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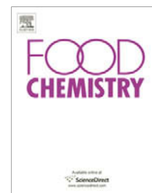

The effect of glycation on foam and structural properties of β-lactoglobulin

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ABSTRACT

The goal of the present work was to evaluate whether the Maillard reaction, with glucose and lactose as substrates, improves the foaming properties of β-lactoglobulin. Lactose led to the lowest degree of modification without significant differences by reaction time and by protein:sugar molar ratio. However, in the case of glucose, the degree of glycation increases with reaction time and molar ratio. The results obtained by UV fluorescence, surface hydrophobicity and differential scanning calorimetry clearly showed differences in the degree of folding of β-lactoglobulin upon modification with different sugars or thermal treatment, with changes in the foaming capacity of β-lactoglobulin. All the modified samples exhibited a significant increase ($\alpha \leqslant 0.05$) in draining stability (Kg) as compared to the non-thermally treated sample. In addition, foams formed by lactose-glycated samples were more stable than those formed by glucose-glycated ones. A significant increase ($\alpha \leqslant 0.05$) of foam stability with reaction time was also detected, particularly in glucose-glycated samples.

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1. Introduction

During recent years, a great deal of attention has been focussed on improving the functional properties of whey proteins by different methods, including the Maillard reaction. During this reaction, the conjugation of a reducing carbohydrate to the ε-amino group of lysine occurs spontaneously under heating conditions without the utilization of toxic chemical products (Chevalier, Chobert, Dalgalarrondo, & Haertle, 2001a).

It is 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, Leonil, Molle, & Bouhallab, 1997). Recent studies have shown that functional properties of β-lactoglobulin, such as thermal stability and emulsifying and foaming capacity, are improved after modification by the Maillard reaction, depending on the sugar used during modification (Chevalier, Chobert, Popineau, Nicolas, & Haertle, 2001b; Moreno, Lopez-Fandiño, & Olano, 2002). Glycated β-lactoglobulin used as a food ingredient may also diminish oxidative reactions and/or can influence cellular and microbial growth (Morgan, Leonil, Molle, & Bouhallab, 1999a; Nakamura, Kato, & Kobayashi, 1991). β-Lactoglobulin has been conjugated with mono- and disaccharides to improve its functional properties, such as solubility and emulsifying capacity (Chevalier, Chobert, Dalgalarrondo, Choiset, & Haertle, 2002; Fenaille, Morgan, Parisod, Tabet, & Guy, 2003; Matsuda, Kato, & Nakamura, 1991; Rada-Mendoza, Villamiel, Molina, & Olano, 2006).

Since structural changes, such as those involved in denaturation processes, may influence the reactivity of proteins, it is important to know how different conditions may affect the susceptibility of different proteins to the Maillard reaction. The goal of the present study was to investigate the effect of glycation of β-lactoglobulin with glucose and lactose and the influence of different conditions of reaction (temperature, time, molar ratio) on the structural changes of the molecules and its foaming properties.

2. Materials and methods

2.1. Materials

β-Lactoglobulin isolate from Davisco (Le Sueur, MN) was used in all experiments performed. All other chemicals and reagents were of analytical grade from Sigma Chemical Co. (St. Louis, MO).

2.2. β-Lactoglobulin glycation

Glycation was performed with glucose (G) and lactose (L) in the following conditions. β-Lactoglobulin (β-Lg) (0.91 mM and 0.091 mM) was dissolved in 9.1 mM glucose in water, and the pH was adjusted to 7 with 50 mM phosphate buffer. After being freeze-dried, the protein–sugar powders were kept under 65% relative humidity (saturated KI solution) and 50 °C for periods of 51 and 96 h (1:10 96 h, β-Lg:G (sample 4); 1:10 51 h, β-Lg:G (sample 5); 1:100 96 h, β-Lg:G (sample 6); 1:100 51 h, β-Lg:G (sample 7)).

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The same protocol was followed for lactose treatment (1:10 96 h, β-Lg:L (sample 8); 1:10 51 h, β-Lg: L (sample 9); 1:100 96 h, β-Lg:L (sample 10); 1:100 51 h, β-Lg:L (sample 11)). After these treatments the powders were dissolved in distilled water and the free glucose or lactose were removed by extensive dialysis (molecular mass cut-off: 8–10 kDa) against 20 mM ammonium carbonate at 5 °C. The dialyzed solutions were freeze dried and the powders were stored at −20 °C until used. Control samples used in the present study were obtained by incubation of the protein without G and L during the same reaction times (51 and 96 h, respectively) (β-Lg c tt 51 h (sample 2), β-Lg c tt 96 h (sample 3)). β-Lactoglobulin without heat treatment was used too as a control (β-Lg stt (sample 1)). All glycation experiments were performed in triplicate.

2.3. Determination of the extent of glycation by MALDI-TOF mass spectrometry

For MALDI-TOF-MS analysis, samples were diluted in a saturated solution of R-cyano-4-hydroxycinnamic acid in 0.1% TFA with 33% acetonitrile. An aliquot of this mixture was spotted onto a stainless steel target, air-dried, and subjected to mass determination using a Voyager DE-PRO MALDI-TOF-MS (Perspective/Applied Biosystems, EUA). The instrument was equipped with a nitrogen laser (λ337 nm) and a reflector. Measurements were performed using delayed extraction. Laser-desorbed positive ions were analysed after acceleration by 19 kV in the reflector mode. External calibration was performed by use of a standard peptide/protein mixture. Each digest was spotted on at least three individual target positions, and 100 individual spectra of each spot were averaged to produce a mass spectrum. The monitored mass range was m/z 10,000–40,000.

2.4. Surface hydrophobicity

Surface hydrophobicity of native and glycated β-Lg was evaluated using ANS (8-anilino-1-naphthalenesulfonic acid) as fluorescent probe. Spectrofluorometric measurements were taken at pH 7.5 on an Aminco-Bowman Series 2 Luminescence spectrometer. The fluorescence intensity of the blank (FIb) and of the ANS-protein conjugate (FIe) were recorded at λ_{ex}: 363 nm and λ_{em}: 475 nm, using 5-nm emission and excitation slit widths. The surface hydrophobicity (S_o) was obtained graphically using the Kato and Nakai equation (Kato & Nakai, 1980). A plot of (FI%) versus (PC) was drawn, where (PC) is the protein concentration; (FI%) = (FIN)/(FI$_{max}$); (FIN) = (FIe) − (FIb); FI$_{max}$ is the maximum fluorescence measured from the total binding of ANS in methanol.

2.5. UV fluorescence

Spectra were obtained with a Luminescence spectrometer Aminco Bowman Series 2 (USA). A spectral scanning was performed between 310 and 560 nm using an excitation wavelength of 295 nm and a scanning rate of 300 nm/min. The maximal emission wavelength of the samples and the maximal fluorescence intensity were determined using tryptophan as standard. Samples of modified and unmodified protein were dissolved in 0.01 M sodium phosphate buffer pH 7.5 to obtain protein concentrations of 0.03 mg/ml. All the assays were performed in triplicate.

2.6. Thermal stability and degree of native conformation measurement by differential scanning calorimetry (DSC)

DSC was performed with a Polymer Laboratories PL – DSC (Rheometric Scientific DSC 2, Ltd, Epsom, England) device previously calibrated with indium. The thermal behaviour of β-lactoglobulin subjected or not to thermal treatment and with different degrees of glycation was studied. All determinations were performed at least in duplicate, using aqueous dispersions of the samples (20% w/v). Hermetically sealed aluminium pans were prepared to contain 12–16 mg of freeze-dried samples suspended in water (200–250 mg l^{-1}). The samples were scanned at 10 °C min^{-1} from 25 to 120 °C. As a reference, a pan with β-Lg, previously subjected to the DSC run was used. After each run, the pans were punctured and their dry matter content determined by leaving the pans overnight in an oven at 105 °C. The denaturation onset temperature (T_o), maximal deflection temperature (T_d), and denaturation enthalpy calculated as the area under the thermogram limited by the baseline were obtained by analysing second peak of each thermograms with a Software Plus V5.41.

2.7. Electrophoresis

Electrophoretic runs were performed on polyacrylamide gels with Tricine–Sodium Dodecyl Sulfate (Tricine–SDS–PAGE) according to (Schagger & Von Jagow, 1987). Stacking, spacer and separating gels of 4%, 10% and 16.5% acrylamide, respectively, were prepared. Protein samples were dissolved in sample buffer containing 4% SDS, 12% glycerol (w/v), 50 mM Tris, 2% mercaptoethanol (v/v), and 0.01% Serva Blue adjusted with HCl to pH 6.8. The molecular weight markers (Biorad SDS-calibration kit) were Triosephosphate isomerase (26,625 kDa), Myoglobine (16,950 kDa), Lactoalbumin (14,437 kDa), Aprotinin (6,512 kDa), Insulin b chain oxidized (3,496 kDa) and Bacitracin (1,423 kDa).

2.8. Protein solubility

The solubility index of all the unmodified and glycated samples was calculated as the ratio between the soluble protein concentration (mg/ml) *100 and the total protein concentration (mg/ml). Protein concentration was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and the Kjeldahl method using a nitrogen:protein conversion factor of 6.25. Samples were solubilized in 0.01 M phosphate buffer pH 7.5 during 30 min at room temperature and centrifuged 15 min at 10,000*g* at 5 °C before measuring protein concentration in the supernatant.

2.9. Surface tension

Surface tension (σ) measurements at the air/water interface were performed with a drop volume tensiometer Lauda TVT2 (Lauda Dr. R. Wobser GMBH & CO. KG Lauda-Königshofen Germany) with injection syringe of 2.5 ml. The conditions of the assay were as follows: the run was started with a drop formation interval of 0.07–0.10 s/μl, number of measurement cycles and number of drops per cycle 6 × 3 by the dynamic method with drop retention; the run followed with a drop formation interval of 0.10–0.80 s/μl, number of measurement cycles and number of drops per cycle 9 × 3, dynamic method with drop retention; and finished with a determination in the quasistatic mode without drop retention under the conditions dV_1 = 10.0% and dV_x = 1.0%, maximal measurement time 600 s, number of measurement cycles and number of drops per cycle 9 × 3. The assay was carried out on sample dilutions at 1 mg/ml concentration in 0.01 M phosphate buffer at pH 7.5.

2.10. Colour

The colour of samples was measured with a tristimulus colorimeter, Chromater CR 300 series, and data processor DP-30, Minolta Co. Ltd., Japan. The parameters *L*, *a* and *b* were determined in all cases.

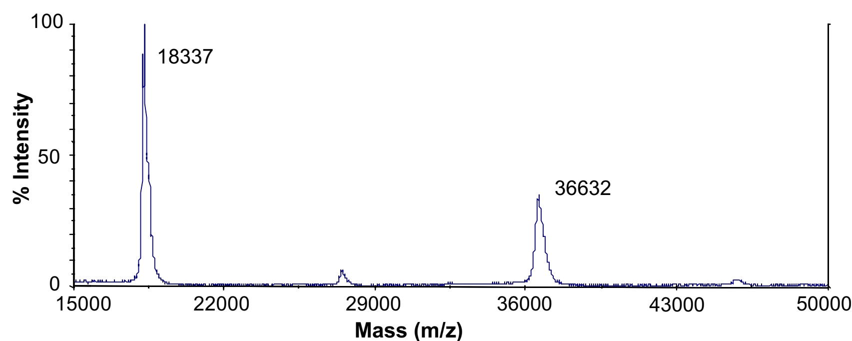

Fig. 1. MALDI-TOF MS spectrum of β-Lg stt.

2.11. Foaming properties

The foaming properties of untreated and glycated β-Lg were determined by conductimetry using the method and device developed by Loisel, Guéguen, and Popineau (1993). Foam was formed by air sparging into the protein solution in a column with a fretted glass disk at the bottom. The foaming solutions were prepared at 1 mg/ml in 0.01 M sodium phosphate buffer pH 7.5. The level of the solution as a function of time was measured by conductimetry with a pair of electrodes located at the base of the column. To evaluate the foaming capacity, the maximal volume of liquid retained in the foam (VLE_{max}) and the initial rate of liquid transfer to the foam (v_0) were measured. Foam stability was estimated from the rate constants of gravitational drainage (k_g) and gas diffusion or disproportion (k_d).

2.12. Statistical analysis

All glycation experiments were performed in triplicate. All glycation samples and control samples were analysed by triplicate. The statistical analysis was established by Variance analysis and Test of minimum significant difference, using statistical program StatgraphicPlus7.0.

3. Results and discussion

3.1. Characterization of the native and glycated β-lactoglobulin

The progress of the Maillard reaction was monitored by measuring the mean value of carbohydrates linked to β-Lg after treatment under the different assay conditions. Such values were obtained by calculating the difference between the spectra of the sample and the control (β-Lg stt (1)). The MALDI-TOF MS spectrum of β-Lg stt (Fig. 1) exhibited the peaks corresponding to the monomeric (18,300 kDa) and the dimeric (36,705 kDa) forms characteristic of β-Lg at pH 7.4.

Table 1 shows the mean number of sugar residues bound per β-Lg monomer under each assay condition. A clear difference can be observed between the behaviour of samples modified with glucose and those modified with lactose. For glucose modification, the number of sugar residues per protein molecule increased significantly with the molar ratio and the reaction time (from 7 to 15 and from 8 to 19, respectively). For lactose modification, in contrast, none of the parameters analysed was related to the number of sugar residues per protein molecule; the mean number of residues was 7 in the four experimental conditions used. The later value agrees with those reported by French, Harper, Kleinholz, Jones, and Green-church (2002) and Morgan et al. (1997) for similar assay conditions (50 °C and 96 h of reaction time).

Table 1
Mean number of sugar residues bound per β-lactoglobulin monomer, surface hydrophobicity (S_o), denaturation onset temperature (T_o), denaturation enthalpy (ΔH) and maximal deflection temperature (T_d) of the monomer in each assay condition

Sample	Mean number of sugar residues bound per β-lactoglobulin monomer	S_o	T_o (°C)	T_d (°C)	ΔH (mJ/mg)
β-Lg s tt	–	37 ± 1^a	66 ± 2^a	74 ± 2^a	9 ± 2^a
β-Lg c tt 96h	–	28 ± 3^b	62 ± 1^a	72 ± 1^a	8 ± 2^a
β-Lg c tt 51h	–	$30 \pm 3^{a,b}$	64 ± 1^a	73 ± 1^a	8 ± 2^a
1:10 96 β-Lg:G	8	12 ± 4^c	62 ± 3^a	78 ± 3^b	3 ± 1^b
1:10 51 β-Lg:G	7	17 ± 4^c	70 ± 4^a	79 ± 3^b	9 ± 1^a
1:100 96 β-Lg:G	19	16 ± 4^c	63 ± 2^a	78 ± 1^b	5 ± 2^b
1:100 51 β-Lg:G	15	14 ± 4^c	70 ± 4^a	86 ± 1^c	5 ± 2^b
1:10 96 β-Lg:L	7	35 ± 1^a	68 ± 1^a	$82 \pm 1^{b,c}$	9 ± 1^a
1:10 51 β-Lg:L	6	40 ± 5^a	68 ± 3^a	$80 \pm 1^{b,c}$	8 ± 1^a
1:100 96 β-Lg: L	8	$30 \pm 5^{a,b}$	68 ± 4^a	85 ± 1^c	5 ± 1^b
1:100 51h β-Lg:L	7	38 ± 8^a	68 ± 4^a	77 ± 1^b	6 ± 1^b

Values are means of triplicates.
Mean value with different letters were significantly different ($P < 0.05$).

The lower number of lactose residues than glucose residues bound to β-Lg may be attributed to the number of lysine residues available for reaction with lactose in the β-Lg molecule (9 residues) in relation to the total number of lysine residues (15 residues) (Fogliano, Monti, Visconti, Randazzo, & Facchiano, 1998).

3.2. Colour

To evaluate the colour of modified and unmodified samples, the following parameters were determined:

$$H^* = t_g - 1(b/a), \quad C^* = (a^2 + b^2)^{1/2}$$

As shown in Fig. 2, colour intensity (C^*) increased significantly ($\alpha \leqslant 0.05$) with reaction time especially in the presence of glucose and less S_o in the presence of lactose. Glycated compounds exhibited a greater shift to red (a) and yellow (b).[1] The generation of coloured compounds was maximal at the longest reaction time assayed (96 h) and did not change when the molar concentration of sugar was increased. Thermal treatment did not modify the colour of the protein.

3.3. Characterization of modified samples by surface hydrophobicity (S_o), UV fluorescence, DSC and electrophoresis

The S_o values of the different samples are shown in Table 2. Binding of glucose to β-Lg produced a significant ($\alpha \leqslant 0.05$) reduc-

[1] For interpretation of color in Fig. 2, the reader is referred to the web version of this article.

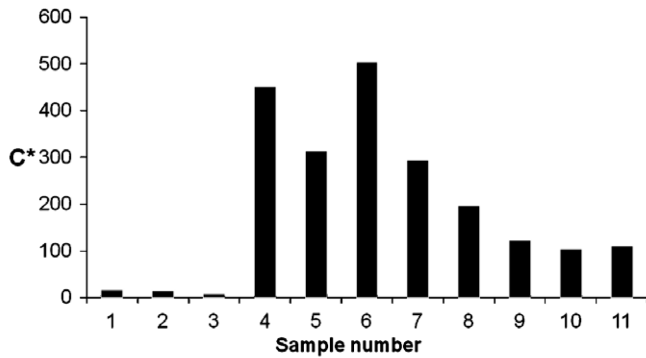

Fig. 2. Colour intensity, C^*, corresponding to the different samples assayed: **1.** β-Lg s tt, **2.** β-Lg c tt 51 h, **3.** β-Lg c tt 96 h, **4.** 1:1 0 96 β-Lg:G, **5.** 1:10 51 β-Lg:G, **6.** 1:100 96 β-Lg:G, **7.** 1:100 51 β-Lg:G, **8.** 1:10 96 β-Lg:L, **9.** 1:10 51 β-Lg:L **10**. 1:100 96 β-Lg:L, **11.** 1:100 51 β-Lg:L.

tion of S_o (two-fold to three-fold as compared to unmodified protein) but this did not happen with lactose binding. This fact suggests that the number of sites on the protein surface available for binding the fluorescent probe is lower in the case of lactose, which may imply the existence of protein aggregation mediated by the creation of disulfide bonds and, consequently, the folding of the β-Lg molecule.

Fig. 3 shows the UV fluorescence spectra as a function of wavelength. The insert depicts the variation in maximal UV fluorescence intensity of the analysed samples as a function of wavelength. A diminished intensity of maximal fluorescence for the samples treated with glucose was observed, which is probably due to a masking of aromatic amino acid residues by the aggregation phenomenon mentioned above. A shift of the maximal emission wavelength from 331 to 334 nm was also detected. The location of the emission maximum of tryptophan residues in the fluorescence spectrum ranges between 307 and 353 nm. Proteins in their native state present a tryptophan emission maximum at a low wavelength (307 nm), while its shift to higher wavelengths (353 nm) indicates protein denaturation. These results are according to those described by (Hattori, Ametani, Katakura, Shimizu, & Kaminogawa, 1993 and Kaminogawa et al., 1989), who observed a slight red shift of the maximum emission of β-Lg glycated attributed to conformational changes around the tryptophan residues (Trp19 and Trp61). The maximal fluorescence intensity of β-Lg samples treated with lactose did not change significantly as compared to control samples, and the same happened regarding surface hydrophobicity. Since UV fluorescence spectra differed significantly ($\alpha \leqslant 0.05$), it can be speculated that, while the number of hydrophobic groups is equivalent, the folding of the protein after lactose binding may differ from that produced after glucose binding.

The characteristic β-Lg thermogram showed two endotherms at 40 °C and 73 °C, respectively, which are attributed to the dimeric and monomeric forms present at pH 7.5 (data non shown). The dissociation of the dimeric form occurs at the lowest temperature, followed by denaturation of the monomeric forms. These results agree with those reported by other authors (Akita & Nakai, 1990; Morgan, Venien, Bouhallab, Molle, & Leonil, 1999b). The temperature of maximal deflection corresponding to the second endotherm is shown in Table 2. An increase of the T_d of the monomer ($\alpha \leqslant 0.05$) with glycation can be observed, especially when lactose is used as substrate. The denaturation enthalpy (ΔH) diminished significantly ($\alpha \leqslant 0.05$) in the sample treated with glucose, which may be related to the folding and aggregation state of β-Lg in these

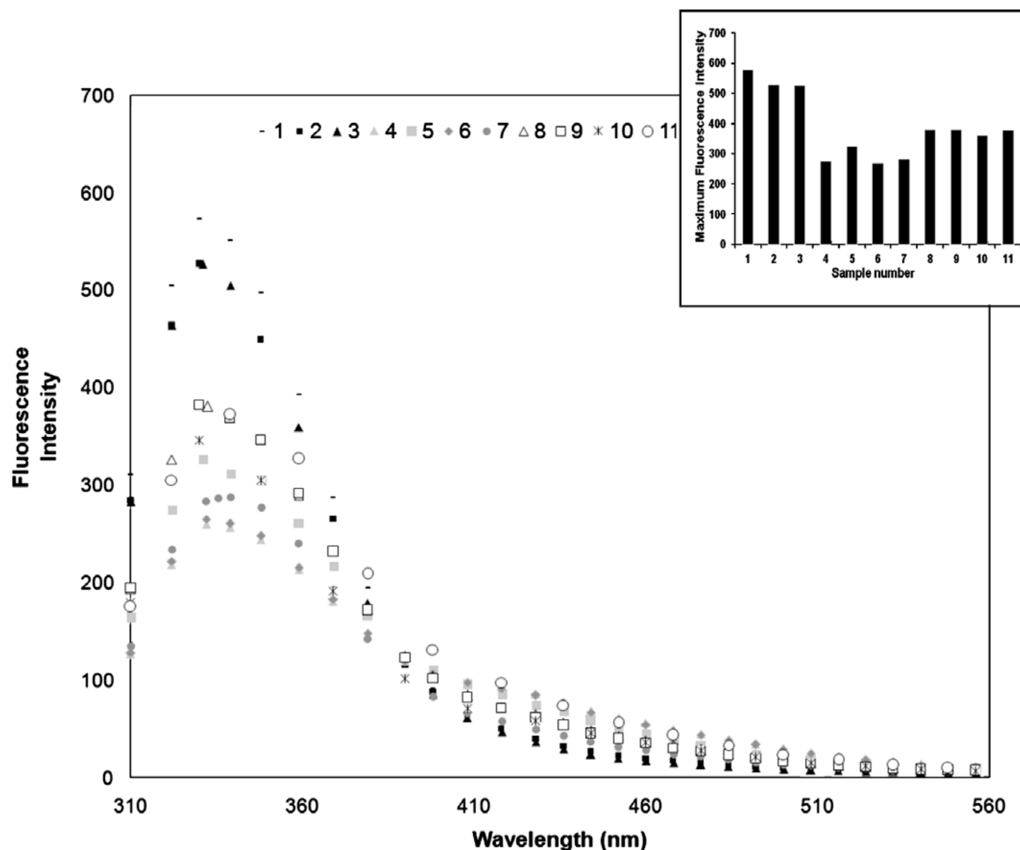

Fig. 3. Fluorescence emission spectra and maximum fluorescence intensity (insert) of the different samples assayed: 1. β-Lg s tt 2. β-Lg c tt 51 h 3. β-Lg c tt 96 h 4. 1:10 96 β-Lg:G 5.1:10 51 β-Lg:G 6.1:100 96 β-Lg:G 7.1:100 51 β-Lg:G **8.** 1:10 96 β-Lg:L **9.**1:10 51 β-Lg:L **10.**1:100 96 β-Lg:L **11.**1:100 51 β-Lg:L.

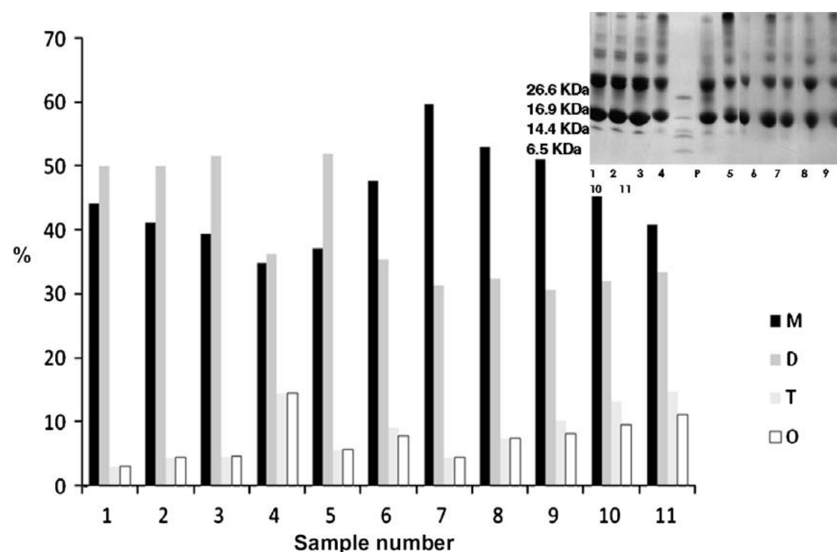

Fig. 4. Electrophoretic runs (Tricine–SDS–PAGE) and percentage of monomers, dimers, tetramers and octamers calculated from the electrophoretic runs – shown in the insert- of the different samples assayed: **1.** β-Lg s tt, **2.** β-Lg c tt 51 h, **3.** β-Lg c tt 96 h, **4.** 1:1 0 96 β-Lg:G, **5.** 1:10 51 β-Lg:G, **6.** 1:100 96 β-Lg:G, **7.** 1:100 51 β-Lg:G, **8.** 1:10 96 β-Lg:L, **9.** 1:10 51 β-Lg:L **10**. 1:100 96 β-Lg:L, **11.** 1:100 51 β-Lg:L.

assay conditions. For lactose modification, significant differences ($\alpha \leqslant 0.05$) of T_d are also observed with reaction time. In addition, the denaturation enthalpy decreased significantly ($\alpha \leqslant 0.05$) when the protein:glucose molar ratio was 1:100.

Fig. 4 shows the percentage of monomers, dimers, tetramers and octamers calculated from the electrophoretic runs depicted in the insert of the figure. Glucose binding at the lowest molar ratio produced a decrease of dimers and monomers in favor of the formation of tetramers and octamers, while at the higher molar ratio dimers were diminished and the other molecular forms were increased. The percentage of octamers was significantly higher ($\alpha \leqslant 0.05$) when the protein was modified with lactose.

3.4. Functional properties of modified and unmodified samples

3.4.1. Solubility

The solubility index of β-Lg and the glycated β-Lg samples was 72% equivalent in all the reaction conditions assayed ($\alpha \leqslant 0.05$) (results not shown).

3.4.2. Surface tension

The experimental data were adjusted with a first order equation with two exponential components developed by Panizzolo (2005), and the first order rate constants for the adsorption (K_a) and rearrangement (K_r) processes of the proteins in the air–water interface were estimated:

$$\gamma_t = \gamma_a e^{-k_a t} + \gamma_r e^{-k_r t} + \gamma_e$$

where k_a and k_r: first order rate constants for adsorption and rearrangement of proteins at the air/water interface. γ_a, γ_r and γ_e: correlation factors between surface tension and protein concentration for the different conformational states of adsorption, rearrangement and equilibrium in the interface.

The surface tension under equilibrium (σ_e) was also determined, which in the case of water at room temperature was 72 mN/m, and in the case of β-Lg, either thermally treated or not, dropped to 49–50 mN/m. When the assay was applied to the samples modified with glucose or lactose no significant differences of surface tension were observed between these samples or in comparison to β-Lg thermally treated or not ((β-Lg stt (1), β-Lg c tt 51 h (2), β-Lg c tt 96 h (3)). Binding of lactose or glucose to β-Lg did not modify its capacity to reduce the surface tension in the air–water interface. In all cases, the calculated k_a value was 100 times higher than that of k_r, indicating that the major contribution to the reduction of surface tension comes from the adsorption process and not from the rearrangement of the protein in the interface.

Adsorption rate values for β-Lg samples subjected or not to thermal treatment are shown in Fig. 5. A significant diminution of ka ($\alpha \leqslant 0.05$) with heating time can be observed for β-Lg c tt 51 h (2), β-Lg c tt 96 h (3), which suggests that adsorption to the interface, is slowed down respect to those corresponding to the β-Lg stt (1). In addition, the k_a/k_r value of the thermally treated β-Lg was also significantly lower ($\alpha \leqslant 0.05$), indicating that the reduction of surface tension during rearrangement of β-Lg c tt in the interface continued for a longer time and contributed more to the reduction of interface tension that in the case of β-Lg s tt (not shown).

The proteins modified with glucose and lactose at a lower reaction time and the lower molar ratio, 1:10 51 h, β-Lg:G (5); 1:10 51 h, β-Lg:L (9), did not exhibit significant differences ($\alpha \leqslant 0.05$) with the non glycated sample submitted to an equal thermal treatment

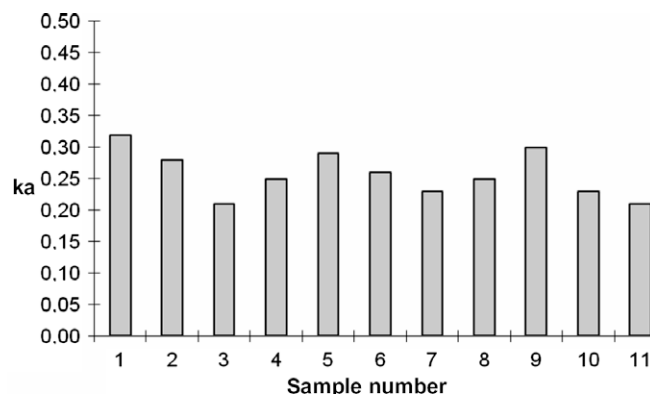

Fig. 5. Calculated values of the protein adsorption rate to the air/water interface, K_a, for the different samples assayed: **1.** β-Lg s tt, **2.** β-Lg c tt 51 h, **3.** β-Lg c tt 96 h, **4.** 1:1 0 96 β-Lg:G, **5.** 1:10 51 β-Lg:G, **6.** 1:100 96 β-Lg:G, **7.** 1:100 51 β-Lg:G, **8.** 1:10 96 β-Lg:L, **9.** 1:10 51 β-Lg:L **10**. 1:100 96 β-Lg:L, **11.** 1:100 51 β-Lg:L.

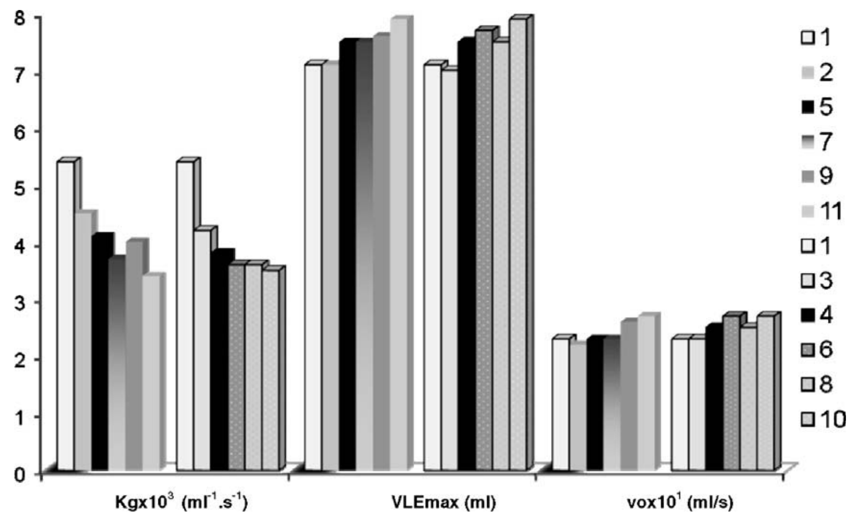

Fig. 6. Parameters related to the foaming capacity, vo and VLEmax, and foam stability, calculated kg values, corresponding to the foams formed by different samples assayed: 1. β-Lg s tt, **2**. β-Lg c tt 51 h, **5**. 1:10 51 β-Lg:G, **7**. 1:100 51 β-Lg:G, **9**. 1:10 51 β-Lg:L, **11**. 1:100 51 β-Lg:L, **1**. β-Lg s tt. **3**. β-Lg c tt 96 h, **4**. 1:1 0 96 β-Lg:G, **6**. 1:100 96 β-Lg:G, **8**. 1:10 96 β-Lg:L, **10**. 1:100 96 β-Lg:L.

($\alpha \leqslant 0.05$). However, significant differences were detected between these samples, 1:10 51 h, β-Lg:G (5); 1:10 51 h, β-Lg:L (9), and the rest of glycated proteins being their k_a value higher than the rest. Samples 1:10 96 h, β-Lg:G (4); 1:100 96 h, β-Lg:G (6); 1:100 51 h, β-Lg:G (7); 1:10 96 h, β-Lg:L (8); 1:100 96 h, β-Lg:L (10); 1:100 51 h, β-Lg:L (11) did not presented significant differences ($\alpha \leqslant 0.05$) in the rate of adsorption to the interface.

3.4.3. Foaming properties

In order to estimate the stability of the foams formed, experimental data were adjusted to the second order equation of two terms proposed by Panizzolo (2005)

$$V(t) = V_g^2 k_g t/(V_g k_g t + 1) + V_d^2 k_d t/(V_d k_d t + 1)$$

where k_g and k_d are the rate constants corresponding to the gravitational draining and gas diffusion or disproportion, respectively.

Fig. 6 shows the parameters related to the foaming capacity, vo and VLEmax, and the calculated values of k_g of foams formed with glycated β-Lg to different extents. Regarding foaming capacity no significant differences were found in the maximal retained liquid volume (VLE_{max}) or the initial rate of liquid to foam passage (v_0) between β-Lg s tt (1) and the samples submitted to the thermal treatment, β-Lg c tt 51 h (2) and β-Lg c tt 96 h (3). However, VLE_{max} and v_0 were affected by the binding of lactose or glucose to β-Lg, the difference being significantly higher ($\alpha \leqslant 0.05$) when protein was modified with lactose for the shorter time period (51 h).

With respect to the foam stability the results obtained shown that thermal treatment resulted in a significant reduction ($\alpha \leqslant 0.05$) of the drainage rate constant (k_g), which implies increased foam stability. All the modified samples exhibited also a significant increase ($\alpha \leqslant 0.05$) in the stability to drainage (k_g) as compared to the unheated sample (Fig. 6). In addition, samples modified with lactose formed foams significantly more stable ($\alpha \leqslant 0.05$) than those modified with glucose. The increase in reaction time shows an equivalent tendency in the stability of the foams formed.

4. Conclusion

The modification of β–lactoglobulin with glucose or lactose in the model systems employed resulted in products with improved foaming properties as compared to systems prepared without carbohydrates (β-Lg stt (1), β-Lg c tt 51 h (2), β-Lg c tt 96 h (3)).

The addition of lactose residues to β–lactoglobulin improved the formation of foams, especially due to an increased adsorption of the modified proteins to the air/water interface, and resulted also in foams with higher stability to gravitational drainage. These changes can be attributed to structural differences induced in β–lactoglobulin by lactose binding. Such binding leads to an increase in exposed hydrophobicity and molecular unfolding, and induces protein stabilization as monomer, with simultaneous formation of a higher number of tetramers and octamers.

Glucose binding to β–lactoglobulin seems to provoke a higher protein aggregation as revealed by a diminution of the exposed hydrophobicity, the denaturation enthalpy associated to the thermally-induced denaturation process, and the UV fluorescence intensity (which indicates the degree of exposure of Trp, Tyr and Phe residues to the polar environment). These modifications result in foams more resistant to drainage than the non-glycated protein but less stable than lactose-modified proteins.

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References

Akita, E. M., & Nakai, S. (1990). Lipophilization of β-lactoglobulin: effect on hydrophobicity, conformation and surface functional properties. *Journal of Food Science, 55*(3), 711–717.

Chevalier, F., Chobert, J. M., Dalgalarrondo, M., & Haertle, T. (2001a). Characterization of the Maillard reactions products of β-lactoglobulin glucosylated in mild conditions. *Journal of Food Biochemistry, 25*(1), 33–55.

Chevalier, F., Chobert, J. M., Popineau, Y., Nicolas, M. G., & Haertle, T. (2001b). Improvement of functional properties of β-lactoglobulin glycated through the Maillard reaction is related to the nature of the sugar. *International Dairy Journal, 11*(3), 145–152.

Chevalier, F., Chobert, J. M., Dalgalarrondo, M., Choiset, Y., & Haertle, T. (2002). Maillard glycation of β-lactoglobulin induces conformation changes. *Nahrung, 46*(1), 58–63.
Fenaille, F., Morgan, F., Parisod, V., Tabet, J.-C., & Guy, P. A. (2003). Solid-state glycation of β-lactoglobulin monitored by electrospray ionisation mass spectrometry and gel electrophoresis techniques. *Rapid Communications in Mass Spectrometry, 17*(13), 1483–1492.
Fogliano, V., Monti, S., Visconti, A., Randazzo, G., & Facchiano, A. (1998). Identification of a β-lactoglobulin lactosylation site. *Biochimica et Biophysica Acta, 1388*(2), 295–304.
French, S., Harper, J., Kleinholz, N., Jones, R., & Green-church, K. (2002). Maillard reaction induced lactose attachment to bovine β-lactoglobulin: electrospray ionization and matrix-assisted laser desorption/ionization examination. *Journal of Agricultural and Food Chemistry, 50*(4), 820–823.
Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlations with surface properties of proteins. *Biochimica et Biophysica Acta, 624*(1), 13–20.
Hattori, M., Ametani, A., Katakura, Y., Shimizu, M., & Kaminogawa, S. (1993). Unfolding/refolding studies on bovine β-lactoglobulin with monoclonal antibodies as probes: Does a renatured protein completely refold. *Journal of Biological Chemistry, 268*(30), 22414–22419.
Kaminogawa, S., Shimizu, M., Ametani, A., Hattori, M., Ando, O., & Hachimura, S. (1989). Monoclonal antibodies as probes for monitoring the denaturation process of bovine β-lactoglobulin. *Biochimica et Biophysica Acta, 998*(1), 50–56.
Loisel, W., Guéguen, J., & Popineau, Y. (1993). A new apparatus for analyzing foaming properties of proteins. In K. D. Schwenke & R. Mothes (Eds.), *Food proteins: Structure and functionality* (pp. 320–323). Weinheim, Germany: VCH.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin-phenol reagent. *Journal of Biological Chemistry, 193*(1), 265–275.
Rada-Mendoza, M., Villamiel, M., Molina, E., & Olano, A. (2006). Effects of heat treatment and high pressure on the subsequent lactosylation of β-lactoglobulin. *Food Chemistry, 99*(4), 651–655.
Matsuda, T., Kato, Y., & Nakamura, R. (1991). Lysine loss and polymerization of bovine β-lactoglobulin by amino-carbonyl reaction with lactulose (4-*O*-beta-galactopyranosyl-D-fructose). *Journal of Agricultural and Food Chemistry, 39*(7), 1201–1204.
Morgan, F., Leonil, J., Molle, D., & Bouhallab, S. (1997). Nonenzymatic lactosylation of bovine β-lactoglobulin under mild heat treatment lead to structural heterogeneity of the glycoforms. *Biochemical and Biophysical Research Communications, 236*(2), 413–417.
Morgan, F., Leonil, J., Molle, D., & Bouhallab, S. (1999a). Modification of bovine β-lactoglobulin by glycation in a powdered state or in an aqueous solution: Effect on association behavior and protein conformation. *Journal of Agricultural and Food Chemistry, 47*(1), 83–91.
Morgan, F., Venien, A., Bouhallab, S., Molle, D., & Leonil, J. (1999b). Modification of bovine β-lactoglobulin by glycation in a powdered state or in an aqueous solution: Immunochemical Characterization. *Journal of Agricultural and Food Chemistry, 47*(11), 4543–4548.
Moreno, F. J., Lopez-Fandiño, R., & Olano, A. (2002). Characterization and functional properties of lactosyl caseinmacropeptide conjugates. *Journal of Agricultural and Food Chemistry, 50*(18), 5179–5184.
Nakamura, S., Kato, A., & Kobayashi, K. (1991). New antimicrobial characteristics of lysozyme–dextran conjugate. *Journal of Agricultural and Food Chemistry, 39*(4), 647–650.
Panizzolo, L.A. (2005). Modificación de proteínas por vía enzimática. Análisis de la relación estructura-funcionalidad de los productos de hidrólisis. Tesis, Facultad de Química. Universidad de la República, Uruguay.
Schagger, H., & Von Jagow, G. (1987). Tricine-sodium dodecyl sulfate-polyacrilamide gel electrophoresis for the separation of proteins in the range from 1 to 100 KDa. *Analytical Biochemistry, 166*(2), 368–379.