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Secondary structure and conformational change of mushroom polyphenol oxidase during thermosonication treatment by using FTIR spectroscopy

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ABSTRACT

To understand the conformational changes of mushroom PPO, the secondary structural change of the enzyme during thermosonication treatment at different power (60, 80 and 100%), temperature (20–60 °C) and time (0–30 min) combinations was investigated by using FTIR spectroscopy and compared with the change in enzyme activity. The enzyme inactivation higher than 99% was obtained at 100% amplitude at 60 °C for 10 min. FTIR studies showed that marked spectral changes were noted after ultrasound treatment at 20 °C. The α-helix and β-sheet contents decreased, while aggregated β-sheet, turns and random coil contents increased as temperature increased up to 60 °C during thermosonication treatment for 10 min indicating protein denaturation. Aggregated bands located at 1683 and 1616 cm^{-1} became evident after ultrasound treatment at 40 °C. When temperature was lowered back to 25 °C, from ultrasound treatment at 60 °C, these bands were still observed, indicating the irreversible change in the structure.

1. Introduction

Non-thermal preservation technologies have been used as alternative processing option to conventional thermal treatment. Ultrasound is the one of the non-thermal technology which has attracted attention in the food industry in the last few decades. Ultrasound has gained importance for the inactivation of microorganisms and enzymes at mild temperature conditions to produce higher quality foods with very similar to the natural flavor and taste. A combination of heat and ultrasound (thermosonication) is reported to enhance the inactivation rates of various food enzymes related to food quality (O'Donnell, Tiwari, Bourke, & Cullen, 2010; Terefe, Buckow, & Versteeg, 2015).

Polyphenol oxidase (PPO), a food quality related enzyme, catalyzes the oxidation of phenolic constituents in the presence of oxygen that causes enzymatic browning in fresh fruit and vegetables products during processing. This enzymatic browning leads to deterioration of color, flavor and nutritional quality in food products. In order to prevent the enzymatic browning, this enzyme should be inactivated during processing of foods. Although, a number of studies have been reported on the effects of ultrasound processing on food quality-related enzymes (Cruz, Vieira, & Silva, 2006; De Gennaro, Cavella, Romano, & Masi, 1999; Wu, Gamage, Vilkhu, Simons, & Mawson, 2008), ultrasound application is very limited on PPO (Cheng, Soh, Liew, & Teh, 2007; Cheng, Zhang, & Adhikari, 2013; Lopez et al., 1994). In these studies, the inactivation of enzymes generally represented with simple kinetic approaches. By contrast, there is relatively little information on the mechanisms and conformation changes of food enzymes during ultrasound treatment.

There are several analytical tools to study protein conformation. Among them, Fourier transform infrared spectroscopy (FTIR) is a versatile tool to study protein conformation. This technique is rapid, non-destructive and easy to perform. It gives high-quality spectra with very small amount of protein. It is especially useful for the analysis of secondary structural characterization of proteins because characteristic protein bands (e.g. the amide I region) in an IR spectrum are very sensitive to conformational changes in the protein (Haris & Severcan, 1999). It is possible to define secondary structure of proteins in solution (Goormaghtigh, Gasper, Bénard, Goldsztein, & Raussens, 2009; Severcan, Haris, & Severcan, 2004; Severcan, Severcan, & Haris, 2001) and in biological systems with FTIR spectroscopy (Cakmak, Zorlu, Severcan, & Severcan, 2011; Garip, Yapici, Simsek Ozek, Severcan, & Severcan, 2010; Turker, Ilbay, Severcan, & Severcan, 2014). Furthermore, it is possible to study morphological and molecular patterns and to distinguish

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different tissue types with their chemical ingredients such as lipids, phospholipids, proteins, carbohydrates and nucleic acids by using FTIR spectroscopic imaging (Kucuk Baloglu, Garip, Heise, Brockmann, & Severcan, 2015; Pallua et al., 2012).

In the literature, there are very few studies about the structural and conformational changes of PPO during food processing such as thermal and ultrasound treatment. Recently, Yu, Zeng, and Lu (2013) investigated the effects of ultrasound on both monophenolase and diphenolase activities of tyrosinase with the determination by ultraviolet–visible spectrometry, FTIR spectroscopy and atomic force microscopy (AFM). Tyrosinase was inactivated in the ultrasonic bath for 2.5 h. It was suggested that both monophenolase and diphenolase activities of tyrosinase could be activated by ultrasound and β-sheet conformation was predominant in the tyrosinase under ultrasound treatment. The effect of ultrasound on the activity and conformation of α-amylase, papain and pepsin was investigated and it was reported that secondary structural components, especially β-sheet, of these three enzymes were significantly influenced by ultrasound (Yu, Zeng, Zhang, Liao, & Shi, 2014).

In our previous study, conformational and secondary structural changes of PPO were analyzed by using FTIR spectroscopy during thermal treatment in the temperature range 25–80 °C (Baltacıoğlu, Bayındırlı, Severcan, & Severcan, 2015). FTIR spectra revealed dramatic changes in secondary structure elements after 40 °C with the occurrence of new bands due to aggregated β-sheet structures which indicated protein denaturation. In addition an irreversible change in the structure was reported after thermal treatment. Moreover, detail analysis of secondary structural components by using neural network (NN) and curve-fitting analysis based on amide I band (1700–1600 cm^{-1}) was investigated for the first time. According to the study, significant decrease in the α-helix and β-sheet contents, and significant increase in the aggregated β-sheet, turns and random coil contents were reported as the temperature increased (Baltacıoğlu et al., 2015).

The purpose of the current study was to investigate the effect of thermosonication treatment not only on the changes in the activity of PPO but also on the structural changes of the enzyme. In order to understand the conformational changes of PPO after thermosonication treatment, secondary structural content of the enzyme in solution was determined using FTIR spectroscopy. To achieve quantitative data, curve-fitting analysis based on amide I band (1700–1600 cm^{-1}) was used. To gain a better insight of the interaction of ultrasound with enzymes, the inactivation mechanism of a model enzyme, mushroom PPO, during thermosonication treatment was monitored. Thereby, this work will provide insight for researchers to predict the behavior of enzyme inactivation in a real food system.

2. Materials and methods

2.1. Chemicals

Mushroom PPO (E.C 1.14.18.1), catechol (⩾99%purity), D_2O (99.9 atom% D) were purchased from Sigma (St. Louis, MO, USA). The enzyme was used without further purification. All chemicals were obtained from commercial sources at the highest grade of purity available.

2.2. Enzyme preparation and activity assay

Lyophilized PPO was dissolved in 50 mM phosphate buffer (pH 6.5). Catechol was used as substrate for the determination of the enzyme activity. 2 ml of 50 mM potassium phosphate buffer (pH 6.5) and 0.3 ml of 0.2 M catechol solution in the phosphate buffer were incubated in a test tube at 25 ± 1 °C. Then the tube content was transferred to a plastic cuvette (path length, 10 mm) and 0.3 ml of the enzyme solution (0.08 mg/ml) was added to the cuvette to initiate the enzyme reaction. PPO activity was determined using a UV–vis spectrophotometer (BOECO Model S22, Germany) at 420 nm at room temperature (25 ± 1 °C). Absorbance was read every 5 s for 3 min. Enzyme activity was calculated from the slope of the initial linear section of the absorbance versus time curves. 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 residual activity (Bayındırlı, Alpas, Bozoglu, & Hizal, 2006; Sun & Song, 2003; Weemaes et al., 1997).

2.3. Thermosonication treatment

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 60, 80 and 100% (125, 170 and 210 μm). Sonication was carried out in temperature controlled dry block heater (HBR-48, DaihanScientific Co. Ltd., Seoul, Korea) at various temperatures ranging 20–60 °C with a 10 °C increment for 0, 5, 10, 15, 20, 25 and 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 (Kadkhodaee & Povey, 2008).

2.4. FTIR spectroscopy

Infrared spectra were collected using Perkin-Elmer Spectrum 100 FTIR spectrometer (Perkin-Elmer Inc., Norwalk, CT, USA) equipped with a MIR TGS detector. The sample compartment was continuously purged with dry air to minimize atmospheric water vapor absorbance, which overlaps in the spectral region of interest, and carbon dioxide interference. The spectrum of air was recorded as background and subtracted automatically by using appropriate software.

For inactivation studies, measurements were performed in D_2O buffer. Prior to infrared experiments, the enzyme was dissolved in 50 mM phosphate buffer (prepared with D_2O) to yield a final protein concentration of 70 mg/ml for FTIR measurements. Enzyme solution was allowed to stand 24 h prior to measurement to allow H-D exchange. Enzyme solution was put 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% (210 μm). Sonication was carried out in temperature controlled dry block heater (HBR-48, Daihan Scientific Co. Ltd., Seoul, Korea) at various temperatures ranging 20–60 °C with a 10 °C increment for 10 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 10 μl of sample was put between CaF_2 windows having a 50 μm path length and then windows were placed into the temperature controller unit. Each spectrum of enzyme solutions and buffer were collected in the 1400–2200 cm^{-1} region. A total of 128 scans were taken for each interferrogram at 2 cm^{-1} resolution.

2.5. Data analysis for FTIR spectra

Collections of spectra and data manipulations were carried out using Spectrum 100 software (Perkin-Elmer). For protein secondary structure determination, OPUSNT data collection