1 **Short communication**

2 Presence of mono-, di- and galactooligosaccharides in 3 commercial lactose-free UHT dairy products

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11 Abstract

12 In this work a study of mono-, di- and galactooligosaccharide (GOS) content as well as
13 the determination of thermal indicators of different commercial lactose-free UHT (LF-
14 UHT) milks and dairy drinks has been carried out. Moreover, GOS formation during
15 hydrolysis of lactose in a commercial UHT milk using Lactozym® pure 6500 was also
16 studied. Presence of GOS was detected in all analyzed samples ranging from 950–4350
17 mg/L in LF-UHT milks and from 600–2260 mg/L in LF-UHT dairy drinks. Their GOS
18 contents correlate roughly with the remaining lactose content of samples. During
19 enzymatic hydrolysis of lactose in commercial UHT milk, the GOS formed reached a
20 maximum about 10000 mg/L when 75–90% of lactose was hydrolyzed and then
21 gradually decreased to values considerably lower than 5000 mg/L when over 99% of the
22 lactose was hydrolyzed. Therefore, GOS formation during low lactose milk

manufacture might be improved by controlling enzymatic lactose hydrolysis so that it would be possible to elaborate low-lactose milk products able to satisfy the physiological requirements of the majority of intolerant groups and with a GOS content high enough to confer beneficial effect as prebiotic.

Keywords: Lactose-free UHT; Milks; Dairy drinks; Lactozym®; GOS; Prebiotics; Furosine; Food composition; Food analysis

Abbreviations

GOS	Galactooligosaccharides
LF-UHT	Lactose-free ultra-high temperature
TMSO	Trimethyl silylated oximes

1 Introduction

In recent years lactose-free UHT (LF-UHT) milk has become popular in many countries since there are a significant number of people who are affected by lactose intolerance, because they lack the required lactase on the intestinal brush borders to hydrolyze lactose to glucose and galactose and to be absorbed through the digestive tract. As a result, lactose reaches the colon where it is fermented, producing symptoms such as gas, bloating and diarrhea. The deficiency of lactase in varying degrees (hypolactasia) affects nearly 70% of the world population being the most common enzyme deficiency in humans (Lomer et al., 2008).

As the interest of consumers for lactose-free (LF) milk grows, the number of dairies producing this type of milk also increases. However, despite the increasing consumption of LF milk, few data are available on the characterization and quality assessment of

these commercial milks (Adhikari et al., 2010; Messia et al., 2007; Rada-Mendoza et al., 2005).

LF-UHT milks present large amounts of reducing monosaccharides which makes them more susceptible to deterioration than ordinary UHT milk, since monosaccharides are more reactive than lactose when participating in the Maillard reaction that takes place not only in the heating process but also during storage of milk (Braekman et al., 2001; Burvall et al., 1978; Tossavainen & Kallioinen, 2008). Moreover, there are several procedures involved in the manufacture of LF-UHT milks such as removal (or not) of some lactose prior to enzymatic hydrolysis; hydrolysis performed before or after heating; use of enzymes from different origins, in soluble or immobilized form, etc. (Harju, 2003; Jelen & Tossavainen, 2003; Jokar & Karbassi, 2011). These different processes used in the production of LF-UHT milks can lead to a wide variability of hydrolysis and heating markers such as lactulose and furosine, found in different commercial samples of LF-UHT milks (Messia et al., 2007). It has been described that heating prior to hydrolysis of lactose is suggested to avoid a considerable loss of available lysine, an essential amino acid, as a result of the Maillard reaction (Rada-Mendoza et al., 2005).

During manufacture of LF-UHT milks, formation of galactooligosaccharides (GOS) can also take place by transglycosylation reactions. GOS are produced from lactose by glycosyl transfer of one or more D-galactosyl units onto D-galactose moiety of lactose catalyzed by β-galactosidases (Mahoney, 1998). The amounts and composition of the GOS fraction formed can be influenced by a number of factors, including enzyme source, pH and temperature (Boon et al., 1998).

GOS are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health (Gibson & Roberfroid, 1995). Although there are some studies that show GOS formation during enzymatic hydrolysis of milk (Indyk et al., 1996; Mahoney, 1998; Mozaffar et al., 1985; Rustom et al., 1998), data about GOS composition and variability of content in commercial LF-UHT milks are not available. It would be interesting to know the GOS composition of LF products in order to develop products that satisfy the physiological requirements of intolerant groups, that would contain enough prebiotics to be beneficial. Therefore, the aim of this work was to study carbohydrate composition (mono- and disaccharides and GOS) and the thermal damage of commercial LF-UHT dairy products, as well as to research GOS formation during controlled hydrolysis of lactose in milk.

2 Materials and methods

2.1 Standards

Lactose was acquired from Scharlau (Barcelona, Spain), D-galactose and D-glucose from Fluka (Steinheim, Germany). Fructose, tagatose, β-1,6-galactobiose and phenyl-β-glucoside were purchased from Sigma (St. Louis, MO, USA). Allolactose, 6'-galactosyl-lactose and 4'-galactosyl-lactose were standards previously synthesized in our laboratory (Cardelle-Cobas et al., 2008; Cardelle-Cobas, 2009; Martínez-Villaluenga et al., 2008).

2.2 Samples

Samples of UHT milk (n=1), LF-UHT milks (n=7) and LF-UHT dairy drinks (n=5), from different manufacturers were purchased from different Spanish supermarkets, stored at refrigeration temperature and analyzed before the sell-by date. Labeling of

92 samples showed lactose content lower than 100 mg/L. Protein content, determined using
93 the Kjeldahl method (AOAC, 1990) was in the ranging from 26–31 g/L product.

94 2.3 Enzymatic hydrolysis of lactose from milk

95 Hydrolysis of lactose from a commercial UHT milk was performed by β-galactosidase
96 (EC 3.2.1.23) activity from *Kluyveromyces lactis* of the commercial enzymatic
97 preparation, Lactozym® pure 6500 (U/mL) (Novozymes, Dittingen, Switzerland). For
98 incubations, 0.3 U/mL of this preparation was added to an UHT milk sample at 30 ºC in
99 aseptic conditions. Reactions (in duplicate) were carried out in individual Eppendorf
100 tubes and incubated in an orbital shaker at 600 rpm at different times for 30 h and
101 immediately immersed in boiled water for 5 min to inactivate the enzyme. The samples
102 were stored at -18 ºC for subsequent analysis.

103 2.4 Determination of carbohydrates

104 Before analysis, samples were deproteinized using anhydrous methanol (1:10, v:v) for
105 24 h. Supernatant was collected, centrifuged at 10,000 rpm for 5 min and stored at 4 ºC
106 for further analysis. All preparations were carried out in duplicate.

107 *2.4.1 High-performance anion exchange chromatography with pulsed*
108 *amperometric detection (HPAEC-PAD) analysis*

109 Mono-, disaccharides and GOS were analyzed by HPAEC-PAD in an ICS2500 Dionex
110 system (Dionex Corporation, Sunnyvale, CA) consisting of a GP50 gradient pump, and
111 ED50 electrochemical detector with a gold working electrode and Ag/AgCl reference
112 electrode. Data acquisition and processing was performed with Chromeleon version 6.7
113 software (Dionex Corporation). For preparation of eluents, MilliQ water (Milli-Q
114 Synthesis A10 system; Millipore, Billerica, Mass., USA), NaOH (50%, w/v) and

115 NaOAc (Fluka, Germany) were used. All eluents were degassed by flushing with
116 helium for 25 min.

117 Separations were performed following the method described in Splechtna et al. (2006).
118 Elution was at room temperature on a CarboPac PA-1 column (250×4mm) connected to
119 a CarboPac PA-1 (50×4mm) guard column. Eluent A (100 mmol/L NaOH) and eluent B
120 (100 mmol/L NaOH and 50 mmol/L NaOAc) were mixed to form the following
121 gradient: 100% A from 0–20 min and 0–100% B from 20–70 min. After each run, the
122 column was washed for 10 min with 100% of eluent C (100 mmol/L NaOH and 1 mol/L
123 NaOAc) and re-equilibrated for 15 min with the starting conditions of the employed
124 gradient.

125 Two milliliters of methanolic extracts were evaporated, reconstituted in 1mL of
126 bidistilled water and filtered through a nylon Millipore FH membrane (0.22 μm)
127 (Bedford, MA) before injection (20 μL). Separations were performed at a flow rate of 1
128 mL/min. Quantification of GOS was performed by external calibration using a standard
129 solution of raffinose being the regression coefficient of calibration curve higher than
130 0.99. All analyses were carried out in duplicate and data were expressed as mean ±
131 standard deviation (SD).

132 ### *2.4.2 Gas chromatographic (GC) analysis*

133 Trimethyl silylated oximes (TMSO) of monosaccharides (glucose, galactose, fructose
134 and tagatose) and disaccharides (allolactose, lactose, β-1,6-galactobiose and sucrose)
135 were determined by GC following the method of Cardelle-Cobas et al., (2009).
136 Chromatographic analysis was performed on Agilent Technologies gas chromatograph
137 (Mod 7890A) equipped with a flame ionization detector (FID). Separation was carried
138 out in a fused silica capillary column HP-5MS (5% phenyl methylsilicone, 25 m x 0.32

mmol/L x 0.25 μm thickness; J & W Scientific, Folsom CA, USA). Nitrogen was used as carrier gas at a flow rate of 1 mL/min. Injector and detector temperatures were 280 and 315 ºC, respectively. The oven temperature was programmed from 180–315 ºC at a heating rate of 3 ºC/min and held 20 minutes. Injections were made in the split mode (1:20). Data acquisition and integration was done using Agilent ChemStations Reb. 4B. 03.01 software (Wilmington, DE, USA).

The oximes were formed following the method of Brobst & Lott (1996). First, 1 mL of methanolic extract (4 mg of sugars) was added to 0.4 ml of internal standard (I. S.) solution (0.5 mg/mL phenyl-β-glucoside). Afterwards, the mixture was dried at 38–40 ºC in a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland). Sugar oximes were formed by adding 200 μL hydroxylamine chloride (2.5%) in pyridine and heating the mixture at 70 ºC for 30 min and then silylated with hexamethyldisilazane (200 μL) and trifluoroacetic acid (20 μL) and kept at 50 ºC for 30 min. Reaction mixtures were centrifuged at 7000 x *g* for 2 min at room temperature. Supernatants were injected in the GC or stored at 4 ºC prior to analysis. All analyses were carried out in duplicate and data were expressed as mean ± standard deviation (SD).

Response factors were calculated after the triplicate analysis of 5 standard solutions (galactose, glucose, fructose, tagatose and lactose) over the expected concentration range in samples.

2.5 Furosine determination

Determination of furosine in the LF-UHT milks and LF-UHT dairy drinks was performed by ion-pair RP-HPLC following the method of Resmini et al. (1990). Before analysis, samples (2 mL) were hydrolyzed with 6 mL of 10.6 N HCl under inert conditions at 110 ºC for 24 h in a Pyrex screw-cap vial with PTFE-faced septa. The

hydrolyzate was filtered through Whatman Nº 40 filter paper and 0.5 ml of filtrate was applied to a previously activated (methanol and water) Sep-Pak C_{18} cartridge (Millipore). Furosine was eluted with 3 mL of 3N HCl and 50 μl were injected into the chromatograph.

RP-HPLC analysis of furosine was carried out in a C_8 column (250 mm x 4.6 mm, 5 μm) (Alltech furosine-dedicated Nicolasville, KY) maintained at 35 ºC using a linear binary gradient at a flow rate of 1.2 mL/min. Mobile phase was constituted by solvent A, 0.4% acetic acid, and solvent B, 0.3% KCl in phase A. The elution program was as follows: 100% A from 0–12 min, 50% A from 20–22.5 min, and 100% A from 24.5–30 min. Detection, was performed using a variable wavelength UV detector at 280 nm (LDC Analytical, SM 4000 Salem, NH). Acquisition and processing of data were achieved with a HPChem Station (Hewlett-Packard) software. Quantification was performed by the external standard method, using a commercial standard of pure furosine (Neosystem Laboratories, Strasbourg, France). All analyses were done in duplicate, and the data are the mean values expressed as milligrams of furosine per kg of protein.

3 Results and discussion

The HPAEC-PAD profile of carbohydrates from a commercial LF-UHT milk is shown in Fig. 1. As expected, glucose and galactose (peak 1) were the main components due to the action of β-galactosidase. It was possible to identify the peak 2 as the disaccharide β-1,6-galactobiose and lactose which co-eluted with allolactose (peak 3). The most abundant GOS present was 6'-galactosyl-lactose (peak 4). Small amounts of 4'-galactosyl-lactose (peak 5) were also detected in all studied samples. Moreover, a series of unidentified galactooligosaccharides (GOS) (marked with an asterisk) were detected

as a result of transglycosylation catalyzed by added β-galactosidase. Commercial LF-UHT dairy drinks showed similar HPAEC-PAD profiles of carbohydrates.

Due to coelution of galactose and glucose as well as lactose and allolactose their quantification was performed by GC and only 6'-galactosyl-lactose and 4'-galactosyl-lactose as well as the unidentified GOS were quantified by HPAEC-PAD.

Fig. 2 shows a GC-FID chromatogram of TMSO derivatives of carbohydrates of a commercial LF-UHT milk. After derivatization, the reducing carbohydrates give rise to two different derivatives corresponding to the *syn* (*E*) and the *anti* (*Z*) isomers (Molnár-Perl & Horváth, 1997). The panel I shows the complete profile where it can be observed: tagatose (1) and fructose (2) (see panel Ia), glucose (3), galactose (4), internal standard (I.S.) and disaccharide region where it was detected, lactose (5), allolactose (6), and β-1,6-galactobiose (7) (panel Ib). Commercial LF-UHT dairy drinks showed similar GC-FID profiles of carbohydrates.

3.1 Lactose-free UHT milks (LF-UHT milks)

Table 1 shows the content of carbohydrates (mono-, disaccharides and GOS) found in the studied samples. The glucose and galactose level varied between 19881 and 25050 mg/L, and 17297 and 23464 mg/L, respectively. The large amount of both monosaccharides in all samples indicates that lactose was not removed from milks prior to enzymatic hydrolysis. The lower content of galactose with respect to glucose in all samples was due to the formation of GOS during the transgalactosylating action of β-galactosidase, as it was previously reported for enzymatic hydrolysis of milk (Indyk et al., 1996, Messia et al., 2007; Rada-Mendoza et al., 2005). The lactose content in the studied samples varied from 33 mg/L–291 mg/L and the average value was about 96 mg/L. These values corresponded to a degree of hydrolysis higher than 99%. Only one

211 sample (number 2) showed a lactose content higher than the amount stated on the label
212 (lactose content < 100 mg/L).

213 The total content in GOS varied among samples in the range from 956–4350 mg/L as
214 can be observed in Table 1. The GOS content increased with the remaining lactose
215 content so that sample 2 showed the highest levels of GOS and lactose whereas sample
216 6 showed the lowest levels of both.

217 Regarding heating markers, lactulose was not detected in any of the samples examined
218 in our study (LOD = 10 mg/L). Given that lactulose is formed from lactose by
219 isomerization during the heat treatment of milk (López-Fandiño & Olano, 1999;
220 Martinez-Castro & Olano, 1978), the absence of lactulose is understandable considering
221 the low content of residual lactose in samples. Moreover, the absence of lactulose is
222 independent whether the UHT-treatment is performed before or after the enzymatic
223 hydrolysis since lactulose is formed from isomerization of lactose during heating of
224 milk. Thus, if UHT treatment is given after lactose hydrolysis, lactulose cannot be
225 formed in appreciable amounts. On the other hand, if hydrolysis is performed after UHT
226 treatment, the lactulose formed from lactose is hydrolyzed by the action of the β-
227 galactosidase. These results are in agreement with those obtained by Rada-Mendoza et
228 al. (2005) for lactose hydrolyzed UHT milks. On the contrary, Messia et al. (2007)
229 reported lactulose contents of up to 401 mg/L in commercial lactose hydrolyzed milks
230 but the lactose contents detected in that samples were approximately up to one hundred
231 times higher than those found in the present study.

232 Isomerization of glucose to fructose and galactose to tagatose during UHT treatment of
233 LF-UHT milks was also detected since appreciable amounts of fructose (from 76 mg/L–
234 185 mg/L) and tagatose (from 36 mg/L–98 mg/L) were found in all samples (Table 1).

The presence of fructose is independent whether the UHT-treatment is performed before or after the enzymatic hydrolysis since fructose may be originated either during enzymatic hydrolysis of lactulose present in UHT-treated milk or from glucose isomerization during UHT treatment of hydrolyzed milk. The presence of tagatose in LF-UHT milks is other indication that lactose has been hydrolyzed before UHT treatment since galactose is only present in very small quantities in milk and tagatose has not been detected in UHT milk (Troyano et al., 1992).

Fig. 3 shows the level of furosine found in LF-UHT milks samples. The values ranging from 2442–4109 mg/kg protein were considerably higher than those reported for UHT milks (Corzo et al., 1994), as it was previously observed in lactose-hydrolyzed milks (Marconi et al., 2002; Messia et al., 2007; Rada-Mendoza et al., 2005; Tossavainen & Kallioinen, 2008). As was inferred from the presence of tagatose, high furosine contents are also an indication that the UHT treatment was given after the lactose hydrolysis, since lactose-hydrolyzed milk, with high content of reducing monosaccharides, can be more susceptible to the Maillard reaction than natural (non-hydrolyzed) milk (Evangelisti et al., 1999).

Therefore, in agreement with results obtained by Rada-Mendoza et al. (2005), it can be proposed that in order to avoid an excessive formation of furosine and, consequently, loss of available lysine and changes in the organoleptic characteristics, it may be advisable to hydrolyze lactose under aseptic conditions after thermal treatment conditions.

3.2 Lactose-free UHT dairy drinks (LF-UHT dairy drinks)

Table 2 shows the carbohydrate composition of commercial LF-UHT dairy drinks. As it can be observed, with the exception of sample 2, the rest of samples showed lower

monosaccharide, disaccharide and GOS content than LF-UHT milks Also, small amounts of sucrose were detected in all samples.

Furosine content (Fig. 3B) ranged from 1547–4477 mg/kg protein and the interval of variation was higher than in LF-UHT milks. Samples 1 and 5 which showed a level of furosine considerably higher than that of the rest of samples, also displayed the highest tagatose content. Elevated values of furosine in LF-UHT dairy drinks may be due to different causes, including excessive heating, prolonged storage or addition of milk powder; however tagatose is only formed from galactose during heat treatment so that the presence of elevated amounts of both compounds may be due to excessive heating during the manufacture of LF-UHT dairy drinks.

3.3 Formation of GOS during enzymatic hydrolysis of lactose from milk

The consumption of GOS has unquestionably been shown to have significant health benefits in humans (Macfarlane et al., 2008). GOS have been used to simulate human milk in infant formulas, and a low level (2400 mg/L) can improve stool frequency, decrease fecal pH and stimulate intestinal bifidobacteria and lactobacilli as in children fed with human milk (Ben et al., 2008). Various health benefits of these carbohydrates have been reported by different research groups, so that currently the main focus among prebiotics is on their production and use as a component of functional foods (Sangwan et al., 2011).

Previous studies on the formation of oligosaccharides during lactose hydrolysis in milk showed that maximum content of GOS was achieved within the range 40–95% hydrolysis of lactose and then gradually decreased with the lactose content (Mahoney, 1998). Fig. 4 depicts the formation of oligosaccharides originated from lactose

283 hydrolysis of a commercial UHT milk. Formation of total GOS reached a maximum
284 about 10000 mg/L (about 20% of total carbohydrates present) when percentage of
285 hydrolyzed lactose ranged from 75–90% of lactose was hydrolyzed and then they
286 gradually decreased to values below 5000 mg/L when over 99% of the lactose was
287 hydrolyzed. According to these data, for a residual lactose content lower than 1000
288 mg/L (about 2% of total carbohydrates), milks with a GOS content around 7800 mg/L
289 can be obtained. This GOS content would be enough to exert a beneficial effect on the
290 consumer's health..

291 4 Conclusion

292 Although extreme intolerance may require an exclusively lactose-free milk, most people
293 with hypolactasia, if given appropriate advice, can tolerate some lactose-containing
294 foods without symptoms (Lomer et al., 2008). It is generally accepted that 50–80%
295 lactose reduced milk will satisfy the physiological requirements of the majority of
296 intolerant groups (Indyk et al., 1996) so that it would be possible to elaborate a low-
297 lactose milk with a noticeable GOS content. Thus, the functional properties of low-
298 lactose milk can be improved by controlling the final stages of the enzymatic hydrolysis
299 of lactose.

300

301 Acknowledgements

302 This work has been financed under a R+D program of the Spanish Ministry of Science
303 and Innovation, Consolider Ingenio 2010 (FUN-C-FOOD): CSD 2007-00063; R + D
304 program of the Comunidad de Madrid, project ALIBIRD 2009/AGR-1469 ;R + D
305 program of the Comunidad de Castilla-La Mancha POII10-0178-4685 and IBEROFUN

P109AC0302 (CYTED). A. I. Ruiz-Matute thanks her JAE-Doc contract from CSIC. M. Corzo-Martínez thanks Danone Institute for a grant. We are grateful to Ramiro Martinez (Novozymes A/S, Spain) for providing us with Lactozym® pure 6500.

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Figure captions

Fig. 1. HPAEC-PAD chromatogram of carbohydrates present in a commercial lactose-free UHT (LF-UHT) milk. 1: galactose + glucose; 2: β-1,6-galactobiose; 3: allolactose + lactose; 4: 6'-galactosyl-lactose; 5: 4'-galactosyl-lactose; * unknown galactooligosaccharides

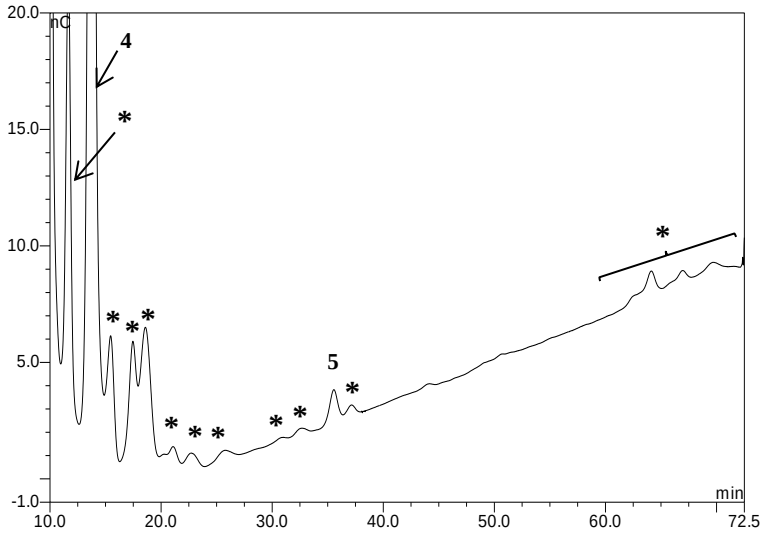

Fig. 2. GC-FID profile of TMSO derivatives of carbohydrates present in a commercial lactose-free UHT (LF-UHT) milk. I) Complete chromatogram: Ia (1) tagatose and (2) fructose; (3): glucose; (4): galactose; I.S: internal standard (phenyl-β-glucoside); Ib: (5) lactose, (6) allolactose; (7) β-1,6-galactobiose.

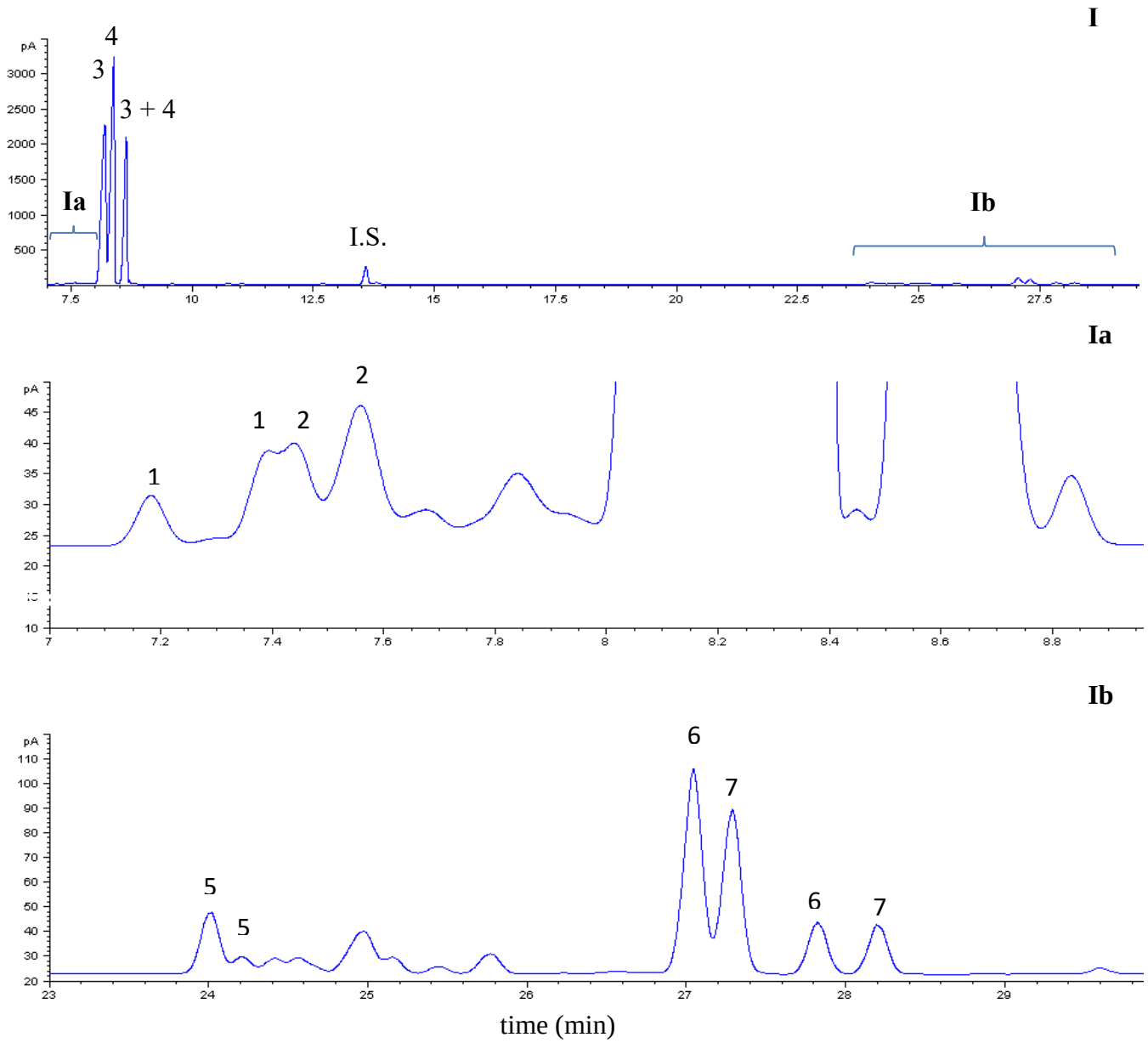

Fig. 3. Content of furosine (ε-2-furoyl-methyl-lysine) of commercial A) lactose-free UHT (LF-UHT) milks (Samples 1-7); and B) lactose-free UHT (LF-UHT) dairy drinks (Samples 1-5).

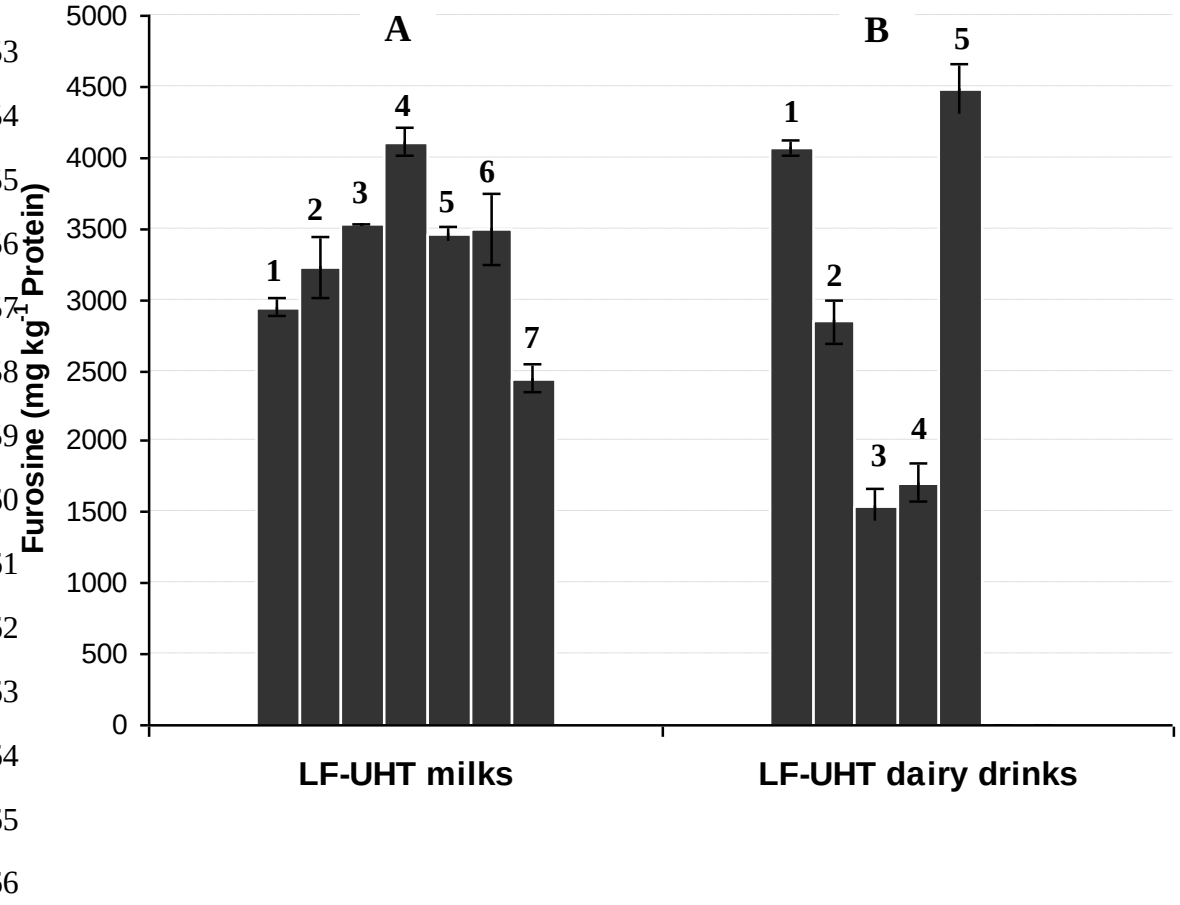

Fig. 4. Galactooligosaccharide (GOS) formation during enzymatic hydrolysis for 30 h of a commercial UHT milk using Lactozym® pure 6500 (0.3 U/mL) at 30 ºC and aseptic conditions (6'-β-gal-lac: 6'-galactosyl-lactose, 4'-β-gal-lac: 4'-galactosyl-lactose, Unk GOS: unknown galactooligosaccharides)

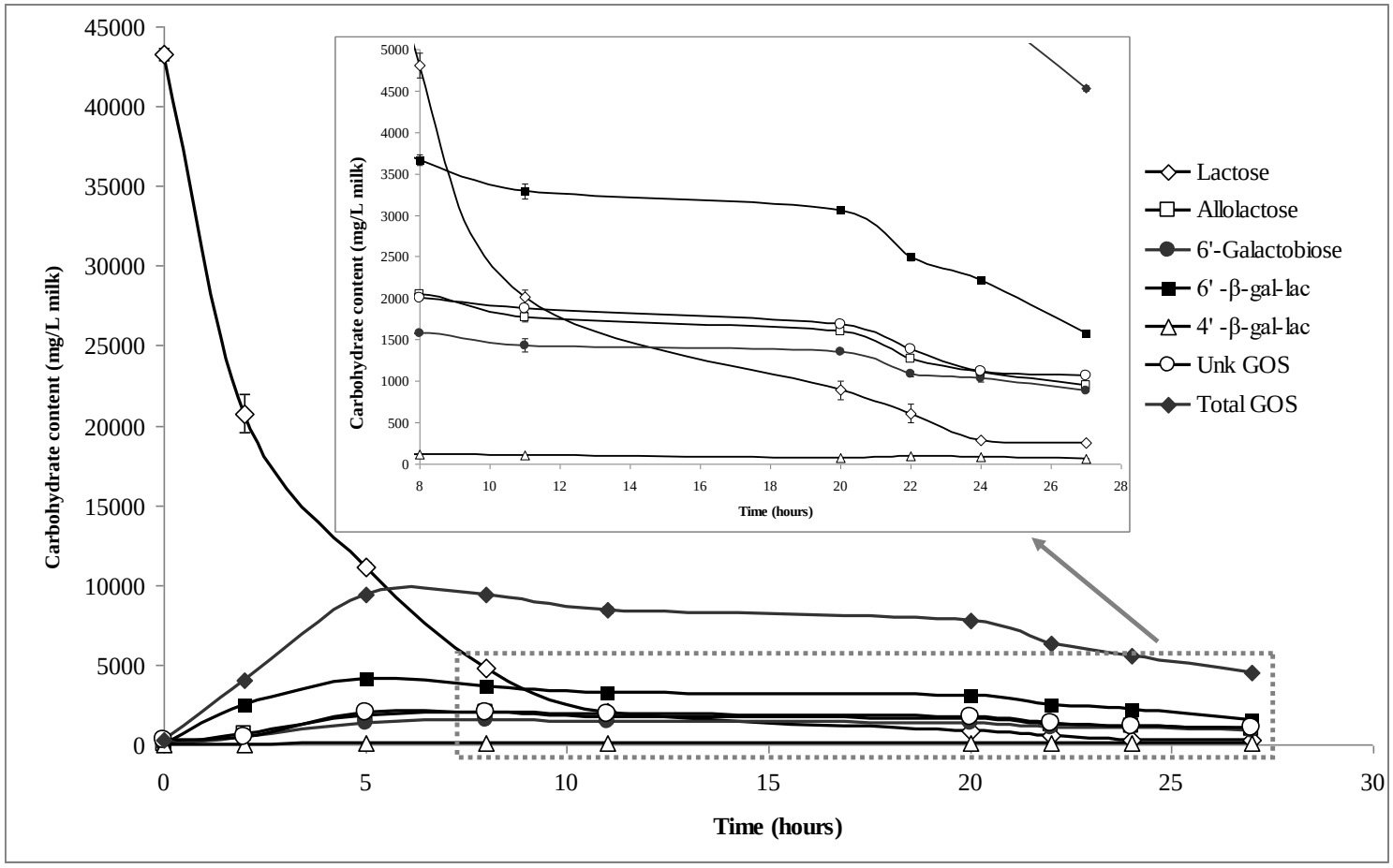

Table 1

Content of mono- and disaccharides and galactooligosaccharides (GOS) found in commercial lactose-free UHT milks (LF-UHT milks).

	Commercial lactose-free UHT milks							
Carbohydrates (mg/L milk)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean** ± SD
Galactose	23464	17297	20848	21386	21493	20211	22115	20974 ± 2044
Glucose	25050	19881	22901	22979	23227	21522	23712	22753 ± 1777
Fructose	132	111	76	185	118	106	94	117 ± 33
Tagatose	88	79	65	75	98	94	36	76 ± 20
Lactose	66	291	101	69	74	33	38	96 ± 83
Allolactose	283	841	446	229	349	139	151	348 ± 226
β-1-6-Galactobiose	339	747	409	248	335	166	191	348 ± 183
6'-galactosyl-lactose	936	1703	1070	687	805	271	302	825 ± 460
4'-galactosyl-lactose	42	39	31	254	28	31	32	65 ± 77
Unidentified GOS	635	1020	669	546	487	349	355	580 ± 220
Total GOS[a]	2235	4350	2625	1735	2004	956	1031	2134 ± 1080

[a] Total GOS includes quantification of: allolactose, β-1-6-galactobiose, 6'-galactosyl-lactose, 4'-galactosyl-lactose and unidentified GOS (Marked as * in figure 1). Samples were prepared and analyzed in duplicate.

Table 2

Content of mono- and disaccharides and galactooligosaccharides (GOS) found in commercial lactose-free UHT dairy drinks (LF-UHT dairy drinks).

	Lactose-free UHT dairy drinks					
Carbohydrates (mg/L milk)	**1**	**2**	**3**	**4**	**5**	**Mean** ± SD
Galactose	16254	22277	13519	16168	12669	16177 ± 3436
Glucose	17372	23478	14355	17455	13534	17238 ± 3575
Fructose	389	39	309	348	187	255 ± 128
Tagatose	192	Tr	72	68	188	131 ± 61
Lactose	38	60	34	21	25	36 ± 14
Sucrose	9	209	180	83	247	146 ± 88
Allolactose	97	142	74	75	68	91 ± 29
β-1-6-Galactobiose	110	172	83	87	135	117 ± 43
6'-galactosyl-lactose	320	445	141	59	135	220 ± 146
4'-galactosyl-lactose	14	88	20	16	141	56 ± 51
Unidentified GOS	272	1418	524	365	695	655 ± 410
Total GOS[a]	813	2265	842	602	1048	1114 ± 597

[a] Total GOS includes quantification of: allolactose, β-1-6-galactobiose, 6'-galactosyl-lactose, 4'-galactosyl-lactose and unidentified GOS (Marked as * in figure 1). Samples were prepared and analyzed in duplicate. Tr: trace (LOD = 5 mg/L)