2013 4th International Conference on Food Engineering and Biotechnology
IPCBEE vol.50 (2013) © (2013) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2013. V50. 12

Inactivation of Mushroom Polyphenoloxidase (PPO) by Thermosonication

Hande Baltacıoğlu[1, 2 +], Alev Bayındırlı[1] and Feride Severcan[3]

[1]Department of Food Engineering, Middle East Technical University, 06800, Ankara, Turkey

[2]Department of Food Engineering, Ondokuz Mayis University, 55139, Samsun, Turkey

[3]Department of Biological Sciences, Middle East Technical University, 06800, Ankara, Turkey

[1]**Abstract.** In this study the combined effect of heat and ultrasound (thermosonication) on the inactivation of mushroom PPO was investigated. Inactivation of mushroom PPO was performed at 100% power for different temperature (20-60 ℃) and time (0-30 min) intervals. The activity of mushroom PPO dropped slightly at 20 ℃. However, higher mushroom PPO inactivation was observed after treatments between 30 ℃ and 60 ℃ with ultrasound compared to the activity in untreated samples. Complete inactivation was achieved at 60 ℃ for 10 min during thermosonication inactivation. As a result it was reported that thermosonication treatment was effective way to inactivate the mushroom PPO enzyme at low temperatures.

Keywords: Mushroom PPO, thermosonication, inactivation

1. Introduction

Thermal treatment is the most common and widely employed technique for the inactivation of microorganisms and enzymes in the food industry. However, heat destroys nutritional components of foods and affects physical characteristics such as texture, color, and flavor. These effects have encouraged the researchers to use minimal thermal treatment methods. Non-thermal preservation technologies or the combination of these technologies with heat treatment at low temperatures have gained importance for the inactivation of microorganisms and enzymes for the productions of foods with higher quality if compared with that of thermal processing of foods at high temperatures. Methods such as high hydrostatic pressure, supercritical carbon dioxide, ultrasound, and pulsed electric field destroy microorganisms and enzymes. One "new" or emerging non-thermal technology receiving a great deal of attention is ultrasound. Ultrasound or thermosonication is reported to be effective against various food enzymes related to food quality such as lipoxygenase, peroxidase, polyphenoloxidase, lipase and protease [1].

Polyphenoloxidase (PPO) is a copper-containing enzyme which is responsible for enzymatic browning in fresh fruits and vegetables products. Enzymatic browning is the result of oxidation of *o*-diphenols into unstable quinones by PPO enzyme in the presence of molecular oxygen. *o*-Quinones are highly reactive compounds and react with other phenols and non-phenolic compounds to give brown pigments. This enzymatic browning affects color, flavor and nutritional quality of foods. Therefore, this enzyme is inactivated during food processing in order to prevent browning [2].

The inactivation of enzymes has been extensively studied over the past years and the inactivation of enzymes is generally represented with simple kinetic approaches in the literature. The purpose of this research is the investigation of changes in activity of the PPO at different operation parameters such as non-

+ Corresponding author. Tel.: + (90-312-210-5793). Fax: + (90-312-210-2767).
E-mail address: bhande@metu.edu.tr

thermal process main variable, temperature and time. Ultrasound application at low temperatures is the technique used in this study.

2. Materials and Methods

2.1. Enzyme Preparation and Activity Assay

Mushroom PPO (E.C 1.14.18.1) was purchased from Sigma. Lyophilized PPO was dissolved in 50 mM phosphate buffer (pH 6.5). To 0.3 ml of the enzyme solution, 2 ml of 50 mM potassium phosphate (pH 6.5) and 0.3 ml of 0.2 M catechol solution in the phosphate buffer was added. PPO activity was determined using a UV-VIS spectrophotometer (BOECO Model S22, Germany) at 420 nm at room temperature (25 $\pm$ 1^{o}C). Enzyme activity was calculated from the slope of the linear section of the ΔA420 curve. One unit of enzyme activity was defined as the amount of the enzyme which caused a change of 0.001 in absorbance unit per minute. Enzyme activities were measured 3 times and expressed as a relative percentage of the activity of the control [3] [4].

2.2. Thermosonication Treatment

The ultrasound application at low temperatures was performed at Non-Thermal Technology Laboratory at Food Engineering Department of Middle East Technical University, Ankara, Turkey. The ultrasonic processor (UP400S, Dr. Hielscher GmbH, Germany) with titanium alloy sonotrode (H3, Dr. Hielscher, GmbH, Germany) was used for the application of high-power ultrasonic vibration. In this study PPO solution was added to the potassium phosphate buffer (50 mM, pH 6.5) in a glass tube with an inner diameter of 16 mm and a depth of 50 mm. The tip of horn was immersed about 5 mm into 5 ml solution. The ultrasonic amplitude was chosen as 100%. Sonication was carried out in temperature controlled circulating water bath (Wise Circu WCB-6, Germany) at various temperatures ranging 20-60 ^{o}C for 0-30 min. The temperature of the solutions was recorded before and after the process. Immediately after inactivation, the tubes were removed and cooled in an ice bath and the residual enzyme activity was measured.

3. Results and Discussion

3.1. PPO Inactivation

PPO activity can be determined by measuring the rate of substrate disappearance or the rate of product formation. The product formation can be determined spectrophotometrically by measuring the optical density of the colored compounds formed from quinones. The use of spectrophotometer to follow colored compound formation from quinones is the easiest method for the measurement of the reaction rates. Catechol is the most suitable substrate for PPO [5]. 0.2 M catechol concentration in substrate solution was selected for enzyme activity determination as in indicated in the literature [4].

The residual enzyme activity of PPO after ultrasound treatment at different temperatures was represented in Figure 1. The activities of PPO dropped slightly during the period of 30 min at 20 ℃. The maximum inactivation was about 18% after 30 min treatment at 20 ℃. In fact, higher PPO inactivation was observed after treatments between 30 ℃ and 60 ℃ with ultrasound compared to the activity in untreated samples. Accordingly, the residual activity was approximately 59% after inactivation at 30 ℃ for 30 min, 46% after inactivation at 40 ℃ for 30 min and 6% after inactivation at 50 ℃ for 30 min. However, complete inactivation was achieved at 60 ℃ for 10 min.

Enzyme inactivation by ultrasound is widely reported in literature. The ultrasonic inactivation of different types of enzymes such as pectinmethylesterase, PPO, lipoxygenases and peroxidases responsible for deterioration of fruit & vegetable juice have been studied. It was reported that positive results for the inactivation of PPO in model buffer system with manothermosonication [6]. A linear decrease in log D values for an increase in ultrasound amplitude level over the range 35-145 μm was observed. They reported that heat or pressure assisted ultrasonic processing of juice can substantially reduce enzyme resistance and the heat treatment required for inactivation. The effect of thermal and thermosonic treatment on the inactivation kinetics of PPO from mushroom was studied in 55-75 ℃ temperature range. They reported that the D values varied from 57.8 $\pm$ 6.1 min to 0.88 $\pm$ 0.05 min during thermosonic inactivation at the same

temperature range. Moreover it was reported that combined effect of ultrasound and heat was found to synergistically enhance the inactivation kinetics of PPO [7].

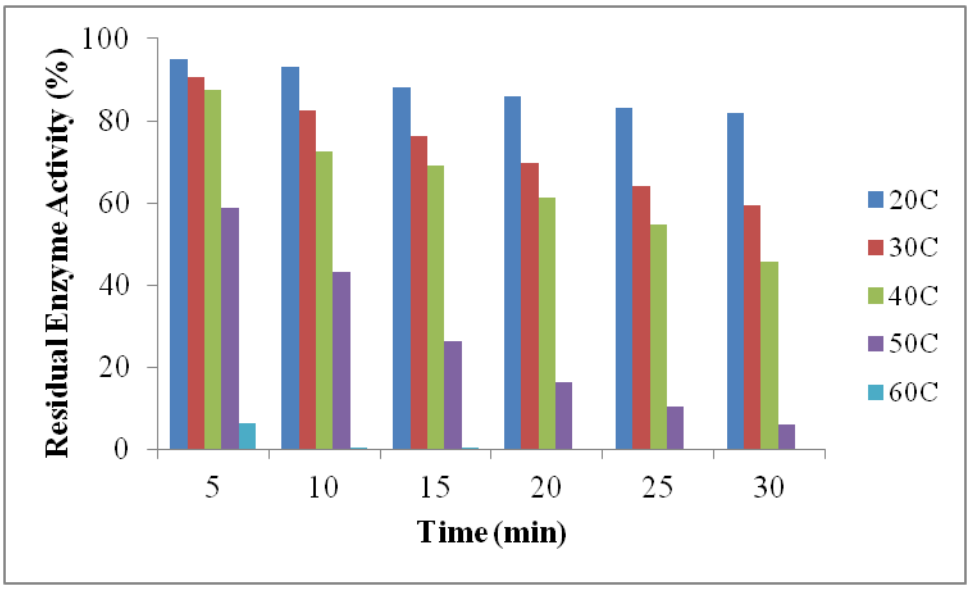

Fig. 1. Residual PPO activities after ultrasound treatment at 100% amplitude at different temperatures.

A similar result was also observed for lemon pectinesterase, known as the most heat resistance cloud destabilizing enzymes in lemon juice. The activities of PE dropped slightly during the period of 1 hour in the temperature range between 40 and 50 ℃. After heating at 50 ℃ without the ultrasound the residual activity was only 30% decreased whereas with the ultrasonic treatment for 63 min, the residual activity was 83% decreased [8]. The thermosonication treatment was also found to be better than the heat blanching process for the inactivation watercress peroxidase, known as heat resistance enzyme [9]. In another study tomato juice was subjected to thermosonication treatment and reduced PME activity by 90% at 60 ℃, 65 ℃ and 70 ℃ for 41.8, 11.7 and 4.3 min exposure, respectively [10].

4. Conclusion

The effect of thermosonication on the inactivation of mushroom PPO was studied at 100% power for different temperature and time intervals. As temperature and time increased higher inactivation rate was obtained. Moreover, our results showed that complete inactivation was achieved at 60 ℃ for 10 min during inactivation. The thermosonication treatment was found to be effective in inactivating the mushroom PPO. This method may be developed as an alternative food pasteurization method.

5. References

[1] C. P. O'Donnell, B.K. Tiwari, P. Bourke and P.J. Cullen. Effect of ultrasonic processing on food enzymes of industrial importance. *Trends Food Sci.Tech.* 2010, **21:** 358-367.

[2] L. Ludikhuyze, A. Van Loey, I.C. Smout and M. Hendrickx. Effects of Combined Pressure and Temperature on Enzymes Related to Quality of Fruits and Vegetables: From Kinetic Information to Process Engineering Aspects. *Crit. Rev. Food Sci.* 2003, **43**(5): 527–586.

[3] C. Weemaes, P. Rubens, S. De Cordt, L. Ludikhuyze, I. Van Den Broeck, M. Hendrickx, K. Heremans and P. Tobback. Temperature Sensitivity and Pressure Resistance of Mushroom Polyphenoloxidase. *J. Food Sci.* 1997, **62**(2): 261-266.

[4] N. K. Sun and K. B. Song. Effect of Nonthermal Treatment on the Molecular Properties of Mushroom Polyphenoloxidase. *J. Food Sci.* 2003, **68**(5): 1639-1643.

[5] C. Queiroz, M. L. Mendes Lopes, E. Fialho and V. L. Valente-Mesquita. Polyphenol Oxidase: Characteristics and Mechanisms of Browning Control. *Food Rev. Int.* 2008, **24**:361–375.

[6] P. Lopez, F. J. Sala, J. L. Fuente, S. Condon, J. Raso and J. Burgos. Inactivation of peroxidase, lipoxygenase and polyphenol oxidase by manothermosonication. *J. Agric. Food Chem.* 1994, **42**(2), 253–256.

[7] X.-f. Cheng, M. Zhang and B. Adhikari. The inactivation kinetics of polyphenol oxidase in mushroom (*Agaricus bisporus*) during thermal and thermosonic treatments. *Ultrason. Sonochem.* 2013, **20**:674-679.

[8] J. Kuldiloke, M. Eshtiaghi, M. Zenker and D. Knorr. Inactivation of Lemon Pectinesterase by Thermosonication. *Int. J. Food Eng.* 2007, **3**(2): Art. 3.

[9] R. M. S. Cruz, M. C. Vieira and C. L. M. Silva. Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (*Nasturtium officinale*). *J. Food Eng.* 2006, **72**(1), 8–15.

[10] J. Wu, T.V. Gamage, K.S. Vilkhu, L.K. Simons and R. Mawson. Effect of thermosonication on quality improvement of tomato juice. *Innov. Food Sci. Emerg.* 2008, **9**: 186–195.