# REVIEW

# Fructosyltransferases: The Enzymes Catalyzing Production of Fructooligosaccharides*

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Received 30 March 2001

This article reviews the information about fructosyltransferases, the enzymes producing important prebiotic compounds, fructooligosaccharides. The chemical structure, safety and physiological effects, preparation and application of fructooligosaccharides are discussed. Plant and microbial sources of fructosyltransferases are presented. A detailed description is devoted to the mechanisms of action, substrate specificities, and regioselectivities of fructosyltransferases. Their physicochemical and catalytic properties are also discussed. Finally, the methods of purification of fructosyltransferases reported in literature are summarized in respect to the yield and factor of purification.

Fructooligosaccharides (FOS's) are nowadays considered natural food ingredients in most European countries. Because of their beneficial effects in proliferating bifidobacteria in human colon, FOS's are classified as prebiotics. Several reviews about their occurrence, preparation, and properties have been published so far [1—5].

Fructooligosaccharides can be synthesized in nature by the catalytic action of enzymes with transfructosylating activity. They are classified as $1^F$-fructosyltransferases (FTases, E.C. 2.4.1.9, E.C. 2.4.1.99, and E.C. 2.4.1.100), or $\beta$-fructofuranosidases (FFases, E.C. 3.2.1.26) [4]. These enzymes occur in many higher plants or can be produced by some microorganisms (*Aureobasidium* sp., *Aspergillus* sp.). The catalytic and physicochemical properties of FTases and FFases such as the optimal conditions for FOS's production, substrate specificity, regioselectivity, relative molecular mass, and degree of glycosylation vary from source to source, even from strain to strain. Plant fructosyltransferases are known to have various regioselectivities and many of them can produce oligosaccharides with different structure. The production of plant FTases is limited by seasonal conditions and the production yield of FOS's prepared by these enzymes is relatively small. The investigation of microbial fructosyltransferases is focused mainly on those producing $1^F$-type fructooligosaccharides. However, complex information about the properties and preparation of fructosyltransferases is still missing.

For that reason, the importance, catalytic and physicochemical properties, and methods of purification of fructooligosaccharide-producing enzymes are reviewed in this publication. Besides, the most important properties and preparation of fructooligosaccharides are presented, too.

## FRUCTOOLIGOSACCHARIDES

### Chemical Structure

Fructooligosaccharides are short oligosaccharides, which are members of a fructan group. Fructan is a general name used for fructose polymers having a general structure of glucose unit linked to multiple fructose units. There are several types of fructans present in nature. These types are distinguished on the basis of the glycosidic linkages by which the fructose residues are linked to each other. They can be divided into three groups. The first group are inulins, which are linear fructans, where the fructose units are mostly linked *via* a $\beta(2\rightarrow1)$ bond. The second group

*Presented at the 28th International Conference of the Slovak Society of Chemical Engineering, Tatranské Matliare, 21—25 May 2001.

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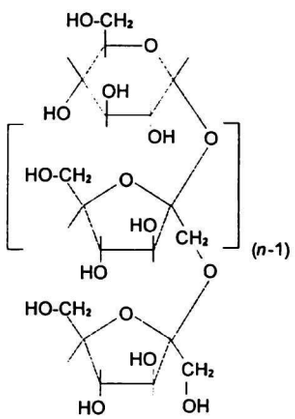
are levans, which are also linear fructans, but where the fructose units are mostly linked *via* a $\beta(2\rightarrow6)$ bond. The third group are the fructans of mixed type, which are also referred to as the graminan type. These fructans have both $\beta(2\rightarrow1)$ and $\beta(2\rightarrow6)$ linkage bonds between the fructose units, and thus contain branches. The term fructooligosaccharides (FOS's) is used for short inulin-type fructans with a terminal glucose group in which 2—4 fructosyl units are bound together *via* $\beta(1\rightarrow2)$-glycosidic bonds. Their structural formula is $\alpha$-D-glucopyranosyl-$(1\rightarrow2)$-[$\beta$-D-fructofuranosyl-$(1\rightarrow2)$-]$_n$, ($GF_n$)




where $n$ is typically 2—4. Individual FOS's are $n-1=1$, 1-kestose ($GF_2$), $n-1=2$, nystose ($GF_3$), and $n-1=3$, $1^F$-fructofuranosylnystose ($GF_4$), respectively.

## Safety and Physiological Effects

Numerous studies have been conducted to evaluate the potential toxicity of fructooligosaccharides to animals and men [3]. Their results provided no evidence that FOS's possessed any toxicological or carcinogenic material. FOS's are officially recognized as natural food ingredients in most European countries and have got a GRAS (generally regarded as safe) status in the USA [2].

Fructooligosaccharides are not significantly hydrolyzed by gastric acid and pancreatic enzymes [3, 6] and most of them reach the colon where they provide an energy source for beneficial bifidobacteria (*Bifidobacterium* sp.). Even a small amount of FOS's in the colon leads to an increase in bifidobacteria [1, 7]. Conversely, pathogenic and putrefactive bacteria including *Escherichia coli*, *Clostridium perfringens*, *Clostridium difficle*, and others have been shown to be unable to utilize the FOS's. FOS's are metabolized to short-chain fatty acids, which results in lowering the pH in the colon, which in turn restricts the growth of pathogenic bacteria. Because of this beneficial power of FOS's to proliferate bifidobacteria growth they are classified as prebiotics. The physiological properties of FOS's are reviewed by *Hidaka* [1], *Yun* [4], *Spiegel* [3], and *Roberfroid* [2, 5].

Because of the nondigestibility of FOS's in the small intestine and their fermentation in the colon, FOS's have a mildly laxative effect and a mild flatulence is frequently observed with subjects being fed by FOS's. This effect is not specific only for the FOS's but common to all nondigested/fermented dietary carbohydrates. Based on the published data about dose-dependent symptoms of intestinal discomfort, *Roberfroid* recommended a maximal daily dose of 20 to 30 g of FOS's [2] when the dose should be split into a few individual servings. In Japan, which is the world leader in the industrial production of FOS's and their application as food ingredients, an approved acceptable daily intake is about 0.8 g per kg of body mass [4].

## Applications

The energy value of FOS's is 4.2—9.5 kJ $g^{-1}$ [2, 6]. The sweet taste of FOS's is very similar to those of sucrose and the relative sweetness of 1-kestose, nystose, and $1^F$-fructofuranosylnystose to 10 % sucrose solution is 31 %, 22 %, and 16 %, respectively [4]. FOS's are highly hygroscopic and the viscosity of FOS's solutions is relatively higher than that of sucrose at the same concentration. The thermal stability of FOS's is also higher than that of sucrose [4]. These properties are utilized in food products such as yoghurts, functional dairy drinks, ice creams, biscuits, bakery products, *etc.*

## Occurrence and Preparation

Fructooligosaccharides naturally occur in many higher plants, for example, chicory, onion, wheat, barley, asparagus, and Jerusalem artichoke. FOS's can also be prepared from sucrose by the action of enzymes with fructosyltransferase activity when glucose and a small amount of fructose are formed as by-products. Fructooligosaccharides of different chain length constitute the major part of the mixture whereas glucose, fructose, and unconverted sucrose represent about 40—45 % of total sugars of commercial FOS's sirups [8—13]. The conditions of the preparation of fructooligosaccharides and properties of the enzymes having fructosyltransferase activity will be discussed in next sections.

# ENZYMES WITH TRANSFRUCTOSYLATING ACTIVITY

## Occurrence

The enzymes catalyzing the production of FOS's are classified as fructosyltransferases (FTase, E.C. 2.4.1.9), or $\beta$-fructofuranosidases (FFase, E.C. 3.2.1.26).

They occur in many higher plants such as asparagus [14], chicory [15], onion [16], Jerusalem artichoke [17—19], and grasses [20—27], or can be produced by some microorganisms. The microbial sources are fungi *Aureobasidium pullulans* [28—33], *Aspergillus niger* [13, 28, 34—37], *Aspergillus japonicus* [10, 11, 38, 39], *Aspergillus foetidus* [40, 41], *Aspergillus oryzae* [42], *Aspergillus sydowi* [43], *Fusarium oxysporum* [44], *Penicillium rugulosum* [45], *Scopulariopsis brevicaulis* [46], yeasts *Saccharomyces cerevisiae* [47], and bacteria *Bacillus macerans* [48].

## Mechanism of Action of β-Fructofuranosidase and Fructosyltransferase

### *β-Fructofuranosidase*

β-Fructofuranosidase (invertase) is an enzyme mainly hydrolyzing sucrose into glucose and fructose, but, depending on its origin, it can exhibit a significant transfructosylating activity at appropriate reaction conditions. There are two ways of the production of fructooligosaccharides by action of the β-fructofuranosidase on sucrose: reverse hydrolysis and transfructosylation.

The *reverse hydrolysis* is an equilibrium process in which the reaction equilibrium is shifted from the hydrolysis towards the synthesis of oligosaccharides according to the following scheme

$$\begin{array}{l} \mathrm{A{-}H + E \rightleftharpoons E{-}A \xrightleftharpoons{+B{-}OH} A{-}B + E + H_2O} \\ \qquad\qquad \uparrow\downarrow + H_2O \\ \qquad\qquad \mathrm{E + A{-}H} \end{array} \qquad (A)$$

where A is the fructosyl group of a donor, B the glycosyl group of an acceptor (sucrose, or a FOS), E the enzyme β-fructofuranosidase, A—B the product (a FOS by one fructosyl unit larger than the acceptor molecule).

In the first step of the reaction, an activated complex enzyme—donor is formed, which reacts with a hydroxyl group of an acceptor in the next step. The acceptor is either water (hydrolysis), or sucrose (reverse hydrolysis). The amount of produced oligosaccharide depends on the equilibrium constants of the two transformation reactions of the E—A complex to a free enzyme and fructose, or kestose. The increase of the substrate concentration and consequently the decrease of the water activity can increase the final FOS's yield.

In the case of *transglycosylation*, a fructosyl group is transferred from an activated donor, which is sucrose, to an appropriate acceptor. The reaction takes place in two steps when an activated complex donor—enzyme is formed in the first step

$$\begin{array}{l} \mathrm{A{-}B + E \rightleftharpoons B{-}OH + E{-}A \xrightleftharpoons{+C{-}OH} A{-}C +} \\ \qquad\qquad \uparrow\downarrow + H_2O \qquad\qquad + E + H_2O \\ \qquad\qquad \mathrm{E + A{-}H} \end{array} \qquad (B)$$

where A—B is the donor of fructosyl group (sucrose), B—OH glucose, C—OH the acceptor (sucrose, or a FOS), E the enzyme β-fructofuranosidase, A—C the product (a FOS by one fructosyl unit larger than the acceptor molecule).

Transglycosylation is usually a kinetically controlled process, as the product A—C is a potential substrate of fructofuranosidase action. Therefore, the fructooligosaccharides concentration depends on the rates of synthesis and hydrolysis reactions. *Hirayama* [34] observed that amount of total FOS's rose at the beginning of the reaction, then it reached a plateau and then dropped. This effect was significant especially in diluted solutions and at high conversion of sucrose. The high yields of FOS's are achieved by applying high sucrose concentration.

### *Fructosyltransferase*

Fructosyltransferase catalyzes the transfer of a fructosyl group to a molecule of sucrose or a fructooligosaccharide when a FOS with a chain longer by one fructosyl unit is formed, according to the following scheme

$$\mathrm{A{-}B + E \rightarrow B + E{-}A \xrightarrow{+C} A{-}C + E} \qquad (C)$$

where A—B is the donor (sucrose, or a FOS), E the enzyme fructosyltransferase, C the acceptor (sucrose, or a FOS), A—C the product (a FOS by one fructosyl unit larger than the acceptor molecule).

Fructosyltransferase shows a little affinity towards water as an acceptor, which means that the hydrolase activity of fructosyltransferases is very low. The yields of FOS's prepared by the action of the FTases are generally high even in dilute substrate solutions.

It was clearly demonstrated by many authors [15, 18, 19, 25, 49, 50] that in plants different enzymes are responsible for hydrolytic and transfructosylating activities, respectively. However, the nomenclature of fructooligosaccharide-producing enzymes of microbial origin remains still in dispute. Some authors use the term fructosyltransferase [31, 32, 35, 37, 40, 51, 52] whereas others designate it as β-fructofuranosidase (invertase) [9, 11, 30, 34, 38, 42, 46, 53—55]. Yeast invertase is able to synthesize fructooligosaccharides from sucrose probably by the reverse hydrolysis mechanism. This hypothesis was confirmed by *Andersen* [56] who observed the formation of a difructoside by the action of a pure fructofuranosidase on fructose. The second evidence should be that the yields of FOS's produced by invertase are low (maximum 4 % of total saccharides) even in high sucrose solutions [57]. Fructofuranosidases with high transfructosylating activity have different catalytic properties than the yeast invertase. The main difference is that FFases with high transfructosylating activity have a much higher affinity to sucrose or FOS's as acceptors of fructosyl

residue than to water. It means that they produce fructooligosaccharides probably by transfructosylating mechanism. This hypothesis has been supported by many studies [9, 11, 13, 34, 35, 38, 53] in which 0.5 mass % sucrose solution was used as a substrate and a mold FFase as an enzyme. The results showed that, in the initial stages of the reaction, 1-kestose and later also nystose were formed but the proportion of FOS's decreased at high degree of sucrose conversion and an equimolar ratio of glucose and fructose was achieved at the end of reaction. However, the purity of enzyme preparations used may have influenced these results. Owing to the similar nature of the enzymes with hydrolytic and transfructosylating activity, it is difficult to separate them and consequently, the two activities may appear to belong to a single enzyme. *L'Hocine* [37] successfully separated two enzymes, a fructosyltransferase and β-fructofuranosidase, from *Aspergillus niger* AS0023. The fructosyltransferase (E.C. 2.4.1.9) catalyzed exclusively fructosyl transfer reaction, while the β-fructofuranosidase (E.C. 3.2.1.26) did not show any transfer activity. Similarly, *Hayashi* [29, 30] isolated two extracellular and two intracellular fructosyltransferases, which did not show any hydrolytic activity.

It is obvious from the presented information that the results differ from one author to another and from one microorganism studied to another, and it is troublesome to make a clear conclusion. For that reason an accurate nomenclature of fructooligosaccharide-producing enzymes is still in question.

## Substrate Specificities of Fructosyltransferases

The substrate specificities of fructosyltransferases depend on the source of the enzyme. In plants, the long-chain fructooligosaccharides are synthesized from sucrose by synergic action of two fructosyltransferases; sucrose:sucrose $1^F$-β-D-fructosyltransferase ($1^F$-SST, E.C. 2.4.1.99) and 1,2-β-D-fructan:1,2-β-D-fructan $1^F$-β-D-fructosyltransferase ($1^F$-FFT, E.C. 2.4.1.100) [15, 19]. On the other hand, a single enzyme, 1-fructosyltransferase (1-FTase, E.C. 2.4.1.9), is present in most microorganisms.

$1^F$-SST catalyzes the irreversible transfer of a fructosyl unit from a donor molecule of sucrose (GF) to an acceptor molecule of sucrose when 1-kestose ($GF_2$) and glucose (G) are formed

$$GF + GF \xrightarrow{1^F\text{-SST}} GF_2 + G \qquad (D)$$

The elongation of FOS chain is performed by the action of $1^F$-FFT on fructooligosaccharides with at least two fructosyl units in the chain ($GF_n$, $n \geq 2$) which are both donors and acceptors. These reactions can be represented by the following stoichiometric equation

$$GF_n + GF_m \xrightarrow{1^F\text{-FFT}} GF_{n-1} + GF_{m+1}$$

$$n \geq 2,\ m \geq 2 \qquad (E)$$

1-Fructosyltransferase of microbial origin catalyzes the transfer of fructosyl residue in a series of disproportionation reactions where one molecule of sucrose or FOS serves as a donor and another acts as an acceptor

$$GF_n + GF_m \xrightarrow{\text{1-FTase}} GF_{n-1} + GF_{m+1} \quad n, m \geq 1 \qquad (F)$$

In this case, a mixture of fructooligosaccharides with a different chain length and glucose as a by-product are formed.

## Regioselectivity

The position of glycosidic bonds between fructosyl residues in resultant oligomer chain depends on the regioselectivity of fructosyltransferase. Regioselectivity is a specific ability of the enzyme to bind a transferred fructosyl residue into an exact position of the acceptor. In addition to the already mentioned $1^F$-fructosyltransferases, which catalyze the transfer of fructosyl groups to the primary hydroxyl of the terminal fructofuranoside, the $6^G$- and $6^F$-fructosyltransferases were identified in some plants and microorganisms (Table 1). $6^F$-FTases (*e.g.* levansucrase, E.C. 2.4.1.10) produce levan-type oligosaccharides with β(2→6) bonds between fructosyl units. Action of $6^G$-FTases on sucrose results in the formation of branched oligosaccharides with internal glucose. Branched fructans of graminan type result from the simultaneous action of $1^F$-, $6^F$-, and $6^G$-FTases on sucrose.

## Optimal Conditions for the Production of FOS's

High initial concentrations of sucrose (up to 850 g $dm^{-3}$) are recommended since a sirup containing high amount of FOS's is obtained in this way and evaporation cost for final processing is reduced. The yields of produced fructooligosaccharides range from 50 to 60 % of the total amount of saccharides at the initial sucrose concentration of 500—850 g $dm^{-3}$ [10, 11, 13, 34, 35, 37, 38, 54]. The high substrate concentration is also very useful, because it increases the ratio of transfructosylating and hydrolyzing activities of these enzymes when β-fructofuranosidases are used in enzyme reactions.

Operating temperatures for the FOS production by microbial fructosyltransferases range from 50 to 60 °C. At these temperatures, the viscosities of concentrated sucrose solutions are low enough, which significantly improves their processing. Elevated temperatures moreover favourably influence the reaction rate. A study of thermal stability of FTase from *Aureobasidium pullulans* [58] showed that sucrose has a strong

**Table 1.** Summary of Various Sources of Enzymes with Fructosyltransferase Activity and Oligosaccharides Produced by the Action of these Enzymes on Sucrose

| Source | Enzyme | Oligosaccharide | Ref. |
|---|---|---|---|
| *Aspergillus niger* | 1-FTase | 1,2-$\beta$ FOS's | [13, 34] |
| *Aspergillus japonicus* | 1-FTase | 1,2-$\beta$ FOS's | [11, 39] |
| *Aureobasidium pullulans* | 1-FTase | 1,2-$\beta$ FOS's | [66, 67] |
| *Aspergillus oryzae* | $1^F$-FTase | 1,2-$\beta$ FOS's | [42] |
| *Penicillium rugulosum* | $1^F$-FTase | 1,2-$\beta$ FOS's | [45] |
| *Aspergillus sydowi* | $1^F$-FTase, $6^G$-FTase | 1,2-$\beta$ FOS's, graminan-type fructans** | [43] |
| *Bacillus macerans* | $1^F$-FTase | $1^F$-fructofuranosylnystose | [48] |
| *Bacillus subtilis* | $6^F$-FTase | 1-kestose, levans | [68] |
| *Scopulariopsis brevicaulis* | $1^F$-FTase | 1,2-$\beta$ FOS's | [46] |
| Asparagus, onion | 1-SST, 1-FFT, $6^G$-FFT | 1,2-$\beta$ FOS's, 6-kestose*, graminan-type fructans** | [14, 16] |
| Chicory | 1-SST, 1-FFT | 1,2-$\beta$ FOS's | [15] |
| Grasses (wheat, barley, ryegrass) | 1-SST, 1-FFT, $6^G$-FFT, $6^F$-FFT | 1,2-$\beta$ FOS's, 2,6-$\beta$ FOS's, graminan-type fructans** | [22, 24, 25] [20, 21, 23, 69, 70] |
| Jerusalem artichoke | 1-SST, 1-FFT | 1,2-$\beta$ FOS's | [17—19] |

*6-Kestose has the chemical structure $\alpha$-D-glucopyranosyl-(1→2)-$\beta$-D-fructofuranosyl-(6←2)-$\beta$-D-fructofuranoside.
**Graminan-type FOS's have the chemical structure $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_m\text{-}6^G(1\text{-}\beta\text{-D-fructofuranosyl})_n$-sucrose, $m \geq 0$, $n \geq 1$.

stabilizing effect on the FTase activity. The optimal pH for the production of FOS's ranges from 5.0 to 6.5 with the exception of the enzymes from *Aspergillus oryzae*, *Aspergillus phoenicis*, and *Scopulariopsis brevicaulis* [42, 46, 59] which have optimal pH's of 8—9. Plant fructosyltransferases isolated from tubers of Jerusalem artichoke had optimal pH's and temperatures 3.5—5 and 20—25 °C in the case of 1-SST, and 5.5—7 and 25—35 °C for 1-FFT, respectively [17, 18].

## Inhibition

The production of FOS's by the action of fructosyltransferases is competitively inhibited by glucose which is formed as a by-product in the transfer reaction [60]. As a result, most commercial FOS products contain 50—60 % of FOS's, 25—30 % of glucose, 10—15 % of unconverted sucrose, and a small amount of fructose. An approach to improve the sucrose conversion and increase the yield of FOS's was proposed by *Yun et al.* [61] and *Jung et al.* [51]. They used a mixed enzyme system of fructosyltransferase and glucose oxidase for FOS's synthesis. Due to the conversion of glucose to gluconic acid by the action of glucose oxidase the amount of free glucose in the reaction mixture decreased and its inhibition effect was diminished. The FOS's content in product sirup after gluconic acid removal was 98 % [61] and 89 % [51], respectively.

Cations of some metals such as $Cu^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $Ag^{+}$, $Zn^{2+}$, and $Fe^{2+}$ are known to act as non-competitive inhibitors [11, 29—31, 34, 37, 46, 53, 54, 62]. The sensitivity of various FTases to particular cations depends mainly on the source of enzyme. The treatment of microbial fructosyltransferases and fructofuranosidases with sulfhydryl reagents such as *N*-bromosuccinimide and *p*-chloromercuribenzoate results in their inhibition or even inactivation [30, 31, 37, 53, 54, 63]. These results indicate that some thiol groups are probably located at or near the active sites of the enzymes.

## Physicochemical Properties

The relative molecular mass, number of monomers in an active protein molecule, and degree of glycosylation of fructofuranosidases and fructosyltransferases depend on the source of a particular enzyme. The native forms of FFases and FTases originated from *Aureobasidium pullulans*, *Aspergillus niger*, *Aspergillus japonicus*, *Aspergillus foetidus*, and *Fusarium oxysporum* have the relative molecular mass between 180 000 and 600 000 as is shown in Table 2. These enzymes are homopolymers with two, three, four, or six monomer units each having $M_r$ from 90 000 to 125 000 [11, 34, 37, 41]. But, the fructofuranosidase isolated from *Aspergillus oryzae* is a monomer with $M_r$ of 87 000—89 000 [42, 53]. Plant fructosyltransferases are generally smaller than the microbial ones, with $M_r$ of about 60 000—85 000. They are formed from two different subunits having $M_r$ of about 50 000—60 000 and 22 000—27 000, respectively [17—19, 22, 24, 25, 50].

Microbial and plant FFases and FTases are glycoproteins containing 20—50 % of carbohydrates in

**Table 2.** Methods of Purification and Selected Properties of Microbial and Plant Fructosyltransferases

| Source | $M_r$<br>Ternary structure<br>pI | Purification steps | Yield/%<br>(Purification factor*/fold) | Ref. |
|---|---|---|---|---|
| *Aureobasidium pullulans*<br>ATCC 20524, intracellular | P1#: 318 000<br>P2: 346 000<br>Dimers<br>N.D. | Ethanol and calcium acetate precipitation, chromatography (ion-exchange, size-exclusion) | P1: 3.8 (267)<br>P2: 2.3 (94) | [30] |
| *Aureobasidium pullulans*<br>ATCC 20524, extracellular | E1#: 304 000<br>E2: 315 000<br>Dimers<br>N.D. | Ethanol and ammonium sulfate precipitation, chromatography (ion-exchange, size-exclusion) | E1: 2.6 (84)<br>E2: 0.7 (74) | [29] |
| *Aureobasidium pullulans*<br>C-23, intracellular | 190 000 (monomer)<br>N.D.<br>3.7 | Ethanol precipitation, ion-exchange chromatography, gel electrophoresis | 10 (197) | [31] |
| *Aspergillus japonicus*<br>TIT-KJ1, intracellular | 230 000<br>Dimer<br>N.D. | Acetone precipitation, size-exclusion chromatography, isoelectric focusing | 46 (8.8) | [11] |
| *Aspergillus japonicus*<br>TIT-KJ1, intracellular | 230 000<br>Dimer<br>4.2 | Acetone precipitation, isoelectric focusing | 14 (24) | [9] |
| *Aspergillus japonicus*<br>MU-2, intracellular | 304 000<br>N.D.<br>N.D. | Calcium acetate and ammonium sulfate precipitation, chromatography (ion-exchange, size-exclusion) | 2 (83) | [54] |
| *Aspergillus niger*<br>ATCC 20611, intracellular | 300 000<br>Trimer<br>4.5 | Acetone precipitation, isoelectric focusing | 14 (23) | [9] |
| *Aspergillus niger*<br>ATCC 20611, intracellular | 300 000<br>Trimer<br>N.D. | Calcium acetate and ammonium sulfate precipitation, chromatography (ion-exchange, size-exclusion) | 10 (51.6) | [34] |
| *Aspergillus niger* AS0023,<br>intracellular | 190 000 (monomer)<br>Di-, tetra-, or hexamer<br>N.D. | Ammonium sulfate precipitation, chromatography (ion-exchange, size-exclusion, ConA affinity) | 16 (78.5) | [37] |
| *Aspergillus niger*,<br>extracellular | N.D.<br>N.D.<br>N.D. | Ammonium sulfate precipitation, ion-exchange chromatography | 76 (138) | [35] |
| *Aspergillus foetidus*,<br>extracellular | 180 000<br>Dimer<br>N.D. | Ammonium sulfate precipitation, chromatography (ConA affinity, ion-exchange) | 27.7 (87.5) | [41] |
| *Aspergillus oryzae*<br>ATCC 76080, extracellular | 87 000<br>Monomer<br>N.D. | Ion-exchange chromatography, isoelectric focusing, size-exclusion chromatography | 10 (37.7) | [53] |
| *Aspergillus oryzae*<br>(Denazyme AP) | 89 000<br>Monomer<br>N.D. | Ammonium sulfate and acetone precipitation, chromatography (size-exclusion, ion-exchange) | 12 (37) | [42] |
| *Aspergillus sydowi*<br>IAM 2544, intracellular | 50 000 (monomer)<br>N.D.<br>N.D. | Chromatography (ion-exchange, hydrophobic interaction) | 44 (25) | [62] |
| *Scopulariopsis brevicaulis*<br>N-01, intracellular | 220 000<br>Dimer<br>N.D. | Chromatography (hydrophobic interaction, ion-exchange, size-exclusion, hydroxyapatite) | 4.6 (1 100) | [46] |
| Barley (*Hordeum vulgare*)<br>1-SST | 72 000<br>Dimer with the $M_r$ of subunits 50 000 and 22 000<br>4.93—4.99 | Precipitation by ammonium sulfate, chromatography (ConA affinity, hydroxyapatite), isoelectric focusing | 10.3 (196.5) | [24] |

**Table 2** (Continued)

| Source | $M_r$<br>Ternary structure<br>pI | Purification steps | Yield/%<br>(Purification factor*/fold) | Ref. |
|---|---|---|---|---|
| Barley (*Hordeum vulgare*)<br>1-SST, 6-SST, INV | N.D.<br>N.D.<br>N.D. | Precipitation by ammonium sulfate, chromatography (anion-exchange, hydrophobic interaction)<br>only partial purification | 1-SST: 103 ( – )<br>6-SST: 93 ( – )<br>INV I: 44 ( – )<br>INV II: 25 ( – ) | [25] |
| Jerusalem artichoke (*Helianthus tuberosus*)<br>1-FFT | 70 000<br>N.D.<br>4.5—5.0 | Precipitation by ammonium sulfate, chromatography (ion-exchange, hydrophobic interaction, size-exclusion) | 24.6 (205) | [17] |
| Jerusalem artichoke (*Helianthus tuberosus*)<br>1-SST | 90 000<br>Dimer with the $M_r$ of subunits<br>55 000 and 27 000<br>N.D. | Precipitation by ammonium sulfate, chromatography (ion-exchange, hydrophobic interaction, ConA affinity, hydroxyapatite) | 3 (655) | [18] |
| Jerusalem artichoke (*Helianthus tuberosus*)<br>1-SST | 81 000<br>Dimer with the $M_r$ of subunits<br>59 000 and 26 000<br>4.89—5.09 | Precipitation by ammonium sulfate, chromatography (ConA affinity, hydroxyapatite, hydrophobic interaction, ion-exchange) | 0.07 (100) | [19] |
| Rye grass (*Lolium rigidum*)<br>SST | 84 000<br>N.D.<br>N.D. | Precipitation by ammonium sulfate, chromatography (ConA affinity, size-exclusion, ion-exchange)<br>only partial purification | 0.2 (214) | [22] |
| Rye grass (*Lolium rigidum*)<br>1-FFT<br>$6^G$-FFT | 50 000—60 000<br>N.D.<br>4.6—4.7 | Precipitation by ammonium sulfate, chromatography (ConA affinity, size-exclusion, ion-exchange), isoelectric focusing | 1-FFT 0.08 (319)<br>$6^G$-FFT 0.07 (292) | [50] |

N.D. – not detected.
* Purification factor is calculated as the ratio of specific activities at the end and beginning of purification process.
#P1, P2, E1, and E2, respectively, denote identification codes of different enzyme forms isolated from the same source.

the mass of their molecules. The glycosylic part of the enzymes is formed mainly by glucose and mannose oligomers, which results in their strong affinity towards the lectin concanavalin A. Deglycosylation causes an increase of susceptibility of the enzymes to the action of inorganic and organic inhibitors but has no effect on the activity and specificity of FTases [63—65].

## Isolation and Purification

Detailed studies of physicochemical and catalytic properties of enzymes necessitate the use of the enzymes free from other enzymes and substances that are contained in a source material. The methods of isolation (*e.g.* getting the enzyme into a solution) of fructosyltransferases and fructofuranosidases depend mainly on the character of source material. The isolation of plant enzymes requests the mashing of plant tissues and extraction of soluble proteins by suitable aqueous solutions. In the case of microbial FTases, the isolation methods depend on whether the enzyme is in an extracellular or intracellular form. A crude extract of extracellular enzyme is recovered from the fermentation broth using typical processes for cell removal. If the enzyme is inside the cells, it must be released into solution by mechanical or enzymatic disintegration of cells. Solids are then removed by centrifugation or filtration. Thereafter it can be concentrated by dialysis, ultrafiltration or protein precipitation by salts (ammonium sulfate, calcium acetate) or organic solvents (ethanol, acetone). Some examples of application of mentioned methods are shown in Table 2.

Proteins in solution are further purified using liquid chromatography and/or electrophoresis. The chromatographic modes useful for protein separation are ion-exchange, size-exclusion, affinity, and hydrophobic interaction chromatography. As an affinity ligand lectin concanavalin A can be effectively used because there are strong interactions between the lectin and mannose and glucose groups in the glycosylic moieties of FTases. From electrophoretic techniques, the applicable ones are nondenaturing electrophoresis and isoelectric focusing. As can be seen from a summary of applications of the methods of purification of mi-

crobial and plant fructosyltransferases in Table 2, the yields of recovered enzyme expressed through enzyme activities range from 4.6 % to 46 % for microbial FTases and from 0.07 % to 24.6 % for plant FTases. It is also obvious from this table that the higher the purification factor, the lower the yield of the enzyme since the multiple purification steps applied result in the loss of active enzyme.

*Acknowledgements. This work was supported by the Slovak Grant Agency for Science VEGA (Grant No. 1/7343/20).*

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