Foods 2025 Conference

The 6th International Electronic Conference on Foods

28–30 October 2025 | Online

MDPI

Ultrasound-Assisted Extracted Optimization of Soluble Dietary Fiber from *Pleurotus ostreatus* Spent Mushroom Substrate Using Box-Behnken Desing

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INTRODUCTION & AIM

Spent mushroom substrate (SMS) is the main co-product of *Pleurotus ostreatus* cultivation. It is mostly used for composting, which poses management and environmental challenges. Due to its high dietary fiber content, SMS is a promising source of bioactive compounds, especially polysaccharides with potential functional properties.

Conventional extraction methods are being replaced by innovative and sustainable approaches, such as **ultrasound-assisted extraction (UAE).** The UAE utilizes acoustic cavitation to disrupt cell structures, thereby enhancing mass transfer and extraction efficiency.

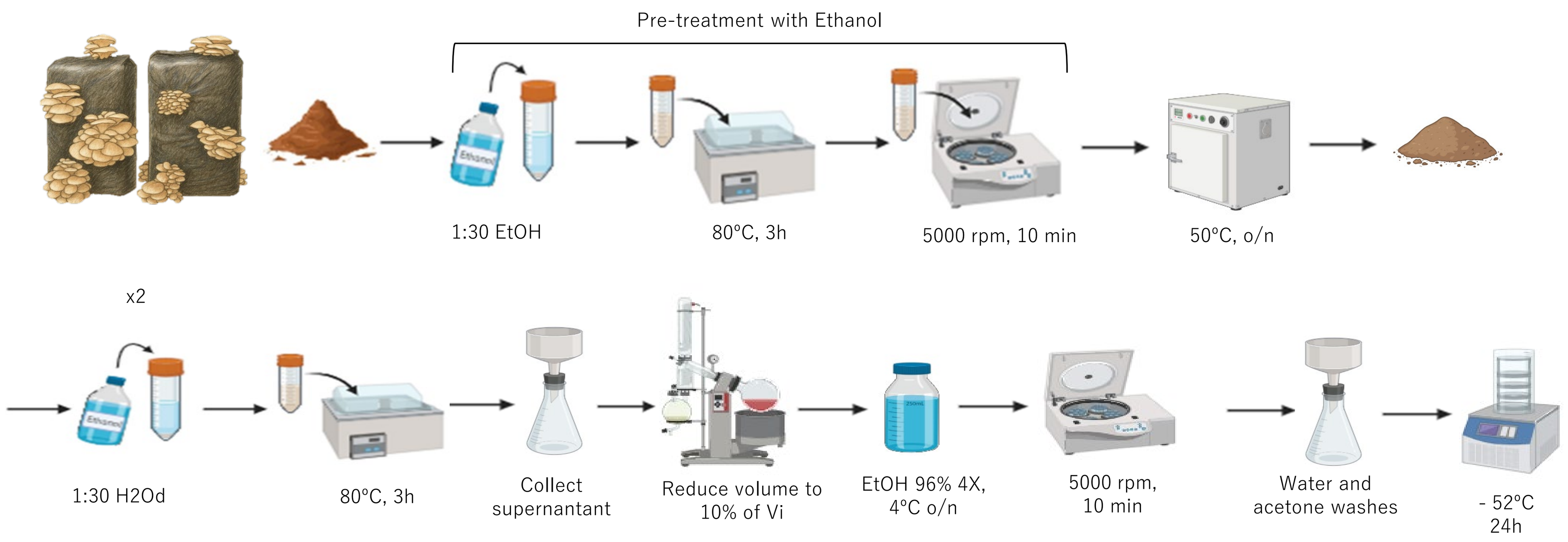
This study aimed to optimize the extraction of **soluble dietary fiber (SDF)** from *P. ostreatus* SMS using UAE. A **Box–Behnken design (BBD)** was applied to assess the effects of solid-to-liquid ratio (15–40 g/L), ultrasonic power (100–1200 J/mL), and particle size (0.25–4 mm). Data were modeled with a second-order polynomial equation using multiple regression analysis in Statgraphics 19.0.

The objective of the present study is to optimize the extraction of soluble dietary fiber (SDF) from *Pleurotus ostreatus* spent mushroom substrate using ultrasound-assisted extraction (UAE), by evaluating the effects of solid-to-liquid ratio, ultrasonic power, and particle size through a Box–Behnken design.

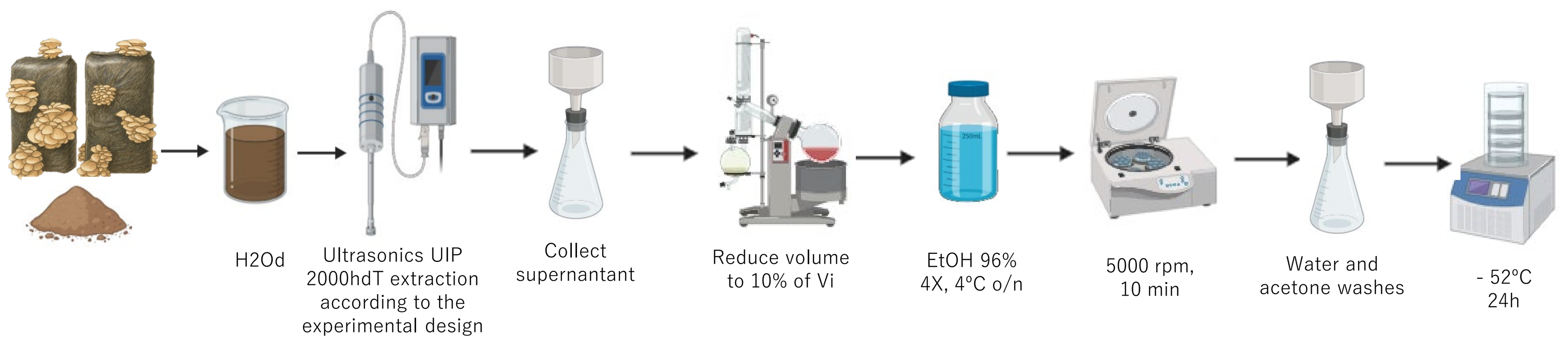
METHOD

SMS processing: SMS bags from *Pleurotus ostreatus* cultivated in CTICH were collected after the second and final mushroom flush, once the fructification process was completed. The SMS was then dried at 40ºC, and ground to 2mm, and stored at 4ºC for further use.

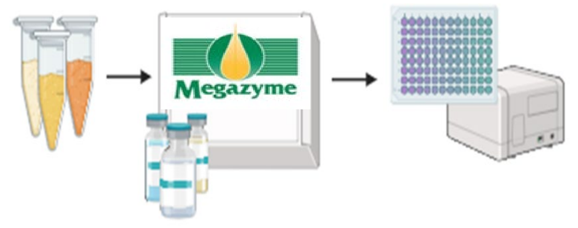
Traditional polysaccharide extraction:



UAE polysaccharide extraction:



Total dietary fiber determination:



RESULTS & DISCUSSION

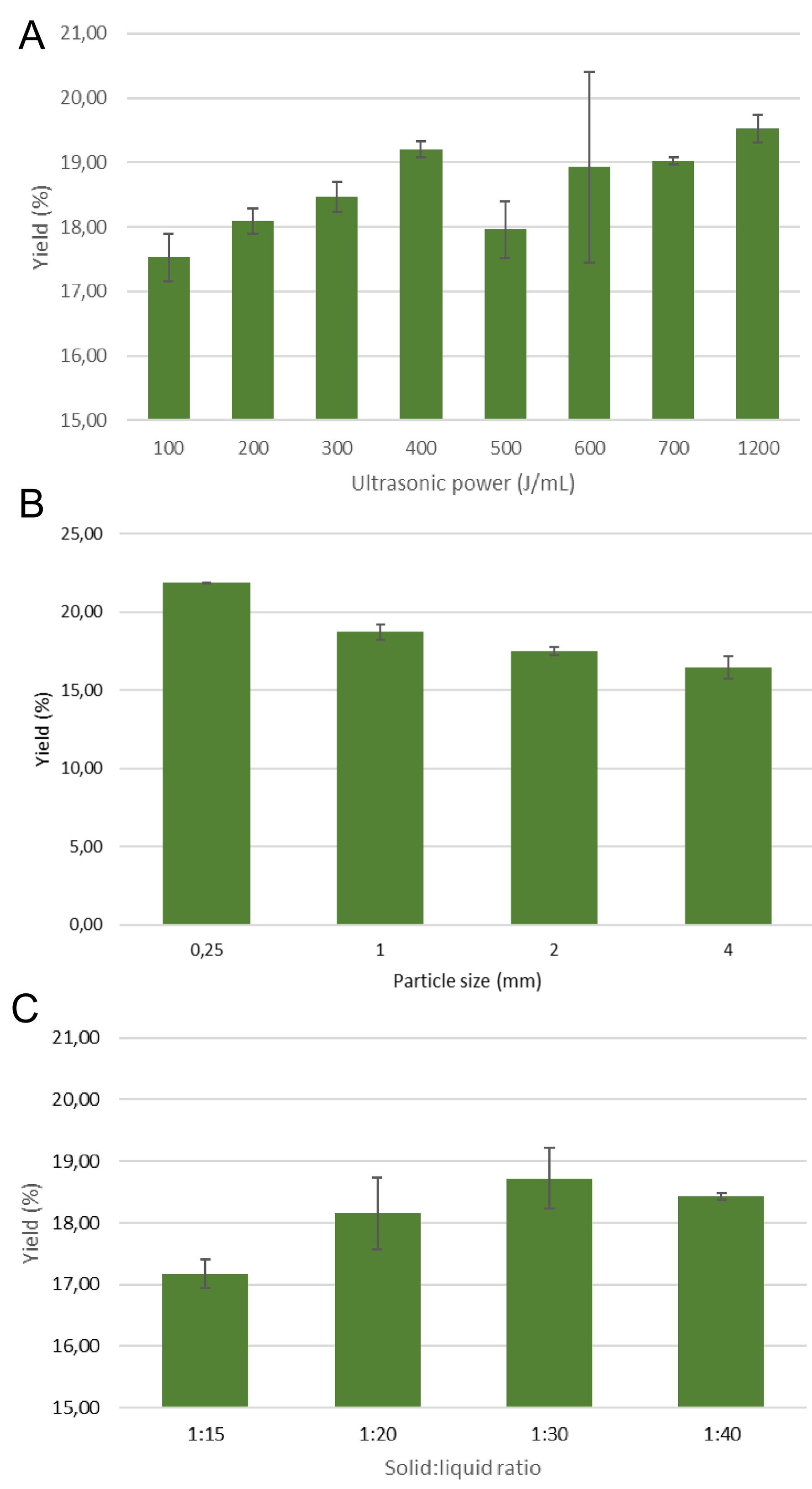


Figure 1. Polysaccharide extraction yield (%) from SMS according to (A) ultrasound power, (B) particle size; and (C) solid:liquid ratio. Values represent the mean of two independent experiments, and error bars indicate the standard error of the mean (SEM).

Run	X1	X2	X3	Yield (%)	SDF (%)	g fiber / 100g SMS
1	200	0.25	27.5	19.89±0.93	29.87±0.25	5,94
2	200	2	27.5	18.71±0.60	39.71±0.65	7,43
3	500	0.25	27.5	17.74±0.90	33.84±0.35	6,00
4	500	2	27.5	17.21±1.16	38.28±0.20	6,59
5	350	0.25	15	16.52±0.71	40.90±0.53	6,76
6	350	2	15	16.56±0.57	38.26±0.59	6,33
7	350	0.25	40	19.10±0.81	29.95±1.18	5,72
8	350	2	40	18.25±0.88	36.42±0.14	6,65
9	200	1.125	15	13.65±0.27	31.67±0.65	4,32
10	500	1.125	15	14.46±0.55	32.23±0.11	4,66
11	200	1.125	40	14.89±0.09	31.60±0.33	4,70
12	500	1.125	40	16.24±0.09	23.27±0.19	3,78
13	350	1.125	27.5	15.44±0.41	23.86±0.49	3,68
14	350	1.125	27.5	15.88±0.05	31.51±1.07	5,00
15	350	1.125	27.5	15.73±0.88	30.72±0.35	4,83
Flour of SMS	-	2	-	-	59.23±1.18	-
Traditional	-	2	30	11.79±0.82	29.48±0.28	3.48
Optimized	200	2	27	18.86±0.31	39.82±0.52	7.51

Table 1. Box–Behnken experimental design for *P. ostreatus* SMS. Independent factors: X_1, ultrasonic power (J/mL); X_2, particle size (mm); X_3, solid-to-liquid ratio (%). SDF was measured as the response variable. Data are presented as mean ± SEM of three determinations..

CONCLUSION

Soluble dietary fiber (SDF) was identified as the response variable best fitting the model (R^2 = 80.8%), with both solid-to-liquid ratio and particle size significantly influencing SDF extraction from *Pleurotus ostreatus* SMS. Optimal conditions (200 J/mL ultrasonic energy, 1:27 solid-to-liquid ratio, 2 mm particle size) yielded a predicted dietary fiber content of 39.5%, experimentally validated and confirming the model's predictive capacity. UAE extraction produced 7.51 g fiber/100 g SMS, approximately doubling the yield of the conventional method (3.48 g/100 g).

These results demonstrate that ultrasound-assisted extraction (UAE) enhances extraction efficiency, increases soluble fiber recovery by ~10%, reduces processing time, and improves the nutritional quality of the product.

Additionally, UAE primarily recovers **water-soluble polysaccharides**, which are associated with **greater bioactivity** (antioxidant, immunomodulatory). However, **insoluble polysaccharide fractions**—retained in the extraction residue—still represent a significant portion of the total content and may contribute **prebiotic and structural benefits**.

FUTURE WORK

Future work will focus on integrating both soluble and insoluble fractions to fully harness the functional potential of *Pleurotus* polysaccharides. Additionally, scaling up the process to a pilot or industrial level will allow assessment of economic feasibility and evaluation of the functional and physicochemical properties of the extracted soluble fiber in food formulations.

The Project leading to this report has recieved funding from European Regional Development Fund (ERDF) , granted to the Autonomous Community of La Rioja within the ERDF Operational Programs 2021-2027 (Project number: CT24_05). This research was also funded by Grants for Industrial Doctorates in the Autonomous Comunity of La Rioja, Spain.