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Determination of mono- and disaccharides in milk and milk products by high-performance anion-exchange chromatography with pulsed amperometric detection

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Abstract

A simple and sensitive liquid chromatographic method for the separation and quantification of mono- and disaccharides in raw- and processed-milk is described. Samples of cows', buffalos', sheeps' and goats' milk were analyzed upon clarification and appropriate dilution for the quantification of lactose, galactose, glucose and *N*-acetylglucosamine (GlcNAc). The separation was accomplished by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD), using a gold working electrode and dilute alkaline eluents modified by a millimolar concentration of barium acetate. The eluent composition employed was designed to provide optimum separation with respect to the selected sample, without interference from the matrix components. The analytical method was successfully employed for the determination of mono- and disaccharides naturally occurring in dairy milk, mozzarella cheese and whey samples, with high sensitivity and accuracy.
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1. Introduction

Quality control of milk and its derivatives is a very demanding field and the need for sensitive, time-saving and accurate analytical methods to be developed is pressing. Among the relevant number of compounds present in whole milk (e.g. proteins, fats, vitamins, hormones, etc.), it is desirable to characterize the sugar profile of naturally occurring compounds both to augment the knowledge of their physiological and biochemical functions in different mammalian species, and to optimize the industrial processes of milk along with its quality control [1–3]. Carbohydrates are water-soluble compounds, and generally, they lack groups which are necessary for UV and fluorescence detection. The various low and high cost analytical methods used for carbohydrate analysis are mass spectrometry [4], ^{1}H-nuclear magnetic resonance (NMR) spectroscopy [5], polyacrylamide gel electrophoresis (PAGE) [6], along with gas-chromatography [7–9] and high-performance liquid chromatography [10] upon precolumn derivatization.

In the last two decades, the elevated capabilities of high-performance anion-exchange chromatography (HPAEC) in the separation of polar aliphatic compounds (e.g. alditols, sugars, alkanolamines, amines, aminoacids, etc.) have been well established and consolidated [11–17]. HPAEC allows high resolution of

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closely related carbohydrates, and in conjunction with pulsed amperometric detection (PAD) is one of the most suitable methods for carbohydrates analysis because it is quantitative and does not need pre- or postcolumn derivatization. Both these last issues represent the major advantages of PAD over other detection methods, such as differential refractometric and evaporative light scattering (ELSD). Indeed, refractive index (RI) measurement lacks sensitivity, moreover depends on temperature and flow-rate, and it is incompatibile with gradient elution. As far as the ELSD, it is strongly affected by the sample matrix. PAD allows very high signal response reproducibility, using well-designed potential waveforms which are suitable to detect electroactive compounds, along the cleaning and reactivation of the electrode surface [18]. HPAEC–PAD fundamentals and applications has been extensively reviewed [12,14,17,19,20]. In a previous paper, we reported a very effective and accurate quantitative determination of lactulose in heat-treated milks by HPAEC in conjunction with pulsed amperometry [21]. However, no simple and sensitive methods are as yet available to detect the presence of natural minor sugars in raw- and processed-milk.

Here, we demonstrate the usefulness of HPAEC–PAD for characterizing the mono- and disaccharide profile of milk relevant to some common mammalian species. Although cows' milk is the predominant type in several countries, buffalo, goat, and sheep milks are also consumed. The presence of galactose in mozzarella and mozzarella surrogate samples was also evaluated. The alkaline eluent composition employed was designed to provide optimum separation with respect to the selected sample matrix and to match the requirement for direct electrochemical detection at a gold working electrode.

2. Experimental

2.1. Chemicals

Sodium hydroxide, 50% solution in water (1.515 g/ml), $Ba(OAc)_2$ 99%, 2-deoxy-D-glucose (dGlc) 99%, D-galactose 99%, D-glucose 99.5%, lactose monohydrate 97%, were purchased from Aldrich Chemical Co. (St. Louis, MO, USA), NaN_3, *N*-acetyl-D-glucosamine (GlcNAc) 98%, *N*-acetyl-D-galactosamine (GalNAc) 98% were from Sigma Chemical Co. (Steinheim, Germany). Other chemicals were from Carlo Erba (Milan, Italy). All reagents were used as received. Doubly distilled deionized water was used throughout for preparing solutions. Sodium hydroxide solutions used as eluents were prepared by dilution of a carbonate-free 50% (w/w) NaOH solution in water, previously filtered with 0.45 μm membrane and degassed with helium. The exact concentration of hydroxide ions in the mobile phases was determined by titration against a standard solution of hydrochloric acid.

2.2. Apparatus

All experiments were performed using a Dionex system (Dionex, Sunnyvale, CA, USA) composed of a metal-free isocratic pump Model IP20 with on-line degas and a pulsed amperometric detector (Model ED40). Compounds under investigation were injected with a metal-free rotary injection valve Model RH9125 (Rheodyne, Cotati, CA, USA) equipped with a 10 μl injection loop and separated with a CarboPac PA1 column (250 mm × 4 mm i.d.) coupled with a guard column (50 mm × 4 mm i.d.). The flow-through detection cell (Dionex) is made from a 1.0 mm diameter gold working electrode and a pH-Ag|AgCl combination reference electrode; the titanium body of the cell served as the counter electrode. The chromatographic system was interfaced, via proprietary network chromatographic software (PeakNet^{TM}), to a personal computer, for instrumentation control, data acquisition and processing (Dionex). The potential waveform parameters applied at the gold working electrode are given in Table 1. As column temperature

Table 1
Details of the potential waveform used in this study for carbohydrate detection by HPAEC–PAD[a]

Time (s)	Potential (V) vs. Ag|AgCl		Current integration
0.00	E_{DET}	+0.05	
0.24		+0.05	Start
0.44		+0.05	End
0.45	E_{OX}	+0.80	
0.63		+0.80	
0.64	E_{RED}	−0.22	
1.00		−0.22	

[a] Gold working electrode in alkaline solution.

influences the anion-exchange separation, the column was kept at a constant temperature 20 °C using a home-made water jacket coupled with a circulating water bath model WK4DS from Colora (Colora, Messtechnik GmbH, Germany). A centrifuge model ALC Refrigerated Centrifuge PK 120 R (ALC International, Milan, Italy) was used for sample preparation.

2.3. Eluent preparation

The alkaline eluents were prepared by following the procedure developed in this laboratory. Briefly, pure water for the eluent preparation was degassed before use by flushing helium for about 20 min. Barium acetate was dissolved and, upon addition of the proper amount of labeled carbonate-free 50% (w/w) NaOH, the eluent solution was kept in plastic bottles and a Dionex eluent organizer (EO1) was used to saturate it with N_2 gas to minimize CO_2 adsorption. Elution was performed isocratically at a flow-rate of 1.0 ml/min. The analytical column was periodically regenerated (usually every 2–3 weeks) by pumping 5×10^{-3} M HCl, water, 200 mM NaOH and then water, in that order over a period of 20 min at a flow-rate of 1.0 ml/min.

2.4. Samples

A range of raw samples from cow's, buffalo's, sheep's and goat's milk were purchased at local farms located in the Basilicata region (south of Italy). Samples were stored in acid-washed polypropylene containers without preservatives, and they were frozen at −18 °C until analysis. All samples were prepared in such a way as to protect the analytical columns. Milk samples were spiked by dGlc used as an internal standard (IS); peak area was used for quantification work. Milk proteins were precipitated by using Carrez solutions I (2.7 g $K_4Fe(CN)_6$ in 100 ml) and II (5.5 g $Zn(OAc)_2$ in 100 ml) [22] and centrifuging the samples at 4000 rpm for 30 min at 4 °C in order to remove fats [23]. Before column injection the supernatant was diluted with water and filtered on 0.2 μm nylon membranes (Whatman plc, Kent, UK). About 10 g of milk derivates were weighed, mixed with 10 ml water, homogenized, sonicated for 15 min and then filtered on nylon membrane. The peaks identification was made performing spiking experiments.

3. Results and discussion

3.1. Quantitative determination of milk sugars

The use of dilute alkaline eluents (e.g. 10–20 mM NaOH) in anion-exchange chromatography suffers from a continuous shortening of retention time on consecutive injections. As already demonstrated, this is an effect of carbonate uptakes and its interferences during chromatographic elution [24]. For this reason, the analytical column needs to be repeatedly flushed with a 0.1 M NaOH and then equilibrated after each chromatographic run. In previous works, it has been shown [25,26] that the separation of closely related monosaccharides is possible using 10–12 mM NaOH modified with barium acetate 1–2 mM.

Fig. 1 shows typical chromatograms obtained in HPAEC–PAD of four raw milk samples from bovine, buffalo, sheep and goat, panels (A)–(D), respectively, using optimized elution conditions on an anion-exchange column (CarboPac PA1): 10 mM NaOH, 1 mM $Ba(OAc)_2$ eluted at 1.0 ml/min, and column temperature at 20 °C. At least four samples of milk from each animal type from different producers were examined. As can be seen, these interference-free chromatographic profiles exhibit significant difference in terms of sugars content, which are not the consequence of microbial contamination, seasonal variations or simple differences in feeding patterns. In addition to lactose (peak 5), at a retention time of ca. 12 min, and except for the internal standard (peak IS), plot (A) exhibits four well-separated peaks (peaks 1–4) whose retention times match those of galactose (peak 1), glucose (peak 2), *N*-acetylgalactosamine (GalNAc) and *N*-acetylglucosamine (GlcNAc) (peak 3), and fructose (peak 4) along with several other minor peaks, which have not yet been assigned. As previously noted, the present chromatographic conditions do not allow the separation of *N*-acetylhexosamines, namely GalNAc and GlcNAc [25], and the quantification of peak 3 was accomplished considering it as due to GalNAc. For retention times greater than 15 min, in each chromatographic profile of Fig. 1, some additional peaks were revealed, which still need to be identified. Further investigations in this direction are currently under way.

The mono- and disaccharide content of whole milks as determined by HPAEC–PAD is summarized

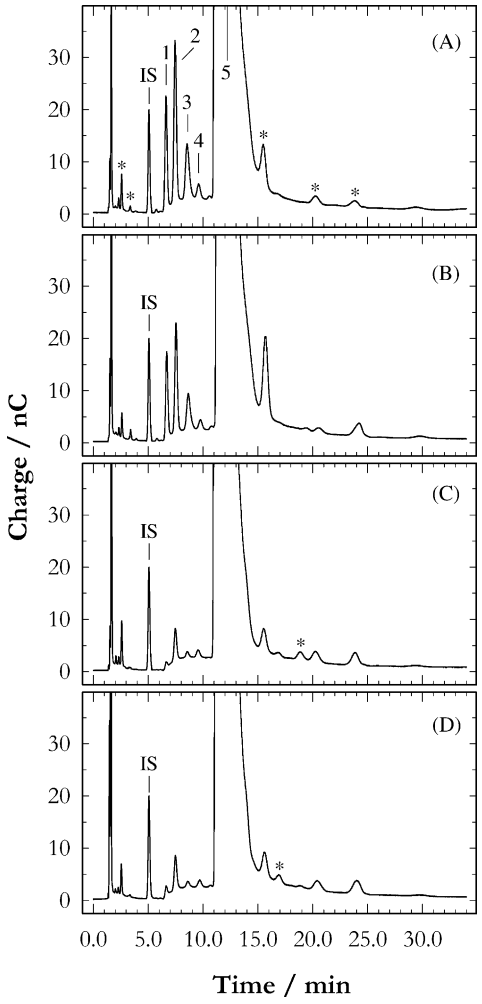

Fig. 1. Separation of raw milk samples with HPAEC–PAD: (A) cow's, (B) buffalo's, (C) goat's, and (D) sheep's milk. All samples were diluted (1:20); eluent, 10 mM NaOH + 1 mM $Ba(OAc)_2$ at a flow-rate of 1.0 ml/min. Column and guard column (Dionex CarboPac PA1). Peaks: (IS) dGlc; (1) galactose; (2) glucose; (3) GalNAc + GlcNAc; (4) fructose; and (5) lactose. Column temperature: 20 °C. Pulsed amperometric detection using the potential waveform reported in Table 1. Peaks marked with asterisk belong to unidentified compounds.

in Table 2. While lactose concentrations in milk samples from goat, buffalo, sheep and cow, ranged from 41 to 52 mg/l, large difference of galactose, glucose and *N*-acetyl hexosamines can be noted. Due to the large amounts of lactose compared with the amount of the minor milk sugars, accurate quantification was made possible only upon dilution 1:100 of the samples. Indeed, the linear range extends up to two orders of magnitude above the limit of detection [25]. Samples from cow's and buffalo's milk are not easily distinguishable, and the same applies when goat's and sheep's milk are compared. Interestingly, milks from bovine and buffalo contained significantly more *N*-acetylhexosamines than other mammals. The higher content of free GalNAc in these raw milks is consistent with data reported by Troyano et al. [27]. A galactose concentration higher than 3 mg/100 ml was determined in cow's and buffalo's milk. Moreover, such milks contained a glucose level lower than 3 mg/100 ml, a value that was in good agreement with that reported by Giescke et al. [28]. We found a total content of galactose, glucose and GlcNAc in cow's milk equal to 11.65 mg/100 ml, which is in good agreement with that stated by Renner [29], who reported a free monosaccharide content not higher than 12 mg/100 ml. Galactose and glucose were also detected in goat's and sheep's milks but at a much lower concentration (galactose <0.6 mg/100 ml and glucose <1.55 mg/100 ml). It is speculated that the differences between the minor sugars of cows', ewes' and goats' milk may be used as supplementary evidence to determine the composition of milk mixtures.

3.2. Analytical performance of HPAEC–PAD

The above examples illustrate the successful detection of sugars in milk by HPAEC–PAD. The mean retention times and the relative standard deviations (R.S.D.s) evaluated for the selected minor sugars normally found in milk of the most common mammalian species are listed in Table 3. There was no negative or positive trending in the peak area or retention times over repetitive injections, thus suggesting that the sample was not fouling either the column or the working electrode. Reproducibility of the present analytical method applied to real matrices was evaluated by measuring the peak chromatographic area five times on the same sample of raw cow's milk. Injection repeatability was calculated as the R.S.D. of peak area for each analyte, and the average value for all compounds was determined to be lower than 4.2%. The intra-assay precision was estimated with repeated analysis of a sample that has been independently prepared, over 1 day, yielding an average R.S.D. of 3.9%

Table 2
Sugar content (mean value ± S.D., $n = 3$) in whole milk of the most common mammalian species evaluated by HPAEC–PAD[a]

	Galactose (mg/100 ml)	Glucose (mg/100 ml)	GalNAc (mg/100 ml)	Lactose (g/100 ml)
Cow's milk	4.04 ± 0.09	2.52 ± 0.07	5.09 ± 0.05	4.1 ± 0.1
Buffalo's milk	3.32 ± 0.05	2.72 ± 0.07	6.57 ± 0.07	4.8 ± 0.1
Goat's milk	0.56 ± 0.05	1.55 ± 0.07	<0.5	5.2 ± 0.1
Sheep's milk	0.31 ± 0.02	0.32 ± 0.05	<0.5	4.1 ± 0.1

[a] Dionex CarboPac PA1 plus guard column; eluent, 10 mM NaOH + 1 mM $Ba(OAc)_2$ at a flow-rate of 1.0 ml/min. Sample injection volume: 10 μl.

and the between-day variance was assessed to be 4.6%. The extent of recovery was determined analyzing three samples of cow milk to which standard amounts of galactose, glucose, GalNAc and lactose were added and mixed. After processing the samples were analyzed by HPAEC–PAD and the average recovery obtained from triplicate samples was 98.3 ± 2.5%.

3.3. Carbohydrate content in milk derivates

In recent years, there has been an increasing demand for mozzarella and its surrogates having longer shelf-lives and decreased costs. The mozzarella surrogates are low-cost products obtained by blending shredded natural cheeses of different types and degrees of maturity, and dairy and non-dairy ingredients with emulsifying agents [30]. The raw materials used in processed cheese highly affect the course of the cooking process and main features of the final product, especially its appearance, flavor, texture and keeping quality. The Italian legislation does not allow the presence of large amounts of compounds formed as a result of heat-treatment; for example the furosine content has to be lower than 12 mg for 100 g of proteins [31]. At the same time, low levels of galactose are required for these surrogates, since this monosaccharide, like many other simple sugars, reacts with the ε-ammino groups of lysine-rich proteins during the cooking of pizza leading to thermally induced non-enzymatic browning, also known as Maillard reactions [32–34]. Fig. 2 (plot (B)) shows the typical separation obtained in HPAEC–PAD of mono- and disaccharides occurring in mozzarella surrogate samples. For a direct comparison, plot (A) of the same figure shows the separation of a standard sugar mixture: IS, galactose,

Table 3
Retention times of sugars occurring in milk as determined by HPAEC–PAD[a]

Sugar	Retention time (min)	R.S.D.[b] (%)
IS (dGlc)	5.07	2.2
Galactose	6.62	2.3
Glucose	7.43	2.5
GlcNAc	8.53	2.7
GalNAc	8.55	3.3
Fructose	9.58	2.2
Lactose	12.35	4.1

[a] Dionex CarboPac PA1 plus guard column; eluent, 10 mM NaOH + 1 mM $Ba(OAc)_2$ at a flow-rate of 1.0 ml/min. Column temperature: 20 °C. The waveform employed for PAD is described in Table 1.

[b] Relative standard deviation evaluated from five repetitive injections.

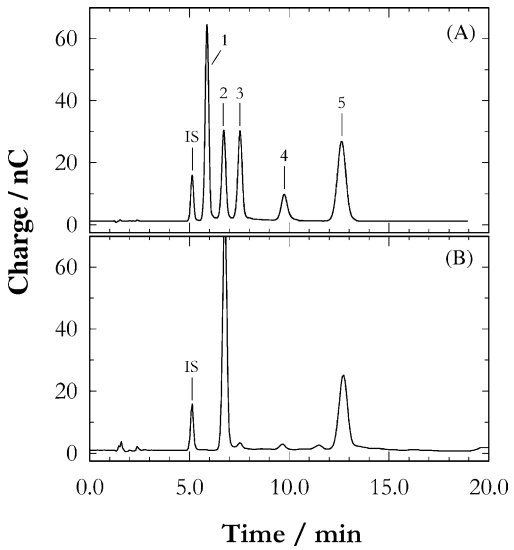

Fig. 2. HPAEC–PAD chromatograms obtained for (A) a standard sugar mixture, and (B) a sample of mozzarella surrogates Pizzottella, Prealpi S.p.A. (Varese, Italy). Peaks: (IS) dGlc; (1) galactose; (2) glucose; (3) GalNAc, (4) fructose; and (5) lactose. Other conditions are as in Fig. 1.

glucose, *N*-acetylgalactosamine, fructose and lactose. While the chromatographic profile reveals the presence of glucose (peak 2) and lactose (peak 5) in significant amounts, no free galactose (peak 1) was found.

Upon lactose hydrolysis the glucose moiety of lactose is metabolized via glycolysis, while galactose is released into the external medium as milk microorganisms are deficient in the metabolic Leloir pathway [35,36]. The Leloir pathway enzymes are responsible for the conversion of galactose to glucose-1P [37]. Browning in mozzarella surrogate is likely to be due to galactose undergoing Maillard reactions. Gopal and Richardson [23] reported that grated Parmesan cheese, manufactured in New Zealand, undergoes a significant browning after packaging and over its shelf-life when the concentration of galactose is greater than 0.08% (w/w). Fig. 3 shows two chromatograms of aqueous extracts from the best selling mozzarella cheeses manufactured in Italy, known as "pasta filata" of bovine's and buffalo's milk, plots (A) and (B), respectively. Galactose (peak 1) in these samples can be detected without any interference from coexisting electroactive compounds. Peak identification was confirmed by the addition of authentic standards. The levels of galactose were found between 5 and 7 mg/100 ml of aqueous extract (i.e. approximately 0.007% (w/w)).

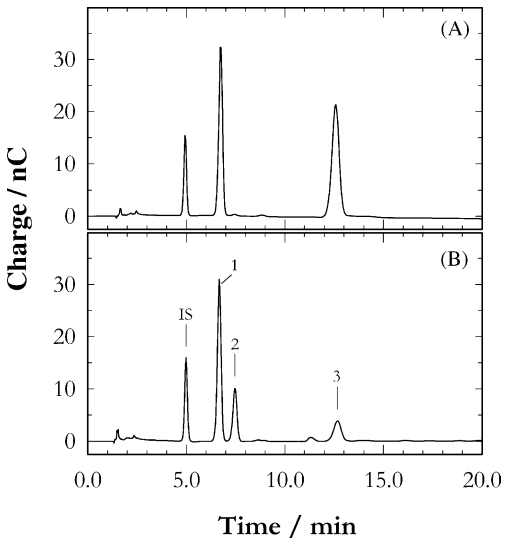

Fig. 3. HPAEC–PAD chromatograms of two samples of mozzarella from cow's milk (A) and buffalo's milk (B). Peaks: (IS) dGlc; (1) galactose; (2) glucose; and (3) lactose. Other conditions are as in Fig. 1.

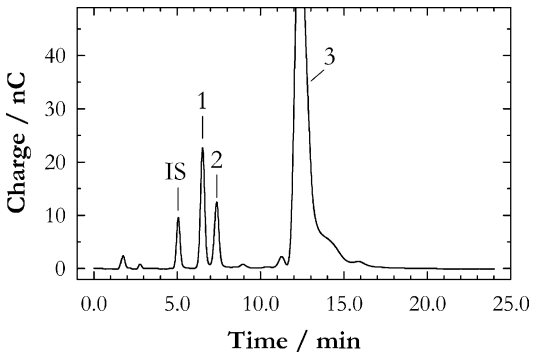

Fig. 4. Whey permeate liquid sample (diluted 1:80) separated by HPAEC–PAD. Peaks: (IS) dGlc; (1) galactose; (2) glucose; and (3) lactose. Other conditions are as in Fig. 1.

3.4. Analysis of sugars in bovine whey

As the by-product of dairy industry, liquid whey poses serious environmental concerns worldwide because of its high biochemical and chemical oxygen demand [38,39]. Whey contains about 50% of the nutrients originally present in milk and whenever possible it is processed and used as nutritional ingredient in animal feeds and food products. The use of modern membrane technology offers a means of producing rich-lactose whey, and this notably holds for a full utilization of such a product. Fig. 4 shows the chromatogram obtained from a sample of liquid whey permeate whereby the content of lactose, galactose and glucose was found equal to 44.26, 1.05 and 0.58 g/l, respectively. As can be seen the minor sugars profile is almost similar to that obtained for milk samples, suggesting the relatively low impact of the cheese-making processes, at least when the soluble fraction of milk is considered. Interestingly, the possible utilization of whey will likely attract increasing interest, and HPAEC–PAD may offer a very simple tool for studying changes in the carbohydrate content.

4. Conclusions

Despite the complexity of milk and the large concentration imbalances between lactose and minor sugar compounds, finely balanced elution conditions (i.e. column, mobile phase composition and temperature) allow the quantification of minor milk sugars

by HPAEC–PAD with high sensitivity. A minimum sample treatment (clean-up, dilution and filtration) prior to injection is required. The application of this method to the analysis of milk samples from the most common mammals has been demonstrated. It can be also employed as a valuable tool to routinely analyze milk derivates. Further applications may include raw and commercial processed liquid milk, powdered milk and sweet whey powders.

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References

[1] M.A. de la Fuente, Trends Food Sci. Technol. 9 (1998) 281.
[2] C. Romero, F.J. Morales, S. Jiménez-Pérez, Food Res. Int. 34 (2001) 389.
[3] J. Belloque, M. Villamiel, R. López-Fandiño, A. Olano, Food Chem. 72 (2001) 407.
[4] A.R. Laine, Methods Enzymol. 193 (1990) 539.
[5] R. Barker, H. Nunez, P. Rosevear, A.S. Serianni, Methods Enzymol. 83 (1990) 58.
[6] C.M. Starr, R.I. Masada, C. Hauge, E. Skop, J.C. Klock, J. Chromatogr. A 720 (1996) 295.
[7] H. Björndal, B. Lindberg, S. Svensson, Carb. Res. 5 (1967) 433.
[8] G.C. Hansson, Y.-T. Li, H. Karlsson, Biochemistry 28 (1989) 6672.
[9] L.M. Chiesa, L. Radice, R. Belloli, P. Renon, P.A. Biondi, J. Chromatogr. A 847 (1999) 47.
[10] M.H. Gey, K.K. Unger, Fresenius J. Anal. Chem. 356 (1996) 488–494.
[11] Y.C. Lee, Anal. Biochem. 189 (1990) 151.
[12] D.C. Johnson, W.R. LaCourse, Anal. Chem. 62 (1990) 589.
[13] D.A. Dobberpuhl, D.C. Johnson, J. Chromatogr. A 694 (1995) 391.
[14] W.R. LaCourse, Pulsed Electrochemical Detection in High-Performance Liquid Chromatography, Wiley, New York, 1997.
[15] A.P. Clarke, P. Jandik, R.D. Rocklin, Y. Liu, N. Avdalovic, Anal. Chem. 71 (1999) 2774.
[16] V.P. Hanko, J.S. Rohrer, Anal. Biochem. 283 (2000) 192.
[17] T.R.I. Cataldi, C. Campa, G.E. De Benedetto, Fresenius J. Anal. Chem. 368 (2000) 739.
[18] D.C. Johnson, W.R. LaCourse, Anal. Chem. 62 (1990) 589.
[19] D.C. Johnson, W.R. LaCourse, in: Z. El Rassi (Ed.), Carbohydrate Analysis—High Performance Liquid Chromatography and Capillary Electrophoresis, Elsevier, Amsterdam, 1995 (Chapter 10).
[20] Y.C. Lee, J. Chromatogr. A 720 (1996) 137.
[21] T.R.I. Cataldi, M. Angelotti, S.A. Bufo, Anal. Chem. 71 (1999a) 4919.
[22] G.R. Andrews, J. Soc. Dairy Tech. 37 (1984) 3.
[23] P.K. Gopal, R.K. Richardson, Int. Dairy J. 6 (1996) 399.
[24] T.R.I. Cataldi, C. Campa, G. Margiotta, S.A. Bufo, Anal. Chem. 70 (1998) 3940.
[25] T.R.I. Cataldi, C. Campa, M. Angelotti, S.A. Bufo, J. Chromatogr. A 855 (1999) 539.
[26] K. Kaiser, R. Benner, Anal. Chem. 72 (2000) 2566.
[27] E. Troyano, A. Olano, I. Martínez-Castro, J. Dairy Res. 62 (1995) 147.
[28] W.H. Giescke, A.M. Durand, I.M. Petzer, Onderstepoort, J. Vet. Res. 51 (1984) 15.
[29] E. Renner, Micronutrients in Milk and Milk-Based Products, Elsevier, Amsterdam, 1989.
[30] L.J. Kiely, S.L. McConnell, P.S. Kindstedt, J. Dairy Sci. 74 (1991) 3568.
[31] Gazzetta Ufficiale Italiana, Quantità massime ammissibili di furosina in mozzarella ed altri formaggi freschi a pasta filata, Decreto Ministeriale, No. 69, 24 March 1994.
[32] J. O'Brien, in: P.F. Fox (Ed.), Heat-Induced Changes in Lactose: Isomerisation, Degradation, Maillard Browning, second ed., International Dairy Federation, Brussels, 1995, pp. 134–170.
[33] M. Friedman, J. Agric. Food Chem. 44 (1996) 631.
[34] M.A.J.S. van Boekel, Food Chem. 62 (1998) 403.
[35] P.A. Frey, FASEB J. 10 (1996) 561.
[36] K. Bettenbrock, C.A. Alpert, Appl. Environ. Microbiol. 64 (1998) 2013.
[37] B. Grossiord, E.E. Vaughan, E. Luesink, W.M. de Vos, Lait 78 (1998) 77.
[38] A.J. Mawson, Biores. Technol. 47 (1994) 195.
[39] M.I. Gonzàles Siso, Biores. Technol. 57 (1996) 1.