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High-performance liquid chromatographic determination of Maillard compounds in store-brand and name-brand ultra-high-temperature-treated cows' milk

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Abstract

Furosine and furfural products of the Maillard reaction are used as specific indicators of the effect of heating treatments on milk quality. Their contents were measured in representative samples of store- and name-brand ultra-high-temperature-treated milks using RP-HPLC with UV detection. Furosine contents ranged from 40.32 to 50.67 and from 65.48 to 310.58 mg/100 g protein in name- and store-brand milks, respectively. Of the furfurals, only hydroxymethylfurfural was detected. The free hydroxymethylfurfural contents of store-brand milks ranged from 0.22 to 1.70 mg/100 g protein. Total hydroxymethylfurfural contents ranged from 0.29 to 0.41 and from 0.72 to 2.21 mg/100 g protein, for name- and store-brands, respectively.

Keywords: Milk; Food analysis; Furosine; Furfurals

1. Introduction

The quality of the raw material and the heating treatments applied can affect the quality of ultra-high-temperature-treated (UHT) milk. As a consequence of the heating treatments, milk undergoes chemical and biochemical changes that affect different components, mainly proteins, carbohydrates and vitamins. This gives rise to physical modifications such as changes in colour and particle size that make it difficult to evaluate the changes produced.

The quality of milk is affected by heating treatments as a consequence of the: interactions between the amino acid lateral groups, degradation reactions of lateral chains of the proteins, restructuring of –SH and S–S– groups, insolubilization of whey proteins, and interactions between κ-casein and β-lactoglobulin, interactions with lipids, and interaction between carbohydrates and proteins (Maillard reaction) [1].

Maillard's reaction is of special interest in studying the effect of heat treatment on milk quality because it affects the nutritional value of proteins, can give rise to antinutritive and toxic compounds and modifies the organoleptic and functional properties of milk. On the other hand, the high ratio between lactose and protein contents of milk, the heating treatments (UHT, sterilization, evaporation, . . .) applied to ensure the safety of milk and to extend shelf life, the quality of the raw material, and the storage conditions favour Maillard reaction. Therefore, analytical methods for monitoring the effect of heating treatments on milk quality are needed.

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Furosine (FUR) and furfural compounds can be considered indicators of the extent of the Maillard reaction related to the type and intensity of the food processing conditions, as well as to the storage conditions. Therefore, FUR and furfural compounds are suitable indicators of the quality of dairy products [2,3].

FUR content gives an estimate of the blocked and therefore non-reactive lysine [4–7] and is considered the most specific and earliest indicator of the Maillard reaction [8].

Furfurals are intermediary compounds in the formation of pigments (melanoidins) in the most advanced stages of the Maillard reaction [9]. The furfural group includes four different compounds: hydroxymethylfurfural (HMF), furfural (F), furylmethylcetone (FMC) and methylfurfural (MF). In fact, when milk is subjected to heating treatment and storage at inadequate temperatures, different furfural derivatives (HMF, F, FMC and MF) can be generated, and these compounds serve as indicators of the extent of the Maillard reaction [10–12].

Several analytical techniques have been used to measure FUR [GC, ion-exchange chromatography (IEC), TLC and HPLC] and furfurals (Vis and HPLC) in milk, but HPLC is now the technique of choice because it provides a good detection limit and the time of analysis is short [13].

Today a wide variety of store-brand products are available. The term store-brand is applied to products that carry the name of the supermarket or hypermarket selling the product or to products subcontracted to another company. The advantage of these store-brands is their low price, but this is sometimes interpreted by the consumer to mean a lack of quality. Products carrying brand names offer the advantage of brand image and consumer fidelity [14].

Given that the consumption of store-brand products is rising the purpose of our work was to evaluate the quality of store-brand UHT milks in comparison with those of name-brands. To do this, FUR, an early indicator of the Maillard reaction, and furfural compounds, an indicator of the advanced Maillard reaction, were measured in order to compare the values obtained and to detect possible differences in quality between the two types of products.

2. Experimental

2.1. Material and methods

2.1.1. Samples

Ten samples of UHT whole cow milk (B_1–B_{10}) belonging to store-brands, three samples of UHT whole cow milk (C_1–C_3) belonging to name-brands, and two more samples of semi-skimmed UHT cow milk (C_4, C_5), one a name-brand product with a low lactose content (C_5), were bought at different supermarkets in Valencia. Their expiration dates ranged from September 5 to September 25, 1999.

Three cartons from the same batch of each milk were bought. The cartons were kept at room temperature until opened, then were stored at 4°C for a maximum of 4 days.

2.1.2. Apparatus

The chromatographic system (Shimadzu, Kyoto, Japan) consisted of two LC-10AD pumps controlled by a CBM-10a, a Model 7725i manual injection valve (Rheodyne, Cotati, CA, USA) equipped with a 20-μl sample loop and an SPD-10AD UV–visible detector. Data were collected and analysed using the CLASS LC-10W/S software package.

Solvents were filtered using a Millipore (Milford, MA, USA) system with 0.22-μm membrane filters (47 mm) and samples were filtered using a Millipore system with 0.22-μm membrane filters (13 mm).

A TU 6060 air-circulation drying oven (Heraeus, Hanau, Germany) was used in the FUR hydrolysis step, and a vacuum freeze dryer (Heto, FD4-85, Gydevang, Denmark) was used to remove liquids.

2.1.3. Chemicals and materials

Materials were sourced as follows: acetonitrile 99.8% and methanol 99.8% HPLC quality from J.T. Baker (Deventer, The Netherlands), hydrochloric acid 37% from Merck (Darmstadt, Germany), formic acid and 1-heptanesulfonic acid from Sigma (St. Louis, MO, USA), oxalic acid dihydrate 99.5% and trichloroacetic acid (TCA) 99.5% from Fluka (Buchs, Switzerland), ethanol 95–96% from Prolabo (Fontenay s/Bois, France), FUR from Neosystem (Strasbourg, France), and HMF (5-hydroxymethyl-2-furaldehyde), F (2-furaldehyde), FMC (2-furyl-

methylketone) and MF (5-methyl-2-furaldehyde from Fluka. Sep Pak C_{18} cartridges were from Waters (Milford, MA, USA). All aqueous solutions were prepared with high-purity water produced with a Millipore Milli Q system. All reagents were of analytical reagent grade unless the contrary is stated.

2.2. Procedures

2.2.1. FUR determination

FUR was measured using RP-HPLC with UV detection at 280 nm according to Delgado et al. [15].

Acid hydrolysis: In a 10-ml screw-capped Pyrex tube, an aliquot of sample corresponding to 40–50 mg of protein (1.5 ml of milk) was hydrolysed in the presence of 8 ml of 8 *M* HCl [16]. After bubbling with nitrogen for 1 min the closed tube was kept at 110°C for 23 h. After hydrolysis the tubes were weighed and sufficient 8 *M* HCl was added, if needed, to recover to the mass prior to hydrolysis.

Sample preparation [17]: The hydrolysate was filtered and diluted with 3 *M* HCl to obtain a protein content of 1–2 $\mu g\ \mu l^{-1}$ (1 ml of hydrolysate and 4 ml of 3 *M* HCl). To minimize contamination, the solid-phase extraction prior to the chromatographic analysis was performed as follows: 0.5 ml of hydrolysate was added to a pre-wetted (5 ml ethanol and 10 ml water) Sep-Pak C_{18} cartridge; the eluted liquid was discarded, and FUR was then eluted with 3 ml of 3 *M* HCl.

RP-HPLC conditions: An ion-pair reversed-phase was used in the analysis. A Spherisorb ODS2 C_{18}, 5-μm column (250×4.6 mm, I.D.) (Teknokroma, Barcelona, Spain) operating at room temperature was used. The mobile phase system consisted of 5 m*M* sodium heptanesulfonate with 20% acetonitrile as the organic modifier and 0.2% of formic acid. The flow-rate was 0.8 $ml\ min^{-1}$. Detection was at 280 nm. The injection volume was 20 μl.

Calibration curves (0.1, 0.2, 0.4, 0.6 and 0.8 μg FUR ml^{-1}) were carried out by plotting absorbance, expressed in area units vs. μg of FUR.

Analytical parameters of the method: Interday (mean$\pm\sigma_{n-1}$) $x=0.251\pm0.03$ $mg\ l^{-1}$, precision [relative standard deviation, RSD (%)]=12%, $n=6$; recovery (%), 87.06%, $n=3$; detection limit on column=12.6 ng, detection limit in assay=0.63 $\mu g\ ml^{-1}$, detection limit in sample=1.26 $mg\ l^{-1}$ milk. Linearity was checked in the range of the analysed sample contents.

2.2.2. Determination of free and total furfural compounds (HMF, F, FMC and MF)

Total and free furfurals of UHT milk were measured using RP-HPLC with UV detection at 280 nm, according to the method proposed by Albalá-Hurtado [18]. Total furfurals include free furfurals, furfurals bound to proteins (as Amadori products) and furfurals formed from the precursors (or novo furfurals).

Sample preparation was based on the Boekel and Rehman procedure [19].

(1) Total Furfurals: 15 g of milk was mixed with 5 ml of 0.15 *M* oxalic acid (prepared fresh daily) in a sealed tube to prevent evaporation. The tube was heated in a boiling water bath for exactly 25 min. After letting it cool at room temperature, 3 ml of a 40% (w/v) TCA solution was added, and the mixture was stirred (magnetic stirring plate) thoroughly for 15 min. It was then centrifuged at 2000 *g* for 15 min and two phases were obtained. The supernatant was collected and 10 ml of 4% (w/v) TCA was added to the solid residue, mixed thoroughly for 10 min and centrifuged at 2000 *g* for 15 min. The solid-phase was discarded, and the two supernatants were combined. The volume was measured, and then the mixture was filtered through a 0.45-μm filter.

(2) Free furfurals: The sample was prepared in the same way as for total furfurals but the heating in the boiling water bath was omitted.

RP-HPLC conditions: A Spherisorb ODS2 C_{18} 5-μm column (250×4.6 mm. I.D.) was used. Separations were carried out isocratically at room temperature using as the mobile phase a mixture of acetonitrile–water (5:95, v/v) at a flow-rate of 1 $ml\ min^{-1}$. Detection was in the wavelength gradient at 284 nm for HMF and F, 274 nm for FMC, and at 293 nm for MF. The injection volume was 20 μl.

Furfurals were quantified by interpolation in a calibration curve in the range 0.05–0.5 $\mu g\ ml^{-1}$ of HMF, F, FMC and MF.

The analytical parameters of the method are reported in Table 1.

Table 1
Analytical parameters of furfural compounds: hydroxymethylfurfural (HMF), furfural (F), furylmethylcetone (FMC) and methylfurfural (MF)[a]

	HMF	F	FMC	MF
Interday precision, $n=6$				
μg/100 ml milk	13.1±1.4	4.4±0.6	12.8±1.2	14.3±2.1
RSD (%)	11.2	14.5	9.4	14.4
Recovery (%) $n=3$	98.5	96.3	84.4	86.7
Detection limit				
μg/100 ml milk	1.0	2.0	2.0	2.0
μg ml^{-1} assay	0.005	0.01	0.01	0.01
Linearity	0.01–0.5 μg ml^{-1} assay 2–500 μg/100 ml milk			

[a] *n*, number of samples.

2.2.3. Determination of total protein

The Kjeldahl method was used to measure total nitrogen [20]. To convert the nitrogen values to protein the factor 6.25 was applied. The analyses were carried out in triplicate.

3. Results and discussion

The results obtained in the determination of furosine and free and total HMF are reported in Tables 2 and 3, respectively.

The structure of FUR and the chromatograms corresponding to (a) standard, (b) store-brand milk, and (c) name-brand milk, are included in Fig. 1. The structures of furfurals and the chromatograms corresponding to (a) standard, (b) sample milk, and (c) spiked sample milk, are included in Fig. 2.

No F, MF and FMC were detected in the analysed samples, because the first furfural compound formed during the Maillard reaction is HMF. The others, F, MF and FMC, are products of the most advanced stages of the Maillard reaction, or are formed by interconversion between them as a consequence of stronger heating conditions or of longer storage periods. Therefore, given that neither the processing nor the storage conditions applied are strong enough to give rise to these compounds, it is normal for them not to be detected.

FUR contents ranged from 40.32 to 50.67 and from 65.48 to 310.58 mg/100 g protein in name- and store-brand milks, respectively.

On the other hand, FUR contents of 187.43 and 1453.46 mg/100 g protein, respectively, were found in the name-brand semi-skimmed and low-lactose milks. This agrees with the report of Finot et al. [21] who indicated an increase in the blockage of lysine (from 0–2% to 55%) in low-lactose milks where lactose has been replaced by glucose.

The FUR contents in our study were in the same

Table 2
Furosine contents (expressed as mg l^{-1} of milk and mg/100 g protein) of the analysed samples

Sample	mg l^{-1} milk[a]	mg/100 g protein[a]
B_1	24.19±0.83	80.64±2.78
B_2	30.01±0.80	100.06±2.65
B_3	23.75±0.90	79.21±2.97
B_4	27.14±0.64	90.48±2.13
B_5	27.22±1.42	90.74±4.76
B_6	65.45±13.7	218.19±45.9
B_7	75.21±19.6	250.72±65.5
B_8	19.64±0.62	65.48±2.08
B_9	30.41±1.40	101.39±4.69
B_{10}	93.17±13.60	310.58±45.52
C_1	12.09±0.31	40.32±1.04
C_2	15.20±0.06	50.67±0.20
C_3	12.36±1.18	41.20±3.94
C_4	56.22±7.17	187.43±23.92
C_5	436.03±159.56	1453.46±531.88

[a] Mean±SD.

Table 3
Total and free HMF contents (expressed as μg/100 ml of milk and mg/100 g proteins) of the analysed samples[a]

Sample	μg/100 ml milk[b]		mg/100 g protein[b]	
	Total HMF	Free HMF	Total HMF	Free HMF
B_1	58.44±2.05	9.21±1.58	1.95±0.07	0.22±0.13
B_2	66.44±6.96	15.83±3.44	2.21±0.23	0.53±0.11
B_3	29.26± 3.26	23.72±5.08	0.98±0.11	0.79±0.17
B_4	45.70±7.78	9.74±0.61	1.52±0.26	0.32±0.02
B_5	22.88±3.70	7.40±1.15	0.76±0.12	0.25±0.04
B_6	54.35±0.65	50.93±4.81	1.81±0.02	1.70±0.16
B_7	53.61±7.61	34.32±9.20	1.79±0.25	1.14±0.31
B_8	21.65±5.00	8.24±0.31	0.72±0.17	0.27±0.01
B_9	55.58±4.26	12.03±3.03	1.85±0.14	0.40±0.10
B_{10}	50.67±9.04	46.56±2.65	1.69±0.30	1.55±0.09
C_1	12.15±0.43	N.D.	0.41±0.01	N.D.
C_2	11.84±0.33	N.D.	0.39±0.01	N.D.
C_3	8.73±1.35	N.D.	0.29±0.04	N.D.
C_4	59.43±7.63	39.15±2.39	1.98±0.25	1.30±0.08
C_5	317.81±56.89	65.22±2.76	10.59±1.90	2.17±0.09

[a] N.D., not detectable, that is the value is lower than the detection limit.
[b] Mean±SD.

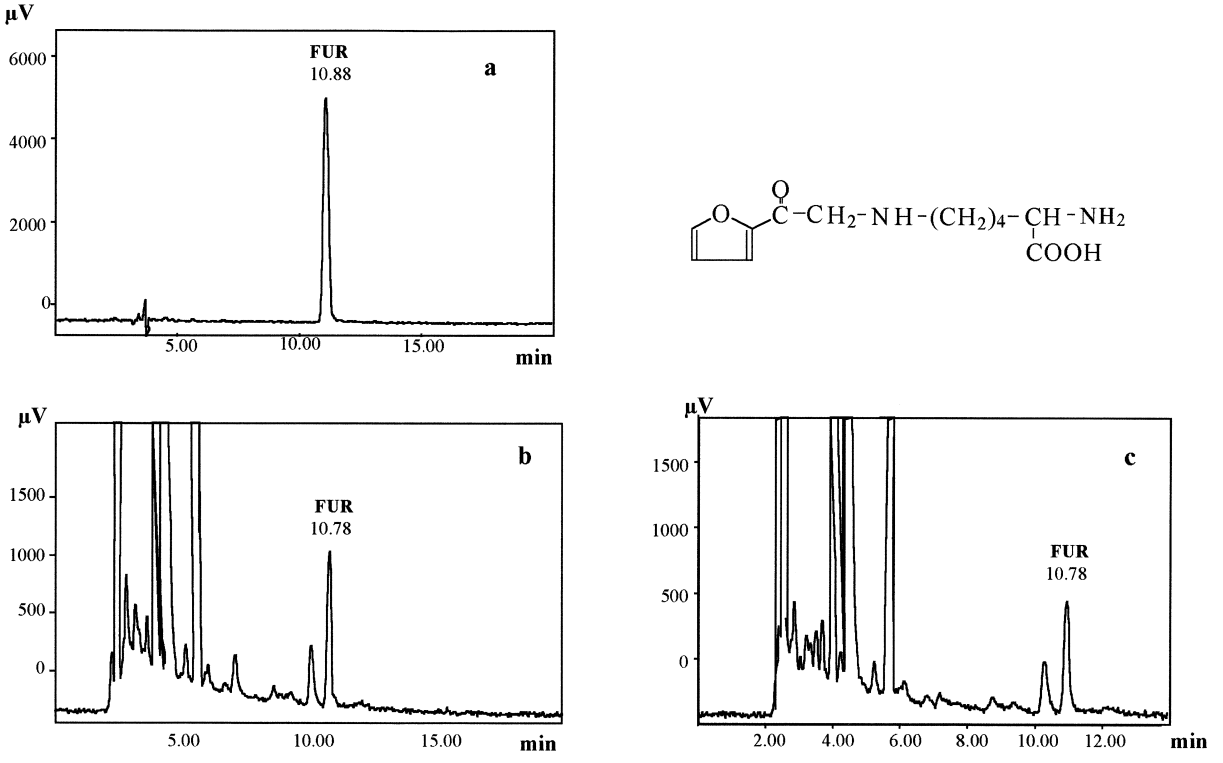

Fig. 1. The structure of FUR and the chromatograms corresponding to: (a) standard milk; (b) store-brand milk; and (c) name-brand milk.

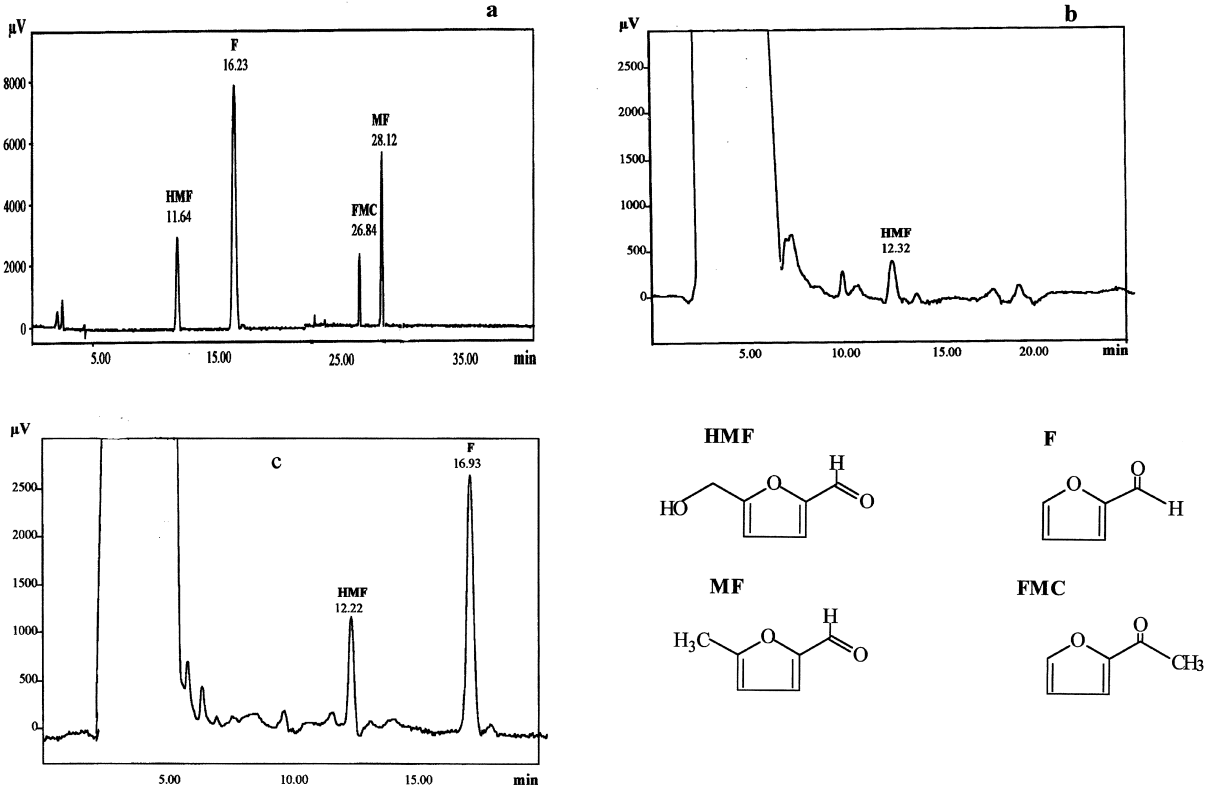

Fig. 2. The structures of furfurals and the chromatograms corresponding to: (a) standard sample; (b) milk sample; and (c) spiked milk sample.

range as those reported by different authors (see Table 4).

Free HMF was not detected in name-brand UHT milks, except for semi-skimmed milk and the low-lactose milk of the same brand, which had free HMF contents of 39.15 and 65.22 μg/100 ml milk, respectively. Store-brand milks had free HMF contents ranging from 9.21 to 50.93 μg/100 ml milk, contents that are in the range (7–75 μg/100 ml milk) reported for UHT milks [22].

The total HMF contents of whole milk of name-brands ranged from 8.73 to 12.15 μg/100 ml milk, and were lower in all cases than those corresponding to store-brand milks (21.65–66.44 μg/100 ml milk). The semi-skimmed and the low-lactose milks had total HMF contents of 59.43 and 317.81 μg/100 ml milk, respectively.

Table 4
Furosine contents in UHT cow milk reported by different authors

Heat treatment	mg l^{-1}	mg/100 g protein	References
UHT		56–220	[17]
	1.6–63		[29]
		50–180	[31]
	15.7–53.4		[33]
	12.1–93.2	40–310	This study
Direct UHT		40–100	[2]
		35–109	[3]
	30.21		[15]
	17.8–50.5		[30] [32]
		50–170	[34]
	8–14		[35]
Indirect UHT		130–210	[2]
		118–193	[3]
	39.44		[15]
	14.6–89.1		[30]
	17.6–85.4		[32]
		150–300	[34]
	15–53		[35]

The total HMF contents found in our study are consistent with those reported by other authors: 57.5–161.5 μg/100 ml milk [9], 149–420 μg/100 ml milk [22], 44.13–107.19 μg/100 ml milk [23], and 119.8–1445.2 μg/100 ml milk [24] in UHT milks. In all these reports the range of contents is wide.

A comparison of the individual values of the total HMF and FUR contents of each sample shows that FUR and total HMF contents of name-brand milks are lower than those of store-brand milks, and that the former have total HMF contents, indicators of the advanced step of Maillard reactions, lower than store-brand milks

Special attention should be paid to the high FUR and total HMF contents in relation to the free HMF content found in low-lactose-content milk in comparison with the values obtained in a semi-skimmed milk of the same brand. These differences could be ascribed to the treatment used to remove lactose (enzymatic hydrolysis with β-galactosidase, prior to or after thermal treatment of milk) [25]. This hydrolysis releases galactose and fructose, carbohydrates that undergo the Maillard reaction to a greater extent than lactose [21,26–28]. It would therefore be necessary, in further studies, to monitor the quality of the commercialized low-lactose UHT milks.

4. Conclusion

Differences were observed in our study between the FUR and HMF contents of store- and name-brand UHT milks. The values of both Maillard reaction indicators were higher in store-brand UHT milks than in name-brand, and this indicates poorer quality of the former.

Therefore, attention should be paid to the quality of store-brand UHT milks, because of the detected differences between name-brand and store-brand milks.

Although the formation of Maillard compounds in store-brands can reflect the use of a low-quality raw product and of stronger processing and storing conditions than in name-brand milks, the type of products formed and their amounts do not involve any risk for the consumers.

References

[1] C. Pompei, M. Rossi, F. Mare, J. Food Quality 10 (1987) 375.
[2] L. Pellegrino, I. De Noni, P. Resmini, Int. Dairy J. 5 (1995) 647.
[3] R. Van Renterghem, J. De Block, Int. Dairy J. 6 (1996) 371.
[4] R.F. Hurrell, P.A. Finot, J.E. Ford, Br. J. Nutr. 49 (1983) 343.
[5] J.M. O'Brien, P.A. Morrissey, Bull. Int. Dairy Fed. 238 (1989) 53.
[6] F. Evangelisti, C. Calcagno, P. Zunin, J. Food Sci. 59 (1994) 335.
[7] L. Pizoferrato, P. Manzi, V. Vivanti, I. Nicoletti, C. Corradini, E. Cogliandro, J. Food Prot. 61 (1998) 235.
[8] N. Corzo, T. Delgado, Alimentación, Equipos Tecnol. 6 (1991) 73.
[9] F.J. Morales, C. Romero, S. Jimenez-Pérez, Chromatographia 33 (1992) 45.
[10] E. Guerra-Hernandez, B. Garcia-Vilanova, J. Montilla-Gomez, J. Liq. Chromatogr. 15 (1992) 2551.
[11] F. Lo Coco, L. Ceccon, C. Valentini, V. Novelli, J. Chromatogr. 590 (1992) 235.
[12] S. Albalá-Hurtado, M.T. Veciana-Nogués, A. Mariné-Font, M.C. Vidal-Carou, J. Agric. Food Chem. 46 (1998) 2998.
[13] E. Ferrer, A. Alegría, R. Farré, P. Abellán, F. Romero, Food Sci. Tech. Int. 5 (1999) 447.
[14] M.P. Fernández-Albalat, B. Guerra, J. Méndez, Alimentaria 29 (1997) 29.
[15] T. Delgado, N. Corzo, G. Santa-Maria, M.L. Jimeno, A. Olano, Chromatographia 33 (1992) 7.
[16] W. Büser, H.F. Erbersdobler, J. Chromatogr. 343 (1985) 363.
[17] P. Resmini, L. Pellegrino, G. Battelli, Ital. J. Food Sci. 3 (1990) 173.
[18] S. Albalá- Hurtado, M.T. Veciana- Nogués, M. Izquierdo-Pulido, M.C. Vidal- Carou, J. Agric. Food Chem. 45 (1997) 2128.
[19] M.A.J.S. Van Boekel, Z.U. Rehman, Neth. Milk Dairy J. 41 (1987) 297.
[20] Association of Official Analytical Chemists (AOAC), Official Methods of Analysis, 15th Edition, Washington, DC, 1980, p. 807.
[21] P.A. Finot, R. Deutsch, E. Bujard, Prog. Fd. Nutr. Sci. 5 (1981) 345.
[22] M. Rossi, C. Pompei, Ital. J. Food Sci. 1 (1991) 39.
[23] R. Fink, H.G. Kessler, Milchwissenschaft 41 (1986) 638.
[24] M.M. Hewedy, C. Kiesner, K. Meissner, J. Hartkopf, H.F. Erbersdobler, J. Dairy Res. 61 (1994) 305.
[25] A.H. Varnam, J.P. Sutherland, in: Leche y Productos Lácteos, Acribia, Zaragoza, 1995, p. 75, Chapter 2.
[26] J.C. Wolf, D.R. Thompson, J. Food Sci. 42 (1977) 1540.
[27] S.H. Aszhor, J.B. Zent, J. Food Sci. 49 (1984) 1206.
[28] W.M. Baisier, T.P. Labuza, J. Agric. Food Chem. 40 (1992) 707.
[29] H.F. Erbersdobler, B. Dehn-Müller, Bull. Int. Dairy Fed. 238 (1989) 62.

[30] R. Lopez-Fandiño, N. Corzo, M. Villamiel, T. Delgado, A. Olano, M. Ramos, J. Food Prot. 56 (1993) 263.
[31] T. Henle, Z. Günter, H. Klostermeyer, Z. Lebensm. Unters. Forsch. 200 (1995) 235.
[32] N. Corzo, T. Delgado, E. Troyano, A. Olano, J. Food Prot. 57 (1994) 737.
[33] N. Corzo, R. López- Fandiño, T. Delgado, M. Ramos, A. Olano, Z. Lebensm. Unters. Forsch. 198 (1994) 302.
[34] P. Resmini, L. Pellegrino, Bull. IDF 298 (1994) 31.
[35] H.F. Ersbersdobler, B. Dehn, A. Nangpal, H. Reuter, J. Dairy Res. 54 (1987) 147.