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review

Fructose Syrup: A Biotechnology Asset

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Summary

In response to the growing demand for the consumption of natural, healthy and low-calorie food, a large number of so-called alternative sugars has emerged since the early 80s, among them fructose. This sugar is a ketohexose, known as D-fructose or levulose, and is considered the sweetest sugar found in nature. Currently, fructose is mostly produced through the acid hydrolysis of sucrose, or through the multi-enzymatic hydrolysis of starch. Processes involving specific enzymes like inulinases, acting on widely available fructose polysaccharides such as inulin, have been studied as alternatives to the current approaches, in order to reduce time, complexity and costs involved in this process. Fructose syrup is used worldwide, mainly because of its sweetening power and functional properties. The present work aims to provide an overview of the properties of fructose and of the present and envisaged production processes, within the scope of a biotechnological approach.

Key words: fructose, syrup, fructooligosaccharides, microorganisms

Introduction

In the last decades particular care has been given to the impact of nutritional habits on public health. Concomitantly with such growing concern, developed countries have put considerable efforts in order to understand the links between diet and health. Policies and guidelines have been adopted to provide suitable information to the consumer and also to adequately influence food product composition and technological approaches for food processing (*1*). Given the widespread presence of sweeteners in common diet, particular consideration has been given to these compounds (*2*). The industrial use of sugars, particularly in the liquid form, is also of relevance, since food manufacturers often prefer to use sugar in the form of syrup, mostly due to the ease and efficiency of manipulation of liquids, and to the favoured process economics. Sugar syrups consist mostly of sucrose syrup; of invert sugar syrup; of blends of more or less complex carbohydrates, including oligosaccharide syrups (and particularly fructooligosaccharides); and of fructose-rich syrup (*3–5*). Oligosaccharides are mostly used due to their functional properties, namely their prebiotic nature, rather than their sweetness, which is relatively low (*6,7*). Sweeteners produce pleasant flavour, and occasionally cooling sensations, enhance shelf-life properties, and may simultaneously provide energy, in which case they are termed nutritive. If they do not provide energy, they are termed nonnutritive. Nutritive sweeteners encompass several natural sugars, such as sucrose or fructose, which are considered GRAS (Generally Recognized As Safe) by the FDA (Food and Drug Administration, USA) (*8*). Among nutritive sweeteners, fructose

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is clearly fit to be used as a sweetener in a wide number of food and drinking goods, since this non-allergenic carbohydrate is the sweetest of all naturally occurring carbohydrates. As such, it has been established as an alternative sweetener to sucrose (*9*,*10*). The sweetening power of fructose enables formulations to be adequately sweetened with small amounts of fructose, without compromising flavour quality, hence allowing the manufacture of dietetic products. These are thus termed since they have lower calorie values when compared to similar products formulated with either glucose or sucrose (*11*). Fructose also has functional properties that enhance flavour, colour, and product stability, and synergizes the sweetening power of sucrose and some nonnutritive sweeteners (*8*). The currently favoured processes for the commercial production of pure fructose rely on glucose isomerization, where the aldohexose itself is obtained from the multi-enzyme hydrolysis of starch; and on the hydrolysis of sucrose, to produce an equimolar mixture of fructose and glucose, from which fructose is recovered. An alternative for the production of pure fructose is the hydrolysis of inulin using inulinases (*5*,*12*). Fructose is a suitable raw material for the production of 2,5-dimethylfuran, which can be used as a chemical synthon (*13*). The present work aims to provide an overview of fructose as a biotechnology product, with particular emphasis on its characteristics, methods of production and industrial applications.

Oligosaccharides and Fructooligosaccharides

Oligosaccharides are short chain carbohydrates, usually containing up to 20 monosaccharide units, bound by glycosydic linkages (*14*). Oligosaccharides are water-soluble and have a relatively low sweetness. They are about 0.3 to 0.6 times as sweet as sucrose, depending on the chain length and sugar residues, but usually sweetness decreases alongside with increased chain length (*15*,*16*). Their low sweetness can be advantageously used when food formulations containing a bulking agent, also acting as a flavour enhancer, are required. Incorporation of oligosaccharides also changes the freezing temperature of foods, prevents browning due to Maillard reactions in high temperature-processed foods, prevents excessive drying, and contributes to the reduction of microbial contamination, given the low water activity (*16*). Oligosaccharides can be used in the stabilization of active substances and can also act as soluble dietary fibres, which simultaneously stimulate the growth of probiotic microorganisms such as *Bifidobacterium* spp. and *Lactobacillus* spp., hence having a prebiotic role (*17*). Probiotic microorganisms are relevant in the prevention of gastrointestinal infections, and in the suppression of pathogenic bacteria by lowering the pH in the intestine through the action of acidic metabolites resulting from the metabolization of nutrients, while facilitating digestion and stimulating the immune system (*18*). Fructooligosaccharides (FOS) emerge among the most relevant carbohydrates within naturally occurring functional oligosaccharides, mostly from plant sources (*19*,*20*). FOS are water-soluble, non-caloric, non-cariogenic and indigestible sweeteners. As typical oligosaccharides, FOS have sweetness of about 20 to 60 % of sucrose, and they flow through the human and animal gastrointestinal tract, where they are used by lactic acid and bifidobacteria. Given such features, FOS have had a significant impact on the sugar industry in relatively recent years (*16*,*21*–*26*). Most commonly available commercial FOS consist of 1-kestose, nystose and fructofuranosyl nystose (Fig. 1). In these oligosaccharides, which are enzymatically synthesized from sucrose on a multi-tonne scale, one to three fructosyl units, respectively, are bound to the β-2,1 position of sucrose. The resulting molecules combine one glucose and several fructose units, and are

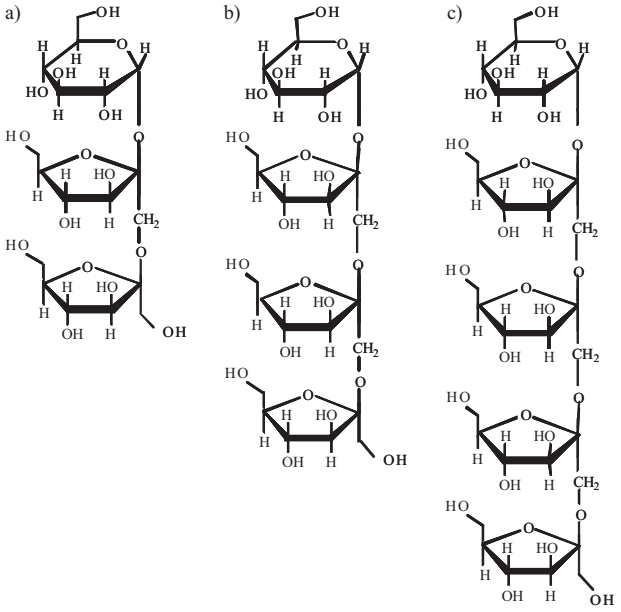

Fig. 1. Chemical structure of relevant fructooligosaccharides: a) 1-kestose (GF_2), b) nystose (GF3), c) 1-β-fructofuranosyl nystose (GF4). Fructosyl units are linked at position β-2,1 of sucrose

referred to as GF_n (*27*–*29*). The enzymes used for the production of FOS are fructosyl transferases and β-fructofuranosidases, which are obtained from bacterial, fungal and yeast sources (*26*,*30*–*32*). FOS production typically evolves from sucrose to 1-kestose initially, then to 1-nystose, and finally to 1-fructofuranosyl nystose (*26*,*33*). FOS yields are relatively low, around 60 %, since unwanted hydrolytic activity is also present, leading to fructose and glucose as by-products. Glucose, furthermore, inhibits enzyme activity (*26*,*30*,*34*). FOS can also be obtained from the controlled enzymatic hydrolysis of inulin, which results in a product containing about 75 % of fructose-only chains, with degrees of polymerization ranging from 2 to 7, and the remaining 25 % of the product in the GF_n form (*35*–*37*). Nutraflora® (from Corn Products International, Inc., Westchester, IL, USA) and Raftilose® (from BENEO-Orafti, Tienen, Belgium) are examples of commercially marketed compounds produced from sucrose or inulin, respectively (*22*,*38*).

Fructose

Fructose, a ketohexose also known as levulose or D-fructose, was isolated for the first time in the mid 19th century from cane juice (*39*). The sweetest natural sugar, its sweetening power nevertheless changes according to the formulation, a feature common to all sweeteners, but

it can be up to 1.8 times sweeter than sucrose (Table 1; *3,40*). Fructose is present in plenty of fruits (namely raisins, apples and grapes), honey (where it can reach roughly 40 % by mass) and, to a lesser extent, in vegetables, namely raw carrots and onions. The wide availability of fructose allows therefore for a regular intake of this carbohydrate by mammals (*9,41,42*). Table 2 displays data regarding the fructose and glucose content in some vegetables and fruits. Fructose can also be found in nature as a forming unit of sucrose, raffinose, stachyose and inulin. Inulin can be isolated from Jerusalem artichoke, dahlia tubers, chicory roots, garlic, asparagus root and salsify (*43*).

The crystalline form of fructose, β-D-fructopyranose, undergoes rapid mutarotation in a solution, leading to a mixture of different tautomers, again β-D-fructopyranose and β-D-fructofuranose, the two most abundant in aqueous media (*44*), alongside α-D-fructofuranose, α-D-fructopyranose and the open-chain keto form, with lower and diverse sweetening power (Fig. 2) (*9,40,45*). The sweet taste of fructose in aqueous solution can be controlled by adequate manipulation of pH, temperature and concentration. In the particular case of fructose, relatively low temperatures and pH environment reduce the formation of the furanose tautomers (*46*).

Table 1. Sweetening power of fructose formulations and other commonly used carbohydrate-based sweeteners, adapted from Godshall (*3*) and White (*40*)

Sweetener	Relative sweetening power/%
fructose (crystalline α-D-fructopyranose anomeric form)	180
fructose (5 to 15 % aqueous solution)	115 to 125
high-fructose corn syrup	90 to 130
invert sugar syrup	105
sucrose (crystalline)	100
sucrose (10 % aqueous solution)	100
glucose (crystalline)	74 to 82
glucose (10 % aqueous solution)	65
glucose (50 % aqueous solution)	90 to 100
xylitol (10 % aqueous solution)	100
maltose	50

Table 2. Occurrence of fructose and glucose in some foods, adapted from Hallfrisch (*41*)

Fruits	*w*(fructose)/%	*w*(glucose)/%
apple	7.3	2.6
banana	2.7	4.2
cherries	6.2	8.1
pineapple	2.1	2.9
grape	7.6	6.5
Vegetables		
carrots	1.0	1.0
corn	0.3	0.5
onion	0.9	2.4
tomato	1.4	1.1
beans	1.4	1.6
lentils	0.1	0
peanuts	0	0.2

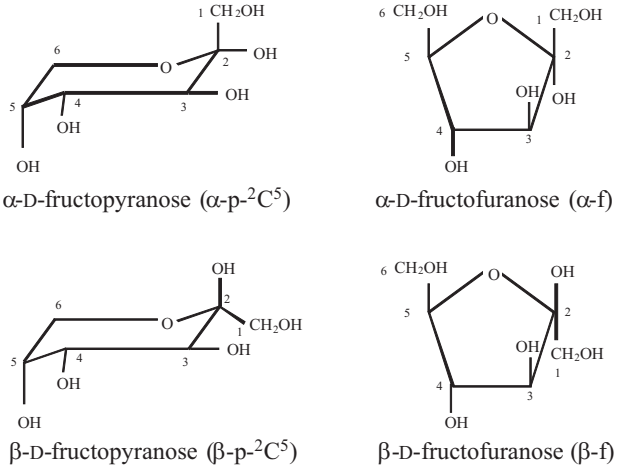

Fig. 2. Tautomeric forms of fructose in a solution

Besides its sweetness, fructose displays considerable synergy with several high-intensity artificial sweeteners and bulk sweeteners. Other properties that contribute to the success of industrial applications of fructose are the high water solubility, roughly 4 g of fructose per gram of water at 25 °C; the tendency to crystallize, which minimizes hardening in nutrition bars; high humectancy, contributing to the improvement of the shelf-life of baking and similar goods; high osmotic pressure; considerable flavour enhancement power; and high freezing point depression ability, which helps to formulate ice creams suitable for consumption in winter. Incorporation of fructose in foods also decreases water activity, thus reducing the risk of microbial contamination without the removal of water, which could result in altered texture of the processed good (*10,11,47,48*). Fructose and fructose syrup are therefore widely used in the food and pharmaceutical industries, *e.g.* in the production of carbonated soft drinks, fruit beverages, yogurts, ice cream, bakery goods, puddings, dairy products and baby food, and as excipient in pharmaceutical formulations (tablets, syrups, and solutions), given its flavouring and sweetening properties (*10,48,49*). Despite being available to the food industry since the 1980s, the use of crystalline fructose is still relatively restricted. On the other hand, the high solubility of fructose in aqueous media was also the major barrier to the production of fructose in a stable, crystalline form. This limitation was overcome by the introduction of carefully controlled processes that allowed for the efficient crystallization from aqueous solutions, rather than from solvent-based systems (*49*). Fructose is thus usually available in blends with other sugars, namely as high-fructose corn syrup (HFCS) and as invert sugar syrup (*40*). Production of HFCS is possibly the largest enzyme-based process implemented at a commercial scale (*4,50*). Since its inception in the market in the late 1960s, the demand

for HFCS has consistently increased, establishing this product as one of the most successful food ingredients. The well established acceptance of HFCS is closely related to its easy handling as a result of the liquid form, and to the stability in food that has a slightly acidic nature, such as, for instance, carbonated beverages. When sucrose is used in these goods as a sweetener, inversion tends to ensue, particularly when a prolonged exposure to room temperature takes place, hence leading to a sugar composition that is ultimately quite similar to HFCS (*40*). Actually, HFCS designation is somehow illusive, since most of the syrups under this designation have a fructose content (on a mass basis) of either 42 (HFCS 42) or 55 % (HFCS 55), the remaining being glucose and small amounts of oligosaccharides, roughly up to 5 %. HFCS 42 is used in baked goods, confectioneries and in several soft drinks, while HFCS 55 is the preferred form for most sweetened drinks. A third type of HFCS commonly marketed is HFCS 90, which has 90 % fructose, and it is obtained by large scale chromatographic processing of HFCS 42. Using liquid chromatography, glucose and fructose are separated, and the fructose-rich fraction is blended with HFCS 42, leading to HFCS 55 (*40*,*51–54*). Most of the production and consumption of HFCS take place in the USA (*55*). The USA is actually the main producer of fructose-rich syrups, and in the 1980s this country was accountable for a little over 70 % of the world production of fructose. This pattern can partly be related to the USA position as a corn producer (over 40 % of the world production). Japan is the second in the world ranking in fructose syrup production and consumption, but unlike the USA, Japan imports both corn and sugar (*56–59*).

Fructose and public health

Since the 1970s the sweetener intake *per capita* has been increasing worldwide, more noticeably in developing countries than in the developed world, where a trend of decrease in sugar consumption *per capita*, together with an increase in the consumption of other sweeteners, has been occasionally observed (*59*). The early success of fructose as a sweetener was partly related to its better adequacy to the diet of obese and diabetic patients, since fructose is sweeter, more soluble, and less glucogenic than sucrose or glucose (*41*). Fructose is actually absorbed more slowly through the intestine when compared to other common sweeteners. Besides, when nutritive sweeteners are considered, fructose has the lowest glycemic index. This parameter describes the rate at which glucose enters the bloodstream after consumption of a given food, and is therefore particularly useful in the preparation of dietetic food products and athletic beverages (*10*). Unlike glucose, which is stored as glycogen, fructose is converted into triglycerides by the liver. Excess fructose is likely to lead to metabolic disfunctions. As an outcome of excessive consumption of fructose (*e.g.* 20 % of ingested calories), collateral effects may occur such as an increase in cholesterol levels (*60*). Specialists in diabetes currently do not fully agree on the role of fructose in the diet of diabetic patients. Available data do not suggest that under similar isocaloric amounts, the glycemic response to fructose and other nutritive sweeteners differs from that to dietary starch. Thus, high daily intake of fructose may not adversely affect glycemic or lipid response in patients with type 2 diabetes, a chronic condition characterized by high levels of glucose in the blood caused by either the lack of suitable insulin production or because the cells ignore the insulin, hence insulin fails to take glucose into the cell and sugar builds up in the blood (*61*). However, given the concern for increased blood lipid levels with high intakes of fructose, addition of fructose as a sweetening agent is not currently suggested by some specialists for people with diabetes (*8*).

The confirmed increase in the consumption of nutritive sweeteners, particularly in snacks and beverages, has been related to excess energy intake and to lower quality diets. Still, the mechanisms underlying the interaction between sweeteners, among them fructose, and excess calorie intake are not wholly understood, turning this issue into a highly controversial matter. The sole recommendations advised by official health stakeholders are to limit the addition of sugars as simple calories to 10 to 25 % of the daily energy intake (*10*,*59*,*62*). In particular, the relatively recent trend to relate some of these nutritive sweeteners, namely fructose syrups, to obesity (*63*) has been strongly contested, particularly claims that sweeteners are a unique cause of such output (*9*,*64*).

Enzyme-based processes for fructose production

Fructose production using enzymes is performed through three different approaches, namely starch hydrolysis and isomerization of the resulting glucose residues, hydrolysis of sucrose, and inulin hydrolysis. The two former processes are somehow indirect methods, since the main product from the production process is HFCS or invert sugar syrup, respectively. Still, they are the processes currently implemented at industrial scale (*5*, *46*,*65*). Both HFCS and invert sugar syrup have significant, roughly equimolar, amounts of glucose, thus requiring a specific separation step to obtain pure fructose. Usually a chromatographic separation is used to achieve this goal, although other approaches have been tested, such as membrane separation, extraction with ionic liquids or selective precipitation (*46*,*65–74*). On the other hand, inulin hydrolysis can be specifically designed for the production of either fructose or fructans, where one of these carbohydrates is the targeted product, thus making it a promising approach (*5*,*75*,*76*).

Fructose from HFCS

Starch is the main reserve polysaccharide of many higher plants, up to 75 % of the dry mass in cereal grains, and up to 65 % in potato tubers. Corn, in particular, contains on average 71.1 % of starch (*77*). This carbohydrate is really a mixture of amylose and amylopectin. They are both polymers of α-D-glucopyranosyl units, but amylose is mostly a linear polymer whereas amylopectin is highly branched. Amylose is a polymer composed of 800 to 1500 glucose units which are mostly bound by α-D-(1→4) glucosidic linkages, combined with a limited amount of branching through α-D-(1→6) glucosidic linkages at the branch points. In amylopectin, which is virtually water insoluble, one in 20 to 25 glucose units is bound to another chain through a α-D-(1→6) glucosidic linkage. The

result is a tree-like structure that contains a wide amount of glucose residues, from 5000 to 40 000. Like amylose, amylopectin contains only one reducing end, *viz*. an anomeric hydroxyl group. The ratio of amylose to amylopectin varies considerably with the source of the starch, but typical values can be found within the range of 20 to 25 % of amylose to 75 to 80 % of amylopectin (*27,78, 79*). The production of HFCS usually starts with a 40 % suspension of starch, with pH adjusted to about pH=6.0, to which calcium ions and a thermostable α-amylase are added. Incubation at 105 °C for 5 to 8 min allows gelatinization, where intramolecular bonds of starch residues are broken down. The resulting mixture is then cooled to 95 °C and incubated at this temperature for roughly 2 h. This encompasses hydrolysis of starch to dextrins, which are later further hydrolyzed to glucose units, under incubation in the presence of glucomylase (and preferably also pullulanase). This process, termed saccharification, is carried out at 55 to 60 °C, under acidic conditions (pH=4.0 to pH=5.5). The resulting glucose-rich solution is then processed in a chromatographic step for colour and calcium removal. Magnesium ions are added to the glucose-rich effluent and glucose is isomerized to fructose, using immobilized xylose (glucose) isomerase. Glucose isomerase has been immobilized in a wide array of supports and using different techniques (*80–82*). Isomerization is performed in a packed bed reactor, under pH=7.5 to pH=8.2, and 55 to 60 °C. The effluent of the packed bed reactor contains about 42 to 45 % fructose. This isoglucose syrup is then fractioned using moving-bed cation-exchange chromatography to produce a 90 % (or higher) fructose syrup, which is then blended with the 42 % fructose syrup to yield a 55 % fructose syrup (*4,51,65,83*). This multienzyme approach is somehow limited by the diverse operational conditions required by the different enzymes (*82*). Efforts are therefore actively being made in order to obtain modified enzymes that: (*i*) can carry out their catalytic activity under more common operational conditions, namely temperature (are more thermostable) and pH (can operate in acidic environments); (*ii*) are more stable; and (*iii*) are less prone to substrate/product inhibition (*51,84–86*). This search for modified/novel enzymes that comply with such criteria is common to all enzymatic approaches designed for the production of fructose syrups. Other suggestive improvements of the enzymatic production of HFCS are at process level. The most relevant merge liquefaction and saccharification in a single step (*87*), merge saccharification and isomerization in a single step by co-immobilization of glucoamylase and glucose isomerase (*88*), or use reactive simulated moving bed technology to improve the fructose yield up to 90 % in the isomerization step (*67,89*).

Fructose from inverted sugar syrup

Fructose can also be produced from inverted sugar syrup obtained from the hydrolysis of sucrose, promoted by immobilized invertase. The inverted sugar syrup is more easily incorporated into industrial preparations and has more added value than sucrose (*75*). Sucrose hydrolysis catalyzed by immobilized invertase produces a high quality product with low amount of ashes and hydroxymethylfurfural. Enzymes can thus be considered as catalysts for the design of industrial scale processes aiming at the transformation of sucrose into inverted sugar syrup (*90–92*). Actually, invertase immobilized in bone char was used for the large-scale production of inverted sugar syrup during World War II, given the acid shortage. Once acid became available again, the enzymatic approach was discontinued (*93*). Virtually every method and support for enzyme immobilization has been tested on invertase (*51,94*). In typical processes, a sucrose solution is added to a packed bed/fluidized bed/membrane reactor, where enzymatic hydrolysis is performed at 40 to 60 °C and in a pH range from pH=4 to pH=6 (*89,95,96*). The resulting invert syrup is further processed in a chromatographic step in order to separate fructose and glucose. A fructose syrup can thus be obtained (*51,92,97*). Ion chromatography can also be used to remove residual intermediate products that are often formed during the enzymatic hydrolysis of sucrose (*98*). High substrate concentrations tend to lead to product inhibition, but on the other hand, they stabilize invertase (*99*).

Fructose from inulin

Inulin is a polysaccharide consisting of linear α-2,1--linked polyfructose units (*43*), most commonly ending with a glucose residue through a sucrose-type linkage at the reducing end (*100,101*). Inulin is a reserve carbohydrate found in roots and tubers of plants, vegetables and cereals, but the main sources of inulin are dahlia, chicory and Jerusalem artichoke (Table 3; *98,102*). Most of the inulin currently produced on industrial scale derives from chicory (*103*). The degree of polymerization, which defines the number of fructosyl residues, varies with the source, but it can range from 2 to 60, with an average of 12 in plant inulin (*104*). Plant inulin has a very small degree of branching (*5*), but again this feature varies according to the source of the fructan. The amount of β-(2→6) branches in inulin from chicory and dahlia is 1 to 2 % and 4 to 5 %, respectively (*105*). The linear chain in inulin is composed of either α-D-glucopyranosyl-(β-D-fructofuranosyl)$_{n-1}$-β-D-fructofuranoside or β-D-fructopyranosyl-(β-D-fructofuranosyl)$_{n-1}$-β-D-fructofuranoside (*7*). The solubility of inulin in water varies significantly according to the source and how inulin is processed. At 25 °C, standard chicory inulin has a water solubility of 125 g/L, whereas high performance chicory inulin a water solubility of 25 g/L, and artichoke inulin of 5 g/L (*102,106*).

Table 3. Occurrence of inulin in plants adapted from Farine *et al.* (*98*) and Coussement (*102*)

Inulin source	*w*(inulin)/%
Jerusalem artichoke	16 to 20
chicory	15 to 20
dahlia	10 to 12
leek	3 to 16
garlic	9 to 11
salsify	4 to 10
onion	2 to 10

Inulinases are enzymes that catalyze the hydrolysis of O-glycosyl bonds, and have fructans as typical substrates. Two classes of inulinases are active, endoinulinases (2,1-β-D-fructan fructanohydrolase, EC 3.2.1.7), which promote the endohydrolysis of 2,1-β-D-fructosidic linkages in fructans, and exoinulinases (β-D-fructan fructohydrolase, EC 3.2.1.80), which promote the hydrolysis of terminal, non-reducing 2,1- and 2,6-linked β-D-fructofuranose residues (*107*). The latter reaction is typical of invertase, hence inulinases are also active on sucrose, whereas invertase (β-fructofuranosidase, EC 3.2.1.26) has no noticeable hydrolytic activity on inulin (*108–110*). Inulinases can be found in plants, namely in tubers and roots, filamentous fungi, bacteria and yeasts. Microorganisms are the most favoured sources for the production of inulinases in a commercial scale, given the high yields of enzyme and the ease of cultivation (*111,112*). Among microorganisms, those that provide extracellular inulinases are preferred, since enzyme recovery and purification are usually easier and cheaper, when compared to processes involving the production of intracellular or periplasmic enzymes (*113*). The production of inulinases by microorganisms has been extensively reviewed recently (*113–115*). Within the microbial producers of inulinases, fungi and yeast are usually preferred, since the levels of inulinase are higher than in bacteria. However, the ability of many bacteria to endure high temperatures makes them eligible candidates for the screening of thermally stable inulinases (*114*). *Aspergillus* spp. cells are typically recognized as the most known and versatile producers of inulinase, and highly thermostable inulinases have been isolated from such sources, particularly from *A. fumigatus* (*112,114,116–119*). Production of inulinases on a large scale currently relies on *A. niger*, a GRAS microorganism. Yeasts also present some attractive features, namely their unicellular nature and relatively simple requirements for growth. *Cryptococcus aureus* G7a (*120*), *Pichia guilliermondii* (*121*) and *Kluyveromyces marxianus* (*122*) are promising yeast sources of inulinase, particularly the last one, which is recognized as GRAS and is accepted by the FDA for food processing (*123*). Intensive research has therefore been performed in order to improve inulinase production with *K. marxianus*. Particular efforts have been made at process level. These efforts include: (*i*) the selection of suitable medium compositions and operational parameters (*124–129*), (*ii*) process integration (*130*), (*iii*) mode of operation and reactor design (*131*), and (*iv*) downstream processing (*125,132*).

Most bacterial inulinases are exoenzymes, the major source of these enzymes being *Aspergillus* spp., *Kluyveromyces* spp. and *Streptomyces* spp. (*5,114*). It is difficult to assess whether the two forms of inulinase coexist, and given the similar properties of the two forms, their complete separation through conventional methods is difficult, requiring chromatographic steps and preparative electrophoresis (*133,134*). Given the synergistic action of endo- and exoinulinases on inulin, this fructan is easily hydrolyzed to fructose (*135*). Complete hydrolysis of inulin with inulinase may lead to a final mass fraction of fructose of 95 % when performed under optimized conditions, making this a promising approach for fructose production (Fig. 3). Besides, the yield in this one-step enzymatic approach clearly surpasses the roughly 45 % fructose yield from the multi-step starch hydrolysis and glucose isomerization (*5,136,137*). Unlike in acid hydrolysis, where a coloured hydrolyzate is obtained that contains difructose anhydride, a compound nearly devoid of sweetening properties, only a vague colouring is reported in inulin enzymatic hydrolysates, which have only slightly changed taste and aroma (*138*). The major inulooligosaccharides resulting from inulin hydrolysis with endoinulinases are also influenced by the source of the enzyme, substrate concentration and inulin source, but typical products are inulobiose, inulotriose, inulotetraose and inulopentose (*139–146*). In the last 10 years, dedicated research focused on inulinases and their practical applications has led to significant advances at microbiological, molecular biology and engineering levels. Efforts were successful in the isolation of new inulinase producers, among them producers of heat-stable enzymes, namely those with an optimum temperature of 60 °C and above (*114,116,147*), and in the cloning and detailed characterization of genes encoding inulinases from several microorganisms (*148*), as reviewed recently (*112*). On the engineering side, methodologies for the production and purification of inulinases have been improved, as reflected by some recent work (*127,128,130,131,149–153*), and strategies for enhancing fructose production from inulin have been developed or improved, as also re-

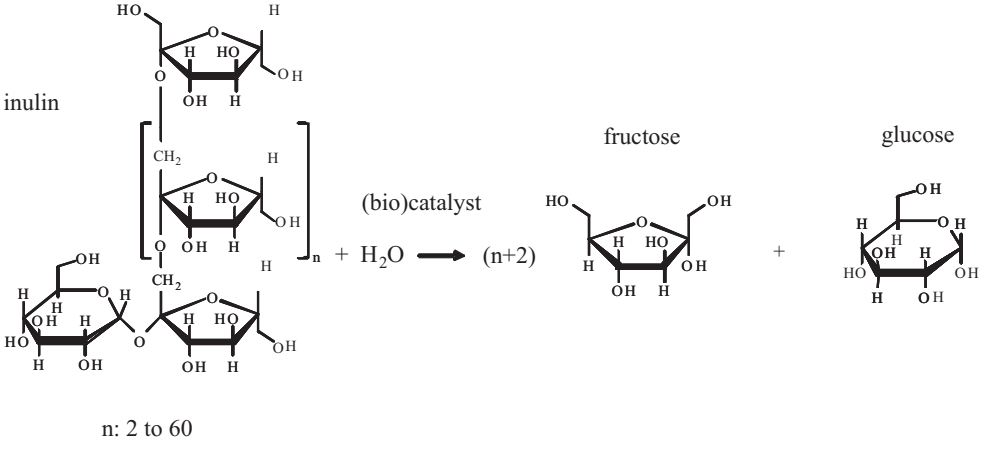

Fig. 3. Full inulin hydrolysis

viewed recently (*5*). Overall improvements of inulinase production processes have focused on: (*i*) the selection of cheap and widely available raw materials for use as substrate; (*ii*) the overproduction of the enzyme and optimization of production media and operational conditions, mode of operation and reactor design; and (*iii*) on suitable downstream processing, mostly involving chromatographic and electrophoretic steps. Alongside, efforts have been made to perform detailed process modelling of inulinase production and purification (*115*). Within the scope of enhancing the enzymatic production of fructose from inulin, recent work has focused on the improvement or introduction of novel immobilization strategies, evaluation of modes of operation and detailed process modelling (*154–159*). These processes allow finding the solutions for the complete hydrolysis of inulin with initial substrate concentration of 50 g/L or above and in the temperature range from 40 to 60 °C. Within the scope of developing such processes, care has to be taken as to identify inulinases with low fructosyl transfer activity and high hydrolytic activity, as occurs with the inulinase from *Kluyveromyces marxianus* var. *bulgaricus* ATCC 16045 (*160*).

Production of Fructose Syrup by Acid Hydrolysis

Fructose can also be obtained through the acid hydrolysis of either sucrose (and subsequent chromatographic separation from fructose) or of inulin, promoted by either homogeneous or heterogeneous catalysis. In the former case an inorganic acid (*viz.* hydrochloric or sulphuric) acts directly on the substrate (*161,162*), whereas in the latter case, the required H^+ ions result from an ion-exchange resins (*163,164*) or from zeolites (*165,166*). The use of sulphonic acid membranes has also been demonstrated (*167*). When homogeneous catalysis or ion-exchange resins are used, considerable formation of undesired, often coloured by-products, *viz.* hydroxymethylfurfural, is observed, due to the acid pH and, also in the case of acid hydrolysis, to the high temperature (*92,168,169*). Lowering the temperature and residence time in the column reduces by-product formation (*170*). The use of zeolites and sulphonic acid membranes as catalysts has been shown to allow for high conversion yields, under relatively mild conditions. Furthermore, the formation of by-products is residual, hence the mentioned catalysts also present an interesting platform for the production of fructose.

Conclusions

This work provides an overview of the relevant chemical, physical, sensorial and physiological aspects of fructose, a naturally occurring sweetener, and an insight into the currently available processes for fructose production, as well as into perspective developments in this field in the near future. The presented data clearly illustrate the key role of biotechnology in the production of this product, as well as the trends of the improvement of current production process strategies, including the development of novel approaches.

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