



A Quantitative Analysis of Antioxidants in Mature and Immature Fruiting Bodies of *Bovista plumbea* Pers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Edible mushrooms are economically important product since they have been used both as food and medicine, and contain diverse organic substances with nutritional and biological properties. Recently they have gained attention as a source of antioxidants as well.

Aim: In terms of antioxidant content, Georgian edible species of mushrooms have not been studied at all. The aim of the presented study was the quantitative study of some antioxidants in wild mushroom *Bovista plumbea* Pers. (puff ball), which has been known as a medicinal remedy in Georgia since ancient times, but today is less popular among population.

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Methodology: The samples of puff ball were collected in village Gremi of Kvareli municipality (Georgia), in vineyards (ripe fruiting bodies); and in township Tianeti, near the coast of r. Iori in the meadow (immature, white fruiting bodies). The content of antioxidants: carotenoids, flavonoids, soluble phenols, proline, as well as total proteins and soluble carbohydrates and total antioxidant activity were investigated in both, immature and mature fruiting bodies of *Bovista plumbea* Pers. (puff ball). Spectrophotometric methods were used for determination of the mentioned indices.

Results: Generally the results obtained on the immature fruiting body of puff ball were significantly higher than the data obtained for the mature one. The content of carotenoids, soluble phenols, and soluble sugars exceeded the results obtained for other edible species. The content of flavonoids, amino acid proline, and total proteins in *B. plumbea* was similar or a bit lower of the minimum values established in other species. The total antioxidant activity of immature and mature fruiting bodies was 90% on average.

Conclusion: The study demonstrates that the wild-grown basidiomycete *B. plumbea* is rich in secondary metabolites with antioxidant properties and can be recommended as an antioxidant-rich and healthy edible mushroom.

Keywords: Antioxidants; *Bovista plumbea*; fruiting body; Georgia.

1. INTRODUCTION

The kingdom of fungi is one of the numerous and challenging among the earth inhabitants, representatives of which can be found everywhere, be it air, soil, water or directly living organisms. Both macro- and microscopic fungi have been widely used by humans since ancient times as food, for making drinks, in medicine, etc. (Li et al. 2016). In recent decades, the interest in fungi has been significantly increased, as they are considered a cheap and dietetic, protein-rich alternative to meat, containing a complete set of essential amino acids and are thought to help the humanity to fill the protein deficiency. Moreover, mushrooms are an important source of biologically active medicinal compounds which are characterized by antifungal, antimicrobial, anti-inflammatory, anti-cancer as well as other positive properties (Erbai et al. 2021 and Dávila- Giraldo et al. 2023). Recently edible mushrooms have gained attention as a source of antioxidants as well (Khatua et al. 2013).

Antioxidants are low-molecular organic compounds of different chemical nature, or enzymes, which provide neutralization of active forms of oxygen (ROS) in living organisms (Abeyrathne et al. 2022 and Noctor et al. 2018). ROS are constantly produced in a living cell in the process of metabolism. A certain balance is established between their formation and transformation. If the balance shifts to the side of ROS accumulation, this leads to oxidative stress, which can be disastrous for the cell and the living system totally (Ray et al. 2012). Today, it has been proven that increased level of free radicals

is related to more than 100 diseases including atherosclerosis, cardiovascular diseases, several kinds of cancer, cirrhosis, diabetes, lung diseases, neurological disorders etc. (Sasaki & Joh 2007 and Lushchak 2014).

Mushrooms may become an alternative source of various antioxidants along with plants, fighting excess formation of ROS. It has been established that mushroom antioxidants, besides direct inhibition of ROS, increase the activity of various antioxidant enzymes and diminish the amount of malondialdehyde (Arslan et al. 2023). Thus, their use in various fields of national economy and medicine is very promising.

In terms of antioxidant content, Georgian fungi, including edible species of mushrooms, have not been studied at all. For this purpose *Bovista plumbea* Pers. (puff ball) was selected; which has been known as a medicinal remedy in Georgia since ancient times (Kupradze et al. 2015 and Sikharulidze et al. 2020). The local, domestic names of the mushroom are conformation to this fact (Kobakhidze 1987, Makashvili 1991, Kobalia 2010). Fruiting bodies of the puff ball were collected by the population for medicinal purposes and kept throughout the year. It was used to stop bleeding - the dusty contents of the fungus were sprinkled on the wound. In Pshavi (region of northern Georgia) mushroom dust soaked in butter was used to treat cattle dug (Khornauli 2000). Ground fruit was also used to treat burns and abscesses (Kupradze et al. 2015 and Kobakhidze 1987). In addition, it is known that women in Kakheti (region in east Georgia) threw the dust of mature *B. plumbea*, which was called "Burnut" (tobacco),

into the nose, probably to relieve headaches and improve mood (narrative of a resident of Kakheti, private person). It is interesting that similar information about the headache-relieving effect of puff ball is given in Hughes' book, in the nineteenth century (Hughes 1968).

Diversely from most edible mushrooms puff ball grows on soil, in open places, mostly in meadows and fields, from spring to late fall; and is spread throughout Georgia. It is also suitable for food; however, due to small size of its fruiting body, the mushroom is less popular as food among people and is used for this purpose only in some regions of Georgia (Sikharulidze et al. 2020 and Ghudushauri 2011).

The quantitative evaluation of some antioxidants of puff ball would raise its nutritional and medical value, as well as popularity as of edible mushroom. Thus, the aim of the presented study was the quantitative investigation of some antioxidants (carotenoids, flavonoids, soluble phenols, proline) as well as total proteins and soluble carbohydrates, and total antioxidant activity in both immature and mature fruiting bodies of the mushroom.

2. MATERIALS AND METHODS

A spherical-round fruiting body of *B. plumbea* (family Lycoperdaceae) reaches 1-4 cm in diameter and is covered with a white, smooth skin. After ripening, the outer white exoperidium bursts and extends beyond the fruiting body; the endoperidium - the inner thin, grayish-brown parchment-like membrane remains, which is opened by a narrow, uneven edged opening in the tip; from which the spores are released. The pulp of the fruiting body (gleba) before ripening is white, fleshy and cheesy in texture. Probably because in Tusheti (east Georgia) it is called "kvavt kveli", that means crow's cheese. Such white balls are peeled and used as food (Sikharulidze et al. 2020 and Ghudushauri 2011). After ripening, the pulp becomes greenish-rusty or purple-black-brown. Spores are spherical or ovoid-elliptic, brownish, thick-walled, almost smooth, 4x6 µm in diameter, with one fatty droplet, and oblong, 8-11 µm long, cylindrical, appendage (sterigma) with a narrow tail at the end.

The samples of puff ball were collected in village Gremi (Kvareli municipality, east Georgia) in vineyards (mature fruiting bodies); and in township Tianeti (Tianeti municipality, east

Georgia) near the coast of r. Iori in the meadow (immature, white fruiting bodies). The identification of the species was carried out by the guides (Ellis & Ellis 1990 and Naskhutsrishvili 2007). Herbarium specimens are stored in the department of fungi and spore bearing plants, of the Institute of Botany, Ilia State University. Analysis were performed both on mature and immature dry fruiting bodies with three replications.

2.1 Biochemical Assays

Carotenoids were determined spectrophotometrically. Optical density of the extract of fruiting bodies in ethanol was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany). Calculations were done by Wettstein formula (Ermakov et al. 1987).

Soluble phenols were determined using Folin-Ciocalteu reagent. Optical density was measured at 765 nm (Ferraris et al. 1987).

Phlavanoids were determined spectrophotometrically, using AlCl₃. The absorbance was measured at 415 nm (Chang et al. 2002).

Proline was investigated after Bates et al. spectrophotometrically, at 520 nm (Bates et al. 1973).

Total proteins were studied after Lowry (Lowry et al. 1951).

Soluble carbohydrates were tested with anthrone reagent at 620 nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germany) (Turkina et al. 1971).

Total antioxidant activity was measured by modified method, using 40 µM diphenyl-picrylhydrazyl (DPPH) solution (Koleva et al. 2002). Optical density was measured at 515nm and the percent of inhibition was calculated.

2.2 Statistical Processing of the Results

One way ANOVA and Tukey's multiple comparison test was used to test differences between the means. All calculations were performed using statistical software Sigma Plot 14.5.

3. RESULTS AND DISCUSSION

According to literary data the chemical composition and the amount of antioxidants of

the mushroom fruiting body changes during ripening process; in particular, it increases until the second stage of maturity, and then sharply decreases (Robaszkiewicz et al. 2010). It seems that we are dealing with the similar case in puff ball; in particular, the content of studied substances in the immature fruiting body of puff ball was significantly higher compared to the mature one.

3.1 Carotenoids

From the obtained results, it is clear that the content of carotenoids in the immature fruiting bodies of the puff ball was almost 3 times higher compared to results of the mature ones ($P>0.05$) (Fig. 1). Results were similar, or in some cases exceeded the data established in other mushrooms (Robaszkiewicz et al. 2010 and Barros et al. 2008). While taking into account the fact that the safe daily norm of carotenoids for humans is 2.7-2.9 mg, it becomes clear that 100 g of dried immature fruiting bodies of puff ball contain the daily norm of these pigments. This is a significant characteristic while evaluating the nutritional and medicinal value of the mushroom.

These tetraterpene pigments are found in all living organisms; however, the ability of their synthesis is characteristic only of photosynthetic bacteria, fungi, algae and plants. Thus, food rich in carotenoids is considered to have high

nutritional value. Carotenoids perform several important functions in living organisms. In animals, they play an important role as precursors of vitamin A, photoprotectors and immunity-enhancing compounds, which also promote reproduction (Maoka 2020). The protective and signaling role of carotenoids against oxidative stress under the influence of various unfavorable factors has also been established (Havaux 2014). Their antioxidant properties guarantee protection against various diseases in animals and humans (Böhm et al. 2021). That is why their content in edible mushrooms is one of the valuable characteristics, while evaluating their nutritional and medical properties. From this point of view the immature fruiting body of puff ball deserves positive estimation, and may be regarded as carotenoid-rich food.

3.2 Soluble Phenols and Flavonoids

The content of flavonoids in *B. plumbea* was similar to the minimum values established in other species (Fig. 1); while the content of total phenolics was high in both immature and mature fruiting bodies (Fig. 2) and exceeded the results obtained for other edible mushrooms (Robaszkiewicz et al. 2010 and Palacios et al. 2011). In addition, it should be noted that the content of phenols in the unripe fruiting body was 2 times higher, compared to the ripe one.

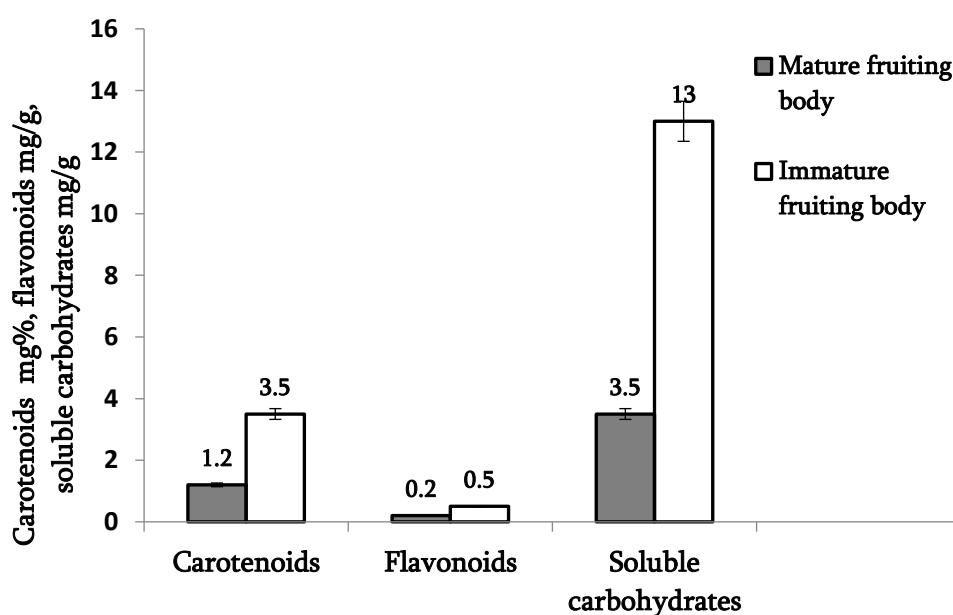


Fig. 1. Content of carotenoids, flavonoids and soluble carbohydrates in mature and immature fruiting body of *Bovista plumbea*

Phenolic compounds - the largest group of phytochemical compounds are the main determinants of antioxidant properties. Flavonoids, from its side, are the largest group of phenolic compounds found in plants and fungi, both in free and glycoside form. Phenols in general have been attributed many health benefits such as: antimicrobial, antimutagenic, anti-arthritis, anti-cancer, anti-diabetic and cardiovascular healing properties, etc. (Mutha et al. 2021). That is why food rich in these substances is regarded as valuable product. High index of phenolic substances in puff ball increases its nutritional and medical value as a food.

3.3 Amino Acid Proline and Total Proteins

According the results is clear that the content of proline in the unripe fruiting body of puff ball was 7 times higher than in the mature one (Fig. 2). However, according to literary data, the result was 3 and more times lower compared to other wild edible mushrooms (Sun et al. 2017).

The role of proline in living organisms in general is complex and multifaceted. As a proteinogenic amino acid, it participates in protein synthesis and cell growth, as well as providing energy. At the same time, it performs a signaling function and is one of the important osmoprotectants and antioxidants in stress conditions (Christgen & Becker 2019). In animals proline is essential for the production of collagen and cartilage. It keeps muscles and joints flexible; that is why, proline supplements may prove useful in the treatment of osteoarthritis and soft tissue strain. Proline is also useful in the treatment of coronary arteries, in combination with ascorbic acid. The recommended daily therapeutic dose of proline ranges from 500 to 1000 mg (<https://www.webmd.com/vitamins/ai/ingredientmono-1620/proline>). So food rich in proline possesses valuable nutritional and medical properties. Though, despite the above-mentioned positive properties of proline, the disadvantages of its excessive intake with food have been also established. Recent studies have shown that eating foods rich in proline can cause severe depression in humans and animals. It is assumed that proline is an endogenous excitotoxin - a compound that causes overstimulation of neuronal receptors like glutamate, and if the nervous system stays in such state for a long time, it can lead to

exhaustion and even death of neurons (Vujanovic et al. 2022).

Thus, the low content of proline in the fruiting body of puff ball can even be considered as a positive feature, while evaluating its nutritional value: application of puff ball as food may be safer compared to other popular edible mushrooms that are rich in proline.

Edible mushrooms are considered as an alternative of a high quality protein food. When compared to animal and vegetable protein sources, it is clear that mushroom proteins contain a complete set of essential amino acids. Studies have shown that mushroom protein concentrates, hydrolysates and peptides have many health benefits (Ayimbila and Keawsompong 2023). According to the available data, the protein content of popular edible mushrooms ranges from 6.6-36.9g/100g, and refers to crude protein in most cases. (Ayimbila and Keawsompong 2023).

The content of total proteins in puff ball (immature fruiting body) approaches the minimum value of edible mushrooms indicated in the literature (Fig. 2). Thus, puff ball may serve as a reliable source of food proteins as well.

3.4 Soluble Carbohydrates

It is generally considered that edible mushrooms have a low glycemic index, which is so important feature for diabetics. The lower the content of soluble sugars in a food item, the lower its glycemic index. This class of organic substances is responsible for the nutritional value and taste of mushrooms, as well as their storage conditions. Moreover, soluble carbohydrates play an important role in the neutralization of ROS (Sławińska et al. 2021 and Rosa et al. 2009).

The total content of soluble sugars in fruiting bodies of puff ball, without identifying individual components has been studied. The content of sugars in the immature fruiting body of the puff ball exceeded the data of the mature variant (2.8 times) (Fig. 1). The total amount of soluble sugars in the immature fruiting body of the mushroom was 1.6-3 times higher of cultivated edible species (*Agaricus bisporus* (Lange) Sing., *Pleurotus ostreatus* (Jacq. Fr.) Kumm) (Sławińska et al. 2021) and was significantly lower compared to some wild mushrooms (Onom et al. 2023).

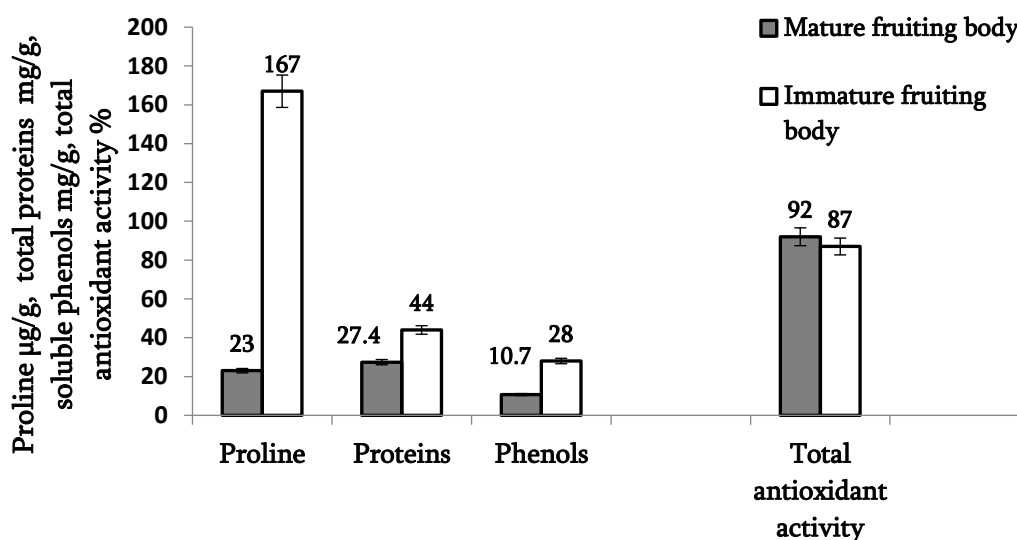


Fig. 2. Total antioxidant activity and content of proline, total proteins and soluble phenols in mature and immature fruiting body of *Bovista plumbea*

So, the low content of soluble sugars in puff ball compared to other wild species should be considered as a positive feature while evaluating its nutritional value: low glycemic index makes puff ball safe for consumers with diabetes, overweighted people and others.

3.5 Total Antioxidant Activity

The total antioxidant activity of immature and mature fruiting bodies of puff ball was found to be similar and was equal to 90% on average, which is considered a high indicator (Fig. 2). This index is an important, integrated characteristic that shows the free radical scavenging ability of hydrophilic antioxidants present in the test material (Arnao et al. 2000). According to some authors, if the total antioxidant activity of the extract exceeds 70%, it is considered highly active; if the value of the sample is within 60-70% - it is characterized by moderate antioxidant activity; and samples with an lower than 60% index are considered to have low antioxidant activity (Luzia & Jorge 2014). High antioxidant index of the puff ball significantly raises its medicinal value as food and must be taken into account while appreciating its nutritional value.

4. CONCLUSION

Investigation of the quantitative composition of antioxidants - carotenoids, flavonoids, soluble phenols, proline, as well as total proteins and

soluble carbohydrates and total antioxidant activity in both immature and mature fruiting bodies of the *B. plumbea* has revealed that the studied indices of the immature fruiting body of the mushroom were significantly higher of the mature one, and were similar, or in some cases exceeded the data established in other edible mushrooms.

In particular, the total antioxidant activity of immature and mature fruiting bodies of puff ball was found to be similar and was equal to 90% on average, which is considered a high indicator.

High content of total phenolics, moderate amount of carotenoids, proline and proteins, and low content of soluble sugars in puff ball should be considered as a positive feature while evaluating its nutritional and medical value.

Thus, the study demonstrates that the wild-grown basidiomycete *B. plumbea* is rich in secondary metabolites with antioxidant properties and can be recommended as an antioxidant-rich and healthy edible mushroom.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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