

The effect of sous-vide cooking on the antioxidant properties of oyster mushroom (*Pleurotus ostreatus* L.)

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SUMMARY

Oyster mushrooms (*Pleurotus ostreatus* L.) are renowned for their antioxidant, antimicrobial, and prebiotic properties. This study explores the antioxidant characteristics, activity, and β -glucan content in freeze-dried mushroom samples, investigating the influence of sous-vide cooking. Uncooked freeze-dried *P. ostreatus* and three pre-cooked freeze-dried samples (70, 80, 90 °C through 4 hours) were analysed for Total Polyphenol Content (TPC), Total Flavonoid Content (TFC), Radical Scavenging (DPPH), Ferric Reducing Antioxidant Power (FRAP), and β -glucans content via HPLC and Total Dietary Fiber (TDF) via enzymatic gravimetric method. Results indicate that uncooked mushroom powder exhibited superior antioxidant capabilities compared to cooked samples. The sous-vide cooked (80 °C) mushrooms displayed the highest total phenolic and flavonoid content. Moreover, pre-cooked (70 °C) mushroom powder demonstrated the highest β -glucan content, significantly surpassing the uncooked control sample. Notably, pre-cooked groups (80, 90 °C) demonstrated significantly higher TDF levels compared to uncooked sample. This research offers valuable insights into the potential use of mushrooms as high-antioxidant, antimicrobial, and prebiotic food or feed supplements, with broad implications across various fields.

Keywords: low temperature cooking methods; nutritional composition; bioactive compounds; β -glucans; oyster mushroom (*Pleurotus ostreatus*)

INTRODUCTION

Pleurotus versatile genus is belonging to the kingdom Fungi, phylum Basidiomycota, class Agaricomycetes, order Agaricomycetes, family Pleurotaceae, and genus *Pleurotus* (Törös et al., 2022, 2023a). Oyster mushrooms are considered to be one of the most cultivated mushrooms all over the world.

There is a growing body of literature that recognizes the importance of bioactive compounds isolated from *P. ostreatus* mushrooms, which are responsible for their antimicrobial, immune-stimulator, prebiotic, anticancer, antidiabetic, antioxidant effects (Törös et al., 2023a).

The literature has emphasized the importance of the presence of organic phytonutrients, such as antioxidants (Akyüz et al., 2022; Tang et al., 2022). Furthermore, this mushroom contains all of the essential amino acids, especially lysine and leucine digestible proteins, carbohydrates, dietary fibers (non-starch polysaccharides), vitamins and minerals (Gallotti, 2019).

Sous-vide processing has been an active research area for food scientists since the 1990s. The sous-vide technology is a water-based, gentle cooking method with several advantages in which vacuum-sealed raw food material is cooked under a precisely controlled temperature, which can enhance several quality attributes (Zavadlav et al., 2020; Cui et al., 2021).

Some recent studies have highlighted the advantages of sous-vide cooking, including enhanced flavor, improved texture, and nutrient retention (Aviles et al., 2020; Bıyıklı et al., 2020; Cui et al., 2021; Thatsarani et al., 2022), furthermore lower chance for the formation of toxic chemicals (acrylamides, heterocyclic aromatic amines, polyaromatic hydrocarbons, advanced glycation end products) (Li et al., 2021; Bai et al., 2022). Baldwin et al. (2012) showed that precise temperature control in sous-vide cooking offers many benefits, including improved texture, flavor, and safety, making it a popular and effective cooking method (Baldwin, 2012).

It is worth mentioning that during sous-vide cooking Maillard reaction can occurred (Li et al., 2022), which leads to the formation of melanoidin compounds (brown macromolecules) (Törös et al., 2023b), which act as antihypertensive, antimicrobial, and antioxidant agent and has several health attributes (Nasirpour and Saeidy, 2021).

The antioxidant activity of oyster mushrooms is believed to be attributable to their phenolic components, including gallic acid, protocatechuic acid, and p-hydroxybenzoic acid (Patel et al., 2012; Rahimah et al., 2019; Mutukwa et al., 2019; Gao et al., 2020; Aliño-González et al., 2022), furthermore their flavonoids, like quercetin, rutin and catechin (Bhekti Rahimah et al., 2023).

A high antioxidant capacity has been observed in oyster mushrooms, which is demonstrated by their

ability to scavenge DPPH radicals (Egra et al., 2019; Akyüz et al., 2022; Aliaño-González et al., 2022) and to combat oxidative stress and protect against free radical damage by FRAP assay (Akyüz et al., 2022; Sołowiej et al., 2023; Tokarczyk et al., 2023).

β -glucans are not recognized as an antioxidant, however, they can have a positive impact on overall health, potentially leading to improved antioxidant capacity by supporting the body's immune and inflammatory responses (Diamantopoulou et al., 2023; Petraglia et al., 2023).

Dietary fiber and antioxidants are both important components of a healthy diet, and they can work together to promote overall health and well-being. Dietary fiber is known for its role in promoting digestive health. It supports a healthy gut microbiome, which can help improve the absorption of antioxidants and other essential nutrients (He et al., 2022; Inyod et al., 2022).

The objectives of this study were to investigate the effect of sous-vide cooking on the content of antioxidants (phenols, flavonoids), β -glucan, dietary fiber, as well as to assess their antioxidant activity using Ferric Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays in freeze-dried *P. ostreatus* mushrooms.

MATERIALS AND METHODS

Sample preparation

For mushroom powder production, fresh I. quality of oyster (*P. ostreatus*) mushrooms were collected from Magyar Gomba Kertész Kft., Demjén. The mushrooms were washed, sliced, and vacuum-packed for the cooking process. The mushroom samples (*P. ostreatus*) were commercially cultivated in Magyar Gomba Kertész Kft. (Demjén, Hungary). Mushroom pieces were separated into a tray as a control sample, and then pre-frozen before lyophilization. For the sous-vide cooking, pre-prepared mushrooms were vacuum-packed and put into three different drying cabinets.

The three cabinets were preheated at 70, 80, and 90 °C, and cooked for 4 hours. Sous-vide and uncooked samples were freeze-dried, then fine powder was made by grinding of the samples.

Throughout the figures and text, the subsequent abbreviations have been employed as the following:

- Freeze-dried oyster mushroom, control sample (-40 °C, 24 hours): **CS**
- Sous-vide cooked (70 °C, 4 hours), then freeze-dried oyster mushroom (-40 °C, 24 hours): **SVS-70**
- Sous-vide cooked (80 °C, 4 hours), then freeze-dried oyster mushroom (-40 °C, 24 hours): **SVS-80**
- Sous-vide cooked (90 °C, 4 hours), then freeze-dried (-40 °C, 24 hours) oyster mushroom: **SVS-90**

Investigation of antioxidant compounds and antioxidant activity

Scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals spectrophotometric measurements referred on Boonsong et al. (2016). 75 μ l of 10% Folin-Ciocalteu reagent was added into 15 μ l of sample, then

7.5 μ l of 60 μ l sodium carbonate solution. The blank well-received 150 μ l of DMSO sample solvent. Subsequently, the mixture was incubated for 10 minutes at 50 °C, and absorbance was measured with UV-VIS Spectrophotometer (Perkin Elmer, Lambda 2S) at 760 nm. The scavenging activity was calculated as a percentage of inhibition (SC%) (Boonsong et al., 2016).

Ferric Reduction Antioxidant Power (FRAP) assay, based on the method by Nemes et al. (2018). 0.5 g of sample was mixed with 5 mL of distilled water, sonicated, and then centrifuged (Hettich Zentrifugen, EBA21). A 10 μ L extract was used for spectrophotometric analysis, and the samples were incubated with the FRAP reagent. Absorbance readings were taken at 593 nm after 8 minutes, and the results were expressed as mg ascorbic acid equivalent per 100 grams (Nemes et al., 2018). Total phenolic content (TPC) of the mushroom samples was analyzed by the method of Ali et al. (2016). 0.1 g of sample as weighed, then 10 mL of 80% methanol was added. The filtered (0.45 μ m Hydrophilic PTFE, Labex Ltd.) a mixture of 2,5 ml 0,2 N Folin reagent, then after 5 minutes of room temperature incubation, 2.0 mL of 7.5 g/100 mL sodium carbonate (Na_2CO_3) added, then shaken and incubated at room temperature for 30 minutes in a dark environment. Absorbance of the mushroom samples was measured at 765 nm using a UV-VIS Spectrophotometer (Perkin Elmer, Lambda 2S) and the results added in mg/100 g for gallic acid equivalent (Ali et al., 2016; Wang et al., 2023).

Total flavonoid content (TFC) was measured according to Xu and Chang (2007). 0.25 mL of the sample was blended with 1.25 mL of distilled water, then 75 μ L of a 5% of sodium nitrite (NaNO_2) solution. After 6 minutes, 150 μ L of a 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution was added and left to stand for an additional 5 minutes before supplementing with 0.5 mL of 1M NaOH. The mixture was then adjusted to a final volume of 2.5 mL using distilled water. The mixture was thoroughly vortexed, and the absorbance was quantified at 510 nm, and the data were expressed as mg /100 g for catechin standard (Xu and Chang, 2007).

Investigation of β -glucan and dietary fiber

The **β -glucan** content of mushroom powders was determined with the HPLC (High-Performance Liquid Chromatography, ECOM ECS05 model manufactured in Czech Republic) method according to Pérez-Vendrell et al. (1995). A 45 mg portion of the standard (49% β -glucan from mushroom and yeast, Megazyme) was utilized, and the dilution was made up to a final volume of 50 mL for β -glucan content measurement. Both the standard and mushroom samples underwent hydrolysis with 12 M sulfuric acid (H_2SO_4) for 2 hours in dry ice, followed by the addition of distilled water, an additional 2-hour incubation at 100 °C, treatment with 8.0 M sodium hydroxide (NaOH), and adjustment of pH to 4.5 using a 200 mM sodium acetate (CH_3COONa) buffer. Detection of β -glucan was accomplished using an Evaporative Light Scattering Detector Evaporative Light Scattering Detector (ELSD,

ESA 301 model, manufactured in France) (Pérez-Vendrell et al., 1995).

Before **Total Dietary Fiber** (TDF) analysis, the sample was subjected to thorough drying to remove any fat or carbohydrates. Three separate 1-gram samples were then mixed with 40 mL of MES–TRIS buffer and underwent enzymatic hydrolysis in the following sequence: 50 μL of heat-resistant α -amylase, maintained in a water bath for 35 minutes, followed by 100 μL of protease, also kept in a water bath at $60\text{ }^\circ\text{C} \pm 1\text{ }^\circ\text{C}$ for 30 minutes. Subsequently, the pH was adjusted within the range of 4.0–4.7, and 300 μL of amyloamylase, in a water bath at $60\text{ }^\circ\text{C} \pm 1\text{ }^\circ\text{C}$ for 30 minutes, was introduced. Soluble fiber was then precipitated using 95% v/v ethanol at $60\text{ }^\circ\text{C}$. The resulting sample was filtered through fritted (sintered) glass filtration crucibles (DURAN®, DWK Life Sciences), of the gooch type, with glass wool serving as the filtration agent. The crucibles, along with the residue, were dried in an oven (Venti line 56 Prime, VWR International Kft.) at $105\text{ }^\circ\text{C}$, allowed to cool in a desiccator, and subsequently weighed (Garbelotti et al., 2003).

Statistical analysis

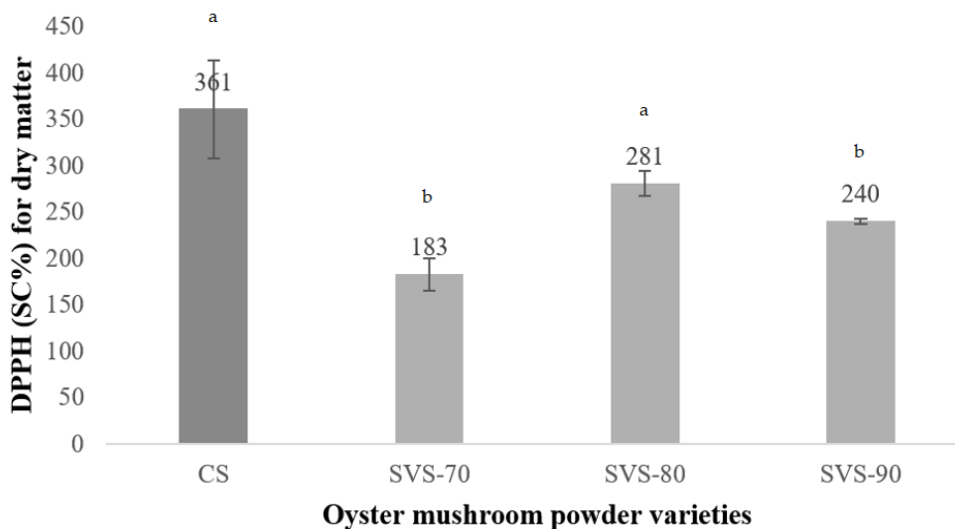
The study involved three repetitions for each experiment, and the data were represented using the mean \pm standard deviation (SD). To analyze the results, the SPSS (IBM, 27. version) statistic software was employed. To assess significant variances in β -glucan (m/m%), DPPH, FRAP, TPC by the non-parametric Kruskal Wallis test ($p < 0.05$).

RESULTS AND DISCUSSION

Determination of antioxidants, antioxidant activity

In *Figure 1*, the highest average DPPH value was observed in the CS sample (361 ± 53 SC%), while the lowest was recorded in SVS-70 (183 ± 17 SC%). Significantly, a marked difference was exhibited by CS in comparison to SVS-70 and SVS-90, although no statistically significant difference was observed when compared CS to SVS-80. Furthermore, the values measured in SVS-80 were found to be significantly higher in comparison to SVS-70.

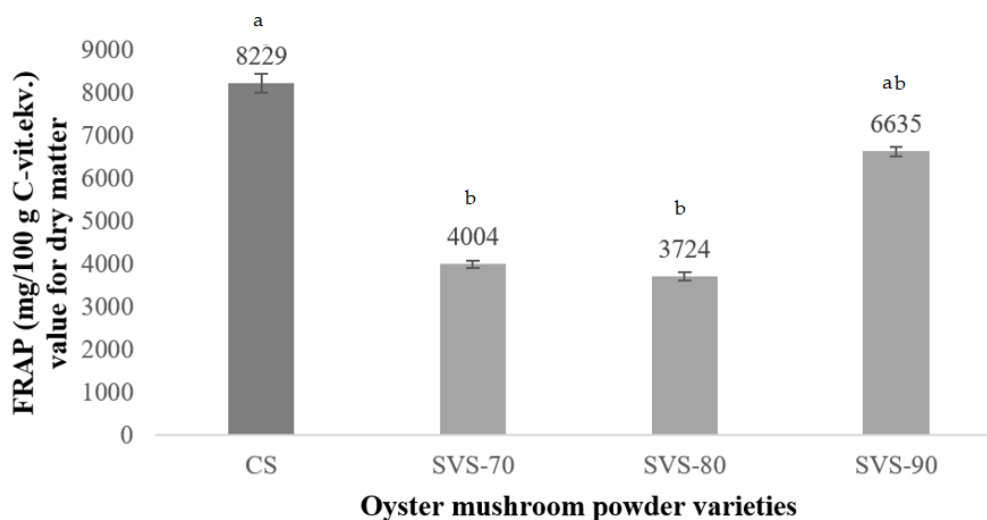
Figure 1. DPPH value of oyster (P. ostreatus) mushroom powders



Notes: Distinct letters within each parameter denote statistically significant variances at the 0.05 significance level ($p \leq 0.05$). Lowercase letters represent the outcomes of the Kruskal-Wallis.

The **FRAP values**, as presented in *Figure 2*, revealed that CS demonstrated the highest FRAP value, with a mean of 8229 ± 214 mg/100 g, while the lowest values were exhibited by SVS-80, measuring at $3724 \pm$

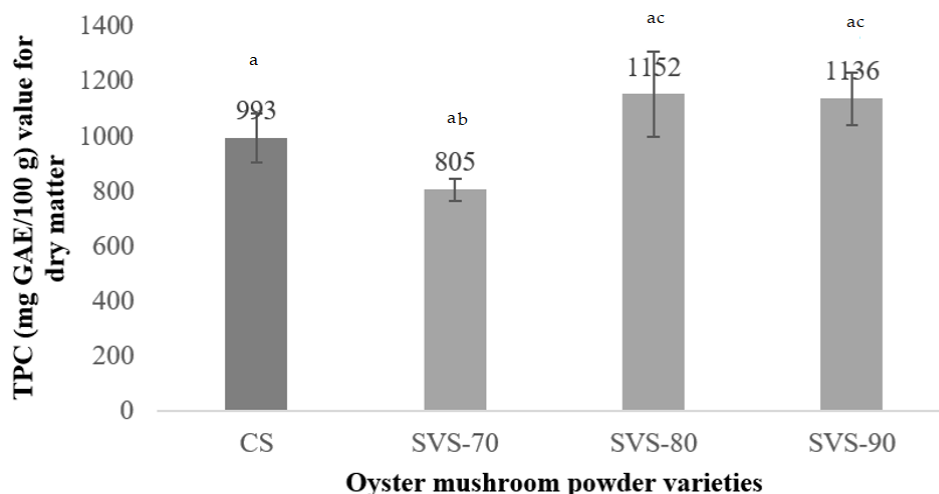
98 mg/100 g. A statistically significant difference was identified between SVS-80 and SVS-90, furthermore, CS was significantly higher than SVS-70 and SVS-80.

Figure 2. FRAP value of oyster (*P. ostreatus*) mushroom powder

Notes: Distinct letters within each parameter denote statistically significant variances at the 0.05 significance level ($p \leq 0.05$). Lowercase letters represent the outcomes of the Kruskal-Wallis test

The **TPC values**, as illustrated in *Figure 3*, demonstrated that SVS-80 and SVS-90 exhibited the highest TPC values, with a mean of 1152 ± 155 mg GAE/100 g and ..., while the lowest values were observed in SVS-70, measuring at 805 ± 41 mg GAE/100 g. A statistically significant difference was

identified between SVS-70 and SVS-80, and furthermore, between SVS-70 and SVS-90. However, no significant difference was found between CS and SVS-70, SVS-80, and SVS-90.

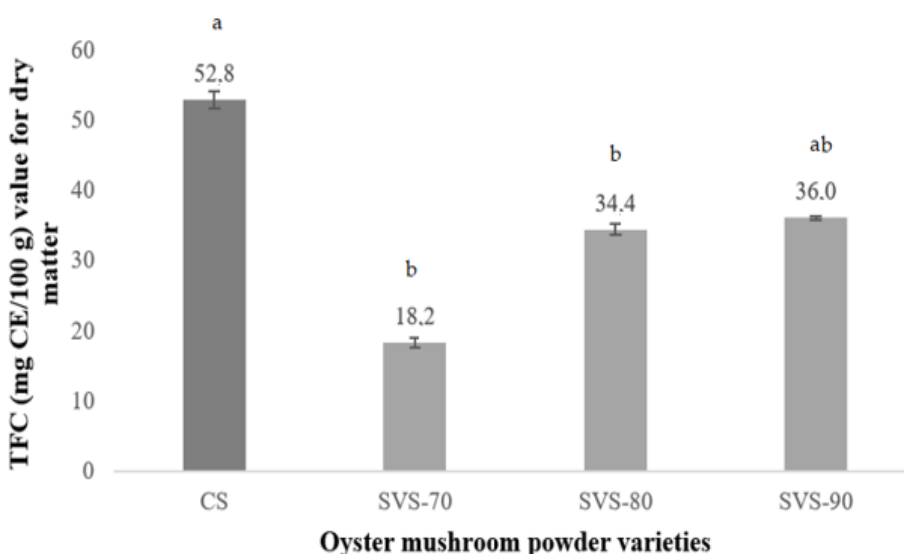
Figure 3. TPC value of oyster (*P. ostreatus*) mushroom powders

Notes: Distinct letters within each parameter denote statistically significant variances at the 0.05 significance level ($p \leq 0.05$). Lowercase letters represent the outcomes of the Kruskal-Wallis test.

The **TFC values**, as presented in *Figure 4*, indicated that CS demonstrated the highest TFC value, with a mean of 52.8 ± 1.2 mg CE/100 g, while the lowest values were exhibited by SVS-70, measuring at 18.2 ± 0.7 mg CE/100 g. CS was significantly higher

than SVS-70 and SVS-80, however, no significant difference was observed with SV-90. It can be said, that SVS-90 was found to be significantly higher than SVS-70.

Figure 4. TFC value of oyster (*P. ostreatus*) mushroom powders



Notes: Distinct letters within each parameter denote statistically significant variances at the 0.05 significance level ($p \leq 0.05$). Lowercase letters represent the outcomes of the Kruskal-Wallis test.

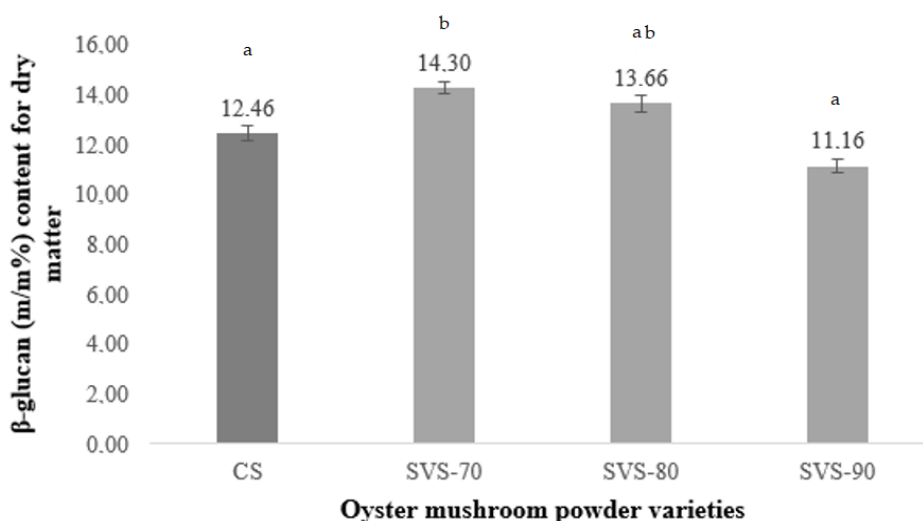
Determination of the β -glucan and dietary fiber content

The **β -glucan content**, depicted in Figure 5, revealed that SVS-70 registered the highest β -glucan levels with a mean of 14.3 ± 0.3 m/m%, whereas the lowest values were observed in SVS-90 at 11.2 ± 0.2 m/m%. Notably, a statistically significant distinction was observed between CS and SVS-70, with SVS-70 exhibiting higher β -glucan content. Furthermore, SVS-90 displayed significantly lower levels compared to

SVS-70 and SVS-80, although no significant difference was found concerning CS.

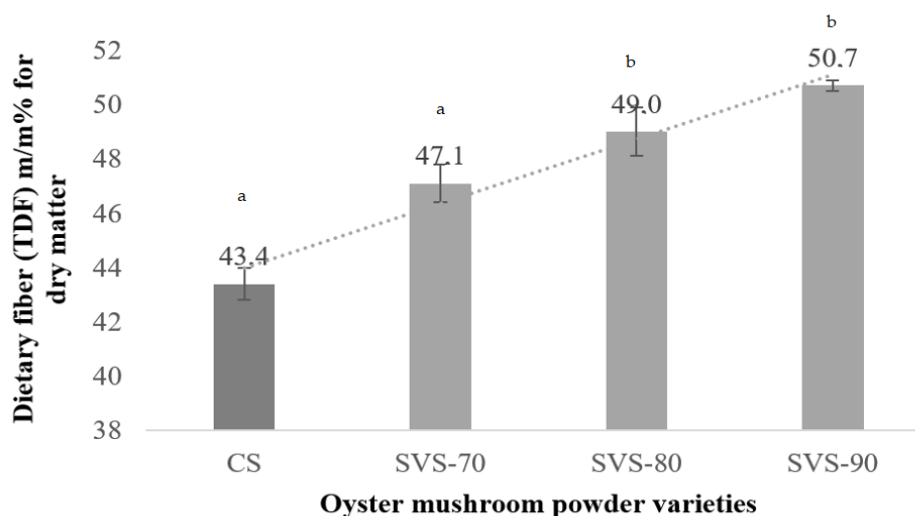
Figure 6 illustrates the levels of **Total Dietary Fiber (TDF)** content and it becomes evident that SVS-90 exhibited the highest dietary fiber content at 50.7 ± 0.2 , while the lowest value of 43.4 ± 0.6 m/m% was recorded for CS. Notably, both SVS-80 and SVS-90 showed significantly higher TDF m/m% levels compared to CS. Furthermore, among the pre-cooked samples, SVS-90 demonstrated a statistically higher TDF content than SVS-70.

Figure 5. β -glucan content of oyster (*P. ostreatus*) mushroom powder



Notes: Distinct letters within each parameter denote statistically significant variances at the 0.05 significance level ($p \leq 0.05$). Lowercase letters represent the outcomes of the Kruskal-Wallis test.



Figure 6. Dietary fiber content of oyster (*P. ostreatus*) mushroom powders

Notes: Distinct letters within each parameter denote statistically significant variances at the 0.05 significance level ($p \leq 0.05$). Lowercase letters represent the outcomes of the Kruskal-Wallis

CONCLUSIONS

Mushrooms are recognized for their significant fungal biomass, which possesses noteworthy antioxidant value. This research seeks to investigate the antioxidant compounds, antioxidant activity, and β -glucan content in freeze-dried mushroom samples. The study focuses on evaluating the impact of a gentle food technology process known as sous-vide on the quality of the final product.

Among the key findings, the CS sample consistently demonstrated higher DPPH and FRAP values, indicating superior antioxidant capabilities, than cooked samples. Regarding TPC and TFC, SVS-80 exhibited the highest values, with CS consistently differing significantly from SVS-70 and SVS-80. However, there were no significant differences between CS and SVS-90 in TPC and TFC.

In addition, the study assessed β -glucan content, with SVS-70 having the highest levels. A statistically significant difference was noted between CS and SVS-70, indicating variability in β -glucan content. SVS-90

had significantly lower levels than SVS-70 and SVS-80, with no significant difference observed when compared to CS. Significantly higher TDF m/m% levels were observed in both SVS-80 and SVS-90 compared to CS, and SVS-90 also exhibited a statistically higher TDF content than SVS-70. These results highlight the diversity in antioxidant properties and β -glucan content among the tested samples, underscoring the importance of selecting specific samples based on their unique attributes for various applications and health benefits. The findings from this investigation have the potential to inform and support future research, possibly justifying their application in various fields, shedding light on their potential as food or feed supplements with high antioxidant activity and potential antimicrobial and prebiotic attributes.

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