Presence of galactooligosaccharides and furosine in special dairy products designed for elderly people

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ABSTRACT

An evaluation of the formation of prebiotic carbohydrates during lactose hydrolysis has been carried out in industrially elaborated dairy preparations designed for elderly people. Due to the hydrolysis of lactose, high levels of galactose and glucose were found together with galactooligosaccharides (GOS), mainly allolactose, 6-galactobiose and 6'-galactosyl lactose. Total GOS content was between 7.1-13.4% of total carbohydrates, depending on lactose hydrolysis extent. In addition, the determination of furosine as indicator of lysine loss during the Maillard reaction (MR) has been also studied. The high content of monosaccharides promoted the progress of the MR during UHT processing, as reflected by the levels of furosine found in samples. After storage at 20°C for 4 months the content of furosine increased by 74-90%. These results underline the importance of controlling lactose hydrolysis, and processing and storage conditions to preserve the quality and increase the bioactivity of dairy preparations designed for elderly people.

Keywords: aging, prebiotic, galactooligosaccharides, furosine, Maillard reaction

1. Introduction

The gradual aging of the population has attracted the interest of investigators in promoting the health of the elderly. Gastrointestinal problems are among the most common pathologies affecting elderly people. During aging, the decrease in levels of β-galactosidase (lactase), responsible for the hydrolysis of lactose in the intestinal mucosa, can give rise to symptoms of lactose intolerance such as gas, bloating, nausea and diarrhea (Casellas, Aparici, Casaus, & Rodríguez, 2013). Therefore, to improve the well-being of elderly people, the consumption of special preparations low in lactose together with a balanced diet is of paramount importance.

Moreover, the decrease of bifidobacteria that also occurs with age can give rise to malnourishment, constipation and alterations in the immune system (Hopkins, Sharp & Macfarlane, 2002). Severe constipation usually involves treatment with laxatives, which can possess side effects; therefore, a preventive action with daily ingestion of dietary fiber is recommended. However, as people get older, food intake may decrease since their energy necessities are reduced and it becomes difficult to ingest the amount of dietary fiber that is recommended to avoid constipation (Taylor, 1990). One option for this is the use of prebiotic oligosaccharides, such as galactooligosaccharides (GOS), which resemble those present in human milk. GOS are usually synthesized by transgalactosylation during the hydrolysis of lactose by the enzymatic activity of β-galactosidases (Villamiel, Montilla, Olano, & Corzo, 2014). Several studies have shown that GOS may not only exert a bifidogenic effect but also improve the symptomatology of constipation and reinforce the immune response in adults and elderly people (Vulevic, Drakoularakou, Yaqoob, Tzortzis, & Gibson, 2008). Recently, it has been reported that, during the process of obtaining low lactose milks using β-galactosidases from different microorganisms, a notable amount of GOS were formed (Ruiz-Matute,

Corzo-Martínez, Montilla, Olano, & Corzo, 2012; Rodríguez-Colinas, Fernandez-Arrojo, Ballesteros, & Plou, 2014).

During lactose hydrolysis elevated concentrations of glucose and galactose are formed. These reducing carbohydrates, more active than the original lactose, can subsequently react more easily with the ε-amino group of lysine and arginine, present in proteins, during the Maillard reaction (MR) forming the corresponding Amadori compound. In low lactose products, furosine has been shown as a useful indicator of the initial steps of this reaction (Evangelisti, Calcagno, Nardi, & Zunin, 1999; Messia, Candigliota, & Marconi, 2007). When the MR is in at the advanced stages, nutritional changes attributed to the participation of an important proportion of essential amino acids such as lysine or reduction of protein digestibility is produced together with the formation of toxic compounds (Corzo-Martínez, Corzo, Villamiel, & del Castillo, 2012). This fact could be especially important for elderly people (Gilani, Xiao, & Cockell, 2012). It has been claimed that to avoid excessive formation of furosine and, consequently, loss of available lysine it may be advisable to hydrolyze lactose after thermal treatments (Messia et al. 2007; Rada-Mendoza, Olano, & Villamiel, 2005; Ruiz-Matute et al. 2012).

To the best of our knowledge, no previous data have been reported on the advance of Maillard reaction and presence of GOS in dairy products intended for elderly people. The aim of this work has been to analyze the furosine and carbohydrate fraction, highlighting GOS, in industrially elaborated dairy products with low concentration of lactose designed for elderly people, in an attempt to guarantee their nutritional quality and to improve the well-being of this sector of the population.

2. Materials and methods

2.1. Standards and samples

Lactose, D-galactose, D-glucose, 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 (Ruiz-Matute et al., 2012).

Six industrially special dairy preparations designed for elderly people (three samples from two batches), kindly provided by a Spanish dairy company (CAPSA, Asturias, Spain) were studied (Table 1). The A samples were semi-skimmed milk with 1% fat content, and were used as controls. The B samples were made of reconstituted buttermilk powder and enriched with vitamins and minerals. The C samples, were semi-skimmed milk with 1% fat content, and were enriched with vitamins and minerals (Table 2). In all samples, hydrolysis of lactose was carried out by β-galactosidase (EC 3.2.1.23) from *Kluyveromyces lactis* of the commercial enzymatic preparation Ha-lactase 5200 (5200 U mL^{-1}) (Chr. Hansen, Denmark). Samples were maintained at 4 °C for 18 h. Subsequently, samples were subjected to direct UHT treatment (150 °C for 7 s). The two industrial batches of products (I and II) were processed 5 months apart; however, they were analyzed at the same time. Samples B-II and C-II were stored in our laboratory in the dark for a period of 4 months at temperatures between 20 °C and 31 °C.

Information on the dry matter (DM), protein content and pH was provided by the manufacturer (Table 3).

2.2. Analytical determinations of furosine and carbohydrates

Determination of furosine in the special dairy preparations was done by ion-pair RP-HPLC following the method of Rada-Mendoza et al. (2005).The data are the mean values expressed as milligrams of furosine per 100 g of protein.

Carbohydrate analysis was carried out by GC-FID according to the method of Ruiz-Matute el al. (2012).

All samples were prepared in duplicate and data were expressed as mean ± standard deviation (SD).

3. Results and discussion

Table 3 shows the pH, DM, protein and total sugars values together with furosine levels of industrially obtained dairy preparations designed for elderly people. Samples had pH values in the 6.4-6.6 range, similar to those showed in the literature for low-lactose products. The only exception were the B samples (I and II), in which carbohydrates and protein values were similar to those of UHT milks with hydrolyzed lactose (Rada-Mendoza et al. 2005). These differences could be due to the different composition of the dairy preparations, since the A and C samples were made with skim milk whereas the main ingredient of the B samples was reconstituted dried buttermilk.

Furosine concentrations (Table 3) ranged from 235.5-819.7 mg/100 g protein. Rada-Mendoza et al. (2005) and Ruiz-Matute et al. (2012) reported levels of furosine from 244.2 to 432.4 mg/100 g protein for lactose-hydrolyzed commercial milks. In this paper, considering the two separate batches, the highest values of furosine were detected in the B samples, (B-I, 819.7 mg/100 g protein and B-II, 454.4 mg/100 g protein), probably due to their higher protein and reducing carbohydrate content. Furthermore, during the manufacture and subsequent storage of dried buttermilk, used for preparation

of B samples, the MR was probably enhanced giving rise to elevated furosine concentrations (Corzo, Delgado, Troyano, & Olano, 1994). Comparing batches, the highest values of furosine were found in batch I, probably due to the fact that, according to the data provided by the manufacturer, these samples were obtained 5 months prior to the other batch (batch II); however, all of them were analyzed at the same time and within the sell-by date. During that period, in batch I, the monosaccharides present could react with proteins during the MR. In addition, the pH values of this batch were slightly lower than those of batch II, probably due to the greater advance of the MR in the former with the consequently higher formation of acidic compounds and the participation of the amino group that could contribute to a slight decrease in pH in the system (Liu, Yang, Jin, Hsu, & Chen, 2008).

As indicated in the Material and Methods section, the B and C samples corresponding to batch II were stored at room temperature for 4 months, immediately after processing, to study the progress of the MR in these products during a controlled storage. As observed in Table 3, the amount of furosine increased after this period by 74 - 90%, confirming the importance of the storage conditions in this type of products to minimize the loss of lysine. These data are consistent with those of lactose-hydrolyzed UHT milks previously reported in the literature. Evangelisti et al. (1999) after the storage of lactose-hydrolyzed UHT milks for 3 months at 20 °C found a nearly 3-fold increase in the amount of furosine; however, hardly any change was observed when samples were stored at 4 °C. In a recent study, Gilani et al. (2012) have also indicated the necessity to control the progress of the MR, since the formation of the MR products can give rise to significant reductions in protein digestibility and bioavailability of lysine and protein quality (Corzo-Martínez, et al., 2012). This fact is especially important in elderly individuals.

Figure 1 shows the GC-profile for the determination of the trimethylsilyl oximes of carbohydrates in the B-I dairy preparation. As observed, mono-, di- and oligosaccharides were detected together with aminosugars and polyols. *N*-acetyl-galactosamine, *N*-acetyl-glucosamine and *myo*-inositol concentrations (Table 4) were similar to those previously found in commercial heat-treated milk with hydrolyzed lactose (Rada-Mendoza et al. 2005). In general, all carbohydrates were in the same ratio, except *N*-acetyl-galactosamine, which was more abundant in the B products (buttermilks). This could be due to the fact that *N*-acetyl-galactosamine is the most abundant sugar in the glycoproteins from the bovine milk fat globule membrane (Kim, Kanno, & Mizokami, 1992).

As expected, the level of lactose (Table 4) was very low (0.22-3.9 g L^{-1}), but it was higher than the data reported by Ruiz-Matute et al. (2012) for lactose-free commercial UHT milks (0.02-0.29 g L^{-1}). As a consequence of lactose hydrolysis the amounts of galactose and glucose (Table 4), were higher compared to UHT milks without lactose-hydrolyzed. As indicated above, these reducing carbohydrates can participate in the MR during the thermal treatment and subsequent storage of samples.

The presence of tagatose and fructose (Table 4) was due to the isomerization of galactose and glucose, respectively, whereas, no presence of lactulose was found due to the low lactose concentration (Rada-Mendoza et al. 2005). Thus, tagatose could be a better heating indicator than lactulose in lactose-hydrolyzed products. Other carbohydrates with prebiotic properties were observed in all dairy preparations intended for elderly people analyzed herein (Figure 1). Thus, together with allolactose, 6-galactobiose, 6'-galactosyl lactose and 4'-galactosyl lactose other unidentified GOS were found. As can be observed in Table 4, the total content of GOS varied in the range from 3.15 to 7.47 g L^{-1} (7.1-13.4% of total carbohydrates) and hardly any change was

observed after storage at room temperature for 4 months, probably due to the high stability of β-glycosidic linkages, (Sangwan, Tomar, Singh, Singh, & Ali, 2011). The values found were higher than those of commercial lactose free UHT milks (0.60-4.35 g L^{-1}) (Ruiz-Matute et al. 2012). Considering the same type of samples (A, B, C), the highest GOS values were detected in samples of batch I which had a higher remaining lactose concentration, indicating the necessity to control the hydrolysis of lactose to obtain the highest formation of GOS. Thus, it has been reported that when the hydrolysis of lactose in UHT milk is controlled (75–90% of lactose hydrolyzed), the level of GOS can reach 10 g L^{-1} (Ruiz-Matute et al. 2012). Rodríguez-Colinas et al. (2014) obtained hydrolyzed milk containing 7.0 g L^{-1} of GOS and 2.1 g L^{-1} of residual lactose.

These data underline the importance of choosing the most suitable β-galactosidase to obtain very low residual lactose content with relatively high amounts of GOS (Boon, Janssen, & van't Riet, 2000). According to Rodríguez-Colinas et al. (2014) the best option is the enzyme derived from *K. lactis,* used in this paper. The profile of GOS here shown (Figure 1) is typical of reaction mixtures treated with this enzyme. Moreover, considering the different lactose content, it is noteworthy that all samples, except C-I, had low lactose content (up to 2.2% of total carbohydrates) whereas sample C-I had a lactose concentration of 8.3%. This result could be due to the slightly lower pH value of sample C-I (6.41) as compared to the other samples with pH values in the range from 6.49-6.64. The impact of pH variations on the ability of the β-galactosidase from *K. lactis* to hydrolyze lactose is well-known, as indicated by Martinez Villaluenga, Cardelle-Cobas, Corzo, Olano, & Villamiel (2009).

4. Conclusion

Due to the elevated monosaccharide concentration, the special dairy products for elderly people presented high levels of furosine, mainly in the case of samples subjected to a storage period at ambient temperature. In addition, and as a result of lactose hydrolysis, high levels of GOS were found. The results obtained in this paper show the importance of controlling all processing conditions during lactose hydrolysis, thermal treatment, and storage conditions to increase the level of prebiotic carbohydrates with low levels of lactose and minimize the loss of lysine of dairy preparations designed for elderly people. The information obtained here can be useful to help in maintaining the health and well-being of this group of population.

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trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. *American Journal of Clinical Nutrition*, *88*, 1438-1446.

Figure caption

Figure 1. GC-FID profile of trimethylsilyl oximes derivatives of carbohydrates present in PR-B-I dairy preparation. Full chromatogram: galactose (peaks 3), glucose (peaks 4) and internal standard (I.S., phenyl-β-glucoside). (A) Tagatose (peaks 1), fructose (peaks 2), (B) *myo*-inositol (peak 5), *N*-acetyl-glucosamine (peak 6), *N*-acetyl-galactosamine (peak 7). (C) lactose (peaks 8), allolactose (peaks 9), 6-galactobiose (peaks 10). (D) 4'-galactosyl-lactose (peak 11), 6'-galactosyl-lactose (peaks 12).*Disaccharides and **trisaccharides unidentified.

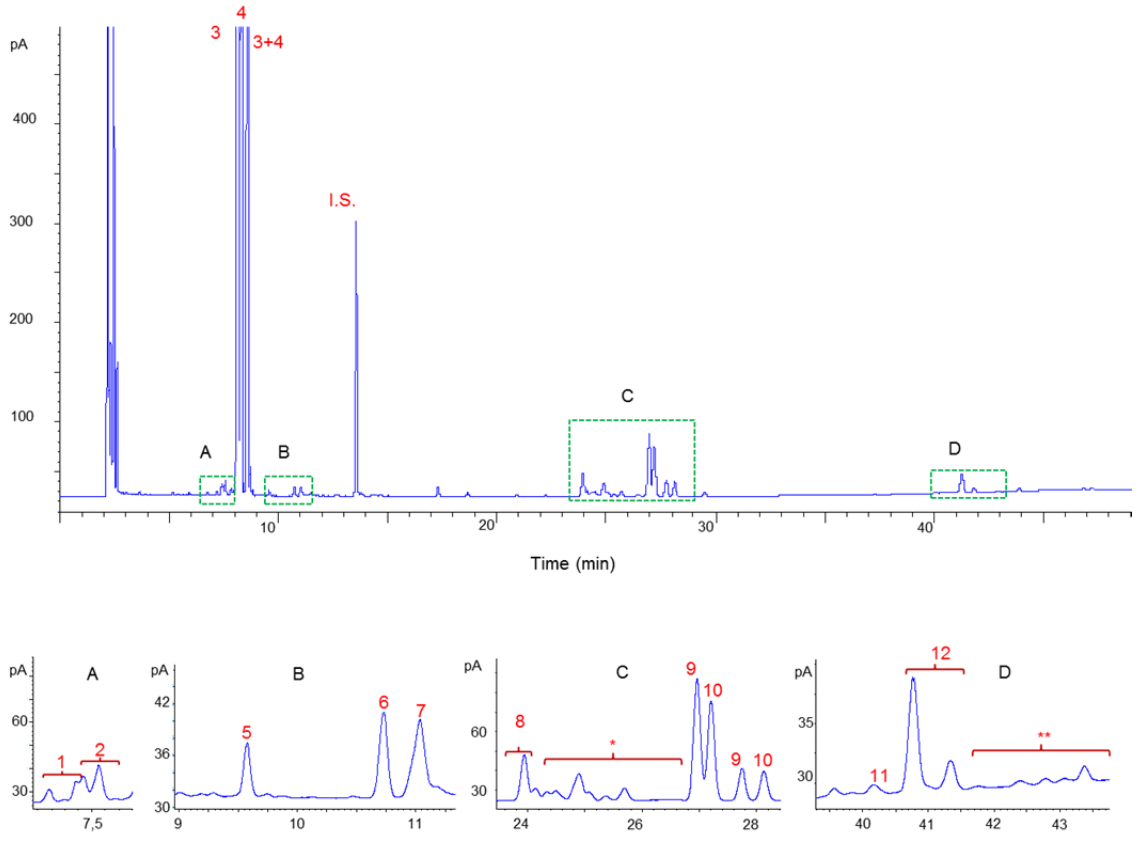

Table 1 Codification of industrially elaborated special dairy preparations designed to aged people under study.

Samples	Batch I	Batch II
Preparation A	PR-A-I	PR-A-II
Preparation B	PR-B-I	PR-B-II
Preparation C	PR-C-I	PR-C-II
Samples stored during 4 months at ambient temperature		
Preparation B	-	PR-B-II-4m
Preparation C	-	PR-C-II-4m

Table 2. Nutritional information per 100 mL of product of dairy preparations B and C designed to elderly people. Data provided by the manufacturer.

Parameter	Preparation B	Preparation C*
Energetic value	46 kcal (193 kJ)	41 kcal (172 kJ)
Carbohydrates	6.4 g	4.7 g
Fat	0.6 g	1.0 g
Fiber	0 g	0 g
Sodium	60 mg	60 mg
Calcium	120 mg	120 mg
Copper	0.36 mg	0.36 mg
Zinc	3.6 mg	3.6 mg
Vit. B5	2.16 mg	2.16 mg
Vit. B6	0.51 mg	0.51 mg
Vit. B9	144 μg	144 μg
Vit. B12	1.8 μg	1.8 μg
Vit. D	3.6 μg	3.6 μg

*This sample contained 9.9 mg of ginseng

Table 3. Values of pH, dry matter (%), content of total sugars (g/L), protein (%) and furosine (mg/100 g protein) in dairy preparations designed to aged people.

Samples	pH	Dry Matter (%)	Total sugars (g/L)	Protein (%)	Furosine (mg/100 g protein)
PR-A-I	6.5	9.6	47.8±4.0	3.2	525.0±1.9
PR-B-I	6.5	11.6	56.5±4.5	3.9	819.7±47.1
PR-C-I	6.4	9.8	47.4±2.7	3.2	516.7±2.5
PR-A-II	6.6	9.3	45.2±1.5	3.1	235.5±6.4
PR-B-II	6.6	11.5	58.3±4.6	3.8	454.4±7.1
PR-C-II	6.6	9.0	44.9±3.9	2.9	243.0±3.6
PR-B-II-4m	6.5	11.6	57.6±3.4	3.8	790.7±5.0
PR-C-II-4m	6.6	9.0	43.8±2.5	2.89	464.2±6.6

Table 4. Content (g/L) of mono- and disaccharides and galactooligosaccharides (GOS) found in dairy preparations designed to aged people.

Samples	PR-A-I	PR-B-I	PR-C-I	PR-A-II	PR-B-II	PR-C-II	PR-B-II-4m	PR-C-II-4m
Galactose	19.0±1.6	22.2±1.8	18.4±1.3	19.4±0.8	24.4±1.9	19.9±1.7	23.6±2.2	18.3±0.6
Glucose	20.5±1.8	24.2±2.0	18.7±0.7	20.4±0.8	26.2±2.2	20.6±1.8	25.7±2.0	19.8±2.3
Tagatose	0.09±0.01	0.11±0.01	0.10±0.01	0.09±0.00	0.11±0.01	0.08±0.01	0.12±0.01	0.08±0.01
Fructose	0.19±0.02	0.26±0.02	0.29±0.04	0.21±0.02	0.28±0.03	0.25±0.03	0.24±0.03	0.25±0.04
Myo-inositol	0.04±0.00	0.06±0.01	0.04±0.00	0.04±0.00	0.07±0.00	0.04±0.00	0.07±0.01	0.05±0.01
N-Acetyl-Glucosamine	0.11±0.01	0.15±0.02	0.11±0.01	0.10±0.01	0.13±0.01	0.09±0.01	0.12±0.01	0.08±0.01
N-Acetyl-Galactosamine	0.06±0.01	0.19±0.02	0.07±0.01	0.05±0.01	0.18±0.03	0.05±0.01	0.14±0.01	0.04±0.01
Lactose	1.05±0.08	1.17±0.11	3.90±0.14	0.49±0.04	0.60±0.05	0.22±0.02	0.71±0.06	0.28±0.03
Allolactose	1.87±0.14	2.22±0.21	1.33±0.05	1.11±0.11	1.62±0.14	0.78±0.08	1.78±0.15	0.99±0.13
6-Galactobiose	1.55±0.12	1.81±0.18	1.16±0.05	0.94±0.09	1.36±0.11	0.64±0.07	1.53±0.17	0.91±0.09
6'-Galactosyl-lactose	1.09±0.12	1.40±0.18	0.96±0.05	0.65±0.07	1.07±0.10	0.61±0.07	1.53±0.17	1.18±0.11
Total GOS*	6.29±0.56	7.47±0.76	5.26±0.47	3.88±0.25	5.64±0.47	3.15±0.27	6.96±0.74	4.87±0.25

*Total GOS includes quantification of: allolactose, 6-galactobiose, 6'galactosyl-lactose and unidentified GOS (marked as * and ** in Fig. 1).