2nd FCUB ERA Workshop

Food Chemistry and Biotechnology

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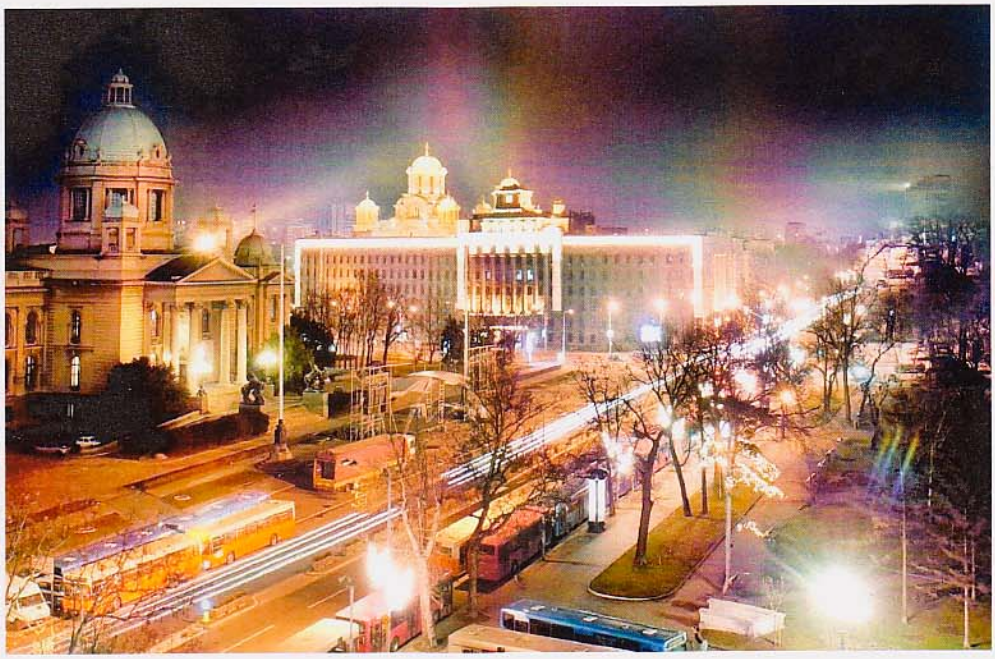

2nd FCUB ERA Workshop

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Content:

P 20. Antioxidative and immunomodulating activities of polysaccharide extracts from the basidiomycetes mushrooms

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Water-soluble polysaccharide-enriched fractions were isolated from the basidiomycetes mushroom species *Agaricus bisporus*, *Agaricus brasiliensis* and *Phellinus linteus* [1,2]. Their antioxidant activities were investigated using *in vitro* assay systems: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric-reducing antioxidant power assay. Immunomodulation was tested *in vitro*, by measuring the synthesis of interferon-gamma (IFN-γ) by enzyme linked immunosorbent assay (ELISA). To explain the possible differences observed, we have measured the total polysaccharide, glucan and total phenolic contents of mushrooms extracts.

With regard to scavenging ability on DPPH radicals, the *P. linteus* extract showed very good scavenging ability as evidenced by their particularly low EC_{50} values (< 0.1 mg/mL). For *A. brasiliensis* and *A. bisporus*, EC_{50} values were 0.27 and 2.0 mg/mL, respectively. *P. linteus* extract with the highest phenol showed the highest scavenging ability.

For reducing power of *P. linteus*, *A. brasiliensis* and *A. bisporus* EC_{50} values were found of 0.47, 3.13 and 14.83 mg/mL, respectively. Regression analysis revealed a strong and significant correlation between the following parameters: EC_{50} (reducing power) and total phenols ($r = -0.706$, $p < 0.05$); EC_{50} and total polysaccharide content ($r = 0.699$, $p \leq 0.05$); total glucan and β-glucan contents ($r = 0.966$; $r = 0.982$, $p < 0.05$).

Measurements of the immunomodulatory activity showed that *A. bisporus*, and *A. brasiliensis* polysaccharides extracts cause an increased synthesis of IFN-γ in human PBMC's, suggesting proinflammatory effects, while *P. linteus* extract showed a decrease of IFN-γ synthesis suggesting an immunosuppressive activity. Modified polysaccharides of *A. bisporus* and *A. brasiliensis* by 1,6 β-glucanase showed a strong enhancement of immunostimulatory activity compared to the native extracts. *P. linteus* lost immunosuppressive effect on PBMC's and IFN-γ synthesis. Results confirmed that the primary structure of polysaccharides is of more importance then the tertiary structure for their immunostimulating activity.

References

[1] Kozarski, M. et al.,(2011), Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus, Agaricus brasiliensis, Ganoderma lucidum* and *Phelinus linteus*. Food Chemistry 129, 1667-1675.

[2] Klaus, A., et al.,(2011), Antioxidative activities and chemical characterization of polysaccharides extracted from the basidiomycete *Schizophyllum commune*. LWT-Food Science and Technology 44, 2005-2011.

Antioxidative and immunomodulating activities of polysaccharide extracts from the basidiomycetes mushrooms

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Introduction-Polysaccharides are potentially useful biologically active ingredients for pharmaceutical use, such as for immune regulation, for anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV and hypoglycemic activities. The mushroom-derived polysaccharides lentinan, schizophyllan, and krestin have been accepted as immunoceuticals in Japan, Korea and China.

Materials and Methods-Water-soluble polysaccharide-enriched fractions were isolated from the basidiomycetes mushroom species *Agaricus bisporus*, *Agaricus brasiliensis* and *Phellinus linteus* [1,2]. Their antioxidant activities were investigated using *in vitro* assay systems: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric-reducing antioxidant power assay. Immunomodulation was tested *in vitro*, by measuring the synthesis of interferon-gamma (IFN-γ) by enzyme linked immunosorbent assay (ELISA). To explain the possible differences observed, we have measured the total polysaccharide, glucan and total phenolic contents of mushrooms extracts.

Results and Discussion- With regard to scavenging ability on DPPH radicals, the *P. linteus* extract showed very good scavenging ability as evidenced by their particularly low EC_{50} values (< 0.1 mg/mL). For *A. brasiliensis* and *A. bisporus,* EC_{50} values were 0.27 and 2.0 mg/mL, respectively. *P. linteus* extract with the highest phenol showed the highest scavenging ability (Fig. 1).

For reducing power of *P. linteus*, *A. brasiliensis* and *A. bisporus* EC_{50} values were found of 0.47, 3.13 and 14.83 mg/mL, respectively. Regression analysis revealed a strong and significant correlation between the following parameters: EC_{50} (reducing power) and total phenols ($r = -0.706$, $p < 0.05$); EC_{50} and total polysaccharide content ($r = 0.699$, $p \leq 0.05$); total glucan and β-glucan contents ($r = 0.966$; $r = 0.982$, $p < 0.05$). The phenolic compounds present in the polysaccharide extracts are primarily responsible for the antioxidant activity of all our mushroom species extracts. The oxidized polyphenols were still present in the extracts after hot water treatment, ethanol precipitation and dialysis.

Measurements of the immunomodulatory activity showed that *A. bisporus*, and *A. brasiliensis* polysaccharides extracts cause an increased synthesis of IFN-γ in human PBMC's, suggesting proinflammatory effects, while *P. linteus* extract showed a decrease of IFN-γ synthesis suggesting an immunosuppressive activity (Fig. 3). Modified polysaccharides of *A. bisporus* and *A. brasiliensis* by 1,6 β-glucanase showed a strong enhancement of immunostimulatory activity compared to the native extracts. *P. linteus* lost immunosuppressive effect on PBMC's and IFN-γ synthesis. Results confirmed that the primary structure of polysaccharides is of more importance then the tertiary structure for their immunostimulating activity.

Fig. 1. Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals of polysaccharide extracts of *bisporus*, *A. brasiliensis* and *P. linteus*. Each value is expressed as mean ± SEM ($n = 3$).

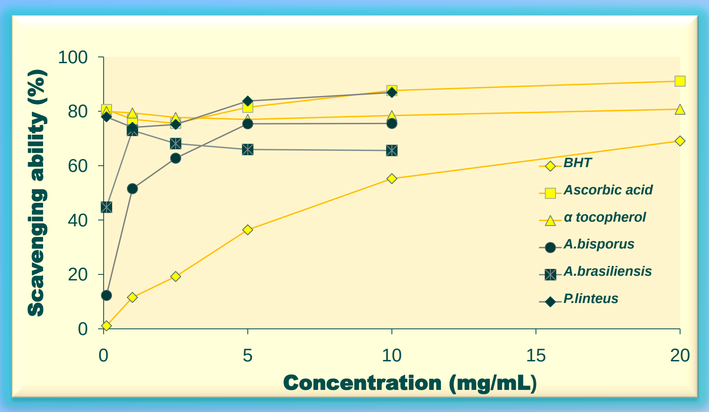


Fig. 2. Reducing power of polysaccharide extracts of *bisporus*, *A. brasiliensis* and *P. linteus*.

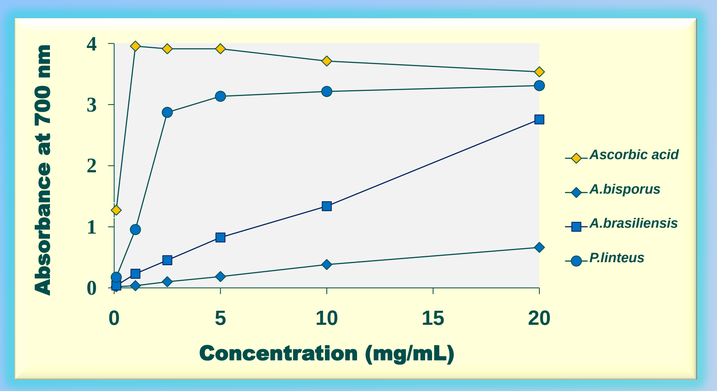
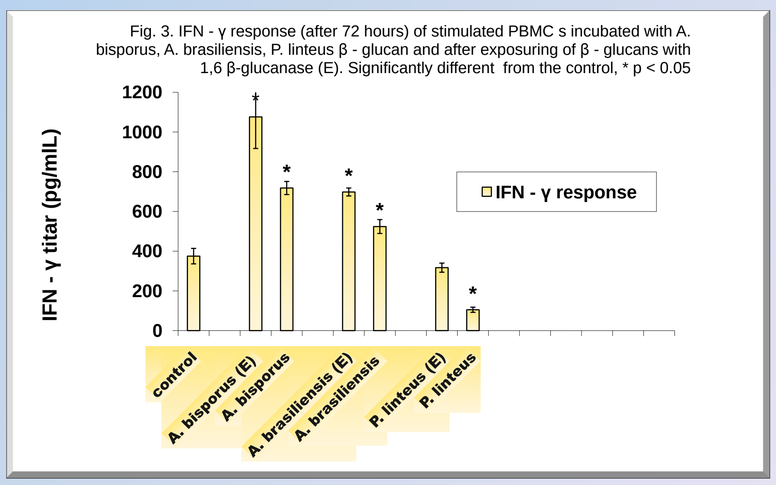


Table 1 EC_{50} values, total phenol, polysaccharide, glucan and β- glucan content of polysaccharide extracts from *A. bisporus*, *A. brasiliensis* and *P. linteus*.

Mushroom species	EC_{50}[a] (mg extract/mL)		Total phenol content (g/100g)	Total polysaccharide content (g/100g)	Total glucan content (g/100g)	Total β-glucan content (g/100g)
	Scavenging ability on DPPH radicals	Reducing power				
Agaricus bisporus	2.00 ± 0.18 A[a]	14.83 ±1.16 A	0.4± 0.04 C	74.4 ± 2.1 A	63.8 ± 0.9 A	58.2 ± 0.9 A
Agaricus brasiliensis	0.27 ± 0.02 B	3.13 ± 0.14 B	1.2 ± 0.2 B	45.9 ± 0.7 C	40.1 ± 1.0 B	22.8 ± 0.4 B
Phellinus linteus	< 0.1	0.47 ± 0.03 C	8.4 ± 0.5 A	62.6 ± 0.3 B	24.5 ± 1.3 C	21.8 ± 1.7 B

[a] EC_{50} value: The effective concentration at which the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were scavenged by 50% and the absorbance was 0.5 for reducing power. EC_{50} value was obtained by interpolation from linear regression analysis.
[b] Means with different letters within a column are significantly different ($p < 0.05$).



[1] Kozarski, M. et al.,(2011), Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus*, *Agaricus brasiliensis*, *Ganoderma lucidum* and *Phelinus linteus*. Food Chemistry 129, 1667-1675.

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