Chapter

Bioconversion of Weedy Waste into Sugary Wealth

Prajakta Prakash Kamble, Suresh Shivaji Suryawanshi, Maheshkumar Vishnu Kore, Nahid Irani, Jyoti Prafulla Jadhav and Yasmin Chand Attar

Abstract

Efforts put in overriding the inulin abundant invader nastiest category I weeds are infeasible that lead into its impermanent confiscation. Hence, their heedful exploitation is obligatory. These invasive weeds have ample amount of inulin, which serves as a renewable, cheap raw substrate for inulinase production. Therefore, they have enticed intention of many researchers toward exploring more idiosyncratic inulinase producing microbial strains that utilize invasive inulin-rich weeds as substrate for fructose liberation. Plenteous industrial applications of inulinases have marked it distinctly crucial in recent biotechnological epoch. This review thus elaborates the literature on infused footprints embedded by the substituted low calorie healthy sweetener in new advancing fields.

Keywords: invasive, weed, inulinases, low calorie, healthy sweetener, fructose

1. Introduction

Weeds are plants that grow luxuriantly in unsolicited places with no special assistance of human. These plants spread rapidly by shading abundant seedlings, making land unfit for agriculture, forestry, and livestock. Their characterized adaptability to extensive range of soils and weathers has proved a boon for their survival at any piece of earth. They also have capabilities of tumbling inherent plant ecosystems and fluctuating natural biota in injurious ways. Due to the competency of piercing and interchanging indigenous flora, majority of them are recognized as environmental weeds or exotic or noxious aggressive invaders [1].

Apart from this negative side, countless constructive purposes of weeds as a part of their control strategies have still remained unnoticed. Therefore, this research theme was needed to be explored and expanded. Consequently, when seen from a different standpoint, such weeds have high inulin in them. Thus, this work explores inulin-rich weeds as a veritable and bioconvertible resource for sugary wealth creation, using efficient inulinase producing microbes.

2. Inulin

Inulin is an allocated polysaccharide mixture composed of α-D-glucopyranosyl-[β-(2,1)-D-fructofuranosyl]-D-fructofuranosides linked by β-(2,1)-D-fructosyl-fructose

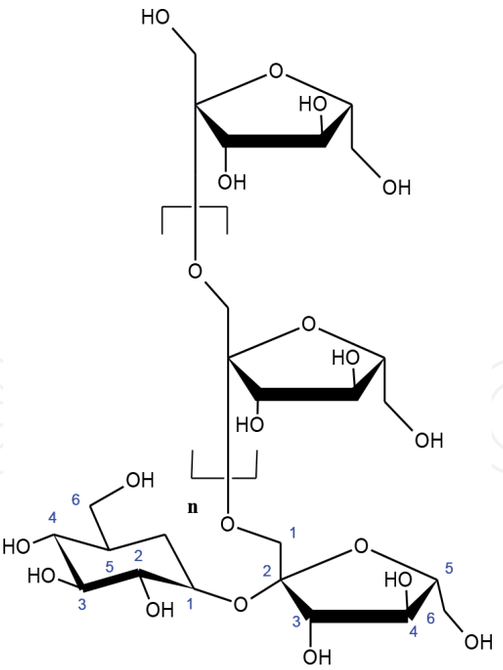

Figure 1.
Inulin structure.

bonds, and each of this chain is terminated by fructose moiety. The linking and bonding in inulin moiety are designated in **Figure 1**. Inulin is a reservoir of nondigestible carbohydrate known as fructans. It constitutes the bulk of glycosidic bonds joining fructosyl-fructose. The inulin-type fructans stored in Dicotyledonous species are connected with linear β(2→1) fructofuranosyl units, whereas monocots encompass branched complex-type fructans [2].

3. Plant sources of fructans

Inulin is abundant in structures such as bulbs, tubers, and tuberous roots of grasses and flowering plants belonging to Liliaceae (3500 species) and Compositae (25,000 species) families. Such plants, for example, asparagus, wheat, rye, and dahlias, mostly lack starch and thus synthesize inulin as energy store house. A wide array of inulin-rich plants with their inulin content is symbolized graphically (**Figure 2**) [3].

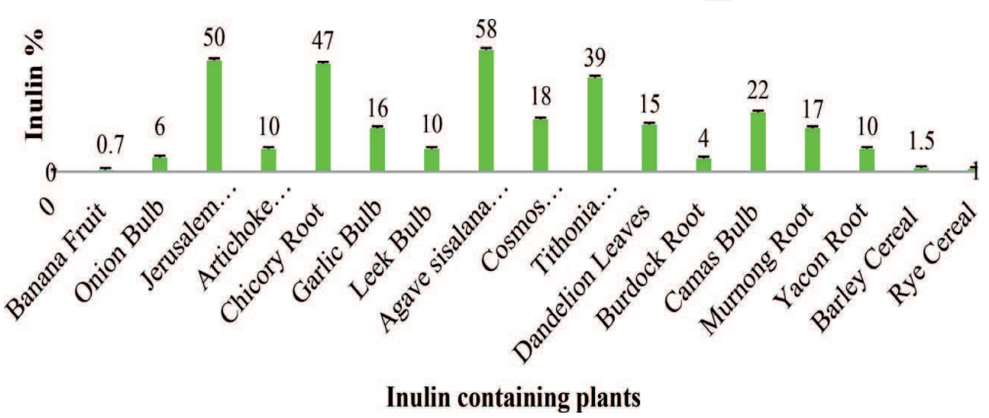

Figure 2.
Schematic depiction of inulin content in variety of inulin consisting plants.

Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus*) are common commercialized inulin source available in market. The fleshy tap root of chicory serves as warehoused of inulin (70–80%) [4]. Depending upon the growth stage of chicory, either inulin or oligofructose can be obtained captivatingly. After full root development and inflorescence axis arrival, endoinulinase hydrolyzes inulin into oligofructose, and exoinulinase further converts it into fructose. Most European countries have officially recognized inulin, oligofructose, and fructose as natural food ingredients, thereby having vast fascinating functional features that are beneficial to satisfy the needs of industries for imminent healthy food formulations. The present work currently focuses on two invasive home-grown (*Tithonia rotundifolia* and *Cosmos bipinnatus*) and one universally studied (*Agave sisalana*) inulin-rich weed species (**Figure 3**).

3.1 Agave

Agave is the most taxonomically diverse members of family Agavaceae. They are been surviving in extreme conditions by adapting themselves morphologically and physiologically. To escape transpirational water loss, they conduct crassulacean acid metabolism, thus liberating fructans as the chief photosynthetic product. *A. sisalana* was the common species found throughout Asia with rich inulin content, thus being used as substrate for alcohol and inulinase synthesis [5].

3.2 *Cosmos bipinnatus*

Cosmos bipinnatus of Asteraceae family is commonly famous as garden cosmos or Mexican aster, which is an inulin comprising weedy annual herb exotic for India. It has acclimatized on infertile, sandy soils along roadsides, exposed slopes, fence lines, hedgerows, or background areas as an ornamental plant getting transmuted into invasive weed [1].

3.3 *Tithonia rotundifolia* (Mill.) S. F. Blake

It belongs to family Asteraceae/Compositae and is commonly known as red sunflower, rooisonneblom, Japanese sunflower, shrub sunflower, and tree marigold. It is rich in inulin [6]. Thus, it serves as renewable raw material for fructose syrup (D-fructose) production. It is also grown as a green manure. But its high propagation frequency has forced to classify it as alien, invasive, competitive, allelopathic [7], noxious category 1 weed. There are reports on these weeds competing with crop plants and shading out native vegetation in the humid and subhumid tropics of South America, South East Asia, and tropical and subtropical Africa. Thus, the overall deleterious impressions put forth by this weed need to be rectified by an ecofriendly way.

Figure 3.
Inulin-rich weeds under present investigation: (a) Agave sisalana*, (b)* Cosmos bipinnatus*, and (c)* Tithonia rotundifolia*.*

4. Weed management strategies

Control majors like manual irradiation of these inulin-rich weeds are a tough job since they induce allergic effects [8]. Chemical practice can be used, but there are reports reviling the incidences of herbicide resistance weed expansion. Additionally, a striking raise in expenditure (>30$ ha^{-1}) of such weed remedy is too observed [9]. Accumulation of chemical scums in groundwater was another problem that emerges by the application of herbicides [10].

Thus, the pressure was to lessen herbicide usage and to reevaluate its environmental safety, development of alternative weed-control options was cheered. The best proposed avenue is to use microbial weed treating strategy, where actively propagating microorganism is subjected on target weed to achieve rapid control by its enzymatic hydrolysis into cost-effective product. Thus, exploiting inulinase producing soil microbes has been crucial tool in our efforts to renovate these weeds into fructose: a profitable calorie condensed sweeteners [11]. Microbial bioconversion finally is the best defense evident against this invasive attack.

5. Inulinase

Inulinases are fructofuranosyl hydrolases that cleave inulin into fructose moieties. Fructo-sugars, fructooligosaccharides (FOSs), or simply oligofructoses are the fructose oligomers formed after the action of inulinase on inulin [12]. Inulinase is an industrially crucial class of enzyme incorporated into glycoside hydrolase families 32 and 91. Based on their mode of action (**Figure 4**) on inulin, inulinases are alienated into dualistic types: (1) exoinulinase (β-D-fructanfructohydrolase, E.C. 3.2.1.80) and (2) endoinulinase (2, 1-β-D-fructanfructanohydrolase, E.C. 3.2.1.7) [13].

5.1 Microbial sources of inulinase

Phylogentically diverse microorganisms comprising bacteria, filamentous fungi, yeasts, and actinomycetes were testified to synthesize inulinase enzyme [14]. Due to

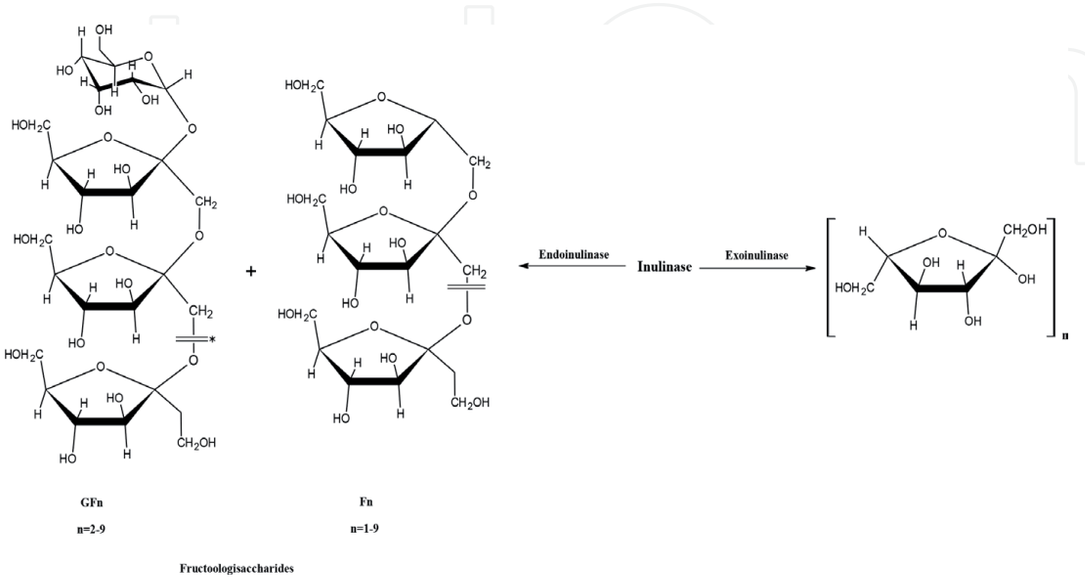

Figure 4.
Enzymatic hydrolysis of inulin-rich weed where (*) signifies site of inulinase activity on repeating β-(1-2)-D-fructosyl units of inulin.

the easy cultivation and higher enzyme yield, bacterial spp. are being commercially exploited to produce inulin hydrolyzing enzymes. The literature published recently [9, 13, 15–21] regarding the inulinase producers yielding maximum enzyme is embodied in **Figure 5**.

5.2 Substrates for inulinase production

Media complexity and culture conditions influence the enzyme production critically. The morphogenesis and metabolic pathway involved in enzyme induction can be noticeably affected by altering the media components and the growth parameters. Therefore, this substitution may accelerate biocatalysis of substrate into desirable products.

Inulin, starch, sucrose, and inulin-rich plant extracts are been widely utilized as exclusive, cheap, and best carbon source for biosynthesis of inulinase by several microbes. This polyfructan along with naturally occurring inulin-rich material and mixed substrates contributes as potent inducers for inulinase production. This plant-derived abundant storage polysaccharide is also present in roots and tubers of Compositae and Gramineae plants and numerous invader weeds. The review mentions a wide substrate used for inulinase production mutant [9, 19]. Dahlia (*Dahlia pinnata*), rhizosphere of Jerusalem artichoke (*H. tuberosus*), chicory (*C. intybus*) roots, kuth (*Saussurea lappa*) roots, *Allium sativum,* and *Allium cepa* have broadly been exploited for this perseverance. Mature *C. intybus* root was found to be the best substrate for receiving maximum extracellular inulinase from *Fusarium oxysporum* [22].

Figure 5.
Glance on varied inulinase producing microorganisms [55–78].

5.3 Inulinase production

Enzyme production is critically influenced by media complexity and culture conditions. Alterations in these two factors noticeably affect the morphogenesis and metabolic pathway involved in enzyme induction. It may accelerate biocatalysis of substrate into desirable products.

Inulinase enzymes are commercially produced consuming synthetic inulin and agroindustrial residues by submerged fermentation as well as by solid-state fermentation (SSF). Microorganisms, substrate, and cultivation method for inulinase production in certain studies reported in the literature [23, 24] are described later. The records show a resilient inclination to substitute high value synthetic inulin by agroindustrial substrates so as to make this enzyme production process cost effective. *Kluyveromyces* genus is reported to be the excellent inulinase producers [25]. Researchers explained that under optimum condition, the *Kluyveromyces marxianus* NRRL Y-7571 extracellular enzyme concentration extended to 391.9 U/g of dry fermented bagasse. Thus, due to the high availability and low rate sugarcane and corn industries, deposits (sugarcane bagasse, molasses, and corn steep liquor) can be economically attractive [26].

5.4 Factorial design

The escalated microbial growth and enzyme yield throughout the fermentation need to be keenly monitored. This is well accomplished by optimizing the fermentation conditions. The single-dimensional traditional simple frequently employed optimization method encompasses fluctuation of one independent variable at given level and maintaining others constant. Since it lacks the possible interactions among factors, it is least preferred. Thus, an effectual experimental scheme like response surface method is adopted to operate optimal conditions for multivariable systems. It aids in appreciating interaction of parameters and recognizing optimal range for higher yield. It also includes variety of statistical techniques used for experimental design and model erection that measures and scrutinizes the optimum conditions. Effective optimization of fungal, bacterial, and yeast inulinase production consuming diverse substrates such as Jerusalem artichoke, sugarcane bagasse, and molasses in submerged or solid-state cultivation was stated in the literature [27]. Diagrammatic depiction of microorganisms and optimized experimental variables is accessible in **Figure 6** [13, 16, 28–33].

5.5 Purification and properties of inulinases

The nature, interaction, and additional specific properties can be well understood in case of pure enzymes than the crude ones. Enzyme purification thus serves as a crucial footstep. The efficacious purification is reliant on complexity, charge distribution, and physicochemical properties of enzyme. Size, polarity, ligand interactions, and solubility are few of the strategic factors that define the choice of purification techniques to be applied for purifying inulinase. Some common purification techniques hired are salt or solvent precipitation, ion exchange, affinity, hydrophobic interaction, gel exclusion chromatography, and ultrafiltration [34].

Implication of ammonium sulfate precipitation method followed with column chromatography, boosted *X. oryzae* endoinulinase recovery by 2.9-folds [35]. Thermostable endoinulinase from *Bacillus smithii* was purified by ammonium sulfate precipitation and ion exchange chromatography. The exoinulinase synthesized by *Arthrobacter* spp., *Arthrobacter globiformis*, *Bacillus stearothermophilus, Pseudomonas mucidolens,* and *Thermotoga maritima* was recovered and purified for further studies. Salt precipitation functioned better in bacterial inulinase

Figure 6.
Highlight on inulinase production by various microbes under specific fermentation conditions.

purification, whereas organic solvent precipitation was preeminent for fungal inulinases. The extraordinary solubility of ammonium sulfate in water makes it more preferential for salt precipitation. This ammonium sulfate after cleavage gets converted into two ionic forms, thus sustaining its top most position in Hofmeister series. Structural integrity of protein is least exaggerated by this salt during the salting out progression. The increased probability of protein repression in organic solvent existence reduces its utility in enzyme purification. Maximum reports on use of ion exchange and gel exclusion chromatography followed by high selective affinity chromatography are noticed for biomolecule purification. The chemical structure and function of bacterial and fungal inulinase decide which purification techniques are to be employed for its purification. These techniques are reliable in convalescing interested protein in short time. The requisite factors like widely oscillating temperature and pH stability of inulinase, along with other vital characters, before being exploited for industrial applications need to be thoroughly inspected.

Physical elements such as molecular weight (M_r), Michaelis-Menten constant (K_m), and maximal velocity (V_{max}) are significantly imperative to characterize an enzyme. Heteromeric structure and any conformational variations are well enlightened by molecular weight studies of an enzyme. K_m and V_{max} values illuminate the enzyme kinetics and also emphasize on the specificity and affinity of inulinase for varied substrates. This affinity is designated by K_m. K_m is the substrate concentration that engages half of enzyme's active site. Lower K_m illustrates higher affinity of enzyme toward specific substrate and vice versa.

5.6 Structural peculiarities of purified inulinases

The molecular masses of bacterial and fungal inulinases oscillate in the range from 28 to 450 kDa as denoted in **Figure 7** [36]. Most of the fungal inulinases have

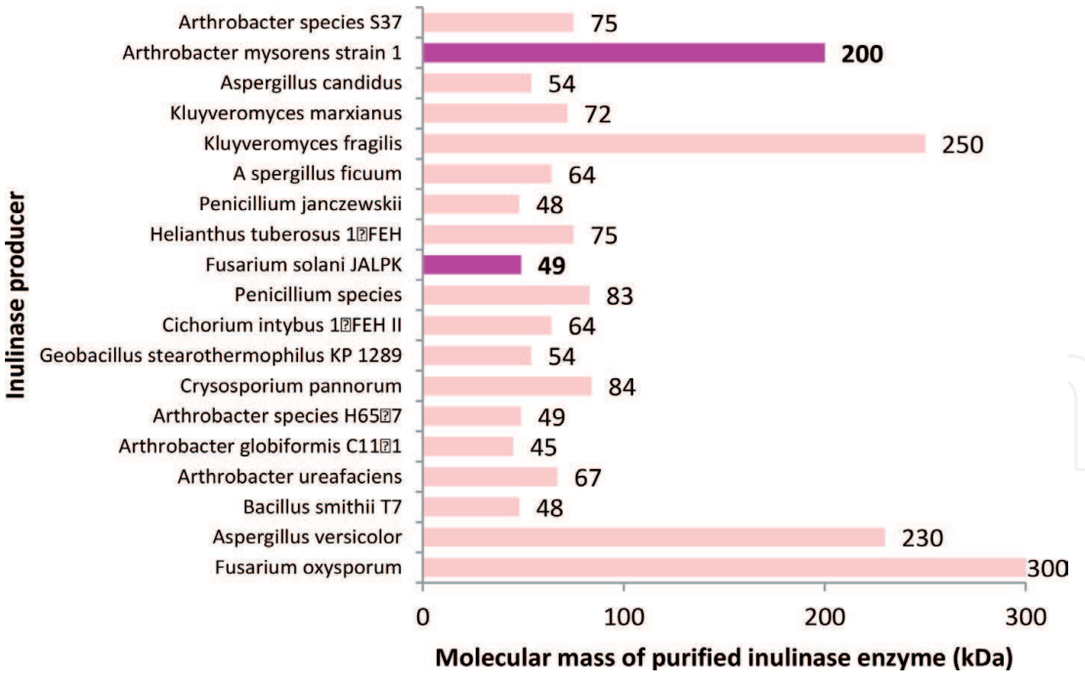

Figure 7.
Comparison of molecular masses of inulinase from numerous microbial sources obtained after SDS-PAGE electrophoresis.

molecular weight exceeding 50.0 kDa. Three inulinases with molecular masses 42, 65, and 57 kDa were isolated and purified from *Kluyveromyces* species Y 85.

Characterization of fungal and bacterial endoinulinases is also investigated after its purification. The purified endoinulinase harvested from *Penicillium* sp. TN-88 has molecular mass of 68.0 kDa [37]. *Arthrobacter* sp. S37 also produced extracellular endoinulinase, which was purified and found to have approximately 75 kDa [38].

5.7 Profitable approach of inulinase efficacy

Owing to the scenarios in food, pharmaceutical and nutraceutical industries, microbial hydrolysis and bioconversion of inulin have established a new source of revenue to several workers [39].

Inulinase offers exciting perceptions in view of the budding need for the Ultrahigh-Fructose Syrup (UHFS) production from inulin. Approximate 95% pure fructose can be obtained by enzymatic hydrolysis of inulin in the presence of inulinase. Thereby, inulinase-producing microbes are been extensively exploited by numerous industries so as to get value-added UHFS from inulin-rich weeds.

Inulinase and inulinase producers along with superfluous microorganism amalgamation are prominently affianced for simultaneous saccharification and fermentation (SSF) of diverse substrates in ethanol production methods [39–41]. Ethanol is the greatest hired liquid biofuel either as a fuel or as a gasoline complement [42]. Agave, chicory, dahlia, Jerusalem artichoke tuber, and many other inulin-rich weeds aid as the finest raw resources for fuel ethanol production. Certain wild-type microbes were mutated to offer maximum yield. Various experimentations were performed on sugar-beet molasses and numerous plant extracts so as to be used as feedstock to gain ethanol.

Inulinases are furthermore broadly subjugated in commercialization of inulo [43], gluconic acid, sorbitol, pullulan, acetone-butanol [44], and other key products.

6. Product formed after inulinolytic hydrolysis

The hydrolysis of inulin feedstock by inulinase yields astonishing amount of fructose in fermented broth. Carbohydrate, particularly fructose, is an indispensible chunk of the human diet. It owes exceptional properties and is nearly 1.5 times sweeter than sucrose, thus enhancing the palate and pleasure of several foodstuffs. It is recovered by passing through carpet bag filters containing activated charcoal and is further crystalized using chilled solvents, ethanol specifically.

Beyond 30 proceeding years, pure crystalline fructose has stood at the heights in the market as a health supplement in food and beverage. Purity is the pivotal feature that draws a distinguishing sharp line between crystalline fructose and high fructose corn syrup (HFCS). Crystalline fructose products are characteristically 100% pure fructose, while HFCS comprehends nearly equivalent shares of fructose and glucose-like sucrose (table sugar). As pure crystalline fructose is bounteously sweeter than sugar, its minor amount is also adequate to accomplish the same level of sweetness. Thus, lower-sugar and trifling calorie foods typically contain pure crystalline fructose. Food genii company also favors pure crystalline fructose as it owns supplementary properties beyond sweetness, which marks it very lucrative in drinks and candy, cakes, and other food industries [45].

6.1 Purification of fructose

The separation of FOS and fructose is frequently accomplished by reckonable chromatographic techniques. In dietetic products, optimal FOS separation is done by implementing glass-packed precoated silica gel with sodium acetate. Liquid chromatography (LC) with acetonitrile as a mobile phase is executed to purify nonstructural carbohydrates such as sugars and FOS with 3–19 degrees of polymerization. Auxiliary cost-effective methods exploiting activated charcoal fixed bed column with 80% degree of purification and 97.8% recovery of Fructose are superfluously proficient [43]. Purified fructose is assessed by diverse techniques such as NMR, MALDI-MS, MALDITOF, GC-MS, and ESI-MS [46]. The prebiotic fructose metabolism in microorganisms can be premeditated through microarrays [43].

6.2 Commercial applications of fructose

Pure fructose along with FOSs is finely specified to exist in voluminous natural foods. Gigantic companies are manufacturing these extensively applicable healthy and calorie-free products via hydrolyzing inulin weeds by exploiting microbial inulinases. Few lucrative applications of fructose emphasized in the review [47].

6.2.1 In food industries

Fructose serves as one of the key ingredients in food products such as energy and sports drinks, flavor boosted water, carbonated sodas and drinks, beverages, low-calorie food options, cereals, oatmeal, and yogurts and baked goods [3].

6.2.2 Fortification of nominated fruit juice beverages

Investigation reveals that sucrose employed as fruit juice sweetener, with no considerably quality loss can be replaced with FOS and fructose.

6.2.3 Fructose in medicine

Fructose is very frequently found as sweetener in cough suppressants, decongestant drops, rubs, and liquids for children and adults. Many pharmaceutical tablets, syrups, and solutions commonly have fructose as an excipient [48].

6.2.4 Proficient sweetener for diabetics

Inulinase from *Aspergillus oryzae* carries out hydrolysis liberating fructose and FOSs. Existence of mono to pentasaccharides without toxic microbial metabolites in the hydrolyzed product was assessed with NMR spectroscopy and LC-MS, thus excavating its application as a food ingredient [49].

6.2.5 Supplementing oral electrolyte solutions as diarrhea control remedy

The retrieval of overall bacterial counts amplified by ingestion of OES and fructose to pigs with acute diarrhea induced by cholera toxin was the most attention grabbing finding [50].

6.2.6 Dietary intonation of the human colonic microbiota

A trifling prebiotic effect with no gastrointestinal distress in pediatric patients with cancer was found to be induced by FOS, especially fructose [51].

6.2.7 Immunomodulatory effect

Clinical trials direct that fructose and FOS supplementation can reduce the influx of clinical inflammation, abridged level in cytokine interleukin (IL)-1α, and necrosis factor-α in ulcerative colitis by *Bacillus longum*. A shoot-up in IL10 positive mucosal dendritic due to inulin, fructose, and FOS intake was displayed in patients with Crohn's illness [52].

6.2.8 Cancer treatment

Incidence of cancer has been rapidly decreasing due to the use of FOS and fructose. Tumor growth, cell differentiation, and upregulate apoptosis were vetoed by the Butyrate manufactured by FOS and fructose [53].

6.2.9 Antibiotic therapy

Damage of normal protective intestinal microflora was a common observation found to be accompanied with acute diarrhea after been treatment with penicillin, cephalosporin, and clindamycin antibiotics. Double-blind randomized controlled trials were set, which efficaciously explain that in the course of antibiotic treatment reoccurrence of diarrhea was shortened in patients ingesting fructose.

6.2.10 Antioxidant properties

Mesa and his coworkers studied protein glycation and cross linking along with the effect of elevated temperature and proteolysis on antioxidant properties of the Maillard reaction mixtures of soy protein isolates, FOS, and fructose with appropriate controls [54].

6.2.11 Enhancing Salmonella vaccine efficacy

An elevation in specific blood immunoglobulin G specific to *Salmonella* and fecal immunoglobulin A was recorded in mice fed on the fructose and inulin encompassing diet as compared with control mice when infected with LD100 of virulent *S. typhimurium* for tolerable time interlude [49].

7. Concluding remark

The current review discloses the elucidations of many global researchers specifically highlighting on the isolation of novel inulinase-producing rhizospheric microbial flora to hydrolyze high inulin content in weeds, thereby serving as a potential, abundant, and profitable avenue of fructose production with vast industrial applications. The food and pharmaceutical preparations with fructose have extended at the top in the market demand list of health conscious modern era. Thereby, the enzyme production expenses linger to be the logjam in understanding its commercial application.

Thus, this review explores the exploitation of inulin containing weeds such as *Tithonia* and *Cosmos* as low-value and efficacious replacement of synthetic inulin as substrates for inulinase production. The research embarked on the health implications of dietary and pharmaceutical fructoses was underlined in the review. Finally, electrifying new uses of fructan polysaccharides such as drug stabilizers, scrupulous release drug delivery systems, and vaccine adjuvants proclaims evolution in pharmaceutical applications of this extremely multipurpose plant-derived sugar.

Acknowledgements

The author thanks the following contemporaries for their service in data assembly: Nishigandha D. Patil, Rohit. R. Joshi, Pramod R. Kamble, Deepali D. Jadhav, Arun D. Jadhav, Samita S. Bhosale, and other Rajaramians. Special thanks to Shivaji University, Kolhapur, for granting Golden Jubilee Research Fellowship to Prajakta P. Kamble, as a financial support to execute the present work.

Conflict of interest

The authors declare that they have no conflicts of interest.

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