

Effect of ultrasonic processing on the properties of polyphenol oxidase from potato (*Solanum tuberosum* L.)

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Abstract. In present work, the effects of ultrasound on activity and conformation of polyphenol oxidase (PPO) from potato were investigated under different ultrasonic gradient for ultrasonic power (240-600 W), time (15-120 min), temperature (20-50°C) and pH (5.4-8.2). The results indicated that potato PPO exhibited a decrease in activity treated by different ultrasonic treatment conditions. Furthermore, the ultrasound significantly down-regulated the expression of PPO gene. The CD analysis demonstrated that the secondary structures such as α -helix were destroyed compared with the pristine PPO. The PPO activity showed a corresponding decrease along with the changes in the secondary structure. This investigation revealed that ultrasound inactivate PPO activity by changing the conformation of enzyme protein and down-regulating gene expression.

Keywords: Ultrasound; PPO; Enzymatic activity; Gene expression; Conformational changes.

1. Introduction

Potato (*Solanum tuberosum* L.), which is rich in nutrients and bioactive compounds, is an important economic crop and among the top 10 most consumed foods in the world [1]. Enzymatic browning is a major reason for decreased shelf life and quality of the potato and derived products [2]. Polyphenol oxidase (PPO) catalyzes the hydroxylation of monophenols to o-diphenols and dehydrogenation of o-diphenols to o-quinones in the presence of oxygen, causing undesirable changes that may hamper its quality in terms of color, taste and nutrition value, especially in potato and apple [3]. Therefore, most of the recent studies are focusing in the inactivation of PPO enzyme to maintain the good quality of fruit and vegetables.

Ultrasound is defined as sound waves at a frequency beyond the range audible to humans (>20 kHz) [4]. It is particularly useful in inactivation of enzyme because of the mechanical and chemical effects of cavitation [5]. Previous studies have reported that ultrasonic treatment can delay the enzymatic browning by inhibiting the activities of PPO and POD [6]. At intensity higher than 200 W, ultrasound caused an inactivation effect and conformational changes of purified PPO from oriental sweet melons [7]. Ultrasound for 10 min inactivated PPO in Taylor's Gold pear, Royal Gala apple and Camarosa strawberry purees [8]. Nevertheless, there are no reports on the impact of ultrasound on the activity and conformation of PPO directly purified from potato. Thus, the present study aims to investigate the effects of ultrasound treatment on activity and conformation of PPO from potato.

2. Materials and methods

2.1 Materials

Potato (*Solanum tuberosum* L.) tubers of the cultivars Holland Seven were harvested from field located at Taian. All the chemicals and reagents used in the experiment were of analytical grade.

2.2 Preparation and Purification of the crude PPO

Preparation and Purification of the crude PPO were prepared based on the previous method according to Marri et al [9].

2.3 Molecular weight, purity of the enzyme and PPO activity

The molecular weight and purity of the purified enzyme were estimated by SDS-PAGE and Native-PAGE according to Laemmli et al [10]. The enzyme activity assay was determined using a spectrophotometric method.

2.4 Protein determination

The protein concentration was measured by the method of Folin phenol. The regression equation was: $y = 0.026x + 0.0069$, $r^2 = 0.9989$.

2.5 Ultrasonic treatment

Airtight test tubes with 1 mL enzyme solutions (pH=6.6) were fixed in an ultrasonic bath and treated for 60 min using the ultrasonic equipment at the power of 240 to 600 W at 30°C. Under the ultrasonic power 600 W and 30°C, the effect of ultrasonic time on the PPO activity was estimated at the different time ranging from 15 to 120 min. Fixing ultrasonic power (600 W) and ultrasonic time (60 min), the effect of ultrasonic temperature (20, 25, 30, 35, 40, 45, 50°C) and ultrasonic pH (from 5.4 to 8.2 at 0.6 intervals) on the PPO activity was investigated, respectively. Enzyme solution without ultrasonic treatments were used as the control.

2.6 Quantitative real-time PCR analysis

The expression level of PPO gene was analyzed by using quantitative real-time PCR. The real-time PCR was carried out using an Mx3000p instrument (Stratagene, La Jolla, CA, USA), and β -tubulin gene was used as the endogenous reference gene. The 50- μ L reaction mixture was prepared according to the manufacturer's instructions of the SYBR Premix Ex Taq kit, and the relative quantification of gene expression was evaluated using the $2^{-\Delta\Delta C_t}$ method.

2.7 Circular dichroism (CD) spectra analysis

CD measurement was performed on a J-715 circular dichroism spectrometer (JASCO Corporation, Tokyo, Japan) with a 1 mm path length quartz cuvette at room temperature ($25 \pm 1^\circ\text{C}$) according to Liu et al [11].

2.8 Fluorescence spectra analysis

Fluorescence emission spectra of PPO solution treated by ultrasound were carried out on a F-7000 Spectrophotometer (Hitachi, Tokyo, Japan) according to Liu et al [12].

2.9 Statistical analysis

Each experiment was carried out in triplicate without special instructions, and the results were presented with mean $\pm$ standard deviations of the mean. Analysis of Variance (ANOVA) was performed using SPSS 20.0 under the significance level of $p < 0.05$.

3. Results

3.1 Purification of potato PPO

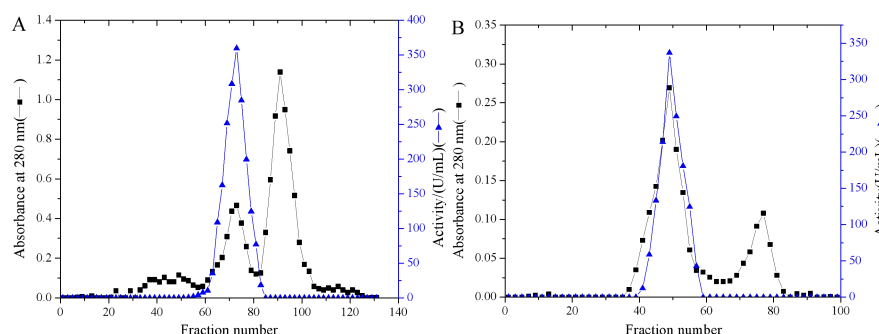


Fig. 1. Chromatographic elution profiles of polyphenol oxidase and enzyme activity from potato.

The results of extraction and purification of potato PPO were shown in Table 1 and Fig.1 A-B. 70.11% non-targeted proteins of the total proteins were removed and the recovery rate of PPO reached 75.61% through the ammonium sulfate fractionation. The protein content in the eluted fraction showed mainly two peaks. PPO activity appeared in one peak and coincided with the first protein peak of the protein (Fig. 1A). No enzymatic activity of the first minor peaks and the third peak was observed. Fractions with high enzymatic activities (61-69) were collected, concentrated and further purified using the Sephadex G-100 gel filtration column. The gel filtration chromatography produced a single peak of PPO activity and two peaks of protein (Fig. 1B). The fractions 47-53 with the highest activity were collected and dialyzed, resulting in a 31.50-fold purification, and the purified PPO had an overall activity yield of 36.11%, with specific PPO activity of 6,439 U/mg (Table 1).

Table 1 Purification procedure of polyphenol oxidase from potato.

Purification steps	Activity total (U)	Protein total (mg)	Specific activity (U/mg protein)	Purification (fold)	Yield (%)
Crude extract	14266	69.8	204.38	1	100
(NH ₄) ₂ SO ₄ precipitation	10786	20.86	517.07	2.53	75.61
Sepharose FF	7583	1.88	4033.51	19.74	53.18
Sephadex G-100c	5151	0.79	6438.75	31.50	36.11

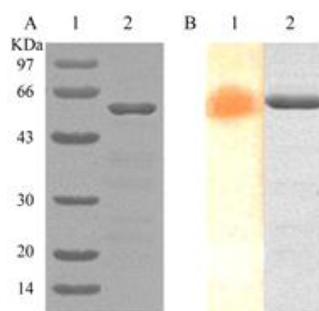


Fig. 2. Denaturing SDS-PAGE and Native-PAGE of purified PPO from potato.

The molecular weight and purity of PPO were estimated by SDS-PAGE and Native- PAGE. The SDS-PAGE of purified PPO revealed a single band with a molecular weight at around 60 kDa (Lane 1 in Fig. 2A). Under Native-PAGE, a single protein band was detected for PPO activity (Lane 1 in Fig. 2B). A single protein band was also observed when stained with Coomassie Brilliant

Blue R-250, and the band was at the same migration distance as the active staining one (Lane 2 in Fig. 2B).

3.2 Effects of ultrasonic treatment on PPO

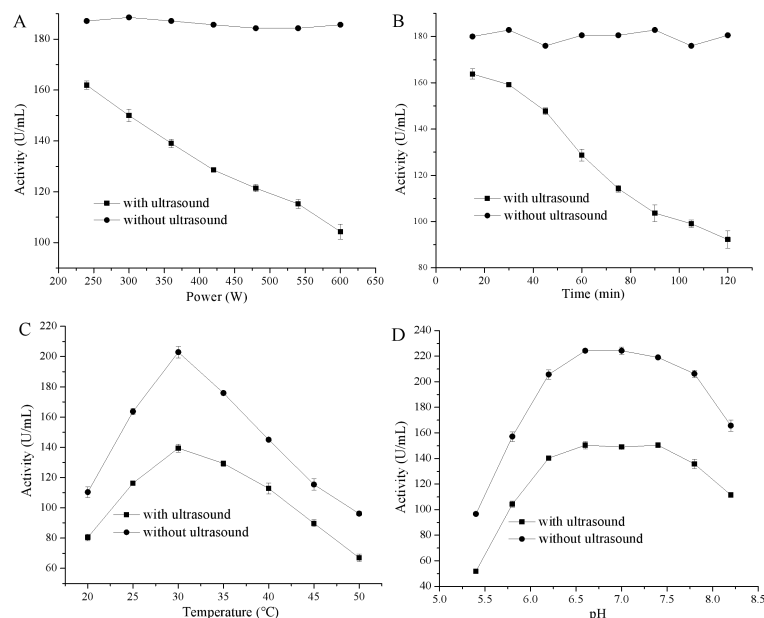


Fig. 3. Effects of ultrasonic treatment on PPO activity from potato.

The influence of ultrasonic treatment on the activity of potato PPO is confirmed. As shown in Fig. 3, the activity of PPO was obviously inactivated by ultrasound treatment and decreased rapidly with the increasing of ultrasound power from 240 to 600 W. With the power up to 600 W, the PPO activity reduced to 129.29 U/ml (Fig. 3A). With the extension of ultrasound time, the PPO activity of blank also remained practically unchanged at approximately 180 U/ml. Meanwhile, the PPO activity with ultrasonic treatment have shown a downward trend. After ultrasonic treatment of 2 h, the PPO activity was significantly decreased to 92.19 U/mL, only about 51.24% in comparison with the control (Fig. 3B). Both of the optimal temperatures of the PPO activity in the presence and absence of ultrasound were 30°C. The activity of PPO by ultrasound treatment was lower than that of control at the optimal temperature, even though at a lower or higher temperature. In the absence and presence of ultrasound at the optimal temperature, the enzyme activities were 202.92 and 180.01 U/mL respectively. The activity was reduced by ultrasound treatment to 88.71% (Fig. 3C). The optimal pH of the PPO activity was range from 6.6 to 7.4. The activity of PPO by ultrasound treatment was lower than that of control. The enzyme activities were decreased by ultrasound treatment to 89.38%, 88.53%, 91.52% at 6.6, 7.0, 7.4, respectively (Fig. 3D).

3.3 PCR analysis of potato PPO

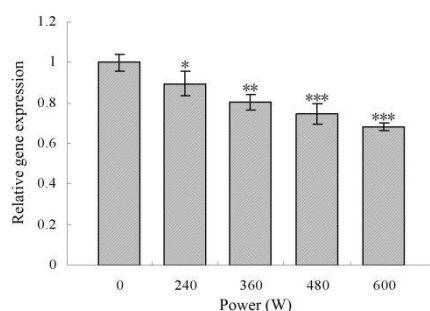


Fig. 4. Effect of ultrasonic treatment on gene expression of PPO from fresh-cut potatoes.

Potato PPO was significantly inactivated by ultrasonic treatment, and one legitimate reason could be that ultrasound down-regulate PPO gene. To confirm this postulate, we evaluated the

expression of PPO gene after treated by ultrasound. The results from Fig.4 indicated that ultrasound significantly down-regulated the expression of PPO gene, and the PPO gene treated by 240 W and 600 W significantly down-regulated with 1.12- and 1.47- folds decrease compared with control.

3.4 CD analysis of potato PPO

Table 2 Secondary structures content of ultrasound treated PPO.

Treatment	α -helix (%)	β -sheet (%)	β -turn (%)	Random coil (%)
Untreated	37.6	24.3	17.4	20.7
240 W	36.1	23.8	16.7	23.4
360 W	34.2	23.7	15.9	26.2
480 W	32.1	23.6	15.2	29.1
600 W	29.6	23.7	14.8	31.9

The effects of under various ultrasound power conditions on the secondary structural compositions of PPO were shown in Table 2. As shown in Table 2, ultrasonic treatment significantly affected the secondary structure of potato PPO. A gradual loss of α -helix content of potato PPO was observed with ultrasound power increased (Table 2). When the power of ultrasound increased to 600 W, the α -helix and β -turn content of PPO decreased obviously, with a concurrent increase of random coil content, while the content of β -sheet did not undergo an enormous change.

3.5 Fluorescence spectra analysis of potato PPO

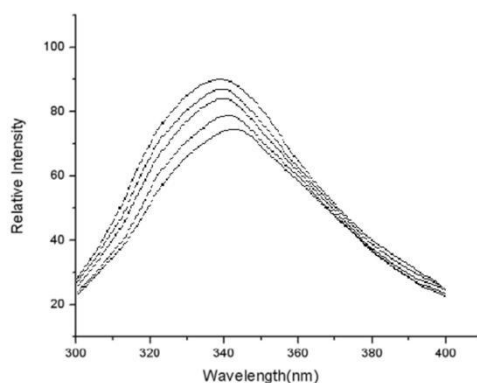


Fig. 5. Fluorescence emission spectra of ultrasound treated PPO. Curves from top to bottom were control and treated at power of 240, 360, 480 and 600 W.

Under a specified excitation light, the aromatic amino acid chain that is an internal fluorescent substance and sensitive to the polarity of microenvironments along the transition could emit fluorescence. To examine further the change on the conformation of PPO treated by ultrasound, fluorescence emission intensity was measured. It could be noted that the ultrasonic treatment caused a decline in relative fluorescence intensity (Fig. 5). Furthermore, with increased in ultrasound power, the fluorescence intensity of potato PPO gradually decreased. The fluorescence intensity of PPO after ultrasonic treatment in low-power (240W) was affected without a shift in the maximum peak wavelength. When the power of ultrasound increased to 600 W, the relative fluorescence intensity of PPO quenched with a red-shift in the maximum peak wavelength. The wavelength of fluorescence emission peak was red-shifted from 339 nm to 343 nm after ultrasonic treatment.

4. Conclusion

After a series of extraction and purification steps, the PPO enzyme has been purified about 31.50-fold with the activity of 6,439 U/mg. Potato PPO consists of a single polypeptide chain with a relative molecular weight of 60 kDa. Ultrasonic treatment down-regulated the expression of PPO gene and reduced the content of α -helix and β -turn. While, the content of random coil of potato PPO was enhanced. The relative fluorescence intensity of potato PPO quenched after ultrasonic treatment and with a red-shift in high-power ultrasonic treatment. Taken together, these findings highlight that ultrasonic processing can inhibit PPO activity by changing the secondary and tertiary structure of potato PPO and down-regulating gene expression.

5. Acknowledgments

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