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Emulsifying and foaming properties of a hydrophobin-based food ingredient from *Trichoderma reesei*: A phenomenological comparative study

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ABSTRACT

The aim of this study was the production of a hydrophobin-based food ingredient (HFB) from submerged cultures of *Trichoderma reesei* and the evaluation of its technological properties in comparison with milk whey (WPC) and egg white (EWP) proteins, widely used in foods for their surface-active properties. *T. reesei* culture medium was formulated without proteins to ease HFB recovery. Culture supernatant after biomass separation was air-bubbled to concentrate HFB at the air-liquid interface, thus reaching a recovery yield of 138 mg/L. HFB, WPC and EWP solutions (1–5 g/L) were used to test the emulsifying activity index (EAI), the creaming stability (CS) and the foaming properties. EAI was almost four times higher than that of WPC and EWP. Also CS was improved in the case of HFB-stabilized emulsions, but only at the lowest concentration. The overrun of the foam obtained with HFB solutions was 1.2–1.9 times higher than that of the foams obtained with the other ingredients; also foam consistency was significantly higher when created with HFB solutions. Overall results indicate that HFB obtained from *T. reesei* showed interesting and promising technological properties, paving the way to possible applications in aerated foods and foamed emulsions.

1. Introduction

Hydrophobins are a family of small globular proteins (<20 kDa) belonging to the most surface-active molecules (Wösten, 2001). They play a variety of functions in fungal growth and development. In particular, they assemble at the medium-air interface, lowering the water surface tension and allowing hyphae to initiate aerial growth (Wösten & Scholtmeijer, 2015). They are known as biosurfactant proteins due to their self-assembling at hydrophilic-hydrophobic interfaces (e.g., between water and air or water and oil). Almost all proteins show amphiphilic properties, since they contain both hydrophobic and hydrophilic amino acids. However, interfacial adsorption in most cases is accompanied by unfolding and loss of function. By contrast, biosurfactant proteins typically retain their structure or undergo specific changes in conformation, because they have developed structural features that favor interfacial adsorption, while maintaining biocompatibility and minimizing aggregation in solution (Cheung & Samantray, 2018). Two classes of hydrophobins have been recognized, class I and class II, with different surface properties and solubility. Class I hydrophobins are relatively large, highly insoluble, and they assemble into a very stable, amyloid-like membrane. On the opposite, class II hydrophobins are smaller, more compact and soluble, and they assemble as a monolayer easily dissociable, with a conformation similar to that in the water-soluble state. All hydrophobins contain eight cysteine residues that form four disulfide bridges, important to give a stable structure and keep the proteins in the soluble state (Burke, Cox, Petkov, & Murray, 2014; Wösten & Scholtmeijer, 2015).

In this study, class II hydrophobins from *Trichoderma reesei* were produced and tested for technological properties. *T. reesei* produces at least three class II hydrophobins, of which HFBI and HFBII can be found in - and easily recovered from - the culture medium (Askolin et al., 2006). They have a molecular weight of about 7 kDa; besides the four intermolecular disulfide bonds, a hydrophobic patch covers 12–19% of the total surface area of the proteins conferring the high surface activity. HFBI is more hydrophobic and stabilizes oil emulsions more effectively, whereas HFBII is more water soluble and a stronger surface-active molecule. At the air/water interphase, HFBII results in mechanically strong films, giving bubbles with excellent resistance toward disproportionation shrinkage. That is the reason why hydrophobins have drawn the attention of food industries, especially for the production of stable foams (Askolin et al., 2006; Burke et al., 2014). In fact, in many food products foams and bubbles play an important structuring role,

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thus affecting texture and sensory properties, as well as consumers' acceptability. However, many factors can lead to foam instability via different mechanisms, with detrimental effects on the quality of the final product (Deotale, Dutta, Moses, Balasubramaniam, & Anandharamakrishnan, 2020). One of the most common methods of counteracting destabilization processes is to modify the gas-liquid interface by adsorption of surface-active molecules (Cox, Aldred, & Russell, 2009). Surface properties of class II hydrophobins alone or in combination with other food proteins have been studied with particular focus on foam stability (Askolin et al., 2006; Cox, Cagnol, Russell, & Izzard, 2007; Cox et al., 2009; Dimitrova, Petkov, Kralchevsky, Stoyanov, & Pelan, 2017). The high surface elasticity of HFBII can stabilize aqueous foams for significant periods of time at a concentration of only 1 g/L and across a range of pH values, when compared to both β-casein and β-lactoglobulin. Quality and stability of the produced foams is not altered by the addition of other proteins, such as β-lactoglobulin, ovalbumin, and bovine serum albumin. The ability of hydrophobins to produce food foams with great stability may lead to improved physical and sensory properties of products such as ice cream, sorbets, and low-fat whipping cream. Moreover, the use of hydrophobins could further lead to the design of new aerated foods (e.g. mayonnaise, shelf-stable milk shakes, smoothies and other beverages, yoghurt, and gelatin-free mousse), with benefits such as fat/calorie reduction or improved/new product textures (Cox et al., 2009).

Class II hydrophobins can also be used as emulsion stabilizers. In particular, Askolin et al. (2006) showed that HFBI is more efficient than HFBII in stabilizing oil droplets in water, when used at a 0.1 g/L concentration. The HFBII emulsions were completely separated after 24 h, whereas emulsions made with HFBI were still stable after 3 days, notwithstanding the appearance of a large cream layer. However, Dimitrova et al. (2016) demonstrated that also HFBII can serve as a valid emulsifier, depending on concentration and the nature of the oil used for emulsion preparation; they demonstrated that HFBII at concentrations higher than 0.05% produces very stable soybean oil-in-water emulsions (at least for 50 days). Moreover, HFBII forms solidified capsules that can be used for retention of soluble and/or volatile compounds (e.g. fragrances, flavors, colors and preservatives) in the aqueous phase.

Based on the high technological potential of hydrophobins, the aim of the present study was the production of a food ingredient concentrated in class II hydrophobins by *T. reesei* and the evaluation of its technological properties at different concentrations (0.1, 0.25, and 0.5 g/100 mL) in comparison with milk whey and egg white proteins, widely used in foods as functional ingredients for their surface-active properties. In the *T. reesei* culture medium, no proteins were used to ease hydrophobin recovery. Although, the utilization of new and emerging fungal enzymes in industrial production could present new occupational exposures (Caballero et al., 2007), *Trichoderma* is non-pathogenic for human beings, does not produce toxins, and is considered a safe and harmless production organism (Nevalainen, Suominen, & Taimisto, 1994).

In order to give a useful practical application guide to possible end-users and to reduce potential production costs, hydrophobins were recovered at a likely Food Grade level (i.e., not the pure isolated protein, but a concentrate recovered with minimal processing) and compared with protein-based food ingredients. A phenomenological approach for the study of the emulsifying and foaming properties was preferred rather than a molecular approach, to provide possible end-users with practical results.

2. Materials and methods

2.1. Protein-based materials

The following protein-based materials were compared: WPC, whey protein concentrate Milacteal 80 (MILEI GmbH, Leutkirch, Germany; protein content: 800 g/kg); EWP, egg white powder (Lactosan-Sanovo Ingredients Group, Zaven/Aspe, Germany; protein content: 800 g/kg); HFB, food grade ingredient enriched in hydrophobins produced from *Trichoderma reesei* as described in the following sections.

Table 1
Formulation of the *Thricoderma reesei* liquid culture media used for the production of the hydrophobin-based food ingredient.

Ingredients	Pre-inoculum medium[a] (g/L)	Standard production medium[a] (g/L)	Protein-free production medium (g/L)
Glucose	20	–	–
Lactose	–	20	20
Bacteriological peptone	4	4	–
Yeast extract	1	1	–
KH_2PO_4	4	4	4
$(NH_4)_2SO_4$	2.8	2.8	5
$MgSO_4$	0.6	0.6	0.6
$CaCl_2$	0.8	0.8	0.8
Microelement solution[b]	20 (μL/L)	20 (μL/L)	1 (μL/L)
Vitamin solution[c]	–	–	1 (mL/L)

[a] From Tchuenbou-Magaia et al. (2009)
[b] Composition (g/L): $FeSO_4$ 0.5, $MgSO_4$ 0.16, $ZnSO_4$ 0.14, $CoCl_2$ 0.37.
[c] Composition (g/L): $MgSO_4$ 200, $MnSO_4$ 38, tiamin 0.2, niacin 0.2, folic acid 0.2, pyridoxal 0.2, pantothenic acid 0.2, cobalamin 0.2.

2.2. Microorganism and maintenance

Trichoderma reesei DSM 769 (DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) was used for HFB production. The strain was routinely maintained on 5 cm

Fig. 1. Air bubbling inside the *Trichoderma reesei* culture filtrate for hydrophobin recovery.

diameter plates containing PDA (Potato Dextrose Agar) culture medium (PDB, Formedium, Hunstanton, UK, supplemented with 15 g/L agar), by depositing on the surface a quarter (around 2 cm^2) of an older (max 2 months) solid culture plate, taken off with a sterile scalpel. Plates were then incubated at 25 °C in the dark; when the mycelium had covered the medium surface, plates were tightly closed with Parafilm (Merck KGaA, Darmstadt, Germany) and stored for a maximum of 2 months at 4 °C until use.

2.3. Hydrophobin production and recovery

Liquid cultures were prepared in 500 mL Erlenmeyer flasks, each containing 100 mL of culture medium. A pre-inoculum medium (Table 1) was inoculated with the biomass grown in one PDA plate obtained as previously reported. After 3 days at 24 °C, cultures were used to inoculate (100 mL/L) a production medium (pH 4.5) modified with respect to the standard formulation reported in the literature (Tchuenbou-Magaia, Norton, & Cox, 2009), avoiding the use of proteins to ease hydrophobin recovery (Protein-free production medium, Table 1). A filter-sterilized multi-vitamins solution was added after medium sterilization at 112 °C for 30 min to complete the formulation. Production cultures were incubated at 24 °C and samples were taken at appropriate intervals up to 14 days, in order to test protein concentration and residual lactose.

After incubation, cultures were filtered to discharge the biomass, and hydrophobins collected as reported by Tchuenbou-Magaia et al. (2009). Specifically, culture filtrate (1 L) containing hydrophobins was inserted into a 5 L separating funnel, and compressed air (1 atm) was bubbled for 5 min through a glass pipette connected to the aeration system with a pipe equipped with a sterile filter (0.2 μm pore size (Merck Millipore KGaA, Darmstadt, Germany) (Fig. 1). The foam was allowed to accumulate on the liquid surface, which was drained away from the bottom of the funnel. The foam was taken off by adding 50 mL of distilled water. The drained culture filtrate was then re-inserted inside the funnel and the bubbling procedure repeated 7 times in order to maximize hydrophobin recovery.

Foam samples were all lyophilized and finally resuspended in distilled water (20 mL/L original culture filtrate) and then stored at −18 °C until use (food-grade ingredient enriched in hydrophobins, HFB).

2.4. Analytical determinations

Protein quantification. Protein concentration of HFB solutions was determined by the Lowry method (Hess, Lees, & Derr, 1978).

Electrophoresis. Electrophoretic protein pattern of HFB solutions was characterized through a NuPAGE® electrophoresis system (Invitrogen by Thermo Fisher Scientific, Monza, Italy) by using NuPAGE® 4–12% Bis-Tris Gel and NuPAGE® MES SDS Running Buffer. Samples were prepared, run and stained in SimplyBlue® SafeStain (Invitrogen by Thermo Fisher Scientific, Monza, Italy), according to standard manufacturer's instructions for reduced condition. Densitometric analysis of electrophoretic gels were carried out by the software GelAnalyzer 19.1 (www.gelanalyzer.com).

MALDI-TOF mass spectrometry. Matrix-assisted laser desorption mass spectrometry (MALDI MS) analysis was carried out on a PerSeptive Biosystems (Framingham, MA, USA) Voyager DE-PRO instrument equipped with a N2 laser (337 nm, 3 ns pulse width). The sample (1 μL) was loaded on the target and dried. Afterwards, 1 μL of a mixture composed of 1 mL/L TFA in H_2O/acetonitrile (1/1, L/L), and 10 mg/mL of matrix (α-cyano-4- hydroxycinnamic acid) were added. For each sample, mass spectrum acquisition was performed in the positive linear mode accumulating 200 laser pulses. The accelerating voltage was 20 kV. External mass calibration was performed with protein standards (PerSeptive Biosystems, Framingham, MA, USA).

Lactose determination. Residual lactose in growth media was determined by a (300 - 8 mm) Sugar SH1821 (Shodex, München, Germany) column, maintained at 50 °C and eluted with 5 mmol/L H_2SO_4 at 0.5 mL/min; chromatographic separations were carried out by an L 7000 HPLC system (Merck Hitachi, VWR International srl, Milan, Italy) equipped with L-7490 Refractive Index and L-7400 UV (210 nm) detectors (Merck Hitachi, VWR International srl, Milan, Italy) serially connected. Data were elaborated through the software EZ-ChromeElite (Merck KGaA, Darmstadt, Germany).

2.5. Technological properties

Every tested protein-based material was used to prepare solutions (1, 2.5 and 5 g/L) in 50 mmol/L sodium phosphate buffer (pH 7), stirring 1 h at 25 °C, to be used for technological property characterization.

Determination of emulsifying activity index (EAI) was carried out according to the method described by Loffredi, Moriano, Masseroni, and Alamprese (2021). Briefly, each protein solution (6 mL) was emulsified with corn oil (2 mL) (Carrefour Classic, Boulogne-Billancourt, France) by using a T25 digital Ultra Turrax (IKA, Staufen, Germany) at 7200 rpm for 5 min. The obtained emulsion (200 μL) was diluted 1:25 in sodium phosphate buffer (10 mmol/L, pH 7) containing 1 g/L sodium dodecyl sulphate (Sigma Aldrich, Saint Louis, MO, USA). Then, the absorbance at 500 nm was measured using a V-650 spectrophotometer (Jasco Europe, Cremella, Italy). EAI (m^2/g) was calculated from absorbance values obtained when protein solution at 5 g/L where used, by following Equation (1):

$$EAI = \frac{2 \times 2.303 \times A \times N}{c \times \varphi \times l \times 10000} \quad (1)$$

where A is the absorbance of the diluted emulsion, N is the dilution factor (25), c is the protein concentration in the initial solution (g/mL), φ is the oil volume fraction of the emulsion (0.25), and l is the optical path length (0.01 m). Results are expressed as the average and standard deviation values of four replicates.

Creaming stability (CS) was determined as described by Moriano and Alamprese (2020), using a dyed (15 mg/kg Oil Red O; Sigma Aldrich, Saint Louis, MO, USA) corn oil (Carrefour Classic, Boulogne-Billancourt, France) to prepare oil-in-water emulsions (20 mL) with 20 mL/100 mL oil phase. Emulsions were prepared as previously described for the EAI determination, then transferred into 10 mL graduated glass cylinders and stored for 1 h at 25 °C. CS was assessed by measuring the separated red creamed layer (Fig. 1S). Results are expressed in percentage, as the average and standard deviation values of two measurements.

Foaming properties were evaluated in triplicate at 20 °C, according to Alamprese, Casiraghi, and Rossi (2012), with slight modifications. Protein-based sample solutions (20 mL each) were whipped for 8 min by using a Cream Tester CT II (Gerber Instrument, Langhag, Switzerland). Foam volume was calculated taking into account the Cream Tester vessel diameter (7.4 cm) and measuring the foam height in four different points by means of a caliper (Fig. 1S). Foaming capacity was expressed as overrun (%), corresponding to the percentage increase of the protein solution volume due to whipping. Foam consistency was expressed by the electric current value (mA) needed by the instrument to maintain a given speed of the rotating elements during whipping. Foam instability (%) was calculated after storage at 4 °C for 2 h in a transparent graduated conical vessel, measuring the protein solution separated from the foam (Fig. 1S).

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was applied to the analytical replicates of technological properties, in order to compare the tested ingredients. The Least Significant Difference (LSD) test ($p < 0.05$) was used to evaluate significant differences among the averages (Statgraphics Centurion 18, Statgraphics Technologies, Inc., The Plains, VA,

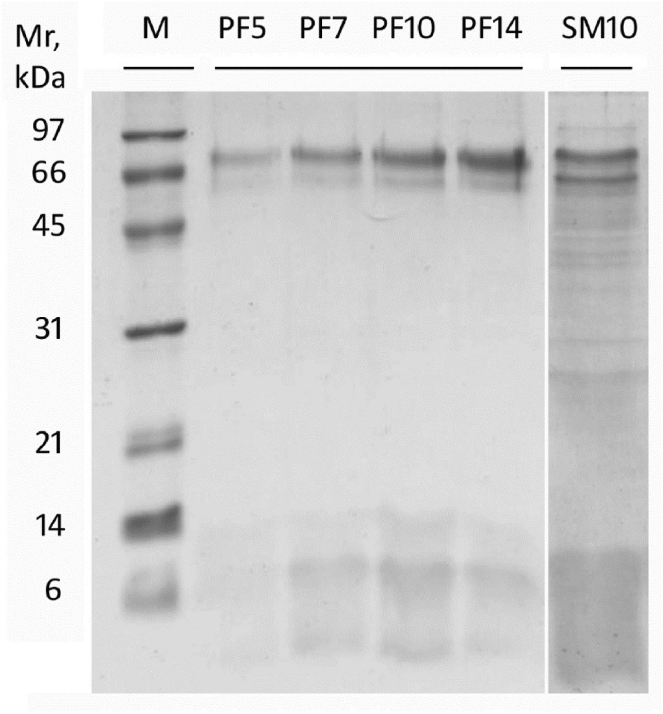

Fig. 2. Gel electrophoresis of the foam samples obtained from supernatant of *T. reesei* at different incubation times using the protein-free and the standard production medium. M: markers; PF5, PF7, PF10, PF14: protein-free medium at 5, 7 10 and 14 days; SM10: standard medium at 10 days.

USA).

3. Results and discussion

3.1. Hydrophobin production, recovery, and characterization

Comparative production trials carried out employing the standard and the new protein-free production medium evidenced predictable differences, highlighted through gel electrophoresis. Samples obtained from the standard medium contained a very broad dispersion of molecular weights coming from residual ingredients of protein origin present inside the medium. On the contrary, the new culture medium provided a "clean" HFB sample, in which the main bands attributable to hydrophobins, around 7 kDa, were visible together with only few contaminant proteins at about 70 kDa (Fig. 2), these latter likely attributable to the intact and the truncated forms of cellobiohydrolase I (CBH I) (Chen, Hayn, & Esterbauer, 1993), also produced when lactose is used as a carbon source (Messner & Kubicek, 1991).

Time course of *T. reesei* growth in the protein-free medium highlighted that a low pH was reached after 14 days of incubation, around 2.7–2.9 *vs.* 5.5 of the standard medium. The residual lactose was 5.7 g/L; taking into account its initial concentration (20 g/L), a final utilization yield of 72% was achieved. The maximum hydrophobin accumulation was reached after 10 days, whereas longer incubation times led to an increase of the 70 kDa contaminant proteins (Fig. 2).

The foaming procedure was effective in the recovery of hydrophobins from the culture filtrate. As shown in Fig. 3, hydrophobins are concentrated in the fraction recovered by foaming, whereas no hydrophobins are visible in the exhausted culture filtrate. Moreover, the 70 kDa contaminants do not accumulate in the foam, suggesting that they have negligible surfactant properties.

The total yield of the HFB production process, in terms of grams of proteins per litre of *T. reesei* supernatant recovered in the foam, was evaluated quantifying the protein content by the Lowry method. The method was applied in the alternative way that includes SDS to solubilize proteins, since virtually insensitive to the atypical molecular features of hydrophobins characterized by an amphipathic structure with a very hydrophobic side opposed to a hydrophilic counterpart (Linder, 2009). These molecular features, that are at the basis of the foaming and emulsifying properties of the protein, lead to the formation of soluble aggregates that may affect the binding of classical probes used in colorimetric methods such as for the Bradford method.

The total recovery yield was assessed in 138 mg/L for the protein-free production medium; note that this result refers to the total protein content in the recovered sample. Although not specifically related to hydrophobins, this quantification is representative of the total protein content of the technological ingredient we are proposing in this paper.

An estimation of the hydrophobin content in the preparation was obtained by the densitometric analysis of the electrophoretic separations (Fig. 3, right panel). According to the relative intensity of the hydrophobins banding at 7 kDa, compared to the proteins banding at 70 kDa, hydrophobins account for about 61% of the total protein content.

Finally, MALDI-TOF mass spectrometry analysis of the HFB sample allowed to confirm the presence of both HFBI (theoretical MW 7532 Da)

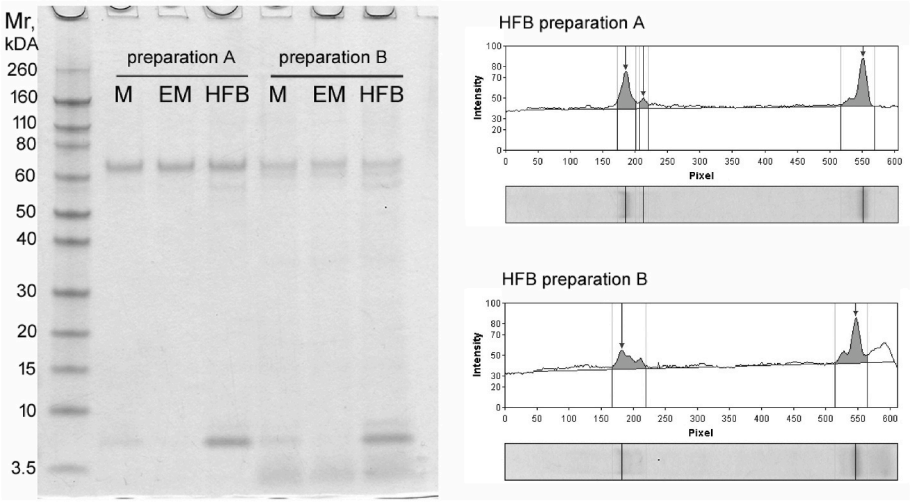

Fig. 3. Electrophoretic characterization of the hydrophobin-based ingredient (HFB) recovery. Left panel: Nu-PAGE of two different independent preparations (named A and B). M: culture filtrate; EM: exhausted culture filtrate; HFB: hydrophobin-based ingredient. Right panel: densitometric analysis of preparation A (top) and B (bottom) HFB lanes (lanes are rotated 90-degree counter clockwise compared to the gel).

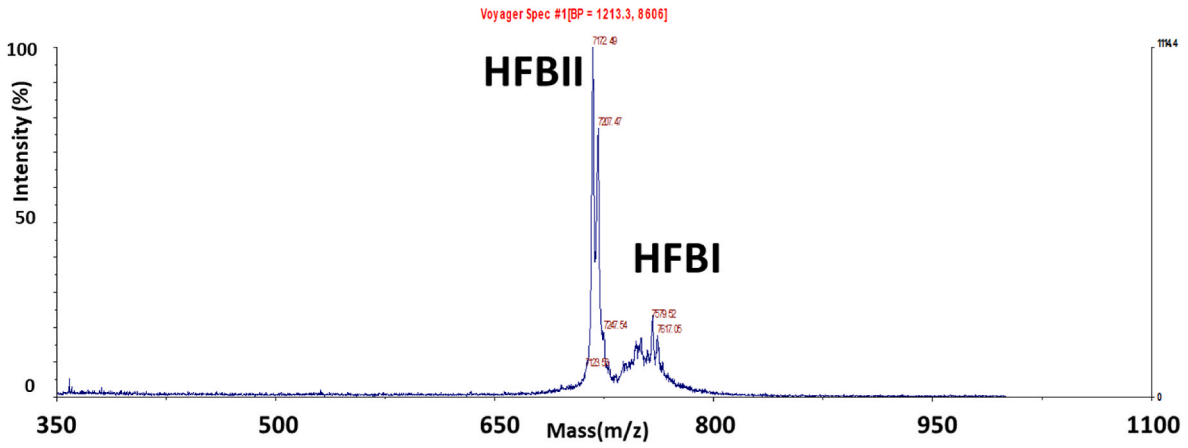

Fig. 4. MALDI-TOF mass spectrometry spectrum of the hydrophobin-based ingredient recovered by foaming *T. reesei* supernatant.

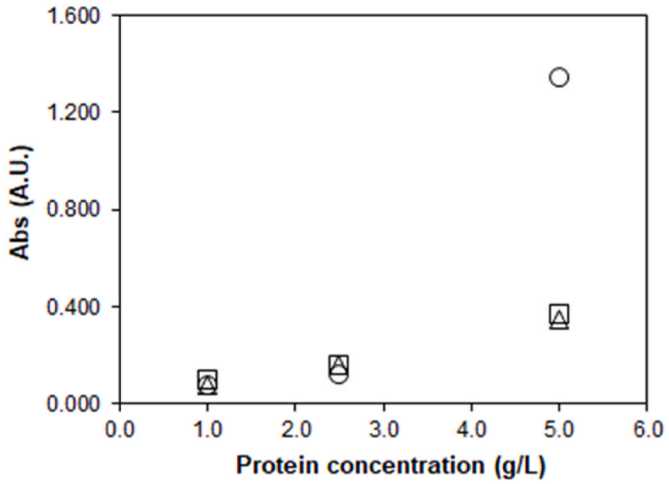

Fig. 5. Emulsifying properties of the tested proteins expressed as absorbance values as a function of protein concentration. Whey protein concentrate, square symbols; egg white powder, triangle symbols; hydrophobin-based ingredient, circle symbols. Standard deviation values (n = 4) ranged from 0.001 to 0.045.

and HFBII (theoretical MW 7182 Da), which were identified through the presence of two family of proteins at 7537.3 ± 5 Da and 7189 ± 4 Da, respectively (Fig. 4).

Kakahi et al. (2019) reported on hydrophobin production employing the traditional submerged fermentation compared with five solid supports in an attempt to obtain a biofilm cultivation to ease the removal of fungal biomass at the end of fermentation processes. The highest hydrophobin production was observed when stainless steel was used as support in the system; nevertheless, production yields were all in the range 28–45 mg/L.

The here obtained production can be considered significantly higher than hydrophobin yields reported by Khalesi, Gebruers, and Derdelinckx (2015) in a review on recent advances on fungal hydrophobin use in industry, in the range 25–30 mg/L.

3.2. Protein technological properties

Emulsions are ubiquitous in foods, and the use of amphiphilic biopolymers, mainly proteins, as emulsifiers can help in satisfying consumers' demand for natural, healthy, and sustainable products (Berton-Carabin & Schroën, 2019). With this in mind, emulsifying properties of HFB were tested and compared to those of WPC and EWP. In order to estimate the protein ability to form an emulsion, EAI was measured as an index of the interfacial area stabilized per unit of protein weight (Pearce & Kinsella, 1978). The significantly ($p < 0.05$) highest value (1239 ± 41 m^2/g) was obtained for HFB, which showed EAI almost four times higher than those of WPC (340 ± 17 m^2/g) and EWP (320 ± 15 m^2/g). Despite the wider use of WPC as food emulsifier compared to EWP, the two protein-based ingredients did not show significantly different EAI values. Similar findings were reported by Aryana, Haque, and Gerard (2002). Since protein concentration can affect the emulsifying capacity (Pearce & Kinsella, 1978), absorbance values obtained in EAI evaluation were plotted against protein solution concentrations (Fig. 5). All protein-based ingredients showed an increase in the absorbance values with the increasing of protein concentration, indicating a higher interfacial area connected to the formation of a high number of little oil droplets. However, the increase was linear for WPC and EWP, but exponential for HFB, confirming the extremely higher emulsifying capacity of HFB at 5 g/L. This result can be ascribed to the higher hydrophobicity of HFB compared to WPC and EWP and to the lack of conformation change at the interface typical of hydrophobins (Wösten & Scholtmeijer, 2015), which make them quick in coating and stabilizing the oil droplets. Indeed, protein emulsifying activity is determined by both surface hydrophobicity, which affects the protein affinity for the oil-water interphase, and molecular flexibility, which influences the ability to unfold and interact with other proteins (Dissanayake & Vasiljevic, 2009). A remarkably high surface elasticity (about 0.5 N/m) has been reported for class II hydrophobins, orders of magnitude higher than that observed for any other surface-active proteins (Linder, 2009).

Table 2
Creaming stability (%) of the emulsions prepared with whey protein concentrate (WPC), egg white powder (EWP), and hydrophobin-based food ingredient (HFB) as a function of the protein concentration (data reported as mean and standard deviation values of two replicates).

Protein concentration (g/L)	WPC	EWP	HFB
1	67 ± 3^a	68 ± 3^a	81 ± 3^b
2.5	73 ± 6^a	70 ± 3^a	80 ± 1^a
5	76 ± 7^a	68 ± 4^a	70 ± 1^a

$^{a\text{-}c}$, different superscript letters in the same row indicate significant differences ($p < 0.05$) among samples.

Stability of the emulsions towards creaming phenomena was significantly ($p < 0.05$) improved in the case of HFB-stabilized emulsions at the lowest concentration (Table 2). The emulsions prepared with 2.5 and 5 g/L HFB showed creaming stability values similar to those of the samples stabilized by the same amount of WPC and EWP. Maybe the very high interfacial area created in these samples (observed in Fig. 5) needs a higher protein content to be stabilized over storage. Actually, as

Table 3
Foaming properties of whey protein concentrate (WPC), egg white powder (EWP), and hydrophobin-based food ingredient (HFB), as a function of protein concentration (data reported as mean and standard deviation values of three replicates).

Protein concentration (g/L)	Foam overrun (%)			Foam consistency (mA)			Foam instability (%)		
	WPC	EWP	HFB	WPC	EWP	HFB	WPC	EWP	HFB
1	112 ± 9^{b}	80 ± 7^{a}	138 ± 14^{c}	146 ± 2^{ab}	147 ± 1^{b}	144 ± 1^{a}	51 ± 2^{b}	29 ± 1^{a}	27 ± 1^{a}
2.5	108 ± 6^{a}	135 ± 2^{b}	183 ± 1^{c}	141 ± 3^{a}	148 ± 4^{b}	151 ± 1^{b}	91 ± 14^{b}	54 ± 3^{a}	44 ± 6^{a}
5	123 ± 1^{a}	177 ± 6^{b}	237 ± 4^{c}	145 ± 1^{a}	151 ± 1^{b}	160 ± 1^{c}	66 ± 1^{a}	65 ± 5^{a}	62 ± 3^{a}

[a-c], for each variable, different superscript letters in the same row indicate significant differences ($p < 0.05$) among samples.

demonstrated by Dimitrova et al. (2016), emulsion stability depends on hydrophobins concentration.

Solid and liquid aerated foods are very appreciated by consumers (Deotale et al., 2020) and stabilization of foams by proteins has attracted great attention in food industries (Wierenga & Gruppen, 2010). Proteins are good foaming agents because they can be strongly adsorbed at the air/water interface and give steric and electrostatic stabilization; moreover, protein-protein interactions at the interface can contribute to structural coherence, thus improving foam stability (Murray, 2007). Due to the self-assembly properties, the high hydrophobicity and surface elasticity (Green, Littlejohn, Hooley, & Cox, 2013), HFB showed foaming properties better than those of WPC and EWP, above all in terms of foam overrun (Table 3). At every tested protein concentration, the foam volume obtained with HFB was significantly higher ($p < 0.05$) than that produced by WPC and EWP (1.2–1.9 times higher), but only at 5 g/L also the foam consistency was significantly ($p < 0.05$) higher. Hydrophobins greatly reduce the air/water surface tension, allowing the formation of smaller air cells; moreover, they coat the air surface quickly and bond to one another, producing an elastic and strong membrane at the interface, preventing distortion of bubbles and coalescence (Green et al., 2013; Wierenga & Gruppen, 2010). The higher foam consistency measured for the highest HFB concentration can account for a denser foam, having smaller bubbles. In this case, also foam instability increased, maybe indicating that the protein amount was not enough to efficiently coat and stabilize the whole water/air interface. Also for EWP, foam instability significantly increased from 1 to 2.5 g/L concentration, being higher the overrun.

4. Conclusions

The food grade hydrophobin–based ingredient obtained from *T. reesei* showed very good technological properties, generally better than those of whey and egg white proteins, which are widespread technological ingredients in many foods.

HFB production costs at lab-scale are more or less 100 times higher than commercial prices of WPC and EWP. However, the evaluation of the real economic impact should consider also the amount of the ingredient needed to obtain the same technological properties as whey and egg white proteins. Actually, HFB emulsifying performance was almost four times higher than that of the other two ingredients. Finally, it is important to notice that WPC and EWP are allergenic ingredients, thus for sensitive consumers their substitution with HFB may represent an added value.

Future trials will be aimed at increasing hydrophobin yields in order to further reduce production costs, thus paving the way to the possibility of extending the applications of hydrophobins in aerated foods and in foamed emulsions to obtain new food structures. Still, the authorization for the use of hydrophobins in food products needs to be elucidated, as there is not a recognized approval so far.

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CRediT authorship contribution statement

Cristina Alamprese: Conceptualization, Investigation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Manuela Rollini:** Methodology, Validation, Visualization, Writing – review & editing. **Alida Musatti:** Formal analysis, Investigation. **Pasquale Ferranti:** Formal analysis, Investigation, Writing – review & editing. **Alberto Barbiroli:** Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.113060.

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