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REVIEW ARTICLE

Inulin Biosynthesis Genes in Gembili (*Dioscorea esculenta*) and Future Applications for Food Industries in Indonesia

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A B S T R A C T

Inulin can be found in abundance in nature, this form of carbohydrate (fructans) is utilized by plants as an energy storage and possibly for other uses. Humans use inulin as a dietary fiber and prebiotics to improve the health of their digestive system or to improve the physicochemical and sensory properties of foods. Gembili (*Dioscorea esculenta*) is a type of yam that grows well in Indonesia and contains inulin up to 14.77% of its dry weight. The current state of inulin production utilizes conventional extraction from tubers; thus, the demands of the market cannot be satisfied. Biotechnological approaches such as incorporating genes that encode the enzymes involved in inulin biosynthesis (1-SST and 1-FFT) into microbial hosts can be exploited to improve yield and sustainability. Therefore, the genetic information of the genes responsible for inulin biosynthesis in gembili needs to be elucidated first.

Keywords: ***1-SST, Inulin, Gembili, Heterologous expression***

H I G H L I G H T S

- Gembili tuber is an Indonesian yam tuber having a high inulin content.
- Inulin is a good source of prebiotic dietary fiber.
- Conventional inulin production is unable to meet the increasing demand.
- Inulin-producing genes from Gembili can be transferred to microbial hosts.
- Lack of genomic information on the inulin-producing genes makes the biotechnological inulin production challenging.

INTRODUCTION

Dietary fiber holds high potential as food additives and functional food ingredients and thus has been widely used in the food and pharmaceutical sectors (Hussain *et al.*, 2020). Many food products such as bakery goods, drinks, and meat products are supplemented with dietary fibers to increase their health benefits such as improving cardiovascular health (Evans, 2020), normalizing blood cholesterol and sugar levels (Zhu *et al.*, 2018), and modulating gut microbiomes (Myhrstad *et al.*, 2020). Dietary fiber is edible plant cell components that are resistant to enzymatic digestion and thus indigestible in the human digestive system (Dhingra *et al.*, 2012). There are two types of dietary fibers based on their water solubility. Insoluble fiber does not dissolve in water and is not fermented by the gut microbiota – this typically includes cellulose, hemicellulose, lignin, and pectin (Swann *et al.*, 2020). Soluble fiber dissolves in water, is generally non-viscous, and is fermentable

by the gut microbiota – some of its examples are inulin, fructooligosaccharides (FOS), and wheat dextrin (McRorie Jr. & McKeown, 2017).

Inulin is labeled by the FDA as a dietary fiber in 2018 (United States Food and Drug Administration, 2018) but has long been studied for its prebiotic properties (Carlson *et al.*, 2018). Inulin is fermentable by *Bifidobacteria* and *Lactobacillus* (Zeaiter *et al.*, 2019; Renye *et al.*, 2021) which are widely known as probiotics in humans. Strains of *Bifidobacterium longum* can increase tight-junction proteins in normal human epidermal keratinocytes (NHEKs) thus reducing pathogen penetration by improving barrier function (Marras *et al.*, 2021). *Lactobacillus plantarum* ECGC13110402 has a high bile salt hydrolase activity and can lower the cholesterol level in hypercholesterolemic adults as recently reported in a controlled randomized trial (Keleszade *et al.*, 2022). The application of inulin in food products such as yogurt and fermented milk increases the viability and probiotic activity of these bacteria in the food matrix (Moghadam *et al.*, 2019). Aside from its prebiotic functions, inulin is also utilized as a fat substitute for several dairy products such as ice cream (Samakradhamrongthai *et al.*, 2021) and cheese (Aydinol & Ozcan, 2017) due to its ability to give a fat-like mouthfeel, sensory, and texture while providing significantly fewer calories (Akbari *et al.*, 2019).

Currently, the demand for inulin for food production in Indonesia is met through import. Indonesian Central Bureau of Statistics (BPS) reported that in January 2022 Indonesia imported ~29,6 kilotons of mill industry products, including inulin, worth US$ 17,330,309.00. These are more than double the export volume (~10,4 kilotons) and value (US$ 7,962,954.41) in the same period (Badan Pusat Statistik, 2022). To reduce the dependency on imports, local plants are currently being explored as a potential source of inulin. Gembili tubers (*Dioscorea esculenta*), a type of yam tubers commonly found in Indonesia, are known to contain 2.8 – 14.77% inulin based on its dry weight, which is one of the highest inulin contents among yam tuber species (Winarti & Saputro, 2013). Studies using fecal samples taken from healthy human volunteers demonstrated that the addition of inulin-containing gembili extract increases the growth of *Bifidobacteria* and *Lactobacillus* and the synthesis of short-chain fatty acids (SCFA) – an indicator of sugar utilization – while reducing the growth of pathogens (*Clostridium* and *Bacteroides*) in anaerobic conditions (Khasanah *et al.*, 2019).

Improving inulin content in source plants and diversification of production methods is crucial to ensure supply and promote sustainability. Modification of growth conditions is possible to enhance inulin content in plants. For example, the addition of potassium fertilizer from 150 to 350 kg ha-1 increases inulin content in Jerusalem artichoke (*Helianthus tuberosus L*.) cv. Topstar by 13.2% (Michalska-Ciechanowska, 2020). However, this approach would be resource-intensive and too reliant on the increasingly unstable supply of fertilizers (Quinn, 2021; Helmore, 2022). The biotechnology approach offers a promising alternative for large-scale inulin production. Tissue culture, for instance, can generate inulin-producing plants at a high speed and large scale (Karimi *et al.*, 2021) or boost inulin accumulation in the plant biomass using specific elicitors (Taha *et al.*, 2012; Ma *et al.*, 2017). Genetic engineering can generate transgenic source plants with an improved inulin yield. Overexpression of inulin biosynthetic enzymes has been successfully applied to chicory (*Cichorium intybus* L. var. *sativum*) cv. 'Melci' (Maroufi *et al.*, 2018). Moreover, transgenic methods allow for inulin production in non-fructan accumulating plants (van Arkel *et al.*, 2013).

Another potential strategy is microbial cell factories engineered for inulin production. The inulin biosynthetic gene sucrose:sucrose 1-fructosyltransferase (1-SST) isolated from Tall fescue (*Schedonorus arundinaceus*), a perennial grass native to Europe, has been expressed in the yeast *Pichia pastoris* (Hernández *et al.*, 2018; Garcia *et al.*, 2020; Pérez *et al.*, 2021). The enzyme was successfully secreted without compromising glycosylation or substrate specificity and caused no cell toxicity (Hernández *et al.*, 2018). Culture supernatants containing the enzyme can be prepared as water-soluble powder stable for one year upon storage (Garcia *et al.*, 2020). Despite these advances, studies on inulin biosynthetic genes in gembili are still scarce due to the unavailability of its whole genomic information. Consequently, its exploration and utilization for biotechnological inulin production are very limited. This paper will discuss inulin, gembili as a

potential source of inulin, expression of inulin biosynthesis genes in heterologous hosts, and recent efforts to isolate inulin biosynthesis genes from gembili tubers. The information provided should be useful to evaluate the potential of sustainable inulin production using biotechnology approaches in Indonesia.

INULIN: SOURCES, STRUCTURE, AND PRODUCTION

Inulin is a type of fructan polysaccharide that is commonly found as the natural energy storage in dicot plants. It is found in approximately 3,000 species of vegetables and fruits such as onion, leek, garlic, banana, and wheat which have been consumed by humans for centuries (Shoaib *et al.*, 2016). Inulin was discovered in the early 1800s by Valentine Rose, a German scientist named, from the roots of Elecampane (*Inula helenium*) upon which it was named inulin by Thomson in 1817 (Shoaib *et al.*, 2016). Several plants considered the ideal natural sources of inulin include chicory roots (*Cichorium intybus* L.), dahlia tubers (*Dahlia* L. spp.), and Jerusalem artichoke tubers (*Helianthus tuberosus* L.). Novel sources such as globe artichoke inflorescence (*Cynara cardunculus* L.) are currently being explored (Shoaib *et al.*, 2016; Redondo-Cuenca *et al.*, 2021). Other than plants, microorganisms such as *Streptococcus* and *Aspergillus* have been reported to be able to produce inulin (Barclay *et al.*, 2010).

Growth conditions, abiotic stresses, and developmental stages affect inulin content in plants to a large extent. Chicory grown at 5 ^{o}C above the ambient temperature has a higher inulin content in their roots but with a reduced total amount of inulin per plant due to inhibition of sugar translocation from leaves to secondary roots (Mathieu *et al.*, 2018). Inulin is known to be actively synthesized in plants under drought and cold stresses and confirmed to confer tolerance to those conditions (de Roover *et al.*, 2000; Kawakami *et al.*, 2008; Livingston III *et al.*, 2009). Inulin stabilizes cellular membranes during drying or freezing by inserting its polysaccharide portion into membrane lipids which helps prevent leakage during water removal from the system (Livingston III *et al.*, 2009). As with other fructans, inulin can serve as reactive oxygen species (ROS) scavengers (Shang *et al.*, 2018). Under stress conditions, abscisic acid (ABA) accumulates in chicory plants which leads to a higher inulin content (Mohammadi *et al.*, 2021). Similar results were observed when exogenous ABA and a high concentration of auxin or ethylene were added. High inulin content is also linked to carbohydrate mobilization during energy-intensive developmental processes, as reported during stalk elongation and grain ripening in the wild cardoon roots (*Cynara cardunculus* L. var. *sylvestris* Lam.) (Branca *et al.*, 2022).

Inulin is a polymer of fructose linked by β(2□1) D-fructosyl-fructose linkage and terminated by a (1↔2) bonded β-D-glucosyl group (Figure 1) (Glibowski & Bukowska, 2011). The chains are composed of 2 to 60 fructose monomers, but shorter ones (up to 10 fructose monomers) are more referred to as oligofructose (Mensink *et al.*, 2015). Inulin is indigestible to humans because their digestive system lacks the enzymes needed to break the β-linkage of the fructose polymer. Inulin is a polydisperse fructan, having a wide molecular weight distribution due to varying degrees of polymerization (DP) (Mensink *et al.*, 2015). The plant species, climatic conditions, time of harvesting, post-harvest storage conditions, and processing methods all affect the polydispersity of inulin, which in turn influences its physicochemical properties and functionality (Van den Ende *et al.*, 2000; Mensink *et al.*, 2015). Plant inulin has a relatively low maximum DP (< 200) and hence a lower molecular weight (Mensink *et al.*, 2015; Shoaib *et al.*, 2016). On the contrary, bacterial inulin has a very high maximum DP (10,000 to 100,000) and the structure is 15% more branched compared to plant inulin (Öztürk, 2016; Shoaib *et al.*, 2016).

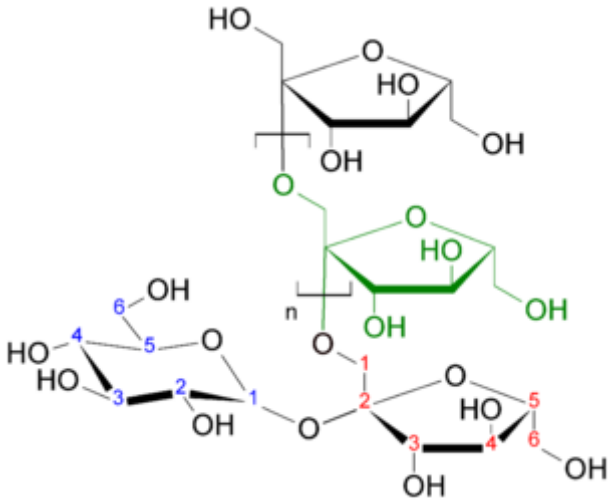

Figure 1. Chemical structure of inulin (Source: Wikipedia).

Until now, commercial inulin is predominately extracted from chicory root due to its high inulin content (14.9–18.3 %) and high tolerance to cold weather (Zhu *et al.*, 2016; Redondo-Cuenca *et al.*, 2021). At the end of the growing season, the inulin content of chicory roots harvested in Western Europe could reach up to 20% of its wet weight or 90% of its dry weight (van Laere & van den Ende, 2002) with a productivity of 5-11 tons/ha (Zhu *et al.*, 2016). The traditional production process of inulin is done in two stages: extraction and purification (Figure 2). In the extraction step, chicory roots are sliced and then boiled at 70–80 °C for 1 to 2 h to obtain an impure juice containing inulin and other water-soluble components. The purification step consists of liming ($CaCO_3$ precipitation) and carbonation – to remove degraded proteins and colloids – followed by filtration. Further refinements are done through demineralization using ion-exchange resins and decolorization using active carbon. The final step includes evaporation and spray-drying to obtain pure inulin powder which is then stored as the end product.

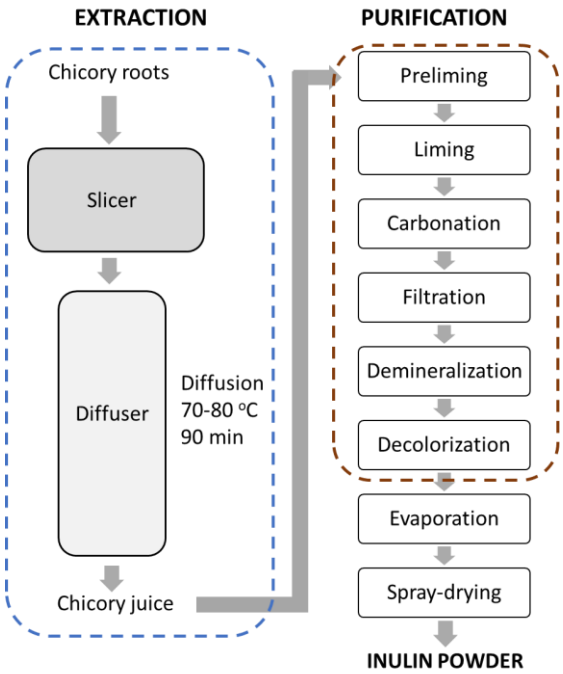

Figure 2. The conventional process for inulin production from chicory roots (adapted from Zhu *et al.*, 2016).

Despite its application for large-scale production, this conventional process is both energy-intensive and time-consuming (Zhu *et al.*, 2016). Therefore, novel procedures such as enzyme-assisted extraction (Chikkerur *et al.*, 2020), ultrasounds (Xu *et al.*, 2021), microwaves (Petkova *et al.*, 2018), and pulsed electric fields (Zhang *et al.*, 2021) are currently being explored for inulin extraction. Ultrasound-assisted extraction, for example, can reduce the power requirement up to 5 times compared to conventional rotary-type extractors, thus lowering the production costs (Yankevich *et al.*, 2022). These improvements would also make it possible to obtain pure inulin with several degrees of polymerization such as native inulin (DP of 10-12), short-chain inulins (DP of 3-10), and a high performance (HP) inulin (DP > 23) (Dehghan *et al.*, 2013).

INULIN APPLICATIONS IN THE FOOD SECTOR

Inulin has various applications in food processing due to its unique properties. The β(2□1) D-frutosyl-fructose bonds between the fructose unit in inulin along with their β configuration of anomeric carbon make it resistant to starch- and sucrose-degrading enzymes in the upper gastrointestinal tract (Apolinário *et al.*, 2014). Therefore, inulin remains intact in the large intestine and can serve as a prebiotic dairy fiber (Teferra, 2021). Compared to complex oligofructoses with different types of glycosidic bonds, inulin is more readily fermentable by the colonic microbiota. The β(2□1) bond in inulin is more accessible to bacterial hydrolysis (Miremadi & Shah, 2021). Colon bacteria with inulinase activity (*Bifidobacteria*, *Lactobacillus*, and *Lactococcus*) convert inulin into SCFA which are then absorbed by the colonic mucosa and helps supply energy molecule to the host (Weitkunat *et al.*, 2015). Inulin fermentation also produces lactate and gases, creating a low pH environment in the gut which enhances the colonization resistance against pathogenic bacteria (Teferra, 2021). A recent randomized, controlled trial on healthy adults reported that the prebiotic intervention using Orafti®, a commercial inulin supplement, led to an increase in *Bifidobacterium* and *Faecalibacterium* and a decrease in the number of *Coprococcus*, *Dorea*, and *Ruminococcus* (Healey *et al.*, 2019). Increased viability of these beneficial microbiomes aids in outcompeting the detrimental strains (van Arkel *et al.*, 2013; Maroufi *et al.*, 2018). Both short (DP < 10) and long (DP ≥ 25) have been reported to exhibit prebiotic effects (Ozturkoglu-Budak *et al.*, 2019; Ruan *et al.*, 2019)

The application of insulin in the food sector is not limited to prebiotic dietary fibers, but also as a bulking agent, sugar replacer, and fat replacer (Mensink *et al.*, 2015). As a bulking agent, inulin helps to relieve constipation and increase stool rate (Shoaib *et al.*, 2016). High DP inulin (DP ≥ 25) can be used as a carbohydrate-based fat substitute because it may form microcrystals and interact to create a smooth creamy texture. These characteristics can increase viscosity, mouthfeel texture, and water-holding capacity when used in food materials (Öztürk, 2016; Iraporda *et al.*, 2019). Due to its indigestibility, inulin has a low caloric value (1.5 kcal/g or 6.3 kJ/g) and provides only 25-35% energy compared to digestible carbohydrates (Roberfroid, 1999). The sweetness level of inulin depends on its polymer length. Low DP inulin (DP <10) is much more soluble and sweeter than the less soluble, long-chain inulin which almost has no taste (Teferra, 2021). The sweetness level of low DP inulin is around 35 to 55% as sweet as sucrose (Teferra, 2021). It could be used to replace sugar in chocolates, dairy products, and meat products (van Arkel *et al.*, 2013; Shoaib *et al.*, 2016). In addition, inulin can also be used as the precursor for alcohol and concentrated fructose syrup production (van Arkel *et al.*, 2013). Unlike other fibers, inulin has no off flavors and contributes very little to viscosity which allows for the formulation of high fiber foods with similar appearance and taste to standard food formulations (Moghadam *et al.*, 2018).

Due to its various uses in the food industry, the demand for inulin is high. The global inulin market is projected to increase with a forecasted CAGR of 9.5% from 2018 to 2026 (Inkwood Research, 2017). This increase is driven by different factors such as the growing popularity of prebiotic ingredients, the growing dairy industry, the increasing number of diabetic patients, and the growing demand for natural ingredients

(Inkwood Research, 2017). There has also been an increased interest in the food industry to produce healthier and low-fat food with the same sensory characteristics (Zhu *et al.*, 2016).

INULIN BIOSYNTHESIS

Inulin is synthesized in plants using sucrose as a precursor. Inulin biosynthesis proceeds at its optimal temperature of 25 °C but could retain its activity even at a lower temperature (van Laere & van den Ende, 2002). The production of inulin in plants is catalyzed by two fructosyltransferase enzymes: sucrose:sucrose 1-fructosyltransferase (EC 2.4.1.99) (1-SST) and fructan:fructan 1-fructosyltransferase (EC 2.4.1.100) (1-FFT) (Barclay *et al.*, 2010). Their reactions are based on the transfer of fructosyl units between reactants. Both enzymes are active in the plant vacuole (Beck, 2017). The third class of enzymes, fructan exohydrolase (EC.3.2.1.153) (1-FEH), catalyzes inulin degradation by hydrolyzing terminal fructosyl units, generating fructose and lower DP inulin (van den Ende *et al.*, 2013).

1-SST catalyzes the first reaction in which two sucrose molecules, each consisting of glucose and fructose are converted into trisaccharide 1-kestose, leaving one glucose as a by-product (Figure 3). 1-kestose is considered the shortest inulin (DP = 3). 1-SST has a high specificity for sucrose with negligible activity against the trisaccharide donor or with glucose (van den Ende *et al.*, 2013). Therefore, the reaction becomes irreversible and 1-SST is unable to elongate the polymer chain. Meanwhile, the glucose by-product can be recycled back either into sucrose or into any other metabolites required for plant growth.

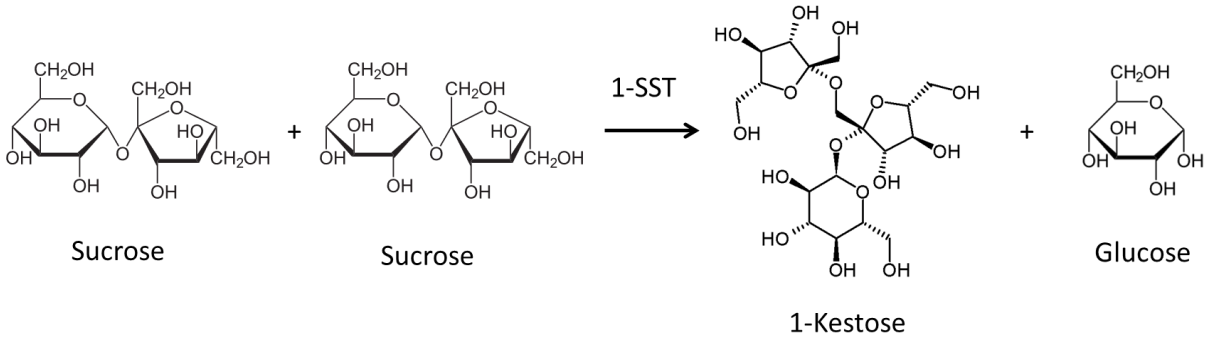

Figure 3. 1-SST rection (adapted from Beck, 2017).

The next enzyme, 1-FFT, then catalyzes the reversible transfer of a single terminal of β(2□1) linked fructosyl residue from 1-kestose onto another 1-kestose. This reaction results in nystose, an inulin oligofructose with DP = 4 (Figure 4). Subsequently, fructose units are shuffled by 1-FFT between 1-kestose and the existing oligofructose, which results in higher DP inulin polymers with only β(2□1) linkages. Through this mechanism, 1-FFT can elongate a pre-existing inulin molecule by transferring an additional fructosyl unit from another oligofructose. The action of the 1-FFT is strongly affected by the relative concentration of reactants and products. Therefore, 1-FFT reaction can generate products with various DPs which can co-exist in an equilibrium (Beck, 2017). Depending on the plant species, 1-FFT can also transfer fructosyl residue to other fructans or a sucrose molecule (van Laere & van den Ende, 2002; van Arkel *et al.*, 2013).

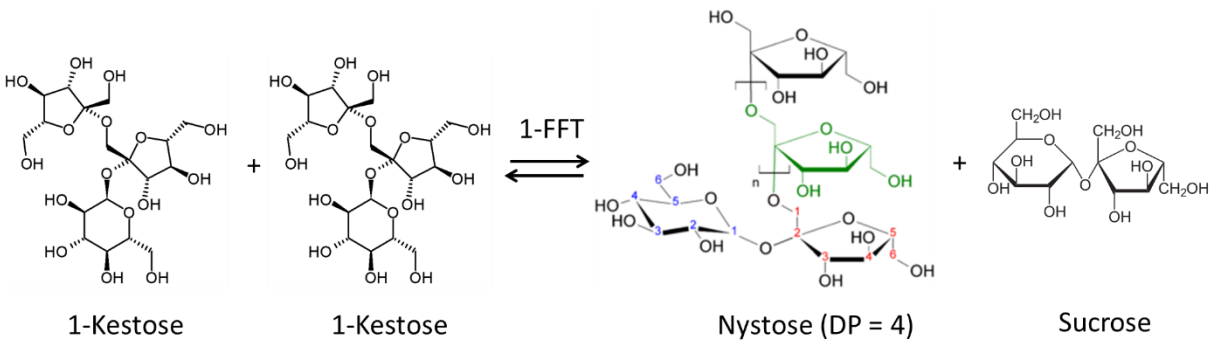

Figure 4. 1-FFT rection (adapted from Beck, 2017).

Another key enzyme called sucrose-fructan 6-fructosyltransferase (6-SFT) is discovered to be also involved in inulin biosynthesis in grasses (Nagaraj *et al.*, 2004). This discovery is originated from the findings that fructans in grasses are structurally more complex. Fructans in barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) consist of β(2→6) linked fructose units with β(2→1) branches (Bancal *et al.*, 1991). 6-SFT itself is homologous to 1-SST. The peptide sequences of barley 6-SFT are mostly identical to that of barley 1-SST except for a short FTNLIQ amino acid sequence (Nagaraj *et al.*, 2004). It was proposed that both 1-SST and 6-SFT are involved to introduce new fructosyl units from sucrose into fructans. This model contrasts with the accepted mechanism where fructosyl units in the fructan are only introduced by 1-SST. *In vitro* gene expression studies suggest that 1-SST is the pacemaker enzyme of fructan synthesis in barley leaves because it is more responsive to regulatory processes compared to 6-SFT (Nagaraj *et al.*, 2004). In the subsequent steps, the structural components can be modified by 1-FFT through shuffling of the fructosyl residues as found in other plants (Beck, 2017).

GEMBILI AS A POTENTIAL INULIN SOURCE

Gembili (*Dioscorea esculenta*) (Figure 5) is a type of yam tuber among the other 600 species of *Dioscorea* spp. which can grow well in Indonesia (Winarti & Saputro, 2013). The tuber has yellow or white flesh which can be cooked and used as a vegetable or even rice analog (Putri, *et al.*, 2017). It has a sweet and pleasant flavor like that of sweet potato or chestnuts. Recently, local gembili in Papua has been explored as an alternative staple food for substituting rice due to its rich carbohydrate content (22.49–31.30 %) (Sabda *et al.*, 2019). Upon processing, the total dietary fibers in boiled, steamed, and fried gembili are 19.01%, 18.15%, and 24.30% of their dry weight, respectively (Rimbawan & Nurbayani, 2013). The insoluble dietary fiber content in gembili ranges from 11.79% to 13.43% (Rimbawan & Nurbayani, 2013). Gembili is also rich in vitamins, protein, and minerals, containing up to 1.10 mg of proteins, 56. 00 mg of calcium, 49 mg of phosphorus, and 66.40 mg of vitamin C per 100 g of tubers (Sabda *et al.*, 2019).

Figure 5. Gembili tubers (Source: Google Images)

Despite its abundance, the use of gembili in the food industry is very scarce. Gembili has the potential to be utilized as the raw material for biscuits, bread, ice creams, and other snacks (Winarti & Saputro, 2013; Ridarineni, 2015). Moreover, gembili is among several *Dioscorea* spp. that contain a high amount of inulin, reaching up to 14.77 % dry weight (Winarti & Harmayani, 2012; Winarti & Saputro, 2013). Ultrasound-assisted extraction can even obtain inulin levels of 21.13% from chipped gembili (Hilman *et al.*, 2018). Inulin extracted from gembili has comparable prebiotic activity to commercial inulin from chicory roots (Fibruline® Instant) in increasing the viable count of probiotic *Lactobacillus casei* in the MRS medium (Zubaidah & Akhadiana, 2013). Crude inulin extract from gembili is also reported to stimulate the growth of probiotic *Bifidobacterium breve* BRL-131, *Bifidobacterium bifidum* BRL-130, *Bifidobacterium longum* ATCC-15707, and *Lactobacillus casei* FNCC-90 while inhibiting the growth of *E. coli* FNCC-195 (Winarti *et al.*, 2013). Fermentation of the inulin extract by *Bifidobacterium* strains on MRS media produced a higher amount of SCFA, including acetic acid, propionic acid, and butyric acid, than commercial inulin (Winarti *et al.*, 2013). In addition, a study on streptozotocin-induced rats showed that inulin from gembili tuber has a potential antidiabetic effect which might be due to modulation of *Bifidobacterium* sp. and *Lactobacillus* sp. (Soetoko *et al.*, 2018). These studies suggest the great potential of gembili as the source of inulin for prebiotic functional foods.

GENETIC ENGINEERING OF INULIN BIOSYNTHESIS IN PLANTS

A better understanding of inulin metabolism in plants can help efforts to improve the inulin yield and tailor its DP. Inulin biosynthesis is upregulated by carbon supplementation, nitrogen starvation, and hormone levels such as salicylic acid, jasmonate, and abscisic acid (Suárez-González *et al.*, 2014; Wei *et al.*, 2016). On the other hand, inulin degradation is induced by stress-related conditions such as cold, flooding, and defoliation upon which the plant remobilize stored carbons (Wei *et al.*, 2017). In chicory, the transcription factor CiMYB17 is crucial in these regulations because not only does it activates promoters of 1-SST and 1-FFT but also activates promoters of 1-FEH (Wei *et al.*, 2017). A study on Jerusalem artichoke suggested that 1-SST prefers inulin biosynthesis more than 1-FFT (Min *et al.*, 2019). However, their expressions can be synchronized to the growth stages and dynamics of inulin accumulation/degradation (Bizzarri *et al.*, 2020). Catalytic breakdown by 1-FFT and 1-FEH typically increases around harvest time (November) which results in a reduced polymer length compared to the early growing season (van den Ende *et al.*, 2013). In another study, low 1-FFT activity after 120 days of seed planting also results in short inulin polymers (DP < 5) (Shoorideh *et al.*, 2018). The decrease in inulin content at harvest time is also reported on asparagus roots (*Asparagus officinalis* L.), another inulin-accumulating plant (Ueno *et al.*, 2020).

This degradation is a major drawback in large-scale inulin production from chicory crops. With the growing market of prebiotic functional food, the conventional production method will be unable to keep up with increasing global demand. Various approaches to solve this problem have been explored, including (i) engineering inulin biosynthesis in chicory to modify DP and increase yield, (ii) engineering inulin production in non-inulin-accumulating plants, and (iii) engineering inulin production in microbial hosts. Details and examples for (i) and (ii) are excellently reviewed by van Arkel *et al.* (2013) but some more recent examples will be added here. Approach (iii) will be discussed in the next section. The scope will be limited only to the utilization of inulin biosynthesis genes isolated from plants.

The yield and DP of chicory inulin can be modified using specific growth elicitors or modifying the expression of inulin biosynthesis genes. As discussed earlier, low DP inulin can be used as sweeteners, whereas high DP inulin is preferable for fat replacement in food products. A study using *in vitro* cell suspension culture of Jerusalem artichoke suggests that three elicitors: chitosan, yeast extract, and $AgNO_3$,

were recommended for producing lower DP inulins (Ma *et al.*, 2017). The study also reported that methyl jasmonate (250 μmol/l) treatment for 15 days led to the highest inulin concentration (2955.27 ± 9.81 mg/l, around a 2.05-fold increase compared to control) (Ma *et al.*, 2017). On the other hand, to obtain high DP inulins, the 1-FEH activity needs to be reduced while the decrease of 1-SST activity should be prevented, especially around the harvest time. Under this strategy, the transgenic chicory plants cultivar 'Melci' has been developed to overexpress 1-SST under the control of the CaMV 35S promoter (Maroufi *et al.*, 2018). The 1-SST gene cloned was originated from dandelion (*Taraxacum officinale*). CaMV 35S is a constitutive promoter commonly used in the transformation of dicot plants due to high levels of expression. In the roots of transgenic chicory, the expression level of 1-SST was 4-fold higher than those in the wild-type whereas the expression level of 1-FFT showed no significant difference (Maroufi *et al.*, 2018). The average DP in transgenic plants was also higher (mean DP = 11.52) compared to the wild-type (mean DP = 9.57 ± 0.58) (Maroufi *et al.*, 2018). The transgenic plants also showed the accumulation of transgenic 1-SST mRNA at harvest time. This stable expression of 1-SST is thought to ensure a sufficient supply of 1-kestose required for 1-FFT to synthesize high DP inulins. However, the role of 1-FEH was not investigated in this study.

Genetic modification of non-inulin-accumulating plants to synthesize inulin is another promising strategy. Examples of plants that have been used are sugar beet (Sévenier *et al.*, 1998), tobacco (Li *et al.*, 2007), maize (Stoop *et al.*, 2007), rice (Kang *et al.*, 2010), and potatoes (Moon *et al.*, 2019). Inulin-producing transgenics often showed low sucrose or starch content along with stunted growth compared to wild type, probably as compensation for the extra energy and substrates need for inulin biosynthesis (van Arkel *et al.*, 2013). Nevertheless, results from this approach demonstrated that the inulin biosynthesis pathway is fully transferable. For example, transgenic potato tubers overexpressing 1-SST from Jerusalem artichoke yielded a high level of 1-kestose (3.36 mg/g), while tubers overexpressing both 1-SST and 1-FFT produced up to 3.14 mg/g short-chain inulins (DP of 3 to 5) (Moon *et al.*, 2019). One of the transgenic lines produced longer inulin chains (DP > 5) up to 1.11 mg/g in their tubers. Since potato cultivation methods and processing chains have been well-established, mass production of inulin using potatoes has a high potential. The integration of transgenic biosynthesis genes with native pathways in non-accumulating species will also make it possible to produce different inulin types and sizes with novel functionalities (van Arkel, 2013).

HETEROLOGOUS EXPRESSION OF INULIN BIOSYNTHESIS GENES IN MICROBIAL HOSTS

A growing body of research has confirmed the possibility of expressing inulin biosynthesis genes from plants in microbial hosts (Table 1). Most of the earlier reports focused on utilizing *Pichia pastoris* to clone and express fructosyltransferases for functional studies such as probing enzyme identity and characterizing its activity (Abe *et al.*, 2009; Ueno *et al.*, 2011). Initially, tobacco protoplasts were used as the model because they do not accumulate fructans and naturally have plant enzymes. However, the native presence of invertases – which also use sucrose as a substrate – has led to inconsistent results (Rehm *et al.* 1998; Luscher *et al.*, 2000). The use of *Saccharomyces cerevisiae* did produce active proteins but with low activity due to hyperglycosylation which prevents optimal protein secretion (Ritsema & Smeekens, 2003). *Pichia pastoris* lacks invertase and – in addition to having established tools for genetic manipulation – has thus been widely used as a suitable host for heterologous expression of fructosyltransferases.

In the studies reviewed, functional studies of plant fructosyltransferases using *Pichia pastoris* follow a general methodology. They typically involve (i) gene or RNA isolation from plant materials, (ii) construction of a cDNA library, (iii) cDNA cloning and transformation to *Pichia pastoris*, (iv) recombinant protein expression, and (vi) enzymatic assays. Protein sequencing can be used to elucidate the partial protein sequence which can later be used to design degenerate primers for cDNA library construction (Luscher *et al.*, 2000). If the genomic information is limited, primers can also be designed from the predicted conserved

sequences of the target gene. For instance, the isolation of the 1-SST gene from agave (*Agave tequilana* Weber var. azul.) utilized a set of primers based on the conserved region of 1-SST alignment from several inulin-accumulating plants such as *Allium cepa*, *Allium sativum*, and *Taraxacum officinale* (Avila-Fernandez *et al.*, 2007).

Recombinant protein expression and secretion in *Pichia pastoris* should be carefully regulated. Methanol is used to induce expression since *Pichia pastoris* is a methylotrophic yeast. The expression plasmids used typically contain the *Saccharomyces cerevisiae* α-factor signal peptide to enable secretion of the recombinant protein. Enzymatic assays can characterize the activity and stability of the purified recombinant protein. For example, recombinant 1-FFT cloned from 105 days old tuber of Jerusalem artichoke and expressed in *Pichia pastoris* X-33 was able to synthesize nystose when incubated with 1-kestose but not with sucrose (Ngampanya & Boonchoo, 2016). In a recent study, recombinant proteins rAoFT2 and rAoFT3 isolated from the cDNA library of asparagus roots were probed as 1-FFT and 1-SST, respectively, based on their enzymatic reaction profiles after being expressed in *Pichia pastoris* (Ueno *et al.*, 2020).

Recent efforts on heterologous expression of inulin biosynthesis genes in microbial hosts are also geared towards overproduction in fermentation conditions. A study by Hernández *et al.* (2018) reported a successful expression of recombinant 1-SST from Tall fescue in *Pichia pastoris*. The 548-aa mature polypeptide of 1-SST was expressed under the control of the constitutive GAP promoter and the methanol-inducible AOX1 promoter. Secretion driven by the *S. cerevisiae* α-factor sequence resulted in a high extracellular release (62%) compared to periplasmic retention (38%). When tested in a fed-batch fermentation at pH 5.5 and 28°C using cane sugar, the 1-SST-expressing *Pichia pastoris* achieved 106 g/l dry weight of biomass after 72 h with a total activity of 102.1 U/ml. Inulin produced from sucrose comprises 55–60% of the overall carbohydrates in the supernatant with a 9:1 ratio of 1-kestose to nystose. Subsequent characterization revealed that the purified recombinant 1-SST has optimum activity at pH 5.0–6.0 and 45–50 ºC. Its specificity and product profile showed no significant difference across varying substrate and enzyme concentrations. One key finding is that increasing gene copies to nine led to improved 1-SST productivity, reaching 1422.2 U/l/h after 72 h without inhibiting the cell growth. Therefore, it is clear that enzymatic inulin production can be improved through genetic engineering.

In recent years, more development on the overexpression of plant fructosyltransferases was reported by the same research group. The culture supernatant containing recombinant 1-SST can be turned into water-soluble powder using a combination of ultrafiltration and lyophilization. The powder displayed a high enzymatic activity (>8000 U/g) and high protein purity (>50 %) while remaining stable after 1-year storage at 4 and 25 ºC (Garcia *et al.*, 2020). A follow-up study showed the potential of cell immobilization to reduce the cost of enzymatic inulin production. Calcium-alginate beads were used to entrap the *Pichia pastoris* cells harboring nine copies of the 1-SST from Tall fescue. Permeabilization of the bead materials using toluene increased the activity of recombinant 1-SST in the periplasm possibly due to improvement in the substrate and products diffusion (Pérez *et al.*, 2021). When tested in a fed-batch fermentation using refined or raw sugar (600 g/l) at 30°C and pH 5.5, the toluene-treated immobilized cells yielded 55% inulin with 1-kestose and nystose in a ratio above 8:2 and the productivity of 7.3 g/l/h (Pérez *et al.*, 2021). Further characterization showed that after 15 repetitive 30-day cycles of inulin production, the beads kept 80% of the initial 1-SST activity (Pérez *et al.*, 2021). It is worth mentioning, however, that the inulin yield decreased to 45% when cheaper carbon sources such as sugarcane syrup and molasses were used in the fermentation. Engineering of substrate specificity or catalytic activity of the recombinant 1-SST might therefore be required. In addition, these studies did not indicate whether longer DP inulins (DP > 4) were also produced by the recombinant 1-SST nor the possibility to tailor the fermentation conditions to generate these commercially attractive fructans.

INULIN BIOSYNTHESIS GENES FROM GEMBILI: PROSPECTS AND CHALLENGES

Successful expression of 1-SST from inulin source plants in various heterologous hosts suggests that biotechnological inulin production using inulin biosynthesis genes from gembili is possible. However, the whole genome information of gembili, including genetic and protein sequence for 1-SST, is not yet available. Typically, primers are designed according to the whole genome sequences (WGS), cDNA sequences, conserved regions from similar genes on other plants, or sequences from close relatives. The WGS for two species in the *Dioscorea* genus, water yam (*Dioscorea alata* L.) and white Guiena yam (*Dioscorea rotundata*), is publicly available (Saski *et al.*, 2015; Tamiru *et al.*, 2017). Previous studies have also reported that it is possible to isolate the 1-SST gene from plants using primers designed for other species (Lasseur *et al.*, 2006; Ávila-Fernández *et al.*, 2007).

A recent study by Heriawan *et al.* (2020) has investigated the possibility of using existing primers from literature to isolate the putative 1-SST gene in gembili but was unsuccessful. The primers were designed based on the sequence of glycoside hydrolases family 32 domain found on chromosomes 6 and 11 of *Dioscorea rotundata*. Initial PCR using 6B primers (Forward: GGTTGAGTGTTTGGTGTTGTATT, Reverse AGGAGATGGATGTTGGGATTG, product size = 1129 bp) resulted in a visible 1-kb band in the onion DNA sample but only a faint one in gembili DNA sample (Heriawan *et al.*, 2020). The optimized annealing temperature of 50 ^{o}C was successful in obtaining a distinct band at 1 kb but later this is showed to be a false positive. Multiple sequence alignment of the PCR product using BLAST returned *Steroidobacter denitrificans* DSM 18526, a Gram-negative bacterial species, as the highest result but with a very low identity (69%) (Heriawan *et al.*, 2020). Another primer set, called 11A (Forward: AGATCCGAATGGTGAGTTCTTC, Reverse: AAGAGAGAGAAGCGTGAATGG, product size = 1004 bp), was also successful in isolating 1-kb products from gembili with BLAST results suggest a close relationship to beta-fructofuranosidase-1 from perennial ryegrass (*Lolim perenne*) and 1-SST from Tausch's goatgrass (*Aegilops tauschii*). However, the percent identity was also low (>75%) and therefore cannot be taken as a true positive result (Heriawan *et al.*, 2020). It is safe to conclude that both primer sets failed to isolate the putative 1-SST gene from gembili.

Therefore, biotechnological inulin production using inulin biosynthesis genes from gembili is still very challenging. Primer design based on multiple sequence alignment of 1-SSTs from closely related species to gembili is not without issues. The WGSs of *Dioscorea alata* and *Dioscorea rotundata* are still lacking annotations thus making it difficult to determine the location of the genes without more genomic data (Heriawan *et al.*, 2020). Exploration of genomic information from other inulin-producing species is therefore still needed to develop specific primers for 1-SST isolation from gembili (Moon *et al.*, 2019; Ueno *et al.*, 2020). Alternatively, gene synthesis can be utilized to explore the genetic sequence space which might generate 1-SST – or other inulin biosynthesis enzymes – with superior activity (Halewood *et al.*, 2018). As a cutting-edge technology, gene synthesis offers an advantage in the possibility of designing novel sequences that previously did not exist in nature (Hughes & Ellington, 2017). For example, synthetic gene synthesis combined with rational bioinformatics and high-throughput screening has been successful in generating variants of glycoside hydrolases (GHs) and polysaccharide lyases (PLs) with novel substrate specificities (Helbert *et al.*, 2019). Therefore, it will be possible to engineer enzymes with improved catalytic activity, yield, and productivity when expressed in heterologous hosts.

CONCLUSION

Inulin has been widely used as a prebiotic dairy fiber and fat substitute in food processing. Commercial inulin is primarily extracted from the roots of the chicory plant (*Cichorium intybus*). The demand for inulin is increasing but cannot be satisfied with conventional production methods. Biotechnological approaches have the potential to achieve large-scale, sustainable inulin production and met the increasing demand. Genes involved in the inulin biosynthesis pathway such as 1-SST and 1-FFT can be isolated, cloned,

and transformed into non-inulin accumulating organisms. Numerous studies have reported the successful expression of 1-SST from inulin source plants in heterologous hosts, including the yeast *Pichia pastoris*, a widely used "working horse" for biotechnological production. Gembili (*Dioscorea esculenta*), a type of yam tuber commonly found in Indonesia, contains a high inulin content (14.77%). However, isolation of 1-SST gene from gembili is still unsuccessful mainly due to the lack of published genomic information. Efforts using primers obtained from previous literature or designed from genome sequences in close relatives – which has been successful in other studies – also failed to give positive results. Whole-genome sequences of other inulin-accumulating species and gene synthesis technologies could be explored to obtain the inulin biosynthesis genes. These approaches are hoped to bring sustainable inulin production using the biotechnological approach in Indonesia closer to reality.

REFERENCES

Abe, M., Ueno, K., Ishiguro, Y., Omori, T., Onodera, S., & Shiomi, N. (2009). Purification, Cloning and Functional Characterization of Fructan: Fructan 1-Fructosyltransferase from Edible Burdock (*Arctium lappa* L.). *Journal of Applied Glycoscience (1999)*, *56*(4), 239–246. https://doi.org/10.5458/jag.56.239

Akbari, M., Eskandari, M. H., & Davoudi, Z. (2019). Application and functions of fat replacers in low-fat ice cream: A review. *Trends in Food Science & Technology*, *86*, 34–40. https://doi.org/10.1016/j.tifs.2019.02.036

Apolinário, A. C., de Lima Damasceno, B. P. G., de Macêdo Beltrão, N. E., Pessoa, A., Converti, A., & da Silva, J. A. (2014). Inulin-type fructans: a review on different aspects of biochemical and pharmaceutical technology. *Carbohydrate Polymers*, *101*, 368–378. https://doi.org/10.1016/j.carbpol.2013.09.081

Ávila-Fernández, Á., Olvera-Carranza, C., Rudiño-Piñera, E., Cassab, G. I., Nieto-Sotelo, J., & López-Munguía, A. (2007). Molecular characterization of sucrose: sucrose 1-fructosyltransferase (1-SST) from *Agave tequilana* Weber var. azul. *Plant Science*, *173*(4), 478–486. https://doi.org/10.1016/j.plantsci.2007.07.009

Badan Pusat Statistik. (2022). Bps.Go.Id. Retrieved March 10, 2022, from https://www.bps.go.id/exim/

Bancal, P., Henson, C. A., Gaudillère, J. P., & Carpita, N. C. (1991). Fructan chemical structure and sensitivity to an exohydrolase. *Carbohydrate Research*, *217*, 137–151. https://doi.org/10.1016/0008-6215(91)84124-w

Barclay, T., Ginic-Markovic, M., Cooper, P., & Petrovsky, N. (2010). Inulin: A versatile polysaccharide with multiple pharmaceutical and food chemical uses. *J Excipients Food Chem*, *1*(3), 27–50. http://doi.org/10.1021/jf030383v

Beck, R. H. F. (2017). Statistic thermodynamic analysis of fructans – Part 4: Modeling inulin biosynthesis as a non-equilibium thermodynamic process. *Zuckerindustrie*, 206–211. https://doi.org/10.36961/si18245

Bizzarri, M., Delledonne, M., Ferrarini, A., Tononi, P., Zago, E., Vittori, D., Damiani, F., & Paolocci, F. (2020). Whole-transcriptome analysis unveils the synchronized activities of genes for fructans in developing tubers of the Jerusalem artichoke. *Frontiers in Plant Science*, *11*, 101. https://doi.org/10.3389/fpls.2020.00101

Branca, F., Argento, S., Paoletti, A. M., & Melilli, M. G. (2022). The Physiological Role of Inulin in Wild Cardoon (*Cynara cardunculus* L. var. *sylvestris* Lam.). *Agronomy (Basel, Switzerland)*, *12*(2), 290. https://doi.org/10.3390/agronomy12020290

Carlson, J. L., Erickson, J. M., Lloyd, B. B., & Slavin, J. L. (2018). Health effects and sources of prebiotic dietary fiber. *Current Developments in Nutrition*, *2*(3), nzy005. https://doi.org/10.1093/cdn/nzy005

Chalmers, J., Johnson, X., Lidgett, A., & Spangenberg, G. (2003). Isolation and characterisation of a sucrose: sucrose 1-fructosyltransferase gene from perennial ryegrass (*Lolium perenne*). *Journal of Plant Physiology*, *160*(11), 1385–1391. https://doi.org/10.1078/0176-1617-01107

Chikkerur, J., Samanta, A. K., Kolte, A. P., Dhali, A., & Roy, S. (2020). Production of short chain fructo-oligosaccharides from inulin of chicory root using fungal endoinulinase. *Applied Biochemistry and Biotechnology*, *191*(2), 695–715. https://doi.org/10.1007/s12010-019-03215-7

De Roover, J., Vandenbranden, van Laere, A., & van den Ende, W. (2000). Drought induces fructan synthesis and 1-SST (sucrose:sucrose fructosyltransferase) in roots and leaves of chicory seedlings (*Cichorium intybus* L.). *Planta*, *210*(5), 808–814. https://doi.org/10.1007/s004250050683

De Sadeleer, E., Vergauwen, R., Struyf, T., Le Roy, K., & Van den Ende, W. (2015). 1-FFT amino acids involved in high DP inulin accumulation in *Viguiera discolor*. *Frontiers in Plant Science*, *6*, 616. https://doi.org/10.3389/fpls.2015.00616

Dehghan, P., Pourghassem Gargari, B., & Asgharijafarabadi, M. (2013). Effects of high performance inulin supplementation on glycemic status and lipid profile in women with type 2 diabetes: a randomized, placebo-controlled clinical trial. *Health Promotion Perspectives*, *3*(1), 55–63. https://doi.org/10.5681/hpp.2013.007

Dewanti, F. K. (2013). Substitusi Inulin Umbi Gembili (*Dioscorea Esculenta*) pada Produk Es Krim Sebagai Alternatif Produk Makanan Tinggi Serat dan Rendah Lemak. Universitas Diponegoro. Retrieved from http://eprints.undip.ac.id/41812/1/546_FANNY_KARINA_DEWANTI_G2C009006.pdf

Dhingra, D., Michael, M., Rajput, H., & Patil, R. T. (2012). Dietary fibre in foods: a review. *Journal of Food Science and Technology*, *49*(3), 255–266. https://doi.org/10.1007/s13197-011-0365-5

Evans, C. E. L. (2020). Dietary fibre and cardiovascular health: a review of current evidence and policy. *The Proceedings of the Nutrition Society*, *79*(1), 61–67. https://doi.org/10.1017/S0029665119000673

Glibowski, P., & Bukowska, A. (2011). The Effect of pH, Temperature and Heating Time on Inulin Chemical Stability. *Acta Sci. Pol., Technol. Aliment, 10*(2), 189–196. Retrieved from www.food.actapol.net

Halewood, M., Chiurugwi, T., Sackville Hamilton, R., Kurtz, B., Marden, E., Welch, E., Michiels, F., Mozafari, J., Sabran, M., Patron, N., Kersey, P., Bastow, R., Dorius, S., Dias, S., McCouch, S., & Powell, W. (2018). Plant genetic resources for food and agriculture: opportunities and challenges emerging from the science and information technology revolution. *The New Phytologist*, *217*(4), 1407–1419. https://doi.org/10.1111/nph.14993

Healey, G., Murphy, R., Butts, C., Brough, L., Whelan, K., & Coad, J. (2018). Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: a randomised, double-blind,

placebo-controlled, cross-over, human intervention study. *The British Journal of Nutrition*, *119*(2), 176–189. https://doi.org/10.1017/s0007114517003440

Helbert, W., Poulet, L., Drouillard, S., Mathieu, S., Loiodice, M., Couturier, M., Lombard, V., Terrapon, N., Turchetto, J., Vincentelli, R., & Henrissat, B. (2019). Discovery of novel carbohydrate-active enzymes through the rational exploration of the protein sequences space. *Proceedings of the National Academy of Sciences of the United States of America, 116*(13), 6063–6068. https://doi.org/10.1073/pnas.1815791116

Helmore, E. (2022, March 21). Fertiliser prices hit new highs as multiple problems affect global supplies. *The Guardian*. https://www.theguardian.com/global-development/2022/mar/21/fertiliser-prices-hit-new-highs-as-multiple-problems-affect-global-supplies

Heriawan, G., Anjani, D., Devanthi, P. V. P., Kemal, R. A., & Jessica, P. (2020). Primer design for isolation of sucrose:Sucrose 1-fructosyltransferase (1-SST) gene from gembili (*Dioscorea esculenta*). *KnE Life Sciences*. https://doi.org/10.18502/kls.v5i2.6445

Hernández, L., Menéndez, C., Pérez, E. R., Martínez, D., Alfonso, D., Trujillo, L. E., Ramírez, R., Sobrino, A., Mazola, Y., Musacchio, A., & Pimentel, E. (2018). Fructooligosaccharides production by *Schedonorus arundinaceus* sucrose:sucrose 1-fructosyltransferase constitutively expressed to high levels in *Pichia pastoris*. *Journal of Biotechnology*, *266*, 59–71. https://doi.org/10.1016/j.jbiotec.2017.12.008

Hilman, A., Harmayani, E., & Cahyanto, M. N. (2018). Inulin extraction and characterisation of fresh and chip gembili (*Dioscorea esculenta*) extract by ultrasound-assisted extraction. *Proceedings of the International Conference of Science, Technology, Engineering, Environmental and Ramification Researches*.

Hughes, R. A., & Ellington, A. D. (2017). Synthetic DNA synthesis and assembly: Putting the synthetic in synthetic biology. *Cold Spring Harbor Perspectives in Biology*, *9*(1), a023812. https://doi.org/10.1101/cshperspect.a023812

Hussain, S., Jõudu, I., & Bhat, R. (2020). Dietary fiber from underutilized plant resources—A positive approach for valorization of fruit and vegetable wastes. *Sustainability*, *12*(13), 5401. https://doi.org/10.3390/su12135401

Inkwood Research. (2017). Global Inulin Market Forecast 2018-2026. Retrieved January 13, 2019, from https://www.inkwoodresearch.com/reports/inulin-market/

Iraporda, C., Rubel, I. A., Manrique, G. D., & Abraham, A. G. (2019). Influence of inulin rich carbohydrates from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers on probiotic properties of *Lactobacillus* strains. *Lebensmittel-Wissenschaft Und Technologie [Food Science and Technology]*, *101*, 738–746. https://doi.org/10.1016/j.lwt.2018.11.074

Kang, K.-K., Song, B.-H., Lee, G.-A., Lee, H.-J., Park, J.-H., Jung, Y.-J., & Cho, Y.-G. (2010). Increment of fructan biosynthesis in rice by transformation of 1-sst and 1-fft genes isolated from jerusalem artichoke (*Helianthus tuberosus* L.). *Journal of Plant Biotechnology*, *37*(1), 102–109. https://doi.org/10.5010/jpb.2010.37.1.102

Karimi, A. A., Naghavi, M. R., Peyghambari, S. A., & Rasoulnia, A. (2021). Inulin content and expression of related genes in different tissues and cell suspension culture of Taraxacum kok-saghyz. *In Vitro Cellular & Developmental Biology. Plant: Journal of the Tissue Culture Association*. https://doi.org/10.1007/s11627-021-10180-6

Kawakami, A., & Yoshida, M. (2002). Molecular Characterization of Sucrose:Sucrose 1-Fructosyltransferase and Sucrose:Fructan 6-Fructosyltransferase Associated with Fructan Accumulation in Winter Wheat during Cold Hardening. *Bioscience, Biotechnology, and Biochemistry, 66*(11), 2297–2305. https://doi.org/10.1271/bbb.66.2297

Kawakami, A., & Yoshida, M. (2005). Fructan:fructan 1-fructosyltransferase, a key enzyme for biosynthesis of graminan oligomers in hardened wheat. *Planta*, *223*(1), 90–104. https://doi.org/10.1007/s00425-005-0054-6

Kawakami, A., Sato, Y., & Yoshida, M. (2008). Genetic engineering of rice capable of synthesizing fructans and enhancing chilling tolerance. *Journal of Experimental Botany, 59*(4), 793–802. https://doi.org/10.1093/jxb/erm367

Keleszade, E., Kolida, S., & Costabile, A. (2022). The cholesterol lowering efficacy of *Lactobacillus plantarum* ECGC 13110402 in hypercholesterolemic adults: a double-blind, randomized, placebo controlled, pilot human intervention study. *Journal of Functional Foods*, *89*(104939), 104939. https://doi.org/10.1016/j.jff.2022.104939

Khasanah, Y., Nurhayati, R., Miftakhussholihah, Btari, S., & Ratnaningrum, E. (2019). Isolation oligosaccharides from gembili (*Dioscorea esculenta* Lour. Burkill) as prebiotics. *IOP Conference Series. Materials Science and Engineering*, *633*(1), 012006. https://doi.org/10.1088/1757-899x/633/1/012006

Lasseur, B., Lothier, J., Djoumad, A., De Coninck, B., Smeekens, S., Van Laere, A., Morvan-Bertrand, A., Van den Ende, W., & Prud'homme, M.-P. (2006). Molecular and functional characterization of a cDNA encoding fructan:fructan 6G-fructosyltransferase (6G-FFT)/fructan:fructan 1-fructosyltransferase (1-FFT) from perennial ryegrass (*Lolium perenne* L.). *Journal of Experimental Botany*, *57*(11), 2719–2734. https://doi.org/10.1093/jxb/erl034

Li, H.-J., Yang, A.-F., Zhang, X.-C., Gao, F., & Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: sucrose 1-fructosyltransferase gene from *Lactuca sativa*. *Plant Cell, Tissue and Organ Culture*, *89*(1), 37–48. https://doi.org/10.1007/s11240-007-9213-8

Livingston, D. P., 3rd, Hincha, D. K., & Heyer, A. G. (2009). Fructan and its relationship to abiotic stress tolerance in plants. *Cellular and Molecular Life Sciences: CMLS*, *66*(13), 2007–2023. https://doi.org/10.1007/s00018-009-0002-x

Lothier, J., Van Laere, A., Prud'homme, M.-P., Van den Ende, W., & Morvan-Bertrand, A. (2014). Cloning and characterization of a novel fructan 6-exohydrolase strongly inhibited by sucrose in *Lolium perenne*. *Planta*, *240*(3), 629–643. https://doi.org/10.1007/s00425-014-2110-6

Lüscher, M., Hochstrasser, U., Vogel, G., Aeschbacher, R., Galati, V., Nelson, C. J., Boller, T., & Wiemken, A. (2000). Cloning and functional analysis of sucrose:sucrose 1-fructosyltransferase from tall fescue. *Plant Physiology*, *124*(3), 1217–1228. https://doi.org/10.1104/pp.124.3.1217

Ma, C., Zhou, D., Wang, H., Han, D., Wang, Y., & Yan, X. (2017). Elicitation of Jerusalem artichoke (*Helianthus tuberosus* L.) cell suspension culture for enhancement of inulin production and altered degree of polymerisation. *Journal of the Science of Food and Agriculture*, *97*(1), 88–94. https://doi.org/10.1002/jsfa.7686

Maroufi, A., Karimi, M., Mehdikhanlou, K., & De Loose, M. (2018). Inulin chain length modification using a transgenic approach opening new perspectives for chicory. *3 Biotech, 8*(8), 349. http://doi.org/10.1007/s13205-018-1377-x

Marras, L., Caputo, M., Bisicchia, S., Soato, M., Bertolino, G., Vaccaro, S., & Inturri, R. (2021). The role of bifidobacteria in predictive and preventive medicine: A focus on eczema and hypercholesterolemia. *Microorganisms*, *9*(4), 836. https://doi.org/10.3390/microorganisms9040836

Mathieu, A.-S., Tinel, C., Dailly, H., Quinet, M., & Lutts, S. (2018). Impact of high temperature on sucrose translocation, sugar content and inulin yield in *Cichorium intybus* L. var. *sativum*. *Plant and Soil*, *432*(1–2), 273–288. https://doi.org/10.1007/s11104-018-3802-7

McRorie, J. W., Jr, & McKeown, N. M. (2017). Understanding the physics of functional fibers in the gastrointestinal tract: An evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. *Journal of the Academy of Nutrition and Dietetics*, *117*(2), 251–264. https://doi.org/10.1016/j.jand.2016.09.021

Mensink, M. A., Frijlink, H. W., van der Voort Maarschalk, K., & Hinrichs, W. L. J. (2015). Inulin, a flexible oligosaccharide I: Review of its physicochemical characteristics. *Carbohydrate Polymers, 130,* 405–419. http://doi.org/10.1016/J.CARBPOL.2015.05.026

Min, C. W., Jung, W. Y., Park, H. J., Moon, K.-B., Ko, H., Sohn, J.-H., Jeon, J.-H., Kim, H.-S., Gupta, R., Kim, S. T., & Cho, H. S. (2019). Label-free quantitative proteomic analysis determines changes in amino acid and carbohydrate metabolism in three cultivars of Jerusalem artichoke tubers. *Plant Biotechnology Reports*, *13*(2), 111–122. https://doi.org/10.1007/s11816-019-00518-3

Moghadam, B. E., Keivaninahr, F., Fouladi, M., Mokarram, R. R., & Nazemi, A. (2019). Inulin addition to yoghurt: Prebiotic activity, health effects and sensory properties. *International Journal of Dairy Technology*, *72*(2), 183–198. https://doi.org/10.1111/1471-0307.12579

Moghadam, B. E., Keivaninahr, F., Fouladi, M., Rezaei Mokarram, R., & Nazemi, A. (2019). Inulin addition to yoghurt: Prebiotic activity, health effects and sensory properties. *International Journal of Dairy Technology*, *72*(2), 183–198. https://doi.org/10.1111/1471-0307.12579

Mohammadi, F., Naghavi, M. R., Peighambari, S. A., Khosravi Dehaghi, N., Khaldari, I., Bravi, E., Marconi, O., & Perretti, G. (2021). Abscisic acid crosstalk with auxin and ethylene in biosynthesis and degradation of inulin-type fructans in chicory. *Plant Biology (Stuttgart, Germany)*, *23*(4), 636–642. https://doi.org/10.1111/plb.13252

Moon, K.-B., Ko, H., Park, J.-S., Sohn, J.-H., Cho, H.-S., Park, Y.-I., Kim, H.-S., & Jeon, J.-H. (2019). Expression of Jerusalem artichoke (Helianthus tuberosus L.) fructosyltransferases, and high fructan accumulation in potato tubers. *Applied Biological Chemistry*, *62*(1). https://doi.org/10.1186/s13765-019-0481-x

Moon, K.-B., Ko, H., Park, J.-S., Sohn, J.-H., Cho, H.-S., Park, Y.-I., Kim, H.-S., & Jeon, J.-H. (2019). Expression of Jerusalem artichoke (*Helianthus tuberosus* L.) fructosyltransferases, and high fructan accumulation in potato tubers. *Applied Biological Chemistry*, *62*(1). https://doi.org/10.1186/s13765-019-0481-x

Myhrstad, M. C. W., Tunsjø, H., Charnock, C., & Telle-Hansen, V. H. (2020). Dietary fiber, gut Microbiota, and metabolic regulation-current status in human randomized trials. *Nutrients*, *12*(3), 859. https://doi.org/10.3390/nu12030859

Nagaraj, V. J., Altenbach, D., Galati, V., Lüscher, M., Meyer, A. D., Boller, T., & Wiemken, A. (2004). Distinct regulation of sucrose: sucrose-1-fructosyltransferase (1-SST) and sucrose: fructan-6-fructosyltransferase (6-SFT), the key enzymes of fructan synthesis in barley leaves: 1-SST as the pacemaker. *New Phytologist, 161*(3), 735–748. https://doi.org/10.1111/j.1469-8137.2004.00995.x

Ngampaya, B. & Boonchoo, K. (2016). Cloning of 1– fructan: fructan fructosyltransferase gene and expression of recombinant 1– fructan: fructan fructosyltransferase in yeast. *KKU Research Journal, 22*(1): 356-365.

Öztürk, B. (2016). a Rising Star Prebiotic Dietary Fiber: Inulin and Recent Applications in Meat Products. *Journal of Food and Health Science, 3*(1), 12–20. http://doi.org/10.3153/JFHS17002

Ozturkoglu-Budak, S., Akal, H. C., Buran, İ., & Yetişemiyen, A. (2019). Effect of inulin polymerization degree on various properties of synbiotic fermented milk including *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12. *Journal of Dairy Science, 102*(8), 6901–6913. https://doi.org/10.3168/jds.2019-16479

Pérez, E. R., Martínez, D., Menéndez, C., Alfonso, D., Rodríguez, I., Trujillo, L. E., Sobrino, A., Ramírez, R., Pimentel, E., & Hernández, L. (2021). Fructooligosaccharides production by immobilized *Pichia pastoris* cells expressing *Schedonorus arundinaceus* sucrose:sucrose 1-fructosyltransferase. *Journal of Industrial Microbiology & Biotechnology*, *48*(5–6). https://doi.org/10.1093/jimb/kuab036

Petkova, N., Genchev, G., Vassilev, D., Koleva, M., & Krastanov and Panteley Denev, A. (2018). Microwave-assisted isolation and acetylation of inulin from Helianthus tuberosus L tubers. *Journal of Renewable Materials*, *6*(7), 671–679. https://doi.org/10.32604/jrm.2018.00001

Putri, D. N., Haqqyana, H., Purwanti, L., Azizah, N., & Astuty, R. M. (2017). BALI (Beras Analog Umbi Gembili): The Utilization of Gembili Tuber (*Dioscorea esculenta*) as The Raw Material for Rice Analogue Production. *UI Proceedings on Science and Technology, 1*(0). Retrieved from http://proceedings.ui.ac.id/index.php/uipst/article/view/122

Quinn, R. (2021, December 17). *Sanctions could affect world potash supply in 2022*. DTN Progressive Farmer. https://www.dtnpf.com/agriculture/web/ag/crops/article/2021/12/17/sanctions-affect-world-potash-supply

Redondo-Cuenca, A., Herrera-Vázquez, S. E., Condezo-Hoyos, L., Gómez-Ordóñez, E., & Rupérez, P. (2021). Inulin extraction from common inulin-containing plant sources. *Industrial Crops and Products*, *170*(113726), 113726. https://doi.org/10.1016/j.indcrop.2021.113726

Rehm, J., Willmitzer, L., & Heyer, A. G. (1998). Production of 1-kestose in transgenic yeast expressing a fructosyltransferase from *Aspergillus foetidus*. *Journal of Bacteriology*, *180*(5), 1305–1310. https://doi.org/10.1128/JB.180.5.1305-1310.1998

Renye, J. A., Jr, White, A. K., & Hotchkiss, A. T., Jr. (2021). Identification of *Lactobacillus* strains capable of fermenting fructo-oligosaccharides and inulin. *Microorganisms*, *9*(10), 2020. https://doi.org/10.3390/microorganisms9102020

Ridarineni, N. (2015). Inulin dari Gembili akan Jadi Produk Unggulan DIY. Retrieved November 25, 2018, from https://www.republika.co.id/berita/nasional/daerah/15/03/19/nlgblu-inulin-dari-gembili-akan-jadi-produk-unggulan-diy

Ritsema, T., & Smeekens, S. C. M. (2003). Engineering fructan metabolism in plants. *Journal of Plant Physiology*, *160*(7), 811–820. https://doi.org/10.1078/0176-1617-01029

Roberfroid, M. B. (1999). Caloric Value of Inulin and Oligofructose. *The Journal of Nutrition*, *129*(7), 1436S-1437S. https://doi.org/10.1093/jn/129.7.1436s

Ruan, M., Wu, T., Cheng, M., Yu, J., Wang, H., & Liu, Z. (2019). Separation and probiotic effect of fructose with different polymerization degrees in inulin. *E3S Web of Conferences, 131*, 01016. https://doi.org/10.1051/e3sconf/201913101016

Sabda, M., Wulanningtyas, H. S., Ondikeleuw, M., & Baliadi, Y. (2019). Characterization of potential local gembili (*Dioscorea esculenta* L) from Papua as alternative of staple food. *Buletin Plasma Nutfah*, *25*(1), 25. https://doi.org/10.21082/blpn.v25n1.2019.p25-32

Samakradhamrongthai, R. S., Jannu, T., Supawan, T., Khawsud, A., Aumpa, P., & Renaldi, G. (2021). Inulin application on the optimization of reduced-fat ice cream using response surface methodology. *Food Hydrocolloids*, *119*(106873), 106873. https://doi.org/10.1016/j.foodhyd.2021.106873

Saski, C. A., Bhattacharjee, R., Scheffler, B. E., & Asiedu, R. (2015). Genomic resources for water yam (*Dioscorea alata* L.): Analyses of EST-sequences, De Novo sequencing and GBS libraries. *PLoS One*, *10*(7), e0134031. https://doi.org/10.1371/journal.pone.0134031

Sévenier, R., Hall, R. D., van der Meer, I. M., Hakkert, H. J., van Tunen, A. J., & Koops, A. J. (1998). High level fructan accumulation in a transgenic sugar beet. *Nature Biotechnology*, *16*(9), 843–846. https://doi.org/10.1038/nbt0998-843

Shang, H.-M., Zhou, H.-Z., Yang, J.-Y., Li, R., Song, H., & Wu, H.-X. (2018). In vitro and in vivo antioxidant activities of inulin. *PLoS One*, *13*(2), e0192273. https://doi.org/10.1371/journal.pone.0192273

Shoaib, M., Shehzad, A., Omar, M., Rakha, A., Raza, H., Sharif, H. R., Shakeel, A., Ansari, A., & Niazi, S. (2016). Inulin: Properties, health benefits and food applications. *Carbohydrate Polymers*, *147*, 444–454. https://doi.org/10.1016/j.carbpol.2016.04.020

Shoorideh, H., Peighambari, S. A., Omidi, M., Naghavi, M. R., & Maroufi, A. (2018). Spatial expression of genes in inulin biosynthesis pathway in wild and root type chicory. *Journal of Agricultural Science and Technology*, *20*(5), 1049–1058. https://jast.modares.ac.ir/article-23-20034-en.html

Soetoko, A. S., Safitri, A. H., Isradji, I., Wibowo, K. A. T., Nandira, A., Rafiq, D. Z. A., Savita, S., Al-Baari, A. N., Budi, Y., Fatmawati, D. (2018). Antidiabetic Effect of Inulin from *Dioscorea esculenta* in Streptozotocin-Induced Diabetic Rats. *International Journal of Health Medicine and Current Research, 3*(3), 953-957

Stoop, J. M., Van Arkel, J., Hakkert, J. C., Tyree, C., Caimi, P. G., & Koops, A. J. (2007). Developmental modulation of inulin accumulation in storage organs of transgenic maize and transgenic potato. *Plant Science: An International Journal of Experimental Plant Biology*, *173*(2), 172–181. https://doi.org/10.1016/j.plantsci.2007.04.011

Suárez-González, E. M., López, M. G., Délano-Frier, J. P., & Gómez-Leyva, J. F. (2014). Expression of the 1-SST and 1-FFT genes and consequent fructan accumulation in *Agave tequilana* and *A. inaequidens* is differentially induced by diverse (a)biotic-stress related elicitors. *Journal of Plant Physiology*, *171*(3–4), 359–372. https://doi.org/10.1016/j.jplph.2013.08.002

Swann, O. G., Kilpatrick, M., Breslin, M., & Oddy, W. H. (2020). Dietary fiber and its associations with depression and inflammation. *Nutrition Reviews*, *78*(5), 394–411. https://doi.org/10.1093/nutrit/nuz072

Taha, H. S., Abd El-Kawy, A. M., & Fathalla, M. A. E.-K. (2012). A new approach for achievement of inulin accumulation in suspension cultures of Jerusalem artichoke (*Helianthus tuberosus*) using biotic elicitors. *Journal, Genetic Engineering & Biotechnology*, *10*(1), 33–38. https://doi.org/10.1016/j.jgeb.2012.02.002

Tamiru, M., Natsume, S., Takagi, H., White, B., Yaegashi, H., Shimizu, M., Yoshida, K., Uemura, A., Oikawa, K., Abe, A., Urasaki, N., Matsumura, H., Babil, P., Yamanaka, S., Matsumoto, R., Muranaka, S., Girma, G., Lopez-Montes, A., Gedil, M., … Terauchi, R. (2017). Genome sequencing of the staple food crop white Guinea yam enables the development of a molecular marker for sex determination. *BMC Biology*, *15*(1). https://doi.org/10.1186/s12915-017-0419-x

Teferra, T. F. (2021). Possible actions of inulin as prebiotic polysaccharide: A review. *Food Frontiers*, *2*(4), 407–416. https://doi.org/10.1002/fft2.92

Ueno, K., Ishiguro, Y., Yoshida, M., Onodera, S., & Shiomi, N. (2011). Cloning and functional characterization of a fructan 1-exohydrolase (1-FEH) in edible burdock (*Arctium lappa* L.). *Chemistry Central Journal*, *5*(1), 16. https://doi.org/10.1186/1752-153X-5-16

Ueno, K., Sonoda, T., Yoshida, M., Kawakami, A., Shiomi, N., & Onodera, S. (2020). Decreased expression of fructosyltransferase genes in asparagus roots may contribute to efficient fructan degradation during asparagus spear harvesting. *Plant Physiology and Biochemistry*, *156*, 192–200. https://doi.org/10.1016/j.plaphy.2020.09.007

United States Food and Drug Administration. (2018). The Declaration of Certain Isolated or Synthetic Non-Digestible Carbohydrates as Dietary Fiber on Nutrition and Supplement Facts Labels; Guidance for Industry; Availability. Retrieved from https://www.fda.gov/FoodGuidances

van Arkel, J., Sévenier, R., Hakkert, J. C., Bouwmeester, H. J., Koops, A. J., & van der Meer, I. M. (2013). Tailor-made fructan synthesis in plants: a review. *Carbohydrate Polymers*, *93*(1), 48–56. https://doi.org/10.1016/j.carbpol.2012.02.001

van den Ende, W., Michiels, A., Van Wonterghem, D., Vergauwen, R., & van Laere, A. (2000). Cloning, developmental, and tissue-specific expression of sucrose:sucrose 1-fructosyl transferase from *Taraxacum officinale*. Fructan localization in roots. *Plant Physiology, 123*(1), 71–80. https://doi.org/10.1104/PP.123.1.71

van Laere, A., & van den Ende, W. (2002). Inulin metabolism in dicots: Chicory as a model system. *Plant, Cell and Environment, 25*(6), 803–813. http://doi.org/10.1046/j.1365-3040.2002.00865.x

Wei, H., Bausewein, A., Greiner, S., Dauchot, N., Harms, K., & Rausch, T. (2017). CiMYB17, a stress-induced chicory R2R3-MYB transcription factor, activates promoters of genes involved in fructan synthesis and degradation. *The New Phytologist*, *215*(1), 281–298. https://doi.org/10.1111/nph.14563

Wei, H., Bausewein, A., Steininger, H., Su, T., Zhao, H., Harms, K., Greiner, S., & Rausch, T. (2016). Linking expression of fructan active enzymes, cell wall invertases and sucrose transporters with fructan profiles in growing taproot of chicory (*Cichorium intybus*): Impact of hormonal and environmental cues. *Frontiers in Plant Science*, *7*, 1806. https://doi.org/10.3389/fpls.2016.01806

Weitkunat, K., Schumann, S., Petzke, K. J., Blaut, M., Loh, G., & Klaus, S. (2015). Effects of dietary inulin on bacterial growth, short-chain fatty acid production and hepatic lipid metabolism in gnotobiotic mice. *The Journal of Nutritional Biochemistry*, *26*(9), 929–937. https://doi.org/10.1016/j.jnutbio.2015.03.010

Winarti, S., & Saputro, E. A. (2013). Karakteristik Tepung Prebiotik Umbi Uwi (*Dioscorea spp*). *Jurnal Teknik Kimia, 8*. Retrieved from http://eprints.upnjatim.ac.id/4871/1/4_erwan_(_belum_revisi_).pdf

Winarti, S., Harmayani, E., & Nurismanto, R. (2012). Karakteristik dan Profil Inulin Beberapa Jenis Uwi (*Dioscorea* spp.). *AgriTECH, 31*(4). https://doi.org/10.22146/agritech.9647

Winarti, S., Harmayani, E., Marsono, Y., & Pranoto, Y. (2013). Effect of inulin isolated from lesser yam (*Dioscorea esculenta*) on the growth of probiotics bacteria and SCFA formation during fermentation. *International Research Journal of Microbiology, 4*, 53-63.

Xu, H., Gunenc, A., & Hosseinian, F. (2021). Ultrasound affects physical and chemical properties of Jerusalem artichoke and chicory inulin. *Journal of Food Biochemistry*, e13934. https://doi.org/10.1111/jfbc.13934

Yankevich, S. V., Malinin, G. V., & Baevskiy, V. V. (2022). A new technology for producing inulin with high degree of polymerization. *IOP Conference Series. Earth and Environmental Science*, *979*(1), 012095. https://doi.org/10.1088/1755- 1315/979/1/012095

Zeaiter, Z., Regonesi, M. E., Cavini, S., Labra, M., Sello, G., & Di Gennaro, P. (2019). Extraction and characterization of inulin-type fructans from artichoke wastes and their effect on the growth of intestinal bacteria associated with health. *BioMed Research International*, *2019*, 1083952. https://doi.org/10.1155/2019/1083952

Zhang, R., Vorobiev, E., Zhu, Z., & Grimi, N. (2021). Effect of pulsed electric fields pretreatment on juice expression and quality of chicory. *Innovative Food Science & Emerging Technologies: IFSET: The Official Scientific Journal of the European Federation of Food Science and Technology*, *74*(102842), 102842. https://doi.org/10.1016/j.ifset.2021.102842

Zhu, Y., Chu, J., Lu, Z., Lv, F., Bie, X., Zhang, C., & Zhao, H. (2018). Physicochemical and functional properties of dietary fiber from foxtail millet (*Setaria italic*) bran. *Journal of Cereal Science*, *79*, 456–461. https://doi.org/10.1016/j.jcs.2017.12.011

Zhu, Z., He, J., Liu, G., Barba, F. J., Koubaa, M., Ding, L., Bals, O., Grimi, N., & Vorobiev, E. (2016). Recent insights for the green recovery of inulin from plant food materials using non-conventional extraction technologies: A review. *Innovative Food Science & Emerging Technologies: IFSET: The Official Scientific Journal of the European Federation of Food Science and Technology*, *33*, 1–9. https://doi.org/10.1016/j.ifset.2015.12.023

Zubaidah, E., & Akhadiana, W. (2013). Comparative Study of Inulin Extracts from Dahlia, Yam, and Gembili Tubers as Prebiotic. *Food and Nutrition Sciences, 4,* 8–12. http://doi.org/10.4236/fns.2013.411A002

APPENDIX

Table 1. Heterologous expression of inulin biosynthesis genes from plants in *Pichia pastoris*

Gene cloned or enzyme assayed	Plant origin	Estimated size	Catalytic activity			Enzyme expression and inulin production			Ref.
			Substrate	Inulin products detected	DP of inulin products	Activity	Productivity	Maximum inulin yield	
1-sst	Tall fescue (*Festuca arundinacea*)	82 kDa	Sucrose (100 mM)	1-kestose	3	9.065 ± (0.278) nkat/ml	n.r.	n.r.	Lüscher *et al.* (2000)
			Sucrose (100 mM)	Nystose	4	0.103 ± (0.006) nkat/ml	n.r.	n.r.	
			1-kestose (50 mM)	Nystose	4	1.352 ± (0.025) nkat/ml	n.r.	n.r.	
			Sucrose (100 mM) 1-kestose (50 mM)	Nystose	4	0.468 ± (0.005) nkat/ml	n.r.	n.r.	
			Nystose	1-kestose	3	0.788 ± (0.022) nkat/ml	n.r.	n.r.	

			(50 mM)						
1-sst	Ryegrass (*Lolium perenne*)	653 a.a. (70 kDa)	Sucrose (100 mM)	1-kestose Nystose	3 4	n.r.	n.r.	n.r.	Chalmers *et al.* (2003)
1-fft	Wheat (*Triticum aestivum* L.)	648 a.a.	Sucrose (200 mM)	1-kestose	up to 6	n.r.	n.r.	n.r.	Kawakami & Yoshida (2005)
			1-kestose (200 mM)	Oligomers	up to 6	n.r.	n.r.	n.r.	
			1,1-kestotetraose (200 mM)	Oligomers	up to 6	n.r.	n.r.	n.r.	
			1,1,1-kestopentaose (200 mM)	Oligomers	up to 7	n.r.	n.r.	n.r.	
1-sst (putative)	Ryegrass (*Lolium perenne*)	645 a.a. (60.6 kDa)	Sucrose (100 mM)	1-kestose	3	n.r.	n.r.	n.r.	Lasseur *et al.* (2006)
			Sucrose (100 mM)	Nystose	4	n.r.	n.r.	n.r.	

			1-kestose (50 mM)						
			1-kestose (50 mM)	Nystose	4	n.r.	n.r.	n.r.	
1-sst	Blue agave (*Agave tequilana* Weber var. *azul*.)	560 a.a. (75 kDa)	Sucrose (300 mM)	1-kestose	4	73 mU/mL (MES buffer, pH 5.5) 85 mU/mL (Citrate buffer, pH 5.5)	n.r.	n.r.	Ávila-Fernández *et al.* (2007)
1-fft	Edible burdock (*Arcitum lappa* L.)	617 a.a.	1-kestose (100 mM)	Oligomers	up to 8	4.57 U/mg (1-ffta) 8.12 U/mg (1-fftb)	n.r.	n.r.	Abe *et al.* (2009)
1-feh	Edible burdock (*Arcitum lappa* L.)	581 a.a. (80 kDa)	Nystose (100 mM)	1-kestose	3	n.r.	n.r.	n.r.	Ueno *et al.* (2011)
			1-kestose (100 mM)	Nystose	4	73.4 U/mg	n.r.	n.r.	
1-sst	Ryegrass (*Lolium perenne*)	580 a.a. (60.9 kDa)	Sucrose (3 mM)	n.r.	n.r.	2% (relative to 6-kestotriose as a substrate)	n.r.	n.r.	Lothier *et al.* (2014)

			1-kestose (3 mM)	n.r.	n.r.	11%	n.r.	n.r.	
			1,1-Kestotetraose (3 mM)	n.r.	n.r.	11%	n.r.	n.r.	
			Inulin (5%)	n.r.	n.r.	1%	n.r.	n.r.	
1-fft	Jerusalem artichoke (*Helianthus tuberosus* L.)	n.r.	1-kestose (50 mM)	Nystose	4	3.33 U/l	n.r.	n.r.	Ngampanya & Boonchoo (2016)
			Inulin (50 nM)	Nystose	4	n.r.	n.r.	n.r.	
1-sst (1 copy)	Tall fescue (*Schedonorus arundinaceus*)	80-90 kDa	Glycerol (fed-batch)	n.r.	n.r.	2.3 ± 0.3 U/ml (extracellular enzyme)	54.9 U/l/h	n.r.	Hernández *et al.* (2018)
1-sst (6 copies)			Glycerol (fed-batch)	n.r.	n.r.	6.6 ± 0.4 U/ml (extracellular enzyme)	154.8 U/l/h	n.r.	

1-sst (9 copies)			Glycerol (fed-batch)	n.r.	n.r.	19.0 ± 0.2 U/ml (extracellular enzyme)	437.2 U/l/h	n.r.	
1-sst (9 copies)			Sucrose (fed-batch)	n.r.	n.r.	63.2 ± 5.9 U/ml (extracellular enzyme)	1422.22 U/l/h	n.r.	
Purified 1-SST			Sucrose (600 g/l)	1-kestose	3	n.r.	n.r.	308.2 g/l	
			Sucrose (600 g/l)	Nystose	4	n.r.	n.r.	53.1 g/l	
1-fft	Asparagus (*Asparagus officinalis* L.)	624 a.a. (68.8 kDa)	1-kestose (100 mM)	1-kestose Nystose	3 4	n.r.	n.r.	n.r.	Ueno *et al.* (2020)
			Nystose (50 mM)	1-kestose Nystose Oligomers	3 4 up to 6	n.r.	n.r.	n.r.	
			1-kestose (100 mM) Nystose (100 mM)	1-kestose Nystose Oligomers	3 4 up to 5	n.r.	n.r.	n.r.	

1-sst	Asparagus (*Asparagus officinalis* L.)	628 a.a. (70.7 kDa)	Sucrose (100 mM)	1-kestose	3	n.r.	n.r.	n.r.	Ueno *et al.* (2020)
			1-kestose (50 mM)	1-kestose Nystose	3 4	n.r.	n.r.	n.r.	
			Sucrose (100 mM) Neokestose (100 mM)	1-kestose Oligomers	3 4	n.r.	n.r.	n.r.	
1-sst (1 copy)	Tall fescue (*Schedonorus arundinaceus*)	654 a.a.	Glycerol (fed-batch)	n.r.	n.r.	3955 U/L	55 U/L/h	n.r.	Hernández-García *et al.* (2020)
1-sst (9 copies)			Glycerol (fed-batch)	n.r.	n.r.	60 U/mL	n.r.	n.r.	
Purified 1-SST			Sucrose (fed-batch)	1-kestose Nystose	3 4	n.r.	n.r.	360 g/l (total)	

1-sst (9 copies)	Tall fescue (*Schedonorus arundinaceus*)	80-90 kDa	Molasses (fed-batch)	n.r.	n.r.	120.7 U/ml (extracellular enzyme)	2368.0 U/l/h	n.r.	Pérez *et al.* (2021)
Toluene-treated imobilized recombinant cells			Refined sugar (fed-batch)	1-kestose Nystose	3 4	19.51 U/g	7.3 g/l/h	58.2% (g inulin/g sucrose) 3.5% (g inulin/g cells) 81.2% (g 1-kestose/g inulin)	
			Raw sugar (fed-batch)	1-kestose Nystose	3 4	19.51 U/g	6.9 g/l/h	55.4% (g inulin/g sucrose) 3.3% (g inulin/g cells) 84.5% (g 1-kestose/g inulin)	

Molasses (fed-batch)	1-kestose	3	19.51 U/g	5.7 g/l/h	46.4% (g inulin/g sucrose)
	Nystose	4			2.8% (g inulin/g cells)
					86.7% (g 1-kestose/g inulin)
Sugarcane syrup (fed-batch)	1-kestose	3	19.51 U/g	5.8 g/l/h	45.5% (g inulin/g sucrose)
	Nystose	4			2.7% (g inulin/g cells)
					88.7% (g 1-kestose/g inulin)

n.r. = not reported