Received: 29 May 2021 | Revised: 10 October 2021 | Accepted: 21 November 2021

DOI: 10.1111/jfpe.13949

ORIGINAL ARTICLE

Journal of Food Process Engineering WILEY

Production of stable emulsions using β-glucans extracted from *Pleurotus ostreatus* to encapsulate oxidizable compounds

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Funding information
Fondazione Cariplo, Grant/Award Number: 2016-0740

Abstract

The basidiomycete *Pleurotus ostreatus* is a sustainable food source known to be rich in β-glucans, functional compounds in mushrooms recognized for their numerous health, nutraceutical, and physicochemical properties. The aim of the present study was to extract β-glucans from *P. ostreatus* powder and to study the feasibility of using the extract to prepare oil-in-water emulsions formulated for the encapsulation of lipophilic antioxidants by spray drying, for example, with an oil droplet size about 2 μm, a dry matter content of 40% w/w, and with a physical stability of at least 2 hrs. Hot-water soluble β-glucans fraction was extracted from *P. ostreatus* powder following a method adapted from literature. Emulsions were prepared by rotor-stator homogenization using maltodextrin as wall material, β-glucans extract as emulsifier, and commercial sunflower oil as a model for lipophilic active compounds. The emulsion stability was estimated from the evolution over time of the oil droplet size distribution measured by laser light diffraction and experimental data were investigated using one-way ANOVA. Extracts containing up to 27% w/w water-soluble β-glucans were produced. Physically stable mono-modal emulsions were obtained when maltodextrin/β-glucans weight ratio was lower than about 500 to avoid depletion and β-glucans/oil weight ratio was more than 0.014 to stabilize oil droplets. The usage of emulsifiers made-up by polymeric constituents with inherent bioactivity, such as mushroom β-glucans, could be a sustainable and healthy alternative to common emulsifiers.

Practical applications

A great deal of interest has been expressed in a unique dietary fiber, named β-glucans, from the basidiomycete *Pleurotus ostreatus*, a mushroom which can grow efficiently on various clean by-products of food processing, thus representing a sustainable food source. β-Glucans' functionality is associated with their many healthy and physicochemical properties, for instance emulsifying properties. The usage of emulsifiers made-up by polymeric constituents with inherent bioactivity, such as mushroom β-glucans, could be a sustainable and healthy alternative to common emulsifiers.

1 | INTRODUCTION

Bioactive lipophilic compounds, such as fatty acids, aroma, and flavor compounds, vitamins, antioxidants, phytosterols, and essential oils are widely used in the food industry due to their multifunctional roles. However, these compounds are also involved in chemical processes linked to degradation; therefore, it is important to protect these active compounds against factors promoting their degradation such as oxygen, humidity, light, and so on (Lavelli, D'Incecco, & Pellegrino, 2021; Meléndez-Martínez et al., 2021). To this aim, bioactive lipophilic compounds can be encapsulated by spray drying. The first step of lipophilic compounds encapsulation by spray drying consists in the preparation of an oil-in-water liquid emulsion with formulation, microstructure, and properties suitable for further spray drying and use (Hernandez-Sanchez, Cuvelier, & Turchiuli, 2015). Moreover, the emulsion needs to be physically stable until and during spray drying in order to ensure correct oil distribution within the matrix of encapsulation: this means that no evolution of the size distribution should occur due to emulsion destabilization or shear stress during atomization (Munoz-Ibanez et al., 2016; Turchiuli, Gallotti, Hernandez-Sanchez, & Cuvelier, 2017). The selection of an efficient combination of wall materials and emulsifier agent with the suitable ratio of each constituent in the blend has therefore to be carefully studied. Wall materials must be soluble in water, allow forming solutions with a proper viscosity at high solids concentration (e.g., up to 40–60%w/w dry matter) in order to be pumped and sprayed, and bring to a stable powder without sticking during drying. Due to their high solubility in water and low viscosity at high solids concentrations, hydrophilic carbohydrate molecules, such as maltodextrins (MD), are suitable wall materials. However, these components have no interfacial properties and must be used in association with emulsifier or surfactant molecules to provide protection from physical destabilization of emulsions (Turchiuli, Jomenez Munguia, Hernandez Sanchez, Cortes Ferre, & Dumoulin, 2014).

Since the field of encapsulation is expanding, research is required to study the possibility of using alternative and naturally occurring compounds, with health effects and available at low cost (e.g., from local production, by-products, etc.), as support materials or emulsifiers for a wide variety of active compounds (Falco, Sotres, Rascón, Risbo, & Cárdenas, 2017). In this scenario, the genus *Pleurotus* can make a valuable contribution, because these mushrooms are able to grow on by-products with a limited capital investment and technical skills. Besides their nutritional value, it has been demonstrated that *Pleurotus* spp. has health promoting benefits, mainly due to their unique dietary fiber fraction. Among the constituents of this fraction, β-glucans are the major component and among the most studied functional compounds (Lavelli, Proserpio, Gallotti, Laureati, & Pagliarini, 2018). β-Glucans from different sources have different linkage types, branching manners and molecular weight. In mushrooms, these polysaccharides have a backbone of D-glucose-linked β-(1 → 3) with no branches or variable amounts of β-(1 → 6) branches; these glucose chains are twisted and create a single or a triple helix stabilized by inter-chain hydrogen bonds. Besides their recognized healthy effects, β-glucans' functionality is also associated with their physicochemical properties, such as thickening, stabilizing, emulsifying, foaming, and gelation properties (Zhu, Du, & Xu, 2016). These characteristics have been widely studied for β-glucans obtained from different cereals (Kontogiorgos, Biliaderis, Kiosseoglou, & Doxastakis, 2004; Valoppi, Wang, Alt, Peltonen, & Mikkonen, 2021) but poorly investigated for β-glucans from yeasts and mushrooms (Umaña, Turchiuli, Rosselló, & Simal, 2021). Thammakiti, Suphantharika, Phaesuwan, and Verduyn (2004) discovered that β-glucans obtained from brewer's yeast can be used in food products as a thickening, water-holding, or oil-binding agent and emulsifying stabilizer. Since the yeast and fungal glucans share a common structure, it can be assumed that they also share the same physical and chemical properties.

Taking into account this information, the aim of the present work was to propose formulations and protocols to produce stable oil-in-water emulsions with an oil droplet size around 2 μm and a dry matter content suitable for spray drying. Sunflower oil, rich in polyunsaturated fatty acids (PUFAs) and vitamin E, was used as a model oil phase containing active lipophilic compounds. Two protocols were tested for the preparation of the β-glucans extract. Emulsions formulations were tested at lab scale in terms of both emulsifier over oil (β-glucans/oil) and wall material over emulsifier (MD/β-glucans) weight ratio in order to find the suitable β-glucans content allowing to obtain physically stable emulsions with high dry matter content while avoiding depletion phenomena. A protocol for the production of emulsions at pilot scale was also tested.

2 | MATERIALS AND METHODS

2.1 | Materials

Commercial sunflower oil containing 11% w/w saturated, 29% w/w monounsaturated and 60% w/w polyunsaturated fatty acids, and 0.05% w/w α-tocopherol (Cora, France) was used as model oil for lipophilic compounds encapsulation. Maltodextrin DE 12 (MD) (Glucidex, Roquette, FR) was used wall material for the encapsulation process. The β-glucans-rich extract obtained from *Pleurotus ostreatus* powder (IoBoscoVivo, Vergiate, IT; see Section 2.2.1) was used to stabilize the emulsions.

2.2 | Methods

2.2.1 | Extract preparation

Fractions of water-soluble polysaccharides were isolated from *P. ostreatus* powder according to modified Synytsya's method (Synytsya et al., 2009) (Figure 1a). Three grams of powder were washed with 60 ml of ethanol 80% (w/v) by keeping the suspension under magnetic stirring overnight; then the supernatant was removed by centrifugation (14,000 rpm for 1 hr at 20°C). The volume of ethanol extract removed was replaced with the same volume of distilled

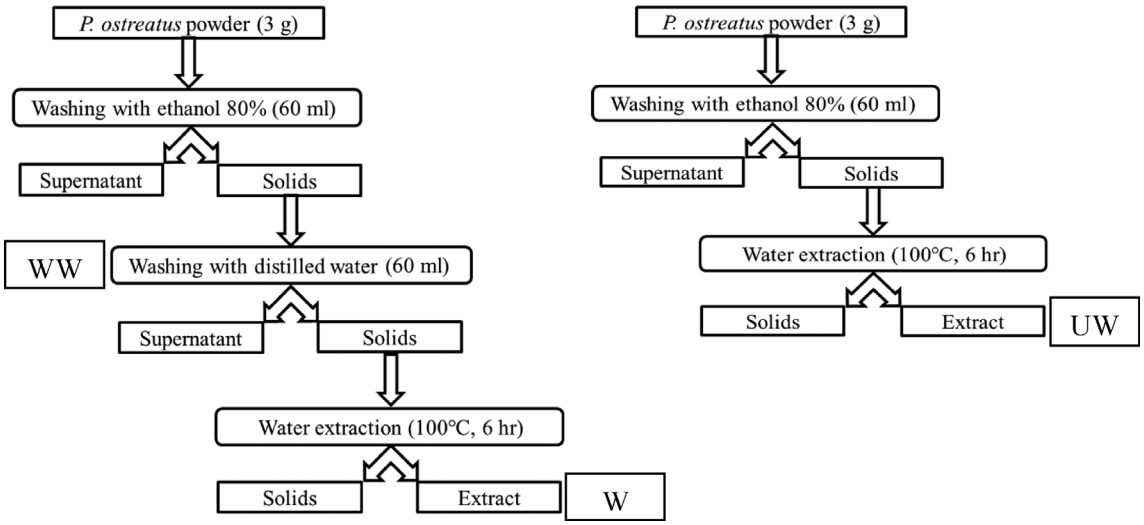

FIGURE 1 Steps to obtain the W and UW β-glucans-rich extracts from *P. ostreatus*

water for another centrifugation under the same conditions in order to wash the powder from the remaining solvent. Again, the aqueous washing solution (WW) was separated and replaced with the same volume of distilled water and the tubes containing the powder in water were placed in boiling water for 6 hrs to extract hot water-soluble compounds remaining in the powder (especially β-glucans). The resulting extract (extract from washed solids, W) was isolated by centrifugation and used to prepare emulsions E1_lab_W, E2_lab_W, E3_lab_W, E4_lab_W, E4_pilot_W, and E5_lab_W (see Section 2.2.3).

This method was further modified by removing the washing step with distilled water, since the results showed that this step of the extraction process caused an important loss of cold water-soluble β-glucans (Figure 1b). The extract obtained without the washing step (extract from unwashed solids, UW) was used to prepare emulsions E4_pilot_UW and E6_lab_UW (see Section 2.2.3).

2.2.2 | Evaluation of β-glucans content

The amount of β-glucans in the mushroom powder, in the extracts and in the spray dried powders was measured with an enzymatic kit purchased from Megazyme (Bray, Co. Wicklow, Ireland). Briefly, total glucans (plus free glucose and glucose from sucrose) was measured using controlled acid hydrolysis with H_2SO_4 and the glucose released was measured using glucose oxidase/peroxidase reagent. α-Glucans (starch/glycogen) plus free glucose and glucose from sucrose were specifically measured after hydrolysis of starch/glycogen to glucose with glucoamylase and sucrose to glucose plus fructose with invertase and the glucose specifically measured with GOPOD reagent (glucose oxidase plus peroxidase and 4-aminoantipyrine dissolved in *p*-hydroxybenzoic acid and sodium azide). β-Glucans were determined by the difference. The same method was used to measure the glucans content of *P. ostreatus* powder, in order to calculate the yield of extraction with the following Equation (1):

$$\%\text{Extraction yield} = 100 \times \frac{\text{glucans (total or }\beta\text{) in extract (g)}}{\text{glucans (total or }\beta\text{) in powder (g)}} \quad (1)$$

2.2.3 | Preparation of emulsions

To prepare emulsion at lab scale (100 ml of volume) and pilot scale (1 L of volume) a two-step protocol was used. An aqueous phase was prepared by slow dissolution of wall material (MD) in the extracts containing β-glucans and distilled water at 35°C under mechanical stirring [with a three-bladed propeller stirrer (Eurostar, IKA, FR) at pilot scale; with magnetic stirrer at lab scale]. A glass container instead of a stainless steel one was used to avoid contamination by metal ions, which can start oxidation. Then, in order to obtain an emulsion with the required oil droplet size (e.g., about 2-μm diameter), sunflower oil was added in the aqueous solution under homogenization. At lab scale a Polytron PT 3100 D model homogenizer (KINEMATICA AG, Switzerland) with a PTG 36/4 stator was used at 10,000 rpm from 10 to more than 30 min. At pilot scale, a rotor-stator homogenizer (AXR Silverson Machine Ltd, FR) was used at 3,900 rpm for 10 min.

2.2.4 | Oil droplet size distribution and emulsion stability

The oil droplet size distribution was measured by LASER light diffraction (Mastersizer 2000, Malvern, FR) in wet mode (Hydro 2000) after dispersion in purified water. The refractive index value used for the oil droplets was 1.475. From the volume size distribution obtained, characteristic diameters were deduced: d_{50} (median diameter), d_{10}, and d_{90}, diameters with, respectively, 50, 10, and 90% of the particles with a smaller size. The span was calculated as $(d_{90} - d_{10})/d_{50}$. The higher is the span value, the wider is the distribution.

To estimate the emulsion stability, the size distribution measurements were repeated after a 2 hrs rest period at room temperature.

2.2.5 | Statistical analysis of data

Experimental data were analyzed using one-way ANOVA with the least significant difference (LSD) as a multiple range test, and by linear regression analysis using Statgraphics 5.1 (STCC Inc.; Rockville, Maryland).

3 | RESULTS AND DISCUSSION

3.1 | Characterization of β-glucans extracts

Pleurotus ostreatus is considered an important source of β-glucans among the most cultivated species of mushrooms worldwide (e.g., *Agaricus bisporus*, *Lentinula edodes*, and *P. ostreatus*; Correa, Brugnari, Bracht, Peralta, & Ferreira, 2016). Indeed, McCleary and Draga (2016), who have developed the method used in this work for the evaluation of β-glucans, reported that the amounts of β-glucans for *A. bisporus*, *L. edodes*, and *P. ostreatus* were 6.0 g/100 g d.w., 23.5 g/100 g d.w., and 32.3 g/100 g d.w., respectively. Using the same approach, a study on 16 different strains of *P. ostreatus* revealed that the total glucans content varied in the range 14–25 g/100 g d.w., with β-glucans in the range of 10.9–22.9 g/100 g d.w. (Koutrotsios, Kalogeropoulos, Stathopoulos, Kaliora, & Zervakis, 2017). For the strain of *P. ostreatus* selected for this work the total glucans content measured was 36 g/100 g d.w. with 35 g/100 g d.w. corresponding to β-glucans. These values were used to calculate the yield of extraction (Table 1). The content of total glucans and β-glucans in extract W was 20.1 g/100 g d.w. and 13.4 g/100 g d.w., respectively, corresponding to low extraction yields.

The analysis of the aqueous washing (WW) showed that it contained a high amount of β-glucans that were lost. Extract UW presented higher contents of total glucans (26.2 g/100 g d.w.) and β-glucans (21.6 g/100 g d.w.) and also higher yields of extraction (20.4% for total glucans instead of 11.2 and 17.2% for β-glucans instead of 7.7%).

3.2 | Emulsions stability

When elevated concentrations of high molecular weight polysaccharides are present in the aqueous phase of an emulsion, the depletion phenomena decrease the number of emulsifying molecules adsorbed at the oil/water interphase, causing flocculation and consequently the coalescence of oil droplets. The polymer concentration must therefore remain below the critical flocculation concentration (CFC; Grundy, McClements, Ballance, & Wilde, 2018). Since this value depends on the emulsifier nature and the oil content, many different formulations of oil-in-water emulsions were tested (Table 2) at lab scale, using both the W and UW extracts, while maintaining the same percentage of dry matter (e.g., 40% w/w) but varying the amount of oil, β-glucans, and MD.

The oil droplet size distribution was measured after different homogenization procedures, carried out at lab scale and pilot scale,

TABLE 1 Characterization of the β-glucans-rich extracts obtained from *P. ostreatus*

Extract code	% dry matter	% yield total glucans	Total glucans (g/100 g d.w.)	% yield β-glucans	β-glucans (g/100 g d.w.)
W	1.0 ± 0.0[a]	11.2 ± 0.6[a]	20.1 ± 1.1[a]	7.7 ± 0.6[a]	13.4 ± 1.1[a]
UW	1.4 ± 0.1[b]	20.4 ± 1.1[b]	26.2 ± 1.3[b]	17.2 ± 0.8[b]	21.6 ± 1.1[b]

Note: Data are average ± *SD*. Values in the same column with differing superscripts are significantly different (LSD, $p < .01$).

TABLE 2 Composition of the different emulsions tested (% w/w), with 40% w/w dry matter including oil, MD, and β-glucans from the *P. ostreatus* extracts

Emulsion code	% oil	% β-glucans	% MD	$\frac{\beta-\text{glucans}}{\text{oil}}$	$\frac{\text{MD}}{\beta-\text{glucans}}$
E1_lab_W	4.0	0.03	35.8	0.008	1,193
E2_lab_W		0.04	35.7	0.010	893
E3_lab_W		0.06	35.5	0.015	592
E4_lab_W		0.08	35.4	0.020	443
E4_pilot_W		0.09	35.4	0.023	393
E4_pilot_UW		0.09	35.6	0.023	396
E5_lab_W	8.0	0.06	31.5	0.008	525
E6_lab_UW		0.13	31.4	0.016	242
E6_pilot_UW		0.17	31.2	0.021	184

TABLE 3 Homogenization conditions and size distributions at the time of preparation ($t = 0$) and after 2 hr ($t = 2$ hrs) (—: not measured for non-stable emulsions at time of production)

	Homogenization		Size distribution ($t = 0$)				Size distribution ($t = 2$ hrs)			
Emulsion code	Time (min)	Speed (rpm)	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	Span	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	Span
E1_lab_W	>30	10,000	1.37	6.11	15.3	2.3	—	—	—	—
E2_lab_W	30	10,000	0.99	3.97	11.01	2.5	—	—	—	—
E3_lab_W	>30	10,000	1.01	3.14	11.22	3.3	—	—	—	—
E4_lab_W	10	10,000	1.02	2.02[b]	3.77	1.4[a]	1.02	2.02[b]	3.83	1.4[a]
E4_pilot_W	10	3,900	1.28	2.51[b]	4.55	1.3[a]	1.39	2.56[b]	4.42	1.2[a]
E4_pilot_UW	10	3,900	1.44	2.50[b]	4.19	1.1[a]	1.42	2.59[b]	4.51	1.2[a]
E5_lab_W	>30	10,000	1.46	7.89	17.57	2.0	—	—	—	—
E6_lab_UW	10	10,000	1.30	2.79[b]	5.55	1.5[a]	1.50	2.96[b]	5.58	1.4[a]
E6_pilot_UW	10	3,900	1.55	2.72[b]	4.61	1.1[a]	1.66	2.83[b]	4.75	1.1[a]

Note: Values in the same row with differing superscripts are significantly different (LSD, $p < .01$).

with the purpose to obtain a median diameter (d_{50}) around 2 μm (Table 3). In some cases (E1_lab_W, E2_lab_W, E3_lab_W, and E5_lab_W), even after a homogenization step of more than 30 min and at 10,000 rpm, the distribution of the emulsions was still bi-modal and with a d_{50} bigger than 3 μm. These parameters are associated to a low stability and hence the evolution of their size distribution with time was not checked.

The other emulsions (E4_lab_W and E6_lab_UW) showed instead a mono-modal distribution and a d_{50} around 2 μm at the time of preparation. Since these properties were satisfying, the size distribution of the liquid emulsions was also measured after 2 hrs storage at room temperature. The results obtained show that no significant changes have occurred in the microstructure of the emulsions, confirming that they were physically stable. Consequently, it was possible to establish that an emulsion with 4% w/w of oil was stable when the weight ratio between β-glucans and oil was bigger than 0.020 and the weight ratio between MD and β-glucans was lower than 443 (Figure 2a). Taking into account that the amount of emulsifier required depends on the oil quantity, it was necessary to increase the percentage of β-glucans up to 0.13% w/w in the formulation of emulsions with 8% w/w of oil to maintain the stability: in this case, a stable emulsion was obtained with a ratio between β-glucans and oil of 0.016 and a ratio between MD and β-glucans of 242 (Figure 2b). E4_lab_W and E6_lab_UW were selected to scale up the protocol at pilot scale (Figure 3). Changing the homogenization procedure, the diameters d_{10}, d_{50}, and d_{90} increased but they remained stable after 2 hrs from the preparation; the span value varied from 1.1 to 1.5 at time 0 and from 1.1 to 1.4 at time 2 hrs, corresponding to relatively narrow distributions. The oil droplet size distributions of the emulsions homogenized at pilot scale (Figure 3) were mono-modal with comparable d50 both at time 0 and after 2 hrs.

Thus, the size of these droplets seems to be compatible with a good oil encapsulation in the powder particles produced with a pilot

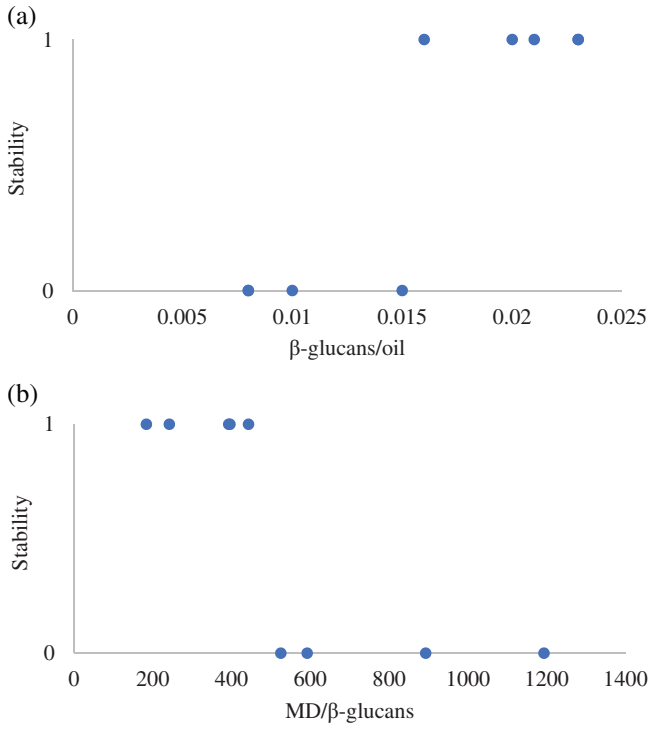

FIGURE 2 Emulsion physical stability as a function of β-glucans/oil and MD/β-glucans ratio (0: unstable; 1: stable)

spray dryer that generally have diameters between 20 and 50 μm (Turchiuli, Lemarie, Cuvelier, & Dumoulin, 2013). It is noteworthy that, even if the extract W contained just hot-water-soluble β-glucans and UW contained also β-glucans soluble in water at T_{amb}, thus with different molecular weight, their emulsifying behavior was the same: however, since the extract UW has a better yield of extraction, it can be chosen over W.

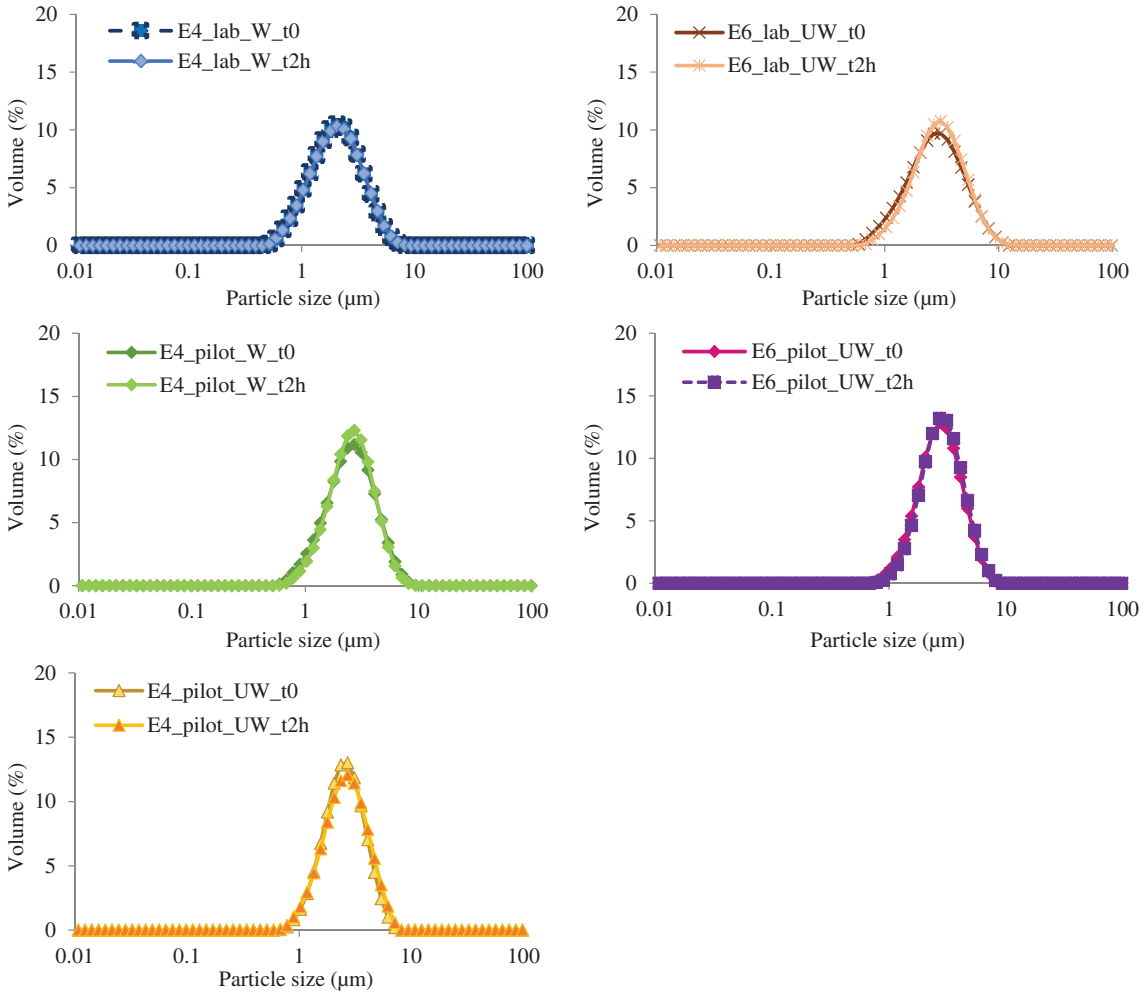

FIGURE 3 The oil droplet size distribution of the emulsions E4_lab_W, E4_pilot_W, E4_pilot_UW, E6_lab_UW, and E6_pilot_UW after production (t_0) and after 2 hrs storage (t_{2h})

4 | CONCLUSIONS

The usage of emulsifiers made-up by polymeric constituents with inherent bioactivity, such as mushroom β-glucans, could be a sustainable and healthy alternative to common emulsifiers. Their efficiency regarding spray drying and protection has been evaluated (Gallotti, Lavelli, & Turchiuli, 2020).

ACKNOWLEDGMENTS

This work was supported by the Fondazione Cariplo, Milan, Italy (Project ReMarcForFood n. 2016-0740). Thanks to UMR Ingénierie Procédés Aliments, AgroParisTech Massy (France) for hosting of F. Gallotti and scientific and technical support provided.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Francesca Gallotti: Conceptualization; data curation; formal analysis; investigation. **Christelle Turchiuli:** Conceptualization; resources; supervision. **Vera Lavelli:** Funding acquisition; project administration; resources; supervision.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available.

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How to cite this article: Gallotti, F., Turchiuli, C., & Lavelli, V. (2021). Production of stable emulsions using β-glucans extracted from *Pleurotus ostreatus* to encapsulate oxidizable compounds. *Journal of Food Process Engineering*, e13949. https://doi.org/10.1111/jfpe.13949