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Effect of pH and substrates on polyphenol oxidase activity of Japanese processing potatoes

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国産加工用ジャガイモのポリフェノールオキシダーゼ活性におよぼすpHおよび基質の影響

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ABSTRACT

The enzyme activity of polyphenol oxidase (PPO) for Japanese processing potatoes, Toyoshiro (bruise-resistant variety) and Norin No.1 (susceptible one), was measured at different pHs or substrates to investigate pH or substrate-specificity dependence. The pH-optimum of Toyoshiro PPO was pH 6.2-6.6, whereas that of Norin No.1 was 5.4. In addition, the PPO activity level of Norin No.1 was approximately 6 times higher ($p<0.01$) than that of Toyoshiro, when the specific activities were determined by using tyrosine as a substrate. This result implies that less tyrosine content of potatoes could reduce blackspot bruise. This information may be useful for breeders in developing new blackspot-bruise-resistant varieties.

［Key words］ potato tuber, PPO, pH, substrate

和文摘要

国産加工用ジャガイモの品種であるトヨシロ（内部損傷抵抗性）および農林1号（感受性）のポリフェノールオキシダーゼ（ＰＰＯ）活性におよぼすpHおよび基質の影響を検討した。その結果，トヨシロおよび農林１号の至適pHは6.2-6.6および5.4であった。さらに，チロシンを基質として酵素活性を測定した場合，内部損傷感受性の農林１号はトヨシロに比べ６倍の高い活性を示した。この結果は，ジャガイモのチロシン含量を減少させると，内部損傷を低減できることを意味し，内部損傷抵抗性品種の開発に有用な情報を与えるものである。

キーワード：ジャガイモ，ポリフェノールオキシダーゼ，pH，基質

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Introduction

Potato tubers are largely parenchymatous, lacking specialized secondary thickened tissues (Peterson et al. 1985). As a result, they are susceptible to various forms of damage during commercial production including external and internal defects. Internal damage (blackspot bruise) resulting from the effects of impact on tubers during harvesting operations alone may cause losses in excess of 20% (Storey et al. 1992). Although it may be a simple matter to grade out tubers showing external damage, blackspot bruise is not visible until after peeling (McGarry et al. 1996). Thus, blackspot bruise is a major quality problem that causes substantial economic damage to potato breeders and the potato processing industry worldwide (Stevens et al. 1997).

Studies associating enzymes with blackspot susceptibility have been concerned primarily with PPO (Mondy et al. 1960; Weaver et al. 1970). Several researchers reported that a positive correlation had been found between PPO and blackspot incidence (Vertregt 1968;Mapson et al. 1963).

The reaction rates for enzymatic blackening depend on pH and substrate specificity. Although there are many reports about pH-dependence and substrate specificity of potato PPO in many countries, these properties of the Japanese processing potato PPO has not been determined. Thus, this study was initiated to investigate the properties of PPO activity for two Japanese processing potato varieties.

MATERIALS AND METHODS

Source and tuber preparation

Two varieties of processing potatoes were used: Toyoshiro (bruise-resistant variety) and Norin No.1 (susceptible one). Toyoshiro and Norin No.1 potatoes were harvested on Sep. 17, 1996 and Oct. 9, 1996, respectively. After harvesting, these potatoes were stored at 6℃ (above 90%RH). Both potato varieties approximately 150g in size were used for experiment.

Sample preparation

Four potatoes were selected for analysis at random, washed, and the basal portion of the tuber was cut for analysis. Then, the potatoes were peeled and diced into approximately 5 mm cubes.

Preparation of crude homogenate

Preparation of the crude homogenate was utilized the method described by Hsu et al. (1988). Potato cubes (25g) were homogenated in a waring blender at high speed for 90 sec with 25 ml of M/15 cold sodium phosphate buffer (pH 6.8). The homogenate was rapidly filtered through four layers of cheese cloth. The filtrate was centrifuged at 18,000×g for 10 min at 0℃. The resulting supernatant was served as the crude homogenate.

Sephadex G-25 chromatography

Two ml of crude homogenate were rapidly applied to a Sephadex G-25 column (i.d. 1.5 cm×10 cm), and eluted with M/15 of sodium phosphate buffer, pH 6.8 (flow rate: 1 ml/min.). After chromatography, the void fractions were combined and used to determine PPO activity and protein content.

Protein assays

Protein content was determined to find the specific PPO activity. Protein content was determined using CBB

(Coomassie Brilliant Blue G250) regent as described by Bradford (1976). One half ml of the void fractions were diluted with up to 5 ml of distilled water. To 1 ml of this solution, 5 ml of CBB regent were added. The mixture was shaken well, and allowed to stand for 10 min. At 595 nm, the absorbance was measured. The protein content was determined by using bovine albumin as the standard.

Determination of PPO activities

The procedure described by Hsu et al. (1984) and Weaver et al. (1970) was used to determine the PPO activity. PPO activity was determined using tyrosine, chlorogenic acid and 3, 4-Dihydroxyphenyl alanine (DOPA) as substrates. The reaction mixture with tyrosine was 3.0 ml of M/15 sodium phosphate buffer, pH 6.8, containing 6.7 μM of tyrosine. The reaction mixture was added into glass cell containing 0.75 ml of enzyme solution. The changes in optical density at 410 nm were followed at 15 min intervals for 150 min (Weaver et al. 1970) at room temperature (20℃). With chlorogenic acid as the substrate, the reaction mixture was 3.0 ml of the same phosphate buffer (pH 6.8) containing 21.4 μM of chlorogenic acid. The reaction mixture was added into a glass cell containing 0.75 ml of the enzyme solution, the changes in absorbance at 390 nm (Weaver et al. 1970) were determined at 1 min intervals for 11 min at room temperature (20℃). Using DOPA as the substrate, the reaction mixture was 3.0 ml of the phosphate buffer at different pHs (M/15: pH 5.0 , 5.4, 5.8, 6.2, 6.6, 6.8 and also 7.0) contained 15.0 μM of DOPA. The mixture was added into a glass cell containing 0.75 ml of the enzyme solution, the change in absorbance at 420 nm was determined at 1 min intervals for 11 mins at room temperature (Hsu et al. 1988). Under these conditions, one unit is defined as the change in absorbance divided by the determination time.

RESULTS

pH-optimum of PPO activity

Fig. 1 shows the effect of pH levels on PPO activity of the Toyoshiro variety. The change in activity shows a maximum from pH 6.2 to 6.6. The activity decreased

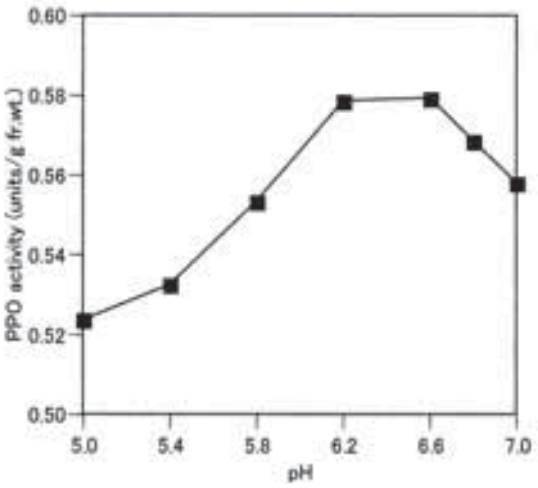

Fig.1. Effect of pH on PPO activity(Toyoshiro)

below pH 6.2 and above pH 6.6. Fig. 2 shows the effect of pH levels on PPO activity of the Norin No.1 variety. The activity levels peaked at pH 5.4, and gradually declined until pH 6.6, and then rapidly dropped above pH 6.6.

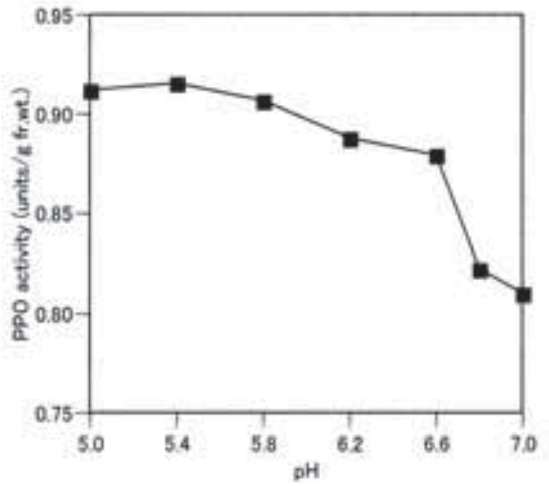

Fig.2. Effect of pH on PPO activity(Norin No.1)

Table 1. Substrate specificity of specific PPO activity (units/mg protein)

Substrate	Toyoshiro	Norin No.1
o-Diphenols		
Chlorogenic acid	0.752a	0.662a*
DOPA	0.460b	0.607a
Monophenol		
L-tyrosine	4.43×10^{-3}b	2.42×10^{-2}a

*Means in each row with same letter are not significantly different ($p<0.01$)

Substrate specificity of processing potato PPO activity

Table 1 lists the substrate specificity of the PPO of processing potatoes. During the course of reaction, brown, red and finally black pigments were formed by tyrosine and DOPA, whereas lighter colored (yellow-green) pigments were produced by the chlorogenic acid.

The PPO activities of Norin No.1 variety were considerably higher ($P<0.01$) than those of Toyoshiro variety excepting the activities with chlorogenic acid,. In particular, when tyrosine was used as a substrate, the activity of Norin No.1 variety was about 6 times higher than that of Toyoshiro variety.

DISCUSSION

Figs 1 and 2 indicated the pH-optimum of PPO activity. PPO of Toyoshiro variety showed a maximum activity from pH 6.2 to 6.6, and Norin No.1 variety peaked at pH 5.4. These plots are similar in shape to the one obtained by Alleghina (1964) and Suresh (1965). It is well known that enzymatic browning is more rapid at higher pHs, whereas it is slower at lower pHs (Muneta, 1977). However, in the Norin No.1 variety, maximum activity was observed at pH 5.4. This result is in agreement with the results reported in an earlier study on the Kennebec variety (Suresh et al. 1965).

Table 1 indicated the substrate specificity of the PPO. The specific activity of both varietys with chlorogenic acid and DOPA (O-diphenols) as the substrate was higher than those with tyrosine (monophenol). This supports the results reported by Suresh (1965). The conversion of tyrosine to DOPA by PPO takes several minutes and chlorogenic acid oxidized almost immediately. PPO shows two different enzyme activities in vitro (Takeuchi et al. 1996). One is the oxidation activity and the other is hydroxylate activity. PPO oxidizes O-diphenols to O-quinones in the absence of an electron donor, while it hydroxylates various monophenols to diphenols in the presence of an electron donor such as L-ascorbic acid. The PPO of Norin No.1 (bruise-susceptible variety) had about a 6 times higher activity level for tyrosine than that of Toyoshiro (bruise-resistant one) (Table 1). Thus, these results can propose that hydroxylate activity of PPO is a key activity for enzymatic browning in the potato. Hughes (1974) reported that the major phenolic substrates in potatoes are chlogenic acid and tyrosine. Therefore, less tyrosine content of potatoes could reduce blackspot bruise. This information may be useful for breeders in developing new processing potato varieties resistant to blackspot bruise.

SUMMARY

The enzyme activity of PPO for Japanese processing potatoes, Toyoshiro and Norin No.1, was measured at different pHs or substrates to investigate pH or substrate-specificity dependence. The pH-optimum of Toyoshiro PPO was pH 6.2-6.6, whereas that of Norin No.1 was 5.4.

The PPO activity level of Norin No.1 was approximately 6 times higher ($P<0.01$) than that of Toyoshiro when the specific activities were determined by using tyrosine as substrate.

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