Research Progress on Functions and Biosynthesis of D-Psicose

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Abstract D-Psicose, a naturally occurring rare sugar, exhibits a sweetness approximately 70% that of sucrose. It possesses high solubility, antioxidant activity, anti-inflammatory properties, and the ability to regulate cholesterol levels and enhance insulin sensitivity. However, D-psicose is relatively scarce in nature, making large-scale extraction and utilization impractical. Consequently, the development of cost-effective synthetic strategies for D-psicose is pivotal for its industrial application. In recent years, the Izumoring strategy has emerged as an efficient alternative to chemical synthesis for producing D-psicose. Nonetheless, limitations in the biotransformation of D-psicose, primarily governed by the conversion rate of D-psicose 3-epimerase (DPEase) and enzyme yield, continue to pose challenges in achieving economically viable production. Enzyme engineering and the establishment of high-level expression systems remain crucial avenues for reducing the overall biosynthesis costs.

Key words D-Psicose; Biosynthesis; High-throughput screening

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In recent years, many studies[1] have focused on the adverse effects of sugar drinks on human health, because these drinks have made great contributions to the intake of total added sugar or free sugar, and their consumption rate has increased rapidly. Generally speaking, sugared drinks are the largest source of sugar, including carbonated and non-carbonated drinks, fruit drinks, sports and energy drinks. According to previous surveys, the consumption of sugary drinks in many developed countries is still at a high level, but it is decreasing. However, in many developing countries, the consumption of sugary drinks is still increasing[2], which may be due to the increase in the supply of sugary drinks with the development of economy. The annual report of Coca-Cola Company in 2007 showed that the consumption of white sugar drinks in India and China increased by 14% and 18% respectively in one year. In 2018, a cross-sectional survey of primary school, junior high school and senior high school students in China showed that sugary drinks accounted for 10% - 15% of the total calories consumed by students. According to the report of National Health and Nutrition Examination Survey (NHANES), in 2009 - 2010, the energy intake of American children from sugary drinks was 8%, and that of adults from sugary drinks was 6.9%. In addition, the 2010 world survey showed that 180 000 people around the world died from eating sweet food. These findings have promoted global policies to restrict sugar consumption, including sugar tax, food labeling law, advertising and marketing restrictions[3-4]. Moreover, the World Health Organization (WHO), the United States Department of Agriculture, the United States Department of Health and other national and international institutions suggest reducing the consumption of added sugar to less than 10% of the daily energy intake. An investigation report (EPIC) from Europe shows that drinking sugary drinks is related to the increase in the incidence of type 2 diabetes, but has nothing to do with obesity. Although artificially-sweetened drinks and fruit juices are also positively correlated with the incidence of type 2 diabetes, the research results may be biased[5].

It seems to be a good choice to use artificial sweeteners as sugar substitutes for sugary drinks, but some recent studies speculate that artificial sweeteners decoupled from calories and content will partially but not completely activate food reward channels, which may further encourage food-seeking behaviors, such as craving for sweetness and dependence on sugar in other foods. In a controlled experiment, aspartame plus sugar water increased the subjective appetite score of normal-weight adult men. A similar phenomenon has also been found in rats, and supplementing saccharin without glucose addition will significantly increase total energy consumption and body weight. A recent study found that commonly used non-calorie artificial sweeteners can lead to glucose intolerance by changing intestinal microflora. Antibiotic therapy can alleviate these effects, and these effects can be completely transferred to sterile mice by fecal transplantation of the microbial community of mice with consumption of non-calorie artificial sweeteners. These observations make people worry about the widespread use of non-calorie artificial sweeteners[6].

Recently, natural rare sugars have become another hot category of sweeteners[7]. The content of these monosaccharides and their derivatives in nature is very small, and their potential advantages include good palatability, no offensive aftertaste and low calorie[8], because they will not be metabolized by the human body or the degree of metabolism is lower than that of natural sugars[9]. Among more than 50 kinds of rare sugars in nature, D-psicose, D-tagatose, D-sorbose and D-allose[10-11] have been studied as sugar substitutes, among which D-psicose has the most market potential.

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Physiological Effects of D-Psicose

D-Psicose is an isomer of D-fructose[12-13], and its sweetness is 70% of sucrose. Because of its high solubility[14] and antioxidant activity[15], it has become a good additive in food industry[16-19]. In addition, D-psicose can inhibit the α-glucosidase in the intestine of rats, and inhibit the blood glucose response after carbohydrate intake[20-21]. In a small clinical trial, after ingesting 75 g of maltodextrin, continuing to ingest 5 g of D-psicose can reduce the plasma glucose and insulin concentrations[22]. D-Psicose can inhibit the activity of intestinal sucrase and maltase and improve postprandial hyperglycemia[23]. In addition, D-psicose has anti-hyperglycemia effect on patients with glucose intolerance and rat models of type 2 diabetes[24-25].

In terms of maintaining the body's function, it is found that D-psicose has anti-inflammatory effect[26-27], which can reduce the levels of proinflammatory cytokines TNF-α, IL-6 and MCP-1 in serum[28]. D-Psicose can also scavenge reactive oxygen species[29] and prevent cell apoptosis and testicular damage[30-32] through anti-oxidation[33-34]. In addition, D-psicose can enhance the activities of SOD and CAT, which may help to prolong life[35-38]. It was also found that D-psicose can reduce the level of Pcsk9 and affect the serum cholesterol level of hamsters[39]. In addition, the insulin sensitivity of rats was improved after ingesting D-psicose[40]. In a word, D-psicose has many physiological functions, such as resisting inflammation and oxidation[41], regulating cholesterol level and improving insulin sensitivity[42-44].

Biosynthesis of D-Psicose

Production of D-psicose by D-psicose 3-epimerase

D-Psicose is a substance with many beneficial physiological functions, but it cannot be widely used because of its rarity. The traditional chemical synthesis method[45] requires complex reaction steps and produces a large number of by-products[46], so it is challenging to obtain a single product configuration. On the contrary, biotransformation method has the advantages of mild reaction conditions, few by-products, simple purification and environmental friendliness, so it has become the main method to synthesize D-psicose. At present, one of the main methods used in the biological preparation of D-psicose is the Izumoring strategy[47].

Through the Izumoring strategy, D-psicose can be produced from D-tagatose by using enzymes of D-tagatose 3-epimerase family[48]. In recent years, the Izumoring strategy is very effective in the biosynthesis of rare sugars. The enzymes of DTEase family, especially D-psicose 3-epimerase (DPEase), play a vital role in the biosynthesis of D-psicose from D-fructose[49]. DPEase has high specificity for D-psicose. Since the first DTE enzyme was identified from *Pseudomonas* sp. ST-24 in 1993, several other DTE enzymes[50] have been isolated from different species, such as DTEase family enzymes from Pseudomonas sp., *Clostridium cellulolyticum* H10[51-52], *Sinorhizobium* and *Rhodopirellula baltica* SH 1[53-59]. It has also been reported that 90 g of D-allose was produced from 500 g of D-fructose by immobilizing DTEase from *Pseudomonas* sp. on chitosan beads. Since then, people have been interested in the method of immobilizing DTEase. Various studies have proved that immobilized enzyme has better thermal stability and storage stability[60-61].

Biosynthesis of D-psicose from cheap materials by DTEase enzyme

The utilization of by-products (such as fruit and vegetable residues) has always been a topic of concern because they will cause serious agricultural problems. These residues are usually buried or burned. However, they contain a lot of dietary fiber and sugars, including sucrose, D-glucose and D-fructose. Transforming dietary fiber and sugar into high-value products is considered to be an important step to coping with agricultural challenges.

Compared with the traditional step-by-step synthesis method, cascade catalysis is regarded as an attractive method. This technique is often used to produce unusual sugars from low-cost materials such as sucrose, jerusalem artichokes, inulin and fruit/vegetable residues. It is observed that D-psicose can be efficiently synthesized from sucrose by using purified recombinant invertase, D-xylose isomerase and DTE enzymes. In addition, cascade solutions can be combined with simulated moving bed (SMB) chromatography to produce pure D-psicose (99.9%) with considerable yield (89%). D-Psicose can also be synthesized by two-step cascade reaction, including hydrolysis of jerusalem artichokes, cruciferous vegetable residues and inulin. Zhu *et al.* developed a one-pot double-enzyme reaction system, which may reduce the production cost and avoid the need for enzyme purification. In their research, they used a new type of exoinsulinase from *Bacillus weihenstephanensis* (BvInu) and DAEase from *Ruminococcus* sp. to produce D-allose from jerusalem artichoke. Exo-insulinase and DAEase were expressed in *Bacillus subtilis* and secreted into the supernatant without purification. According to the study by Zhu *et al.*, 10.3 g/L D-psicose could be obtained from 50 g/L inulin at the optimal ratio of BvInu/RDAE (80∶40 U/g inulin) and 50 ℃ for 2 h[62].

Biosynthesis of D-psicose using microorganisms

Compared with one-pot enzymatic cascade reaction, whole-cell biocatalytic reaction has several advantages. (1) Cells containing enzymes are easy to obtain, and complicated enzyme purification is not needed. (2) Cell environment provides suitable microenvironment and cofactor regeneration (ATP, NAD +/NADH). (3) Cell wall and cell membrane protect enzymes from adverse reaction conditions. (4) The co-location of multiple enzymes in cells increases the local concentration of enzymes and reduces the diffusion of intermediates in cascade reactions[62].

Escherichia coli is widely used in engineering strains producing D-psicose. The co-expression of DAEase and D-glucose isomerase (GIase) in *E. coli* can transform D-glucose into D-psicose, and the co-expression of DAEase and xylose isomerase (XI) can transform D-glucose into D-isopentose. However, due to endotoxin, *E. coli* is not suitable for food industry. Therefore, DAEase is also expressed in food-grade strains, such as *Bacillus subtilis*,

Saccharomyces cerevisiae and *Corynebacterium glutamicum*. Subsequent research found that the fusion of DAEase with the C-terminal of CotZ can improve its thermal stability, which is applied in the expression of DAEase on the spore surface of *B. subtilis*. In addition, a variety of DAEase and invertase (INV) are overexpressed in glutamic acid yeast, and the engineered cells immobilized with alginic acid are cascaded in a one-pot two-step reaction system to produce D-psicose from cane molasses. After 8 h of reaction, 61.2 g/L of D-psicose can be obtained, accounting for 17.4% of the total monosaccharide. At present, the Izumoring strategy is the main method for industrial production of D-psicose, and it is also the simplest and most commonly used method for biosynthesis of D-psicose. However, the limitation of thermodynamic equilibrium is the bottleneck restricting the large-scale application of D-psicose in food industry[63].

Structure and Catalytic Mechanism of D-Psicose 3-Epimerase

DPEase from *Agrobacterium tumefaciens* and the crystal structure (2HK0) of its complex with real substrate D-fructose shows that DPEase is a tetramer, and each monomer belongs to TIM barrel folding. The active site in each subunit is different from those of other TIM barrel enzymes, which use phosphorylated ligands as substrates. It contains a metal ion with octahedral coordination, which is completely conserved with two water molecules and four residues, and these residues are absolutely conserved in the whole DTEase family. After combining with D-fructose, the substrate displaces water molecules in the active site, and its conformation is similar to that of intermediate cis-ene diester. Subsequently, Trp112 and Pro113 in β4-α4 ring undergo significant structural changes, blocking the active site. Structural evidence and site-directed mutagenesis of putative catalytic residues show that metal ions play a key role in catalysis by anchoring D-fructose, and Glu150 and Glu244 undergo epimerization at C-3 position. In the forward reaction, the deprotonation of fructose at C-3 seems to be realized by Glu244, because the hydrogen atom of the bonded D-fructose at C-3 faces the carboxylic acid group of Glu244. The interaction observed around the metal binding site indicates that sp2C-3 is oriented towards the optimal direction of Glu150, and D-psicose is produced. In the reverse reaction, catalysis begins with the removal of protons from Glu150, and follows the same stereochemical mechanism as that suggested in the forward reaction[64].

Modification of D-Psicose 3-Epimerase

At present, D-psicose 3-epimerase has been isolated and identified from various microorganisms[65]. However, all reported D-psicose 3-epimerase shows low conversion rate and thermal stability, which brings challenges to the industrial production of D-psicose[66]. In 2006, Kim *et al.* reported a new DPEase from *A. tumefaciens*, which can effectively catalyze the isomerization of D-fructose and D-psicose. Immobilized free enzyme is an effective method for enzyme recycling and improvement of cost-effectiveness. Takeshita *et al.*[67] improved the large-scale production of D-psicose by immobilizing *P. cichorii* DTEase in a continuous bioreactor, with a conversion rate of 25%. After 60 d, about 20 kg of pure D-psicose was obtained by treatment with baker's yeast and crystallization with ethanol. In 2015, Bosshart *et al.*[68] reported the directional differentiation and evolution of constant temperature mutant (Var8) of *P. cichorii* DTEase, and realized two efficient external chimeras at C-3 position. Eight-loci mutant IDF8 was obtained by iterative randomization and screening around substrate binding sites, and Kcat was increased by 9 times during surface polymerization of D-fructose[69]. Zhang *et al.*[70] obtained a two-loci mutant (Y68I/G109P) of *C. bolteae* DAEase, and its catalytic efficiency and thermal stability were improved. They further obtained a triple mutant (F154Y/E191D/I193F) by site-directed mutation, and the t1/2 value of the mutant increased by 5.4 times at 50 ℃. Although scholars tried to use above strategies to improve the activity and thermal stability of the enzyme, new strategies are still needed to overcome the limitations of low yield, low conversion rate and poor thermal stability of D-psicose 3-epimerase, so as to realize the industrial production of D-psicose[71].

Construction of High-level Expression System of D-Psicose 3-Epimerase

In 2013, Chen *et al.* artificially modified AAL45544.1 gene from *A. tumefaciens* str. C58, and constructed it into recombinant *E. coli*, thus realizing high-level extracellular production of D-psicose 3-epimerase (DPEase). It was found that the addition of glycine had a positive effect on the extracellular production of DPEase. In the presence of 150 mM glycine, the extracellular activity (3.5 U/ml) and protein concentration (408 mg/L) reached the highest. After optimizing the medium composition, induction temperature and inducer concentration, the total DPEase activity reached 3.96 U/ml. Furthermore, the total DPE activity and extracellular DPE activity were 5.08 and 3.11 times higher after applying the two-stage glycerol feeding strategy based on the specific growth rate before induction and the residual glycerol after induction in a 3 L fermentor than those in shake flasks. The extracellular and intracellular DPE activity reached 10.9 and 13.2 U/ml[72].

In 2016, Zhang *et al.* successfully expressed RDPE (DPEase from *Ruminococcus* sp. 5_1_39BFAA) in *B. subtilis*, and compared three sugar-induced promoters. Xylose-induced promoter PxylA was proved to be the most effective promoter for DPEase production. Based on the analysis of inducer concentration and DPEase expression, it is clear that there is a very close correlation between intracellular RDPE expression and xylose accumulation level. Subsequently, the utilization of xylose was successfully blocked by the lack of lignin proteins (xylA and xylB) in *B. subtilis*, thus keeping the intracellular and extracellular xylose concentration constant. More importantly, the xylose level in cells increased to about 80 mg/g, which was significantly higher than that

of the parent strain, resulting in the increase of DPEase expression level in cells. As a result, the intracellular and extracellular expression of RDPE were significantly enhanced. Meanwhile, the optimal concentration of xylose was reduced from 4.0% to 0.5%. Finally, the secretion level of DPEase reached 95 U/ml and 2.6 g/L in a 7.5 L fermentor[73].

In 2019, Fu *et al.* cloned DPEase from *Ruminococcus* sp. (RDPE) and expressed it in *B. subtilis* A311. Through two-step pH regulation of staged fermentation, the yield of RDPE was significantly increased and the fermentation cost was reduced. The two-step regulation included the first step of keeping the pH value at 7.0 for 24 h, and the second step of slowly regulating the pH value to 7.5 and keeping it for 24 h. Finally, the yield of DPEase increased to 74 U/ml, which was about 2.5 times compared with the control group[74].

Prospects

D-Psicose 3-epimerase has been isolated and identified from various microorganisms. However, all reported D-psicose 3-epimerase shows low conversion rate and thermal stability, which brings challenges to the industrial production of D-psicose. At present, studies show that strategies such as enzyme immobilization, rational design and semi-rational design transformation of enzymes and directed evolution can improve the activity and thermal stability of enzymes. Constructing high-level expression systems is an important means to improve the yield of D-psicose 3-pimerase.

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Plastic waste causes serious pollution to the environment; therefore, sustainable packaging materials and packaging designs need to be researched and explored to minimize the negative impacts on the environment.

In addition, the choice of ingredients for prepared dishes is also relevant to the issue of sustainability. Some prepared dishes may use large amounts of pesticides, chemical fertilizers and genetically modified ingredients, which have negative impacts on the ecosystem and human health. Therefore, examining the sustainability concerns of prepared dishes should also take into account the choice and origin of ingredients in order to promote a healthier and environmentally friendly food supply chain.

Finally, the consumption of prepared dishes is also relevant to sustainability challenges. Consumers' choices and purchasing behavior towards prepared dishes also have an impact on the development and sustainability of the market. Therefore, research on sustainability concerns should also include investigations and analyses of consumer awareness, preferences and purchase motivations.

Conclusions

Looking ahead, the market for prepared dishes still has much room for development. With the popularity of healthy diet and changes in lifestyle, the prepared dishes market will gradually develop in the direction of health, nutrition and convenience. Meanwhile, with the progress and application of technology, the prepared dishes market will also usher in more innovation and change.

In conclusion, through this research and analysis, we can see that most of the interviewed college students show positive attitudes towards the prepared dishes market and are willing to try to buy and consume prepared dishes products, but also put forward higher requirements for the quality and trust of the products. Therefore, for prepared dishes companies, the prepared dishes market needs to further enhance transparency and information disclosure in order to increase college students' trust in their products, and needs to be improved in terms of product quality, hygiene supervision, healthy dishes and brand promotion. Through the implementation of these measures, it is believed that the prepared dishes market will have a broader market prospect among college students.

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