Effect of Pulsed Light Treatment on Inactivation of Horseradish Peroxidase

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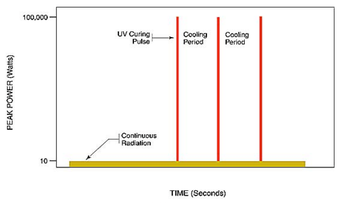
Abstract

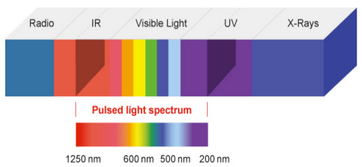
Peroxidases are a group of enzymes that cause enzymatic browning in pear juice. Pulsed light (PL) was reported as useful tool to inactive peroxidases due to its high energy intensity. Horseradish peroxidase (HRP) has the highest activity among peroxidases and the inactivation of HRP could ensure inactivation of all peroxidase. Therefore, HRP was taken as a model to study the mechanism of peroxidase inactivation by using PL. The objective of this research was to investigate the effect of PL treatment on the activity and conformation of HRP. Influences of PL treatment on surface microcosmic, secondary and tertiary structures of HRP were also studied. The results showed that PL treatment could effectively inactivate HRP by destroying secondary and tertiary structures at the active center of enzyme.

Background

China is the biggest pear producer in the world. Extraction of pear juice is an important approach to alleviate the problem of post-harvest spoilage. Enzymatic browning of pear juice caused by peroxidases greatly threatens the juice quality. Peroxidase inactivation methods widely used by the industries such as heating and chemical treatments have negative impacts on flavor, color and nutritional value of juice. Therefore, a non-thermal and highly effective processing approach without use of chemicals for enzyme inactivation is needed. PL is an FDA-approved non-thermal technology which has a high potential to inactivate the enzyme by changing structures of its active center.

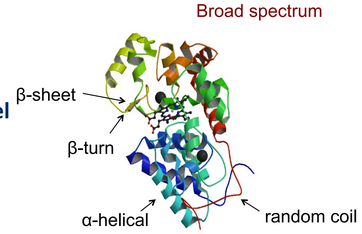


High power



Broad spectrum

HRP was used as a model for studying peroxidase inactivation.



Objective

1. To study the effectiveness of PL treatment to prevent browning of pear juice.
2. To investigate the effect of PL treatment on the activity and protein conformation of HRP.

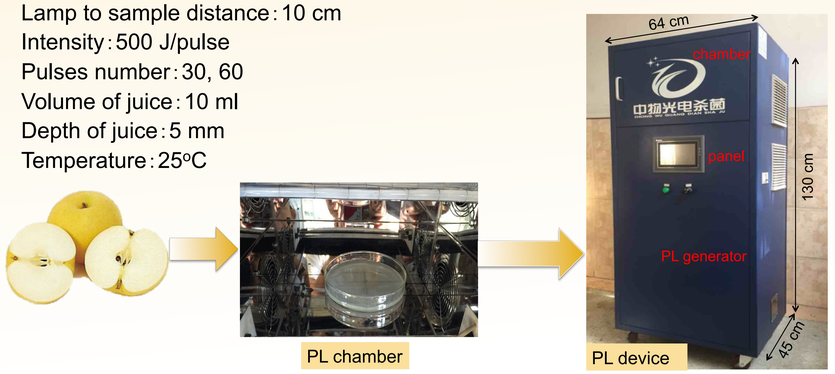
Materials and Methods

PL treatment on pear juice

The juice extracted from fresh pears of *Crown* variety was filtered and centrifuged prior to PL treatment

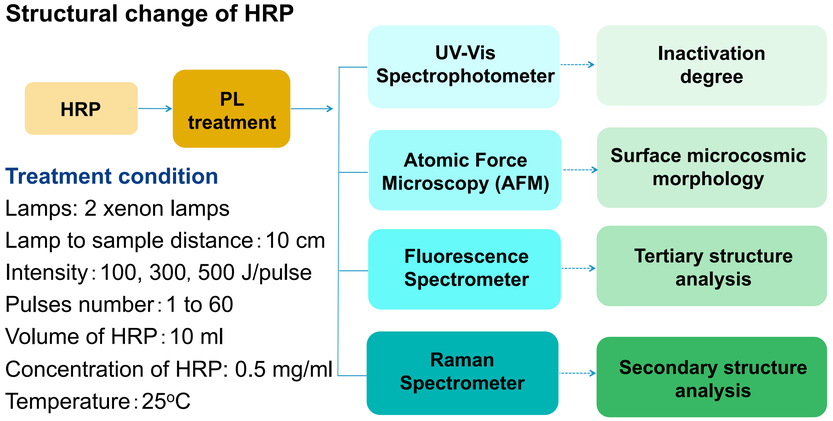
Treatment condition

Lamps: 2 xenon lamps
Lamp to sample distance: 10 cm
Intensity: 500 J/pulse
Pulses number: 30, 60
Volume of juice: 10 ml
Depth of juice: 5 mm
Temperature: 25°C



PL chamber

Structural change of HRP



Treatment condition

Lamps: 2 xenon lamps
Lamp to sample distance: 10 cm
Intensity: 100, 300, 500 J/pulse
Pulses number: 1 to 60
Volume of HRP: 10 ml
Concentration of HRP: 0.5 mg/ml
Temperature: 25°C

Results and Discussion

PL treatment inhibited the browning of pear juice (Fig. 1). The activity of HRP enzyme was eliminated after 10 pulses of PL (Fig. 2). AFM showed that PL treatment lead to assembling or segregation of HRP molecules (Fig. 3 & 4). Fluorescence spectroscopy analysis exhibited that PL treatment destroyed the tertiary structures and led to the unfolding of molecules (Fig. 5). Raman spectra showed that the PL irradiation significantly changed the content of secondary structure of HRP (Fig. 6). The β-sheet was decreased by 14.05% while β-turn and random coil were increased by 6.20% and 6.51%, respectively, after PL treatment of 60 pulses (Table 1).

Results and Discussion (Continued)

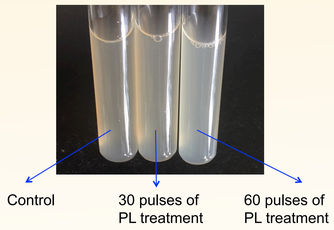


Fig. 1 PL treated pear juice

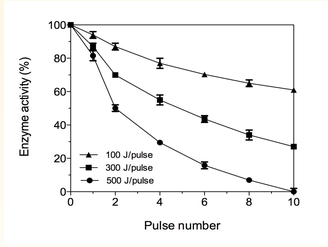


Fig. 2 Enzyme activity of HRP after PL treatment

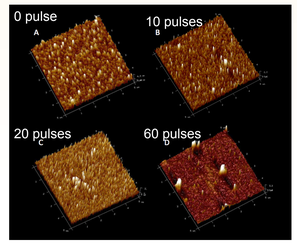


Fig. 3 3D AFM image of HRP protein

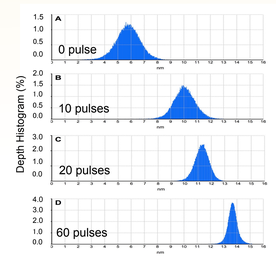


Fig. 4 Height distribution of HRP protein particles

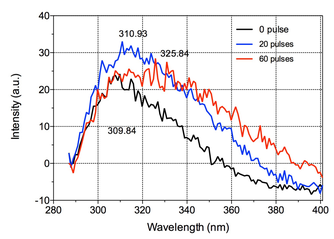


Fig. 5 Fluorescence emission spectra of HRP

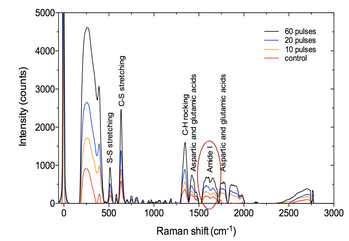


Fig.6 Raman spectra of HRP

Table 1 Contents of secondary structure of HRP

Treatment	α-helix (%)	β-sheet (%)	β-turn (%)	Random coil (%)
0 pulse	26.79	29.89	10.40	32.91
10 pulses	27.48	19.05	19.33	34.15
20 pulses	25.38	17.30	18.20	39.13
60 pulses	28.14	15.84	16.60	39.42

Conclusions

PL treatment effectively inhibited the browning of pear juice by destroying secondary and tertiary structures at the active center of peroxidase. PL treatment can be used in juice processing to prevent enzymatic browning without affecting the quality of juice.

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