Content of Antioxidants in Mature and Immature Fruiting Bodies of Puff ball - *Bovista plumbea* Pers.

General comments:

1. Language barrier, grammatical error better if modified especially punctuations.
2. Rewrite your abstract
3. The introduction needs the arrangement because has no logical flow.
4. The methodologies need modification
5. The results were not discussed well and not identified from the discussion. Therefore, analysis your data or results well and discuss your investigation with other authors for comparison in your discussion.
6. Write strong conclusion based on your investigation.
7. Check your citation and references it should be in consistence through your manuscript. Some references are too old try to search recent.

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Abstract

Introduction:

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Aim: Edible mushrooms have recently gained attention as a source of antioxidants. In terms of antioxidant content, Georgian fungi, including edible species of mushrooms, have not been studied at all. The aim of the presented study was the quantitative study of some antioxidants in mushroom *Bovista plumbea* Pers. (common name puff ball).

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). Spwctrophotometric methods were used for determination of the mentioned indices.

Results: ~~Generally~~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, 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.

Key words: Antioxidants, Bovista plumbea, Fruiting body, Georgia

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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. [1]. In recent decades, the interest in fungi has been significantly increased. This is due to the fact that, on the one hand, fungi, especially edible mushrooms, are a cheap and dietetic, protein-rich alternative to meat, containing a complete set of essential amino acids and are thought to help humanity fill the protein deficiency; on the other hand, fungi are an important source of biologically active and medicinal compounds [2]. Bioactive compounds of mushrooms comprise terpenoids, polyphenols, polysaccharides, etc. which are characterized by antifungal, antimicrobial, anti-inflammatory, anti-cancer as well as other positive properties [3].

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Edible mushrooms have recently gained attention as a source of antioxidants as well [4].

Antioxidants are low-molecular organic compounds of different chemical nature, or enzymes, which provide neutralization of active forms of oxygen (ROS) in living organisms [5, 6].

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 [7]. Today, it has

been proven that many diseases are the result of harmful effects of oxidative stress on one or another organ, be it heart disease, hypertension, atherosclerosis, diabetes, cancer etc. [8, 9].

Plant Antioxidants are the most popular means of protecting the body against oxidative stress [5]. However, fungi, including mushrooms, may become an alternative source of various antioxidants. 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 [10]. 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. Accordingly, the aim of the presented study was the quantitative study of some antioxidants in mushroom *Bovista plumbea* Pers. (common name puff ball). 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 the *B. plumbea*.

2. Material and Methods

2.1. Research Object

B. plumbea Pers. (family. Lycoperdaceae) - puff ball has been known as a medicinal remedy in Georgia since ancient times. 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 [11, 12].

The local, domestic names of *B. plumbea* are the conformation to the fact that the mushroom has been known in all sites of Georgia since ancient times. In Khevi (a northeastern Georgia) it is called Kuskusela, in Kakheti (east Georgia)- Burnuta, in Imereti (region in west Georgia)- Tsuana soko (farting mushroom), in Guria (region in west Georgia) - Kovt satsuara (crows fart) or Dzilguda (sleepyhead), in Kartli (central-to-eastern Georgia) - Eshmakis burnuti (devil's tobacco), in Samegrelo (east Georgia) - Rshin or Lurishguda, in Svaneti (northwestern part of Georgia) - Kokoshol, Mavla tu, Khevsureti – Kvavt kudi (crows tail), in Tusheti (notheast Georgia)- Kvavt kveli (crow's cheese), in Racha (highland area in western Georgia)– Dzilguda (sleepyhead),, Pshokura, Gudapshuta [13-17].

Fruiting bodies of the mushroom were collected by the population for medicinal purposes and kept throughout the year. It was used to stop bleeding. For this purpose the dusty contents of the fetus were sprinkled on the wound. In Pshavi (region of northern Georgia), mushroom dust soaked in butter was used to treat cattle dug [18]. Ground fruit was also used to treat burns and abscesses [11, 15,19]. In Samegrelo, ripe fruiting bodies were put under the pillow of children to sleep well [17]. In addition, it is known that women in Kakheti 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 nineteeth century [20].

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A spherical-round fruiting body of *B. plumbea* 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 it is called kvavt kveli, that means crow's cheese. Such white balls are peeled and used as food [12,13, 21]. 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. Puff ball grows on soil, in open places, mostly in meadows and fields, as well as in forest edges; from spring to late fall; it is spread throughout Georgia.

The samples of puff ball were collected in village Gremi of Kvareli municipality, in vineyards (ripe fruiting bodies); and in township Tianeti, near the coast of r. Iori in the meadow (immature, white fruiting bodies). The identification of the species was carried out by the guides [22, 23]. Herbarium specimens are stored in the department of fungi and spore bearing plants, of the Institute of Botany, Ilia State University. Analyzes were performed both on mature and immature dry fruiting bodies with three replications.

2.2 Biochemical Assays

2.2.1. 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 [24].

2.2.2.Soluble phenols were determined using Folin-Ciocalteu reagent. Optical density was measured at 765 nm [25](Ferraris et al., 1987).

2.2.3. Phlavonoids were determined specrtophotometrically, using $AlCl_3$. The absorbance was measured at 415 nm [26].

2.2.4. Proline was investigated after Bates at al. spectrophotometerically, at 520 nm [27](Bates et al., 1973).

2.2.5. Total proteins were studied after Lowry [28](Lowry et al., 1951).

2.2.6. Soluble carbohydrates were tested with anthrone reagent at 620 nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germany) [29](Turkina and Sokolova, 1971).

2.2.7. Total antioxidant activity was measured by modified method, using 40 µM diphenyl-picryl-hydrazyl (DPPH) solution [30]. Optical density was measured at 515nm and the percent of inhibition was calculated.

2.3. 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

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Generally it must be noted that the results obtained on the immature fruiting body of puff ball were significantly higher than the data obtained for the mature one. 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 [31]. It seems that we are dealing with a similar case in puff ball.

3.1. Carotenoids

These are tetraterpene pigments found in all living organisms; however, the ability of their synthesis is characteristic only of photosynthetic bacteria, fungi, algae and plants; in animals, these compounds are either accumulated from food, or are found in a partially changed form as a result of metabolic reactions. Carotenoids perform several important functions in living organisms: in the photosynthetic apparatus of plants, they act as light-collecting pigments, and at the same time, they protect chlorophylls from photooxidation. They also participate in growth regulation and serve to attract pollinating organisms; in animals, carotenoids play an important role as precursors of vitamin A, photoprotective and immunity-enhancing compounds, which also promote reproduction [32]. The protective and signaling role of carotenoids against oxidative stress under the influence of various unfavorable factors has also been established [33]. Their antioxidant properties guarantee protection against various diseases in animals and humans [34]. That is why it is interesting to study their content in edible mushrooms.

From the obtained results, it is clear that the content of carotenoids in the immature fruiting body of the puff ball is almost 3 times higher compared to results in the mature one (P>0.05) (Fig. 1). Results are similar, or in some cases exceed the same index established in other fungi [31,34]. In addition, if we take into account the fact that the safe daily norm of carotenoids for humans is 2.7-2.9 mg, 100 g of dried immature fruiting bodies of puff ball contain the daily norm of these pigments, which is a significant fact while evaluating the nutritional value of the mushroom.

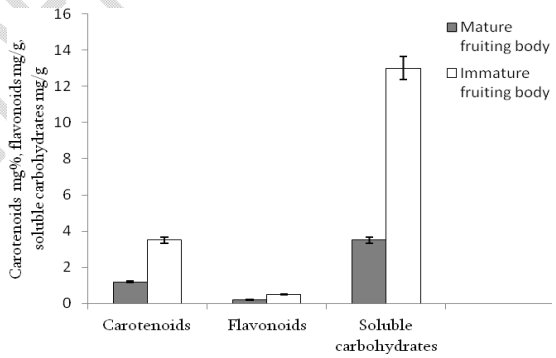

Fig. 1. Content of carotenoids, flavonoids and soluble carbohydrates in mature and immature fruiting body of *Bovista plumbea*

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3.2. Soluble phenols and flavonoids

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. Half of the 8,000 phenolic compounds found in plants are flavonoids. Phenols in general have been attributed many health benefits such as: antimicrobial, antimutagenic, anti-arthritic, anti-cancer, anti-diabetic and cardiovascular healing properties, etc. [36].

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 species [31, 37]. 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.

3.3. Amino acid proline and total proteins

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 [38]. 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 [39] (https://www.webmd.com/vitamins/ai/ingredientmono-1620/proline).

From experimental 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 amino acid content is 3 and more times lower compared to other, wild edible mushrooms [40].

The low content of proline in the fruiting body of puff ball can even be considered as a positive sign while evaluating the nutritional value of the mushroom. In particular, despite the above-mentioned positive properties of proline the disadvantages of its excessive intake with food has 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 [41]. From this point of view, application of puff ball as food may be even safer compared to other popular edible mushrooms that are rich in proline.

Edible mushrooms are 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 [42]. 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. [42].

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From the obtained results, it is clear that the content of total proteins in puff ball (immature fruiting body) is approximately 1.7 times lower than the minimum value indicated in the literature (Fig. 2). If we take into account that this indicator refers to the true protein, and is also calculated on dry material, then it is not so low. Thus, puff ball may serve as a reliable source of food proteins as wll.

3.4. Soluble carbohydrates

This class of organic substances is responsible for the nutritional value and taste of mushrooms, as well as their storage conditions. It is known that the dry biomass of mushrooms consists mainly of complex and simple carbohydrates. Typical components of soluble sugars are glucose, trehalose and the sucrose alcohol - mannitol. [43].

Sugars create a low water potential in the cell, which is a necessary condition for absorbing water from a highly mineralized soil solution. Soluble carbohydrates also play an important role in the neutralization of ROS. A number of works demonstrate the accumulation of soluble carbohydrates in plants in response to various stresses. In general, it is established that the metabolism of soluble carbohydrates under stressful conditions is a dynamic process and simultaneously includes the reactions of ROS decomposition and synthesis [44, 45].

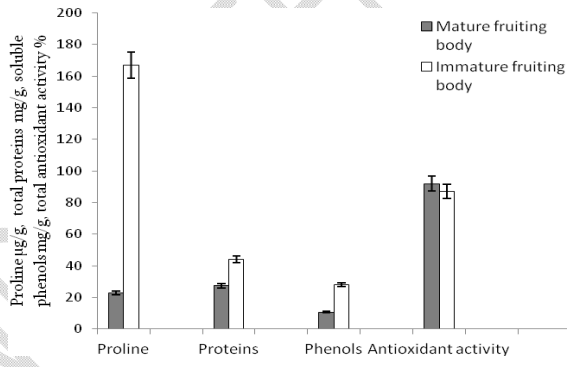

Fig. 2. Total antioxidant activity and content of proline, total proteins and soluble phenols in mature and immature fruiting body of *Bovista plumbea*

The total content of soluble sugars, without identifying individual components was 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 puff ball was 1.6-3 times higher than that of cultivated edible species (*Agaricus bisporus* (Lange) Sing., *Pleurotus ostreatus* (Jacq. Fr.) Kumm) [43], and is significantly lower compared to wild mushrooms [46].

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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. So, compared to other wild species, the low content of soluble sugars in puff ball should be considered as a positive feature while evaluating its nutritional value.

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3.5. Total antioxidant activity

This index is an important, integrated characteristic that shows the free radical scavenging ability of hydrophilic antioxidants present in the test material [47]. 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 [48]. 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).

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4. 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.

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References:

1. Li D-W, Castañeda-Ruiz RF, and La Mondia J. Evolution of Fungi and Update on Ethnomycology. Chapter 11. In: D.-W. Li (ed.), Biology of Microfungi, Fungal Biology. Springer International Publishing Switzerland. 2016. DOI 10.1007/978-3-319-29137-6_11

2. Vetter J. Mushrooms as Functional Foods. In: Kandikere R.S., Deshmukh S.K., editors. Advances in Macrofungi: Pharmaceutical and Cosmeceuticals. Taylor Francis Group LLC; London, NY, USA. 2021; pp. 139–174.

3. Dávila- Giraldo LR, Pérez Jaramillo CC, Méndez Arteaga JJ, Murillo-Arango W. Nutritional value and antioxidant, antimicrobial and cytotoxic activity of wild macrofungi. Microorganisms. 2023; 11, 1158. https://doi.org/10.3390/microorganisms11051158

4. Khatua S, Paul S, Acharya K. Mushroom as the Potential Source of New Generation of Antioxidant: A Review Research J. Pharm. and Tech. 2013; 6(5):496-505.

5. Abeyrathne EDNS, Nam K, Huang X, Ahn DU. Plant- and Animal-based antioxidants' structure, efficacy, mechanisms, and applications: a review. Antioxidants. 2022: 11, 1025. https://doi.org/10.3390/antiox11051025

6. Noctor G, Reichheld JP, Foyer CH. ROS-related redox regulation and signaling in plants. Semin. Cell Dev. Biol. 2018; 80:3-12. doi: 10.1016/j.semcdb.2017.07.013. Epub 2017 Jul 18. PMID: 28733165.

7. Ray PD, Huang B-W, Tsuji Y. Reactive oxigen species (ROS) homeostasis and redox regulation in cellular signalling. Cell. Signal. 2012; 24, 981–990.

8. Sasaki M, Joh T. Oxidative stress and ischemia-reperfusion injury in gastrointestinal tract and antioxidant protective agents. J. Clin. Biochem. Nutr. 2007; 40, 1–12.

9. Lushchak VI. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact. 2014; 224:164-75. doi: 10.1016/j.cbi.2014.10.016.

10. Arslan NP, Dawar P, Albayrak S, Doymus M, Azad F, Esim N, Taskin M. Fungi-derived natural antioxidants. Crit Rev Food Sci Nutr. 2023; 29:1-24. doi: 10.1080/10408398.2023.2298770. Epub ahead of print. PMID: 38156661.

11. Kupradze I, Jorjadze A, Arabidze A, Beltadze T, Batsatsashvili K, Paniagua -ambrana N. Y, Bussmann RW. Ethnobiological Study of Svaneti Fungi and Lichens: History of Research, Diversity, Local Names and Traditional Use. American Journal of Environmental Protection. Special Issue: Applied Ecology: Problems, Innovations. 2015; 4(3-1):101-110. doi: 10.11648/j.ajep.s.2015040301.26

12. Sikharulidze Sh, Kikodze D, Khutsishvili M, Chelidze D, Maisaia I, Jorjadze A, Batsatsashvili K. Wild plants used for “Mkhali” and edible mushrooms of Georgia. Akademiuri Tsigni, Tbilisi, Georgia. 2020.

13. Oniani A. Collection of Svanetian names of trees and shrubs (on Lashkuri dialect), Materials on Japhetic linguistics, vol. VIII. Petrograd: Academy of Sciences Press. 1917; (Russian)

14. Ghlonti Al. Dictionary of the Georgian dialects. Vol. I., Tbilisi, Ganatleba Publishing. 1974. (Georgian)

15. Kobakhidze A. Dictionary of the Rachian dialect.Tbilisi, Metsniereba Publishing. 1987. (Georgian).

16. Makashvili A. Botanical dictionary. Metsniereba, Tbilisi. 1991. 246p.(Georgian).

17. Kobalia A. The megrelian dictionary. Tbilisi, Artaniji Publishing. 2010. 748p.

18. Khornauli G. Pshavian dictionary. Tbilisi, 2000. 324p. (Georgian)

19. Chkonia I. Collection of idiomatic expressions of the Georgian dialects. Petersburg. 1910. (Russian)

20. Hughes RAW. Manual of pharmacodynamics. London. 1868. 1111p. Retrieved 2008-11-25.

21. Ghudushauri I. Mokhevian dialect dictionary. A. Orbeliani society, Tbilisi. 2011. https://www.ice.ge/liv/liv/moxeur.php (Georgian)

22. Ellis MB and Ellis JP. Fungi without Gills (Hymenomycetes and Gasteromycetes) An identification Hand- book. Chapman and Hall, London. 1990.

23. Naskhutsrishvili I. Mushrooms of Georgia. Tbilisi, “Buneba printi” and the Georgian nature support Center. 2007; 256p. ISBN 99940-856-1-1 (Georgian).

24. Ermakov AI, Arasimovich VV, Iarosh VV, Peruanskiy IP, Lugovnikova GA and Ikonnikova MI. Methods of Plant Biochemical Investigation. Agropromizdat, Leningrad. 1987. (Russian)

25. Ferraris L, Abbatista-Gentile I and Matta A. Variations of phenolics concentrations as a consequence of stress that induce resistance to Fusarium wilt of tomato. J Plant Dis. Protect. 1987; 94, 624-629.

26. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10(3):178–182.

27. Bates LS, Waldren RP and Treare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973; 39, 205-207.

28. Lowry OH, Rosebrough NT, Far AL and Randall RJ. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 1951; 139, 256-275.

29. Turkina MV and Sokolova SV. Methods of determination of mono- and olygosacharids. In: Pavlinova O.A. ed. Biokhimicheskie metody v fiziologii rasteniy [Biochemical methods in plant physiology]. Nauka, Moscow. 1971; 20-26. (Russian).

30. Koleva II, van Beek TA, Linssen JPH, Groot A & Evstatieva LN. Screening of Plant Extracts for Antioxidant Activity: a Comparative Study on Three Testing Methods. Phytochemical Analysis. 2002; 13(1):8–17. Willey. https://doi.org/10.1002/pca.611

31. Robaszkiewicz A, Bartosz G, Lawrynowicz M, Soszyński M. The role of polyphenols, β-carotene, and lycopene in the antioxidative action of the extracts of dried, edible mushrooms. J Nutr Metab. 2010; 2010:173274.

32. Maoka T. Carotenoids as natural functional pigments. J Nat Med. 2020; 74(1):1-16. doi: 10.1007/s11418-019-01364-x. Epub 2019 Oct 1. PMID: 31588965; PMCID: PMC6949322.

33. Havaux M. Carotenoid oxidation products as stress signals in plants. Plant J. 2014; 79:597–606. doi: 10.1111/tpj.12386.

34. Böhm V, Lietz G, Olmedilla-Alonso B, Phelan D, Reboul E, Bánati D, Borel P, Corte-Real J, et al. From carotenoid intake to carotenoid blood and tissue concentrations – implications for dietary intake recommendations. Nutrition Reviews. 2021; 79(5):544–573.

35. Barros L, Venturini BA, Baptista P, Estevinho LM, Ferreira ICFR. Chemical composition and biological properties of Portuguese wild mushrooms: a comprehensive study. Journal of Agricultural and Food Chemistry. 2008; 56(10):3856–3862;

36. Mutha RE, Tatiya AU & Surana SJ. Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. Futur J Pharm Sci 2021; 7(1):25

37. Palacios I, Lozano M, Moro C, D'arrigo M, Rostagno MA, Martínez JA., et al. Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chem. 2011; 128, 674-678, 10.1016/j.foodchem.2011.03.085

38. Christgen ShL and Becker DF. Role of proline in pathogen and host interactions. Antioxidants and redox signaling. Mary Ann Liebert, Inc. 2019; 30(4):683-709. DOI: 10.1089/ars.2017.7335

39. https://www.webmd.com/vitamins/ai/ingredientmono-1620/proline

40. Sun L, Liu Q, Bao C, Fan J. Comparison of Free Total Amino Acid Compositions and Their Functional Classifications in 13 Wild Edible Mushrooms. Molecules. 2017; 22(3):350. doi: 10.3390/molecules22030350. PMID: 28245582; PMCID: PMC6155212.

41. Vujanovic S, Vujanovic J,Vujanovic V. Microbiome-driven proline biogenesis in plants under stress: Perspectives for balanced diet to minimize depression disorders in humans. Microorganisms. 2022; 10, 2264.

42. Ayimbila F, Keawsompong S. Nutritional quality and biological application of mushroom protein as a novel protein alternative. Curr Nutr Rep. 2023; 12(2):290-307.

43. Sławińska A, Jabłońska-Ryś E & Stachniuk A. High-performance liquid chromatography determination of free sugars and mannitol in mushrooms using corona charged aerosol detection. Food Anal. Methods. 2021; **14**, 209–216. https://doi.org/10.1007/s12161-020-01863-8

44. Rosa M, Prado C, Podazza G, Interdonato R, González SJ, Hilal M, Prado F. Soluble sugars - metabolism, sensing and abiotic stress. A complex network in the life of plants. Plant signaling & behavior. 2009; 4(5): 388-93. 10.4161/psb.4.5.8294.

45. Mohammadkhani N, Heidari R. Drought induced accumulation of soluble sugars and proline in two maize varieties. World Applied Sciences Journal. 2008; 3(3), 448-453.

46. On-nom N, Suttisansanee U, Chathiran W, Charoenkiatkul S, Thiyajai P, Srichamnong W. Nutritional security: carbohydrate profile and folk remedies of rare edible mushrooms to diversify food and diet: Thailand case study" Sustainability. 2023; 15(18):1-9.

47. Arnao M, Cano A, & Acosta M. Methods to measure the antioxidant activity in plant material. A comparative discussion. Free radical research. 2000; 31, 589-96.

48. Luzia DM, Jorge N. Study of antioxidant activity of non-conventional Brazilian fruits. J Food Sci Technol. 2014; 51(6):1167-72. doi: 10.1007/s13197-011-0603-x. Epub 2011 Dec 29. PMID: 24876651; PMCID: PMC4033744.