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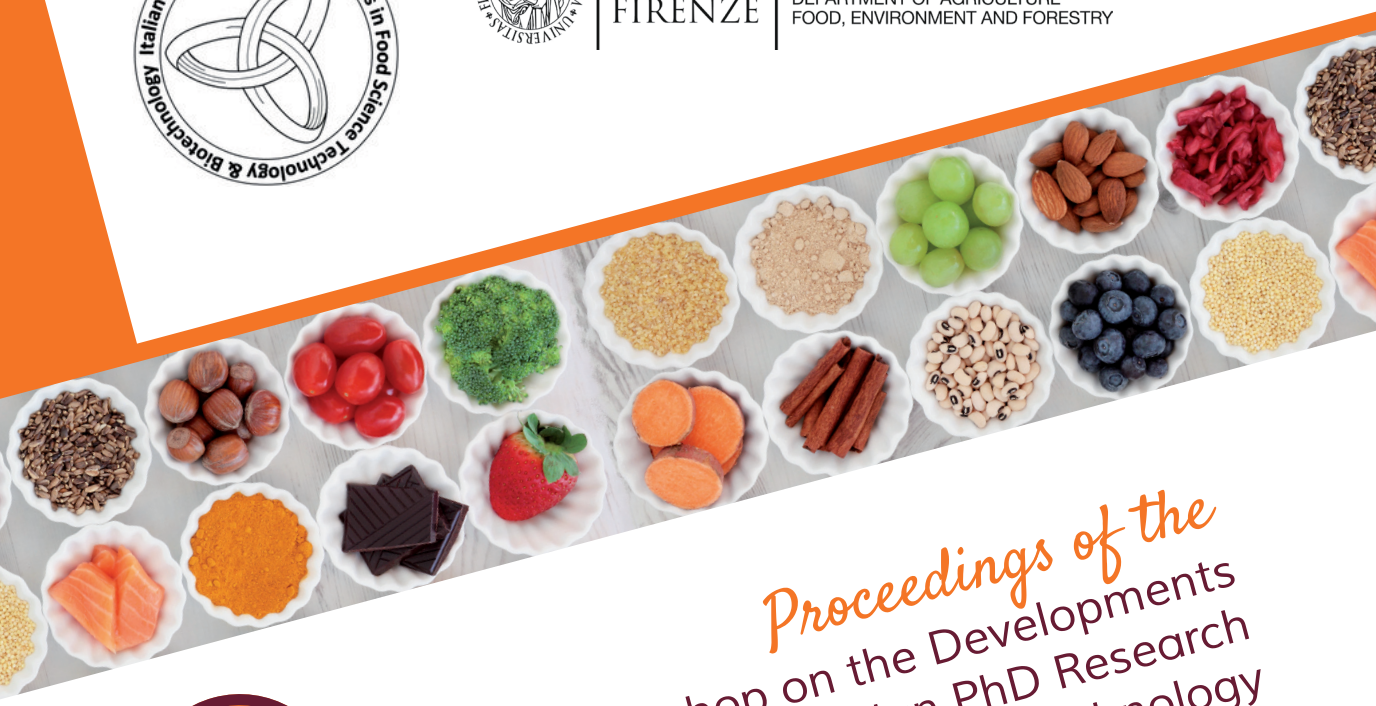

Proceedings of the XXIV Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology

Florence, 11-13 September 2019

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frullaidee@gmail.com

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Production of Functional Ingredients Using Bioactive Compounds from *Pleurotus Ostreatus*

Francesca Gallotti (francesca.gallotti@unimi.it)
Dept. of Food, Environmental and Nutritional Sciences (DeFENS),
University of Milan, Italy
Tutor: Prof. Vera Lavelli

The first activities of the PhD thesis project are described. Firstly, the major bioactive compounds of *Pleurotus ostreatus*, namely: β-glucans, ergosterol, vitamin D_2 and phenolics were evaluated. Secondly, a food grade extraction procedure for the recovery of β-glucans was developed by measuring the yield and antioxidant activity. β-glucan-rich extract was used as emulsifying agent for the encapsulation of an oxidisable compound. The stability of oil-in-water (O/W) emulsions was determined checking the variation of oil droplets size distribution over time. The left-over insoluble solids could be exploited as a source of vitamin D_2 and ergosterol.

Produzione di ingredienti funzionali usando composti bioattivi da *Pleurotus ostreatus*

Le prime attività del progetto di tesi di dottorato sono descritte. In primo luogo, sono stati misurati i principali composti bioattivi di *P. ostreatus*, ovvero: β-glucani, ergosterolo, vitamina D_2 e fenoli. Successivamente, è stata messa a punto una procedura di estrazione dei β-glucani misurando la resa e l'attività antiossidante. I β-glucani sono stati utilizzati come emulsionanti per l'incapsulamento di un composto ossidabile. La stabilità delle emulsioni olio-in-acqua (O/W) è stata determinata attraverso la variazione della distribuzione dimensionale delle gocce d'olio nel tempo. Il residuo di estrazione può essere utilizzato come fonte di ergosterolo e vitamina D_2.

Key words: *Pleurotus ostreatus*, β-glucans, emulsion stability, encapsulation, vitamin D_2, sustainability.

1. Introduction

This PhD project aims at the development of applications of *P. ostreatus* biomass grown on a "sustainable" substrate as an ingredient in added-value foods. In accordance with the PhD thesis project previously decribed (Gallotti, 2018), this poster reports the main results of the first two activities concerning:

(A1) Evaluation of the major bioactive compounds, namely: β-glucans, ergosterol, vitamin D_2 and phenolics;

(A2) Development of a "food grade" extraction procedure of β-glucans and application of the extract for the stabilization of O/W emulsions.

2. Materials and Methods

For the study, a selected commercial strain of *P. ostreatus* rich in β-glucans was dried at 40 °C and grinded to a fine powder before use. Vitamin D_2 and its precursor ergosterol were extracted according to the procedure of Sławinska et al. (2016) and identified by the HPLC procedure by Huang et al. (2015). Hot-water soluble β-glucans fraction was extracted from *P. ostreatus* powder following a method adapted from Synytsya et al. (2009) and then modified as described by Gallotti et al. (2019). The resulting extracts are, namely: extract from Washed solids, W; extract from Uwashed solids, UW, obtained

removing a washing step with distilled water before the extraction. The amount of β-glucans in the mushroom powder and in the extracts was measured with an enzymatic kit purchased from Megazyme (Bray, Co. Wicklow, Ireland). The phenolic content was determined spectrophotometrically by the Folin-Ciocalteu assay, while the antioxidant activity was determined by the ferric ion reducing antioxidant power (FRAP) method. The O/W emulsions were prepared using maltodextrin with dextrose equivalents of 12 (MD, DE12) as wall material, β-glucans extract as emulsifier and commercial sunflower oil with 500 mg/kg of α-tocopherol as a model for lipophilic active compounds. To prepare the emulsions, a two-step protocol with first the production of a pre-emulsion without wall material and then the addition of an aqueous solution of MD and emulsifying agent was used. Emulsions were homogenized at lab scale (100 mL of volume) by a Polytron homogenizer and at pilot scale (1L of volume) by a rotor-stator homogenizer. The emulsion stability was estimated from the evolution of the oil droplet size distribution measured by laser light diffraction with a Mastersizer at the time of preparation and after 2 hours. From the volume size distribution obtained, the median diameter d50 and d10 and d90, corresponding to the diameters for which respectively 50, 10 and 90% of the particles have a smaller size, were determined and the span was calculated as span = (d90 – d10)/d50. Experimental data were analyzed by one-way ANOVA using LSD (Least Significant Difference) as a multiple range test.

3. Results and Discussion

3.1 Characterization of the biomass of *P. ostreatus* and its extracts

The major bioactive compounds of *P. ostreatus* biomass are reported in Table 1. The levels of these compounds in the selected strain were relatively high compared to those reported in the literature (Lavelli et al., 2018)

Table 1 *Characterization of P. ostreatus biomass.*

Total glucans g/100g d.w.	β-glucans g/100g d.w.	Vitamin D_2 mg/100g d.w.	Ergosterol mg/100g d.w.	Total phenolics mg gallic acid equivalents/100g d.w.
36.0 ± 0.9	35.0 ± 0.9	0.26 ± 0.09	154 ± 23	272 ± 19

Data are average ± SD.

3.2 Encapsulation of an oxidisable target using *Pleurotus* β-glucans as an emulsifyng agent

The total glucans and β-glucans contents (Tab. 1) were used to calculate the yield of extraction reported in Table 2. The extraction method for β-glucans previously proposed by Synytsya et al. (2009) was modified by removing a washing step in order to increase β-glucan yield from 7.7 to 17.2 % (Tab. 2). Different formulations of emulsions were then tested (Tab. 3) at lab and pilot scale, using both the W and UW extracts, maintaining the same percentage of dry matter (aroud 40%w/w). The results about stability (Table 3) show that no significant changes have occurred in the microstructure of the emulsions, confirming that they were physically stable when the percentages of β-glucans was more than 0.08% for the emulsions with 4%w/w of oil. The amount of emulsifier required depends on the oil quantity, thus for emulsions with 8%w/w of oil was necessary to increase the percentage of β-glucans up to 0.13%w/w to maintain the stability; less percentages of β-glucans resulted in not stable emulsions (data not shown). In conclusion, the water-soluble extract

of P. ostreatus can act as an efficient emulsifying agent, while the insoluble solids could be exploited as a source of vitamin D_2 and ergosterol.

Table 2 *Characterization of the β-glucans-rich extracts obtained from P. ostreatus.*

Extract	Dry matter %	Yield total glucans %	Total glucans g/100g d.w.	Yield β-glucans %	β-glucans g/100g d.w.	FRAP µmol Fell eq./100g d.w.
W	$1.0^{a} \pm 0.0$	$11.2^{a} \pm 0.6$	$20.1^{a} \pm 1.1$	$7.7^{a} \pm 0.6$	$13.4^{a} \pm 1,1$	$0.07^{a} \pm 0.01$
UW	$1.4^{b} \pm 0.1$	$20.4^{b} \pm 1.1$	$26.2^{b} \pm 1.3$	$17.2^{b} \pm 0.8$	$21.6^{b} \pm 1.1$	$0.13^{b} \pm 0.00$

Data are average ± SD. Values in the same column with differing superscripts are significantly different (LSD, $p < 0.01$).

Table 3 *Formulations of emulsions and size distributions at the time of preparation (t = 0) and after two hours (t = 2h)*

Emulsion	Formulation			Size distribution			
	Oil %	MD %	β-glucans %	d50 (t = 0) (µm)	d50 (t = 2h) (µm)	Span (t = 0)	Span (t = 2h)
E4_lab_W	4.0	35.4	0.08	2.02^{a}	2.00^{a}	1.4^{b}	1.4^{b}
E4_pilot_W	4.0	35.4	0.09	2.49^{a}	2.56^{a}	1.4^{b}	1.2^{b}
E4_pilot_UW	4.0	35.6	0.09	2.50^{a}	2.59^{a}	1.1^{b}	1.2^{b}
E6_lab_UW	8.0	31.4	0.13	2.84^{a}	2.96^{a}	1.4^{b}	1.4^{b}
E6_pilot_UW	8.0	31.5	0.13	2.76^{a}	2.83^{a}	1.1^{b}	1.1^{b}

D50 and span values in the same row with differing superscripts are significantly different (LSD, $p < 0.01$).

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