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Effect of blanching and cooking on antioxidant capacity of cultivated edible mushrooms: A comparative study

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Abstract

Three common species of cultivated edible mushrooms were analyzed in terms of their antioxidant capacity and total phenolics content. Fruiting bodies of *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes* were subjected to blanching in a citric acid solution for five minutes and boiling in water for fifteen minutes. Both ethanolic and water extracts were obtained from the mushrooms and their antioxidant abilities were evaluated with FRAP and DPPH assays. The content of total phenolic compounds in the extracts was determined. Among the three analyzed species, *A. bisporus* possessed the highest antioxidant capacity and total phenolics content, whereas *L. edodes* had the lowest. In general, the processing resulted in the decrease of the antioxidant activity and the species which was the most vulnerable to the hydrothermal treatments was *P. ostreatus*.

Introduction

Number of studies suggests that oxidative stress, caused by free radicals, can damage vital cellular molecules like lipids, proteins or DNA. As a consequence, it may contribute to the development of chronic degenerative disorders such as cancer or cardiovascular diseases (Limón-Pacheco and Gonsebatt 2009; Birben *et al*. 2012). Both prokaryotic and eukaryotic organisms possess enzymatic systems which provide protection against the oxidative stress. However, this innate protection may not be sufficient enough to neutralize the excess of free radical species. Therefore, it is believed that consumption of food that is rich in natural antioxidants may boost organism's protection against negative impact of reactive oxygen species (Lotito and Frei 2006; Blasa *et al.* 2010; Wang *et al*. 2011).

Edible mushrooms, including three the most popular cultivated species, i.e. *Agaricus bisporus, Pleurotus ostreatus* and *Lentinula edodes,* accumulate various antioxidant metabolites. Among these metabolites phenolic compounds are the most abundant and thus are primarily responsible for the total antioxidant capacity of mushrooms (Ferreira *et al.* 2009; Karaman *et al.* 2010). Other antioxidants which are considered to be minor include tocopherols, vitamin C and ergothioneine (Sapozhnikova *et al.* 2014; Cheung 2010).

Mushroom fruiting bodies are rarely eaten raw and some kind of culinary processing is usually applied before ingestion. This may include cutting, boiling, frying, stewing or microwave cooking. Moreover, mushrooms which are intended to be stored for a period of time (dried, frozen, pickled or fermented) are often subjected to blanching. Several previous studies have reported that treatments of vegetables, especially these where elevated temperature is involved, may lead to substantial changes in the chemical composition and the antioxidant activity (Galgano *et al*. 2007; Yao and Ren 2011; Ahmed and Ali 2013). However, little attention has been paid to the impact of hydrothermal processing on the antioxidant capacity of mushrooms. Moreover, the existing studies have focused solely on alcoholic extracts. Therefore, this study aims to compare the influence of blanching (in citric acid solution) and boiling on the antioxidant potential and total phenolics content of both water and ethanolic extracts obtained from mushrooms. The comparative study was done on three common cultivated species: A. *bisporus, P. ostreatus* and *L. edodes*.

Material and Methods

Mushroom samples

Three mushroom species were selected for this

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study: *Agaricus bisporus, Pleurotus ostreatus* and *Lentinula edodes*. Mushroom fruiting bodies of every species were obtained directly from commercial growers and belonged to one crop. The samples were stored at 5°C prior to the treatments. The mushrooms were processed within five hours after the harvest.

Processing of mushrooms

The samples of all the species were subjected to two hydrothermal treatments. They were blanched in 0.5% (w/v) citric acid solution for five minutes at 95°C and boiled in water for 15 minutes at 100°C. Fruiting bodies belonging to the control group were not subjected to any treatment. All the samples were then lyophilised with an Alpha 1-2LD plus freeze dryer (Christ, Germany), vacuum-sealed and kept at room temperature before further analysis.

Solvent extraction

Dried fruiting bodies were ground in a WŻ-1 mill (Społem, Poland). One gram of the powdered fruiting bodies were extracted with 30 mL of water and 80% (v/v) ethanol. The extractions were performed for 60 minutes with a rotary shaker at 80°C and 175 rpm. The samples were then centrifuged at the speed of 3755 x g for 20 minutes and the supernatants were referred as water extracts and ethanolic extracts. The extractions were done in triplicate and the extraction yields (expressed as a percent of dry weight) were determined after complete evaporation (48 h, at 70°C) of 1 mL of the extracts.

Determination of polyphenolics concentration

The content of total phenolics was quantified according to the procedure described by Singleton and Rossi (1965), later modified (Dubost *et al.* 2007). An aliquot of each extract (0.2 mL) was mixed with 0.8 mL of Folin and Ciocalteu's phenol reagent which was ten times diluted. After 3 minutes, 1.25 mL of 7% Na_2CO_3 was added to the mixtures. The mixtures were incubated at ambient temperature in the dark for 30 minutes and the absorbance was recorded at 725 nm (Helios Gamma, Thermo, USA). The reference antioxidant compound used was gallic acid and total phenolics amount was expressed as gallic acid equivalent (GAE) per 1 g of mushroom dry weight (dw).

DPPH radicals scavenging activity

The scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was measured with the methodology developed by Blois *et al* (1958). The samples (0.2 mL) were added to 0.8 mL of 0.2 mM DPPH ethanolic solution and the mixtures were shaken with a vortex before they were left to stand for 15 minutes in the dark. The absorbance of the remaining DPPH radicals was recorded at 520 nm against a blank sample and compared with Trolox (synthetic analogue of vitamin E) standard curve. The antioxidant capacity was expressed as μmoles Trolox equivalent (TE) per 1 g of mushroom dry weight.

Ferric-reducing antioxidant power assay

The ferric-reducing antioxidant power (FRAP) of the mushroom extracts was determined by adapting the procedure developed by Benzie and Strain (1996). FRAP reagent was prepared as follows: 300 mM acetate buffer (pH 3.6) was mixed with 10 mM 2,4,6-tripyridyl-striazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3 \bullet 6H_2O$ (10:1:1 ratio). The samples (0.1 mL) were added to 1.9 mL of FRAP reagent and the mixtures were incubated at 37°C in the dark for 15 minutes and the absorbance was read at 593 nm. The results were compared with the standard curve using Trolox and were reported as μmoles Trolox equivalent (TE) per 1 g of mushroom dry weight.

Statistical analysis

Statistical evaluation was carried out with Statistica v.10 (Statsoft, Poland). All the measurements were done in triplicate and the data were expressed as the mean ± SD (standard deviation). The data were assessed using analysis of variance ANOVA with a level of significance set at $p<0.05$. The LSD (least significance difference) test was employed to compare the statistically different data.

Results and Discussion

Extraction yields

The yields of water and ethanolic extracts of mushroom fruiting bodies are summarized in Fig 1a and Fig 1b, respectively. The amounts of extracts obtained from the non-processed mushrooms depended on the solvent type and was notably higher in the case of water extraction. This finding is consistent with those of Tsai *et al*. (2007) who reported that water extraction of wild-growing mushrooms produced much higher yields comparing to ethanolic extraction. This discrepancy may be explained by the fact that water extracts contain solubilized polysaccharides which are not soluble in ethanolic solution (Lee *et al*. 2007).

The yields of water extracts obtained from the non-processed mushrooms were species-dependent. The highest value was observed for *A. bisporus* (59.2 ± 1.4%), while the lowest for *L. edodes* (41.4 ± 1.2%).

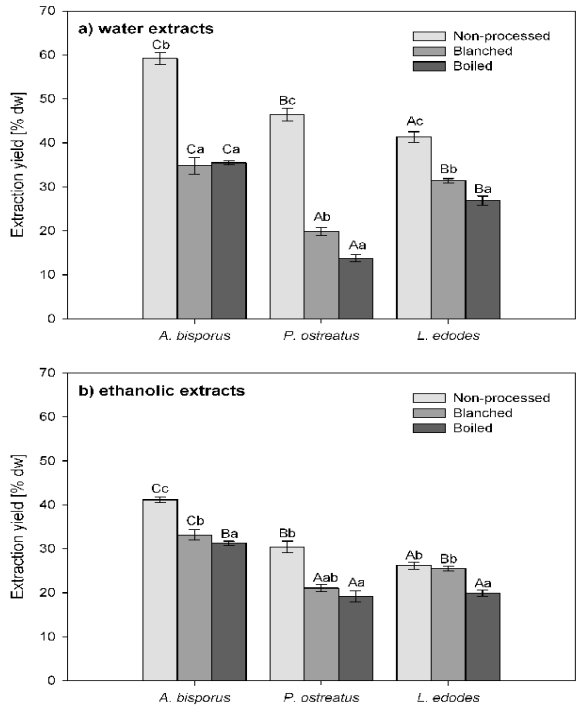

Figure 1. Extraction yields of water (a) and ethanolic (b) extracts. Mean values with different capital letters indicate significant differences among the species within the same treatment. Mean values with different lowercase letters represent differences among the treatments within the same mushroom species ($p<0.05$, LSD test)

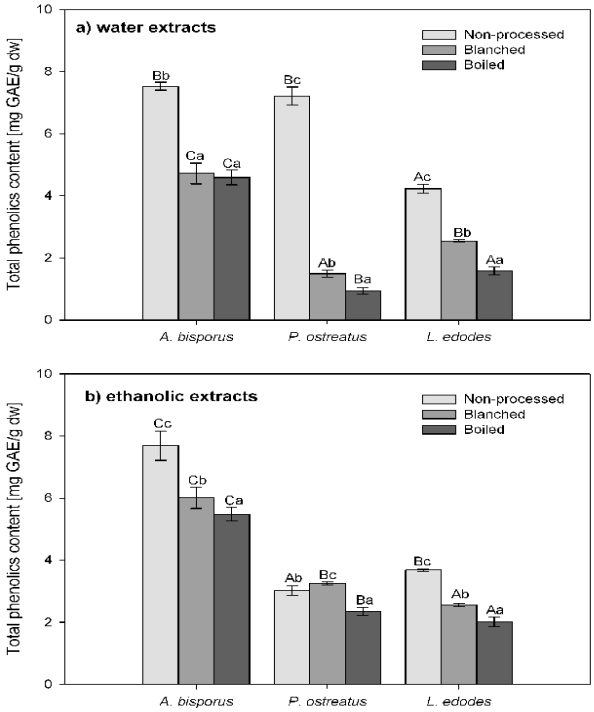

Figure 2. Total phenolics content in water (a) and ethanolic (b) extracts. Mean values with different capital letters indicate significant differences among the species within the same treatment. Mean values with different lowercase letters represent differences among the treatments within the same mushroom species ($p<0.05$, LSD test)

Blanching and boiling led to the significant decrease of the yields, regardless of the analyzed species. However, the loss of soluble compounds caused by boiling was usually greater than by blanching. The highest drop (~70%) was observed for boiled *P. ostreatus*, whereas the lowest (~35%) for blanched *L. edodes*.

A similar trend to water extracts was noticed for ethanolic extracts. Among the non-processed samples, the highest ethanolic yield was observed for *A. bisporus* (41.2 ± 0.7%), while the lowest for *L. edodes* (26.2 ± 0.8%). A marked decline in yields was noticed in fruiting bodies that were submitted to the processing. However, the decrease was not as evident as in the case of water extracts and ranged from ~37% for cooked *P. ostreatus* to ~2.7% for blanched *L. edodes*.

Content of polyphenolic compounds

The data on polyphenolics content in water and ethanolic extracts are illustrated in Fig 2. Both solvents used led to fair extraction of phenolic compounds. In general, water extracts which were obtained from non-processed mushrooms contained noticeably more phenolic compounds. The exception was *A. bisporus*, in which their content was at a similar level. This fact is consistent with the results of other experiments that were conducted on mushrooms (Radzki *et al.* 2014; Puttaraju *et al.* 2006; Sudha *et al.* 2012). However, water extracts may contain compounds (e.g. proteins or vitamin C) which interfere with the reagents and thus the results may be overestimated.

Figure 2a shows that the content of polyphenolics in water extracts differs among the analyzed species. With regard to the non-processed samples, the highest amount was reported for *A. bisporus* (7.53 ± 0.13 mg/g dw) and *P. ostreatus* (7.21 ± 0.29 mg/g dw), while the lowest for *L. edodes* (4.23 ± 0.14 mg/g dw). The processing caused a rapid drop in water soluble polyphenolics content. This effect was especially evident in *P. ostreatus* where ~79% and 87% decrease was noticed in blanched and boiled samples, respectively. Boiling led to higher loss of polyphenolics compared with blanching with an exception of *A. bisporus* where no statistical difference was found between these two processing techniques.

The data on ethanolic extracts is provided in Figure 2b. The concentration of polyphenolics in the non-processed mushrooms varied from 7.69 ±

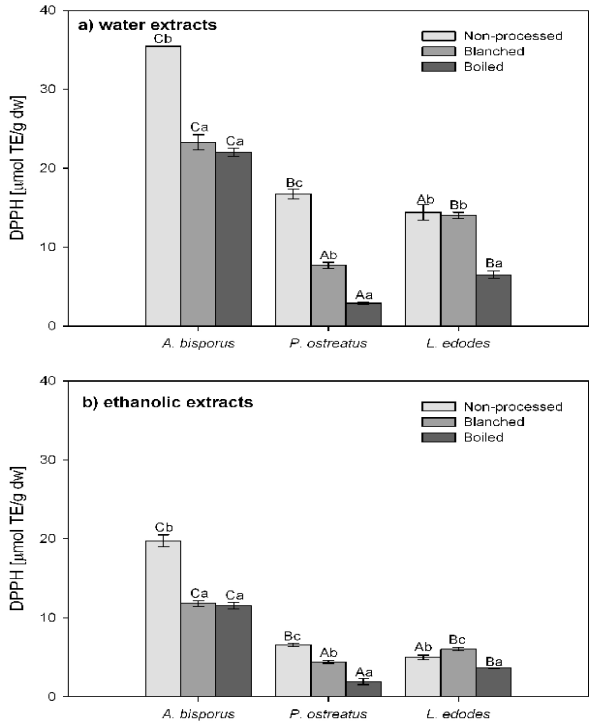

Figure 3. Scavenging effect on DPPH radicals of water (a) and ethanolic (b) extracts. Mean values with different capital letters indicate significant differences among the species within the same treatment. Mean values with different lowercase letters represent differences among the treatments within the same mushroom species ($p<0.05$, LSD test)

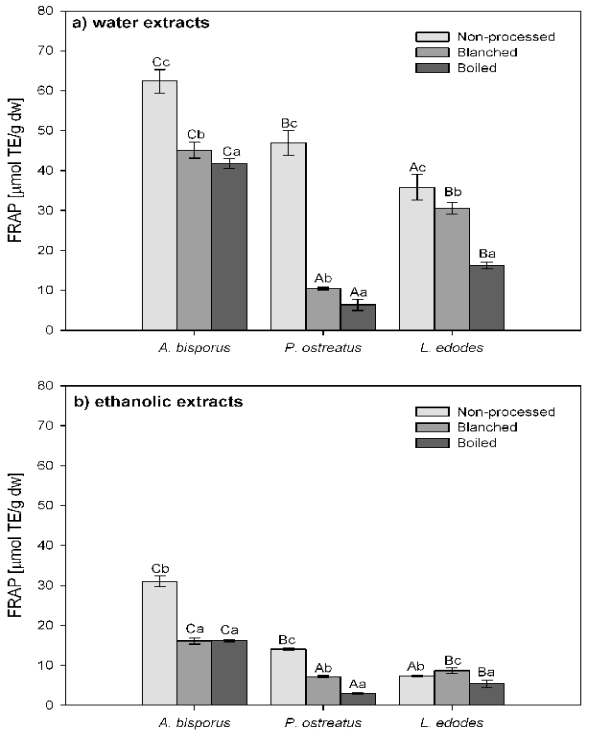

Figure 4. Ferric-reducing antioxidant power of water (a) and ethanolic (b) extracts. Mean values with different capital letters indicate significant differences among the species within the same treatment. Mean values with different lowercase letters represent differences among the treatments within the same mushroom species ($p<0.05$, LSD test)

0.47 mg/g dw *(A. bisporus)* to 3.02 ± 0.15 mg/g dw *(P. ostreatus)*. Interestingly, unlike water extract, ethanolic extract from *P. ostreatus* contained slightly less phenolic compounds than the extract from *L. edodes*. These findings are consistent with an earlier study by Dubost *et al.* (2007), where ethanolic extracts from *A. bisporus, P. ostreatus* and *L. edodes* were compared. Blanching resulted in the decrease of polyphenolics which amounted to ~32% in *L. edodes* and ~22% in *A. bisporus.* Similarly to water extracts, boiling had a more deleterious effect on polyphenolics than blanching. This is in good agreement with Jagadish *et al.* (2009) who reported that boiled *A. bisporus* contained significantly less polyphenolics compared with the non-processed sample. The detrimental effect of the treatments on the concentration of polyphenolics could be attributed mainly to a leaching effect and alterations of structure of these compounds (Barros *et al.* 2007).

Scavenging effect on DPPH radicals

The antioxidant potential of water and ethanolic extracts was evaluated using the DPPH assay (Figure 3). It is apparent that water extracts exhibited higher antioxidant capacity, regardless of species and processing techniques. This is partially consistent with previous findings in which, water extracts from *A. bisporus* and *L. edodes* exerted much higher DPPH scavenging activity than alcoholic extracts (Piljac-Žegarac *et al.* 2011). However, in contrast to the current study, water extracts from *P. ostreatus* had lower activity.

As shown in Figure 3a the antioxidant capacity of water extracts obtained from non-processed fruiting bodies was in the descending order of *A. bisporus* (35.46 ± 0.06 µmol TE/g dw), *P. ostreatus* (16.74 ± 0.60 µmol TE/g dw), *L. edodes* (14.39 ± 0.97 µmol TE/g dw). Along with the decrease of total phenolics content, the decline in DPPH scavenging activity was noticed due to the applied hydro-thermal processing. A very high linear correlation between the total phenolics content in water extracts and the ability to neutralise DPPH radicals was established (R=0.849). Blanching resulted in ~34% decrease in *A. bisporus* and ~54% decrease in *P. ostreatus*, whereas it had no influence on *L. edodes*. Boiling caused further reduction in the activity in case of *P. ostreatus* (~83%) and affected the activity of *L. edodes* (~55% decrease). Interestingly, boiling produced similar effect to blanching in the case of *A. bisporus*.

The ability to neutralize DPPH radicals by ethanolic extracts is presented in Figure 3b. Similarly to water extracts, the non-processed *A. bisporus* showed the highest antioxidant potential (19.73 ± 0.78 µmol TE/g dw), whereas the lowest activity was exerted by *L. edodes* (5.01 ± 0.27 µmol TE/g dw). The fact that alcoholic extracts from *A. bisporus* exhibited remarkably higher DPPH radicals scavenging activity compared with the other two analyzed species was reported in earlier studies (Reis *et al.* 2012; Fu *et al.* 2002; Ferrari *et al*. 2012). The impact of the processing on the fruiting bodies was similar to those reported for water extracts. Blanched fruiting bodies exhibited lower activities in the case of *A. bisporus* and *P. ostreatus* where the decrease rate amounted to ~34% and ~54%, respectively. To the contrary, ethanolic extract from *L. edodes* showed ~20% higher antioxidant capacity. Similar effect was reported by Choi *et al*. (2005) and can be attributed to the formation of secondary compounds with the increased antioxidant capacity. However, this effect was not noticed in boiled *L. edodes*, where ~27% decrease was observed. The highest decline in the antioxidant capacity among the boiled mushrooms was noticed in *P. ostreatus* (~71%), followed by *A. bisporus* (~42%). Our experiment confirmed the findings by other authors who observed that hydrothermal processing resulted in the substantial drop of the antioxidant activity (Soler-Rivas *et al.* 2009; Nguyen *et al.* 2012). According to these authors, this negative impact could be explained by the disruption of mushroom cell walls and releasing water-soluble antioxidant compounds into surrounding water. The correlation between the total phenolics content and DPPH scavenging activity was found to be 0.951.

Ferric-reducing antioxidant power

FRAP analysis is commonly employed to assess the antioxidant activity of various food samples, including mushrooms. As shown in Fig 4, water extracts displayed significantly higher reducing potential than ethanolic extracts, which was similar to the previous assay. This finding is in agreement with Gan's findings (2013) which showed that reducing power of a water extract from A. bisporus displayed approximately twofold higher activity compared with an ethanolic extract.

The activity of water extracts obtained from the non-processed samples ranged from 62.39 ± 2.92 µmol TE/g dw *(A. bisporus)* to 35.83 ± 3.16 µmol TE/g dw *(L. edodes)* (Figure 4a) and the values correlated well with the content of total phenolics (R=0.945) and the DPPH scavenging activity (R=0.942). Blanching led to the substantial changes of the activity in *A. bisporus* and *P. ostreatus* (~28% and ~78% decrease, respectively), whereas in *L. edodes* the noticed decrease was rather minor and amounted to ~15%. As the result of boiling, further reduction in the activity was observed in all the species.

The highest antioxidant potential of ethanolic extracts among the non-processed fruiting bodies exerted *A. bisporus* (31.01 ± 1.35 µmol TE/g dw), followed by *P. ostreatus* 14.11 ± 0.28 µmol TE/g dw) and *L. edodes* (7.31 ± 0.13 µmol TE/g dw). This comparison is consistent with the findings reported in previous work (Piljac-Žegarac *et al.* 2011). Blanched fruiting bodies of *A. bisporus* and *P. ostreatus* displayed lower ability to reduce Fe^{3+}-TPTZ complex, compared with the non-processed samples (the decrease amounted to ~48% and ~49%, respectively). However, the exception was *L. edodes*, where the slight increase (~19%) of the activity was observed. With regard to boiled samples, the drop of the reducing power was noticed in all the analyzed species. The highest decrease was observed in *P. ostreatus* (~79%) and the lowest in *L. edodes* (~26%). Interestingly, there was no difference in the antioxidant activity between boiled and blanched *A. bisporus*. FRAP activity of ethanolic extracts correlated well with the content of phenolics (R=0.898) and the ability to scavenge on DPPH radicals (R=0.975).

Conclusions

Our findings allowed us to conclude that in general hydro-thermal processing could lead to the alterations of total phenolics content and the antioxidant capacity of mushrooms. Boiling for 15 minutes had deleterious effect in all the tested species, regardless of the solvent type that was used for preparing the extracts. On the other hand, blanching in citric acid solution for five minutes caused the increase of alcohol-soluble phenolics in *P. ostreatus* and improved antioxidant potential in *L. edodes.* The research has also shown that generally the loss of polyphenolics due to the applied procedures was much higher in water extracts compared with the ethanolic ones. Moreover, the results revealed that the three tested species differed significantly in the susceptibility to the processing. The species which seemed to be the most vulnerable to the applied treatments was *P. ostreatus.*

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