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Comprehensive nutritional profiling and antioxidant capacity assessment of indigenous mushrooms *Pleurotus ostreatus* and *Agaricus bisporus*

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Abstract: The global demand for high-quality, nutritious food is increasing, and mushrooms have gained popularity as a healthy dietary option; mushroom consumption is increasing in Pakistan owing to their rich nutritional profile, phytochemicals presence and antioxidant capacity. This study was carried out to determine comprehensive nutritional analysis, antioxidant parameters, amino acids, and fatty acid composition of commonly grown mushroom varieties oysters (*Pleurotus ostreatus*) and white buttons (*Agaricus bisporus*) in Pakistan. The study results indicated mushrooms are perishable, high in protein and fibre content, along with a low caloric value. Also, they are rich in essential minerals like potassium, phosphorus, iron, and water-soluble vitamins. In addition, both mushrooms demonstrated significant antioxidant activity, with oyster mushrooms having a higher potential. Additionally, profiling of fatty acids and amino acids showed mushrooms having low caloric value and powerful nutrition composition that makes them suitable for developing healthy dietary choices for better human health.

Keywords: amino acids; edible mushrooms; healthy foods; nutritional composition; phytochemicals

Edible mushrooms have long been recognised for their nutritional and health benefits, and among thousands of mushroom species, only 25 are widely consumed as food. In Pakistan, button and oyster mushrooms are the most common (Bederska-Łojewska et al. 2017; Dilfy et al. 2020). Mushrooms are considered one of the functional foods categories with higher nutritional content, in addition to getting attention due to their overall rich nutritional profile, consumers' acceptability and medicinal properties (Valverde et al. 2015). Mushrooms are a longstanding superfood, rich in macronutrients like carbohydrates, fats, and essential micronutrients (vitamins and minerals) vital for health. *Pleurotus* varieties, in particular, offer robust health benefits as both food and medicine (Adebayo et al. 2018; Corrêa et al. 2020). China is the world's largest producer of edible mushrooms (Tsing 2015). Despite their potential, mushroom consumption in Pakistan is limited due to a lack of knowledge about their nutritional value.

Mushrooms are nutrient-dense potential sources of macronutrients such as carbohydrates, fats, proteins, and adequate amounts of dietary fibre (Farooq et al. 2021). Mushrooms are an excellent protein source, mushroom proteins offer all essential amino acids for growth, making them a viable meat substitute for vegans (Kakon et al. 2012). *Pleurotus ostreatus* is one of the nutritionally rich varieties of mushrooms, offering ample amounts of nutrients and bioactive compounds (Muszynska et al. 2018). *Agaricus*

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bisporus is also a rich source of essential nutrients, vitamins, and minerals, lacking in many other plant-based foods (Owaid and Ibraheem 2017). Mushroom varieties abundantly provide these essential macro and micronutrients and abundance of polysaccharides offering numerous health benefits in disease control (Dilfy et al. 2020).

Currently, comprehensive data on nutritional composition, fatty acids, phytochemicals, antioxidant capacity along with amino acids profiling of locally produced mushrooms in Pakistan is lacking and not well researched. Hence, it is essential to assess nutritional composition to determine their suitability for health benefits. In this context, the present study aimed to explore the nutritional and phytochemicals of oyster and white button mushrooms in Pakistan.

MATERIAL AND METHODS

The presented research was conducted in the Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences, Lahore to assess nutritional characteristics, amino acid profile, fatty acid composition, and phytochemicals of mushrooms *P. ostreatus* and *A. bisporus*.

Procurement of research materials and chemicals. Freshly procured mushroom samples from Lahore local market and O Mushrooms farm were cleaned, weighed, and divided. One portion was used for moisture determination, while the rest were dehydrated at 105 °C for 12 h, ground into fine powder, and vacuum-sealed for analysis. High-quality chemicals from a reputable source were used for analysis in the Food Analysis Laboratory, Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan.

Proximate analysis of mushrooms. Proximate composition of mushroom samples was determined by following AOAC 2000 standard methods. Moisture content is determined by drying 5 g samples at 105 °C for 24 h. Ash measured by incinerating 2 g charred samples at 550 °C. The total nitrogen content (N) of mushrooms was calculated using the Kjeldahl method procedure, and protein was calculated as total N × 6.25. The fat was extracted from the samples using the Soxhlet apparatus over 5 h. Crude fibre is determined by acid and alkali treatments, followed by ignition. Nitrogen-free extract calculated by subtraction method using 100 – Σ(protein, fat, fibre, ash). Additionally, the total carbohydrate content was found using the difference method (Dida et al. 2018), and calorie estimation was based on Atwater factors (16.7 $kJ \cdot g^{-1}$ for protein, 37.4 $kJ \cdot g^{-1}$ for fat, and 16.7 $kJ \cdot g^{-1}$ for carbohydrates).

Determination of minerals. The minerals of mushroom samples were determined using atomic absorption spectrophotometer by deploying acid digestion using a combination of nitric acid and perchloric acid following the procedure outlined by (Feldsine et al. 2002).

Vitamins analysis. The vitamins (B, C, and D_2) of mushroom samples were determined using AOAC (2000) and (Mattila et al. 1994) methods. Accordingly, the mushroom samples were subjected to saponification, extraction, and High Performance Liquid Chromatography (HPLC) analysis for vitamin D_2. Also, vitamin C was determined by enzymatic oxidation to dehydroascorbic acid, followed by fluorescent derivative formation.

Amino acid profiling. Amino acid profiles of mushrooms were analysed using High-Speed Amino Acid Analyser (Hitachi, Japan), following the procedure of the Ullah et al. (2017) method. Samples underwent defatting with petroleum spirit in Soxhlet extractor, protein hydrolysis, and amino acid extraction. The concentrated extract was derivatised and analysed with amino acid analyser.

Fatty acids analysis. Mushroom samples' fatty acid composition was analysed following Saini et al. (2021) method. Lyophilised mushroom powder (1 g) was mixed with sodium ascorbate (150 mg) and an isopropyl alcohol-cyclohexane mixture (22 mL). After sonication and shaking, lipids were extracted, dried, and quantified. A 500 µL sample was hydrolysed and used for analysis.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The mushroom extracts scavenging free radicals activity was assessed using the protocol of Azieana et al. (2017). for the analysis, various sample concentrations (1 to 100 $\mu g \cdot mL^{-1}$) were mixed with DPPH solution, and after a 30-min incubation, absorbance was measured at 517 nm. Higher absorbance meant lower scavenging activity of the free radicals.

Ferric reducing antioxidant power (FRAP) assay. The mushroom antioxidant activity was assessed through the FRAP assay following Azieana et al. (2017) method. Extracts and ascorbic acid were mixed with reagents and incubated, and then their absorbance at 700 nm was measured.

Total phenolic content. Total phenolic content of mushrooms determined using the Folin-Ciocalteu method as described by Mattila et al. (1994). Accordingly, the sample extract was mixed with reagents, incubated, and its absorbance was measured at 517 nm

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whereas the TPC value was calculated in gallic acid equivalent (mg GAE·g^{-1} of extract).

Statistical analysis. The collected data were analysed using statistical software Statistical Package for Social Sciences (SPSS, version 12). Descriptive stats and one-way analysis of variance (ANOVA) were employed. Results are reported as means ± standard deviation (SD) with significance at ($P < 0.05$).

Graphical abstract. The Figure 1 shows the graphical abstract of the study.

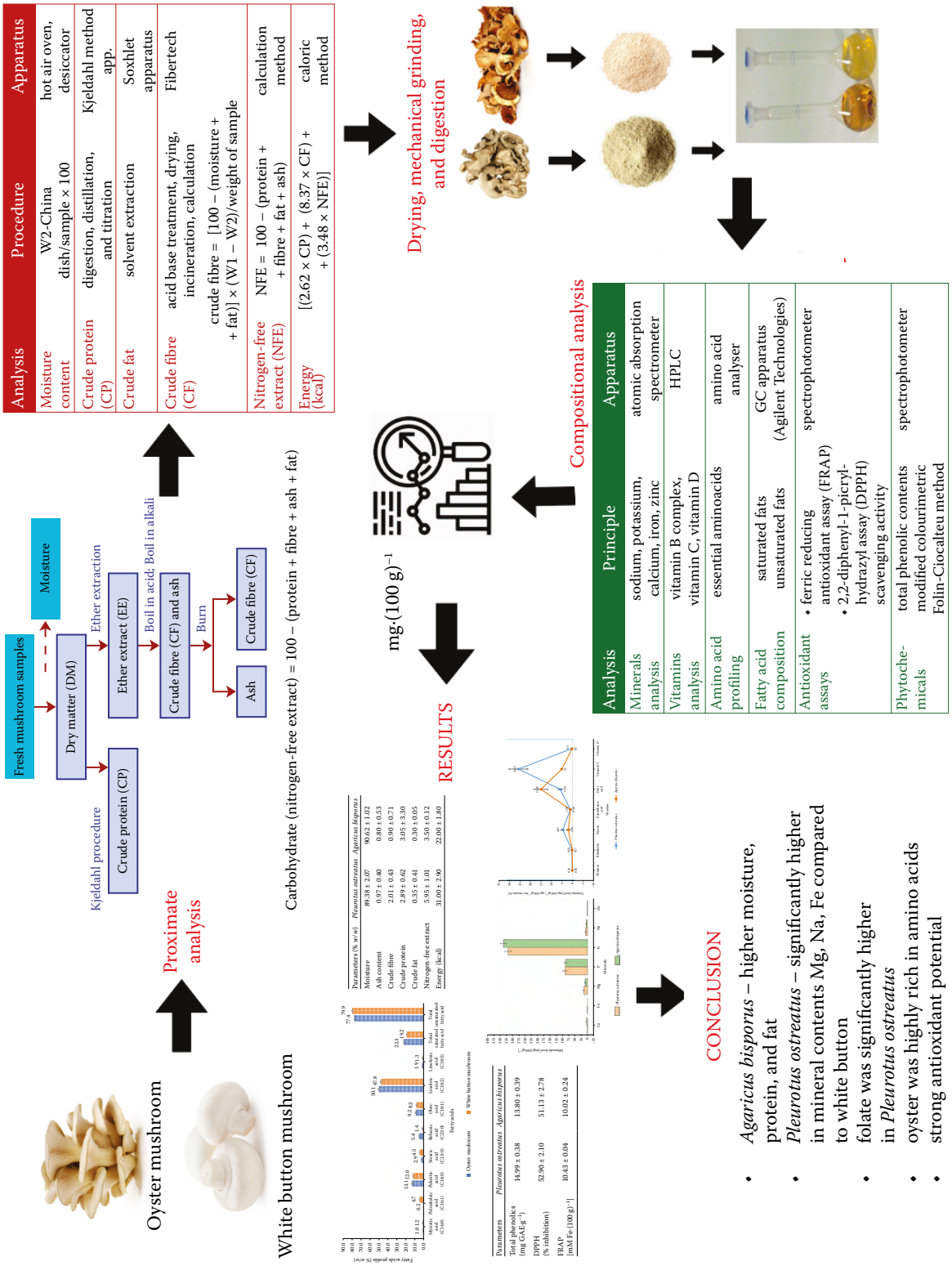

Figure 1. Graphical abstract of the study

HPLC – High Performance Liquid Chromatography; GC – gas chromatography

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Table 1. Compositional analysis of indigenous *Pleurotus ostreatus* and *Agaricus bisporus* mushrooms of Pakistan

Parameters	*Pleurotus ostreatus*	*Agaricus bisporus*
Moisture (%)	89.38 ± 2.07	90.62 ± 1.02
Ash content (% *w*/*w*)	0.97 ± 0.40	0.80 ± 0.53
Crude fibre (% *w*/*w*)	2.01 ± 0.43	0.90 ± 0.71
Crude protein (% *w*/*w*)	2.89 ± 0.62	3.05 ± 3.30
Crude fat (% *w*/*w*)	0.35 ± 0.41	0.30 ± 0.05
Nitrogen-free extract (% *w*/*w*)	5.95 ± 1.01	3.50 ± 0.12
Energy (kcal)	31.00 ± 2.90	22.00 ± 1.80

RESULTS

Proximate composition of mushrooms

The results (Table 1) reported nutritional analysis of *P. ostreatus* and *A. bisporus* mushrooms with moisture content as 89.38 ± 2.07% in *P. ostreatus* and 90.62 ± 1.02% in *A. bisporus.* Higher protein was reported in *A. bisporus* (3.05 ± 3.30% *w*/*w*), whereas higher values for carbohydrates and fibre were reported in oyster mushrooms as 2.01 ± 0.43% *w*/*w*. Non-significant differences for ash and fat content were reported in both varieties whereas, in terms of energy content, *P. ostreatus* was (31.00 ± 2.90 kcal), while *A. bisporus* was (22.00 ± 1.80 kcal).

Fatty acid composition of mushrooms. Figure 2 results showed fatty acids level of selected mushrooms which indicated that oyster mushroom had lower myristic acid (1.0 ± 0.01% *w*/*w*) compared to white button mushroom (1.2 ± 0.02% *w*/*w*). In contrast, white button mushroom exhibited higher palmitoleic acid as (4.7 ± 0.74% *w*/*w*) than oyster mushroom (0.2 ± 0.01% *w*/*w*). Palmitic acid was slightly higher in oyster (13.1 ± 0.30% *w*/*w*) than white button mushroom. Stearic acid content was (2.9 ± 0.12% *w*/*w*) in oyster mushroom and (4.5 ± 0.7% *w*/*w*) in white button mushroom. Furthermore, oyster mushroom exhibited notably higher content of behenic acid (5.4 ± 0.78% *w*/*w*) than white button mushroom (1.4 ± 0.03% *w*/*w*). In terms of unsaturated fatty acids, oyster mushroom contained (9.2 ± 1.09% *w*/*w*) oleic acid and (50.1 ± 4.09% *w*/*w*) linoleic acid while white button mushroom had (8.2 ± 1.00% *w*/*w* and 47.8 ± 3.63% *w*/*w*), respectively. Oyster mushroom also showed a slightly higher content of linolenic acid (1.9 ± 0.03% *w*/*w*) compared to white button mushroom (1.3 ± 0.03% *w*/*w*). The total saturated fatty acid content was (22.3 ± 2.05% *w*/*w*) in oyster mushroom and (19.2 ± 2.02% *w*/*w*) in white button mushrooms. Conversely, total unsaturated fatty acid content was lower in oyster mushroom (77.4 ± 5.01% *w*/*w*) compared to (79.9 ± 5.02% *w*/*w*) white button mushroom.

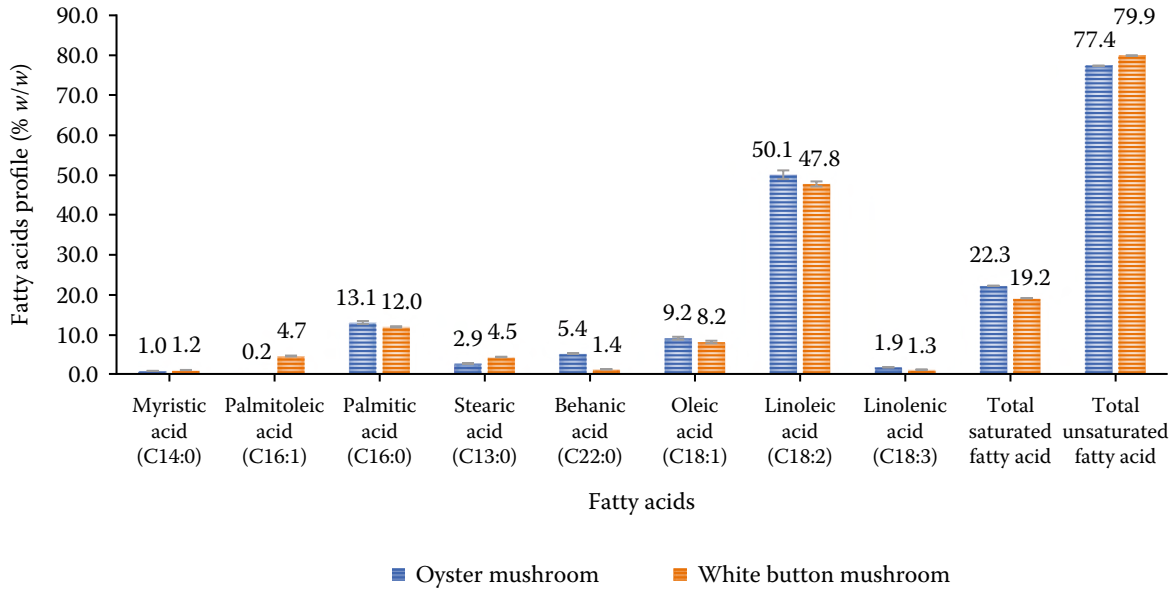

Figure 2. Fatty acids composition of indigenous oyster and white button mushrooms of Pakistan

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Mineral composition. The results in Figure 3 provided insights into mineral composition of *P. ostreatus* and *A. bisporus* mushrooms, which indicated that calcium content in *A. bisporus* [3.78 ± 1.02 mg·(100 g)$^{-1}$] is significantly higher compared to *P. ostreatus* [1.50 ± 0.07 mg·(100 g)$^{-1}$]. Also, *P. ostreatus* demonstrated significantly higher iron content [0.94 ± 0.04 mg·(100 g)$^{-1}$] compared to *A. bisporus* [0.11 ± 0.03 mg·(100 g)$^{-1}$]. Also, magnesium content displayed a significant difference between *P. ostreatus* [15.50 ± 2.73 mg·(100 g)$^{-1}$] and *A. bisporus* [9.50 ± 2.31 mg·(100 g)$^{-1}$]. In terms of phosphorus, potassium, sodium, and zinc, significant differences were reported in both mushrooms, indicating that their contents in these minerals were comparable. Furthermore, the selenium content also showed a non-significant difference, with *P. ostreatus* [5.04 ± 0.15 mg·(100 g)$^{-1}$] and *A. bisporus* [4.78 ± 0.11 mg·(100 g)$^{-1}$].

Amino acid profiling of mushrooms. The results (Figure 4) showed a comparative analysis of amino acid profiles in *P. ostreatus* and *A. bisporus* that showed cysteine content was significantly higher in *P. ostreatus* [23.50 ± 1.22 mg·(100 g)$^{-1}$] compared to *A. bisporus* [17.35 ± 0.98 mg·(100 g)$^{-1}$]. Histidine was also significant in *P. ostreatus* [43.92 ± 1.03 mg·(100 g)$^{-1}$] compared to *A. bisporus* [49.30 ± 1.45 mg·(100 g)$^{-1}$]. Isoleucine content in *P. ostreatus* [84.29 ± 1.75 mg·(100 g)$^{-1}$] was significantly higher than in *A. bisporus* [56.78 ± 1.11 mg·(100 g)$^{-1}$]. Leucine [143.43 ± 3.11 *vs.* 116.77 ± 2.87 mg·(100 g)$^{-1}$], lysine [112.38 ± 2.75 *vs.* 101.88 ± 2.42 mg·(100 g)$^{-1}$], methionine [39.58 ± 1.02 *vs.* 30.28 ± 1.35 mg·(100 g)$^{-1}$], phenylalanine [83.11 ± 1.65 *vs.* 75.18 ± 1.56 mg·(100 g)$^{-1}$], threonine [90.67 ± 1.82 *vs.* 81.90 ± 1.78 mg·(100 g)$^{-1}$], tyrosine [66.52 ± 1.44 *vs.* 45.20 ± 1.15 mg·(100 g)$^{-1}$], alanine [115.85 ± 3.01 *vs.* 109.02 ± 2.87 mg·(100 g)$^{-1}$], and arginine [131.18 ± 2.98 *vs.* 100.30 ± 2.36 mg·(100 g)$^{-1}$] also exhibited significantly higher levels in *P. ostreatus*. Valine content in *A. bisporus* [68.45 ± 0.97 mg·(100 g)$^{-1}$] was significantly higher than *P. ostreatus* [9.50 ± 1.26 mg·(100 g)$^{-1}$].

Vitamins analysis. The Figure 5 results showed vitamin levels of oyster and white button mushrooms, which indicated *P. ostreatus* had significantly higher levels of niacin content as 4.67 ± 0.81 mg·(100 g)$^{-1}$ and vitamin C 25.75 ± 3.24 mg·(100 g)$^{-1}$ compared to *A. bisporus* as 1.98 ± 0.84 mg·(100 g)$^{-1}$ and 2.31 ± 0.98 mg·(100 g)$^{-1}$, respectively. Also, *P. ostreatus*

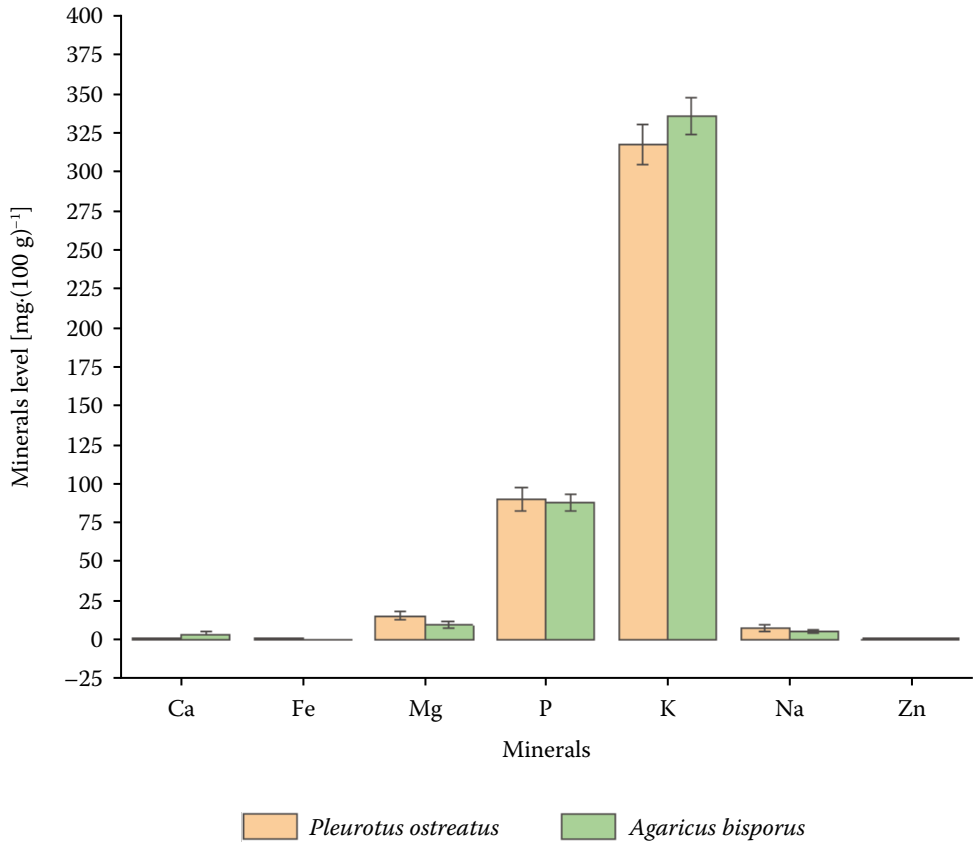

Figure 3. Minerals including calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), and zinc (Zn) levels of of indigenous mushrooms grown for human consumption in Pakistan

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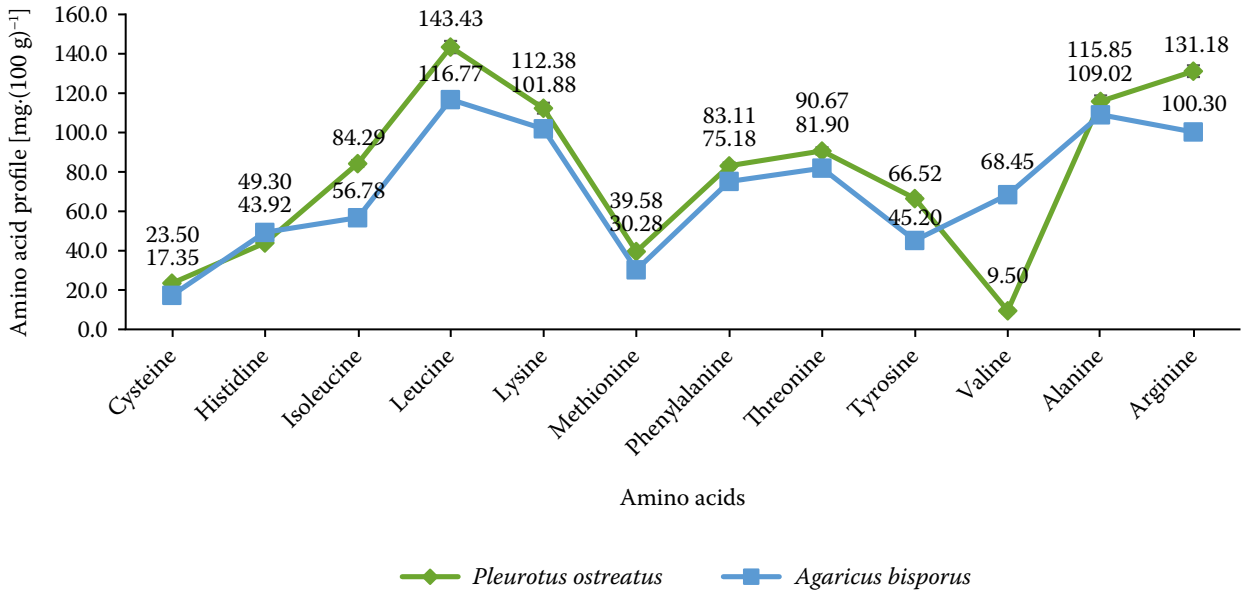

Figure 4. Amino acid profile [mg·(100 g)$^{-1}$ of foods] of indigenous oyster and white button mushrooms of Pakistan

also had higher thiamin [0.15 ± 0.03 mg·(100 g)$^{-1}$], riboflavin as 0.24 ± 0.01 mg·(100 g)$^{-1}$ and pantothenic acid as 1.04 ± 0.02 mg·(100 g)$^{-1}$ compared to *A. bisporus* as 0.05 ± 0.01, 0.17 ± 0.01, and 0.98 ± 0.04 mg·(100 g)$^{-1}$, respectively. Additionally, *A. bisporus* had significantly higher folic acid level as 15.01 ± 3.12 mg·(100 g)$^{-1}$ com-

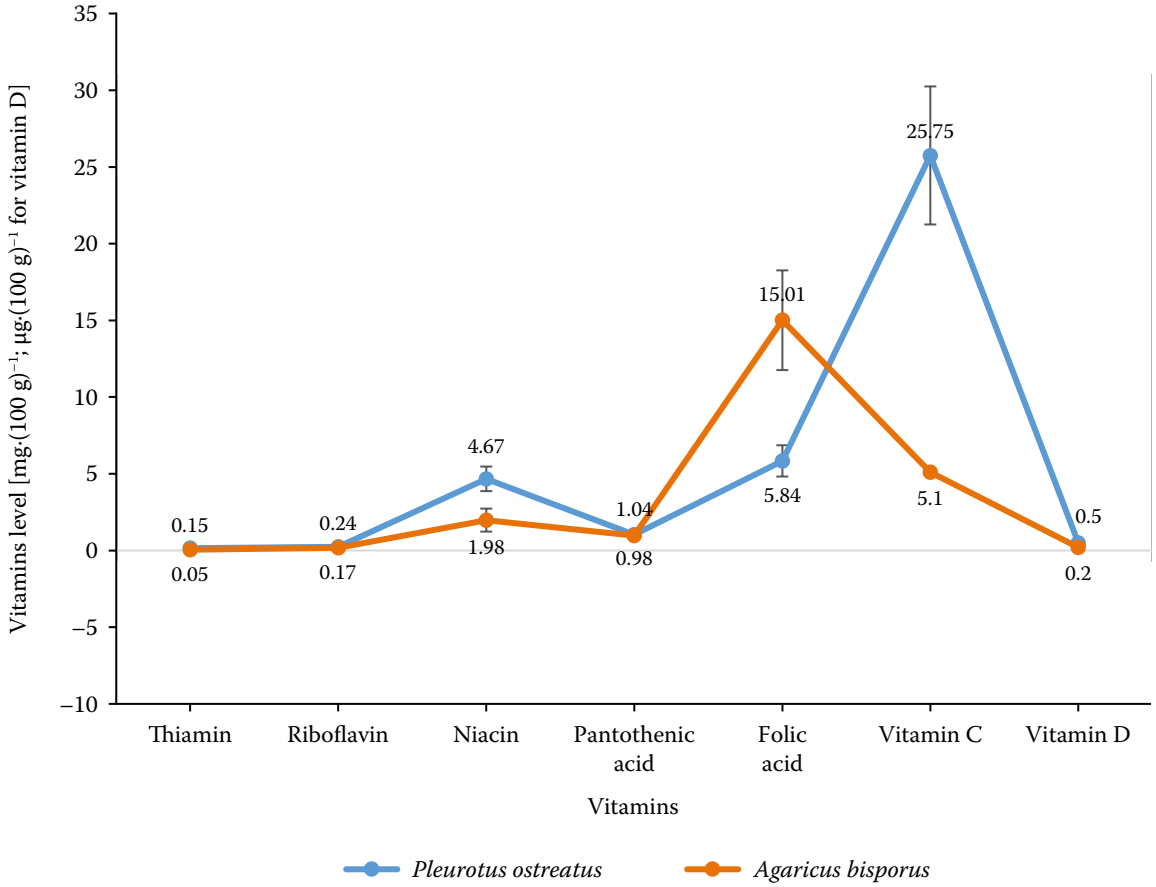

Figure 5. Vitamin levels of *Pleurotus ostreatus* and *Agarcus bisporus* mushrooms grown in Pakistan

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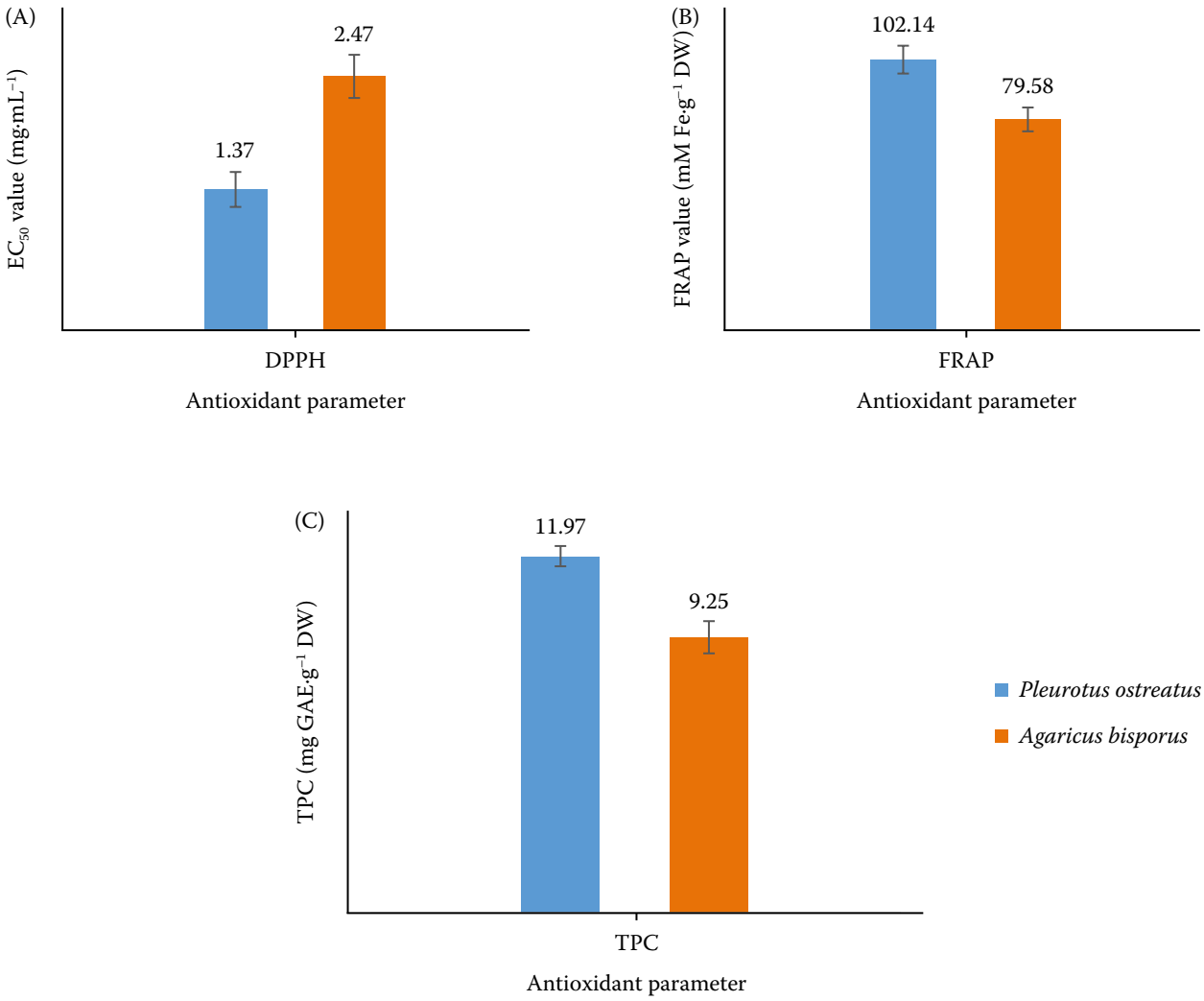

Figure 6. Antioxidant assays of *Pleurotus ostreatus* and *Agarcus bisporus*: (A) 2,2-diphenyl-1-picrylhydrazyl (DPPH), (B) ferric reducing antioxidant power assay (FRAP), and (C) total phenolic content (TPC)

EC_{50} – half maximal effective concentration; DW – dry weight; GAE – gallic acid equivalent

pared to *P. ostreatus* as 5.84 ± 1.05 mg·(100 g)$^{-1}$. However, both mushrooms had similar vitamin D as *P. ostreatus* contained 0.5 ± 0.02 µg·(100 g)$^{-1}$, while *A. bisporus* had 0.2 ± 0.01 µg·(100 g)$^{-1}$.

Phytochemical analysis of mushrooms. The results (Figure 6) reveal significant differences in antioxidant activity between *P. ostreatus* and *A. bisporus*. In the DPPH assay, *P. ostreatus* demonstrated a significantly lower value (1.37 ± 0.17 mg·mL^{-1}) compared to *A. bisporus* at 2.47 ± 0.21 mg·mL^{-1}, suggesting higher antioxidant activity in *P. ostreatus*. Similarly, the FRAP assay showed significantly higher value for *P. ostreatus* as 102.14 ± 3.05 mM Fe·g^{-1} DW compared to *A. bisporus* as 79.58 ± 2.15 mM Fe·g^{-1} DW, indicating a greater capacity for ferric ion reduction. Additionally, the total phenolic content (TPC) was higher in *P. ostreatus* as 11.97 ± 0.34 mg GAE·g^{-1} DW compared to *A. bisporus* as 9.25 ± 0.54 mg GAE·g^{-1} DW, highlighting richer phenolic composition in *P. ostreatus*.

DISCUSSION

This present study examined the nutritional composition of indigenous *P. ostreatus* and *A. bisporus*, encompassing vitamins, minerals, and antioxidant capacity. Proximate analysis revealed both mushrooms are high in moisture, with *P. ostreatus* having slightly less humidity and more crude fibre than *A. bisporus*, as supported by (Idrees et al. 2019). Both mushrooms have similar ash content, reflecting the mineral composition of both mushrooms. Oyster mushrooms had higher levels of nitrogen-free extract (carbohydrates) was

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higher than white button mushrooms, making them a potential choice for those seeking carbohydrate-rich. Dietary fibre is known for its role in promoting digestive health and aiding in weight management (Idrees et al. 2019). The higher crude fibre content in *P. ostreatus* suggests it may be a richer dietary fibre source than *A. bisporus*. (Rachappa et al. 2020). Both edible mushrooms are considered as low caloric food as *P. ostreatus* gives 31 kcal and *A. bisporus* 22 kcal, making it good choice for energy restriction condition. Our results are in agreement with previous researches on the nutritional composition (Lebeque et al. 2018; Shams et al. 2022) showing that *P. ostreatus* are nutritional rich mushrooms which provides good support for the potential health benefits of consuming mushrooms as part of a healthy diet.

Mushrooms are typically low in fat and contain essential fatty acids (Kakon et al. 2012). *P. ostreatus* showed higher content of unsaturated fatty acids, including oleic acid, linoleic acid, and linolenic acid (Atila et al. 2017; Törős et al. 2022). *A. bisporus* had a relatively higher content of saturated fatty acids such as palmitic acid and stearic acid than *P. ostreatus*. The presence of essential fatty acid in both edible mushrooms make them healthy and beneficial diet source for overall well-being (Muszynska et al. 2018).

The mineral analysis of *P. ostreatus* and *A. bisporus* mushrooms revealed that both are rich source of phosphorus and potassium along with other minerals like calcium, iron, megnesiumsodium, zinc and selenium, which are present in less amount relatively. These findings are consistent with (Siwulski et al. 2022), as *P. ostreatus* exhibited significant levels of iron than *A. bisporus* and sodium and potassium are also im high quantity compared to *A. bisporus* (Ogundele et al. 2017). *A. bisporus* had significantl higher levels potassium than *P. ostreatus*. Other minerals such as zinc and selenium are also present in both mushrooms. These findings are supported by (Zahid et al. 2020) highlight the variability of mineral composition in mushrooms.

The amino acid composition analysis of oyster mushroom and white button mushroom underscores their potential as rich sources of high-quality protein, aligning with previous studies (Reis et al. 2020). The higher content of essential amino acids in *P. ostreatus*, known for its potential health benefits, supports the notion that mushrooms, beyond being flavourful additions to culinary dishes, offer good protein source too. *P. ostreatus* has significantly higher levels of cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, valine, alanine, and arginine compared to *A. bisporus*, consistent with findings of (Amp et al. 2022). Higher valine content in *A. bisporus* compared to *P. ostreatus*, provides valuable insights into the unique amino acid profiles of different mushroom varieties and supports the idea that each mushroom species possesses a distinct nutritional profile. These finding are supported by Qing et al. 2021.

Mushrooms are also a ample source of vitamins (Majesty et al. 2019). The results revealed that *P. ostreatus* demonstrated high levels of folic acid and vitamin C in comparison to *A. bisporus*, and these results are in agreement with the findings of Atila et al. (2017). Concerning thiamin, *A. bisporus* displayed higher levels than *P. ostreatus*. The results highlighted that *A. bisporus* exhibited significantly greater levels of riboflavin (vitamin B_2) than *P. ostreatus*. Niacin (vitamin B_3) was detected in higher concentrations in *P. ostreatus* that consistent with the observations of Rathod et al. (2021). Additionally, *P. ostreatus* showed higher levels of vitamin D compared to *A. bisporus*, by the study by Keflie et al. (2019), which reported increased vitamin D content in *P. ostreatus*. *P. ostreatus* and *A. bisporus* are rich in bioactive compounds, including total phenols, known for their health benefits like antioxidants (Zalewska et al. 2021). Both mushrooms have similar total phenol content, making them good sources of bioactive compounds, as reported by (Wang et al. 2021). DPPH assay shows strong free radical scavenging ability, indicating robust antioxidant activity (Mohana and Sumathi 2020). In the FRAP assay, *A. bisporus* displays higher antioxident potential, aligning with (dos Santos Garcia et al. 2022) on white button mushroom's potent antioxidant capacity. *P. ostreatus* and *A. bisporus* exhibit significant antioxidant activity.

CONCLUSION

In conclusion, this comparative study of oyster and white button mushrooms highlights differences in their nutritional profiles. *P. ostreatus*, with slightly lower moisture and higher fibre content, presents a potential for firmer textures in culinary applications and a notable contribution to digestive health. The micronutrient analysis reveals that oyster mushrooms are richer in folic acid, vitamin C, niacin, and certain minerals, emphasising their potential benefits for immunity and energy metabolism. Conversely, *A. bisporus*, with higher levels of thiamin and riboflavin, provides essential nutrients for energy conversion, skin health, and vision maintenance. Both mushrooms exhibit an-

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tioxidant activity, underlining their role in reducing oxidative stress. The presence of essential amino acids in both varieties underscores their significance as valuable protein sources, especially for individuals adhering to vegetarian diets. This comprehensive understanding of the nutritional distinctions between *P. ostreatus* and *A. bisporus* informs dietary choices and culinary practices, catering to diverse nutritional needs and preferences and product development along with ingredients for healthy food product development.

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