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# Effect of Lactic Acid Fermentation on Antioxidant Properties and Phenolic Acid Contents of Oyster (*Pleurotus ostreatus*) and Chanterelle (*Cantharellus cibarius*) Mushrooms

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**Abstract** Fruiting bodies of *Pleurotus ostreatus* (oyster) and *Cantharellus cibarius* (chanterelle) mushrooms underwent acid fermentation using 3 strains of lactic acid bacteria (LAB) as starter cultures. Polyphenol contents, antioxidant activities, and phenolic acid contents in fresh, blanched, and fermented mushrooms were investigated. Fruiting bodies of oyster mushrooms exhibited higher total phenolic contents than chanterelle mushrooms. Blanching caused a decrease in polyphenol contents and antioxidant activities in both mushroom types. No important differences were observed in total phenolic compound contents (measured using Folin-Ciocalteau reagent) in mushrooms using different LAB strains. *Lactobacillus plantarum* was the most useful microorganism for lactic acid fermentation of fruiting bodies for reduction of the pH value. The highest concentrations of single phenolic acids: gallic, homogentisic, and ferulic acids were present in mushrooms fermented using *L. plantarum*.

**Keywords:** *Pleurotus ostreatus*, *Cantharellus cibarius*, phenolic compound, antioxidant activity, fermented mushroom

## Introduction

Mushrooms have been a part of the human diet for thousands of years as flavoring components in soups and sauces, typically in fresh and dried forms. Mushrooms are appreciated not only due to flavor ingredients, but also due to nutritional value, bioactive compound contents, and medicinal properties (1). One of the important properties of edible mushrooms is an ability to scavenge free radicals due to contained phenolic compounds (2). The main phenolic compounds found in mushrooms are the phenolic acids: *p*-hydroxybenzoic, protocatechuic, gallic, gentisic, homogentsic, *p*-coumaric, caffeic, and ferulic acids (2-6). Also, phenolic acids exert antibacterial, antiviral and antifungal properties (7). In addition, phenolic acids play an important role in control of cancer and other human diseases (8).

Oyster mushrooms (*Pleurotus ostreatus*) are popular in Europe, probably due to sensory value, low price and broad availability throughout the year. Chanterelle mushrooms (*Cantharellus cibarius*) are a wild edible mushroom species popular in Poland, Portugal, and Spain (2,9). Fruiting bodies are used for preparation of soups and sauces, and dishes are also prepared using fresh, dried, frozen, or freeze-dried fruiting bodies. Chanterelle mushrooms are also main or additional components of marinades in an acetic acid water solution, and are also consumed with cold processed meats. However, a superior alternative to marinades are products obtained subsequent to lactic acid fermentation.

Edible mushrooms have a short shelf life due to rapid post-harvest changes. Therefore, lactic acid fermentation of wild and cultivated mushrooms is a cheap and efficient method of preservation. This method is in domestic use in Eastern Europe and in Asia, but only a limited number of studies in this field are known (10-17). In this studies as a substrate for lactic acid fermentation, *Agaricus bisporus* and *Pleurotus ostreatus* have usually been used. Addition of either bacterial cultures or brine from fermented sauerkraut is necessary for initiation of fermentation. Also, a higher quality final product has been obtained when mushrooms were blanched prior to fermentation. Bacterial strains tested for addition include *Lactobacillus plantarum*, *L. casei*, *L. brevis*, *and L. helveticus* (12-17).

Many studies of the antioxidant properties of cultivated and wild edible mushrooms are available. However, no studies concerning antioxidant activities and phenolic acid profiles of fermented mushrooms are known. In this study, antioxidant activities, total phenolic contents, and phenolic acid profiles of 2 edible mushroom types after lactic acid fermentation are presented for the first time.

## Materials and Methods

**Materials** Oyster mushrooms were purchased from a local market in Lublin, Poland in July of 2013. Chanterelle mushrooms were collected in the Janowskie Forests of Lublin Voivodeship, Eastern Poland (50°68'N, 22°30'E) in July of 2013. Fresh fruiting bodies were cleaned of forest debris and mushroom specimens were stored at 5°C in refrigerator (G-08S; Geco, Bochnia, Poland) before experimental use. Oyster mushrooms were processed on the same day of purchase and chanterelle mushrooms were processed within 6 h of harvest. Starter cultures of *L. plantarum* Ib, *L. casei* Lby, and *L. helveticus* $K_1$Lb were obtained from the culture collection held by the Department of Biotechnology, Human Nutrition and Food Commodity Science, University of Life Sciences in Lublin, Poland and were propagated twice in MRS broth (Biocorp, Warszawa, Poland) and incubated (TK-2; Cabrolab, Warszawa, Poland) overnight at 30°C. After centrifugation (MPW 350-R; MPW, Warszawa, Poland) at 1,400×*g* for 10 min, microbial cells were harvested and washed twice in sterile 0.9% NaCl (P.O.Ch., Gliwice, Poland) before inoculation.

**Preparation of fermented mushrooms** The fermentation procedure was based on a previous study (17). Before fermentation, mushrooms were washed in cold tap water of approximately 15°C and blanched in boiling water for 4 min. After cooling in the fridge (by putting at 5°C in a monolayer), 2% (w/w) NaCl and 1% (w/w) sucrose were added prior to individual inoculation at $10^7$ cfu/mL with starter cultures of *Lactobacillus* strains. Mushrooms were mixed with inocula and placed tightly (to remove air) in 500 mL glass jars. Then, 70 mL of a 2% salt solution was added, and jars were then closed. Lactic fermentation proceeded for 8 days at 21-22°C (TK-2; Cabrolab), after which fermented mushrooms were stored in the same, sealed, intact jars at 5°C for 5 weeks to proceed maturation. In total, after 43 days, fermented mushroom samples were collected for analyses. The fermentation process was performed in triplicate.

**Determination of pH** Evolution of pH was monitored using an HI 2211 pH/ORP Meter (Hanna Instruments, Cluj-Napoca, Romania) at days 0, 1, 2, 3, 4, and 8 of fermentation and after 15 and 43 days of storage.

**Analysis of dry weight** Calculations of total phenolic contents, antioxidant activities, and phenolic acid contents were performed on a dry weight (DW) basis. Mushroom samples were dried in a laboratory dryer (SML 32/250; Zalmed, Warszawa, Poland) at 105°C until constant weight was achieved for determination of moisture levels.

**Analysis of total phenolic contents and antioxidant activities**

***Mushroom sample preparation*:** The extraction procedure was based on a method described by Radzki *et al.* (18). Fresh, blanched, and fermented mushrooms were frozen at −20°C for 24 h, then lyophilized in an Alpha 1-2 LD plus freeze-dryer (Martin Christ, Osterode am Harz, Germany) for 72 h. The condenser temperature was set at −60°C, the vacuum was maintained at 0.8 mbar, and the shelf temperature was set at 25°C. Lyophilized materials were ground in a Społem Wż-1 mill (Społem, Warszawa, Poland), then 1 g of mushroom sample was subjected to extraction using 30 mL of 80% (v/v) methanol in a shaker (Elpan 357; Elpan, Lubawa, Poland) at 80°C and 175 rpm for 1 h. Obtained extracts were centrifuged (MPW 350-R; MPW) at 4,800×*g* for 15 min, then used for determination of antioxidant activities and analysis of total phenolic compounds.

***Determination of total phenolic contents*:** Methanol extract samples of 0.2 mL were mixed with 0.8 mL of Folin-Ciocalteu reagent (P.O.Ch.) previously diluted with water to 1:10 (v/v). After 3 min, 1.25 mL of 7% $Na_2CO_3$ (P.O.Ch.) was added. The mixture was vortexed (TK3S; Kartell, Noviglio, Italy) for 15 s and allowed to stand in the dark for 30 min. The absorbance was then measured at 765 nm using a Helios Gamma apparatus (Thermo Fisher Scientific, Waltham, MA, USA) (19). Gallic acid was used for preparation of a standard curve with a calibration range of 0-100 μg/mL (coefficient of determination $R^2$= 0.996), and results were expressed as mg of gallic acid equivalents (GAE) per 1 g of mushroom on a dry weight (DW) basis.

***Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities*:** The DPPH radical scavenging activity was assayed following the method of Choi *et al.* (20). Each 0.2 mL methanol extract was mixed with 0.8 mL of a 0.2 mM DPPH methanol solution. The mixture was shaken (TK3S; Kartell) for 15 s and left to stand for 15 min in the dark. The absorbance was measured at 520 nm using a Helios Gamma apparatus (Thermo Fisher Scientific). Trolox (Sigma-Aldrich, St. Louis, MO, USA), an analog of vitamin E, was used for preparation of a standard curve with a calibration range of 0-400 μM, ($R^2$=0.999), and the antioxidant activity was expressed as μmol of Trolox equivalents (TE) per 1 g of mushroom (DW).

***Determination of the ferric reducing antioxidant power (FRAP)*:** The reducing power of mushroom extracts was also determined using a FRAP assay following the method of Thetsrimuang *et al.* (21) with modification. A FRAP reagent was prepared by mixing a 300 mM acetate buffer at pH 3.6 with a 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) (Sigma-Aldrich) solution (10 mM TPTZ in 40 mM HCl), and a 20 mM $FeCl_3 \cdot 6H_2O$ solution at a 10:1:1 ratio. Assay solutions were prepared by mixing 1.9 mL of FRAP reagent with 0.1 mL of a mushroom extract. After 15 min of incubation (water bath W610; LABOPLAY, Bytom, Poland) at 37°C in the dark, the absorbance was measured at 593 nm using a Helios Gamma apparatus (Thermo Fisher Scientific). Trolox was used for preparation of a standard curve with a calibration range of 0-500 μM ($R^2$=0.999) and results were reported as μmol of Trolox equivalents (TE) per 1 g of mushroom (DW).

**Analysis of phenolic acids**

***Extraction of free phenolic acids*:** Samples of lyophilized mushrooms (0.5 g) were ground in a mill (Wż-1; Społem), then were subjected to extraction with a solution containing 6.7 mL of methanol (Sigma-

Aldrich), 0.3 mL of glacial acetic acid (Sigma-Aldrich), and 20 mg of butylated hydroxyanisole (BHA) (Sigma-Aldrich). The mixture was sonicated in a MXB 14 Ultrasonic Water Bath (Grant Instruments, Royston, UK) for 30 min, then 3 mL of double deionized water (DDI) was added. One mL of extract was centrifuged (MPW 350-R; MPW) at 4,000×*g* for 10 min, then filtered through a 0.22 μm disposable LC filter disc (Cameo PTFE syringe filters; Sigma-Aldrich) prior to HPLC analysis.

***HPLC-UV analysis of phenolic acids*:** An HPLC-UV system consisting of 2 Smartline 100 pumps, a 0.1 mL loop, a dynamic mixer, and a Retriever 500 fraction collector (Knauer, Berlin, Germany) was used. Separations were performed using a Hypersil ODS-2 C18 column (250 mm×4.6 mm, 5 μm) coupled with a pre-column of the same kind (5 μm i.d., 8.0×20 mm) (Thermo Fisher Scientific).

Chromatograms were recorded at 280 nm for hydroxybenzoic acid and 320 nm for hydroxycinnamic acid using a Linear 200 UV-VIS detector (Linear Instruments, Palo Alto, CA, USA) coupled to an Interface IF-2 (Knauer). Chromatographic data were analyzed using EuroChrom Basic Edition software ver. 3.05 (Knauer). The mobile phase consisted of 1% (v/v) formic acid in DDI as eluent A, and 1% (v/v) formic acid in acetonitrile as eluent B (all these reagents were purchased from Sigma-Aldrich). A 1 mL/min gradient elution scheme (A:B ratio) of 100%:0% for t=0-5 min, 70%:30% for t=5-60 min, 40%:60% for t=60-80 min, and 100%:0% for t=80-85 min was used.

Phenolic acids were identified based on retention times compared with commercial standards (phenolic acids were purchased from Sigma-Aldrich). A calibration curve was obtained by injection of known concentrations of different standard compounds for quantitative analysis of phenolic acids. Standards used were gallic acid with a calibration range of 0-100 μg/mL ($R^2$=0.999), homogentisic acid with a calibration range of 0-100 μg/mL ($R^2$=0.997), *p*-hydroxybenzoic acid with a calibration range of 0-100 μg/mL ($R^2$=0.989), vanillic acid with a calibration range of 0-100 μg/mL ($R^2$ =0.982), *p*-coumaric acid with a calibration range of 0-100 μg/mL ($R^2$=0.999), ferulic acid with a calibration range of 0-100 μg/mL ($R^2$=0.981), and sinapic acid with a calibration range of 0-100 μg/mL ($R^2$=0.982). Results were expressed as μg per g of mushroom (DW).

**Statistical analysis** All analyses were performed in triplicate. Results were recorded as mean values±standard deviation (SD) and were compared using a one-way analysis of variance (ANOVA) at $p$<0.05 with STATISTICA 9 (StatSoft; Dell Software, Round Rock, TX, USA). Correlations between polyphenol contents and antioxidant activities were calculated using Office Excel 2007. The significance of the correlation coefficients was calculated using a method described by Stanisz (22).

## Results and Discussion

**The pH** The fastest acidity increase in fermented mushrooms for *P. ostreatus* and *C. cibarius* was observed using *L. plantarum* (Fig. 1A and 1B). After 3 or 4 days of fermentation for chanterelle and oyster mushrooms, respectively, in the presence of *L. plantarum,* pH values reached 3.5 and remained unchanged throughout the storage period. Reduction of pH values in fermented mushrooms reflected elevation of the lactic acid content. The titratable acidity and the pH value of fermented foods are important factors for extension of product shelf life and microbial safety. Therefore, it is important to obtain a fast and large reduction in the pH value (an elevation of the titratable acidity) in fermented food products. At low temperatures of 5°C, the fermented product was stable for 5 weeks with no sign of microbial spoilage, after which mushroom samples were collected for final analysis. In this study, fermented mushrooms were not stored for a long period of time. However, previous studies have shown that fermented mushrooms were microbiologically stable for 6 months under continuous storage at 5°C after fermentation (14).

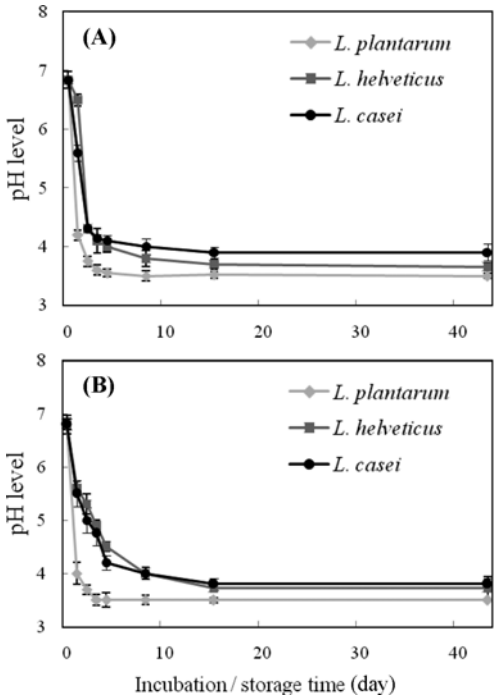


**Fig. 1.** Evolution of pH values of fermented *P. ostreatus* (A) and *C. cibarius* (B) mushrooms.

**Total phenolic contents and antioxidant activities** Total phenolic contents in mushrooms are shown in Table 1. Fruiting bodies of *P. ostreatus* exhibited higher total phenolic contents than *C. cibarius* with values of 2.10 vs. 1.88 mg of GAE/g (DW), respectively. The blanching process contributed to a significant ($p$<0.05) decrease in polyphenol contents in the fruiting bodies of both mushrooms. The lactic acid fermentation process exerted no significant ($p$>0.05) effect on total phenolic contents in fermented fruiting bodies (measured using Folin-Ciocalteau reagent), compared with blanched mushrooms. Also, use of different lactic acid bacterial strains had no significant

**Table 1.** Total phenolic contents, scavenging activities against DPPH radicals, and the ferric-reducing antioxidant power (FRAP)

| | total phenolics mg GAE/g DW | FRAP μmol TE/g DW | DPPH μmol TE/g DW |
|---|---|---|---|
| *P. ostreatus* | | | |
| fresh | 2.10±0.07[b2)] | 9.71±0.44[c] | 18.69±0.93[b] |
| blanched | 1.24±0.13[a] | 4.53±0.29[a] | 15.47±1.06[a] |
| fermented by[1)]: | | | |
| *L. plantarum* | 1.23±0.15[a] | 5.09±0.60[ab] | 15.60±1.01[a] |
| *L. helveticus* | 1.14±0.18[a] | 5.05±0.63[ab] | 15.33±0.29[a] |
| *L. casei* | 1.27±0.08[a] | 6.05±0.74[b] | 15.54±0.62[a] |
| *C. cibarius* | | | |
| fresh | 1.88±0.13[b] | 16.56±1.27[b] | 34.08±0.22[c] |
| blanched | 1.30±0.15[a] | 5.77±0.35[a] | 19.72±0.60[ab] |
| fermented by[1)]: | | | |
| *L. plantarum* | 1.08±0.13[a] | 7.57±0.70[a] | 20.87±0.68[b] |
| *L. helveticus* | 1.15±0.17[a] | 6.30±0.52[a] | 19.57±0.68[ab] |
| *L. casei* | 1.09±0.19[a] | 6.71±0.62[a] | 18.75±0.56[a] |

[1)]Mushroom samples were collected after 43 days from the start of the experimental period.

[2)]Differences among mean values denoted using different letters are statistically significant ($p$<0.05).

($p$>0.05) influence on levels of total phenolics in fermented mushrooms of all types. It is obvious, that there is significant ($p$<0.05) difference in total phenolics in fresh and corresponding, fermented mushrooms.

Two methods were used for evaluation of antioxidative activities of fermented mushrooms. The DPPH method is based on neutralization of a stable DPPH radical by antioxidants present in fermented mushrooms. The FRAP method was used for measurement of the reducing activity of mushrooms. Blanching caused a significant ($p$<0.05) decrease in antioxidant activities of both mushrooms, compared with fresh ones. An increase in FRAP values after fermentation was observed in the case of oyster mushrooms (statistically significant, $p$<0.05). For both mushroom types, lactic acid fermentation had no significant ($p$>0.05) influence on antioxidant activities measured using DPPH, in comparison with blanched mushrooms. However, for chanterelle mushrooms, significant ($p$<0.05) differences between fermented mushrooms obtained using individual bacterial strains were observed (Table 1). Chanterelle mushrooms fermented using *L. plantarum* exhibited a higher antioxidant activity (20.87 μmol TE/g DW) in comparison to mushroom samples fermented using other *Lactobacillus* strains ($p$<0.05).

Strong correlations were obtained between the total phenolic content and the scavenging activity using the DPPH radical, and between the total phenolic content and the ferric-reducing antioxidant power for *P. ostreatus* ($R$=0.997, and $R$=0.970, respectively), and for *C. cibarius* ($R$=0.956, and $R$=0.922, respectively). Phenolic compound contents in fresh mushrooms have been reported to be in the range 1.39-5.8 mg of GAE/g (DW) for *P. ostreatus* (2,7,15), and 2-2.8 mg of GAE/g (DW) for *C. cibarius* (2,18,23).

Barros *et al.* (24) reported that heating during cooking can destroy the chemical structures of polyphenols and can cause a decrease in the antioxidant activities of mushrooms. On the other hand, heat treatment can increase the extractability of polyphenols due to disruption of the cell wall, leading to increased release of polyphenols from mushroom tissues, in comparison with raw materials (20). Skąpska *et al.* (15) reported that blanching of button and oyster mushrooms caused a 60-79% decrease in total polyphenol contents, and a 54-79% decrease in antioxidant activities, with further decreases during ongoing fermentation. However, after 3 weeks of refrigerated storage, a significant increase was found.

**Phenolic acid profile** Phenolic acid contents of mushrooms are summarized in Table 2. A strong correlation was observed between total phenolic contents (measured using Folin-Ciocalteau reagent) and phenolic acid contents ($R$=0.833, and $R$=0.857 for *P. ostreatus* and *C. cibarius,* respectively). The 2 benzoic acid derivatives gallic acid and homogentisic acid, and the 2 cinnamic acid derivatives *p*-coumaric acid and ferulic acid were identified in both mushroom varieties. Sinapic acid was only detected in *P. ostreatus*. Vanillic acid was found only in fermented samples of *C. cibarius*, and 4-OH-benzoic acid was not detected in any mushroom sample. Homogentisic acid was the most abundant phenolic acid in both mushroom varieties. Fresh samples of *P. ostreatus* and *C. cibarius* contained 507.93 and 626.47 μg of homogentisic acid per g (DW), respectively. A similar observation was reported by Palacios *et al.* (2), who detected homogentisic acid in *P. ostreatus* at 622.89 μg/g (DW) and in *C. cibarius* at 316.76 μg/g (DW). Kim *et al.* (3) reported a considerably lower homogentisic acid content in *P. ostreatus* at 16 μg/g (DW).

Gallic acid was the second most common phenolic acid component in both oyster and chanterelle mushrooms in amounts of 333.37 μg/g (DW) and 431.94 μg/g (DW) in *P. ostreatus* and *C. cibarius*, respectively. Palacios *et al.* (2) reported that gallic acid contents were 161.83 and 290.34 μg per g (DW) in *C. cibarius* and *P. ostreatus*, respectively**.** In comparison to results obtained by Palacios *et al.* (2), a higher gallic acid content in *P. ostreatus* was reported by Karaman *et al.* (7) at 1,010 μg per g (DW). On the other hand, a significantly lower content in comparison to Palacios *et al.* (2) was reported by Kim *et al.* (3) of 7 μg per g (DW) and by Woldegiorgis *et al.* (25) of 13 μg per g (DW). *p*-Coumaric acid was reported in *P. ostreatus* at a level of 76.91 μg per g (DW) and in *C. cibarius* at 15.26 μg per g (DW). Previously, *p*-coumaric acid was only identified by Palacios *et al.* (2) who reported a *p*-coumaric acid level in dried *P. ostreatus* of 11.15 μg/g, and by Valentäo *et al.* (4) who reported a 1.6 μg level of *p*-coumaric acid per g (DW) in *C. cibarius*.

Ferulic acid has been reported both in *P. ostreatus* at 5.30 μg per g (DW) and in *C. cibarius* at 2.20 μg per g (DW). Palacios *et al.* (2) reported levels of ferulic acid for *P. ostreatus* and *C. cibarius* of 20.16 μg per g (DW) and 10.38 μg per g (DW), respectively. Muszyńska *et al*. (6) reported that the ferulic acid content in *P. ostreatus* was 0.46 μg per g (DW). However, Woldegiorgis *et al*. (25) reported that ferulic acid was not detected in the *P. ostreatus* fruiting body.

**Table 2.** Free phenolic acid contents (μg per g of DW)

| | gallic | homogentisic | 4-OH-benzoic | vanillic | *p*-coumaric | ferulic | sinapic | total |
|---|---|---|---|---|---|---|---|---|
| *P. ostreatus* | | | | | | | | |
| fresh | 333.37±26.72[c2)] | 507.93±62.62[c] | ND[3)] | ND | 76.91±8.78[b] | 5.30±0.33[a] | 0.88±0.59[ab] | 924.39 |
| blanched | 66.33±9.10[ab] | 483.14±7.15[c] | ND | ND | 23.79±5.60[a] | 1.57±1.09[a] | 1.55±0.44[b] | 576.38 |
| fermented by[1)]: | | | | | | | | |
| *L. plantarum* | 116.71±29.66[b] | 304.20±47.88[b] | ND | ND | 41.23±8.68[a] | 44.36±6.83[b] | ND | 506.50 |
| *L. helveticus* | 89.71±47.41[ab] | 253.08±20.70[b] | ND | ND | 34.32±2.46[a] | 44.04±12.31[b] | 1.58±0.17[b] | 422.73 |
| *L. casei* | 38.68±12.47[a] | 98.37±12.11[a] | ND | ND | 32.81±7.00[a] | 47.93±7.56[b] | 1.33±0.18[b] | 219.12 |
| *C. cibarius* | | | | | | | | |
| fresh | 431.94±27.71[d] | 626.47±61.82[b] | ND | ND | 15.26±1.59[c] | 2.20±0.34[a] | ND | 1,075.87 |
| blanched | 178.38±18.98[c] | 153.21±21.30[a] | ND | ND | 7.92±1.82[b] | 1.65±0.13[a] | ND | 341.16 |
| fermented by[1)]: | | | | | | | | |
| *L. plantarum* | 125.21±7.16[b] | 289.44±30.89[a] | ND | 134.86±13.65[a] | 0.49±0.16[a] | 10.93±2.34[b] | ND | 560.93 |
| *L. helveticus* | 112.27±7.37[b] | 239.31±23.12[a] | ND | 200.50±23.22[b] | 0.93±0.15[a] | 1.52±0.48[a] | ND | 554.53 |
| *L. casei* | 64.20±6.11[a] | 172.25±22.20[a] | ND | 140.88±17.56[a] | 2.72±0.64[a] | 1.23±0.25[a] | ND | 381.28 |

[1)]Mushroom samples were collected after 43 days from the start of the experimental period.
[2)]In each column, different letters denote statistically significant ($p$<0.05) differences.
[3)]ND, not detected.

In this study, sinapic acid was present only in *P. ostreatus* at 0.88 μg per g (DW), whereas Muszyńska *et al*. (6) reported sinapic acid in both *P. ostreatus* at 2.11 μg per g (DW) and *C. cibarius* at 3.04 μg per g (DW). Generally, in comparison with other reports, mushrooms studied herein contained higher amounts of phenolic acids. Compositions of mushrooms may be associated with cultivation, in the case of *P. ostreatus*, or with natural environmental factors for wild mushrooms, such as *C. cibarius*. Woldegiorgis *et al*. (25) reported that high amounts of caffeic acid might be due to a coffee waste substrate used for growth of *P. ostreatus* mushrooms.

In this study, blanching caused a significant ($p$<0.05) decrease in gallic (80%) and *p*-coumaric (69%) acid contents in *P. ostreatus*, and gallic (59%), homogentisic (76%), and *p*-coumaric (48%) acids in *C. cibarius,* compared with non-blanched mushrooms, probably due to use of high temperatures and washing out of water-soluble compounds. No study of heat treatment effects on phenolic acid contents in mushrooms is known. However, heat treatment effects have been analyzed for other organisms. Effects of boiling and steaming on phenolic compound contents and antioxidant activities of whole yellow and black soybeans were investigated by Xu and Chang (26). Compared with raw soybeans, pressure steaming treatment caused a significant increase in gallic acid and 2,3,4-trihydroxybenzoic acid contents, whereas all heat treatments caused significant decreases in chlorogenic acid and trans-cinnamic acid contents, and the sum of total phenolic acids in both yellow and black soybeans.

Effects of fermentation on phenolic acid contents in oyster and chanterelle are first reported herein. After fermentation, changes in phenolic acid profiles and concentrations were observed that were dependent on the mushroom species and on the lactic acid bacterial strain used (Table 2). Comparison of fermented *P. ostreatus* mushroom samples with blanched non-fermented mushroom samples revealed an increase in ferulic and *p*-coumaric acid contents. Sinapic acid contents were similar in blanched and fermented oyster mushroom samples, with the exception of mushroom samples fermented using *L. plantarum* in which sinapic acid was not detected. Gallic and homogentisic acid contents of fermented mushroom samples were dependent on the bacterial strain used. The highest contents were found in oyster mushrooms fermented using *L. plantarum* with values of 304.20 and 116.71 μg per g (DW) for homogentisic and gallic acids, respectively. The lowest homogentisic and gallic acid contents were observed in oyster mushrooms fermented using *L. casei* with values of 98.37 and 38.68 μg per g (DW), respectively.

For fermented *C. cibarius* mushrooms, significant ($p$<0.05) decreases in gallic and *p*-coumaric acid contents were observed, in comparison with blanched, non-fermented mushrooms. Ferulic acid contents were similar in all chanterelle mushroom samples, with the exception of mushrooms fermented using *L. plantarum* where a significant ($p$<0.05) (more than 6x) increase was observed, in comparison with fresh or blanched mushrooms. Ferulic acid exerts a number of positive biological effects, including antioxidant, anti-inflammatory, anticoagulant, and antithrombotic activities, and protective effects for the liver. Therefore, consumption of ferulic acid is beneficial (27). The homogentisic acid content in blanched and fermented chanterelle mushrooms was similar, therefore the fermentation process had no influence on the content of this phenolic acid in the final product. Vanillic acid was only detected in fermented samples of *C. cibarius* at 134.86-200.50 μg per g (DW). Vanillic acid was reportedly present in fresh fruiting bodies at 3.32 μg per g (DW) (6), perhaps because of maceration during the fermentation process due to microbial activity, which caused release of bound phenolic compounds, as reported by Othman *et al*. (28) who analyzed the influence of fermentation on phenolic acid contents in olives.

Phenolic acid contents in fermented mushrooms are a result of

many factors related to the mushroom species, the LAB strain, and conditions applied during fermentation. In some cases, an increase in phenolic acid contents was observed in fermented mushrooms in comparison with fresh or blanched mushrooms. The content of ferulic acid in fermented oyster was elevated in comparison to the content of this phenolic acid in the fresh or blanched oyster.

It is possible that hydrolytic enzymes produced by LAB during fermentation released cell wall phenolics or bound phenolics due to breakdown of cellular constituents, resulting in increased extraction of polyphenols. Svensson *et al*. (29) reported that fermentation using LAB affects polyphenol contents and can influence the nutritional value of sorghum, and that some enzymes produced by single cultures of lactobacilli contributed to metabolism of phenolic acids. Similarly, Rodríguez *et al*. (30) investigated the ability of *Lactobacillus plantarum* CECT 748T to metabolize 19 food phenolic acids. *p*-coumaric, caffeic, ferulic and *m*-coumaric acids were metabolized by *L. plantarum*. Therefore, decreases in phenolic acid contents in fermented mushrooms could have been due to phenolic acid metabolism by the starter cultures used in this study. However, derivative products were not analyzed herein. Transit of phenolic acids from the fruiting bodies of mushrooms to the brine, resulting in loss of phenolic acids from the solid parts that are consumed, may also have occurred (28). Brine and water compositions after blanching were not analyzed in this study.

In conclusion, *Lactobacillus plantarum* Ib was the most useful strain for fermentation of mushroom fruiting bodies. This strain most effectively reduced the pH value of fermented mushrooms. Also, the highest concentrations of gallic (for *P. ostreatus* and *C. cibarius*), homogentisic (for *P. ostreatus*), and ferulic acids (for *C. cibarius*) were found in mushrooms fermented using of the *Lactobacillus plantarum* Ib.

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## References


1. Stachowiak B, Reguła J. Health-promoting potential of edible macromycetes under special consideration of polysaccharides: A review. Eur. Food Res. Technol. 234: 369- 380 (2012)
2. Palacios I, Lozano M, Moro C, D'Arrigo M, Rostagno MA, Martinez JA, García-Lafuente A, Guillamón E, Villares A. Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chem. 128: 674-678 (2011)
3. Kim MY, Seguin P, Ahn JK, Kim JJ, Chun SC, Kim EH, Seo SH, Kang EY, Kim SL, Park YJ, Ro HM, Chung IM. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. J. Agr. Food Chem. 56: 7265-7270 (2008)
4. Valentäo P, Andrade PB, Rangel J, Ribeiro BR, Silva BM, Baptista P, Seabra RMJ. Effect of the conservation procedure on the contents of phenolic compounds and organic acids in chanterelle (*Cantharellus cibarius*) mushroom. J. Agr. Food Chem. 53: 4925-4931 (2005)
5. Barros L, Dueñas M, Ferreira ICFR, Baptista P, Santos-Buelga C. Phenolic acids determination by HPLC-DAD-ESI/MS in sixteen different Portuguese wild mushrooms species. Food Chem. Toxicol. 47: 1076-1079 (2009)
6. Muszyńska B, Sułkowska-Ziaja K, Ekiert H. Phenolic acids in selected edible *Basidiomycota* species: *Armillaria mellea*, *Boletus badius*, *Boletus edulis*, *Cantharellus cibarius*, *Lactarius deliciosus* and *Pleurotus ostreatus.* Acta Sci. Pol. 12: 107-116 (2013)
7. Karaman M, Jovin E, Malbaša R, Matavuly M, Popović M. Medicinal and edible lignicolous fungi as natural sources of antioxidative and antibacterial agents. Phytother. Res. 24: 1473-1481 (2010)
8. Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J. Med. Plants Res. 5: 6697-6703 (2011)
9. Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira ICFR. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem. Toxicol. 46: 2742-2747 (2008)
10. Kreß M, Lelley J. Preservation of oyster mushrooms by lactic acid fermentation. pp. 665-671. In: Science and Cultivation of Edible Fungi. Maher MJ (ed). Taylor and Francis, Rotterdam, Netherlands (1991)
11. Stojanovic M, Niksic M, Veres M, Petrovic L. Preservation of edible mushrooms (*Agaricus bisporus*) by lactic acid fermentation. Microbiologia 21: 16-21 (1994)
12. Joshi VK, Kaur M, Thakur NS. Lactic acid fermentation of mushroom (*Agaricus bisporus*) for preservation and preparation of sauce. Acta Aliment. Hung 25: 1-11 (1996)
13. Niksic M, Stojanovic M, Zivanovic S, Veljic S. Ecological approach in preservation of edible mushrooms by lactic acid fermentation. pp. 217 - 223. In: Ecology in Food Industry and Biotechnology. Baras J (ed). Poslovna zajednica "Vrenje", Belgrade, Serbia (1997)
14. Jabłońska-Ryś E, Kalbarczyk J, Sztaba A. The use of starter cultures of lactic and propionic acid bacteria in the process of button mushrooms fermentation. pp. 23-24. In: Abstracts: 5th Jubilee Scientific Conference, Food Quality and Safety: Determinants of Raw Materials, Technology, Manufacturing and Legal. November 17-18, Białobrzegi, Poland (2005)
15. Skąpska S, Owczarek L, Jasińska U, Hasińska A, Danielczuk J, Sokołowska B. Changes in the antioxidant capacity of edible mushrooms during lactic acid fermentation. Food Sci. Technol. Qual. 59: 243-250 (2008)
16. Milanovič N, Davidovič A, Savič A. Lactic acid fermentation of mushroom (*Agaricus bisporus*) with *Lactobacillus plantarum*. pp. 338-345. In: 9th Savjetovanje hemičara i tehnologa Republike Srpske. November 12-13, Banja Luka, Bosnia and Herzegovina (2010)
17. Jabłońska-Ryś E, Sławińska A. Possibilities of use of lactic fermentation in bio conservation of edible mushrooms fruit bodies. p. 185. In: Abstracts: 2nd International Conference and Workshop, Plant-the source of research material. October 18-20, Lublin, Poland (2012)
18. Radzki W, Sławińska A, Jabłońska-Ryś E, Gustaw W. Antioxidant capacity and polyphenolics content in dried wild growing edible mushrooms. Int. J. Med. Mushrooms 16: 65-75 (2014)
19. Dubost NJ, Ou B, Beelman RB. Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. Food Chem. 105: 727-735 (2007)
20. Choi Y, Lee SM, Chun J, Lee HB, Lee J. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. Food Chem. 99: 381-387 (2006)
21. Thetsrimuang C, Khammuang S, Chiablaem K, Srisomsap C, Sarnthima R. Antioxidant properties and cytotoxicity of crude polysaccharides from *Lentinus polychrous* Lév. Food Chem. 128: 634-639 (2011)
22. Stanisz A. Basics of statistics for scientific research. Part 21. The correlation analysis. Med. Prakt. 10: 176-181 (2000)
23. Witkowska AM, Zujko ME, Mirończuk-Chodakowska I. Comparative study of wild edible mushrooms as sources of antioxidants. Int. J. Med. Mushrooms 13: 335-341 (2011)
24. Barros L, Baptista P, Correia DM, Morais JS, Ferreira ICFR. Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. J. Agr. Food Chem. 55: 4781-4788 (2007)
25. Woldegiorgis AZ, Abate D, Haki GD, Ziegler GR. Antioxidant property of edible mushrooms collected from Ethiopia. Food Chem. 157: 30-36 (2014)
26. Xu B, Chang SKC. Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. J. Agr. Food Chem. 56: 7165-7175 (2008)
27. Zhao Z, Moghadasian MH. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. Food Chem. 109: 691-702 (2008)
28. Othman NB, Roblain D, Chammen N, Thonart P, Hamdi M. Antioxidant phenolic compounds loss during the fermentation of chétoui olives. Food Chem. 116: 662-669 (2009)
29. Svensson L, Sekwati-Monang B, Lutz DL, Schieber A, Gänzle MG. Phenolic acids and flavonoids in nonfermented and fermented red sorghum (*Sorghum bicolor* (L.) Moench). J. Agr. Food Chem. 58: 9214-9220 (2010)
30. Rodríguez H, Landete JM, de las Rivas B, Muñoz R. Metabolism of food phenolic acids by *Lactobacillus plantarum* CECT 748$^T$. Food Chem. 107: 1393-1398 (2008)