An insight into *in vitro* antioxidant activity of *Cantharellus cibarius* hot water extract for the potential application in meat products

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An insight into *in vitro* antioxidant activity of *Cantharellus cibarius* hot water extract for the potential application in meat products

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Abstract. The current research was undertaken to estimate the *in vitro* antioxidant activity of *Cantharellus cibarius* mushroom extracted by boiling in water for 30 minutes. Several previous studies have shown that the addition of edible mushrooms in meat products affects the reduction of lipid oxidation and prolongs the shelf-life of the final products. Antioxidant capacity of *C. cibarius* was measured using the following methods: reducing power ability, lipid peroxidation assay, cupric ion reducing antioxidant capacity (CUPRAC) and DPPH free radical scavenging activity. Reducing power and antioxidant activity assays of *C. cibarius* hot water extract showed low antioxidant activity. CUPRAC assay demonstrated positive effect only at the concentration of 10 mg/mL, whereas DPPH radical scavenging activity showed moderate antioxidant activity in comparison with culinary-medicinal mushrooms, with the effective concentration (EC_{50}) from 7.41 mg/mL.

1. Introduction

Mushrooms have various quality characteristics that have been defined by Djekic *et al*. [1] and they are particularly respected for their taste and texture [2]. A plenty of protein, fiber, vitamins and minerals are contained in mushrooms. Typically, dried mushrooms comprise of 22% protein, which contains most of the essential amino acids, 5% fat, mostly in the form of linoleic acid (the essential fatty acid not

synthesized in the human organism), 63% carbohydrates including fiber and 10% good source of minerals counting thiamin, riboflavin, niacin and biotin [3].

Mushrooms have a tendency to gather a variety of secondary metabolites including phenolic compounds, polypeptides, terpenes, steroids, etc. Their phenolic compounds have been found to be an excellent antioxidants [4]. It is very important due to the fact that oxidation is one of the most important processes of food deterioration since it may affect food safety, color, flavor and texture [5].

Antioxidants or molecules with radical scavenging capacity are believed to exert a potential defending effect against free radical destruction. Methanol and/or water extracts from common button (*Agaricus bisporus*), shiitake (*Lentinus edodes*), straw (*Volvariella volvacea*), oyster (*Pleurotus* sp.), winter (*Flammulina velutipes*), ear (*Auricularia* sp. and *Tremella* sp.) mushrooms [6,7] have displayed important antioxidant activities [8]. Presently, not many reports can be found depicturing the bioactive effects of wild edible mushrooms usually found in European woods [9].

The chanterelle *Cantharellus cibarius* is broadly viewed as among the most desired of wild edible mushrooms [10]. It is presumably the best known species of the genus *Cantharellus*, if not of the complete family of *Cantharellaceae*. *C. cibarius* is world famous not only as palatable food, but also because of its spreading from Scandinavia to the Mediterranean in Europe [10].

Mushroom decoctions contain the hot water dissolvable components from the fruiting body. Thus, squash or small pieces of the fruiting body are boiled and the descend decoction is consumed. Besides that, mushrooms are generally not eaten raw, but subjected to various food processing procedures in order to be more readily assimilated by digestion [11]. Hence, it can be thought that preparation of hot water extracts simulate cooking conditions - the characteristic manner of how edible mushrooms are consumed or how the product of interest (cooked sausages) are processed. Therefore, the objective of our investigation is to examine whether *Cantharellus cibarius* water decoction exerts antioxidant activity *in vitro*, through the following methods: the reducing power, inhibition of lipid peroxidation, CUPRAC (cupric reducing antioxidant capacity) and the scavenging capacity of the radical DPPH. In relation to this, it will be decided whether this mushroom could be used for the production of cooked sausages in order to potentially extend the shelf-life of the final product.

2. Materials and methods

2.1. Preparation of mushroom decoction

Milli-Q water, obtained from a Milli-Q water purification system (Merck, Darmstadt) was used. In order to obtain a decoction (hot aqueous mushroom extract), a mixture of dry powdered mushroom and MQ water (1:10) was heated at 100ºC, 30 min. The resulting decoction was subjected to the assays determining the reducing power, inhibition of lipid peroxidation, CUPRAC (cupric reducing antioxidant capacity) and the scavenging capacity of the radical DPPH *in vitro*.

2.2. Reducing power (FRAP)

The reducing power was determined according to Petrović *et al.* [12].

2.3. Lipid peroxidation

Conjugated diene method according to Lingnert *et al.* was used [13].

2.4. Cupric reducing antioxidant capacity (CUPRAC)

The ability of samples to reduce cupric ion was determined according to method described by Öztürk *et al.* [14]. Solutions of Cu(II) (10 mM, 0.05 mL), neocuproine (7.5 mM, 0.05 mL), NH_4Ac buffer (1 M, pH 7.0, 0.06 mL) and serial dilutions of mushroom decoction prepared in MQ water (0.04 mL), were added to

a 96-well microplate and incubated for 1h, at 30 °C. The absorbance was measured at microplate reader (Lab Companion, VM-96, Korea) at 450 nm, against blank solution (water was added instead of sample solution).

2.5. DPPH free radical scavenging activity

The method was performed according to Vunduk *et al*. [15]. Extract solutions were prepared in MQ water (Merck, Darmstadt).

2.6. Statistical analysis

The data were analysed by one-way ANOVA using the SPSS software version 23 (Chicago, Illionis USA). Differences between the means were compared by Tukey's comparative test. A significance level of $P<0.05$ was used for evaluations.

2. Results and discussion

3.1. Reducing power ability

The capability of mushroom extracts to donate electrons could be assessed using the reducing power assay. In the presence of antioxidants, the Fe^{3+}-ferricyanide complex is reduced to the ferrous form, Fe^{2+} and the latter can be monitored by measuring the formation of Perl's Prussian blue at 700 nm; higher absorbance indicates better reducing power [16]. The ability of mushroom extract and control (ascorbic acid and butylated hydroxyanisole) to reduce Fe^{3+} to Fe^{2+} at different concentrations are shown in Table 1.

Table 1. Reducing power of *C. cibarius* hot water extract

Concentration (mg/mL)	*C. cibarius*
0.625	0.031 ± 0.010^{A}
1.25	0.040 ± 0.000^{A}
2.5	0.102 ± 0.021^{A}
5	0.280 ± 0.000^{A}
10	0.534 ± 0.022^{B}
Positive controls*	
Butylated hydroxyanisole (BHA)	2.506 ± 0.054^{C}
Ascorbic acid	2.380 ± 0.197^{C}

Notes: Values are mean ± standard deviation. Means in the same column with different capital letters are significantly different ($P<0.05$)
*Absorbance values for positive controls were measured at the concentration of 0.50 mg/mL.

At the concentration of 0.50 mg/mL, the positive controls that is BHA and ascorbic acid displayed high-reducing ability of 2.506 and 2.380, respectively, which are distinctively higher than that obtained from any *C. cibarius* extracts. Mushroom extracts exhibited a variable reducing capacity and general, the reducing capacity increased with increasing concentration, but only at the concentration of 10 mg/mL, it was significantly higher. In comparison to the other investigations of hot water extracts of mushrooms, Tsai *et al*. [17] claimed that reducing powers of *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis* were determined at 0.83, 0.86 and 1.15 at 5 mg/mL, respectively. It is significantly higher than the absorbance obtained from *C. cibarius* even at the concentration which is twice lower. Other authors reported that hot water extracts from Ling chic exhibited reducing powers of 0.48 and 0.44 at 1 mg/mL and 1.08 and 1.04 at 5 mg/mL, respectively. Also, at 5 mg/mL, *P. citrinopileatus* displayed a high reducing power of 1.10 [18] in comparison to our mushroom which absorbance is about twice lower in

twice higher concentration. Apparently, comparing to the other commercial and medicinal mushrooms, *C. cibarius* showed lower reducing power ability for hot water extracts.

3.2. Lipid peroxidation

The results obtained using the conjugated diene method for antioxidant activity are shown in Table 2.

Table 2. The ability of *C. cibarius* extract and commercial antioxidants to prevent the peroxidation of linoleic acid

Concentration (mg/mL)	*C. cibarius*	Ascorbic acid	α-tocopherol
0.1	$0 \pm 0^{a,A}$	$78.33 \pm 0.91^{b,A}$	$82.77 \pm 0.75^{c,A}$
1	$0 \pm 0^{a,A}$	$79.50 \pm 0.75^{b,A}$	$82.88 \pm 0.06^{c,A}$
2.5	$0 \pm 0^{a,A}$	$79.60 \pm 0.9^{b,A}$	$81.53 \pm 0.11^{c,A,B}$
5	$11.75 \pm 1.98^{a,B}$	$80.84 \pm 0.72^{b,A,B}$	$80.94 \pm 0.06^{b,B}$
10	$22.36 \pm 1.34^{a,C}$	$82.73 \pm 0.8^{b,B}$	$82.36 \pm 1.11^{b,A}$

Notes: Values are mean ± standard deviation. Means in the same column with different capital letters and means in the same row with different lowercase letters are significantly different ($P<0.05$).

The conjugated diene method is based on the ability of the substance to slow the oxidation of conjugated dienes, which can be formed only by polyunsaturated fatty acids. At the concentration of 5 and 10 mg/mL, mushroom extract showed low antioxidant activity, while in lower concentration did not show any effect at all. Widely used commercial antioxidants, ascorbic acid and α-tocopherol expressed high antioxidant activity at the investigated concentrations. Hot water extracts from wild edible mushrooms, such as *Boletus edulis* showed high antioxidant activity (85.7%) at 5 mg/mL [17]. Similarly, at 5 mg/mL, *H. marmoreus* showed moderated antioxidant activities (38.6%) [19]. Regarding the method of slowing the oxidation of conjugated dienes, our mushroom has not achieved the expected effect.

3.3. CUPRAC assay

The CUPRAC assay utilized copper (II)–neocuproine (Cu(II)-Nc) reagent as the chromogenic oxidizing agent. It is based on the monitoring of the formation of stable complex between neocuproine and copper (I) by measurement of absorbance at 450 nm [16]. CUPRAC of the mushroom extracts was assessed and compared to that of the positive controls and shown in Table 3.

Table 3. CUPRAC of *C. cibarius* and commercial antioxidants

Concentration (mg/mL)	*C. cibarius*	Butylated hydroxytoluene (BHT)	α-tocopherol
0.1	$0 \pm 0^{a,A}$	$0.90 \pm 0.1^{b,A}$	$0.44 \pm 0.04^{c,A}$
1	$0 \pm 0^{a,A}$	$1.54 \pm 0.13^{b,B}$	$0.69 \pm 0.01^{c,B}$
2.5	$0 \pm 0^{a,A}$	$2.52 \pm 0.02^{b,C}$	$1.11 \pm 0.01^{c,C}$
5	$0 \pm 0^{a,A}$	$3.33 \pm 0.1^{b,D}$	$1.48 \pm 0.08^{c,D}$

10	$1.545 \pm 0.2^{a,B}$	$4.08 \pm 0.04^{b,E}$	$2.06 \pm 0.04^{c,E}$

Notes: Values are mean ± standard deviation. Means in the same column with different capital letters and means in the same row with different lowercase letters are significantly different (P<0.05)

Testing the antioxidative ability of mushroom extract using the CUPRAC method, it was observed that only at the concentration of 10 mg/mL *C. cibarius* demonstrated the antioxidative activity. However, it is worth mentioning that antioxidative potential of *C. cibarius* determined in CUPRAC assay for the highest tested concentration, was only 25% lower than the same determined for the model antioxidant α-tocopherol. Presently, CUPRAC assay is not frequently used among researchers working on antioxidant studies and little has been published on the assessment of cupric ion-reducing ability of mushroom, especially for the hot aqueous extracts [20, 21]. In the study of Abdullah *et al*. [20], the minimum and maximum absorbance from 14 culinary-medicinal mushrooms, at the concentration of 10 mg/mL were in range from 1.739 ± 0.222 to 2.778 ± 0.015, which is higher in comparison to our result.

3.4. Scavenging activity of DPPH radical

One of the most common procedures for determination of antioxidant capacity is the DPPH free radical scavenging activity assay [20]. DPPH assay is based on the measurement of the scavenging capability of antioxidants toward the stable radical DPPH. The DPPH radical is reduced to the matching hydrazine when it reacts with hydrogen donors [22].

The results of *C. cibarius* hot water extract and commercial antioxidants scavenging ability are shown in Table 4.

Table 4. Scavenging ability of hot water extract from *C. cibarius* and commercial antioxidants

Concentration (mg/mL)	*C. cibarius*	Ascorbic acid	Butylated hydroxytoluene (BHT)
0.1	$12.72 \pm 10.02^{a,A}$	$81.33 \pm 1.11^{b,A}$	$5.56 \pm 1.17^{c,A}$
1	$13.43 \pm 2.95^{a,A}$	$83.65 \pm 0.09^{b,B}$	$10.51 \pm 0.70^{a,B}$
2.5	$20.57 \pm 1.85^{a,A,B}$	$83.38 \pm 0.15^{b,B}$	$21.10 \pm 1.00^{a,C}$
5	$26.08 \pm 9.15^{a,B}$	$84.38 \pm 0.26^{b,B}$	$32.93 \pm 1.81^{a,D}$
10	$31.20 \pm 1.10^{a,B}$	$81.07 \pm 0.49^{b,A}$	$54.12 \pm 1.00^{c,E}$

Notes: Values are mean ± standard deviation. Means in the same column with different capital letters and means in the same row with different lowercase letters are significantly different (P<0.05)

As shown in Table 4, scavenging ability of mushroom extracts was dependent on the concentration of the extract. In comparison to ascorbic acid, *C. cibarius* had significantly lower values for each concentration, whereas with BHT, mushroom exhibited almost identical ability except for the concentration 10 mg/mL, when BHT expressed significantly higher value.

The scavenging activity of mushroom extracts towards DPPH free radicals can also be expressed in term of EC_{50}. EC_{50} (mg/mL) is the effective concentration of the mushroom extract that are required to show 50% antioxidant properties. A lower EC_{50} value corresponds to higher antioxidant activity of the mushroom extract [10]. Puttaraju *et al*. [23] showed that a hot water extract from *C. cibarius* among 23 mushrooms naturally grown in India took 21st place with EC_{50} value 6.40 (mg/mL). Our result (7.41 mg/mL) for EC_{50} corresponds to the investigation from Puttaraju *et al*. [23] and also it would take the same place among investigated mushrooms. On the other hand, Abdullah *et al*. [20] investigated the

scavenging activity from 14 culinary-medicinal mushrooms and showed the EC_{50} effect in the range 5.28-39.05 (mg/mL). Only *G. lucidum* showed better scavenging activity than our mushroom with the value for EC_{50} 5.28 mg/mL.

The capability of hot water extract of other mushrooms to quench free radicals has been described earlier. Hot water extracts of mature and baby Ling chih (*Ganoderma tsugae Murrill*) exhibited excellent antioxidant activities with low EC_{50} of 0.30 and 0.40 mg/mL, respectively [24]. In their study, Chirinang *et al*. [25] noticed that the radical scavenging activity of water extract of *Pleurotus ostreatus* (EC_{50} = 11.56 mg/mL) was better than that of *P. sajorcaju* (EC_{50} = 13.38 mg/mL) presumably due to higher content of phenolic compounds and dietary fibres. *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis* displayed moderate DPPH scavenging activities with EC_{50} of 13.75, 26.98 and 15.78 mg/mL, respectively [17]. It has been reported that the EC_{50} of hot water extract of *Hypsizygus marmoreus* was 4.19 mg/mL [19], while the white mutant strain of the same species was less effective with an EC_{50} of 18.85 mg/mL. In total, hot water extract of the mushroom tested by the DPPH method showed a moderate scavenging ability in relation to the other edible mushrooms researched.

There is a few studies about the addition of mushrooms in meat products. Pil-Nam *et al.* [26] proved that the adding of shiitake improved the sensory quality of frankfurters and slowed the lipid oxidation and aerobic bacteria growth during storage. In addition, Van Ba *et al.* [27] examined the effect of addition of shiitake (*Lentinula edodes*) extract on the quality characteristics of fermented sausages and decided that the addition of mushroom reduced lipid oxidation and retarded the growth of spoilage bacteria, as well as controlled the growth of pathogens. Also, Alnoumani *et al.* [28] showed remarkable inhibition of formation of lipid oxidation compounds when dried *Agaricus bisporus* powder was added to ground beef.

4. Conclusion

Future research is needed with other types of mushrooms and meat products in order to come with a general conclusion about the feasibility of their application in meat products in general.

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