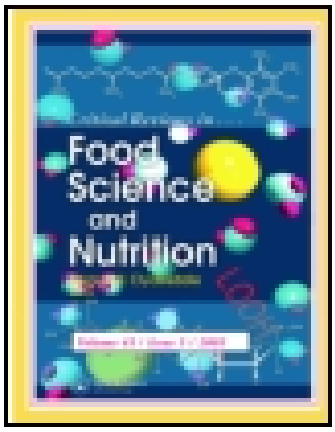

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New trends and technological challenges in the industrial production and purification of fructo-oligosaccharides

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New trends and technological challenges in the industrial production and purification of fructo-oligosaccharides

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ABSTRACT

An increased commercial interest in fructo-oligosaccharides (FOS) has emerged in the last decade due to their prebiotic activity. At large scale, the FOS are produced by microbial enzymes from sucrose. A mixture of FOS and other saccharides is obtained in this process. The presence of such saccharides reduces the prebiotic, caloric and cariogenic value of the final product. Therefore, many efforts have been conducted to obtain a product with increased FOS purity. This review comprises the most important technological and physicochemical aspects including FOS production and recovery processes; safety, dose and health claims concerning its intake; and commercially available FOS.

Keywords: Oligosaccharides; Microbial treatment; Membrane technology; Activated charcoal; Ion exchange chromatography; Simulated Moving Bed.

INTRODUCTION

Several gastrointestinal (GI) disorders ranging from discomfort or colitis to cancer can be prevented and/or treated by promoting the selective growth of commensal beneficial bacteria, named probiotic bacteria, and keeping pathogenic species at low levels. Therefore, the modulation of large bowel microflora through a regular consumption of prebiotics has been largely studied in recent years (De Preter *et al.*, 2011; Delgado *et al.*, 2010; Gibson *et al.*, 2004).

Carbohydrates constitute the main substrate for gut bacteria and some have been reported as potential prebiotics. Nevertheless, only fructans (inulin and fructo-oligosaccharides (FOS)) and galacto-oligosaccharides (GOS/TOS) fulfill all the criteria required to be considered as prebiotics (Roberfroid, 2007; Torres *et al.*, 2010).

Apart from their nutritional value, FOS also have great physicochemical properties that make them powerful ingredients for improving the organoleptic quality of food (Crittenden & Playne, 2002; Franck, 2002).

Most commercial FOS products are not pure but mixtures containing different amounts of FOS, mainly due to the low yields of their industrial production processes. Typically, these commercial products contain about 5% of other sugars, although products with 55 to 99% of purity can be found in the market.

The presence of the small chain saccharides in the mixture decreases the prebiotic, caloric and cariogenic value of the final product, preventing the incorporation of these mixtures in health, dietetic and diabetic foods. Therefore, it is of utmost importance to obtain purer FOS mixtures. Moreover, if pharmaceutical applications or specific studies on physicochemical and functional

properties (e.g. *in vitro* prebiotic activity) of individual FOS are envisaged, pure oligosaccharides should to be used.

Currently, the isolated FOS are only available for analytical purposes and their prices are prohibitive. Indeed, even pure FOS mixtures are still very expensive. Besides, there are very few reports available regarding the FOS purification. Therefore, the isolation and purification of FOS constitutes an actual challenging and important task for the scientific community.

This review aims to provide an overview of the currently available downstream techniques for the isolation and purification of FOS from fermentative broths. The most important technological and physicochemical aspects involving FOS separation are discussed. Issues on safety, dose and health claims concerning FOS intake are reviewed. Also, a compilation of the commercially available FOS, as well as the main FOS production technologies is provided. Finally, the separation techniques used for FOS purification are presented and discussed.

PHYSICOCHEMICAL AND TECHNOLOGICAL PROPERTIES

Fructans are a category of carbohydrates that include inulin and oligofructose. They consist in fructose (F) molecules linked to each other that can or cannot have a glucose (G) residue in their initial configuration. Oligofructose are short chain molecules, that can be a mixture of GF_n and FF_n molecules ($2<DP<7$, $DP_{av}=4$), and FOS enzymatically synthesised from sucrose, that are all GF_n type ($2<DP<4$, $DP_{av}=3.6$), where *n* represents the fructose units and DP the degree of polymerization. Inulin comprises sugars with higher DP, varying between 2 and 60 units.

FOS compounds include kestose (GF_2), nystose (GF_3) and fructofuranosylnystose (GF_4). They are very hygroscopic sugars and their viscosity is relatively higher than that of sucrose at the

same concentration. The thermal stability of FOS is also superior to sucrose. They are extremely stable at the normal pH range found in food (4.0–7.0) and at refrigerated temperatures over the course of a year. The solubility, freezing and boiling points, and crystal data of FOS seem to be very similar to sucrose (Yun, 1996). Some physicochemical properties of the sugars involved in the FOS production are summarized in Table 1.

Sugars are highly polar due to the presence of many OH groups, and are non-soluble in organic solvents. However, when certain conformations are adopted, oligosaccharides can generate apolar surfaces which are capable of interacting with non polar solutions and adsorbents, such as activated charcoal. The hydrophobicity of sugars is determined by several factors such as CH-surface area, hydration effect of the OH groups and molecular planarity and rigidity (Yano *et al.*, 1988). Since the hydrophobicity is related with the extent of the CH and -CH_2 moieties, it increases with the extension of the chain length, provided that the configuration and the glycosidic bond stereochemistry are favorable (Sundari & Balasubramanian, 1997). Therefore, the hydrophobicity of di- and higher saccharides increases with the number of monosaccharide units that constitute the oligosaccharide (Yano *et al.*, 1988).

The steric orientation of the polar OH group determines the ability of sugars to form hydrogen bounds and to establish complexes with other substances. The stability of the complex formed depends on the eq-ax sequences present on sugars. Sucrose and FOS have no pairs of eq-ax oriented OH groups, while fructose and glucose have different relative compositions of eq-ax pairs (Table 2).

Concerning the caloric value, FOS have only 25 to 50% of the caloric value of digested sugar molecules as fructose, glucose or sucrose (Gibson & Roberfroid, 1995). The relative sweetness

of FOS, estimated on the basis of 10% (w/v) sucrose solution, corresponding to 100%, is 31, 22, and 16%, for GF_2, GF_3 and GF_4, respectively (Yun, 1996).

FOS are the most studied oligosaccharides and the best implemented in the European market. These compounds are used as food ingredients not only for their nutritional value, but also for their great technological properties, such as improving the organoleptic quality of products; decreasing microbial development and thus increasing products shelf life; enabling fibre incorporation within liquid foods; and controlling the amount of browning due to Maillard reactions (Crittenden & Playne, 1996).

FOS have been commonly used as ingredients in functional food such as biscuits; baking products; fillings, drinks; yoghurts; dairy products; breakfast cereals; tablets; frozen desserts; infant formulae; fruit preparations; dietetic products; meal replacers; and sweeteners (Crittenden & Playne, 1996; Franck, 2002). Generally FOS are added to food in doses that vary between 2 and 50% (Franck, 2002). Detailed descriptions of FOS technological functionalities have been reviewed by several authors (Crittenden & Playne, 1996; Franck, 2002).

SAFETY, DOSE AND HEALTH CLAIMS

Fructans naturally occur in many edible fruits and vegetables; as such they are regularly ingested by humans as part of a normal diet. Hence, these sugars do not pose any safety concerns to consumers. Furthermore, many *in vitro* and *in vivo* studies have provided scientific evidences of the FOS prebiotic features (Gibson *et al.*, 2004; Yen *et al.*, 2011).

The values that have been suggested as the adequate dose of a daily intake of fructans vary from 3 to 40 g/day (Bruhwyler *et al.*, 2009; Gibson & Shepherd, 2010; Pereira & Gibson, 2002).

Several health claims have been based on the fructans prebiotic activity. Current claims on the reduction of the risk of diseases are very promising. Although many studies have been conducted *in vitro* with successful results, more clinical trials are needed to unequivocally prove the benefits for humans. The health benefits of fructans have been extensively reviewed (De Preter *et al.*, 2011; Delgado *et al.*, 2010; Kelly, 2009; Qiang *et al.*, 2009; Roberfroid & Delzenne, 1998).

COMMERCIAL OLIGOSACCHARIDES

In Japan, FOS produced by enzymatic processes are in the market since 1984 (Crittenden & Playne, 1996). The first FOS industrial producer was the Meiji Seika Kaisha, Ltd. with the product named "Meioligo" (Crittenden & Playne, 1996; Oku *et al.*, 1984). Meioligo products include several product types with different purity level. Currently in Japan, FOS can be found in more than 500 food products (Macfarlane *et al.*, 2008). In 2007, the retail FOS market was estimated to be about US$200/kg (Macfarlane *et al.*, 2008).

Other companies also commercializing FOS obtained by enzymatic processes are GTC Nutrition, Cheil Foods and Chemicals and Victory. These FOS products are provided with a purity level above 95% (*data obtained from the suppliers*).

In Europe, FOS obtained by enzymatic processes are only commercialized by Beghin-Meiji Industries. These FOS are produced from sucrose by the company TEREOS sugar group in France. Beghin-Meiji commercializes FOS for two industry segments: the Actilight® for food industry, and the Profeed® for animal feed industry. Actilight® is available in powder and liquid forms, with a fibre content ranging from 55% to 95%.

Inulin and oligofructose extracted from the chicory root are produced in Europe by Beneo-Orafti, Cosucra and Sensus. By varying the contents in dietary fibre and sugar, these companies can offer more than one type of product.

In Mexico, Nutriagaves de Mexico S.A. de C.V. (Namex) produces and commercializes inulin and oligofructose extracted from the desert succulent blue agave hearts of the Tequiliana Weber variety. A summary of the fructans currently produced and commercially available is presented in Table 3.

As previously mentioned, individual FOS molecules with purities varying between 80 to 99% are only available for analytical purposes. The main companies supplying GF_2, GF_3 and GF_4 are Sigma Aldrich, Megazyme and Wako Chemicals GmbH.

FOS PRODUCTION

FOS are naturally present in more than 36.000 plants and vegetables (Havenaar *et al.*, 1999). However, the FOS content in these foods is very low and most probably not enough to produce a prebiotic effect in the host. Furthermore, these FOS-containing foods are season-limited. Therefore, for large scale applications, FOS have been synthetically produced.

The different FOS production processes have been widely reviewed by several authors (Sangeetha *et al.*, 2005; Yun, 1996). Guio and collaborators (2009) revised the patents related with FOS production and their applications. Also, the microbial production of fructosyltransferase was discussed by Maiorano and collaborators (2008).

Briefly, FOS can be produced by chemical glycosylation and *de novo* synthesis using glycosidase and glycosyltransferase activities (Barreteau *et al.*, 2006). The chemical FOS

synthesis using this method is a laborious multi-step endeavor, uses hazardous/expensive chemicals and provides low production yields (Palcic, 1999; Prapulla *et al.*, 2000). Therefore, its implementation at an industrial scale is not economically feasible (Barreteau *et al.*, 2006).

Industrially, FOS are produced by the transfructosylation of sucrose. By transferring between one to three molecules of fructose to the fructose residue in sucrose, Fructosyl Transferase enzymes (FTase) produce firstly GF_2, then GF_3 and finally GF_4 releasing F, G, GF and GF_2.

Various microorganisms have been reported to produce FTase. Mostly are fungi strains, such as *Aspergillus* sp., *Aureobasidium* sp., *Fusarium* sp., *Penicillium* sp., among others (Chen & Liu, 1996; Prata *et al.*, 2010; Sangeetha *et al.*, 2004; Yun, 1996). Additionally, there are some bacteria that have also been reported as FTase producers, as *Arthrobacter* sp., *Zymomonas mobilis* and *Bacilus macerans*; as well as some yeasts strains including *Kluyveromyces*, *Candida* and *Saccharomyces cerevisiae* (Caicedo *et al.*, 2009; Sangeetha *et al.*, 2005; Yun, 1996). The enzymes used for the industrial FOS synthesis are produced by the fungi *A. pullulans* and *Aspergillus niger* (Maiorano *et al.*, 2008).

FOS can be produced using whole cells of a given microorganism, suspended or immobilized (Mussatto *et al.*, 2009; Sangeetha *et al.*, 2004), or more frequently using the extracted enzymes in free or immobilized conditions (Sangeetha *et al.*, 2005). The enzymes involved in FOS production can be either intra or extracellular.

The maximum theoretical yield for FOS produced by microbial FTases is around 55-60% based on the initial sucrose concentration (Sangeetha *et al.*, 2005; Yun & Song, 1993). This yield cannot be increased due to the high amounts of glucose co-produced during the fermentation which inhibits the transfructosylating reactions (Yun & Song, 1993). Therefore, at the end of the

fermentation, FOS are obtained as mixtures with high levels of other contaminant sugars, mostly glucose, but also fructose and residual sucrose.

FOS PURIFICATION

Several downstream techniques currently available could be used for the isolation and purification of FOS obtained in industrial fermentative processes. Some of these techniques will be discussed in detail below.

Increase of the fermentation yields

The presence of glucose in the final fermentative mixture resulting from the FOS production process inhibits the transfructosylating activity of the FOS-producing enzymes (Yun & Song, 1993). Therefore, in order to achieve higher fermentation yields, many authors have studied the impact of the continuous removal of glucose and residual sucrose from the medium during the FOS conversion. Consequently, by increasing the fermentation yields a purer final product could be obtained. The use of enzymes and membrane reactors has been proposed for the glucose and sucrose removal (Lin & Lee, 2008; Nishizawa *et al.*, 2001). Purification of FOS from commercial mixtures could also be achieved using the same approach.

Some authors used mixed enzyme systems with β-fructofuranosidase and glucose oxidase to increase the FOS production yields in fermentative processes (Sheu *et al.*, 2002; Yun & Song, 1993; Yun *et al.*, 1994). The process can be conducted with enzymes or microorganisms containing β-fructofuranosidase and/or glucose oxidase enzymes. Lin & Lee (2008) used calcium alginate-immobilized mycelia from *A. japonicus* (β-d-fructofuranosidase producer) and *A. niger*

(glucose oxidase producer). The enzyme β-fructofuranosidase has the ability to synthesize FOS from sucrose, while glucose oxidase enzymes are able to convert glucose into gluconic acid that can be afterwards removed from the broth by adsorption onto ion-exchange resins or precipitation with calcium carbonate solutions (Sheu *et al.*, 2002). Therefore, the combination of the two enzymes has been successfully used and up to 98% FOS mixtures have been obtained (Yun *et al.*, 1994). However, Yun *et al.* (1994) found that a higher content of GF_3 was accumulated and only a trace amount of GF_4 was detected when using the combination of the two enzymes as compared to that produced by Ftase/furanosidases.

Reactor systems equipped with nanofiltration membranes, through which glucose permeates but not sucrose and FOS, can also be used to remove glucose continuously during FOS production. Studies showed an increase of FOS production up to 90% (Nishizawa *et al.*, 2001).

Furthermore, by preventing the occurrence of oligosaccharides hydrolysis during the enzymatic synthesis, an increase of the fermentation yield can be obtained. Based on that, an activated charcoal column connected to a fermenter has also been used to remove oligosaccharides from the fermentative broth during the enzymatic synthesis (Ajisaka *et al.*, 1987; Boon *et al.*, 2000) with promising results.

Microbial treatment

S. cerevisiae and *Z. mobilis* are able to ferment some common mono- and disaccharides, but since these organisms do not possess carbohydrases they are unable to hydrolyze most of the oligosaccharides. Therefore, their use to eliminate small saccharides from broth mixtures with oligosaccharides has been reported (Crittenden & Playne, 2002). Both microorganisms convert

glucose, fructose and sucrose to ethanol and carbon dioxide. During sucrose fermentation by *Z. mobilis* a minimal amount of by-products, such as sorbitol and FOS can also be formed. In a study conducted with a commercial FOS mixture (Meioligo G), containing 57% of FOS in total sugars, no degradation of FOS was observed and glucose, fructose and sucrose were completely fermented without the formation of by-products (Crittenden & Playne, 2002).

Studies conducted with *S. cerevisiae* showed that the yeast is able to completely remove fructose, glucose, galactose and sucrose from a mixture of sugars while oligosaccharides with four or more monosaccharide units were not found to be fermented (Goulas *et al.*, 2007; Hernandez *et al.*, 2009; Yoon *et al.*, 2003).

The microbial treatment seems to be a good alternative for increasing the percentage of FOS in a mixture by removing mono- and disaccharides. Moreover, the process can even be used during the enzymatic synthesis of FOS. However, the use of microbial treatments implies a further step of purification for the removal of biomass and metabolic products formed during the fermentation, in order to obtain a FOS product with few contaminants. Also, the use of yeast treatment was found to modify the oligosaccharides composition (Sanz *et al.*, 2005).

Ultra and Nanofiltration

Membrane technology, mainly ultrafiltration using different operational modes, and nanofiltration, has been used to fractionate and purify oligosaccharides (Pinelo *et al.*, 2009). Leiva & Guzman (1995) used cross-flow filtration to concentrate GOS produced from the enzymatic hydrolisys of lactose. The retention factors obtained were 15.4% for glucose and galactose, 37% for lactose and 74.7% for GOS. Sarney and co-workers (2000) also used cross-

flow filtration for the recovery of oligosaccharides from human milk. Goulas and collaborators (2002) used cross-flow filtration, in a continuous diafiltration module, obtaining yields of 81 and 98% for raffinose and GOS, and 14-18% and 59-89%, for mono- and disaccharides, respectively. Later, the same authors studied the fractionation of several commercial oligosaccharide mixtures using diafiltration in a 'dead-end' filtration cell and the same yields were obtained after four filtration steps (Goulas *et al.*, 2003). In order to reduce the dilution water, Li and co-workers (2004) introduced a variable volume of diafiltration (VVD), during which dilution water flux was different to that of permeate, obtaining the same purity of FOS with a lower water volume consumed. Kamada and collaborators (2002b) were able to reduce from 9.0 to 2.6% the content of fructose, glucose and sucrose from the commercial product Raftiline (oligosaccharides from chicory) using nanofiltration in a dead-end module.

Numerous types of membranes have been used to separate oligosaccharides by nanofiltration. A low-pressure plasma modified cellulose acetate (CA) membrane was used by Gulec and collaborators (2010) to separate highly concentrated sugar mixtures (40%, w/v) of lactose, glucose and galactose. CA membranes were modified by means of plasma polymerization (PlzP) using low-frequency (LF) excitation. Very high retention (>94%) was obtained with the LF/PlzP-modified CA membrane for lactose, while the retention for monosaccharide was rather low (<73%) at the same reaction time. Moreover, Botelho-Cunha and colleagues (2010) studied the nanofiltration potential for fractionation of GOS mixtures by CA membranes submitted to annealing treatments. Starting with a 150 $g.L^{-1}$ sugar feed solution they were able to totally retain the trisaccharides. More recently, Kuhn and co-workers (2011) demonstrate the potential of diafiltration using a NP030 (Microdyn Nadir, Germany) membrane in the purification of FOS

from mixtures containing mono and disaccharides by obtaining a concentration up to 80% in FOS.

Other related works reported the use of membrane technology for recovery of non-digestible saccharides from yacon rootstock (mainly FOS and inulo-poysaccharide) (Kamada *et al.*, 2002a); caprine milk (Martinez-Ferez *et al.*, 2006), rice husks xylan (Vegas *et al.*, 2008) and unwanted compounds coming from lactic acid recovered from fermentative broths derived from apple pomace (Gullón *et al.*, 2011).

Activated charcoal

Sugars are physically adsorbed onto the activated charcoal in a reversible process due to Van der Walls' forces. The major surface area of the activated charcoal is non polar or hydrophobic. Therefore, as much hydrophobic is the solute more adsorbed it will be (Abe *et al.*, 1983). Since the hydrophobic character of the sugar is related with the extent of the CH groups, sugars will be adsorbed according to their molecular weight, provided that the configuration and the glycosidic link stereochemistry are favorable (Sundari & Balasubramanian, 1997). Hence, FOS are more adsorbed onto the activated charcoal than the other small saccharides as sucrose, fructose and glucose, enabling their separation. The activated charcoal has been used in slurry or in packed columns as a single adsorbent or in combination with Celite for the recovery of oligosaccharides. The most common process used to purify oligosaccharides with activated charcoal is divided in three main steps. First, the activated charcoal is loaded with the oligosaccharide mixture. Next, the non-retained compounds, as monosaccharides and salts, are washed out with pure water. Finally, the adsorbed sugars are selectively recovered using ethanol gradients. Disaccharides and

oligosaccharides have been recovered with 5-10% (v/v) and 15-50% (v/v) of ethanol in water, respectively (Hidaka *et al.*, 1988; Morales *et al.*, 2006; Nobre *et al.*, 2012; Sanz *et al.*, 2005; Swallow & Low, 1990; Whistler & Durso, 1950).

Whistler and Durso (1950) were the first to propose the use of activated charcoal mixed with Celite for sugars separation. The activated charcoal has been used to separate many different sugars in mixtures including: glucose, maltose, raffinose, sucrose and melibiose (Whistler & Durso, 1950); GF_2, GF_3, pentaose and hexaose from a *Lycoris radiata* bulbs FOS extract (Uchiyama *et al.*, 1985); GOS from fermentative broths (Sai Prakash *et al.*, 1989); COS from a culture medium (Samain *et al.*, 1997); monosaccharides and oligosaccharides from honey (Morales *et al.*, 2008; Morales *et al.*, 2006; Sanz *et al.*, 2005; Swallow & Low, 1990; Weston & Brocklebank, 1999); oligosaccharides from human milk (Priem *et al.*, 2002); oligosaccharides from a fermented fruit and vegetable beverage (Kawazoe *et al.*, 2008); and mono- and disaccharides from a commercial GOS product (Hernandez *et al.*, 2009).

Furthermore, activated charcoal columns have been used to purify FOS mixtures (Kaplan & Hutkins, 2000; Kaplan & Hutkins, 2003). FOS produced by *A. niger* were purified and fractioned by Hidaka *et al.* (1988) using a preparative activated charcoal column. GF_2, GF_3 and GF_4 were recovered with purities of 70, 69 and 44%, respectively. Kuhn & Maugeri (2010) also evaluated the purification of FOS from a fermentative broth using an activated charcoal fixed bed column with ethanol as eluent. Results showed that with 15% ethanol at 50 ºC, 80% recovery of FOS with about 97.8% purity could be obtained. Nevertheless, a poor efficiency for separation of FOS from sucrose was found. Although the process seemed promising, the authors

found that column efficiency varies deeply with the type of charcoal used. Consequently, the method was found to be non-reproducible between the different batches conducted.

Recently, Nobre et al. (2012) developed a simple and efficient process to purify FOS from a fermentative broth using a single activated charcoal column. Fermentative broth mixtures containing 50.6% (w/w) of FOS were purified to 92.9% (w/w) with a FOS recovery of 74.5% (w/w). The process showed that by elution with water, up to 93% (w/w) of the monosaccharides could be removed. The non-oligosaccharides were desorbed using a gradual increase of ethanol concentration, up to 5% (v/v). And finally, FOS were recovered using ethanol solutions ranging from 10 to 40% (v/v). Moreover, with the proposed process, fractions with purities up to 97% (w/w) of FOS were recovered. The process was also found to be efficient in the desalting of the fermentative broth.

Sanz and co-workers (2005) compared nanofiltration, yeast treatment and activated charcoal methods regarding their potential to separate monosaccharides from oligosaccharides in honey, and concluded that the activated charcoal was the most efficient method. In another study, Hernandez et al. (2009) compared diafiltration, yeast treatment, activated charcoal and size exclusion chromatography for GOS fractionation. Although size exclusion was the best technique to fractionate GOS at an analytical scale, the activated charcoal was also the most appropriated method to remove mono- and disaccharides.

FOS synthesized by microorganisms are obtained as mixtures containing not only small saccharides, but also large amounts of salts that must be removed to guarantee certain quality parameters of the final product. One advantage of using activated charcoal to purify FOS from fermentative broths, rather than other separation techniques, is the potential that charcoal has to

simultaneously desalt solutions (Nobre *et al.*, 2012; Samain *et al.*, 1997; Whistler & Durso, 1950).

Ion exchange chromatography

Several authors studied the potential of using ion exchange resins to separate carbohydrates. Chromatographic separation is based on the molecular differences of carbohydrates instead of their macroscopic properties. Therefore, this technique allows the separation of very similar carbohydrates, such as isomers, and shows a very high potential for FOS separation.

At an industrial scale, ion exchange resins have been used as adsorbents in Simulated Moving Bed (SMB) chromatography. Ion exchange resins of sulfonated poly(styrene-*co*-divinylbenzene) (PS-DVB) have been largely used due to their chemical inertness, higher capacity and selectivity (Luz *et al.*, 2008; Okada, 1995; Tiihonen *et al.*, 2002). PS-DVB resins are obtained by cross-linking the linear chain of the styrene polymer with DVB. This cross-linking increases the molecular weight of the average polymer chain length, which decreases the polymer solubility and increases its mechanical stability (Fritz & Gjerde, 2009). Gel-type resins normally contain less than 12% of DVB, while macroporous resins have DVB contents greater than 20%. Macroporous resins are characterized by a permanent well-developed porous structure and do not shrink. Since these resins have a high content in DVB, they are resistant to degradation caused by osmotic shock and oxidation. On the other hand, gel-type resins are soft, compressible and are able to swell with the appropriated solvent. However, these properties may restrict their use in chromatographic applications in which the back pressure may reach high values (Sherrington, 1998).

PS-DVB resins can be functionalized with counter-ions which are able to form complexes with the OH groups of sugars. The strength of the complex formed will depend on the hydration and ionic radius of the counter-ion, and the number and orientation of the OH group (Angyal, 1989; Tiihonen *et al.*, 2002). Therefore, the sugar conformation and the counter-ion involved determine the cation-sugar affinity, and consequently, the degree of adsorption. The stability of the complex formed depends on the number of pairs of eq-ax oriented OH groups. Since fructose has more pairs than glucose (Table 2**Table**), it is expected to be more adsorbed. Sucrose and FOS have no eq-ax oriented group, so they are excluded from the resin. As a result, the separation of the high molecular size sugars occurs mainly by size exclusion.

The separation mechanism involved in sugar purification with ion exchange-resins is mainly based in three phenomena: size exclusion, partition and ligand exchange based on complex formation (Churms, 1996).

The water contained in the resin is present in two forms, namely as hydration water (water contained in the hydration shell of the counter-ion available for complex formation) and as hygroscopic water (free water inside the resin available for partition). The total amount of water contained in the resin is therefore dependent on the resin type, cross-link density and ionic form.

Since the gel-type resins are able to swell, their water retention capacity is greater compared to the macroporous resins, resulting in faster kinetic diffusions (Sherrington, 1998). The cross-linking of the resin decreases its elasticity. Therefore, the ability to swell and retain water for partition also decreases. On the other hand, the amount of ions per volume of unit water increases with the DVB content. Hence, the adsorption by complex formation may increase. The hydration number of the counter-ion increases with the ionic valence (Tiihonen *et al.*, 2002).

Consequently, univalent cations only form weak complexes with sugars. As such, for these resins the separation mechanism is mainly based on the combination of size exclusion and restricted diffusion effects. In contrast, ions with increasing ionic valence may form strongest complexes with sugars. Nevertheless, the water uptake decreases with the increase of ionic valence (Tiihonen *et al.*, 1999).

As previously mentioned, several studies have been carried out to evaluate the performance of ion-exchange resins in the separation of sugars. The characteristics of resins as stability, resistance, selectivity and adsorption capacity have been deeply investigated. Also, studies were conducted to evaluate the influence on the adsorption of the resin type, ionic form and cross-link density. The influence of temperature and additional sugars in the mixture has been also studied. A summary of the most relevant and recent studies in adsorption equilibrium of sugars on ion-exchange resins is presented in Table 4.

Resin type

The strong acid cation (SAC) resins are the most commonly used for industrial sugar separations. Therefore, SAC are the best well studied type of resins (Lei *et al.*, 2010; Luz *et al.*, 2008; Nobre *et al.*, 2009; Pedruzzi *et al.*, 2008). Recently, Saari *et al.* (2010) evaluated the use of weak acid cation (WAC), weak base anion (WBA), and strong base anion (SBA) ion-exchange resins for the separation of sugars from lignocellulosic biomass hydrolysates. Results showed that SBA resins in SO_4^{2-} form could be an alternative to separate xylose and rhamnose from biomass hydrolysates (Saari *et al.*, 2010). WBA resins in Cl^- form have also been used to separate lactic

acid from oligosaccharides found in fermentative broths derived from apple pomace (Gullón *et al.*, 2010) .

Nobre *et al.* (2009) compared the adsorption equilibrium of fructose, glucose and sucrose onto gel-type (K^+) and macroporous (Na^+) resins. Based on selectivity results, the gel-type (K^+) resin was found to be the best choice for the separation of the three sugars.

Ionic form

The separation of glucose from fructose is the most well-known sugar separation process. As Ca^{2+} cations are able to form strong complexes with fructose enabling the isomers separation, Ca^{2+} resins have been widely reported (Beste *et al.*, 2000; Howard *et al.*, 1988; Luz *et al.*, 2008; Nowak *et al.*, 2009; Saska *et al.*, 1992). However, the separation mechanism of monovalent counter-ions provides higher adsorption kinetic rates compared with resins that form strong complexes with sugars. Therefore, counter-ions such as K^+ and Na^+ have been studied for sugar separation processes. Vente *et al.* (2005) studied the influence of K^+, Na^+ and Ca^{2+}, as the counter-ions of a SAC resin, in the adsorption equilibrium of mono- and disaccharides that are relevant for the separation of oligosaccharides. Results showed that Ca^{2+} resin provided the highest selectivity values for fructose/oligosaccharides separation, due to the complexes formed with fructose. On the other hand, for glucose/oligosaccharides separation, all resins studied showed the same selectivity. However, with the K^+ resin, a higher capacity compared to Na^+ and Ca^{2+} resins could be obtained. Therefore, the K^+ resin seems to be most suitable for the envisaged separation. Accordingly, Nobre *et al.* (2009) and Dendene *et al.* (1995) also found better results for the separation of mono- and disaccharides, with a resin in K^+ form rather than

with a Na^+ one. Furthermore, Pedruzi *et al.* (2008) elected a resin in K^+ form for the separation of lactobionic acid from a mixture of sugars, instead of resins in H^+ and Ca^{2+} forms. Nevertheless, the resin in Ca^{2+} form showed higher selectivity to separate sorbitol/lactose and fructose/sucrose. In a study conducted with four commercial resins in Na^+ and Ca^{2+} forms for FOS purification, Gramblicka *et al.* (2007) found almost no selectivity of sucrose/kestose for Na^+ resins. On the other hand, Ca^{2+} resins showed higher selectivity values. Recently, in a study on the separation of glucose, xylose and arabinose, the adsorbance of sugars onto the resins varied in the following order $K^+ > Ca^{2+} > Fe^{3+}$; however Ca^{2+} resin was elected as the best due to its higher selectivity (Lei *et al.*, 2010).

Cross-link density

According to the sugar molecules involved different resin DVB content should be selected so as to obtain a successful separation. A 6% DVB showed to be more suitable to separate arabinose/xylose, while 8% DVB was more suitable for the xylose/glucose separation (Lei *et al.*, 2010). Pedruzzi *et al.* (2008) found that a 4% DVB resin was better to separate lactobionic acid/lactose as compared to the one with 8% DVB. However, the resin with higher DVB content presented higher selectivity values for the separation of sorbitol/lactose and fructose/sorbitol. The effects of the DVB content and ionic form of cation-exchange resins on the chromatographic separation of MOS was evaluated by Adachi *et al*. (1989). In this study, the resin with higher DVB content gave the smaller coefficient of distribution. Also, as the radius of the hydrated ion became larger and the distribution coefficient was reduced (Adachi *et al.*, 1989a).

Temperature

The high concentration of sugar leads to an increased viscosity of the liquid phase, with a consequent increase in pressure drop and a decrease in solubility of sugars. As a result, many authors used temperatures above 70ºC to promote a decrease of viscosity and an increase of solubility (Luz *et al.*, 2008). Besides, the use of high temperatures also avoids microbial growth.

The temperature effect in sugar adsorption equilibrium onto gel-type resins, using single-component mixture of sugars has been widely investigated (Dendene *et al.*, 1995; Mostafazadeh *et al.*, 2011; Nobre *et al.*, 2009; Pedruzzi *et al.*, 2008). These works reported a decrease on the adsorption capacity at high temperatures. It is important to notice that studies conducted with multi-component sugar mixtures are scarce. In this case, the temperature effect seems to vary according to the resin, sugar and range of temperatures tested. Nowak *et al.* (2009) studied the influence of temperature on the adsorption of a mixture of sucrose, glucose and fructose on a Lewatit MDS 1368 in Ca^{2+} form. Experiments were conducted at 60ºC and 80ºC. Results showed no temperature dependency of the adsorption of glucose and sucrose, while for fructose a decreased adsorption was found for increasing temperatures (Mostafazadeh *et al.*, 2011; Nowak *et al.*, 2007). However, Best *et al.* (2000) studied the temperature effect (25ºC to 80ºC) in the adsorption of a mixture of fructose and glucose onto the same resin, and an increased adsorption behavior of glucose was found with increasing temperatures. Regarding the adsorption of fructose, the authors reported a decrease for temperatures up to 60ºC, and an increase for 80ºC. On the other hand, a gel-type (K^+) and a macroporous (Na^+) resin were used to study the adsorption behavior of a mixture of fructose, glucose and sucrose. The temperature increases

conducted to an increased adsorption, however with a decreased selectivity for the K^+ resin (Nobre *et al.*, 2009).

Multi-component mixtures

Nowak *et al.* (2007) studied the competitive loading of fructose, glucose and sucrose onto a gel type resin in Ca^{2+} form, at 60ºC and 80ºC, by adding the individual sugars to a mixture. Results showed that the presence of additional sugars in solution lead to a synergistic effect in the adsorption, *i.e.* the increased concentration of one sugar conducted to the increased adsorption of the others. Accordingly, Nobre *et al.* (2009) also found a synergistic effect at 40ºC; however, the authors reported a reverse effect when using a lower temperature (25ºC). Nonetheless, Vankova *et al.* (2010b) observed a competitive effect for all sugars, except fructose, in the adsorption of binary mixtures of fructose, glucose, sucrose and FOS, working at 60ºC, with a different gel-type resin in Ca^{2+} form.

SMB chromatography

PS-DVB resins have been largely and successfully used in SMB plants for sugar separations (Beste *et al.*, 2000; Coelho *et al.*, 2002; Luz *et al.*, 2008). SMB chromatography consists in multiple chromatographic columns connected in series and a complex valve arrangement that allows an appropriated shift of injection and collection points. The system works in continuous counter-current motion of the solid phase relatively to a liquid phase, without the real motion of the adsorbent. Instead, the movement of the adsorbent is simulated by the valves that move the position of two inlet (feed and eluent) and two outlet streams (raffinate and extract) by switching

one column in the direction of the liquid phase flow, at a fixed interval of time (Charton & Nicoud, 1995).

The main advantages of this separation method are that it works as a continuous system and enables very difficult separations (even with components presenting low selectivity). Compared to elution chromatography, higher productivities are obtained with lower solvent consumption and it becomes less expensive for large scale separations (Gomes *et al.*, 2006; Mazzotti *et al.*, 1997). However, the design, operation, optimization and control of the process are complex requiring longer time for the start up.

The number of reports on oligosaccharides adsorption onto ion-exchange resins for SMB applications is very limited. Ion-exchange resins for MOS purification were extensively studied by Adachi *et al.* (1995, 1989b). Kawase *et al.* (2001) studied the simultaneous production and separation of lactosucrose from glucose in a SMB reactor. Geisser and collaborators (2005) were the only authors reporting the separation of a complex mixture of oligosaccharides using a SMB plant. The separation of lactose from the human milk oligosaccharides mixture was studied. Results showed that an almost complete lactose separation was possible under stable conditions. Gramblicka *et al.* (2007) selected, among four commercial resins, the Amberlite CR1320 resin in Ca^{2+} form for FOS purification, based on the adsorption equilibrium studies of fructose, glucose, sucrose and FOS. Later, the authors studied the adsorption equilibrium of binary mixtures (Vankova *et al.*, 2010b) and modelled a fixed-bed adsorption of these sugars onto the same resin (Vankova *et al.*, 2010a). In another work, the separation of FOS from mono- and disaccharides using a single-column resulted in a 86% recovery of FOS, with an increase of purity from 61.7 to 82% (Vankova & Polakovic, 2010). Furthermore, Vankova and co-workers have demonstrated

the feasibility of scaling-up this process. Moreover, very good productivity, low product dilution and low eluent consumption was obtained from design simulations of a SMB chromatography unit to separate FOS (Vankova & Polakovic, 2012).

Taking into account all the results reported so far by several researchers, the SMB chromatographic process seems to be the most promising for the industrial purification of FOS.

CONCLUSIONS

Nowadays, consumers tend to seek food that can provide some additional heath benefits. Food products are increasingly supplemented with bioactive ingredients such as prebiotics, thus resulting in a great commercial interest in FOS. This increased interest in FOS has driven the research efforts during the last decade, namely towards the development of efficient recovery processes.

Continuous removal of glucose or FOS from fermentative broths during the production process has been proposed as a way to increase production yields. Fermentations carried out with mixed enzymes, reactor systems using nanofiltration membranes and charcoal columns connected to bioreactors have also been evaluated for this purpose. Moreover, the recovery of FOS from mixtures of sugars using several separation techniques has been described, namely microbial treatment, ultra- and nanofiltration and activated charcoal systems. All these techniques present advantages and drawbacks that must be considered in the design of an industrial FOS production process.

Several types of ion-exchange resins have recently been evaluated for use in SMB chromatography systems. The SMB chromatography works in a continuous mode; does not

require the use of organic solvents, and resins are very stable. Therefore, although it is a complex system, its application at an industrial scale is advantageous compared to batch activated charcoal columns. This technique has already proved to be suitable to separate binary sugar mixtures and some complex oligosaccharide mixtures efficiently. Hence, SMB chromatography is expected to play an important role on the large scale purification of FOS.

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Table 1. Physicochemical properties of sugars involved in FOS production.

FOS	Molecular formula	Molecular weight	Melting point (ºC)	Solubility*		
				Water	Alcohol	Ether
F	$C_6H_{12}O_6$	180	95-105	Very soluble	8 (at 18ºC)	
G	$C_6H_{12}O_6$	180	146 (α-D-Glucose) 150 (β-D-Glucose)	82 (at 17.5ºC)	Slightly soluble	Insoluble
GF	$C_{12}H_{22}O_{11}$	342	170-186	179 (at 0ºC)	0.9	Insoluble
GF_2	$C_{18}H_{32}O_{16}$	504	199-200			
GF_3	$C_{24}H_{42}O_{21}$	666				
GF_4	$C_{30}H_{52}O_{26}$	828				

Data from (Liley *et al.*, 1997) and (Crittenden & Playne, 1996).

(*Solubility is given in parts by weight of the sugars per 100 parts by weight of the solvent).

Table 2. Equilibrium composition of D-fructose and D-glucose in water (mole percentage) (adapted from (Angyal, 1991)).

Sugar	T (ºC)	Pyranose				Furanose			
		α	#Pairs	β	#Pairs	α	#Pairs	β	#Pairs
D-Fructose	30	2	1	70	2	5	0	23	1
D-Glucose	27	38.8	1	60.9	0	0.14	1	0.15	0

Table 3. Fructans currently produced and available in the market*.

Company	Country	Product name	Type of Fructan
Meiji Seika Kaisha	Tokyo, Japan	Meioligo	FOS
GTC Nutrition	Golden, Colorado, US	NutraFlora®	FOS
Cheil Foods and Chemicals	Seoul, Korea	Oligo-Sugar	FOS
Victory Biology Engineering	Shangai, China	Beneshine™ P-type	FOS
Beghin-Meiji Industries	Paris, France	Actilight® Profeed®	FOS
BENEO-Orafti	Brussels, Belgium	Orafti®	Inulin and oligofructose
Cosucra	Warcoing, Belgium	Fibruline® Fibrulose®	Inulin and oligofructose
Sensus	Roosendaal, Netherlands	Frutafit® inulin Frutalose® oligo-fructose	Inulin and oligofructose
Nutriagaves de Mexico S.A. de C.V.	Ayotlan, Jalisco, Mexico	OLIFRUCTINE-SP®	Inulin and oligofructose

*Data obtained from a survey of the major worldwide FOS manufacturers

Table 4. Summary of the studies reported on the adsorption equilibrium of sugars on ion-exchange resins.

Sugars	Resin	Process conditions	Adsorption isotherms/Method	Main observations	Reference
Fructose (F) Glucose (G)	Purolite PCR642 Ca^{2+}	▪ C: [10 – 70] $g.L^{-1}$; ▪ T: 30 and 60 ºC; ▪ Single-component mixtures; and syrup	▪ Linear isotherms ▪ Adsorption-desorption in batch and in column	▪ Adsorption capacity decrease with increasing T ▪ Results suggest that F and G may be successfully separated from syrup	(Mostafazadeh *et al.*, 2011)
Fructose (F) Glucose (G) Sucrose (S) FOS	Amberlite CR 1320 Ca^{2+}	▪ C: [0 – 400] $g.L^{-1}$; ▪ T: 60 ºC; ▪ Single-component and binary mixtures	▪ Linear isotherms ▪ Frontal analysis	▪ Binary adsorption equilibrium showed that the distribution coefficients of all saccharides besides F were slightly lower in the presence of another saccharide	(Vankova *et al.*, 2010b)
Glucose (G) Xylose (X) Arabinose (A)	Resin 001*4 Ca^{2+} Resin 001*6 Ca^{2+} Resin 001*8 Ca^{2+} Resin 001*6 K^{+} Resin 001*6	▪ C: [10 – 100] $g.L^{-1}$; ▪ T: 25 ºC ▪ Single-component mixtures	▪ Linear isotherms ▪ Adsorption-desorption in batch	▪ Sugars adsorption: K^{+} > Ca^{2+} > Fe^{3+} ▪ Resin in Ca^{2+} form more suited to separate the mixture ▪ A 6% DVB more suitable to separate A/X; while 8% DVB more suitable	(Lei *et al.*, 2010)

	Fe^{3+}			for X/G	
Arabinose (A) Fructose (F) Galactose (Ga) Glucose (G) Mannose (M) Rhamnose (R) Sucrose (S) Xylose (X)	Finex CS 11 GC Na^+ (SAC) Finex CA 12 GC Na^+ (WAC) Finex AS 510 GC SO_4^{2-} (SBA) Finex AA 12 GC FB SO_4^{2-} (WBA)	▪ C: [0 – 350] $g.L^{-1}$; ▪ T: 65 °C ▪ Single-component mixtures	▪ Linear isotherms ▪ Adsorption-desorption in batch	▪ Use of WAC exchange resin was restricted due to the decomposition of sugars ▪ WBA exchange resin showed high selectivity for X and A ▪ SBA exchange resin showed good selectivity for X and R, which implies that these could be separated from biomass hydrolyzates	(Saari *et al.*, 2010)

Fructose (F) Glucose (G) Sucrose (S)	Dowex Monosphere 88 Na^+ Dowex Monosphere 99 Ca^{2+}/320	▪ C: [5 – 250] $g.L^{-1}$; ▪ T: 25 and 40 ºC; ▪ Single- and multi-component mixtures	▪ Linear isotherms ▪ Adsorption-desorption in batch	▪ Competitive effect on the adsorption at 25ºC; Synergetic effect on the adsorption at 40ºC ▪ Adsorption capacity decreased on mono-component mixtures; and increased on multi-component mixtures with increasing T ▪ Dowex Monosphere 99Ca/320 was more suited for F, G, S separation	(Nobre *et al.*, 2009)
Lactobionic acid (La) Fructose (F) Lactose (L) Sorbitol (So)	Dowex Monosphere 99 Ca^{2+}/320 Dowex 50WX8-400 (H^+ and K^+ form) Dowex 50WX4-400 H^+ and K^+ form)	▪ C: [5 – 130] $g.L^{-1}$; ▪ T: 20, 40 and 60 ºC; ▪ Single-component mixtures	▪ Linear isotherms: F, L and So; Anti-Langmuir model: La. ▪ Frontal Analysis; Adsorption-desorption in column	▪ Best results of retention factor and separation factor obtained with K^+ resins ▪ Dowex 50WX4-400 in K^+ form showed better resolution to separate La ▪ Adsorption capacity decreased, and mass transfer coefficient increased with increasing T	(Pedruzzi *et al.*, 2008)
Fructose (F) Glucose	Lewatit MDS 1368 Ca^{2+}	▪ C: [0– 600] $g.L^{-1}$; ▪ T: 60 and 80	▪ Anti-Langmuir model.	▪ Synergetic effect on the competitive	(Nowak *et al.*, 2007)

(G) Sucrose (S)		ºC; ▪ Single- and multi-component mixtures	▪ Frontal Analysis; Adsorption-desorption in column	adsorption ▪ Adsorption capacity decreased for F with increasing T. G and S were not affected	
Fructose (F) Glucose (G) Sucrose (S) FOS	Diaion UBK 530 Na^+ Dowex Monosphere 99 Ca^{2+}/320 Lewatit S 2568 Na^+ Amberlite CR 1320 Ca^{2+}	▪ C: [0 – 450] $g.L^{-1}$; ▪ T: 60 ºC; ▪ Single-component: F, G, S; and multi-component: FOS	▪ Linear isotherms: F, G and FOS; Concave isotherm model: S. ▪ Adsorption-desorption in batch	▪ Amberlite CR 1320 Ca^{2+} resin was more suited to purify FOS	(Grambli cka & Polakovi c, 2007)
Fructose (F) Glucose (G) Sucrose (S) Galactose (Ga) Lactose (L)	Dowex 50WX4-400 (K^+, Na^+ and Ca^{2+} forms)	▪ C: [0 – 400] $g.L^{-1}$; ▪ T: 60 ºC; Single-component: F, G, S; and multi-component: FOS	▪ Linear isotherms: F, G, Ga and L; Concave isotherm model: S ▪ Frontal Analysis; Adsorption-desorption in batch	▪ Order of sugars adsorption: K^+> Na^+ > Ca^{2+} ▪ Resin in K^+ form was more suited to separate G from oligosaccharides. ▪ Resin in Ca^{2+} form was more suited to separate F from oligosaccharides	(Vente *et al.*, 2005)