

# Pleurotus ostreatus bio-residues: a source of bioactives with antioxidant and antimicrobial activity

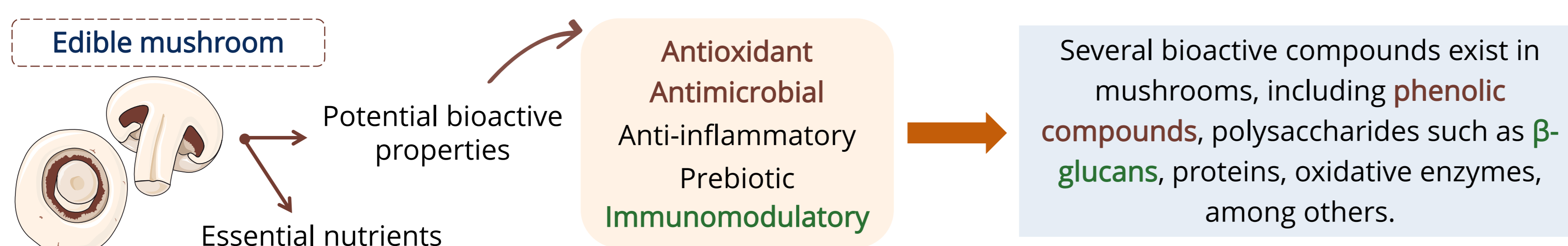
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## Introduction



Mushrooms are important sources of natural bioactive compounds. Many bio-residues are generated during mushroom's production, with a high environmental impact and managing costs to the industry. These residues are promising sources of valuable compounds, which may be used for their functional properties and nutritional value.

The valorisation of mushroom bio-residues provides an opportunity for the development of innovative high-value products and can lead to an increase of the sustainability of mushroom productive chain.

## Objectives

In the present work, the total phenolic compounds content, the  $\beta$ -glucans content and bioactivity properties of bio-residues obtained from *Pleurotus ostreatus*, one of the most cultivated species worldwide, were evaluated in order to develop a circular bioeconomy approach.



Figure 1. Examples of mushroom bio-residues

Bio-residues include caps, stalks, mushrooms of irregular dimensions and shape and basal material

## Methods

*P. ostreatus* bio-residues were provided by Voz da Natureza, Lda and were cleaned and frozen after harvest. Before extraction, the dry matter (DM) of *P. ostreatus* bio-residues was determined (24 g/ 100 g DM). The extraction was performed using water as the only solvent, in order to obtain a process as green as possible and with minimal costs. In addition, it can be easily scaled up at industrial level. The aqueous extracts were obtained according to two different methods (1 and 2). Extracts obtained from each method were freeze-dried.

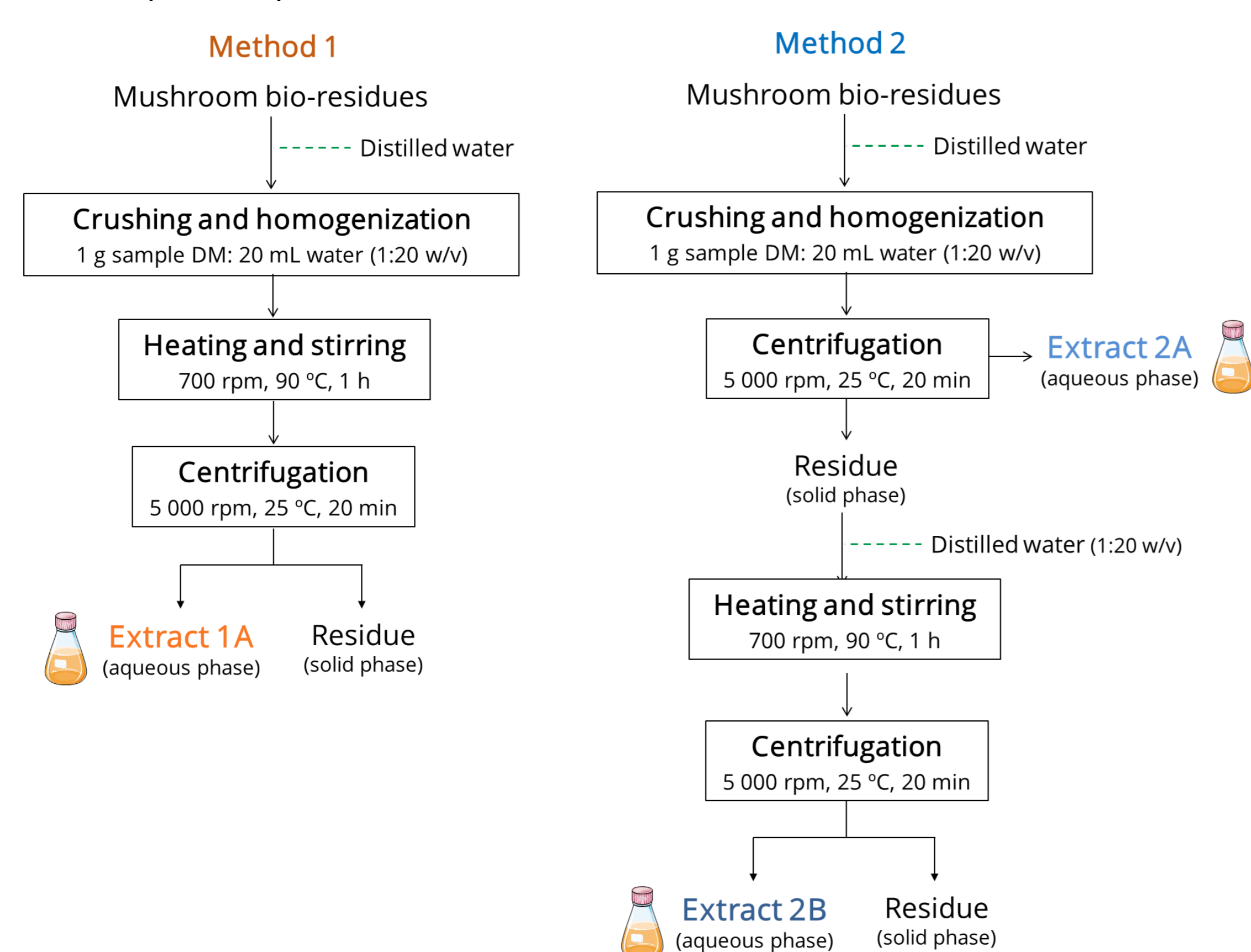


Figure 2. Extraction methods

- The total phenolic content (TPC) of aqueous extract was determined using Folin-Ciocalteu colourimetric method. The total antioxidant activity of the *P. ostreatus* extracts was measured through ABTS, DPPH and ORAC assays.
- $\beta$ -glucans content was performed by " $\beta$ -Glucan Assay Kit (Yeast & Mushroom)" assay kit (Megazyme Cat. No. K-YBGL).
- The antibacterial activity against Gram-positive and Gram-negative strains was evaluated using broth microdilution to determine the minimum inhibitory concentration (MIC).
- The cytotoxicity (PrestoBlue) and mutagenicity (Ames) of all extracts were also evaluated.

Regarding statistical analysis, one-way ANOVA test ( $p < 0.05$ ) was used to compare the means of extracts from *P. ostreatus*.

## Results

### Total phenolic compounds (TPC) and antioxidant activity (AA)

Table 1 TPC and antioxidant activity of extracts from *P. ostreatus* (mean  $\pm$  SD) (n=9).

	Aqueous Extracts from <i>P. ostreatus</i> bio-residues	Extracts		
		Extract 1A	Extract 2A	Extract 2B
Reducing Power	Folin-Ciocalteu (mg GAE/ g dry extract)	11.94 $\pm$ 1.50 <sup>a</sup>	9.95 $\pm$ 0.50 <sup>b</sup>	13.42 $\pm$ 0.46 <sup>c</sup>
	ABTS assay (mg AAE/ g dry extract)	9.50 $\pm$ 0.97 <sup>a</sup>	11.38 $\pm$ 0.39 <sup>b</sup>	13.21 $\pm$ 0.86 <sup>c</sup>
Scavenging activity	DPPH assay (mg TE/ g dry extract)	3.28 $\pm$ 0.28 <sup>a</sup>	1.10 $\pm$ 0.19 <sup>b</sup>	4.17 $\pm$ 0.26 <sup>c</sup>
	ORAC assay (mg TE/g dry extract)	44.28 $\pm$ 2.38 <sup>a</sup>	39.82 $\pm$ 3.01 <sup>b</sup>	66.81 $\pm$ 2.96 <sup>c</sup>

In each row different letters (a-c) mean significant differences between extracts ( $p < 0.05$ ). Abbreviations: GAE - Gallic acid equivalent; AAE - Ascorbic acid equivalent; TE - Trolox equivalent.

All extracts were good sources of natural phenolic compounds and showed antioxidant capacity. In general, the AA was higher in the extract 2B than in other extracts

### $\beta$ -glucans

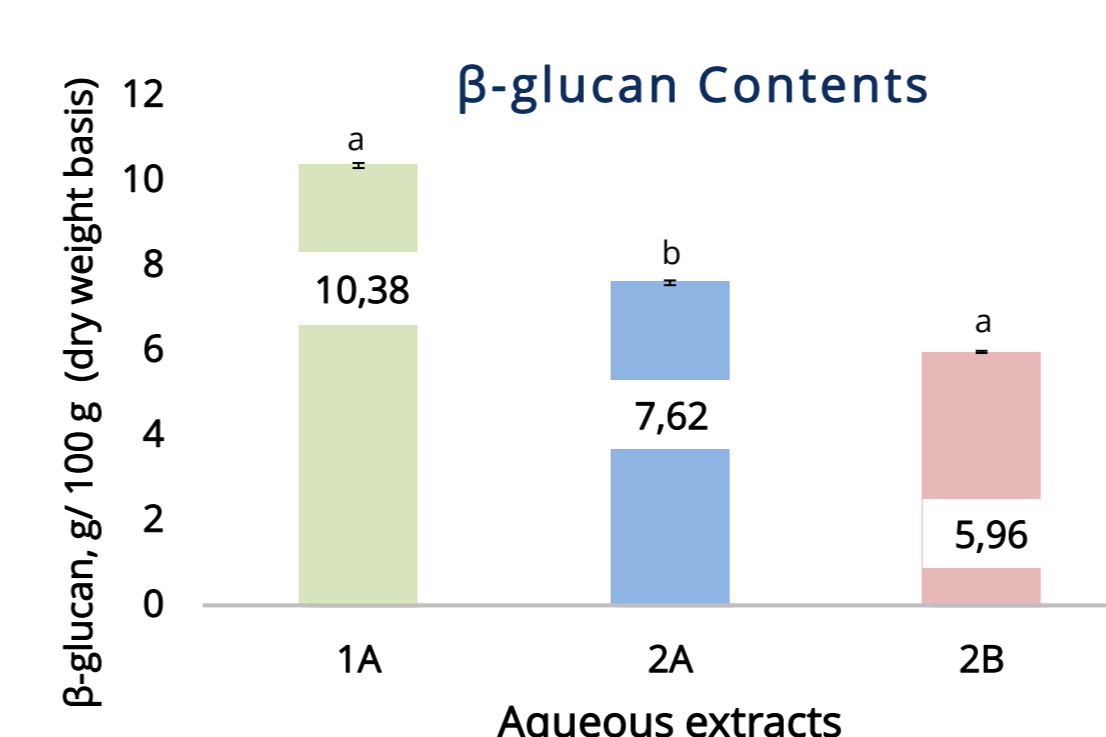


Figure 3. Extraction yields of aqueous extracts. Each value is expressed as mean  $\pm$  standard deviation (n=4) and different letters indicate significant difference  $p < 0.05$  between extracts

$\beta$ -glucans represent 4-13% of the total dietary fiber. Extract 1A had higher  $\beta$ -glucan content, which allows to predict a potential natural source of these compounds with immunological activity.

### Toxicity

The extracts of *P. ostreatus* (40 mg/mL) did not have a cytotoxic or genotoxic effect, demonstrating their safety for application in food products.

### Antibacterial activity

Table 2. MIC values (mg/mL) of the aqueous extracts against Gram-positive bacteria.

Samples	<i>Bacillus cereus</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus aureus</i>
1A	20	40	40
2A	10	10	10
2B	40	40	40

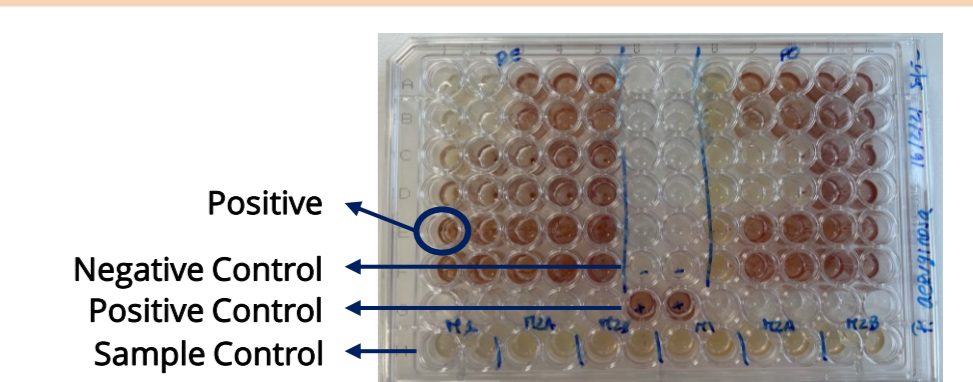


Figure 4. Example of an antimicrobial test microplate

Table 3. MIC values (mg/mL) of the aqueous extracts against Gram-negative bacteria.

Samples	<i>Salmonella enterica</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas tolaasii</i>	<i>Pseudomonas agarici</i>	<i>Yersinia enterocolitica</i>
1A	20	40	20	40	40	40	20
2A	10	10	10	20	10	10	10
2B	20	40	20	40	> 40	20	20

In general, all aqueous extracts of *P. ostreatus* inhibited bacterial growth, and the extract with the best results and lowest concentration (10 mg/mL) was 2A. None of the extracts tested was bactericidal.

## Conclusions

According to the results, these aqueous extracts of *P. ostreatus* bio-residues can be considered a natural source of phenolic compounds,  $\beta$ -glucans and compounds with antioxidant and antimicrobial activities. Thus, the valorization of these bio-residues is viable by developing "green" value-added products with great potential for application in nutraceutical and food products, contributing to a circular economy.

## Bibliography

Marçal, S., Sousa, A. S., et al. (2021). Impact of postharvest preservation methods on nutritional value and bioactive properties of mushrooms. *Trends in Food Science & Technology*.

## Acknowledgements

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