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# Distribution of Polyphenol Oxidase in some Cruciferae Vegetables

[1]Andi Nur Faidah Rahman, [2]Februadi Bastian, [3]Jumriah Langkong and [4]Shuji Fujita

[1,2,3]*Department of Food Science and Technology,*
*Faculty of Agriculture, Hasanuddin University, Makassar 90245 Indonesia*
[4]*Laboratory of Food Science, Faculty of Agriculture, Saga University, Saga 840-8502 Japan*

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Corresponding Author:
Andi Nur Faidah Rahman
Department of Food Science and Technology, Faculty of Agriculture, Hasanuddin University, Makassar 90245 Indonesia
Email: faidah83@yahoo.com

**Abstract:** Enzymatic oxidation of polyphenolic compounds in damaged tissue of many fruits and vegetables induced undesirable browning as results in decreased marketability of products. A new type of polyphenol oxidase (PPO) that is "Phloroglucinol Oxidase (PhO)" have been reported in turnip and cabbage, and the purified enzymes also had strong peroxidase (POD) activity. Distribution of PPO from different plant source of cruciferae vegetables were observed. Cabbage, broccoli, turnip and cauliflower PPOs from Indonesia and Japan strongly oxidized 1,3,5-trihydroxybenzenes such as phloroglucinol (Phl) and phloroglucinol carboxylic acid, but not oxidized 1,2,3-trihydroxybenzenes and *o*-diphenol. On the other side, Chinese mustard, Chinese cabbage, shingensai, Japanese radish root, mizuna, takana, leaf mustard, katsuona and komatsuna also strongly oxidized 1,3,5-trihydroxybenzenes and little oxidized 1,2,3-trihydroxybenzenes such as pyrogallol and gallic acid, but not oxidized *o*-diphenol. These results indicated that the PPOs in all cruciferae vegetables are a group of a new type of PPO, however, the level activity of 7 varieties of vegetables from Indonesia and 15 varieties from Japan tested showed different activity level of PhO and POD.

**Keywords:** Cruciferae Vegetables, Polyphenol Oxidase, Peroxidase

## Introduction

Cruciferae vegetables are vegetables of the family brassicaceae. These vegetables contain high phytochemicals compound such as polyphenols to prevent common human cancers. Oxidation of phenolic compounds by polyphenol oxidase (PPO) in the damaged tissue induced undesirable browning, resulting decrease the nutritional value of vegetables. Generally, PPO oxidized *o*-diphenols, such as found in Japanese butterbur (Han *et al*., 2009) and edible burdock (Han *et al*., 2006) strongly oxidized chlorogenic acid; edible yam (Fujita *et al*., 2006) strongly oxidized dopamine; broccoli florest (Gawlik-Dziki *et al*., 2007), mango (Wang *et al*., 2007) and mamey (Palma-Orozco *et al*., 2011) strongly oxidized catechol; garland chrysanthemum (Nkya *et al*., 2003) strongly oxidized catechol and epicatechin . On the other hand, the purified enzyme of soybean (Toiguchi *et al*., 1989) and one of the purified enzymes of edible burdock (Murao *et al*., 1993) not oxidized *o*-diphenols, but oxidized pyrogallol (1,2,3-trihydroxybenzene) and phloroglucinol (1,3,5-trihydroxybenzene). In addition, a new type of PPO that is "Phloroglucinol Oxidase (PhO)" which only oxidizes 1,3,5-trihydroxybenzene such as phloroglucinol and phloroglucinol carboxylic acid was found in Satsuma mandarin (Fujita and Tono, 1980a) and cruciferae vegetables such as turnip (Fujita and Tono, 1980b) and cabbage (Fujita *et al*., 1995; 1997), and the purified enzymes also had strong POD activities. PPO and POD play roles in darkening of cruciferae vegetables, therefore, the experiments were performed to report the distributions of PPOs of cruciferae vegetables.

## Materials and Methods

### *Materials*

Variation of cruciferae vegetables were grown and purchased from a local market in Makassar, Indonesia (Table 1) and Saga, Japan (Table 2). Reagents used were obtained from Chemical Market in Makassar, Indonesia and from Wako Pure Chemical Co., Osaka, Japan.

### *Enzyme Extraction*

The fresh (fresh weights 500 g) of various cruciferae vegetables were stirred with 0.1 M phosphate buffer (pH 7.0) at 5°C. The filtrate then centrifuged at 10,300× g for 20 min at 5°C and the supernatant was salted out to 80% saturation with ammonium sulfate for 24 h. The precipitated protein was collected by centrifugation (10,300×g) and dissolved in a small quantity of 0.1 M phosphate buffer (pH 7.0) then dialyzed against the same buffer for 36 h at 5°C with four times changed of buffer and centrifuged again at the same condition. The supernatant was used as the enzyme solution.

### *Assay of Enzyme Activity*

### *PhO Activity*

PhO activity was determined by a spectrophotometric method (Fujita *et al*., 1993). The reaction mixture contained 0.5 mL of 20 mM aqueous solution of phloroglucinol, 1.4 mL of 0.1 M potassium phosphate/0.1 M sodium hydrogen phosphate buffer (pH 7.0) and 0.1 mL of the enzyme solution: it was incubated at 30°C, after 10 min of incubation, 0.5 mL was taken out and added to 4.5 mL of distilled water and then measured immediately at 272 nm against an enzyme blank. One unit of enzyme activity was expressed as △A272 of 0.1 per min and per mL of the enzyme solution (1.0 cm light path).

### *PPO Activity*

PPO activity was measured by a colorimetric method (Yang *et al*., 2000). The mixture to be tested consisted of 0.5 mL of 10 mM aqueous solution of various polyphenols, 4.0 mL of 0.1 M phosphate buffer (pH 7.0) and 0.5 mL of the enzyme solution: it was incubated at 30°C for 5 min and reaction mixture was measured as absorbance at 420 nm (△A420). One unit of enzyme activity was expressed as △A420 of 0.1 per min and per mL of the enzyme solution (1.0 cm light path).

### *POD Activity*

POD activity was measured by a colorimetric method (Fujita *et al*., 1995). The reaction mixture contained 0.5 mL of 0.1 M aqueous solution of guaiacol, 4.1 mL of 0.1 M phosphate buffer (pH 6.0), 0.2 mL of 0.1% hydrogen peroxide and 0.2 mL of the enzyme solution: it was incubated at 30°C for 2 min and reaction mixture was measured as absorbance at 470 nm (△A470). One unit of enzyme activity was defined as a change in absorbance of the mixture at 470 nm (△A470) of 0.1 per min and per mL enzyme solution (1.0 cm light path).

### *Determination of Protein*

Protein was determined by the method of Hartree (1972) using Bovine Serum Albumin (BSA, fraction V; Katayama Chemical Company, Osaka, Japan) as standard. Protein was determined as absorbance at 280 nm.

## Results and Discussion

Polyphenol oxidase (PPO) from different plant source of cruciferae vegetables were extracted by 80% ammonium sulfate saturation. Table 1 and 2 showed substrate specificity of crude PPO from various Indonesia's and Japan's cruciferae vegetables. Generally, PPO oxidized *o*-diphenols, as listed in Table 3, garland chrysanthemum (Nkya *et al*., 2003) strongly oxidized chlorogenic acid and epicatechin; Japanese butterbur (Han *et al*., 2009) and apple (Murata *et al*., 1992) strongly oxidized chlorogenic acid; banana pulp and peel (Yang *et al*., 2000; 2001) strongly oxidized dopamine; red Swiss chard leaves strongly oxidized L-DOPA (Gao *et al*., 2009); and broccoli florest (Gawlik-Dziki *et al*., 2007), butter lettuce (Gawlik-Dziki *et al*., 2008) and cherry pulp (Jia *et al*., 2011) strongly oxidized catechol. However, substrate specificity of crude PPO from various Indonesia's and Japan's cruciferae vegetables (Table 1 and 2) showed different activity, all the cruciferae vegetables PPOs tested did not show activity toward *o*-diphenol. From 7 varieties of Indonesia's vegetables tested showed cabbage, broccoli and cauliflower PPOs strongly oxidized 1,3,5-trihydroxybenzenes such as phloroglucinol and phloroglucinol carboxylic acid, but not oxidized 1,2,3-trihydroxybenzenes and *o*-diphenols. On the other hand, PPOs of Chinese mustard, Chinese cabbage, shingensai and Japanese radish root PPO also strongly oxidized 1,3,5-trihydroxybenzenes, but little oxidized 1,2,3-trihydroxybenzenes such as pyrogallol. In addition, the PPOs from 15 varieties of Japan's cruciferae vegetables such as cabbage, turnip, broccoli and cauliflower PPOs also strongly oxidized 1,3,5-trihydroxybenzenes such as phloroglucinol and phloroglucinol carboxylic acid, but not oxidized 1,2,3-trihydroxybenzenes and *o*-diphenol. On the other hand, PPOs of mizuna, takana, leaf mustard, katsuona and komatsuna also strongly oxidized 1,3,5-trihydroxybenzenes, but have small activity toward pyrogallol. In addition, Japanese radish root PPO has small activity toward pyrogallol and gallic acid (Table 2). The results indicated that the PPOs from different plant source of cruciferae vegetables are a group of a new type of PPO that is "Phloroglucinol Oxidase (PhO)".

Distribution of PPO in the cruciferae vegetables from Indonesia and Japan showed in Table 4 and 5. The enzyme showed dual activities of phloroglucinol oxidase (PhO) and peroxidase (POD) and it showed wide variation in PhO and POD activities. The level activities of PhO from 7 varieties of vegetables from Indonesia (Table 4) in order as follows (unit/mg protein): Japanese radish root (22.0) > cauliflower (20.7) > broccoli (14.4) > cabbage (13.6) > shingensai (1.70) > Chinese mustard (0.64) > Chinese cabbage (0.14). In addition, the level activities of POD in order as follows (unit/mg protein): Cabbage (60.0) > Japanese radish root (52.0) > broccoli (41.0) >

cauliflower (40.0) > shingensai (9.0) > Chinese mustard (5.8) > Chinese cabbage (3.4). On the other side, the level activity of PhO from 15 varieties of vegetables from Japan (Table 5) in order as follows (unit/mg protein): Japanese radish root (127.0) > turnip (96.8) > cabbage (37.0) > broccoli (36.0) > cauliflower (16.8) > broccoli sprout (5.4) > nabana (4.6) > Chinese cabbage (3.1) > shingensai (2.7) > daikon sprout (2.0) > mizuna (1.9) > takana (1.1) > komatsuna (1.0) > Chinese mustard (0.9) > katsuona (0.3). On the other hand, the level activities of POD in order as follows (unit/mg protein): Turnip (432.0) > cabbage (292.0) > Japanese radish root (216.0) > broccoli (45.0) > cauliflower (41.3) > nabana (26.5) > broccoli sprout (19.3) > mizuna (10.5) > daikon sprout (9.5) > Chinese cabbage (9.4) > shingensai (9.0) > takana (7.8) > Chinese mustard (5.8) > komatsuna (5.2) > katsuona (1.7).

Table 1. Substrate specificity of crude PPO from various Indonesia'scruciferae vegetables

| | Cabbage | Broccoli | Chinese mustard | Chinese Cabbage | Shingensai | Cauliflower | Japanese radish root |
|---|---|---|---|---|---|---|---|
| **1,3,5-trihydroxybenzenes:** | | | | | | | |
| phloroglucinol | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 |
| Phloroglucinol carboxylic acid | 64±2.0 | 71±5.0 | 20±1.0 | 15±1.0 | 20±2.0 | 31±2.0 | 36±1.0 |
| **1,2,3-trihydroxybenzenes:** | | | | | | | |
| Pyrogallol | 0±0.0 | 0±0.0 | 14±2.0 | 11±1.0 | 20±2.0 | 0±0.0 | 2.0±0.5 |
| Gallic acid | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |
| ***o*-diphenols:** | | | | | | | |
| catechol | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |
| Chlorogenic acid | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |
| DL-DOPA | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |
| Dopamine | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |
| *m*-diphenols resorcinol | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |

Above data were averages of three trials

Table 2. Substrate specificity of crude PPO from various Japan'scruciferae vegetables

| | Relative activity (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Substrates | Mizuna | Takana | Cabbage | Turnip | Broccoli | Leaf mustard | Katsuona | Komatsuna | Cauliflower | Japanese radish root |
| **1,3,5-trihydroxybenzenes:** | | | | | | | | | | |
| phloroglucinol | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 |
| phloroglucinol carboxylic acid | 45±3.0 | 99±8.0 | 71±7.0 | 47±5.0 | 86±2.0 | 34±2.0 | 25±2.0 | 40±4.1 | 42±3.0 | 46±5.2 |
| **1,2,3-trihydroxybenzenes:** | | | | | | | | | | |
| pyrogallol | 15±1.0 | 18±2.0 | 0±0.0 | 0±0.0 | 0±0.0 | 4±0.5 | 2±0.2 | 26±2.0 | 0±0.0 | 2.5±0.2 |
| gallic acid | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0.4±0.1 |
| ***o*-diphenols:** | | | | | | | | | | |
| catechol | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |
| chlorogenic acid | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |
| DL-DOPA | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |
| dopamine | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |
| *m*-diphenols resorcinol | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 | 0±0.0 |

Above data were averages of three trials

Table 3. Substrate specificity of PPO from fruits and vegetables

| | Relative activity (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Substrates | Garland chrysanthemum (Nkya *et al*., 2003) | Banana pulp (Yang *et al*., 2000) | Banana peel (Yang *et al*., 2001) | Broccoli florest (Gawlik-Dziki *et al*., 2007) | Butter lettuce (Gawlik-Dziki *et al*., 2008) | Japanese butterbur (Han *et al*., 2009) | Apple (Murata *et al*., 1992) | Red Swiss chard leaves (Gao *et al*., 2009) | Cherry pulp (Jia *et al*., 2011) |
| ***o*-diphenols** | | | | | | | | | |
| catechol | 76 | 54 | 34 | 100 | 100 | 39 | | 86 | 100 |
| 4-methylcatechol | | | | 62.76 | 88.5 | | | | |
| Chlorogenic acid | 100 | 24.5 | 5.3 | 1.89 | 53.4 | 100 | 100 | 80 | |
| DL-DOPA | 72 | 12.3 | 8 | | | 2 | 9.5 | | |
| L-DOPA | | | | | | | | 100 | |
| dopamine | 74 | 100 | 100 | | | 9 | 10.2 | | |
| resorcinol | 0 | 0 | 0 | | | 0 | 0 | | 0 |
| Caffeic acid | | 2 | 0.7 | 5.92 | 2.3 | 42 | | | |
| D-catechin | 70 | 35.6 | 11.5 | | | 37 | 12.9 | | |
| Epicatechin | 100 | 22.7 | 9.3 | | | 94 | 14.4 | | |
| *p*-dimethylphenol | | | | | | | | 10 | |
| *m*-dimethylphenol | | | | | | | | 11 | |
| Ferulic acid | | | | 1.1 | 0.2 | | | | |
| **1,2,3-trihydroxybenzenes:** | | | | | | | | | |
| Pyrogallol | 70 | 5.5 | 1.4 | | | 0 | 11.9 | | |
| Gallic acid | 72 | 0 | 0 | | | 0 | | 73 | 70.1 |
| **1,3,5-trihydroxybenzenes:** | | | | | | | | | |
| Phloroglucinol | 0 | 0 | 0 | 0.16 | | 0 | 0 | | 0 |

Table 4. Distribution of PhO and POD in the cruciferae vegetables from Indonesia

| | Specific activity (unit/mg protein) | |
|---|---|---|
| Various of cruciferae vegetables | PhO | POD |
| Chinese cabbage (*Brassica campestris* L.) | 0.14±0.01 | 3.4±0.01 |
| Chinese mustard (*Brassica campestris* L.) | 0.64±0.02 | 5.8±0.01 |
| Shingensai (*Brassica rapa*L. (Chinensis Group)) | 1.70±0.10 | 9.0±1.00 |
| Cauliflower (*Brassica oleracea* L.) | 20.7±1.00 | 40.0±2.00 |
| Broccoli (*Brassica oleracea* L.) | 14.4±1.10 | 41.0±4.00 |
| Japanese radish root (*Raphanussativus* L.) | 22.0±3.00 | 52.0±6.00 |
| Cabbage (*Brassica oleracea* L.) | 13.6±0.51 | 60.0±8.00 |

Above data were averages of three trials

Table 5. Distribution of PhO and POD in the cruciferae vegetables from Japan

| | Specific activity (unit/mg protein) | |
|---|---|---|
| Various of cruciferae vegetables | PhO | POD |
| Komatsuna (*Brassica rapa* var. perviridis) | 1.0±0.1 | 5.2±0.5 |
| Mizuna (*Brassica rapa* var. nipposinica) | 1.9±0.1 | 10.5±1.0 |
| Takana (*Brassica juncea* Czern. et Coss. (Integlifolia Group)) | 1.1±0.2 | 7.8±1.2 |
| Turnip (*Brassica compestris* L.) | 96.8±12.0 | 432.0±20.0 |
| Chinese cabbage (*Brassica campestris* L.) | 3.1±0.1 | 9.4±1.2 |
| Nabana (*Brassica napus*L.) | 4.6±0.5 | 26.5±2.0 |
| Chinese mustard (*Brassica campestris* L.) | 0.9±0.1 | 5.8±1.2 |
| Shingensai (*Brassica rapa* L. (Chinensis Group)) | 2.7±0.5 | 9.0±1.0 |
| Katsuona (*Brassica juncea*Czern.) | 0.3±0.1 | 1.5±0.5 |
| Daikon sprout (*Raphanussativus* L.) | 2.0±0.4 | 9.5±2.0 |
| Broccoli sprout (*Brassica oleracea* L.) | 5.4±1.0 | 19.3±3.0 |
| Cauliflower (*Brassica oleracea* L.) | 16.8±3.0 | 41.3±10.0 |
| Broccoli (*Brassica oleracea* L.) | 36.0±7.1 | 45.0±6.0 |
| Japanese radish root (*Raphanussativus* L.) | 127.0±15.0 | 216.0±19.0 |
| Cabbage (*Brassica oleracea* L.) | 37.0±2.0 | 292.0±18.0 |

## Conclusion

Distribution of PPO from different plant source of cruciferae vegetables were determined. The PPO from all various cruciferae vegetables tested showed a group of a new type of PPO that is "Phloroglucinol Oxidase (PhO)" which were strongly oxidized 1,3,5-trihydroxybenzene such as phloroglucinol and phloroglucinol carboxylic acid, and the purified enzymes also had strong POD activities. However, the level activity of 7 varieties of vegetables from Indonesia and 15 varieties from Japan tested showed different activity level of PhO and POD.

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## Author's Contributions

**Andi Nur Faidah Rahman:** Participated in all experiments.

**Februadi Bastian and Jumriah Langkong:** Coordinated the mouse work

**Shuji Fujita:** Designed the research plan and organized the study.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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