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Fungal bioconversion of brewery by-products: assessment of fatty acids and sterols profiles

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Abstract

Waste management of brewery by-products is economically and environmentally problematic. In the frame of bio-recycling, this study aims to investigate the bioconversion of brewery by-products by filamentous fungi. *Pleurotus ostreatus* and *Lentinula edodes* were grown on different substrates based on brewer's spent grains (fresh and dry). Afterwards, fatty acids and sterols were determined. Following the selection of the suitable substrate composition for fungal growth, results showed that fatty acids composition of fungal biomasses varied significantly as a function of substrate and fungal strain. Interestingly, fungal fat might be used for human nutrition due to low SFA/UFA ratios (~0.2–0.4) within the same range of vegetal oils. Sterols profile of fungi biomass revealed the predominance of ergosterol. Also, it was found that the fungi growing on by-products slightly reduced the cholesterol contents. As such, this approach focusing on the bioconversion of by-products using fungi can provide biomasses with a fat composition suitable for feed and human consumption.

Keywords: bioconversion; fatty acids; *Lentinula edodes*; *Pleurotus ostreatus*; sterols

Introduction

By-products from the agro-industrial sector present crescent environmental and economic problems because handling large amounts of organic material requires high expenses and can negatively affect the environment (Barcelos *et al.*, 2020; Correddu *et al.*, 2020; Radenkovs *et al.*, 2018). In most cases, this material is reutilized as fertilizers in the field, or burnt or drained as a waste (Bhuvaneshwari *et al.*, 2019; Duque-Acevedo *et al.*, 2020; Puglia *et al.*, 2021). The proper valorization of by-products can efficiently reduce their environmental impact and obtain new strategic solutions to increase the productivity and sustainability of agro-industrial systems (Boukid *et al.*, 2021a; Ferreira *et al.*, 2022; Pauletto *et al.*, 2020; Hamam *et al.*, 2021; Pauletto *et al.* 2020; Boukid *et al.*, 2021). In the European Union, the use and valorization of waste and by-products are considered the key elements in the Work Programme 2021–2022 (EU 2021).

The brewing industry is an important global business with huge annual revenues (US$ 651,398 million in 2021) with a compound annual growth rate of 7.4% from 2021 to 2025 (Statistica 2021). On the other hand, malting and brewing generate high amounts of by-products including brewer's spent grain, hot trub, wastewater, spent hops, and residual brewer's yeast (Jaeger *et al.*, 2020; Karlović *et al.*, 2020). Brewer's spent grain, the main by-product (~85%), is known for its rich composition of proteins (20–30%), fiber (30–70%), lipids (5–10%), vitamins, and minerals (Fărcaş *et al.*, 2017). Given this nutritious composition and affordability, the biotechnological valorization of such by-products is deemed necessary for economic and environmental motives (Berglund *et al.*, 2016;

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Karlović *et al*., 2020; Rachwał *et al*., 2020; Severini *et al*., 2015). From a circular economy viewpoint, an integral bioremediation and valorization of brewery by-products relies on implementing bioreactors and identifying suitable microorganisms (e.g., yeast and fungi) to degrade this material and produce valuable compounds (Bianco *et al*., 2020; Gmoser *et al*., 2020; Mohajeri *et al*., 2021; Xiros and Studer 2017).

Filamentous fungi were demonstrated to be efficient decomposers of different substances and complexes biomass (Dai *et al*., 2018). Fungi could produce numerous extracellular enzymes, mainly hydrolases and oxidoreductases. Phytase, laccase, pectinase, cellulases, xylanases, lipases, and tannase are among the most important enzymes produced by fungi (Campioni *et al*., 2019; Linhartová *et al*., 2020; Londoño-Hernandez *et al*., 2020). One of the main activities of these enzymes is the bioconversion of a wide variety of agricultural and/or industrial biomass (El-Gendi *et al.,* 2022; Fernandes *et al*., 2020; Xu *et al*., 2018). Thus, fungi can be considered as cell factories that produce high-value compounds including cellulose, antibiotics, organic acids, pectin, inulin, proteins, and lipids (Liu *et al*., 2020). Furthermore, fungal enzymes are of high value and dominate nearly half of the enzyme market due to their availability, compatibility, cost-efficiency, and versatility of applications (e.g., medicine, biotechnology, food, leather, and textile (El-Gendi *et al*., 2022; Meyer *et al*., 2020; Pellegrino *et al*., 2022; Wang *et al*., 2018). Therefore, the use of fungi is a promising green approach *toward* the production of sustainable compounds, reduction in the cost of production, and mitigation of the impact of wastes on the environment which contributes to bio-economies and human health (Boukid and Gagaoua 2022; Meyer *et al*., 2020).

Brewer's spent grains are lignocellulosic biomass rich in arabinoxylan (22–28%), cellulose (17–25%), and lignin (12–28%) (Fărcaş *et al*., 2017; Reis *et al*., 2015), and thus represent a good source of carbon for fungal growth. Several studies focused on the use of this by-product as a low-cost substrate for edible mushrooms (saprophytic fungi) able to degrade lignocellulosic materials (Fărcaş *et al*., 2017; Rachwał *et al*., 2020). Thus, the bioconversion of brewer's spent grain using fungi can be considered a promising sustainable strategy (Dias *et al*., 2018; Marcus and Fox 2021). The most cultivated edible fungi are *Lentinula* (shiitake and relatives), *Pleurotus* (oyster mushroom), *Auricularia* (wood ear mushroom), and *Agaricus* (button mushroom and relatives) (Kumla *et al*., 2020). Fungi such as *Rhizopus* spp., *Trichoderma* spp., and *Mucor* spp. have been used to hydrolyze brewer's grain spent, which resulted in producing nitrogen and carbon and releasing proteins and sugars for their growth that required no extra nutrients (Bekatorou *et al.,* 2015; Cooray and Chen 2018). *Aspergillus brasiliensis and Aspergillus ibericus* produced lignocellulolytic enzymes using brewer's grain spent as substrate (da Silva Menezes *et al*., 2017; Sousa *et al*., 2018). The enzymes such as xylanase and cellulase contribute to the increase in the release of primary and secondary metabolites (Verni *et al*., 2020). The nutritional value of the by-products was improved in terms of increasing protein content and producing value-added products such as lignocellulolytic enzymes and antioxidant peptides (da Silva Menezes *et al*., 2017; Sousa *et al*., 2018). Fungi *Neurospora intermedia* and *Rhizopus oryzae* were used to produce new protein-enriched products out of this by-product (Gmoser *et al*., 2020).

Past studies on the bioconversion of brewery by-products using fungi focused on the impact of substrate on fungal growth, yield, and nutritional composition of the biomass with emphasis on proteins, amino acids, fibers, and minerals (Hoa *et al*., 2015; Koutrotsios *et al*., 2014; Wang *et al*., 2001), while lipids were rarely targeted. Noteworthy, fungal lipids are gaining attraction in production of healthier and more sustainable alternatives for edible plant and/or animal lipids (Athenaki *et al*., 2018; Zhang *et al*., 2022). The interest in fungi as a source of proteins is not new and keeps increasing (Ciani *et al*., 2021; Ibarruri *et al*., 2021). This can be attributed to their white color and bland taste, and most importantly their filamentous texture that suits meat analog applications such as Quorn™ made using biomass fermentation (*Fusarium venenatum)* (Boukid *et al*., 2021b). Further understanding of the fat component of edible fungi could be of interest as a potential fat replacer to make healthy and sustainable plant-based products.

Therefore, this work aims to expand the knowledge on fungal efficiency and the bioconversion of by-products to fatty acids and sterols using edible fungi. Remarkably, *Pleurotus ostreatus* and *Lentinula edodes*, two widely used edible fungi, were cultivated using substrates made using brewery by-products. The first step consisted of the selection of suitable substrates for fungal growth. Afterward, the focus was attributed to assessing fatty acids and sterols profiles as a function of selected substrates.

Material and Methods

Material

For the purposes of this study, the fungal strains *P. ostreatus* (Jacq.) P. Kumm. (common name: Winter Oyster Mushroom) *M2191* and *L. edodes* (Berk.) Pegler (common name: Shiitake) *M3790* were purchased from Mycelia BVBA® (Veldeken, Belgium). Brewer's spent grains were collected from a local brewery (Mahou San

Miguel, Spain). Fresh brewer's spent grain contained 75.3% moisture, 2.4% total fat, and 7.6% protein, while dried brewer's spent grain contained 6.7% moisture content, 8.6% total fat, and 32.4% protein.

Substrate preparation and mushroom cultivation process

For cultivation, heat-resistant polypropylene bags (Sac O2®) were used. The formulation of substrates is reported in Table 1. All culture bags were filled with a final quantity of 4 L of substrate and autoclaved at 100°C for 2 h. The mushroom cultivation was performed with an inoculum concentration of 10% (dwt/dwt) (Hultberg *et al.*, 2018). In all cases, the inoculation ratio used was 1:30 (inoculum:substrate, v:v). The applied dose was higher than that usually used in the industrial cultivation of mushrooms, which allowed to shorten the incubation times (Aranaz *et al.*, 2021; Hultberg *et al.*, 2018). The viability of all the inoculums was verified in each experimental batch by seeding an aliquot on 3% malt extract agar plates.

Once inoculated, the bags were incubated at a temperature of 22–25°C for 5–7 weeks. After the incubation period, the substrate and the apparent absence of contaminants were verified, and the bags were transferred to fruiting chambers (temperature: 15 ± 3°C; relative humidity: 80–90%) to produce mushrooms. In all the experiments, a commercial substrate of edible mushrooms was used as a control. For each experiment, four replications were carried out.

Fatty acids profile

Fatty acids profile was assessed using a method reported in a previous work by Riudavets *et al.*, (2020). In brief, samples (500 mg) were extracted using a mixture of chloroform and methanol (2:1, v/v), derivatized with a mixture of toluene and 3 N HCl in methanol (1:4, v/v) at 80°C for 1 h and added with 10% NaCl in water and hexane (10:3, v/v). Fatty acids methyl esters were recovered in the organic phase and then separated on a gas chromatograph, Agilent 6890 Series II (Hewlett Packard SA, Barcelona, Spain), equipped with a capillary column DB23 (30 m × 0.25 mm i.d., 0.25 μm; Agilent, Santa Clara, USA), a split or splitless injector, and a flame ionization detector. Identification of single methyl esters was performed by comparing retention time of the peaks with those of pure standards; while quantification was carried out using tripentadecanoin (Merck KGaA, Darmstadt, Germany) as an internal standard.

Table 1. Formulation of substrates based on selected by-products.

Substrate	Composition
Control	Chestnut wood chips (1500 mL) + chestnut sawdust (1000 mL) + cereal seeds (equal parts of corn, barley, and wheat) (92.6 g) adjusted to 60% humidity and a pH of 5.5–6
A*	Dry brewer's spent grain (3 L) + H_2O (1.8 L) + control (1:3, v:v)
B*	Dry brewer's spent grain (1 L) + yeast lysate (500 mL) + H_2O (500 mL) + control (1:3, v:v)
C*	Fresh brewer's spent grain + control (1:3, v:v)
D*	Fresh brewer's spent grain + control (1:1, v:v)

*The pH of the substrates made with brewer's spent grain was adjusted by adding calcium carbonate (40 g/kg substrate) to reach 5.4.

Sterol profile

The lyophilized fungi (500 mg) were hydrolyzed in Soxcap (FOSS IBERIA, S.A., Barcelona, Spain) with 4 N HCl for 5 h. Subsequently, an extraction was carried out with 350 mL of hexane: diethyl ether (2:1, v/v). The internal standard (5α-cholestan-3β-ol) was added to the evaporated extract, and saponification was carried out using 8 mL of 9 N KOH for 3 h at 80°C. For the extraction of the unsaponified fraction, three aliquots of 12 mL of hexane and diethyl ether (2:1) and 4 mL of ethanol were added. The apolar fractions of the three extractions were dried and then derivatized with 50 μL of silanizing solution (Silylating mixture I according to Sweeley, Sigma Aldrich, Missouri, US) for 1 h at 80°C. The derivatized sample was dried using nitrogen flow, resuspended with 1 mL isooctane and 2-propanol and injected into the chromatographic equipment.

For chromatographic analysis, a CP-3800 gas chromatograph (Varían) equipped with a DB-5MS column (length: 30 m, diameter: 0.250 mm diameter, film thickness: 0.25 μm; Agilent Technologies, Santa Clara, US) was used. For chromatographic separation, an initial oven temperature of 80°C was used, with a gradient of 10°C/min up to 160°C, a gradient of 5°C/min up to 250°C, and a gradient of 1°C/min up to 285°C.

Statistical analysis

Fatty acids and sterols composition was determined in triplicate, and data were expressed as means ± standard deviations (SD). The Kruskal–Wallis test was performed to verify the substrate on fatty acids, sterols, and lipid indices as well as sterols. These tests were performed at a significance level of α = 0.05. Finally, to verify the influence of fungi on fatty acids profile, principal components analysis was performed. All the statistical analyses were

performed using IBM SPSS 24 statistical software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Substrate selection

Fungi showed different behaviors toward the substrates as illustrated in Table 2. *P. ostreatus* was able to grow on all substrates and yielded higher biomass than the control. This fungus is known to produce enzymes including lipase, phytase, xylanase, β-galactosidase, and cellulase that can break down organic biomass and use for its growth (Naim *et al*., 2020). The quantity of biomass cultivated on dry brewer's spent grains was higher than that that on fresh brewer's spent grains. This can be explained by the higher amount of nutrients in dry substrate compared to that in the fresh (less dry amount) one. Particularly, Substrate D ensured the highest biomass production. On the other hand, *L. edodes* was able to grow on all substrates except B. Substrate C made with a ratio of 1:3 (v/v) yielded the highest biomass, while the remaining substrates (A and D) had low yields. Compared to *P. ostreatus, L. edodes* showed lower growth rate, which can be attributed to the higher enzymatic activity in *P. ostreatus* favoring substrate degradation and thus fungal growth (Challa *et al*., 2019).

In the following sections, brewery by-products substrates yielding the highest biomass were retrieved and compared to that of the control. Therefore, for *P. ostreatus*, C and D are considered, and C was considered for *L. edodes*.

Impact of selected substrates on fatty acids profile

Irrespective to the substrates, six fatty acids (C14:0, C15:0, C16:0, C18:0, C18:1 (n-6c), and C18:2 (n-6c)) were identified in *P. ostreatus* biomass (Table 3). The main fatty acids found were C18:2 (n-6c) followed by C16:0 and C18:2 (n-6c), which was consistent with the previous works (Cardoso *et al*., 2020; Gnanwa *et al*., 2021; Pedneault *et al*., 2007). Results showed variations in the percentages of the single components and their concentrations, without significantly modifying the general profiles. More specially, substrates did not impact the percentage of fatty acids (C14:0, C15:0, C16:0, C18:1[n-9c] and C18:2[n-6c]) except C18:0. This indicates that by-products did not change the fatty acids as compared to the control. The concentration of C14:0 was significantly higher in the control than in biomasses grown on brewery by-products, while C16:0 was higher in C than in the control and D. As for C18:2(n-6c), C had significantly high values than that of the control and D. Total fatty acids varied significantly with higher values in C than those of the control and D, which showed that lower concentration of fresh spent grains favored formation of fatty acids. Similar trend was observed for monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and ω6. Total saturated fatty acid (SFA) was significantly higher in C. No significant differences were found in UFA and SFA/UFA as a function of substrates. The study results revealed PUFA as the dominant fatty acids, and this finding aligned with the previous studies focused on the same fungus but using different substrates (Cardoso *et al*., 2020; Gnanwa *et al*., 2021). Overall, growing *P. ostreatus* on brewery by-products did not affect the composition of fatty acids, suggesting their potential use instead of others expensive substrates. Noteworthy, all SFA/UFA were lower than 0.5, indicating that the fat of these biomasses could be a good candidate for use in human and/or animal foods (Sinanoglou *et al*., 2015; Wołoszyn *et al*., 2020). Indeed, the fatty acids profile was found comparable to that of soybean (C16:0: 5.5%; C18:2 [n-6c]: 33.2%) and peanut oils (C16:0: 9.55%; C18:2 [n-6c]: 65.8%) (Adjepong *et al*., 2017).

Table 2. Biomass resulting from fungi growth on different by-products substrates.

Substrates	*Pleurotus ostreatus*		*Lentinula edodes*	
	Growth	Biomass (g)	Growth	Biomass (g)
Control	+	173	+	217
A	+	243	±	99
B	+	208	-	0
C	+	300	+	293
D	+	324	±	84

+ Growth and production comparable to control; ± Growth and production much lower than control; - No growth or production.

Table 4 shows the fatty acids profile and lipid indices of *L. edodes* as a function of different substrates. Regardless of substrates, of the eight fatty acids (C14:0, C15:0, C16:0, C16:1 [n-7c], C18:0, C18:1 [n-9c], C18:2 [n-6c], and C18:3 [n-6c]), C18:2 (n-6c) was the most abundant, followed by C16:0. This aligns with the previous study findings of *L. edodes* cultivated using oak tree sawdust and rice (or wheat) bran (Chung *et al*., 2020). No significant differences were found among the percentages of fatty acids as a function of substrate. Nevertheless, the biomass of concentrations C16:0, C18:0, and C18:1(n-9c) was found to be significantly higher when grown on the control than on by-products. Total fatty acids, PUFA, ω6, and UFA were found to be unaffected by the substrate. As for SFA and MUFA, the control had higher values than the biomass grown on brewery by-products. Noteworthy, low

Table 3. Percentages and concentration of fatty acids and lipid indices in *Pleurotus ostreatus* biomasses grown on different substrates.

Fatty acids	Control	C	D	Significance
C14:0 (%)	0.281 ± 0.001	0.332 ± 0.071	0.401 ± 0.070	NS
C15:0 (%)	4.12 ± 0.69	3.75 ± 0.21	4.08 ± 0.11	NS
C16:0 (%)	18.09 ± 1.75	20.9 ± 0.65	21.65 ± 1.83	NS
C18:0 (%)	1.75 ± 0.45[b]	0.96 ± 0.13[a]	1.1 ± 0.08[a]	*
C18:1(n-9c) (%)	9.88 ± 0.89	6.17 ± 0.35	6.32 ± 0.35	NS
C18:2(n-6c) (%)	65.87 ± 2.25	67.88 ± 1.19	66.45 ± 2.37	NS
C14:0 (mg/100 mg)	0.002 ± 0.001[a]	0.004 ± 0.001[b]	0.004 ± 0.001[b]	*
C15:0 (mg/100 mg)	0.036 ± 0.003	0.042 ± 0.004	0.036 ± 0.003	NS
C16:0 (mg/100 mg)	0.157 ± 0.008[a]	0.234 ± 0.023[b]	0.194 ± 0.030[a]	*
C18:0 (mg/100 mg)	0.015 ± 0.004	0.011 ± 0.002	0.010 ± 0.001	NS
C18:1(n-9c) (mg/100 mg)	0.086 ± 0.015	0.069 ± 0.010	0.057 ± 0.007	NS
C18:2(n-6c) (mg/100 mg)	0.579 ± 0.039[a]	0.757 ± 0.062[b]	0.594 ± 0.017[a]	*
Lipid indices				
Total	0.875 ± 0.106[a]	1.116 ± 0.097[b]	0.895 ± 0.059[a]	*
SFA	0.210 ± 0.005[a]	0.290 ± 0.028[a,b]	0.245 ± 0.035[b]	*
MUFA	0.086 ± 0.015	0.069 ± 0.011	0.057 ± 0.007	NS
PUFA	0.579 ± 0.039[a]	0.757 ± 0.062[b]	0.594 ± 0.017[a]	*
ω6	0.580 ± 0.150[a]	0.760 ± 0.06[b]	0.5911 ± 0.017[a]	*
UFA	0.665 ± 0.089	0.827 ± 0.072	0.651 ± 0.024	NS
SFA/UFA	0.321±0.041	0.351 ± 0.017	0.375 ± 0.039	NS

MUFA = sum of all monounsaturated fatty acids; PUFA = sum of all polyunsaturated fatty acids; SFA = sum of all saturated fatty acids; UFA = MUFA + PUFA; SFA/UFA = ratio of total saturated fatty acids/total monounsaturated fatty acids + total polyunsaturated fatty acids. Values are shown as means ± standard deviation.
Means within a row with different letter differ significantly, *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; NS = not significant.

SFA/UFA values (around 0.2) were obtained, which were within the same range of that of canola oil (Tartrakoon *et al.*, 2016). Further investigation is needed to study the application of *L. edodes* grown on brewery by-products as potential animal-free fat substitute.

Principal component analysis based on fatty acid concentrations and lipid indices enabled a better understanding of the impacts of substrate and fungi simultaneously. The overall variability explained by the first two components was 89%, where the first component (PC1) accounted for 55% and the second (PC2) for 34%. As illustrated in Figure 1A, PC1 was explained as a function of C18:3(n-6c), SFA/UFA, C15:0, C16:1(n-7c), C18:1(n-9c), MUFA, ω6, PUFA, C18:2(n-6c) and UFA; whereas PC2 was a function of SFA, C16:0, C18:0, total fatty acids, and C14:0.

The projection the substrate and fungal strains on the factorial spaces created by the first two components showed that each fungus took a side of PC1 (*L. edodes was* on the positive side *and P. ostreatus* on the negative side) (Figure 1B). Variability between fatty acid compositions of fungal biomass can be attributed to different enzyme activities and types (lipase) responsible for fatty acid release and degradation (Chandra *et al.*, 2020; López-Fernández *et al.*, 2020). *L. edodes* control and that grown on by-products were located in opposite sides of PC2, where the control had the highest values of C18:3(n-6c), C16:1(n-7c), ω6, SFA, PUFA, C18:0, C14:0, C16:0, C18:2(n-6c), UFA, and total fatty acids, contrary to fungus grown on C. As for *P. ostreatus,* three biomasses were characterized by high amounts of C15:0, MUFA, SFA/UFA, and C18:1(n-9c). These results confirmed the variability between substrates and fungus, yet no clear clustering was found.

Impact of selected substrates on sterols profile

Sterol profiles of fungal biomass are summarized in Tables 5 and 6. Regardless of the used fungus, no significant differences were found among sterols as a function of substrate except for estigmastanol in the case of *P. ostreatus*. In this case, the control had higher value than that of biomass grown on by-products. In *L. edodes,* only cholesterol and campesterol contents were significantly different from the control. Cholesterol was found higher

Table 4. Percentages and concentration of fatty acids and lipid indices in the biomasses of *Lentinula edodes* grown on different substrates.

Fatty acids	Control	C	Significance
C14:0	0.190 ± 0.040	0.260 ± 0.031	NS
C15:0	1.68 ± 0.73	1.48 ± 0.28	NS
C16:0	14.24 ± 1.41	13.75 ± 0.72	NS
C16:1(n-7c)	0.340 ± 0.071	0.361 ± 0.030	NS
C18:0	1.57 ± 0.45	0.75 ± 0.04	NS
C18:1(n-9c)	1.62 ± 0.24	1.75 ± 0.35	NS
C18:2(n-6c)	79.88 ± 2.73	81.01 ± 1.42	NS
C18:3(n-6c)	0.47 ± 0.21	0.63 ± 0.02	NS
C14:0 (mg/100 mg)	0.003 ± 0.001	0.003 ± 0.001	NS
C15:0 (mg/100 mg)	0.029 ± 0.011	0.017 ± 0.001	NS
C16:0 (mg/100 mg)	0.246 ± 0.002	0.164 ± 0.025	*
C16:1(n-7c) (mg/100 mg)	0.006 ± 0.001	0.004 ± 0.001	NS
C18:0 (mg/100 mg)	0.027 ± 0.005	0.009 ± 0.001	*
C18:1(n-9) (mg/100 mg)	0.028 ±0.001	0.021 ± 0.000	*
C18:2(n-6c) (mg/100 mg)	1.388 ± 0.194	0.973 ± 0.217	NS
C18:3(n-6c) (mg/100 mg)	0.008 ± 0.004	0.008 ± 0.002	NS
Lipid indices			
Total	1.735 ± 0.184	1.199 ± 0.246	NS
SFA	0.304 ± 0.013	0.193 ± 0.027	*
MUFA	0.034 ± 0.002	0.025 ± 0.001	*
PUFA	1.397 ± 0.199	0.981 ± 0.218	NS
ω6	1.4 ± 0.2	0.98 ± 0.22	NS
UFA	1.43 ± 0.197	1.005 ± 0.219	NS
SFA/UFA	0.215 ± 0.039	0.194 ± 0.015	NS

MUFA = sum of all monounsaturated fatty acids; PUFA = sum of all polyunsaturated fatty acids; SFA = sum of all saturated fatty acids; UFA = MUFA + PUFA; SFA/UFA = ratio of total saturated fatty acids/total monounsaturated fatty acids + total polyunsaturated fatty acids. Values are shown as means ± standard deviation.
Significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; NS = not significant.

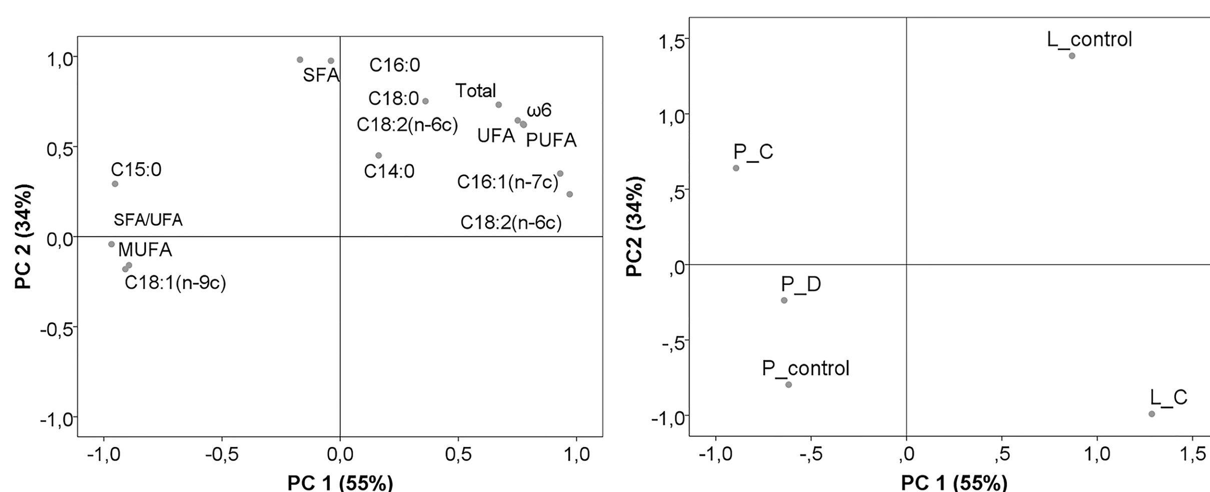

Figure 1. Scattering the data of fatty acids and lipid indices by the first two principal components (PC1 and PC2) analysis of fungi grow on brewery by-products. (A) Biplot of the first two components created, considering fatty acids and lipid indices; (B) Rotated principal scores of fungi and their corresponding substrates projected into the first two principal components. P_control: P. ostreatus grown on the control substrate; P_C: *P. ostreatus* grown on C substrate; P_D: *P. ostreatus* grown on D substrate; L_control: *L. edodes* grown on control substrate; L_C: *L. edodes* grown on C substrate.

Table 5. Concentration (mg/g) of phytosterols and cholesterol in *Pleurotus ostreatus* biomasses as a function of substrate.

Control	C	D	Significance	
Cholesterol	0.043 ± 0.032	0.032 ± 0.018	0.027 ± 0.006	NS
Ergosterol	0.252 ± 0.035	0.190 ± 0.064	0.223 ± 0.020	NS
Campesterol	0.059 ± 0.019	0.025 ± 0.006	0.038 ± 0.014	NS
Stigmaesterol	0.002 ± 0.003	n.d.	n.d.	NS
ß-sitoesterol	0.018 ± 0.003	0.009 ± 0.001	0.013 ± 0.012	NS
Estigmastanol	0.02 ± 0.011^{b}	0.001 ± 0.002^{a}	0.003 ± 0.002^{a}	**

Values are shown as means ± standard deviation.
Means within a row with different letter differ significantly, *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; NS = not significant.

Table 6. Concentration (mg/g) of phytosterols and cholesterol in *Lentinula edodes* biomasses as a function of substrate.

Control	C	Significance	
Cholesterol	0.048 ± 0.006	0.011 ± 0.004	*
Ergosterol	0.225 ± 0.117	0.274 ± 0.072	NS
Campesterol	0.070 ± 0.001	0.124 ± 0.018	*
Stigmaesterol	n.d.	0.019 ± 0.018	NS
ß-sitoesterol	0.056 ± 0.032	0.063 ± 0.019	NS
Estigmastanol	0.017 ± 0.015	0.029 ± 0.004	NS

Values are shown as means ± standard deviation.
Significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; NS = not significant.

in the control, indicating that growing fungi on by-products ensured a low cholesterol, possibly due to the degradation of cholesterol deriving from the substrate by fungi to use for their metabolism (El-Gendy *et al.*, 2016; Hyde *et al.*, 2019). This aligns with the evidence from previous studies that indicated the effectiveness of fungi in lowering cholesterol levels (Keong 2015).

Irrespective of substrate, *P. ostreatus* profile was characterized by the predominance of ergosterol and campesterol, and traces of other phytosterols and cholesterol similar to that of *L. edodes*. The amount of ergosterol was double that of fungi cultivated on sugarcane bagasse–based substrates (Cardoso *et al.*, 2020), this finding was in alignment with that of the data reported in a systematic review by Weete *et al.*, (2010). Ergosterol is a metabolite synthesized by fungi, which cannot be synthesized by plants (Baur *et al.*, 2016). This suggests the potential valorization of these biomasses as a source of vitamin D in contrary to most vegetable oils (e.g., peanut, soybean, rapeseeds, and olives) characterized by β-sitosterol and campesterol as the predominant phytosterols (Yang *et al.*, 2019). Furthermore, both fungi had relevant amounts of total phytosterol comparable to soybean, peanut, and olive oils (~0.300 mg/100 mg) but lower than rice bran (1.89 mg/100 mg), corn (0.990 mg/100 mg), and rapeseed oils (0.893 mg/100 g) (Yang *et al.*, 2019).

Conclusion

To conclude, the use of brewery by-products as substrates was efficient to ensure the fungal growth. Fatty acids composition was significantly impacted by fungal strain and substrate formulation. In *P. ostreatus* biomass, six fatty acids were found, where C18:2 (n-6c) was the most dominant. As for *L. edodes*, eight fatty acids were identified, with C18:2 (n-6c) being the most abundant. Interestingly, both fungi used in this study resulted in different fatty acids profiles that are comparable to vegetable oils with the advantage of having low SFA/UFA ratios. Sterols profile showed that regardless of the fungal strain, no significant differences were found among sterols as a function of substrate except for estigmastanol (*P. ostreatus*). Ergosterol was found as the primary sterol suggesting the potential valorization of these biomasses as a source of vitamin D. In addition, fungal biomass showed a low cholesterol level suggesting the cholesterol-lowering effect of fungus. This might be used as a mitigation strategy and thus valorize a wider range of by-products having a high initial cholesterol. Overall, these findings confirm the efficiency of fungi as bioconvertors of agro-industrial by-products to produce fats with interesting properties. More investigations are required to determine their functionality and applicability as a nonanimal fat substitute in food reformulation.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

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