Food Chemistry 190 (2016) 922–927

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Inhibitory effect of rice bran extracts and its phenolic compounds on polyphenol oxidase activity and browning in potato and apple puree

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ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form 2 June 2015
Accepted 4 June 2015
Available online 6 June 2015

Keywords:
Rice bran
Browning
Polyphenol oxidase
Potato
Apple

ABSTRACT

Full-fatted and commercially defatted rice bran extracts (RBE and CDRBE) were evaluated for their ability to inhibit enzymatic browning in potato and apple. RBE showed more effective inhibition of polyphenol oxidase (PPO) activity and browning in potato and apple as compared to CDRBE. Five phenolic compounds in RBE and CDRBE (protocatechuic acid, vanillic acid, *p*-coumaric acid, ferulic acid and sinapic acid) were identified by HPLC. They were then evaluated for their important role in the inhibition using a model system which found that ferulic acid in RBE and *p*-coumaric acid in CDRBE were active in enzymatic browning inhibition of potato and apple. *p*-Coumaric acid exhibited the highest inhibitory effect on potato and apple PPO ($p \leqslant 0.05$). Almost all phenolic compounds showed higher inhibitory effect on potato and apple PPO than 100 ppm citric acid.

1. Introduction

The browning reaction is recognized as a main problem of fruit and vegetable processing and is mainly due to polyphenol oxidase (PPO: EC 1.14.18.1) which catalyzes the oxidation of phenolic compounds into dark colored pigments. In potato, the browning develops during the peeling and the subsequent storage of fresh peeled tuber (Kaaber, Martinsen, Bråthen, & Shomer, 2002) and this reaction also leads to development of off-flavors and nutritional quality losses in potatoes (Severini, Baiano, De Pilli, Romaniello, & Derossi, 2003). The browning reaction also limits the commercial shelf life of apple products (Kim et al., 2001). Several chemical browning inhibitors have been assessed for their inhibition of PPO activity in fruits and vegetables; sulfites are among the most effective browning inhibitors (Chen, Mehta, Berenbaum, Zangerl, & Engeseth, 2000). However, the use of sulfites for this purpose has been restricted by the U.S. Food and Drug Administration due to adverse health effects (Coetzer, Corsini, Love, Pavek, & Tumer, 2001). In recent years, there has been increasing interest in finding natural antibrowning agents. Examples of alternative natural PPO inhibitors investigated by several researchers are honey (Chen et al., 2000), pineapple juice (Chaisakdanugull, Theerakulkait, & Wrolstad, 2007), onion (Kim, Kim, & Park, 2005), and green tea (Soysal, 2009).

Rice (*Oryza sativa* L.) is one of the most staple diet items for humans, especially in Asian countries (Sereewatthanawut et al., 2008). Rice bran, a by-product of the rice milling process, is a good source of phytochemicals and antioxidative compounds such as oryzanols, tocopherols, phytosterols, tocotrienols, squalene, polycosanols, phytic acid, ferulic acid, inositol, hexaphosphate (Chotimarkorn, Benjakul, & Silalai, 2008). Commercially defatted bran of rice, the predominant by-product of rice bran oil extraction, is also a good source of phytic acid, inositol and vitamin B (Devi, Jayalekshmy, & Arumughan, 2007). The heat treatment during the commercial extraction of oil from rice bran reduces the phenolic compound content of commercially defatted rice bran. Additionally, the high temperature of extraction promotes the degradation of some phenolic compounds (Liazid, Palma, Brigui, & Barroso, 2007). Commercially defatted rice bran was also reported to have antioxidative compounds such as oryzanols, tocols, and ferulic acid (Devi et al., 2007; Wiboonsirikul et al., 2008). Previously, we have shown that rice bran extract and commercially defatted rice bran extract could inhibit enzymatic browning in potato (Boonsiripiphat & Theerakulkait, 2009; Kaewka, Portongkum, & Theerakulkait, 2009; Sukhonthara & Theerakulkait, 2012). Rice bran and commercially defatted rice bran are cheap, valuable by-products containing high concentrations of nutritional components with a longer shelf-life than vegetable products. They also provide an opportunity for the utilization as a supplement in food products to enhance their nutritional value.

However, the effect of full-fatted and commercially defatted rice bran extracts and their phenolic compounds on enzymatic browning of potato and apple puree has not been previously studied. Therefore, the main objective of this research was to

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http://dx.doi.org/10.1016/j.foodchem.2015.06.016
0308-8146/

investigate the inhibitory effect of full-fatted and commercially defatted rice bran extracts and their phenolic compounds on PPO activity and browning in potato and apple puree.

2. Materials and methods

2.1. Materials and chemicals

The full-fatted rice bran from milling of rice cultivars, namely *Oryza sativa* L. CV. Khao Dawk Mali-105, and its commercially defatted rice bran was obtained from Patum Rice Mill and Granary Public Co. Ltd., Thailand. Potatoes (*Solanum tuberosum L.*) and 'Fuji' apple (*Malus domestica*, Borkh.) were purchased from a local market in Bangkok, Thailand. Catechol, ascorbic acid, citric acid, sodium metabisulfite (SMS) and protocatechuic acid were obtained from Sigma Chemical Co. Ltd. (St Louis. MO, USA). Vanillic acid, *p*-coumaric acid, ferulic acid and sinapic acid were HPLC grade obtained from Fluka Chemika, USA.

2.2. Preparation of rice bran extracts

Full-fatted or commercially defatted rice bran (100 g) was milled and screened past a 50 mesh sieve, homogenized with distilled water (300 mL for full-fatted rice bran, 400 mL for commercially defatted rice bran) by using an overhead stirrer at 700 rpm at 40 °C for 30 min. The homogenate was then centrifuged at 8000×*g* for 20 min at 20 °C. The supernatant obtained after centrifugation was filtered through a cheese cloth and was defined as full-fatted rice bran extract (RBE) and commercially defatted rice bran extract (CDRBE). RBE and CDRBE were evaluated for inhibitory effect on browning of potato and apple puree and PPO activity as described below.

2.3. PPO activity inhibition study

2.3.1. PPO preparation

Fifty grams of potato or apple were homogenized for 30 s with 50 ml of a cold 0.1 M sodium phosphate buffer (pH 7.0, 4 °C) containing 10.0 g/L polyvinylpyrrolidone and 5.0 g/L Triton X-100. The homogenates were centrifuged for 30 min (4 °C) at 8000×*g* (Sorvall RC 5 C Refrigerated Centrifuge, Du Pont, Newtown, CT). The supernatant was collected and stored at −30 °C for use as crude PPO enzyme.

2.3.2. PPO activity inhibition

Potato or apple PPO activity was determined by measuring the increase in absorbance at 420 nm with a spectrophotometer (Cecil instrument model CE 292, Cambridge, UK) at 25 °C. 0.1 mL of the enzyme was added to the mixture containing 0.9 mL of 0.05 M sodium phosphate buffer at pH 7.0, 1.0 mL of 0.2 M catechol substrate solution in 0.05 M sodium phosphate buffer at pH 7.0, and 1.0 mL of RBE or CDRBE as an inhibitor. The change in absorbance at 420 nm was recorded for 1 min. As a control, 1.0 mL of distilled water was mixed with the same substrate solution before adding the enzyme. One unit of enzyme activity was defined as the amount of enzyme responsible for a change of 1 absorbance unit at 420 nm/min at 25 °C and pH 7.0. The percent inhibition was calculated as follows (Chaisakdanugull et al., 2007):

$$\%\ \text{PPO inhibition} = [(\text{activity of control} - \text{activity of treatment})/\text{activity of control}] \times 100.$$

All determinations were performed in triplicate.

2.4. Determination of browning in potato and apple Puree

Potatoes or apple were peeled and blended with RBE, CDRBE or distilled water (DW, control) at 1:1 (w/w) ratio for 20 s at room temperature. The color L^*, a^*, and b^* values of the samples were measured with a Spectrophotometer (CM-3500D, Minolta, Osaka, Japan) at 0, 1, 2, 3, 4, 5 and 6 h after storage at room temperature.

2.5. HPLC analysis

2.5.1. Sample preparation

Rice bran extract samples were centrifuged at 10,000×*g* (20 °C) for 20 min. The supernatant was filtered through a 0.45 μm membrane filter (Ø 47 mm, Vertipore, Bangkok, Thailand), and the permeate was then further subjected to solid-phase extraction (SPE) using a Sep-Pac® Vac 3 mL (500 mg) C_{18} cartridge (Waters, Milford, MA, USA) following the modified method of Tian, Nakamura, Cui, and Kayahara (2005); first the cartridge was preconditioned by sequentially passing 3 mL of methanol and 6 mL of acidified water (pH 2.72 with 1% acetic acid). Then, 1.5 mL of rice bran extract sample was loaded. The cartridge was washed first with 3 mL of acidic water–methanol (9:1, v/v) and then the phenolic compounds were eluted with 1.5 mL of acidic water–methanol (1:9, v/v). The latter fraction was collected and filtered through a 0.45 μm membrane filter before analysis by HPLC.

2.5.2. HPLC analysis

The HPLC system consisted of an Agilent 1100 Series high-performance liquid chromatograph equipped with a diode array detector. The column used was SB-C_{18} column (4.6 mm × 100 mm; Agilent, Santa Clara, CA, USA). Isocratic elution with the solvent system, 1% acetic acid (v/v): acetonitrile (91:9, v/v) was used at a flow rate of 1 mL/min. Protocatechuic acid and vanillic acid were monitored at 280 nm, and *p*-coumaric acid, ferulic acid and sinapic acid were monitored at 325 nm. The phenolic compounds were identified by matching the retention time and UV absorption spectrum against authentic standards of protocatechuic acid, vanillic acid, *p*-coumaric acid, ferulic acid and sinapic acid. The standard curve for quantitative analysis were generated by plotting each authentic standard with five dilutions ranging from 4 to 20 mg/mL.

2.6. Effect of phenolic compounds from rice bran extracts on potato and apple PPO inhibition

The phenolic compounds in RBE or CDRBE (protocatechuic acid, vanillic acid, *p*-coumaric acid, ferulic acid and sinapic acid) were prepared in model system experiments at the same concentration of RBE and CDRBE from HPLC analysis results. The phenolic compounds were evaluated for their inhibitory effect on potato and apple PPO as described in the method 2.4.

2.7. Statistical analysis

Statistical analyses were carried out by using SPSS version 12.0 (SPSS Inc., Chicago, Illinois, USA). Statistically significant differences of data were assessed using one-way analysis of variance. Significant differences ($p \leqslant 0.05$) among various treatments were detected by using Duncan's multiple range tests.

3. Results and discussion

3.1. Effectiveness of rice bran extract in enzymatic browning inhibition of potato and apple puree

The L^*, a^* and b^* values of potato and apple puree blended with RBE, CDRBE and DW after storage at 25 °C for 1–6 h are shown in