Use of whey permeate containing in situ synthesised galacto-oligosaccharides for the growth and preservation of *Lactobacillus plantarum*

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Galacto-oligosaccharides (GOS) are prebiotics that have a beneficial effect on human health by promoting the growth of probiotic bacteria in the gut. GOS are commonly produced from lactose in an enzymatic reaction catalysed by β-galactosidase, named transglycosylation. Lactose is the main constituent of whey permeate (WP), normally wasted output from the cheese industry. Therefore, the main goal of this work was to optimise the synthesis of GOS in WP using β-galatosidase from *Aspergillus oryzaea*. WP and whey permeate enzymatically treated (WP-GOS) were used as culture media of *Lactobacillus plantarum* 299v. *Lb. plantarum* 299v attained the stationary phase in approximately 16 h, reaching 3·6 and 4·1 × 10^8 CFU/ml in WP and WP-GOS, respectively. The in situ synthesised GOS were not consumed during growth. No significant differences were observed in the growth kinetics of microorganisms in both media. After fermentation, microorganisms were dehydrated by freeze-drying and spray-drying and stored. The recovery of microorganisms after fermentation, dehydration and storage at 4 °C for at least 120 d was above 10^8 CFU/g. These studies demonstrated that WP is an appropriate substrate for the synthesis of GOS and the obtained product is also adequate as culture medium of *Lb. plantarum* 299v. The coexistence of GOS and dehydrated viable probiotic microorganisms, prepared using an effluent as raw material, represents the main achievement of this work, with potential impact in the development of functional foods.

Keywords: Galacto-oligosaccharides, freeze-drying, spray-drying, *Lactobacillus*, whey permeate.

The development of novel functional foods, containing probiotic lactic acid bacteria and prebiotics, is of utmost importance because of their widely known health benefits (i.e.: immunological stimulation, improvement of digestion and absorption, synthesis of vitamins, decrease of cholesterol levels, inhibition of growth of potential pathogens, reduction of gas distension and recovery of the normal flora after antibiotic therapy (Gibson & Roberfroid, 1995; Manning & Gibson 2004; Wang, 2009; Wallace et al. 2011).

Prebiotics have been defined as non-digestible food components that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health.

These compounds include oligosaccharides such as inulin, lactulose and fructo, gluco or galacto-oligosaccharides (Playne & Crittenden 2004, 2009). Gibson & Roberfroid (1995) introduced the concept of 'Synbiotic' for the synergic effect produced on the gut microbiota by the ingestion of prebiotics and probiotics in functional foods.

Galacto-oligosaccharides (GOS) are present in human breast milk and their presence in infant formulas have been described as responsible for the stimulation of bifidobacteria and lactobacilli in a similar way to oligosaccharides in human breast milk (Boehm & Stahl 2003; Boehm et al. 2003; Weaver, 2003). GOS are composed of a variable number of galactose units linked to a terminal glucose. The degree of polymerisation is variable (from 2 to 8 monosaccharide units), tri and tetra-saccharides being the main components and also those with the highest prebiotic effect (Panesar et al. 2006). In addition to their prebiotic properties, the ability of

GOS to act as cryoprotectants has been recently demonstrated (Tymczyszyn et al. 2011, 2012).

The synthesis of GOS consists of a kinetically controlled enzymatic reaction of lactose transgalactosylation catalysed by β-galactosidases (E.C.3.2.1.23). In this reaction, the produced galactosyl-enzyme intermediate is either hydrolysed or attacked by another nucleophile acceptor (lactose in this case) to form new glycosidic bonds leading to GOS (Splechtna et al. 2006; Neri et al. 2009; Vera et al. 2011a, b).

Whey is a by-product of cheese making usually managed as a waste that, having a high biochemical oxygen demand (BOD), is costly to remove. Hence, the use of whey represents a very interesting option to give an added value to effluents (Marwaha & Kennedy 1988). Whey proteins are generally separated from cheese whey by ultrafiltration, and employed as food additives or protein supplements. Therefore, the permeate remaining after whey protein recovery, mostly composed by lactose and salts, can be dried and used for pig feeding (Nessmith et al. 1997). Due to the high lactose concentration, cheese whey permeate (WP) can also be used to produce different added-value products, such as GOS (Vera et al. 2012), lactic acid, ethyl alcohol, or even as a culture medium for probiotic bacteria (Cui et al. 2012).

Probiotic cultures for food applications are most frequently provided in frozen and dried forms. Drying techniques to obtain dehydrated probiotic microorganisms in a viable state have proven to be useful. Despite its high cost, freeze-drying (lyophilisation) has been the most widely used technique. Spray-drying is a lower cost technique and therefore, it is more convenient for producing large quantities of bacterial probiotic cultures (Desmond et al. 2001; Silva et al. 2002; Corcoran et al. 2004; Golowczyc et al. 2010, 2011).

With this background, the aim of this work was to optimise the enzymatic synthesis of GOS in WP using β-galatosidase from *Aspergillus oryzae* as biocatalyst. WP and enzymatically treated WP containing GOS (WP-GOS) were used as culture media for *Lactobacillus plantarum* 299v, a microorganism with recognised probiotic properties (Ducrotté et al. 2012). The composition of WP-GOS before and after fermentation was determined by HPLC. The fermented WP and WP-GOS were dehydrated by freeze-drying or spray-drying and stored to obtain a low cost product with promising applications in functional foods.

Materials and methods

Synthesis of GOS from WP

Reactions were carried out at a constant temperature of 37 °C in 200 ml Erlenmeyer flasks using *A. oryzae* β-galactosidase as biocatalyst. The β-galactosidase preparation employed in this study is traded as Enzeco Fungal lactase® and was kindly donated by Enzyme Development Corporation (EDC, New York, NY). The enzyme was stored at 4 °C and remained fully active throughout this

investigation. Enzyme has a transgalactosylation activity of 56 000 IU_T/g of enzyme preparation. One international unit of transgalactosylation (IU_T) was defined as the amount of β-galactosidase that catalyses the transgalactosylation of 1 μmol galactose per minute at 40 °C, pH 4·5 and 40% (w/w) initial lactose monohydrate concentration (Vera et al. 2011a, b).

WP was donated by a local dairy industry (Ilolay S.A, Sante Fe, Argentina). It was obtained by drying desprotenised sweet whey and contains approximately 80% (w/w) lactose, 6% (w/w) of ashes and 3% (w/w) of proteins (Leon Pelaez 2013).

A proper amount of WP was mixed with 100 mM McIlvaine citrate-phosphate buffer at pH 4·5, in order to obtain a reaction medium containing 20 to 60 g solids/100 g of suspension. The previous suspension was heated over 95 °C to promote lactose dissolution and then the temperature was adjusted to 37 °C. Afterwards, 10 g of enzyme solution were added to start the reaction of synthesis, so that the enzyme dosage was 100 IU_T per gram of lactose. The progress of synthesis was followed by taking samples at regular time intervals and the reaction was stopped by adding 0·5 ml 200 mM NaOH (Chemical Co., St. Louis, MO). The oligosaccharides content was analysed by HPLC (see section *HPLC Analysis* below). After analysis, the WP-GOS composition containing the maximum concentration of GOS was selected as growth medium for the microorganism. At these conditions, the reaction was stopped by boiling. All experiments were done in duplicate, differences among replicates never exceeding 5%. The following parameters were considered:

- **Conversion (X)**: represents the percentage of initial lactose consumed during the synthesis.

$$X = [(L_0 - L)/L_0] \times 100 \tag{1}$$

where L_0 and L represents the initial and final concentration of lactose, respectively.

- **Yield (Y)**: represents the mass of total GOS obtained per unit mass of initial lactose. For the purpose of this work, it was evaluated at the maximum GOS concentration.

$$Y = (M_{GOS}/L_0) \times 100 \tag{2}$$

where M_{GOS} represents the mass of total GOS produced.

- **Specific productivity (π)**: represents the mass of total GOS produced per unit of enzyme mass and unit of reaction time. For the purpose of this work, it was evaluated at the maximum GOS concentration.

$$\pi = M_{GOS}/E \times t \tag{3}$$

where E represents the enzyme mass and t, the time of reaction at which maximum GOS is obtained.

HPLC analysis

The content of oligosaccharides and monosacharides was determined by HPLC in a Jasco RI 2031 HPLC equipment,

provided with refractive index detector, isocratic pump (Jasco PU2080) and autosampler (Jasco AS 2055), using BP-100 Ag^+ columns (300 mm × 7·8 mm) for carbohydrate analysis (Benson Polymerics, Reno, NV, USA). Samples were eluted with mili-Q water at a flow-rate of 0·5 ml/min. Column and detector temperatures were 80 and 40 °C, respectively. Chromatograms were integrated using the software ChromPass. Composition of samples was determined by assuming that the area of each peak is proportional to the percentage of the corresponding sugar. The accuracy of such assumption was checked by making a material balance. Retention times were determined using standards of galactose, glucose, galactobiose, lactose and 3α–4β–3α galactotetraose supplied by Sigma (St. Louis, MO).

Bacterial strain

Lb. plantarum 299v, deposited at the Deutsche Sammlung von Mikroorganismen (DSM) under the code DSM 9843, was used in this work. The strain was maintained frozen at − 80 °C in 120 g/l non-fat milk solids. Microbial cells were reactivated in MRS broth (Biokar Diagnostics, Beauvais, France) (De Man et al. 1960) at 37 °C before conducting the experiments. This microorganism was selected because of its probiotic properties (Ducrotté et al. 2012) and preliminary results demonstrated that it can grow in WP suspensions at high concentrations of solids.

Growth conditions

WP and WP-GOS, containing 40 and 20 g solids/100 g suspension, were neutralised with NaOH to pH 7, and autoclaved for 15 min at 121 °C. The content of GOS, determined by HPLC, was not affected by this process ($P > 0·05$) (data not shown). WP and WP-GOS were inoculated with *Lb. plantarum* 299v and incubated at 37 °C. For spray-drying, WP and WP-GOS were centrifuged and the insoluble solids were removed before inoculating *Lb. plantarum* 299v.

Culture aliquots were taken at different times of growth (0, 8, 12, 16, 20, 24 and 36 h) to determine viable cells by plate count (see section *Bacterial plate counts*) and microorganism sugar consumption by HPLC.

Freeze-drying procedure

Lb. plantarum 299v was cultivated for 24 h in WP and WP-GOS at 37 °C, at the best concentration determined above (see *Synthesis of GOS from WP* and *HPLC* subsections). Aliquots of 1 ml of culture containing *Lb. plantarum* 299v and GOS, neutralised or not, where then transferred into 5 ml glass vials under aseptic conditions and frozen for 48 h at − 80 °C. The freeze-drying process was carried out at − 50 °C using a Heto FD4 freeze drier (Heto Lab Equipment, Denmark) and lasted 48 h.

Spray-drying procedure

Lb. plantarum 299v was cultivated for 24 h at 37 °C in WP and WP-GOS (prepared as described above). Microorganisms were dried directly from the growth medium (WP and WP-GOS). A laboratory-scale spray-dryer (model B290 Büchi mini spray-dryer) was used to process the samples at a constant air inlet temperature of 180 °C and an outlet temperature of 65–70 °C.

Bacterial plate counts

Viable bacterial plate counts were determined before and after freeze-drying and spray-drying and during storage at 4 °C. Freeze-dried samples were rehydrated in 1 ml salt solution (0·85 g NaCl/100 ml). One gram of spray dried powder was rehydrated in 9 ml of the salt solution, homogenised for 1 min in a vortex mixer and maintained at room temperature for 30 min. Bacterial suspensions were serially diluted and plated on MRS agar plates (Biokar Diagnostics, Beauvais, France). Bacterial counts were determined after 48 h incubation at 37 °C of samples stored for 120 d at 4 °C. The recovery of microorganisms after different times of storage was analysed by plate counts.

Water activity measurements

Water activity was determined after drying the samples using an Aqualab water activity instrument (Aqualab, Model Series 3TE, USA). The equipment was calibrated using standard salt solutions provided by the manufacturer.

Reproducibility of the results

All experiments were done on duplicate samples using three independent cultures of bacteria. The relative differences were reproducible independently of the cultures used. Analysis of variance (ANOVA) of the viable counts corresponding to the different treatments was carried out using the statistical program Statistix 8 Software (Analytical Software, Florida USA). Comparison of means by Tukey methods were tested, and if $P < 0·05$ then the difference was considered statistically significant.

Results

Optimisation of synthesis of GOS from WP

In a first stage, the conditions of the enzymatic reaction leading to WP-GOS were optimised. Maximum Y was 27·3 ± 0·28 g GOS/100 g lactose, and was obtained with 40 g solids/100 g suspensions (Fig. 1). A remarkable increase of 35% in Y was obtained when increasing WP concentration from 20 to 40 g solids/100 g suspension. Over 40 g/100 g, a decrease in Y was detected. From Fig. 1, it can also be noticed that an increase in the WP concentration
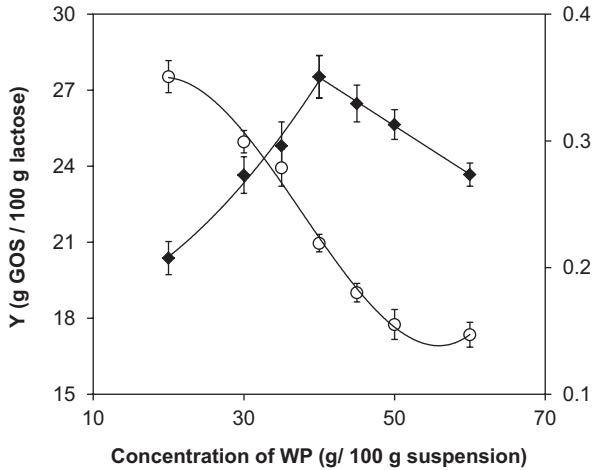
Fig. 1. Effect of the concentration of WP on the synthesis of GOS at 37 °C and pH 4·5, using *A. oryzae* β-galactosidase as biocatalyst. (♦) Yield (Y) and (○) specific productivity (π).

from 20 to 60 g solids/100 g suspension produced a decrease in π of 67%. These results allowed us to define the best conditions for further experiments. Then, a concentration of 40 g solids/100 g of suspensions, where the percentage of total GOS with respect to total sugars is maximum, was selected.

Figure 2 shows the kinetics of synthesis of GOS. The decrease in the concentration of lactose, concomitant with the increase in the concentration of total GOS, galactose and glucose is presented in Fig. 2a. Figure 2b shows that the highest concentration of GOS was produced when X is close to 57% conversion, reaching a total GOS concentration of 27 ± 0·15% of the total sugar content. Therefore, the reaction was stopped at this point and the product obtained was used as culture medium for *Lb. plantarum* 299v in the following experiments.

Fermentation of WP and WP-GOS

The growth kinetics of *Lb. plantarum* 299v in WP and WP-GOS containing 40 and 20 g solids/100 g suspensions was analysed at pH 7. Taking into account that fermented products will be dehydrated by freeze-drying or spray-drying, low concentrations of solids are not suitable for these processes and thus, they were not tested. The highest bacterial counts were obtained when the strain was grown in WP and WP-GOS containing 20 g solids/100 g suspensions after 24 h of incubation. Concentrations of 40 g solids/100 g suspensions inhibited microbial growth.

No significant differences between the growth kinetics of *Lb. plantarum* 299v in WP and WP-GOS ($P > 0·05$) were observed (Fig. 3). *Lb. plantarum* 299v reached stationary phase in approximately 16 h in both culture media, attaining bacterial counts higher than 10^8 CFU/ml.

The composition of GOS in WP-GOS before and after fermentation was analysed by HPLC. Table 1 shows the
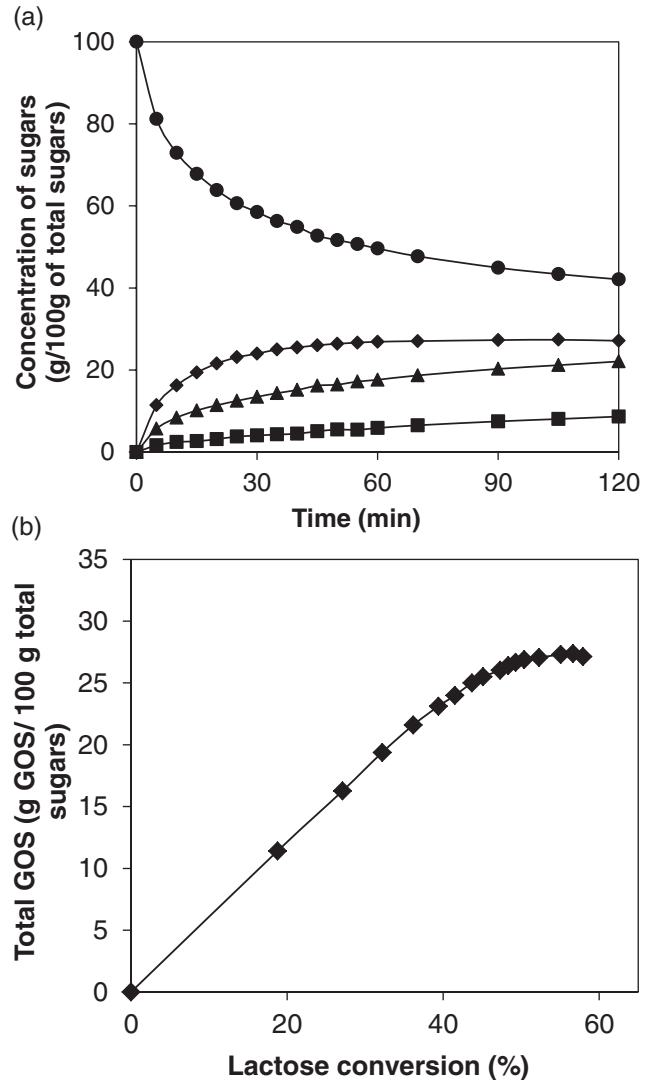

Fig. 2. Kinetics of synthesis of GOS from of WP using *A. oryzae* β-galactosidase as biocatalyst. (a): Composition of the reaction product. (●) lactose, (■) galactose, (▲) glucose and (♦) total GOS. (b) Production of total GOS as a function of the conversion rate in the enzymatic reaction. The enzymatic reaction was carried out with an initial concentration of 40 g WP/100 g of suspension, at 37 °C and pH 4·5.

relative composition of sugars in WP-GOS prior to fermentation. The GOS present in WP were composed of trisaccharides (GOS 3), tetrasaccharides (GOS 4) and oligosaccharides with degree of polymerisation ⩾ 5 (GOS 5). GOS 3 were those in higher proportion.
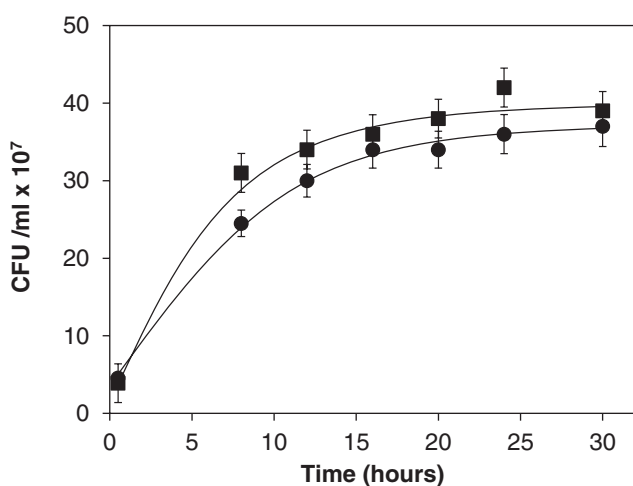
Figure 4 shows the evolution of the relative concentration of GOS 3, 4 and 5 during growth of *Lb. plantarum* 299v in WP-GOS. GOS 3 showed a clear increase in relative concentration after 20 h of fermentation. Also after 20 h fermentation, the concentration of GOS 4 slightly increased and that of GOS 5 slightly decreased at such conditions.

Table 1. Sugar composition of whey permeate enzymatically treated (WP-GOS) containing 20 g solids/100 g of suspensions

	WP-GOS	
	% †	g sugar/100 g suspensions
Glucose	21·1	3·38
Galactose	8·1	1·29
Lactose	43·4	6·94
GOS 3	17·9	2·86
GOS 4	6·6	1·06
GOS 5 or higher	2·9	0·47
Total GOS‡	**27·4**	**4·39**

† g sugar/100 g of total sugar
‡ Total GOS denotes GOS 3, GOS 4, GOS 5 and GOS of higher degree of polymerisation

Fig. 4. Evolution of Galacto-oligosaccharide concentration during the growth of *Lb. plantarum* in WP-GOS: (■) GOS 3, (♦) GOS 4 and (○) GOS 5.

Fig. 3. Growth kinetics of *Lb. plantarum* in WP (■) and WP-GOS (●) at 37 °C.
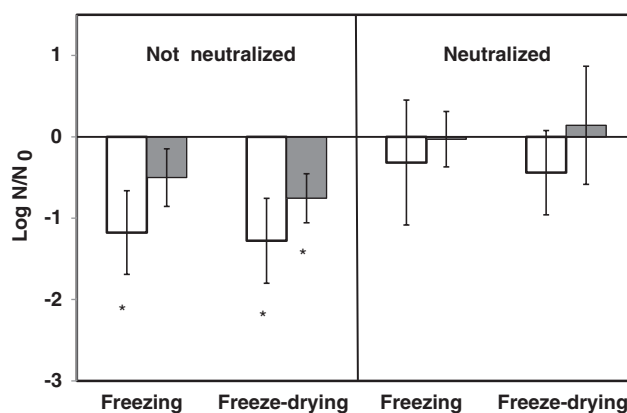
Fig. 5. Logarithm of N/N_0 of *Lb. plantarum* 299v grown in WP (white bars) and WP-GOS (grey bars) after freezing and freeze-drying, with or without neutralisation. *Significant differences respect to control (non-dried microorganisms) ($P < 0.05$).

Dehydration process

In the following step, we studied the survival of *Lb. plantarum* 299v in the freeze-drying and spray-drying processes and subsequent storage, as well as the effect of GOS as protectants.

Lb. plantarum 299v grown in WP and WP-GOS containing 20 g solids/100 g suspensions at 37 °C and pH 7, was harvested in the stationary phase (as described in Fig. 3) and freeze-dried. The recovery after freezing at − 80 °C and after freeze-drying was analysed. The effect of neutralising or not the fermentation medium was also studied. Figure 5 depicts the decrease in the number of viable cells (N/N_0) after freezing and freeze-drying. No significant differences ($P > 0.05$) were observed when the fermented WP-GOS medium was neutralised before dehydration. For non-neutralised fermentation media, a decrease of one log cycle in the CFU/ml was observed. This indicates that it is the acidity of the growth medium and not the preservation process, which is the principal factor affecting the bacterial survival.

In parallel experiments, WP and WP-GOS were fermented for 24 h, neutralised and then spray-dried. The final a_w in both media was 0·262 and 0·250, respectively. No significant differences between the survival of *Lb. plantarum* 299v grown and dehydrated in WP and WP-GOS were observed ($P > 0.05$). The high counts of viable microorganisms obtained after spray-drying (above 10^8 per gram) indicate that *Lb. plantarum* 299v is a thermo-resistant microorganism (Table 2).

Storage stability

Bacterial survival was assessed at different times at 4 °C, the temperature usually employed in the industry for the storage of starters. Figure 6 shows the storage stability of *Lb. plantarum* v299 dehydrated by freeze-drying or

Table 2. Survival and water activity (a_w) of *Lb. plantarum* 299v grown in whey permeate (WP) and in whey permeate containing GOS (WP-GOS) after spray-drying. SD = ±0·50 × 10⁸ CFU

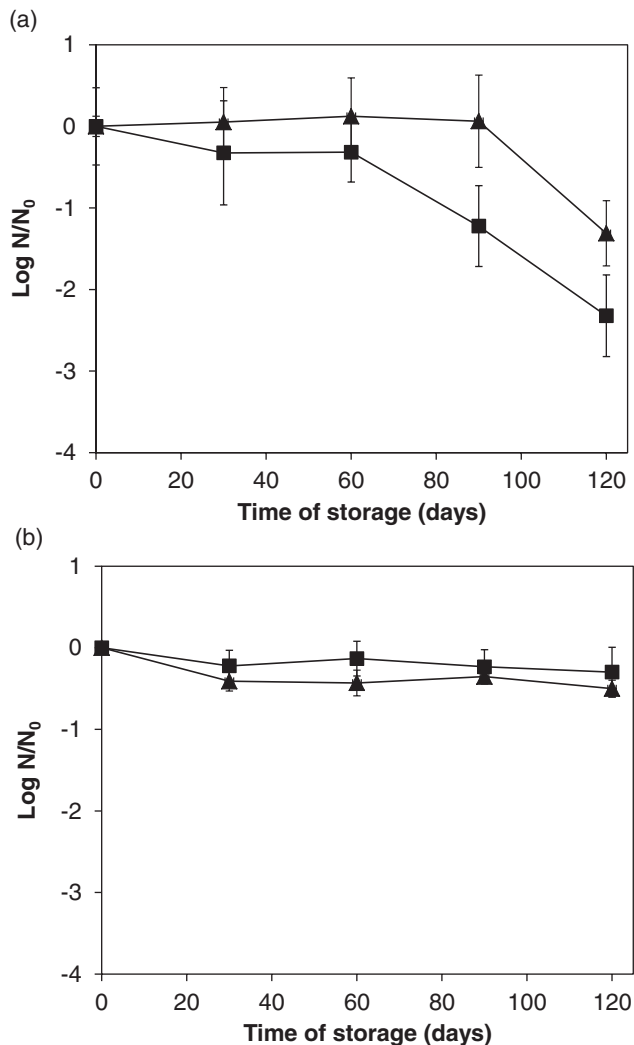| Culture media	Bacterial counts before spray drying (CFU/ml)	Bacterial counts after spray drying (CFU/g)	a_w
WP	$1·65 × 10^8$	$4·20 × 10^8$	0·262
WP-GOS	$1·52 × 10^8$	$4·38 × 10^8$	0·250

(a)

(b)

Fig. 6. (a) Stability of freeze-dried *Lb. plantarum* 299v as a function of the time of storage at 4 °C. ■: grown and dehydrated in WP, ▲: grown and dehydrated in WP-GOS. (b) Stability of spray-dried *Lb. plantarum* v299 as a function of the time of storage at 4 °C. ■: grown and dehydrated in WP, ▲: grown and dehydrated in WP-GOS.

spray-drying. For the freeze-drying process (Fig. 6a), no significant differences in the viability of *Lb. plantarum* 299v grown in WP-GOS were observed up to 90 d storage. However, in the same period of storage, the viability of freeze-dried *Lb. plantarum* 299v grown in WP decreased

significantly. The viability of spray-dried *Lb. plantarum* v299 grown in both WP and WP-GOS was stable during storage up to 120 d (Fig. 6b).

Discussion

Whey proteins are widely used for several industrial purposes and therefore, the remaining whey permeate, mainly composed of lactose, represents the final effluent of cheese production. For this reason, strategies addressed to generate added value products are constantly being developed. The use of whey permeate as a source of lactose for the synthesis of GOS and its subsequent use as a culture medium for probiotic bacteria, as carried out in this work, appears as an appropriate way to add value to this effluent.

The synthesis of GOS was firstly optimised to obtain an appropriate culture medium. The increase in GOS yield observed at WP concentrations up to 40 g solids/100 g suspensions can be explained considering the higher initial lactose concentration required to favour the reaction of synthesis over the reaction of hydrolysis (Neri et al. 2009). Despite this, a decrease in GOS yield was observed over 40 g of solids/100 g suspensions, which is in agreement with the results reported by Das et al. (2011). This can be explained considering that at high WP concentrations, the concentration of salts is also high and the water activity is low. In this regard, van Griethuysen et al. (1985) highlighted the inhibitory effect of whey salts on *A. oryzae* β-galactosidase activity. In the same way, the decrease in π at high WP concentrations is probably the consequence of low water activities and intensification of the inhibitory effect of WP salts. Cruz-Guerrero et al. (2006) have reported that the reaction rate of synthesis of GOS decreases significantly with a reduction in the water activity, thus explaining the decrease observed in π at higher WP concentrations.

The concentrations of total GOS synthesised from WP are comparable to those obtained using pure lactose as substrate (Neri et al. 2009; Vera et al. 2011a, b). At optimum conditions for the synthesis of GOS, Y was only 4% lower than the value obtained using pure lactose (Vera et al. 2012). In addition, the relative composition of oligosaccharides with different degrees of polymerisation is similar to that obtained using *A. oryzae* β-galactosidase and pure lactose as reagent, GOS 3 being the prebiotic in higher concentration (Guerrero et al. 2011).

It has been reported that several species of lactic acid bacteria are capable of growth in WP 5 g/100 ml (Cui et al. 2012). WP used in this work as culture medium has a much higher content of solids (20 g solids/100 g suspension), a clear adverse condition for bacterial growth. In spite of that, WP was used as culture medium because of two facts: (a) they were the best ones for GOS synthesis and (b) the content of solids is required for the subsequent dehydration processes. Even under these unfavourable conditions, bacterial counts of *Lb. plantarum* 299v in WP and WP-GOS were comparable to those obtained using MRS containing

GOS or glucose as the only carbon source (Hernandez-Hernandez et al. 2012). This represents a significant economic advantage in the growth of this microorganism at industrial level.

It must be pointed out that the relative increase in the concentration of GOS 3 and GOS 4 during growth in WP-GOS is due to the consumption of glucose as carbon source, since the absolute concentration of GOS remained unchanged. In this way, GOS remain available for their consumption by the gut microflora. Numerous health claims have been made on behalf of prebiotics from investigations undertaken in vivo and in vitro. Most of the research has been focused on the fermentability and bifidogenicity activity of the GOS. Many authors have reported that GOS are substrate for the growth of human faecal flora, including bifidobacteria and lactobacilli (Bouhnik et al. 1997; Gopal et al. 2001; Amaretti et al. 2007; Cardelle-Cobas et al. 2009; Ignatova et al. 2009; Hernandez-Hernandez et al. 2012).

GOS are mainly known because of their prebiotic properties, but little attention has been paid to other interesting properties already demonstrated in vitro. For example, it has been reported that GOS can mimic surface receptors of eurkaryotic cells inhibiting the attachment of certain pathogens to intestinal cells (Shoaf et al. 2006). It was also reported that the growth of some *Lactobacillus* strains in the presence of different prebiotics may increase the bacterial resistance to gastrointestinal conditions (Hernandez-Hernandez et al. 2012). Considering this, the appropriate growth of *Lb. plantarum* 299v in WP-GOS reported in this work could enhance its probiotic properties. Since *Lb. plantarum* 299v was grown and directly dehydrated in WP-GOS, the high concentration of GOS in the culture supernatants contributes to obtain a dried product with high concentration of probiotics and prebiotics in a single step.

Preservation of probiotic bacteria is necessary for potential applications in functional foods. Spray drying and freeze drying have proved to be suitable methods to obtain viable dehydrated microorganisms, although their success is strain-dependent. *Lb. plantarum* 299v grown in both WP and WP-GOS proved to be highly resistant to both dehydration processes, and also to storage. The high stability ($>10^8$ CFU/g) of spray-dried can be ascribed to the fact that water activity was close to the optimal values reported by Laroche et al. (2005).

This indicates that spray-drying is a highly efficient process for dehydration and storage of *Lb. plantarum* v299. Considering that spray-drying is about 10-fold cheaper than freeze drying (Ananta et al. 2004), the use of this process is highly recommended for obtaining a synbiotic product at a lower cost.

The higher stability during storage of freeze-dried *Lb. plantarum* 299v grown in WP-GOS than grown in WP (without GOS) could indicate a protective effect of GOS during storage, as already demonstrated in *Lactobacillus delbrueckii* subsp. *bulgaricus* (Tymczyszyn et al. 2011, 2012).

Finally, the strategy developed in this work may contribute to expand the applications of GOS derived from WP in the elaboration of products containing both prebiotics and probiotics, which is important in the design of new functional foods and feeds.

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