

$\beta$ -Amylase is used in the production of maltose syrup and preventing retrogradation of rice cake confectionery. However, until now the supply source for  $\beta$ -amylase has been limited to edible plants such as barley, wheat and soybean. Considering the current worldwide food crisis, it is the responsibility of enzyme producers to explore alternative sources for a stable and constant enzyme supply going forward. It was not long ago that the trend toward bioethanol as a petroleum fuel alternative caused a steep rise in grain prices and made it more difficult to obtain food grain.

Microbial  $\beta$ -amylase was first described in the 1970s and despite many subsequent studies performed, proved difficult to produce at a large scale. For this reason, Amano Enzyme Inc. initiated a new screening procedure to find a more thermostable  $\beta$ -amylase compared to the enzyme from barley or wheat, and recently succeeded in its commercialization efforts through a joint development project with Daiwa Kasei K.K., one of the Amano Enzyme Group companies.

#### ■ History of $\beta$ -amylase and the status of industrial production

$\beta$ -Amylase was initially found among amylases in malt as an enzyme producing  $\beta$ -maltose and was named accordingly. Detailed studies started when a crystalline enzyme was prepared from sweet potatoes in 1946. More recently, the enzyme was found in cereal crops such as barley and wheat and leguminous crops such as soybean, and is now known as an enzyme widely distributed in higher plants. Studies with these plant enzymes have elucidated their functional mode and activation mechanisms: for instance, the X-ray crystal structure for the enzyme derived from soybeans was elucidated in 1993. Plant-derived  $\beta$ -amylase has been the source of choice for industrial production because the enzyme is abundant in plant seeds, which allows for relatively low-cost production. The barley-derived enzyme produced in Europe presently accounts for the majority of the global market. In Japan, the production of a soybean-derived enzyme began in the 1960s; however, because of a change in the extraction process for soybean oil, the supply of the enzyme has been uncertain. The production and sales of a competing wheat-derived enzyme started in the late 1980s. Despite the fact that microorganisms were not believed to contain  $\beta$ -amylase, a microbial  $\beta$ -amylase was found in Japan in 1974. Since then, a number of microbial enzymes have been discovered. Nevertheless, the industrial production of  $\beta$ -amylase from microorganisms has not been successful until the present time because of the insufficient thermostability of the enzyme, poor productivity on an industrial level, and the unsuitability of the microorganism as a production strain for food enzymes.

#### ■ Industrial applications of $\beta$ -amylase

Enzymes are used in a broad range of industrial applications and the starch processing industry in particular is one of the industries most actively using enzymes.

The enzymes used for starch processing are mainly bacterial  $\alpha$ -amylase (such as KLEISTASE L), used as a starch liquefaction enzyme, fungal glucoamylase (such as Gluczyme), used as a saccharification enzyme, and bacterial pullulanase (such as Pullulanase "Amano"), used as a debranching enzyme. Among saccharification enzymes, glucoamylase is used in the production of glucose and isomerized sugar, whereas  $\beta$ -amylase is used in the production of maltose syrup. Although  $\alpha$ -amylase from *Aspergillus oryzae* (which accumulates relatively high amounts of maltose) is also used for the same purpose, its application is limited because it also produces a significant amount of glucose. Maltose is used as a sweetener in candy, confectionery, "tsukudani" (food boiled in soy sauce), ice cream and other food because maltose, compared to glucose, has a full-bodied taste, has a lower Maillard reaction rate, and is resistant to crystallization. Since maltose has a refined and mild type of sweetness and a low level of coloring, it is particularly indispensable as a sweetener for Japanese confectionery.

$\beta$ -amylase can also be used to inhibit the retrogradation of starch. It is believed that  $\beta$ -amylase shortens the  $\alpha$ -1,4-linkage in the straight chain starch molecule utilizing its exo-type activity and thereby reduces the intermolecular association of the straight-chain portion of amylose, which is known as the main cause for starch retrogradation. In addition, the moisturizing effect of maltose produced by the enzyme is also thought to contribute to the softness of starch-containing foods. Thus,  $\beta$ -amylase is mainly used in Japan for preventing the retrogradation of rice cake. Also in baking, the  $\beta$ -amylase naturally present in wheat flour is considered to be effective in the inhibition of retrogradation; although the barley-derived enzyme may be used on some occasions, its use is limited because of the issue of thermostability.

#### ■ Discovery of microbial $\beta$ -amylase and elucidation of its features

In order to develop a stable supply of  $\beta$ -amylase for industrial applications, Amano Enzyme undertook to screen for  $\beta$ -amylase activity in microorganisms; as a result we found a thermostable  $\beta$ -amylase produced by a strain belonging to the genus *Bacillus*. The purification of the enzyme and the cloning of the gene revealed that the enzyme is produced and secreted as a monomer comprising 515 amino acids with a molecular weight of 57.6 kDa. The primary structure demonstrated 43%–80% homology to the previously reported  $\beta$ -amylases derived from the genus *Bacillus*.

The optimum reaction temperature and thermostability of this enzyme was found to be 10° C higher compared to the barley- or wheat-derived enzymes, which account for the majority of  $\beta$ -amylase industrial use in the world, and equivalent to the thermostable soybean-derived enzyme.

## Application of the microbial $\beta$ -amylase

### 1) Production of maltose syrup

Figure 1 and Table 1 show the results of experiments producing maltose from liquefied starch. The microbial enzyme was demonstrated to be superior to the barley- or wheat-derived enzymes, which are currently used in the production of maltose syrup. Not only is more maltose produced using a smaller amount of enzyme, but also there is less concern about possible microbial contamination during the reaction because of the higher reaction temperature. Moreover, this enzyme has a neutral optimum pH (around pH 6.0), which is more suitable for the starch liquefaction process that takes place prior to maltose saccharification by  $\beta$ -amylase; in contrast, the barley- or wheat-derived enzymes have more acidic optimum reaction pHs. Glucose formation was very low in the sugar composition of the produced syrup, which is desirable for maltose syrup products.

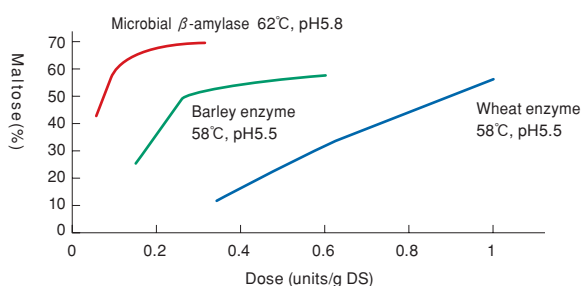


Figure 1. Maltose production from liquefied starch

Table 1. Sugar composition of maltose syrup

Reaction time : 42hr

origin	Temperature	pH	Dose (units/g-DS)	Sugar composition (%)				
				G1	G2	G3	G4	G5 $\equiv$
Microbial	62°C	5.8	0.3	0.2	59.1	7.2	0.7	32.8
Barley	58°C	5.5	0.7	0.2	56.1	7.2	0.8	35.9
Wheat	58°C	5.5	1.0	0.2	54.2	7.2	1.1	37.4

### 2) Retrogradation in starch based food

Figures 2 and 3 show the inhibitory effect of the enzyme on retrogradation in rice cake. Rice cake containing this enzyme remained soft even after storage at 15° C for 3 days. In this application, even a very small quantity of  $\alpha$ -amylase contamination in the enzyme preparation can cause problems – for instance, the rice cake can become sticky during storage. Fortunately, the bacterial strain used for production of  $\beta$ -amylase does not contain  $\alpha$ -amylase, which is one of the main advantages of this strain as an industrial production strain for  $\beta$ -amylase.



Figure 2. Inhibitory effect on retrogradation of rice cake

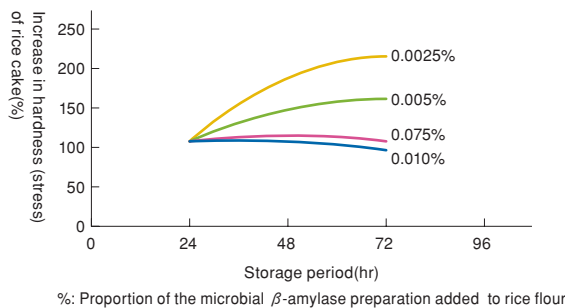


Figure 3. Inhibitory effect on retrogradation of rice cake

In addition, this enzyme also possesses the unique ability to work on raw starch (Table 2). Plant  $\beta$ -amylases, including the enzyme derived from soybeans, cannot act on raw starch and therefore can not work at a temperature below the gelatinization temperature of starch (up to 60° C–70° C). However, the microbial enzyme is active at a lower temperature than gelatinization, and thus has the potential for a broader range of applications, including those in baking.

One of the advantages of the microbial enzyme compared to plant-derived enzymes is that it can bypass labeling requirements on regulated foods. Allergen-related labeling is mandatory for foods that use soybean-derived enzymes. The need for gluten-free food has also recently risen especially in the West. The microbial enzyme has an advantage in comparison to cereal-derived enzymes in that it can be used in the production of gluten-free food.

Table 2. Composition of activity on raw starch

origin	Hydrolysis activity on raw starch (units/g)
Microbial	800.3
Barley	1.6
Wheat	0.1
Soybean	0.0

Amano Enzyme Group has successfully commercialized the microbial enzyme by applying efforts to improve productivity through a traditional mutation, optimization of cultural conditions, and to develop optimal downstream process procedures in order to produce the enzyme at an industrial level.

Finally, the conceptual features of this product are summarized below (Table 3).

Table 3. Conceptual features of microbial  $\beta$ -amylase as a product

- ◆ The world's first microbial  $\beta$ -amylase produced at an industrial scale.
- ◆ The microbial enzyme can be produced to meet the industrial demand, unlike the plant-derived enzymes.
- ◆ Thermostability is greater than for the barley and wheat enzymes and equivalent to the soybean enzyme.
- ◆ Capable of hydrolyzing raw starch which opens the potential for a wide range of food applications.
- ◆ Does not require allergen-related labeling and can be utilized for gluten-free food applications.
- ◆ The microbial enzyme is a kosher- and halal-compatible, non-GMO enzyme.