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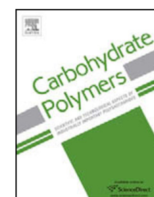

Review

Inulin-type fructans: A review on different aspects of biochemical and pharmaceutical technology

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ABSTRACT

Inulin is a natural storage polysaccharide with a large variety of food and pharmaceutical applications. It is widely distributed in plants, being present as storage carbohydrate in more than 30,000 vegetable products. Due to their wide distribution in nature and significant role in industry, the extraction, isolation and characterization of inulin-type fructans are gaining attention in recent years. Inulin sources have recently received increasing interest as they are a renewable raw material for the production of bioethanol, fructose syrup, single-cell protein and single cell oil, obtainment of fructooligosaccharides and other useful products. This review focuses on the state-of-the-art of biochemical and pharmaceutical technology of inulin-type fructans.

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1. Introduction

Inulin is a natural, plant-derived storage polysaccharide with a large variety of food and pharmaceutical applications. It is a substitute for sugar or fat having a very low caloric value, acts in a similar way as dietary fibers and contributes to improve gastrointestinal system conditions. Among other possible pharmaceutical applications are its use as an orally delivered drug targeting the colon, to delay absorption of drugs with adverse effects on stomach, or the treatment of diseases that show a peak in symptoms in the early morning (Barclay, Ginic-Markovic, Johnston, Cooper, & Petrovsky, 2012). Because of these properties, food and pharmaceutical industries have been finding applications of inulin and its derivatives such as fructooligosaccharides (FOS) in the production of functional foods, nutritional composites and drugs (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2010; Barclay et al., 2012; Cummings, Macfarlane, & Englyst, 2001; Judprasong, Tanjor, Puwastien, & Sungpuag, 2011; Laparra, Tako, Glahn, & Miller, 2008; Matusek, Meresz, Le, & Oersi, 2009; Morris & Morris, 2012).

Inulin is widely distributed in a variety of plants as storage carbohydrate, being present in more than 30,000 vegetable products (Wichienchot et al., 2011), among which are the tubers of *Helianthus tuberosus* (Jerusalem artichoke), *Cichorium intybus* (chicory), *Dahlia pinnata* (dahlia) and *Polymnia sonchifolia* (yacon) (Braz de Oliveira et al., 2011). It was discovered by the German scientist Valentine Rose, in the early 1800s, as a carbohydrate source from the roots of *Inula helenium*, and after named inulin by Thomson in 1817. The plant physiologist Julius Sachs, who was a pioneer in fructan research, was able in 1864 to detect, using a microscope, the spherocrystals of inulin in the tubers of *D. pinnata*, *H. tuberosus* and *I. helenium* after precipitation with ethanol (Franck & De Leenheer, 2005). It was shown to be a mixture of oligo- and/or polysaccharides composed of fructose units with β-configuration of the anomeric C_2, which makes inulin-type fructans resistant to hydrolysis by human intestinal digestive enzymes that have specificity for α-glycosidic bonds. For this reason all these compounds have been classified as nondigestible oligosaccharides (Roberfroid, 2007).

Plant inulin has chains incorporating from 2 to 100 fructose units, whose length, composition and polydispersity depend on the plant species, the phase in its life cycle, the harvesting date and the extraction and post-extraction procedures (Barclay et al., 2010; Ronkart et al., 2007). Inulin can be hydrolyzed by both endo- and exo-inulinases. The exo-inulinases remove the terminal fructose residues from the non-reducing end of chain, while the endo-inulinases act on the internal linkages (Braz de Oliveira et al., 2011; Ertan, Ekinci, & Aktac, 2003; Ronkart et al., 2007).

This review focuses on the state-of-the-art of biochemical and pharmaceutical technology of inulin-type fructans with emphasis in their biosynthesis. Moreover, the methods for isolation and characterization of inulin from different vegetal species are described and biotechnological applications of these carbohydrates are related.

2. Fructans: origin and role in plants

Inulin-type fructans are water-soluble fructose-based polymers that result from extended sucrose metabolism (Weyens et al., 2004). In plants, they are frequently stored in leaves and others organs acting as carbohydrate reserve (Ritsema & Smeekens, 2003). These fructan-containing plant species are found in a number of mono and dicotyledonous families such as Liliaceae, Amaryllidaceae, Gramineae and Compositae. In Liliaceae, Amaryllidaceae and Compositae, inulins are usually stored in bulbs, tubers and tuberous roots (Braz de Oliveira et al., 2011). Besides this, fructans have been reported to play a fundamental role also in osmoregulation, to act as protectants against dehydration induced by drought or freezing and to be involved in abiotic stress–tolerance (Livingston, Premakumar, & Tallury, 2006; Ritsema & Smeekens, 2003).

These substances play an important role in the quality control of fruits because several pathways that link the synthesis and breakdown of these carbohydrate reserves are in dynamic equilibrium and determine fruit quality during storage. Alterations in the pattern of soluble sugars are often associated with increased cold hardiness in a wide range of plant species (Gibson, 2005). The stress/tolerance response by changes in FOS accumulation in tablet grape was monitored after high CO_2 treatment, during low temperature storage, and the results showed an increasing FOS accumulation (Blanch, Sanchez-Ballesta, Escribano, & Merodio, 2011)

Some species growing in arid habitats develop photosynthetic adaptive processes such as the crassulacean acid metabolism (CAM) that allow them to efficiently uptake CO_2 at night and use water. Fructans are photosynthetic product of CAM and act as osmoprotectants during drought (Borland, Griffiths, Hartwell, & Smith, 2009). Fructans of these species include inulin, levans, neo-series inulin and highly branched structures (Waleckx, Gschaedler, Colonna-Ceccaldi, & Monsan, 2008), whose main function is energy storage and to act in abiotic stress tolerance in plants (Arrizon, Morel, Gschaedler, & Monsan, 2010; López, Mancilla-Margalli, & Mendoza-Diaz, 2003; Leach & Sobolik, 2010).

A large number of agave species possess a CAM, which explains the recent efforts to achieve inulin-type fructans from them (López & Urías-Silvas, 2007). Heads of plants belonging to the Agave genus have high contents of fructan oligomers, composed mainly of fructose units linked to a sucrose molecule, which can be easily degraded by thermal or enzymatic treatments leading to free sugars, mainly fructose. Many patents have been granted on the use of fructans from Agave species as a raw material for many purposes (Narvávez-Zapata & Sânchez-Teyer, 2009).

3. Chemical structure of fructans

Fructans are present in plants as heterogeneous mixtures with different degrees of polymerization (DP) and various chemical structures. The type of fructans found in plants (oligomeric or polymeric molecules) and the presence of a specific type of fructan are species-dependent and related with the environmental conditions and developmental stage of the plant (Mancilla-Margalli & Lopez, 2006). Five types of fructans with different structures were described in higher plants: inulin-type fructans (1-kestose), levan-type fructans (6-kestose), fructans of the inulin neoseries (neokestose), mixed-type levans (bifurcose) and fructans of the levan neoseries also called mixed-type levans (mixed-type F_3 fructan), whose shortest representatives, mentioned between brackets, have their chemical structures illustrated in Fig. 1 (Van Laere & Van den Ende, 2002).

These authors reported that inulin-type fructans are fructose polymers that have mostly or exclusively β-(2→1) fructosylfructose linkages, whereas levan-type fructans have mostly or exclusively β-(2→6) fructosylfructose linkages. Although these fructan types are essentially linear molecules, a low degree of branching can occur through β-(2→6) linkages in the case of inulins or β-(2→1) linkages in levans. In case the terminal glucose molecule is absent (Fn-type fructans), there are reducing compounds in contrast to the regular type fructans (G-Fn), and the terms inulo-n-oses [β-(2→1) linkages] and levan-n-oses [β-(2→6) linkages] are used.

Without any doubt, inulin is the best-known and studied fructan (Van Laere & Van den Ende, 2002), and the

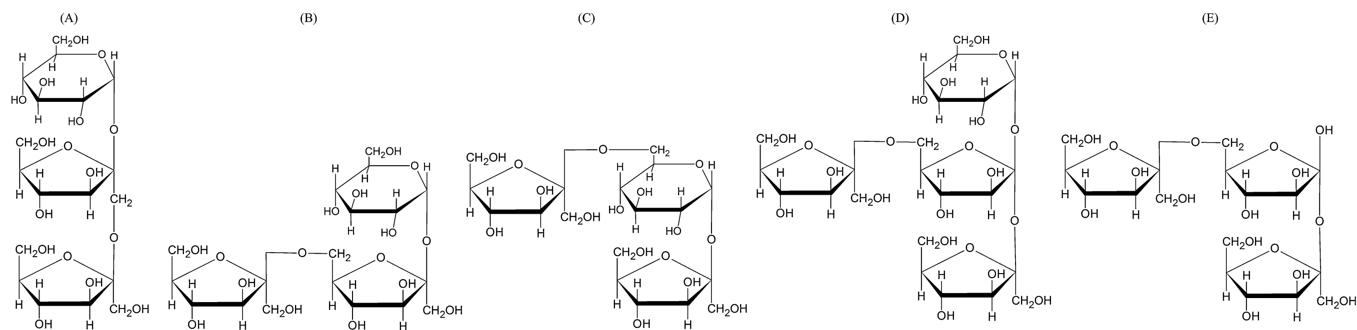

Fig. 1. Different types of fructans from higher plants according to the classification of Van Laere and Van den Ende (2002): (A) 1-kestose; (B) 6-kestose; (C) neokestose; (D) bifurcose; (E) mixed type F3 fructan.

number and distribution of different oligomers were shown to be characteristic of the inulin producing plant (Kiss & Forgo, 2011). Native inulin always is extracted from fresh plants, taking precautions either to inhibit the plant own inulinase activity or to prevent acid hydrolysis, but no fractionation procedure is applied to eliminate the smaller oligosaccharides and monomers that are naturally present, as it occurs for commercially available products (Franck & De Leenheer, 2005).

Inulin has not a simple structure; its chain constituted by a variable number of fructose units, linked by β-(2→1) D-fructosyl-fructose bonds, usually terminates with only one glucose unit linked through an α-D-glucopyranosyl or α-(1→2) bond as in sucrose (Bruyn, Alvarez, Sandra, & De Leenheer, 1992). Inulins with a terminal glucose unit are called α-D-glucopyranosyl-[β-D-fructofuranosyl]$_{n-1}$-D-fructofuranosides (or FOS), while those made up only of fructose fructopyranosyl-[α-D-fructofuranosyl]$_{n-1}$-D-fructofuranosides (or inulooligosaccharides) (Ronkart et al., 2007).

Based on the polymeric structure described above, it is evident that the main aspect of inulin structure is associated with its β-(2→1) bonds, which prevent inulin from being digested like a typical carbohydrate and are responsible for its low caloric value along with its behavior as a dietary fiber (Roberfroid & Slavin, 2000).

The physico-chemical and functional properties of inulin are linked to DP as well as the presence of branches. The short-chain fraction, oligofructose, is much more soluble and sweeter than native and long-chain inulin, and can contribute to improve mouthfeel because its properties are closely related to those of other sugars. For example, owing to a sweetness profile similar to that of sucrose, but lower caloric content (1–2 kcal/g) and sweetening power (30–35%), it can be useful to partially replace sucrose or to replace it totally when combined with other non-caloric sweeteners (Guggisberg, Cuthbert-Steven, Piccinali, Bütikofer, & Eberhard, 2009; Tárrega, Rocafull, & Costell, 2010). The long-chain fraction is less soluble, more viscous and more thermostable than native inulin and can act in rheological and sensory properties of dairy products as a fat substitute in low-fat or reduced-fat products; in these cases inulin acts as a filler or as breaker of structure in the same way as fat globules do (Guggisberg et al., 2009). It was reported that long-chain inulin, when sheared in water or milk, has the ability to form microcrystals, which can interact to form a smooth creamy texture and provide a fat-like mouth sensation (López-Molina et al., 2005).

Other physico-chemical properties that are influenced by DP include the melting, glass transition temperature, the capability of gel formation and gel strength (Bot, Erle, Vreeker, & Agterof, 2004). The interaction with other food components such as starch or hydrocolloids is also influenced (Meyer, Bayarri, Tarrega, & Costell, 2011). Therefore, fractions with variable DP can be used to formulate specialty food products (Hernalsteens & Maugeri, 2008; Yi, Zhang, Hua, Sun, & Zhang, 2010).

4. Biosynthesis of inulin

Fructan synthesis starts when photosynthesis exceeds the demand and sucrose reaches a critical level in sink organs, but differences exist among species regarding linkages, branching patterns and sizes (Livingston, Hincha, & Heyer, 2009).

Fructan biosynthesis in plants is catalyzed by three different classes of enzymes: sucrose:sucrose 1-fructosyltransferase (EC 2.4.1.99) (1-SST), fructan:fructan 1-fructosyltransferase (EC 2.4.1.100) (1-FFT) and fructan exohydrolase (EC 3.2.1.153) (FEH). Inulin is synthesized from a starting molecule of sucrose, which explains the presence of only one glucose unit in its chain. The relative inertness of glucose confers certain protection to the polymer, because it does not break down spontaneously. Enzymes then gradually transfer fructose from another sucrose molecule to perform the polymerization. The attachment of the incoming fructose takes place on the relatively reactive primary hydroxyl group linked to the anomeric carbon through the methylene group at C_1 of fructose moiety of sucrose (Barclay et al., 2010). In 1968, it was proposed a model for the biosynthesis of inulin in *H. tuberosus* that assumes no phosphorylated precursor, sucrose as the only substrate and the action of both 1-SST and 1-FFT. 1-SST transfers a fructose moiety from sucrose to the C-1 of a fructose in another sucrose molecule yielding the trisaccharide 1-kestose (GF + GF → GFF + G) in an essentially irreversible reaction, and similar transferases lead to 6-kestose and neokestose (Edelman & Jeeeord, 1968). Then, 1-FFT transfers fructose moieties from 1-kestose (or larger fructans) to sucrose or other fructans (GFn + GFm → GF(n + 1) + GF(m − 1) with $n \geq 1$, $m \geq 2$) (Van Laere & Van den Ende, 2002).

A further model was proposed (Livingston et al., 2009) for the synthesis in plants (Fig. 2) of the earlier five types of fructans from sucrose illustrated in Fig. 1.

It involves other two fructosyltransferases in addition to 1-SST, FEH and 1-FFT, specifically a sucrose:fructan 6-fructosyltransferase (6-SFT) and a fructan:fructan 6G-fructosyltransferase (6G-FFT). 6-SFT is able to catalyze the synthesis of (a) 6-kestose from sucrose and its subsequent elongation to higher levans, (b) fructans of levan neoseries from neokestose, (c) bifurcose from 1-kestose and sucrose, and (d) mixed-type levans from bifurcose. On the other hand, 1-SST catalyzes the synthesis of 1-kestose from sucrose, 6G-FFT that of neokestose from 1-kestose and sucrose, and 1-FFT the elongations of (a) neokestose to higher fructans of the inulin neoseries, (b) 1-kestose to inulin, and (c) bifurcose to mixed-type levans. Finally, levan-type fructans can also be produced from bifurcose by the concerted action of 6-SFT, 1-FFT and FEH.

Some researchers drew attention to differences between inulins of bacterial or fungal origin, which range from high molecular-weight fructans to oligosaccharides, and inulins of plant origin. The synthesis of inulin takes place in bacteria and fungi spores by transfer of additional fructose moiety of sucrose to the terminal fructose unit of 1-kestose or higher inulin-type fructans and is performed by

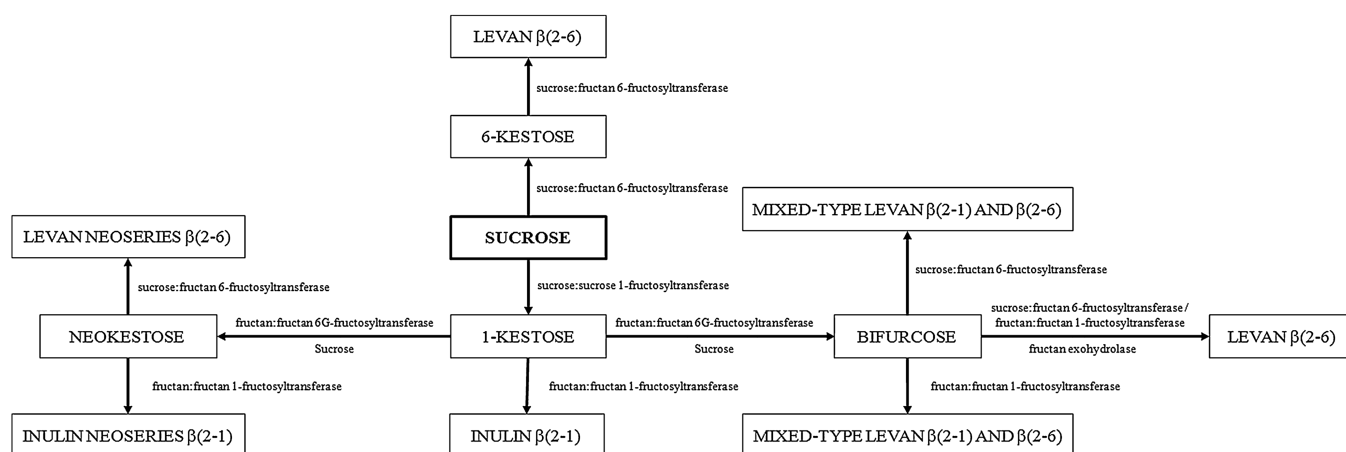

Fig. 2. Model proposed by Livingston et al. (2009) for the synthesis of fructans in plants involving four fructosyltransferases and a fructan exohydrolase.

sucrases (inulosucrase or levansucrase). Since the acceptor reactivity increases with the length of its chain, inulin from these sources usually has very high DP (Franck & De Leenheer, 2005).

5. Natural factors influencing the chemical structure and content of inulin-type fructans

The chemical structure of fructan greatly depends on the species. Inulin from garlic has a 2,1-linked β-D-fructosyl back bone with 2,6-linked β-D-fructosyl side chains, and the same applies to *Agave tequilana* (Ávila-Fernández, Galicia-Lagunas, Rodríguez-Alegría, Olvera, & López-Munguía, 2011). In addition, plants that naturally accumulate fructan can also degrade it in order to remobilize the stored carbon, which is the major drawback for inulin recovery.

The inulin DP depends upon different factors: plant species, climate and growing conditions, harvesting maturity and storage time after harvest (Chi et al., 2011). For instance, inulin stored by chicory has a rather low mean DP. Higher-DP inulins are found in artichoke, globe thistle (*Echinops ritro*) and *Viguiera discolor*. Variation in the chain length of inulin polymers in different Asteraceae species could be the result of different enzymatic characteristics (Vergauwen, Van Laere, & Van den Ende, 2003). Throughout the period of artichoke storage there occur a decrease in inulin content and mean DP, owing to its depolymerisation (Leroy, Grongnet, Mabeau, Le Corre, & Baty-Julien, 2010).

It has been observed in chicory that inulin DP in the early growing season is much higher than at harvest, because the activities of 1-FFT and 1-FEH depend on both plant and environmental factors. Several solutions for this breakdown problem have been proposed, including attempts to obtain new fructan-accumulating plants by genetic modification of crops that originally do not synthesize fructan. The main advantage of using highly productive non-fructan-accumulating plant species with well-established husbandry and processing chain is that plants that naturally accumulate fructan can also degrade the polymer in order to remobilize stored carbon. Moreover, the introduction of fructan biosynthesis in non-fructan species would render new types of fructan with different DP not yet present in natural fructan-producing plants, i.e. the so-called tailor-made fructan (Van Arkel et al., 2013).

When the water-soluble sugar content of 2-, 4- and 6.5-year old *A. tequilana* plants for linkage analysis was investigated, the youngest plants exhibited the highest levels of free monosaccharides and low molecular weight fructans (DP = 3–6) with potential application as prebiotics, while the DP reached a maximum of 3–30 in 4-year-old plants and then decreased to 4–24 in the oldest ones (Arrizon et al., 2010). Besides, it was established a relationship between plant age and fructan synthesis in *Agave atrovirens* Karw by identifying changes in fructan composition in its leaves at three ages (3, 6 and 9 years) and quantifying the fructosyltransferase enzymatic complex activity, thus determining the growth stage with the highest fructan content (Leopoldo, Maria Eugenia, Aurea, & Rosalva, 2011). The content of non-structural carbohydrates was the highest in the leaves of the youngest plants and decreased by 86% in those of the oldest ones. The main non-structural carbohydrate was sucrose in the youngest plants, while inulin-type fructans, glucose and fructose predominated in the oldest ones.

6. Inulin extraction and precipitation

Due to their wide distribution in nature and significant role in industry, the extraction, isolation and characterization of inulin-type fructans are still gaining attention in recent years (Yang, Hu, & Zhao, 2011). Many investigations were developed to set optimum extraction conditions in order to improve inulin extraction from plants, and temperature, extraction time, and solvent/solid ratio were identified as the most important factors influencing the yield (Abou-Arab, Talaat, & Abu-Salem, 2011; Abozed, Abdelrashid, El-Kalyoubi, & Hamad, 2009; Paseephol, Small, & Sherkat, 2007; Saengkanuk, Nuchadomrong, Jogloy, Patanothai, & Srijaranai, 2011; Toneli, Park, Ramalho, Murr, & Fabbro, 2008). Solubility of inulin in water remarkably increases with temperature, being almost insoluble at 25 °C and reaching about 35% (weight/volume) at 90 °C; therefore, the industrial production process is based on diffusion in hot water (Kim, Faqih, & Wang, 2001).

Following this concept almost all of inulin extraction methods described in the literature make use of hot water as a solvent, with only small differences in temperature and extraction time. For example, inulin was extracted from Jerusalem artichoke ground tubers by a pretreatment step involving boiling water treatment for 10–15 min (Paseephol et al., 2007), from dry chicory roots by hot water diffusion at an average temperature of 80 ± 2 °C for 1 h with continuous stirring (Toneli et al., 2008), from globe artichoke by distilled water (80 °C) at pH 6.8 (by NaOH) to avoid inulin hydrolysis at pH < 6 (Ronkart et al., 2007), and from *H. tuberosus* L. by hot deionized water at 85 °C for 1 h (Saengthongpinit & Saijaanantakul, 2005).

Ultrasound-assisted extraction has been recently proposed to improve inulin extraction yield compared to the above traditional methods, the main independent variables being sonication amplitude, temperature and time. In the extraction of inulin from the body of Burdock root (*Arctium lappa*), a raise in the amplitude or

the extraction time increased the yield, while temperature had a minor effect, and the optimum extraction conditions were shown to be a sonication time of 25 min, a sonication amplitude of 83.22% and a temperature of 36.76 °C (Milani, Koocheki, & Golimovahhed, 2011). However, caution is needed in using sonication to extract inulin, because some low-molecular-weight fragments are formed by the direct action of ultrasounds and changes occur in the chemical composition of the inulin; therefore, their direct use has been suggested for inulin depolymerization to get a diffluent short inulin, while indirect sonication would be more suited to extract natural inulin (Lingyun et al., 2007). In the direct method a probe is directly inserted into a sample vessel, whereas the indirect sonication is performed by immerging the sample in an ultrasound cleaning bath and shaking it periodically in orbital shaker.

A new method has recently been proposed to extract inulin from Jerusalem artichoke tubers, consisting in a three-stage homogenate extraction that ensured an extraction yield (16.39 g per 100 g of tuber) about 14% higher than the conventional hot water extraction (Li, Meng, & Sun, 2012). After tuber washing with pressurized water to remove oils, drying in air at ambient temperature and treating with hot water at 80 °C for 10 min to inactivate the polyphenol oxidases, they were suspended in distilled water, shredded and extracted three times, and the resulting filtered liquors subjected to a clarification process.

Inulin recovery usually starts with its precipitation after extraction, which can be performed by either lowering temperature or using different solvents and involves variables such as centrifugation speed and time (Abozed et al., 2009; Lingyun et al., 2007). Due to its low solubility at low temperature, when an inulin concentrated solution is cooled or frozen it undergoes a process of phase splitting. Toneli et al. (2008) proposed a new method of inulin precipitation by cooling or freezing the extract followed by centrifugation and spry drying to obtain inulin in powder form. However, since this method implies significant energy expenditure, the liquid extract must be concentrated by evaporation before drying, and the temperature lowered to favor inulin precipitation. Freezing/thawing is another method proposed to precipitate inulin followed by centrifugation (Yang et al., 2011).

Long chain inulin can be alternatively precipitated from aqueous solutions in the presence of high concentrations of organic solvents such as methanol, ethanol, propanol, acetonitrile and acetone among others. Acetone was shown to be the best solvent to keep the natural DP, followed by ethanol and methanol. The strong precipitating power of acetone with regard to polysaccharides was ascribed to its ability to remove the water of solvation of these biomolecules, thereby promoting dehydration and subsequent precipitation (Dalonso et al., 2009). In addition, it has a very low boiling point (56.5 °C), which allows it to be easily recovered by distillation (Moerman, Van Leeuwen, & Delcour, 2004). Even though ethanol and acetone were shown to be the best solvents to precipitate inulin from Jerusalem artichoke tubers (Abozed et al., 2009), in general acetonitrile and acetone are more effective than ethanol for most inulins (Ku, Jansen, Oles, Lazar, & Rader, 2003). Nonetheless, for safety reasons, ethanol is recognized to be the best choice in food applications (Paseephol et al., 2007). With ethanol, recovered chicory inulin had a DP of 25 and the dahlia one of 40 (Moerman et al., 2004).

Table 1 summarizes the most significant methods of inulin extraction from different sources.

7. Purification methods

The extraction and precipitation methods described above usually result in solutions containing a mixture of crude inulin, other polysaccharides, non-carbohydrate compounds, particulate and colloidal matter (i.e. pectin, proteins, and cell wall materials), which have to be further purified to isolate the specific polysaccharide of interest (Izydorczyk, 2005). To remove these impurities and purify inulin, various physicochemical methods as well as modern and expensive chromatographic techniques can be used.

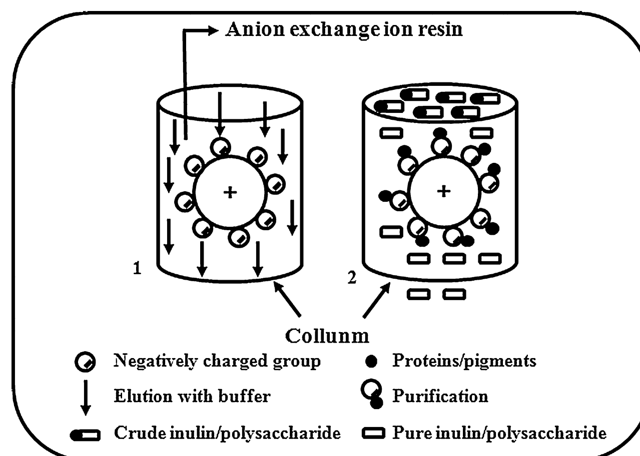

Fig. 3. Schematic of inulin purification by anion-exchange resins. 1. The DEAE cellulose anion-exchange resin (made up of a cationic nucleus and external negative chains) is sequentially eluted and equilibrated with buffer. 2. The supernatant (crude inulin) resulting from centrifugation of re-dissolved precipitate in water or buffer is fractionated using ion-exchange chromatography resin and the pure inulin/polysaccharide is obtained in a clear solution. Further steps can also be used using different buffers to obtain various fractions of polysaccharides.

The initial step common to almost all methods is dissolving the precipitate in distilled water and centrifuging it to remove any insoluble material (Fang, Jiang, & Wang, 2006; Holderness et al., 2011). Chemical treatments usually precede the separation through chromatographic techniques. Among these, deproteination with trichloroacetic acid has been reported by different authors (Chen et al., 2011; Fang et al., 2006; Feng, Jia, Shi, & Chen, 2010). In some cases, crude polysaccharides were further treated with cetylpyridinium chloride, amyloglucosidase and protease to remove almost completely proteins, uronic acids, starch-like α-D-glucans and hexosamines (Li et al., 2012). To obtain sulfated polysaccharide from *Sargassum pallidum* (Turn.) C. Ag., and two acidic polysaccharide fractions from *Polygala tenuifolia*, 10% $CaCl_2$ was added and kept overnight to precipitate tannins (Ji et al., 2011; Xin et al., 2012).

Several types of chromatographic techniques can be used to separate inulins from each other and/or from non-carbohydrate contaminants. Among them is the ion-exchange chromatography, whose effectiveness is well known to be influenced by pH, ionic strength of the buffer, nature of the counter-ion, flow rate and temperature. For inulin purification, the most commonly used anion-exchange resins are diethylaminoethyl (DEAE) cellulose (Fig. 3), DEAE Sepharose and DEAE Sephacel.

A large number of studies describe the isolation and purification of polysaccharides by means of DEAE cellulose. By this method, single water-soluble polysaccharides were obtained from the roots of *Cudrania tricuspidata* (Carr) (Lei, 2010) and *Ophiopogon japonicus*, a traditional Chinese medicinal herb (Chen et al., 2011), from the fruit of açai (*Euterpe oleracea*) (Holderness et al., 2011), from *Inonotus obliquus*, a well-known medicinal plant traditionally used for its antihyperglycemic effects, from *Grifola frondosa*, which belongs to the family of Meripilaceae and is marketed in China, Japan and other Asian countries as a medicinal and edible fungus (Chen, Ma, Liu, Liao, & Zhao, 2012), and from *Ginko biloba sarcotesta* (Wu et al., 2011); two different polysaccharide fractions were isolated and purified from the aqueous extract of *Prunella vulgaris* L. (PV) (Feng et al., 2010); and even three fractions were successfully purified

Table 1
Inulin type-fructans extraction methods from different sources.

Plant	Plant's treatment	Extraction	Extract's treatment	Reference
Helianthus tuberosus L.	Fresh frozen globes were thawed and then grated into slices	Twelve kg of plants were extracted by 50 L distilled water (80 °C) at pH 6.8 (by NaOH)	The extract was filtered through 1 mm and 5 μm filters, frozen/thawed and the precipitate was centrifuged at 3000 × *g* for 20 min	Ronkart et al. (2007)
Helianthus tuberosus L.	Tubers were washed to remove undesirable materials and cutted into slices. In order to avoid enzymatic browning, the slices were dipped in boiling water acidified with ascorbic acid and boiled for 2–3 min. Then, the slices were kept in polyethylene bags and stored in freezer at −10 °C until used	One kg of tubers was transferred into a warming blender and extracted with five-fold excess of hot water (70 °C) for 60 min at 70 °C with constant stirring	The suspension was filtered and residue was re-extracted using the same steps	Abou-Arab et al. (2011)
Cichorium intybus	Dried grounded root and root were used as the starting material	Batch extraction was performed at 70 °C with continuous stirring. Distilled water and alcoholic solutions were tested as solvent for inulin extraction	The suspension was filtered through a cloth	Dobre et al. (2008)
Helianthus tuberosus L.	Tubers were washed and soaked in 0.038 M sodium hypochlorite for 30 min to eliminate soil and reduce micro-organisms. The remaining tubers were packed in sealed polyethylene bags and kept in duplicates at 5, 2, and −18 °C	Eighty-five grams of deionized water at 85 °C were added to 11.5 g of crushed tubers, and the slurry was shaken at 130 rpm at 85 °C for 1 h in a water bath	After cooling to room temperature, the total weight was adjusted to 100 g with deionized water and the slurry was then centrifuged for 20 min at 12,000 × *g*	Saengthongpinit and Saijaanantakul (2005)
Helianthus tuberosus L.	To extract fructans of the tubers, 2 kg lots of peeled tubers were chopped into fine pulp in 10 L of hot water containing 100 ppm sodium metabisulphite to minimize browning at 95–98 °C for 10 min	Batch extraction was performed at 70 °C with continuous stirring. Distilled water and alcoholic solutions were tested as solvents for inulin extraction	The resulting extract was filtered through muslin cloth and then concentrated to 50% of the original volume using a single-stage climbing film evaporator	Paseephol et al. (2007)
Helianthus tuberosus L.	Tubers were washed with tap water and any deteriorated parts were removed, then the tubers were sliced. The sliced tubers were immersed immediately in boiling water for 5 min, following by immediate dipping in cold acetic acid solution (2%) to inhibit polyphenoloxidase activity. After that, slices were dried in electronic air oven	The dried powdered tubers were mixed with water at different powder tubers/water ratio (1:2.5, 1:5, 1:10, 1:15 and 1:20, w/v) at different temperatures (65, 75, 85 and 95 °C) as well as for different times (40, 50, 60 and 70 min)	The suspension was filtered	Abozed et al. (2009)
Morina officinalis	The dried roots were powered	Twenty g of material were extracted with 95% ethanol (400 mL) for 1.5 h at 100 °C	Once filtered, the extracts were concentrated to 20 mL under reduced pressure at 50 °C. The residue was then mixed with 20 mL water	Yang et al. (2011)
Agave tequilana	Fifty g of pulp were produced from the transversal cutting of six mature *A. tequilana* heads	The heads were placed in a mixer with 1.5 L of distilled water at 80 °C and agitated for 5 min	The obtained suspension was then filtered in preparation for analysis	Waleckx et al. (2008)

from the crude polysaccharide of *Dendrobium denneanum* (Fan et al., 2009).

DEAE Sepharose was successfully employed in other efforts. A water-soluble polysaccharide was isolated from the rhizome of *Menispermum dauricum* DC (Menispermaceae), a traditional Chinese medicinal herb that is widely used for treating sore throats, colitis, dysentery and rheumatic arthralgia (Lin et al., 2013); polysaccharides were purified from the seed of Longan (*Dimocarpus longan* Lour.), a non-climacteric subtropical fruit with high commercial value (Jiang et al., 2013); inulin-type oligosaccharides (DP < 10) were isolated from *Morinda officinalis*, a plant of the traditional Chinese herbal medicine (Yang et al., 2011); sulfated polysaccharides were purified from *Turbinaria conoides* (Chattopadhyay et al., 2010); and a crude polysaccharide was isolated from the rhizome and roots of *Rhodiola rosea* L. and fractionated (Cai et al., 2012).

Although less commonly used, also DEAE Sephacel was successful to isolate and fractionate similar polysaccharides from the roots of *Polygala tenuifolia* and from *Glycyrrhiza glabra* L., a ligneous perennial shrub growing in the Mediterranean region, Asia Minor and Middle East, which is also cultivated widely in southern Russia (Wittschier, Faller, & Hensel, 2009).

In these studies, the type and concentration of the buffer to equilibrate the resin in the column were varied so as to give rise different polysaccharide fractions. For instance, using 0.2 mol/L NaCl, Hu, Liu, Ni, & Lu (2012) isolated a complete and single fraction from *Inonotus obliquus* that might have been a single compound, while multiple fractions were obtained with 0.5 mol/L NaCl, with

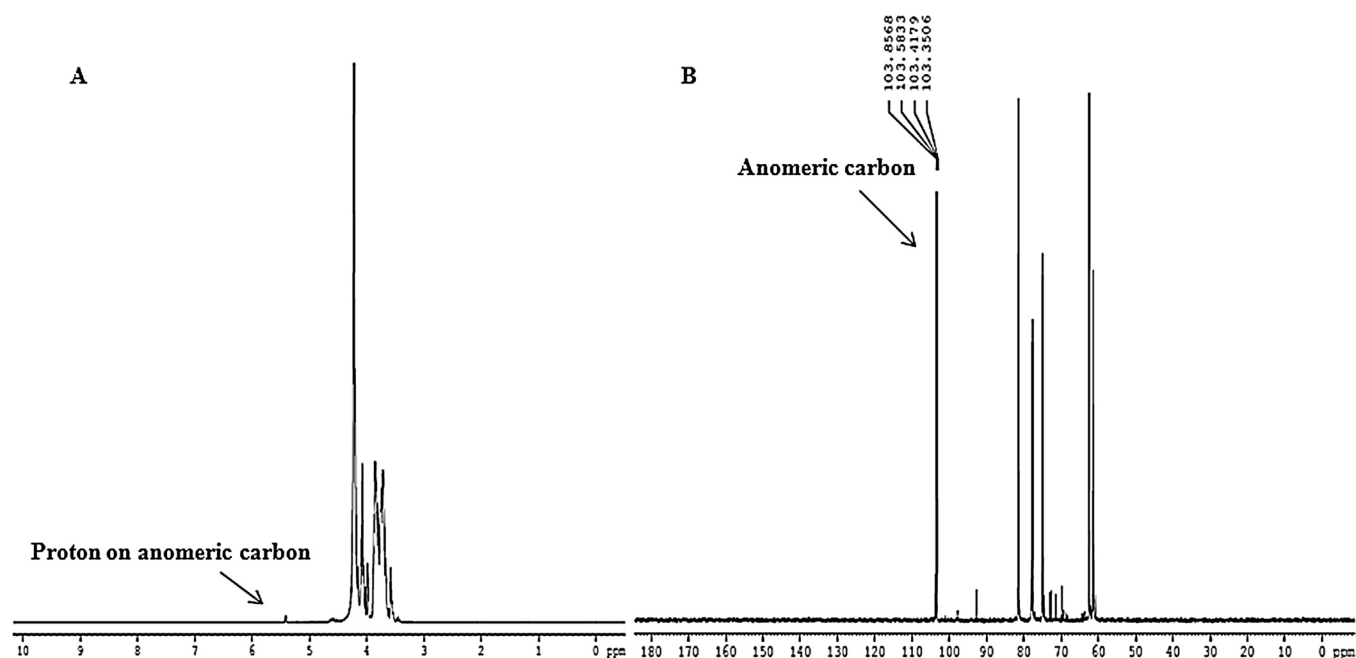

Fig. 4. Typical ^{13}H NMR (A) and ^{13}C NMR (B) spectra of inulin HP®.

the second peak displaying the highest polysaccharide concentration. Polysaccharides were successfully recovered from leaves of *Panax ginseng* CA Meyer using a DEAE Cellulose column eluted by a stepwise gradient of NaCl solutions (0.0, 0.1, 0.25 and 0.5 mol/L), and the appropriate fractions pooled, dialyzed and lyophilized to give four fractions (Ni et al., 2010). Polysaccharides from powdered açai were passed through a DEAE cellulose column and sequentially eluted with 0.05 mol/L Tris–HCl buffer and 2 mol/L NaCl, with minimal loss (Holderness et al., 2011).

8. Analytical techniques

Quantification of inulin type-fructans may be performed in the extracts so as to provide a preliminary assessment of plant fructan contents. Since fructans are usually found as complex mixtures of carbohydrates with different DP, monomer composition and glycosidic linkages, their analysis is a fundamental step to acquire basic information on the polysaccharide itself as well as to deepen understanding of its action mechanism, which is dependent on its chemical structure. However, the separation of complex mixtures of oligosaccharides is not straightforward, because of structural and molecular weight similarity; in addition, their identification is also hampered by the lack of available commercial standards (Brokl, Hernández-Hernández, Soria, & Sanz, 2011).

Gas chromatography–mass spectrometry (GCMS), nuclear magnetic resonance (NMR), and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry have been successful to obtain structural information on fructans, mainly DP. Fig. 4, panel A, illustrates a typical ^{1}H NMR spectrum of inulin HP®, in which one can see the chemical shift of glucose anomeric carbon, while panel B clearly shows four signals from 10.35 to 103.86 ppm of the ^{13}C NMR spectrum that suggest a high DP (>20). The presence of β-D-fructofuranosyl linkage is confirmed by the signal at 81.43 ppm. On the other hand, Fig. 5 shows the MALDI-TOF spectrum of inulin HP®, where the sharp peaks correspond to different values of molecular weight and then of DP.

Thin-layer chromatography (TLC) can be used to assess both the level and the composition of fructans in plant tissues. However, the last technique has limited resolution and low sensitivity and accuracy when used for quantitative purposes. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has been accepted as the most powerful method for direct determination of inulin, for it furnishes not only the content of inulin but also the DP profiles (López et al., 2003).

Although the absence of a chromophore or fluorophore group in oligosaccharide structures limits their direct detection by conventional spectrophotometric detectors (Brokl et al., 2011), some indirect spectrophotometric methods have been developed, which are based on inulin hydrolysis, derivatization of the released fructose and glucose with various reagents such as dinitrosalicylic acid (DNS) and p-hydroxybenzoic acid hydrazide (PHBAH), phenol and anthrone, and final measurement of the reaction products. Many reports on enzymatic hydrolysis and detection by different analytical methods have been published (Arrizon et al., 2010; Lingyun et al., 2007; Paseephol et al., 2007). The inulin content of different plant materials is finally measured as the difference between total carbohydrate and reducing sugars, which are often preliminarily quantified in their extracts.

To provide only a few examples, an indirect method was proposed (Paseephol et al., 2007) to quantify the fructan content of a concentrated Jerusalem artichoke extract, where total carbohydrate was assayed colorimetrically by the phenol–sulfuric acid method and reducing sugars spectrophotometrically using PHBAH. The same was done by Lingyun et al. (2007) on tubers of the same plant, but using the DNS method for reducing sugar determination. The same way, Arrizon et al. (2010) determined the fructan content of *A. tequilana* using the anthrone and DNS methods to assess its total carbohydrate and free reducing sugar contents, respectively. Another indirect spectrophotometric method was developed and validated to determine the inulin content of Jerusalem artichoke, which is based on the oxidation of released fructose by excess periodate and subsequent quantification of the remaining reactant by measuring the absorbance at 350 nm of the triiodide complex formed by the addition of potassium iodide (Saengkanuk et al., 2011).

Nonetheless, attempts with direct, conventional spectrophotometric methods on extracts are also reported in the scientific literature. For example, inulin content of dry and grounded chicory

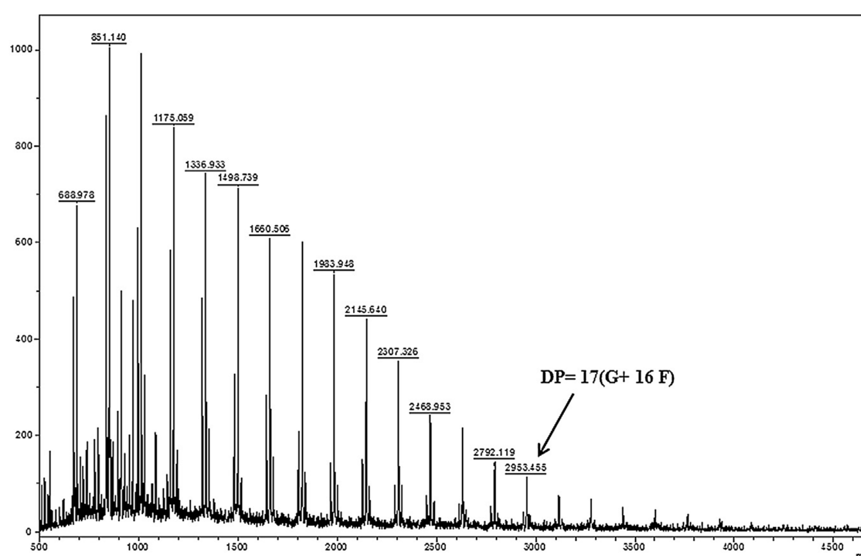

Fig. 5. Positive ion MALDI-TOF-MS spectrum of inulin HP® recorded with 2,5-dihydroxybenzoic acid as the matrix.

root was determined by a simple method based on the fact that vanillin, in presence of concentrated sulfuric acid, forms with inulin a deep red color complex that yields a characteristic absorption (Dobre, Stroescu, Stoica, Draghici, & Antohe, 2008), and the same was done for *A. sisalana* juice using HCl and resorcinol reagent (Sharma & Varshney, 2012).

Inulin oligomers have been analyzed by high-performance liquid chromatography (HPLC) using different detection techniques, however UV/vis detection gave poor results in terms of sensitivity, mainly due to the weak UV-absorbing properties of native carbohydrate derivatives. HPAEC–PAD has been applied to the analysis of inulin samples along with refractive index (RI) detection methods. However, these methods require special chromatographic arrangements, because PAD and RI detections are sensitive to eluents and the applied gradient elution (Saengthongpinit & Saijaanantakul, 2005).

A reliable HPLC method was set up using a carbohydrate column and evaporative light scattering detection (HPLC-ELSD) for proper qualitative and quantitative analysis of inulin oligomers, in order to determine the composition of thermally treated samples of chicory, Jerusalem artichoke and food products, as well as to lay the basis of assessment of the prebiotic effect (Kiss & Forgo, 2011). Thermal treatments were carried out at two high temperatures (180 and 210 °C) so as to characterize diversely inulin thermal degradation, and the released oligomers were identified by HPLC–MS and mass spectrometric detection using atmospheric pressure chemical ionization in positive ion mode.

These spectrophotometric techniques can also be used in combination to get more reliable information. For instance, Arrizon et al. (2010) investigated the effect of *A. tequilana* age on its fructan content and structure for economical purposes by HPLC, HPAEC-PAD, MALDI-TOF-MS and GC–MS, with HPLC allowing for separation of polymerized and non-polymerized sugars, HPAEC-PAD for determination of distribution of fructans with approximate DP ranging from 3 to 6 and longer fructans compared with Dahlia tubers inulin, MALDI-TOF-MS for detection of differences in oligosaccharide distribution induced by plant age, and GC profile for linkages analysis.

Despite of the increase in the inulin yield from Jerusalem artichoke tubers by direct sonication, by means of HPAEC-PAD it was verified the reduction of inulin DP and of some low-molecular-weight compounds (Lingyun et al., 2007). By the same technique, five FOS were identified in table grapes, namely 1-kestose, neokestose, nystose, nystose and kestopentaose, whose patters were shown to be differently influenced by storage and CO_2 treatment (Blanch et al., 2011). Researchers isolated inulin-type oligosaccharides with different DP from the traditional Chinese medicine plant *Morina officinalis* by size-exclusion chromatography, and determined their purity by HPLC–ELSD equipped with cyclodextrin-bond column (Yang et al., 2011). The elution flow rate was shown to be a crucial factor influencing the separation of oligosaccharides, in that an increase from 0.2 to 0.4 mL/min narrowed the fraction array from DP < 10 to DP < 6.

Magnetic nucler resonance (NMR) has recently been used to determine DP of fructans in aqueous extracts of roots and leaves of *Stevia rebaudiana* (Bert.) Bertoni that belongs to the family of Asteraceae (Oliveira et al., 2011). Whereas the ^{1}H NMR spectrum of FOS from roots showed the presence of one signal in the anomeric region, all resonances present in the ^{13}C NMR spectrum of leaves could be assigned to FOS. Moreover, the C-2 resonance of fructofuranose indicated ketose residues, while chemical shifts of ^{1}H and ^{13}C of the main residues in the 2D NMR spectra were fully assigned, based on literature data, to D-fructofuranosyl units with β-configuration. The same technique has been successful in combination with MS and literature data to confirm the structures of inulin-type oligosaccharides from *M. officinalis* (Yang et al., 2011). Analyses showed that glucose was present in both α-glycopyranose and β-glycopyranose forms, while fructose as α-fructofuranose, β-fructofuranose and β-fructopyranose. ^{1}H NMR and ^{13}C NMR spectra of oligosaccharides with 2 < DP < 10 were similar to those of sucrose and revealed they were inulin-type oligosaccharides made up of the same α-glycopyranose and β-fructofuranose residues, differing in the number of fructofuranose residues. Finally, NMR has recently been proposed in a new method to monitor inulin hydrolysis in real time (Barclay et al., 2012) based on the progressive variation of its DP.

9. Biotechnological applications of inulin oligosaccharides

Inulin sources have recently received increasing interest as they are a renewable raw material for production of bioethanol, fructose syrup, single-cell protein (substitute for protein-rich foods) and single cell oil (transesterification of triacylglycerols from renewable biomass), obtainment of FOS and other useful products (Chi et al., 2011). Functional foods have been developed by addition of inulin to increase their dietary fiber content (Komatsu et al., 2013). The effect of inulin, as one of the most attracting prebiotics in functional food preparation, on the fermentation patterns either

of pure cultures of *Streptococcus thermophilus* and *Bifidobacterium lactis* or in co-culture was investigated by Oliveira, Perego, Oliveira, and Converti (2012). These authors showed that the addition of inulin significantly reduced the time to complete the fermentation, enhanced biomass growth and increased the levels of lactic and acetic acids, diacetyl and acetoin in both pure cultures and co-cultures.

Inulin is a promising source for oligosaccharide production as a result of the action of inulinases. According to their mode of action, such enzymes can be classified into endoinulinases (2,1-β-D-fructan fructanohydrolases; EC 3.2.1.7), which specifically hydrolyze bonds between fructose units located away from the ends of inulin network releasing fructooligosaccharides, and exoinulinases (β-D-fructohydrolases; EC 3.2.1.80), which split terminal fructose units in sucrose, raffinose and inulin releasing fructose. Strong endoinulinases act on inulin in the absence of exoinulinase or invertase activities. Inulin can be easily hydrolyzed to fructose by exoinulinase that progressively removes the terminal fructose units from the non-reducing end of inulin in only one step. Taking into account that exoinulinases are encoded by only one gene in most of microorganisms, it is evident the interest of food industry for this biopolymer as raw material for fructose production (Pessoa & Vitolo, 1998, 1999). Since fructose is significantly sweeter than table sugar (sucrose) and glucose, the possibility to obtain high-fructose-corn-syrup (HFCS) from inulin-containing materials rather than by isomerization of corn starch hydrolyzates (Sirisansaneeyakul, Worawuthiyanan, Vanichsriratana, Srinophakun, & Chisti, 2007) would be an interesting challenge for the food industry.

In the last decades a large number of fungal, yeast and bacterial strains were used for inulinase production, among which *Kluyveromyces marxianus* (Yépez Silva-Santisteban, Converti, & Maugeri Filho, 2006; Yépez Silva-Santisteban, Converti, & Maugeri Filho, 2009) and *Aspergillus niger* (Paixão, Teixeira, Silva, Teixeira, & Alves, 2013) were reported as the most common and preferred sources. Inulinases catalytic properties are greatly influenced by the molecular weight, optimum pH, optimum temperature and stability, which in turn depend especially upon their provenience (Neagu & Bahrim, 2011; Singh & Gill, 2006). The simultaneous occurrence of inulinase (I) and invertase (S) activities in many cases could be explained by an enzymatic complex, which would mainly function as inulinase or invertase when $I/S > 10^{-2}$ or $<10^{-4}$, respectively (Sharma & Varshney, 2012).

Some studies have been focused on the development of enzymatic processes to reduce energy consumption and increase sugar recovery from *A. tequilana* fructans (ATF). In a study comparing the ATF hydrolysis by fructozyme (a mixture of endo and exoinulinases from *A. niger*) with that of inulin, it was found a lower enzyme specificity for ATF (V_{max} = 32.1 U/mL, k_M = 27 mmol/L) than for inulin (V_{max} = 34.1 U/mL, k_M = 7.2 mmol/L), which was ascribed to the complex ATF structure with high branching degree as well as to a different ratio of β-(2→1) and β-(2→6) linkages (Munõz-Gutiérrez, Rodríguez-Alegría, & Munguía, 2009).

Direct fermentation of inulin extracts into ethanol has been investigated using several inulinase-producing yeasts mainly belonging to the *Kluyveromyces* and *Saccharomyces* genera. In another interesting two-step approach, the inulin extract was first hydrolyzed by bacteria or fungi and then fermented to ethanol (Bonciu, Tabacaru, & Bahrim, 2010). To this purpose, yeast strains from different sources were isolated and characterized for their ability to growth in medium having fructose as a carbon source and to ferment inulin hydrolyzates.

Another promising application of inulin is the production of difructose anhydrides (DFAs,) cyclic disaccharides consisting of two fructose units linked at their reducing carbons with potential function as food additives (Saito & Tomita, 2000). Two kinds

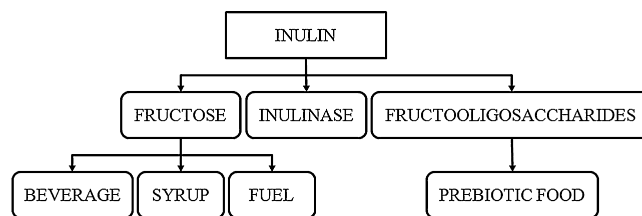

Fig. 6. Possible biotechnological applications of the inulin obtained from plants.

of DFAs have been produced by inulin degradation by microbial enzymes, namely DFA I (α-D-fructofuranose-β-D-fructofuranose-1,2′:2,1′-dianhydride) by DFA I-forming inulin fructotransferase (EC 4.2.2.17) and DFA III (α-D-fructofuranose-β-D-fructofuranose-1,2′:2,3′-dianhydride) by DFA III-forming inulin fructotransferase (EC 4.2.2.18). DFA III was shown to have half the sweetness but only 1/15 the calories of sucrose, thus enhancing the absorption of calcium and other minerals in the small and large intestines of rats and in humans (Zhao et al., 2011).

The flowsheet in Fig. 6 illustrates some biotechnological applications of inulin from plants.

10. Conclusions

In this review it was shown that the aspects of the biosynthetic origin of inulin type-fructans and their chemical structures are already well known. The process of hot-water extraction, followed by precipitation, is equally widespread in the literature; however, due to the large number of variables involved in this preliminary processing step, a lot of optimization studies based on statistical tools were carried to find the best temperature and solvent/raw material ratio as well as the most effective recovery technique. One of the bottlenecks in terms of cost and standardization is the purification of these products. The analyzed studies devoted to their purification describe methods consisting of a variety of different operations, but in general anionic exchange resins are the most widely and successfully employed. Analytical techniques have their complexity justified by the fact that the DP of oligosaccharides may largely vary depending on the plant species or even on the phase of its reproductive cycle, thus requiring sensitive techniques to highlight this aspect clearly. Regarding the broad applications of inulin type-fructans, to which a specific chapter of this review was addressed, there is a clear need for additional efforts to disseminate low-cost methods of extraction, purification and analysis reproducible on an industrial scale.

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