Research article

Impact of Pre-drying Treatments on Antioxidant Activities and Quality of Dried Golden Oyster Mushroom (*Pleurotus citrinopileatus*)

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Abstract

The effects of three pre-drying treatments including blanching in hot water (60ºC-70ºC for 3 and 5 min), soaking in NaCl (1-4% w/v for 10 and 20 min), and soaking in citric acid (0.2-0.8% w/v for 10 and 20 min) on dried Golden oyster mushroom (GOM) were studied. Color values (L^*, a^*, and b^*), browning index (BI), polyphenol oxidase (PPO) activity, protein content, flavonoid content (FC), total phenolic content (TPC), and antioxidant activities (metal chelating activity and DPPH radical scavenging activity) of dried mushrooms were evaluated. All pretreated mushrooms had significantly higher lightness (L^*) and lower a^* and b^* values compared to those of un-pretreated ones. Decreases in BI and PPO activity were observed in pretreated mushrooms. Higher blanching temperatures, concentrations of NaCl and citric acid, and time yielded lower protein content, TPC, and FC. Among the pretreatments, the highest antioxidant activities were observed in citric acid treated GOM. Thus, blanching and chemical pretreatments could enhance the lightness and lower BI of dried GOM, although they lowered the nutrient levels and antioxidant activities of the dried GOM.

Keywords

golden oyster mushroom;
polyphenol oxidase;
drying pretreatment;
browning index;
antioxidant activity

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1. Introduction

Edible mushrooms have been traditionally consumed worldwide in both food and medicine due to their flavor and texture as well as their nutritional and chemical properties [1]. Golden oyster mushroom, also called Yellow oyster mushroom (*Pleurotus citrinopileatus*), is one of the most popular mushrooms in Vietnam and other South East Asian countries. Golden oyster mushrooms are a rich source of protein, and are higher in protein than nuts such as soybeans, peanuts, and other plant-based protein sources [2]. In addition, Golden oyster mushroom is also highly valued for its large quantities of minerals (Ca, P, Fe, Zn), vitamins (niacin, riboflavin, and thiamin), carbohydrates, and low-fat content [3]. Moreover, several *in vitro* studies have also shown that *Pleurotus* spp. possess high free radical scavenging and antioxidant activities. In particular, when human immunological repair systems are inadequate to prevent the DNA damage and cell malignancy [4-7]. However, oyster mushrooms contain high water content (87-95%) and a range of browning reaction enzymes, especially phenolase enzymes (including polyphenol oxidase, catecholases, or tyrosinases). These enzymes are responsible for the browning of plant and seafood products, and the resultant decrease in the food organoleptic values [7, 8]. Therefore, the oyster mushrooms spoil easily and deteriorate under atmospheric storage conditions [4]. It is necessary to have suitable postharvest methods to maintain the nutritional value and prolong the shelf life of these kinds of mushrooms. The dehydration process has been known as the most practical techniques to extend the shelf life of many kinds of food products. However, the high temperature of the hot air drying process has been known to promote enzymatic browning reactions that reduce the sensory and nutritional qualities of dried products [9]. Thus, various pre-treatment methods using thermal processing and the use of different chemical agents have been studied and implemented.

Many studies have shown that polyphenol oxidase (PPO) can be inhibited under thermal processing conditions (65ºC-90ºC) depending on the enzyme sources [10, 11]. In addition, PPO activity can also be retarded by soaking in NaCl solution (1-4%), and in organic acids (citric acid, ascorbic acid) at concentrations from 0.2 to 1% depending on the kinds of materials [12, 13]. In Vietnam, depending on the type of mushrooms, there are different pre-treatment methods. Oyster mushrooms are normally pretreated before drying by immersing in salt solution, lemon juice, or by blanching in hot water. However, there is a lack of knowledge on the impact of pre-treatment conditions on the antioxidant activities and quality of dried Golden oyster mushrooms. Hence, this experiment was conducted to find the most favorable pretreatment processes to increase the sensory quality and antioxidant capacities of dried Golden oyster mushrooms.

2. Materials and Methods

2.1 Materials and chemicals

Golden oyster mushrooms (*Pleurotus citrinopileatus*) or GOM were cultivated and collected in the membrane house of the Department of Biotechnology, Faculty of Applied Biological Sciences, Vinh Long University of Technology Education. The mushrooms had been cultivated for 2 weeks before the study was conducted. The selected mushroom is a 2-day fruiting body in which the size of the mushroom cap is 3 cm. The freshly harvested mushrooms, without visible flecks, were kept in plastic bags and stored at 4ºC within 2 h before the experiments.

All chemicals used in the experiments were analytical grade and purchased from Merck KGAA Co. (Darmstadt, Germany). Included were Folin-ciocalteu, $FeSO_4$, catechol, Ferrozine, and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2.2 Pre-drying treatments and drying process

The pretreatments given to Golden oyster mushrooms before drying are shown in Table 1: (A) blanching in hot water at 60ºC, 65ºC, and 70ºC for 3 and 5 min; or (B) soaking in a NaCl solution at concentrations of 1%, 2%, 3%, and 4% for 10 and 20 min; or (C) immersing in solutions containing 0.2 %, 0.4%, 0.6%, and 0.8% citric acid. The samples were then drained and subjected to a drying process. Untreated mushrooms (U) were used as a control.

Table 1. Different pre-drying treatments for Golden oyster mushroom

Pretreatment Methods	
U	**Control (untreated)**
A: Blanching in hot water	A1: 60 ºC, 3 min A2: 60 ºC, 5 min A3: 65 ºC, 3 min A4: 65 ºC, 5 min A5: 70 ºC, 3 min A6: 70 ºC, 5 min
B: Soaking in a NaCl	B1: 1% NaCl, 10 min B2: 1% NaCl, 20 min B3: 2% NaCl, 10 min B4: 2% NaCl, 20 min B5: 3% NaCl, 10 min B6: 3% NaCl, 20 min B7: 4% NaCl, 10 min B8: 4% NaCl, 20 min
C: Immersing in citric acid	C1: 0.2% citric acid, 10 min C2: 0.2% citric acid, 20 min C3: 0.4% citric acid, 10 min C4: 0.4% citric acid, 20 min C5: 0.6% citric acid, 10 min C6: 0.6% citric acid, 20 min C7: 0.8% citric acid, 10 min C8: 0.8% citric acid, 20 min

The treated and untreated mushrooms were hot-air dried at 55ºC for 8 h with airflow rates of 1.5 m/s using a tray dryer (DRC-16T, Vietnam). Materials were loaded on the trays and moisture content was continuously determined using a moisture analyzer (MOC-63U Shimadzu, Japan).

2.3 Analyses

The dried mushrooms were crushed using a blender (Philips Blender HR2221, China). After sieving through a 16 mesh screen (wire diameter 0.457 mm), the powder was then kept in polyethylene bags and placed in a -18ºC freezer before the analysis was done.

2.3.1 Determinations of moisture and protein contents

The moisture content (%, w/w) and protein content (%, w/w) of the dried GOMs were determined following the oven method and the Kjeldahl method, respectively [14].

2.3.2 Determinations of color and browning index

The colors of dried mushroom samples were measured using a colorimeter (MSEZ-4500 L, HunterLab, USA) and expressed in CIE system. L^*, a^*, and b^* values represent lightness, redness, and yellowness, respectively.

The browning index (BI) of samples was analyzed following the method of Lidhoo and Agrawal [15]. Five grams of the dried mushrooms were mixed with 100 mL of 60% ethanol and shaken thoroughly at ambient temperature for 6 h. Then, the mixture was passed through a filter paper (Whatman no. 2). Thereafter, the filtrate was read at 440 nm using a UV/Vis spectrophotometer (2602 Labomed, USA).

2.3.3 Determinations of FC and TPC

The TPC (total phenolic content) and FC (flavonoid content) of the dried mushroom samples were analyzed as described by the methods of Piskov *et al*. [7]. To extract phenolic and flavonoid compounds, 1 g sample was mixed with 50 mL absolute methanol. After extracting for 24 h using a shaker at 150 rpm in the dark, the mixture was centrifuged at 4,000 rpm for 20 min and decanted. The liquid extract was then evaporated until dry by nitrogen gas at 40°C. The residue was dissolved again in absolute methanol to a concentration of 50 mg/mL and kept at 4°C.

For the TPC assay, the Folin-Ciocalteau reagent procedure was used. Briefly, 1.8 mL of ten-fold diluted Folin-Ciocalteau reagent was mixed with 40 µL of the dried mushroom extract in an aluminum foil-covered test tube for 5 min. Thereafter, 1.2 mL of sodium bicarbonate solution (7.5% w/v) was added and thoroughly vortexed. The reaction was kept in the dark for 1 h, and the absorbance of the solution was measured at 765 nm by a spectrophotometer. The TPC of the samples was expressed as mg gallic acid equivalents (mg GAE)/g mushroom.

For the FC procedure, the dried mushroom extract (0.5 mL) was mixed with 10% $AlCl_3$ (0.1 mL), 1 M sodium acetate (0.1 mL), and distilled water (2.8 mL). Then, the mixture was kept at ambient temperature in the dark for 30 min. The absorbance ($A_{415\ nm}$) of the reaction mixture was recorded using a spectrophotometer. FC was presented as mg quercetin equivalents (mg QE)/g mushroom.

2.3.4 Determination of antioxidant activities

1) DPPH radical scavenging activity

The DPPH radical scavenging activity (%) of the dried mushrooms was conducted as per the protocol of Elmastas *et al*. [1]. For methanol extraction, the samples (10 g) were added into absolute methanol (100 mL) and shaken at 150 rpm for 24 h at room temperature. This step was repeated until the extracting solvent became uncolored. The extract obtained was filtered over Whatman no. 2 paper. Then, methanol was removed from the filtrate using a rotary evaporator (WEV-1010, Daihan, Korea) at 40°C. The dried extract was kept in an amber bottle and stored at 4°C until analysis to inhibit oxidation. To analyze DPPH radical scavenging activity, the dried extract (10 mg) was dissolved in absolute methanol (10 mL). One milliliter of 0.1 mM DPPH freshly prepared in absolute methanol was then mixed with 3 mL of the dried mushroom extract. The mixture was stirred well and kept in the dark for 30 min at room temperature. The absorbance ($A_{517\ nm}$) was recorded on a spectrophotometer. The activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1)/A_0]\times 100$$

where A_0 is $A_{517\ nm}$ of the blank (without sample extract), and A_1 is $A_{517\ nm}$ of sample extract.

2) Metal chelating activity

The metal chelating activity (%) of the dried mushrooms was done following the method of Wong *et al*. [13]. Briefly, 0.1 mM $FeSO_4$ (0.2 mL) and 0.25 mM ferrozine (0.4 mL) were thoroughly stirred with the dried mushroom extract (0.2 mL). After incubation in the dark for 30 min at room temperature, the absorbance of the mixture was recorded at 562 nm using a spectrophotometer. The metal chelating activity was calculated according to the following formula:

$$\text{Metal chelating activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is absorbance of the control (without sample extract), and A_1 is absorbance of sample extract.

2.3.5 Determination of polyphenol oxidase (PPO) activity

The protocol of PPO activity analysis was documented by Rivas and Whitaker [16]. Generally, 1 g of dried mushroom sample was added to sodium phosphate buffer (pH = 6.4), and the mixture was then centrifuged at 10,000 rpm at 4°C for 20 min to obtain an enzyme extract. Thereafter, 1 mL of sodium phosphate buffer (0.2 M, pH = 6.4), 0.9 mL of distilled water, 1 mL of catechol (0.05 M), and 0.1 mL of enzyme extract were vigorously mixed. The increase in absorbance at 420 nm (A_{420}) after 10 min incubation at 35°C was determined using a spectrophotometer. One unit of PPO was defined as the enzyme causing an increase in A420 nm by 0.001/min. PPO activity of the samples was expressed as units/mL.

2.4 Statistical analysis

All the experimental results of triplicate measurements were shown as mean±standard deviation (STD). Data were compared as variances across the means of different groups using analysis of variance (ANOVA) and Duncan's multiple range test [17]. Statistical analysis was conducted using the Statistical Package for Social Science for windows (SPSS 11.0, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1 Effect of different pretreatments on color of dried GOM

Table 2 shows the effect of different pretreatment conditions on the color of the dried GOMs. It was observed that dried mushrooms treated with blanching in hot water, soaking in NaCl, or citric acid solution had significantly higher lightness *(L*)*, compared to the control dried mushroom ($P<0.05$). The increases in temperatures of hot water, NaCl, citric acid concentrations, and treatment time yielded increases in the *L** values of the dried mushrooms ($P<0.05$). The highest *L** values were obtained when the mushrooms were pretreated by blanching in hot water at 70°C for 5 min, soaking in a 4% NaCl for 20 min, and soaking in 0.8% citric acid for 20 min. The increased *L** value was in line with the decreased *a** and *b** values of the dried mushrooms. The increases in both *a** and *b** values of the dried mushrooms were observed when the levels of pretreatment conditions and time increased ($P<0.05$). For *a** value, mushrooms treated with citric acid showed a slightly lower value than those treated with hot water or NaCl solution. Citric acid has been considered a better inhibitor of enzymatic and non-enzymatic browning reactions than blanching and salt pretreatments. Thus,

Table 2. Color values (*L*, a*,* and *b**) of dried GOMs treated by different methods

Treatments	*L**	*a**	*b**
U	36.38±0.61^{l*}	3.76±0.16^{ab}	21.72±1.75^{ab}
A1	41.04±1.14^{k}	3.45±0.14^{cd}	25.51±1.07^{ab}
A2	44.83±1.30^{j}	3.31±0.05^{de}	24.24±0.66^{b}
A3	47.65±1.34^{hi}	3.36±0.16^{d}	26.17±0.77^{a}
A4	52.26±0.36^{fg}	2.55±0.24^{gh}	27.19±0.42^{ab}
A5	57.32±0.52^{bc}	2.37±0.07^{hij}	26.84±0.47^{ab}
A6	60.77±1.14^{a}	2.08±0.12^{kl}	21.38±1.00^{b}
B1	42.83±2.94^{k}	3.96±0.15^{a}	23.69±1.00^{b}
B2	46.00±0.95^{ij}	3.65±0.07^{bc}	26.19±0.93^{ab}
B3	47.58±1.54^{hi}	3.36±0.15^{d}	28.16±2.36^{ab}
B4	52.41±1.53^{fg}	3.12±0.07^{ef}	25.16±1.98^{ab}
B5	52.12±0.45^{fg}	2.68±0.13^{g}	22.07±0.65^{b}
B6	55.08±0.51^{de}	2.66±0.10^{g}	23.95±0.58^{b}
B7	56.87±0.71^{bcd}	2.62±0.14^{g}	24.70±1.22^{b}
B8	60.43±3.15^{a}	2.34±0.06^{hij}	28.09±1.74^{ab}
C1	48.21±0.36^{h}	2.98±0.01^{f}	22.44±0.33^{b}
C2	50.23±0.80^{g}	1.94±0.10^{l}	21.22±1.22^{b}
C3	52.12±0.45^{fg}	2.13±0.06^{jkl}	22.86±0.52^{b}
C4	55.32±0.52^{cd}	2.18±0.10^{ijk}	20.34±0.38^{b}
C5	53.20±0.56^{ef}	2.38±0.23^{hi}	22.45±1.81^{b}
C6	56.28±0.86^{cd}	2.23±0.14^{ijk}	22.85±1.08^{b}
C7	58.87±1.17^{ab}	2.18±0.07^{ijk}	25.25±0.33^{ab}
C8	60.87±0.41^{a}	2.75±0.14^{g}	25.17±1.64^{ab}

*Data are expressed as mean±standard deviation (n= 3); Different superscripts in the same column indicate significant differences of data in the same column (P≤0.05)

the lowered redness of pretreated samples was observed [18]. However, there were no differences in *b** values between dried mushrooms treated with blanching in hot water, soaking in NaCl, or citric acid solution at all levels and treatment times (P>0.05). Martínez-Soto *et al.* [8] reported that blanching oyster mushrooms (*Pleurotus ostreatus*) in hot water at 80ºC for 3 min decreased the *L** value. However, the dried mushrooms had a higher *L** value than those treated with 1 or 5 g/L sodium metabisulphite and 1 or 5 g/L citric acid for 10 min [8]. The increase in *L** and decreases in *a** and *b** values of dried mushrooms were due to the inactivation of PPO by heat, NaCl, and citric acid solutions. Visual appearances of fresh and dried GOMs reinforce the color differences in comparison with the control sample and dried mushrooms pretreated with different methods (Figure 1). There was an increase in brown color intensities observed in untreated mushrooms with a decrease in white color. The reduction of yellow and brown color rates observed in pretreated mushrooms could be a result of the inhibition of the enzymatic and non-enzymatic browning reactions (Maillard reactions). In line with these observations, Almeida and Nogueira [19] documented that the enzyme PPO from mushrooms was quickly denatured by heat treatment at temperatures over 70°C. Moreover, Mishra and Gautam [20] reported that several acidulants including phosphoric, malic, and citric acids are able to reduce the pH of a system, therefore yielding the activation of PPO, while halide salts including NaCl and $CaCl_2$ at concentrations in the range of 2-4% (w/v) are widely used for the prevention of browning in food products. The inhibition by halide salts could involve the action of the halide ion on the copper atom of the active site of PPO [21]. Thus, pretreatments could enhance the brightness of the dried GOMs.

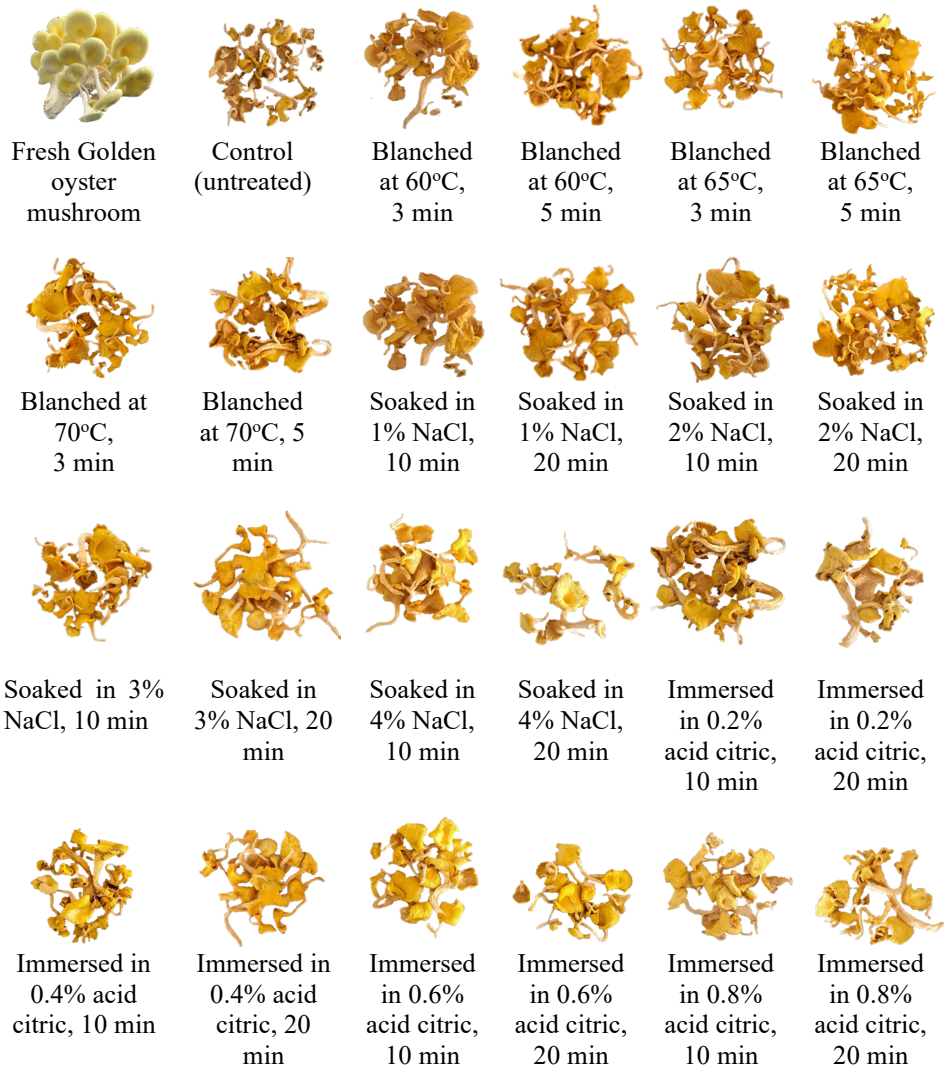

Figure 1. The difference in appearance compared to the control sample and the dried mushrooms pretreated by different methods

3.2 Effect of different pretreatments on PPO activity and browning index (BI) of dried GOM

The PPO activity (units/mL) and browning index (BI) of dried GOMs pretreated by different methods are shown in Table 3. The highest BI was found in the control dried mushrooms (0.43±0.02). The BI decreased when the soaking time, concentrations of NaCl and citric acid solutions, and heating time increased ($P<0.05$). The mushrooms soaked in citric acid showed higher BI values than those immersed in NaCl solution and blanched in hot water. The formation of browning in dried products, which eventually reduces the sensory characteristics of the products, is

Table 3. Browning index (BI) and PPO activity (units/mL) of dried GOMs treated by different methods

Treatments	Browning Index (BI)	PPO Activity (units/mL)
U	$0.43\pm0.02^{a*}$	93.83 ± 8.67^{a}
A1	0.31 ± 0.01^{cdef}	61.31 ± 1.35^{efg}
A2	0.27 ± 0.01^{efg}	54.90 ± 4.02^{gh}
A3	0.26 ± 0.01^{efgh}	50.79 ± 0.06^{hi}
A4	0.25 ± 0.01^{fghi}	47.69 ± 2.60^{ij}
A5	0.22 ± 0.00^{ghijk}	46.97 ± 2.15^{ij}
A6	0.20 ± 0.00^{hijk}	41.40 ± 2.01^{jk}
B1	0.37 ± 0.02^{abc}	78.87 ± 1.33^{b}
B2	0.32 ± 0.01^{cde}	72.78 ± 3.17^{bc}
B3	0.30 ± 0.01^{def}	73.14 ± 4.16^{bc}
B4	0.25 ± 0.01^{fgh}	71.72 ± 2.90^{c}
B5	0.21 ± 0.01^{ghijk}	63.12 ± 5.81^{def}
B6	0.19 ± 0.01^{hijk}	69.59 ± 1.75^{cd}
B7	0.18 ± 0.01^{ijk}	56.51 ± 2.32^{fgh}
B8	0.18 ± 0.01^{jk}	57.67 ± 4.11^{fge}
C1	0.41 ± 0.01^{ab}	67.70 ± 2.31^{cde}
C2	0.40 ± 0.01^{ab}	66.05 ± 0.96^{cde}
C3	0.35 ± 0.01^{bcd}	52.76 ± 2.53^{hi}
C4	0.30 ± 0.02^{cdef}	46.84 ± 1.54^{k}
C5	0.26 ± 0.00^{efgh}	35.45 ± 2.97^{ij}
C6	0.24 ± 0.01^{fghij}	40.88 ± 1.97^{jk}
C7	0.17 ± 0.01^{k}	39.43 ± 2.31^{k}
C8	0.13 ± 0.01^{ghijk}	36.96 ± 1.39^{k}

*Data are expressed as mean±standard deviation (n= 3); Different superscripts in the same column indicate significant differences of data in the same column ($P \leq 0.05$)

the result of the reaction between PPO enzymes and phenolic compounds [20]. In the presence of oxygen, the enzymatic reaction results in the formation of quinone which autopolymerises to produce a melanin-like brown pigment [20]. The decreased BI was also related to the reduction of TPC (the substrate for enzymatic browning reactions) in the pretreated samples (Table 4). Maray *et al*. [22] revealed that blanching at 90ºC for 3 min and immersing in a 0.5% citric acid for 30 min lowered the BI of dried oyster mushrooms (*Pleurotus ostreatus*). The result from this study was in agreement with the findings of Hassan and Medany [23], who showed that dried *Pleurotus ostreatus* and *Pleurotus eryngii* steeped in 0.1% $Na_2S_2O_5$, 0.1% NaCl, and 0.1% citric acid for 10 min had lower BI than that of untreated mushrooms. Lower BI in blanched, NaCl, and citric acid-treated mushrooms can probably be related to inactivation of browning enzymatic reactions.

PPO (EC1.10.3.1) is a Cu-containing enzyme that is classified in the group of oxidoreductases presenting in the tissues of animals and plants, and especially in vegetables, fruits and mushrooms [11]. A dramatic decrease in the PPO activities of dried GOMs was observed when all pretreatment methods were applied (Table 3). The results revealed that the untreated mushrooms had the highest PPO activity (93.83±8.67 units/mL) ($P<0.05$). When the mushrooms were pretreated by blanching in hot water at 60ºC for 3 min, the PPO activity remained at 61.31±1.35 units/mL, reduced by 34.66% activity compared to the control sample. Higher temperatures and longer soaking time caused an increase in the inhibition rate of PPO. The lowest PPO activity was found in mushrooms treated with 0.6% citric acid for 10 min (35.45±2.97 units/mL) ($P<0.05$). Cheng *et al.*

[24] revealed that the inactivation of mushroom PPO occurred quickly when temperatures higher than 65°C were applied. The remaining activity of PPO in mushrooms (*Agaricus bisporus*) decreased by almost 46% and 92% when the mushrooms were heated at 55°C and 60°C, respectively. Additionally, soaking in halide salts was also an effective method to retard the PPO activity in fruit and vegetable materials. The PPO activity in the dried mushrooms also decreased by immersing the mushrooms in NaCl. Among the NaCl-treated samples, the lowest PPO activity was in the mushroom immersed in 4% NaCl for 10 and 20 min (56.51±2.32 and 57.67±4.11 units/mL, respectively). However, in this present study, dipping GOM in citric acid was the most effective treatment for the inactivation of PPO activity. Approximately 62.22% activity of PPO was lost after the mushrooms were soaked in 0.6% citric acid for 10 min (35.45±2.97 units/mL). Nevertheless, the inhibition capacity of PPO was not enhanced when the levels of citric acid were increased to 0.8% for 10 and 20 min ($P>0.05$). Citric acid is an antioxidant agent but its inhibiting effect could be related to the PPO copper-chelating activity [25]. Liu *et al.* [26] documented that PPO from *Agaricus bisporus* mushrooms was markedly inactivated (remaining 4.3%) when the mushrooms were soaked in 60 mM citric acid. The conformation of PPO was gradually unfolded, which was reflected in the decrease of α-helix contents, increase of β-sheet, and exposure of aromatic amino acid when the concentration of citric acid was increased [26]. Therefore, pretreatments could reduce the activity of PPO from Golden oyster mushrooms, which contributed to the reduction of the browning index of the dried mushrooms, and this effect was especially pronounced for dipping in citric acid at a concentration of 0.6% for 10 min.

3.3 Effect of different pretreatments on protein content, flavonoid content (FC), and total phenolic content (TPC) of dried Golden oyster mushrooms

The protein content, FC, and TPC of dried GOMs as affected by different pretreatments were studied and the results are presented in Table 4. Fresh Golden oyster mushrooms were rich in protein content (26.96±2.98%, data not shown). After the drying process, this component did not significantly change (27.97±0.55%). However, the pretreatments caused a dramatic reduction in the protein content of the dried mushrooms ($P<0.05$). The lowest protein content was recorded in mushrooms heated in hot water at 70°C for 5 min (20.74±0.41%) and immersed in 4% NaCl for 10 and 20 min (20.92±0.10% and 20.38±0.16%, respectively). Nevertheless, citric acid at a concentration of 0.2% had no significant effect on the protein content in the dried mushrooms (27.69±0.12%) ($P>0.05$). The lower protein content was noticeable when the mushrooms were treated at higher concentrations of citric acid. It was noted that blanching in hot water and soaking in NaCl solution yielded a lower protein content compared to that of mushrooms treated with citric acid. The decreased protein content of dried oyster mushrooms was related to leaking out of proteinaceous materials during blanching or immersing in brine and citric acid. Moreover, the higher levels of protein denaturation and solubilization yielded lowered protein content when a longer brining time was applied [2]. The decreased protein content in pretreated GOMs was also likely responsible for lower BI values (Table 3) due to the partial elimination of substrate for Maillard reactions. Hassan and Medany [23] documented that mushrooms steamed or blanched with hot water at high temperatures caused weight and nutritional composition losses and were also fundamentally correlated to undesirable changes in product texture.

Table 4. Protein content, total phenolic content (TPC), and flavonoid content (FC) of dried GOMs treated by different methods

Treatments	Protein Content (%, dry matter)	Total Phenolic Content (TPC, mg GAE/g dry weight)	Flavonoid Content (FC, mg QE/g dry weight)
U	27.97±0.55^{a*}	0.77±0.03^{a}	70.19±5.01^{a}
A1	25.37±0.31^{bc}	0.71±0.02^{b}	41.23±0.83^{ij}
A2	25.26±0.23^{cd}	0.60±0.05^{cd}	45.46±0.51^{fg}
A3	23.73±0.36^{g}	0.55±0.01^{e}	39.31±0.51^{jkl}
A4	22.94±0.54^{h}	0.47±0.03^{f}	36.23±0.60^{lm}
A5	22.48±0.53^{h}	0.45±0.02^{f}	29.44±0.64^{o}
A6	20.74±0.41^{i}	0.38±0.01^{g}	27.80±1.03^{o}
B1	25.53±2.22^{h}	0.63±0.01^{c}	50.81±0.25^{cd}
B2	26.32±0.43^{b}	0.63±0.01^{c}	52.91±0.64^{bc}
B3	24.62±0.60^{def}	0.55±0.01^{e}	47.28±0.40^{ef}
B4	24.79±0.12^{de}	0.53±0.02^{e}	44.99±0.40^{fgh}
B5	23.06±0.26^{h}	0.55±0.04^{e}	41.79±0.77^{hij}
B6	22.40±0.21^{h}	0.47±0.03^{f}	38.85±0.38^{jkl}
B7	20.92±0.10^{i}	0.32±0.02^{h}	35.39±0.24^{lm}
B8	20.38±0.16^{i}	0.32±0.02^{h}	32.72±0.27^{n}
C1	28.45±0.69^{a}	0.73±0.01^{ab}	44.38±0.46^{fghi}
C2	27.69±0.12^{a}	0.61±0.05^{c}	53.98±0.31^{b}
C3	26.11±0.45^{b}	0.56±0.01^{de}	49.51±0.50^{de}
C4	24.95±0.20^{de}	0.48±0.03^{f}	46.17±0.55^{fg}
C5	24.50±0.18^{ef}	0.46±0.02^{f}	43.46±1.26^{ghi}
C6	24.30±0.23^{fg}	0.39±0.01^{g}	39.63±1.08^{jk}
C7	23.77±0.32^{g}	0.32±0.01^{h}	36.45±0.65^{klm}
C8	23.03±0.26^{h}	0.25±0.01^{i}	33.77±0.70^{lm}

*Data are expressed as mean±standard deviation (n= 3); Different superscripts in the same column indicate significant differences of data in the same column (P≤0.05)

The FC and TPC of fresh GOMs were 63.17±3.51 mg QE/g and 0.24±0.02 mg GAE/g, respectively (data not shown). The slightly higher TPC and FC contained in the dried mushrooms were 0.77±0.03 mg GAE/g and 70.19±5.01 mg QE/g, respectively (data not shown). It was previously documented that the bound phenols contained in plant cellular matrices are liberated during the thermal process. Thus, these phenol compounds in dried samples are released to a greater extent in the extracting solvent [27, 28]. However, the TPC and FC of the dried mushrooms dramatically decreased after pretreatment processes were applied (P<0.05). The lowest TPC was analyzed in the mushrooms treated in 0.8% citric acid for 20 min (0.25±0.01 mg GAE/g). Nevertheless, the mushrooms blanched in hot water at 70ºC for 5 min had the lowest FC compared to those of mushrooms immersed in brine and citric acid at the highest treatment levels (P<0.05). Additionally, the decreases in TPC and FC contributed to the lower BI of pretreated GOMs (Table 3) because of the reduction of substrates for browning enzymatic reactions. However, Mutukwa *et al.* [28] found that *P. ostreatus* that had received chemical pretreatments (lemon juice, potassium bisulfite, and vinegar), and un-blanched mushrooms had more TPC and FC compared to blanched samples. Hence, pretreatments might have caused a decrease in phytochemicals, especially bioactive compounds, in dried oyster mushrooms.

3.4 Effect of different pretreatments on antioxidant activities of dried GOM

The antioxidant activities of dried GOMs in terms of DPPH radical scavenging activity (DRSA, %) and metal chelating activity (MCA, %) as affected by different pretreatment methods are presented in Table 5. The fresh mushrooms possessed high DRSA and MCA, which were 10.14±2.83% and 1.77±0.50%, respectively (data not shown). Khatun *et al*. [29] found that *Pleurotus florida, Pleurotus citrinopileatus*, and *Pleurotus pulmonarius* possessed high reducing power and chelating activity for Fe^{2+}. The chelating activities of *P. citrinopileatus* extracted with a concentration of 1.0 mg/mL was 78.14% [29]. The drying process decreased the DRSA of the dried mushrooms (6.91±0.47%), while the MCA of the dried mushrooms was not significantly affected (1.92±0.07%). Pre-drying treatments continuously reduced the antioxidant activities of the dried Golden oyster mushrooms. Blanching in hot water and soaking in brine solution caused a higher reduction of the DRSA than those of mushrooms immersed in citric acid ($P<0.05$). Compared to the untreated dried mushrooms, more than 80% of the DRSA was lost when the samples were soaked in 4% NaCl for 20 min followed by blanching in hot water at 70ºC for 5 min (66.28%). The lowest reduction was recorded in the mushrooms treated with 0.8% citric acid for 20 min (53.69%). The same trend was observed in MCA when the mushrooms were pretreated at higher levels and time. The lowest MCA was found in mushrooms soaked in brine solution. The mushrooms immersed in citric acid obtained the highest MCA compared to the ones treated by blanching in hot water and soaking in NaCl ($P<0.05$). The decreases in the antioxidant activities were in agreement with the decreases in TPC and FC in the dried mushrooms (Table 4). Phenolic substances are accountable for antioxidant activity as they are singlet oxygen quenchers, hydrogen donors, and redox properties [27]. Moreover, polyphenols are essential plant components because their hydroxyl groups possess scavenging ability [1].

4. Conclusions

From the present study, it can be concluded that pre-drying treatments such as blanching in hot water, soaking in NaCl, and soaking in citric acid had effects on the colors, chemical composition, and antioxidant activities of the dried GOMs. All pretreatment methods enhanced the lightness of the dried mushrooms. However, they caused the leaching out of proteins and phytochemicals from the dried mushrooms. Among the pretreatments, immersing mushrooms in 0.6% citric acid for 10 min can be recommended as a pretreatment condition before further processing because it resulted in more enhanced color, and helped to maintain the composition and antioxidant activities of the dried GOMs.

Table 5. Antioxidant activities of dried GOMs treated by different methods

Treatments	DPPH Radical Scavenging Activity (DRSA, %)	Metal Chelating Activity (MCA, %)
U	6.91±0.47^{a*}	1.92±0.07^{a}
A1	4.29±0.10^{fg}	1.34±0.06^{efg}
A2	4.16±0.19^{fg}	1.34±0.08^{efg}
A3	2.93±0.19^{hi}	0.87±0.08^{ij}
A4	2.63±0.15^{ij}	0.80±0.07^{j}
A5	2.41±0.23^{i}	0.88±0.08^{ij}
A6	2.33±0.01^{i}	0.68±0.05^{j}
B1	5.60±0.35^{d}	1.65±0.01^{efg}
B2	4.84±0.38^{e}	1.65±0.04^{bc}
B3	4.45±0.17^{ef}	1.23±0.02^{fgh}
B4	4.41±0.15^{ef}	0.78±0.05^{j}
B5	3.90±0.17^{g}	0.73±0.03^{j}
B6	2.28±0.15^{j}	0.65±0.05^{jk}
B7	1.30±0.12^{k}	0.46±0.06^{kl}
B8	1.32±0.02^{k}	0.31±0.03^{l}
C1	6.83±0.25^{a}	1.836±0.08^{ab}
C2	6.46±0.09^{ab}	1.594±0.11^{cd}
C3	6.22±0.14^{bc}	1.463±0.10^{cde}
C4	5.87±0.11^{cd}	1.437±0.08^{cdef}
C5	5.52±0.41	1.395±0.05^{defg}
C6	4.26±0.212^{fg}	1.305±0.10^{efg}
C7	3.98±0.25^{fg}	1.201±0.05^{gh}
C8	3.20±0.14^{h}	1.058±0.08^{hi}

*Data are expressed as mean±standard deviation (n= 3); Different superscripts in the same column indicate significant differences of data in the same column ($P \leq 0.05$)

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