Hanguk Nonghwahak Hoechi
(J. Korean Agric. Chem. Soc.)
35(3), 170~172(1992)

Some characteristics of *Ligularia fischeri* polyphenol oxidase

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Abstract : Four types of polyphenol oxidase were isolated from the crude extract of a *Ligularia fischeri* by gel filtration on Sephadex G-150. Optimum pH and temperature for the activity of partially purified enzyme were 7.5 and 25 ℃, respectively. It was stable at temperature 40 ℃ when examined at pH 7.5 for 5 min, and lost 90% of its activity at 70 ℃ in 30 min at pH 7.5. The enzyme has good activity on catechol and chlorogenic acid but was inactive on dopamine(Received 3, March, 1992, accepted 6, May 1992).

Polyphenol oxidase is also known as phenol oxidase, tyrosinase, O-diphenol oxidase, catechol oxidase, phenolase and chlorogenic acid oxidase. Polyphenol oxidase (EC 1.10.3.1) is a copper-containing enzyme which catalyzes either one or two reactions involving molecular oxygen.[1]

Brown discoloration of edible mountain herb and its concentrate was found to be related to the enzymatic browning that takes place before or during processing.[2,3]

We previously reported some properties of polyphenol oxidase from *Spuriopimpinella bracycarpa*[4] and *Aster scaber*.[5] Since polyphenol oxidase in *Ligularia fischeri* has not been adequately investigated, the purpose of this investigation was to purify the enzyme of *Ligularia fischeri* and to clarify some properties and specificity.

Materials and Methods

An edible mountain herb, "*Ligularia fischeri*" was purchased from the local market, and cut into slices, rinsed with tap water and moistened with acetone; they were chilled to 4 ℃.

The protein concentration in enzyme solution was determined by the method of Lowry.[6] Measurement of enzyme activity and enzyme unit determined as described previously.[5,7] All the procedures of enzyme purification, preparation of crude enzyme and enzyme extraction were carried out as described previously.[4,5]

Results and Discussion

Four types of polyphenol oxidase were partially isolated from the crude extract of a *Ligularia fischeri* by gel filtration on Sephadex G-150. Our previous papers[4,5] have reported that polyphenol oxidase from *Spuriopimpinella bracycarpa* and *Aster scaber* appeared three and five peaks by the same chromatography.

The optimum pH and temperature for the activity of enzyme was found to be arround 7.5 and 25 ℃ (Fig. 1). And the enzyme was stable at 50 ℃ up to 5 min but completely inactivated by the treatment at 80 ℃ for 30 min (Fig. 2). In comparasion with the above values reported for the enzyme from *Spuriopimpinella bracycarpa* and *Aster scaber*, the values of these enzyme were similar to one another, though small difference found among them.

The enzyme was inhibited by treatment with potassium cyanide (0.05, 0.1 and 0.5 mM), ascorbic

Key words : *Ligularia fischeri* polyphenol oxidase
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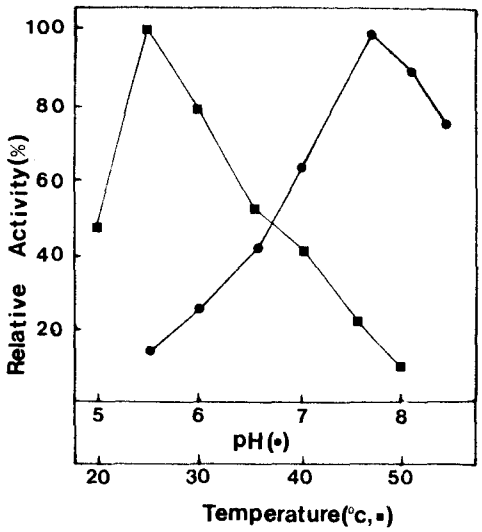

Fig. 1. Effect of pH and temperature on *Ligularia fischeri* polyphenol oxidase activity.

Table 1. Effect of various inhibitors on *Ligularia fischeri* polyphenol oxidase activity.

Inhibition (mM)	Relative activity (%)			
	Concentration(mM)			
	0.05	0.1	0.5	1.0
None	100	100	100	100
L-cysteine	89	71	0	0
Ascorbic acid	93	57	9	0
Glutathione	80	72	0	0
Potassium cyanide	44	27	9	0
EDTA	86	72	63	29

acid (0.5 mM) and EDTA (1 mM), and completely inhibited by treatment with L-cystine(0.5 and 1 mM), ascorbic acid (1 mM) (Table 1). On the other hand, *Spuriopimpinella bracycarpa* enzyme was partially inactivated by ascorbic acid, glutathione and potassium cyanide (0.1 mM), and was completely inhibited by L-cysteine, ascorbic acid, glutathione and potassium cyanide (0.5 and 1 mM).

The enzyme showed good activity on catechol and chlorogenic acid but did not hydrolyze dopamine (Table 2). In comparasion with the substrate specificity of the enzyme from *Spuriopimpinella bracycarpa* and *Aster scaber*, *Spuriopimpinella bracycarpa* enzyme has good activity on catechol and 3,4-

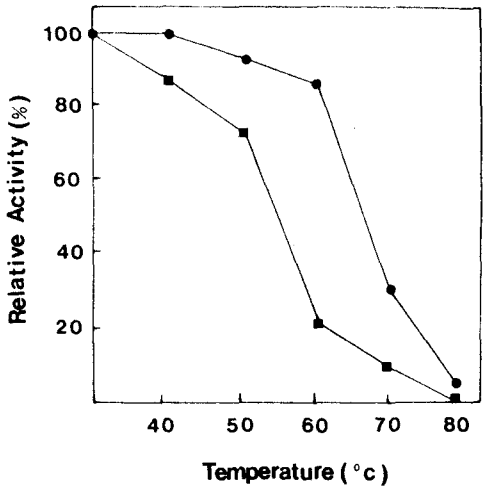

Fig. 2. Thermal stability of *Ligularia fischeri* polyphenol xoidase.
The enzyme solution were heated at various temperature (40~80 ℃) for 5 min and 30 min. After heating, the remaining enzyme activities were determined with catechol as substrate at pH 7.5 and 30 ℃. ●—● : 5 min, ■—■ : 30 min

Table 2. Substrate specificity of *Lingularia fischeri* polyphenol oxidase activity

Substrate (10 mM)	Relative Activity (%)
Catechol	100
Chlorogenic acid	100
3,4-Dihydroxytoluene	71.4
Pyrogallol	28.6
Hydroxyhydroquinone	85.6
Dopamine	7.4
DL-Dopa	14.3

dihydroxytoluene but was strongly inactivated on pyrogallol, dopamine and DL-dopa. And also, *Aster scaber* enzyme has good activity on chlorogenic acid but was inactive on DL-dopa.

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곰취 Polyphenol oxidase의 효소화학적 성질

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초록 : Sephadex G-150 column chromatography에 의해 부분 정제된 곰취 polyphenol oxidase의 최적 pH와 최적온도는 7.5와 25℃였으며, pH 7.5에서 5분간 처리시에는 40℃에서 안정하였으나, 30분간 처리시에는 70℃에서 약 90% 실활하였다. polyphenol oxidase는 ascorbic acid와 potassium cyanide(0.5 mM)에 의해 불활성화 되었으며, L-lysine, glutathione (0.5 and 1 mM), ascorbic acid와 potassium cyanide(1 mM)에서는 완전 실활하였다. catechol과 chlorogenic acid의 기질은 높은 특이성을 나타낸 반면 dopamine은 7.4%로서 가장 낮았다.