Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 910–917
Food Chemistry
www.elsevier.com/locate/foodchem

Assessment of quality and technological characterization of lactose-hydrolyzed milk

M.C. Messia, T. Candigliota, E. Marconi *

DISTAAM, Università del Molise, Via De Sanctis, 86100 Campobasso, Italy

Received 4 August 2006; received in revised form 12 October 2006; accepted 18 December 2006

Abstract

The intensity and sequence of heat and hydrolytic treatments as well as storage stability of lactose-hydrolyzed milk was assessed during processing and storage in 15 different commercial samples by monitoring the glycidic fraction (glucose, lactose and galactose) and selected thermal treatment markers (furosine, lactulose and fructose). The use of an additional indicator (fructose) together with classical process indicators (lactulose and furosine), was useful to better understand the quality of this dietetic milk and the processing procedures utilized. The results confirmed the high reactivity of lactose-hydrolyzed milk to the Maillard reaction and the more limited chemical stability of this milk typology when stored at 20 °C. In addition, a wide variability in the quality of commercial samples of lactose-hydrolyzed milk was found, which underlines the necessity to establish definite thresholds for this milk to defend both consumers and product quality.

Keywords: Lactose-hydrolyzed milk; Process and quality markers; Storage stability

1. Introduction

Lactose, a disaccharide found in milk at concentrations from 4.5 to 5.0 g/100 ml, is absorbed after conversion to its monosaccharide units glucose and galactose by lactase. In certain populations, there are many individuals in which the activity of lactase is insufficient to produce satisfactory lactose hydrolysis, provoking bloating, abdominal cramps, flatulence, nausea, diarrhea and loss of appetite, known as lactose intolerance (Di Stefano & Veneto, 2001; Hourigan, 1984; Torun, Solomons, & Viteri, 1979).

In recent years, the interest of the dairy industry in the enzymatic hydrolysis of lactose has progressively increased throughout the world as a result of newly acquired knowledge indicating the extent of lactose malabsorption and the potential market for modified milk products (Harju, 2003; Jelen & Tossavainen, 2003; Zadow, 1992, 1993). In fact, more than 70% of the world population suffers from the inability to use lactose or lactose-containing products due to lactose intolerance caused by the lack of β-galactosidase activity (Harju, 2003; Jelen & Tossavainen, 2003; Mahoney, 1997; Sloan, 1999; Vasiljevic & Jelen, 2003).

In this context, the food industry is responding to consumer demands by offering lactose-hydrolyzed milk and lactose-free milk in which some lactose has been removed physically and the rest is hydrolyzed to obtain the same sweetness as ordinary milk (Harju, 2003; Jelen & Tossavainen, 2003; Zadow, 1993). Various lactose-hydrolysis technologies are available for the production of lactose-hydrolyzed milk: for example, hydrolysis with soluble or immobilized enzymes can be performed either before or after heating (pasteurization, UHT) (Harju, 2003; Jelen & Tossavainen, 2003; Lanzarini, 1998; Morisi, 1977; Pastore, 1977; Roger, Thapon, Maubois, & Brule, 1976; Zadow, 1992). Considerable efforts have been dedicated to the qualitative, technological and nutritional characterization of ordinary milks (pasteurized, UHT, in container sterilized), whereas characterization of the processing and quality assessment of these dietetic milks has received very little

* Corresponding author. Tel.: +39 0874 404616; fax +39 0874 404652.
E-mail address: marconi@unimol.it (E. Marconi).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.12.045

attention. Several papers, in fact, have reported various process markers (furosine, lactulose, hydroxymethylfurfural, α-lactalbumin and β-lactoglobulin) with well defined thresholds for assessing ordinary pasteurized and sterilized milk (Claeys, Van Loey, & Hendrickx, 2002; Pellegrino, Resmini, & Luf, 1995).

The aim of this work was characterization of lactose-hydrolyzed milk during processing and storage by analyzing the glycidic fraction (lactose, glucose and galactose) and specific processing markers (furosine, fructose and lactulose) to evaluate the intensity and sequence of heat and hydrolytic treatments as well as storage stability. Moreover, several commercial lactose-hydrolyzed milks were assessed.

2. Materials and methods

2.1. Assessment of lactose-hydrolyzed milk during processing

Samples of lactose-hydrolyzed milk (semi-skimmed) from three successive batches, obtained at the pilot plant of the Research and Development Centre of Parmalat (Sala Baganza, Parma, Italy), were collected at different stages in the production process. The skimmed raw milk was mildly pasteurized (72 °C for 15 s, in continuous system), hydrolyzed by adding soluble yeast β-galactosidase (Maxilact LX-5000; DSM, Gist Brocades, Delft, The Netherlands) for 36 h at 4 °C and lastly treated by direct UHT (infusion system).

2.2. Glucose isomerization test

For the glucose isomerization test, samples of fresh pasteurized semi-skimmed milk, with and without the addition of glucose (2.0 g/100 ml, the amount generally found in lactose-hydrolyzed milk) were sterilized in an autoclave (model 760 Sacco srl Cadorago, Como, Italy) at 121 °C for 15 min.

2.3. Assessment of storage stability in lactose-hydrolyzed milk

Storage tests were carried out on two commercial semi-skimmed samples of UHT lactose-hydrolyzed milk (A-UHT-HD and B-UHT-HD) produced by two different technologies and on the corresponding UHT milk (A-UHT and B-UHT) as a control sample. A-UHT-HD was obtained by a direct (infusion) UHT treatment performed after the hydrolytic treatment, while B-UHT-HD was produced by an indirect UHT treatment made before the hydrolytic treatment. Milk samples were stored at 4 °C and at 20 °C for 1, 2, 3 and 4 months.

2.4. Quality assessment and technological characterization of commercial samples of lactose-hydrolyzed milk

A total of 14 different commercial samples of semi-skimmed UHT lactose-hydrolyzed milk (1-UHT-HD–14-UHT-HD) from different countries (Sweden, Canada, Spain, Dominican Republic and Italy) were used in addition to one commercial sample of fresh pasteurized lactose-hydrolyzed milk (1-PAST-HD). The milk samples were portioned and stored at −20 °C until the analysis.

2.5. Methods

2.5.1. Glucose, lactose and galactose determination

Glucose, lactose and galactose were determined using enzymatic-spectrophotometric kits, from R-Biopharm Gmbh, (Darmstadt, Germany). Absorbance measurements were made with a Varian DMS UV–Visible spectrophotometer (Varian Inc., Walnut Creek, CA, 94598, USA) using a 1 cm path length.

2.5.2. Lactulose and fructose determination

Lactulose and fructose were determined according to the sensitive and rapid enzymatic-spectrophotometric methods standardized by Marconi et al. (2004) for the analysis of lactulose in milk. Fructose dehydrogenase (FDH) from *Gluconobacter* sp. (EC 1.1.99.11, 112 Units/mg), β-D-galactosidase from *Aspergillus orizae* (EC 3.2.1.23, 9 Units/mg) and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

A milk sample of 10 ml was deproteinized by gradually adding 1.75 ml of Carrez I and 1.75 ml of Carrez II; the resulting solution was stirred for 2–3 min, and 6.5 ml of citric/phosphate buffer was added. The solution was then thoroughly mixed for 2–3 min, left to rest for 30 min and filtered through filter paper eliminating the first 2–3 ml of filtrate. Lactulose present in the deproteinized filtered milk sample was hydrolyzed to D-galactose and D-fructose with β-galactosidase (Reaction 1). The free D-fructose and 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) produced a colored compound (MTT formazan) that absorbed at 570 nm as a result of fructose dehydrogenase (FDH) and the electron carrier phenazine methosulfate (PMS) (Reaction 2):

$$\text{Lactulose} \xrightarrow{\beta\text{-galactosidase}} \text{D-fructose} + \text{D-galactose} \quad (1)$$

$$\text{D-fructose} + \text{MTT} \xrightarrow[\text{PMF}]{\text{FDH}} \text{5-keto-D-fructose} + \text{MTT formazan} \quad (2)$$

2.5.2.1. Lactulose determination. In the UHT milk sample, all the fructose was derived from Reaction 1 (from the hydrolysis of lactulose), while in the case of lactose-hydrolyzed milk, since free fructose may already be present, the sample had to be analyzed both with and without adding β-galactosidase in order to subtract the absorbance value, obtained without the addition of β-galactosidase.

2.5.2.2. Fructose determination. Fructose content in milk and the lactose-hydrolyzed milk samples was determined using Reaction 2. Regarding the analytical performances for fructose determination, using 2 units of FDH, the

smallest differentiating absorbance for the procedure is 0.010 absorbance units. This corresponds to a maximum sample volume of 0.5 ml having a fructose concentration of 0.5 mg/l. The linear range was between 1 and 110 mg/l. The detection limit of 1 mg/l is derived from the absorbance difference of 0.020 and a maximum sample volume of 0.5 ml.

2.5.3. Furosine determination

Two millilitres of milk were hydrolyzed with 6.0 ml of 10.6 N HCl at 110 °C for 23 h under nitrogen. After hydrolysis, 0.5 ml of the hydrolysate was purified on a Sep-pak cartridge (Waters Corp., Milford, MA), and furosine was determined according to a HPLC procedure standardized by Resmini, Pellegrino, and Battelli, 1990 (ISO, 2003). HPLC determination was carried out using a Waters HPLC system (Milford, MA) equipped with two pumps (model 510), a diode array (model 991) and an injector with a 50 μl loop (Rheodyne, Cotati, CA). Analytical separation was performed with a dedicated furosine column (Alltech, Deerfield, IL) at 280 nm. The furosine standard was purchased from Neosystem Laboratoire (Strasbourg, France). The results were expressed as mg of furosine per 100 g of protein. The protein content was determined according to AOAC Method 991-20 (2000, Chap. 33).

All determinations were carried out in triplicate.

3. Results and discussion

3.1. Assessment of lactose-hydrolyzed milk during processing

With regards to hydrolysis indicators, the evolution of the glycidic fraction (lactose, glucose and galactose) during technological processing was considered. As shown in Table 1, the lactose content of heated milk corresponds to the amount generally found in milk (4.5–5.0 g/100 ml). The glucose and galactose content of heated milk was negligible (<0.01 g/100 ml). The lactose content after enzymatic hydrolysis decreased to an average value of 0.6 g/100 ml, while the glucose and galactose contents increased, reaching values of 2.3 g/100 ml and 2.1 g/100 ml, respectively.

The lower amount of galactose found was due to the formation of galacto-oligosaccharides due to the transgalactosylating action of β-galactosidase (Burvall, Asp, & Dhalqvist, 1979; Dhalqvist, Asp, Burvall, & Rausing, 1977; Gopal, Sullivan, & Smart, 2001; Mahoney, 1998; Zarate & Lopez-Leiva, 1990) and is consistent with the findings of Chen, Hsu, and Chiang (2002), Corradini, Canali, Nicoletti, Biondi, and Vinci (2001), Mendoza, Olano, and Villamiel (2005). Galactose, in fact, is generally more involved in oligosaccharide formation than glucose (Kwak & Jeon, 1986; Zadow, 1992).

Lactose values of 0.5 g/100 ml in UHT milk, corresponding to a degree of lactose hydrolysis of about 90%, meet the values required for this type of milk, in which at least 75% of the original lactose should be hydrolyzed (<1.0 g/100 ml) (Corradini et al., 2001; Harju, 2003; Mendoza et al., 2005; Modler, Gelda, Yaguchi, & Gelda, 1993).

A further reduction of glucose and galactose could occur after UHT treatment for the isomerization of glucose to fructose and of galactose to tagatose (Adachi, 1958; Andrews, 1986; Mendoza et al., 2005; Troyano, Villamiel, Olano, Sanz, & Martinez-Castro, 1996) and due to the occurrence of the Maillard reaction (Krause, Knoll, & Henle, 2003; Martins, Jongen, & van Boekel, 2001; Pellegrino, Resmini et al., 1995; van Boekel, 1998).

With regards to the heating markers (Table 2), the amount of furosine after preheating treatment was lower than the 8.6 mg/100 g protein threshold limit set by Italian law for "fresh pasteurized milk" (Ministerial Decree of 15/12/2000; Official Journal no. 31 7/02/01). "Fresh pasteurized milk" is the product "obtained from raw milk by means of a unique heating treatment (at least 71.7 °C for 15 s or any equivalent combination) characterized by a negative reaction to the phosphatase test, a positive reaction to the peroxidase test and a soluble whey protein content ⩾14.0% of total protein content" (Italian Lex no. 169 03/05/1989, Official Journal no. 108 11/05/89).

Thermal treatment, applied before hydrolysis, has the aim of microbiologically stabilizing the milk and favoring the enzymatic process because part of the ionic calcium is bound, because of the release of sulphydryl groups for the denaturation of whey protein, particularly β-lactoglobulin, and because of the ability of the native β-lactoglobulin to bind to the lactase (Chen et al., 2002; Harju, 2003; Jimenez-Guzman et al., 2002; Jimenez-Guzman et al., 2006).

The increase in furosine after lactose hydrolysis (16.0 mg/100 g protein) and, in particular, after UHT treatment (234.6 mg/100 g protein) was due to the Maillard reaction and to considerable amounts of glucose and galactose. In fact, the amount and nature of the reducing

Table 1
Evolution of lactose, glucose and galactose at different stages of the lactose-hydrolyzed milk process

Sample	Lactose (g/100 ml)		Glucose (g/100 ml)		Galactose (g/100 ml)	
	Mean[a]	RSD	Mean[a]	RSD	Mean[a]	RSD
Raw milk	4.7	0.9	<0.01	–	<0.01	–
Heated milk	4.8	1.2	<0.01	–	<0.01	–
Lactose-hydrolyzed milk	0.6	9.1	2.3	0.0	2.1	2.8
Lactose-hydrolyzed UHT milk	0.5	12.4	2.3	2.5	2.0	0.0

[a] Mean of three different batches.

Table 2
Furosine, fructose and lactulose amount at different stages of the lactose-hydrolyzed milk process

Sample	Furosine (mg/100 g of protein)		Fructose (mg/100 ml)		Lactulose (mg/100 ml)	
	Mean[a]	RSD	Mean[a]	RSD	Mean[a]	RSD
Raw milk	7.4	12.5	0.17	0.26	–	–
Heated milk	8.1	3.1	0.19	0.30	1.88	1.70
Lactose-hydrolyzed milk	16.0	10.6	0.42	1.49	1.40	1.43
Lactose-hydrolyzed UHT milk	235	15.8	7.61	1.47	7.79	1.75

[a] Mean of three different batches.

carbohydrates have an important influence on the Maillard reaction (Abrahamsson, Bengtsson, Hambraeus, & Holm, 1979; Finot, Deutsch, & Bujard, 1981; O'Brien & Morrissey, 1989). When lactose is split into glucose and galactose, the number of reducing carbohydrate groups able to take part in the reaction is doubled. Furthermore, monosaccharides are more reactive than lactose (Braekman, Mortier, Van Renterghem, & De Block, 2001; Burvall, Asp, Bosson, San Josè, & Dhalqvist, 1978; Evangelisti, Calcagno, Nardi, & Zunin, 1999).

The greater susceptibility of lactose-hydrolyzed milk is demonstrated by the higher values of furosine found in commercial lactose-hydrolyzed UHT milks, ranging from 160 to 603 mg/100 g protein as reported by Evangelisti et al. (1999), Mendoza et al. (2005) and Messia et al. (this paper), compared to ordinary UHT milk, ranging from 35 to 230 mg/100 g protein (Feinberg, Dupont, Efstathiou, Louapre, & Guyonnet, 2006; Van Renterghem & De Block, 1996). The substantial nutritional damage to liquid and powdered lactose-hydrolyzed milk was also demonstrated by the high values of blocked lysine found by several authors (Burvall et al., 1978; Dhalqvist et al., 1977; Evangelisti et al., 1999; Finot et al., 1981; Mittal, Hourigan, & Zadow, 1989). For the above reasons, mild UHT treatment (such as infusion) or UHT treatment prior to lactose hydrolysis generally based on the injection of sterile filtered enzyme into each milk pack before sealing (Zadow, 1992), should be adopted.

With regards to fructose content, negligible amounts (<0.2 mg/100 ml) were found in pasteurized milk, as also found by Cataldi, Angelotti, and Bianco (2003) using high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The slight increase of fructose (0.42 mg/100 ml) found in milk samples, after hydrolysis with β-galactosidase, resulted from the hydrolysis of lactulose formed during the previous pasteurization treatment. The considerable increase in fructose after UHT treatment may be due to the isomerization of glucose provoked by thermal treatment (Krause et al., 2003; Martins et al., 2001; O'Brien & Morrissey, 1989).

Glucose isomerization to fructose in the milk samples was confirmed by adding glucose to a pasteurized milk, which was subsequently submitted to sterilization (Table 3). The amount of fructose formed in sterilized milk was low (1.11 mg/100 ml), whereas in milk sterilized after glucose addition it was considerable (53.7 mg/100 ml).

Table 3
Glucose, fructose and lactulose content of milk sterilized with and without adding glucose (2 g/100 ml)

Sample	Glucose (g/100 ml)	Fructose (mg/100 ml)	Lactulose (mg/100 ml)
Sterilized milk	<0.01	1.11 ± 0.01	117 ± 1.0
Sterilized milk + glucose	1.99 ± 0.28	53.7 ± 0.12	102 ± 4.2

With regards to lactulose, Table 2 shows that it decreases slightly in hydrolyzed samples from 18.8 to 14.0 mg/l due to the action of β-galactosidase that can cleave both lactose and lactulose (Harju, 1986a, 1986b). UHT treatment provoked an increase in lactulose content up to 77.9 mg/l due to the isomerization of the residual lactose (0.6 g/100 ml; Table 1). This amount of lactulose was less than that found in the various typologies of ordinary UHT milk, including those obtained from the infusion system (89–120 mg/l) (Marconi et al., 2004), since lactose isomerization is reduced by the lower concentration of lactose (Andrews, 1986).

3.2. Assessment of storage stability in different typologies of lactose-hydrolyzed milk

The effect of storage conditions (4 months either at 4 °C or at 20 °C) on two commercial samples of lactose-hydrolyzed milk produced by different technologies and in the respective UHT milk samples (as control) is shown in Table 4. The different technologies used for the production of two typologies of lactose-hydrolyzed milk were demonstrated by the furosine and lactulose content of A-UHT samples (45.1 mg/100 g protein and 11.0 mg/100 ml respectively), typical of a direct UHT system, and of B-UHT (235 mg/100 g protein and 34.0 mg/100 ml respectively), which is typical of indirect-UHT treatment (Feinberg et al., 2006; Marconi et al., 2004; Resmini & Pellegrino, 1994; Resmini, Pellegrino, & Cattaneo, 2003).

The higher initial value of furosine in A-UHT-HD than A-UHT is strictly related to the hydrolysis carried out before the thermal treatment, which produced greater amounts of more reactive reducing sugars (Finot et al., 1981; Resmini & Pellegrino, 1994). The similar furosine values found in B-UHT-HD and B-UHT (234 and 235 mg/100 g protein) confirm that thermal treatment was carried out before hydrolysis. The lower lactulose content (4.45 mg/100 ml) found in A-UHT-HD compared to

Table 4
Furosine, lactulose and fructose in lactose-hydrolyzed UHT milk and UHT milk samples stored at 4 °C and 20 °C for 4 months

Storage (months)	A-UHT-HD[a]		A-UHT[b]		B-UHT-HD[a]		B-UHT[b]	
	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C
Furosine (*mg/100 g of protein*)								
0	151 ± 9.2		45.1 ± 0.83		234 ± 14.0		235 ± 6.9	
1	158 ± 0.7	230 ± 7.0	48.2 ± 3.99	60.7 ± 1.57	242 ± 4.9	300 ± 9.2	240 ± 2.0	253 ± 1.8
2	164 ± 0.3	298 ± 8.0	50.9 ± 0.41	78.6 ± 0.68	245 ± 1.5	374 ± 7.3	255 ± 1.7	265 ± 2.4
3	170 ± 1.8	393 ± 2.1	51.1 ± 0.74	104 ± 1.46	251 ± 1.8	435 ± 4.5	262 ± 7.4	279 ± 0.8
4	195 ± 1.7	480 ± 9.2	52.9 ± 1.14	126 ± 5.07	284 ± 1.8	562 ± 0.1	271 ± 4.8	310 ± 5.6
Lactulose (*mg/100 ml*)								
0	4.45 ± 0.11		11.0 ± 0.00		11.7 ± 0.20		34.0 ± 0.00	
1	4.42 ± 0.14	5.04 ± 0.11	11.1 ± 0.11	12.0 ± 0.20	11.6 ± 1.84	11.2 ± 0.09	33.9 ± 0.10	37.8 ± 0.13
2	4.48 ± 0.19	5.36 ± 0.10	11.1 ± 0.09	13.3 ± 0.27	11.6 ± 1.07	10.7 ± 0.11	34.0 ± 0.08	38.7 ± 0.29
3	4.51 ± 0.16	5.89 ± 0.12	11.2 ± 0.13	14.6 ± 0.24	11.6 ± 0.87	10.1 ± 0.18	34.2 ± 0.09	37.3 ± 0.28
4	4.59 ± 0.19	6.56 ± 0.18	11.2 ± 0.77	15.7 ± 0.22	11.5 ± 1.84	9.28 ± 0.10	34.2 ± 0.16	38.1 ± 0.18
Fructose (*mg/100 ml*)								
0	9.12 ± 0.28		2.36 ± 0.21		8.92 ± 0.22		2.42 ± 0.18	
1	8.85 ± 0.07	9.29 ± 0.14	2.48 ± 0.12	2.50 ± 0.00	9.01 ± 0.00	11.7 ± 0.14	2.33 ± 0.20	2.25 ± 0.12
2	9.08 ± 0.21	9.45 ± 0.35	2.45 ± 0.07	2.68 ± 0.10	9.11 ± 0.14	13.1 ± 0.07	2.48 ± 0.15	2.75 ± 0.15
3	9.13 ± 0.21	10.7 ± 0.22	2.40 ± 0.00	2.83 ± 0.19	9.18 ± 0.07	15.3 ± 0.21	2.55 ± 0.13	2.95 ± 0.07
4	9.24 ± 0.14	11.8 ± 0.21	2.50 ± 0.28	3.00 ± 0.14	9.17 ± 0.00	16.9 ± 0.07	2.58 ± 0.14	3.23 ± 0.00

[a] A-UHT-HD and B-UHT-HD = UHT-lactose hydrolyzed milks.
[b] A-UHT and B-UHT = UHT milk (control samples).

the reference sample A-UHT (11.0 mg/100 ml) can be attributed to the reduced quantities of lactose present in milk since this milk typology was hydrolyzed before thermal treatment. The fructose content of 9.12 mg/100 ml in A-UHT-HD is due to the isomerization of glucose during UHT treatment.

The lower lactulose content of B-UHT-HD compared to the reference sample B-UHT (11.7 versus 34.0 mg/100 ml respectively) is due to the action of β-galactosidase, performed after thermal treatment that hydrolyzes lactulose into fructose and galactose as demonstrated by the levels of fructose (8.92 mg/100 ml). The constant decrease of lactulose in B-UHT-HD during storage at 20 °C (from 11.7 to 9.28 mg/100 ml) and the constant increase of fructose (from 8.92 to 16.9 mg/100 ml) is due to the activity of β-galactosidase added after the thermal treatment.

The amount of furosine in milk samples stored for 4 months at 4 °C increased to about 50 mg/100 g protein in lactose-hydrolyzed milk (from 151 to 195 mg/100 g of protein in A-UHT-HD and from 234 to 284 mg/100 g protein in B-UHT-HD) and to <20 mg/100 g protein in non-hydrolyzed milk (A-UHT and B-UHT). However, furosine increased significantly (about 320 mg/100 g protein) in both lactose-hydrolyzed milk samples when stored at 20 °C for 4 months (from 151 to 480 mg/100 g of protein in A-UHT-HD and from 234 to 562 mg/100 g protein in B-UHT-HD). In the UHT control milk samples, the furosine increase is lower (about 90 mg/100 g protein) from 45.1 to 126 mg 100/g of protein in A-UHT and from 235 to 310 mg/100 g protein in B-UHT. Similar furosine values were also reported by Corradini et al. (2001), Evangelisti et al. (1999), and Mendoza et al. (2005). These findings confirm the high susceptibility to the Maillard reaction of UHT lactose-hydrolyzed milk during storage at room temperature. For the reduced chemical stability of this milk typology, attributed to the different amount and type of the reducing carbohydrates, manufacturers recommend that they should be stored at 4 °C (Evangelisti et al., 1999; Giardina, Cattaneo, & Barzaghi, 2003).

While the early Maillard reaction was approximately constant during milk storage and was dependent on the storage temperature (Pellegrino, De Noni, & Resmini, 1995), contradictory results have been reported on the effect of milk storage on lactulose formation (Andrews, 1989; Claeys et al., 2002; Nangpal & Reuter, 1990). Andrews (1989) as well as Berg and van Boekel (1994) have affirmed that lactose isomerization depends on the initial concentration of lactose and no effect of milk storage at room temperature on the lactulose concentration has been observed. There is likely to be a balance between formation and degradation of lactulose since lactulose, as for lactose, is involved in the Maillard reaction (Berg & van Boekel, 1994; O'Brien, 1997). This balance depends on the external conditions and composition (Claeys et al., 2002). In the present report, the lactulose does not significantly vary during storage at 4 °C and 20 °C in both milk typologies (hydrolyzed and non-hydrolyzed).

3.3. Quality assessment and technological characterization of commercial lactose-hydrolyzed milk

Different commercial samples of lactose-hydrolyzed milks were assessed by the combined use of hydrolysis and heating markers (Table 5). All samples showed lactose concentrations that were typical of this category of dietetic milk with a lactose reduction about 75% of the initial value.

Table 5
Hydrolysis markers (lactose, galactose, glucose) and heating markers (furosine, lactulose and fructose) in lactose hydrolyzed milk

Sample	Hydrolysis markers			Heating markers		
	Lactose (g/100 ml)	Galactose (g/100 ml)	Glucose (g/100 ml)	Furosine (mg/100 g protein)	Lactulose (mg/100 ml)	Fructose (mg/100 ml)
1 UHT-HD	1.2 ± 0.03	1.8 ± 0.05	1.8 ± 0.03	1071 ± 72.6	12.6 ± 0.16	18.4 ± 0.87
2 UHT-HD	1.1 ± 0.04	1.7 ± 0.07	2.0 ± 0.04	942 ± 44.4	6.6 ± 0.09	22.6 ± 1.14
3 UHT-HD	0.6 ± 0.02	2.1 ± 0.04	2.1 ± 0.04	397 ± 16.9	38.2 ± 1.08	65.5 ± 1.89
4 UHT-HD	1.2 ± 0.03	1.8 ± 0.04	1.8 ± 0.05	220 ± 5.6	25.4 ± 2.05	12.6 ± 0.76
5 UHT-HD	0.7 ± 0.02	2.1 ± 0.05	2.2 ± 0.04	174 ± 4.6	4.1 ± 0.56	11.0 ± 0.56
6 UHT-HD	0.9 ± 0.01	2.1 ± 0.03	2.0 ± 0.03	327 ± 9.0	37.7 ± 0.98	10.0 ± 0.91
7 UHT-HD	0.9 ± 0.03	1.9 ± 0.04	1.9 ± 0.05	162 ± 3.8	1.8 ± 0.65	9.8 ± 0.31
8 UHT-HD	0.9 ± 0.02	1.9 ± 0.05	2.2 ± 0.03	319 ± 10.4	0.0	39.1 ± 2.05
9 UHT-HD	0.9 ± 0.02	1.9 ± 0.03	2.0 ± 0.06	393 ± 16.4	10.6 ± 1.16	14.7 ± 1.04
10 UHT-HD	0.8 ± 0.03	2.2 ± 0.06	2.1 ± 0.05	188 ± 2.9	13.9 ± 0.95	5.6 ± 0.65
11 UHT-HD	0.4 ± 0.01	2.3 ± 0.04	2.4 ± 0.04	554 ± 11.4	40.1 ± 1.98	8.8 ± 0.79
12 UHT-HD	0.3 ± 0.01	2.5 ± 0.05	2.5 ± 0.02	603 ± 9.8	30.9 ± 2.45	7.3 ± 0.15
13 UHT-HD	0.3 ± 0.01	2.2 ± 0.06	2.3 ± 0.04	484 ± 12.5	26.5 ± 0.87	5.2 ± 0.76
14 UHT-HD	0.9 ± 0.02	2.1 ± 0.07	2.1 ± 0.05	512 ± 15.2	18.7 ± 1.65	4.7 ± 0.27
Min	0.3	1.7	1.8	162	0.0	4.7
Max	1.2	2.5	2.5	1071	40.1	65.5
Mean	0.8	2.0	2.1	453	19.1	16.8
CV%	37.8	10.8	9.9	60.8	73.7	99.2
1 PAST-HD	0.4 ± 0.01	2.3 ± 0.04	2.4 ± 0.02	10.9 ± 0.99	0.0	0.5 ± 0.03

The degree of lactose hydrolysis in commercial lactose-free milk samples showed a wide variability with residual content ranging from 0.3 to 1.2 g/100 ml (Table 5). This variability is related to the hydrolytic treatment used (enzyme in solution or immobilized prior to or after heating). The lowest values of lactose (0.3–0.4 g/100 ml) and the highest contents of galactose (2.2–2.5 g/100 ml) and glucose (2.3–2.5 g/100 ml) found in milk samples 11 UHT-HD, 12 UHT-HD, 13 UHT-HD and 1 PAST-HD, could be the result of the activity of β-galactosidase added in a soluble form after the thermal treatment, as indicated by the producers on the package.

The amount of furosine showed a wide variability ranging from 162 to 1071 mg/100 g of protein (CV = 60.8) due to the different heat and hydrolytic treatment that milks (from different countries) undergo. Moreover, furosine values >900 mg/100 g protein of samples 1 UHT-HD and 2 UHT-HD are due to their prolonged conservation beyond the time recommended for this commercial category (330 and 150 days, respectively, versus 90 days) at room temperature. The low levels of furosine (about 174 mg/100 g protein) of 5 UHT-HD, 7 UHT-HD and 10 UHT-HD indicate that a very mild UHT treatment (infusion) was carried out after the hydrolysis as attested by the low content of lactulose and the significant presence of fructose (derived from residual lactose epimerization and from the isomerization of glucose, respectively).

The absence of lactulose and the presence of fructose (39.1 mg/100 ml) in sample 8 UHT-HD could indicate that indirect UHT treatment was carried out subsequent to hydrolysis, as also shown by the furosine levels that were significantly higher than in 5 UHT-HD and 7 UHT-HD. The fructose content of samples 4 UHT-HD, 6 UHT-HD and 9 UHT-HD could be derived from the action of β-galactosidase on lactulose produced by the indirect UHT thermal treatment carried out before the hydrolytic treatment.

Furosine (mean value 538 mg/100 g protein) and lactulose (mean value 29.1 mg/100 ml) levels in samples 11 UHT-HD, 12 UHT-HD, 13 UHT-HD and 14 UHT-HD were significantly higher than the other samples, which could be the result of a severe thermal treatment (indirect UHT) made before the hydrolytic treatment.

The high content of furosine (397 mg/100 g protein), lactulose (38.2 mg/100 ml) and fructose (65.5 mg/100 ml) in sample 3 UHT-HD is indicative of severe thermal treatment carried out before and after the lactose hydrolysis, which allows the formation of lactulose from the epimerization of lactose and the production of fructose from the isomerization of glucose. The absence of lactulose and the low content of furosine in 1 PAST-HD sample confirmed that this sample was subjected to pasteurization. The qualitative difference (lower nutritional damage) between pasteurized and UHT milk is more accentuated in dietetic milks compared to ordinary milks for the high susceptibility of hydrolyzed milk to the Maillard reaction both during thermal treatment and storage.

4. Conclusions

The technology used in the production of lactose-hydrolyzed milk (intensity and sequence of thermal and hydrolytic treatments) and the quality of hydrolyzed milk can be better characterized by the combined use of conventional markers (furosine and lactulose) as well as the more innovative marker (fructose). The classical lactulose and furosine threshold and ratio fixed for ordinary UHT milk (Corzo, Delgado, Troyano, & Olano, 1994; Montilla,

Calvo, Santa Maria, Corzo, & Olano, 1996; Pellegrino, De Noni et al., 1995) are not appropriate to lactose-hydrolyzed milk because the kinetics of the Maillard reaction and the isomerization of lactose are modified in relation to the different amount and nature of the reactants. This finding and the wide variability of various hydrolysis and heating markers found in different commercial samples of lactose-hydrolyzed milk denote the necessity to assess other markers (tagatose, β-lactoglobulin, oligosaccharides) and to fix appropriate thresholds for this milk typology in order to defend both consumers and product quality.

Acknowledgements

The authors are grateful to Dr. Ivana Gandolfi and Dr. Claudia Vatteroni of the Research and Development Centre of Parmalat (Sala Baganza, Parma, Italy) for providing milk samples.

References

Abrahamsson, L., Bengtsson, O., Hambraeus, L., & Holm, H. (1979). Protein quality of milk-cereal based foods for infants and children in relation to processing methods and composition of the products. *Journal of Food Technology, 14*, 429–440.

Adachi, S. (1958). Formation of lactulose and tagatose from lactose in strongly heated milk. *Nature, 181*, 840–841.

Andrews, G. R. (1986). Formation and occurrence of lactulose in heated milk. *Journal of Dairy Research, 53*, 665–680.

Andrews, G. (1989). Lactulose in heated milk. *Bulletin of International Dairy Federation, 238*, 45–52.

AOAC (2000). Dairy products. In W. Horwitz (Ed.). *Official methods of analysis of the Association of Official Analytical Chemists, Method 991.20* (Vol. II, pp. 10–11). Gaithersburg, Maryland, USA: AOAC International.

Berg, H., & van Boekel, M. (1994). Degradation of lactose during heating of milk. I. Reaction pathways. *Netherlands Milk & Dairy Journal, 48*, 157–175.

Braekman, A., Mortier, L., Van Renterghem, R., & De Block, J. (2001). The influence of batch-pasteurisation on Maillard reaction in sweetened milk products with modified carbohydrate content. *Milchwissenschaft, 56*, 3–6.

Burvall, A., Asp, N. G., Bosson, A., San Josè, C., & Dhalqvist, A. (1978). Storage of lactose-hydrolysed dried milk: effect of water activity on the protein nutritional value. *Journal of Dairy Research, 45*, 381–389.

Burvall, A., Asp, N. G., & Dhalqvist, A. (1979). Oligosaccharide formation during hydrolysis of lactose with *Saccharomyces lactis* lactase (Maxilact R): Part-1. Quantitative aspects. *Food Chemistry, 4*, 243–250.

Cataldi, T. R. I., Angelotti, M., & Bianco, G. (2003). Determination of mono- and disaccharides in milk and milk products by high-performance anion-exchange chromatography with pulsed amperometric detection. *Analytica Chimica Acta, 485*, 43–49.

Chen, C. S., Hsu, C. K., & Chiang, B. H. (2002). Optimization of the enzymic process for manufacturing low-lactose milk containing oligosaccharides. *Process Biochemistry, 38*, 801–808.

Claeys, W. L., Van Loey, A. M., & Hendrickx, M. E. (2002). Intrinsic time temperature integrators for heat treatment of milk. *Trends in Food Science and Technology, 13*, 293–311.

Corradini, C., Canali, G., Nicoletti, I., Biondi, A., & Vinci, G. (2001). Caratterizzazione di latte UHT delattosato con tecniche analitiche innovative. *Industrie Alimentari, XL*, 261–266 (271).

Corzo, N., Delgado, T., Troyano, E., & Olano, A. (1994). Ratio of lactulose to furosine as indicator of quality of commercial milks. *Journal of Food Protection, 57*, 737–739.

Dhalqvist, A., Asp, N. G., Burvall, A., & Rausing, H. (1977). Hydrolysis of lactose in milk and whey with minute amounts of lactase. *Journal of Dairy Research, 44*, 541–548.

Di Stefano, M., & Veneto, G. (2001). Lactose malabsorption and intolerance in the elderly. *Scandinavian Journal of Gastroenterology, 36*, 1274–1278.

Evangelisti, F., Calcagno, C., Nardi, S., & Zunin, P. (1999). Deterioration of protein fraction by Maillard reaction in dietetic milks. *Journal of Dairy Research, 66*, 237–243.

Feinberg, M., Dupont, D., Efstathiou, T., Louapre, V., & Guyonnet, J. P. (2006). Evaluation of tracers for the authentication of thermal treatments of milks. *Food Chemistry, 98*, 188–194.

Finot, P. A., Deutsch, R., & Bujard, E. (1981). The extend of the Maillard reaction during the processing of milk. In C. Eriksson (Ed.). *Progress in food and nutrition science* (Vol. 5, pp. 345–355). London: Pergamon Press.

Giardina, C., Cattaneo, T. M. P., & Barzaghi, S. (2003). Study of modifications in delactosated milk during shelf-life by NIR and FT-IR spectroscopy. *Milchwissenschaft, 58*, 363–366.

Gopal, P. K., Sullivan, P. A., & Smart, J. B. (2001). Utilisation of galacto-oligosaccharides as selective substrates for growth by lactic acid bacteria including *Bifidobacterium lactis* DR10 and *Lactobacillus rhamnosus* DR20. *International Dairy Journal, 11*, 19–25.

Harju, M. (1986a). Lactulose as a substrate for beta-galactosidases.1. Materials and methods. *Milchwissenschaft-Milk Science International, 41*, 281.

Harju, M. (1986b). Lactulose as a substrate for beta-galactosidases.2. Results and discussion. *Milchwissenschaft-Milk Science International, 41*, 349.

Harju, M. (2003). Chromatographic and enzymatic removal of lactose from milk. *International Dairy Federation Bulletin, 389*, 4–8.

Hourigan, J. A. (1984). Nutritional implications of lactose. *The Australian Journal of Dairy Technology, 39*, 114–120.

ISO/DIS 18329/IDF 193. (2003). *Milk and milk products – Determination of furosine content – Ion-pair reverse-phase high performance liquid chromatography method*. IDF Standard (final draft), ISO, Geneve, CH.

Jelen, P., & Tossavainen, O. (2003). Low lactose and lactose-free milk and dairy products – prospects, technologies and applications. *The Australian Journal of Dairy Technology, 58*, 161–165.

Jimenez-Guzman, J., Cruz-Guerrero, A. E., Rodriguez-Serrano, G., Lopez-Munguia, A., Gomez-Ruiz, L., & Garcia-Garibay, M. (2002). Enhancement of lactase activity in milk by reactive sulfhydryl groups induced by heat treatment. *Journal of Dairy Science, 85*, 2497–2502.

Jimenez-Guzman, J., Sarabia-Leos, C., Cruz-Guerrero, A. E., Rodriguez-Serrano, G., Lopez-Munguia, A., Gomez-Ruiz, L., et al. (2006). Interaction between β-lactoglobulin and lactase and its effect on enzymatic activity. *International Dairy Journal, 16*, 1169–1173.

Krause, R., Knoll, K., & Henle, T. (2003). Studies on the formation of furosine and pyridosine during acid hydrolysis of different Amadori products of lysine. *European Food Research Technology, 216*, 277–283.

Kwak, H. S., & Jeon, I. J. (1986). Effect of various conditions on the formation of oligosaccharides in milk treated with β-galactosidase. *Journal of Dairy Science, 69*, 2785–2790.

Lanzarini, G. (1998). Applications of the immobilized biocatalysts for food industry. In: *Proceedings of the second workshop on the developments in the Italian PhD in food biotechnology*, Bertinoro (FO), 29 September–2 October 1997, (pp. 99–128).

Mahoney, R. R. (1997). Lactose-enzymatic modification. In P. F. Fox (Ed.). *Advanced dairy chemistry, lactose, water, salts and vitamins* (Vol. 3, p. 116). New York, NY: Chapman & Hall.

Mahoney, R. R. (1998). Galactosyl-oligosaccharide formation during lactose hydrolysis: a review. *Food Chemistry, 63*, 127–154.

Marconi, E., Messia, M. C., Amine, A., Moscone, D., Vernazza, F., Stocchi, F., et al. (2004). Heat-treated milk differentiation by a sensitive lactulose assay. *Food Chemistry, 84*, 447–450.

Martins, S. I. F. S., Jongen, W. M. F., & van Boekel, M. A. J. S. (2001). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science and Technology, 11*, 364–373.

Mendoza, M. R., Olano, A., & Villamiel, M. (2005). Chemical indicators of heat treatment in fortified and special milks. *Journal of Agriculture and Food Chemistry, 8*, 2995–2999.

Mittal, S. B., Hourigan, J. A., & Zadow, J. G. (1989). Effect of UHT treatment, storage time and temperature on the available lysine of UHT recombined and UHT recombined lactose hydrolysed milk. *The Australian Journal of Dairy Technology, 11*, 88–90.

Modler, H. W., Gelda, A., Yaguchi, M., & Gelda, S. (1993). Production of fluid milk with a high degree of lactose hydrolysis. *International Dairy Federation Bulletin, 289*, 57–61.

Montilla, A., Calvo, M., Santa Maria, G., Corzo, N., & Olano, A. (1996). Correlation between lactulose and furosine in UHT-heated milk. *Journal of Food Protection, 59*(10), 1061–1064.

Morisi, F. (1977). Enzimi immobilizzati in fibre in applicazioni nutrizionali. *Latte, 2*, 449–454.

Nangpal, A., & Reuter, H. (1990). Reference diagram for lactulose content in UHT milk. *Kieler Milchwirtschaftliche Forschungsberichte, 42*, 77–86.

O'Brien, J. (1997). Reaction chemistry of lactose: non enzymatic degradation pathways and their significance in dairy products. In P. Fox (Ed.), *Advanced dairy chemistry*. *Lactose water, salts and vitamins* (Vol. 3, pp. 155–213). London: Chapman & Hall.

O'Brien, J. M., & Morrissey, P. A. (1989). The Maillard reaction in milk products. *International Dairy Federation Bulletin, 238*, 53–61.

Pastore, M. (1977). Produzione industriale di latti delattosati mediante β-galattosidasi immobilizzata in fibre. *Latte, 2*, 456–460.

Pellegrino, L., De Noni, I., & Resmini, P. (1995). Coupling of lactulose and furosine indices for quality evaluation of sterilized milk. *International Dairy Journal, 5*, 647–659.

Pellegrino, L., Resmini, P., & Luf, W. (1995). Assessment (indices) of heat treatment of milk. In P. F. Fox (Ed.), *Heat-induced changes in milk* (pp. 409–453). Brussels, Belgium: International Dairy Federation.

Resmini, P., & Pellegrino, L. (1994). Maillard reaction compounds as marker of technological processes in food. In: *Proceedings of a round table on: Maillard reaction in food* (pp. 37–45). RAISA CNR Raisa.

Resmini, P., Pellegrino, L., & Battelli, G. (1990). Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Italian Journal of Food Science, 2*, 173–183.

Resmini, P., Pellegrino, L., & Cattaneo, S. (2003). Furosine and other heat-treatment indicators for detecting fraud in milk and milk products. *Italian Journal of Food Science, 4*(15), 473–480.

Roger, L., Thapon, J. L., Maubois, J. L., & Brule, G. (1976). Hydrolyse du lactose contenu dans l'ultrafiltrat de lait ou de lactosérum en réacteur enzymatique à membrane. *Le Lait, 551*, 56–75.

Sloan, E. (1999). The new market: foods for the not-so-healthy. *Food Technology, 53*, 54–60.

Torun, R., Solomons, N. W., & Viteri, F. E. (1979). Lactose malabsorption and lactose intolerance: implications for general milk consumption. *Archivos Latinoamericanos de Nutricion, 29*, 445–494.

Troyano, E., Villamiel, M., Olano, A., Sanz, J., & Martinez-Castro, I. (1996). Monosaccharides and myo-inositol in commercial milks. *Journal of Agriculture and Food Chemistry, 44*, 815–817.

van Boekel, M. A. J. S. (1998). Effect of heating on Maillard reactions in milk. *Food Chemistry, 62*, 403–414.

Van Renterghem, R., & De Block, J. (1996). Furosine in consumption milk and milk powders. *International Dairy Journal, 6*, 371–382.

Vasiljevic, T., & Jelen, P. (2003). Retention of β-galactosidase activity in crude cellular extracts from *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842 upon drying. *International Journal of Dairy Technology, 56*(2), 111–116.

Zadow, J. G. (1992). Lactose hydrolysis. In J. G. Zadow (Ed.), *Whey and lactose processing* (pp. 371–387). Elsevier Science Publishers Ltd.

Zadow, J. G. (1993). Economic considerations related to the production of lactose and lactose by-products. *International Dairy Federation Bulletin, 289*, 10–15.

Zarate, S., & Lopez-Leiva, M. H. (1990). Oligosaccharide formation during enzymatic lactose hydrolysis: a literature review. *Journal of Food Protection, 53*, 262–268.