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Effect of the lactose source on the ultrasound-assisted enzymatic production of galactooligosaccharides and gluconic acid

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ABSTRACT

It is well known that one of the main problems in galactooligosaccharide production (GOS) via tranglycosylation of lactose is the presence of monosaccharides that contribute to increasing the glycaemic index, as is the case of glucose. In this work, as well as studying the effect of ultrasound (US) on glucose oxidase (Gox) activation during gluconic acid (GA) production, we have carried out an investigation into the selective oxidation of glucose to gluconic acid in multienzymatic reactions (β-galactosidase (β-gal) and Gox) assisted by power US using different sources of lactose as substrate (lactose solution, whey permeate, cheese whey). In terms of the influence of matrix on GOS and GA production, lactose solution gave the best results, followed by cheese whey and whey permeate, salt composition being the most influential factor. The highest yields of GOS production with the lowest glucose concentration and highest GA production were obtained with lactose solution in multienzymatic systems in the presence of ultrasound (30% amplitude) when Gox was added after 1 h of treatment with β-gal. This work demonstrates the ability of US to enhance efficiently the obtainment of prebiotic mixtures of low glycaemic index.

1. Introduction

Production of galactooligosaccharides (GOS), non-digestible carbohydrates with commonly recognised proven health benefits for humans [1], has been the subject of study in recent decades for both production [2] and purification [3]. Different amounts of lactose, galactose and glucose are commonly present in commercial GOS, and have a negative effect on the glycaemic index and the caloric content [4]. These commercial GOS are not suitable for certain population groups such as diabetics, the lactose intolerant and overweight people. To fulfil the needs of these populations, purification of the GOS is required. With respect to food manufacturing industries, GOS are very suitable ingredients due to their desirable properties such as a low pH and high temperature tolerance which makes them compatible with different food matrixes, mainly fruit derivatives (juices, jams and bakery products, among others) and dairy products [5].

The most common GOS production method is transgalactosylation of lactose in presence of β-galactosidase (β-gal, EC 3.2.1.23), where the latter hydrolyses lactose and transfers a galactose moiety to another lactose or carbohydrate molecule [6]. β-Gal from *Kluyveromyces lactis* is one of the most frequently used mesophilic enzymes in the dairy industry to produce lactose-free products [7], however it exhibits both transgalactosylation and hydrolytic activity. During GOS formation, about 40% of glucose from this enzyme is released to media as an undesirable by-product that must be removed [8].

As high purity lactose is an expensive raw material, different sources rich in this compound have been assayed. Fischer and Kleinschmidt [9] and Lisboa et al. [10] evaluated optimal conditions for GOS production using cheese whey. Eskandarloo and Abbaspourrad [11] obtained GOS from whey permeate at a maximum yield of 39% at 60 °C and 40% lactose concentrations with immobilised thermostable β-gal.

Several attempts have been made to purify GOS from media; among them, Córdova et al. [12] used three-stage serial nanofiltration units to separate GOS from monosaccharides, achieving purity above 50%. Sen et al. [13] evaluated the effect of ethanol precipitation properties to separate GOS from a reaction mixture. Although GOS purity was higher than 90% in this assay, the amounts of solvents required were excessive. Guerrero et al. [3] used a subsequent yeast fermentation step after the transgalactosylation reaction. After biomass separation processes, GOS with 90% purity were obtained.

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D-Gluconic acid (GA) and its salts are produced by oxidation of the first carbon of β-D-glucose to a carbonyl group by chemical or enzymatic transformation. The enzyme used is glucose oxidase (Gox, EC.1.1.3.4) [14]. Like other organic acids, GA and its salts have very interesting physicochemical properties such as low toxicity, high temperature resistance, low corrosiveness and high capability to form water-soluble complexes with divalent and trivalent metal ions, making GA suitable for several industrial applications. They have been used in different processes for pharmaceutical, food, detergent, textile, leather, photographic, and other industries [15,16]. So, in the food industry, regarding the most important field of application considering GA as a complement of GOS, this acid and the gluconates are recognised as food ingredients (E574–E580), and are used as acidity regulators, sequestering agents, stabilizers, thickeners and for other purposes. They are commonly added to dairy products and soft drinks to preserve and/or enhance their sensory properties imparting a bitter but refreshing taste. Due to their chelating capacity, GA prevents cloudiness in beverages [14,17].

The multienzyme synthesis process has been used to produce lacto-N-neotetraose and its sialyl and fucosyl derivatives, essential components of human milk oligosaccharides [17]. More specifically, in the GOS synthesis process, multienzyme systems have enhanced properties like the sweetening power of the mixture by converting glucose present in fructose sweetener [18]. Rico-Rodríguez et al. [19] evaluated the effect of time and the addition of individual enzymes (β-gal and Gox) in a multienzyme system to produce GOS and GA. Sequential use of enzymes was found to be the best strategy for this biologically important compound production.

Emerging technologies like power ultrasound (US) (20–100 kHz) have been used in biocatalytic processes. In many cases, this technology has led to fruitful results, improving yields of enzymatic reactions [20]. In these procedures, US irradiation can be applied in different enzymatic systems (biopolymer hydrolysis, compound synthesis) and live biological systems (cell cultures, microbial fermentations), and the systems can be homogeneous or quasi-homogeneous, heterogeneous or biphasic [21]. In this regard, reaction systems with Gox could be considered quasi-homogeneous since the presence of oxygen is necessary and its solubility in aqueous media is low. It has been proven that the collapse of acoustic bubbles can dissolve oxygen [22], increasing available oxygen concentration in the reaction medium. So, in applications such as wastewater treatment, where oxygen is necessary as a reactant, it has been observed that the rate of oxygen absorption increases with the application of ultrasound [23]. On the other hand, it has been reported that acoustic cavitation produced by US waves is capable of having an indirect effect on enzyme reactions, generating sufficient energy to modify vibrational and rotational molecular states [24]. Under adequate process parameters, US cavitation has mechanical oscillation and magnetostrictive effects, which can change the conformation of the enzymes and enhance the contact between the enzyme and the substrate [25].

Although a multienzymatic sequential system combined with US has been shown to be superior for production of GOS enriched with GA using lactose as a substrate, to the best of our knowledge, no studies have been reported for this multienzyme system involving various US conditions and other sustainable lactose sources like whey permeate (WP) and cheese whey (CW). Thus, the aim of this work was to evaluate the impact of different US conditions on the US-assisted enzymatic production of GOS and GA using the multienzyme system formed by β-gal and Gox and different lactose sources (cheese whey, cheese whey permeate and pure lactose) to obtain a prebiotic mixture with enhanced functional properties.

2. Materials and methods

2.1. Reagents and enzymes

Commercial β-galactosidase (β-gal) HA-Lactase™ 5200 from *K. lactis* was provided by CHR Hansen (Bogotá, Colombia), commercial glucose oxidase (Gox) Gluzyme® Mono 10.000 BG from *Aspergillus oryzae* was a generous gift from Novozymes (Bagsvaerd, Denmark). Glucose (Glc), lactose (Lac), Gluconic acid (GA) galactose (Gal), bovine serum albumin (BSA), sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate, and sodium hydroxide were purchased from Sigma–Aldrich (St. Louis, MO).

Enzymes used, β-gal and Gox, had a total protein content of 4.8 ± 0.1% and 7.0 ± 0.1%, determined with the method of Bradford, using BSA as standard, [26] and activity of 5172 U/mL and 10,270 U/mL, corresponding at 108 and 147 U/mg of protein respectively. Enzyme activities were measured as previously reported by Rico-Rodríguez et al. [19].

2.2. Physico-chemical characterisation of substrates

For WP and CW, physical–chemical characterization (moisture, protein, lipids and ashes) was made according to the Association of Official Analytical Chemistry (AOAC) [27]. The mineral composition was measured in an ICP-MS Elan 6000 Perkin-Elmer Sciex instrument from the Service Interdepartmental Research (SIdI-UAM) in Madrid according to methodology described by Zuluaga et al. [28]. A semi-quantitative analysis and quantitative analysis of the elements of interest using the external calibration method and internal standards to correct instrumental drift were carried out. As for carbohydrates, they were measured by HPLC, as will be descried bellow.

2.3. Enzyme reactions

Three sources of lactose were evaluated: pure lactose, cheese whey permeate (WP) and cheese whey (CW) powders (see Table 1). All assays were carried out in 50 mL falcon tubes with effective volume of 15 mL (40% w/w of lactose equivalent) of the respective sugar source dissolved in phosphate buffer solution 0.05 M at pH 7.0 and temperature of 40 ± 1 °C.

2.3.1. US conditions

US assays were done using a 450 digital Sonifier (Branson Ultrasonics Corp., Danbury, CT), equipped with a temperature sensor (error ± 0.1 °C) and a tip of 3 mm diameter directly attached to a disruptor horn (20 kHz, 400 W full power) and immersed 2 cm in depth with respect to the liquid surface. US wave amplitude were 0, 15 and 30%. Pulsed US of 3 s on/7 s off was the operating mode. Throughout US treatments, temperature was kept between 40 and 45 °C using a water–ice bath. All sonicated samples were stored at −20 °C for further

Table 1
Composition of different lactose sources.

Component	Lactose	Whey permeate	Cheese whey
Protein (%)	–	2.9	12.5
Lipids (%)	–	< 1	1.3
Carbohydrates (%)	99.9	87.7	71.7
Lactose (%)	99.9	87.7	71.5
Glucose (%)	–	–	< 1
Galactose (%)	–	–	< 1
Ash (%)	0.1	8.6	7.5
Sodium (mg/100 g)	–	708	818
Potassium (mg/100 g)	–	2815	748
Calcium (mg/100 g)	–	289	1311
Magnesium (mg/100 g)	–	126	63
Moisture (%)	–	< 1	7.2

Table 2
Parameters for multienzyme reactions.

Design parameters		
	Lactose source	Lactose (99.9%*) WP (87.7%) CW (71.7%)
	US intensity (%)	0 15% 30%

*Lactose purity (w/w).

analyses.

Acoustic power applied (W) was measure recording the temperature as a function of time by the equation [29]:

$$P = m.\ C_p.\ \left(\frac{\partial T}{\partial t}\right) \quad (1)$$

where m is the mass of the sonicated liquid (g); C_p is its specific heat at a constant pressure (kJ/kg K), and ∂T/∂t represents the slope at the origin of the curve. Ultrasound intensity (UI) is expressed in watts per unit area of the emitting surface (W/cm^2), according to equation (2)

$$UI = \frac{4.\ P}{\pi.\ D^2} \quad (2)$$

For the treatments with US (15 and 30% of amplitude), the acoustic power estimated was 14 ± 1.4 and 30 ± 1.6 W and the UI was 203.4 and 423.1 W/cm^2.

2.3.2. Galactooligosaccharides production

To evaluate the effect of lactose sources and US treatment (single effect and interactions) on GOS production (Table 2), a 3^2 factorial design (total 9 assays) was performed. For each assay, 1.7 U/mL of β-gal was added into 15 mL of lactose source solution. Afterwards, samples were incubated at 40–45 °C and stirred (800 rpm for samples without US assistance) or treated with US horn disruptor. Samples were taken by duplicate at 0, 20, 40, 60, 90, 120, 180 and 300 min to analyse and obtain the evolution of the reaction with time.

2.3.3. Gluconic acid production

Glucose solutions (10% w/w) were prepared in sodium phosphate buffer (pH 7.0). For each assay, 3.2 U/mL of Gox was added into 15 mL of glucose solution. Immediately, samples were incubated at 40–45 °C, air in excess (10 mL/min) as oxygen source and stirred (800 rpm for samples without US assistance) or treated with US horn disruptor at different US wave amplitude (0, 15 and 30%). The reactions were carried out at the conditions above indicated. Pulsed US and sampling were operated as previously mentioned.

2.3.4. Multienzyme system

Once the effect of lactose source and US intensity was evaluated, a multienzyme system was proposed in sequential addition where Gox (3.2 U/mL) was added 60 min after GOS reaction started by β-gal (1.7 U/mL), as the best condition previously reported by Rico-Rodríguez et al. [19]. Multienzymatic reactions were carried out in a 3^2 factorial array. As previously mentioned, samples were incubated at the reaction temperature range. The reactions were carried out at the conditions above indicated.

2.4. Carbohydrate quantitation

Samples from reactions were diluted in water, filtered using a 0.22 µm syringe filter and therefore analysed in an Agilent Technologies 1260 Series HPLC system (Boblingen, Germany). Monosaccharides were firstly quantified by high performance liquid chromatography (HPLC-IR) using a Shodex Ionpak KS801 (4.6 mm × 300 mm, 6 µm particle size) (New York, USA) at 50 °C. The mobile phase was degassed sulfuric acid (0.025 M) and the flow rate was 0.7 mL/min. This column could separate monosaccharides (GA, glucose and galactose), di-, tri- and tetrasaccharides. To separate lactose from other disaccharides, samples were diluted in acetonitrile/water (70:30) and then filtered using a 0.22 µm syringe filter. Analyses were carried out on a Kromasil® column (100-NH_2; 250 mm × 4.6 mm, 5 µm particle size) (New York, USA) using acetonitrile/water (75:25 v/v) as mobile phase and elution in isocratic mode at a flow rate of 0.7 mL/min. The injection volume was 20 µL. Data acquisition and processing were performed using Agilent ChemStation software. Quantitation was made through standard curves in appropriate dilution of corresponding carbohydrate correlated with peak area in the chromatograms.

2.5. Statistical analysis

Minitab v17® (State College, PA: Minitab, Inc. USA) was employed to generate and analyse the results of the multienzyme system though a multivariate analysis. A randomised complete block design was used in a split-plot treatment arrangement with nested factors (time within US levels). From the combination of three lactose sources, with three UI, there were 9 treatments. Experimental period was 300 min with sampling at 0, 20, 40, 60, 90, 120, 180 and 300 min. The Tukey test was used to determine differences between means at a $p < 0.05$ significance level.

3. Results and discussion

3.1. Effect of US on the formation of gluconic acid

As glucose is one of the main by-products in GOS production, its oxidation to GA was proposed. However, this reaction is longer (10 to 12 h) than GOS production (1 to 5 h) without US [23,24]. Fig. 1 presents the production of GA and glucose consumption under different US intensities. Control reaction (0% US) shows a linear trend; however, as US intensity increases, so does GA production speed, as observed in

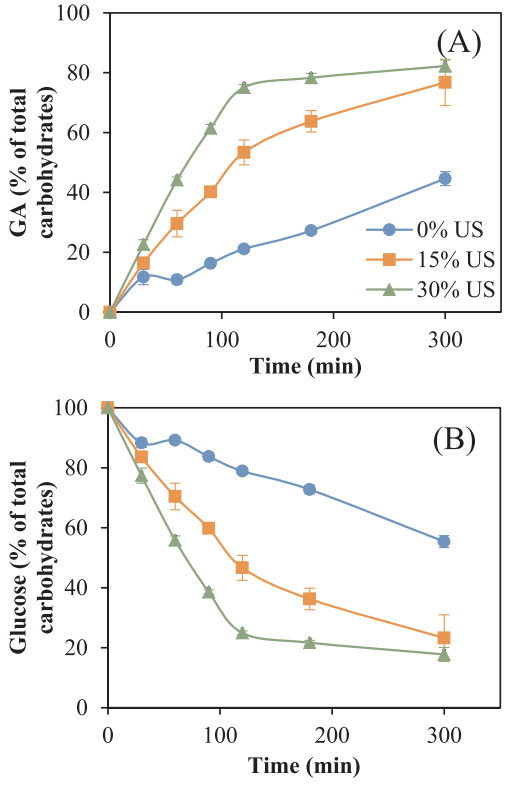

Fig. 1. Gluconic acid production (A) and glucose consumption (B) with assistance of US at 40 °C, pH 7.0, 10% (w/w) of initial glucose and Gox 3.2 U/mL.

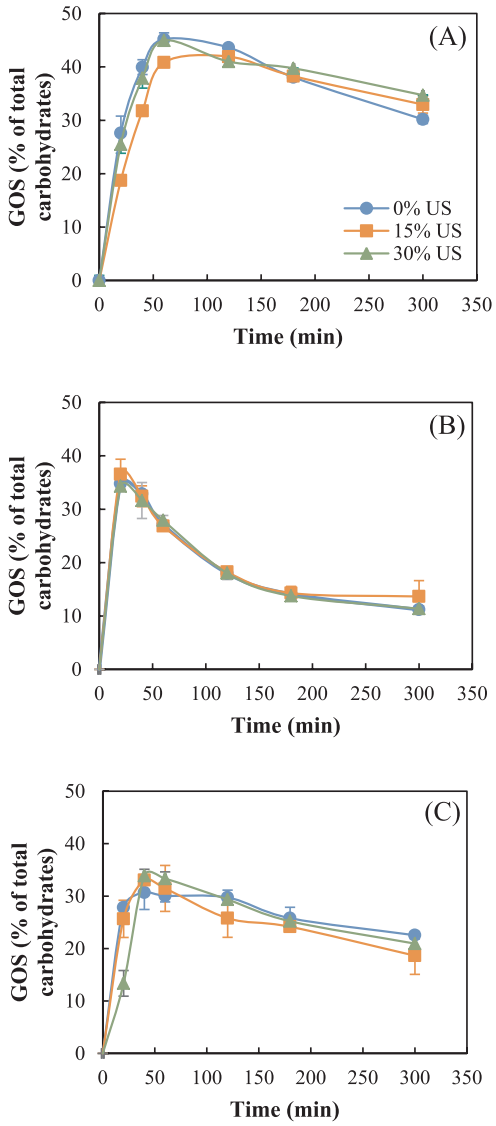

Fig. 2. GOS production using (A) lactose; (B) whey permeate; (C) cheese whey as substrate (40% w/w of initial lactose) and 1.7 U/mL of β-gal at 40 °C, pH of 7.0 and different US amplitudes, 0% (○) 15% (□) and 30% (Δ).

Fig. 1A. This behaviour is related to the amount of glucose consumed in the reaction (Fig. 1B) and can be attributed to US mechanisms like cavitation, helping to increase molecular mass transfer (air and substrate) towards the enzyme [20,30] and improving the reaction rate. This is a very important parameter, since under normal conditions (without US) it takes 15 h to finish the reaction [31]. In this study, conversion of glucose into GA to nearly 80% was achieved in 2 h in presence of US (Fig. 1A). Lower reaction times were accomplished by Önal et al. [32] and Witonska et al. [33], although they used chemical catalysts and high temperatures instead of enzyme and US. Nonetheless, this technique leads to generation of undesirable by-products.

3.2. Effect of US and lactose source on GOS synthesis using β-galactosidase

Fig. 2A shows that transgalactosylation in lactose media presented no significant differences ($p > 0.05$) regardless of the US treatments employed. US has no apparent effect on GOS production with β-gal from *K. lactis*, obtaining a higher yield (44.9%) after 60 min hours of reaction, concurring with the earlier results of Rico-Rodríguez et al., [19]. In this earlier work, the incidence of US on the stability of β-gal was evaluated by means of intrinsic fluorescence and its effect on the secondary structure of the enzyme. Small changes were found, indicating the existence of minor non-conformational changes in the structure of the enzyme caused by US pulses during the reaction process. No changes in enzymatic activity were observed, while Gox showed evident variations in both secondary structure and activity. However, Demirhan et al. [34] reported that US had a positive effect on lactose hydrolysis present in milk. In their work, lactose consumption reached 92% in presence of US with an acoustic power of 20 W, 24% higher than control without US, while increasing the acoustic power from 20 to 100 W caused the lactose hydrolysis degree to decrease.

On the other hand, the source of lactose has a significant influence ($p < 0.05$) on GOS formation, so with WP (Fig. 2B) it showed a maximum production (36.5% of total carbohydrates) at 20 min of reaction. Subsequently, a rapid reduction in GOS content was observed until the end of the reaction. Besides, CW (Fig. 2C) had a similar evolution than with lactose solution, although GOS concentration was lower (34% of total carbohydrates after 60 min) than that present in the latter (45% of total carbohydrates), but at the same reaction time. Nevertheless, GOS hydrolysis at the end of reaction with CW did not occur as fast as those in WP. These results were related to the potassium content of CW (748 mg/100 g) and WP (2815 mg/100 g), which was higher in the latter (Table 1). It is known that potassium enhances hydrolysis activity of β-gal from *K. lactis* [35]. Although there is abundant evidence of the role of potassium and sodium in β-gal activity, there is scant information on the mechanism of action of these ions on the enzyme. It is known that β-gal is a metallo-enzyme which requires divalent ions to increase its catalytic activity. With respect to the monovalent ion effect, this may be attributable to different orientations caused by the type of ion affecting the formation of the complex enzyme-substrate [36]. More recently, Souza et al. [37] have indicated that potassium increased hydrolytic activity by reducing the flexibility of the polypeptide backbone, thereby increasing the stability of the enzyme.

In general, lower results were reported by Fischer and Kleinschmith [6] in a review on GOS synthesis in milk, whey and permeate when β-gal from *K. lactis* were used (average 20.6%, range 5–36%), for example, Lisboa et al. [10] and Fischer and Kleinschmith [9] reported similar GOS yields (30–33%) after 3.5–4 h of reaction using CW as substrate.

3.3. Effect of US and lactose source on multienzyme reactions

3.3.1. Lactose, monosaccharides and GOS

The effects of different US treatments were evaluated in a multienzyme system for simultaneous GOS and GA production. Fig. 1S shows lactose consumption over reaction time. Regardless of the US intensity or the source of lactose, all reactions had similar trends, and lactose concentration dropped below 10% w/w after 60 min, which indicates high β-gal efficiency to use the substrate. The reaction had the characteristic behaviour of transgalactosylation reactions without the assistance of US reported by other authors [38,39]; therefore, according to our results, US intensity does not have a significant effect ($p > 0.05$) on β-gal activity or lactose consumption, under the assay conditions. Regarding the source of lactose, WP presented the fastest consumption of lactose of all treatments, with the highest content of potassium salts (2.8%). At 40 min of reaction, concentrations dropped to 5% w/w, whereas in pure lactose solutions and CW, this concentration was reached after 90 min of reaction.

To have a comprehensive view of the transgalactosylation reaction, galactose content must be determined, since, as previously mentioned, GOS are units of galactose. For this reason, concentration of this monosaccharide may act as indicator of transgalactosylation activity in reaction media. Production of this monosaccharide (Fig. 1S) was statistically different ($p < 0.05$) from all the lactose sources. Of the three types of samples studied, WP released the largest amount of galactose (26.6% of total carbohydrates), indicating that hydrolytic activity of β-gal was greater with this substrate. However, in pure lactose solutions

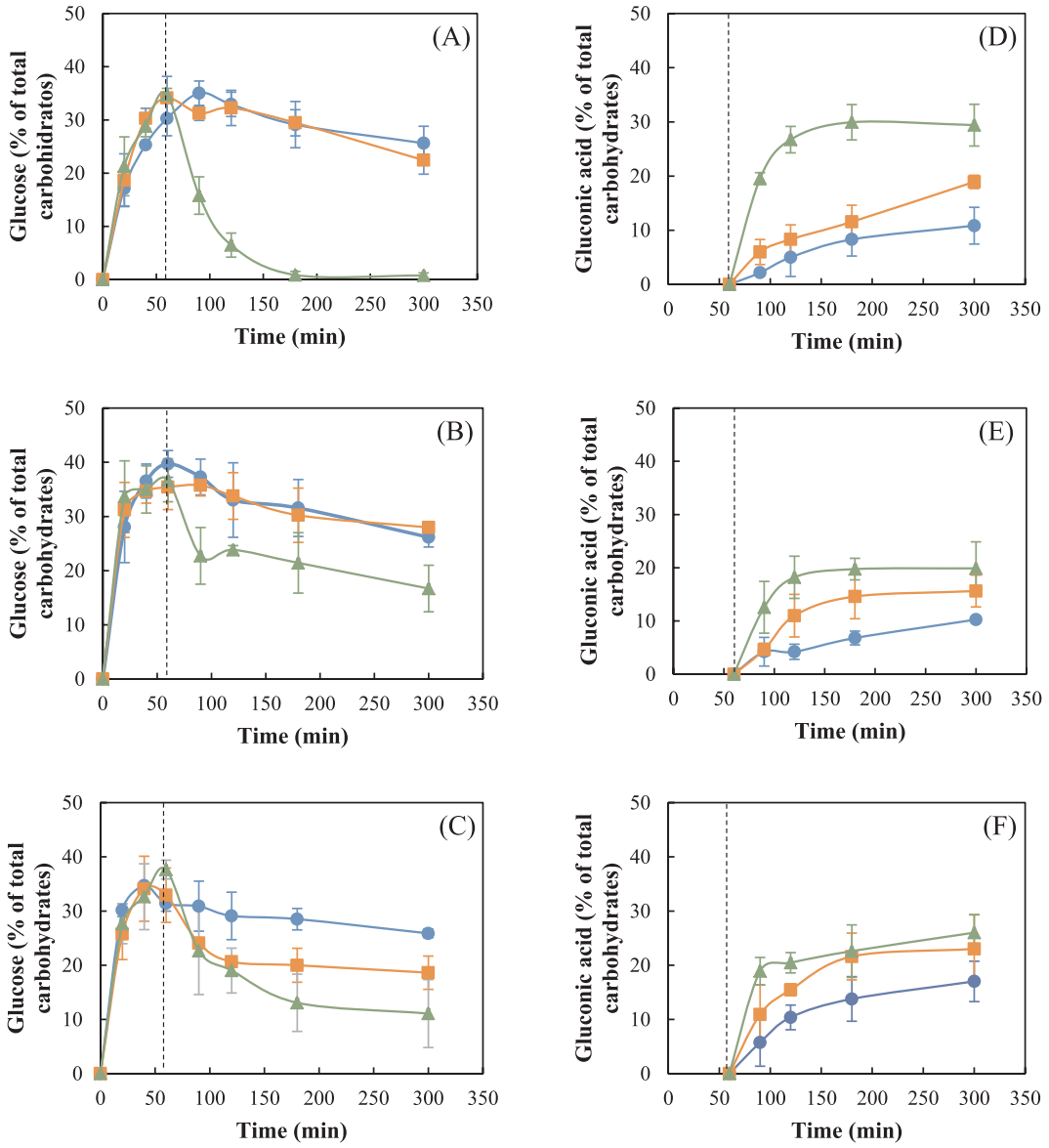

Fig. 3. Glucose and gluconic acid content in a multienzyme system with β-gal (1.7 U/mL) and Gox (3.2 U/mL) at 40 °C, pH 7.0 and initial lactose concentration of 40% (w/w). A and D lactose; B and E whey permeate; C and F cheese whey. (○) 0% US, (□) 15% US, (Δ) 30% US.

the concentration of galactose was the lowest of all the reactions (below 20% w/w) which indicates that a higher proportion of galactose was used in GOS formation. This behaviour is characteristic of transgalactosylation reactions with β-gal from *K. lactis*, reported in other works [7,9]. It is noteworthy that with pure lactose as substrate, the only cation that enhances transgalactosylation activity is sodium from the buffer, while CW and WP have also potassium, which favours hydrolysis activity; therefore, equilibrium was established between both activities [35]. No significant effect ($p > 0.05$) was found for galactose production in presence of any of the US intensities evaluated. Galactose content is usually used to define the effectiveness of transgalactosylation. As GOS are mainly composed of galactose moieties, the amount of this monosaccharide released to media may help to explain aspects of enzymatic behaviour; whether the conditions of reaction favour hydrolysis or transgalactosylation, or even the size of GOS synthetized.

Regarding glucose, it was released very quickly during the first 60 min of reaction when β-gal acts alone (Fig. 3A-C), and its evolution was highly correlated to lactose hydrolysis. Although the maximum amount of glucose was produced in WP media (40% of total carbohydrates) the lactose source did not have a significant ($p > 0.05$) effect on glucose release. For all treatments in the multienzyme system, glucose started its depletion after 60 min of reaction when Gox was added and GA was formed. However, only in reactions with pure lactose and US treatment at 30% amplitude was glucose totally consumed after 180 min. This process also had a greater effect on Gox activity; however after 300 min of reaction, 16% and 11% of glucose remain unreacted for WP and CW, respectively. In contrast, 15% of US treatment had a analogous kinetic behaviour in processes without US for lactose and WP, and an intermediate glucose consumption for CW. Similar glucose behaviour in lactose hydrolysis reactions was also reported by other authors [40,41], in reactions with β-gal, Gox and catalase.

Fig. 4A, 4B and 4C show GOS kinetics during 5 h of reaction. Of all the sources of lactose; lactose solution had the highest yields (45–47%), followed by CW (36%) and WP (33%). For lactose and CW, high yields were obtained after 60 min of reaction, while for WP this was after 40 min. Fischer and Kleinschmith [40] and Mueller et al. [41] reported lower yields for GOS production (22.4 and 31.5%, respectively) when a tri-enzymatic system (β-gal, Gox and catalase) and lactose solutions were used as substrate, 57% of disaccharide remaining unreacted in the former study.

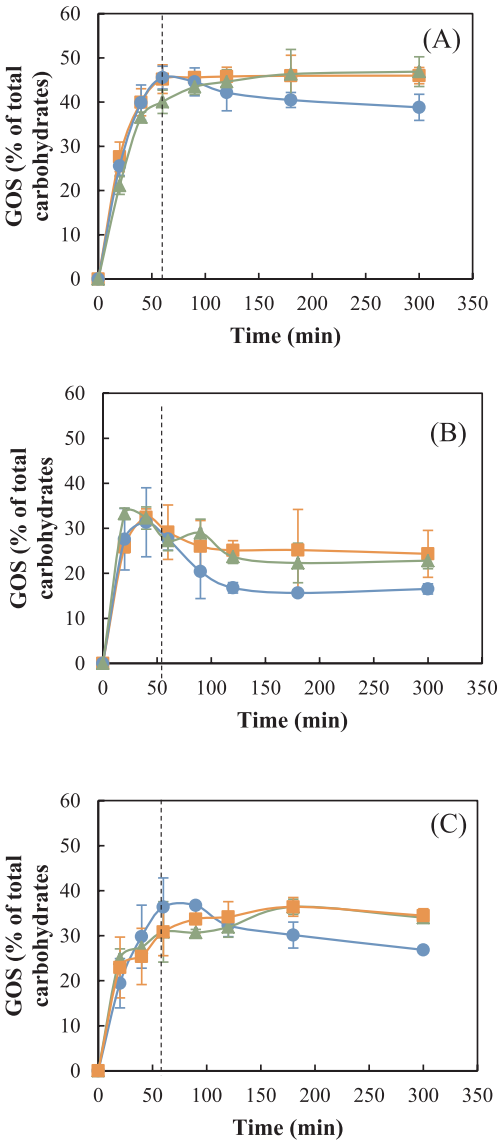

Fig. 4. GOS production through multienzyme system with β-gal (1.7 U/mL) and Gox (3.2 U/mL), and different US intensities at 40 °C, pH 7.0 and initial lactose concentration of 40% (w/w). A lactose; B whey permeate; C Cheese whey. (○) 0% US, (□) 15% US, (Δ) 30% US.

3.3.2. GA production

Multienzyme assays resulted in successful GA production after a significant amount of glucose was released from β-gal reaction (Fig. 3D, 3E and 3F). With respect to GA production without US assistance, the effect of lactose source was not significant ($p > 0.05$). This result suggests that lactose source composition did not have effect on Gox activity. Fischer and Kleinschmith [40] produced GOS in presence of Gox and catalase obtaining a total conversion of glucose at GA, however, as mentioned above, a large amount of lactose (57%) remains unreacted. And, although the authors reported glucose oxidation until the end of reaction, GA was not quantified. Mueller et al. [41] also used the same enzymatic system (β-gal/Gox/catalase), but in their results glucose oxidation was very low due to limited oxygen solubility in the reaction media. In those works, enzymes were added simultaneously. However, in this paper, Gox was added after there was a considerably high concentration of glucose in media (60 min) and no catalase supplement was necessary for Gox reaction. Besides, reaction times were notably shorter in our assays.

Regarding reactions with US assistance, the higher the US intensity, the higher the amount of oxidised glucose, and a significantly positive effect ($p < 0.05$) of US intensity was found in both single and multienzyme reactions with Gox. For GA production (Fig. 3D, 3E and 3F), which started after 60 min of reaction (vertical dotted line), Gox behaved in the same way as in the single enzyme reaction, where US accelerated glucose conversion to GA in the first 120 min, although it is noteworthy that the greatest GA production took place during the first 60 min of Gox addition. On the other hand, while the lactose source had no effect on GA production in reactions without US, when these were applied, maximum concentration of GA (30%) was found in lactose solution, while in CW and WP the final concentrations were 26 and 20%, respectively. These results may be explained by the research of Ahmad et al. [42]; these authors observed that neutralization of negative charges in the side chain carboxyl groups in Gox leads to a significant loss of enzymatic activity. It is possible that US may increase the interaction between the enzyme and the monovalent ions of WP and CW, partially inactivating the enzyme. In all cases, the highest amounts of GA were found when US intensity was 30%. While, using 15% US, GA production had a linear trend for 300 min of reaction. In the literature, the maximum amount of GA produced by Gox in pure glucose solutions has been reported to occur after 12 h of reaction [31,43]. However, as reported in a previous work, it was established that in presence of appropriate US intensities, GA might be produced from glucose in about 2 h of reaction [19]. This option has been shown to be a very good alternative for reducing reaction times in GOS production with low amounts of high glycaemic index carbohydrates.

Fig. 5 summarises the best GOS formation yields for single and multienzyme systems (Fig. 5A and 5B), and GA (Fig. 5C). Lactose solution produced the best results both for single and multienzyme reactions, leading to yields of between 46 and 52%, statistically higher ($p < 0.05$) than those obtained in other lactose sources such as WP (34–36%) and CW (34–40%). These results suggest that other components, such as cations, of CW and WP have an important effect on GOS synthesis. On the other hand, in GA production, yields increased significantly ($p < 0.05$) when US were applied, indicating the usefulness of US technology for glucose oxidation of Gox.

4. Conclusions

A multienzyme system composed of β-gal and Gox was successfully assayed as a strategy to reduce glucose concentration during transgalactosylation of lactose. All the lactose sources studied (lactose solutions, whey permeate, cheese whey) were shown to be adequate for GOS production, lactose solutions being the medium where the highest yields of these prebiotics were produced. The application of US did not affect the activity of β-gal, however, US significantly affected Gox activity and, consequently, yields of glucose oxidation to GA were increased and times were reduced, regardless of the lactose source under all the studied conditions. We also studied the impact of US on Gox in a glucose solution to evaluate GA formation under the effects of different US power. In conclusion, it the usefulness of US for the production GA, an important ingredient, has been shown, as that the use of a multienzyme system to produce GOS from different lactose sources is a feasible alternative streamline purification by removing glucose through oxidation reactions, mainly in the case of US assistance to enzymatic reactions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

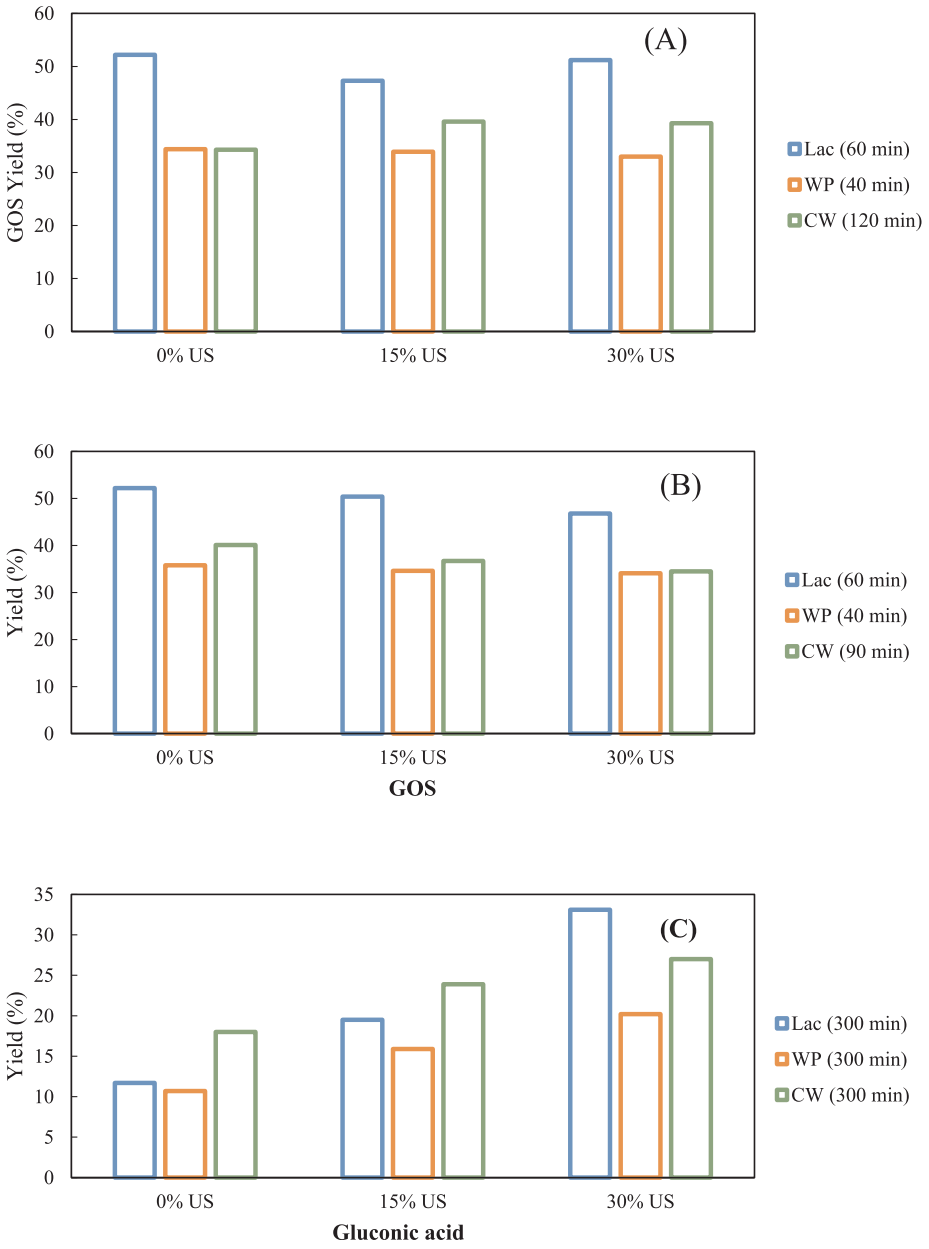

Fig. 5. Yields of GOS and GA production in single and multienzyme reactions at higher production times. (A) β-gal reaction; (B) multienzyme reaction; (C) sugar ratio of compounds of interest related to galactose released and lactose consumed for single enzyme reactions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2019.104945.

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