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Production of lactulose oligosaccharides by isomerisation of transgalactosylated cheese whey permeate obtained by β-galactosidases from dairy *Kluyveromyces*

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β-Galactosidases from *Kluyveromyces lactis* and *Kluyveromyces marxianus* isolated from artisanal ewes' milk cheeses, were used to transgalactosylate lactose from cheese whey permeate (WP). The content of galactooligosaccharides (GOS) obtained by transgalactosylation was comparable with that formed using pure lactose as substrate. In order to obtain a mixture with higher prebiotic oligosaccharide content, isomerisation of the transgalactosylated WP was carried out using sodium aluminate as catalyst. The transgalactosylated mixtures at 6 h of reaction contained amounts of prebiotic carbohydrates (tagatose, lactulose, GOS and oligosaccharides derived from lactulose, OsLu) close to 50 g/100 g of total carbohydrates for all the strains tested, corresponding to 322 g prebiotics/kg whey permeate. Thus, the suitability of this methodology to produce mixtures of dietary non-digestible carbohydrates with prebiotic properties from WP has been demonstrated, which is interesting for the food industry since it increases the value and the applicability of this by-product from cheese manufacture.

Keywords: Cheese whey permeate, transgalactosylation, isomerisation, *Kluyveromyces*, prebiotic oligosaccharides.

Introduction

Nowadays, the development of new bioactive oligosaccharides is gaining attention for their potential use as prebiotic compounds (Figueroa-González et al. 2011). Galactooligosaccharides (GOS) and lactulose are recognised as prebiotic carbohydrates and they are widely used in Japan, Europe and the United States (Tuohy et al. 2005). GOS are usually produced by transgalactosylation of lactose using microbial β-galactosidases, and in addition to their prebiotic character, other health benefits such as improvement of mineral absorption, prevention of intestinal infections and enhancement of immune function among others have been described (Pérez-Conesa et al. 2006; Arslanoglu et al. 2008; Vulevic et al. 2008; Ebersbach et al. 2010). Lactulose, a synthetic disaccharide manufactured by lactose isomerisation in basic media, was the first carbohydrate commercialised with recognised beneficial effects on gut bifidobacteria (Méndez & Olano, 1979; Rycroft et al. 2001). This disaccharide has also been proposed as an enzymatic substrate to synthesise prebiotic oligosaccharides (OsLu) (Cardelle-Cobas *et al.* 2008a, 2011a, b; Martínez-Villaluenga *et al.* 2008). Another strategy for OsLu synthesis is the isomerisation of GOS reaction mixtures obtained from transglycosylation of lactose solutions using commercial β-galactosidases (Cardelle-Cobas et al. 2008b).

Whey is the major by-product of the cheese making industry and presents important environmental problems since its disposal is highly contaminating (Gänzle et al. 2008). Ultrafiltration of cheese whey yields whey protein concentrate used in the food industry, and whey permeate (WP), comprising mainly lactose and salts, with low market value. Thus, the possibility of using lactose from a

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waste material, such as WP, to obtain GOS is particularly interesting for the food industry (Lamsal, 2012).

In different studies, the feasibility of commercial yeast β-galactosidases to produce GOS from WP has been described (Pocedičová et al. 2010; Klein et al. 2013; Lorenzen et al. 2013). On the other hand, a new methodology to obtain mixtures of GOS and OsLu from WP by a combination of two reactions, isomerisation using basic catalysts and transgalactosylation using commercial *Bacillus circulans* β-galactosidases, has been recently proposed (Corzo-Martínez et al. 2013). The use of both reactions is a feasible strategy to obtain a mixture of prebiotic carbohydrates with a wide diversity of structural features.

The potential use of β-galactosidases from *Kluyveromyces lactis* and *K. marxianus* strains isolated from artisanal cheeses (Padilla et al. 2014), to transgalactosylate buffered solutions of pure lactose and lactulose has been demonstrated (Padilla et al. 2012). Reaction mixtures with different levels of individual oligosaccharides were obtained. However, oligosaccharide production from WP using these β-galactosidases was not assayed and it is known that permeate ingredients such as mineral salts may hamper transgalactosylation reactions.

Therefore, in the present work, the feasibility of the above mentioned β-galactosidases from *K. lactis* and *K. marxianus* to produce prebiotic oligosaccharides from WP was explored. First, WP was submitted to transgalactosylation by *Kluyveromyces* β-galactosidases to obtain GOS mixtures, and in a second step transgalactosylated WP was isomerised using a basic catalyst with the aim of obtaining reaction mixtures of prebiotic carbohydrates with a wide diversity of structural features (GOS and OsLu). The use of different experimental conditions to obtain prebiotic carbohydrates may provide new ingredients with improved functionalities.

Materials and methods

Chemicals

Lactose was obtained from Scharlau (Barcelona, Spain). D-Galactose, D-glucose, D-fructose, lactulose, raffinose, β-1,6-galactobiose, phenyl-β-D-glucoside and *o*-nitrophenyl β-D-galactopyranoside (*o*NPG) were purchased from Sigma-Aldrich Co. (Steinheim, Germany). D-Glucose and lactose for yeast culture media were obtained from Panreac (Barcelona, Spain), bacteriological peptone was acquired from Cultimed (Barcelona, Spain) and yeast extract and agar were purchased from Pronadisa (Madrid, Spain). Ultrapure water (18·2 M Ω-cm, with levels of 1–5 ng/ml total organic carbon and <0·001 EU/ml pyrogen) produced in-house with a laboratory water purification system (Milli-Q Synthesis A10, Millipore, Billerica, MA, USA) was used throughout.

Yeast strains

Two yeast strains belonging to *K. lactis* and *K. marxianus* species (CECT 13121 and CECT 13122, respectively) were isolated from artisanal ewes' milk cheeses produced in Cheese Company 'Los Corrales' from rural Castelló province (Spain) (Padilla et al. 2014). In addition, *K. lactis* CECT 1961^T was obtained from the Spanish Type Culture Collection and was included in the study as a control.

Kluyveromyces crude cell extracts (CCEs)

Yeasts were grown overnight in medium GPY (glucose 2%, peptone 0·5% and yeast extract 0·5%) at 28 °C. Afterwards, yeast cells were transferred to LPY medium (lactose 2%, peptone 0·5% and yeast extract 0·5%) and incubated overnight at 28 °C. CCEs preparation was performed as described elsewhere (Padilla et al. 2012).

Oligosaccharide synthesis from cheese whey permeate (WP)

Industrial bovine cheese WP powder with a lactose content of 81·6% (w/w dry matter) was kindly supplied by the dairy company Reny Picot (Navia, Spain). Physical and chemical composition of this WP was determined in a previous work (Díez-Municio et al. 2012). WP was reconstituted with ultrapure water at a lactose concentration of 250 g/l. The pH was measured using a pH meter (MP 230, Mettler-Toledo, Barcelona, Spain).

A solution of the reconstituted WP powder was prepared for transgalactosylation reaction. Enzymatic synthesis of oligosaccharides from cheese WP using different *Kluyveromyces* CCEs was performed under the defined reaction conditions of 250 g/l substrate at pH 6·5, temperature of 50 °C and 6 U β-galactosidase activity/ml (Padilla et al. 2012). Enzymatic reactions were performed in duplicate in a final volume of 10 ml and were incubated under agitation. After 4 h, the reaction was stopped by immersing the reaction mixture in boiling water for 5 min to inactivate the enzyme. An aliquot of 600 µl was withdrawn and stored at −20 °C until further analysis and the rest of the sample was submitted to isomerisation reaction.

Isomerisation reaction of transglycosylated WP

Isomerisation assays (in duplicate) were carried out in cheese WP transgalactosylation mixtures containing 1 g carbohydrates. Sodium aluminate (0·7 g) was added as catalyst and then samples were diluted to 10 ml with Milli-Q water. Afterwards, samples were immersed into a water bath adjusted to the required temperature (40 °C) and maintained for a time period of 24 h (Cardelle-Cobas et al. 2008b). Aliquots of 2 ml were withdrawn from the reaction mixtures at 0, 2, 4, 6, and 24 h.

The reaction was stopped by placing the tubes in an ice bath and then adding a few drops of H_2SO_4 (25%) to decrease the pH up to 3·5–4·5. In order to assist the precipitation of the formed salts, $CaCO_3$ (40%) was added until pH increased to 6·5–7·5. Then, sample was centrifuged at 7000 ***g*** for 6 min and the supernatant was collected, filtered using a

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0·45 µm syringe filter (Symta, Madrid, Spain) and diluted to a final volume of 10 ml with water. All assays were performed in duplicate.

Chromatographic determination of carbohydrates

Carbohydrates in reaction mixtures were analysed by gas chromatography (GC). A volume of 300 µl of supernatant was added to 0·4 ml of internal standard (IS) solution, containing 0·5 mg/ml of phenyl-β-D-glucoside. The mixture was dried at 38–40 °C in a rotatory evaporator (Büchi Labortechnik AG, Falwil, Switzerland).

Previous to GC analysis of carbohydrates, oximes of trimethylsilyl derivatives (TMSO) must be prepared (Brobst & Lott, 1966). First, oximes were obtained by addition of 250 µl of a solution of 2·5% hydroxylamine chloride in pyridine to the carbohydrate mixture after 30 min at 70 °C. Subsequently, the oximes were silylated with hexamethyldisilazane (250 µl) and trifluoroacetic acid (25 µl) at 50 °C for 30 min. Then, reaction mixtures were centrifuged at 10000 ***g*** for 2 min. This derivatisation procedure gives rise to a single chromatographic peak for non-reducing sugars, corresponding to their trimethylsilyl ethers, whereas two peaks are detected for reducing sugars, corresponding to their *syn-* (*E*) and *anti-* (*Z*) oxime isomers.

GC analysis of derivatised samples was carried out using an Agilent Technologies 7890A gas chromatograph (Wilmington, DE, USA) equipped flame ionisation detector (FID). A commercial fused silica capillary column SPB-17, crosslinked phase (50% diphenyl / 50% dimethylsiloxane; 30 m × 0·32 mm *i.d.* × 0·5 µm film thickness) (Supelco, Bellefonte, PA, USA) was used. The initial oven temperature was 200 °C, increasing to 230 °C at a rate of 4 °C/min, and finally increased to 290 °C at 2 °C/min and held for 25 min. The injector and detector temperatures were set at 280 °C and 290 °C, respectively. Injections were carried out in split mode (1:30) using nitrogen at 1 ml/min as carrier gas. Data acquisition and integration were performed using Agilent ChemStation Rev. B.03·01 software.

Quantitative analysis was carried out through the IS method. Response factors relative to IS (phenyl-β-D-glucoside) were calculated from the analysis of standard solutions containing tagatose, fructose, glucose, galactose, lactose and lactulose, prepared over the expected concentration range in the samples. Also, raffinose was used as a standard to quantify trisaccharides. The identities of oligosaccharides produced after transglycosylation and isomerisation of WP were confirmed by comparison with relative retention times of standards previously synthesised, purified and characterised in our laboratory (Cardelle-Cobas *et al.* 2008b, c, 2009; Martínez-Villaluenga *et al.* 2008; Cardelle-Cobas, 2009). The amounts of lactose, lactulose, glucose, galactose, tagatose, fructose and other sugars remaining in the transgalactosylation and isomerisation mixtures were calculated as grams per 100 g of the total carbohydrate content. All analyses were performed in duplicate

Statistical Analysis

Fisher's Least Significant Difference (LSD) test was used for mean comparison at 95% confidence level (StatGraphics Plus 5·1, StatPoint, Herndon, VA).

Results and discussion

Transgalactosylation of lactose from WP

In this study, the feasibility of dairy *Kluyveromyces* CCEs to hydrolyse and transgalactosylate lactose present in cheese WP to produce GOS was evaluated. The conditions used to hydrolyse lactose from cheese WP were selected taking into account previous reported results, where the optimal production of GOS from pure lactose solutions employing CCEs from dairy *Kluyveromyces* was reached after 4 h of reaction (pH 6·5, 50 °C) (Padilla et al. 2012). Figure 1 shows the chromatographic profile of carbohydrates found in the transgalactosylated reaction mixture of lactose in cheese WP by β-galactosidase activity of *K. lactis* CECT 13121. It can be observed the presence of released monosaccharides (galactose and glucose, peaks 1 and 2) as well as unreacted lactose (peaks 3 and 4). Moreover, the formation of GOS (di- and trisaccharides) obtained by transgalactosylation reaction was also detected. Allolactose (β-1,6-galactosyl glucose, peaks 5 and 7), β-1,6-galactobiose (peaks 6 and 8), 4′-galactosyl lactose (peak 9) and 6′-galactosyl lactose (peaks 10 and 11) could be identified. These assignments were made by comparing relative retention times to those of authentic standards and to those found in previous studies (Cardelle-Cobas et al. 2009). Different unknown di- and trisaccharides were also detected (labelled with an asterisk in Fig. 1). For the other two studied strains the GC profiles obtained were very similar.

Quantitative composition of the reaction mixtures originated by β-galactosidase activity of the three studied strains after 4 h of reaction is depicted in Table 1. During the production of GOS from lactose, significant amounts of free glucose and galactose were released as a consequence of lactose hydrolysis although considerable lactose content remained unaltered. GOS yield (consisting of di- and trisaccharides) above 30 g/100 g total carbohydrates for the three CCEs tested was found, in agreement with previous results using pure lactose solutions as substrate (Padilla et al. 2012) and commercial β-galactosidase from *K. lactis* (Martínez-Villaluenga et al. 2008). These results indicate that the salts present in WP did not seem to have an effect on transgalactosylation reactions. Regarding other experiments conducted with cheese WP and commercial *K. lactis* β-galactosidases, final GOS yields are difficult to compare, as reaction conditions are highly variable among different reported studies. Lisboa et al. (2012) found a similar maximum yield using WP and Lactozym 3000 L from *K. lactis*.

Isomerisation of transgalactosylated WP

Galactose, glucose and unreacted lactose present in transgalactosylation reaction mixtures from WP do not have

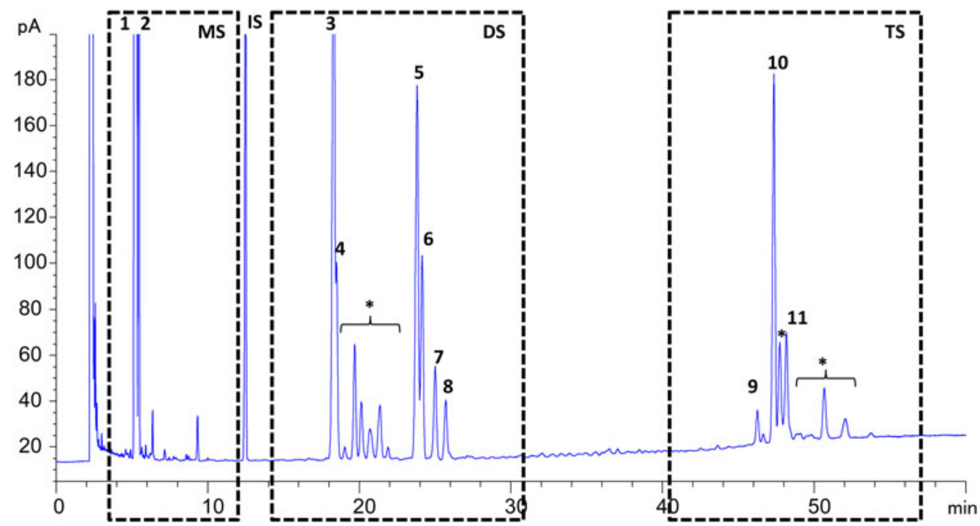

Fig. 1. GC-FID profile obtained for the transgalactosylated reaction mixture of lactose from cheese WP by β-galactosidase activity of *K. lactis* CECT 13121 after 4 h at pH 6·5, 50 °C. Peaks: (**1**) galactose (**2**) glucose, (**3**) lactose *E*, (**4**) lactose *Z*, (**5**) allolactose *E*, (**6**) β-1,6-galactobiose *E*, (**7**) allolactose *Z*, (**8**) β-1,6-galactobiose *Z*, (**9**) 4′-galactosyl lactose, (**10**) 6′-galactosyl lactose *E*, (**11**) 6′-galactosyl lactose *Z* and (*) unknown GOS. **MS:** monosaccharides; **DS:** disaccharides; **TS:** trisaccharides.

prebiotic properties because they are absorbed in the small intestine and are not selectively fermented by intestinal microbiota. Moreover, glucose in reaction mixtures increases the glycemic index. Isomerisation reaction of lactose and galactose leads to lactulose and tagatose, respectively, which are carbohydrates considered as prebiotics (Bertelsen et al. 1999; Olano, & Corzo, 2009). Therefore, isomerisation of transgalactosylated WP containing mono-, disaccharides and GOS (allolactose, β-1, 6-galactobiose and; 4′- and 6′-galactosyl lactose) to corresponding ketoses can contribute to enrich them in prebiotic carbohydrates. Additionally because glucose is converted into fructose, a decrease of glycemic index of the final product can occur. Figure 2 shows mono-, di- and trisaccharide GC-FID profiles obtained before (0 h) and after isomerisation reaction (6 and 24 h) of the transgalactosylated mixture from WP. In the monosaccharide region (Fig. 2a), the products resulting from isomerisation of glucose and galactose (peaks 4, 5 and 6) to fructose and tagatose (peaks 1, 2 and 3), respectively are observed. In the disaccharide region (Fig. 2b) after 6 and 24 h of reaction, besides the isomerisation of lactose (peaks 8 and 9) to lactulose (peaks 7 and 8), two peaks corresponding to allolactulose can be observed (peaks 15 and 16). The occurrence of an unknown disaccharide (peak 17), probably derived from lactulose, was also detected. Moreover, during the isomerisation the disappearance of some unknown peaks present in the sample at time 0 h (such as peaks 10, 11 and 12) could be observed. The trisaccharide region (Fig. 2c) at 6 and 24 h of isomerisation shows the presence of 4′-galactosyl lactulose (peak 23), 6′-galactosyl lactulose (peaks 25 and 26) as well as other oligosaccharides which could be derived from lactulose (peaks 22, 29 and 30). Peaks corresponding to 4′- and 6′-galactosyl lactose (peaks 24 and 28) were not detected after 24 h of reaction, except the peak 26 corresponding to 6′-galactosyl lactulose, indicating a complete isomerisation.

The time course of carbohydrate isomerisation from transgalactosylated WP followed up to 24 h is depicted in Figures 3 & 4. Fig. 3 shows the evolution of the released glucose, galactose and unreacted lactose during transgalactosylation of WP as well as the formation of their corresponding isomerised carbohydrates. Lactose was rapidly isomerised (Fig. 3a) into lactulose (Fig. 3b) which levels increased during 6 h of reaction reaching concentrations ranging from 4 to 10 g/100 g total carbohydrates. The level of lactose found in mixtures from *K. marxianus* was lower than in the other two tested strains and, therefore, less lactulose was formed during isomerisation. Additionally, glucose (Fig. 3c) and galactose (Fig. 3e) decreased over time since they were converted into fructose (Fig. 3d) and tagatose (Fig. 3f), respectively. The latter, increased during reaction achieving levels of approximately 20–30 g/100 g total carbohydrates.

In Fig. 4, the evolution of GOS isomerisation in transgalactosylated WP (di- and trisaccharides, Fig. 4a and c, respectively) to form OsLu (di- and trisaccharides, Fig. 4b and d, respectively) is represented. Total GOS content (Fig. 4e) decreased during reaction time in all the mixtures while total OsLu content (Fig. 4f) increased during isomerisation, reaching a maximum yield of trisaccharides after 6 h for the three CCEs tested. Levels of GOS and OsLu found in the isomerised mixtures after 6 h of reaction were in the range of 12–14 and 16–18 g/100 g total carbohydrates, respectively. It is important to remark that the initial mixture obtained by *K. marxianus* CCE contained less lactose and GOS and consequently, when the catalyst agent acts, less lactulose and OsLu were formed. The formation of prebiotic carbohydrates after 6 h of

Table 1. Carbohydrate composition (g/100 g of total carbohydrates) of the transgalactosylated reaction mixtures of cheese WP by *Kluyveromyces* β-galactosidases after 4 h at pH 6·5, 50 °C

	Monosaccharides		Disaccharides				Trisaccharides		
Strains	Galactose	Glucose	Lactose	Unknown galactose derivatives	Allolactose	β-1, 6-galactobiose	Unknown galactose derivatives	6′-Galactosyl lactose	Total GOS†
K. lactis CECT 1961^{T}	21·5 ± 1·4^{a}‡	32·4 ± 1·9^{a}	12·4 ± 2·7^{b}	2·5 ± 0·3^{a}	9·8 ± 0·2^{b}	5·2 ± 0·0^{b}	5·2 ± 0·1^{b}	10·5 ± 0·3^{a}	33·2 ± 0·5^{b}
K. lactis CECT 13121	22·1 ± 0·3^{a}	33·1 ± 0·3^{a}	12·7 ± 0·2^{b}	2·1 ± 0·2^{a}	9·4 ± 0·0^{a}	5·0 ± 0·0^{a}	5·1 ± 0·1^{ab}	9·8 ± 0·6^{ab}	31·3 ± 0·9^{ab}
K. marxianus CECT 13122	25·2 ± 0·3^{b}	35·8 ± 0·2^{a}	6·1 ± 0·3^{a}	2·1 ± 0·2^{a}	9·5 ± 0·0^{a}	5·4 ± 0·0^{c}	4·9 ± 0·5^{a}	8·5 ± 0·5^{c}	30·3 ± 1·6^{a}

†These values include: disaccharides (Unknown galactose derivatives, allolactose, β-1,6-galactobiose) and trisaccharides (Unknown galactose derivatives and 6′-galactosyl lactose)
‡Different letters indicate significant differences for the carbohydrate group (LSD test; $P < 0·05$) ($n = 2$)

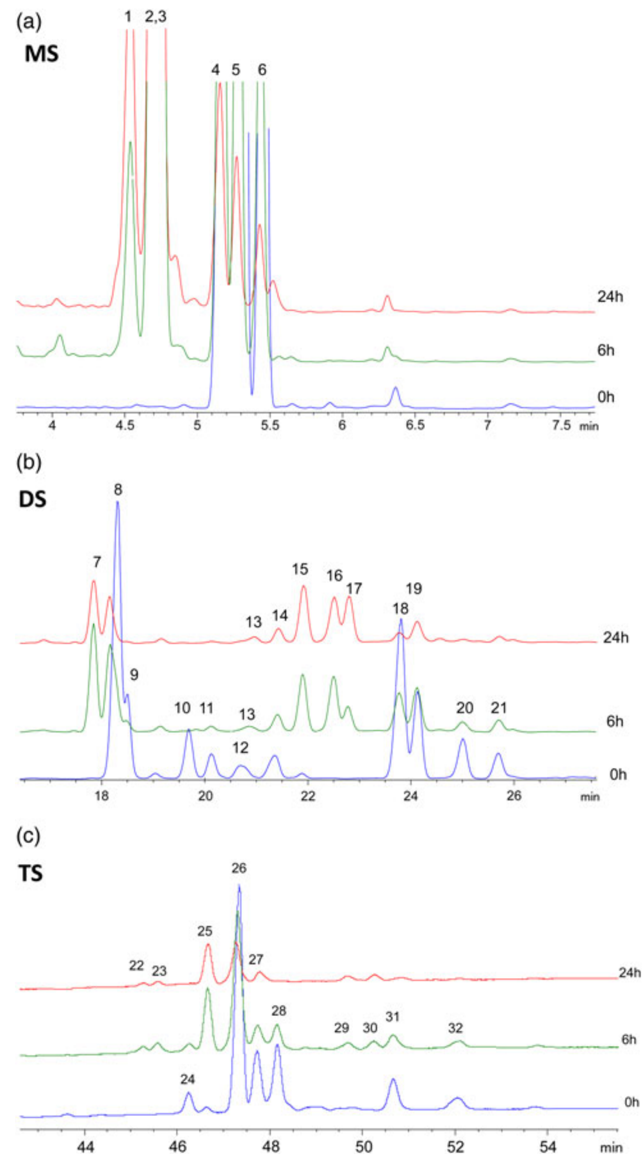

Fig. 2. Mono- (a), di- (b) and trisaccharide (c) GC-FID profiles obtained before (0 h; *blue line*, (*on-line version*)) and after isomerisation reaction (6 h, *green line (on-line version)* and 24 h, *red line* (*on-line version*)) of transgalactosylated WP. Peaks: (**1**) *tagatose* 1, (**2**) *tagatose* 2 + *fructose* 1, (**3**) *fructose* 2, (**4**) galactose *E*, (**5**) glucose *E*, (**6**) galactose *Z* + glucose *Z*, (**7**) *lactulose* 1, (**8**) *lactulose* 2 + lactose *E*, (**9**) lactose *Z*, (**10, 11, 12, 13** and **14**) unknown lactose disaccharides, (**15**) *allolactulose* 1, (**16**) *allolactulose* 2, (**17**) unknown lactulose disaccharides, (**18**) allolactose *E*, (**19**) β-1,6-galactobiose *E*, (**20**) allolactose *Z*, (**21**) β-1,6-galactobiose *Z*, (**22**), (**29**) and (**30**) *unknown lactulose trisaccharides,* (**23**) *4′-galactosyl lactulose*, (**24**) 4′-galactosyl lactose, (**25**) *6′-galactosyl lactulose* 1, (**26**) *6′-galactosyl lactulose* 2 + 6′-galactosyl lactose *E*, (**27**), (**31**) and (**32**) unknown lactose trisaccharides, (**28**) 6′-galactosyl lactose *Z*. *In italics: products resulting from isomerisation.*

isomerisation, taking into account tagatose, lactulose, GOS and OsLu, reached levels of 44·4–50·4 g/100 g total carbohydrates (Fig. 4e).

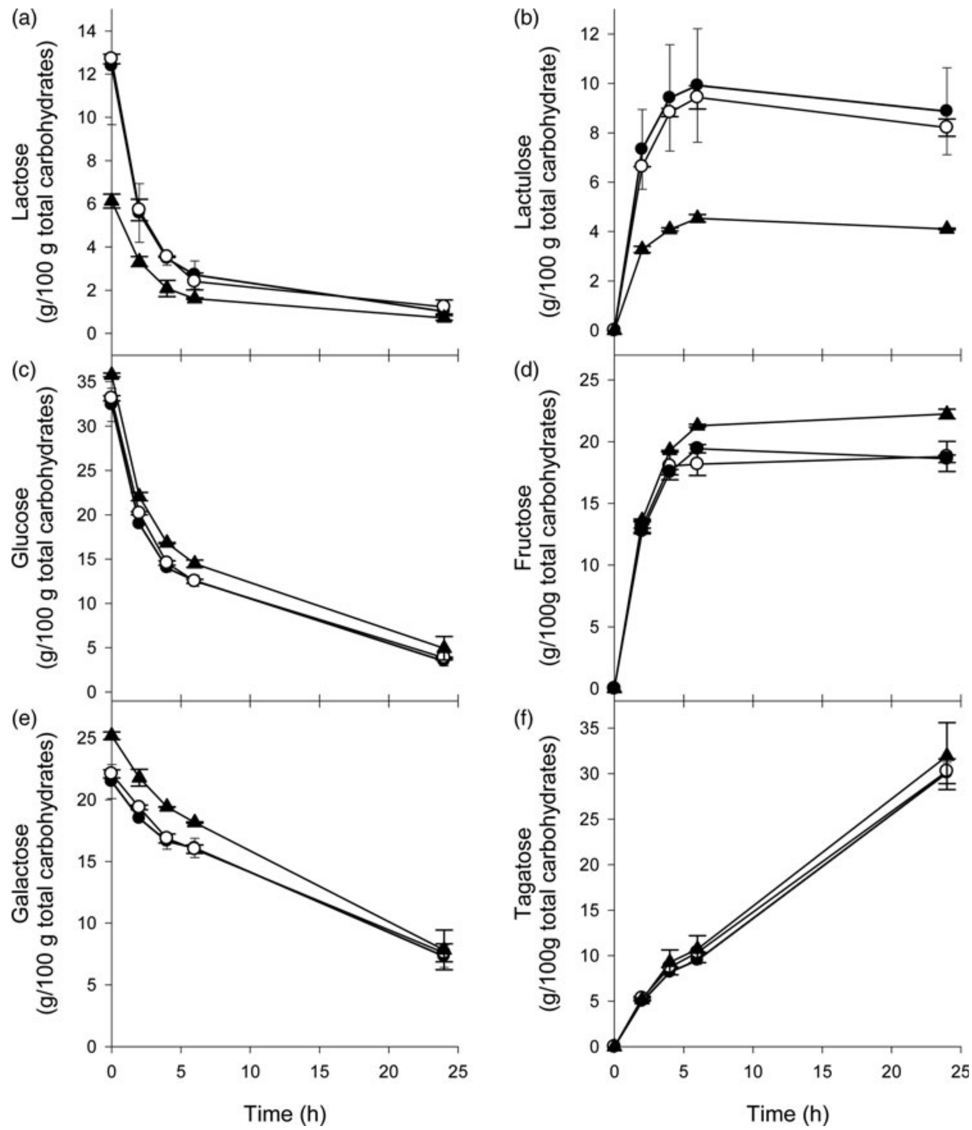

Fig. 3. Carbohydrate yields during isomerisation with sodium aluminate at 40 °C of the transgalactosylated whey permeate (WP) (250 g/l carbohydrates) obtained by β-galactosidase activity of *Kluyveromyces CCEs: K. lactis* CECT 1961ᵀ (–●–); *K. lactis* CECT 13121 (–○–) and *K. marxianus* CECT 13122 (–▲–).

Results obtained in the present study show that the combined reactions of transgalactosylation of lactose from cheese WP using β-galactosidase from dairy *Kluyveromyces* (*K. lactis* and *K. marxianus* from cheese origin) and subsequent isomerisation lead to mixtures containing a high concentration of prebiotic carbohydrates (50 g/100 g total carbohydrates, resulting in a total of 322 g prebiotics/kg whey permeate). Cardelle-Cobas et al. (2008c), obtained similar results when transgalactosylation reaction was performed using pure lactose solutions and commercial β-galactosidase from *K. lactis* and subsequent isomerisation using the same catalyst (sodium aluminate). Therefore, it has been demonstrated that all tested *Kluyveromyces* CCEs will be suitable for prebiotic synthesis, being *K. lactis* CCEs slightly best producers.

It should be pointed out that isomerisation reaction, apart from enriching the reaction mixtures in oligosaccharides of high polymerisation degree, produced a decrease of lactose, glucose and galactose concentrations, lowering the final caloric value of the mixture and making the product suitable for diabetics or subjects with lactose intolerance.

Additionally, GOS as well as OsLu have been proved to be an excellent alternative to simple carbohydrates to promote the growth of *Bifidobacterium* and *Lactobacillus* (Cardelle-Cobas *et al.* 2011a, b, 2012; Hernández-Hernández *et al.* 2012; Marín-Manzano *et al.* 2013). Regarding tagatose, health benefits related to its consumption have been described, such as beneficial effects on postprandial hyperglycaemia and hyperinsulinaemia as well as prebiotic and antioxidant activities (Lu et al. 2008; EFSA, 2010).

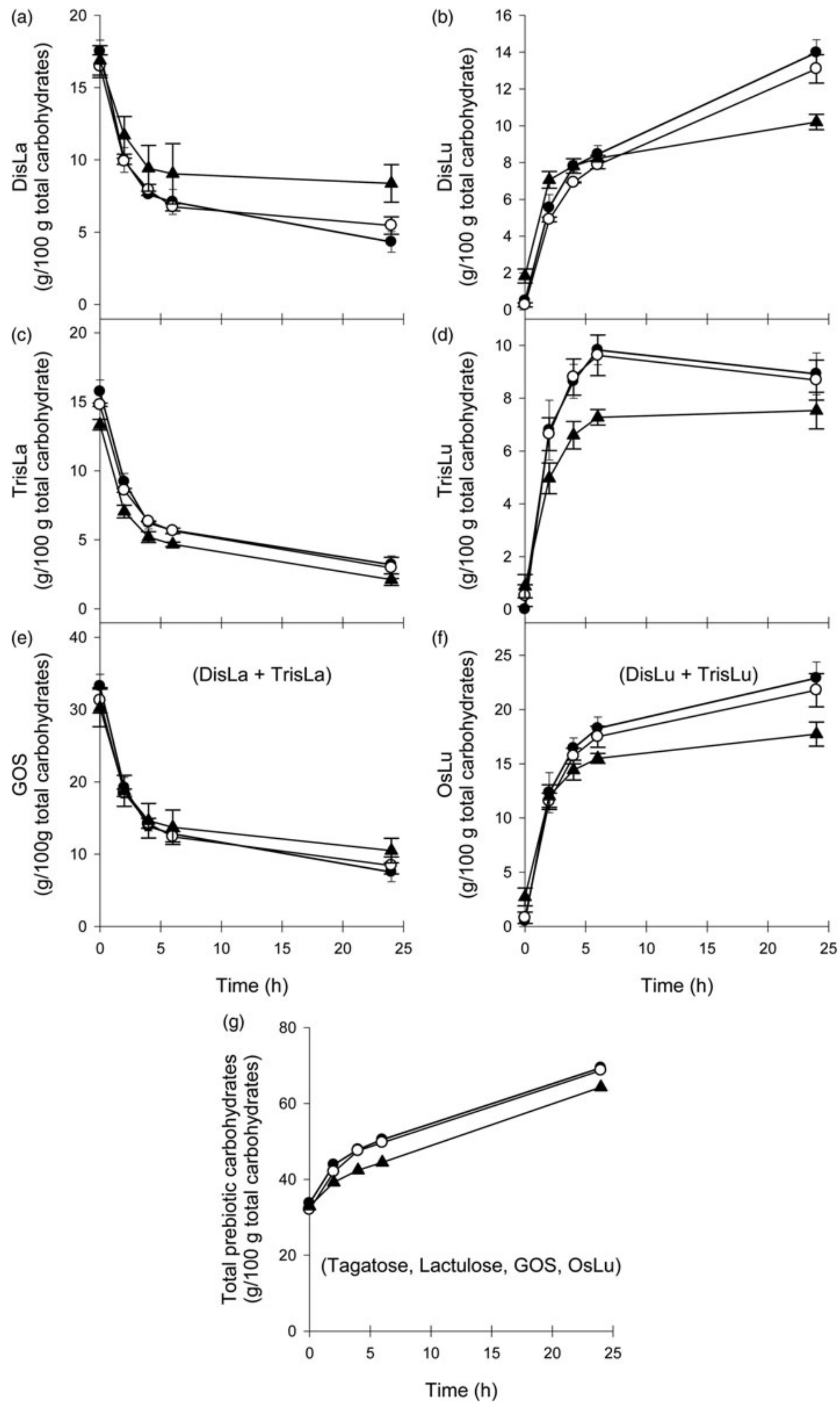

Fig. 4. Oligosaccharide yields during isomerisation with sodium aluminate at 40 °C of the transgalactosylated whey permeate (WP) (250 g/l of carbohydrates) obtained by β-galactosidase activity of *Kluyveromyces CCEs: K. lactis* CECT 1961[T] (–●–); *K. lactis* CECT 13121 (–○–) and *K. marxianus* CECT 13122 (–▲–) GOS: oligosaccharides derived from lactose. DisLa: allolactose, 6-galactobiose and other unknown disaccharides. TrisLa: 4′- and 6′-galactosyl lactose and other unknown trisaccharides. OsLu: oligosaccharides derived from lactulose. DisLu: allolactulose and unknown disaccharides; TrisLu: 6′-galactosyl lactulose and unknown trisaccharides. Total prebiotic oligosaccharides: tagatose, lactulose, GOS and OsLu.

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Conclusions

The results presented here demonstrate the feasibility of using β-galactosidases from *K. lactis* and *K. marxianus* isolated from ewe's milk cheese to transgalactosylate lactose from cheese WP and thus to increase the value of this by-product. The subsequent isomerisation enhanced the diversity of potentially prebiotic carbohydrates present in the mixture (50 g/100 g total carbohydrates) composed of tagatose, lactulose, GOS and OsLu, suggesting the suitability of this method to produce novel mixtures of dietary non-digestible carbohydrates. Moreover, the procedure proposed here (transgalactosylation and isomerisation of WP) yield 322 g prebiotics /kg whey permeate. Therefore, in this work a new strategy to obtain prebiotic oligosaccharides derived from lactulose using an inexpensive raw material such as cheese whey permeate has been proposed.

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