

BIOACTIVE COMPOUNDS IN LATVIAN WILD EDIBLE MUSHROOM *BOLETUS EDULIS*

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Abstract

Considering the interest for mushrooms and the demand to search for natural antioxidants and other sources of bioactive-compounds, the aim of this study was to investigate the content of bioactive compounds of two widely used wild edible mushrooms *Boletus edulis f. beticola* and *Boletus edulis f. pinicola* collected at Jelgava and Riga regions in Latvia. Ash amount was determined to characterize the mineral content; protein was determined by Lowry method (325–526 mg g⁻¹ of dried mushrooms). Using HPLC the phenolic compounds like gallic acid, caffeic acid, catechin, epicatechin and rutin were detected and quantified. β -carotene and lycopene were determined. DPPH assay was used to evaluate free radical-scavenging activity. In water extracts titratable acidity (0.22–0.26 mmol of NaOH per g of dry mushroom matter) and formol number (0.74–1.40 mmol NaOH per g of dry mushroom matter) were determined. The total content of phenols (TP) as determined by Folin-Ciocalteu assay was higher in the water extracts (11.2–12.5 mg of gallic acid equivalents GAE per 1 g of dry mushroom matter) than in methanol extracts (7.3–8.0 mg of GAE per 1 g of dry mushroom matter). The total content of flavonoids (TF) was higher in the water extracts (0.33–0.37 mg of QE per 1 g of dry mushroom matter) than in methanol extracts (0.13 mg of quercetin equivalents QE per 1 g of dry mushroom matter).

Keywords: wild edible mushrooms, bioactive compounds

Introduction

Mushrooms have been widely known and used as a source of food from ancient time. Many species of mushrooms are used also as medicine (Barros et al., 2007a, Dembitsky et al., 2010). Mushrooms are very appreciated, not only for their texture, flavour, but also for their nutritional properties. Mushrooms have been demonstrated antitumor, antifungal, antibacterial activities (Chirinang et al., 2009) and are useful in preventing diseases such as hypertension, hypercholesterolemia, atherosclerosis and cancer (Ribeiro et al., 2006).

The mushrooms showed antioxidant, free radical-scavenging activity (Vidovic et al., 2010). These functional characteristics are mainly due to their chemical composition (Bernas et al., 2006). *Boletus* mushrooms are valuable food source, low in calories, lipids and high in vegetable proteins, minerals and vitamins. A study of the amino acid composition has showed *Boletus edulis* to have the highest total amino acid content of all the mushrooms tested. The major amino acids found were glutamine, alanine, glycine, serine, proline (Ribeiro et al., 2008). The major saturated fatty acid was 16:0, the major unsaturated fatty acid was 18:1(9) (Dembitsky et al., 2010). *Boletus edulis* presents a qualitative profile composed by oxalic, aconitic, citric, malic, quinic, fumaric acids. The major acids are malic and quinic acids (Ribeiro et al., 2006). Mushrooms are characterized by a high level of well assimilable mineral constituents. Potassium and phosphorus compounds were most abundant. *Boletus edulis* contains appreciable amount of selenium. Mushrooms are an important source of vitamins. The vitamins of group B are abundant, particularly thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, folic acid, cobalamin, as well as other vitamins, such as phylochinon, tocopherols, ergosterol (Bernas et al., 2006). Mushrooms have powerful antioxidant properties derived from compounds such as selenium, ergothioneine, phenols (Vidovic et al., 2010).

Considering the interest for mushrooms and the demand to search sources of bioactive-compounds, the aim of this study was to investigate the bioactive compounds content of two widely used Latvian wild edible mushrooms – king boletus *Boletus edulis f. beticola* and *Boletus edulis f. pinicola*.

Materials and Methods

Samples of *Boletus edulis f. beticola* and *Boletus edulis f. pinicola* were collected at Jelgava and Riga regions in Latvia in late summer 2010. After collection, the mushrooms were freeze-dried in order to obtain dry matter (Christ Freeze Dryer Alpha 1-2 LD plus). All dried mushroom samples were grounded in a blender and then stored in air-tight bags at the room temperature. Gravimetric method for the determination of ash amount was used as described by Mortensen et al. (1989). The content of proteins in mushroom dry matter was determined by Lowry procedure.

Mushroom powder sample (1 g) was extracted with 50 ml of methanol at 25 °C for 24 h. Water extract was prepared as follows: 1 g of powdered mushroom was boiled in 50 ml of water for 30 min. The mixture was centrifuged (3000×g, room temperature for 10 min), and supernatant portioned and kept frozen at -23 °C until analysis (Ribeiro et al., 2006, Barros et al., 2007a).

For all spectrophotometrical analysis Jenway 6405 UV/Vis. spectrophotometer were used. The total content of phenol compounds in water and methanol extracts was determined by Folin-Ciocalteu assay. Gallic acid (0-0.75 mg ml⁻¹) was used as a standard to produce the standard curve. The absorbance of the reaction mixture was measured at 765 nm. The total content of phenol compounds was expressed as milligrams of gallic acid equivalents (GAE) per gram of mushroom dry matter (Barros et al., 2007a).

The total content of flavonoids (TF) in water and methanol extracts was determined as described previously (Jia et al., 1999, Barros et al., 2007a, Robaszekiewicz et al., 2010). The absorbance of the supernatant was read at 515 nm against a blank. Quercetin (0-0.4 mg ml⁻¹) was used as a standard. The results were expressed as milligrams of quercetin equivalents QE per gram of mushroom dry matter. To determine polyphenol compounds HPLC analysis (Shimadzu LC-20 prominence) of the extracts was performed as described by Vidovic et al. (2010).

The concentration of the content of β-carotene and lycopene was determined spectrophotometrically. Obtained methanol extract was evaporated and 100 mg of the remaining dry matter were stirred with 10 ml of acetone-hexane mixture and filtered. The absorbance was measured at 453, 505, 645 and 663 nm. The content of β-carotene and lycopene was calculated according to the following equations (Barros et al., 2007b):

$$Lycopene(mg \cdot 100ml^{-1}) = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453} \quad (1)$$

$$\beta - carotene(mg \cdot 100ml^{-1}) = 0.216A_{663} - 0.304A_{505} + 0.452A_{453} \quad (2)$$

The results were expressed as milligrams of carotenoid per gram of dry matter.

The free radical scavenging activity of mushrooms in water and methanol extracts was determined with 1,1-diphenyl-2-picrylhydrazyl DPPH by measuring absorbance at 517 nm (Vidovic et al., 2010, Wang et al., 2010). Radical scavenging activity (% RSA) was calculated by the following equation (3):

$$\% RSA = 100 - \frac{(A_{sample} \cdot 100)}{A_{blank}} \quad (3)$$

In water extract titratable acidity and formol number was determined as described by Tanner and Brunner (1987).

Results are presented as the mean ± standard deviation of three measurements.

Results and Discussion

The yields of dry matter of *Boletus edulis f. beticola* and *Boletus edulis f. pinicola* samples were $10.5 \pm 0.4\%$ and $9.2 \pm 0.6\%$ accordingly.

The highest content of protein was found for *Boletus edulis f. pinicola* – 526 ± 2 mg per 1 g of mushroom dry matter (Table 1).

Table 1

The content of protein and ash in Latvian mushroom dry matter

Mushroom	Protein, mg g ⁻¹ in mushroom dry matter	Ash, % in mushroom dry matter
<i>Boletus edulis f. beticola</i>	325 ± 2	5.56 ± 0.06
<i>Boletus edulis f. pinicola</i>	526 ± 2	6.07 ± 0.03

Ash amount was determined to characterize the mineral content. Mineral content for *Boletus edulis f. pinicola* was higher than for *Boletus edulis f. beticola* (Table 1).

Titrateable acidity (total amount of acid in the solution as determined by the titration using a standard solution of sodium hydroxide) was determined in water extracts of mushroom dry matter and results expressed as mmol of NaOH per 1 g of mushroom dry matter. The total amount of acids for *Boletus edulis f. pinicola* and *Boletus edulis f. beticola* was very similar (Table 2). Amino acids present in water extract of mushroom dry matter were determined by formol titration and expressed as a formol number. The results were expressed as mmol of NaOH per 1 g of mushroom dry matter. Formol number for *Boletus edulis f. pinicola* was about 2 times higher than for *Boletus edulis f. beticola* (Table 2).

Table 2

Titrateable acidity (TA) and formol number (FN) in water extracts of Latvian mushroom dry matter

Mushroom	TA, mmol of NaOH g ⁻¹ of mushroom dry matter	FN, mmol of NaOH g ⁻¹ of mushroom dry matter
<i>Boletus edulis f. beticola</i>	0.22 ± 0.01	0.74 ± 0.02
<i>Boletus edulis f. pinicola</i>	0.26 ± 0.01	1.40 ± 0.02

The content of total phenols in water extracts of mushroom dry matter was higher for *Boletus edulis f. pinicola* (12.5 ± 0.3 mg of gallic acid equivalents GAE per 1 g of mushroom dry matter) than that for *Boletus edulis f. beticola* (11.2 ± 0.1 mg of GAE g⁻¹). Such tendency was observed also in methanol extracts of mushroom dry matter: for *Boletus edulis f. pinicola* (8.0 ± 0.1 mg of GAE per 1 g of mushroom dry matter) and for *Boletus edulis f. beticola* (7.3 ± 0.1 mg of GAE g⁻¹) (Figure 1).

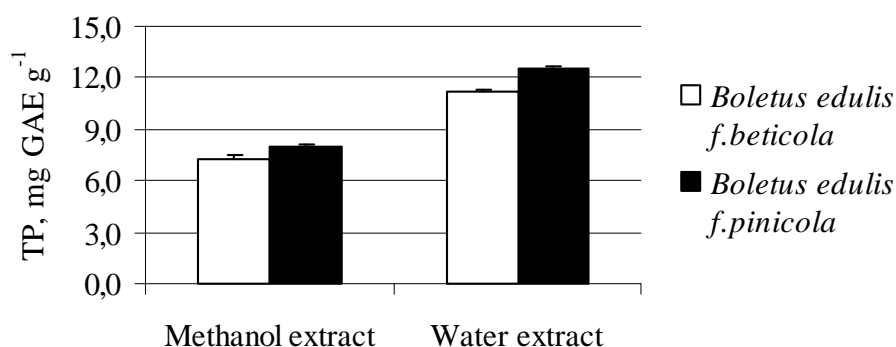


Figure 1. The content of total phenols (TP) in Latvian mushroom dry matter

In general, the concentration of total phenols was higher in water than in methanol extracts (Figure 1). The content of total flavonoids was identical for *Boletus edulis f. pinicola* and *Boletus edulis f. beticola* (0.13±0.1 mg of quercetin equivalents QE per 1 g of mushroom dry matter) in methanol extracts of mushroom dry matter and the same was observed in water extracts (0.37±0.1 and 0.33±0.1 mg of QE per 1 g of mushroom dry matter respectively). The concentration of total flavonoids was higher in water than in methanol extracts (Figure 2).

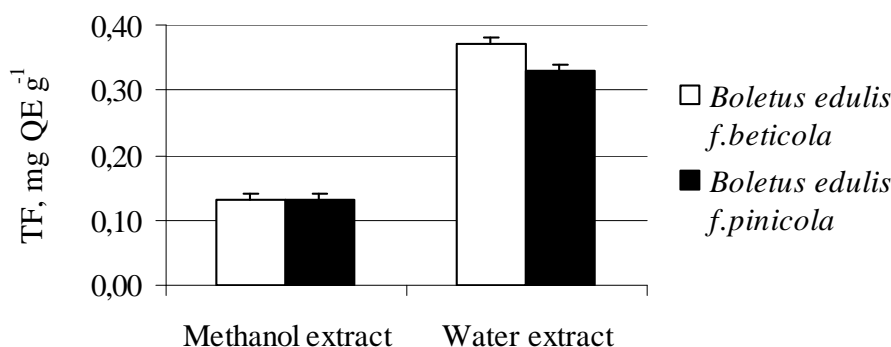


Figure 2. The content of total flavonoids (TF) in Latvian mushroom dry matter

Using HPLC the phenol compounds like catechin, epicatechin, gallic acid, caffeic acid and rutin were detected and quantified (Table 3).

Table 3

Content of phenol compounds in methanol extracts of Latvian mushroom dry matter

Phenols	<i>Boletus edulis f. beticola</i>	<i>Boletus edulis f. pinicola</i>
Catechin, $\mu\text{g g}^{-1}$ dry matter	1701.2	765.5
Epicatechin, $\mu\text{g g}^{-1}$ dry matter	2.3	0.0
Gallic acid, $\mu\text{g g}^{-1}$ dry matter	9.5	7.1
Caffeic acid, $\mu\text{g g}^{-1}$ dry matter	15.6	17.3
Rutin, $\mu\text{g g}^{-1}$ dry matter	0.0	1129.0

The content of β -carotene was almost 2 times higher than the concentration of lycopene. The highest content of β -carotene and lycopene was found in methanol extracts of *Boletus edulis f. pinicola* (Table 4).

Table 4

The content of β -carotene and lycopene in methanol extracts of Latvian mushroom dry matter

Mushroom	β -carotene, $\mu\text{g g}^{-1}$ of mushroom dry matter	Lycopene, $\mu\text{g g}^{-1}$ of mushroom dry matter
<i>Boletus edulis f. beticola</i>	26.2±0.1	13.8±0.1
<i>Boletus edulis f. pinicola</i>	43.5±0.3	23.3±0.2

The radical scavenging activity (RSA) of mushroom extracts was tested against 1.1-diphenyl-2-picrylhydrazyl (DPPH). The RSA of water extracts of mushrooms was found to be higher than those of methanol extracts. The RSA was higher for *Boletus edulis f. pinicola* than that for *Boletus edulis f. beticola* (Figure 3).

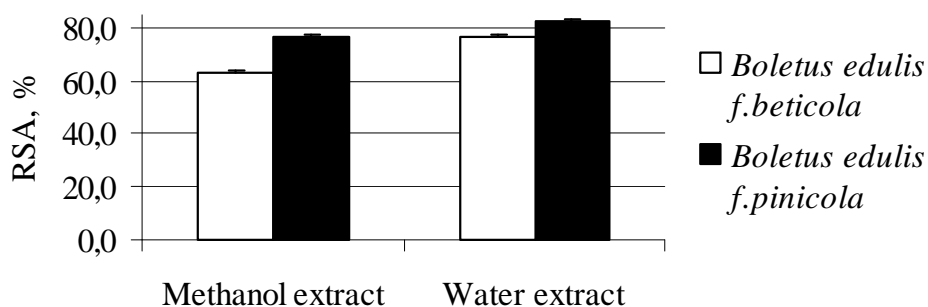


Figure 3. Radical scavenging activity (%RSA) of Latvian mushroom dry matter

The antioxidant activity of the mushroom extracts highly depends on the concentration of active compounds – phenols, flavonoids, carotenoids etc. Our results are consistent with previous reports (Robaszekiewicz et al., 2010, Vidovic et al., 2010).

Conclusions

Both studied edible boletus mushrooms *Boletus edulis f. pinicola* and *Boletus edulis f. beticola*, collected in Latvia are important source of protein, minerals, phenols and carotenoids.

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