A NOVEL LATENT POLYPHENOLOXIDASE FROM SAGO LOGS (*METROXYLON SAGU*): PURIFICATION, ACTIVATION AND SOME OF ITS PROPERTIES

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Introduction

Sago palm (*Metroxylon sagu*) is considered as a main source of starch in South East Asia. The use of the starch in food and food based industries is high. It is either incorporated into food as an ingredient or as a major raw material for manufacturing high fructose syrup, monosodium glutamate etc. However, the starch extracted from the palm suffers the phenomenon of colour modification during industrial processing and storage. These colour alterations are due to either formation or degradation of pigmented compounds usually present in the plant itself and are mediated by endogenous enzymatic activities such as polyphenoloxidase. One unusual and intriguing characteristic of this enzyme is its ability to exist in an inactive or latent state. Therefore, the aim of this study was to purify and characterise a novel latent polyphenoloxidase (LPPO) from the pith and to investigate the implication of its properties on the browning reaction. The enzyme was found to be one of the major factors affecting the final quality of the starch produced.

Materials and Methods

Sago (*Metroxyllon sagu*) pith was collected from a young tree aged between 2 - 4 years old from Batu Pahat, Malaysia. The membrane-bound enzyme was isolated and purified from the fresh sago pith following the method of Sanchez-Ferrer et al. (1989) with some modifications. Latent polyphenoloxidase activity was determined spectrophotometrically using the substrate 4-methyl-cathecol. The molecular weight was determined by SDS-PAGE. Protein concentration was determined by Hatree method (1972) using bovine serum albumin as a standard.

Results and Discussion

This study revealed that full extraction of LPPO required the use of a detergent. The extractability was significantly enhanced by using non-ionic detergents (Triton X-114, Triton X-100 and Tween-80) compared to the enzyme dissociated by ionic detergents and buffer. Among the nonionic detergents tested, Triton X-114 was the most effective where it enhanced the enzyme extraction by 2136.7 %. Tween-80 was found to be less efficient. With the ionic detergents, the extractability was around 557.2 - 443.7 %. Further purification of the enzyme by Temperature-Induced Phase Separation Method yielded a 4.1-fold increase in specific activity with a recovery of 70 %. Native-PAGE of the purified enzyme revealed the presence of three protein bands when stained with Commassi Blue. When the gel was stained for activity staining with 0.05 M catechol and 0.1 M pyrogallol, two and three activity bands, respectively were observed. The molecular weights of the three isoforms were estimated to be 53 and 40 and 37 kD. The purified LPPO was strongly activated by trypsin followed by sodium sodecyl sulfate, linoleic acid and ethanol. The activation of LPPO by SDS suggests that SDS could have caused a conformation change in the protein structure that may induce the increase of the enzymatic activity. The purified enzyme was very reactive towards diphenolics such as 4-methyl-catechol, epicatechin, catechol and chlorogenic acid as well as towards triphenolics such as pyrogallol. No activity was observed with monophenols. pH was found to have a profound effect on the activity.

Conclusions

Latent polyphenoloxidase (LPPO), a major membrane-bound enzyme responsible for the browning reaction of sago starch during processing and storage was successfully isolated and purified from sago pith. Using activity staining, the purified enzyme was found to exist in two or three different isoforms. The enzymes were very reactive toward diphenols such as 4-methyl-catechol, epicatechin, catechol and chlorogenic acid. Further works are required for detail characterisation of the enzyme.

References

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