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REVIEW

Biocatalysis for the Production of Industrial Products and Functional Foods from Rice and Other Agricultural Produce

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Many industrial products and functional foods can be obtained from cheap and renewable raw agricultural materials. For example, starch can be converted to bioethanol as biofuel to reduce the current demand for petroleum or fossil fuel energy. On the other hand, starch can also be converted to useful functional ingredients, such as high fructose and high maltose syrups, wine, glucose, and trehalose. The conversion process involves fermentation by microorganisms and use of biocatalysts such as hydrolases of the amylase superfamily. Amylases catalyze the process of liquefaction and saccharification of starch. It is possible to perform complete hydrolysis of starch by using the fusion product of both linear and debranching thermostable enzymes. This will result in saving energy otherwise needed for cooling before the next enzyme can act on the substrate, if a sequential process is utilized. Recombinant enzyme technology, protein engineering, and enzyme immobilization are powerful tools available to enhance the activity of enzymes, lower the cost of enzyme through large scale production in a heterologous host, increase their thermostability, improve pH stability, enhance their productivity, and hence making it competitive with the chemical processes involved in starch hydrolysis and conversions. This review emphasizes the potential of using biocatalysis for the production of useful industrial products and functional foods from cheap agricultural produce and transgenic plants. Rice was selected as a typical example to illustrate many applications of biocatalysis in converting low-value agricultural produce to high-value commercial food and industrial products. The greatest advantages of using enzymes for food processing and for industrial production of biobased products are their environmental friendliness and consumer acceptance as being a natural process.

KEYWORDS: Amylases; amylopectin; amylose; bioethanol; biofuel; bifunctional amylopullulanase, ethanol; immobilized enzymes, maltose; *Picrophilus torridus;* protein engineering; recombinant enzyme technology; starch; starch hydrolysis; thermostable enzymes; trehalose; trehalose synthase; transgenic rice

INTRODUCTION

Starch, a polyglycan (polymer of D-glucose units) and the major storage polysaccharide in plants, is composed of amylose and amylopectin. Amylose is mainly a linear polymer of D-glucosyl units (up to 6000 units) joined together through α-1,4 glycosidic linkages, and amylopectin is a highly branched polymer of D-glucosyl units (up to 2 million) joined together through α,1-4 and α,1-6 glycosidic linkages. The degree of polymerization in starch is denoted as DP. Rice (*Oryza sativa* L.) is a cereal grain rich in starch and protein, but not as high in starch or commercially used for starch products as other grains like corn, wheat, tapioca, and potato. Rice is a major staple food in many continents of the world (Asia, Africa, and Middle East). Rice grain can be processed to obtain oil, starch, and proteins for various food and industrial applications that may require further processing or conversion to the desired product.

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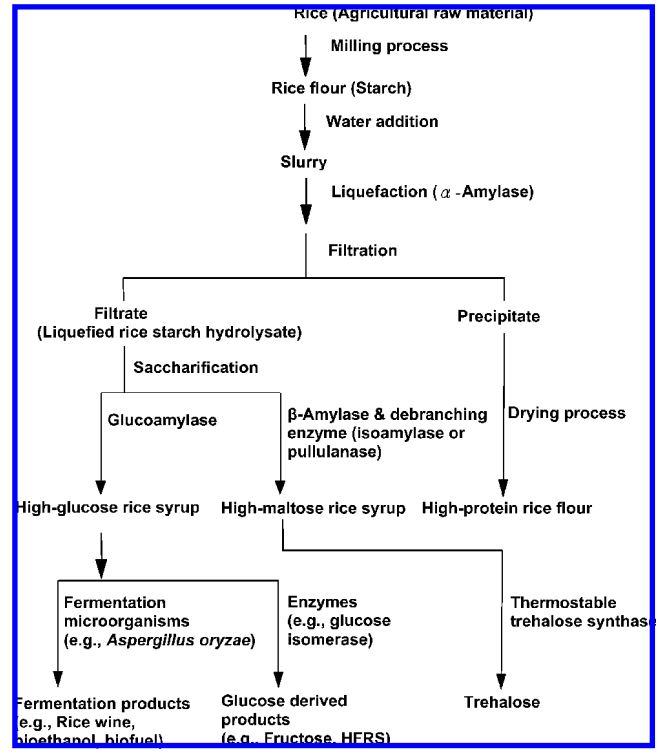
Rice starch is used in foods as a thickener, stabilizer, or filler. Starch is a major constituent of the human diet and can be chemically and enzymatically processed to obtain other products such as starch hydrolysates, glucose, maltose and fructose syrups, maltodextrins, and cyclodextrins. Starch is degraded or hydrolyzed through liquefaction and saccharification processes catalyzed by amylases. The main aim of liquefaction is to convert granular starch into soluble dextrins of lower molecular weight expressed as dextrose equivalents (DE). Usually, a thermostable α-amylase is added and the starch slurry heated to 105−110 °C for 5−7 min, then cooled to 95 °C and held for 1−2 h to complete the liquefaction process (*1*). On the other hand, the aim of saccharification is to hydrolyze the oligosaccharides (mainly 8−12 glucose units) to form maltose syrup catalyzed by *β*-amylase or glucose syrup catalyzed by glucoamylase (*2*). Before saccharification process, the temperature must be cooled down to 60 °C and pH adjusted to 4.2−4.5 for the glucoamylase from *Aspergillus niger* to catalyze the reaction efficiently. The heating and cooling processes and pH adjustment are not cost-effective with respect to energy and time consumption. A preferred and more desirable approach would be to use a combination of individual enzymes that would work under the same temperature and pH, possibly without cofactor requirements, or to use recombinant enzyme technology to fuse two or more thermostable enzymes to catalyze the sequential hydrolysis of starch to various products at the same pH and temperature without temperature adjustments. This will save energy, be cost-effective, and increase the enzyme productivity and desired product yield. The following sections will be used to illustrate and discuss these possibilities.

INDUSTRIAL PRODUCTS-EXAMPLE BIOFUEL

Starch, biomass, cellulose, and switch grass are some examples of renewable resources that can be converted to ethanol or bioethanol through fermentation and/or biocatalytic conversions. The renewed interest in other sources of energy such as biodiesel, bioethanol or cellulosic ethanol is due to the uncertainty in petroleum energy supply from unstable regions of the world, the possibility of fossil fuel exhaustion, and the need to decrease ozone depletion, reduce greenhouse gases, and have a more viable program for energy security. Low value agricultural products can be converted to biofuel and other industrial chemicals with the aid of enzymes. Enzymes are considered natural biocatalysts with high turnover number, high efficiency and specificity, energy savings, and low downstream processing cost for the product, and they can catalyze multistep reactions, can be reused in immobilized forms, are more thermophilic after immobilization, can be genetically modified to improve efficiency, and catalyze new reactions. Therefore, they are excellent candidates for biofuel production through biorefining. Biorefinery is a system that combines all the available technologies necessary to convert renewable low-value agricultural raw materials to high-value-added products such as chemicals, food ingredients, or biofuel. Other researchers have discussed biorefining and possible renewable biomass raw materials (*3, 4*). According to Turner et al. (*4*), biorefineries utilize the activities of microbial cells and their enzymes to convert biomass into target products. Similarly, both whole cells and thermostable enzymes can be used to convert raw rice starch or any starch and cell wall materials into bioethanol for fuel. As depicted in **Scheme 1**, it is possible to convert rice starch to glucose which in turn can be converted to ethanol through fermentation with the help of appropriate enzymes/enzyme systems and microorganisms or whole cells. For example, in rice wine production

Scheme 1. Schematics of the Process for Converting Raw Material Rice Starch to Industrial and Functional Food Products[a]



[a] HFRS = high fructose rice syrup (*32*).

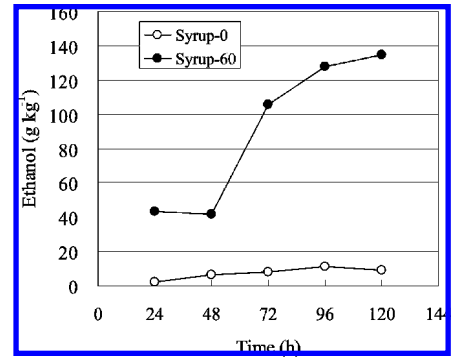
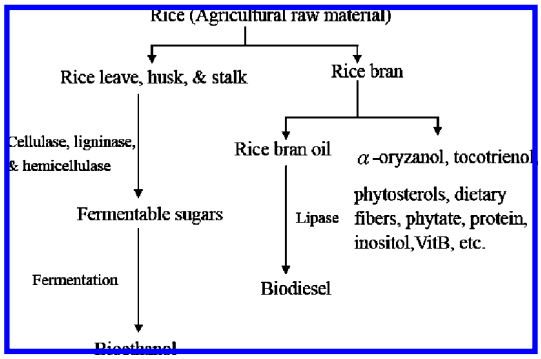


Figure 1. Time courses for bioethanol production from high-glucose rice syrups produced from 60 min (Syrup-60) or 0 min (Syrup-0) of glucoamylase-catalyzed hydrolysis (*32*).

from high-glucose syrup (105 mg mL^{-1} of glucose concentration) derived from the soluble rice starch hydrolysate, the ethanol concentration obtained in the current process was 13.5-fold higher than the original starch hydrolysate (11 mg mL^{-1} of glucose concentration) in anaerobic fermentation conditions at 30 °C for 5 days (**Figure 1**). In addition, the rice leaf, husk and stalk can be converted to bioethanol by enzymatic hydrolysis and fermentation processes. The rice bran oil is a specialty oil from which several valuable nutraceuticals such as oryzanol and vitamin E can be extracted, and the nonedible part of rice bran oil can be converted to biodiesel by lipase-catalysis reaction to maximize the exploitation of rice in full (*5*). The enzymatic process for converting various raw materials to biofuel products is depicted in **Scheme 2**.

Thermostable enzymes (discussed below) will play important role in the industrial production of biofuel, especially in the starch liquefaction and saccharification processes, avoiding chemical pretreatment, to produce sugars from starch and convert sugars to ethanol. Other possible raw materials include

Scheme 2. Schematics of the Enzymatic Process for Converting Raw Material Rice Leaf, Husk, Stalk and Bran to Functional Industrial Products



rice, sorghum, tapioca, and wheat starch, and sugar cane. In the U.S., the Department of Energy, venture capital groups, environmental groups, and oil companies such as Chevron are providing the financial resources for research in bioethanol production. The cost of available commercial enzymes is also coming down. Indeed, the cost of enzymes that would break down and ferment cellulose has decreased from $5/gallon to about 20 cents/gallon, making the biocatalysis approach to ethanol production cost-effective (*6*). Novozymes North America (Franklinton, NC) and Diversa (San Diego, CA) are currently working on providing commercially more efficient and effective enzymes (*6*).

FUNTCIONAL FOODS

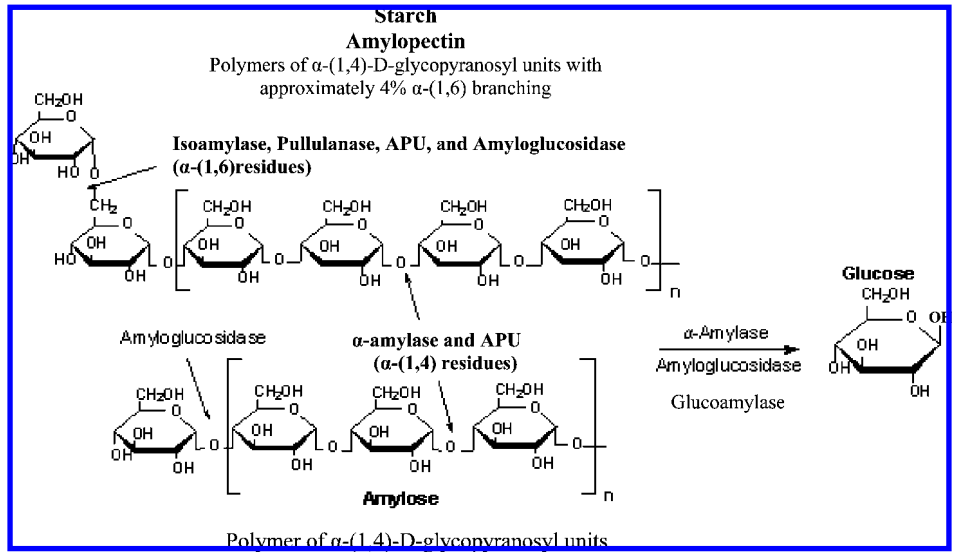
Many food and pharmaceutical ingredients and functional foods can be derived from starch. Examples include sugar, sugar alcohols, maltose, glucose, fructose, maltodextrins, cyclodextrins, and syrups. Acid hydrolysis is generally used to hydrolyze starch after it is isolated from the starch-containing agricultural produce. Some of the chemicals used to process starch may be undesirable and the process can be costly. Fermentation products such as wine, vinegar, and ethanol have commercial value. Microorganisms are used to convert starch to fermentable sugars; this process usually takes long, and the rate of conversion is slow. Rice protein content is low (6−10 wt %) compared to other grains, but rice flour has high nutritional value and has been used to formulate various food products such as pudding, instant milk and baby foods. A biocatalysis process to produce starch-derived and high-protein products from rice has been successfully developed. The starch hydrolysate products or fermentation products include high-glucose rice syrup and high-maltose rice syrup can be further converted to high fructose rice syrup (HFRS) and trehalose, respectively. We present here the current process for the conversion of various functional products from rice starch (**Scheme 1**). The enzymes/enzyme systems and microorganisms were able to simultaneously produce two valuable products, namely maltose and glucose, or their derivatives such as trehalose and fructose or fermentation products from glucose such as rice wine. High-protein rice flour was also produced from rice as a byproduct with high nutritive value for further health food applications. The major advantages of our biocatalysis process were significantly shortened the fermentation time, reduced the pollution or fermentation waste, and reduced their production cost.

To produce glucose-rich syrup and other syrups from rice, the rice starch can be treated into slurry with starch hydrolases such as α-amylase (EC 3.2.1.1), isoamylase (EC 3.2.1.68), pullulanase (EC 3.2.1.41), and amylopullulanase, APU (EC 3.2.1.41 or 3.2.1.1) that hydrolyze starch to linear oligosaccharide. α-Amylase will hydrolyze starch to oligosaccharides of varying chain length and α-limit dextrin that constitute branched oligosaccharides. Debranching enzymes such as isoamylase will hydrolyze only α,1-6 bond in amylopectin and pullulanase hydrolyzes α,1-6 glycosidic bond in pullulan and amylopectin. APU will hydrolyze α,1-4 and α,1-6 glycosidic bonds to yield mainly maltose and maltotriose. Glucoamylase (EC 3.2.1.3) was also added to hydrolyze starch or oligosaccharides to glucose (**Scheme 3**). The rice slurry can also be treated with a converting enzyme such as glucose isomerase (EC 5.3.1.5) also known as D-xylose-ketol isomerase or a microorganism from *Moniliella* PTA-2862 to convert glucose to other monosaccharides such as glucose, fructose, xylose, sorbose, mannitol, erythritol, sorbitol, and xylitol. For example, a thermostable α-amylase was used to hydrolyze starch-containing rice slurry at elevated temperature to a soluble starch hydrolysate and to coagulate protein. After coagulated proteins were removed, the filtrate containing oligosaccharides, DP around 7, was treated with *Rhizopus* sp. glucoamylase and incubated at 55 °C. The addition of glucoamylase resulted in increased glucose concentration to 114 mg/mL compared to 11 mg/mL in the absence of the enzyme (**Figure 2**). High glucose rice syrup can thus be converted to high fructose rice syrup (HFRS) which is twice as sweet as glucose. High fructose corn syrup (HFCS) and HFRS can be used as sweeteners in many beverages, dairy products, and baked and canned food products. Monosaccharide-rich syrup may contain more than 3% or preferably 5 or 10% monosaccharide. Both starch hydrolases and converting enzymes can be obtained commercially, or isolated from natural sources such as microorganisms, animals, plants, or prepared by recombinant enzyme technology. Other starch-containing agricultural raw materials such as corn, potato, sorghum, cassava or tapioca, wheat, and barley can be used to make starch slurry and the enzymes described above used to hydrolyze and convert them to useful high-value functional foods.

Disaccharide-rich syrup was obtained by treating the rice starch with a thermostable α-amylase first to obtain soluble oligosaccharides and then the starch hydrolysate solution was treated with *β*-amylase (EC 3.2.1.2) to produce high maltose syrup (a disaccharide) (*7, 8*). High-maltose syrup will find application in candy, ice cream, and pancakes. It is mildly sweet, has low solution viscosity, low hygroscopicity, and good heat stability. Further treatment of maltose syrup with a thermostable trehalose synthase, TSase (EC 5.4.99.16), resulted in the formation of trehalose-rich syrup (0.5% trehalose). Trehalose-rich syrup can be used directly as a food ingredient. Trehalose (α-D-glucopyranosyl α-D-glucopyranoside) is a naturally occurring nonreducing disaccharide (i.e., no free acetal at the anomeric carbon) found in sugar beet and a number of organisms such as bacteria, yeast, fungi, and insects.

To prepare high-protein flour from the same agricultural raw material (rice), a thermostable α-amylase was used to hydrolyze starch to soluble oligosaccharides in an autoclave. Heat coagulated protein was separated by filtration, centrifugation, or decantation, and then recovered as high-protein. The final protein content of the rice flour was increased from 9.2−10.9% in the raw material to 25−53% in the high-protein product. However, the starch content was reduced from 77−84% to 16−42%. The protein content of raw rice was low (6−10%) and may not supply adequate protein to young children who require 20−25% protein (*9*). The product obtained by the current process has enough protein to satisfy the recommended protein amount for young children. A genetic attempt to modify the protein content

Scheme 3. Schematics of the Actual Chemistry Steps for Glucose-Rich Syrup and Other Syrups Produced from Rice[a]



[a] APU = amylopullulanase.

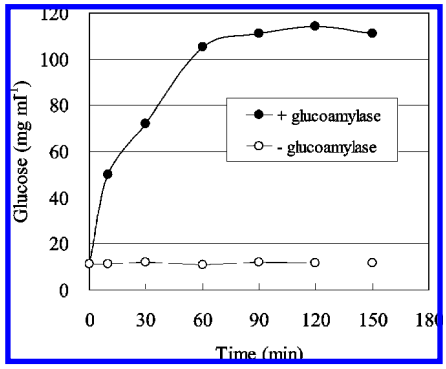


Figure 2. Time course for glucoamylase-catalyzed hydrolysis of α-amylase-liquefied rice starch product to yield glucose (*32*).

of rice only resulted in 10−15%, which is not adequate as stated above. Enzymatic hydrolysis of starch is an excellent alternative method to produce protein-enriched flour with improved nutrition (*7, 10*). Shih (*11*) reviewed the methods for rice protein processing that include the traditional alkaline extraction, enzyme-assisted extraction, and the novel uses of physical treatment before water extraction, and how processing affects the functional and nutritional properties of rice protein. Rice protein extracts such as glutelin may have poor solubility, emulsifying and foaming properties in aqueous system, but the gelling properties which is desirable in food applications is good (*12*). Enzymes have been used to produce protein-rich rice flour in 76% yield (*13*−*15*).

Overall, the biocatalytic process is natural and therefore desirable for producing food grade high-maltose, high-glucose syrups, wine, ethanol, and trehalose from raw agricultural material without using chemicals such as acids, bases, and surfactants. Some of the advantages of using enzymes for food processing are high efficiency, natural and environmentally friendly (green process), energy saving, less equipment cost, and high specificity with less unwanted byproducts.

ENZYME IMMOBILIZATION

Processing of starch to value-added commodities can substantially be improved by the use of immobilized enzymes. Immobilized enzyme processes have several advantages over the use of soluble free enzymes and nonimmobilized whole cells. Immobilization would allow semicontinuous and continuous hydrolysis and conversion of starch to desired products; immobilized enzymes are more pH stable and thermostable; enzyme reuse is possible and hence overall processing cost is low; more than one enzyme can be immobilized on a single support to perform multistep processes at a single pH; recovery of the end product is easy; there is no need for use of much equipment; inactivation of the enzyme by heat at the end of the process is not necessary; operational stability is high; high enzyme loading is possible for faster reaction; productivity is improved and specific activity is high; risk of microbial contamination of the product is reduced; energy and time are saved because cooling and reheating may not be needed; less side products are formed because enzymes are specific; and downstream processing cost for product recovery and purification is low. Enzymes can be immobilized by simple adsorption to a support matrix, entrapment, encapsulation, covalent attachment to mono- or bifunctional reagents, and by cross-linking.

Atia et al. (*16*) used coimmobilized β-amylase and pullulanase to reduce the saccharification time of starch to 50 h with improved yield of maltose. Both enzymes were immobilized onto poly(acrylamide-acrylic acid) resin [P(AAm-AAc)] using 1-ethyl-3-(3-dimethylaminopropyl) carbodimide hydrochloride (EDC), and they exhibited improved thermostability and pH stability and could be used for commercial starch hydrolysis. Trypsin, α-amylase, and lipase were coimmobilized together onto nonwoven polyester surfaces, and uniform distribution of the different enzymes was achieved (*17*). α-Amylase was entrapped in cross-linked κ-carrageenan beads and the shelf life of the immobilized enzyme increased to 3.53 years compared to 0.99 years for nonimmobilized enzyme (*18*). Sweet potato β-amylase and pullulanase from *Bacillus brevis* were separately immobilized onto chitosan beads (Chitopearl BCW 3505) and used in a semicontinuous production of maltose from high concentrated solution (40% potato starch hydrolysate, Pine-Dex #1) in 71% yield after hydrolysis at 60 °C for 14 d at pH 6.0 (*19*). Maltose was produced industrially by batch reactions, but immobilized enzymes are now used by the industry to lower cost because they are reused several times. Chitosan is nontoxic and cheap, with low rate biodegradability, and should easily be adopted by the food industry as enzyme support matrix.

Shewale and Pandit (*20*) recently immobilized *Bacillus licheniformis* α-amylase on a superporous cross-linked cellulose matrix (CELBEADS) and used it to produce maltodextrins with different saccharide contents. The immobilized enzyme was more thermostable than the free enzyme and retained 100% activity after 8 batches of use at 55 °C with initial starch concentration of 90 mg/mL. Each batch reaction was for 8 h. Immobilized enzymes can also be used for bioethanol (biofuel) production as alternative fuel. Jamai et al. (*21*) used calcium alginate-immobilized *Candida tropicalis* to increase the rate of ethanol production by fermentation from soluble corn starch with 96% conversion compared to 9% conversion with exogenous α-amylase. This microorganism can be used as a biocatalyst to produce ethanol without any starch saccharification step and therefore will save time and energy for reducing production cost. The above review on the use of immobilized enzymes for starch hydrolyses and conversions is in no way meant to be exhaustive, but just to illustrate the many advantages and the potentials for this technology in increasing the yield of functional food ingredients and industrial products.

RECOMBINANT ENZYME TECHNOLOGY AND PROTEIN ENGINEERING

The future of industrial enzymes will depend on their ability to be cost-effective, to catalyze single or multistep reactions, and to catalyze reaction in combination with other enzymes individually or as fusion enzymes, on their large scale production of enzyme, and on their ease of genetic manipulation and expression in heterologous organisms. Biotechnology will play a major role in achieving these goals. Therefore, recombinant enzyme technology and protein engineering are tools available to improve the catalytic efficiency, thermostability, pH stability, substrate selectivity, and productivity of enzymes involved in starch hydrolysis and conversion to useful products such as food, feed, and industrial chemicals and products.

One of the pathways to produce trehalose involves the direct conversion of maltose by trehalose synthase (TSase) that catalyzes the intramolecular rearrangement of the α,1-4 linkage of maltose to the α,1-1 linkage of trehalose (*22*). This pathway utilizes an inexpensive maltose as substrate, and therefore could be used to produce large quantities of trehalose for commercial use in food, cosmetic, and pharmaceutical industries. Of the 6 known TSases, the enzyme isolated from *Thermus aquaticus* is highly thermostable but the enzyme yield in the original organism is low. It is desirable to increase the enzyme yield for this process to be economical in trehalose manufacture. Recently, the recombinant TSase from *Picrophilus torridus* (PTTS) has been cloned and purified (*23*), a hyperacidophilic, thermophilic, heterotrophic, and absolutely aerobic archaea that grows optimally at 60 °C and pH 0.7 (*24*), that exhibited optimum pH and temperature of 6.0 and 45 °C, respectively. TSase gene was synthesized by overlap PCR and transformed into *Escherichia coli* for expression. This enzyme showed higher preference for maltose (even in high maltose concentration) than trehalose resulting in 71% yield of trehalose at 20 °C. The recombinant PTTS appeared to use the same hydrolysis mechanism as the α-amylase family of enzymes to break α,1-4 glycosidic linkage of maltose. The results also showed that residues of catalytic importance for the α-amylase family of enzymes were conserved in PTTS. This recombinant TSase can be used for the efficient and economical production of high-value trehalose from low-priced agricultural produce such as rice (**Scheme 1**). Because the hydrolysis and conversion of starch involves more than one enzyme, it would be more benefit to construct a bifunctional enzyme system of recombinant thermostable β-amylase and TSase for the conversion of starch to trehalose. Previously, a DNA fragment that contains two genes encoding TSase from *Thermus thermophilus* and β-amylase (BAase) (E.C. 3.2.1.2) from *Clostridium thermosulphurogenes* has been successfully constructed in our laboratory. The recombinant bifunctional fusion enzymes, TSBA and BATS, catalyzed the sequential reactions for the production of trehalose from starch, an abundant and cheap raw material. The catalytic efficiency of TSBA or BATS was 3.4 or 2.4 times higher than that of a mixture of individual enzymes, respectively. The TSBA showed higher catalytic efficiency over BATS and this might be due to the conformational change of TSase in the TSBA construct. This suggested that TSBA configuration might have a more rigid structure which was formed by BAase fusion with the C terminus of TSase to decrease glucose formation as a minor side reaction and to enhance trehalose formation efficiency. This resulted in a thermostable and thermophilic fusion enzyme that has highly attractive advantages under high temperature conditions to solve some severe problems of carbohydrate industry, such as reduction of microbial contamination and lowering of medium viscosity. This idea of fusing two or more enzymes with multifunctional properties should have great application potential in industrial biocatalysis and protein engineering of enzymes (*25*).

APPLICATION OF TRANGENIC PLANTS

For cost reduction in enzyme production from microorganisms and to simplify the production process, the starch degrading enzyme genes can be further transferred into agricultural produce to be used as transgenic rice seeds. APU from *Thermoanaerobacter ethanolicus* 39E, which harbors both α-amylase and pullulanase activities, is capable of hydrolyzing both α-1,4 and α-1,6 bonds of polysaccharides, and is heat stable with a catalytic optimum at 90 °C (*26, 27*). Transgenic rice seeds composed of this thermostable and bifunctional APU would, theoretically, facilitate liquefaction and saccharification of starch simultaneously at high temperature without adding exogenous α-amylase and pullulanase. Such an application would be desirable for health food production, simplifying the sugar and protein production process, improving the efficiency of starch bioprocessing, and significantly reducing production cost.

A transgenic "sweet rice" containing the APU gene (*28, 29*) was highly expressed in both mature and germinated transgenic sweet rice seeds under the control of rice glutelin and α-amylase gene promoters. It demonstrated that sugar syrup and protein-enriched flour can be produced simultaneously from complete autohydrolysis of starch in flour derived from transgenic seeds upon heating at 85 °C for only a few hours. In comparison with conventional enzymatic methods, this novel approach significantly reduces the incubation time in processing, leading to complete autohydrolysis of starch into sugar syrup within few hours (*7*). This is important for optimal recovery of sugar syrup and proteins by avoiding chocking of filters during processing. APU rice seeds could be used directly or as a source of APU in place of microbial amylolytic enzymes, to convert starch into liquefied starch hydrolysate for the industrial production of sweeteners or fermentation products.

Starch, a major ingredient in various agricultural products (i.e., rice, sweet potato, etc.), is composed of two different glucan chains, amylose and amylopectin. Amylose is a linear polymer of glucosyl residues linked via α-1,4 glucosidic linkages, whereas amylopectin exists as a branched α-1,4;α-1,6 D-glucan polymer. The relative amount of amylose and

amylopectin affects the unique physical and chemical properties of starch, which confers specific functionality and could be of biotechnological importance (*30*). Modification of levels and properties of the starch biosynthetic enzymes has been accomplished by the identification and transgenic technology of starch biosynthetic mutants, but these approaches generally led to a significantly lower yield (*30, 31*). It is surprising to find that amylose content, a key determinant of the eating and cooking quality of rice, in transgenic rice seeds was reduced without affecting starch content, plant growth, or grain yield. High amylose levels are usually associated with dry, fluffy and nonsticky rice grains, indicating the characteristics of rice quality were significantly affected by amylose content for industrial use (*32*).

Recently, a novel APU-containing rice seed has been developed (*28, 29*) with three unique features: (i) complete starch autohydrolysis within a few hours of incubation at 85−95 °C; (ii) can be processed efficiently and cost effectively for simultaneous production of protein-enriched flour and sugar syrup for human consumption and for broad industrial applications; and (iii) reduction of amylose contents without a significant impact on grain yield. APU expressed with a signal peptide could be targeted to amyloplasts where this enzyme affects activity of granule-bound starch-metabolizing enzymes and starch biosynthesis. The result demonstrates the technical feasibility of improving nutritional and commercial values of rice through a biotechnological approach. Similar approaches could also be applied to other agricultural produces, e.g., maize, potato, and cassava, which might offer even lower production cost than rice. Other enzyme genes that can further convert starch hydrolysate into other industrial products, such as maltose, trehalose, glucose and fructose, can also be included in the transgenic seeds, which can directly produce the desirable products by a simple and economical method. The yield of proteins from the four randomly selected transgenic lines ranged between 30−50% of rice flour after starch hydrolysis. It shows that the processing of APU rice seeds for simultaneous production of protein-enriched flour and sugar syrup is simple, efficient, and inexpensive. It may also provide people in developing countries with an easily accessible and important source of nutritious proteins in their diets (*33*).

In conclusion, enzymatic hydrolysis of starch offers an alternative method for the production of protein-enriched flour with improved nutrition (*7, 10*). Use of enzymes as biocatalysts offers many advantages: (i) high efficiency and specificity; (ii) natural and green (low pollution) process; and (iii) energy savings and less instrumentation cost requirement. The development of immobilized enzymes would allow semicontinuous and continuous hydrolysis and conversion of starch to desired products to lower overall processing cost. The application of enzymes to convert low-value agricultural produce into high-value-added industrial products such as health foods, sweeteners, and fermentation products is highly feasible, and it has great commercial potential to improve farmers' income simultaneously. New biotechnology, including gene cloning and expression, recombinant enzyme technology, and transgenic plant technology, are expected to further develop a more simple, efficient, and inexpensive approach to improve the application potential of enzymes for producing high-value-added products or agricultural produce with an easily accessible and important nutrition source.

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