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Enzymatic Synthesis of Galacto-Oligosaccharides from Concentrated Sweet Whey Permeate and Its Application in a Dairy Product

David Orrego * and Bernadette Klotz-Ceberio

Alpina Productos Alimenticios S.A. BIC, Sopó 251001, Colombia
* Correspondence: david.orrego@alpina.com

Abstract: Valorization of by-products in the dairy industry as a strategy to promote circular economies has become a priority around the globe. Cheese whey and its derivates from filtration technologies offer a source of valuable molecules such as proteins, fat, lactose, and minerals. For this study, after protein concentration and recovery through ultrafiltration, lactose remaining in the permeate was furtherly concentrated with nanofiltration, resulting in a retentate used as substrate for the enzymatic production of galacto-oligosaccharides (GOS). The kinetics of GOS generation with a commercial β-galactosidase, was carried out, quantifying the carbohydrate composition by HPAEC-PAD. Results showed that at 0.5 h, GOS yield reached a maximum of 74% (g GOS/g lactose) with a lactose utilization of 63%. Under these conditions, a GOS syrup (75% soluble solids) was generated and applied in a porridge for blind paired comparison test, including a control without the syrup. No differences were identified in color and odor between porridges; however, flavor and mouthfeel of the GOS-added sample improved according to the comments of panelists. This study presents an alternative process for the valorization of whey permeate to produce GOS ingredients that can be used directly in day-to-day dairy products.

Keywords: whey permeate; lactose; β-galactosidase; galacto-oligosaccharides; porridge

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1. Introduction

By 2019, cheese whey production globally reached a total of 21,600,000 tons, and even though several strategies are applied to take advantage of this whey, there is still a concern about environmental problems generated by its unproper disposition, especially due to the high biological oxygen demand (BOD) of this stream [1]. Among the components in cheese whey, whey protein, lactose, fat, and minerals represent the majority of solids present. Fat is normally removed in equipment to defat milk, which is common in dairy-processing plants; however, separation of protein, lactose and minerals represent a bigger challenge.

Having in mind the technological and economic advantages of fractioning the components in whey, tangential filtration gives the opportunity to separate said components, from lab scale to industrial set-ups. Using ultrafiltration membranes, with a molecular weight cut-off (MWCO) of 10 kDa, protein concentration in whey became possible, enabling opportunities to use this stream as an ingredient in dairy industries or as raw material for production of protein concentrates such as whey protein concentrate (WPC), whey protein isolate (WPI) or whey protein hydrolysate (WPH) [2].

The permeate from ultrafiltration, rich in lactose (4–5%) and minerals can be concentrated by nanofiltration, where the size of the pores in the membranes allows us to retain the lactose and some of the minerals, increasing the concentration of the carbohydrate up to 20–25% [3,4]. This stream is underutilized by many dairy industries; however, lactose can be used as a substrate for multiple fermentative and enzymatic processes to valorize this by-product.

One of the options explored for utilization of whey permeate is the production of galacto-oligosaccharides (GOS) from lactose [5,6]. GOS are oligomers of glucose connected to galactose chains of different lengths, normally between 3 and 10 monomers per molecule [7]. Production of this oligomers has been evaluated through chemical synthesis [8] with poor opportunities for industrial scale-up; hence, the use of β-galactosidase has become the most popular method to generate GOS. β-galactosidases are enzymes commonly used by the food industry to hydrolyze lactose in dairy products, looking to reach lactose intolerant consumers. However, these enzymes have an alternative transferase activity known as transgalactosylation, able to use lactose, or other carbohydrate, as an acceptor of galactosyl units, forming oligomers with different degrees of polymerization (DP) [9]. Different microorganisms have been studied as a source of β-galactosidases for GOS production, including strains of *Kluyveromyces* [10], *Aspergillus* [11], *Bacillus* [12], and *Bifidobacterium* [13]. However, commercial enzymes designed for GOS production (Novozymes, IFF) are now available to be used in dairy products, aiming to reduce lactose content and generate prebiotic fiber.

A prebiotic effect has been attributed to GOS molecules given the specific effect on growth promotion of probiotic bacteria, primarily strains from the *Bifidobacterium* and *Lactobaccillus* genus [14]. Several studies have evaluated different prebiotic effects generated by the consumption of GOS, including the improvement of colonic health in breast-fed infants, and reduction in colon cancer risk and enhanced immunity in older consumers [15–18]. Specifically, infant food products such as formulas, include a mixture of GOS and FOS (fructo-oligosaccharides) given the resemblance on the gut microbiota formation when compared to breastfed infants [19]. Besides the prebiotic benefits of GOS, some technological advantages when applying GOS ingredients in food products have been identified, including improvements in mouth feel, creaminess, and sweetness [20,21].

Given the attributes of GOS, in this study, an alternative process to generate an unrefined GOS syrup from concentrated whey permeate has been developed, and its application on a dairy product was tested to identify possible organoleptic changes and determine its potential as an ingredient in the food industry. A lactose-rich medium was produced by nanofiltration of whey permeate in an industrial set-up, serving as substrate for GOS formation using a commercial enzyme. The GOS yield and length distribution of the oligomers generated were determined to select the conditions that maximize GOS production. These conditions were replicated to generate a GOS syrup that was applied in a dairy porridge and evaluated in a blind sensory test comparing with a control product. In-house generation of functional ingredients such as GOS, using by-products with low or no cost to manufacturers could represent a game-changing strategy in sustainable food fractionation for food industries in developing countries and/or emerging businesses.

2. Materials and Methods

2.1. Materials

All chemicals and carbohydrates including glucose, galactose, lactose, maltose, maltotriose, and maltotetraose were purchased from Merck (Kenilworth, NJ, USA). A commercial GOS producing enzyme from *Bifidobacterium bifidum*, with a reported β-galactosidase activity of 3000 LAU-C/g, was used in this study.

2.2. Concentrated Whey Permeate Preparation

Concentrated whey permeate (CWP) was obtained as a liquid from Alpina Productos Alimenticios S.A. BIC (Sopó, Colombia). Initially, defatted sweet cheese whey was passed through an ultrafiltration (UF) device (spiral membrane, MWCO 10 kDa) to remove protein and remaining fat in the whey; the permeate, containing carbohydrates (lactose), minerals, and water was then passed through a nanofiltration (NF) device (spiral membrane, 100 Da) to concentrate carbohydrates (rejection coefficient 1), and, to a lesser extent, minerals (rejection coefficient 0.6). The retentate from the NF process (CWP) was used as raw material for the following steps in this study.

Before proceeding with the enzymatic reaction to generate GOS, CWP solids were further concentrated by evaporation in a rotovap at 50 mbar, 50 °C, and 40 rpm, until reaching a lactose concentration close to 30% *w/w* on a wet basis; the evaporated CWP (E-CWP) was used as a substrate for the enzymatic production of GOS. According to Fisher and Kleinschmidt [22], lactose concentration of 30% or higher maximizes the GOS yield production when a β-galactosidases from *Aspergillus oryzae* is used on dairy substrates (milk, whey).

2.3. GOS Production Kinetics

Enzymatic reaction for GOS production was performed in a 10 L bioreactor (Centricol, Medellín, Colombia), with a working volume of 7 L. Experimental conditions such as enzyme dose, temperature, agitation speed, as well as enzyme inactivation conditions, were defined according to recommendations from the enzyme manufacturer. pH was not adjusted at time 0, starting the reaction at 6.1, a value close to the optimum pH of the enzyme used (6.5). The reactor was equipped with two Rushton turbines separated by 7 cm, and an external jacket for temperature control with steam. Experiments (duplicate) were conducted with 7 L of E-CWP at 30% lactose, and the enzyme was added at 57 LAU/g lactose. The reaction was run at 55 °C, 200 rpm, for 2 h, taking samples every 0.5 h to build the kinetics of GOS production. Samples were treated in a water bath at boiling temperature until reaching 90 °C internally and were kept at this condition for 5 min to inactivate the enzyme and avoid hydrolysis of the recently formed GOS. Inactivated samples were stored at −20 °C until analysis. An analysis of variance (ANOVA), with a *p*-value of 0.05, was implemented to determined significant differences in GOS production of the samples taken.

2.4. Carbohydrate Quantification

High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a gold electrode was used for the quantitative analyses of GOS, as well as glucose, galactose, and lactose. The analyses were performed with a ICS-5000 DP pump, AS-AP autosampler, DC column compartment and ED electrochemical detector (Thermo Scientific, Waltham, MA, USA). Then, a 5 µL sample was injected on a Carbopac PA-1, 250 mm × 2 mm, column (Thermo Scientific) thermostated at 30 °C. The GOS were eluted at a flow rate of 0.25 mL/min with a linear gradient of 44 mM sodium hydroxide + 10 mM sodium acetate to 76 mM sodium hydroxide + 80 mM sodium acetate in 48 min.

Data analysis was performed with Chromeleon software version 7.2 (Thermo Scientific). Quantitative analyses were carried out using standard solutions of the mono and oligosaccharides (lactose, galactose, glucose, maltose, maltotriose, maltotetraose from Sigma-Aldrich/Merck) and Biotis GOS (Friesland Campina as control sample).

2.5. Analytical Methods

Protein quantification was performed in a LECO-FP528 (St. Joseph, MI, USA) which measures total nitrogen in samples based on the DUMAS method [23] and a conversion factor of 6.38 [24] was used to calculate total protein. Fat was measured by extraction with petroleum ether (bp 60–80 °C) in a Soxhlet device [25]. The ash content was determined by heating 1 g of the samples in a muffle furnace, at 550 °C, until constant weight [25]. Minerals were quantified by atomic absorption spectrophotometer (Perkin-Elmer model 2380) using hollow cathode lamps [26]. Soluble solids were determined with a digital refractometer ATAGO PAL-1 (Tokyo, Japan), adding 1 g of sample to the device. Color was measured in a spectrophotometer Hunter Lab Colorflex EZ (Reston, VI, USA), using the CIELab coordinates: L, a, and b.

2.6. GOS Application in a Dairy Product

To apply the GOS fiber as an ingredient in a banana porridge product, the GOS syrup was produced in a 10 L bioreactor as described in Section 2.3. The reaction time selected for

the enzymatic process was based on the maximum GOS concentration possible according to the reaction kinetics in Section 2.3. After finishing the enzymatic process, the temperature in the reactor was increased to 90 °C for 5 min to inactivate the enzyme. The resulting solution was concentrated in a rotovap IKA RV 10 (Germany) at 50 mbar, 50 °C, and 40 rpm, until reaching a soluble solids concentration of 75% w/w, to simulate conditions of commercial GOS syrups.

Preparation of the porridge was performed according to the standards in Alpina Productos Alimenticios S.A. BIC (Colombia), in a 15 kg batch. Two version of the products were generated: one with the regular formulation used for the product (control), and a second batch with addition of the GOS syrup to reach a dose of 3 g GOS per portion (100 g). Table 1 presents the composition of the control porridge and the GOS-added porridge.

Table 1. Formulations of control and GOS-added porridge.

Ingredients (%)	Porridge	
	Control	GOS-Added
Water	65.1	58.6
Banana puree	14.4	14.4
Milk	11.4	11.4
Rice flour	6.7	6.7
Starch	2.1	2.1
Whey protein (WPC 80)	0.3	0.3
GOS syrup		6.5
Total	100	100

The rice flour was mixed with water and heated at 70 °C for 5 min, then the banana puree, milk, starch, whey protein, and GOS-syrup were mixed in with the rice flour and water to be heated again, at 70 °C, for another 5 min. Finally, the porridge was poured into glass flasks and sterilized, at 120 °C, for 15 min.

The final products were characterized for pH, soluble solids content, and color, and a blind paired comparison test [27] was performed to evaluate perception of costumers between the control and the GOS-added porridge. Characteristics evaluated were color, odor, flavor, and texture. For the sensory test, ten untrained panelists volunteered to evaluate the samples, which were identified with a 3-digit random number. A scoring sheet was handled to each panelist to select "yes" or "no" if a difference in certain attribute was identified. Finally, a significance test (p-value 0.05) was performed for each attribute to identify statistical differences between the samples presented.

3. Results

3.1. Concentrated Whey Permeate

As a by-product of cheese making, whey is generated after coagulation of caseins and curd formation, leaving a liquid fraction rich in whey proteins, lactose, and a modest amount of fat (depending on the type of cheese). Fat is easily removed by density different in a cream separator, giving the option of using it in different products. Whey proteins can be separated by UF taking advantage of tangential filtration technologies. The retentate from UF can contain around 3% protein and can be used as an ingredient in dairy products, or as raw material for protein-rich powders commonly used for sport nutrition. The permeate resulting from UF still carries the lactose and minerals in whey, then, by a last step of NF, all the lactose and some minerals can be concentrated in the retentate. The retentate CWP, furtherly concentrated by evaporation (E-CWP), was used in this study as a substrate for GOS production.

Table 2 presents the average physicochemical composition of CWP obtained in the production plant of Alpina Productos Alimenticios S.A. BIC, and the E-CWP resulting after evaporation.

Table 2. Composition on a wet basis (wb) of the concentrated whey permeate.

Component, % wb	CWP	E-CWP
pH	6.1	6.1
Soluble solids	25	37
Protein	0.4	0.6
Lactose	20.6	30.5
Ash	1.2	1.8
Potassium	0.28	0.41
Sodium	0.08	0.12
Calcium	0.15	0.22
Phosphorous	0.03	0.04
Others	2.2	3.26
Fat	0	0
Water	75	63

3.2. GOS Production Kinetics

Samples taken during the enzymatic reaction of the E-CWP at 30% lactose, were analyzed for galacto-oligosaccharides DP 2 to DP 8. For GOS DP 2, the following molecules were counted as GOS fiber: Allo-lactose (Gal-[1->6]-Glc), Gal-[1->3]-Gal, Gal-[1->3]-Glc, Gal-[1->2]-Glc. Figure 1 presents the GOS yield, expressed as g GOS/g lactose, at the different time points where samples were taken.

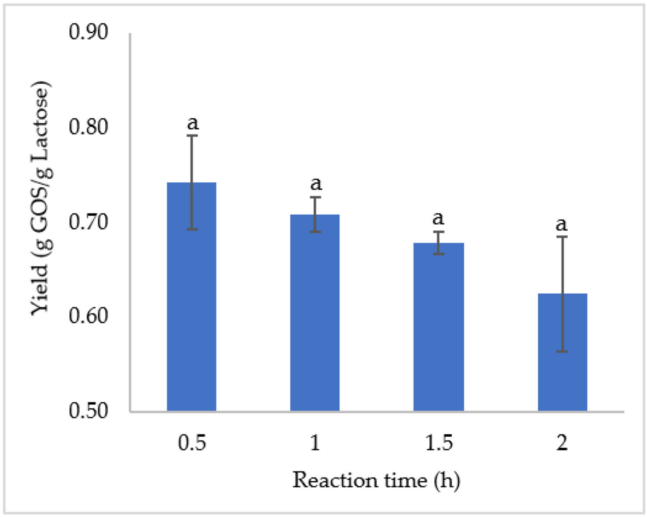

Figure 1. GOS yield reached at different time points during the enzymatic reaction. Bars with different letters represent statistical difference, with a *p*-value of 0.05.

Results presented in Figure 1 indicate that maximum GOS yield in the enzymatic conversion of lactose in concentrate cheese whey was reached at 0.5 h, and after that time, a decrease in GOS fiber is observed at 1, 1.5 and 2 h. Even though the statistical analysis did not detect any significant difference in GOS yield during the reaction, it is evident that, under the reaction conditions defined in this experiment, the reaction time that maximize GOS production is 0.5 h. From time 0.5 h to 2 h, the GOS yield decreased by 16%, which can be explained by the double enzyme activity present in β-galactosidase enzymes, that include transgalactosidase activity, responsible for GOS formation, but also able to hydrolyze lactose and GOS according to the equilibrium of components in solution [28].

The distribution of the carbohydrates present in the syrup at each sampling point is presented in Figure 2. The results are expressed in mg of the carbohydrate per gram of the syrup. The composition presented correspond to the syrups at a soluble solids content of 75% *w*/*w*.

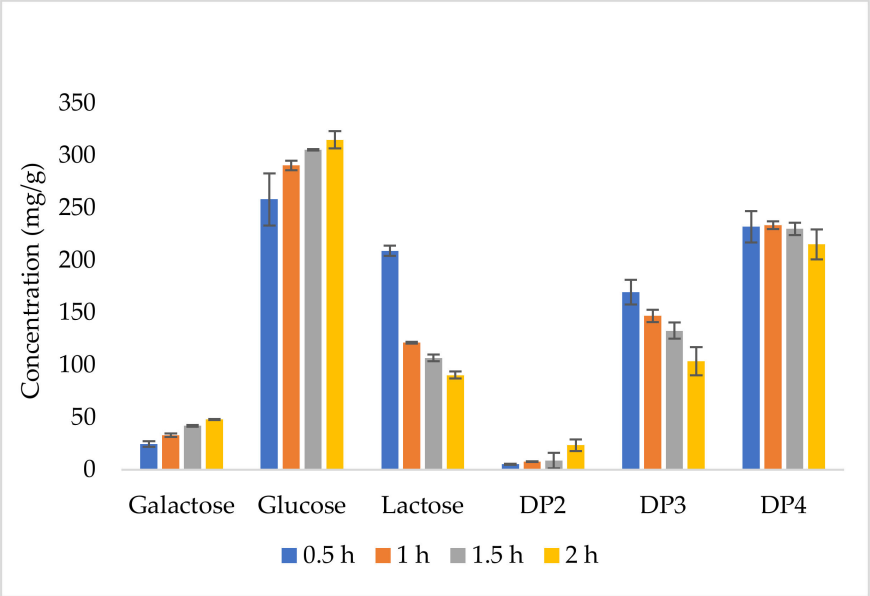

Figure 2. Distribution of carbohydrates in solution during the enzymatic production of GOS. Carbohydrates identified include galactose, glucose, lactose and GOS with degrees of polymerization 2 (DP2), 3 (DP3) and 4 (DP4). No larger oligomers were found in the samples.

As observed in Figure 2, concentrations of galactose and glucose constantly increase in solution, because of the hydrolysis of lactose necessary for GOS production. Glucose concentration is significantly higher than galactose since the former molecule is released from lactose, but at the same time, it is used for GOS formation, while glucose molecules remain free in solution. Lactose concentration decreases over time as result of the hydrolysis process needed for GOS formation. It is evident that after the first 0.5 h, most of the GOS molecules are formed, with a high concentration of GOS DP4, compared to DP2 and DP3. Over time, the concentration of GOS DP4 remains constant, with a slight decrease from 1.5 h to 2 h; however, GOS DP3 content decreases over time, while that of DP2 slightly increases over time, but in a lower concentration compared to DP3 and DP4.

3.3. GOS Application in a Dairy Product

GOS syrup production for the application in a baby porridge was performed following the protocol mentioned in the section before, with a reaction time of 0.5 h to guarantee a maximum GOS concentration in solution. The syrup was concentrated to 75% (*w*/*w*) soluble solids and was applied as is in the product (Figure 3). Composition of the GOS syrup is presented in Table 3.

Figure 3. GOS syrup at 75% *w*/*w* of soluble solids.

Table 3. Composition on a dry basis (db) of the GOS syrup.

Component	% db
GOS *	40.7
Lactose	20.9
Glucose	25.8
Galactose	2.5
Protein	1.9
Ash	3.7
Fat	0.1
Others	4.4

* Includes all oligomers detected (DP2, DP3, DP4).

For the formulation of the GOS-added porridge, a content of 3 g GOS per portion of 100 g of porridge was stablished. Formulation of the original porridge (control) and the GOS-added porridge is presented in Table 1.

Physicochemical characteristics, including pH, soluble solids, and color (Lab coordinates) of the control and GOS-added porridge is presented in Table 4.

Table 4. Physicochemical characteristics of original porridge (control) and GOS-added porridge.

			Color		
	pH	**% SS ***	**L**	**a**	**b**
Control	5.97	10.7	63.05	6.29	15.31
GOS-added	5.87	16	66.55	5.12	16.72

* Percentage of soluble solids.

The pH of the control was slightly higher than the GOS-added porridge due to the lower pH of the GOS syrup (5.8) compared to the porridge matrix (5.9–6); however, this change did not significantly modify the organoleptic characteristics of the porridge with GOS. The color also remains very similar between the control and the test, with a small variation on the Lab coordinates that were not perceived by the human eyes. Concentration of soluble solids was the major difference between the samples, increasing by 50% when the GOS syrup is added. This is an expected result since the amount of syrup added, at a soluble solids concentration of 75%, was balanced by removing water (0% soluble solids) from the formulation, which resulted in a higher concentration of soluble solids in the GOS-added porridge.

To evaluate the effect of the changes in pH, color, and soluble solids concentration on the organoleptic characteristics of the control and GOS-added porridge, a blind paired comparison test was performed with ten panelists. Statistically, no perceived difference was noticed in color and odor between the two samples presented (Figure 4). However, with a 0.05 significance level, differences in flavor and texture were noticed by the panelists. Some comments on the flavor of the GOS-added porridge included: "fruit notes are more intense;" "better balance in flavor;" "sweeter, more flavor." On the other hand, for the texture test, the only comment said "*thicker*". This outcome was expected given the sweetener capacity of the syrup, as it contains a significant amount of lactose, glucose, and GOS, all of them with a relative sweetness between 0.3 to 0.7 (when compared to sucrose, 1). Additionally, the higher concentration of soluble solids in the GOS-added porridge, necessarily affects the texture and mouthfeel of the product, which was perceived as an improvement by the panelists. These results are similar to those reported by van Leusen and others [29], where a yoghurt was supplemented with 6% db of a GOS syrup. The sensory characteristics of a regular yoghurt and GOS-added yoghurt were analyzed by a trained panel through a quantitative descriptive analysis (QDA), resulting in a statistically higher qualification of creaminess, sweetness, and mouth feel for the GOS-added product.

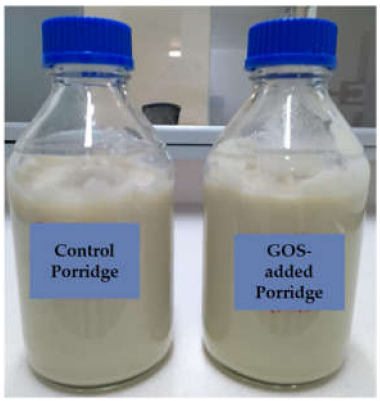

Figure 4. Final products of control and GOS-added porridge.

In general, the results obtained from the sensory evaluation can be consider successful, since parameters such as color and odor were not negatively affected by the application of the GOS syrup; on the contrary, texture and flavor were improved by the addition of this functional ingredient. Application of the GOS syrup can enhanced the nutritional value of the finished product by adding prebiotic fiber, and could improve the organoleptic characteristic of certain products, especially from the dairy industry.

4. Discussion

By-products of the dairy industry are used as raw materials for the generation of added-value ingredients containing whey protein, phospholipids, minerals, among others. Thanks to separation technologies such as tangential filtration, these elements can be easily concentrated at industrial level based on molecular size, widening the possibilities of exploitation. Lactose is the main carbohydrate in milk and can be found in cheese whey (4–5% *w/w*), and even though its commercial value is not as attractive as that of protein and fat, biotechnology tools can be applied to transform and valorize it [30].

Different studies have focused on the production of GOS from by-products generated by the dairy industry, specifically milk and cheese whey and its derivates from filtration processes, evaluating different enzymes in suspension or immobilized, as well as different process parameters such as pH, temperature and reaction time, always looking to increase GOS yields [29–33]. In this research, a GOS yield of 74% (g GOS/g lactose*100) was achieved after 0.5 h of reaction, converting 63% of the lactose available. DP distribution of the oligomers generated includes 57% GOS DP4, 42% GOS DP3, and 1% GOS DP2. After 0.5 h, GOS yields decrease to 71% (1 h), 68% (1.5 h), and 62% (2 h). The reduction in the GOS yield can be attributed to the hydrolase activity of the β-galactosidase used, able to break down GOS molecules formed initially, if not inactivated promptly [34]. The work presented by Duan et al. [35] evaluated the production of GOS from lactose (20% *w/v*) in dairy whey with a final yield of 44.7%, using a β-galactosidase from *Lactobacillus bulgaricus.* In a different study, Eskandarloo et al. [5] used a continuous flow packed-bed reactor to convert lactose in whey permeate into GOS, immobilizing a β-galactosidase from *Aspergillus oryzae* in glass beads. According to their results, a maximum GOS yield of 39.3% was achieved after the 2nd cycle of passing through the packed-bed reactor. An additional study, carried out by Yañez-Ñeco et al. [36], evaluated GOS production in a lactose solution at 40% *w/v*, resulting in a conversion yield of 34%, utilizing 96% of the lactose available.

As observed in the previous paragraphs, most of the methods evaluated for GOS production, under different process conditions as source of enzyme, substrate matrix, type of process (batch or continuous) resulted in GOS yields lower than that obtained in this study. It is worth noting that the enzyme used here is a commercial preparation design for maximum GOS production, with optimum temperature and pH previously determined by the manufacturer. In this case, optimum pH and temperature are 6.5 and 55 °C, respectively. For the tests, the pH was not adjusted in the CWP; however, its natural pH of 6.1, was

not far from the optimum. On the other hand, the temperature of the enzymatic process was adjusted at 55 °C in the bioreactor to meet the optimum conditions of the enzyme. These conditions, plus the additional concentration of the CWP to increase the lactose concentration from 20% to 30%, enhanced the transgalactosylation activity, increasing GOS yields compared to other studies where new enzymes are being tested under process conditions that can be distant from the optimum ones.

The molecular size distribution of the GOS generated during this study shows that short chain oligomers (DP2-DP4) with β(1->3) and β(1->6) linkages are formed preferentially, which is similar to the results reported by Füreder et al. [37], where a β-galactosidase from *B. bifidum* was used to produced GOS from a lactose solution at 40% *w*/*v*. The results indicated a GOS yield of 27% with a lactose conversion of 90%, and a majority of GOS DP2 formed, followed by GOS DP3 and GOS DP4, with a tendency for linkages β(1->3) and β(1->6), and, in a lesser proportion, β(1->4).

According to Böger et al. [38], diverse probiotic strains showed preference for consumption of GOS with DP 2, 3 and 4 including *Lactobacilli* and *Bifidobacteria,* which supports the potential functionality of the syrup generated in this study. Moreover, Kittibunchakul et al. [39] demonstrated that GOS preparation with a high content of oligomers DP3 (60.5%), and linkages β(1->3) and β(1->6) promoted higher fermentation activities in several *Lactobacillus* and *Bifidobacterium* strains. In general, there is an agreement on the better effect exerted by GOS generated with β-galactosidases obtained from probiotic strains, which could be translated to a better prebiotic effect [16,37].

The use of GOS syrups in food applications needs to consider different aspects such as the mild sweetness of the GOS molecules, normally 0.3–0.6 times that of sucrose [14]; however, the syrup generated in this study also contains glucose, galactose and lactose, each with sweetening capacities that will affect the final sweetness of the syrup and the final product. For the GOS syrup obtained in this work, a sweetness of 0.36 time that of sucrose, was quantified. It is also important to determine the calories apported by the syrup according to its composition. In general, the calories of GOS molecules should be around 1.7 kcal/g [40]; then, adding the 4 kcal/g for glucose, galactose and lactose, the GOS syrup in this study presented a caloric content of 2.7 kcal/g, which is still lower than regular digestible carbohydrates, but still able to add sweetness to the final product. These attributes, plus the prebiotic effect of the fiber in the syrup, presents this ingredient as an affordable, functional, natural option for the evolution of day-to-day dairy products, either fermented or not, or refrigerated or not.

The following steps include the validation of the prebiotic effect of GOS added in the porridge, conducting in vitro and in vivo tests in the target public to guarantee a proven beneficial effect that promote wellness in potential consumers. Additionally, a cost analysis of the GOS syrup, including raw materials, enzyme and production expenses should be carried out to determine the impact on the price of the final product and possible effect on the buying intention from consumers. Further separation and purification steps can be applied on the GOS syrup generated in this research to obtain a highly concentrated GOS matrix, reducing the content of caloric carbohydrates (glucose, galactose, lactose) and improving the organoleptic characteristics of the ingredient, such as color and odor, reaching a more neutral syrup or powder.

5. Conclusions

The by-product CWP (concentrated whey permeated), obtained from the nanofiltration of whey permeate, and evaporated to an approximate lactose concentration of 30% *w*/*w*, had been successfully used as substrate for the enzymatic formation of a raw GOS syrup, with an oligomer content of 40%, lactose of 21%, glucose of 26%, and galactose of 2.5%. Even though the syrup was not furtherly purified to concentrate GOS molecules and reduce digestible sugars, its application in a dairy porridge positively affected the perception of the product in a group of panelists, when compared to a regular product. An increase in sweetness and a better mouthfeel were the main advantages of the GOS-added product,

which can be attributed to free sugars in the syrup (glucose, galactose, and lactose), and an increment in soluble solids, respectively. This study presents an enzymatic process that enables the generation of a functional ingredient from a dairy by-product that could be applied in traditional dairy products to elevate their nutritional value.

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