

## 生物機能応用科学専攻

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学位記番号	生博 甲第 110 号
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学位論文題目	Study on Outer-layer Structures of Waxy and Normal Starches Amylopectins by Debranching Enzymes (枝切り酵素によるモチ及びウルチ澱粉のアミロペクチン外層構造に関する研究)
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## SUMMARY

Starch is not utilized for food and energy source only, but also as a high sustainable and earth environmental friendly source that can be utilized for industrial purposes. Unfortunately up to now, the relationship between the molecular structure and physico-chemical properties of each starch is not well recognized, which caused its limited utilization. The main reasons are: (1) starch is a homopolysaccharide, only composed of glucose; (2) starch consists of 15-25% of amylose and 75-85% of amylopectin, but the retrogradation is caused by amylose, and (3) amylopectin has a very high molecular weight with more than 10 millions.

For recognition of the physico-chemical properties of starch, the properties should be studied together with the fine structure of amylopectin. In the previous studies, the high molecular weight structure of whole amylopectin has not been investigated clearly, but it was found that the physico-chemical properties were highly affected by the outer-layer of amylopectin. In the detail results, the immobilized debranching enzymes split the amylopectin from the outer portion. A method was developed to prepare the short-amylose by early debranching reaction. This method offers exactly new and interesting information, but since the procedure for enzyme immobilization is complicated and its properties has lacking as shown in the

preliminary research on the characteristics of the free and immobilized enzymes.

For these aims, on the first part of this research, novel immobilized enzyme and free enzyme were used to investigate the outer-layer of amylopectin structure, and the second part introduced a new method for studying a direct analysis of the outer-layer of amylopectin structure of normal starch without separation of amylopectin and amylose; and on the third part, applications of this methods on several starches were conducted in order

to get general information of outer-layer of amylopectin which can be used as first screening for understanding the characteristic of normal starches.

On the preliminary research, three kinds of chitin-based supports were used for the immobilization of debranching enzymes. All the supports could immobilize isoamylase and pullulanase, but it was found that the immobilized pullulanase hardly hydrolyzed waxy maize amylopectin, while immobilized isoamylase could debranch waxy maize amylopectin. The immobilized enzymes lost almost the half of their activities, and could not hydrolyze the amylopectin completely.

On the first part: various kinds of partial hydrolysates of waxy corn amylopectin were prepared by controlling the debranching actions of immobilized and free isoamylase, and free

pullulanase, and then fractionated by a gel chromatography using Toyopearl HW-65F. About 10-15% of partial hydrolyses were appropriate to collect the shortish chains released from amylopectin. The chain distribution of the smallest fraction from outer-layer of amylopectin consisted of many short-chains (DP6 ~15). The comparative experiments among the debranching enzymes confirm that the partial hydrolyzed materials obtained under low enzyme activity of free isoamylase and pullulanase could be used for studying the outer-layer of amylopectin like as immobilized isoamylase (published on J. Appl. Glycosci., 48 (1), 2001).

On the second part: Short-amylose chains released from normal corn starch with isoamylase were studied to establish an analytical method for getting directly the structural information of amylopectin. The partial hydrolysates of normal corn starch by free isoamylase obtained without previous separation of amylose and amylopectin

were fractionated into three fractions by gel chromatography of Toyopearl HW-50S. Although the short-amylose chains in the fr.3 came not from only amylopectin, but also branched amylose, it was estimated that the chains relating to the branched amylose were minor in the hydrolysates (will be published on J. Appl. Glycosci., 48 (2), 2001).

The analytical method conducting together by controlling the debranching reaction of starch and GPC on Toyopearl HW-50S is convenient for studying the structure of normal starch without previous separation of amylose and amylopectin, and will be worth using as the first screening for understanding the characteristic of normal starches.

General information of amylopectin structures obtained from Japan and South-east Asia regions cultivated starches, especially corn and rice starches, were applied on this method, and the results were shown on the third part of this research.

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学位記番号	生博 甲第 111 号
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学位論文題目	Studies on Hydrogen Production from Chitinous Biomass by <i>Clostridium paraputrificum</i> M-21. ( <i>Clostridium paraputrificum</i> M-21. によるキチン含有バイオマスからの水素生産に関する研究)
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### 要 旨

The studies were carried out to convert chitinous biomass to valuable materials such as hydrogen gas and organic acids by an anaerobic microorganisms.

A strictly anaerobic, mesophilic, chitinolytic

bacteria was isolated and identified as *Clostridium paraputrificum* M-21 (FERM P-16390). Optimum cultivation conditions of the bacterium for gas evolution in GS medium containing N-Acetyl-D-glucosamine (GlcNAc) as a main carbon source