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Evaluation of the inhibitory effect of pistachio (*Pistacia vera* L.) green hull aqueous extract on mushroom tyrosinase activity and its application as a button mushroom postharvest anti-browning agent

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ABSTRACT

The short shelf life of button mushroom is a serious problem in its postharvest distribution. One approach is the application of plant extract (mainly polyphenolic compounds) to inhibit tyrosinase activity. The inhibitory activity of pistachio green hull extract (PGHE), sodium metabisulfite (MET), and ascorbic-citric acids (ASC-CIT) on mushroom tyrosinase was investigated at different concentrations (0.05–0.8% w/v) and IC_{50} value was determined (0.07%). Prevention of button mushroom postharvest browning was investigated by vacuum impregnation pretreatment with PGHE, MET, and ASC-CIT. Physicochemical, organoleptic, and microbial properties of the treated mushrooms were determined over 10 d of storage (at 4 °C). The results of the browning index (BI) showed that the PGHE-treated mushrooms had lower BI than the other samples. On the 10th d, only the mushrooms treated with PGHE had highest phenolic content (10.4%). The antioxidant activity of PGHE-treated mushrooms was higher than the control (4.5%). The mushrooms treated by PGHE had 13.9% higher firmness than the control. The sensory evaluation results showed that the samples treated with PGHE had the highest sensory scores. Also, the main components of PGH extract determined by RP-HPLC-DAD were phloroglucinol, gallic acid, naringin, vanillic acid, catechin, and protocatechuic acid. The inhibitory activity of two main components, pure phloroglucinol, gallic acid and the mixture of them, on tyrosinase were 43.6, 24.4 and 42.5%, respectively. It could be concluded that these compounds have a synergistic effect. It can be concluded that pistachio green hull extract is a new natural tyrosinase inhibitor that can delay browning reactions in mushrooms.

1. Introduction

The short shelf life of mushrooms is a main problem in their postharvest distribution. The main reasons of their limited shelf life include loss of water, high metabolic activity and respiration. Therefore, mushrooms loss their commercial value, in a short time of storage (Khan et al., 2014; Fernandes et al., 2012). The quality of white button mushrooms is determined by their color, cleanliness, maturity, texture, flavor, flush number, and cap opening. Color is a very important parameter and is very effective on customer satisfaction, and mushrooms browning reduce their marketable value (Weijn et al., 2011).

Tyrosinase (EC 1.14.18.1) is a copper-containing enzyme found in animals and plants (Deng et al., 2016). In button mushrooms, tyrosinase plays the main role in the enzymatic browning reaction. Various factors such as concentrations of active polyphenol oxidase (PPO) and phenolic compounds, the oxygen availability of the tissue, pH, and temperature affect the rate of enzymatic browning (Loizzo et al., 2012; Singh et al., 2010).

Chemical treatments, refrigeration, washing (with anti-microbial and anti-browning compounds), coating, modified/controlled atmosphere packaging, use of humectants, use of tyrosinase inhibitors, and ozone treatment are reported for increasing shelf life and market value of mushrooms (Khan et al., 2014; Lagnika et al., 2013; Fernandes et al., 2012; Cliffe-Byrnes and O'Beirne, 2008). Some anti-browning compounds that can be applied to mushrooms are sodium metabisulfite (Brennan et al., 1999), citric acid or hydrogen peroxide (Brennan et al., 2000); however, they can have negative health effects (Bernaś and Jaworska, 2015).

In order to use anti-browning compounds in processing of fruits and vegetables, there are various methods such as vacuum impregnation (VI). Vacuum impregnation is a suitable way for transferring the compounds from an external solution to the plant tissue. This method

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improves the product quality (Lima et al., 2016; Neri et al., 2016).

Use of tyrosinase inhibitors is one of approaches to extend mushrooms` shelf life (Singh et al., 2010; Gao et al., 2014). Polyphenols are one of the tyrosinase inhibitors; hence, plant extracts with high total phenolics have a higher possibility of tyrosinase inhibitors (Mazlan et al., 2013).

Pistachio green hull (PGH) is an abundant agricultural waste in Iran. Iran is among the main producers and exporters of pistachio in the world. Pistachio production of Iran is about 261,000 t $year^{-1}$ (Ahmadi et al., 2016). It is estimated that 35–45 % of the whole fruit is green hull (Barreca et al., 2016). Therefore, pistachio green hull is a cheap and abundant natural source of useful compounds ($> 104.0 \times 10^6$ kg) in Iran that can be used for other useful purposes. Pistachio green hull extract (PGHE) has a high quantity of phenolic compounds and antioxidant capacity, suggesting that it could be a cost effective source of bioactive compounds with health protective potential (Grace et al., 2016; Kilic et al., 2016; Rajaei et al., 2010; Goli et al., 2005). Goli et al. (2005) reported that aqueous and methanolic extracts of pistachio green hull were rich in phenolics compounds and effectively retarded the oxidation of soybean oil with efficiency comparable to the synthetic antioxidant butylated hydroxyl anisole. The growth of Gram positive bacteria may be inhibited by pistachio green hull aqueous extracts (Rajaei et al., 2010). Abolhasani et al. (2018) reported the anti-tyrosinase activity of irradiated (30 kGy) and non-irradiated water extracts of pistachio green hull that it could prevent enzymatic browning in sliced raw potatoes. Additionally, phytochemical analysis of pistachio green hull was carried out by Grace et al. (2016) and the presence of anacardic acids (31.98 g kg^{-1}), fatty acids (15.00 g kg^{-1}), and phytosterols (19.20 g kg^{-1}) as major components were reported. The main phenolic compounds of pistachio green hull (*Pistacia vera* L., variety Bronte) were reported as follows: gallic acid, 4-hydroxybenzoic acid, protocatechuic acid, naringin, eriodictyol-7-O-glucoside, isorhamnetin-7-O-glucoside, quercetin-3-O-rutinoside, isorhamnetin-3-O-glucoside and catechin (Barreca et al., 2016). Garavand et al. (2017) investigated the phytochemicals content and radical scavenging activity of pistachio green hull extract obtained by different solvents and ultrasound-assisted aqueous extraction was more efficient. Also, increased amounts of vanillic acid, *p*-coumaric acid, naringenin and catechin in ultrasound-assisted extracts (in comparison with control that no radiation was applied) have been determined by HPLC/MS. It seems that pistachio's variety, climate, and the geographical origin are effective factors on the phenolic compounds and their quantities in pistachio green hull. Due to the adverse effect of synthetic preservatives on health and demands of consumers for natural products, use of natural compounds as a replacer to chemical preservatives in foods have been increased (Gao et al., 2014; Brennan and Gormley, 1998).

As mentioned above, plant extracts with high total phenolics have an anti-tyrosinase activity, therefore, the goal of the present research was to assay the inhibitory effect of pistachio green hull extract on tyrosinase activity and to inhibit the button mushrooms postharvest browning by vacuum impregnation pretreatment with pistachio green hull aqueous extract as a natural preservative.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Mushroom tyrosinase (T3824) and 2, 2-dipheny l-1-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA). L-Dopa substrate was purchased from ACROS Co. (Geel, Belgium). Sodium metabisulfite CDH Co. (New Delhi, India), citric acid, ascorbic acid, and sodium carbonate were purchased from Merck Chemical Co. (Darmstadt, Germany). Plate count agar (PCA) was purchased from Liofilchem Co. (Roseto, Italy). Other chemicals were analytical grade.

2.1.2. Mushrooms

Mushrooms were harvested in first flush. They were white in color, with caps diameter of 3–4 cm and the veil connecting the pileus and stipe still intact. They were transported by refrigerator vehicle at 2–4 °C during one hour to the laboratory.

2.2. Methods

2.2.1. Preparation of pistachio green hull extract

Pistachio green hull (*Ahmad Aghaei* cultivar) was provided from Yazd Agricultural Research Center, Yazd, Iran. The hulls were dried, ground, and sieved with a 40-mesh sieve and stored at −20 °C until use. 25 g of pistachio green hull was mixed with water (solid to solvent ratio) 1:20 and extracted under continuous stirring for 8 h at ambient temperature. Then, the pistachio green hull extract was centrifuged at 10,500 *g* for 20 min at 20 °C, and the supernatant was filtered by filter paper Whatman No. 40. Finally, the pistachio green hull extract was freeze-dried and stored at −20 °C until use.

2.2.2. Determination of the polyphenolic compounds of pistachio green hull extract by RP-HPLC-DAD

Polyphenolic compounds of the aqueous extract of the pistachio green hull were analyzed according to the procedure described by Barreca et al. (2016) using an Azura HPLC system (Knauer, Berlin, Germany) equipped with a UV–vis photodiode-array detector (DAD 2.1 L, Knauer) and an LC pump (P 6.1 L). The separation of polyphenolic compounds was performed with a 5 µm ODS3 reversed-phase Prodigy column (0.25 × 0.0046 m, particle size 5×10^{-6} m, Phenomenex, USA) using solvent A (water: acetic acid, 97:3, v/v) and solvent B (methanol) under the following gradient condition: 0–3 min, 0% B; 3–9 min, 3% B; 9–24 min, 12% B; 24–33 min, 20% B; 33–43 min, 30% B; 43–66 min, 50% B; 66–81 min, 60% B; 81–86 min, 0% B and equilibrated 4 min for a total run time of 90 min. The column temperature was 25 °C. The flow rate and injection volume were 1.6×10^{-5} L/s and 2×10^{-5} L, respectively. UV–vis spectra were recorded in the wavelength range of 190–700 nm. The peak identification was based on the comparison of retention times and spectral data with those of pure standards. The linear range of the calibration curve was 20–120 µg ml^{-1} for phloroglucinol and 1–50 µg ml^{-1} for other standards (gallic acid: $y = 64.822x + 31.378$, $r = 0.9931$; phloroglucinol: $y = 4.424x - 5.366$, $r^2 = 0.9972$; naringin: $y = 30.445x + 13.662$, $r^2 = 0.9925$; vanillic acid: $y = 37.083x + 1.423$, $r^2 = 0.9920$; catechin: $y = 14.737x + 5.656$, $r^2 = 0.9947$; protocatechuic acid: $y = 79.513x + 37.437$, $r^2 = 0.9925$). The determinations were performed in triplicate.

2.2.3. Anti-tyrosinase activity assay

The anti-tyrosinase activity was measured according to the method of Yang and Ouyang (2012) and Hsu et al. (2007) with some modifications. First, 0.64×10^{-3} L of sodium phosphate buffer (25 mM, pH = 6.8), 0.16×10^{-3} L of pistachio green hull extract at different concentrations (0.05, 0.075, 0.1, 0.2, 0.4 and 0.8% w/v), pure gallic acid and phloroglucinol (at 1 g L^{-1}), and a mixture of them (a mixture of 0.5 g L^{-1} of each compound), 0.04×10^{-3} L of the mushroom tyrosinase enzyme (50 U mL^{-1}) was mixed and incubated at 25 °C for 600 s. Then, 0.16×10^{-3} L of L-Dopa solution (4.5 mM) was added to the mixture, and the formation of dopachrome was immediately monitored by measuring the linear increase in absorbance at 475 nm for 300 s. Deionized water was used as a blank. All experiments were carried out in triplicate. L-ascorbic acid was used as a positive control for the determination of anti-tyrosinase activity. The inhibitory effects of the tested samples on the enzyme activity were represented as:

$$\text{Inhibition (\%)} = (\Delta A - \Delta B)/\Delta A \times 100$$

Where $\Delta A = (A_5 - A_0)/300$ s is the absorbance in the absence of pistachio green hull extract and $\Delta B = (B_5\text{-}B_0)/300$ s is the absorbance in