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The role of polyphenol oxidase and peroxidase in the browning of water caltrop pericarp during heat treatment

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ABSTRACT

The mechanism of browning involving enzymatic browning was investigated in the pericarp of water caltrop, an Asian vegetable popular for its taste and medicinal properties. Polyphenol oxidase (PPO) and peroxidase (POD) activities were determined in pericarp at various times and temperatures. Water caltrop consisted of 44.22% moisture content, 37.23% crude fibre, and 2.63% crude protein. PPO and POD activities dropped from 62 and 38 units/g sample, respectively, as water temperature was increased from 30 to 80 °C. Optimum pH and temperature for PPO activity was at pH 5.0, 25–45 °C, and POD activity peaked at 60 °C. High PPO and POD activities at 40–50 °C resulted in degradation of phenolic compounds, which led to increased aggregation of browning pigments and discolouration (lower *L*-values) of the pericarp. Enzymatic browning was determined as the major factor in the browning discolouration of heat-treated water caltrop pericarp.

1. Introduction

Browning of fruits and vegetables is a major problem in the food industry and is considered to be one of the main causes of quality loss during post harvest handling and processing. Browning is reportedly the result of pigment degradation and/or aggregation caused by enzymatic or non-enzymatic browning and it represents an interesting research area due to its influence on food appearance, nutrition, and health (Dogan & Dogan, 2004; Lee & Lee, 1997; Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001). In plant tissues enzymatic browning, induced by mechanical damage, is generally attributed to polyphenol oxidases (PPO) activity on phenolic compounds, which causes oxidation resulting in a brown colour in fruits, such as apples (Murata, Tsurutani, Tomita, Homma, & Kaneko, 1995), bananas (Yang, Fujita, Ashrafuzzaman, Nakamura, & Hayashi, 2000), peaches (Flurkey & Jen, 1980), grapes (Lamikanra, Sharon, & Mitwe, 1992), and raspberries (Gonzalez, DeAncos, & Cano, 1999). PPO exists widely in nature and is responsible for enzymatic browning reactions that occur during the handling, storage, and processing of fruits and vegetables (Dincer, Colak, Aydin, Kadioglu, & Guner, 2002). In an oxygen environment, polyphenol from fruits and vegetables is transformed into benzoquinone by PPO (Macheix, Fleuriet, & Billot, 1990). Further condensation of quinines leads to brown melanin pigments (Martinez & Whitaker, 1995).

Peroxidase (POD) is a similar oxidative enzyme that exists in the pericarp of fruits such as litchi (Gong & Tian, 2002; Underhill & Critchley, 1995). Researchers have reported that increases in POD activity enhances enzymatic browning during storage (Underhill & Critchley, 1995; Zhang, Pang, Xuewu, Ji, & Jiang, 2005). Postharvest browning of fruit is generally thought to be a rapid degradation of anthocyanins caused by PPO and POD, producing brown by-products (Jiang, Zauberman, & Fuchs, 1997; Zhang et al., 2005). Since enzymatic browning reaction of the products usually results in a negative influence on food quality, preventing or inhibiting enzymatic browning has become a significant tool for improving food quality during food processing.

Water caltrop belongs to the family of *Trapaceae* and is a free-floating plant grown in shallow water fields, ponds or swampy lands in tropical and sub-tropical Asian countries. Mature water caltrops are 3–5 cm wide and 5–6 cm long, with one pair of spines in the shoulder and/or one pair of short spines in the abdomen. The outer pericarp is hard, making it difficult to peel off to obtain the white edible fruit inside (Tulyathan, Boondee, & Mahawanich, 2005). The browning of water caltrop pericarp frequently occurs from either exposure to high temperatures during postharvest storage or heat treatment. The effects of increased temperatures

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on the activities of PPO and POD play a key role in the enzymatic browning of processed fruits. The objective of this study was to investigate the effect of heat treatments on the enzymatic and non-enzymatic browning of water caltrop pericarp. Furthermore, changes in PPO and POD activities, total phenolic content, and enzymatic browning index were studied, in order to investigate the browning reaction mechanism in water caltrop pericarp during heat treatment.

2. Material and methods

2.1. Materials

Water caltrops (*Trapa taiwanensis* Nakai) were obtained from a local farm in Tainan, southern Taiwan. After harvesting, water caltrops were soaked in cold water at 10 °C; immediately peeled, and the pericarps were collected and stored at at 4 °C. One batch of fresh water caltrop pericarps was balanced at ambient temperature for 1 h and used as the control set. The rest of the pericarp samples was divided into 48 heat treatment sets and heated in a water bath at 30, 40, 50, 60, 70, 80, 90, and 100 °C, respectively, for 10, 20, 30, 40, 50, and 60 min, respectively.

2.2. Proximate composition

Analyses for moisture (Method 934.01), protein (Method 984.13), lipid (Method 954.02), fibre (Method 962.09) and ash content (Method 942.05) of water caltrop pericarp were conducted according to the methods of AOAC (1998).

2.3. Effect of pH on PPO and POD enzyme activities

The determination of PPO and POD activities at various pH values was conducted according to the methods of Lin et al. (1988) and Aquino-Bolanos and Mercado-Silva (2004). Universal buffers of various pH values (3.0, 5.0, 7.0, 9.0, and 11.0) were prepared from 0.1 M Citric acid-KH_2PO_4 buffer. The universal buffers replaced the citric acid buffer to determine the changes in PPO and POD activity at the selected pH values.

2.4. Assay of polyphenol oxidase (PPO) activity

Fresh and hot-water-treated caltrop pericarps (100 g) were homogenised for 3 min in 500 mL of 0.05 M phosphate buffer (pH 7.0, 4 °C). The homogenates were immediately centrifuged at 15,000×*g* for 20 min at 4 °C. The supernatants were collected and used as enzymatic extracts of water caltrop pericarp throughout this experiment. A spectrophotometric technique, adopted from Aquino-Bolanos and Mercado-Silva (2004), was used to measure PPO activity. Aliquots containing 285 mL of 0.2 mM phosphate buffer, 50 μL of catechol (0.6 M) as a substrate;and 100 μL of enzymatic extract were measured at 420 nm continuously at 25 °C/min. One unit of PPO activity was defined as a change in absorbance of 0.001 min^{-1}.

2.5. Assay of peroxidase (POD) activity

Fresh and hot-water-treated water caltrop pericarps (60 g) were homogenised in a domestic blender in 300 mL of 0.05 M phosphate buffer (pH 7.0, 4 °C) for 3 min. The homogenates were immediately centrifuged at 10,000×*g* for 20 min at 4 °C and the supernatant was used as enzyme extracts. POD activity, with guaiacol as a substrate, was assayed by using a reaction mixture (3 mL) containing 25 μL enzyme extract, 2.73 mL 0.05 M phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2, and 0.15 mL of 4% guaiacol (Lin et al., 1988). An increase in absorbance at 470 nm, due to the guaiacol oxidation, was recorded for 2 min. One unit of POD activity was defined as a change in absorbance of 0.001 $min^{-}1$.

2.6. Assay of total phenolics

Quantification of total phenolics was carried out using the method proposed by Sato et al. (1996). Five grams of finely diced water caltrop pericarps were homogenised in 20 mL of 80% ethanol for 1 min. The homogenate was then filtered through two layers of cheese cloth, and then the filtrate was centrifuged at 10,000×*g* for 15 min. One milllitre of the supernatant liquid was mixed with 1 mL of Folin Ciocalteu reagent and 10 mL of sodium carbonate (7% Na_2CO_3). The total volume was brought up to 25 mL using distiled water and left to settle for 1 h. The absorbance was then read at 735 nm. The standard curve of gallic acid (0–0.12 g/L) was used for quantification.

2.7. Colour analysis

The CIE-LAB system of colour values *L* (lightness), *a*, (redness–greenness), and *b* (yellowness–blueness) of fresh and hot-water treated samples were recorded using a Colour Quest II – Sphere Colorimeter (Hunter Lab Rston, Va). Chroma values were calculated using the following equation;

$$\text{chroma(C)} = (a^2 + b^2)^{1/2}$$

2.8. Statistical analysis

The data were subjected to an analysis of variance (ANOVA) and where *F*-values were deemed to be significant, the mean value for each parameter was compared using the least significant differences (LSD) procedure as specified by the Statistical Analysis System (SAS, 1988).

3. Results and discussion

3.1. Proximate composition

The moisture content and crude fibre of water caltrop pericarp was recorded at 44.22% and 37.23%, respectively, of the total sample weight. Water caltrop contained 4.265% crude lipid, 2.63% crude protein, and 0.75% ash.

3.2. Effect of pH-value on PPO and POD activity

Enzyme activities of PPO and POD were measured at different pH levels ranging from pH 3.0–11.0, based on the substrates catechol and guaiacol. One hundred percentage relative activity of enzyme was calculated within the pH range of 3.0–11.0. As illustrated in Fig. 1, the optimum pH for enzyme activity was found to be pH 5.0 for both PPO and POD, based on their reactions with catechol and guaiacol, respectively. As pH values increased to 9.0, relative enzyme activity compared to enzyme activity at pH 5.0 decreased to 23% for POD, but remained at 65% for PPO. When the pH value of the buffer was increased to 11.0, the relative enzyme activities of PPO and POD decreased to 41% and 23%, respectively. Optimum pH levels for PPO obtained from various sources are reportedly different; for example, optimum pH values are 5.5 for strawberry (Wesche-Ebeling & Montgomery, 1990) and 6.0 for grape (Lee, Smith, & Pennesi, 1983). The findings of our study indicated that the optimum pH values for both PPO and POD of water caltrop pericarps were close to pH 5.0.