability of interfacial films which facilitate mixing, impart structure and contribute to sensory qualities and consumer perception of food quality (Martínez et al., 2009).

Among milk proteins, bovine sodium caseinate (SC), a more soluble form of casein, is extensively used in food industry as a functional ingredient in a wide variety of food products due to its simple production, excellent nutritional value, and versatile functional properties, including heat stability, water-holding capacity, fat binding, rheological properties (increase of viscosity and gel formation) and foaming, emulsifying, and stabilizing capacities (Dalgleish, 1997; Dickinson, 2003). Particularly, due to its pronounced amphiphilic nature, flexible structure, and relatively small molecule size, SC shows high capacity to adsorb at the air/water interface, lowering the interfacial tension and producing foams with good overrun (Rodríguez Patino and Carrera, 2004; Carrera and Rodríguez Patino, 2005; Rodríguez Patino et al., 2007). Nevertheless, due to the high hydrophobic character of SC, intermolecular interactions at the interface will not be very strong, giving rise to lamellar layers between the air bubbles with a viscosity and rigidity lower than those produced by other proteins able to interact at the interface through disulfide bridges such as β-lactoglobulin (β-Lg). Consequently, SC results in interfaces without a mechanical strength large enough to stabilize the foam, which breaks down shortly after its formation (Dalgleish, 1997). For this reason, SC is not widely used on its own as foaming agent in food formulations. In addition, the use of SC as foaming agent is mainly impaired in foods whose pH is close to its isoelectric point (pI 4.6), since the protein capacity to be adsorbed at the air/water interface can be decreased due to the loss of protein solubility (Damodaran, 1997). In this context, the search for methods that can efficiently improve the foam stabilizing capacity of SC, especially at pH values close to its pI, and, therefore, increase its degree of applicability is of growing interest.

Thus, with the aim of enhancing its foaming properties and expanding its industrial application, physical, enzymatic and chemical modifications have been applied to change SC conformation and physicochemical properties such as flexibility, hydrophilicity, viscosity, and charge. Among the studied chemical modifications, a great deal of attention has been focussed on the covalent interaction protein/carbohydrate via the Maillard reaction (MR) (glycation).

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During this reaction, the conjugation of the carbonyl group of a reducing carbohydrate with an available, unprotonated amino group, mainly the ε-amino group of the lysine, occurs spontaneously under heating conditions, without the need for chemical catalysis (Chevalier et al., 2001a), being, therefore, a food-grade process (Oliver et al., 2006a). Moreover, it is well-known that the Maillard reaction, carried out under dry state and well controlled conditions (temperature, relative humidity and time), is an adequate method for improving functionality of proteins without important structural changes (Morgan et al., 1997). In particular, several studies have shown that glycation of SC under controlled conditions improves its rheological (Oliver et al., 2006b; Corzo-Martinez et al., 2010) and emulsifying properties (Shepherd et al., 2004; Morris et al., 2004; Oliver et al., 2006b; Fechner et al., 2007; Corzo-Martínez et al., 2011). However, to the best of our knowledge, there are no reported works in the literature that evaluate the impact of glycosylation via the MR of SC on the interfacial and foaming properties of this protein.

Concerning the reducing sugar used, particularly interesting could be the case of the conjugation of SC with galactose (Gal), which is isomerised to tagatose (Tag) during the early stages of the MR, giving rise to the corresponding Amadori compound, tagatosyl-lysine. Taking into account the food interest of tagatose, especially as prebiotic sugar, the Amadori compound tagatosyl-lysine derived from glycation with Gal could be expected to have the same or, even, better functional properties than Tag. Structural characterization of glycoconjugates SC:Gal [4 h at 60 °C] and [72 h at 50 °C] have been addressed in a previous work (Corzo-Martinez et al., 2010). Likewise, in another work carried out recently in our research group (Corzo-Martínez et al., 2012a), we have found that SC glycated with Gal at 60 °C for 4 h, with a high content in the Amadori compound tagatosyl-lysine, favored in greater extent than Tag the growth of twelve potential probiotic strains of *Lactobacillus, Streptococcus* and *Bifidobacterium*.

The aim of this work was, firstly, to study the effect of glycation with galactose on the adsorption of SC at the air/water interface and to characterize the rheological properties of the interfacial films at pH values of 7 and 5. Then, an evaluation of foaming properties (foamability and foam stability) of SC glycoconjugates in relation to their interfacial behavior was carried out.

2. Materials and methods

2.1. Materials

Galactose (Gal) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and sodium caseinate (SC) (Rovita FN 5) was obtained from Proveedora Hispano Holandesa, S.A. (Barcelona, Spain). All other reagents were of analytical grade.

2.2. Preparation and purification of sodium caseinate–galactose glycoconjugates

Gal and SC in a weight ratio of 0.2:1 were dissolved in 0.1 M sodium phosphate buffer, pH 7.0 (Merck) and lyophilized (Corzo-Martinez et al., 2010). The SC–Gal powders were kept at 60 and 50 °C for 4 and 72 h, respectively, under vacuum in a desiccator equilibrated at 0.67 a_w (Oliver et al., 2006b), achieved with a saturated solution of $CuCl_2$ (Sigma–Aldrich, St. Louis, MO, USA). In addition, control experiments were performed with SC stored at 50 and 60 °C without Gal during the same periods (control heated SC).

After incubation, the products were reconstituted in distilled water to a protein concentration of 1 mg mL^{-1}. To remove free carbohydrate, 2 mL portions were ultrafiltered through hydrophilic 3 kDa cut-off membranes (Centricon YM-3, Millipore Corp., Bedford, MA) by centrifugation at 1548*g* for 2 h. After removal of free Gal, samples were lyophilized and stored at −20 °C for further analysis.

Incubations were performed in duplicate, and all analytical determinations were carried out at least in duplicate.

2.3. Determination of the surface hydrophobicity of sodium caseinate glycoconjugates

The surface hydrophobicity (S_0) of native, control heated and glycated SC was investigated by binding of 8-anilino-l-naphthalenesulfonate (ANS). The relative fluorescence intensity (FI) of the ligand–protein conjugates was measured on a Shimadzu RF-1501 fluorescence spectrophotometer at room temperature. The wavelengths of excitation (λ_{exc}) and emission (λ_{em}) were 390 and 470 nm, with slit widths of 10 nm. Native, control heated, and glycated SC samples were diluted with 0.1 M sodium phosphate buffer, pH 7.4, to a final concentration of 0.1 mg mL^{-1}. Then 10 μL of ANS solution (8.0 mM in 0.1 M sodium phosphate buffer, pH 7.4) was added to 1 mL of the diluted sample, the resulting solution mixed and equilibrated for 2 min and, finally, the fluorescence intensity measured at room temperature. Solution of ANS in sodium phosphate buffer was prepared daily. All measurements were performed at least in duplicate.

2.4. Solubility of sodium caseinate glycoconjugates

For solubility evaluation, solutions of native, control and glycated SC, dissolved in distilled water (1 mg mL^{-1}), were adjusted to pH 5 and 7 using HCl or NaOH 1 N. After 30 min of stirring at room temperature, the samples were centrifuged for 15 min at 4 °C and 15,000*g*. The protein content in the supernatants was determined by measuring the absorbance at 280 nm (A_{280}) in a Beckman DU 70 spectrophotometer (Beckman Instruments Inc., Fullerton, CA) and the solubility was expressed as the percentage of the total protein content, considering as 100% the A_{280} of native SC.

2.5. Interfacial properties measurement

Interfacial properties of native, control heated and glycated SC were determined at both pH 7 and pH 5. For this, samples were dissolved in trizma–HCl buffer (0.05 M, pH 7.0) or acetic acid–acetate buffer (0.05 M, pH 5) (Sigma–Aldrich, St. Louis, MO) to give a final protein concentration of 5 mg mL^{-1}, a little lower than that required for the interface saturation by sodium caseinate (Rodríguez Niño et al., 2001) and, hence, concentration at which the foaming capacity of a sodium caseinate solution is maximum.

Time-dependent surface pressure and surface dilatational measurements of native, control heated and glycated SC adsorbed films at the air/water interface were performed with an automatic pendant drop tensiometer (TRACKER, IT Concept, Longessaigne, France) as already described (Rodríguez Patino et al., 1999; Rodríguez Niño and Rodríguez Patino, 2002). The method involves a periodic automated-controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume at a given amplitude ($\Delta A/A$) and angular frequency (ω), and the response of the surface pressure (π, mN m^{-1}) is monitored throughout the experiment, being:

$$\pi = \sigma^0 - \sigma \quad (1)$$

where σ^0 is the surface tension of aqueous solution, in the absence of protein (σ^0 = 72.5 mN m^{-1}), and σ (mN m^{-1}) is the surface tension in the presence of protein.

At low surface concentrations, the surface pressure is low (π < 10 mN/m), and molecules adsorb irreversibly by diffusion. In the case of diffusion controlled adsorption, the diffusion is driven