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Characterization and Functional Properties of Lactosyl Caseinomacropeptide Conjugates

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Ovine caseinomacropeptide (CMP) was modified with lactose through Maillard reaction under 44% relative humidity and 40 °C for various periods (0−11 days). Different lactosylated CMP forms were separated by capillary electrophoresis and reversed phase high-performance liquid chromatography (RP-HPLC) and identified by RP-HPLC coupled with electrospray ionization mass spectrometry (ESI-MS). Around 55−60% of CMP was lactosylated under the conditions assayed, with the monolactosylated form being the most abundant one, followed by the di-, tri-, and tetralactosylated species. During the first days of incubation amino acid analyses showed a decrease in lysine and NH_2-terminal methionine, which coincided with an increase in the furosine content. However, from the ninth day of incubation, further degradation of Amadori compounds prevailed over their formation. Solubility, heat stability, and emulsifying capacity of the native and modified CMP were investigated. Lactosylation improved the emulsifying activity, but it did not modify the great solubility and heat stability of native CMP.

KEYWORDS: Caseinomacropeptide; Maillard reaction; lactosylated forms; functional properties

INTRODUCTION

Nonenzymatic glycosylation of peptides and proteins may affect both their functional properties and biological activities (*1*−*3*). Foaming and emulsifying properties of milk proteins are enhanced through glycosylation (*4*−*7*), whereas improvements in emulsifying (*8*) and gelling (*9*) properties of egg white proteins have been achieved by the attachment of carbohydrates during the early stages of the Maillard reaction. Advanced stages of the Maillard reaction of glycated proteins lead to protein polymerization and browning (*10*, *11*); thus, glycosylation of proteins through the Maillard reaction should be performed under controlled conditions to prevent color changes and loss of solubility.

Modification of physical properties such as charge, hydrophilicity, or viscosity may explain the functional changes observed in glycated proteins. Improvements in solubility can be due to an increase in negative net charge and a decrease in surface hydrophobicity caused by the addition of hydrophilic groups (*12*). On the other hand, the exposure of new hydrophobic regions at the surface of modified proteins may improve their adsorption at interfaces, and, therefore, their emulsifying capacity (*5*). Furthermore, the carbohydrate moieties could be oriented to the aqueous phase, inhibiting the flocculation of oil droplets by electrostatic repulsion (*13*).

The C-terminal fragment of κ-casein (residues 106−169 in bovine milk and residues 106−171 in ovine and caprine milks), the so-called caseinomacropeptide (CMP), is present in the cheese whey fraction and consists of a mixture of peptides that differ, mainly, in the degree of glycosylation and phosphorylation. The structural microheterogeneity of bovine CMP, regarding the peptide and oligosaccharide structures, as well as the carbohydrate and phosphorylation sites, is quite well established (*14*). However, less is known about post-translational modifications in CMP from other species. Ovine CMP possesses a lower glycosylation degree than bovine CMP, and its most abundant form is diphosphorylated (*15*).

It has been reported that CMP exhibits biological and functional properties, which may render it a valuable source of protein in the food and pharmaceutical industries (*16*). Furthermore, the *O*-glycosyl residues of CMP are thought to have a significant effect on its functional and biological activities (*17*); thus, controlled glycosylation may give rise to glycomacropeptides with new or improved properties. CMP contains four potential reactive amino groups to be nonenzymatically glycosylated. These sites are constituted by the ϵ-amino groups of the three lysine residues, situated in positions 6, 7, and 11, and the α-amino group of the NH_2-terminal methionine.

In this work, the influence of nonenzymatic lactosylation under controlled conditions on selected functional properties of ovine CMP was studied. We have used capillary zone electrophoresis (CZE) and reversed phase high-performance liquid chromatography (RP-HPLC) to monitor the formation of lactosyl CMP conjugates via Maillard reaction. The number of lactose residues linked to the CMP was determined by RP-HPLC coupled with electrospray ionization mass spectrometry (ESI-MS). Amino acid analysis and determination of furosine after acid hydrolysis of CMP have been performed to evaluate

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the extent of lactosylation. Finally, solubility, heat stability, and emulsifying properties of CMP, native and lactosylated to different degrees, were measured.

MATERIALS AND METHODS

Lactosylation of Caseinomacropeptide. Ovine milk (Manchega) was provided by a local dairy farm from the central region of Spain. Whole caseins and CMPs were prepared as described previously (*15*). Purity of ovine CMP, as calculated from the contents of lysine and methionine (*18*), was ~94%.

Aliquots of a solution consisting of 2.0 mg/mL CMP and 2.5 mg/mL lactose (Scharlau Chemie) (lactose/CMP molar ratio = 24) in 0.1 M sodium phosphate buffer, pH 7 (Merck), were lyophilized. These were kept under vacuum in a desiccator at 40 °C and a water activity of 0.44, achieved with a saturated K_2CO_3 solution (Merck), for various periods (0, 2, 5, 7, 9, and 11 days). In addition, control experiments were performed with CMP stored at 40 °C without lactose during the same periods (heated CMP).

Analysis of Lactosylated Forms. After incubation, the powders were dissolved in distilled water and free lactose was removed by ultrafiltration through a centrifugal filter (Centricon YM-3, 3.000 MW cutoff, Millipore). After removal of lactose, samples were reconstituted in distilled water at 2 mg/mL and analyzed by CZE, RP-HPLC, and RP-HPLC-ESI-MS.

CZE followed the method described by Recio et al. (*19*). Separations were performed using a Beckman P/ACE System 2050 and a hydrophilic coated fused-silica capillary, Supelco Celect P1 (37 cm × 50 μm i.d., with a slit opening of 100 × 800 μm). Electromigrations were run at 45 °C, with a linear gradient from 0 to 50 μA in 10 min, followed by a constant current of 50 μA. Detection was at 214 nm. The electrophoresis buffer (pH 3.0) contained 10 M urea (Sigma), 10 mM citrate buffer (Sigma), and methylhydroxyethyl cellulose 0.05% (w/v) (Serva). Sample buffer contained 10 M urea, 167 mM Tris (Sigma), 42 mM 3-morpholinopropanesulfonic acid (Fluka), and methylhydroxyethyl cellulose 0.083% (w/v).

RP-HPLC and RP-HPLC-ESI-MS followed the methods described by Moreno et al. (*15*). RP-HPLC separations were carried out with a C18 Nucleosil column (300 Å, 250 × 4.6 mm i.d., 5 μm particle size) (Macherey Nagel Düren). Operating conditions were as follows: column temperature, 40 °C; flow rate, 1 mL/min; solvent A, 1% trifluoroacetic acid (TFA) in double-distilled water; solvent B, 1% TFA in HPLC grade acetonitrile (Scharlau Chemie). The elution was performed with a linear gradient by increasing the concentration of solvent B as follows: 0−40 min, 21.6−37.6%; 40−42 min, 37.6−100%. Absorbance was recorded at 214 nm using a Beckman 166 UV detector (Beckman Instruments).

RP-HPLC with on-line ESI-MS separations were performed with a C18 Zorbax column (150 × 2.1 mm i.d., 5 μm particle size) (Hewlett-Packard). The mobile phase, column temperature, and gradient applied were those mentioned above. The flow rate was 0.2 mL/min, and detection was accomplished by using a diode array detector series 1100 (Hewlett-Packard), at 214 nm. ESI-MS analyses were performed on a quadrupole 1100 MSD mass spectrometer (Hewlett-Packard) equipped with an atmospheric pressure ionization ion source (API), and the ES parameters were as follows: needle potential, 4 kV; gas temperature, 330 °C; drying gas, 10.0 L/min; nebulizer pressure, 0.35 MPa. When required, minor peaks were collected from the RP-HPLC system after several analyses, lyophilized, and directly analyzed by ESI-MS through infusion of the sample solution (20 μL) using a flow of 4 μL/min through the electrospray interface.

Amino acid analysis and determination of furosine were carried out after acid hydrolysis of the stored powders. Briefly, 400 μL of 8 N HCl was added to 2 mg of CMP in hydrolysis tubes and heated at 110 °C for 23 h under inert conditions, followed by the addition of 2 mL of 8 N HCl and filtering through Whatman no. 40 filter paper.

For amino acid analysis, 150 μL of the filtered hydrolysate was taken to dryness, dissolved in 900 μL of 0.4 M sodium borate buffer, pH 10 (Merck), and submitted to an automatic precolumn derivatization with *o*-phthaldialdehyde (OPA) (Fluka). The separation of amino acids was carried out on a Novapak C-18 column (60 Å, 4 μm particle size, 150 × 3.9 mm i.d.) at a flow rate of 1.5 mL/min. Solvent A was HPLC grade methanol (Waters)/0.01 M sodium phosphate buffer, pH 7.3/HPLC grade tetrahydrofuran (Waters) (19:80: 1) (v/v/v); solvent B was HPLC grade methanol/0.01 M sodium phosphate buffer, pH 7.3 (80:20) (v/v). The gradient used was described in ref *18*. Detection was performed by fluorescence using 340 and 425 nm for excitation and emission, respectively. Calibration curves of lysine and methionine were built up by using commercial pure standards (2.5−200 nmol/mL) purchased from Sigma.

For determination of 2-(furoylmethyl)lysine (furosine), 500 μL of the filtered hydrolysate was applied to a previously activated Sep-Pak C_{18} cartridge (Millipore). Furosine was eluted with 3 mL of 3 N HCl and 50 μL was injected. Analysis of furosine was by an ion-pair RP-HPLC method using a C_8 (Alltech furosine-dedicated) column (250 × 4.6 mm i.d.) and a variable wavelength detector at 280 nm (LDC Analytical, SM 4000). Operating conditions were as follows: column temperature, 35 °C; flow rate, 1.2 mL/min; solvent A, 0.4% HPLC grade acetic acid (Scharlau Chemie) in double-distilled water; solvent B, 0.3% KCl (Merck) in solvent A (*20*). Calibration was performed by using solutions of known concentrations (from 0.52 to 5.2 mg/L) of commercial pure standard of furosine (Neosystem Laboratories). The presence of furosine in hydrolyzed CMP samples was confirmed by RP-HPLC-ESI-MS using the previously mentioned C_8 Alltech column, maintained at 37 °C and with 2% acetic acid as mobile phase. The flow rate was 0.6 mL/min, and the ES parameters were as follows: fragmentor voltage, 40 V; needle potential, 4 kV; gas temperature, 320 °C; drying gas, 10.0 L/min; nebulizer pressure, 0.28 MPa (*21*).

Functional Properties of Caseinomacropeptide. Measurement of functional properties was always performed after the removal of free lactose as described above.

Solubility. Native, heated, and lactosylated CMPs were dissolved in distilled water or 0.05 M sodium phosphate buffer (1, 2, and 5 mg/mL). The pH was adjusted to 3, 3.6, 5, and 7 by using 1 M HCl or NaOH. After 30 min of stirring at room temperature, the solutions were centrifuged for 15 min at 4 °C and 3500*g*. Solubility was expressed as the percentage of the total CMP concentration, determined by measuring the absorbance at 226 nm in the supernatants and using a standard curve previously prepared. In all cases, before absorbance was measured, CMP solutions were diluted twice with 8 M urea (Sigma) to prevent the interference of nonsoluble solids.

Heat Stability. Native, heated, and lactosylated CMPs were dissolved in distilled water (1 mg/mL) at pH 4 and 7 and heated at different temperatures (80−95 °C) for 15 min. Samples were then cooled to room temperature and centrifuged for 5 min at 4 °C and 3500*g*, to precipitate aggregates. Finally, the CMP content of the supernatants was measured as indicated above, and was compared with that of the corresponding unheated samples.

Emulsifying Properties. Emulsifying activities were measured by spectroturbidity following the method described by Chobert et al. (*22*). To prepare the emulsions, 3 mL of 0.1% CMP aqueous solution and 1 mL of rapeseed oil (ϕ, volume fraction of the dispersed phase = 0.25) was shaken together with an Ultra-turrax type TP 18/10 (Janke and Kunkel), and homogenizations were carried out for 3 min at the maximum power. After 24 h of storage at room temperature and 30 min of heating of the emulsions at 80 °C, turbidity was again measured to determine the emulsifying stability (ΔEAI).

RESULTS AND DISCUSSION

Characterization of Lactosylated Forms of Caseinomacropeptide. CZE electropherograms of CMP incubated with lactose are shown in **Figure 1**. One main peak with a migration time of 26−27 min was observed in the native CMP profile (**Figure 1a**). Analyses of CMP incubated with lactose showed the appearance of several slower migrating peaks, the areas of which increased upon storage while a simultaneous loss of the main peak was observed (**Figure 1b−d**). Such peaks, which were absent from CMP samples incubated in the absence of lactose (results not shown), could correspond to lactosylated forms of CMP. At low pH, CMP is positively charged and, in

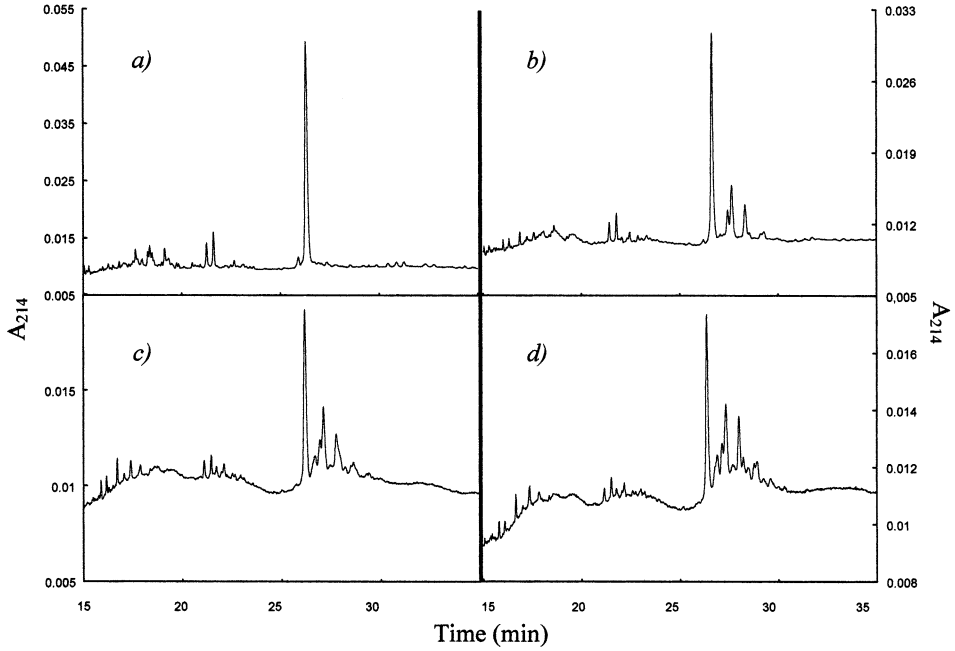

Figure 1. Capillary electrophoresis patterns of ovine CMP incubated with lactose at 40 °C for 0 (a), 2 (b), 7 (c), and 11 days (d).

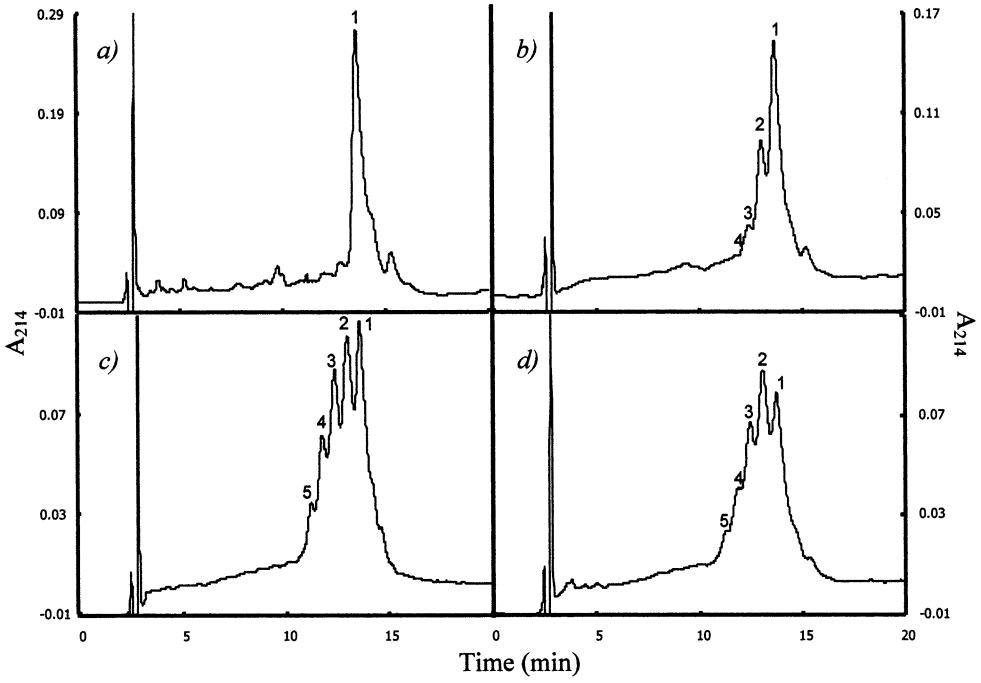

Figure 2. RP-HPLC patterns of ovine CMP incubated with lactose at 40 °C for 0 (a), 2 (b), 7 (c), and 11 days (d).

coated capillaries, migrated toward the cathode according to its ratio of net charge to mass. Therefore, the longer migration times of the lactosylated forms could be due to a lower positive charge or a larger molecular size. Because binding of a sugar to lysine gives rise to a slight change in the pK_a value of the amino group (*23*−*25*), the net positive charge of lactosylated CMP could be slightly lower than that of native CMP at low pH. Moreover, the addition of one or several lactose residues to the peptide chain would lead to an increase of ∼5−18% in the molecular mass of the CMP, reducing the mobility of the lactosylated forms. Otte et al. (*25*) and Fayle et al. (*26*) observed a similar behavior in the analysis by capillary electrophoresis of β-lactoglobulin incubated with lactose and ribonuclease A incubated with dehydroascorbic acid, respectively.

The chromatographic profiles of native and lactosylated CMP obtained by RP-HPLC are shown in **Figure 2**. Native CMP (**Figure 2a**) presented one main peak, designated 1, with a retention time of 13.5 min, which was previously identified as the diphosphorylated carbohydrate-free form of ovine CMP (*15*). The chromatograms corresponding to the CMP incubated with lactose indicated the appearance of a series of peaks less retained than the main peak, designated 2−5 in **Figure 2b**−**d**, that could correspond to lactosylated forms of the CMP. The shorter retention times of these peaks can be attributed to the incorporation of lactose residues into the peptide chain that leads to an increase in CMP hydrophilicity.

The identification of the different lactosyl CMP conjugates was achieved by RP-HPLC-ESI-MS (**Table 1**). The use of ESI-

Table 1. Experimental Molecular Masses of the Different Lactosylated Forms of Ovine Caseinomacropeptide and Assigned Contents of Lactose Residues and Phosphate Groups Derived from the RP-HPLC–MS Analyses

peak[a]	obsd molecular mass[b]	lactose and phosphate content (mol/mol)	calcd molecular mass[c]
1	6965.1 ± 1.3	0 lactose, 2P	6964.4
2	7288.5 ± 0.5	1 lactose, 2P	7288.7
3	7612.8 ± 0.7	2 lactose, 2P	7613.0
4	7937.7 ± 1.4	3 lactose, 2P	7937.3
5[d]	8261.9 ± 1.5	4 lactose, 2P	8261.6

[a] Peak numbers refer to **Figure 2**. [b] Mean ± SD ($n = 3$). [c] Average molecular mass values calculated from the primary amino acid sequence of ovine CMP published by Jollès et al. (*36*). [d] Determined by off-line HPLC-MS.

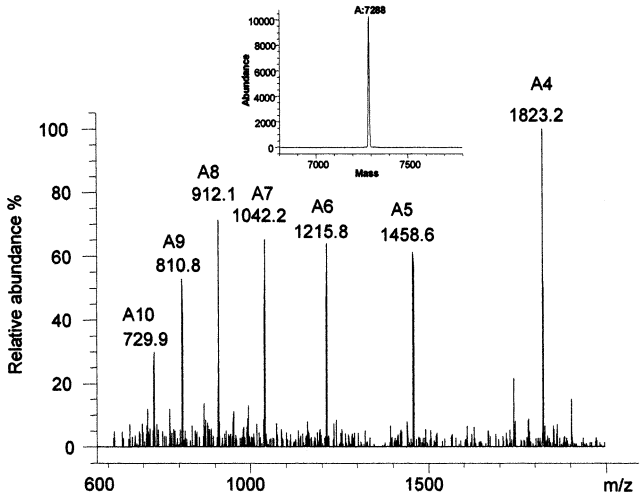

Figure 3. Electrospray mass spectra of the diphosphorylated monolactosylated form of ovine CMP (peak 2 in **Figure 2** and **Table 1**). The mass spectrum is characterized by a series of ions with *m*/*z* values corresponding to their multiply protonated forms (Ax). The inset represents the deconvoluted spectrum after ESI-MS analysis.

MS to study the glycation of several proteins such as β-lactoglobulin (*27*, *28*) and lysozyme (*29*) was recently reported. The increase of 324.3 amu, resulting from attachment of 1 mol of lactose/mol of CMP, allowed the identification of mono- (the mass spectrum of which is shown in **Figure 3**), di-, and trilactosylated species of the CMP, which were contained in RP-HPLC peaks 2, 3, and 4, respectively. The molecular mass of peak 5 could not be determined by RP-HPLC on-line with ESI-MS, and a previous concentration step was required. The molecular mass value obtained (8261.9) was in accordance with the presence of a tetralactosylated form. It should be noted that, even if the lysine residues occupy close positions in the CMP chain, conjugates with all reactive sites lactosylated were found after 5 days of incubation.

Determination of the Extent of Lactosylation. The distribution of the different lactosylated forms of the CMP after certain incubation periods was estimated from the relative areas of their corresponding RP-HPLC peaks, and it is shown in **Figure 4**. A considerable formation of lactosylated compounds was found after 2 days of incubation except for the tetralactosylated form, which was not identified at this incubation time. After the fifth day of incubation, only a slight increase in the percentage of all lactosylated forms was observed, with the monolactosylated form being the most abundant one, followed by di-, tri-, and tetralactosylated species. Assuming that the ultraviolet response at 214 nm is equal for all of the CMP species detected by RP-HPLC, ~55−60% of CMP was lactosylated under the conditions assayed.

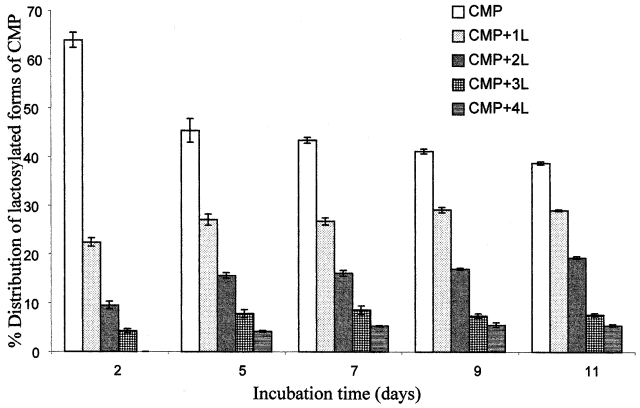

Figure 4. Percentage distribution of the lactosylated forms of ovine CMP incubated with lactose at 40 °C for up to 11 days. The graph shows means ± SD.

Table 2. Contents of Methionine, Lysine, and Furosine Obtained after Acid Hydrolysis of CMP Incubated with Lactose and Determined by RP-HPLC

incubation time (days)	methionine (μmol/100 mg of CMP)	lysine (μmol/100 mg of CMP)	furosine (mg/100 mg of CMP)
0	13.6 ± 0.1[a]	40.8 ± 2.8	0.00
2	8.2 ± 0.3	31.0 ± 0.4	0.61 ± 0.02
5	8.5 ± 0.4	29.4 ± 0.6	0.98 ± 0.06
7	8.5 ± 0.8	28.9 ± 0.6	1.04 ± 0.08
9	7.8 ± 0.7	28.3 ± 0.3	1.22 ± 0.05
11	6.4 ± 0.2	24.2 ± 1.0	1.04 ± 0.02

[a] Incubation was carried out in duplicate, performing two separate chromatographic analyses of each sample.

The contents of lysine, methionine, and furosine of the CMP samples incubated with lactose are shown in **Table 2**. The concentrations of lysine and methionine of native CMP were similar to the theoretical values (43.2 and 14.4 μmol/100 mg of CMP, respectively). An important decrease in methionine and lysine was found after 2 days of incubation with lactose, suggesting that lactosylation took place in both amino acids. This decrease coincided with the increase in furosine observed after 2 days of incubation. From the second to the ninth days of incubation, the content of methionine remained constant while the content of lysine decreased and that of furosine increased slightly. Nevertheless, a considerable decrease in both amino acids took place between 9 and 11 days of incubation, which occurred simultaneously with a decrease in furosine. These observations indicate that, from the ninth day of incubation, the degradation of Amadori compounds prevailed over their formation, also suggesting that methionine and lysine could participate in collateral or advanced stages of Maillard reaction. For instance, it has been described that α-dicarbonyl intermediates derived from the decomposition of Amadori compounds can form different heterocyclic and smaller carbonyl compounds, which are susceptible to reaction with amino acids (*30*, *31*). Furthermore, this α-dicarbonyl intermediate can undergo nucleophilic addition reactions with amino acids via Strecker degradations (*31*).

Functional Properties of Native and Lactosylated Caseinomacropeptides. *Solubility and Heat Stability.* Native ovine CMP was soluble in distilled water at 1 and 2 mg/mL at the pH values studied (3, 3.6, 5, and 7), although a slightly lower solubility

Table 3. Solubility of Native CMP Dissolved in Distilled Water and 0.05 M Sodium Phosphate Buffer as a Function of pH[a]

	solubility (%)			
pH	1 mg/mL of distilled water	2 mg/mL of distilled water	1 mg/mL of 0.05 M sodium phosphate buffer	5 mg/mL of 0.05 M sodium phosphate buffer
7.0	97.8 ± 2.5[a]	98.9 ±1.6	98.6 ± 2.4	99.7 ± 1.5
5.0	90.5 ± 1.6	90.3 ± 0.9	101.5 ± 2.7	100.5 ± 2.8
3.6	88.4 ± 2.3	90.4 ± 2.6	100.6 ± 1.6	99.4 ± 2.7
3.0	91.0 ± 2.6	89.8 ± 1.8	100.2 ± 1.9	99.9 ± 1.7

[a] Solubility is expressed as a percentage of the total CMP in solution. Mean ± SD.

was observed at acid pH (**Table 3**). A similar behavior was reported for bovine CMP dissolved in distilled water at 1 mg/mL (*22*). However, in 0.05 M sodium phosphate buffer, the solubility of CMP was ~100%, even at a concentration of 5 mg/mL and acid pH (**Table 3**), probably due to a salting-in effect that decreased electrostatic attractions between molecules and to the competition of Na^+ with protons (*32*).

The fraction of native CMP insoluble at pH 5 was separated after centrifugation and analyzed by RP-HPLC-ESI-MS, showing the presence of impurities derived from rennet action on casein fractions other than κ-casein. Therefore, these data indicate that the elimination of the fraction insoluble at acid pH would contribute to obtain CMP with a higher degree of purity, when it derives from whole casein or milk.

Evaluation of the heat stability of native CMP showed that it was almost 100% soluble after heat treatments at 80−95 °C, regardless of the temperature and pH.

With respect to heated and lactosylated CMP (for 2, 5, and 9 days), no changes were observed in the values of solubility and heat stability obtained at acid and neutral pH at a concentration of 1 mg/mL as compared to native CMP, indicating the absence of important structural changes such as aggregations and polymerizations. These results suggest that CMP could be incubated with lactose during periods sufficient to obtain highly glycosylated forms without impairing their solubility and heat stability.

Emulsifying Properties. The emulsifying activity index (EAI) of ovine CMP (1 mg/mL) was measured over a range of pH values (3−7). The EAI values obtained for native CMP were comparable to the values reported for bovine CMP, κ-casein (*22*), and β-lactoglobulin (*1*) and superior to those described for β-casein (*1*), thus showing a good emulsifying character. The results obtained proved a good emulsifying activity at pH values >6, whereas at pH 4 and 5 emulsions could not be formed. These pH values are close to its p*I* (pH 3.6), suggesting that a change in the ionizable groups may affect the structure of the CMP and, therefore, its emulsion activity (*22*). Minkiewicz et al. (*33*) indicated that a decrease in pH may lead to a decrease in voluminosity of CMP particles due to suppression of internal electrostatic and steric repulsions. This change in voluminosity could have a significant influence on the conformation of CMP and, therefore, its emulsifying capacity.

No differences were observed between the emulsifying activities of native and heated (for 2, 5, and 9 days) CMP over the range of pH studied, 3−7, probably due to its high heat stability (results not shown). Groubet et al. (*1*) and Chevalier et al. (*2*) observed a lower emulsifying activity in heated β-lactoglobulin over native β-lactoglobulin. This result was attributed to the fact that heating native β-lactoglobulin leads to structural changes, such as polymerizations, that affect its solubility and, therefore, its emulsifying activity.

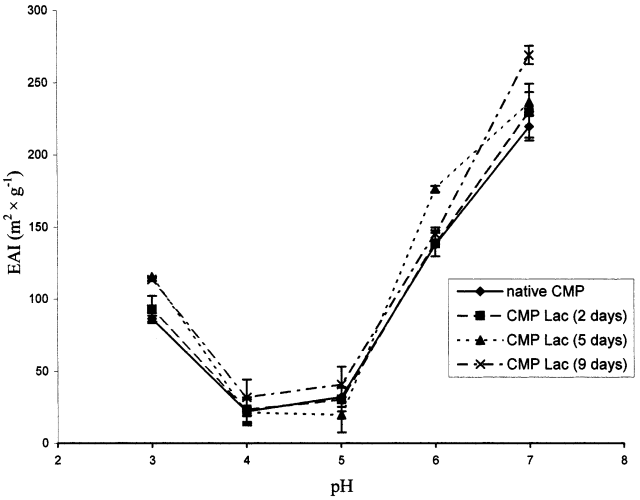

Figure 5. EAI of native and lactosylated (for 2, 5, and 9 days) CMPs as a function of pH. The graph shows means ± SD.

A slight improvement in the emulsifying activity was observed after lactosylation of the CMP. As shown in **Figure 5**, this was more pronounced in the CMP stored for 5 and 9 days than in that stored for 2 days, suggesting that the lactosylation degree of CMP might be related to its emulsifying properties. In fact, the RP-HPLC chromatograms and furosine values had shown an important increase in the lactosylation degree between 2 and 5 days of incubation, which was much less pronounced between 5 and 9 days (**Figure 2** and **Table 2**).

The emulsifying stability values (ΔEAI) of native CMP showed a large pH-dependent decrease from 26.1 to 47.1% with decreasing pH values from 7 to 3. These data are similar to the values reported for bovine CMP by Chobert et al. (*22*), who found a 22−60% decrease in emulsifying stability. Regarding lactosylated CMP, emulsion stability was enhanced only in CMP incubated with lactose for 2 days at pH 3 (24.5%) and pH 7 (14.1%), whereas an increase in the time of incubation (5 and 9 days) did not modify the emulsion stability as compared with native CMP.

The results presented in this work show that CMP can be lactosylated through the Maillard reaction to a considerable glycosylation degree without substantial conformation changes, as monitored with several analytical techniques. Ovine CMP presented a good emulsifying activity that was slightly improved by lactosylation, although the binding of lactose did not change its low emulsion stability properties.

It has been reported that associations of proteins with hydrophilic high molecular weight polysaccharides can lead to substantial improvements in emulsion stability without significant changes in emulsifying capacity (*34*, *35*). The high conformational stability maintained by CMP during glycosylation suggests the feasibility of the formation of conjugates of CMPs with polysaccharides while keeping its excellent solubility and heat stability properties.

ABBREVIATIONS USED

ΔEAI, emulsifying stability; CMP, caseinomacropeptide; CZE, capillary zone electrophoresis; EAI, emulsifying activity index; ESI-MS, electrospray ionization mass spectrometry; RP-HPLC, reversed-phase high-performance liquid chromatography.

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