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Inhibitory Effect of Pine Needle(*Pinus densiflora* S.) Extract on Potato Polyphenol Oxidase

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The inhibitory effect of pine needle (*Pinus densiflora* S.) on potato polyphenol oxidase (PPO) was investigated. The addition of the pine needle extract exhibited a higher inhibitory effect on the potato polyphenol oxidase activity than that of the citric acid or potassium sorbate. The enzyme activity was strongly inhibited in a pH range of 7.0-8.0. When the incubation time of reaction mixture was increased, the potato polyphenol oxidase activity was markedly inhibited. The pine needle extract inhibited the potato polyphenol oxidase non-competitively. And also the pine needle extract subjected to a heat treatment at 100℃ for 10 min or to an acid treatment at pH 2.0, 3.0, and 4.0 for 3 hours still retained inhibitory effect on potato polyphenol oxidase.

Key words – Pine needle extract, polyphenol oxidase, potato

Polyphenol oxidase (PPO, E.C.1.14.18.1) is a copper-containing enzyme which catalyzes the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-dihydroxyphenols to *o*-quinones, utilizing molecular oxygen[1]. Polyphenol oxidase is often associated with deterioration of foods because of its involvement in browning reactions[12]. Although browning reactions, in some food products, result in good appearance in terms of colour, these kinds of reactions, in general, lead to undesirable results with respect to texture, sweetness, and overall flavour[3]. Control of enzymatic browning during processing and storage is important to preserve the original appearance of fruits, thus becoming one of the main goals for food processors and researchers[14]. The PPO-catalysed browning food can be prevented by the addition of bisulfite[4], ascorbic acid and its analogs[15] as well as cysteine[2]. Although the bisulfites are effective to prevent browning, they could be harmful for human health, especially in asthmatic patients[16]. Therefore, the development of alternative safe and efficient polyphenol oxidase inhibitors is needed.

Pine needle (*Pinus densiflora* S.) belongs to the Pinaceae family[7]. It is an evergreen needle-leafed tree indigenous to East Asia. And it has bitter tasting leaves, which are gathered between spring and autumn[8]. Pine needles have long been valued for their medical effects and have been used in popular medicines for the treatment of hepatosis, various neurological disorders, and arteriosclerosis[13]. They are also valued for their flavouring properties: the essential oil of pine needles has found wide commercial use and is a constituent of certain beverages, cookies, detergents, cosmetics, amongst others[11].

Many papers have reported the inhibition activity of polyphenol oxidase, but little research has been conducted on the inhibitory action of the polyphenol oxidase produced by a natural source. Also no reports have been found on the inhibitory effect of pine needle on polyphenol oxidase activity. The objective of this work is to study the inhibitory effect of pine needle extract on potato polyphenol oxidase

Materials and Methods

Materials

Potato (*Solanum tuberosum* L.) was purchased from a local market in Busan, Korea. Pine needle (*Pinus densiflora* S.) was purchased from Kyung-Dong market in Seoul, Korea. Catechol as substrate, ascorbic acid, cysteine, citric acid were obtained from Sigma Chemical Co. Ltd. Sodium pyrosulfite was obtained Yakuri pure Chemical Co. Ltd. And potassium sorbate was obtained Junsei Chemical Co. Ltd.

Pine needle preparation

Pine needle (200 g) was homogenized with 1000 ml of a 50 mM phosphate buffer at pH 6.6 for 3 min. The homo-

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genate was centrifuged at 15,000×g for 20 min, and the supernatant was collected. All steps were carried out at 4℃.

Extraction and assay of potato polyphenol oxidase

Potato (200g) was homogenized with 200 ml of a 50 mM phosphate buffer at pH 6.6 for 3 min. The homogenate was centrifuged at 15,000×g for 20 min, and the supernatant was collected. The supernatant was used as a potato enzyme throughout this experiment. All steps were carried out at 4℃. Potato polyphenol oxidase activity was assayed with 0.2M catechol as a substrate by a spectrophotometric procedure (Ultrospec 3000, Pharmacia Biotech)[17]. The assay mixture contained 0.1 ml of potato PPO, 1.4 ml of a 50 mM phosphate buffer at pH 6.6, 0.5 ml of pine needle and was incubated for 15 min at 25℃. After this incubation, 1 ml of 0.2 M catechol was added to the assay mixture, and the inhibitory effect was measured. The total assay volume was 3 ml. The increase in absorbance at 420 nm and 25℃ was recorded automatically for 1 min.

Results and Discussion

Inhibitory effect of pine needle extract on potato polyphenol oxidase

Table 1 shows the inhibitory effect of various anti-browning agents on potato polyphenol oxidase with catechol as a substrate. The enzyme was strongly inhibited by cysteine and sodium pyrosulfite. The addition of the pine needle extract exhibited by the spectrophotometric method a higher inhibitory effect on the potato polyphenol oxidase activity than that of the citric acid or potassium sorbate. The amount of the pine needle extract was 33 mg/ml. All anti-browning agents was present at a final concentration of 0.0025%. Jang *et al.* reported that the alternative treatment in order to control enzymatic browning due to polyphenol oxidase are usually accomplished by the addition of ascorbic acid, citric acid, benzoic acid, calcium chloride, cinnamic acid, cysteine, glutathione, and various combinations of these compounds[5]. And also Lee *et al.* suggested that the addition of the heated onion extract to potato exhibited a marked inhibitory effect on potato polyphenol oxidase and the formation of a brown color[9]. As shown in Table 2, the inhibitory effect of added pine needle extract amount on potato polyphenol oxidase was investigated. The enzyme activity of potato polyphenol oxidase decreased as the added pine needle extract amount increased.

Table 1. The inhibitory effect of various anti-browning agents on potato polyphenol oxidase

Anti-browning agents	Relative activity(%)
None	100.00
Pine needle extract	63.57±1.54
Ascorbic acid	34.75±1.21
Citric acid	79.25±0.97
Cysteine	0.23±0.09
Sodium pyrosulfite	0.25±0.07
Potassium sorbate	72.21±1.01

The amount of the pine needle extract was 33 mg/ml. All anti-browning agents was present at a final concentration of 0.0025%. The reaction mixture was incubated at 25℃ for 15 min. The enzyme activity was measured at 25℃ for 1 min by the spectrophotometric procedure. Datas are mean±SD values(n=3).

Table 2. The inhibitory effect of added pine needle extract amount on potato polyphenol oxidase

Addition of pine needle	Relative activity(%)
None	100.00
13 mg/ml	71.66±1.63
26 mg/ml	68.70±0.74
33 mg/ml	63.57±1.54
40 mg/ml	53.58±1.98
53 mg/ml	44.92±0.97
66 mg/ml	34.96±1.87

The reaction mixture was incubated at 25℃ for 15 min. The enzyme activity was measured at 25℃ for 1 min by the spectrophotometric procedure. Datas are mean±SD values(n=3).

These results suggested that the pine needle extract had inhibitory effect on potato polyphenol oxidase.

Effect of pH and incubation time on the inhibition of potato polyphenol oxidase

pH is a key factor affecting the enzyme activity, therefore the effect of pH on the inhibition of potato polyphenol oxidase, using catechol as a substrate, was shown in Fig. 1. The polyphenol oxidase activity was determined in a pH range of 3.0 in 0.1M glycine buffer, in a pH range of 4.0-5.0 in 0.1M acetate buffer and in a pH range of 6.0-8.0 in 0.1M phosphate buffer. The pH optima for catechol was found to have a very broad optimum, between 5.0 and 8.0. These results was similar to the optimum for polyphenol oxidase in yali pear(between pH 5.8 and 7.8)[18] and artichoke heads(between 5.0 and 7.0)[1] using catechol as a substrate. The enzyme activity was strongly inhibited in a pH range of 7.0-8.0. As shown in Fig. 2, the effect of incubation time on the inhibition of potato polyphenol oxi-

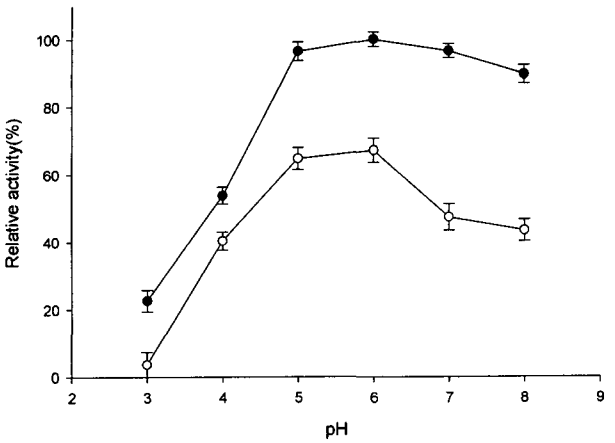

Fig. 1. The effect of pH on the inhibition of potato polyphenol oxidase by pine needle extract. The reaction mixture was incubated at 25℃ for 15 min in various pH. Catechol was used as a substrate. The enzyme activity was measured at 25℃ for 1 min by the spectrophotometric procedure. The amount of the pine needle extract was 33 mg/ml. Control(●-●); Addition of pine needle extract(○-○).

dase was investigated. When the incubation time of reaction mixture was increased, the potato polyphenol oxidase activity was markedly inhibited. Fig. 3 shows that the pine needle extract inhibited the potato polyphenol oxidase non-competitively. Lee *et al.* reported that the onion extract inhibited the potato polyphenol oxidase non-competitively[9]. And also Kim *et al.* reported that the heated

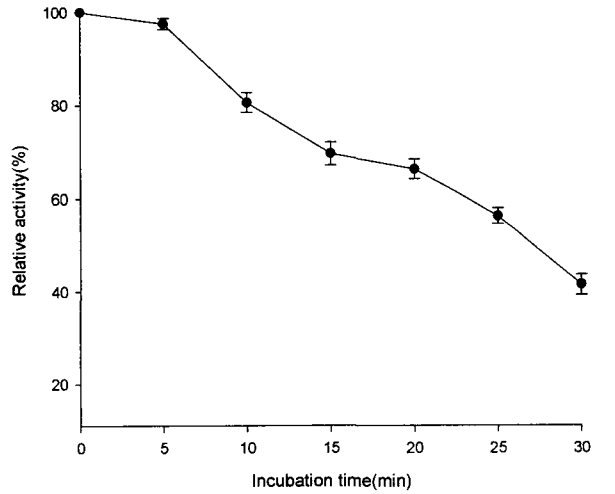

Fig. 2. The effect of incubation time on the inhibition of potato polyphenol oxidase by pine needle extract. The reaction mixture was incubated at 25℃ for various time(5-30 min). Catechol was used as a substrate. The enzyme activity was measured at 25℃ for 1 min by the spectrophotometric procedure. The amount of the pine needle extract was 33 mg/ml.

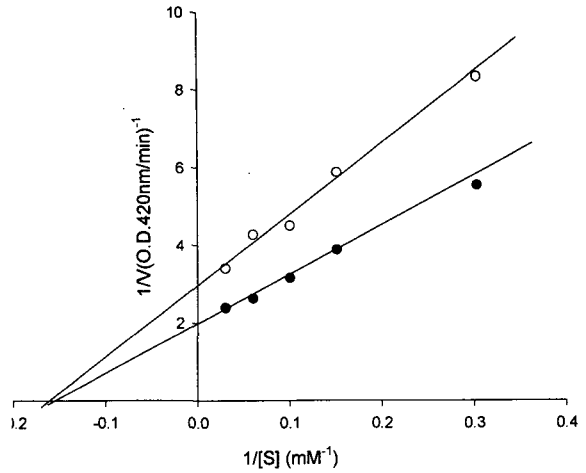

Fig. 3. Lineweaver-Burk plots of potato polyphenol oxidase in the presence of pine needle extract. Catechol was used as a substrate. The enzyme activity was measured at 25℃ for 1 min by the spectrophotometric procedure. The amount of the pine needle extract was 33 mg/ml. Control(●-●); Addition of pine needle extract(○-○).

onion extract inhibited the pear polypheno oxidase non-competitively [6].

Heat and acid stability of pine needle extract

Table 3 shows heat and acid stability of pine needle extract on potato polyphenol oxidase. The pine needle extract subjected to a heat treatment at 100℃ for 10 min or to an acid treatment at pH 2.0, 3.0, and 4.0 for 3 hours still retained inhibitory effect on potato polyphenol oxidase. Therefore, the pine needle extract seems to have been a heat and acid stable substance. Lee and Park reported that the inhibitory action of onion extract toward mushroom tyrosinase activity slightly increased after heat treatment at 100℃ for 10 min and decreased after incubation of the extract at pH 2.3 for 3 hours[10]. Kim *et al.* suggested that both fresh and heated onion extracts decreased pear polyphenol oxidase, and the heated onion extract was more effective in inhibition of pear polyphenol oxidase[6].

Conclusions

In the present work, the inhibitory effect of pine needle on potato polyphenol oxidase was indicated. The addition of the pine needle extract exhibited inhibitory effect on the potato polyphenol oxidase activity. The result support the conclusion that pine needle extract contained certain compounds which is efficient for inhibition of potato polyphenol oxidase activity. Although the effective compound

Table 3. The heat and acid stability of pine needle extract on potato polyphenol oxidase

Condition		Relative activity(%)
Control		63.57±1.54
After heat treatment		65.26±1.23
After acid treatment	pH 2.0	71.26±2.12
	pH 3.0	69.77±1.87
	pH 4.0	67.89±1.67

The pine needle extract was treated at 100℃ for 10 min or at pH 2.0, 3.0 and 4.0 for 3 hours. The reaction mixture was incubated at 25℃ for 15 min. The enzyme activity was measured at 25℃ for 1 min by the spectrophotometric procedure. Datas are mean±SD values(n=3).

in pine needle for inhibitory effect of a potato polyphenol oxidase has not been identified, utilization of pine needle extract will be possible as a natural inhibitor. Further research will be required for the identification of the effective compound.

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초록 : 감자 polyphenol oxidase에 대한 솔잎 추출물의 저해효과

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감자 polyphenol oxidase에 대한 솔잎 추출물의 저해효과가 조사 되었다. 솔잎 추출물은 anti-browning agents 인 potassium sorbate나 citric acid보다도 더 강한 저해효과 를 나타냈으며 pH 7.0-8.0 사이에서 가장 강력한 저해효과가 있음을 알 수 있었다. 또한 반응시간이 증가 할수록 감자 polyphenol oxidase 활성은 현저하게 저해 되었으며 솔잎 추출물은 감자 polyphenol oxidase에 대해 비경쟁적인 저해를 하는 것으로 나타났다. 솔잎 추출물은 100℃ 에서 10분간 열처리시에도 안정 하였으며 pH 2.0, 3.0, 4.0에서 3시간 처리시에도 여전히 감자 polyphenol oxidase 에 대한 저해효과를 보유하고 있어서 산과, 열에 비교적 안정한 물질임을 알 수 있었다.