

Raw Sago Starch Digestion by Amylase from *Penicillium brunneum* No. 24

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Penicillium brunneum No. 24 の産生するアミラーゼによる生サゴ澱粉の分解

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Starch from tropical sago palms (*Metroxylon* sp.) is useful as foodstuffs and as raw industrial materials. Despite its highly possible applicabilities to a variety of industries, however, a large portion of sago starch has not yet been sufficiently utilized.

Hydrolyzation of starch to glucose is an essential process, to which one of the important economic limitations for utilization of this starch has been attributed. To reduce costs in gelatinization which requires high temperature, much interest has been paid to the study of enzymes which are capable of digesting raw starch (Ueda 1974). However, only little investigation has been made on sago starch digesting enzymes (Kainuma et al. 1985).

The authors revealed that a fungal strain had digestion ability of raw sago starch; the isolate No. 24 was selected as the best source of raw sago starch digesting amylase and it was identified as *P. brunneum* No. 24 (Haska and Ohta 1991a). This strain was cultivated in a solid culture medium, consisting of 56% wheat bran, 4% sago starch and 40% water (Haska and Ohta 1991b).

Pretreatment of sago starch by heating at 70°C, below gelatinization temperature, in low pH condition was effective in improving the hydrolyzing activity (2.7 IU/g solid medium, 4 days) (Haska and

Ohta 1991c). Treatment of sago starch granules at 60°C (pH 2.0) resulted in increase in the susceptibility to enzyme action. During this process, however, the structure of starch granules was not changed and spontaneous hydrolysis did not occur. Glucose was mainly produced through hydrolyzation of untreated and treated sago starch granules.

Based on these findings, a simple treatment method which could be easily applied to sago processing areas was developed (Haska and Ohta 1991c). Water baths with 2 cm starch layer in water or buffer in a simple solar heating system was heated to about 60°C after 3 hr. The application of this treatment to the extraction process of sago starch in the drying stage could improve the efficiency of enzymatic hydrolysis of sago starch granules. The combined treatment of partially purified enzyme and commercial cellulase was effective in increasing the ability of the raw sago starch digesting amylase.

Alcohol fermentation from the treated sago starch granules using *P. brunneum* No. 24 and a strain of yeast, *S. cerevisiae* No. 33, could produce ethanol under incubation conditions of 35°C (pH 4.8) (Haska and Ohta 1993).

It is also mentioned that the raw sago starch di-

gesting amylase was partially purified (Haska and Ohta 1994). Purification after several chromatographies gave a raw sago starch digesting activity of 2.07 IU/mg-protein.

Affinity chromatography (α -CD-Sepharose 6B) of the enzyme after DEAE-Cellulose chromatographic fractionation step resulted in a homogeneous glucoamylase. SDS-polyacrylamide gel electrophoresis of purified enzyme showed a single band (a molecular weight of 80,000) for the native glucoamylase from *P. brunneum* No. 24 was identified.

After modification of the native glucoamylase by subtilisin, the molecular weight was reduced to 67,000. It lost the ability to digest and adsorb raw starches but preserved the ability to digest gelatinized starch.

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